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Further Observations on the Cystine Deficiency of Lucerne Proteins and the Effect of Heat and Incubation upon their Growth-Promoting Value.

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LUCERNE is not only high in minerals and vitamins but also in proteins, yet, curiously enough, it is rather low in cystine as determined biologically with rats by Haag (1931). This discovery may be of importance to parts of this and other countries where some domestic animals are, for certain seasons of the year, almost solely subsistent on the lucerne plant. However, it is dangerous to reason by analogy from one species to another, and it cannot be inferred, therefore, that lucerne is also low in cystine for animals such as, for instance, sheep and cattle, in view of the great anatomical and physiological differences in their digestive systems. Furthermore, even though lucerne is low in cystine, the large capacity of ruminants for food may make it possible for them to obtain enough of this essential amino-acid to promote normal growth and production. Another probability is that sheep may be capable of synthesising cystine through a special function of their wool follicles as suggested by Fraser and Roberts (1932), or through a symbiotic action of their intestinal flora and fauna (bacteria, protozoa, etc.), as postulated by Rimington and others (1932, 1933). The latter hypothesis has given impetus to studies in this field, and the preliminary experiments, to be presented in this paper, have been carried out with the object of seeing whether Haag's results could be substantiated and what the effect of heat and incubation with a sheep's "ruminal juice" would be upon the utilization of lucerne-meal* proteins by the rat. Furthermore, because maize, lucerne and teff (*Eragrostis Abyssinica*) form three of the major food materials for animals in this country, it was thought of interest to study also the supplementary values of maize-lucerne and maize-teff proteins.

* By the term "lucerne meal" in this paper is meant only that part of commercial lucerne meal that passed through a 1 mm. sieve.

EXPERIMENTAL.

Albino rats of about three to four weeks old and of the London strain of the Wistar Institute stock were used in these experiments. Each rat was kept in a separate cage with a raised screen bottom. The paired-feeding method of Mitchell and Beadles (1930) was employed, and all pairs were " isogenic ", that is, they were of the same sex, litter and as nearly as possible of the same weight. There were six pairs to each group. The initial and final weights of the rats were the average of weighings taken on three consecutive days. During the rest of the experimental period they were weighed once a week. Fresh distilled water was at all times available to the animals. The method of feeding was the same as that described by Mitchell (1933), namely:—

> In each pair, both rats were given the same amount of food, determined at any time by the rat that was eating the least . . . During the process of equating each day the food intakes of pair mates, the food offered was increased by 1 gram if both pair mates cleaned up, or was left the same as that of the preceding day if only a small amount of food was refused by either rat, or was decreased if either rat left a considerable amount, the size of the reduction being gauged roughly by the amount of orts.

The composition of the rations is given in Table I.

The rations utilized were—

(*a*) lucerne meal with and without 0·1 per cent. cystine;

(*b*) lucerne-yellow maize meal with and without 0·15 per cent. cystine, and

(*c*) teff*-yellow maize meal with and without 0·15 per cent. cystine.

The diets given to pair mates were therefore identical with the only difference that one of them contained in addition a small amount of l-cystine. The lucerne meal-cystine ration consisted of 100 gm. lucerne meal ration supplemented with 0·1 gm. cystine, whereas the corresponding cystine rations of lucerne-yellow maize and teff-yellow maize meal consisted of 100 gm. of the respective basal diets supplemented with 0·15 gm. cystine. The latter had a rotation of $(x)\ ^{27}_{D}$ —213·5 as determined by the method of Andrews (1925) on a 1 per cent. solution in N hydrochloric acid [accepted value $(x)\ ^{27}_{D}$ —209·2 according to the formula of Toennies and Lavine (1930)]. The cod liver oil was a first grade Norwegian oil, ant the butter was filtered butter fat. The brewer's yeast was a dried commercial sample which had been found effective in aiding growth and preventing polyneuritis when added at a 5 per cent. level to a vitamin B-free ration. Its nitrogen, as explained in Table I, was not calculated as protein-nitrogen. The protein contents of the lucerne meal, lucerne-yellow maize and teff-yellow maize meal basal rations were 10·44, 13·94 and 8·37 per cent. respectively.

* Dry teff-hay was ground up into a fine powder and only that part was used that passed through a 1 mm. sieve.

The results are given in Tables II, III, and IV.

TABLE I.

Composition of Rations in Percentage by Weight.

	Nitrogen.	Protein.	Lucerne meal.	Lucerne-yellow maize meal.	Teff-yellow maize meal.
Lucerne meal 1...	3·340	20·88	50·0	—	—
Lucerne meal 2...	3·166	19·79	—	50·0	—
Teff meal.....	1·382	8·64	—	—	50·0
Yellow maize meal	1·620	10·12	—	40·0	40·0
Brewer's yeast....	7·204	(45·02)	2·0	2·0	2·0
Dextrinised starch.	—	—	26·5	—	—
Sucrose.....	—	—	5·0	—	—
Lard.....	—	—	9·0	—	—
Butter fat (filtered)	—	—	4·0	4·0	4·0
Cod liver oil.....	—	—	1·0	1·0	1·0
$NaH_2PO_4.H_2O$....	—	—	1·5	—	—
Bone ash.....	—	—	—	2·0	2·0
NaCl.....	—	—	1·0	1·0	1·0
TOTALS.....	—	—	100·0	100·0	100·0
Total nitrogen..	—	—	1·814	2·375	1·483
Corr. total nitrogen.....	—	—	1·670	2·231	1·339
Total protein...	—	—	10·44	13·94	8·37

Lucerne meal—cystine ration = 100 gm. lucerne meal ration + 0·1 gm. cystine.

Lucerne-yellow maize—cystine ration = 100 gm. lucerne-yellow maize meal ration + 0·15 gm. cystine.

Teff-yellow maize—cystine ration = 100 gm. teff-yellow maize meal ration + 0·15 gm. cystine.

The nitrogen of the yeast was not calculated as protein-nitrogen in view of the fact that Still and Koch (1928) when measuring by Mitchell's method the biological values of diets containing one half of the nitrogen from yeast and one half from casein (total N = 2·9%) found little supplementary relation between the two proteins.

In Table II are given the individual gains in weight of each of six pairs of rats over the experimental period. The periods during which these gains accumulated differ, however, for the different pairs and hence the mean gains per week are rather to be considered. The differences in gain were all in favour of the cystine supplemented ration, with an average difference in gain (m) of 3·533 gm. per week for all the pairs. These differences vary with a standard deviation for each pair difference(s) of 0·51 gm., and hence the standard deviation of the mean difference for all six pairs (Sm.) is equal to 0·2082.

On the assumption that the cystine supplement had no effect on the gain in weight a mean difference in gain for these pairs which does not differ appreciably from zero, should be expected. However,

TABLE II.

The Value of Cystine as a Supplement to the Proteins of Lucerne-meal.

(All weights in grams.)

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.		Mean.
	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	
Initial weight.....	81	81	69	67	65	64	60	59	66	65	75	74	—
Final weight.....	102	137	82	105	93	119	89	113	100	121	106	130	—
Total gain.....	21	56	13	38	28	55	29	54	34	56	31	56	—
Total food.....	423	423	355	355	344	344	340	340	377	378	409	409	—
Weeks on rations.....	8	8	8	8	7	7	8	8	7	7	7	7	—
Comparison of wk. gains.	1	7	1·5	6·5	1	6	2	6	1·5	5·5	0·5	6·5	—
Difference in mean gain per week (cystine- control).....	4·375		3·125		3·857		3·125		3·143		3·571		3·533

TABLE III.

The Value of Cystine as a Supplement to the Proteins of Lucerne-yellow Maize Meal.

(All weights in grams.)

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.		Mean.	
	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.
Initial weight...	88	89	53	53	59	60	56	57	55	57	53	52	60·6	61·3
Final weight....	181	191	134	137	190	184	192	188	136	143	165	194	166·3	172·83
Total gain.....	93	102	81	84	131	124	136	131	81	86	112	142	105·6	111·5
Total food.....	763	763	618	618	762	762	823	823	659	659	819	819	—	—
Weeks on rations	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Comparison of wk. gains.....	3	5	4	4	4	4	4	4	4·5	3·5	2	6	21·5	26·5
Difference between total gains. (Cystine - control).....	9		3		−7		−5		5		30		5·833 (S.D. = 13·303.)	

Table IV.

The Value of Cystine as a Supplement to the Proteins of Teff-yellow Maize Meal.

(All weights in grams.)

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.		Mean.	
	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.
Initial weight...	75	76	66	66	66	68	61	62	56	55	60	59	64·0	64·3
Final weight....	131	134	111	118	138	138	134	137	109	109	111	111	122·3	124·5
Total gain.....	56	58	45	52	72	70	73	75	53	54	51	52	58·3	60·16
Total food.....	621	621	577	577	576	576	580	580	530	530	543	543	—	—
Weeks on rations	6	6	6	6	6	6	6	6	6	6	6	6	—	—
Comparison of wk. gains.....	2	4	3	3	3	3	2	4	2·5	3·5	3	3	—	—
Difference between total gains (Cystine - control).....	2		7		−2		2		1		1		1·83 (S.D. = 2·9268).	

to test the validity of such an hypothesis the mean difference (m) is compared with its standard deviation (Sm) and calculate (Fisher's 1932) $t = \frac{m}{Sm} = 16 \cdot 969$. This value is obviously very significant, showing that the probability of finding a mean difference,, for 6 pairs, on similar rations, nearly 17 times its standard deviation, is exceedingly small. It can thus safely be concluded that the gain in weight of the rats which received added cystine was definitely greater than that of their control mates.

This conclusion is further supported by the fact that in the case of all six pairs, the rat on the cystine diet gained more in weight than the control. If the chances were even the differences should all be in favour of one of the treatments in $2 \times (\frac{1}{2})^6$ of the cases, that is, once in 36 cases, which is less than 4 per cent.

This result is even more strongly brought out if the number of weekly gains in favour of the rats receiving cystine is compared with those in favour of the control animals. There were altogether 45 weekly gains of which 37·5 were in favour of cystine. The number of weekly gains in favour of cystine to be expected if the chances were even is equal to $\frac{45}{2} = 22 \cdot 5$. Hence the deviation from expectation = 15. The standard deviation of this deviation is $\sqrt{\cdot 5 \times \cdot 5 \times 45}$ $(= \sqrt{npq}) = 3 \cdot 35$. The number of weeks being as high as 45, t (calculated as before) $= \frac{22 \cdot 5}{3 \cdot 35} = 6 \cdot 76$ and X the t table is entered with $n = 44$. This gives significance at the 1 per cent. point in favour of the cystine rats.

Table III gives the total gains in weight of 6 pairs of rats over a period of 8 weeks. During this period all the rats received the same lucerne-yellow maize meal ration with a 0·15 per cent. cystine supplement to one animal in each pair. Although the mean difference between the total gains in weight within the six pairs is 5·83 gm. in favour of cystine, the difference is insignificant compared with its standard deviation which is 5·431 gm. Neither is there any significant response to either treatment in the numbers of pair and weekly gains, thus confirming the observations of McCollum, Simmonds and Pitz (1917) that maize and lucerne can correct the deficiencies in one another's proteins.

From Table IV it will be seen that the effect of a cystine supplement to the proteins of a teff-yellow maize meal, on the gain in weight for the six pairs of rats over a period of 6 weeks, is negligible. The mean difference of 1·83 gm. in favour of the cystine supplement is very insignificant compared with a S.D. of a single difference equal to 2·927 gm. Likewise the deviations of the numbers of pair and weekly gains from their hypothetical values, when the chances were even, are well within the ranges that could reasonably be allowed for random sampling errors.

The teff experiment was repeated with a 0·3 gm. cystine supplement but the results duplicate those of the preceding experiment to such a considerable extent that they have been omitted and need not be further described. One is therefore led to the conclusion that a combination of maize and teff proteins is efficient for growth.

TABLE V.

Composition of Rations in Percentage by Weight.

	Nitrogen.	Protein.	Group I.		Group II.	
			Lucerne meal.	Incubated lucerne meal.	Lucerne meal.	Autoclaved lucerne meal.
Lucerne meal I	3·411	21·32	45·0	—	—	—
Incubated lucerne meal I	3·360	21·00	—	45·0	—	—
Lucerne meal II	3·631	22·69	—	—	45·0	—
Autoclaved meal II	3·160	19·75*	—	—	—	45·0
Dextrinised starch	—	—	21·5	21·5	27·5	27·5
Sucrose	—	—	5·0	5·0	5·0	5·0
Lard	—	—	10·0	10·0	10·0	10·0
Butter fat	—	—	4·0	4·0	4·0	4·0
Molasses (cane)	—	—	6·0	6·0	—	—
Brewer's yeast	7·204	(45·02)	5·0	5·0	5·0	5·0
Cod liver oil	—	—	1·0	1·0	1·0	1·0
$NaH_2PO_4.H_2O$	—	—	1·5	1·5	1·5	1·5
NaCl	—	—	1·0	1·0	1·0	1·0
TOTALS	—	—	100·0	100·0	100·0	100·0
Total nitrogen	—	—	1·896	1·872	1·994	1·782
Corr. total nitrogen	—	—	1·536	1·512	1·634	1·422
Total protein	—	—	9·60	9·45	10·21	8·89

* Some of the "juice," containing proteins amongst others, was accidentally lost during the autoclaving process.

The following preliminary experiment is based upon Rimington and Bekker's (1932) hypothesis, and it deals with the effect of incubating lucerne meal with " ruminal juice " of a sheep on the growth-promoting value of the lucerne proteins. It was thought that if, for instance, the bacteria in a sheep's rumen could synthesise cystine to an appreciable extent, it might be possible to demonstrate biologically with rats the increase in cystine. The latter is, as was shown, the limiting factor in the lucerne proteins and, if therefore, an additional amount of it has been synthesised by the bacteria, then, if all other values remained the same, the growth-promoting value of the incubated lucerne ought to be superior to the untreated material.

In order to test the validity of this assumption, an adult merino wether was fed for one month on lucerne hay. It was then slaughtered and the ruminal contents removed immediately. The latter weighed 3·629 Kg. with an average moisture content of 85·5 per cent. The contents were put at once into glass jars and incubated for three days at 37° C. Once daily they were thoroughly mixed by hand in order to ensure an even bacterial development. At the end of this period the contents were diluted with water at about 37° C. and the " juice " strained through muslin. This procedure was repeated once and the combined portions of " juice " were then added to and mixed with 1·5 Kg. of lucerne meal. This in turn was incubated for two days at 37° C. after which it was dried at approximately 70° C. in a hot air oven and incorporated in the rations. Concurrently with this experiment there was run one with rats in which the effect of autoclaving on the growth-promoting value of lucerne proteins was also determined. 1·5 Kg. of lucerne meal was moistened with warm water and autoclaved in glass jars for 1 hour at 1 Kg. pressure (120° C.) and finally dried as described before.

The animals refused to eat the incubated lucerne meal ration for the reason that it carried an acid odour resembling butyric acid as was found by Bechdel, Honeywell, Dutcher and Knutsen (1928). The incorporation of 5 per cent. of brewer's yeast and 6 per cent. of cane molasses in the ration proved to make it palatable, and at the same time supplied an optimum amount of the vitamin-B complex. The composition of the rations finally adopted is given in Table V.

The table shows that the protein contents of the control and incubated rations in group I were 9·60 and 9·45, and of the control and autoclaved rations in group II, 10·21 and 8·89 per cent. respectively. Therefore the protein contents of the rations in group I differed only by 0·15 per cent. in favour of the control ration, whereas those of group II differed by as much as 1·32 per cent. in favour of the control diet. This great difference in protein contents was brought about by an accidental loss of some of the " juice " during the autoclaving process.

The results are given in Tables VI, VII, and VIII.

Table VI.

The Effect of Incubating Lucerne-meal with "Ruminal Juice" of Sheep for Two Days at 37° C. upon the Utilisation of Lucerne-porteins by the Rat.

(All weights in grams.)

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.		Mean.	
	Control	Incubated.	Control.	Incubated.	Control	Incubated.	Control.	Incubated.	Control.	Incubated.	Control.	Incubated.	Control.	Incubated.
Initial weight...	76	75	93	92	80	80	86	87	74	75	92	92	83·5	83·5
Final weight....	92	84	108	102	114	94	98	98	92	75	109	107	102·16	94·16
Total gain.....	16	9	15	10	34	14	12	11	18	0	17	15	18·6	9·5
Total food.....	430	430	454	451	507	507	387	387	374	374	411	411	—	—
Weeks on rations	8	8	8	8	8	8	8	8	8	8	8	8	—	—
Comparison of wk. gains.....	4·5	3·5	4	4	7·5	0·5	4	4	5·5	2·5	5	3	—	—
Difference between total gains (Control - Cystine).....	7		5		20		1		—		2		7 (S.D.= 7·649).	

TABLE VII.

The Effect of Autoclaving Lucerne-meal for One Hour at 1 Kg. Pressure (120° C.) upon the Utilisation of Lucerne-proteins by the Rat.

(All weights in grams.)

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.		Mean.	
	Control.	Autoclaved.	Control.	Autoclaved.	Control.	Autoclaved.	Control.	Autoclaved.	Control.	Autoclaved.	Control.	Autoclaved.	Control.	Autoclaved.
Initial weight...	76	75	72	71	68	69	63	63	63	64	67	68	68·16	68·3
Final weight....	118	98	119	106	98	100	84	79	86	74	80	81	97·5	89·6
Total gain.....	42	23	47	35	30	31	21	16	23	10	13	13	29·3	21·3
Total food.....	519	519	521	522	432	432	392	392	369	369	314	314	—	—
Weeks on rations	8	8	8	8	8	8	8	8	8	8	8	8	—	—
Comparison of wk. gains.....	7	1	5	3	3·5	4·5	5	3	6	2	5	3	—	—
Difference between total gains (Control-Autoclaved).....	19		12		—1		5		13		0		8 (S.D. = 7·95.)	

TABLE VIII.

The Growth-promoting Values of Fresh, Incubated and Autoclaved Lucerne-meal Proteins, as Determined by the Method of Osborne, Mendel and Ferry (1919) over a Period of Eight Weeks.

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.		Mean.
	Control.	Incubated.	Control.	Incubated.	Control.	Incubated.	Control.	Incubated.	Control	Incubated.	Control.	Incubated.	
Protein consumed P (gm.)	41·28	40·63	43·58	42·62	48·67	47·91	37·14	36·57	35·90	35·34	39·46	38·84	—
Percentage of total food intake	9·60	9·45	9·60	9·45	9·60	9·45	9·60	9·45	9·60	9·45	9·60	9·45	—
Weight increase W (gm.)	16·00	9·00	15·00	10·00	34·00	14·00	12·00	11·00	18·00	0·00	17·00	15·00	—
Ratio W/P (or G–P value)	0·387	0·222	0·344	0·204	0·698	0·292	0·323	0·301	0·501	0·00	0·431	0·386	—
Difference in rations W/P (Control-incubated)	0·165		0·140		0·406		0·022		—		0·045		0·1556 (S.D. 0·1526.)
	Control.	Autoclaved.	Control.	Autoclaved.	Control.	Autoclaved.	Control.	Autoclaved.	Control.	Autoclaved.	Control.	Autoclaved.	
Protein consumed P (gm.)	52·99	46·14	53·19	46·40	44·11	38·40	40·02	34·85	37·67	32·80	32·06	27·91	—
Percentage of total food intake	10·21	8·89	10·21	8·89	10·21	8·89	10·21	8·89	10·21	8·89	10·21	8·89	—
Weight increase W (gm.)	42·00	23·00	47·00	35·00	30·00	31·00	21·00	16·00	23·00	10·00	13·00	13·00	—
Ratio W/P (or G–P value)	0·793	0·498	0·884	0·754	0·680	0·807	0·525	0·459	0·611	0·305	0·405	0·466	—
Difference in ratios W/P (Control-Autoclaved)	−0·295		−0·130		−0·127		−0·066		−0·306		−0·061		−0·1015 (S.D. 0·1844).

In Table VI it is important to note that the rat which received incubated lucerne meal in the fifth pair gained nothing in weight. At first its growth rate compared well with that of its pair mate but during the progress of the experiment this rat became ill for some unknown reason and lost all the weight it had previously gained. The fifth pair is therefore ignored in the considerations. The mean difference between the gains in weight, 7 gm. is in favour of the control group, but compared with its standard deviation of 7·65 gm. is insignificant. A difference of this magnitude could be due to random sampling, without any treatment effect, in more than 30 cases out of every 100. Neither is there any significance in the differences between the numbers of pair and weekly gains.

Table VII shows a mean difference between the total gains in weight of rats receiving autoclaved lucerne-meal and the controls of 8 gm. in favour of the latter. The standard deviation of this mean difference is equal to 3·2455 gm. Hence t, which is equal to 2·464, fails to be significant for 5 degrees of freedom. There is also no significance in either the number of pair or weekly gains.

In Table VIII are given the W (weight increase)/P (protein consumed) ratios as calculated from the two preceding tables by the method of Osborne, Mendel and Ferry (1919). Again ignoring the 5th pair in Table VI the mean differences within pairs are equal to 0·1556 and 0·1015 from the two tables respectively. Both differences are insignificant compared with their respective standard deviations of 0·06823 and 0·07527.

It may be concluded, therefore, that incubation and autoclaving had, under the experimental conditions, no effect on the growth-promoting value of lucerne proteins.

DISCUSSION.

More attention has already been given to the study of the lucerne proteins than to those of any other hay or grass. This is due, no doubt, to the good qualities of lucerne, and its extensive use as a hay and green food in the feeding of most domestic animals. Of course, the qualities of a protein depend largely upon its growth-promoting value, and the latter again is dependent upon its essential amino acid-balance. According to Hagg's (1930) and the above observations it seems that lucerne proteins contain enough of all of the essential amino acids (except cystine) for growth in rats. These proteins must be quite low in cystine because Haag (1930) has found that rats on a ration containing 15·04 per cent. of them and only 0·05 per cent. of added cystine made better growth than animals on the same ration without added cystine.

The only paper in the literature in which the cystine content of lucerne is given as determined on the whole plant is that by Woodman, Evans and Norman (1933). They determined the cystine content of lucerne by the method of Evans (1931) which "depends on the assumption that cystine, either in the free or combined state, yields two-thirds of its sulphur as sulphide on treatment with alkali" and found it to range from 0·09 to 0·15 per cent. of the dry matter

with a mean value of 0·12 per cent. This value is of the same order as was found by Evans (1931) for pasture herbage. Dr. Henrici of the Veld Reserve, Fauresmith, was kind enough to determine the cystine content of a sample of lucerne meal according to her method (1932). The sample contained 17·76 per cent. of crude protein, and she found its cystine content to vary from 0·070 to 0·076 per cent. of the dry matter with a mean value of 0·073 per cent.

Although the methods of Woodman *et. al.* and Henrici for determining cystine in grasses are quite different in principle, it is of interest to note that their results show a fair degree of similarity, and were it not that Woodman and co-workers omitted to give the protein contents of their samples, their cystine values might even have been more comparable with those of Henrici. However, the results given above are by far still too divergent to warrant any definite conclusions as to the cystine content of lucerne and, moreover, the various chemical methods for determining cystine in plant materials not only differ much in principle but all of them offer small hope of eliminating all of the many interfering substances present in hays and grasses. The chemical and biological methods available at present for evaluating the cystine content of hays and grasses, therefore, seem to fall in the same category in so far that they only give relative values upon which it will be rather dangerous to found any hypothesis.

If the average cystine values of 0·073 per cent. for lucerne and 0·72 per cent. for top brewer's yeast, as found by Henrici, and Prunty (1933) respectively, are taken as more or less correct, then a synthetic ration containing 50 per cent. of lucerne meal and 2 per cent. of brewer's yeast will only possess 0·05 per cent. of cystine. This value is undoubtedly too low for promoting normal growth in rats and needs no further comment. On the other hand, however, if it is assumed that an adult Merino sheep consumes on an average 4 lb. of dry hay per day with an average cystine content of 0·073 per cent., it will take in 1·07 lb. of cystine per annum. On the further assumption that only 50 per cent. of the cystine in lucerne is actually retained in the animal for purposes of wool-protein synthesis, the animal will still have left enough cystine for producing a 4·1 lb. fleece of clean or about 8·5 lb. of grease wool which is a good average production per head of sheep under good management, and it is clear, therefore, that even though a food substance is low in cystine it may still contain sufficient cystine for normal wool growth.

It should also be pointed out that because the incubation of lucerne meal with the " ruminal juice " of a sheep did not enhance the growth-promoting value of the lucerne proteins, it does not in the least disprove the theory of Rimington and Bekker (1932) that sheep may be capable of synthesising cystine through a symbiotic action of their intestinal flora. It may be that cystine was synthesised by the bacteria but in such minimal amounts that it could not be detected under these experimental conditions. In order to test the validity of the assumption that the bacteria in a sheep's rumen may be capable of synthesising cystine, more delicate methods will have to be resorted to in future, and it may not be out of place to mention here that experiments are in progress in this laboratory in which the

effects of feeding bacteria, isolated from a sheep's rumen and cultivated in an incubator, to young rats on a cystine deficient diet, are studied, and will be presented as soon as more data are available. Furthermore by incubating lucerne meal with a sheep's " ruminal juice " the function of the protozoa has been terminated as they die off very soon after removal from the rumen, and it may be that only they and not the bacteria and yeasts are capable of synthesising cystine.

SUMMARY.

(1) A study has been made of the supplementary value of cystine to the proteins of lucerne, lucerne-yellow maize and teff-yellow maize, and the effect of heat and incubation with a sheep's " ruminal juice " upon the growth-promoting value of lucerne proteins.

(2) Young rats fed upon a ration with lucerne-meal as the only source of protein suffered from a deficiency which was immediately removed by the addition to the ration of 0·1 per cent. of l-cystine. During a period of 6 to 8 weeks the cystine fed rats gained on an average 26·5 gm. more than their control mates.

(3) A combination of maize and lucerne or maize and teff proteins was found adequate for growth and left no amino-acid deficiencies under the experimental conditions. The growth-promoting value of the combined proteins were not enhanced by the addition of 0·15 per cent. of l-cystine.

(4) The incubation for two days at 37° C. with the " ruminal juice " of a sheep or autoclaving for one hour at 1 Kg. pressure (120° C.) had no effect upon the growth-promoting value of lucerne proteins.

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