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Plant Proteins. V.—The Biological Value of Lucerne and Lucerne Supplemented by Cystine in Sheep.

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IN experiments with rats (1937) it was shown that lucerne protein fed at approximately 8 per cent. level, gave the lowest biological value of all the plant proteins so far investigated. This defective utilization of the lucerne protein by rats was then definitely shown to be caused by a deficiency of the amino acid cystine, which, when added to lucerne in minimal quantities, enhanced its biological value considerably. In view of these findings, together with the controversial opinions in the literature as to the rôle of cystine and sulphur-compounds in the nutrition of sheep, it was regarded as essential to attack the question from a somewhat different angle and to ascertain whether the addition of cystine to lucerne has any effect on the biological utilization of its nitrogen by sheep.

Until quite recently the indispensability of cystine in the nutrition of the rat has been recognized universally. Rose (1937) now queries the indispensability of this amino acid and states that it only acts as a stimulant to growth when methionine is present in suboptimal quantities, and that the latter is in reality the indispensable component. However, a number of workers [Haag, T. R. (1933), Mitchell, H. H. (1930), Smuts, D. B. (1937), Schrewsbury and Bratzler (1933), Weichselbaum (1935), and Scoz (1932)] have shown that the addition of cystine enhances the growth-promoting properties of a protein feed deficient in this amino acid, and that it is necessary for normal nutrition. Furthermore, it has been shown that the addition of minute quantities of cystine to a cystine deficient ration markedly increases the utilization of the absorbed nitrogen of such a ration. These observations, it appears, can only be explained by assuming that the added cystine supplemented the existing amino acid complex in the protein molecule, thereby creating an assortment of amino acids more suitable for the selective needs of tissue synthesis. The cystine inclusion therefore avoids that wastage of amino acid nitrogen which, in its absence, would have been incompletely utilized and must consequently be regarded as essential in the process of tissue construction. On the other hand, Jackson and Block (1932) have shown that on a cystine-methionine free diet d-methionine and formyl *l* methionine can stimulate growth in rats. Weichselbaum (1935), on a Sherman-Merril cystine deficient diet, found that rats develop a syndrome which can be prevented by either methionine or cystine and that cystine alone could overcome symptoms when they have definitely appeared.

Whatever explanation may be forthcoming, the preponderance of evidence at present favours the indispensability of cystine in the nutrition of the growing rat. It further appears that apart from methionine no other known amino acid can replace cystine in a ration or that the latter can be synthesized by the rat from simpler organic or inorganic sulphur-containing compounds. Rose and Huddlestin (1926) and Lewis and Lewis (1926) found that taurine, a derivative of cystine, could not supplement a cystine deficient diet for maintenance and growth. Westerman and Rose (1927) obtained no evidence that thioglycollic acid and dithio-dipropionic-acid could be utilized for synthesis of cystine by the rat.

As far as synthesis of cystine from inorganic sulphur is concerned only negative results have been obtained. Daniels and Rich (1918) found inorganic sulphates incapable of covering a cystine deficiency either for growth or maintenance. Geiling (1917) obtained no change in the declining weight of mice on a cystine deficient diet when flowers of sulphur were added to it.

While the indispensability of cystine in the absence of sufficient methionine holds true for rats and other omnivores, it seems quite possible that ruminants with their complicated and differently constructed alimentary tract may react otherwise. In fact, the synthesis of vitamin B by the rumen flora of cattle, as postulated by Bechdell, Honeywell, Durcher and Knutser (1928), strongly indicates that there are distinct differences in the digestive activities of these two types of animals. Reasoning on more or less analogous grounds, Rimington and Bekker (1932) suggested that sheep may be able to synthesize cystine through a symbiotic action of the intestinal flora. Fraser and Fraser-Roberts (1932), in trying to explain the high percentage of cystine in wool suggest that the wool follicles themselves may perform this special function of transformation into cystine. These theories, although probable, can only be solved one way or the other if such factors as the cystine content of the ration, percentage utilization of cystine for maintenance, growth, and wool production, and the cystine content of the fleece, are determined. In the absence of the latter information, it appears but naturel to accept Woodman and Evans' (1932) explanation that there is enough cystine in pasture to satisfy the needs of growth and wool production in sheep.

In our work on the determination of the biological value of lucerne for sheep, it seemed as if we could obtain some information on the cystine problem by supplementing lucerne with cystine and determining its biological value. If cystine is indispensable for sheep and the cystine and methionine content of lucerne too low for maintenance, then one would expect an enhanced biological value with the addition of cystine if no synthesis of this amino acid is accomplished.

EXPERIMENTAL.

Four mature wether merinos were used in this experiment. They were transferred from a standard protein ration to a nitrogen-low ration for 15 days, after which period they were put in metabolism cages. The collection periods lasted ten days, and the preliminary feeding periods of the protein rations also ten days. The feed for each experimental period was weighed out at the start, and from it the daily food requirements were weighed out and placed in paper bags. Representative samples were taken and analysed in triplicate. Sheep were fed once daily and any unconsumed feed of the day collected, air-dried and stored.

Urine was collected daily in 2 per cent. HCl and the urine, together with the washings made up to a known volume, from which a 10 per cent. aliquot was taken and stored. The daily collection of faeces was air-dried, weighed, aliquoted and stored in air-tight containers until analysed. The composition of the rations is given in Table 1. By lucerne hay is meant sun-dried hay which was chopped in convenient lengths to prevent spilling.

TABLE 1.

Composition of Rations.

	A.	B.	C.
Lucerne hay	58·0	56·0	—
Cystine[1]	—	·20	—
Codliveroil[1]	2·0	2·0	2·0
Salt	2·0	2·0	2·0
Bone ash[2]	3·0	3·0	3·0
Dex starch	45·0	36·8	73·0
Agar	—	—	20·0
TOTAL	100·0	100·0	100·0

[1] Cystine was prepared from wool and contains ·114 per cent. N in comparison with the theoretical value of ·116 per cent. N. The latter figure was utilised.

[2] Bone ash contains 42·5 per cent. Ca and 17·04 per cent. P.

EXPERIMENTAL RESULTS.

The results of the metabolism experiments, together with the calculation of the biological values, are given in Table 2. As will be seen, the order in which the different periods appear was as follows: Nitrogen free period, 8 per cent. lucerne protein, 8 per cent. lucerne protein supplemented by cystine, followed by a nitrogen free period. The results of the first nitrogen free period were utilized in the calculation of the biological value of the 8 per cent. lucerne protein ration. The same procedure was followed in calculating the biological value of lucerne supplemented by cystine by using the figures obtained in the second nitrogen free period. In graph 1 the metabolic faecal nitrogen for the two nitrogen free periods is plotted against the corresponding quantities of dry matter intake. These points, except for one, very nearly assume a straight line showing, what is generally accepted, that there is a strong positive correlation between dry matter intake and metabolic faecal nitrogen elimination.

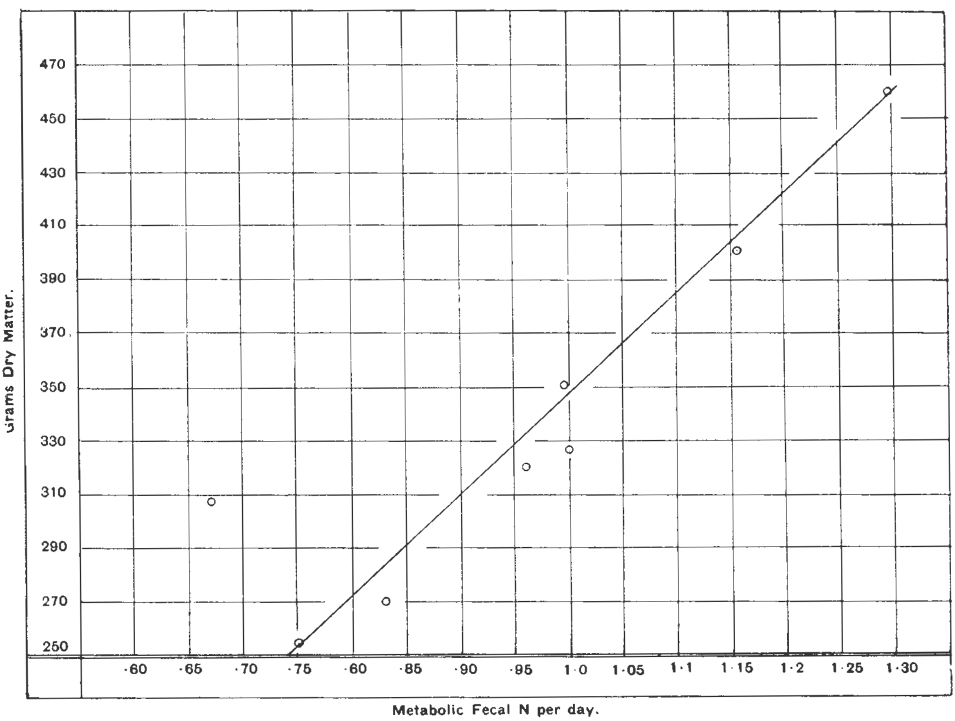
TABLE 2.

The Apparent and True Digestibility of Lucerne and Lucerne Supplemented by Cystine.

Animal No.	Lucerne.		Lucerne Cystine.	
	Apparent.	True.	Apparent.	True.
1	71	90	57	76
2	77	97	59	79
3	74	88	64	82
5	79	87	65	82
	75	91	61	80

GRAPH 1.

Relation between Metabolic Fecal N and Dry Matter Consumed.



The average biological value obtained for lucerne protein at an 8 per cent. level is 60. Sheep 1 and 2 in both periods of protein feeding gave higher biological values than sheep 3 and 5. This fact is not accounted for by a difference in digestibility, but is in all probability due to a better utilization of the absorbed nitrogen, since there is not much difference in the magnitude of endogenous nitrogen excretion between these sheep per Kg. body weight. The fact that this difference in biological values is so consistent in the two different periods show that it must be due to some inherent difference in the physiological abilities of these sheep to utilize the absorbed nitrogen better than their two companions.

The value 60 obtained by us corresponds well with the value obtained for this protein with rats and also agrees very closely with the value reported by Satola (1930) for sheep. Satola's values vary from 53 to 60, with an average of 56. The value reported by Turk, Morrison and Maynard (1934) are much higher and average 79. The average apparent digestibility for lucerne protein as reported in Table 3 is 75 and the true digestibility 91. Our figure approaches that of Satola but is much higher than 56 as reported by Turk and his co-workers.

The addition of cystine to lucerne has no effect on the utilization of the lucerne nitrogen. The average biological value under these conditions is 58, a value very similar to that obtained when lucerne was fed alone. In fact the addition of cystine depresses the apparent digestibility of lucerne protein. This effect also shows up in the true digestibilities. In comparison with the first period of lucerne feeding there is a marked increase in the nitrogen elimination in the faeces when cystine was added. Whether this is due to an incomplete absorption of cystine or an inhibitory effect of cystine on the other nitrogenous constituents, which normally would have been absorbed, is difficult to say. It is, however, evident from these results, that sheep when fed on an 8 per cent. lucerne protein do not need extra cystine. These sheep as was stated were mature wethers and would consequently not have put on new tissue. It may therefore be quite possible that a more liberal need for cystine may occur during periods of active growth.

Furthermore, although this work shows that mature sheep fed on lucerne do not need cystine for maintenance and wool production, it does not exclude the possibility of synthesis of this animo acid. Nevertheless from a practical point of view these results are of great value since they establish that the utilization of lucerne protein for maintenance and wool production is not impaired by a deficiency of cystine.

Conclusions.

1. By means of the balance-sheet method, it has been shown that the biological value of lucerne protein for sheep is 60 and that the addition of cystine does not effect this biological value.

TABLE 3.

Metabolism Data and Calculation of Biological Value (Daily Basis).

Animal No.	Initial Weight.	Final Weight.	Average Weight.	Dry Matter Consumed.	N Intake Daily.	N in feces Daily.	Metabolic Fecal N. Per Gm. Dry Matter.	Metabolic Fecal N. Per Day.	Food N in feces.	N Absorbed.	Total Uninary N.	Endogenous N. Per Kg. Weight.	Endogenous N. Per Dry.	Food in Urine.	N Retained.	Biological Value.
	Kg.	Kg.	Kg.	Gr.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
N FREE RATION.																
1.............	36	31	33	253	—	·756	·0030	—	—	—	1·20	·036	—	—	—	—
2.............	35	35	35	324	—	1·00	·0031	—	—	—	1·70	·048	—	—	—	—
3.............	37	35	36	308	—	·677	·0022	—	—	—	1·26	·035	—	—	—	—
5.............	40	44	42	461	—	1·29	·0028	—	—	—	1·39	·033	—	—	—	—
8 PER CENT. LUCERNE RATION.																
1.............	31	32	31·5	523	8·29	2·44	·0030	1·57	0·87	7·42	3·91	·036	1·13	2·78	4·64	63
2.............	34	35	34·5	511	7·82	1·79	·0031	1·58	0·21	7·61	4·21	·048	1·66	2·55	5·06	66
3.............	35	35	35	523	8·41	2·20	·0022	1·15	1·05	7·36	4·62	·035	1·23	3·39	3·97	54
5.............	41	43	42	461	7·33	1·53	·0028	1·29	0·24	7·09	4·58	·033	1·39	3·19	3·90	55
															Average............	60
N FREE RATION.																
1.............	34	32	33	320	—	·960	·0030	—	—	—	1·10	·033	—	—	—	—
2.............	35	35	35	270	—	·837	·0031	—	—	—	1·40	·040	—	—	—	—
3.............	35	37	36	400	—	1·16	·0029	—	—	—	1·29	·033	—	—	—	—
5.............	44	40	42	350	—	·980	·0028	—	—	—	1·39	·033	—	—	—	—
8 PER CENT. LUCERNE RATION PLUS ·20 PER CENT. CYSTINE.																
1.............	34	36	35	516	8·13	3·47	·0030	1·55	1·92	6·21	3·56	·033	1·16	2·40	3·81	61
2.............	36	36	36	516	8·13	3·33	·0031	1·60	1·73	6·40	3·80	·040	1·44	2·36	4·04	63
3.............	35	37	36	516	8·13	2·94	·0029	1·50	1·44	6·69	4·28	·033	1·19	3·09	3·60	54
5.............	42	42	42	516	8·13	2·88	·0028	1·44	1·44	6·69	4·57	·033	1·39	3·18	3·51	52
															Average......	58

2. The apparent digestibility of lucerne protein has been found to be 75 per cent. and the true digestibility 91 per cent. The addition of cystine depresses the apparent digestibility and to a slight extent the true digestibility, the average figures being 61 and 80 per cent. respectively.

3. It has been shown that cystine is not a limiting factor in lucerne protein when fed to mature sheep.

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