Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 12, Number 2, April, 1939.

Printed in the Union of South Africa by the Government Printer, Pretoria.

The Effect of Supplementing Lucerne with Cystine and Methionine on the Growth of Rats.

By J. S. C. MARAIS and D. B. SMUTS, Section of Nutrition, Onderstepoort.

EARLY in 1938, we submitted for publication two articles, one on "the supplementary effect amongst plant proteins" and the other on "the animo acid deficiencies of plant proteins". These articles are now in the press. In the former paper (1938) we found by means of complete nitrogen metabolism experiments that the addition of ·20 per cent. *l*-cystine to an 8 per cent. lucerne ration markedly increased the nitrogen utilization and enhanced the biological value of lucerne significantly. In the latter paper (1938), we confirmed, by means of paired feeding tests, that lucerne was markedly deficient in cystine. About the same time there appeared a short article by Rose (1938) in "Science", in which he claimed that methionine was the indispensible sulphur containing amino acid and that cystine was dispensible for growth. In the first paper we stated that at present, it is difficult to reconcile this statement of Rose (1938) with the existing data on cystine metabolism. In the following paper, we intimated that the evidence for the dispensibility of cystine must await further confirmation, since it is not fully established that these two amino acids may have a reciprocal function in nutrition.

At present the opinion on the question of the indispensibility of cystine and methionine is divided. (A) Those who obtained a marked supplementation by cystine, (B) others who claim that methionine can entirely replace cystine and that the latter is therefore dispensible; and a third group (C) who adopt an intermediate view, namely, that methionine and cystine go through the same pathways in metabolism.

(A) The majority of the work on sulphur containing amino acids prior to Rose's announcement centred around the indispensability of cystine. Thus John and Finks (1920) demonstrated that when cooked beanmeal was supplemented by 2 per cent. cystine, rats grew much faster, attaining an average weight of 250 grams in 80 days as against 80 grams on the unsupplemented ration. Sherman and Woods (1925) utilized this stimulating power of cystine for the biological assay of the cystine content of feeds. Shrewsbury and Bratzler (1933) found soyabeans deficient in cystine. By means of the paired feeding method Hayward, Steenbock and Bohstedt (1936) as well as Mitchell and Smuts (1932), found soyabeans seriously lacking in cystine.

Similarly Haag (1931), Kellermann (1935), Smuts and Marais (1938) found with rats, that the addition of cystine to lucerne increased its growth-promoting properties significantly. The latter workers also obtained results showing that the addition of cystine enhances the biological value of lucerne by almost 25 per cent. Scolz (1932) and Weichselbaum (1935) obtained poor growth on a diet deficient in cystine. The latter investigator obtained a characteristic syndrome on a Sherman-Merrill cystine deficient ration. Both cystine and methionine prevented this syndrome. Once the syndrome has definitely developed, cystine brought about recovery in some cases, while methionine was without effect. Krohn and Barnwolff (1937) found an increased nitrogen retention after the administration of cystine.

(B) By means of mixtures of purified amino acids fed to rats Rose (1938), concluded that methionine is actually the indispensible amino acid and should be present in suboptimal quantities for cystine to have an effect. Jackson and Block (1933) found that both *d* and *l*-methionine effectively supplement a ration deficient in methionine and cystine. Beach and White (1937) showed that arachin, present in the protein of peanut, is deficient in methionine and that cystine cannot cover this deficiency.

(C) A few papers have appeared, which indicate that these two amino acids may be intraconvertible or follow the same pathways in metabolism. Brand, Block, Kossel and Cohill (1937) in 1937, from observations on a cystinuric patient, found that one of the pathways of methionine catabolism is its conversion into cysteine. The cystine excreted from this type of patient is derived mainly from dietary methionine. White (1937) showed that the incorporation of iodo-acetic acid in the ration markedly restricted growth in rats, but that growth was immediately resumed after the addition of cystine or *d and l*-methionine. By the determination of cystine in the plasma of rabbits Lewis and Brown (1938) found an increased cystine content in the plasma after cystine or methionine administration. In a study on the growth response to sulphur amino acids Brand (1938) stated that the conversion of methionine into cysteine is only one of the pathways of its metabolism and that it has another important function in the animal body. Bennet (1938) maintains that her study indicates that 1 molecule of cystine is metabolically equivalent to two of methionine or cysteine.

In this study we have further investigated the effect of an addition of cystine and methionine to lucerne on the growths of rats. The results of this investigation are reported below.

Experimental.

Rats were selected and paired according to the usual procedure followed for the paired feeding method. In the first comparison of lucerne, lucerne plus cystine and lucerne plus methionine triplicates instead of pairs were used. The standard conditions of feeding and weighing were similar to that practised under paired feeding. Food consumption was equated amongst the three animals comprising a triplicate. Cystine was prepared from wool in the laboratory and the methionine obtained from Dr. Theo. Schulhardt, Goroitz. The composition of the Ration is given in Table I.

TABLE 1.

Composition of Rations.

	A.	B.	C.	D.	E.
Lucerne	55·2	54·2	54·2	46·7	46·7
Cystine	—	0·2	—	0·2	0·4
Methionine	—	—	0·2	0·2	—
Butterfat	8·0	8·0	8·0	8·0	8·0
Codliveroil	2·0	2·0	2·0	2·0	2·0
Yeast extract*	10·0	10·0	10·0	—	—
Harris Yeast	—	—	—	2·0	2·0
Sucrose	10·0	10·0	10·0	10·0	10·0
Salt mixture†	4·5	4·5	4·5	4·5	4·5
NaCl	1·0	1·0	1·0	1·0	1·0
Starch	9·3	10·1	10·0	25·4	25·4
TOTAL	100·0	100·0	100·0	100·0	100·0
Percentage Nitrogen	1·33	1·34	1·34	1·57	1·64

* Yeast Extract was prepared according to the method of Itter, Orent, McCollum, *J.B.C.* Vol. 108, No. 2, pp. 571–577 (1935).

† A modified Osborne and Mendel salt mixture proposed by Hawk, P. B. and Oser, Bl. *Science* Vol. 74, pp. 369 (1931).

RESULTS.

In Table II the data pertaining to the comparison of lucerne supplemented by cystine and methionine is tabulated. It will be seen that lucerne supplemented by methionine gained over lucerne supplemented by cystine in five out of six comparisons, and that these two supplementations gained more than the unsupplemented lucerne in every comparison. However, the significance of such an outcome can only be assessed by a statistical analysis of the data. Before treating the final outcome statistically, it becomes necessary to ascertain whether the error in the outlay of the test may not have been instrumental in causing the difference amongst treatments as noticed. This factor can be eliminated by an analysis of the variance. By means of such an analysis it is found that the chance of such an outcome being due to an inherent error in the test is approximately 1 in 1,000, which naturally is insignificant, and can thus be disregarded. Analysing therefore the difference in average gain in weight, it is found that the S.D. of a mean of six observations is equal to $\frac{3 \cdot 982}{\sqrt{6}}$ and that the S.D. of a difference of two means would therefore be $3 \cdot 982 \sqrt{2/6} = 2 \cdot 299$. On this basis t which is equal to $\frac{\text{Diff.}}{\text{S.E. of diff.}}$ at $N = 10$ is equal to $2 \cdot 228$ at $P = 0 \cdot 05$ and $3 \cdot 169$ at $P = 0 \cdot 01$. Hence for significance at $P = \cdot 05$ difference between means must be equal to or less than $5 \cdot 122$ and for significance at $P = \cdot 01$ the the difference between means must be equal to or less than $7 \cdot 285$.

Table II.

A Comparison of the Effect of Supplementing Lucerne with Cystine and Methionine on its Growth-Promoting Value in Young Rats.

	Triplicate 1.			Triplicate 2.		
	Lucerne plus Cystine.	Lucerne.	Lucerne plus Methionine.	Lucerne plus Cystine.	Lucerne.	Lucerne plus Methionine.
Initial weight, grams..........	93	93	93	68	70	72
Final weight, grams...........	118	113	129	88	70	100
Average gain, grams...........	25	20	36	20	—	28
Total consumption.............	437	437	437	355	355	355

	Triplicate 3.			Triplicate 4.		
	Lucerne plus Cystine.	Lucerne.	Lucerne plus Methionine.	Lucerne plus Cystine.	Lucerne.	Lucerne plus Methionine.
Initial weight, grams..........	77	77	82	105	107	107
Final weight, grams...........	114	98	115	130	115	137
Average gain, grams...........	37	21	33	25	8	30
Total consumption.............	403	403	403	421	421	421

	Triplicate 5.			Triplicate 6.		
	Lucerne plus Cystine.	Lucerne.	Lucerne plus Methionine.	Lucerne plus Cystine.	Lucerne.	Lucerne plus Methionine.
Initial weight, grams..........	95	86	92	76	72	763
Final weight, grams...........	119	91	124	102	84	104
Average gain, grams...........	24	5	32	26	12	31
Total consumption.............	412	412	412	372	372	372

Based on this analysis it can clearly be seen that lucerne and methionine and lucerne and cystine were significantly better than lucerne alone, and that the chances are approximately 1 in 100 that such an outcome is due to chance. Continuing on the same lines of comparison it will be seen that lucerne supplemented by methionine is significantly better than lucerne supplemented by cystine at $P = \cdot 05$. That is, the chances are approximately 1 in 50 that the difference in gains registered is actually due to the methionine supplementation. Hence it must be concluded that in this experiment the addition of methionine enhanced the growth-promoting properties of lucerne, beyond that produced by the addition of cystine.

In Table III data are presented in which lucerne is supplemented by methionine and cystine compared against lucerne supplemented by cystine. Cystine alone was supplemented at the rate of ·40 per cent., while in the other comparison methionine and cystine were supplemented at the rate of ·20 per cent. as shown in the composition of the rations. The S.D. of a mean of six differences is 2·0776 and $t = \cdot 722$. For $N = 5$, P becomes 0·5, which shows that the probability that the difference in gain in favour of cystine is only accidental and may be brought about by chance alone once in every two trials. This result is therefore quite insignificant.

TABLE III.

A Comparison of Lucerne Supplemented by Methionine plus Cystine and Cystine.

	Pair 1.		Pair 2.		Pair 3.	
	Lucerne plus Cystine plus Methionine.	Lucerne plus Cystine.	Lucerne plus Cystine plus Methionine.	Lucerne plus Cystine.	Lucerne plus Cystine plus Methionine.	Lucerne plus Cystine.
Initial weight................	129	118	100	88	115	114
Final weight..................	164	148	141	133	152	147
Average gain..................	35	30	41	45	37	33
Food consumption............	340	340	340	340	340	340

	Pair 4.		Pair 5.		Pair 6.	
	Lucerne plus Cystine plus Methionine.	Lucerne plus Cystine.	Lucerne plus Cystine plus Methionine.	Lucerne plus Cystine.	Lucerne plus Cystine plus Methionine.	Lucerne plus Cystine.
Initial weight................	137	130	124	119	104	102
Final weight..................	158	158	148	146	139	143
Average gain..................	21	28	24	25	35	41
Food consumption............	340	340	340	340	340	340

Discussion.

These results together with our findings on the supplementation of lucerne by cystine are difficult to interpret especially in regard to the new theory of dispensibility of cystine as propounded by Rose (1938). In the case, where we found increased growth as well as an increased nitrogen utilization, after cystine supplementation, it must be assumed in order to fit in with Rose's theory that lucerne contains suboptimal amounts of methionine. Under such conditions the postulation by Brand (1938) that a small portion of the methionine fulfils a certain function in the system, which cannot be replaced by cystine seems very plausible. Furthermore in order to explain the increase of nitrogen utilization of lucerne protein after the addition of cystine it has to be assumed that methionine content of lucerne is minimal and that cystine can replace the major portion of methionine. The data presented in this paper definitely show that methionine supplements lucerne significantly better than cystine. In the paired feeding trial the addition of cystine is slightly superior to that of methionine plus cystine, although not statistically significant. Our findings do not seem to support the statement of Bennet (1938), namely that one molecule of cystine is metabolically equivalent to two of methionine. Because, if that was so, one would expect in the triplicate comparisons a superior gain in weight by the cystine-fed rats.

We are rather inclined to believe that age may have some effect on the methionine and cystine needs. Young rats such as we had in our triplicate test, may need more of that fraction of methionine, which cannot be displaced by cystine. Older rats, of 100 grams or more, as in our paired feeding test may need less of this fraction, and in this case the major portion of methionine and cystine go through the same metabolic pathways, being utilized primarily for supplementing the sulphur moiety in the animo acid complex of lucerne protein, to enable the synthesis of new tissues to take place.

Summary and Conclusions.

By means of the paired feeding method it was shown that methionine supplemented lucerne significantly better than cystine. If lucerne is supplemented by methionine plus cystine and compared with lucerne supplemented by cystine no difference in total gains between the two comparisons is noted..

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