CVI. ANTISCORBUTIC VALUE OF LUCERNE.

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DURING the course of an investigation into the distribution of the antiscorbutic factor in some South African foodstuffs specimens of the common lucerne, or alfalfa (*Medicago sativa*) were examined, and it was found that, as judged by the indophenol reaction, this plant was an outstandingly rich source of vitamin C.

According to the report on the vitamins issued by the Medical Research Council [1932] lucerne is regarded as rich in vitamins A and B and exceptionally rich in E. More recent work, such as that described by Scheunert and Schieblich [1934] and by Hauge [1934], confirms the high values for vitamin A, though the former authors state that vitamins B and D are present only in low concentration. We have been unable, however, to find any reference dealing with the antiscorbutic value of this plant.

Lucerne is also of value since it contains no less than 1-2 % of mineral salts, of which nearly 0.5 % is calcium; in addition about 8 mg. of iron are present per g., or nearly double that found in spinach. It will be agreed, therefore, that lucerne is an interesting plant from the nutritional point of view; moreover, it is growing in favour as a forage crop and is becoming fairly readily available in many parts of the world [see monograph by Klinkowski, 1933]. In view of the foregoing it seemed desirable to investigate its antiscorbutic activity more closely.

Although it is well known that lucerne is occasionally the cause of death in animals, Steyn [1933], after careful examination of the available evidence is of the opinion that there is no experimental evidence to show that this is due to the presence of any poisonous substance in the plant. In all probability death is due to the effects of excessive fermentation, which, it appears, it likely to take place when large amounts are consumed, especially if the plant is in a young, frosted or wilted condition.

We learn from several sources that green lucerne is actually used to some small extent as a human food in parts of Russia, China and America, whilst here in South Africa it is occasionally used as a substitute for spinach. On account of its high mineral and vitamin A contents lucerne meal has been recently incorporated into the special "cereal mixture" devised by Brown and Tisdall [1933] and used by them with conspicuous success in the feeding of small children.

Determination of antiscorbutic activity by means of indophenol.

For this purpose the method of Birch *et al.* [1933] was used. The following are typical values from amongst many samples of lucerne that have been examined.

Table I. *Antiscorbutic value of lucerne as measured by the indophenol reaction.*

Young plants:	mg. of ascorbic acid per g. of fresh lucerne
Leaves only	3.2, 3.3, 3.3, 3.8, 3.8
Leaves and small stalks	2.7, 3.0, 3.1, 3.2, 3.2, 3.3
Shoots only	3.0, 3.8
Flowering stage:	
Leaves only	2.2, 2.2, 2.3, 2.3, 2.3, 2.4, 2.5, 2.6, 2.7, 2.7, 2.7, 2.7, 3.0, 3.1
Another specimen gave:	
Leaves only	4.5
Small stalks	1.8
Fibrous stalks	0.7

As will be seen from the above, the antiscorbutic value is of a high order, being frequently more than four times that of the same weight of orange juice.

Confirmation of antiscorbutic activity by means of animal experiments.

These experiments were carried out upon young guinea-pigs according to the method described by Bracewell *et al.* [1930]. A fresh supply of lucerne was obtained daily and the vitamin content estimated by means of indophenol. Only the leaves and young shoots were used, and the amounts weighed out and given to each animal were adjusted to correspond as nearly as possible to a definite quantity of vitamin C. The animals were divided into three groups, and the amounts of ascorbic acid given *per diem* corresponded approximately to

Fig. 1. A, Negative controls; B, 0.25–0.3 g. lucerne $\equiv$ 0.8 mg. ascorbic acid; C, 0.5–0.6 g. lucerne $\equiv$ 1.6 mg. ascorbic acid; D, 1.0–1.2 g. $\equiv$ 3.2 ascorbic acid.

+ S Died of scurvy.

+ I. Died of intercurrent infection.

K. Killed.

No S. No scurvy.

P Pregnant.

↓ Birth of young.

0.8, 1.6 and 3.2 mg. This represented a daily ration of 0.25–0.3, 0.5–0.6 and 1.0–1.2 g. respectively of the fresh leaf. A double quantity was given on Saturdays. At the conclusion of the experiment the animals were killed and

carefully examined for evidence of scurvy. No such evidence was obtained from any of the animals receiving the lucerne, whereas both the animals receiving the basal diet alone quickly died from this disease. These two animals showed extensive subcutaneous haemorrhages, fragile epiphyses containing numerous areas of haemorrhage in the marrow and beading of the costochondral junctions; the teeth, however, did not appear to be brittle nor was there swelling or haemorrhage into the gums. The charts of the animals' growth are given in Fig. 1.

Some difficulty arises in the quantitative interpretation of these animal experiments, owing to the uncertainty which appears to exist regarding the minimum protective dose for the guinea-pig. According to Birch *et al.* [1933] 0.9 mg. of ascorbic acid *per diem* may be regarded as sufficient to protect the animal from macroscopical evidence of scurvy, whilst Göthlin [1934] claims that protection against macroscopical scorbutic alterations can be reached with values as low as 0.5 mg. of ascorbic acid. Since 0.25–0.3 g. of lucerne leaf *per diem* protected these animals from macroscopical evidence of scurvy, it may be concluded that this amount of leaf contains at least 0.5 mg. of the vitamin. This corresponds to 2.0–3.6 mg. per g. of leaf depending on which value for the minimum protective dose is accepted; in either case the values obtained by the chemical and biological methods are seen to be in very fair agreement.

Variations in the antiscorbutic activity of lucerne.

As will be noticed from Table I, the shoots and leaves tend to be somewhat richer when young than at the flowering stage. There are a very great many different varieties of lucerne in cultivation [see Klinkowski, 1933], and it is quite possible that these varieties may differ very considerably in their antiscorbutic values and in other properties that may be of importance when considering lucerne as a possible green vegetable for human consumption. The variety used for the above experiments is known as Provence; substantially the same values were, however, obtained for a few specimens of two other varieties in common use, namely Chinese and Hunter's River. No difference was found between samples taken at the same time from a variety receiving different fertiliser treatment.

Preservation of the antiscorbutic value of lucerne.

Green lucerne. The changes that are liable to occur after cutting are seen in Table II. In the first case there is an apparent increase in concentration owing to wilting of the leaves, whilst in the second, although wilting was prevented, the lucerne became slightly yellow in colour; in such cases lower values were invariably obtained.

Table II. *Deterioration of antiscorbutic value of lucerne after cutting.*

	mg. ascorbic acid per g. of leaf.	
	Standing without water	Standing in water
Fresh leaves	3.2, 3.2, 3.3	3.1, 2.6, 2.5, 3.0
After 24 hours	3.8, 3.7, 3.6	2.3, 2.2, 2.3, —
After 72 hours	— — —	1.9 — — 2.1

That lucerne, when cooked like spinach, retains its antiscorbutic activity to a very considerable extent is suggested by the following experiment.

10.6 g. of carefully sampled lucerne leaves, corresponding to an ascorbic acid content of 34.0 mg., were boiled for 20 minutes with 50 ml. of water and a

pinch of salt in a covered pyrex beaker. At the conclusion of the experiment very little water remained, and after the contents were cooled 31.7 mg. or 90 % of the original ascorbic acid was found to be present.

Lucerne hay. That ordinary lucerne hay of commerce may contain a good deal of ascorbic acid is seen from the following figures obtained for samples of varying quality found at the local market:

0.09, 0.28, 0.45 and 1.6 mg. per g. of hay.

Lucerne meal. Specimens of commercial meal were also examined and were found to vary considerably in their ascorbic acid contents. Two samples contained negligible amounts, whilst the best, which was of a good green colour and contained much less stalk, gave a value of 0.57 mg. per g.

A sample of meal was also prepared from a bale of lucerne obtained from the market; the leaves when removed from the stalks were found to represent about 37 % of the original weight. On grinding the meal gave a value of 0.79 mg. per g.

It was thought that the rate of destruction of the vitamin during the curing of green lucerne and the preparation of meal would be much reduced if enzyme action could be arrested at an early stage. The following rough experiments support this view and indicate possible methods for the practical preparation of meals possessing really high antiscorbutic activity.

(1) Fresh lucerne plants were plunged into boiling water for 30 seconds and then dried by exposure to air, but away from direct sunlight.

Table III. *Lucerne meal prepared from leaves scalded for 30 seconds.*

	mg. ascorbic acid per g.			
	Initial value		Value after scalding and drying	
	Fresh leaves	Calculated to dry weight	4 days' drying	11 weeks' storage
(a)	—	—	1.5	—
(b)	3.1	10.3	3.8	—
(c)	2.4	8.0	4.4	—
(d)	3.1	10.3	4.0	2.7

(2) Lucerne dried in an air-oven for 6 minutes at 250° gave values of over 7.0 mg. per g.

(3) Lucerne leaves and shoots were dried for 2 days over sulphuric acid *in vacuo*. The excellent preservation obtained is seen from Table IV.

Table IV. *Antiscorbutic value of desiccated lucerne.*

	Initial value		Value after desiccation	
	Fresh leaves	Calculated to dry weight	Immediately after desiccation	After 6 months' storage
	2.3	9.2	8.3	—
	2.5	10.0	7.4	3.0

These three experiments indicate that there should be no great difficulty in devising a practical method for the preparation of a lucerne meal possessing considerable antiscorbutic activity, should such a dry and comparatively non-perishable source of the vitamin be required.

SUMMARY.

1. Lucerne is an outstandingly good source of vitamin C, the concentration in the average leaf examined being about four times that found in *Citrus* juice. This conclusion was first arrived at by means of the indophenol reaction and then confirmed by the biological test.

2. Although the original activity rapidly diminishes when the cells are damaged, preliminary experiments indicate that much of the vitamin remains after cooking, and that it is possible to dry the plant in such a way that lucerne meal of high activity can be obtained.

3. Since lucerne is also a rich source of vitamins A and E and is also unusually rich in minerals such as calcium and iron, the possibility of using certain types of the young plant as a human food, either as green salad, spinach or in the form of dry meal is worthy of consideration.

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