

## Effect of Small Quantities of a Yeast Preparation on the Recovery of Appetite in Sheep

By J. TOSIC

*Rowett Research Institute, Bucksburn, Aberdeenshire*

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In his classical experiments on accessory food factors, Hopkins (1912) showed that not only milk but also protein-free and salt-free extracts of yeast contain 'organic complexes' without which rats failed to grow and often died, if fed on mixtures of purified casein, fat, carbohydrate and salts. Since then it has been established that yeast is a rich source of the members of the vitamin B complex (for a recent review see Van Lanen & Tanner, 1948), and as such it has been used not only as a therapeutic agent, but also as a starting material for the detection and isolation of new members of the vitamin B complex. Furthermore, the value of yeast as the sole source of protein in the diet of rats was demonstrated by Osborne & Mendel (1919); later Macrae, El-Sadr & Sellers (1942) confirmed the high biological value of yeast protein for rats and showed that a small addition of dried yeast to a diet containing only maize protein greatly increased the value of the latter in the rearing of pigs. Ritzman (1945) found yeast protein highly digestible for cows. However, the experiments of Braude, Kon & White (1943, 1944) on the rachitogenic effect of rations containing a relatively high proportion of yeast, and on the adverse effect of yeast on the storage of vitamin A in the liver of pigs, suggest that as a major dietary supplement for farm animals yeast should be used with caution. These, and other aspects of the nutritive value of food yeast, have been discussed in an excellent survey prepared by the Accessory Food Factors Committee (1945) of the Medical Research Council and the Lister Institute.

During the early stages of investigations of microbial activities in the rumen of sheep, the present author observed that one of the sheep, maintained on hay, lost appetite, and that the loss of appetite was accompanied by a loss of weight and condition. Similar observations have been made by W. Thomson (personal communication), who successfully revived appetite by including concentrates in the diet, and by Elsdon (1945) who achieved it by changing the diet from a poor quality hay (adequate calorifically) to a good quality clover hay. No attempts seem to have been made to revive appetite without substantial changes in the diet.

In approaching the problem of fall in appetite in the present work, it was assumed hypothetically that in sheep this condition might be due to a physiological disturbance of the animal related to a deficiency of one or more accessory food factors (Hopkins, 1912), or of some other minor constituent of the diet, which directly, or indirectly through microbial activities in the rumen, might affect the appetite of the animal. To test this hypothesis a yeast preparation was made, which, on introduction into the rumen of the sheep, without otherwise changing the diet, led not only to the recovery

of appetite, but also to an improvement in condition and a gain in weight. During the last 2 years, three sheep maintained on hay at this Institute have been observed to lose appetite, and each has been successfully treated with the yeast preparation. In this paper details of these observations are reported, and some of the unsuccessful attempts which were made to induce experimentally loss of appetite in sheep are mentioned.

## EXPERIMENTAL

*Sheep*

All but one of the sheep used were Suffolk Cross wethers, between 1 and 2 years of age. The exception, no. S1, was a 3½-year-old wether of no specific breed, kindly placed at my disposal by Dr A. T. Phillipson. The sheep were kept in individual pens. They had free access to water and a salt lick, and were maintained exclusively on hay. Trough feeding was *ad lib.*, fresh lots of hay being given twice daily, at 8 a.m. and 8 p.m., except with sheep no. S1 which was fed three times a day, at 7 a.m., 11 a.m., and 4 p.m. The hay consisted of a mixture of clover and rye grass, used as a stock hay at this Institute, and was readily taken by all but those sheep that showed a fall in appetite. Broadly speaking, the quantity of hay consumed by individual sheep was related to the body-weight of the animal.

*Rumen fistulas*

Fifteen sheep were fitted with permanent rumen fistulas (Quin, Van der Wath & Myburgh, 1938; Phillipson & Innes, 1939), modified by the use of a stab wound to exteriorize the cannula (Phillipson, personal communication).

*Preparation of yeast extract*

Fresh baker's yeast, supplied by the Distillers Company Ltd., Glenochil, was broken into small fragments, placed in a sterile flask fitted with a rubber bung and a Bunsen valve, and autolysed at 58° for 7 days. The autolysis could conveniently be initiated by the addition of 0.1% sodium chloride as saturated solution. After 7 days the clear liquid released from the yeast was separated by centrifugation. It was distributed in sterile bacteriological screw-cap bottles, pasteurized for 10 min. at 70°, and stored at 2°. Fresh preparations were made as required. On the average, 340 ml. yeast preparation were obtained from 1 kg. of fresh baker's yeast. The figures in Table 1 show that

Table 1. *Average analytical figures for the fresh yeast and its extract*

Component	Fresh yeast (%) (a)	Extract		(a) as percentage of (b)	Dry matter	
		(g./100 ml.)	(g./34 ml.)* (b)		Yeast (%)	Extract (%)
Dry matter	26.20	11.35	3.86	14.7	—	—
Ash	1.64	1.79	0.61	37.2	6.25	15.82
Nitrogen	2.39	1.28	0.44	18.3	9.14	11.34
Crude protein (N × 6.25)	14.94	8.03	2.73	18.3	57.05	70.08
Nicotinic acid	—	7.20 × 10 <sup>-3</sup>	2.45 × 10 <sup>-3</sup>	—	—	63.5 × 10 <sup>-3</sup>

\* Volume obtained from 100 g. yeast.

the yeast-extract preparation contained 14.7% of the dry matter, 37.2% of the ash and 18.3% of the nitrogen of the yeast used, and that it was rich in nicotinic acid. On a dry-matter basis both the nitrogenous and the mineral constituents of the yeast were more concentrated in the yeast extract than in the whole yeast. Ash and crude protein ( $N \times 6.25$ ) accounted for nearly 86% of the dry matter of the yeast preparation.

### Analytical methods

Nitrogen was estimated according to the method of Chibnall, Rees & Williams (1943). Dry matter was determined at 102–105° and ash by ignition at 470° for 18 hr. Nicotinic acid was assayed microbiologically (Kodicek & Pepper, 1948).

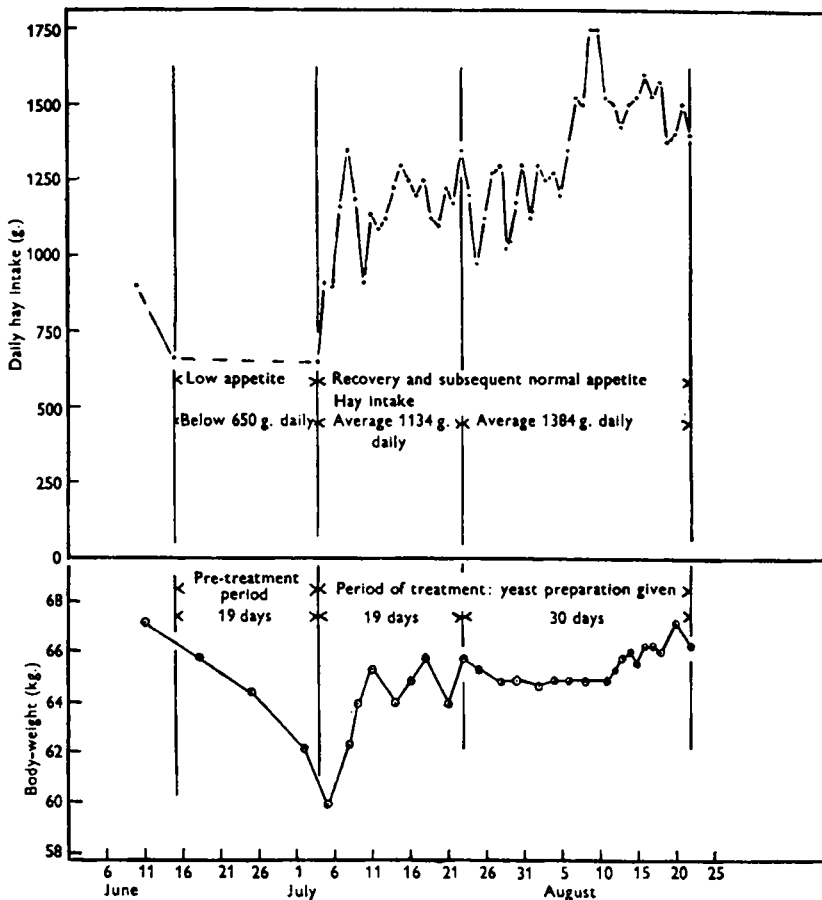


Fig. 1. The daily hay intake and body-weight of sheep no. 118, and the effect of a yeast-extract preparation introduced by rumen fistula.

## RESULTS

### First observation

A 15-month-old Suffolk Cross wether, no. 118, was maintained exclusively on hay, of the following average composition on a dry-matter basis: crude protein ( $N \times 6.25$ ) 11.8, crude fibre 38.0, ash 7.0%. During a period of some 3 weeks the hay intake of

this sheep was below 650 g./day and its condition steadily deteriorated, with the result that within 26 days its weight fell from 67.1 to 59.9 kg. (Fig. 1). At this stage (4 July 1947) it was decided to maintain it on the same hay but to introduce daily into its rumen by way of the permanent fistula a small dose of the yeast preparation (a solution containing 2.3 g. dry matter and 247 mg. nitrogen), with the intention of supplying any factor or factors which might be involved in regulating the appetite, or which might help the microbial population of the rumen to manufacture these factors.

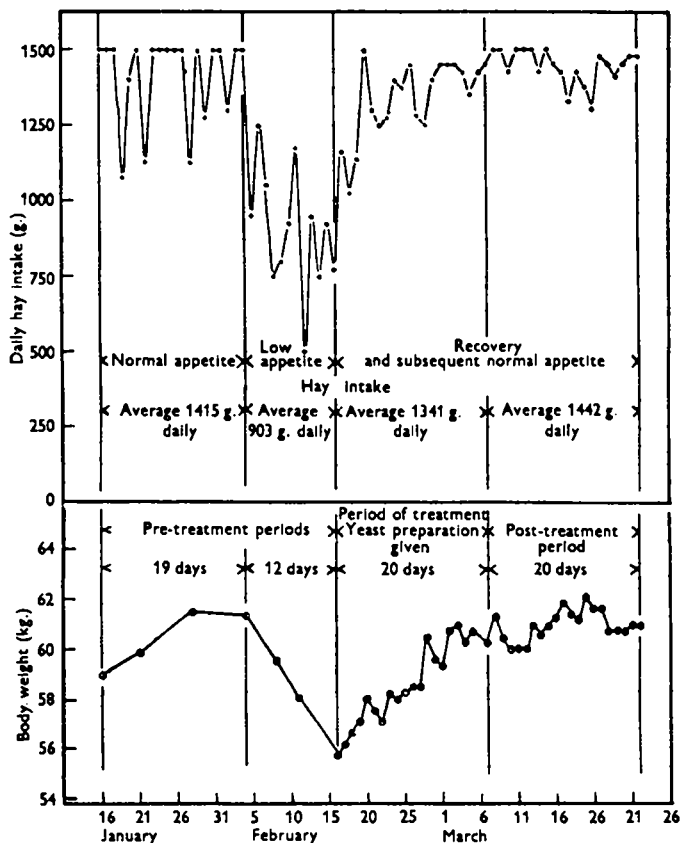


Fig. 2. The daily hay intake and body-weight of sheep no. S<sub>1</sub>, and the effect of a yeast-extract preparation introduced by rumen fistula.

The effect of the yeast preparation was soon demonstrated (Fig. 1) by the sheep's almost doubling its intake of hay and regaining its body-weight. It was intended to discontinue the treatment of this animal towards the end of August to see if a fall in food intake would recur, but after an accident with the cannula it had to be slaughtered.

#### Subsequent observations

In February 1948 a similar case of fall in appetite was noticed (Fig. 2) in a 3½-year-old wether no. S<sub>1</sub>, wholly maintained during the period of observation on a diet of hay containing on a dry-matter basis 1.57% nitrogen and 6.35% ash. Before the fall of

appetite occurred this sheep thrived on the diet, consuming on the average 1415 g./day. This period of normal appetite was followed, however, by a period of lowered appetite, during which the average food intake fell to 903 g./day, a reduction of 512 g./day. The lower food intake was accompanied by a steady loss of weight. On the 13th day of this period, when the daily intake of hay amounted to only 750 g., it was decided to treat the sheep daily for a time with the yeast preparation; 20 ml. containing 2.2 g. dry matter and 230 mg. nitrogen were introduced into the rumen by fistula during the morning feed.

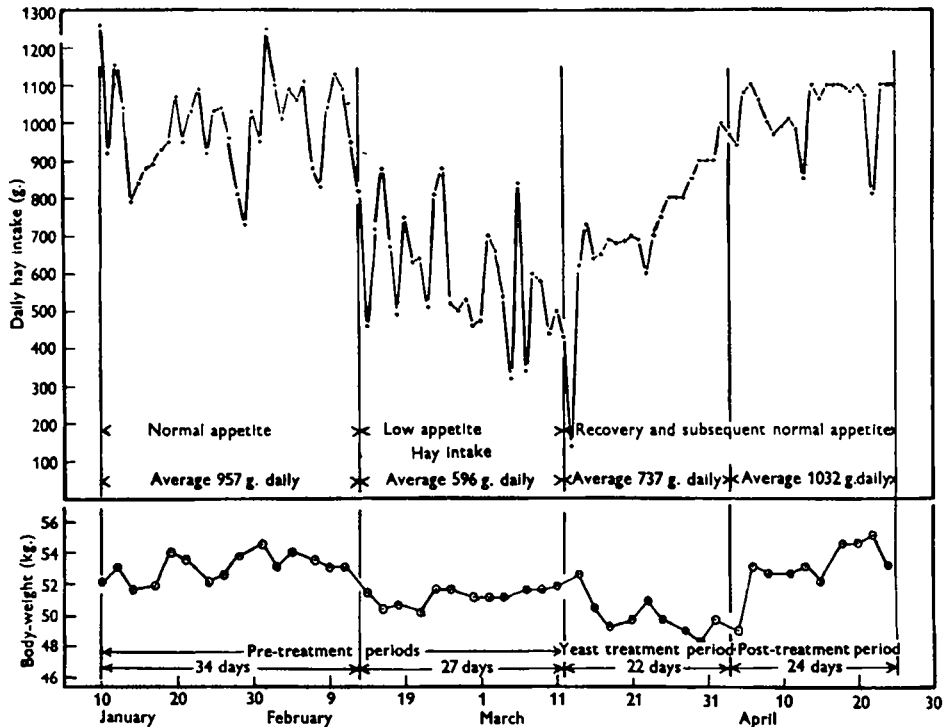


Fig. 3. The daily hay intake and body-weight of sheep no. 337, and the effect of a yeast-extract preparation introduced by rumen fistula.

The response (Fig. 2) to the yeast-extract preparation was very similar to that observed with sheep no. 118 (Fig. 1), consisting not only of recovery of appetite by the sheep, as demonstrated by the average rise in hay intake from 903 to 1341 g./day, but also of a gain in weight of 5 kg. in the 20 days of treatment. The high hay intake and the body-weight were well maintained for at least 20 days after the treatment had been discontinued.

#### *Attempts to produce experimentally a fall in appetite*

In an attempt to produce experimentally a fall in appetite, twelve Suffolk Cross wethers, fitted with permanent rumen fistulas, were maintained exclusively on a diet of hay during 1948-9. This hay contained, on a dry-matter basis, 1.62% nitrogen and 6.85% ash, and was readily taken by all but one of the sheep.

The body-weight and hay-intake curves of sheep no. 337, in which a fall in appetite was noticed during February-March 1949, are given in Fig. 3. A period of normal appetite, lasting several months, of which only the last month is shown in Fig. 3, was followed by a period of lowered hay intake, the average figures for the two periods being 957 and 596 g./day, respectively. This period of lowered appetite was successfully ended by dosing the sheep daily with 20 ml. yeast-extract preparation, containing 2 g. dry matter and 210 mg. nitrogen. The treatment lasted 42 days and was followed by a post-treatment period during which no yeast preparation was given. Hay from the same stock was used throughout the experiment.

The response of this animal to the addition of the yeast preparation amounted to an average rise in hay consumption of from 596 to 885 g./day (1032 g. in the latter part of the period), but comparison of Fig. 3 with Figs. 1 and 2 suggests that the response was somewhat slower with this sheep than with the first two sheep. It is also interesting to note that, in contrast to the other two sheep, little change occurred in the body-weight of this sheep throughout the four periods of observation.

The remaining eleven sheep in the same group, although maintained under comparable conditions and on the same stock hay as sheep no. 337, showed no fall in appetite, and dosing with the yeast preparation caused no increase in their consumption of hay.

The next step in this inquiry was an attempt to induce a fall in appetite in sheep fed on hay, so that the phenomenon could be studied at will. Two preliminary approaches were made.

The first approach, suggested to the author by the late Dr Marjory Stephenson, involved dosing the sheep daily, by way of the permanent rumen fistulas, with a suspension of activated charcoal in distilled water. It was thought that charcoal, with its high absorptive power, might interfere with the utilization of the factor or factors which may affect appetite in sheep. But the experiments, in which three sheep were dosed daily with 2 g. activated charcoal (British Drug Houses Ltd.) for a period of 3 weeks and in which three others were used as controls, showed that under the experimental conditions dosing with charcoal did not affect hay intake or body-weight to any appreciable extent either during or after treatment.

The second approach involved daily dosing of the sheep with one of the sulphonamides, since these chemotherapeutic agents are known to inhibit, in a competitive manner, the utilization of *p*-aminobenzoic acid (Woods, 1940), and so in turn to inhibit growth of certain bacteria by preventing their synthesis of folic acid (Nimmo-Smith, Lascelles & Woods, 1948). If certain of the rumen bacteria produced the hypothetical appetite factor, and yet depended for their growth on the synthesis of folic acid from *p*-aminobenzoic acid, the addition of sulphathiazole would inhibit some of the microbial activities in the rumen, and in turn bring about a deficiency of the postulated factor. In order to test this hypothesis four sheep were dosed daily, by way of rumen fistulas, with 2 g. sulphathiazole for periods of up to 8 weeks, and their hay intakes and body-weights were recorded. With none of the four sheep, as compared with sheep in the control group receiving no sulphathiazole, was there any appreciable fall in either hay intake or body-weight. Food intake and body-weight were not affected when the administration of the drug was discontinued.

## DISCUSSION

The main point arising out of these observations is that only three of fifteen sheep wholly maintained on a hay diet showed a marked fall in appetite as measured by the average daily intake of hay; in two of these the reduced hay intake was accompanied by a marked fall in body-weight; in the third the fall in hay intake was accompanied by a less pronounced change in body-weight. These detrimental changes were successfully arrested and normal appetite and gain in body-weight restored on dosing the sheep with a small quantity of a yeast-extract preparation rich in accessory food factors. On the other hand, in sheep that showed normal appetite, dosing with the yeast preparation produced no significant change in hay intake or body-weight. Thus it appears that some sheep may suffer from a deficiency which can be cured by a factor or factors required in very small quantities and which are found in baker's yeast. In addition to accessory food factors, however, yeast is known to contain certain trace elements (Mitchell & Tosic, 1949*b*), so that it is possible that the deficiency discussed here may also involve these elements in some way. At the same time it is important to observe that in trace-element deficiency diseases such as, for example, 'pining', the incidence of deficiency among the sheep on the deficient diet is very high (Russell, 1944), whereas in the present work only a small proportion of the sheep appeared to suffer. It is well established that the micro-organisms of the rumen synthesize some of the vitamins (for review see Kon & Porter, 1947-8) and that they also assimilate trace elements (Mitchell & Tosic, 1949*a*). It may be, therefore, that in this field a functional link will be found between trace elements and accessory food factors. An indication of this has already been obtained by Ray, Weir, Pope & Phillips (1947), who found that in cobalt-deficient sheep the levels of nicotinic acid and pyridoxin in the blood were significantly lower than in sheep maintained on the same diet but dosed with cobalt.

The literature appears to contain very little about the factors affecting appetite in sheep, but two points arise out of the observations reported in this paper. The first is concerned with the reason why only some of the sheep maintained on the same diet of hay showed deterioration of appetite, and the second is concerned with the mechanism by which the yeast-extract factor(s) functioned in restoring normal appetite.

Attempts to induce experimentally a fall in appetite in sheep maintained on a natural diet, by dosing them by rumen either with activated charcoal or with sulphathiazole, have so far been unsuccessful, and until a fall in appetite can be induced at will, comparable systematic studies of 'the biochemistry of appetite' in sheep will be possible only occasionally, as reported in this communication.

## SUMMARY

1. In an experimental flock of fifteen sheep fitted with permanent rumen fistulas and wholly maintained indoors on hay, only three animals showed a marked fall in appetite, as measured by the average daily intake of hay. In two the reduced hay intake was accompanied by a marked fall in body-weight; in a third the fall in body-weight was less pronounced.



2. Both these detrimental changes were successfully arrested and normal appetite and weight restored simply by dosing the sheep through rumen fistulas with a small quantity of a yeast-extract preparation amounting to about 0.2% of the total solids of the diet.

3. Attempts to induce experimentally a fall in appetite in sheep maintained on a natural diet, by dosing them by rumen either with activated charcoal or with sulphathiazole, have so far been unsuccessful.

4. These observations are discussed in relation to a possible deficiency in some sheep of accessory food factors or trace elements, which are supplied by a yeast preparation and which can cure the deficiency.

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