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FEEDING AND BREEDING OF LABORATORY ANIMALS

II. GROWTH AND MAINTENANCE OF RABBITS WITHOUT FRESH GREEN FOOD

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(With 5 Figures in the Text)

INTRODUCTION

In the first paper of this series (Parkes, 1946) consideration was given to certain general principles of the feeding of laboratory animals, and in particular to the question of wastage. This question is particularly acute in the case of rabbits and guinea-pigs which conventionally receive large quantities of fresh greenstuff and often a wet mash.

Fresh greenstuff is probably the most wasteful, and because of the amount used one of the most expensive of the foodstuffs used in the urban animal house. It is commonly thought, however, to be essential for rabbits and guinea-pigs. In considering this matter a sharp distinction must be drawn between greenstuff as an expensive and messy container for water, and greenstuff as an essential source of accessory food factors. As regards the former, both rabbits and guinea-pigs are often kept in the laboratory without any water other. than that present in the food. Wild rabbits, however, drink water in spite of the availability of unlimited amounts of greenstuff, and Kennaway (1943) found that laboratory rabbits (2.5 kg.) kept on dry diet would readily drink up to 160 c.c. per day. Kennaway's paper brought to light the interesting fact that fanciers in the past habitually gave rabbits water to drink, and that diarrhoea results only when the water is allowed to become foul. Following Kennaway's suggestion we have confirmed that both rabbits and guinea-pigs very quickly learn to drink from a water bottle of the type used for rats, and that with a separate water supply these animals thrive well on a dry diet. In our experience adult rabbits will drink up to 300 c.c. or more a day. Nearly 1 lb. of greenstuff would be required to supply this amount, and it is likely, as Kennaway points out, that most laboratory rabbits, even when a wet mash is given in association with greenstuff, are kept on a quite inadequate allowance of water. The use of a wet mash, moreover, whether to supplement the water supply or for other reasons, is strongly to be deprecated. Such mashes rapidly

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deteriorate by fermentation, so that, if wastage is to be avoided, an accurate amount must be made each day and an accurate amount fed to each animal.

The nutritional necessity for greenstuff is a much more complex problem. Comparatively little relevant work has been published on the nutritional requirements of rabbits. Experiments on the efficacy of simplified diets (Hogan & Hamilton, 1942; Hogan & Ritchie, 1934) are not very helpful, since the present problem relates only to the most effective use of natural foodstuffs. Such experiments, however, have shown that the ascorbic acid requirement of rabbits is of an entirely different order from that of guinea-pigs. Results with the latter will be reported in a later paper.

TECHNIQUE

Animals

Female rabbits of the variety known as Dutch, obtained from the National Institute for Medical Research Farm Laboratories at Mill Hill, were used for experiment. They were started on the experimental diets at about 1000 g. body weight and were kept singly in barred cages $20 \times 15 \times 12$ in. high. The diets were given once daily in earthenware pots, and animals on dry diets were allowed water *ad lib*. from 500 c.c. bottles hung outside the cages with a nozzle projecting inwards. They were given hay or straw daily, and weighed and cleaned twice weekly.

Preparation of diets

The stock diet of the rabbits at the time the experiments were begun was a daily ration of damp sugar-beet mash (about 150 g.), green food (about 100 g.), and hay. The mash was made by soaking dry sugar-beet pulp in water overnight and slightly drying it off in the morning with bran. The mash fermented very rapidly, and the diet in general had a very low protein content (about 7%).

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Small batches of the experimental diets in meal form were prepared at the Institute. Large batches of meal, and all the batches in pellet form were prepared by Messrs J. and H. Robinson, Ltd. The pelleting process involved heat and pressure, and like the cubing process referred to previously (Parkes, 1946) required a total oil content of some 4%. The pellets were approximately 4 mm. in diameter and 4-6 mm. long. The constituents of the diets, together with the theoretical composition obtained from the *Ministry of Agriculture and Fisheries Bulletin*, no. 124, are shown in Table 1. The ascorbic acid content of constituents and diets, where known, are given in Table 2. The problem of

Table 1.	Constituents	of	diets,	and	theoretical	composition
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	Diet no.				
	1 Pellets	2	5 Pellets	7, 10, 12	18
Ingredient	and cubes	Meal	and meal	Pellets	Pellets
Rolled or ground oats	30	_	20		·
Crushed oats		30	·	_	·
Wholemeal flour	35	—		—	
Bran		22	20	15	15
Barley meal				—	20
Sugar-beet pulp	—	25		. 20	—
Ground-nut meal		:		15	15 -
Linseed cake	—		<u> </u>	10	10
Dried yeast	· 5	—	15		
Dried skimmed milk	15		10	<u> </u>	
Dried meat and bone meal	10		. —	8	. 8
Dried grass meal	—	20	30	30	30
Calcium carbonate	1	2	2	1	1
Sodium chloride	1	1	1	· 1	· 1
Nut oil		—	2		<u> </u>
Cod-liver oil	3	—			<u> </u>
	Theoretical a	nalysis	•		
Crude digestible protein (%)	16.9	8.5	16.8	16.0	16.5
Fat (%).	6.3	2.2	$4 \cdot 2$	4.3	4.6
Soluble carbohydrate (%)	44.4	42.0	$35 \cdot 8$	$32 \cdot 8$	33.7
Fibre (%)	1.1	8.7	5.9	9.4	6.7

(i) Diet 1 pellets also contained a trace of ferric citrate and potassium iodide.

(ii) Diet 1 cubes had 2% cod-liver oil and 6% dried yeast.

(iii) Diet 5 pellets had 5 mg. per l. calciferol added to nut oil.

(iv) Diets 10 and 12 had crystalline ascorbic acid added, 11 oz. per ton and 50 mg. per kg. respectively.
 (v) See also Table 2.

Table 2. Ascorbic acid values

Constituent or diet	Theoretical	Analytical		
Grass meal used in diets 2, 5 and 10		5		
Grass meal used in diet 7, batch 1 and diet 12		6		
Grass meal used in diet 7, batch 2		19		
Diet 2	$1 \cdot 0$			
Diet 5	1.5			
Diet 7, batch 1 pellets	1.8			
Diet 7, batch 2 pellets	5.7	3		
Diet 10, batch 2 pellets	31.5	8		
Diet 10, batch 2 pellets after $4\frac{1}{2}$ months storage in air	31.5	3		
Diet 12 meal	6.8			

the destruction of ascorbic acid during the pelleting process, and in stored diets, will be considered in a later paper dealing with the diet of guinea-pigs.

RESULTS

Growth of young rabbits on diets containing grass meal

Growth rates on the original diet were observed in three different experiments (Figs. 1, 2 and 5), and were uniformly poor. The time, and therefore the labour and accommodation, required to increase body weight by 500 g. was about double that taken on diets with a higher protein content. There thus seemed to be every justification for modification of the stock diet, quite apart from questions of wastage.

The first experiment was to feed a dry meal (diet 2) supplemented only by hay and water from a water bottle. Like the original stock diet, this dry diet had a low protein content. A large percentage of grass meal was included to help supply the large quantity of fibre required by rabbits and

The third experiment was therefore planned on more extensive lines. Dried skimmed milk and yeast were replaced by ground nut and linseed meal, and meat and bone meal to give the protein in a cheaper form, and sugar-beet pulp was reintroduced in place of the oats (diet 7). This diet was fed as (a) pellets with water, (b) meal with water, (c) pellets with greenstuff but no water, and (d) meal with 50 mg. of ascorbic acid per kg. (diet 12), calculated to give 5 mg. per day per rabbit, with water. The results were quite clear-cut (Fig. 3). The growth rate on pellets was nearly double that on the meal, fresh greenstuff was not such an effective supplement to the pellets as water, and the addition of ascorbic acid did not improve the poor performance on meal. A repeat experiment with the pellets at a less favourable time of year gave similar though not quite such good results. The incorporation of ascorbic acid with the pellets (diet 10) did not obviously improve the growth rate previously observed with diet 7 pellets (Fig. 4). At a later date barley meal (diet 18) was substituted for the sugar-beet pulp which became unobtainable.

 Table 3. Consumption of pelleted diets by rabbits

Diet no.	No. of rabbits	Approximate average body weight (kg.)	Period of observation (days)	Average daily consumption (g.)
7	12	2.1	6	66
10	. 6	$2 \cdot 3$	6	64
18	18	$2 \cdot 2$	6	74
18	17	$2 \cdot 2$	3-7	61

to provide small amounts of ascorbic acid. The results are shown in Fig. 1, from which it will be seen that over a period of 15 weeks the average increment of the rabbits on the dry diet was rather greater than that of those on the original stock diet. It might have been considerably better except for an unexplained break in the growth curve during the sixth and seventh weeks. The grass meal in the dry diet was the only obvious source of ascorbic acid, and the experiment showed that the ascorbic acid requirement of rabbits was not likely to prove a serious difficulty in the abolition of greenstuff.

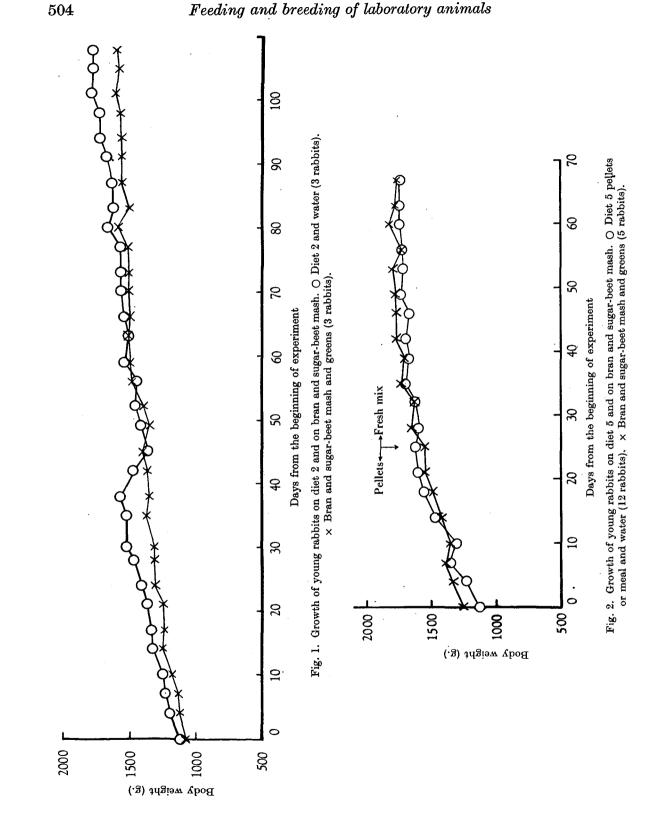
In the next experiment, the protein content of the diet was greatly increased by the addition of dried skimmed milk and yeast (diet 5), sugar-beet pulp being omitted. The first batch of this diet was pelleted, rather unsatisfactorily owing to its small amount, by the ordinary commercial process. While the pellets lasted the growth rate of the rabbits (Fig. 2) was considerably in excess of that of the controls. Subsequently, the rabbits were fed on freshly made small batches of meal of the same composition, and growth fell off. From this experiment it was concluded that the growth rate could be improved by increasing the protein content, and that rabbits did better on pellets than on meal. The first batch of rabbits on diet 7 pellets have now been-maintained for 18 months on this diet (followed by diet 18) and they are still in very good condition at a weight of about 2.5 kg., which is heavy for the breed in question. The breeding capacity of these and of other rabbits similarly maintained is being studied and will be reported upon later.

It seems therefore than in diets 7 and 18 useful diets for stock rabbits have been evolved. They are cheap (\pounds 15 to \pounds 20 per ton in pellet form) and they possess the added advantage at the present time of not containing human foodstuffs.

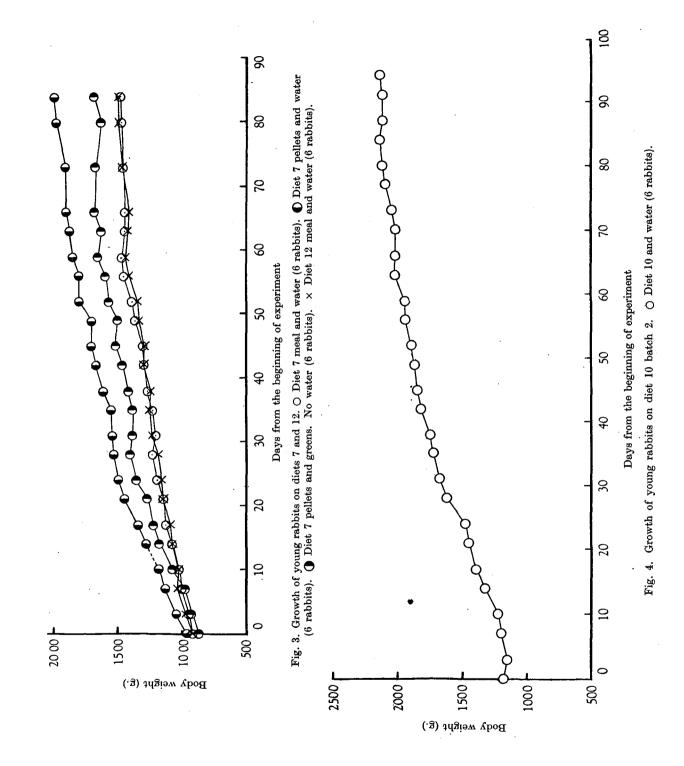
Food consumption

When pellets are fed in open pots the amount spilt is small provided the containers are not filled to the top. Food consumption can therefore be determined with fair accuracy. Three groups of six rabbits each, whose early growth curves are shown in Figs. 3 and 4, at an average body weight of $2 \cdot 5$, $2 \cdot 3$ and $1 \cdot 8$ kg. respectively, were given weighed amounts of pellets (diets 7, 10 or 18) each day and the residue weighed next morning, observations being made on each group over several periods. The combined results are shown in Table 3, from which

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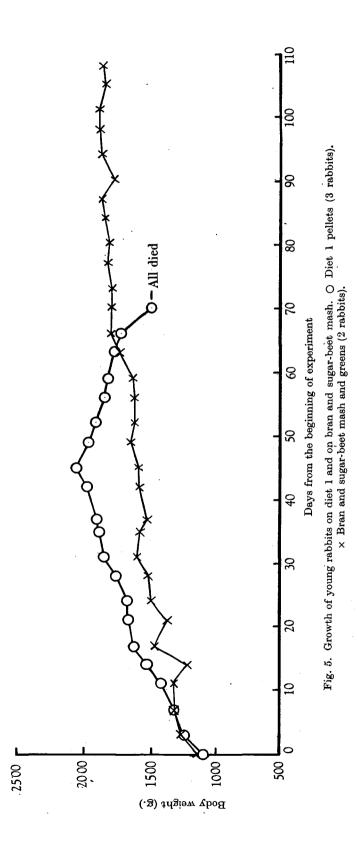


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it will be seen that average daily consumption in the different sets of observations varied from 61 to 77 g. It thus appears that 70 g. may be taken as a rough average daily consumption of pellets of this type by adult Dutch rabbits. There was no obvious correlation of food intake with body weight, within the range examined. At £20 per ton the daily ration of pellets costs about $\frac{1}{3}d$. Rabbits kept on pellets, hay and water therefore cost about $\frac{1}{2}d$. per day, about one-half the cost on the original stock diet.

Growth on diets containing cod-liver oil

An experiment was carried out to test the suitability for rabbits kept without green food of diet 1 pellets as used successfully for rats and mice (Parkes, 1946). Growth, shown in Fig. 5, was extremely good for a period of $6\frac{1}{2}$ weeks, during which time the average increment of three rabbits was nearly 1 kg. During the next three weeks, however, a severe loss of weight and condition occurred and all the animals were dead by the end of the 11th week. The chief symptom was stiffening and loss of muscular control. This result is probably to be ascribed to the effects, which have been described by several writers (Goettsh & Pappenheimer, 1931; Madsen, McCay & Maynard, 1933, 1935) of feeding cod-liver oil to rabbits. These rat pellets contained 3% cod-liver oil, so that with a daily consumption of 70 g. the daily dose of cod-liver oil received by each rabbit would be about 2 c.c. A repeat of this experiment with diet 1 cubes and cross-bred female rabbits (Cross × Dutch) which is at present in progress is not giving such a clear-cut result. The average growth of the three rabbits receiving diet 1 was better than that of two control rabbits (one male and one female) receiving diet 18 for the first $5\frac{1}{2}$ weeks. After this the growth rate of the experimental animals slowed down and is now (after 12 weeks of test) showing a gradual but unmistakable decline. The two control animals are still growing. The food consumption of the animals receiving diet 1 has been of the same order throughout as that of the controls, and does not yet indicate a loss of appetite in spite of the slight decline in weight. The average daily intake per rabbit of diet 1 cubes for a period of declining weight of 33 days is 80 g.; for that of the corresponding period of growth immediately preceding the decline 94 g.

In another experiment two rabbits (Dutch) were given 2 c.c. of cod-liver oil by mouth daily, except on Sundays. These animals did not show any adverse effects for a long period either in body weight or food consumption. One of them, however, has shown a gradual loss of body weight and of appetite from 175 days onwards. The other still remains steady after over 200 days of dosing at a weight of just over 2 kg., which is approaching the maximum for this breed. Madsen et al. (1933) have also reported the tardy appearance of symptoms after prolonged periods of 155 and 197 days. The partial protection from the effects of cod-liver oil feeding afforded by diet 18 may possibly be due to the dried grass in the diet. Morgulis & Spencer (1936) have demonstrated the presence in dry alfalfa of a factor having a protective action against the condition of muscular dystrophy in rabbits.

SUMMARY

1. Fresh greenstuff is not necessary for rabbits, provided that drinking water is supplied.

2. A dry diet has been evolved which gives very satisfactory results with rabbits when supplemented only by hay and water.

3. Dry diet in the form of pellets is much more acceptable to rabbits than an identical diet in the form of meal.

4. The presence in the diet of 30% of even poorquality dried grass seems to supply an adequate amount of ascorbic acid, and the exogenous requirement of rabbits for this vitamin must therefore be very small.

5. Confirmation of the toxic effects of cod-liver oil for rabbits is given.

We are most grateful to Miss M. Olliver, Messrs Chivers and Sons Ltd., who carried out the ascorbic acid determinations and gave valuable advice. We are also indebted to Mr D. J. Short, senior technician in charge of the animals at the Institute, for his willing and valuable co-operation; to Roche Products Ltd., who provided the crystalline ascorbic acid at a nominal price; and to Messrs J. and H. Robinson (Mr Brian Robinson) who prepared all the large-scale batches of diet.

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