THE FEEDING AND BREEDING OF LABORATORY ANIMALS

VII. METHODS OF TESTING THE ADEQUACY OF DIETS FOR BREEDING MICE

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INTRODUCTION

The rat and mouse cubes described in the first paper of this series have now been in continuous use in this laboratory for over 4 years, and give excellent results for growth and maintenance. It was noted, however, from the start, that they were quite unsuitable for breeding animals (Parkes, 1946). First litter production was seriously impaired even in mice which had been fed on the cubes only during the experiment. By contrast, the breeding diet then in use, a freshly prepared meal mixed as required, which contained 27 % full-cream dried milk, gave very good results. This diet, however, was both expensive and wasteful, and work was undertaken to determine the reasons for the failure of the maintenance cubes to support reproduction. The composition of the two diets is as follows:

	Stock breeding diet (diet 20) (%)	Maintenance cubes (diet 1) (%)
Wholemeal flour	50	35
Oats	—	30
Full-cream dried milk	27	—
Dried skimmed milk	—	15
Dried yeast	12	6
Dried meat and bone meal	6	10
Cod-liver oil	3	2
Sodium chloride	1	1
Calcium carbonate	1	1

The main differences between them lie in:

(1) The physical state of the diet.

(2) The nature and amount of the dried milk.

(3) The content of dried yeast and meat and bone meal.

(4) The cereal component.

The problem has been tackled along the lines indicated by these differences, and the results of the investigation are reported here. The differences in the nature of the dried milk were examined in a series of experiments in which each new diet was compared directly with the stock breeding diet. Differences (3) and (4) were investigated factorially (see below). A factorial test simultaneously employs all possible combinations of the dietary factors being investigated; its efficiency is such that each point at issue is examined with as high an accuracy as if it were the sole object of the test (cf. Fisher, 1947).

TECHNIQUE

The housing, care and breeding methods used have been described in an earlier paper (Bruce, 1947). In the first experiments the mice were mated polygynously, one male with four or five females, and the pregnant females were separated to individual cages for the birth of the young. In the final experiment the system of monogamous pairs was used.

The mice were all bred at the National Institute for Medical Research Farm Laboratories, Mill Hill, from the same stock as had been used in the earlier work. Groups of young adult virgin females with males of similar age were used. Records were kept of the date of birth of litters, number of young born, number of young raised to weaning (21 days), and weight of young at weaning. The experimental diets were given from the day on which the animals were paired and the records started from this date. No preliminary feeding period was allowed. The stock breeding diet was regarded as the standard for comparison and was tested in every experiment. Comparisons were made only between diets tested at the same time under identical conditions. The diets compared in any one experiment were always in the same physical condition, i.e. either a freshly prepared meal, or cubes prepared in bulk the stock of which lasted for the whole of the test.

Criteria on which the diets were judged

Statistical criteria

An examination of the internal variation within diets showed that, although tests of fertility based on numbers of young born and weaned might be expected to be based on χ^2 tables, there was usually significant heterogeneity. Variance analysis has therefore been employed throughout, using the square roots of:

(1) The total number of young born to each female in a test period.

(2) The total number of young weaned by each female in a test period.

(3) The total weight of young weaned by each female in a test period.

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The square root transformation should, and does, sufficiently equalize variances for the analysis of variance to be a valid procedure.

In tests employing polygynous mating, there was usually significant box-to-box variation, so that the appropriate mean square for use as the error term is that between boxes and not between mice. With monogamous pairs, boxes and mice become synonymous for the purposes of analysis.

Length of the test period

The fallacy of testing the fertility of a stock of animals by studying the reproduction of a selected group over the period of maximum reproductive capacity has been stressed by King (1924), and the same principle must be applied to testing fertility on experimental diets. Some authors (Deuel, Hallman & Movitt, 1945) surmount the difficulty by breeding animals through several generations on the same diet. Without experimental evidence it is impossible to predict whether a slowly developing deficiency of some factor or factors present in the diet in suboptimal amounts would be more clearly manifest by such a method or by continued breeding from the same animals for longer periods. Animals such as rats, mice, and guinea-pigs, with relatively early maturity, can have three or four generations in reproduction at the same time. Unless special care is taken to ensure that the young selected for breeding from successive generations are chosen at random, there is a serious danger of unconscious selection. It is not, however, practicable in experimental work to allow even small groups of mice to come to the limits of their reproductive capacity before reaching a conclusion as to the value of a diet for a breeding stock. In view of the big differences in breeding performance on different diets the breeding period has been limited to that long enough to include litters from second and third parities of the more rapid breeders, and also to give records of a sufficiently large number of litters to permit valid comparison. At the end of the arbitrarily chosen experimental period the males were removed; existing pregnancies were allowed to terminate and were included in the results.

Mortality

Sporadic deaths are to be expected in any normal breeding colony even in the absence of specific epidemics. In the series of tests described the mortality of the females, from all causes, during the experimental period was about 14 %, a figure in good agreement with that previously recorded for the normal breeding colony (Bruce, 1947). For this reason all records of females which died before the end of the experimental period were omitted from the results. There was no evidence that any of the diets affected male fertility and any male which died during the course of the experiment was immediately replaced by a normal male from stock.

Sterility

Of the females which survived the full test period 8 % had no recorded matings and were regarded as sterile. In the first tests these were included. Later, as it became evident that a small proportion of sterile animals was to be expected in all groups tested and that sterility, as defined above, was not associated with particular diets, such animals were omitted from the calculations of the results.

RESULTS

Physical state of the diet

Any possible influence of the cubing process, which involves exposure to heat, moisture and pressure for short periods, on the nutritional quality of the diet was examined as a preliminary precaution. A batch of diet 20, cubed, was tested against the diet in meal form freshly mixed in small amounts as required. There was no difference in the results obtained with the two forms of diet (Tables 1 and 2).

Dried milk

The first difference studied was that in the nature and quantity of the dried milk used in the two stock diets. Two new diets were designed, diet 24, in which the full-cream dried milk of diet 20 was replaced by dried skimmed milk; diet 21, in which the dried skimmed milk of the maintenance cubes was raised to 27 %. At the same time, an increase was made in the amount of dried yeast. The full composition of these diets is given in Table 3.

Reproduction was apparently impaired when the full-cream dried milk of the breeding diet was replaced by dried skimmed milk, but statistical analysis did not show a significant effect because of the variation between boxes. However, reproduction failed rapidly on diet 21 in spite of the increase in the dried skimmed milk (Tables 1 and 2). The introduction of dried skimmed milk in the place of full-cream dried milk reduces the fat content of the diet from 11·6 to 4·9 %, and it seemed possible that the apparent partial failure of lactation could be corrected by a return of the fat content to its original level. Arachis oil was chosen for the test, and thus diet 30 was derived (Table 3).

At the same time the effect of merely reducing the level of the full-cream dried milk (the most expensive item in diet 20) was tested (diet 32, Table 3).

The first experiment with diets 30 and 32 was abandoned owing to an outbreak of mouse typhoid, and a second test was started using very much larger numbers of animals than usual (seventy females per group at the start). This proved fortunate as

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Table 1. *Total production of young on individual diets (polygynous mating)*

Exp. no.	Diet: No.	Diet: Form	Duration of test (weeks)	No. of females surviving	Total no. of young: Born	Total no. of young: Weaned	Average size of litter weaned (young)	Average wt. of young weaned (g.)
1	20	Meal	15½	18	194	155	6·0	9·8
	20	Cubes	—	16	186	168	6·0	10·8
2	20	Meal	11	22	260	222	6·9	9·9
	24	Meal	—	25	212	188	6·3	7·7
	21	Meal	—	25	80	30	3·3	6·5
3	1st pregnancies							
	20	Meal	3½	65	400	374	6·8	9·9
	30	Meal	—	69	215	120	4·6	7·8
	32	Meal	—	68	350	309	5·6	10·1
	2nd and later pregnancies							
	20	Meal	14	33	421	389	6·6	10·9
	32	Meal	—	35	533	511	7·4	10·3

Table 2. *Average production per female and the significance of differences between diets (polygynous mating)*

Exp. no.	Diet	No. of females	No. of boxes	No. of young born	No. of young weaned	Total wt. of young weaned (g.)	Young born: *t*	Young born: *P*	Young weaned: *t*	Young weaned: *P*	Total wt. of young weaned: *t*	Total wt. of young weaned: *P*
1	20 (meal	18	5	10·8	8·6	84·3	—	—	—	—	—	—
	20 (cubed)	16	5	11·6	10·5	113·1	0·41	—	0·57	—	0·88	—
2	20	22	6	8·2	10·1	99·4	—	—	—	—	—	—
	24	25	6	8·5	7·5	57·6	1·81	—	1·03	—	1·98	—
	21	25	6	3·2	1·2	7·8	5·40	**<0·001**	5·19	**<0·001**	6·20	**<0·001**
3	1st pregnancies											
	20	65	15	6·2	5·8	58·1	—	—	—	—	—	—
	30	69	15	3·1	1·7	13·7	4·20	**<0·001**	6·03	**<0·001**	7·30	**<0·001**
	32	68	17	5·2	4·5	45·1	0·84	—	1·12	—	1·50	—
	2nd and later pregnancies											
	20	33	8	12·8	11·8	128·0	—	—	—	—	—	—
	32	35	8	15·2	14·6	151·0	1·52	—	1·68	—	1·43	—

Table 3. *Percentage constituents of the diets, with theoretical composition calculated from the* Ministry of Agriculture Bulletin, *no.* 124

	Diet no. 20 (stock breeding)	1 (maintenance cubes*)	24	21	30	32
Wholemeal flour	50	35	50	20	50	58
Ground oats	—	30	—	30	—	—
Full-cream dried milk	27	—	—	—	—	20
Dried skimmed milk	—	15	27	27	20	—
Dried brewer's yeast	12	6	12	9	12	12
Dried meat and bone meal	6	10	6	10	6	6
Cod-liver oil	3	2	3	2	2	2
Calcium carbonate	1	1	1	1	1	1
Sodium chloride	1	1	1	1	1	1
Arachis oil	—	—	—	—	8	—
Digestible protein	19·3	16·9	21·3	20·96	19·0	18·4
Soluble carbohydrate	45·3	44·4	48·2	41·8	44·8	47·8
Fat	11·6	6·3	4·9	5·3	11·8	8·9
Fibre	0·5	1·1	0·5	0·96	0·5	0·5

* The original diet as first published (Parkes, 1946) contained 5% dried yeast and 3% cod-liver oil.

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the supply of arachis oil failed about half way through the test and figures were available only for first pregnancies from diet 30. Even so, the results are highly significant and the addition of arachis oil to replace the milk fat did not restore full reproduction.

About half the females, chosen at random, on diets 20 and 32 were allowed to continue for a further period, and a second comparison was made between the later pregnancies on these two diets. Neither comparison showed any significant difference between the diets (Table 2). It was clear that the level of the full-cream dried milk could be reduced to 20 % in the breeding diet without effect on reproduction and diet 32 provisionally became the stock breeding diet for rats and mice at this Institute. Whether the full-cream dried milk could be reduced further or eliminated was uncertain from these results. There was some doubt as to the efficacy of dried skimmed milk as a substitute, but none as to the unsuitability of dried skimmed milk and arachis oil. At this stage attention was turned to the cereal and yeast contents. These experiments are dealt with below.

Marking special diets

Diet 32, like the maintenance diet, was ultimately prepared in cube form, and to distinguish it a small quantity of an edible dyestuff (Edicol Rose B 500—½ lb. per ton) which gives a strong pink colour was incorporated. A note of warning about the use of this particular dye has been sent to us by Dr T. Moore (1948). The dye permeates the tissues of the animals and gives the white fur a pink tinge. On exposure to screened ultra-violet rays a yellow fluorescence is shown by the tissues and faeces and urine, which might be a serious disadvantage in certain types of work. It has not been established whether the young animals obtain the dye through the milk or after they have started to eat solid food. The dye is not, of course, an essential part of the diet and its concentration could be reduced much below ½ lb. per ton without impairing its function as a marker for special cubes. It is of some interest that an edible dye of the kind commonly used in colouring foodstuffs should apparently act as a vital dye. Other edible dyes were tried for marking particular cubes, including Edicol Chocolate NS, Edicol Pea Green H, and Edicol Tartrazine NNS, but results were not satisfactory.

Cereals and dried yeast

The differences in cereal and yeast contents of diets 20 and 1 were studied by an experiment of factorial design. On the basis of the evidence then available it was decided to put the same proportion of dried milk in all experimental diets, namely that of the diet 32, the latter now being regarded as the standard. Modifications in the nature of the cereal and in the quantity of the dried yeast were introduced to make five new diets. Three types of cereal content were examined: wheat alone, wheat and oats in equal parts, and oats alone, together with the two levels of dried yeast (12 and 6 %) as present in diets 20 and 1. The composition of these diets (33–37) is given in Table 4. In addition to the six diets comprising the factorial test, the maintenance cubes and another cubed diet said to permit satisfactory reproduction were also tested. This diet has a high cereal content and contains very little dried yeast and very little dried milk (Table 4).

Deficiency of any factor which can be stored by the female may not be manifest in first litter production. Some vitamin deficiencies, e.g. vitamin E, are notably slow to establish even with synthetic diets. The use of the monogamous mating system

Table 4. *Percentage composition of the diets used in the factorial experiment*

	Diet no.							
	32	33	34	35	36	37	High-cereal cubes	
Cereals: Wheat	58	29	—	58	29	—	Wheat offal	19·2
Oats	—	29	58	—	29	58	Ground wheat	19·2
	—	—	—	—	—	—	Sussex ground oats	19·2
	—	—	—	—	—	—	Ground barley	9·5
	—	—	—	—	—	—	Ground maize	9·5
Milk: Full-cream	20	20	20	20	20	20		
Skimmed	—	—	—	—	—	—	Dried skimmed milk	7·0
Yeast	12	12	12	6	6	6	Dried yeast	1·2
Meat and bone meal	6	6	6	12	12	12	Meat and bone meal	9·5
	—	—	—	—	—	—	White-fish meal	4·7
Cod-liver oil	2	2	2	2	2	2	Cod-liver oil	0·5
Sodium chloride	1	1	1	1	1	1	Sodium chloride	0·5
Calcium carbonate	1	1	1	1	1	1		

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in which there is continuous opportunity for breeding presumably raises the nutritional requirements of the females to a maximum, and such a method should be particularly suitable for the testing of dietary adequacy. Even so, in the factorial test, in which the monogamous system was used, the deficiencies did not appear quite as rapidly as in some of the earlier tests (compare results from diet 30); moreover, the weaning weight of young from all diets in this test was considerably lower than had been found in the groups of mice receiving the standard diet in the earlier tests (Table 6). No adequate explanation of this was found, but as a safeguard the diets on which lactation had been satisfactory in the earlier work were retested during the course of the factorial experiment.

Four diets were examined in the retest. Diets 20 and 32 as freshly prepared meals; diet 32 cubed from the same batch as was used in the factorial experiment; diet 32 cubes recrushed to a meal. The weaning weights of young from all four diets were below those previously found (Table 5), but there was again no significant difference between them ($F = 2{\cdot}3$ with 3 and 31 D.F.; $P > 0{\cdot}05$). In this type of experiment, as in all biological assays, the necessity for the simultaneous testing of a standard diet in all comparisons made cannot be too strongly emphasized, as well as the necessity for adequate statistical analysis which takes into account the variation in individual response within the particular groups concerned.

The results of the factorial analysis and of those from the two diets tested at the same time are given in Tables 6–9. They have been tabulated for three periods of equal length, starting from the date on which the first litter was born. This shows clearly the gradual impairment of reproduction as the length of time for which the mice had received the inadequate diets (diets 36, 37, 1, and high-cereal cubes) is prolonged.

Factorial analysis of the cereal/yeast balance

Since the diets in this test were about equally, if not optimally, adequate for lactation, and the average weight of young did not vary much from diet to diet, the analysis has been carried out without reference to weaning weights.

Table 5. *Litter size and weaning weight of young*
(Standard diets retested. Length of test, 10 weeks.)

No.	Diet	No. of females	Total no. of young		Average size of litter weaned (young)	Average wt. of young weaned (g.)
			Born	Weaned		
20	Freshly prepared meal	8	108	92	5·8	7·7
32	Freshly prepared meal	10	158	119	6·0	6·7
32	Cubes	8	144	106	5·9	8·0
32	Cubes crushed to a meal	9	80	61	4·7	6·6

Table 6. *Production of young on individual diets (monogamous mating)*
(Length of test, 12 weeks.)

Diet	No. of females	Total no. of young		Average size of litter weaned (young)	Average wt. of young weaned (g.)	Average no. of young weaned per female per period			
		Born	Weaned			1	2	3	All
32	16	282	212	5·0	7·7	5·9	4·2	3·1	13·3
33	20	361	290	5·2	8·8	4·7	5·9	3·9	14·5
34	16	221	148	4·2	7·6	2·7	4·5	2·1	9·3
35	21	244	237	5·4	8·1	5·2	3·1	3·0	11·3
36	20	140	95	4·1	7·3	3·4	1·3	0·1	4·8
37	18	124	88	4·6	7·3	4·4	0·5	0·0	4·9
High-cereal cubes	17	215	175	6·0	7·9	4·4	4·1	1·7	10·3
1 (maintenance cubes)	17	202	93	3·4	6·6	2·9	1·8	0·8	5·5

Heterogeneity. As was indicated above, an examination of the variation encountered within diets for each of the three experimental periods shows that χ^2 tests would not be appropriate for deciding the significance of results. Thus, when the individual females are segregated at random into small within-diet within-period groups and the variation in numbers of young produced by such groups is examined, the overall value of χ^2 with 58° of freedom is 136·8 in the case of young born, and 176·6 in the case of young weaned. If there were no significant heterogeneity, the value of χ^2 should in each case approximate to 58·0, and the deviations found are highly significant.

Table 7. *Analyses of variance for diets* 32–37, *young born and weaned*

Source of variation	D.F.	Sum of squares (born)		Mean square	*F*	*P*	Sum of squares (weaned)		Mean square	*F*	*P*
Diets:	5	97·05		19·41	17·1	**<0·001**	74·66		14·93	12·9	**<0·001**
(*a*)	1		1·92	1·92	1·7	—		3·82	3·82	3·3	—
(*b*)	1		0·97	0·97	0·9	—		3·53	3·53	3·1	—
(*c*)	1		32·23	32·23	28·4	**<0·001**		17·80	17·80	15·4	**<0·001**
(*d*)	1		9·90	9·90	8·7	**<0·01**		5·71	5·71	4·9	**<0·05**
(*e*)	1		50·53	50·53	44·5	**<0·001**		42·50	42·50	36·7	**<0·001**
Sum			95·55					73·36			
Between mice within diets (error 1)	105	119·13		1·13	—	—	121·67		1·16	—	—
Periods:	2	67·09		33·55	29·6	**<0·001**	45·93		22·97	21·8	**<0·001**
(*f*)	1		67·02	67·02	59·2	**<0·001**		45·31	45·31	42·9	**<0·001**
(*g*)	1		2·78	2·78	2·5	—		0·24	0·24	0·2	—
Sum			69·80					45·55			
Diet/period interaction:	10	35·56		3·56	3·1	**<0·01**	33·21		3·32	3·2	**<0·01**
(*a*)/(*f*)	1		0·17	0·17	0·2	—		0·62	0·62	0·6	—
(*b*)/(*f*)	1		0·74	0·74	0·7	—		1·51	1·51	1·4	—
(*c*)/(*f*)	1		7·34	7·34	6·5	**<0·05**		8·28	8·28	7·8	**<0·01**
(*d*)/(*f*)	1		0·00	0·00	0·0	—		0·04	0·04	0·0	—
(*e*)/(*f*)	1		9·27	9·27	8·2	**<0·01**		8·50	8·50	8·1	**<0·01**
(*a*)/(*g*)	1		3·08	3·08	2·7	—		3·32	3·32	3·1	—
(*b*)/(*g*)	1		0·10	0·10	0·1	—		0·67	0·67	0·6	—
(*c*)/(*g*)	1		3·41	3·41	3·0	—		2·13	2·13	2·0	—
(*d*)/(*g*)	1		4·26	4·26	3·8	—		1·27	1·27	1·2	—
(*e*)/(*g*)	1		7·94	7·94	7·0	**<0·01**		9·15	9·15	8·7	**<0·01**
Sum			36·31					35·49			
Within mice (error 2)	210	237·81	—	1·13	—	—	221·66	—	1·06	—	—
Total	332	556·64	—	—	—	—	497·13	—	—	—	—

Table 8. *Separate analyses of variance for dietary factors within each period, young born and weaned*

Period	Source of variation	D.F.	Sum of squares (born)	Mean square	F	P	Sum of squares (weaned)	Mean square	F	P
1	Diets:	5	9·90	1·98	1·7	—	8·75	1·75	1·5	—
	(*a*)	1	1·50	1·50	1·3	—	5·91	5·91	5·0	**< 0·05**
	(*b*)	1	0·03	0·03	0·0	—	0·29	0·29	0·2	—
	(*c*)	1	0·37	0·37	0·3	—	0·04	0·04	0·0	—
	(*d*)	1	6·91	6·91	6·0	**< 0·05**	2·86	2·86	2·4	—
	(*e*)	1	0·64	0·64	0·6	—	0·22	0·22	0·2	—
	Sum		9·45				9·32			
	Between mice within diets (error 1)	105	120·66	1·15	—	—	124·92	1·19	—	—
2	Diets:	5	64·17	12·83	10·9	**< 0·001**	55·31	11·06	9·5	**< 0·001**
	(*a*)	1	0·40	0·40	0·3	—	0·13	0·13	0·1	—
	(*b*)	1	0·68	0·68	0·6	—	2·67	2·67	2·3	—
	(*c*)	1	22·91	22·91	19·4	**< 0·001**	13·16	13·16	11·3	*c.* **0·001**
	(*d*)	1	0·02	0·02	0·0	—	0·21	0·21	0·2	—
	(*e*)	1	41·02	41·02	34·8	**< 0·001**	38·86	38·86	33·5	**< 0·001**
	Sum		65·03				55·03			
	Between mice within diets (error 1)	105	123·78	1·18	—	—	121·96	1·16	—	—
3	Diets:	5	58·55	11·71	10·9	**< 0·001**	43·75	8·75	9·5	**< 0·001**
	(*a*)	1	3·27	3·27	3·1	—	1·73	1·73	1·9	—
	(*b*)	1	1·10	1·10	1·0	—	1·18	1·18	1·3	—
	(*c*)	1	19·70	19·70	18·4	**< 0·001**	15·00	15·00	16·3	**< 0·001**
	(*d*)	1	7·24	7·24	6·8	*c.* **0·01**	3·96	3·96	4·3	**< 0·05**
	(*e*)	1	26·07	26·07	24·3	**< 0·001**	21·07	21·07	22·9	**< 0·001**
	Sum		57·38				42·94			
	Between mice within diets (error 1)	105	112·50	1·07	—	—	96·50	0·92	—	—

It was, therefore, decided to continue using the technique of variance analysis with the square root of the number of young produced by each mouse in each period as the variate.

Method of analysis (for diets 32–37 only)

(1) The following dietary factors have been isolated in the analysis:

(*a*) Wheat versus oats with high yeast.

(*b*) Wheat versus oats with low yeast.

(*c*) and (*d*). The difference between the diet containing equal parts of wheat and oats and the average of the two diets with all wheat and all oats, for high and low yeast separately.

(*e*) The overall difference between high and low yeast.

Overall analyses of variance. Table 7 shows these analyses for young born and young weaned. The error mean square for 'between diets' is that derived from variation between mice, the error mean square for 'between periods' and interactions is that derived from variation within the same mice from period to period. It is interesting that the two measures of error are practically identical in each case. The figures for both young born and weaned show the same effects. The sum of the sums of squares for the separately isolated factors would add up to the sum of squares above and to the left in the table in each case, if groups were equal in number. The discrepancy measures the extent to which there is approximation, and is clearly not of importance.

Table 9. *Comparison of high-cereal cubes and diet* 1 *with diets* 32 *and* 33 *combined*

	Mean no. of young per female						High-cereal cubes				Maintenance cubes (diet 1)			
	High-cereal cubes		Maintenance cubes (diet 1)		32 + 33		Born		Weaned		Born		Weaned	
Period	Born	Weaned	Born	Weaned	Born	Weaned	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*
All	12·7	10·2	11·9	5·5	17·9	13·9	2·7	< 0·01	2·2	< 0·05	2·8	< 0·01	4·8	< 0·001
1	5·9	4·4	6·1	2·9	6·5	5·3	0·4	—	0·7	—	0·0	—	2·0	*c.* 0·05
2	5·0	4·1	3·7	1·8	6·7	5·1	1·5	—	1·1	—	1·8	—	3·0	< 0·01
3	1·7	1·7	2·1	0·8	4·6	3·6	3·2	< 0·01	2·4	< 0·05	2·3	< 0·05	3·5	< 0·001

The high-cereal diet fails in the third period (with signs of failure in the second), but with no evidence for lactational failure, while the maintenance cubes fail from the outset, with lactation failure or killing of young as the outstanding feature in addition to fewer births.

(2) The following secular factors have been isolated:

(*f*) The difference between periods 1 and 3.

(*g*) The difference between period 2 and the average for periods 1 and 3.

(3) The ten interactions between the factors in (1) and (2) above have also been isolated, e.g. interaction (*e*)/(*f*) measures the extent to which the difference between high and low yeast was consistent from periods 1 to 3, and so forth.

(4) Since the actual numbers of females on each diet varied, although not by a high percentage, these isolations have been made on the basis of the mean number of females per group (diet), namely 18·5. Tests of significance of the individual factors are therefore approximate, although so very nearly exact that it is not considered necessary to determine the separate actual error term for each factor, in particular since the most important factors have very high levels of significance attached to them.

(5) Following the demonstration of significant interactions, the dietary effects were examined separately within each period, the five factors being isolated as under (1) above.

Conclusions from these overall analyses are:

(1) There is no significant effect of substituting oats for wheat with high yeast.

(2) The substitution of even half of the wheat by oats is highly deleterious with low yeast.

(3) The overall effect of high yeast versus low yeast is thus highly significant, but is due to the diets in which some or all oats is used. A high wheat/low yeast diet is adequate (assuming that any of them is).

(4) The only interactions of diets and periods which attain significance are:

(*c*)/(*f*)—i.e. the difference between wheat and oats was not seen consistently in all periods, since the impairment of reproduction was gradual.

(*e*)/(*f*) and (*e*)/(*g*)—i.e. the difference between high and low yeast, which was also dependent upon a deficiency which developed gradually, was not constant in all periods.

Separate analyses by periods. Table 8 shows the results of investigating the separate dietary effects within periods. From it, the detailed meaning of the interactions is apparent. In short, no significant differentiation between diets occurred in the first

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period, while in the second and third periods the failure of low yeast, oats-containing diets, is seen to be highly significant.

Comparison of the reproduction on a grossly inadequate diet (1), *a slightly deficient diet* (*high cereal*) *and adequate diets* (32 *and* 33). Table 9 gives the results of *t*-tests of the difference between diet 1 or the high-cereal diet, and diets 32 and 33 together. These latter diets were chosen as representing the best of the experimental diets and as having wheat present as in the high-cereal diet and diet 1, although with much more yeast and with full-cream milk. It is seen that the figures for young born and weaned tell a rather different story, indicating lactational failure, in the less satisfactory diet 1. Thus the high-cereal diet fails in the third period (with signs of failure in the second), but with no evidence for lactational failure, while diet 1 fails from the outset, with lactational failure or killing of young as the outstanding feature in addition to fewer births.

DISCUSSION

Factorial design and analysis is particularly suited to the study of dietary effects. The introduction of a new component into a diet, e.g. oats in diets 33, 34, 36, 37, or an alteration in the proportion of any one constituent, e.g. diets 32/35, 33/36, 34/37, may be compensated by decreasing the absolute amounts of all other constituents, keeping their respective ratios constant, or by decreasing the amount of at least one constituent, leaving the others unaltered in absolute amount. In the factorial tests reported here, the dietary factors have been dealt with as reciprocating pairs, the increase of one factor being always at the expense of only one other. Thus, in the six diets in the factorial test, the change in the level of dried meat and bone meal balanced the change in the level of dried yeast (diets 32–34 and diets 35–37) and the resulting effect on reproduction may be due to either factor. It is assumed that the factor concerned is the level of dried yeast, as the impaired performance on those diets low in yeast could only otherwise be explained on the assumption that doubling the meat and bone meal content of a diet has a toxic action. It has thus been possible to measure the effect of two dietary factors, one at three levels and the other at two levels, and to evaluate the interactions between them in a single experiment involving only 144 pairs of mice, which was completed within a few months. In fact, the important conclusions could have been reached with even fewer animals. It must also be noted that the overall differences between the diets were adequately demonstrated by period 3 alone, and that a considerable saving of labour could be effected by taking detailed records for this period only. Deuel, Movitt & Hallman (1944), when studying the effect of different fats in the dietary of breeding rats, took the precaution to rear the animals from weaning until ready for mating on the diets concerned. This practice would undoubtedly help where the deficiency of a factor which the animal can store is in question, but it is time-consuming.

The present limitations in the supply of many of the feeding stuffs in general use for small animal breeding colonies mean constant changes in the diet supplied to the animals. Where a large number of animals are concerned a rapid method of checking the effects of any major change is greatly to be desired. The use of monogamously mated animals where the females are pregnant and lactating at the same time, in an experiment of factorial design where several factors can be tested simultaneously, meets such a need.

The exact nutritional requirements of mice have not been determined, but in general they appear to be similar to those of rats. In a recent publication Morris (1944) reviewed the available knowledge in this field, most of which has been supplied by experiments with synthetic diets. The available knowledge in the more limited field of the influence of the B vitamins on reproduction has been collected in an article by Hertz (1946). The dietary implications of the experiments reported here will be discussed later.

SUMMARY

1. The application of factorial design to the testing of diets for breeding mice is described.

2. The use of a system of monogamous mating, by which the females have a continuous series of pregnancies with concurrent gestation and lactation, is advocated for such tests.

3. Present indications are that a high wheat content or a high yeast content is desirable in the diet of breeding mice.

4. A diet containing a considerable proportion of full-cream milk was very effective for breeding mice, but it is not yet certain that this constituent is necessary.

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* The high cereal cubes referred to on p. 318 had the same composition as those described by Thomson (1936). They were not, however, prepared by the same firm as the Aberdeen cubes, nor were they used in conjunction with fresh green food as considered essential by Thomson.

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