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A study of the protein and amino acid requirements of the growing New Zealand White rabbit with emphasis on lysine and the sulphur-containing amino acids

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1. New Zealand White (NZW) rabbits were given, between 4 and 8 weeks of age, a range of diets, based on oats and fish meal, containing from 104 to 255 g crude protein (nitrogen ×6·25; CP)/kg to establish the level of CP below which growth was retarded.

2. In three experiments each diet was fed to four animals and food intake, growth and N balance were measured over 4 weeks. Body analysis was also carried out after two of the experiments.

3. The rates of food intake and growth of animals increased with dietary CP concentration until a CP concentration of approximately 150 g/kg diet had been reached. Beyond this there was little further improvement. N balance studies showed that once this dietary concentration of CP had been reached, there was a reduced rate of N retention.

4. Good agreement was found between N retention measured by balance methods and by body analysis: body composition showed a tendency towards an increase in fat and a decrease in N as the dietary protein concentration was reduced.

5. Microbial protein produced in the caecum and eaten during coprophagy, was found to supplement the dietary protein by approximately 2 g CP/d, or by only 0·1 of a normal dietary intake of CP.

6. In the second part of the study NZW rabbits were offered, between 5 and 8 weeks of age, diets based on oats containing 150 g CP/kg. The protein supplied by oats was supplemented with maize gluten, gelatin, groundnut meal, casein, soya-bean meal or fish meal.

7. Rabbits offered diets containing casein, soya-bean meal and fish meal gained 40–50 g/d similar, to animals given a well-balanced control diet, while those given diets containing maize gluten, gelatin or groundnut meal gained approximately 30 g/d. This indicated that amino acid balance in dietary protein was important to the growing rabbit.

8. In later experiments, diets based on cereals and groundnut meal supplemented with varying amounts of lysine and methionine were offered during a 3-week post-weaning period in order to assess requirements for those limiting amino acids.

9. The addition of both lysine and methionine improved growth rates. The minimum requirements for normal growth were found to be 6·2 g methionine+cystine and 9·4 g lysine/kg diet.

The rabbit has a large caecum containing a varied microflora. It is also coprophagous, half the faeces appearing as a distinct soft type, rich in protein. These are eaten directly from the anus. For nearly a century the species has consequently been credited with some of the digestive ability of a ruminant (Morot, 1882; Madsen, 1939; Eden, 1940; Taylor, 1940).

Current commercial diets fed to intensively-kept rabbits are based largely on cereals and their by-products, oil-seed meals, and grass meal. Crude protein (nitrogen × 6·25; CP) levels are similar to those in poultry diets but the crude fibre (CF) content is usually higher. According to published estimates the growing rabbit requires amounts ranging from 150 to 230 g CP/kg diet ((US) National Research Council, 1966; Niehaus, 1968; Heckmann & Mehner, 1970; Ministry of Agriculture, Fisheries and Food, 1973). Commercially it is usual for only one type of diet with a CP level of approximately 190 g/kg to be manufactured for all classes of stock.

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The present study was undertaken to determine whether amino acid balance in dietary protein was important to this animal, which can recycle material fermented in the caecum, and if so, which were the limiting amino acids and their requirements.

MATERIALS AND METHODS

The study was divided into two parts. Expts 1–3 were designed to determine that level of protein which was required in a good-quality diet based on cereal and fish meal, to allow the full growth potential of the young growing rabbit to be achieved. Included were studies of N balance and body composition as well as an assessment of the amount of bacterial protein recycled by coprophagy. In Expts 4–7 the effectiveness of supplementary sources of protein other than fish meal were assessed to determine whether coprophagy allowed the young rabbit to be more tolerant of a poor balance of amino acids in dietary protein than are simple-stomached species. Serial supplementation was then carried out with amino acids found to be limiting growth when feeding a poorly-balanced diet containing groundnut. In this way an estimate was made of the growing rabbits' requirements for the limiting amino acids lysine and the sulphur-containing amino acids.

Rabbits

Expts 1–3. Males and females of the New Zealand White (NZW) breed were used throughout. They were weaned at 4 weeks of age but experiments did not begin until they were 5 weeks old and weighed 600–700 g.

Expts 4–7. NZW weanling rabbits, 4 weeks old, were again used. On this occasion they were obtained at 500–600 g live weight from a breeding colony of females established at the Rowett Research Institute under conditions designed to exclude pathogenic organisms. Young produced by this colony had been shown to grow at rates similar to those of animals raised conventionally.

Cages

Sixteen cages used in the first three experiments were designed for balance work and had, beneath each, a stainless-steel screen having 4 mm mesh to retain faeces but allow free passage of urine which was funnelled into a collecting bottle. Both the mesh-screen and collecting-funnel were easily removed for the collection of faeces and for the 'washing-down' of urine residues.

For Expts 4-7 animals were kept individually in commercial wire cages suspended approximately 1 m above the floor. Excreta dropped into sawdust which was removed weekly.

Diets

During each experiment a control diet was fed. This was based on diet SG1 developed by Short & Gammage (1959) and found previously at the Rowett Research Institute to allow normal growth. All diets were fed in the form of pellets 4·7 mm in diameter.

Table 1 gives the range of ingredients used in Expts 1–3 to achieve the CP levels shown in Table 2. The vitamin and mineral mixes added to the final diet contributed the amounts suggested by Adamson & Fisher (1971).

Reductions in the concentrations of CP were made by the replacement of fish meal by oats and starch. As the proportion of oats in the diet was increased, the amount of oat husk added to the diet was reduced and in this way the level of CF was kept relatively constant throughout the series of diets. Similarly the amount of bonemeal was increased to replace minerals lost when the proportion of fish meal was reduced. In this way the major change

Table 1. *Expts* 1–3. *Composition* (*g/kg*) *of experimental and control diets*

	Experimental diets						
	A	B	C	D	E	F	G
Ground oats	689	675	690	660	630	574	444
Ground oat husk	29	30	35	45	55	58	106
Grass meal	48	50	50	50	50	48	48
Fish meal	9·5	10	40	70	100	126	242
Bonemeal	19	20	10	5	0	9·5	0
Dried distillers' solubles	48	50	50	50	50	48	48
Maize oil	19	20	20	20	20	25	29
Starch	57	60	20	15	10	29	1·5
Vitamin mix*	24	25	25	25	25	25	24
Mineral mix*	48	50	50	50	50	48	48
Pellet binder	9·5	10	10	10	10	9·5	9·5
CP in diet (g/kg)	104	112	125	139	156	168	255

Control diet SG1†	
White fish meal	100
Grass meal	200
Bran	400
Ground oats	120
Middlings	180
Vitamins	†
Minerals	†

CP, crude protein (nitrogen × 6·25).

* Contributed to the final diet the amounts suggested by Adamson & Fisher (1971).

† This diet SG1 (Short & Gammage, 1959) was commercially produced and the vitamin and mineral additions were undisclosed.

Table 2. *Expts* 1–3. *Proximate analyses* (*g/kg*) *of the experimental and control diets**

		Crude protein (nitrogen × 6·25)	Crude fibre	Crude fat	Ash	Gross energy (MJ/kg)
Experimental diets	A	104	65	57	50	16·5
	B	112	77	54	80	15·9
	C	125	78	46	84	15·8
	D	139	79	56	85	15·8
	E	156	80	48	89	15·7
	F	168	70	53	74	16·5
	G	255	69	70	102	16·5
Control diet SG1†		183	68	45	96	16·6

* For details, see Table 1.

† Short & Gammage (1959).

from diet to diet was restricted to the level of CP, although there would also have been some change in the balance of amino acids present.

The composition of the basal diet for Expts 4–7 is given in Table 3. The isonitrogenous additions of six sources of protein made to 810 g basal diet for Expt 4 are shown in Table 3, together with the amounts of starch necessary to maintain similar concentrations of CP in each diet. Chemical analyses are also given.

Results from Expt 4 indicated that the diet based on groundnut meal was probably deficient in certain essential amino acids. From experience with pigs and poultry these were likely to be lysine and the S-containing amino acids. Diets in Expt 5 were thereforef ormulate

Table 3. *Expt* 4. *Composition* (*g/kg*) *of experimental and control diets*

Protein source ...	Maize gluten	Gelatin	Ground-nut meal	Casein	Soya-bean meal	Fish meal	Control diets SG1†
Ingredients							
Basal mix*	810	810	810	810	810	810	—
Added protein source	125	99	187	99	187	132	—
Maize starch	65	91	3	91	3	58	—
Analysis							
Crude protein (nitrogen × 6·25)	150	162	144	151	154	148	193
Lysine	4·1	6·8	5·8	9·6	8·0	8·6	9·5
Cystine	2·9	1·4	2·8	1·5	2·3	1·8	3·0
Methionine	2·3	2·0	1·9	3·5	2·3	3·7	4·2
Gross energy (MJ/kg)	16·8	16·3	16·3	16·5	16·3	16·3	16·8

* Basal mix: ground oats 520, ground oat husk 123, grass meal 123, maize oil 37, vitamin mix 62, mineral mix 123, pellet binder 12; the vitamin and mineral mixes contributed to the final diet the amounts suggested by Adamson & Fisher (1971).

† For details, see Tables 1 and 2.

Table 4. *Expts* 5–7. *Additions of lysine hydrochloride* (*LYS.HCl*) *and methionine* (*MET*) *made to groundnut-meal-based diets** (*g/kg diet*)

Expt no. ...	5		6		7	
Amino acid ...	LYS.HCl	MET	LYS.HCl	MET	LYS.HCl	MET
Diet no.						
1	—	—	6·8	—	—	2·2
2	2·0	—	6·8	1·0	2·0	2·2
3	4·0	—	6·8	2·0	3·4	2·2
4	4·0	3·0	6·8	3·0	6·8	2·2

* For details, see Table 3.

to contain 810 g basal mix and 187 g groundnut meal, unsupplemented or with the addition of one of two levels of lysine or with lysine plus methionine, as indicated in Table 4.

A new batch of groundnut meal with a higher CP concentration was used in Expts 6 and 7, therefore only 150 g was added to 810 g basal mix. Each diet was made up to 1 kg with maize starch. In Expt 6 the concentration of lysine was increased well above the estimated requirement level in each diet and the concentration of methionine was gradually increased by the additions of amino acid given in Table 4. In Expt 7 the methionine concentration was increased above the estimated requirement in all diets while the level of lysine was progressively increased by the amounts given in Table 4.

Procedure

Expts 1–3. The control diet SG1 was fed to all animals for 5 d after weaning. Groups of four animals were then selected at random to provide a similar range of weights, and transferred to experimental diets for 2 d before recording began. Animals were weighed daily but the consumption of food was measured weekly while excreta were collected daily, pooled and recorded over 1 week and analysed. Each experiment lasted for 4 weeks.

The daily collections of faeces were stored at −20 °. The 7 d combined collections were sampled and the sample freeze-dried. Previously it had been found that no loss of N occurred when faeces were feeeze-dried.

Urine was held at 1° during the 7 d collection period, but any sample held longer before testing was deep-frozen. Samples of urine showed no reduction in N after such treatment even though they were not acidified.

The sixteen balance cages accommodated up to four groups of four animals given the control diet SG1 and up to three experimental diets. In Expt 1 a wide range of protein concentrations was tested, the CP levels of the three experimental diets being 104, 168 and 255 g/kg diet. Three intermediate CP levels were used in the second experiment, 125, 139 and 156 g/kg diet. In the third experiment, a diet containing 112 g CP/kg diet was used to provide some further results at a level where animals were performing poorly.

To prevent coprophagy and thus obtain soft faeces for analysis, a separate group of animals was fitted with collars for periods of 24 h. These collars were cut from 25 mm fibreboard and were 120 mm in diameter. The soft and normal faeces were easily distinguished and separated.

Before the start of Expt 2 a group of nine animals was killed to obtain an estimate of their total body N. At the end of Expts 2 and 3 all animals were killed for body analysis. The contents of the abomasum, colon, caecum and rectum were weighed and freeze-dried. The empty organs were replaced and the body was frozen at −20°, sawn into pieces and passed through a mincer with a die having 4 mm diameter holes. A sample of the minced body was then freeze-dried and again minced through a similar-sized die before analysis.

Expts 4–7. All animals were offered the control diet SG1 for 6 d after weaning, when all those growing normally were randomly allocated to one control treatment and to the experimental treatments. Experimental diets were introduced without a transition period and were offered *ad lib.* for the next 3 weeks. During this period the weight gain and food consumption of each animal were recorded. A regression analysis of live weight *v.* time for the 3-week period was carried out, using all results obtained after steady growth on the diet had begun. This was usually within 48 h. The regression coefficients obtained were used as estimates of daily weight gain.

In Expt 4, when various sources of protein were compared, ten animals were used for each treatment group. In Expts 5 and 6 groups of twenty animals were used and in Expt 7 sixteen animals/group were used for each treatment.

Analytical methods

N. The sample was digested by the normal macro-Kjeldahl procedure of the Association of Official Analytical Chemists (1970), with mercuric oxide as the catalyst. After the appropriate dilution of the digest, N was estimated colorimetrically as the indophenol blue complex using a Technicon AutoAnalyzer (Technicon Instruments Co. Ltd, Basingstoke, Hants) system as described by Davidson, Mathieson & Boyne (1970).

Body lipids. The chloroform–water–methanol solvent extraction system described by Atkinson, Fowler, Garton & Lough (1972) was used for this estimation.

Ash, crude fat and crude fibre. These components were determined by the procedure described by Association of Official Analytical Chemists (1970), except that in the CF test, a filter-cloth was used in place of the filters described.

α-Diaminopimelic acid (DAPA). The method used was that described by Mason (1969).

Gross energy. Energy values for diets and body samples were estimated using an adiabatic bomb calorimeter (Gallenkamp, London E.C.2).

Amino acid analysis. Samples of finely-ground diet containing approximately 10 mg N were hydrolysed for 18 h in 200 ml 'constant-boiling' 6 M-hydrochloric acid under reflux in an oil-bath maintained at 130°. The resultant hydrolysate was filtered and made up to an appropriate volume. A measured volume containing approximately 1 mg N was taken just to dryness in a rotary evaporator. The residue was diluted with water and taken just to dryness

Table 5. *Expts* 1–3.* *Effect of increasing dietary concentration of crude protein (nitrogen* × 6·25; *CP) on food intake (g), weight increase (g) and the excretion and retention of N (g) over* 28 *d in weanling New Zealand White rabbits*

(Mean values with their standard errors for 4 animals/dietary group)

Expt no. ...	1	3	2	2	2	1	1	1	2	3
Diet† ...	A	B	C	D	E	F	G	Control diet SG1		
CP in diet (g/kg)	104	112	125	139	156	168	255	183	183	183
Weight increased										
Mean	432	705	921	1049	1074	954	1068	1008	962	1008
SE	41·1	70·7	36·4	39·3	52·3	44·8	37·0	70·3	103	23·4
Food eaten										
Mean	1478	2070	2735	2887	3189	2764	3039	3085	3167	2935
SE	18·6	123	32·8	173	138	92·4	83·5	112	246	44·8
N intake										
Mean	24·6	37·1	54·7	64·2	79·6	74·3	124	90·4	92·8	85·9
SE	0·32	2·22	0·68	3·86	3·46	2·49	3·50	3·85	8·04	1·54
N in urine										
Mean	6·91	9·18	16·1	21·9	28·8	25·5	66·9	30·1	38·2	32·5
SE	0·46	0·34	0·60	0·69	0·51	1·74	2·34	1·27	3·95	1·11
N in faeces										
Mean	4·56	7·79	9·88	11·7	15·3	13·8	16·3	24·5	23·7	22·6
SE	0·23	0·53	0·71	1·77	1·05	0·65	0·75	1·59	2·81	0·26
N retained (by difference)										
Mean	13·1	20·1	28·7	30·5	35·4	35·1	40·6	35·9	30·9	30·9
SE	0·36	1·77	1·08	1·87	2·49	1·31	0·62	2·18	1·76	1·90
N retained (by body analysis)										
Mean	—	19·0	26·8	32·3	33·3	—	—	—	28·9	30·8
SE	—	1·80	0·73	1·53	1·84	—	—	—	4·40	1·03

* For details, see p. 604.
† For details, see Tables 1 and 2.

Table 6. *Expts 2 and 3.* Effect of variation in dietary crude protein (nitrogen × 6·25; CP) content on composition of body (g/kg dry matter (DM)) of weanling New Zealand White rabbits*

	Expt 2					Expt 3		
CP content† (g/kg DM)	125	139	156	Control diet SG1		112	Control diet SG1	
	Adjusted treatment means				SE of difference between means	Adjusted treatment means		SE of difference between means
DM in body (g/kg)	337[a]	308[b]	324[b]	323[b]	11·1	321[a]	308[a]	17·8
N in body DM	92·4[a]	101[b]	94·3[a]	94·8[a]	2·7	93·3[a]	104[b]	3·64
Fat in body DM	322[a]	279[b]	292[b]	275[b]	14·6	301[a]	259[a]	24·7
Ash in body DM	103[a]	109[a]	105[a]	105[a]	6·0	117[a]	97·7[b]	8·22

[a, b] Within each Expt mean values with the same superscript letter were not significantly different ($P < 0.05$).
* For details, see p. 605.
† For details of diets, see Tables 1 and 2.

twice more before making up to 10 ml with pH 2·0 buffer containing a standard amount of norleucine. The amino acids in a known volume equivalent to approximately 0·05 mg N were then separated using an amino acid analyser (Technicon NCl; Technicon Instruments Co. Ltd) and estimated as described by Davidson, Boyne, Hepburn & Mackie (1974).

In Expt 6 a sample of basal diet was also hydrolysed and analysed after a preliminary treatment with performic acid (Moore, 1963), thus obtaining more accurate estimates of cystine and methionine.

RESULTS

Expts 1–3

Growth. During these preliminary experiments on protein requirement, maximum live-weight gain occurred when the dietary CP concentration was between 140 and 156 g/kg (Table 5).

Food intake had also reached a maximum at approximately this dietary CP concentration (Table 5), so that the efficiency of conversion of food to live body-weight was 2·8:1 while at the lowest concentration of CP it was 3·4:1. No improvement was found at higher concentrations of CP.

All food conversion efficiency values were increased by approximately 20 % if expressed in terms of 'empty' body-weight, using the weights for gut contents obtained during body analyses.

N balance. N excreted daily in the faeces with the experimental diets increased until the dietary concentration of CP reached 156 g/kg (Table 5) after which there was little or no further increase.

Urinary N, however, increased linearly and more than doubled between dietary concentrations of 168 and 255 g CP/kg.

Where body analysis was carried out (Expts 2 and 3) there was reasonable agreement between the values for N retention obtained by direct and indirect methods.

Body composition. No clearly-defined pattern was evident in the results from body analysis (Table 6). In Expt 3 the proportion of N in the body was significantly less ($P < 0.05$) for

Table 7. *Expt* 4. * *Effect of supplementary protein on rate of weight gain and food consumption of weanling New Zealand White rabbits given a diet based on oats*†, *in which the supplement supplied* 0·6 *of the total crude protein* (*nitrogen* × 6·25)

	Supplementary protein						Control diet SG1‡	SE of difference
	Maize gluten	Gelatin	Ground-nut meal	Casein	Soya bean meal	Fish meal		
Wt gain (g/d)	24·7[a]	29·5[a]	32·0[a]	46·3[b]	42·0[b]	50·1[b]	47·3[b]	4·4
Food intake (g/21 d)	1196[a]	1314[a]	1432[a]	1940[b]	2085[b]	2013[b]	2323[c]	101

[a, b, c] Mean values with the same superscript letter were not significantly different ($P < 0·05$).
* For details, see p. 605.
† For details, see Table 3.
‡ For details, see Table 1.

Table 8. *Expt* 5 * *Effect of additions of lysine* (*LYS*) *and methionine* (*MET*) *on rate of weight gain and food consumption of weanling New Zealand White rabbits when given a diet based on groundnut meal and oats*†

Calculated amino acid concentration (g/kg diet)	Experimental diets				Control diet SG1‡	SE of difference
LYS	5·9	7·4	9·0	9·0		
MET+CYS	4·6	4·6	4·6	7·6		
Wt gain (g/d)	30·6[a]	29·3[a]	28·1[a]	36·2[b]	42·2[c]	1·7
Food intake (g/21 d)	1784[a]	1772[a]	1755[a]	1986[b]	2435[c]	75

[a, b, c] Mean values with the same superscript letter were not significantly different ($P < 0·01$).
* For details, see p. 605.
† For details, see Tables 3 and 4.
‡ For details, see Table 1.

the treatment than for the control, whereas in Expt 2 only the proportion of body fat showed a significant difference ($P < 0·05$). In the latter instance the diet containing the lowest protein concentration gave rise to significantly higher body fat than the other three diets.

Microbial protein. Each 'collared' animal was found to produce a mean wet weight of 28 g soft faeces in 24 h. The mean N content of these faeces was 16·9 g/kg and they contained 0·53 g DAPA/kg.

Weller, Gray & Pilgrim (1958) demonstrated that N derived from DAPA represented 0·57–0·64 % of the total N of mixed rumen bacteria. A corresponding value for the pig was 0·67 % (P. J. Whittle, personal communication). A mean value of 0·62 % was assumed for rabbit faeces. DAPA itself contains 14·7 % N.

If the N in DAPA is 0·62 % of the total bacterial N in soft rabbit faeces, then 0·62 × 100/14·7 g DAPA is equivalent to 100 g bacterial N. The concentration of DAPA in these soft faeces was found to be 0·53 g/kg, which represents 12·6 g bacterial N/kg soft faeces. Thus the 28 g soft faeces produced daily contained 0·353 g bacterial N, which is equivalent to 2·2 g CP or 1·3 g true protein if a value of 60 % amino acid N in bacterial N (Smith & Palmer, 1976) is taken.

Table 9. *Expt* 6.* *Effect of additions of methionine (MET) on rate of weight gain and food consumption of weanling New Zealand White rabbits when given a diet based on groundnut meal and oats supplemented with lysine (LYS)*†

Amino acid concentrations found by analysis (g/kg)	Experimental diets				Control diet SG1‡	SE of difference
LYS	10	10	10	10		
CYS	1·9	2·1	2·1	2·0		
MET	2·0	2·8	3·1	4·6		
CYS§	2·5	(2·5)	(2·5)	(2·5)		
MET§	2·7	(3·7)	(4·7)	(5·7)		
Wt gain (g/d)	28·6[a]	34·2[b]	33·7[b]	35·4[b]	41·4[c]	1·63
Food intake (g/21 d)	1576[a]	1761[b]	1757[b]	1818[b]	2364[c]	71·6

CYS, cystine.
[a, b, c] Mean values with the same superscript letter were not significantly different ($P < 0.01$).
* For details, see p. 605.
† For details, see Tables 3 and 4.
‡ For details, see Table 1.
§ From second basal diet when oxidation with performic acid preceded hydrolysis; values in parentheses assume efficient mixing of added MET.

Table 10. *Expt* 7.* *Effect of additions of lysine (LYS) on rate of weight gain and food consumption of weanling New Zealand White rabbits when given a diet based on groundnut meal and oats supplemented with methionine (MET)*†

Amino acid concentrations in diet found by analysis (g/kg)	Experimental diets				Control diet SG1‡	SE of difference
MET+CYS	6	6	6	6		
LYS	5·0	5·7	7·1	9·4		
Wt gain (g/d)	32·4[a]	39·6[b]	41·6[b]	47·2[c]	49·4[c]	2·0
Food intake (g/21 d)	1362[a]	1626[b]	1575[b]	1824[c]	2186[d]	87

CYS, cystine.
[a, b, c, d] Mean values with the same superscript letter were not significantly different ($P < 0.01$).
* For details, see p. 605.
† For details, see Tables 3 and 4.
‡ For details, see Table 1.

Expts 4–7

Table 7 gives the mean weight gains and food intakes for the groups in Expt 4. The supplementary value of the protein sources could be divided into two categories. Animals given casein, soya-bean meal and fish meal as supplements grew at rates not significantly different ($P < 0.05$) from the value of 47·3 g/d for the control group. Maize gluten, gelatin and groundnut meal were not as effective as supplements, however, for animals given these gained approximately 30 g/d, which was significantly less ($P < 0.05$) than the control group. Food intakes could be divided similarly into two categories which were significantly different ($P < 0.05$).

During Expt 5 the weight gains of animals given diets containing groundnut meal supplemented with lysine remained low unless methionine was also added (Table 8). Weight gains were then significantly improved ($P < 0.01$) but were still inferior to those of the control group. The food consumptions indicate that only when methionine was added was there a significant increase ($P < 0.01$) in the food consumed.

In Expt 6 (Table 9) when all diets contained an adequate concentration of lysine, only the first addition of 1·0 g methionine increased the rate of weight gain significantly

($P < 0.01$). There was no further response to added methionine above this first level of addition. All weight gains were however significantly less ($P < 0.01$) than those of the control group. Food consumption increased significantly ($P < 0.01$) only with the first addition of methionine.

In Expt 7 (Table 10) when there was an adequate level of methionine in all diets, the addition of lysine brought about significant ($P < 0.01$) progressive improvements in the rate of weight gain. With the highest concentration of lysine, 9·4 g/kg, the rate of weight gain was not significantly different from the control group. Food consumption also increased significantly ($P < 0.01$) as the concentration of lysine in the diet increased.

DISCUSSION

In the first three experiments a decrease in food consumption and weight gain occurred when the CP concentration of the diet decreased below approximately 150 g/kg. The reduction in CP level was made by replacing fish meal with oats so the relative concentrations of amino acids would also have been changed. The decrease in food consumption and weight gain might therefore have been less marked if the balance of amino acids could have been maintained while the over-all CP level was reduced. However, any practical diet having this CP level would contain a similar balance of amino acids which could be substantially changed only by the use of purified ingredients.

The food intake of animals given the diet containing 168 g CP/kg was less than would have been expected, lowering the values for growth and N balance (Table 5). There was no obvious reason for the reduced intake, as all animals appeared healthy, but it is possible that this particular diet had become tainted in some way, making it less palatable.

Groups given the control diets SG1 excreted more faecal N than other groups (Table 5). The apparent N digestibility for diet SG1 was 0.74 ± 0.006 (SE) while the mean ($\pm$ SE) for the experimental diets was 0.82 ± 0.006. Of the CP in diet SG1 60 % was derived from bran and grass meal (Table 1) making it apparently less digestible than the CP contributed by oats in the experimental diets.

Although few animals were used in the study of body composition, nevertheless the results in Table 6 reveal differences in fat and N concentration which are consistent with the view that when the dietary concentration of protein is below the optimum there is a tendency for the proportion of fat in the body to increase and the proportion of N to decrease.

Bacterial cells accounted for 74·4 % of the N in the soft faeces. Griffiths & Davies (1963) dissected soft faeces and estimated that without the faecal envelope 81 % of the N came from bacterial debris. The estimate of 2 g CP recycled daily by coprophagy probably represents a little more than 1 g true protein if a value of 60 % true protein N in total N (Smith & Palmer, 1976) is taken for bacteria. Bacterial protein was found to have a value for net protein utilization of 0·57 for the rat (Mason & Palmer, 1971), so the growing rabbit can probably make similar use of this type of protein, and derives only a small proportion of its requirement from this source. It should perhaps be noted, however, that because greater quantities of carbohydrate or fibre reaching the caecum could encourage microbial activity, the nature of the diet may determine the concentration of bacterial debris in the soft faeces.

For optimum food consumption and growth, a minimum concentration of 150 g CP/kg diet seems to be required by the growing NZW rabbit when given a good quality diet based on oats–fish meal. The highest N retention value was obtained during Expt 1, when offering a diet containing 255 g CP/kg. This may indicate that N retention continues to increase, albeit at a slower rate, when rabbits are given diets that contain considerably more CP than that required for optimum growth or food intake. However, it is doubtful if this would warrant the considerable expense involved in providing the extra protein in practical diets.

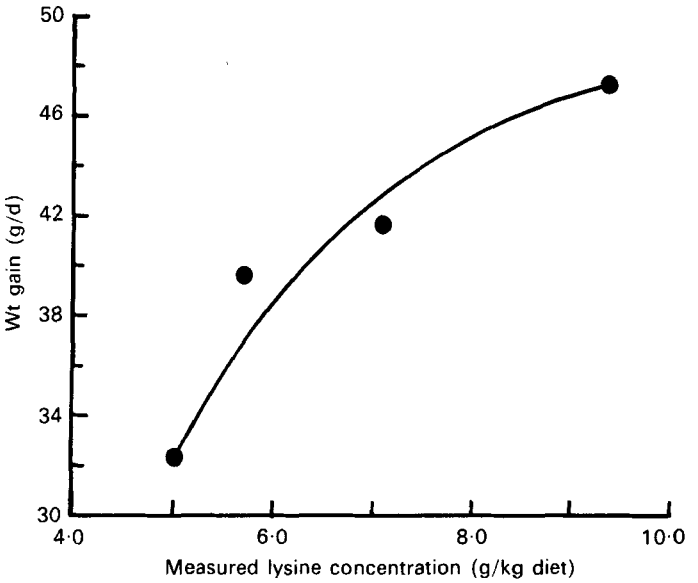

Fig. 1. Expt 7. Effect on growth rate of weanling New Zealand White rabbits of increasing lysine concentration in a diet based on oats and groundnut meal with adequate methionine; for details of experimental procedure, see p. 605 and of diet, see Table 3.

The diet containing 156 g CP/kg was found to have a digestible energy (DE) value of 13 MJ/kg, giving a value for protein:DE of 12 g CP/MJ DE. On a dry matter (DM) basis it contained 185 g CP/kg DM, a value which might be compared with 200 g CP/kg DM given as the requirement for the young pig just before weaning and 185–200 g CP/kg DM for just after weaning (Agricultural Research Council, 1967). Thus even with the protein gained through coprophagy, the young rabbit appears to have a dietary CP requirement similar to that of a simple-stomached species like the pig at the same stage of growth.

The fourth experiment showed that the balance of amino acids in the dietary protein was important to the growing rabbit. As in simple-stomached species, like the pig, growth was best when the diet contained a supplementary protein high in lysine, such as fish meal. Some destruction of unprotected methionine and cystine during normal hydrolysis with 6 M-HCl would have resulted in an under-estimation of these amino acids. A second basal mix for Expt 6 was therefore analysed for methionine and cystine both after normal hydrolysis with HCl as described previously, and after a hydrolysis preceded by an oxidation with performic acid. By the second method methionine and cystine were protected by oxidation, and the total concentration was found to be 5·2 g/kg diet, whereas the equivalent value for the unoxidized sample was 3·9 g/kg. The latter value was the same as that estimated previously for the basal diet.

The higher value for the basal diet was used to calculate the actual methionine and cystine concentrations in the diets used in Expt 6 (Table 9). Thus the estimated requirement for total S-containing amino acids is 6·2 g/kg diet.

The estimated requirement for lysine is 9·4 g/kg diet. This was the highest level incorporated in Expt 7 and it might be argued that a higher concentration would have given further improvement in the rate of weight gain. However in a plot of weight gain *v.* lysine concentration (Fig. 1) the curve suggests that the point of maximum response has been reached.

In Table 11 the concentrations of lysine and methionine plus cystine found to be necessary in the diet are compared with published values. The lysine value of Adamson & Fisher (1973)

Table 11. *Requirement (g/kg diet) of the growing rabbit for lysine (LYS) and methionine and cystine (MET+CYS)*

	Present study	Adamson & Fisher (1973)	Cheeke (1971)	Lebas & Colin (1973)
LYS	9·4	7·0	9·3	9·3
MET+CYS	6·2	6·0	4·5	—

was derived using a mixture of pure amino acids, and the low weight gain of only 19 g/d achieved on this diet may have contributed to the low lysine requirement value obtained. The present assessment of the requirement for S-containing amino acids however, based on estimates after performic acid oxidation of the diet, agrees well with the value given by Adamson & Fisher (1973), feeding mixtures of pure amino acids.

In all experiments food intake was a good indicator of the adequacy or otherwise of amino acid balance. A similar reduction in food intake has been observed in the rat when the quantity or quality of protein in the diet was inadequate to sustain maximum growth rate (Harper, Benevenga & Wohlhueter, 1970). Consumption of the control diet SG1 was higher than that of any of the experimental diets, even when the latter supported rates of weight gain not significantly different from those of the control diet. This was due to the over-all poorer digestibility of diet SG1.

There is no evidence that the growing rabbit can tolerate poor-quality dietary protein deficient in those essential amino acids normally considered important for other species. Proto & Gianini (1969) showed that the soft faeces consumed by coprophagy increased the intake of certain important amino acids like methionine so it must be assumed that the quantities are too small to improve the over-all dietary amino acid balance. From the present work it seems unlikely that more than 0·1 of the daily intake of protein will be recycled in bacterial matter even if the nature of the diet can affect microbial activity in the caecum.

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