The effect of lucerne-protein concentrate in the diet on growth, reproduction and body composition of rats

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1. Protein concentrates were prepared from freshly cut lucerne by the Pirie process and freeze-dried. When supplemented with methionine or cystine and given to rats as the sole source of protein at 120 g protein/kg diet, the adjusted mean protein efficiency ratio was 2·89 (casein standard at 2·50). As a supplement to protein from barley meal the lucerne leaf-protein concentrate (LPC) was similar to casein.

2. To investigate nutritional safety, lucerne LPC supplemented with methionine was given to rats at high levels for 6 months; exposure of these rats to diffuse daylight was avoided to prevent a severe disfiguring photosensitivity reaction. At a dietary protein concentration of 100 g/kg, rats grew equally well with lucerne LPC or casein. When the supplement was given at protein concentrations of 200 or 300 g/kg the rates of body-weight gain of male and female rats were less than those of control rats given casein. However, after 5 months on the diets, body-weights of male rats had nearly reached those of the controls.

3. Apparent protein digestibility ratio was about 0·80 with all three levels of lucerne LPC.

4. Reproduction was normal in seventeen of the eighteen female rats given the lucerne LPC at the three levels; lactation was also normal and litters were successfully raised to weaning.

5. Organ weights, liver histology and blood haemoglobin were normal in male rats given the lucerne LPC for 6 months.

6. Total body lipid of male rats given lucerne LPC was about half that of the control rats given casein. Body protein was slightly increased, and moisture content was higher in rats given lucerne LPC.

7. The 'whey' remaining after precipitation of the protein from lucerne juice strongly inhibited the initial growth of mice given a complete control diet. The mice soon accommodated to the depressive effect of 'whey', and body-weight gains were normal during the 3rd week.

The preparation of protein concentrates from green lucerne leaves and their potential for use in the diets of man and animals were reviewed in detail at a recent international symposium (Pirie, 1971). Byers (1971*a*, *b*) has reported that the amino acid compositions of leaf-protein preparations (LPC) from a variety of plant species were similar, and were characterized by concentrations of lysine and other essential amino acids, except methionine, that were more than adequate for the nutritional needs of simple-stomached animals. Allison (1971) reported collaborative studies correlating factors involved in the preparation of protein concentrates with the biological availability of their lysine, and this work was analysed further by Allison, Laird & Synge (1973). Woodham (1971) showed by rat-feeding tests that methionine was the limiting amino acid.

Recent work has centred on lucerne as the source plant for the commercial production of LPC (Kohler & Bickoff, 1971). However, care must be taken to avoid

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growth depressant factors, possibly saponins (Birk, 1969), in the expressed lucerne juice; Ferrando & Spais (1966) noted that the spray-dried juice was nutritionally inferior to the separated lucerne protein; this was confirmed by Subba Rau, Mahadeviah & Singh (1969). We found that freeze-dried lucerne 'whey' added to a normal diet strongly inhibited the growth of mice.

The lucerne LPC has been shown to support good growth and protein utilization in animals when supplemented with methionine (Mokady & Zimmermann, 1966; Garcha & Kawatra, 1971; Subba Rau & Singh, 1971; Woodham, 1971; Pick-Seng & Kinsella, 1972).

Subba Rau, Ramana & Singh (1972) explored reasons why nine different species of plants, including lucerne, yielded LPC preparations that had similar amino acid compositions but markedly different nutritional values. The general subject of LPC has been reviewed by Allison (1973).

A small pilot plant for the production of lucerne LPC using essentially the same process as that described by Morrison & Pirie (1961) has been in operation at our Christchurch divisional substation. The material prepared at Christchurch has been evaluated for the presence of toxic factors by long-term and high-level feeding to rats.

EXPERIMENTAL

Preparation of lucerne LPC

Lucerne LPC was prepared at intervals during the 1971–2 growing season from freshly harvested lucerne (*Medicago sativa*, L. var. Wairau). Each batch of LPC was obtained from lucerne harvested at approximately the same stage of growth (pre-flowering), always leaving a stubble of 60–100 mm to minimize soil contamination of the herbage. Harvesting to shorter stubbles resulted in a product containing more acid-insoluble ash. The fresh herbage was macerated in a laboratory pulper (Davys & Pirie, 1969), and the juice was expressed on a belt press scaled down and modified from that of Davys & Pirie (1965). Immediately after pressing, the juice was treated with potassium metabisulphite to give a final concentration of 0·5 g/l; no other additions were made. The pH of the juice was 6·3. Protein was precipitated by steam, batchwise, in juice volumes of not more than 10 l, the steam injector creating maximum turbulence and reaching 80° as quickly as possible (about 10 min). Each batch of coagulum was collected by centrifugal filtration, and washed with not less than 30 l hot water to remove growth inhibitors known to be present, e.g. saponins and trypsin-inhibitor activity (Chien & Mitchell, 1970). The protein paste containing about 650 g water/kg was freeze-dried, ground to about 80 mesh, and stored under refrigeration until used.

The lucerne 'whey' (i.e. the fluid remaining after centrifugal separation of the coagulated protein) from one batch was freeze-dried for feeding to mice.

A total of 36 kg of dried lucerne LPC was prepared for the long-term rat-feeding study. A commercially prepared lucerne LPC, X-Pro, was purchased from Batley-Janss Co., Brawley, California.

Table 1. *Composition (g/kg) and proximate analyses of diets containing different protein sources at various concentrations for long-term feeding trials with male and female albino rats*

Approx. protein content	100	200	300	100	200	300
Leaf-protein concentrate	160	320	480	—	—	—
Casein	—	—	—	110	220	330
DL-methionine	3	3	3	3	3	3
Vitamin mix	50	50	50	50	50	50
Salt mix I–H*	50	50	50	50	50	50
Butter oil	40	40	40	40	40	40
Maize oil†	10	10	10	10	10	10
Sucrose	200	200	200	200	200	200
Wheat starch	487	320	167	537	427	317
Nitrogen	15·0	32·5	49·5	15·5	31·2	48·0
Ash	53·0	60·4	66·9	47·0	48·0	49·7
Diethyl ether extract	63	76	89	50	51	53

* Contained (g/kg): $CaHPO_4$ 540, $MgSO_4.3H_2O$ 120, KCl 200, iodized NaCl (stated to contain 40–80 mg KI and 10 g calcium silicate/kg NaCl) 100, basic ferric sulphate (Monsel's salt) 22, ZnO 1·2, $CuCO_3$ 0·4, $MnSO_4.4H_2O$ 1·6, cellulose 14·8.

† 10 mg DL-α-tocopherol/g maize oil.

Diets

The protein contents (N × 6·25) of the dietary protein sources were (g/kg); barley meal 94, 80-mesh lactic casein 871, X-Pro 358, and lucerne LPC 605–641 (for different batches). The composition of the vitamin premix was described by Hove, Fry & Schwarz (1958) and that of the salt mix is given in Table 1. For the estimation of protein efficiency ratios (PER) the diets contained (g/kg): protein source approximately 100 (protein), vitamin mix 50, salt mix 50, dehydrated butter oil 40, maize oil 10 (with 10 mg DL-α-tocopherol/g oil) and wheat starch to 1 kg. Amino acids when used were added at the expense of starch and were included in total protein for calculations. The casein used as the reference protein was prepared specifically for this purpose in a single large homogeneous batch by the Dairy Research Institute of New Zealand at Palmerston North, and stored under cool, dry conditions.

The diets for the long-term study contained approximately 100, 200 and 300 g protein/kg supplied either by lucerne LPC or by the reference casein. As shown in Table 1, all diets were enriched with 3 g DL-methionine/kg.

The control diet given to mice in one experiment to test the effect of freeze-dried 'whey' was made up of ground, whole-wheat flour, commercial dried buttermilk, and commercial dried lactalbumin (72% protein) (2:1:1 by weight). To 800 g portions of these thoroughly mixed ingredients were added 200 g of freeze-dried lucerne 'whey', or the 'whey' + 50 g cholesterol/kg 'whey', or LPC.

Design of experiments

For the short-term PER estimations, five male weanling albino rats, obtained from the Small Animal Research Unit of Massey University, were used in each group. The rats ranged in body-weight from 51 to 67 g. In most instances they were accommodated to the test diets for 5 d, after which body-weight gains and food consumptions were recorded for 3 weeks. A standard reference-casein control group was

included with each series, and the PER of the test group was adjusted to the reference casein PER of 2·50 for that series. The procedure of adjusting to a constant standard control value was introduced by Chapman, Castillo & Campbell (1959), who gave detailed explanations of the advantages in precision thus gained when comparing results obtained at different times and at different laboratories.

For the long-term study, six male and six female rats were assigned to each of the three lucerne LPC diets, and four males and four females were assigned to the three casein control diets. The rats were randomly selected from large batches of weanlings of each sex ranging in body-weight from 50 to 63 g. The animals were housed two rats/cage in wire cages with raised floors. Food and water were available *ad lib.* Body-weights were recorded weekly. After 5 months, males and females receiving the same diet were placed together. For littering, the animals were housed in individual cages with solid bottoms, providing shredded paper for nesting material. The young were raised to weaning. The study was then terminated because of the lack of supplies of lucerne LPC. A period of 30 weeks separated the beginning of the experiment and the final weaning.

About 2–3 weeks after the beginning of the long-term feeding trial many of the rats given lucerne LPC began to show very severe photosensitization reactions. This was the subject of an additional investigation, which is described in a separate publication (Lohrey, Tapper & Hove, 1974). All rats were killed, and a fresh group of weanlings was given the diets, and kept out of daylight to avoid the photosensitizing reaction.

Analytical methods

The nitrogen concentrations of protein sources, diets, faeces and urines were estimated after digestion in selenium-catalysed sulphuric acid digestion mixture using a procedure based on that of Mann (1963) and using the Technicon AutoAnalyzer (Technicon Corporation, Ardsley, New York). The moisture, diethyl ether extract, ash and acid-insoluble ash (i.e. silica) contents of the protein sources, diets and dried faeces were estimated by procedures described by the Association of Official Agricultural Chemists (1965). Cellulose and hemicellulose of LPC were estimated by the methods of Bailey (1967). For fatty acid estimation, 10 g LPC were extracted with chloroform–ethanol (2:1, by volume). The extract was saponified. Methyl esters of the free fatty acids were prepared and portions injected into an F & M gas–liquid chromatograph, model 5750 (Hewlett-Packard Co., Rt. 41 Avondale, Pa., USA) with an EGSSX (Applied Science Laboratories, PO Box 440, State College, Pa., USA) column at 185° with N_2 as the carrier gas; a flame ionization detector was used. The difference between total lipid extract and 1·1 × weight of total fatty acids was considered to be the non-saponifiable fraction. For estimation of the amino acid content of lucerne LPC, samples were hydrolysed at 100° for 24 h in 6 M-HCl in evacuated sealed tubes. Estimations were carried out in a Beckman model 120-C amino acid analyser by the procedure described in Technical Bulletin TB 6089B, sections 5, 6, 7 (October 1965).

Apparent digestibility of dietary components and N balances were calculated

Table 2. *Proximate analyses, essential amino acid and fatty acid contents (g/kg) of lucerne leaf-protein concentrate (LPC) and commercial lucerne-protein concentrate (X-Pro) protein sources in diets given to male albino rats in short-term feeding trials*

	Lucerne LPC	X-Pro
Proximate analyses:		
Protein (N × 6·25)	605	358
Diethyl ether extract (including non-saponifiable lipid)	153	74
Non-saponifiable lipid	118	ND
Ash (including acid-insoluble ash)	44	232
Acid-insoluble ash	8	187
Cellulose	15·7	ND
Hemicellulose	24·0	ND
Moisture	29·0	107
Difference	129·3	229
Essential amino acids (g/kg total amino acids):		
Lysine	63	62
Histidine	23	23
Threonine	40	39
½ Cystine	5	5
Methionine	17	14
Leucine	103	91
Isoleucine	57	54
Phenylalanine	67	56
Tyrosine	37	40
Valine	67	63
Fatty acids (g/kg total fatty acids):		
14:0	7	ND
15:0	31	ND
Unknown*	63	ND
16:0	194	ND
16:1	25	ND
17:0	10	ND
18:0	31	ND
18:1	11	ND
18:2	114	ND
18:3	514	ND

* Fatty acid not identified.
ND, not determined.

from the measured dietary intakes and faecal and urinary excretions of the male rats after 5 months on the respective diets. The rats were placed in standard metabolism cages for two successive 3 d periods. Faeces collected during the 3 d periods were oven-dried at 104° to constant weight; the urine was collected in a container with 5 ml 1 M-HCl and 2 ml toluene; the urines were made to a known volume and portions taken for N determination.

Haemoglobin in blood taken from the tip of the tail was determined by the alkaline haematin method described by Hawk, Oser & Summerson (1947). The standard for comparison was a sample of blood on which the haemoglobin content had been established by an analysis for iron on the separated red blood cells. An EEL Spectra spectrophotometer (Evans Electroselenium Ltd, Halstead, Essex) was used.

At the termination of the experiment male rats were killed 6 h after removal of their food cups. The organs were weighed after they had been examined for gross

Table 3. *Mean values for the protein efficiency ratios* (PER) *of lucerne LPC and casein given alone, supplemented with amino acids and as supplements to barley meal in diets given to groups of five male weanling rats in short-term feeding trials*

Dietary protein source (g protein/kg diet)	Total protein (g/kg diet)	Average 3-week body-wt gain (g)	Adjusted PER (g body-wt gain/g protein eaten)	
			Mean	SE
LPC	80	14·5	1·14	0·07
LPC 53 + barley 27	80	41·8	2·14	0·11
LPC 27 + barley 53	80	60·0	2·24	0·19
Barley	80	41·5	1·75	0·10
LPC + Met 3	83	61·0	2·51	0·09
LPC 53 + barley 27 + Met 3	83	65·5	2·80	0·13
LPC 27 + barley 53 + Met 3	83	50·5	2·06	0·15
Barley + Met 3	83	37·3	1·68	0·09
LPC, 2nd batch	108	42	1·63	0·15
LPC, 3rd batch	121	58	1·92	0·08
LPC, 3rd batch + Met 3	124	106	3·02	0·16
LPC, 3rd batch + Cys 5	126	101	3·13	0·21
LPC 48 + barley 60	108	102	2·61	0·10
LPC 48 + barley 60 + Met 3	111	111	2·88	0·17
X-Pro	100	54·5	1·60	0·09
X-Pro + Met 3	103	99·7	2·23	0·06
Casein 40 + barley 60	100	117	3·01	0·08
Casein control*	96	75	2·50	0·09

LPC, leaf-protein concentrate; X-Pro, commercial lucerne-protein concentrate.

* Actual mean PER values for the casein controls of the four series of assays varied from 2·66 to 3·03; the average was 2·81.

abnormalities. For histological examination, samples of liver, kidney and heart tissue were fixed in phosphate-buffered (pH 7·4) formalin containing 100 ml/l of 40% formaldehyde solution (British Drug Houses). The stomachs were examined for hyperplasia and ulcers. The organ-free carcasses were dried to constant weight for 3 d at 104° for dry-matter estimation. Lipid concentrations were determined by continuous extraction with light petroleum (b.p. 40–60°); body ash was determined by heating the defatted carcass at 550° to constant weight. Protein content was calculated by subtraction of the amounts of lipid + ash from total dry-matter content.

All mean values were tested for statistical significance by Student's *t* test.

RESULTS

Short-term feeding trials

The proximate analyses of lucerne LPC and X-Pro with the fatty acid and amino acid contents are shown in Table 2.

The results in Table 3 suggested that lucerne LPC as the sole source of protein was of indifferent nutritional quality unless supplemented with sulphur amino acids. As a supplement cystine was as effective as methionine, a factor not previously reported. Lucerne LPC was an excellent supplement to barley meal; the combined protein source without methionine supplementation was superior to either of the protein

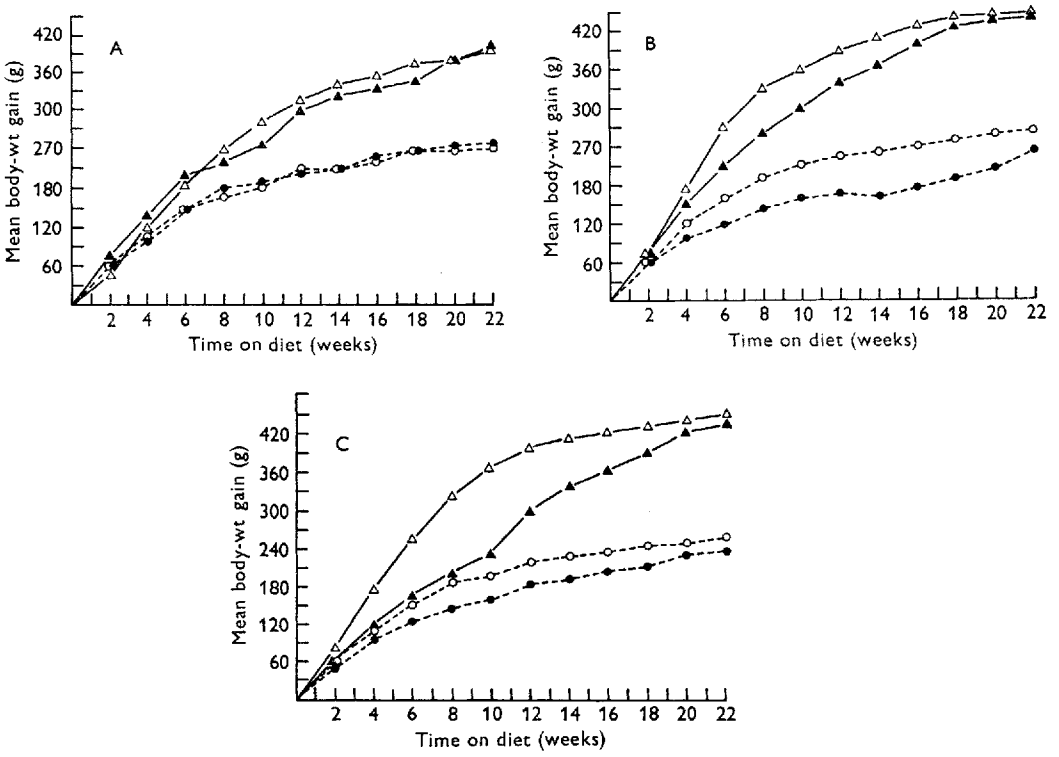

Fig. 1. Mean body-weight gains of male and female albino rats given diets containing (A) 100, (B) 200 or (C) 300 g protein/kg, supplied by lucerne leaf-protein concentrate (▲—▲, males; ●- - -●, females) or by casein (△—△, males; ○- - - ○, females).

sources given alone at the same total protein level. The PER of the commercial lucerne LPC X-Pro was similar to that of our locally produced lucerne LPC.

Long-term feeding trials

Rats given diets containing 100 g protein/kg, supplemented with methionine, grew equally well (as indicated by body-weight gain) when the protein was supplied by lucerne LPC or by casein (Fig. 1 A). But when the LPC content was raised to supply 200 g (Fig. 1 B) or 300 g protein (Fig. 1 C) per kg diet, the rates of body-weight gain of male and female rats were less than those of the casein-fed control rats; at 70 d the differences were significant at $P < 0.05$. However, after 5 months on the diets the male rats given the diets containing lucerne LPC had nearly reached weights similar to those of their casein-fed controls.

Apparent digestibility and N-balance determinations are summarized in Table 4. The digestibility ratio of lucerne LPC protein was about 0·80, which was much below the value for casein protein. The digestibilities of crude lipid and ash in lucerne LPC-fed rats were also much lower than the values for control rats. All rats were in positive N balance and increased their body-weight by 2–6 g/rat during the 3 d collection periods.

Table 4. *Food intake, faecal analyses, apparent digestibility of diet and nitrogen balance of albino rats given lucerne leaf-protein concentrate (LPC) or casein at three different protein concentrations in the diet*

(Mean values for two 3 d collection periods, with six and four male rats/group, respectively, after 5 months on diets)

	Lucerne LPC			Casein		
Approx. protein content (g/kg diet)...	100	200	300	100	200	300
Food intake (g/3 d)	47·1	56·1	56·6	56·1	56·1	52·5
Faeces, dry (g/3 d)	3·53	6·53	8·92	2·04	2·02	1·84
Faecal protein* (g/kg)	262	322	360	223	275	305
Faecal lipid* (g/kg)	95	128	137	44	32	13
Faecal ash* (g/kg)	412	309	259	559	567	545
Apparent digestibility ratio:						
Dry matter	0·92	0·88	0·87	0·97	0·97	0·97
Protein	0·79	0·82	0·81	0·92	0·95	0·96
Crude lipid	0·88	0·80	0·75	0·97	0·97	0·99
Ash	0·42	0·39	0·38	0·57	0·59	0·59
N balance (mg/3 d):						
Food N	705	1820	2800	870	1750	2520
Faecal N	148	326	516	72	91	85
Urinary N	450	1260	2160	620	1380	2200
N balance	+108	+234	+124	+178	+278	+235

* Determined on an oven-dried basis.

Table 5. *Body composition of male albino rats fed for 6 months on diets containing either lucerne leaf-protein concentrate (LPC) or casein as the sole protein source at three concentrations*

(Values are means of four rats per group given as g/kg, wet weight basis)

	Lucerne LPC			Casein		
Approx. protein content (g/kg diet)...	100	200	300	100	200	300
Dry matter	359*	353†	339†	388	419	423
Protein	223*	237*	223*	198	195	198
Diethyl ether extract	89·0†	72·4†	71·4†	134	170	165
Ash	46·4*	43·4*	44·1*	59·0	58·0	60·5

Significantly different from the corresponding casein value: * $P < 0.05$; † $P < 0.01$.

Table 6. *Reproductive performance of albino rats given lucerne leaf-protein concentrate (LPC) for 5 months as the sole protein source at three concentrations in the diet*

(Total no. of pups is given in parentheses; large litters were reduced to eight pups on day of birth)

Approx. protein content (g/kg diet)	No. of females	No. of litters	Average no. of pups/litter	Average weight of pups (g) at week: 0	2	3
Lucerne LPC:						
100	6	5	8·8 (44)	6·1 (30)	26·5 (20)	41·0 (20)
200	6	6	9·5 (57)	6·1 (45)	29·6 (41)	41·9 (41)
300	6	6	11·3 (68)	6·2 (48)	29·5 (42)	40·8 (42)
Stock:						
220	10	10	12·0 (120)	5·8 (100)	—	43·8 (80)

Table 7. *Mean organ weights of male albino rats given lucerne leaf-protein concentrate (LPC) or casein as the sole source of dietary protein at three concentrations for 6 months*

(Mean values with their standard errors for four rats/group)

	Lucerne LPC			Casein		
Approx. protein content... (g/kg diet)	100	200	300	100	200	300
Body-wt (g)	425 ± 15	499 ± 29	482 ± 27	458 ± 8	529 ± 25	533 ± 17
Tissue wt:						
Liver (g)	12·7 ± 0·32	14·3 ± 0·94	14·2 ± 1·05	13·4 ± 1·06	15·1 ± 0·63	15·9 ± 1·10
Kidney (g)	2·44 ± 0·06	2·85 ± 0·19	2·93 ± 0·23	2·72 ± 0·06	3·35 ± 0·18	3·47 ± 0·21
Spleen (g)	0·63 ± 0·03	0·69 ± 0·06	0·68 ± 0·04	0·53 ± 0·03	0·63 ± 0·03	0·56 ± 0·03
Heart (g)	1·37 ± 0·04	1·45 ± 0·06	1·37 ± 0·08	1·48 ± 0·07	1·57 ± 0·07	1·66 ± 0·10
Brain (g)	1·99 ± 0·02	1·99 ± 0·04	1·93 ± 0·06	2·02 ± 0·04	1·98 ± 0·02	2·05 ± 0·02
Testes (g)	3·28 ± 0·04	2·84 ± 0·16	3·34 ± 0·09	3·22 ± 0·24	3·41 ± 0·11	3·59 ± 0·22
Thymus (mg)	267 ± 40	346 ± 44	304 ± 20	351 ± 56	330 ± 58	328 ± 31
Adrenals (mg)	58 ± 2·3	69 ± 9·7	64 ± 5·3	63 ± 1·9	63 ± 5·8	65 ± 2·5

At the end of the 5-month feeding period, females were caged with males for 3 weeks. The males were then killed, subjected to autopsy examination, and body composition was determined (Table 5) while the females raised their young. The protein contents of the male rats fed on lucerne LPC were about 15 % higher than the control values; lipid contents were significantly lower, as were the dry-matter contents.

The reproductive performance of the female rats given lucerne LPC was very good at all three dietary protein concentrations, as shown by results in Table 6. The results for the control females given casein were not as good; those given the diets with the two lower protein concentrations reproduced normally but could not rear their young, and with the highest concentration of casein protein no pregnancies occurred. The performance of a randomly selected group of rats from the stock breeding colony is shown in Table 6 for comparison.

Organ weights and pathology

Mean organ weights of male rats given lucerne LPC or casein as the source of protein were not significantly different (Table 7).

Gross inspection showed that all organs were free from pathological lesions. Histologically prepared sections of liver and kidney stained with haematoxylin–eosin were normal.

Effect of lucerne 'whey' on growth of mice

When freeze-dried lucerne 'whey' was added to a standard mouse diet, growth was depressed (Table 8). The animals lost weight during the 1st week but by the 3rd week they were gaining weight. Cholesterol added to the 'whey' counteracted the depressant effect in males but not in females. The lucerne LPC also showed an initial depressant effect in males.

Table 8. *Initial growth depression of mice given lucerne 'whey', 'whey' + cholesterol or lucerne leaf-protein concentrate (LPC) added at a concentration of 200 g/kg to control diets of wheat, buttermilk, and lactalbumin*

(four mice/group; initial mean body-weight 10·2 g)

	Body-wt gain (g)			
	1st week		3rd week	
Supplement to control diet	♂	♀	♂	♀
None	5·4	1·8	3·1	0·8
Dried 'whey'	−0·4*	−1·3*	2·0	1·1
'Whey' + cholesterol (10 g/kg)	1·9*	−0·4*	1·8*	−0·6*
LPC	2·3*	1·5	3·0	1·3

* Significantly different from value for unsupplemented control diet at $P < 0·05$.

DISCUSSION

Residual amounts of saponins or other antimetabolites may have contributed to the slower gain in body-weight of rats given the diets containing the higher concentrations of lucerne LPC. It is possible that the effect may have been the result of taste and poor acceptability of the diets by the animals. The recovery of the rats and mice from the initial growth depression supports this explanation.

In the formulation of diets for man or animals, lucerne LPC would probably never be included at concentrations as high as 100 g protein/kg diet. This amount was more than adequate to supply the lysine and other essential amino acids (except sulphur amino acids) needed to supplement the proteins of the cereal-grain components of the diet. The results clearly showed that this was a safe and effective concentration for rats not exposed to daylight, and allowed reproduction to proceed normally. When adequate supplies of lucerne LPC are available, lifetime and three-generation feeding studies will need to be done for assurance of safety.

The reason for the low digestibility (0·80) of the lucerne LPC protein was not obvious; the cellulose content was too low to be the cause. It is important to note that the apparent digestibility did not differ with the concentration of protein in the diet; therefore, the effect was not the result of overloading the digestive enzymes. The low value for the digestibility of crude lipid, especially at the higher dietary levels of lucerne LPC, can be explained by the high non-saponifiable content of the lipid fraction with its very high content of chlorophyll and other pigments. The depressed digestibility of minerals was due to the higher mineral content in the diets containing lucerne LPC and to some extent to the small amount of acid-insoluble (silica) ash present.

The lower digestibility of lucerne LPC resulted in increasingly greater amounts of faecal material. Because of the substantial amounts of nutritionally inert material in the diets, a search was made for ulcers or other lesions in the stomach and intestines, but none were found.

The body composition of the adult male rats given lucerne LPC was marked by a

significantly lower total lipid content. The lipid content appeared to be inversely related to the amount of lucerne LPC in the diet, although this relation was not statistically significant. Values for total body lipids of 70–80 g/kg would seem to be well below what is considered normal (150 g/kg). The biological reasons for, and implications of, these results will need to be explored in depth.

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