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'Effects of varying the type and amount of Dietary Protein on Growth and Reproduction in the Golden Hamster and Related Metabolic Studies in the New Zealand White Rabbit'

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ABSTRACT

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Effects of varying the type and amount of Dietary Protein on growth and reproduction in the Golden Hamster and related metabolic studies in the New Zealand White Rabbit.

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Five materials were incorporated individually as the protein constituents of diets fed to golden hamsters during growth and reproduction trials. Dietary protein equivalent to 5% "reference" protein was shown to be unsatisfactory for growth or reproduction. Growth could not be maintained on diets containing fishmeal as the major protein source, also these diets led to reproductive failure. The high level of calcium in this material and the possible production of toxic substances by heat treatment during manufacture have been discussed as adverse factors. Dietary protein equivalent to 10-15% "reference" protein was found to be adequate for normal growth in this species but a higher level was usually necessary for good reproductive performance. Events determining litter size at birth were affected only by the level of dietary protein but subsequently both the level and source of protein influenced survival of offspring

to weaning. The pups which survived to weaning had similar body weights and the insulin secretory response of their pancreases in vitro were also similar. At the termination of the growth trial a positive correlation was shown to exist between fasting serum insulin and body weight.

Congenitally malnourished New Zealand White Rabbits showed poor insulin secretion and poor glucose tolerance at weaning. The pattern of development of tolerance to glucose was shown to be similar in this species to that demonstrated previously in others, but with a normal adult level of only 2.5% min^{-1}. A high sucrose diet was introduced at weaning and insulin insensitivity developed resulting in poor glucose tolerance. A correlation existed between insulin sensitivity and glucose tolerance thus the results support the theory that sensitivity to insulin rather than circulating level of the hormone is the major factor determining glucose tolerance.

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CHAPTER 1. INTRODUCTORY REVIEW

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The Golden Hamster

The golden hamster (Mesocricetus auratus) was introduced into the laboratory in 1930. Notes on its breeding and growth were published by Bruce and Hindle (1934). By this time it was already proving its usefulness as a tool in medical research. Alder and Theodor (1931) had shown that the golden hamster could be used in the study of Kala Azar which was a widespread endemic disease of Mediterranean countries producing symptoms of dysentery and fever. Kala Azar is caused by the protozoa Leishmania which is naturally transmitted via the bites of sandflies. The only species being used in the study of Leishmania was the Chinese hamster and when the golden hamster was shown to be very susceptible to this parasite it was welcomed as an alternative experimental species because it breeds readily in captivity. Bruce and Hindle (1934) reported that spontaneous disease in the golden hamster was rare but that the animal was susceptible to several extraneous diseases. That the golden hamster may be useful in studies involving human virus diseases became apparent in the early 40's. Wheeler and Nungester (1942) used the animal in a study of influenza virus and, in 1941, Lennette had shown its susceptibility to encephalitus virus making it only the third experimental species, after the mouse and the monkey, to show this characteristic. The use of the golden hamster in biomedical research has since increased dramatically and in a review of the subject Fulton (1968) listed the "unique characteristics" of the species which make it such a useful research tool. These include its immunogenetic tolerance to homologous, heterologous and human tumours, parasites, viruses and bacteria, the possession of paired eversible cheek pouches which can serve as natural windows for detailed microscopic investigation of blood flow and growth of transplanted tumours and embryonic organs, susceptibility to man-like dental caries and muscle degeneration from

vitamin E deficiency, a lengthened renal papilla which increases the ease of sampling and the phenomenon of hibernation which is valuable for the experimental study of prolonged low temperature effects.

Because the hamster has become a widely used model in biomedical research it is perhaps suprising that relatively few studies have been made on its nutritional requirements. The level of protein required in the diet for optimum growth was assumed to be similar to that of the albino rat and early workers using basal diets containing purified casein as the protein source at levels of 18% (Cooperman, Waisman and Elvehjem, 1943), 20% (Routh and Houchin, 1942; Hamilton and Hogan, 1944; Clausen and Clark, 1943), and 25% (Granados and Dam, 1950) have studied the qualitative requirements of the hamster for various vitamins. It has been established that vitamin C is not required by this animal (Clausen and Clark, 1943; Cooperman et al, 1943), but the results of studies of other vitamins are contradictory. In the investigations of protein requirements reported in this thesis all the known vitamins, other than vitamin C, were included in the diets.

Protein requirements for growth

Although it was already suspected that the "flesh of animals" was a desirable constituent of diets, it was Magendie in 1816 who demonstrated the profound difference in nutritive value between nitrogenous and non-nitrogenous matter as components of diets. He reported that dogs fed on a diet containing only carbohydrate and fat, in the forms of olive oil and sugar, died within a few weeks. Later proteins were identified as a class of compounds composed of amino acids. It was also recognised that proteins differed in their nutritive value because gelatin was totally inadequate when it was the sole dietary source of nitrogen. In 1905 Kauffman tested the hypothesis that a reduction in nutritive value was associated with deficiencies in amino acids by demonstrating that he could maintain himself in nitrogen balance,

i.e. his nitrogen intake was equal to his nitrogen loss via urine and faeces, when he added tyrosine, cysteine and tryptophan to a diet in which the only other source of nitrogen was gelatin. Although Kauffman's results were refuted by Rona and Müller who carried out a similar experiment in 1906, the link proposed between nutritive value and the amino acid composition was further established by Willcock and Hopkins (1906). They reported that zein was an inadequate protein source for maintaining growth in young mice and although the addition of tryptophan (which is lacking in zein) did not make it capable of supporting growth, it did greatly increase the survival time of the animals on the diet as well as improving their general condition. The addition of tyrosine (which is present in zein) had no such effect. This finding was extended by Osborne and Mendel in 1914, who demonstrated that if both tryptophan and lysine were added to zein the diet was capable of sustaining the growth of young animals. These workers also demonstrated that some amino acids were indispensable constituents of an adequate diet. In the 1930's Rose performed series of expierments in which the sole nitrogen source was in the form of purified amino acid mixtures. By 1938 he was able to classify amino acids as essential - those which cannot be synthesised by the animal at a speed sufficient to meet the requirements for growth - and non-essential, first for the rat and eventually for man. Mitchell and Block (1946) devised a method for predicting the nutritive value of proteins from their amino acid composition. The prediction is based on the assumption that the amino acid which limits the nutritive value for maintainance and growth is the one present in the least amount relative to "requirement" i.e. that amino acid with the greatest percentage deficit compared to a "reference protein". In theory reference protein should contain 16% nitrogen and be completely utilisable for protein synthesis (because it contains exactly the right proportions of amino acids required by the animal). The reference protein used by Mitchell and Block was whole egg protein because it is

highly digestible and almost perfectly utilised in rodent metabolism. So they compared the amino acid composition of the test protein to that of egg protein and the greatest percentage deficit in an essential amino acid they termed the "chemical score" of the protein. A standard has been proposed by the FAO (1957, 1973) which is a theoretically ideal dietary protein and this has replaced egg protein as the reference for calculating protein scores.

Alongside investigations concerned with the relationship of the chemical composition of proteins to their nutritional value, methods were being established to determine nutritional value by biological means. The procedures sought to quantify changes in total body protein and the methods employed to this end monitored either weight gain or used the concept of nitrogen balance - the sum of all the gains and losses of tissue nitrogen in the body, i.e. nitrogen intake minus nitrogen loss via faeces, urine and the skin (the last is normally considered negligible and ignored).

A criticism of the weight gain method is that gain or loss of weight may not always be of a constant composition. However, some workers have reported that the percentage nitrogen in animals is remarkably constant over relatively short term trials (4-6 weeks) (Bender and Doell, 1957; Middleton, Morrison and Cambell, 1960). In 1919 Osborne, Mendel and Ferry introduced the term "Protein Efficiency Ratio". This is still probably the most widely used method employing weight change as the criterion for assessing protein quality. Protein Efficiency Ratio (PER) is defined as the weight gain in grams as a ratio of the protein intake in grams. A criticism of this method is that the ratio will change with the level of protein in the diet and may also be affected by the species and sex of the test animal and the weight or age when the trial commences. When the method was introduced Osborne et al did point out that tests should be run with diets containing various levels of protein and the

highest value achieved used to indicate the nutritional value, because this would be the level at which the protein is optimally utilised for growth. Assay conditions have been specified (Chapman, Castillo and Cambell, 1959) e.g. the use of male rats 21-23 days of age and an assay period of 4 weeks, but whatever conditions are used, interlaboratory variation is reduced if they are all defined. The major criticism of the PER method is that it assumes all protein is being used for growth, thus disregarding the requirements for maintenance. This results in proteins which do not support growth being as assigned a very low value although they may be adequate for maintenance (e.g. wheat gluten). This criticism is valid and it follows that the method can only be used for assessing protein quality in the growing animal and not in the adult. The advantage that nitrogen balance methods have over weight gain methods is that they can be used for the adult animal as well as during growth because it is the gain (or loss) of nitrogen from the body that is measured irrespective of whether gains result in growth. If intake exceeds loss then the body is in a state of positive nitrogen balance and increasing its protein content. If output exceeds intake then there is a negative nitrogen balance showing that the body is losing nitrogen and therefore total body protein.

Thomas (1909) defined the fraction of absorbed nitrogen retained in the body as the Biological Value (BV) of the protein. Thomas calculated absorbed nitrogen by measuring nitrogen intake and subtracting from this the nitrogen excreted in the faeces minus the faecal nitrogen of body origin. Retained nitrogen was calculated by subtracting from the absorbed nitrogen the nitrogen excreted in the urine minus the urinary nitrogen of body origin. He estimated endogenous nitrogen in urine and faeces by measuring nitrogen excretion while feeding a nitrogen-free diet. There is now evidence that the excretion of endogenous nitrogen is not constant. Endogenous faecal nitrogen is not independant of nitrogen intake and endogenous urinary nitrogen is related to

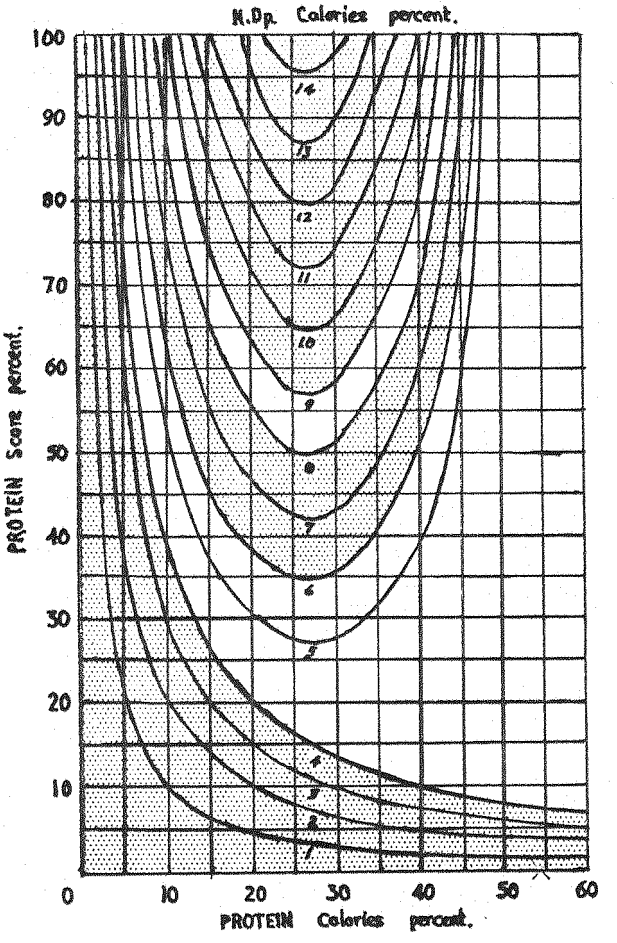
basal metabolic rate. This method has been extensively reviewed and modified since its introduction (e.g.Mitchell 1923-4; Allison, Anderson and Seeley, 1946).

Bender and Miller (1953) described a method of estimating protein quality which they stated was similar to that for Biological Value being a measure of nitrogen retained in the body, but as a proportion of nitrogen intake as opposed to nitrogen absorbed. This makes Net Protein Utilisation (NPU), as it was called, much less laborious to measure than Biological Value. Because "Digestibility" is that fraction of nitrogen intake which is absorbed, it follows that NPU = Digestibility x BV. NPU is measured by determining the body nitrogen of the test group on diet, the body nitrogen of a group offered a protein-free diet (to correct for the protein requirements for maintenance) and the nitrogen intake of the test group, then NPU = $\frac{\text{body nitrogen of test group} - \text{body nitrogen of non-protein group}}{\text{Nitrogen intake of test group.}}$

It is now well known that the nutritive value of a protein varies with the protein level used. When the maximum value is achieved the amino acids are being utilised as fully as possible (100% in the case of reference protein)for protein synthesis. As the level of protein is increased the amino acids in excess of requirements are used as an energy source so the nutritive value of the protein is decreased. This was one criticism of the PER method but it applies to all biological determinations. It is also established that other factors in the diet and the level of food intake affects the value of protein as a nitrogen source (Allison,1958;Munro,1951). For example if the food intake of an animal is restricted it will be ingesting insufficient energy to meet demands and the protein will be used as a source of energy, so the animal will be experiencing protein deficiency.If fat or carbohydrate is added to the diet so that energy demands are met the protein will then be used as a source of amino acids and the animal will no longer be protein deficient even though no protein has been added to the diet. It is evident, therefore, that

it is not the level of protein in the diet which is important but the amount which can be utilised as a nitrogen source. This is dependent on the presence of other dietary constituents as well as the level of protein and its quality in terms of amino acid composition. Because the quality of a protein is not constant Net Protein Utilization was developed as a technique using standard conditions with respect to level of protein, amounts of fat, vitamins and minerals in the diet (Miller and Bender, 1955). Later Platt and Miller (1959) referred to the values obtained when using standard conditions, including a protein level at maintenance requirements, as NPU standardized (NPU_{st}). If higher levels of protein are used the values obtained are termed NPU operative (NPU_{op}). Some prediction of NPU_{st} can be made from the amino acid composition of proteins (FAO, 1957) but when practical diets are considered it has been shown that NPU_{op} is not directly related to amino acid composition (Drury and Miller, 1959), so direct biological assay would be necessary. In 1961 Miller and Payne devised a method for predicting the effects of protein concentration in the diet on protein value. First they expressed the protein concentration of their diets, not as percentage dry weight, but as the percentage of the total energy of the diet derived from utilisable protein, pointing out that diets containing the same utilisable protein at equal energy content must have the same dietary value. This product of quantity and quality was termed Net Dietary Protein Calories per cent (NDpCal%) ($=NPU_{op}$ x protein calories%) and is now referred to as net dietary protein energy (NDpE). They then established, by feeding diets containing protein sources of widely varying NPU_{st}, included in amounts that supplied varying levels of protein calories, that rats maintained weight with diets of 4.0 NDpCal%. Using this as a constant it was possible to calculate the % of protein calories in a diet required for maintenance for any given NPU_{st}. They determined NPU_{op} for proteins at several levels above

maintenance and showed that the relationship between NPU_{op} and protein calories % was essentially linear and the rate of fall depended on the protein source. Using the regression equations for the different lines, they derived a general equation relating to NDpCal %, protein content and NPU_{st} (which can be predicted from chemical scores). A nomograph was constructed showing the inter-relations.



Normograph for the prediction of the protein value of diets. The parabolas are lines of equal NDpCals %.

The vast majority of experiments designed to investigate the effect of protein level on growth have involved use of the rat as the experimental model. Stewart and Sheppard (1971) demonstrated that, after three months, female rats weaned onto oatmeal-based diets with a NDpCal% of 5 or 10 (which contained 4 or 22% of the dry weight of the diet as casein) had mean body weights of 127g and 190g respectively, which were significantly different to a high degree ($P < 0.001$). Similarly, Dickerson,

Hughes and McAnulty (1972) weaned rats on to diets containing 5 or 25% casein and after 4 weeks on diet the mean weight gain of the former group was 7g whereas that of the high protein group was 116g. Kirsch, Brock and Saunders (1968) using isoenergetic diets containing protein derived from a mixed source at levels of 5, 8, 12 and 20% of the dry weight reported a gradual step-wise reduction in weight gain which was proportional to the protein content of the diet, those of the 5% and 20% groups being significantly different. The groups given diets containing 8% and 12% protein increased their food intake relative to the high protein control group but the group offered the 5% protein diet did not. They proposed, therefore, that if diets were marginally deficient in protein the animals would raise their food intake to compensate to some degree but that if the deficiency was more severe they could not. Included in the experimental design was a group given the 20% protein diet in the amount eaten by the 5% protein group i.e. a pair fed control group. The 5% protein group had a significantly lower weight gain compared to this control group and because they were eating the same amount of energy the difference in weight gain was ascribed to the lower protein intake of the 5% group. Another trial based on the pair feeding method, as opposed to ad libitum feeding, was reported by Hogan and Pilcher (1933), who used diets varying in protein content from 7 - 33%. They also found that rats on the higher levels of protein made better gains than those on the lower levels even though the metabolisable energy intakes were the same. McCoy (1940) also used high levels of protein, in the form of casein, in his diets, these being 15, 25 and 40% of the dry weight of the diet. When the diets were offered ad libitum there was very little difference between the weight gains of rats on the 25% and 40% protein diets but they were both superior to those of rats given the diet containing 15% of protein.

So it has been established that an increase in the level of protein in diets similar in other respects will

increase the growth rate until the minimum level of the protein which will produce the maximum growth is reached. The addition of more protein will have no more effect on growth, until at very high levels of the dietary protein a reduction in the rate of growth is observed (Horwitz and Waisman, 1966; Wang and Waisman, 1961). However, although there is an increase in weight gain as the dietary protein is increased to optimal level, there is an associated reduction in the efficiency of utilisation of the proteion.

There has been one report of the protein requirement for growth in the hamster using purified protein (Arrington, Platt and Shirley,1966). The protein used was casein at levels of 8-20% of the dry weight of the diet. It was concluded that with this protein source the level of protein required for optimal growth is between 12 and 16%. These workers also compared the effects of diets containing 16% of either casein or purified soybean protein ("Assay Protein C-1") and the results indicated that the reponse was similar in each case. A further investigation by Banta, Warner and Robertson (1975) using a mixture of protein sources indicated that the protein requirement for optimum growth lies between 15% and 20%. The protein in their diets was derived from a mixture of feed ingredients including ground corn, wheat and oats, soybean, fish and alfalfa meals and brewers' dried yeast. They reported weight gains superior to those of Arrington et al (1966). Arrington et al (1966) compared the effects of a natural diet ("Laboratory Chow") containing 25% protein and a diet containing 25% protein derived from casein but did not comment on any growth differences, only on the difference in food intake which was slightly lower with the purified protein source, probably because of the higher energy density.

A factor which cannot be overlooked when considering the protein nutrition of the golden hamster is that it is a polygastric animal, albeit having the simplest forestomach of all such animals, and not a monogastric animal like the rat. Matsumoto (1955) compared the effect of adding 1% urea to a diet

containing naked barley flour as the major source of nitrogen in hamsters and in rats, on the growth, nitrogen retention, and biological value of the protein. The nitrogen content of the diets were 204mg (without urea) and 250mg (including urea) per 100g diet. A nitrogen-free diet was fed before the trial and between the two test diets. In both species the nitrogen intake was increased when fed the diet containing urea. There was also an increase in faecal nitrogen (but small in both species), urinary nitrogen (small in the hamster) and absorbed nitrogen. Apparent absorption of urea-nitrogen was 89.6% in the rat and 95.2% in the hamster. However there was no increment of retained nitrogen in the rat whereas the hamsters retained 70.9% of the urea nitrogen. The result was that the addition of urea to the diet increased the biological value from 38% to 46% for the hamster but decreased it from 53% to 40% for the rat. The mean increase in body weight for the rats during 5 days was 6.3g with the unsupplemented diet but only 1g when urea was added. The hamsters increased their mean weight gain in the same period from 1.7g to 4.3g. The author concluded that the addition of urea to the ration was useful for the hamster but rather hamful for the rat.It was even suggested that the hamster may be a useful small animal model for ruminant nutrition. A further study was reported by Banta et al (1966) in which 3 diets were fed to groups of hamsters and rats. These contained (1) 10% protein; (2) 10% protein supplemented with sufficient amino acids to meet the requirement for rat growth as stated by the National Research Council (Washington DC); (3) 20% protein. Rats supplied with the 10% protein diet had weight gains of only 60% of those given the 20% protein diet but when the 10% protein was supplemented with amino acids the growth was increased to that of the 20% control group. On the other hand the supplementation of the 10% protein diet in hamsters did not have any effect on the growth performance. Just as the use of urea in ruminant nutrition is well established (Loosli, Williams, Thomas, Ferris and Maynard, 1949) so it

is also known that feeding unprotected amino acid supplements to ruminants has litte nutritive value (Oltjen, 1969). Banta et al concluded that this explanation can be applied to the observations in the hamsters given the diet containing 10% protein supplemented with amino acids.This group of workers also gave rats and hamsters diets containing equal amounts of wheat gluten, soybean protein and fish protein concentrate . Wheat gluten was an inferior protein source for both species in terms of absolute weight gain and the protein efficiency ratio which was achieved. In the rats the fish protein concentrate supported better growth and gave a higher PER value than the soybean protein whereas in the hamster the reverse was true. They interpret this result as being at least suggestive of an alteration in protein quality in the forestomach.

There is no doubt that the quality of protein is important for the golden hamster. Even the ruminant with its voluminous forestomach, which in the adult contains 90 - 97% of the total stomach content (Warner and Platt, 1965) is not totally independent of protein quality. The forestomach of the mature hamster contains about 37% of the total stomach content (Hoover, Manning and Sheerin, 1969) and this animal may be less dependent on protein quality than the rat and certainly the quality of protein may be different in this species.

Therefore in the absence of more than minimal data, experiments were set up to examine the effect of both the amount and the type of protein in the diet on the growth response in the golden hamster.

Protein Requirements for Reproduction.

It is well known that maternal nutrition is of very great importance in determining the outcome of pregnancy. Before and during the 1939-45 war, a number of surveys showed that the food policy, which was to give preferential treatment to pregnant women was accompanied by a sharp decrease in the stillbirth and neonatal mortality rates

(Thompson, 1957). It has been reported thar successful reproductive performance increases from social classes IV and V up to social classes II and I when judged by perinatal mortality rate, mean birth weight or the frequency of maternal and foetal complications (Smithells, Ankers, Carver, Lennon, Schorak and Sheppard, 1977). Of course it is not only maternal nutrition which determines the size of the offspring, a major influence is the genetic make-up of both parents and particularly, at birth, the size of the mother which has been demonstrated by Walton and Hammond, (1938) with their well known reciprocal cross matings between shire horses and shetland ponies. The growth potential of the offspring is also greatly influenced by these factors. Thompson and Billewicz, (1963) showed a correlation between stature of women and socio-economic class which must be at least partly nutritional in origin. A poor dietary regime, perhaps imposed by economic necessity and continued by custom will lead to a stunting of offspring (Chow & Rider,1973) who will in turn give birth to smaller babies. It is likely that the babies will be reared on the same diet thus resulting in the correlation of Thompson and Billewicz, (1963). Factors which cannot be discounted which also vary with social groupings include the general level of heath and hygiene but the former must also be influenced by the general diet. In a survey in Toronto the reproductive performance of mothers classified as being on a "poor diet" (mean 56g protein in 170kcal/day) were compared to those on a "good diet" or a "supplemented poor diet" (mean 94g protein in a 2400kcal/day). The "poor" group had high incidence of miscarraige (6%), still birth (3.4%), prematurity (8%) smd 'difficulty in labour' (24%). The other two groups had no miscarriages or still births, 2.2% premature births and 3% difficult labours. The health of the babies during the first 6 months was also adversely affected by the "poor" diet with an increase in the incidence of pneumonia, colds, influenza, rickets and

other illnesses. The "poor" and "poor supplemented" dietary groups came from the same section of the community and the survey shows the dramatic improvement in reproductive efficiency whoch can be achieved by dietary supplementation as late as the 5th month of gestation (Ebbs, Tisdall and Scott, 1941). Many cases of more severe maternal malnourishment are widespread in underdeveloped countries. In poor Indian communities 20% of pregnancies are terminated in abortion, miscarriage or still birth and it is possible that this may be a low estimate because of the high incidence of unrecorded early abortion. (Venkatachalam, 1963; Gopalan, 1962).

The maternal diet should be adequate in all respects, that is with regard to energy, protein, vitamins, minerals etc. and the effects of many dietary restrictions have been investigated in several animal species. This report is concerned with the protein requirements for reproduction in the golden hamster and, while recognising the importance of adequate intakes of all nutrients, only those reports dealing with protein restrictions will be recorded here.

El-maraghi, Platt and Stewart, (1966), using rats as the experimental model, maintained females from weaning on diets with net protein values of 5.1 or 10.2 (8 or 25% casein). They terminated pregnancies and noted an increase in the number of resorption spots in the uteri, a reduced number of foetuses per litter and slightly lighter foetuses in the low protein group. Stewart and Sheppard, (1971) reported a study using similar diets of net protein values 5 and 10 (4 and 22% casein). They also maintained their rats on the diets from weaning and, after mating at 3 months, the pups produced by the low protein mothers were significantly lighter but they reported no change in litter size. The fact that lighter litters are produced by the low protein females is not suprising since their mean weight at mating was 127g as opposed to a mean of 190g in the high protein group. Resorptions were more frequent and

more animals in the low protein group failed to conceive. Feeding a diet to rats from weaning containing 8% casein, as compared to a control group supplied with a diet containing 25%, increases perinatal mortality of the offspring from 26% to 73% and reduces the number of litters with live pups at weaning from 78% to 33% (Turner, 1973). The effect of supplying rats with a diet containing 9% protein from 4 weeks of age compared to a stock diet with 18% protein is to delay the onset of puberty (as measured by the opening of the vagina) from 6 weeks to 8 weeks, to reduce fertility and to reduce litter size. The low protein diet also impaired the lactation performance of the mothers as measured by the reduced growth of the offspring in the first three weeks of life. The subsequent reproductive performance of a group of rats severely deprived of protein (6% in the diet) from 4 weeks of age then rehabilitated with the stock diet at 13 weeks of age was not impaired in terms of litter size, fertility and number of litters produced but the onset of puberty was delayed until the animals were offered the stock diet (Widdowson and Cowen, 1972).

The observations recorded so far have all been the result of long term trials, that is the dietary regimens were imposed from weaning. Investigations have also been carried out where the restriction is imposed over more than one generation. Similar results are reported but they may be more severe because the effects are somewhat additive, that is the earlier the unsatisfactory regimen is imposed (even if it is before the lifespan of the individual under consideration) the more serious the effects will be (Platt and Stewart, 1968). It has been suggested that the foetus is basically a parasite, or at least has a very high priority for nutrients available (Naismith, 1969; Hammond, 1944). It has been shown in dogs that although a severe nutrient deficiency is shared by mother and foetus, one which is less severe is borne by the mother (Platt and Stewart, 1967, 1968), that is, she can compensate for the deficiency at the

expense of her body reserves. If pregnacy is entered in a state of nutritional well-being the mother is more able to protect the foetus than if her reserves are already sub-optimal.

A different approach to the problem of protein requirements during gestation is the use of short term trials where the restriction is imposed only at the time of mating. In this case the females are comparable in their nutritional history at the beginning of gestation and therefore are of a similar size and potential fertility, thus the number of factors affecting the reproduction performance is reduced.

Curtiss, (1953) reported that 52% of dams fed, from mating,on a diet containing 5% protein in the diet did not complete pregnancy whereas the failure rate was only 8.5% with a 24% protein control diet. Also, although there were no differences in energy intake between the two groups, 95% of the protein restricted animals lost weight during the first two weeks of gestation. Nelson and Evans, (1953) fed diets containing protein derived from casein at varying levels from 0 to 25% of the dry weight of the diet. They observed no adverse effects on reproduction when the level of protein was decreased from 25 to 15% with maternal weight gains of 120g and a mean birth weight of 6g. With 10% protein in the diet the maternal weight gain dropped to 82g and the pups were lighter than 6g. However the number of foetuses per litter after 12 days of gestation were similar to the higher protein groups (Kenney, (1975) also has reported that a diet containing 5% protein, fed from mating, did not affect litter size after 12 days). When the protein content of the diet was only 5% the mean maternal weight gain was reduced to 11g, the young weighed less than 5g at birth and 2% were still born. With a protein level of only 2.5% the mean birth weight was only 3.8g, 70% of the dams resorbed their foetuses and the females completing gestation lost a mean of 32g. Nelson and Evans concluded that 5% was the critical protein level for satisfactory reproduction which agrees

with the estimate made by Guilbert and Goss, (1943). Again using rats as the experimental model, Venkatachalam and Ramanathan (1964) supplied one group with a diet containing 7% wheat protein, intended to simulate the diet common among poor Indian women, and compared their performance with a group supplied with a diet containing 18% protein from a mixed source. The high protein group showed good survival but no low protein pups survived to the end of lactation although they were of a similar weight to the controls at birth and litter sizeswere also similar. If the pups from low protein mothers were suckled by high protein mothers the mortality to weaning was only 40% but the weanling weight was reduced by about half. When high protein offspring were suckled by low protein mothers the mortality was increased to 78% and the weanling weights were reduced still further. A similar comparison was carried out using casein at levels of 6% and 24% of the diet (Zeman, 1967). No offspring born to low protein mothers (who had lower weight gains through gestation than the controls) survived more than 7 days, whereas survival of the high protein offspring was 81%. If high protein pups were suckled by low protein mothers none survived more than 11 days but if the situation was reversed 52% of the offspring survived to weaning. Therefore it is possible for gestation to be completed, albeit not totally successfully, on diets which are inadequate for subsequent lactation. It is possible for rats to complete gestation even if they are offered a protein free diet during part of gestation and although the litter sizes at term are not significantly different from a control group receiving 18% protein throughout gestation, the offspring from mothers deprived during the second or third week have reduced birth weights and weaning weights are reduced whether deprivation is during the first, second or third week. Also with deprivation during the first week ¼ of the mated females failed to conceive (Venkatachalam and Ramanathan, 1966). It has been reported that rats will still bear live young if

they are given a nitrogen free diet after only eight days of gestation, (Seegers, 1937). Rats given a nitrogen free diet from mating invariably absorb their litters before the end of gestation. However, if they receive injections of progesterone and estrone they can be made to produce live young with a success rate of 100% (Nelson amd Evans, 1954). The idea that the poor reproductive reformance in protein restricted rats is the result of poor pitutary performance has been supported by the data of other workers.

So in experiments carried out to estimate the optimal and minimal protein requirements for reproduction in rats with a previously good dietary history, it is found that it is possible for pregnancy to proceed to term in the absence of protein in the diet (although not under normal physiological conditions), but that a good reproductive performance is obtained when the diets contain of the order of 16 - 25% of a good protein (Macomber, 1933; Nelson and Evans, 1953; Platt and Miller, 1957).

The only reported investigation of nutritional requirements during reproduction in the golden hamster is that of Hamilton and Hogan (1944) who concluded that hamsters could reproduce successfully on synthetic diets.Their diets all contained 20% protein derived from casein, the main object of the experiments being to determine vitamin requirements. The present studies were undertaken to investigate the effects of different protein levels derived from various sources on the reproduction of the golden hamster.

Diet and Glucose Tolerance.

The last few decades have seen a growth of interest in the changes in carbohydrate metabolism which accompany malnutrition at both extremes of the nutritional spectrum - that is, undernutrition leading to kwashiorkor and marasmus, through moderate protein energy malnurition to excessive dietary intakes of nutrients particularly from refined sources, which have been implicated in "diseases of civilisation", such as diabetes and coronary heart disease.

It is convenient to divide the regulation of carbohydrate metabolism into two parts; 1) factors affecting blood glucose homeostasis; and 2) the regulation of carbohydrate metabolism at the cellular level through changes in the affinity of the enzyme for its substrate, activation of enzymes and changes in the rate of enzyme synthesis. These mechanisms are subject to hormonal influence (Harper and Mayes, 1969).

The division of carbohydrate regulation into these two parts is somewhat arbitrary since they are functionally related. In this thesis work on the maintenance of blood glucose levels is reported.

Blood glucose is derived from three major sources. Firstly from dietary carbohydrate which is for the most part digested to glucose and fructose, the latter being readily converted to glucose in the liver under normal physiological conditions. Secondly from the release by the liver of glucose derived from gluconeogenesis. The precursors for this process are either non-carbohydrate, such as amino acids, or compounds which have themselves resulted from the metabolism of glucose such as lactate and glycerol. Lastly glucose which has been converted to glycogen for storage can be released from the liver having undergone glycogenolysis.

The efficiency of the glucose homeostatic control may be ascertained by measuring glucose tolerance, which is the rate of removal of excess glucose from the circulation. Glucose tolerance is evaluated by administering a glucose load in an amount related to body weight and monitoring the fall in blood, plasma or serum glucose levels after the rapid rise. The glucose load can be given orally (oral glucose tolerance test (GTT) or directly into the blood via a vein (intravenous (iv) GTT). The intravenous method avoids intestinal factors. For example a 'flat' response is seen in malnourished humans and pigs (Politzer, 1955; Platt, Heard and Stewart, 1964) if the oral GTT is used but this should not be interpreted as rapid removal of

glucose from the circulation (i.e. good tolerance) because if the i.v. GTT is performed removal of blood glucose in malnourished humans is seen to be very slow. Thus the response seen with the oral test must be due to poor absorption from the gut, analogous to that demonstrated by James, (1968). Also the intravenous method of administration is far easier to perform with experimental animals and is a more rapid procedure. Glucose tolerance tests are carried out when blood glucose is at a fasting level, normally after an overnight fast. Heard, (1978) has argued that it is preferable to monitor plasma rather than blood glucose levels in determining glucose tolerance because the equilibration of glucose between plasma and red blood cells which takes a short time under normal physiological conditions, is markedly impaired under certain nutritional conditions such as beri-beri and severe protein energy malnutrition. This results in observing a rise in blood glucose levels for approximately the first fifteen minutes of the GTT while if plasma levels are monitored, the first sample after the injection shows, as usual, the highest glucose concentration. After the first fifteen minutes plasma and blood glucose concentrations give the same glucose tolerance values.

In normal pigs and dogs glucose tolerance is poor in the young animal and increases during growth reaching a peak in early adult life, thereafter declining slowly with increasing age (Heard, Turner and Platt, 1964). It has also been shown in man that tolerance to glucose is very low in the normal newborn (Baird and Farquhar, 1962) and the general pattern of development is similar but in man 'adult' levels are reached by six months of age (Loeb, Chapenois and Conard, 1961, cited by Heard, 1978). This normal pattern of development is affected by nutritional factors. Offspring of normal dogs weaned onto a low protein diet at 6 weeks of age exhibit the peak in tolerance to glucose within 4 instead of 12 months and offspring of protein malnourished dogs have

high glucose tolerance levels at weaning (Heard and Turner, 1967). In the human situation, the offspring of diabetic mothers have abnormally high glucose tolerance levels at birth (Baird and Farquhar, 1962). In later life also it has been shown that adverse nutritional states will impair glucose tolerance. Several authors have reported that children suffering from kwashiorkor show impaired tolerance to glucose loading (Baig and Edozien, 1963; Hadden, 1967; James and Coore, 1970; Milner, 1971). It has also been shown in experimental situations using rats (Cohen and Teitelbaum, 1966),dogs (Heard and Turner, 1967) and pigs (Heard, 1966) that feeding protein-energy deficient diets results in poor glucose tolerance. Himsworth, (1940) showed that impaired glucose tolerance develops if a diet is low in carbohydrate and that in some cases poor glucose tolerance can be improved by increasing the carbohydrate content of the diet. It has since been demonstrated both in man and rats that the nature of the dietary carbohydrate is important when considering this effect. Cohen, (1967), using two groups of human subjects who had been accustomed to a typical "western" diet in which fat supplied 36% of the total energy, showed that by replacing 60% of the fat with bread, such that it supplied 60% of the energy of the diet, glucose tolerance was improved within four to six weeks. If sucrose was substituted for the fat, however, glucose tolerance was slightly impaired. When the diets of the two groups were interchanged the effects were reversed and the new high bread group showed improved tolerance to glucose while that of the new high sucrose group deteriorated.The different effects on glucose tolerance of feeding diets containing sucrose, as opposed to starch, as the source of dietary carbohydrate have been demonstrated in rats by Cohen and Teitelbaum, (1964). They used synthetic diets containing either 67% starch, 67% sucrose or a mixture of the two which included sucrose at levels of 40% and 33% of the dry weight of the diet. Glucose tolerance on the high

starch diet was similar to that in rats given the stock diet, which was a natural ration containing 60% carbohydrate and 5% fat. However glucose tolerance became impaired after 50 to 100 days when the diet contained 33% sucrose, after 40 days with 40% sucrose in the diet and after 21 to 40 days with the 67% sucrose in the diet. After feeding the high sucrose diet for 78 days the impaired tolerance was shown to be reversible if the animals were then given the high starch diet for 15 days. However on subsequent refeeding with the high sucrose diet only 6 days were necessary for impairment of glucose tolerance to redevelop. The reason for this enhanced susceptibility to sucrose is not clear. Uram, Friedman and Kline (1958) also noted poor tolerance to an oral glucose load after feeding diets containing 66 or 25% of the total energy as sucrose compared to that observed after feeding a natural stock ration.

The maintenance of stable levels of glucose in the blood is a very finely regulated homeostatic mechanism in which the liver, extrahepatic tissues and several hormones play a part. Liver cells appear to be freely permeable to glucose (Cahill, 1959) whereas those of the extrahepatic tissues are relatively impermeable. Thus it is the activity of certain enzymes and concentrations of key intermediates which exert a direct effect on uptake and output of glucose from the liver whereas in the extrahepatic tissues the passage of glucose through the cell membranes is the rate-limiting step. Nonetheless the concentration of glucose in the blood (and thus glucose tolerance) is an important parameter in determining the net rate of glucose uptake in both liver and extrahepatic tissues.

The hormone insulin has a central role in the regulation of blood glucose concentration. Produced in the beta cells of the islets of Langerhans in the pancreas, it is secreted into the blood in response to hyperglycaemia and the result is a lowering of blood glucose levels. Insulin enhances the transport of glucose across the cell membrane in tissues such as muscle and adipose tissue. A direct effect of insulin

on glucose uptake in liver has not been demonstrated but after in vivo administration of insulin, liver slices show enhanced glucose uptake, presumably as a result of changes in the activity of liver enzymes. Furthermore Mortimore, (1963) and Exton, Jefferson, Butcher and Park, (1966) have shown, using isolated perfused liver, that insulin suppresses glucose output by the liver and Miller (1965), using a similar technique, demonstrated reduced oxidation of glucose U -^{14}C to $^{14}CO_2$ in the presence of insulin. Thus in the liver also insulin has a direct influence on glucose metabolism.

The anterior pituitary gland secretes hormones that tend to elevate blood sugar levels and thus are antagonistic to the action of insulin. These include growth hormone and adrenocorticotrophic hormone (ACTH, corticotropin). The major effect on carbohydrate metabolism of the latter is through its stimulation of the secretion of the adrenal cortex. The adrenal cortex secretes a number of steriod hormones of which the glucocorticoids, including cortisol and hydrocortisol, are important in carbohydrate metabolism. By their actions of increasing protein catabolism in peripheral tissues and activating key gluconeogenic enzymes, the hepatic uptake of amino acids and their conversion to glucose is enhanced. In addition glucocorticoids inhibit the utilisation of glucose in extrahepatic tissues, thus they also are antagonistic to the actions of insulin.

Glucagon, a hormone secreted by the alpha cells of the pancreas, activates liver (but not muscle) phosphorylase thus stimulating glycogenolysis. It also enhances gluconeogenesis from amino acids and lactate. All the hormones so far mentioned (excluding insulin) are secreted directly in response to hypoglycaemia. Hypoglycaemia also causes a discharge in sympathetic nerves. One result of which is the secretion of adrenalin from the adrenal medulla. Adrenalin stimulates breakdown of glycogen in a similar way to glucagon but in both liver and muscle. Hence, as long

as glycogen is present, this results in enhanced release of glucose from the liver and production of lactate in muscle which diffuses into the blood, is carried to the liver and in turn converted to glucose by gluconeogenesis.

Thyroid hormone should also be considered as affecting blood sugar levels. In hyperthyroid patients the fasting blood glucose level is elevated but the rate of glucose utilisation is normal. In hypothyroid patients fasting blood glucose is decreased, as is the ability to utilise glucose and, in addition,hypothyroid patients are much less sensitive to insulin than normal or hyperthyroid individuals. These effects on carbohydrate metabolism of thyroid hormone may be related to differences in end-organ response, in rates of destruction of insulin, or both.

Thus there are other hormones besides insulin (and only the major ones have been outlined here) as well as the other factors mentioned which have a controlling effect on blood glucose levels. However, while recognising it as an over simplification, the object of work reported later in this thesis was to study the effect of diet on insulin and its capacity to lower blood glucose levels.

Because insulin secretion is directly affected by blood glucose levels, it is obvious when considering animals in the fed state that their circulating insulin levels will depend on the length of time since they last ingested food as well as on the nature of the food consumed. However, the different dietary regimens which have been shown to influence glucose tolerance, also affect fasting insulin levels and insulin secretion in response to a glucose load.
Hadden (1967) reported normal to high fasting levels of serum insulin in children suffering from kwashiorkor. However, Lunn, Whitehead, Hay and Baker, (1973) found levels of 6.3 μU/ml instead of the 20μU/ml observed in their controls. Lunn et al (1973) did find a few cases where the insulin levels

were very high and explained the variance between their results and those of Hadden in terms of the severity of the disease . They proposed that if the children were severely affected their fasting insulin levels were decreased but that if the condition was more moderate they could be increased. The effect of PEM in the children studied by Hadden, however,were sufficient to cause impaired glucose tolerance. Low plasma levels, both fasting and in response to a glucose load, have also been observed in children suffering from kwashiorkor by other workers and as indicated earlier is generally accepted that such children also show poor tolerance to glucose. Fasting levels of insulin and its secretion are also reduced in marasmic children (Hadden, 1967) and this may be accompanied by normal or abnormal glucose tolerance (Oxman,Maccioni, Zúñiga, Spada and Mönckberg, 1968). Thus although insulin secretion has been shown to be affected in different states of malnutrition the changes are not parallel to those in glucose tolerance. Analogous findings are reported in relation to the changes in glucose tolerance which may be induced by feeding diets high in sucrose. Szanto and Yudkin (1969) found raised serum insulin levels, both fasting and in response to a glucose load, after feeding subjects diets containing 50% of the total energy as sucrose for two weeks compared to their levels after consuming diets containing only 18% of the total energy as sucrose. Dunnigan , Fyfe, McKiddie and Crosbie (1969) however,found no change in insulin levels after subjects received diets containing 32% of the total energy as sucrose for four weeks. The differences in sucrose content of the diet may account for the discrepancy in observations by the two groups of workers but the interesting point is that glucose tolerance was unchanged in the subjects of Szanto and Yudkin, (1969) as well as in those of Dunnigan et al, (1969). Using the rat as the experimental animal, reduced levels of plasma insulin-like-activity (determined by

the effect of the serum on glucose uptake by isolated hemidiaphragms) have been observed after offering a diet containing 67% sucrose by weight (62% of the total energy) for 24 days (Blasquez and Quijada, 1969) and no change was found in fasting serum insulin levels after offering a diet containing 70% of the total energy as sucrose after 50 days (Vrána, Slabochová, Kazdová and Fábry, 1971). However, no evidence has been reported which disagrees with the observation that sucrose at concentrations similar to these in the diets of the rat leads to impaired glucose tolerance in as little as 20 to 25 days (Cohen and Teitelbaum, 1966; Uram, Friedman and Kline, 1958).

Himsworth, (1940) proposed that the major factor governing the efficiency of insulin action in the body was tissue sensitivity to the hormone rather than the circulating levels. He had devised a method for evaluating sensitivity to insulin in vivo which was similar to the glucose tolerance test (Himsworth, 1936). Immediately after an oral glucose load he gave an intravenous dose of insulin, which was related to body weight, and measured the change in blood glucose levels. He performed the test on diabetic patients whose responses fell into two distinct types. In those he subsequently termed insulin-sensitive the rise in blood glucose was suppressed by the insulin and in the others the insulin had no effect on the glucose tolerance curve. These he termed insulin-insensitive. The test has since been modified such that both metabolite and hormone are given intravenously. The rise in blood glucose occurs as in the i.v. GTT but the return to basal levels should be accelerated by the injected insulin.It is possible to perform the glucose-insulin tolerance test immediately following an i.v. GTT so that relationships between the two parameters can be investigated. It has been shown that the first test should not influence the second, if performed after basal glucose levels have been regained, because two consecutive i.v. GTTs

give similar results (Samols and Marks, 1965; Heard and Henry, 1969). In more recent years more evidence has been presented that glucose tolerance may be related as much to tissue sensitivity as to insulin release. Cerasi and Luft (1967) and Martin, Pearson and Stocks (1968) came to this conclusion by studying the possible inter-relationships between insulin secretion and glucose tolerance after a glucose load and blood glucose levels in response to exogenous insulin in apparently healthy siblings of diabetics. They found that a combination of both factors, insulin release and tissue sensitivity, was needed to account for the observed glucose tolerance. Heard and Henry, (1969) reported that in pigs and dogs, both normal and protein malnourished, the increase in glucose tolerance with age was paralleled by a similar increase in insulin sensitivity but not in circulating insulin levels. Further, when the two tests were performed on the same animals, they showed positive correlations between glucose tolerance and insulin sensitivity, thus concluding that, except in conditions of absolute insulin deficiency, sensitivity to insulin was the primary factor in determining glucose tolerance. No reports have been presented where the changes in glucose tolerance resulting from feeding sucrose-rich diets experimentally were accompanied by changes in insulin sensitivity. However insulin sensitivity has been evaluated in vitro in such circumstances. Blasquez and Quijada, (1969) found reduced sensitivity to insulin, assessed by its affect on glucose uptake, of both adipose (epididymal fat) and muscle (diaphragm) tissue from rats which were fed a high sucrose (68% by weight) diet from weaning for 24 days compared to a control group on a standard diet. Reiser and Hallfrisch, (1977) measured the effectiveness of insulin in increasing the production of carbon dioxide from glucose by adipose tissue and found that its action was impaired in tissue from rats fed on a diet containing 54% sucrose, as opposed to 54% carbohydrate from mixed cooked starches, for 12

weeks. Similarly Vrána, Slabochová, Kazdová and Fábry, (1971) found that feeding a high sucrose diet to rats markedly reduced the insulin sensitivity of adipose tissue as compared with rats fed a diet with the same proportion of carbohydrate as wheat starch and that the effect was roughly proportional to the amount of dietary sucrose. Bruckdorfer, Kang and Yudkin, (1974) fed rats for 30 days on diets containing 68% by weight carbohydrate as starch, glucose, sucrose or fructose. At the end of the period they measured the ability of insulin to increase incorporation of glucose into lipid and observed that its action was impaired in the sucrose and fructose fed rats thus confirming the effect of sucrose and also implicating the fructose component of the molecule.

Against this background of information a long term experiment was set up, using the New Zealand White rabbit as the model, to investigate the relationship between and dietary effects on glucose tolerance and insulin sensitivity as indicators of carbohydrate metabolism.

CHAPTER 2. GROWTH AND FOOD INTAKE OF THE GOLDEN HAMSTER : THE EFFECT OF VARYING THE LEVEL AND SOURCE OF DIETARY PROTEIN

CHAPTER 2: GROWTH AND FOOD INTAKE OF THE GOLDEN HAMSTER : EFFECT OF VARYING THE LEVEL AND SOURCE OF DIETARY PROTEIN

Introduction

The purpose of these experiments was to investigate the effect on growth and food intake of different levels of protein in the diet of the golden hamster. Also to determine whether the response varied when the protein was derived from different dietary materials.

The protein sources selected for study were:

1) Casein - a commonly used isolated protein source;
2) Fishmeal - another feed stuff in common usage with a much lower nitrogen content;
3) Soya protein in the form of a full fat soya flour;
4) Soya protein in the form of a protein isolate;
5) A mould (Fusarium graminearium) - a material of special interest as a novel dietary source of nitrogen.

In order that comparisons could be made between groups given the different dietary proteins at the various levels included in the diets, it was necessary to make some allowance for protein quality which would vary between the protein sources depending on their amino acid composition. Therefore, on the basis of the chemical score of the proteins, the protein sources included in the diets were calculated to supply similar amounts of reference protein, which means all proteins provided the same quantity of limiting amino acid at each level. An alternative method of overcoming the problem would have been to supplement proteins of poorer quality with essential amino acids such that their quality, as determined by amino acid content, would have been improved but supplementation with "unprotected" amino acids has been shown to have no value in this species.

Two experiments were undertaken. In the first

the growth response in male and female hamsters housed in groups was evaluated. At the end of this trial determinations of various fasting serum metabolites were made so that normal levels in the hamster colony could be established and the effect of the experimental diets monitored. Only male animals were used in the second growth trial. They were housed individually to investigate more fully relationships between growth and nutrient intake.

Methods and materials

The animals used in this study were taken from the randomly bred stock colony at Southampton University. Animals were taken at weaning (28 days) and allocated to 48 experimental groups, 24 groups of female and 24 groups of male animals, on the basis of weight and litter of origin such that the mean weight of all groups were similar and litter mates were in different groups. Each group contained 6 animals housed together in a wire mesh cage. The temperature of the room was maintained at 22^{o}C and humidity maintained by flooding the floor daily. Artificial light was supplied for 9 hours each day to supplement poor natural lighting. Sterilised woodwool was supplied as bedding material.

Each group of animals was supplied with stock diet (Porton Mouse Diet, Christopher Hill Ltd., Poole, Dorset) for the first week after selection then each of the 20 experimental diets described below was offered to 1 group of females and 1 group of male animals. The remaining 4 groups of each sex were maintained on the stock diet throughout. Individual body weights and mean food intake for each group of 6 hamsters were measured for 6 weeks after introduction of the experimental diets.

Food Intake Measurements

Food was supplied ad libitum in a hopper at the front of the cage and food intake measured by weight

difference with correction for spillage. When food intake measurements were made the cages were inspected for hoarded food and also the pouches of each animal examined. If food pellets were present they were expelled and included with the spillage. When powdered diets were given, storage in the cheek pouches still occurred and it was difficult to expel the material quantitatively. Therefore in all of the experimental work described food was given in the form of pellets.

Experimental diets.

The proteins used in the experimental diets were:

1) Casein - "Casumen":(sodium caseinate) (Prideaux, Evercreech, Somerset);
2) White Fishmeal - "Provimi 66" (Prideaux, Evercreech, Somerset);
3) Mould - "A 3/5" (Fusarium graminearium) (Lord Rank Research Centre, High Wycombe);
4) Soya Bean, sample 1- "Trusoy" (full fat soya flour) (British Soya Products Ltd., Puckeridge, Herts);
5) Soya Bean, sample 2- "Promine D" (isolated soya bean protein) (Oppenheimer Casing Co. London).

Each protein source was used in 4 experimental diets in varying amounts calculated to supply protein equivalent to approximately 5% (Diet 1),10% (Diet 2), 15% (Diet 3) or 20% (Diet 4) of reference protein. In each case a fraction of the protein was derived from wheat bran,which was included in the diet as a source of fibre.

The amino acid composition of the protein sources is shown in Table 2.1, together with the FAO recommended 'ideal' protein which is used as the reference for calculating the protein scores. The amount of protein added to the diets is shown in Table 2.2. Using the figures in Table 2.1 and 2.2 the amino acid composition of the mixed protein (i.e the major protein source + the wheat bran) was calculated and the score for each

Table 2.1 Essential Amino Acid content (mg/g protein) of the dietary proteins.

	Casein[1]	Fishmeal[1]	Mould[2]	Soya[2] protein (1)	Soya[2] protein (2)	Bran[3]	FAO[4]
Methionine + Cystine	34	38	33	32[2+3]	19	24	35
Lysine	93	68	83	62	57	33	55
Trytophan	22	10	17	15	11	18	10
Threonine	42	41	54	40	36	25	40
Valine	67	45	60	52	45	47	50
Leucine	87	67	79	75	78	60	70
Isoleucine	64	41	49	52	46	40	40
Phenylalanine + Tyrosine	91	67	84	66[2+3]	88	57	60

Sources [1] Commonwealth Bureau of Animal Nutrition Techn.Comm.no.19

[2] Supplied by manufacturers.

[3] "Nutrition of the Chicken" ed.Scott Nesheim & Young 1969

[4] World Health Organisation Tech. Rep. ser.1973 no.522

Table 2.2 Protein (g/kg) included in diets. 15g/kg in each case is derived from wheat bran.

Protein Source.	Casein + Bran	Fishmeal + Bran	Mould + Bran	Soya protein (1) + Bran	Soya protein (2) + Bran
Diet					
1	56	57	56	57	86
2	112	111	108	111	189
3	165	165	161	166	282
4	219	219	213	219	375

Table 2:3 Chemical Score of protein mixture.

Protein Source	Casein + Bran	Fishmeal + Bran	Mould + Bran	Soya protein (1) + Bran	Soya protein (2) + Bran
Diet					
1	90	91	87	85	57
2	93	91	91	88	55
3	95	90	92	89	55
4	95	90	92	90	55
Limiting amino acid	met + cys	val	met + cys	met + cys	met + cys

Table 2.4 Reference protein levels in diets (%)

Major protein source.	Casein	Fishmeal	Mould	Soya protein (sample 1)	Soya protein (sample 2)
Diet					
1	5.2	5.2	4.9	4.9	4.9
2	10.4	10.1	9.8	9.8	10.4
3	15.3	14.9	14.8	14.7	15.5
4	20.8	19.7	19.6	19.7	20.6

Table 2.5 Composition of diets in g/kg dry weight.

[1]Vitamins	10
[2]Minerals	40
[3]Corn oil	100
Sucrose	100
Wheat bran	100
Protein source)	650
Maize starch)	

[1]to supply(mg/kg diet)

Biotin	0.039
Folic acid	0.39
Inositol	39
Nicotinic acid	9.75
CaPanthothenate	11.6
Pyridoxine HCl	0.39
Riboflavine	1.93
Thiamine HCl	0.58
Para-amino benzoic acid	110
Menaphthone K	2
Vitamin B_{12}	0.01
Choline HCl	110

IU/kg diet:	
Vit.A	25,000
Vit.D_3	5,000
Vit.E	12.5

[2]to supply (mg/kg diet)

Co	0.214
Cu	4.593
Zn	4.68
Mn	49.39
Fe	204.8
Mg	431.9
I	22.81
(g/kg diet)	
K	4.21
Na	2.08
Ca	5.75
Cl	3.17
CO_3	8.63
SO_4	2.17
PO_4	10.24

[3]In the diets containing soya protein (sample 1) the level of corn oil was adjusted to allow for the fat content (20%) of the protein source.

Table 2.6 Calculated metabolizable energy of the diets (kJ/g dry weight)

Major protein source	Casein	Fishmeal	Mould	Soya protein (1)	Soya protein (2)
Diet					
1	17.1	16.7	16.7	16.7	17.1
2	17.1	16.3	15.5	16.3	16.7
3	16.7	15.5	14.2	15.5	16.7
4	16.7	15.1	14.2	14.6	16.3

Table 2.7 Composition of the stock diet (PMD - Porton Mouse Diet)

	g/kg		g/kg
Barley	56.3	Soya	100
Wheat	200	Roller dried skim milk	75
Maize	100	Unext dried yeast	25
Oats	181.3	Salt	2.5
Wheat feed	200	Labvit A	5
		Labmin K	5

Metabolizable energy 12.23 kJ/g. Protein 201.1 g/kg

Chemical score of protein 94.8 Reference protein 191 g/kg

mixture evaluated by comparison with the FAO Standard (Table 2.3). The exact level of reference protein in each diet is shown in Table 2.4. The remaining constituents of the diets are shown in Table 2.5 and the metabolisable energy content of each diet in Table 2.6. The composition of the stock diet is shown in Table 2.7.

A second experiment was carried out which was designed to investigate more fully the relationship between nutrient intake and body weight gain of the animals. Male hamsters were selected at weaning, allocated to groups and housed in the conditions already described except that in this trial they were housed individually. Groups of 7 or 8 animals were given one of 15 diets, these being Diets 1, 2 and 3 of each protein source as described above. Individual weight gains and food intakes were monitored weekly.

Fasting Serum 'Metabolites'

After 6 weeks on the experimental diets the animals from the first growth trial were subjected to an overnight (16 h) fast and ~~killed by cervical dislocation~~ anaesthetized with ether. Blood was removed by cardiac puncture and kept in ice for 1 hour. The serum was collected by centrifugation and used for the determinations described below.

1. Insulin

Insulin was measured by a radio immunoassay method based on that of Hales and Randle (1963). The principle of the test is the reaction of a limited, fixed quantity of anti-insulin serum with a mixture of the sample of insulin to be assayed together with a constant amount of radioactive insulin. After completion of the reaction, the antibody-bound insulin is separated from free insulin and the distribution of radioactivity determined. The binding of labelled insulin to the antibody is progressively inhibited by increasing amounts of unlabelled insulin owing to competition

for specific binding sites on the antibody and the concentrations of insulin in the serum under test may be determined by reference to a standard curve prepared at the same time. The separation of bound and free insulin in this method is by use of a double antibody such that the 2 factors are of sufficiently different size to be separated by filtration.

Materials

Stock buffer : 0.05 M phosphate buffer.

Buffer A : Stock buffer + 0.5% bovine serum albumin + 0.025% thiomersal.

Buffer B : Buffer A + 0.9% NaCl.

Buffer C : Stock buffer:horse serum (Wellcome diagnostic research laboratories Hithergreen, London) 50:50 v/v (made up immediately prior to use).

Insulin Binding Reagent (Wellcome) reconstituted in deionized distilled water. This reagent is prepared from guinea pig anti-insulin serum and rabbit anti-guinea pig-globulin serum (a precipitating serum) which combined form an immune precipitate — the double antibody mentioned above.

Standard insulin (Wellcome) reconstituted in Buffer B

^{125}I - insulin (Radio Chemical Centre, Amersham) containing 1μCi/ml and diluted 1:7 in buffer A.

Glass fibre membrane filter papers (diameter 2.5cm) (Oxoid Ltd) used in conjunction with a 'Millipore' 'Pyrex' microanalysis filter holder (Millipore Filter Corp., Bedford, Massachusetts). The membranes were soaked in Buffer C before use.

3 ml. polystyrene tubes.

Method

Standards: the top standard used was a solution of 200μU insulin /ml. 5 serial doubling dilutions were made giving concentrations down to 6.25μU/ml. 0.1 ml of each standard was added to 0.1 ml Binding Reagent in triplicate.

Wash blanks consisted of 0.1ml Buffer A + 0.1 ml Buffer B.

Zero tubes, which were assumed to ~~theoretically~~ undergo 100% binding, consisted of 0.1 ml Buffer B + 0.1 ml Binding Reagent.

Samples: 0.1 ml of each serum sample was added to 0.1 ml Binding Reagent in duplicate.

All procedures were carried out at $4^{o}C$ on a bench refrigerator. The tubes were mixed on a vortex mixer and incubated at $4^{o}C$ for 6 hours. 0.1 ml ^{125}I - insulin was added to all tubes which were mixed again and left incubating at $4^{o}C$ for 18 hours.

The contents of each tube, plus two washings of the emptied tubes with 0.5 - 1 ml portions of Buffer C, were filtered through glass fibre membranes on a 'Millipore' holder under suction. Using forceps the filter papers were wrapped in foil and placed in counting vials to be counted in a gamma counter.

Standard and sample counts were expressed as a percentage of the zero count after all had been corrected for the wash blank counts. A standard curve was constructed and used to determine the concentration of insulin in the samples.

2. Glucose

Glucose was measured by a method based on that of Flemming and Peglar (1963). It employs the following reactions:

1) Glucose + glucose oxidase → gluconic acid + H_2O_2;
2) H_2O_2 + peroxidase + colour reagent → coloured product. The glucose concentration is

then determined colorimetrically.

Materials

Zinc sulphate solution : 5g $ZnSO_4$ in 100 mls water.

Barium hydroxide solution : approximately 0.3N such that 1 ml of this exactly neutralises 1 ml zinc sulphate solution (using phenolphthalein as the indicator).

Colour reagent : 100 ml tris-glycerol buffer containing 100 mg glucose oxidase (Sigma chemicals) 1 mg peroxidase (Sigma chemicals) & 10mg o-dianisidine dihydrochloride (Sigma chemicals)

Tris-glycerol buffer : prepared by dissolving 61 gm tris-(hydroxymethyl) amino methane in 85 ml of 5N hydrochloric acid, making up to 1 litre with water then adding 600 ml glycerol and adjusting the pH to 7.

Method

0.5 ml sample was added to 1 ml 5% $ZnSO_4$ solution and shaken on a vortex mixer. 1 ml 0.3N $Ba(OH)_2$ solution was added. The tubes were centrifuged for 15 minutes in a bench centrifuge. 0.5 ml of the supernatant was added to 2 ml colour reagent and incubated for 30 minutes at 37°C. After the addition of 4 ml 5N sulphuric acid the tubes were mixed and the contents read at a wavelength of 525 nm in a Pye-Unicam SP600 spectrophotometer. An appropriate standard curve was constructed by treating standard glucose solutions in a similar way and used to determine glucose concentration in the samples.

3. Cholesterol

Total serum cholesterol was determined using the method of Zlatkis, Zak and Boyle (1953). This determination, made colorimetrically, is claimed to be 4 to 5 times more sensitive than the original Liebemann-Burchard colour reaction on which it is based.

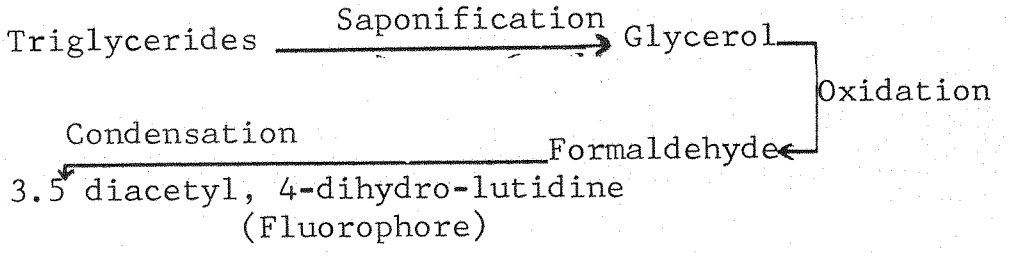
0.1ml serum was added to 3.0 ml glacial acetic acid in a test tube. 2.0 ml colour reagent (0.25 ml of a

saturated solution of ferric chloride in glacial acetic acid added to 50 ml concentrated sulphuric acid) was carefully added and the contents of each tube mixed. The test tubes were left at room temperature for 30 minutes. The contents of the tubes were read in a Pye-Unicam SP600 spectrophotometer at 560nm wavelength in glass cuvettes of 1cm light path. Standard curves were constructed using 0.1 - 0.5 ml of a standard cholesterol solution (1 mg/ml in glacial acetic acid) made up to 3 ml with glacial acetic acid then adding 0.1 ml distilled water and 2.0 ml colour reagent.

All spectrophotometric readings were made against a distilled water (0.1 ml water, 3 ml glacial acetic acid and 2.0 ml colour reagent)blank.

4. Triglyceride

Triglyceride levels were determined using an automated method on a Technicon Auto Analyzer II. The procedure was based on the reaction:



Isopropanol extracts of triglyceride were pumped into an air-segmented stream of ethanol and potassium hydroxide. Saponification of the triglycerides was performed in a 50°C heating bath and the glycerol was oxidised and condensed in a second 50°C heating bath. The stream of samples passed through a fluorometer of 2mm internal diameter (Technocon Automated Clinical Methods No.24 Mar.1972).

Extraction of triglyceride

100 µl aliquots of the serum were added to 1.9ml 99% isopropanol in disposable plastic test tubes. The tubes were capped and mixed vigorously for 30 seconds on a vortex

mixer. 0.4g zeolite mixture (hydrated magnesium silicate, Technicon Ltd) was added to remove the phospholipids and the tubes again shaken for 30 seconds. The batch of sample tubes so prepared was allowed to stand for 30 minutes, the sediment being resuspended at 10 min. intervals. The tubes were then centrifuged at 2500g for 5 mins. and the supernatant decanted into sample cups for the Auto Analyzer (Technicon Instrument Corp, Tarrytown, New York).

Statistical analysis of results

1. The standard error of the mean for each group of results was calculated from the formula :-

$$\text{Standard Error (SE)} = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n(n-1)}}$$

Where x is the observation.
$\bar{x}$ is the mean of the observation in the group.
n is the number of observations in the group.

2. The statistical difference between two groups of observations (1 and 2) was evaluated by the students t-test where:-

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(SE_1)^2+(SE_2)^2}}$$

the value of P, the probability that the two groups of observations were statistically different, was obtained from the appropriate tables.

3. The estimated correlation coefficient (r) was calculated by the formula :-,

$$r = \frac{\Sigma(x-\bar{x})(y-\bar{y})}{\sqrt{\Sigma(x-\bar{x})^2\Sigma(y-\bar{y})^2}}$$

the value of P, the probability that r was statistically different from the true correlation, which was assumed to be zero, was obtained from appropriate tables. (ref. Bailey 1960)

RESULTS

Weight gain and food intake.

Stock control group.

The mean body weight of 24 male and 24 female hamsters reared on stock diet are shown in Table 2.8. The mean food intake of the groups expressed as g/animal/d are shown in Table 2.9a and energy intakes per metabolic mass (kJ/kg $^{.75}$/d) in Table 2.9b. The number of observations in Table 2.9a and b is 4 because the 24 animals were housed in groups of 6 and only group food intakes were recorded. The mean food intake of both sexes during the 6 week period week 6-week 11 was 6.5g/100g body weight/d (Table 2.9a) and mean energy intake for the same period was approximately 480 kJ/kg$^{.75}$/d (Table 2.9b).

The data collected on the stock control groups are considered to be typical of animals in the Southampton colony housed in the conditions described and handled routinely and will be used therefore, as the reference point in discussion of the observations made on groups offered experimental diets.

Groups offered diets containing casein

The mean body weights of animals given casein diets are shown in Table 2.10 and the mean weight gains after being given experimental diet are shown with those of the stock control groups in Figures 2.1 (females) and 2.2 (males). In both male and female groups the rate of growth with Diet 3 (15% reference protein) was comparable to that of the stock control group. In the groups offered Diet 4 (20% reference protein) growth was reduced in the latter part of the trial such that body weight was significantly lower, by about 15% on day 49, in the female group than in the relevant stock group. However neither in males nor females did growth differ significantly between groups given Diet 4 or Diet 3. In both male and female groups the animals given diets containing 5% reference protein (Diet 1) and 10% reference protein

Table 2.8 The mean body weights(g ± SEM) at different ages of 24 female and 24 male hamsters given stock diet.

Age in days	Female	Male
28 (weaned)	63.8 ± 1.9	67.4 ± 1.7
35	77.8 ± 1.7	79.3 ± 1.4
42	87.5 ± 1.9	90.4 ± 1.6
49	97.3 ± 2.1	98.6 ± 1.6
56	105.3 ± 1.9	105.5 ± 2.0
63	112.8 ± 2.3	109.5 ± 2.5
70	117.5 ± 2.4	112.7 ± 2.9
77	120.4 ± 2.6	116.1 ± 3.3
Mean gain days 35-77	42.5 ± 2.5	37.5 ± 3.4

Table 2.9a Mean food intake (g/animal/d) during growth of 4 groups of 6 female- and 6 male hamsters weaned at 4 weeks of age.

Week	Female	Male
5	5.9 ± 0.8	7.7 ± 0.7
6	5.9 ± 1.0	8.0 ± 0.5
7	5.4 ± 0.6	5.5 ± 0.7
8	6.0 ± 1.2	7.2 ± 0.6
9	7.2 ± 0.6	7.1 ± 0.6
10	6.2 ± 1.0	6.2 ± 1.1
11	7.4 ± 0.5	6.4 ± 0.9
Mean weeks 6-11:	6.4 ± 0.3 (6)	6.7 ± 0.5 (6)
Expressed as g/100g body weight/day		
Mean weeks 6-11:	6.2 ± 0.3 (6)	6.7 ± 0.6 (6)

Table 2.9b Metabolisable energy intake ($kJ/kg^{0.75}$ /day) during growth of 4 groups of 6 female and 6 male hamsters weaned at 4 weeks of age.

Week	Female	Male
5	600.3 ± 81.1	738.6 ± 85.7
6	512.9 ± 71.1	650.0 ± 46.0
7	412.6 ± 45.6	437.2 ± 58.5
8	448.5 ± 86.9	536.3 ± 27.2
9	507.5 ± 25.1	507.4 ± 33.4
10	422.2 ± 61.9	427.6 ± 60.2
11	496.2 ± 21.3	543.4 ± 69.4
Mean weeks 6-11	466.5 ± 18.2 (6)	517.0 ± 33.3 (6)

Table 2.10 Mean body weights (g ± SEM) at different ages of female and male hamsters given diets containing casein (6 animals/group) or stock diet (24 animals/group)

	Age in days	Diet 1	2	3	4	Stock
FEMALES	28 (weaned)	62.4 ± 0.9	62.4 ± 1.7	62.4 ± 2.2	62.5 ± 2.1	63.8 ± 1.9
	35 (given experimental diet)	72.8 ± 2.3	76.7 ± 1.8	77.3 ± 3.7	75.7 ± 2.3	77.8 ± 1.7
	42	74.3 ± 1.4**	88.6 ± 1.8	91.1 ± 3.3	88.2 ± 1.8	87.5 ± 1.9
	49	77.7 ± 2.2***	93.6 ± 1.7	103.3 ± 4.1	96.5 ± 1.3	97.3 ± 2.1
	56	78.3 ± 3.6***	90.7 ± 3.4**	107.0 ± 5.2	100.7 ± 1.6	105.3 ± 1.9
	63	75.7 ± 4.4***	87.4 ± 4.5***	118.3 ± 6.4o	102.2 ± 2.3*	112.8 ± 2.3
	70	74.0 ± 5.5***	79.2 ± 7.0***	115.8 ± 7.1	101.8 ± 3.7**	117.5 ± 2.4
	77	74.0 ± 7.2***	75.3 ± 7.3***	113.0 ± 7.5	102.3 ± 4.4**	120.4 ± 2.6
Mean weight gain days 35-77		1.2 ± 7.4	-1.3 ± 6.5	35.7 ± 6.8	26.7 ± 5.8	42.5 ± 2.5

Table 2.10 continued

	Age in days	Diet 1	2	3	4	Stock
MALES	28 (weaned)	66.1 ± 1.1	66.1 ± 1.2	66.6 ± 1.0	66.4 ± 1.1	64.7 ± 1.7
	35 (given experimental diet)	84.0 ± 1.8	81.2 ± 2.3	80.5 ± 1.1	83.0 ± 1.2	79.3 ± 1.4
	42	90.3 ± 1.6+	90.5 ± 5.1	95.9 ± 1.6	94.0 ± 2.1	90.4 ± 1.6
	49	94.3 ± 2.0●+++	99.6 ± 5.3	106.3 ± 1.6	103.8 ± 2.3	98.6 ± 1.6
	56	93.4 ± 1.9**●●●+++	99.7 ± 5.3+	113.5 ± 2.5	108.5 ± 1.9	105.5 ± 2.0
	63	90.7 ± 2.3***●●●+++	94.2 ± 5.8●++	116.2 ± 2.7	110.2 ± 1.9	109.5 ± 2.5
	70	85.8 ± 3.4***●●●+++	85.2 ± 6.2***●●++	116.2 ± 2.9	111.3 ± 2.4	112.7 ± 2.9
	77	81.8 ± 5.4***●●●+++	79.5 ± 6.6***●●+++	119.3 ± 2.9	112.5 ± 3.0	116.1 ± 3.3
Mean weight gain days 35-77		-0.5 ± 7.4***●●●+++	-1.6 ± 4.6***●●●+++	38.8 ± 3.1	29.5 ± 3.2	37.5 ± 3.4

Significant differences from stock groups * P<0.05 ** P<0.01 *** P < 0.001

from group 4 ● P<0.05 ●● P<0.01 ●●● P < 0.001

from group 3 + P<0.05 ++ P<0.01 +++ P < 0.001

from group 2 ǂ P<0.05 ǂǂ P<0.01 ǂǂǂ P < 0.001

Figure 2.1 : Weight gains from 35 days of age of female hamsters given diets containing casein (6 animals/group) or stock diet (24 animals).

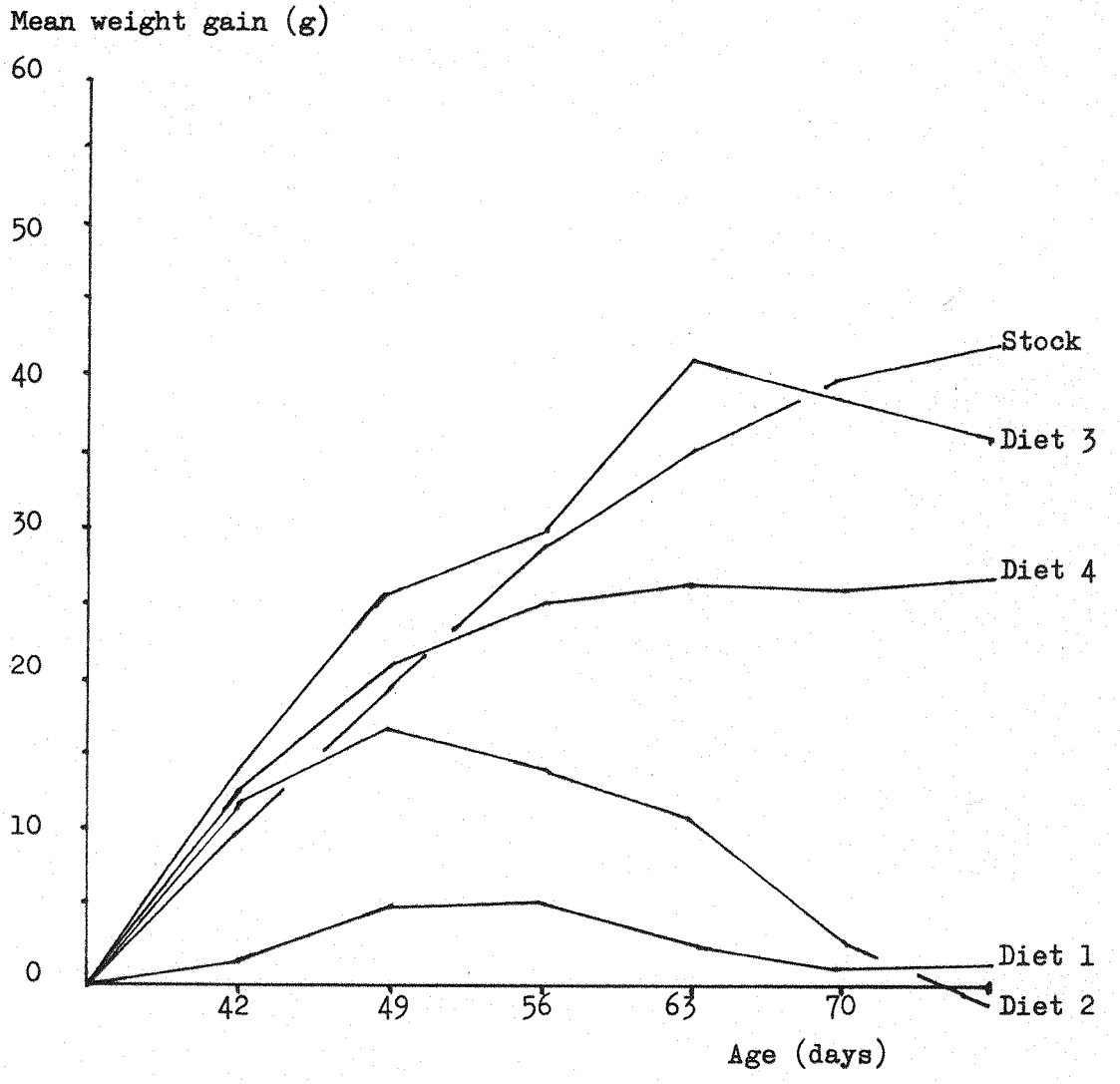


Figure 2.2 : Mean weight gains from 35 days of age of male hamsters given diets containing casein (6 animals / group) or stook diet (24 animals).

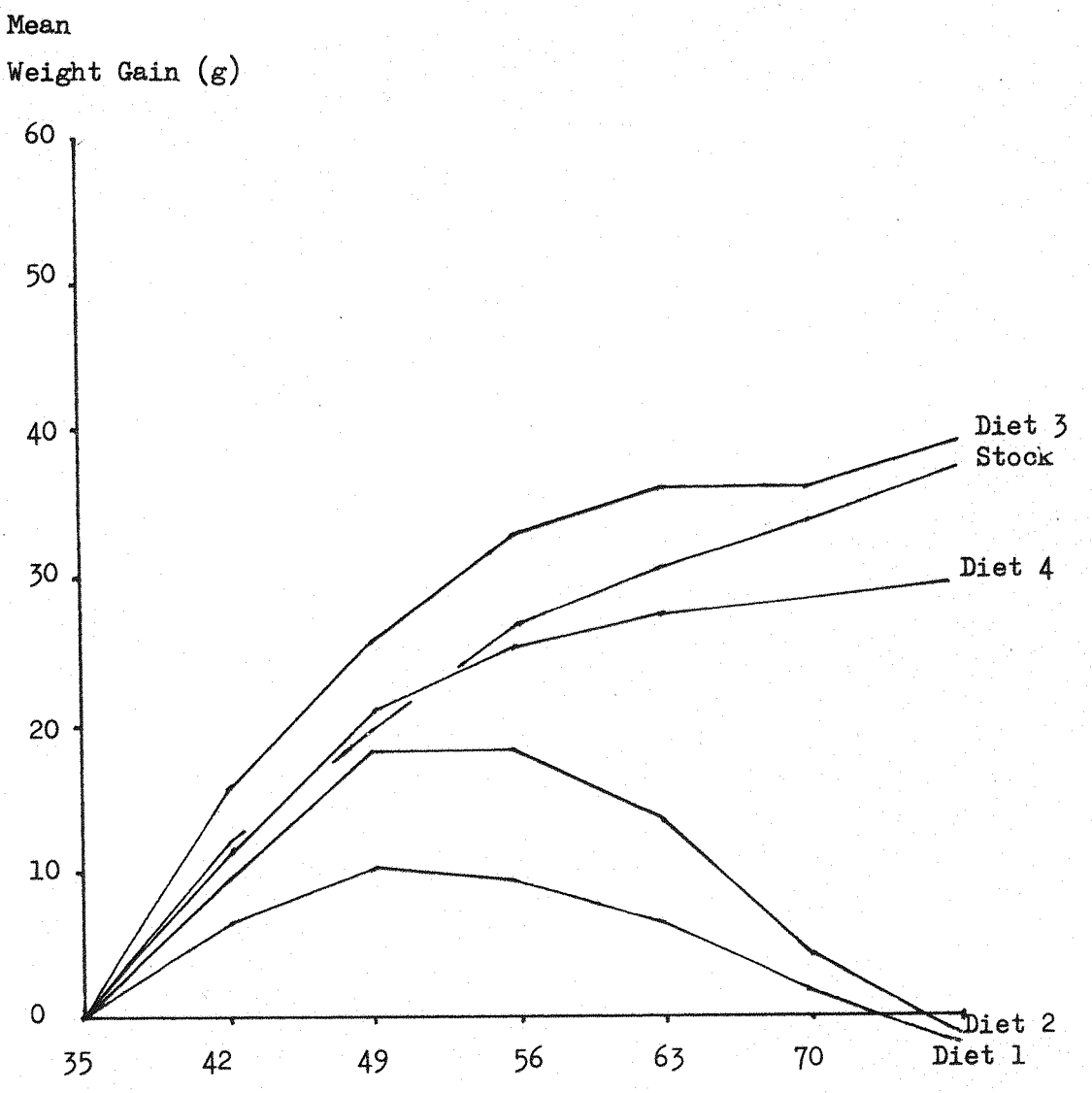


Table 2.11a Mean food intake (g/animal/day) during growth of female and male hamsters given diets containing casein (6 animals/group) or stock diet (4 groups of 6 animals/group for each sex).

	Week	Diet 1	2	3	4	Stock
FEMALES	5 (Stock diet)	6.5	7.4	7.2	7.3	5.9 ± 0.8
	6	7.4	7.0	6.3	6.8	5.9 ± 1.0
	7	5.9	5.8	6.5	5.9	5.4 ± 0.6
	8	5.4	5.0	6.3	5.3	6.0 ± 1.2
	9	5.1	4.4	6.3	5.2	7.2 ± 0.6
	10	4.5	3.8	5.9	5.7	6.2 ± 1.0
	11	4.8	3.7	5.1	5.3	7.4 ± 0.5
Mean weeks 6 - 11		5.5 ± 0.5	5.0 ± 0.6	6.1 ± 0.4	5.7 ± 0.3	6.4 ± 0.3
Expressed as g/100g body weight /day						
Mean weeks 6 - 11		7.3 ± 0.6	5.6 ± 0.6	5.9 ± 0.4	5.9 ± 0.5	6.2 ± 0.3

Table 2.11a contined

	Week	Diet 1	2	3	4	Stock
	5 (Stock diet)	9.1	8.3	7.9	8.6	7.7 ± 0.7
	6	9.0	7.4	8.1	7.6	8.0 ± 0.5
	7	6.2	6.8	7.5	7.0	5.5 ± 0.7
MALES	8	6.1	6.7	6.7	6.1	7.2 ± 0.6
	9	4.5	5.4	5.6	5.9	7.1 ± 0.6
	10	4.5	3.7	5.5	5.4	6.2 ± 1.1
	11	4.0	2.7	5.5	5.8	6.4 ± 0.9
Mean weeks 6 - 11		5.7 ± 0.8	5.5 ± 0.8	6.5 ± 0.5	6.3 ± 0.4	6.7 ± 0.5
Expressed as g/100g body weight /day						
Mean weeks 6 - 11		6.4 ± 0.9	6.0 ± 0.8	6.2 ± 0.7	6.1 ± 0.6	6.7 ± 0.6

Table 2.11b Metabolisable energy intake ($kJ/kg^{0.75}$ /day) during growth of female and male hamsters given diets containing casein (6 animals/group) or stock diet (4 groups of 6 animals/group for each sex)

	Week	Diet 1	2	3	4	Stock
	5 (Stock diet	602.3	669.2	642.5	666.7	600.3 ± 81.1
	6	896.2	780.8	668.4	731.9	512.9 ± 71.1
	7	696.0	597.3	626.6	591.5	412.6 ± 45.6
FEMALES	8	631.6	509.5	565.6	498.7	448.5 ± 86.9
	9	594.8	459.0	543.8	479.0	507.5 ± 25.1
	10	534.2	423.4	489.5	525.8	422.2 ± 61.9
	11	580.2	426.8	433.5	486.1	496.2 ± 21.3
Mean weeks 6 - 11		655.4 ± 53.1**	533.0 ± 56.4	554.7 ± 35.2	552.2 ± 39.7	466.5 ± 18.2

Table 2.11b continued

	Week	Diet 1	2	3	4	Stock
	5 (Stock diet)	772.1	721.1	683.4	736.5	738.6 ± 85.7
	6	961.0	798.4	836.8	780.4	650.0 ± 46.0
	7	639.1	676.3	697.2	661.3	437.2 ± 58.5
MALES	8	611.1	645.4	586.9	548.0	536.3 ± 27.2
	9	465.2	536.3	476.5	514.1	507.4 ± 33.4
	10	472.3	384.6	472.8	472.3	427.6 ± 60.2
	11	440.2	296.8	453.1	499.1	543.4 ± 69.4
Mean weeks 6 - 11		598.2 ± 79.8	556.4 ± 76.9	587.3 ± 62.3	579.4 ± 48.5	517.0 ± 33.3

Significant differences from stock group **$P < 0.01$

(Diet 2) gained significantly less weight than the stock control groups and the groups given the higher levels of casein. Females were affected earlier than the males by the low levels of protein, the difference from the stock group becoming significant at a younger age. Also, for a period of 3 weeks the females on Diet 1 were significantly lighter than those on Diet 2.

The differences in weight gain of the experimental groups are not a reflection of differences in food intake which is similar in all dietary groups when expressed in g/100g body weight/d (Table 2.11a)and the mean energy intakes from week 6 to week 11 in $kJ/kg^{.75}/d$ are similar to the stock group in all cases (Table 2.11b) with the exception of the females on Diet 1 (5% reference protein) whose mean intake was 40% greater than that of the stock animals ($P < 0.01$)

Groups offered Diets containing White Fishmeal.

The only female group to achieve a rate of growth comparable to that of the stock control group was the one supplied with 15% reference protein (Table 2.12 and Figure 2.3). At the highest level of reference protein (Diet 4) the animals started to lose weight after the second week on diet and were 18% lighter than the stock group of animals and those given experimental Diet 3 after a further 2 weeks, and also at days 42 and 49 they were no longer significantly heavier than the animals on the two lowest levels of protein (Diets1 and 2).

The mean food intake for the 6 week period on experimental diet is lower than that of the stock group in all but the group given Diet 3 (Table 2.13a) but not if the size of the animals are taken into account i.e. if the data is expressed in g/100g body weight/d (Table 2.13a). The mean energy intake per metabolic mass $kJ/kg^{.75}/d$) for weeks 6-11, although reduced in the high protein group, was not significantly different from that of the stock group (Table 2.13b). Of the male groups offered

Table 2.12 Mean body weights (g ± SEM) at different ages of female and male hamsters given diets containing fishmeal (6 animals/group) or stock diet (24 animals/group)

	Age in days	Diet 1	2	3	4	Stock
FEMALES	28 (weaned)	62.4 ± 2.0	62.4 ± 2.3	62.4 ± 2.0	62.4 ± 1.8	63.8 ± 1.9
	35 (given experimental diet)	76.7 ± 1.0	72.2 ± 2.3	76.2 ± 1.2	77.8 ± 1.4	77.8 ± 1.7
	42	76.4 ± 0.8 **●●● +++	78.9 ± 1.7 *●● ++	88.0 ± 1.6	89.3 ± 1.7	87.5 ± 1.9
	49	80.0 ± 1.7 ***●●● +++	83.3 ± 1.2 **●●● +++	96.3 ± 2.5	96.8 ± 1.3	97.3 ± 2.1
	56	75.3 ± 1.8 ***●●● +++	78.7 ± 0.8 ***●●● +++	107.2 ± 3.0●	96.5 ± 1.6*	105.3 ± 1.9
	63	73.8 ± 2.2 ***●● +++	76.8 ± 2.0 ***●● +++	112.0 ± 3.0●●	92.0 ± 3.7***	112.8 ± 2.3
	70	69.7 ± 2.5 ***+++	74.2 ± 4.5 ***+++	117.2 ± 4.4●●	85.5 ± 6.8***	117.5 ± 2.4
	77	66.2 ± 3.7 ***+++	76.2 ± 7.0 ***+++	127.0 ± 5.4●●	79.8 ± 9.7***	120.4 ± 2.6
	Mean weight gain days 35-77	10.5 ± 3.6 ***+++	5.0 ± 8.8 ***++	50.8 ± 6.0●●	2.0 ± 9.7***	42.5 ± 2.5

Table 2.12 continued

	Age in days	Diet 1	2	3	4	Stock
MALES	28 (weaned)	62.3 ± 2.2	62.3 ± 3.0	62.3 ± 3.0	62.3 ± 2.3	64.7 ± 1.7
	35 (given experimental diet)	79.8 ± 3.3	78.9 ± 2.8	80.8 ± 2.7	80.2 ± 2.3	79.3 ± 1.4
	42	81.2 ± 2.8 *●●	90.0 ± 3.5	89.7 ± 1.5	91.3 ± 3.0	90.4 ± 1.6
	49	84.3 ± 3.3 ***●●●+++	101.3 ± 3.9	103.3 ± 1.2	105.3 ± 3.0	98.6 ± 1.6
	56	85.3 ± 3.5 ***●●+++	105.8 ± 4.6	108.8 ± 2.0	110.3 ± 4.6	105.5 ± 2.0
	63	84.3 ± 4.2 ***●●●+++	109.0 ± 5.1	112.0 ± 2.3	113.2 ± 4.6	109.5 ± 2.5
	70	79.2 ± 4.5 ***●●●+++	107.5 ± 6.0	111.3 ± 2.7	111.0 ± 4.6	112.7 ± 2.9
	77	75.2 ± 5.7 ***●●●+++	104.8 ± 7.4	108.7 ± 2.6	109.5 ± 4.0	116.1 ± 3.3
Mean weight gain days 35-77		-4.2 ± 4.2 ***●●●+++	26.0 ± 5.9	28.2 ± 4.5	29.3 ± 4.8	37.5 ± 3.4

Significant differences from stock groups * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

from group 4 ● $P < 0.05$ ●● $P < 0.01$ ●●● $P < 0.001$

from group 3 + $P < 0.05$ ++ $P < 0.01$ +++ $P < 0.001$

from group 2 ǂ $P < 0.05$ ǂǂ $P < 0.01$ ǂǂǂ $P < 0.001$

Figure 2.3 : Mean weight gains from 35 days of age of female hamsters given diets containing fishmeal (6 animals / group) or stock diet (24 animals).

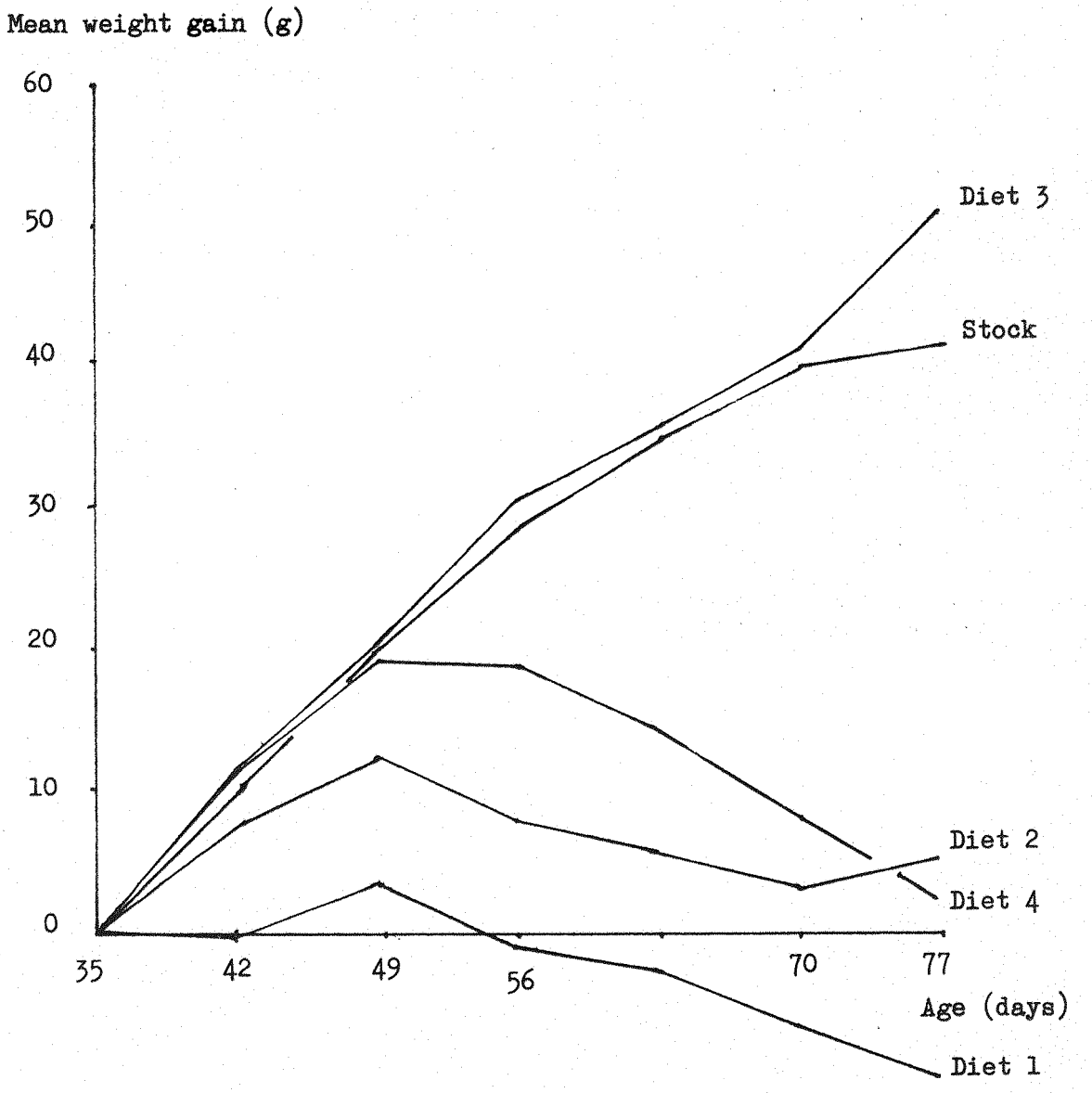


Figure 2.4 : Mean weight gains from 35 days of age of male hamsters given diets containing fishmeal (6 animals / group) or stock diet (24 animals).

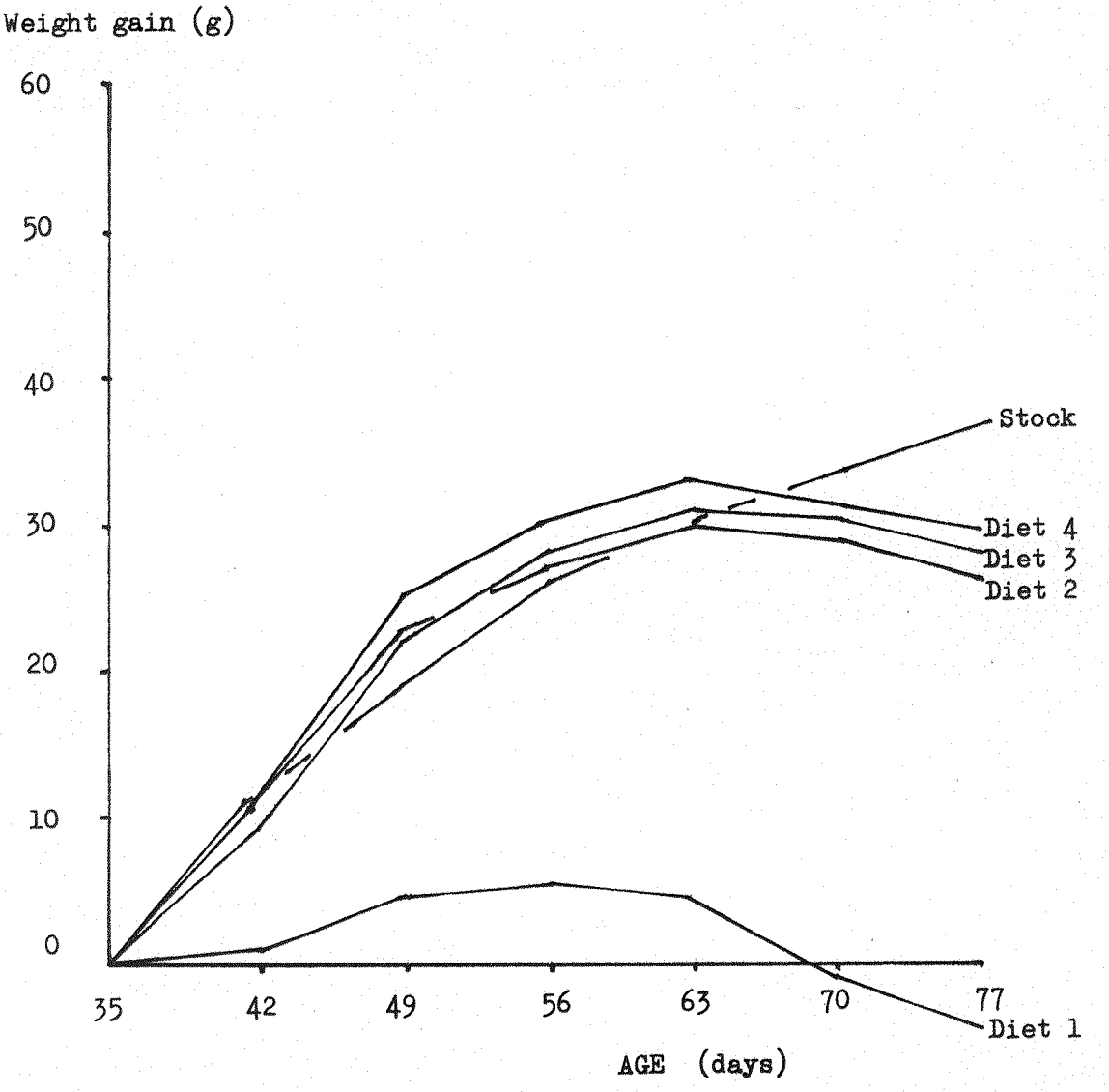


Table 2.13a Mean food intake (g/animal /day) during growth of female and male hamsters given diets containing fishmeal (6 animals/group) or stock diet (4 groups of 6 animals/group for each sex).

	Week	Diet 1	2	3	4	Stock
	5 (Stock Diet)	7.0	5.9	7.0	6.7	5.9 ± 0.8
	6	4.4	6.0	5.6	5.5	5.9 ± 1.0
	7	4.8	4.8	5.5	5.2	5.4 ± 0.6
FEMALES	8	3.1	4.1	6.1	4.5	6.0 ± 1.2
	9	4.1	4.2	5.3	5.9	7.2 ± 0.6
	10	3.8	4.5	5.9	3.8	6.2 ± 1.0
	11	5.5	2.5	6.2	0.9	7.4 ± 0.5
Mean of weeks 6-11		4.3 ± 0.3**	4.4 ± 0.5*	5.8 ± 0.2	4.3 ± 0.8*	6.4 ± 0.3
Expressed as g/100g body weight/day Mean of weeks 6-11		5.7 ± 0.5	5.7 ± 0.6	5.7 ± 0.3	4.7 ± 0.8	6.2 ± 0.3

Table 2.13a continued

	Week	Diet 1	2	3	4	Stock
	5 (Stock diet)	8.2	8.1	8.3	8.2	7.7 ± 0.7
	6	8.7	7.2	7.0	7.9	8.0 ± 0.5
	7	8.0	7.6	6.5	6.9	5.5 ± 0.7
MALES	8	2.3	5.1	5.2	5.3	7.2 ± 0.6
	9	4.2	5.4	5.3	5.1	7.1 ± 0.6
	10	3.5	4.6	4.5	4.5	6.2 ± 1.1
	11	4.1	5.1	4.9	3.7	6.4 ± 0.9
Mean of weeks 6-11		5.1 ± 1.1	5.8 ± 0.6	5.6 ± 0.5	5.6 ± 0.6	6.7 ± 0.5
Expressed as g/100g body weight/days Mean of weeks 6-11		6.5 ± 0.6	6.5 ± 0.7	6.5 ± 0.3	5.3 ± 0.9	6.7 ± 0.6

Significant differences from stock groups: *P < 0.05 ; **P < 0.01

Table 2.13b Metabolisable energy intake (kJ/kg $^{0.75}$ /day) during growth of female and male hamsters given diets containing fishmeal (6 animals/group) or stock diet (4 groups of 6 animals/group for each sex)

	Week	Diet 1	2	3	4	Stock
	5 (Stock diet)	629.1	547.2	631.2	600.7	600.3 ± 81.1
	6	505.4	681.8	561.4	532.5	512.9 ± 71.1
	7	537.1	514.1	507.0	461.5	412.6 ± 45.6
FEMALES	8	380.8	436.4	521.7	392.9	448.5 ± 86.9
	9	483.2	462.7	430.1	518.3	507.5 ± 25.1
	10	455.2	510.8	464.8	354.5	422.2 ± 61.9
	11	684.3	285.1	463.6	86.9	496.2 ± 21.3
Mean of weeks 6 - 11		507.9 ± 41.4	482.0 ± 52.7	491.6 ± 19.2	391.3 ± 67.3	466.5 ± 18.2
	5 (Stock diet)	731.5	720.2	736.9	730.3	738.6 ± 85.7
	6	965.2	749.5	683.4	751.6	650.0 ± 46.0
	7	866.5	716.9	584.4	585.6	437.2 ± 58.5
MALES	8	244.5	457.7	433.9	426.8	536.3 ± 27.2
	9	449.4	467.7	427.6	393.3	507.4 ± 33.4
	10	385.4	407.1	361.2	351.5	427.6 ± 60.2
	11	462.3	449.4	395.4	292.6	543.4 ± 69.4
Mean of weeks 6 - 11		562.2 ± 116.6	541.3 ± 61.5	481.1 ± 51.0	466.9 ± 69.8	517.0 ± 33.3

the fishmeal diets only that given Diet 1 (5% reference protein) showed a reduced growth rate compared to the stock group (Table 2.12 and Figure 2.4). This group was also consistently lighter than the three male groups given higher levels of fishmeal in the diet and at the end of the trial were 30% lighter than any other experimental group. The mean weights of the groups offered Diets 2, 3 and 4 were never statistically different from those of the stock control group but they reached a plateau and even tended to lose weight towards the end of the trial. There were no differences from the stock control group in overall food intake (Table 2.13a) or metabolisable energy intake (Table 2.13b) in any of the male experimental groups.

Groups offered Diets containing Mould

Both male and female groups offered the diet containing 5% reference protein (Diet 1) had significantly reduced mean weights after 2 weeks on diet compared to the stock control (Table 2.14, Figures 2.5 and 2.6) (female $P < 0.001$, male $P < 0.02$). The degree of significance between the males on Diet 1 and the stock group increased through the trial. The female group 1 was significantly lighter than the group given Diet 3 after only one week on diet, but this significance was not observed in the corresponding male groups until the third week of the trial. The smaller weight gains of the two low protein groups were not due to a reduction in food intake (Table 2.15a) or metabolisable energy intake (Table 2.15b) which was actually increased compared to the stock group level in the female group on Diet 1 ($P < 0.001$).

Growth comparable to or greater than that of the stock groups was achieved in all other groups offered diets containing mould as the source of protein. The females given the 10% reference protein diet (Diet 2) achieved this with an increase by 30% in their

Table 2.14 Mean body weights (g ± SEM) at different ages of female and male hamsters given diets containing mould (6 animals/group) or stock diet (24 animals/group)

	Age in days	Diet 1	2	3	4	Stock
FEMALES	28 (weaned)	66.8 ± 2.6	66.7 ± 2.4	66.8 ± 3.7	66.7 ± 4.5	63.8 ± 1.9
	35 (given experimental diet)	82.2 ± 2.2	81.2 ± 1.7	80.3 ± 3.8	80.7 ± 4.6	77.8 ± 1.7
	42	82.0 ± 2.6+	89.3 ± 3.2	93.3 ± 3.8	93.0 ± 4.9	87.5 ± 1.9
	49	78.7 ± 3.5***	95.3 ± 2.3	103.8 ± 4.1	105.8 ± 4.8	97.3 ± 2.1
	56	80.5 ± 2.6***	101.5 ± 2.9+	114.7 ± 4.9*	114.7 ± 4.7*	105.3 ± 1.9
	63	85.5 ± 2.0***	107.8 ± 3.4	122.2 ± 5.9	121.3 ± 5.0	112.8 ± 2.3
	70	84.8 ± 2.5***	117.2 ± 4.4	132.5 ± 7.2*	127.7 ± 5.9	117.5 ± 2.4
	77	89.5 ± 2.9***	123.3 ± 5.0	139.5 ± 7.3**	133.7 ± 5.6*	120.4 ± 2.6
	Mean gain days 35-77	7.3 ± 2.0***	42.1 ± 4.8	57.4 ± 7.3*	54.1 ± 3.7*	42.5 ± 2.5

Table 2.14 continued

	Age in days	Diet 1	2	3	4	Stock
MALES	28 (weaned)	65.7 ± 2.5	65.6 ± 2.9	65.7 ± 2.8	65.6 ± 3.5	64.7 ± 1.7
	35 (given experimental diet)	78.1 ± 2.9	78.8 ± 4.5	79.3 ± 1.9	76.3 ± 4.3	79.3 ± 1.4
	42	85.0 ± 3.1	91.7 ± 4.6	91.3 ± 2.2	89.7 ± 4.2	90.4 ± 1.6
	49	88.7 ± 3.4*	100.0 ± 5.2	99.0 ± 2.2	96.1 ± 4.3	98.6 ± 1.6
	56	90.3 ± 3.5** ‡‡	110.6 ± 6.2	101.7 ± 1.3	102.3 ± 4.2	105.5 ± 2.0
	63	88.3 ± 3.9*** ●● +++	117.6 ± 7.0	109.7 ± 1.4	108.0 ± 4.4	109.5 ± 2.5
	70	88.2 ± 3.7*** ●● ‡‡‡‡	125.9 ± 7.3	118.7 ± 1.5	115.2 ± 4.7	112.7 ± 2.9
	77	88.5 ± 4.6*** ●● ‡‡‡‡	131.8 ± 6.4	124.3 ± 2.8	117.8 ± 5.3	116.1 ± 3.3
	Mean gain days 35-77	10.5 ± 5.1*** ●● ‡‡‡‡	52.0 ± 2.9	44.0 ± 3.5	41.7 ± 5.8	37.5 ± 3.4

Significant differences:

from stock control group	*P < 0.05	**P < 0.01	***P < 0.001
from group 4	●P < 0.05	●●P < 0.01	●●●P < 0.001
from group 3	+P < 0.05	++P < 0.01	+++P < 0.001
from group 2	‡P < 0.05	‡‡P < 0.01	‡‡‡P < 0.001

Figure 2.5 : Mean weight gain from 35 days of age of female hamsters given diets containing mould (6 animals / group) or stock diet (24 animals).

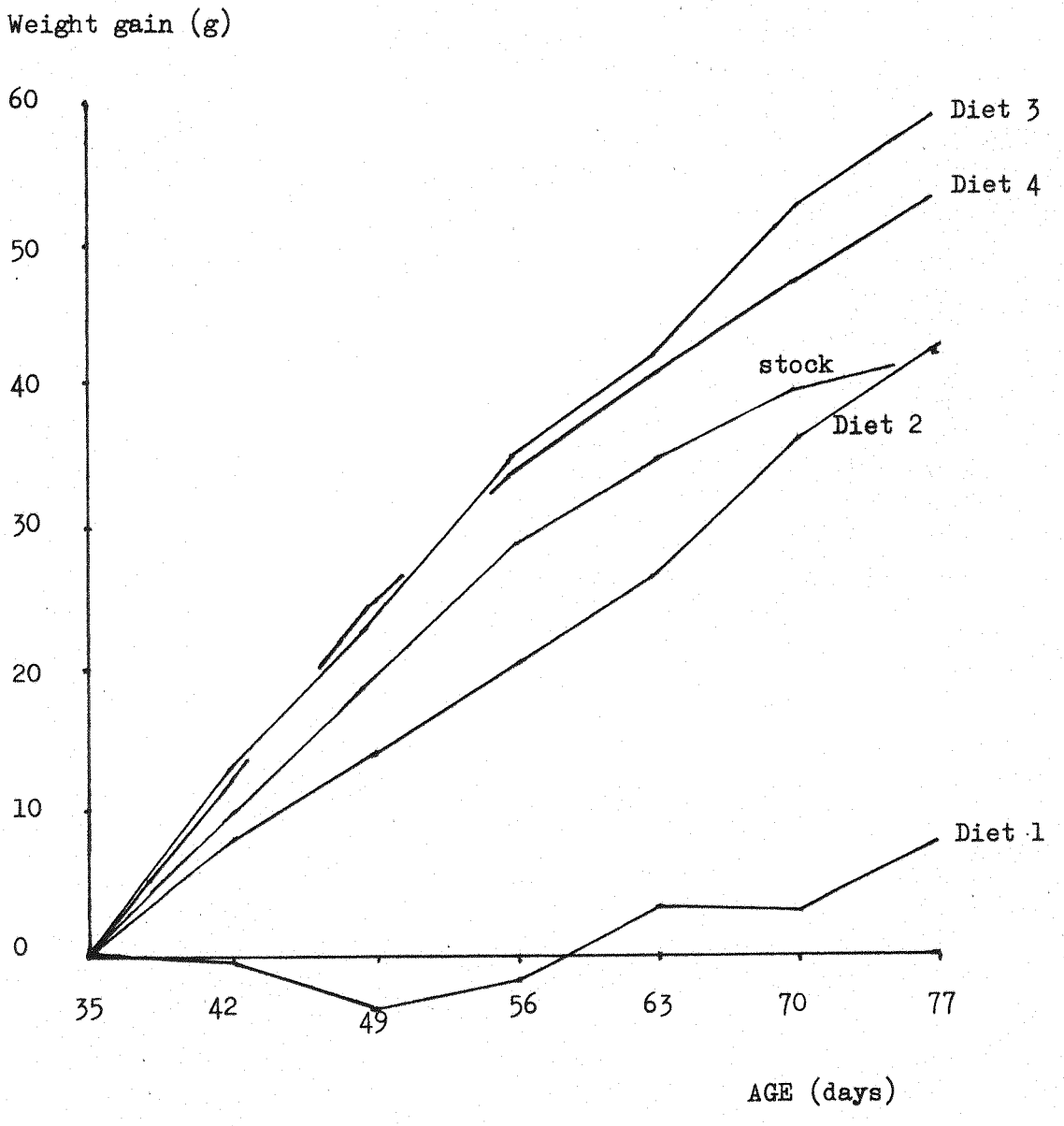


Figure 2.6 : Mean weight gains from 35 days of age of male hamsters given diets containing mould (6 animals / group) or stock diet (24 animals).

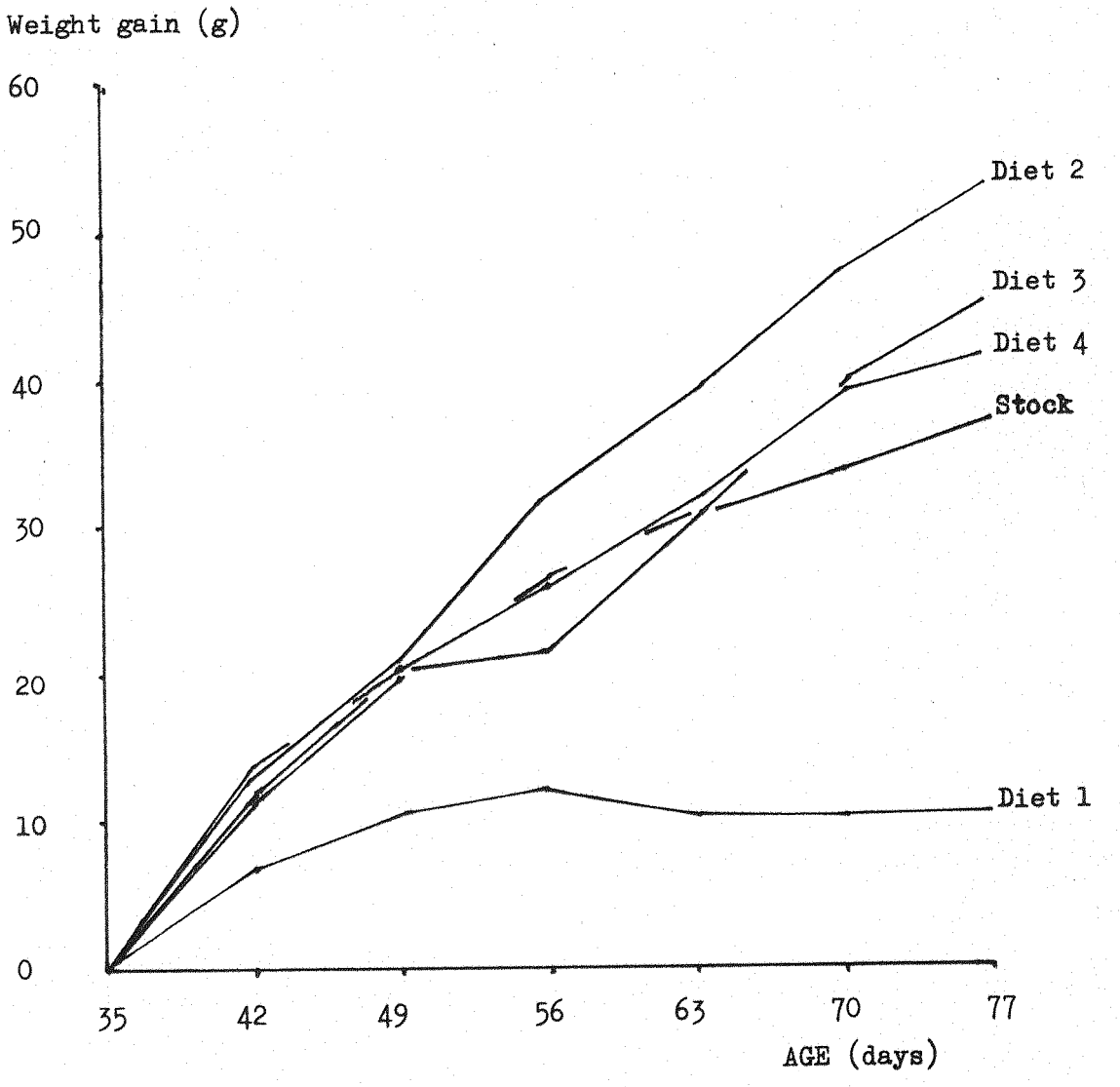


Table 2.15a Mean food intake (g/animal/day) during growth of female and male hamsters given diets containing mould (6 animals/group) or stock diet (4 groups of 6 animals/group for each sex)

	Week	Diet				
		1	2	3	4	Stock
FEMALES	5 (Stock diet)	6.3	5.8	6.2	6.2	5.9 ± 0.8
	6	5.8	7.1	7.3	6.6	5.9 ± 1.0
	7	5.5	7.0	7.5	6.1	5.4 ± 0.6
	8	6.3	7.6	7.3	7.1	6.0 ± 1.2
	9	6.1	7.1	5.3	5.2	7.2 ± 0.6
	10	5.4	7.1	6.3	5.5	6.2 ± 1.0
	11	6.1	7.0	6.6	6.2	7.4 ± 0.5
Mean of weeks 6-11		5.9 ± 0.3	7.2 ± 0.3	6.7 ± 0.4	6.1 ± 0.3	6.4 ± 0.3
Expressed as g/100g body weight/day Mean of weeks 6-11		7.0 ± 0.3	7.1 ± 0.4	6.2 ± 0.7	5.6 ± 0.5	6.2 ± 0.3

Table 2.15a continued

	Week	Diet 1	2	3	4	Stock
	5 (Stock diet)	6.3	6.4	6.4	6.2	7.7 ± 0.7
	6	6.8	7.7	7.4	6.7	8.0 ± 0.5
	7	6.3	7.6	6.7	6.3	5.5 ± 0.7
MALE	8	6.2	7.0	6.3	6.4	7.2 ± 0.6
	9	7.0	6.3	5.8	6.1	7.1 ± 0.6
	10	4.3	6.0	5.9	5.9	6.2 ± 1.1
	11	5.1	3.8	5.7	5.7	6.4 ± 0.9
Mean of weeks 6-11		6.0 ± 0.5	6.4 ± 0.7	6.3 ± 0.4	6.2 ± 0.3	6.7 ± 0.5
Expressed as g/100g body weight/day Mean of weeks 6-11		6.8 ± 0.5	6.2 ± 0.9	6.2 ± .6	6.2 ± 0.4	6.7 ± 0.6

Table 2.15b Metabolisable energy intake ($kJ/kg^{0.75}$ /day) during growth of female and male hamsters given diets containing mould (6 animals/group) or stock diet (4 groups of 6 aminals/group for each sex)

	Weeks	Diet				
		1	2	3	4	Stock
FEMALES	5 (Stock diet)	545.1	500.8	532.5	533.0	600.3 ± 81.1
	6	632.4	699.3	648.3	587.7	512.9 ± 71.1
	7	603.6	651.7	603.6	488.6	412.6 ± 45.6
	8	705.6	666.3	545.5	522.1	448.5 ± 86.9
	9	656.7	599.4	374.5	363.2	507.5 ± 25.1
	10	569.7	568.1	417.6	372.9	422.2 ± 61.9
	11	632.9	534.2	420.1	403.8	496.2 ± 21.3
Mean of weeks 6-11		633.7 ± 18.8***	619.3 ± 25.9***	501.6 ± 46.0	456.5 ± 37.2	466.5 ± 18.2

Table 2.15b continued

	Weeks	Diet 1	2	3	4	Stock
	5 (stock diet)	551.3	563.9	562.6	425.1	738.6 ± 85.7
	6	731.9	752.0	664.6	616.1	650.0 ± 46.0
	7	652.9	679.3	555.9	529.2	437.2 ± 58.5
MALES	8	628.3	590.2	499.1	510.8	536.3 ± 27.2
	9	711.4	494.1	441.8	468.6	507.4 ± 33.4
	10	446.4	450.2	429.7	437.2	427.6 ± 60.2
	11	525.8	272.1	392.1	404.6	543.4 ± 69.4
Mean of weeks 6-11		616.1 ± 45.1	539.6 ± 70.6	497.4 ± 41.0	494.5 ± 30.9	517.0 ± 33.3

Significant difference from stock group: *** $P < 0.001$

metabolisable energy intake (Table 2.15b) There were no differences in food intake from the stock group on the other groups fiven Diets 2, 3 and 4.

Groups supplied with Diets containing Soya Bean protein (sample 1)

The female groups offered diets containing 5% reference protein (Diet 1) or 10% reference protein (Diet 2) were approximately 13% lighter than the stock control group after one week on diet (Table 2.16, Figure 2.7) and the difference increased particularly in the Diet 1 group in spite of an overall increase in energy intake of 20% (Diet 2)and 30% (Diet 1) (Table 2.17b). These groups were also significantly lighter than the animals on Diet 3 from day 21 onwards and by the end of the trial animals given Diet 1 were lighter than all other female groups by 20-30%. Although the animals given Diet 4 grew less well than those on Diet 3 this difference was not significant and these two female groups were comparable to the stock control group both in terms of their growth response and in terms of food (Table 2.17a) and energy (Table 2.17b) intakes.

Male animals given Diet 1 (5% reference protein) were significantly lighter than the stock group ($P < 0.01$) after 2 weeks on diet and also lighter than the other three experimental groups (Table 2.16) These differences were maintained throughout the trial. Although mean food intake for weeks 6-11 in g/animal/d was reduced in the Diet 1 animals compared to the stock group ($P < 0.05$) (Table 2.17a) when it is expressed per 100g body weight (Table 2.17a) it is the same as that of the other groups being approximately 6g/100g body weight/d. Energy intake per metabolic mass was similar to the stock group in all 4 groups (Table 2.17b). Towards the end of the trial, animals given Diets 2,3 or 4 were of a similar weight to the stock control group even though at certain points during the growth curve animals on Diets 2 and 3 were heavier than the stock group (Table 2.16 Figure 2.8). Diet 3 animals were 10% heavier than those given Diet 4 for most of the trial ($P < 0.05$) (Table 2.16).

Table 2.16 Mean body weights (g ± SEM) at different ages of female and male hamsters given diets containing soya protein (sample 1) (6 animals/group) or stock diet (24 animals/group)

	Age in days	Diet 1	2	3	4	Stock
FEMALES	28(weaned)	60.3 ± 1.7	60.2 ± 1.3	60.2 ± 1.6	60.2 ± 1.5	63.8 ± 1.9
	35(given experimental diet)	73.7 ± 2.0	76.3 ± 3.4	78.3 ± 1.8	74.0 ± 2.1	77.8 ± 1.7
	42	74.7 ± 1.7**	77.0 ± 4.5*	88.7 ± 3.0	84.5 ± 3.8	87.5 ± 1.9
	49	77.2 ± 1.6***	86.7 ± 4.6*	100.7 ± 3.2	94.3 ± 4.0	97.3 ± 2.1
	56	80.8 ± 2.9***	92.5 ± 5.2**	111.5 ± 3.3	101.3 ± 4.7	105.3 ± 1.9
	63	79.0 ± 3.4***	94.8 ± 5.8**	119.0 ± 3.6	105.8 ± 6.2	112.8 ± 2.3
	70	81.3 ± 3.8***	98.3 ± 6.4**	122.2 ± 2.7	109.5 ± 7.5	117.5 ± 2.4
	77	83.0 ± 4.0***	104.2 ± 6.7*	130.5 ± 1.6	114.3 ± 8.9	120.4 ± 2.6
Mean gain days 35-77		9.3 ± 4.7***	27.8 ± 4.2*	52.2 ± 1.3	40.8 ± 8.0	42.5 ± 2.5

Table 2.16 continued

	Age in days	Diet 1	2	3	4	Stock
MALES	28(weaned)	62.3 ± 2.3	62.3 ± 2.4	62.3 ± 3.3	62.3 ± 2.6	64.7 ± 1.7
	35(given experimental diet)	76.7 ± 2.8	78.1 ± 2.5	83.8 ± 2.4	78.5 ± 2.0	79.3 ± 1.4
	42	83.7 ± 3.1 ǂǂǂ	98.2 ± 2.1 * ●	99.2 ± 2.2 * ●	89.7 ± 2.2	90.4 ± 1.6
	49	84.5 ± 5.2 ** ● +++ ǂǂǂ	106.7 ± 2.3 * +	110.8 ± 2.4 ** ●	99.2 ± 2.8	98.6 ± 1.6
	56	85.5 ± 6.5 *** ● ǂǂǂ	107.7 ± 1.3 +	116.8 ± 3.2 * ●	106.5 ± 3.1	105.5 ± 2.0
	63	86.5 ± 7.1 *** ● ǂǂǂ	114.7 ± 2.7	121.0 ± 3.4 * ●	110.2 ± 2.9	109.5 ± 2.5
	70	88.3 ± 7.2 ** ● ǂǂǂ	117.2 ± 2.9	123.0 ± 4.0 ●	111.0 ± 2.8	112.7 ± 2.9
	77	90.0 ± 7.6 ** ● ǂǂǂ	120.8 ± 2.8 ●	123.2 ± 4.3 ●	109.8 ± 3.3	116.1 ± 3.3
Mean gain days 35-77		13.3 ± 6.5 ** ● ǂǂǂ	42.5 ± 1.6 ●	39.5 ± 2.5	31.5 ± 3.2	37.5 ± 3.4

Significant difference: from stock group *P < 0.05 **P < 0.01 ***P < 0.001

from group 4 ●P < 0.05 ●●P < 0.01 ●●●P < 0.001

from group 3 +P < 0.05 ++P < 0.01 +++P < 0.001

from group 2 ǂP < 0.05 ǂǂP < 0.01 ǂǂǂP < 0.001

Figure 2.7 : Mean weight gains from 35 days of age of female hamsters given diets containing soya protein (sample 1) (6 animals / group) or stock diet (24 animals).

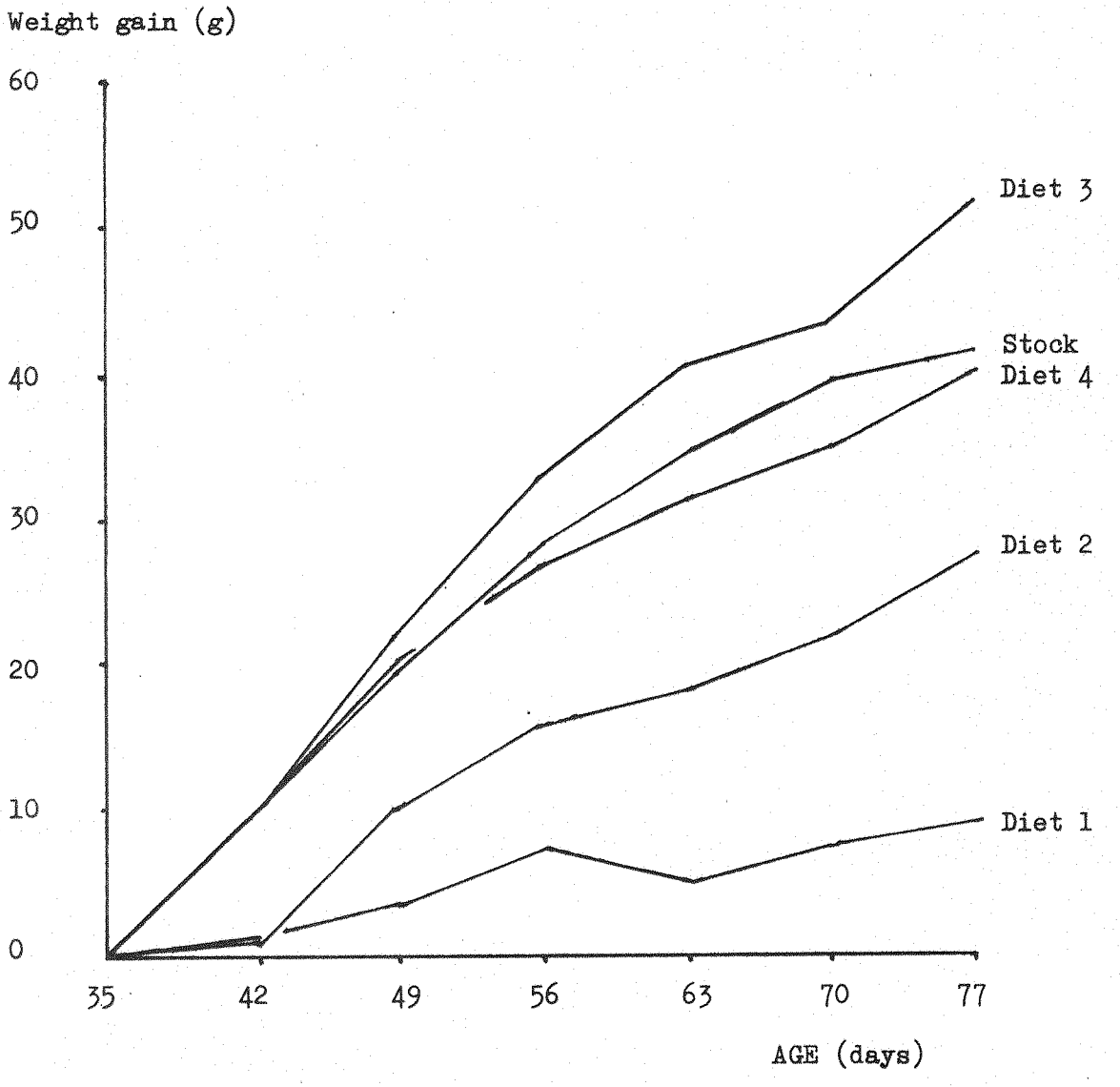


Figure 2.8 : Mean weight gains from 35 days of age of male hamsters given diets containing soya protein (sample 1) (6animals / group) or stock diet (24 animals).

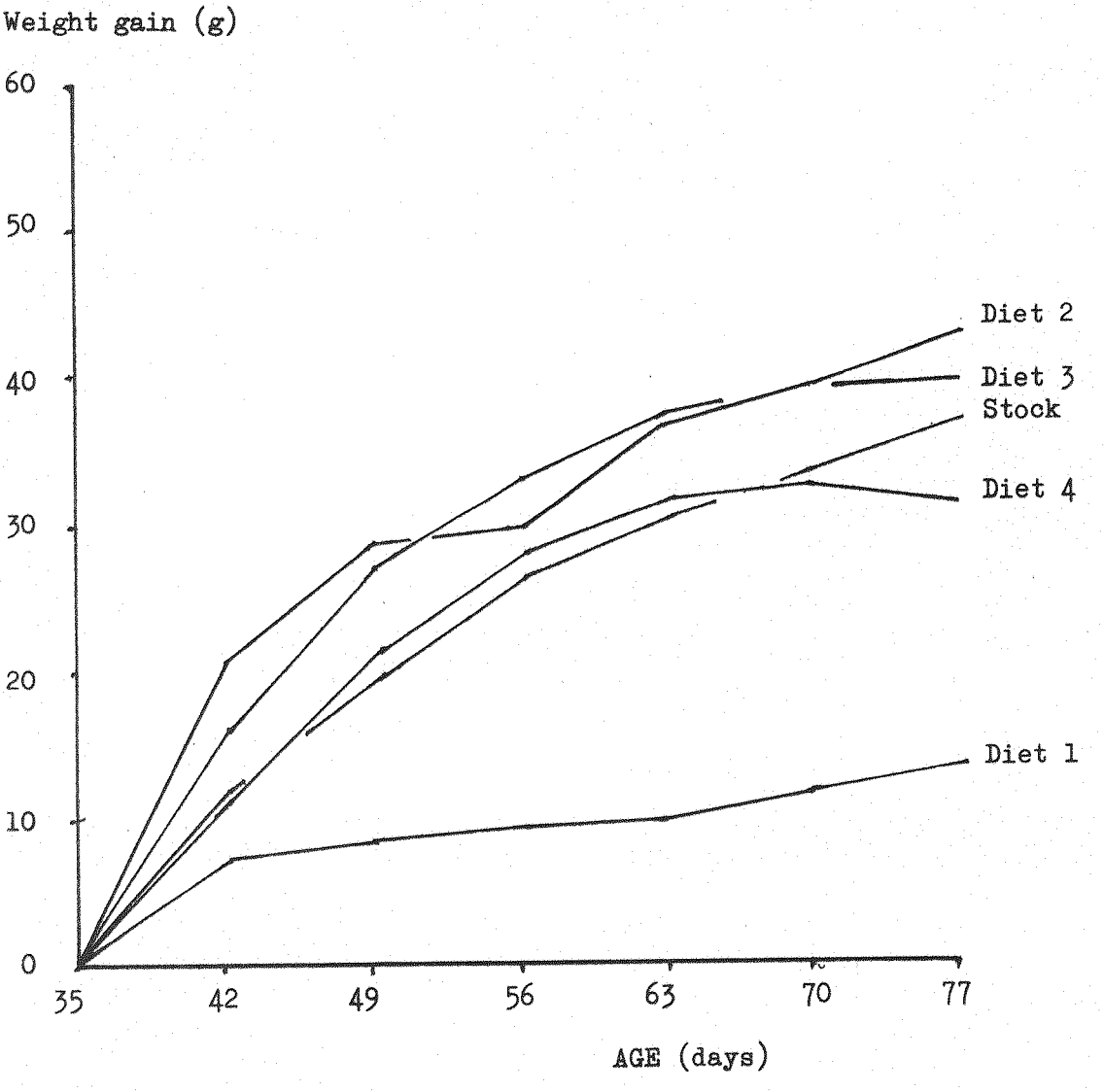


Table 2.17a Mean food intake (g ± SEM) during growth of female and male hamsters given diets containing soya protein (sample 1) (6 animals/group) or stock diet (4 groups of 6 animals/group for each sex)

	Week	Diet				
		1	2	3	4	Stock
FEMALES	5(Stock diet)	6.7	7.0	7.6	6.8	5.9 ± 0.8
	6	4.7	5.5	6.0	5.9	5.9 ± 1.0
	7	5.6	5.0	6.0	5.1	5.4 ± 0.6
	8	6.4	5.9	5.7	5.6	6.0 ± 1.2
	9	4.1	5.6	5.3	5.2	7.2 ± 0.6
	10	5.3	5.6	5.2	5.1	6.2 ± 1.0
	11	6.7	6.5	6.7	5.9	7.4 ± 1.0
Mean of weeks 6-11		5.5 ± 0.4	5.7 ± 0.4	5.8 ± 0.4	5.5 ± 0.3	6.4 ± 0.3
Expressed as g/100g body weight/day Mean of weeks 6-11		7.5 ± 0.6	6.8 ± 0.2	5.9 ± 0.5	6.2 ± 0.5	6.2 ± 0.3

Table 2.17a continued

	Weeks	Diet				
		1	2	3	4	Stock
	5(stock diet)	8.2	8.0	8.3	7.8	7.7 ± 0.7
	6	7.0	7.6	8.0	7.0	8.0 ± 0.5
	7	5.8	3.6	6.0	5.3	5.5 ± 0.7
MALES	8	4.2	5.3	5.3	5.3	7.2 ± 0.6
	9	4.7	5.1	5.0	4.9	7.1 ± 0.6
	10	2.5	6.1	5.0	4.3	6.2 ± 1.1
	11	2.9	3.8	5.5	6.5	6.4 ± 0.9
Mean of weeks 6-11		4.5 ± 0.8*	5.3 ± 0.7	5.8 ± 0.6	5.6 ± 0.5	6.7 ± 0.5
Expressed as g/100g body weight/day						
Mean of weeks 6-11		5.7 ± 0.9	5.2 ± 0.8	5.6 ± 0.8	5.9 ± 0.6	6.7 ± 0.6

Significant differences from stock group *$P < 0.05$

Table 2.17b Metabolisable energy intake (kJ/kg$^{0.75}$/day) during growth of female and male hamsters given diets containing soya protein (sample 1) (6 animals/group) or stock diet (4 groups of 6 animals for each sex).

	Week	Diet 1	2	3	4	Stock
	5(Stock diet)	622.0	638.3	690.1	630.8	600.3 ± 81.1
	6	552.6	619.5	640.4	573.5	512.9 ± 71.1
	7	649.6	529.6	582.7	452.7	412.6 ± 45.6
FEMALES	8	712.7	586.9	510.4	466.1	448.5 ± 86.9
	9	450.6	543.0	441.4	412.2	507.5 ± 25.1
	10	590.6	530.9	418.8	393.3	422.2 ± 61.9
	11	732.3	596.1	524.6	443.1	496.2 ± 21.3
Mean of weeks 6-11		614.9 ± 43.1*	566.8 ± 15.6**	519.6 ± 34.3	456.9 ± 25.9	466.5 ± 18.2

Table 2.17b continued

	Week	Diet 1	2	3	4	Stock
	5(Stock diet)	744.0	713.9	725.7	697.2	738.6 ± 85.7
	6	776.6	772.1	796.3	650.0	650.0 ± 46.0
	7	621.2	323.1	539.2	656.0	437.2 ± 58.5
MALES	8	448.9	466.5	446.0	428.0	536.3 ± 27.2
	9	494.9	429.7	408.8	378.3	507.4 ± 33.4
	10	257.5	500.4	400.9	324.8	427.6 ± 60.2
	11	302.2	305.1	434.3	493.2	543.4 ± 69.4
Mean of weeks 6-11		483.6 ± 79.8	466.1 ± 69-0	504.1 ± 61.9	455.2 ± 45.6	517.0 ± 33.3

Significant differences from stock group: *$P < 0.05$ **$P < 0.01$

Groups supplied with diets containing Soya Bean Protein (sample 2)

The mean weights of animals given diets containing soya protein (sample 2) as the main source of protein are shown in Table 2.18 and their weight gains from the time they were given experimental diet compared with those of the stock group in Figures 2.9 (females)and 2.10 (males).

The only male group which did not achieve a growth rate comparable to that of the stock control group was the Diet 1 (5% reference protein) group which was significantly lighter from day 21 onwards and lighter than all other experimental groups from day 28 onwards. Although the metabolisable energy intake per metabolic mass of the 4 experimental groups were all similar to the stock group (Table 2.19b) the overall food intake in g was reduced in the group given Diet 1 compared to the stock group intake even when body size was taken into account (i.e. when expressed per 100g body weight) (Table 2.19a). However, the overall intake for this group per 100g body weight was not significantly lower than the intakes of the other three experimental groups.

Within the female experimental animals those offered Diet 1 also had a very poor growth response compared to the stock animals (Table 2.18) and this occurred with no difference in overall food intake per 100g body weight (Table 2.19a) or energy intake (Table 2.19b). The other three groups showed a difference in response from the male groups in that although for the first two weeks on diet the growth rates were comparable to those of the stock group, the weight gains were not maintained. The group supplied with 10% reference protein (Diet 2) lost weight from that time onwards and groups given diets containing higher levels of protein reached plateaux after 3 weeks (Diet 3) or 4 weeks (Diet 4) (Fig.2.10). The result was that at theend of the trial the weights of the animals on Diet 2 and 3 were reduced to 78% and 88%

Table 2.18 Mean body weights (g ± SEM) at different ages of female and male hamsters given diets containing soya protein (sample 2) (6 animals/group) or stock diet (24 animals/group).

	Age in days	Diet 1	2	3	4	Stock
FEMALES	28(Weaned)	67.2 ± 1.9	67.4 ± 1.7	67.0 ± 1.8	67.1 ± 1.5	63.8 ± 1.9
	35(Given experimental diet)	82.7 ± 1.5	81.5 ± 0.8	80.5 ± 2.0	83.3 ± 1.9	77.8 ± 1.7
	42	86.7 ± 2.0●‡‡	97.3 ± 1.3*	92.8 ± 2.4	94.8 ± 1.7	87.5 ± 1.9
	49	87.2 ± 2.3*●●‡‡‡	104.7 ± 2.1	102.0 ± 2.3	105.2 ± 1.6	97.3 ± 2.1
	56	87.8 ± 2.0***●●‡‡	102.3 ± 8.0	106.0 ± 3.5	109.3 ± 1.6	105.3 ± 1.9
	63	85.2 ± 1.7***●●‡‡	100.0 ± 9.1	105.1 ± 5.9	114.3 ± 3.7	112.8 ± 2.3
	70	84.6 ± 1.7***●●‡‡	95.7 ± 8.1**●	104.8 ± 4.9*	114.0 ± 5.1	117.5 ± 2.4
	77	86.0 ± 3.2***●●‡‡	93.8 ± 7.9***	105.0 ± 3.8*	113.8 ± 5.7	120.4 ± 2.6
Mean gain days 35-77		3.3 ± 4.1***●●‡‡	12.3 ± 7.3***	24.5 ± 4.3**	30.3 ± 5.5*	42.5 ± 2.5

Table 2.18 continued

	Age in days	Diet 1	2	3	4	Stock
	28(Weaned)	70.0 ± 2.6	70.0 ± 2.9	70.0 ± 2.5	70.1 ± 2.8	64.7 ± 1.7
	35(Given experimental diet)	79.4 ± 3.0	79.6 ± 2.9	78.3 ± 2.8	82.9 ± 1.9	79.3 ± 1.4
	42	86.8 ± 3.5	95.7 ± 2.9	91.8 ± 2.7	95.0 ± 2.3	90.4 ± 1.6
	49	89.8 ± 3.4 *●‡	104.7 ± 3.8	99.0 ± 2.7	103.7 ± 2.4	98.6 ± 1.6
MALES	56	92.8 ± 4.4 **●‡‡	113.5 ± 4.3	104.8 ± 2.6	111.0 ± 2.5	105.5 ± 2.0
	63	90.9 ± 4.8 **●●‡‡‡	118.0 ± 5.0	109.8 ± 2.3	115.8 ± 2.5	109.5 ± 2.5
	70	91.2 ± 5.3 **●●‡‡‡	122.2 ± 5.7	114.2 ± 1.8	119.1 ± 2.5	112.7 ± 2.9
	77	89.0 ± 6.0	122.5 ± 6.2	114.8 ± 2.1o	123.8 ± 2.6	116.1 ± 3.3
Mean gain days 35-77		9.5 ± 3.3 ***●●●‡‡‡‡	42.8 ± 4.8	36.5 ± 3.2	41.3 ± 2.6	37.5 ± 3.4

Significant differences from stock groups *P < 0.05 **P < 0.01 ***P < 0.001

from group 4 ●P < 0.05 ●●P < 0.01 ●●●P < 0.001

from group 3 +P < 0.05 ++P < 0.01 +++P < 0.001

from group 2 ‡P < 0.05 ‡‡P < 0.01 ‡‡‡P < 0.001

Figure 2.9 : Mean weight gains from 35 days of age of female hamsters given diets containing soya protein (sample 2) (6 animals / group) or stock diet (24 animals).

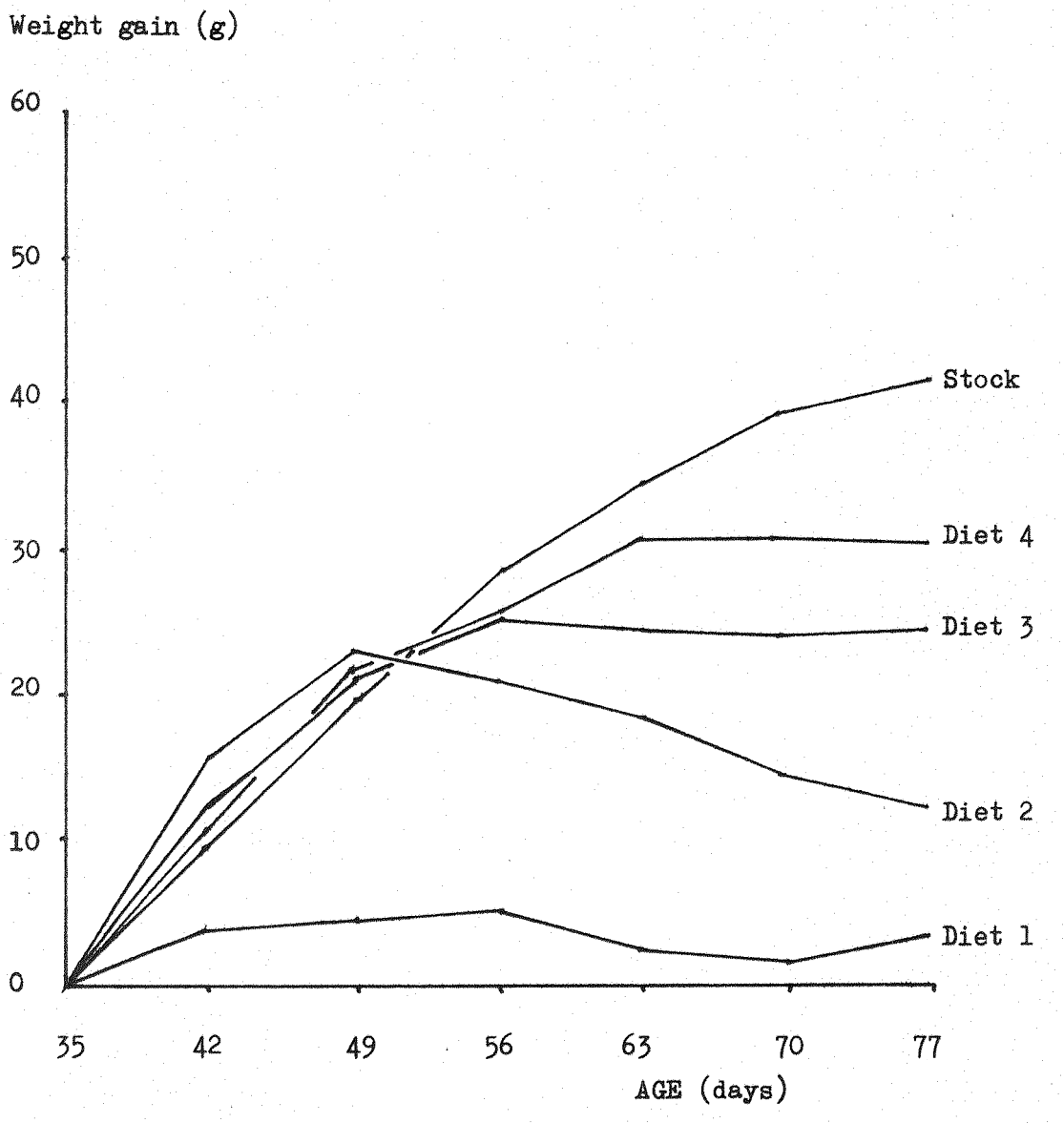


Figure 2.10 : Mean weight gains from 35 days of age of male hamsters given diets containing soya protein (sample 2) (6 animals / group) or stock diet (24 animals).

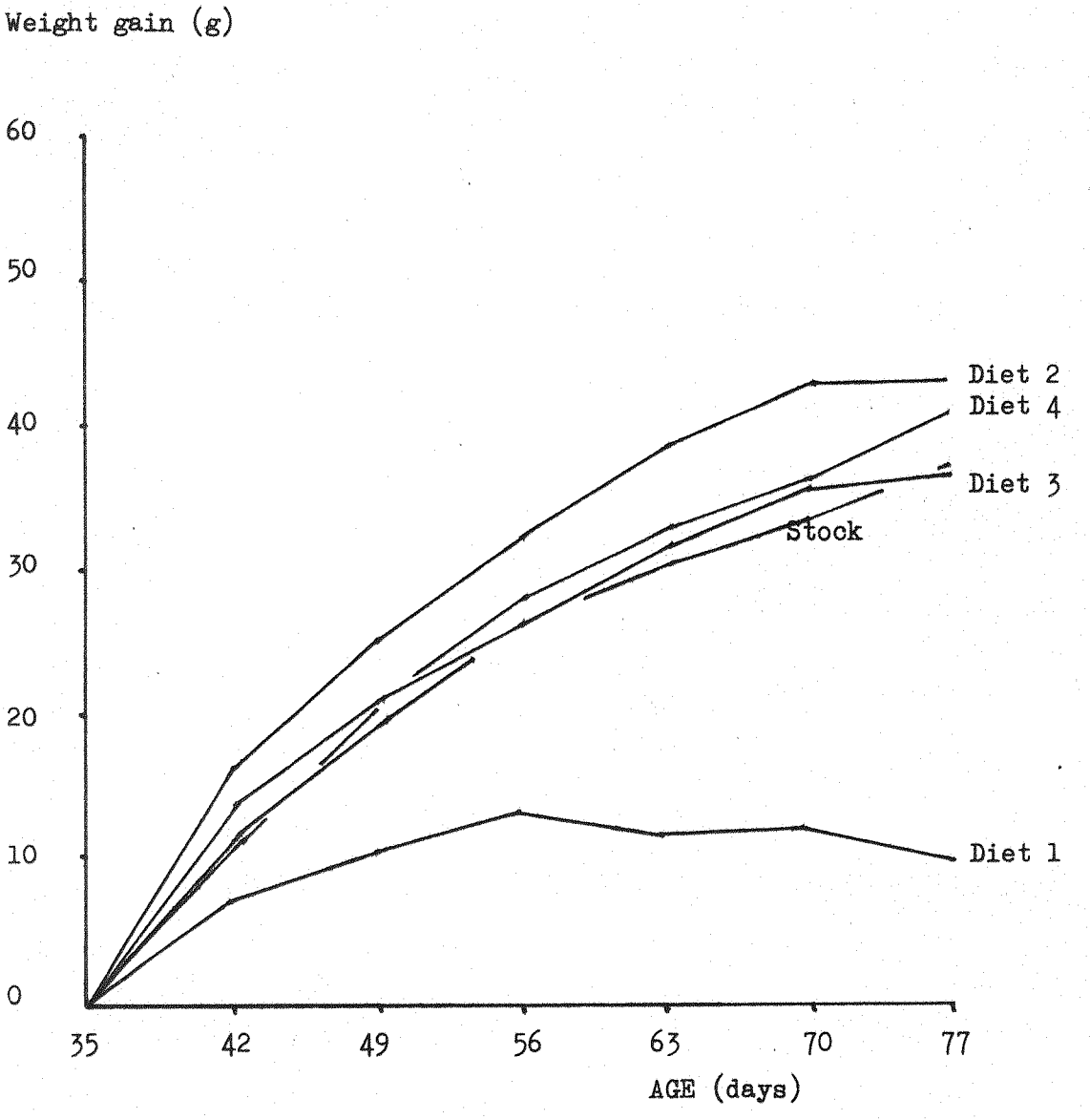


Table 2.19a Mean food intake (g ± SEM) during growth of female and male hamsters given diets containing soya protein (sample 2) (6 animals/group) or stock diet (4 groups of 6 animals/group for each sex).

	Week	Diet				
		1	2	3	4	Stock
FEMALES	5(Stock diet)	5.2	7.1	4.0	5.2	5.9 ± 0.8
	6	5.3	8.9	5.7	6.4	5.9 ± 1.0
	7	5.9	5.9	5.4	6.4	5.4 ± 0.6
	8	5.9	5.7	6.1	5.9	6.0 ± 1.2
	9	4.1	3.1	5.0	5.5	7.2 ± 0.6
	10	4.1	3.7	4.6	5.4	6.2 ± 1.0
	11	4.6	2.9	5.1	5.3	7.4 ± 1.0
Mean of weeks 6-11		5.0 ± 04*	5.0 ± 1.0	5.3 ± 0.4	5.8 ± 0.4	6.4 ± 0.3
Expressed as g/100g body weight/day Mean of weeks 6-11		5.8 ± 0.4	5.3 ± 1.2	5.4 ± 0.4	5.5 ± 0.5	6.2 ± 0.3

Table 2.19a continued

	Week	Diet 1	2	3	4	Stock
	5(Stock diet)	6.1	6.4	6.4	6.4	7.7 ± 0.7
	6	5.0	7.7	6.6	6.3	8.0 ± 0.5
	7	5.6	6.7	6.6	6.9	5.5 ± 0.7
MALES	8	5.5	6.3	6.2	6.8	7.2 ± 0.6
	9	3.9	5.9	5.4	5.9	7.1 ± 0.6
	10	4.0	5.4	5.7	5.7	6.2 ± 1.1
	11	4.2	4.9	5.7	6.4	6.4 ± 0.9
Mean of weeks 6-11		4.7 ± 0.4*	6.2 ± 0.5	6.0 ± 0.2	6.3 ± 0.4	6.7 ± 0.5
Expressed as g/100 g body weight/day						
Mean of weeks 6-11		5.0 ± 0.4*	5.6 ± 0.6	5.7 ± 0.5	5.7 ± 0.4	6.7 ± 0.6

Siginifcant differences from stock group *$P < 0.05$

Table 2.19b Metabolisable energy intake ($kJ/kg^{0.75}$/day) during growth of female and male hamsters given diets containing soya protein (sample 2) (6 animals /group) or stock diet (4 groups of 6 animals for each sex)

	Week	Diet				
		1	2	3	4	Stock
FEMALES	5(Stock diet)	440.6	606.9	345.3	448.1	600.3 ± 81.1
	6	580.2	910.0	594.0	640.8	512.9 ± 71.1
	7	626.2	555.1	517.9	587.7	412.6 ± 45.6
	8	623.7	520.0	559.3	510.4	448.5 ± 86.9
	9	435.1	284.2	455.6	464.8	507.5 ± 25.1
	10	441.0	351.1	417.2	451.0	422.2 ± 61.9
	11	497.8	281.3	464.8	437.2	496.2 ± 21.3
Mean of weeks 6-11		516.2 ± 34.7	483.6 ± 97.8	501.6 ± 27.6	515.4 ± 33.4	466.5 ± 18.2

Table 2.19b continued

	Week	Diet 1	2	3	4	Stock
	5(Stock diet)	520.8	549.3	552.6	540.1	738.6 ± 85.7
	6	558.9	804.2	697.6	631.2	650.0 ± 46.0
	7	591.1	624.5	640.4	640.0	437.2 ± 58.5
MALES	8	566.4	554.7	574.8	588.1	536.3 ± 27.2
	9	398.4	501.2	481.5	489.9	507.4 ± 33.4
	10	408.4	442.7	493.2	462.3	427.6 ± 60.2
	11	439.7	391.7	482.0	507.9	543.4 ± 69.4
Mean of weeks 6-11		493.7 ± 3.59	553.0 ± 60.2	561.8 ± 37.2	553.4 ± 31.4	517.0 ± 33.3

respectively of the stock control group weights. Food and energy intakes of female groups on Diets 2, 3 and 4 were all similar to the stock group intakes (Table 2.19a and b).

Summary of all dietary groups

Tables 2.20a and b are a summary of the weight gain and nutrient intake data of all dietary groups. Table 2.20a shows data for the whole period of the trial, from 35 to 77 days of age. Some of the groups, for example those given casein Diets 1 and 2 and fishmeal Diet 1, have a small positive or even a negative weight gain overall. This perhaps misrepresents the results since there were bigger weight gains in these groups indicating that the diets did support growth but the weight losses which followed had a cancelling effect. Therefore in Table 2.20b the data summarising the first two weeks of the trial has been presented. In almost all cases these data represent the period of most rapid growth and they should, therefore, provide the best indication of the relative value of the different diets.

Therefore, having observed differences in growth rates between dietary groups which apparently were not due to different mean energy intake, an experiment was set up in which the animals were individually housed to investigate the relationship between nutrient intake and growth more fully.

Results of the Second Growth Trial

These data are presented in Tables 2.21a - e and 2.22a - d. The mean weight gains of all groups are shown in Figure 2.11. Within the mould dietary groups (Table 2.21c) although the mean weight gain of Diet 1 group is only 10g whereas it is about 20g in the groups given the higher levels of protein, this is not a significant difference. With all the other protein sources the Diet 1 animals gained significantly less weight over the 4 week trial than those on Diet 3 and in all cases except the casein group, Diet 1 animals

Table 2.20a Summary of data from growth trial from d 35 to d 77. Values are means for the whole period. (F=female, M=male)

	Diet 1		2		3		4	
	F	M	F	M	F	M	F	M
				Weight gain (g)				
Casein	1.2	-.05	-1.3	-1.6	35.7	38.8	26.7	29.5
Fishmeal	-10.5	-4.2	5.0	26.0	50.8	28.2	2.0	29.3
Mould	7.3	10.5	42.1	52.0	57.4	44.0	54.1	41.7
Soya protein (1)	9.3	13.3	27.8	42.8	52.2	39.5	40.8	31.5
Soya protein (2)	3.3	9.5	12.3	42.8	24.5	36.5	30.3	40.9
Stock	42.5	37.5						
				Metabolisable Energy intake (kJ)				
Casein	564	585	513	564	611	651	571	631
Fishmeal	431	511	430	567	539	521	390	507
Mould	591	601	670	595	571	537	520	528
Soya protein (1)	551	451	557	518	539	539	482	491
Soya protein (2)	513	482	501	561	531	601	567	616
Stock	470	492						

Table 2.20a continued

				Protein intake (g)				
Casein	1.85	1.92	3.33	3.66	6.01	6.42	7.49	8.28
Fishmeal	1.47	1.74	2.93	3.86	5.74	5.54	5.65	7.36
Mould	1.98	2.02	4.67	4.15	6.47	6.09	7.80	7.92
Soya protein (1)	1.88	1.54	3.80	3.53	5.78	5.78	7.23	7.36
Soya protein (2)	2.58	2.42	5.67	6.35	8.97	10.15	13.05	14.18
Stock	7.72	8.08						
				Reference protein intake (g)				
Casein	1.72	1.78	3.09	3.40	5.57	5.95	7.11	7.86
Fishmeal	1.34	1.59	2.67	3.51	5.19	5.01	5.08	6.61
Mould	1.73	1.76	4.23	3.76	5.95	5.59	7.17	7.29
Soya protein (1)	1.62	1.32	3.35	3.12	5.12	5.12	6.50	6.62
Soya protein (2)	1.47	1.38	3.12	3.49	4.93	5.58	7.17	7.79
Stock	7.32	7.66						

Table 2.20b Summary of data from growth trial from d35 to d49. Values are means for the 2 week period. (F = female, M = male)

	Diet 1		2		3		4	
	F	M	F	M	F	M	F	M
				Weight gain (g)				
Casein	4.9	10.3	16.9	18.4	26.0	25.8	20.8	20.8
Fishmeal	3.3	4.5	11.1	22.4	20.1	22.5	19.0	25.1
Mould	-3.5	10.6	14.1	21.2	23.5	19.7	25.1	19.8
Soya protein (1)	3.5	7.8	10.4	28.6	22.4	27.0	20.3	20.7
Soya protein (2)	4.5	10.4	23.2	25.1	21.5	20.7	21.9	20.8
Stock	19.5	19.3						
				Metabolisable Energy Intake (kJ)				
Casein	227	260	219	243	214	261	212	244
Fishmeal	154	279	176	241	172	209	162	223
Mould	189	219	219	237	210	200	180	185
Soya protein (1)	172	214	171	183	186	217	161	180
Soya protein (2)	192	181	247	240	185	220	209	215
Stock	138	165						

2.20b continued

		Protein intake (g)						
Casein	0.74	0.85	1.43	1.59	2.11	2 57	2.78	3.20
Fishmeal	0.52	0.95	1.20	1.64	1.83	2.23	2.34	3.24
Mould	0.63	0.73	1.52	1.65	2.38	2.27	2.71	2.77
Soya protein (1)	0.59	0.73	1.17	1.24	1.99	2.32	2.41	2.69
Soya protein (2)	0.96	0.91	2.80	2.72	3.13	3.72	4.80	4.95
Stock	2.27	2.71						
		Reference protein intake (g)						
Casein	0.69	0.79	1.33	1.48	1.96	2.39	2.64	3.04
Fishmeal	0.48	0.87	1.09	1.49	1.65	2.01	2.11	2.92
Mould	0.55	0.64	1.38	1.50	2.21	2.09	2.49	2.55
Soya protein (1)	0.50	0.63	1.03	1.10	1.76	2.06	2.17	2.42
Soya protein (2)	0.55	0.52	1.54	1.50	1.72	2.05	2.64	2.72
Stock	2.15	2.57						

gained significantly less weight than those on Diet 2 which had similar weight gains to the respective Diet 3 groups. When casein was the main protein source animals on Diets 1 and 2 had statistically similar weight gains, both being less than those of the Diet 3 group (Table 2 .21a).

Energy intakes for the 4 week period were similar for Diets 1, 2 and 3 when the major protein source was casein (9MJ), fishmeal (8.5MJ) or soya protein (sample 1) (9.5MJ). There was a difference in energy intake between animals on Diets 1 (8MJ)and 3 (10MJ) when the major protein source was soya protein (sample 2) ($P < 0.05$). This probably results from an increase in the Diet 3 group intakes rather than a decrease in the Diet 1 group if the values are looked at relative to those of all 15 groups. With mould as the main protein source the animals on the highest level of protein (Diet 3) showed a reduced energy intake over the four week period (6.5MJ). This was the lowest energy intake of all groups and was significantly less ($P \ll 0.01$) than animals given the other two mould diets which had mean intakes of 8MJ/ hamster . Therefore the differences in weight gain described above are not a result of differences in food or energy intake but rather a reflection of differences in the efficiency of utilization of the food for growth. Increasing the reference protein level from 10% to 15% did not promote significantly greater weight gains over the 4 week trial in the non-casein groups.

Nutrient efficiency ratios of all the groups for the 4 week period are shown in Table 2.21a,b,c and d. Food conversion (g weight gain/g food intake) (Table 2.22a) in the group given the lowest level of fishmeal was poor compared to the other Diet 1 groups being significantly less than that of the two soya protein groups. The larger increase in food conversion efficiency ratios occurred when the reference protein level was increased

from 5% to 10% except when casein was the major protein source when the more substantial increase accompanied the increase in reference protein level from 10% to 15%. The increase from the Diet 1 ratio in the mould groups was not significant ($P > 0.05 < 0.1$) but the trend was similar to the other non-casein groups.

Utilisation of energy in terms of weight gain is shown in Table 2.22b. The pattern of results is similar to those of the weight gain/food intake with the fish-meal group having the lowest ratio in the Diet 1 groups, the casein at the Diet 2 level of protein and all groups being similar with 15% reference protein in the diet. Because the diets were approximately isocaloric it was to be expected that the energy and food utilisation ratios would show similar trends.

Protein efficiency ratio (g weight gain/g protein intake) is shown in Table 2.21c. Due to the relatively poor growth promoted by the casein Diet 2, the protein efficiency ratios for all of the casein groups again do not fit the general pattern of results observed with the other protein sources. With all the other protein sources there was a decrease in protein efficiency ratio when the level of reference protein in the diet was increased from 10% to 15% of the dry weight of the diet. This is only statistically significant in the group given diets containing soya protein (sample 1). With fishmeal as the major protein source the protein efficiency ratio of Diet 1 was only 55% that of Diet 2 due to poor growth response of the Diet 1 animals. With either mould or soya protein (sample 1) as the main protein source the protein efficiency ratios for Diets 1 and 2 were similar. There was a decrease of 20% in the ratio when the reference protein level was increased from 5% to 10% and the major protein source was soya protein (sample 2).

Table 2.21a Feed intake and weight gain of male hamsters given diets containing casein for 4 weeks

	Diet 1	Diet 2	Diet 3
No. of animals	7	8	7
Initial weight (g)	68.7 ± 3.8	65.4 ± 2.6	69.9 ± 2.6
Total gain (g)	9.0 ± 2.9***	13.9 ± 2.9**	26.1 ± 2.6
Total food intake (g)	520.8 ± 28	506.8 ± 11.2	565.6 ± 36.4
Total metabolisable energy intake (MJ)	8.91 ± 0.48	8.67 ± 0.19	9.45 ± 0.61
Total protein intake (g)	30.2 ± 1.6***●●●	56.8 ± 1.3***	93.3 ± 6.0
Reference protein intake (g)	27.1 ± 1.5***●●●	52.7 ± 1.2***	86.5 ± 5.6

Table 2.21b Feed intake and weight gain of male hamsters given diets containing fishmeal for 4 weeks

	Diet 1	Diet 2	Diet 3
No. of animals	7	7	8
Initial weight (g)	68.1 ± 3.6	69.6 ± 4.2	66.9 ± 4.4
Total gain (g)	5.9 ± 2.4**●	20.1 ± 5.7	25.4 ± 4.8
Total food intake (g)	526.4 ± 64.4	506.8 ± 36.4	551.6 ± 39.2
Total metabolisable energy intake (MJ)	8.79 ± 1.08	8.26 ± 0.59	8.55 ± 0.61
Total protein intake (g)	30.0 ± 3.7***●●●	56.3 ± 4.0***	91.0 ± 6.5
Reference protein intake (g)	27.4 ± 3.4***●●●	51.2 ± 3.7***	82.2 ± 5.8

Table 2.21c Feed intake and weight gain of male hamsters given diets containing mould for 4 weeks

	Diet 1	Diet 2	Diet 3
No. of animals.	7	8	8
Initial weight (g)	66.1 ± 4.2	67.6 ± 3.5	65.5 ± 2.7
Total gain (g)	10.3 ± 4.6(●)	21.4 ± 3.3	19.6 ± 3.6
Total food intake (g)	481.6 ± 22.4	509.6 ± 25.2	459.0 ± 14.0
Total metabolisable energy intake (MJ)	8.04 ± 0.37**	7.90 ± 0.39**	6.52 ± 0.20
Total protein intake(g)	27.0 ± 1.3***●●●	55.0 ± 2.7***	73.9 ± 2.3
Reference protein intake (g)	23.6 ± 1.1***●●●	49.9 ± 2.5***	68.0 ± 2.1

Table 2.21d Feed intake and weight gain of male hamsters given diets containing soya protein (sample 1) for 4 weeks.

	Diet 1/7	2/8	3/7
No. of animals			
Initial weight (g)	66.6 ± 2.9	66.0 ± 3.1	70.9 ± 2.3
Total gain (g)	16.6 ± 2.0**●●	30.1 ± 3.0	26.1 ± 1.1
Total food intake (g)	590.8 ± 39.2	610.4 ± 19.6	596.4 ± 25.2
Total metabolisable energy intake (MJ)	9.87 ± 0.65	9.95 ± 0.32	9.24 ± 0.39
Total protein intake (g)	33.7 ± 2.2***●●●	67.8 ± 2.2***	98.4 ± 4.2
Reference protein intake (g)	28.9 ± 1.9***●●●	59.8 ± 1.9***	87.7 ± 3.7

Table 2.21e Feed intake and weight gain of male hamsters given diets containing soya protein (sample 2) for 4 weeks.

	Diet 1/8	2/8	3/8
No. of animals			
Initial weight (g)	67.2 ± 3.1	66.6 ± 2.8	65.4 ± 3.6
Total gain (g)	15.3 ± 3.1**●●	28.5 ± 2.7	36.6 ± 4.5
Total food intake (g)	529.2 ± 22.4*	585.2 ± 14.0	610.4 ± 19.6
Total metabolisable energy intake (MJ)	9.05 ± 0.38*	9.77 ± 0.23	10.19 ± 0.33
Total protein intake (g)	45.5 ± 1.93***●●●	110.6 ± 2.7***	172.1 ± 5.1
Reference protein intake (g)	25.9 ± 1.1***●●●	60.9 ± 1.5***	94.6 ± 3.

Significant differences from group 3: *P < 0.05 **P < 0.01 ***P < 0.001

Significant differences from group 2: (●)P > 0.05 < 0.1 ●P < 0.05 ●●P < 0.01 ●●●P < 0.001

Figure 2.11 : Weight gain from 35 days of age of male hamsters given experimental diets for 4 weeks and housed individually.

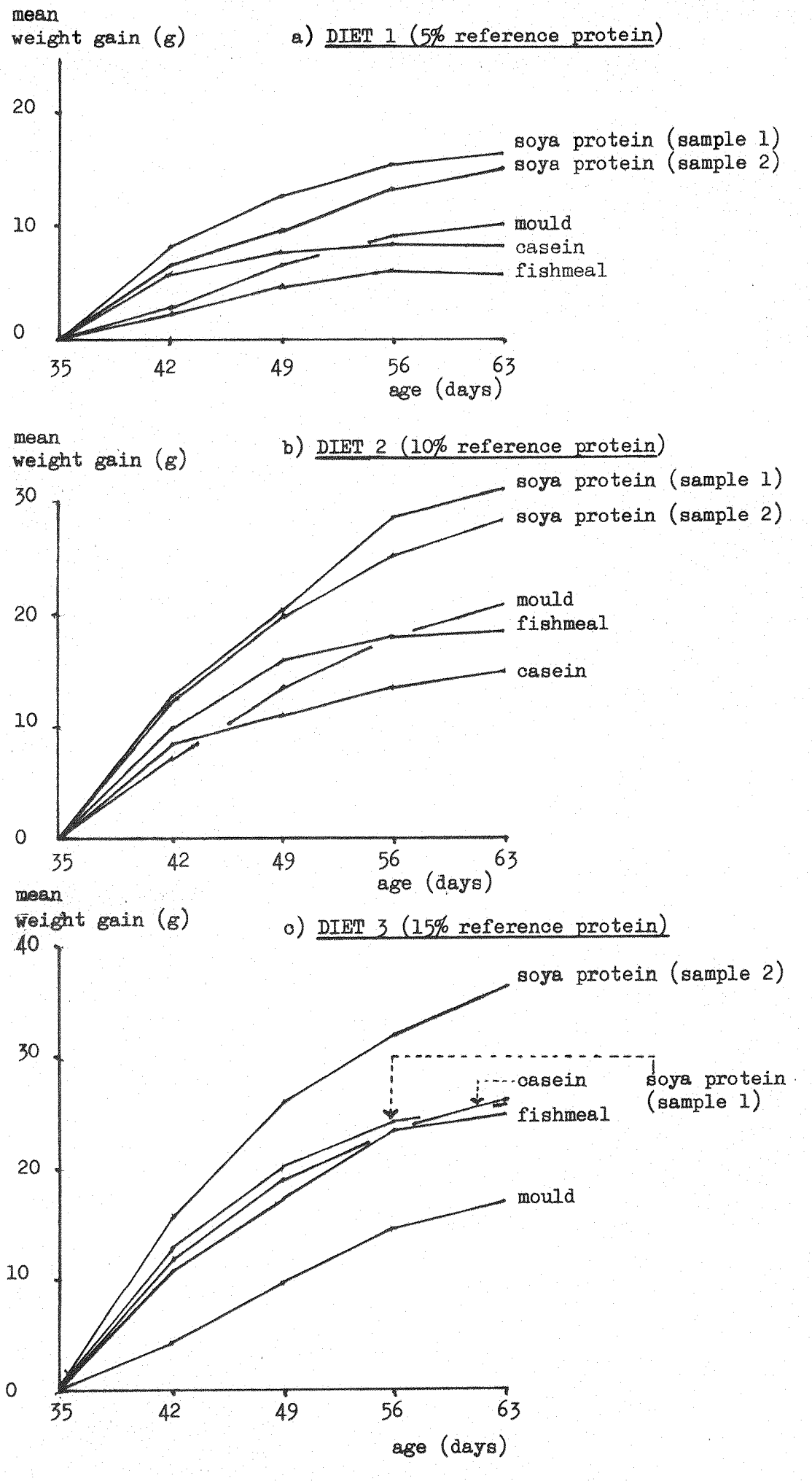


Table 2.22 Feed efficiency ratios of male hamsters (7 or 8 per group) after 4 weeks on experimental diet.

a) Food Conversion Efficiency Ratio :
Weight gain(g) x 100/food intake(g)

Main protein source	Diet 1	Diet 2	Diet 3
Casein	1.7 ± 0.6**AB	2.7 ± 0.6*A	4.6 ± 0.5
Fishmeal	1.1 ± 0.5**●A	4.0 ± 1.2 AB	4.6 ± 0.9
Mould	2.1 ± 0.9(*)● AB	4.2 ± 0.7 AB	4.3 ± 0.8
Soya protein (sample 1)	2.8 ± 0.4**●●B	4.9 ± 0.5 B	4.4 ± 0.3
Soya protein (sample 2)	2.9 ± 0.6**● B	4.9 ± 0.5 B	6.0 ± 0.8

b) Energy Conversion Efficiency Ratio :
Weight gain(g) / metabolisable energy intake(MJ)

Main protein source	Diet 1	Diet 2	Diet 3
Casein	1.01 ± 0.33** AB	1.60 ± 0.34*A	2.76 ± 0.33
Fishmeal	0.67 ± 0.29**● A	2.43 ± 0.71AB	2.97 ± 0.60
Mould	1.28 ± 0.58(*)● AB	2.71 ± 0.44AB	3.01 ± 0.59
Soya protein (sample 1)	1.68 ± 0.23**●● B	3.03 ± 0.32 B	2.82 ± 0.17
Soya protein (sample 2)	1.69 ± 0.35**● B	2.92 ± 0.28 B	3.59 ± 0.46

c) Protein Efficiency Ratio x 10:
Weight gain(g) x 10/protein intake(g)

Main protein source	Diet 1	Diet 2	Diet 3
Casein	2.98 ± 0.97 AB	2.45 ± 0.51 A	2.80 ± 0.33
Fishmeal	1.97 ± 0.84 A	3.57 ± 1.04AB	2.79 ± 0.56
Mould	3.81 ± 1.71 AB	3.87 ± 0.63AB	2.65 ± 0.52
Soya protein (sample 1)	4.93 ± 0.67**B	4.44 ± 0.47**B	2.65 ± 0.16
Soya protein (sample 2)	3.36 ± 0.70 AB	2.58 ± 0.25 A	2.13 ± 0.27

Table 2. 22 continued

d) Weight gain (g) x 10 / reference protein intake (g)

Main protein source	Diet 1	Diet 2	Diet 3
Casein	3.32 ± 1.08 AB	2.64 ± 0.55 A	3.02 ± 0.36
Fishmeal	2.15 ± 0.92 A	3.93 ± 1.15 AB	3.09 ± 0.62
Mould	4.36 ± 1.96 AB	4.29 ± 0.70 AB	2.88 ± 0.56
Soya protein (sample 1)	5.74 ± 0.79**B	5.03 ± 0.53**B	2.98 ± 0.18
Soya protein (sample 2)	5.91 ± 1.22 B	4.68 ± 0.46 B	3.87 ± 0.49

Significant differences within protein group (horizontally)

from diet 3: (*)P > 0.05 < 0.1 *P < 0.05 **P < 0.01 ***P < 0.001
from diet 2: (●)P > 0.05 < 0.1 ●P < 0.05 ●●P < 0.01 ●●●P < 0.001

Significant difference within Protein level (vertically)
values without similar letters are significantly different ($P < 0.05$)

Table 2.22d shows the ratios of all groups of weight gain in g to reference protein intake in g for the 4 week period. Expressed in this way the growth of animals given soya protein (sample 2) at all levels is comparable to that of those given diets containing soya protein (sample 1). Again the only groups to perform badly relative to the others at the same level of reference protein are the Diet 1 fishmeal group and Diet 2 casein group.

Fasting Serum Metabolites

1. Serum Insulin levels

The mean fasting serum insulin levels for all dietary groups are shown in Table 2.23. The male stock group level (20.9 μU/ml) is approximately double that of the female group (9.2 μU/ml) which is a highly significant difference ($P<0.001$). In the groups given the experimental diets containing higher levels of protein there is also a tendency for the male levels to be higher than those of the female equivalents although the difference is only significant in a few instances. At the lowest level of protein (Diet 1 groups) male and female data are very similar. Animals given soya protein (sample 1) as the protein source had the lowest serum insulin levels ($\simeq$ 5.5 μU/ml) and animals given diets containing mould the highest ($\simeq$ 13 μU/ml). In general the highest serum insulin values observed were in animals given diets containing mould and the lowest in animals given casein and fishmeal diets.

Although not often significant, the data indicate that the serum insulin levels tend to increase with the level of protein in the diets. The mean fasting insulin levels were plotted against the mean body weight at sacrifice and the regression lines for each sex are shown in Figure 2.12. A highly significant correlation ($P<0.001$) is seen within the male groups but the relationship between these two factors in the female groups is less well defined ($P<0.05$).

Table 2.23 Mean Fasting Serum Insulin Levels (μU/ml $\pm$ SEM(n)) of female and male hamsters given experimental diet or stock diet for 6 weeks.

Major dietary protein	Diet 1	Diet 2	Diet 3	Diet 4
FEMALES				
Casein	6.6 $\pm$ 0.4(6) $^{AC}_{1}$	8.3 $\pm$ 0.5(6) $_{2}$	7.9 $\pm$ 1.4(6) $^{AC}_{12}$	6.6 $\pm$ 0.9(6) $^{A}_{12}$
Fishmeal	10.2 $\pm$ 1.7(6) $^{A}_{12}$	6.8 $\pm$ 0.5(6) 1	8.8 $\pm$ 0.7(6) $^{AC}_{2}$	—
Mould	13.3 $\pm$ 1.6(6) ^{B}	19.3 $\pm$ 6.1(6) **	19.4 $\pm$ 2.9(6) ^{B} ***	22.0 $\pm$ 5.7(6) ^{B} ***
Soya protein (sample 1)	5.9 $\pm$ 0.9(6) C	6.0 $\pm$ 0.3(6)	6.8 $\pm$ 1.1(6) A	10.2 $\pm$ 2.2(6) B
Soya protein (sample 2)	12.6 $\pm$ 2.1(6) B	14.5 $\pm$ 6.9(6)	13.6 $\pm$ 2.5(6) CB	16.2 $\pm$ 2.6(6) ^{B} **
Stock	9.2 $\pm$ 1.0(24)			
MALES				
Casein	6.8 $\pm$ 0.8(4) $^{AC}_{1}$	9.5 $\pm$ 2.9(6) $^{AC}_{12}$	12.0 $\pm$ 1.5(6) $^{AC}_{2}$	10.5 $\pm$ 1.5(6) $^{A}_{12}$
Fishmeal	9.5 $\pm$ 0.9(4) A	7.4 $\pm$ 1.1(6) C	9.6 $\pm$ 1.4(6) AC	17.0 $\pm$ 4.2(6) AC
Mould	12.6 $\pm$ 1.7(6) B	31.9 $\pm$ 5.4(5) B	40.2 $\pm$ 7.4(5) * $\bullet$ B	31.8 $\pm$ 6.5(6) BC
Soya protein (sample 1)	5.1 $\pm$ 0.9(4) $^{C}_{1}$	15.8 $\pm$ 1.9(4) $^{\bullet\bullet\bullet}_{AD\ 12}$	14.3 $\pm$ 1.4(4) $^{C}_{\bullet\bullet\ 2}$	20.4 $\pm$ 5.6(6) $^{ACD}_{12}$
Soya protein (sample 2)	10.6 $\pm$ 1.0(6) $^{B}_{1}$	26.2 $\pm$ 4.1(6) $^{BD}_{2}$	21.2 $\pm$ 3.8(5) $^{BC}_{2}$	30.1 $\pm$ 3.9(6) $^{BD}_{\bullet\ 2}$
Stock	20.9 $\pm$ 2.8(23) $\bullet\bullet\bullet$			

Significant differences from stock group *P$<$0.05 **P$<$0.01 ***P$<$0.001

Significant differences from corresponding female group $\bullet$P$<$0.05 $\bullet\bullet$P$<$0.01 $\bullet\bullet\bullet$P$<$0.001

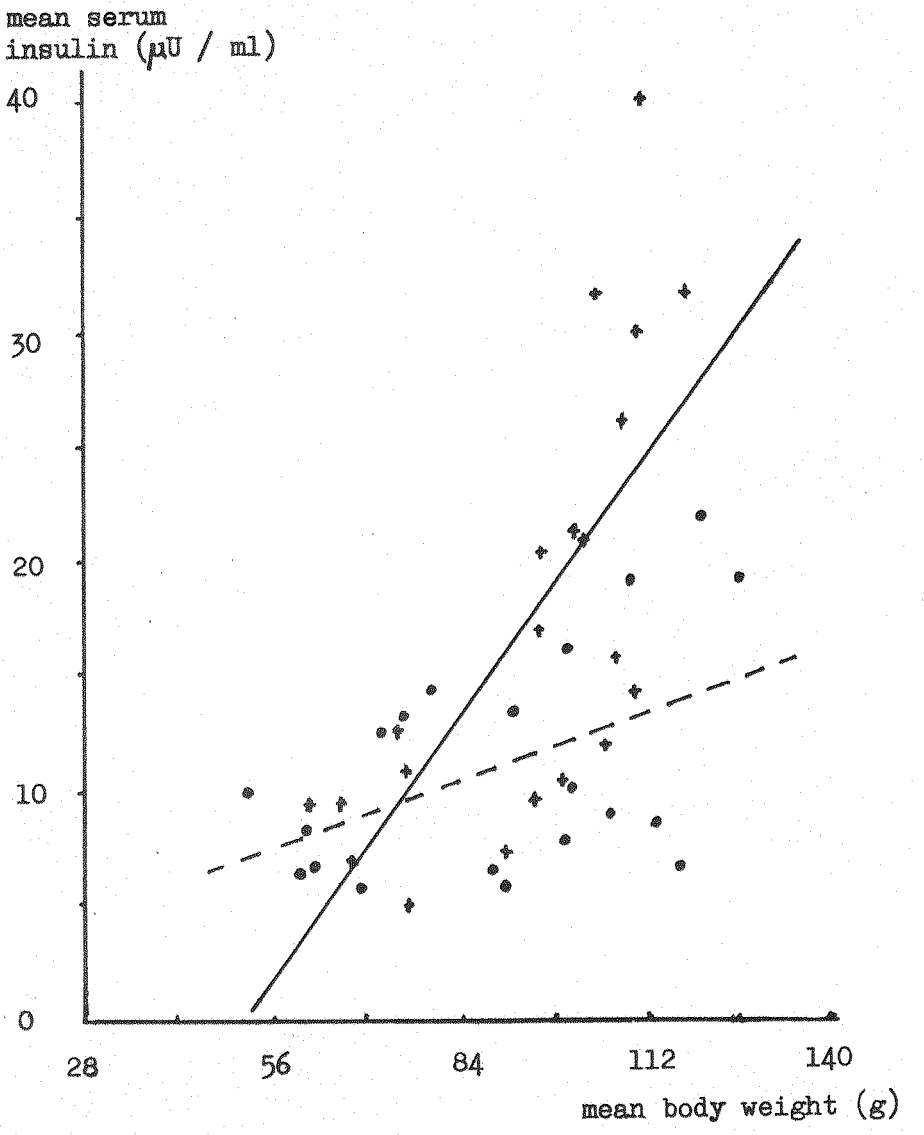
A B C D - comparing protein sources at each level, values without common superscripts are significantly different (P$<$0.05)

12 - within protein source, values significantly different if they do not have similar superscripts (P$<$0.05)

Figure 2.12 : Correlation of mean fasting serum insulin levels with mean body weight at sacrifice of male and female hamsters.

+ ——— male groups. Y=-26.5±0.41X r=0.70 n=21 P< 0.001

• ------ female groups. Y= 0.2±0.11X r=0.47 n=20 P< 0.05



2. Serum glucose levels

These data are shown in Table 2.24. There was no significant difference between males and females with the mean values for the stock groups being 4.57 and 5.02 mM/l respectively. In the experimental groups there were no overall variations in glucose levels with the level of protein in the diet. In both the male and female groups the mould and soya protein (sample 2) diets produced the highest serum glucose values - these containing the only means which were significantly different from the stock group values. In both males and females, where the data was available, the groups given diets containing soya protein (sample 1) had the lowest glucose levels and these were consistantly significantly lower than those of the mould and soya protein (sample 2) groups although not significantly below those of the stock control groups.

3. Serum Cholesterol levels

The mean serum cholesterol levels are shown in Table 2.25. There was no apparent difference between the sexes with values being 2.51 amd 2.28mM/l for the male and female stock groups respectively. In both male and female animals all groups given diets containing casein or soya protein (sample 2) had mean serum cholesterol levels similar to those of the stock group. The animals given diets containing fishmeal had mean cholesterol levels significantly higher than those of the stock groups at the two higher levels of protein (Diets 3 and 4) in the males and at all levels of protein in the female groups. The mean level of serum cholesterol increased with the level of fishmeal in the diet and was 4.16 and 5.58mM/l in the female and male Diet 4 groups. When the dietary protein source was soya protein (sample 1) serum cholesterol levels were significantly lower than those of the respective stock group in both male and female animals. This was with the exception of the low protein male group which had a mean level of 2.21mM/l and was

Table 2.24 Mean fasting Serum Glucose levels (m mole/l) of female and male hamsters given experimental diets or the stock diet for 6 weeks.

	Major dietary protein	Diet 1	Diet 2	Diet 3	Diet 4	Mean level for protein source
FEMALES	Casein	4.88 ± 0.20(4)A	4.63 ± 0.26(4)A	5.04 ± 0.18(4)AB	4.36 ± 3.9(5)A	4.71
	Fishmeal	-	5.03 ± 0.68(4)AC	5.82 ± 0.96(4)AB	-	5.43
	Mould	6.63 ± 0.12(6)B**	6.09 ± 0.28(6)BC*	5.66 ± 0.51(5)AB	5.88 ± 0.38(6)B	6.08
	Soya protein(1)	-	-	3.93 ± 0.51(5)A	3.89 ± 0.14(4)A	3.92
	Soya protein(2)	5.70 ± 0.39(4)A	6.14 ± 0.22(4)BC	6.63 ± 0.71(6)B**	6.31 ± 0.26(6)B**	6.26
	Stock	5.01 ± 0.23(21)				
MALES	Casein	5.61 ± 0.69(4)AB	3.92 ± 0.06(4)AC	3.99 ± 0.52(4)A	4.71 ± 0.49(4)AC	4.56
	Fishmeal	3.96 ± 0.18(4)A	4.83 ± 0.41(4)BC	5.50 ± 0.82(4)AB	5.17 ± 0.42(4)ABC	4.87
	Mould	6.48 ± 0.13(5)B**	5.82 ± 0.36(5)BC*	5.87 ± 0.25(6)B	6.22 ± 0.33(6)B**	6.09
	Soya protein(1)	3.97 ± 0.31(5)A	3.61 ± 0.20(5)A	3.93 ± 0.65(5)A	3.57 ± 0.38(5)A	3.77
	Soya protein(2)	5.08 ± 0.34(4)AB	5.22 ± 0.35(4)B	4.94 ± 0.54(4)AB	6.05 ± 0.76(6)BC*	5.41
	Stock	4.56 ± 0.27(19)				

Significant differences from stock group *$P<0.05$ **$P<0.01$

ABC Comparing protein sources at same levels:values without common superscripts are significantly different ($P<0.05$)

Table 2.25 Mean fasting Serum Cholesterol levels (m mole/l) of female and male hamsters given experimental diets or stock diet for 6 weeks.

	Major dietary protein	Diet 1	2	3	4
FEMALES	Casein	2.49 ± 0.16(6)A	2.77 ± 0.10(5)A	2.30 ± 0.07(6)A	2.60 ± 0.15(6)A
	Fishmeal	3.05 ± 0.22(6)A**	3.11 ± 0.13(5)A**	3.50 ± 0.11(6)B***	4.16 ± 0.30(6)B***
	Mould	2.47 ± 0.16(6)A	2.19 ± 0.03(6)B	1.95 ± 0.04(6)C	1.81 ± 0.05(6)C*
	Soya protein (1)	1.77 ± 0.08(6)B*	1.57 ± 0.09(6)C**	1.65 ± 0.08(6)D**	1.64 ± 0.08(5)C*
	Soya protein (2)	2.48 ± 0.23(5)A	2.17 ± 0.10(5)B	1.96 ± 0.14(5)CD	2.22 ± 0.09(6)A
	Stock	2.28 ± 0.11(23)			
MALES	Casein	2.57 ± 0.20(6)A	2.17 ± 0.11(5)AB	2.35 ± 0.05(6)A	2.25 ± 0.07(6)A
	Fishmeal	2.73 ± 0.26(5)A	2.63 ± 0.17(6)A	3.91 ± 0.15(6)B***	5.58 ± 0.27(6)B***
	Mould	2.29 ± 0.23(6)A	2.07 ± 0.15(5)B*	2.32 ± 0.09(6)A	1.81 ± 0.10(5)C***
	Soya protein (1)	2.21 ± 0.12(5)A	1.98 ± 0.11(6)B**	2.03 ± 0.08(5)C**	1.98 ± 0.07(6)C**
	Soya protein (2)	2.19 ± 0.14(6)A	2.22 ± 0.05(6)B	2.10 ± 0.14(6)AC	2.23 ± 0.08(6)A
	Stock	2.51 ± 0.08(24)			

Significant differences from stock group *$P<0.05$ **$P<0.01$ ***$P<0.001$

ABC comparing protein sources at similar levels - values without common superscripts are significantly different ($P<0.05$)

Table 2.26 Serum Triglyceride Levels of female and male hamsters given experimental or stock diet for six weeks (m g/100 ml).

Major dietary protein	Diet 1	Diet 2	Diet 3	Diet 4	Mean for protein source
FEMALES					
Casein	138.3 ± 10.3(6)A	140.2 ± 7.4(5)A	138.7 ± 8.8(6)A	153.0 ± 15.8(6)A	142.7
Fishmeal	205.8 ± 21.3(5)BC	194.0 ± 18.3(5)BC	213.5 ± 26.6(6)B*	244.5 ± 30.6(4)B**	212.9
Mould	206.3 ± 12.2(6)B*	205.5 ± 11.2(6)B*	195.5 ± 9.1(6)B	199.7 ± 8.8(6)BC	201.8
Soya protein (sample 1)	104.6 ± 2.0(5)D	115.7 ± 16.3(6)A*	126.4 ± 11.2(5)A	166.8 ± 16.0(5)AC	127.8
Soya protein (sample 2)	148.5 ± 16.3(4)AC	149.2 ± 1.5(5)AC	144.5 ± 1.5(6)A	152.2 ± 17.2(6)A	148.6
Stock	153.0 ± 12.1(22)				
MALES					
Casein	180.2 ± 32.9(5)AB	167.3 ± 30.5(4)AB	162.2 ± 16.1(6)	158.3 ± 27.8(6)A	166.3
Fishmeal	175.8 ± 13.4(4)A	184.8 ± 24.8(5)AB	218.2 ± 26.7(6)	241.7 ± 15.0(6)B	208.9
Mould	157.8 ± 19.3(6)AB	163.6 ± 16.6(5)AB	175.3 ± 6.2(6)	177.5 ± 12.5(6)A	205.5
Soya protein (sample 1)	117.8 ± 6.8(5)B	133.0 ± 6.8(5)A	151.5 ± 20.3(6)	145.8 ± 22.3(6)A	138.1
Soya protein (sample 2)	148.8 ± 16.4(6)AB	205.2 ± 11.6(6)B	195.5 ± 29.4(6)	201.4 ± 17.9(5)AB	187.1
Stock	182.3 ± 18.5(22)				

Significant differences from stock group: *$P < 0.05$; **$P < 0.01$.

A B C D comparing different protein sources at the same level - values without common superscripts are significantly different ($P < 0.05$)

statistically similar to the stock group. Within the female groups also the animals given the diet with the lowest level of protein (Diet 1) had the highest mean serum cholesterol level (1.17mM/l) of the four groups but this was still sigificantly lower than the stock group value. A similar pattern was seen in the groups given diets containing mould with the Diet 1 groups having similar levels to those of the stock groups and sigificant reductions from this level appearing as the level of protein in the diet increased.

4. Serum Triglyceride levels

These data are shown in Table 2.26. There was a considerable amount of individual variation within some of the groups. No significant sex difference was apparent and there were few significant deviations from the stock control group values within the experimental groups - none in the case of male animals. The mean value for male and female animals supplied with each protein source are shown in the Table. The animals contributing to the highest protein means were those offered diets containing fishmeal and mould where the values for males and females were in the region of 200-210 mg/100 ml. serum. The lowest levels in both cases were observed in the animals given soya protein (sample 1) where the means for all animals were 138 mg/100ml in the male group and 128 mg/100ml in the females.

Discussion

The first general observation from the results of the growth trials described here is that the diets containing the equivalent of only 5% reference protein were inadequate for supporting growth in the hamster. This was true for all protein sources including soya protein (sample 2) where the total level of protein included (86g/kg) was more comparable to the 10% reference protein diets from the other protein sources. This

suggests that the method of predicting protein value appears to be valid as an indicator of potential growth performance. The energy intake of these low protein groups, when expressed per unit of metabolic mass of the animals, was never less than that of the stock group, in fact, when the protein source was mould, casein or soya protein (sample 1) the female groups increased their energy intakes by 30-40%. This increase in energy intake observed when the diet was deficient in protein is similar to that reported by Kirsch, Brock and Saunders (1968) but no explanation can be offered as to why the increase was confined to the female groups. The poor growth response was not, therefore, a result of restricted energy intake but a reduction in the ability to utilise the nutrients for growth. Indeed the data from the second growth trial show that the conversion efficiency ratios (i.e. weight gain:food or energy intake) were lower in the groups given only 5% reference protein than in those where the same protein source is supplied at a higher level. However, the ratios relating weight gain to protein or reference protein intake were generally similar within each protein source whether the diet contained 5% or 10% reference protein. Thus the poor growth reflects low protein intake.

A second general observation from the data presented relates to the difference in behaviour between some male and female groups given the same diet. Growth and food intake in the male and female stock control groups were similar as was growth on the low protein diets, as already discussed. But between these levels of 'good' and 'poor' growth, differences between the sexes in the growth response were observed. In all diets containing 15% or 20% reference protein, regardless of the protein source, growth in the male groups was comparable to that of the stock group and similar responses were also observed in four of the five sources (casein excluded)

when only the equivalent of 10% reference protein was supplied. Within the female groups, however, with the exception of groups given the mould diets, growth was not greatly improved by increasing the reference protein level from 5% to 10% and even at higher protein levels growth was sometimes impaired relative to thr stock group. In addition, when a diet resulted in impaired growth in both male and female animals compared to the relevant stock control group, the effect was observed at a younger age or to a greater extent in the female group. In the females given diets containing mould an interesting anomaly was observed. Not only did all three groups grow as well as the stock group , but also at the two higher levels of protein the animals became significantly heavier than the stock control group. It has previously been reported that female hamsters grow faster and bigger than males (Cooperman, Waisman and Elvehjem, 1943) but this was only shown here statistically when the animals were supplied with diets containing a high level of mould. It is interesting to speculate that the female of this species has a greater growth potential which becomes apparent when the diet is entirely satisfactory but which is associated with an increased susceptibility to dietary deficiencies.

A difference between the sexes was also observed in fasting serum insulin levels. When a high level of dietary protein was supplied, whether in stock or experimental groups, there was a tendency for the male animals to have higher levels than the females given the same diet, although this was not always significant. When the protein content of the diet was only equivalent to 5% reference protein the fasting serum insulin levels in the male groups were similar to those in the corresponding female groups. Low plasma insulin levels have previously been reported in protein malnourished children (James and Coore, 1970) and these low levels

might have been expected in the hamsters given low protein diets. It was interesting however, that the levels in the female hamsters did not increase to the same extent as in the males when the dietary protein content was increased. This was with one notable exception - the female given the high levels of mould had significantly increased serum insulin levels, although these were still lower than their male counterparts. It is tempting, therefore, to draw parallels between growth and fasting serum insulin levels. A correlation, similar to that previously reported in man (Bierman, Bagdade and Porte, 1968), was shown to exist in male animals but in the females the relationship was less well defined and had a lower degree of significance and a less steep slope for the regression line. The reason for this difference between the sexes is not readily apparent.

Diets furnishing fishmeal as the dietary source of protein at all but the lowest level produced a growth response in the male groups which was comparable with that of other high protein groups. However during the last two weeks of the trial the mean weight of all these groups decreased. The difference from the stock group did not become significant but with no other protein source was this behaviour in the male groups observed. The adverse effect of fishmeal in the female groups was also noticeable since it was the only protein source where the animals given the highest level of protein became significantly lighter than those given a lower level. With both the high protein diet and the two supplying the lowest levels of protein the weight gains achieved after the two weeks on diet were negated by subsequent weight loss such that the animals receiving the equivalent of 5% reference protein experienced a net weight loss throughout the trial. Furthermore it was only within the fishmeal group that the protein efficiency ratio increased to any degree when the reference protein level was

increased from 5 to 10% of the diet, thus emphasising the poor growth, both absolute and relative to that with the other proteins, supported by the low protein fishmeal diet. This behaviour with fishmeal was somewhat surprising since it is a protein source commonly used in commercial feedstuffs, although in such circumstances it would usually be one of a mixture of protein sources. Fish protein concentrate has previously been used, at a level of 100g/kg in hamster diets and although the weight gains achieved were inferior to those when soya meal was the protein source, no adverse effects were reported (Banta, Warner and Robertson, 1975). One point to be noticed is that these workers used fish protein concentrate whereas here white fishmeal was used. In looking for an explanation for this poor response to the fishmeal diets, it is perhaps possible to implicate the high levels of calcium in the protein source. White fishmeal, produced from waste, inevitably has high levels of calcium and phosphorus from the bones which are included. The other four protein sources used had low levels of calcium (6 to 2.2 g/kg) and phosphorus (5 to 10 g/kg) whereas the levels of the two constituents in the fishmeal were 79 and 36 g/kg respectively. It has been estimated that the requirements of the golden hamster include 6g calcium and 3.5g phosphorus per kg diet (Festing, 1972). These requirements are adequately met by the mineral mix included in the experimental diets, so any further amounts derived from the protein sources are in excess. Hypercalcemia in infants is accompanied by arrest of growth and even weight loss (Morgan, Mitchell, Stowers and Thomson, 1956; Rhaney and Mitchell, 1956). This condition is also often accompanied by elevated serum cholesterol levels (Leitch, 1964). Whether or not the increased levels of dietary calcium were sufficient to cause hypercalcemia, even if this condition exists in the golden hamster, which resulted in the observed weight

losses is a matter of speculation. It could explain the serum cholesterol levels in these groups which increased with the level of dietary protein source. Although the calcium intake would be increased at the higher levels of fishmeal, the animals given the lower levels would still have the additional stress of a low protein diet and thus exhibit a poor growth response. This explanation however does not allow for the fact that the female group given the equivalent of 15% reference protein derived from fishmeal experienced no weight loss.

If the foregoing reasoning is not applicable then other explanations for the high serum cholesterol levels in response to the fishmeal diets must be sought. It is likely that the cause lies in the nature of the fishmeal rather than in fish itself as a protein source, because the oil from fish (which is very low in white fishmeal) has previously been shown to lower serum cholesterol levels (Bronte-Stewart, Antonis, Eales and Brock, 1956; Keys, Anderson and Grande, 1957). It has even been suggested that fish may be used to improve the rather monotonous diet of atherosclerotic patients (Lovern,1958)

Apart from the high levels with fishmeal, the serum cholesterol levels in response to the dietary sources can be divided easily into two groups. When the protein was derived from casein or soya protein (sample 2) which were the two protein isolates, all serum cholesterol levels were similar to those of the stock control groups. However when the protein source was soya protein (sample 1) serum cholesterol levels were significantly reduced, more so at higher protein levels. This behaviour was also seen with mould diets but only at high levels of protein. These two materials have a protein content of approximately 45% only and another constituent present in substantial amounts is unavailable carbohydrate. This material, or 'dietary fibre', as it is commonly referred to, has been shown to lower serum cholesterol levels in man and experimental animals

(Trowell, 1972) and this could be the effect observed here, particularly since the effect was greater when higher levels of the protein sources were included. The mould specifically has previously been shown to prevent increases in serum cholesterol induced by dietary means (Owen, Munday, Taylor and Turner, 1975). Also in addition to the possible effect of dietary fibre the oil of soya beans, which is included in a preparation of the protein source used here, has high levels of polyunsaturated fats (Sinclair, 1964) which also reduce serum cholesterol levels (Lovern, 1958).

The growth response of the groups supplied with soya protein as the major protein source were generally comparable, whether it was in the form of the 'full fat flour' (sample 1) or the protein isolate (sample 2). With both sources growth in the male groups was comparable to the stock control group at all but the lowest level of protein whereas in the female groups growth was impaired unless higher levels of protein were supplied. Also these two protein sources produced comparable ratios of weight gain: reference protein intake at each level of protein. This also suggests that the method of predicting protein quality was valid.

The only protein source with which observations in the male and female groups were similar was casein. Poor growth in the males when the equivalent of 10% reference protein was suppled resulted in food and energy conversion ratios similar to those when 5% reference protein was suppled rather than when 15% reference protein was supplied - which was the situation found with all other protein sources. The response to casein here is similar to that reported by Arrington, Platt and Shirley (1966) who found a significant difference in growth when the diets contained 12% or 16% protein. The fact that the first growth trial animals supplied with the equivalent of 10% reference protein gained

weight at a rate comparable to the higher protein groups initially then lost weight whereas in the second trial the weight gain was slower throughout is surprising. In fact, the weight loss observed in the original trial is in itself a cause for concern particularly since there is no apparent explanation: the animals appeared healthy with no clinical signs of disease.

The response to different levels of protein derived from various sources, in terms of growth, in golden hamsters has been evaluated. An attempt was made to equalise the diets in terms of protein quality but differences in the responses of the dietary groups were due to the protein sources as well as to the level of protein in the diet. The major observations resulting from the work can be summarised as follows:

1) Dietary protein equivalent to 5% reference protein was unsatisfactory for growth although energy intake was never reduced;

2) Growth and food intake were similar in male and female hamsters given the stock diet but, in general, higher levels of dietary protein were necessary for female animals than male animals to produce growth comparable to the stock control groups;

3) Differences in the fasting serum insulin levels between male and female animals seemed to relate to differences in growth and furthermore a positive correlation was shown to exist between fasting serum insulin levels and body weight at sacrifice;

4) Growth was not maintained when fishmeal was the protein source and the high level of calcium in this foodstuff has been discussed as a possible influencing factor;

5) Growth performance was similar with two samples of soya protein whether it was in the form of a protein isolate with a high nitrogen content but poor protein

score or a soya flour with a low nitrogen content but relatively high protein score;

6) Casein compared unfavourably to the other protein sources in that a higher level of protein was necessary to support growth in the male animals;

7) Dietary protein equivalent to 10 - 15% reference protein was required for normal growth in male and female hamsters.

CHAPTER 3. REPRODUCTIVE PERFORMANCE OF THE GOLDEN HAMSTER: THE EFFECT OF VARYING THE LEVEL AND SOURCE OF DIETARY PROTEIN

CHAPTER 3: REPRODUCTIVE PERFORMANCE IN THE GOLDEN HAMSTER: The effect of varying the level and source of dietary protein.

Introduction

It is known from experiments in other species that varying levels of protein in the maternal diet during gestation and lactation will affect reproductive performance in terms of resorption rate (Curtiss, 1953; Nelson and Evans, 1953), mean birth weight and litter size (Nelson and Evans, 1953) and survival of the offspring (Zeman, 1967; Venkatachalam and Ramanathan, 1964). The number of effects observed and their severity depends on the degree of protein restriction which is influenced by the source from which the protein is derived (Turner and Munday, 1974) as well as by the amount of protein in the diet. The work reported here was undertaken to evaluate the response in the golden hamster to different levels of dietary protein which was derived from various sources. Five protein sources were used in the experimental diets, the same as those employed in the growth trials (Chapter 2): casein, fishmeal, mould and two samples of soya protein.

Two experiments were carried out. In the first the females were given the experimental diets at mating and the response to the diets was evaluated in terms of the offspring produced and their growth and survival to weaning. In 82 cases direct evidence was found of mothers eating all or some of their pups, and since some litters had only 1 or 2 pups after birth the actual number of litters affected by this phenomenon could have been much larger. Therefore the data concerning litter size at birth was considered unsatisfactory and a parallel trialwas carried out but the pregnancies were terminated one day prior to term in order to collect reliable data regarding litter size at the end of the gestation and also to evaluate the loss in utero.

Methods and Materials

Animals and diets

Experiment 1

240 virgin female hamsters were allocated at 7 - 8 weeks of age to 16 groups, such that the mean weight and range of weights in each group were similar and litter mates were in different groups. The females were housed individually and offered ad libitum either stock diet (Porton Mouse Diet) or one of 15 experimental diets. The diets used were 1, 2 and 3 incorporating the five protein sources (+ wheat bran) as described in Chapter 2 (p33). A male was introduced for 4 days, this being the length of the estrous cycle. After removal of the male the females were weighed daily and food intake recorded throughout gestation until the day of birth, which is denoted as G16. Animals not pregnant at term were discarded. These together with a few other accidental losses, such as animals escaping which resulted in unknown food intakes, resulted in 8 to 13 animals per group as is shown in the results section. The day of mating (G0) was determined as the 16th day before birth, the length of gestation in the hamster being very constant. The notation used throughout is G0 - G16 for the 16 days of gestation, then L0 - L21 for the 21 days of lactation, G16 and L0 both being the day of birth. Pup and maternal data were recorded throughout lactation until day L21. The surviving pups were sacrificed and insulin secretion from their pancreases measured in vitro.

Experiment 2

A parallel trial was carried out with the same selection procedure and dietary groupings. The day of mating was determined by the appearance of a vaginal plug in the female. The pregnancies were terminated on day G15 by cervical dislocation of the mother and the foetuses were removed, counted, weighed and measured. The number of resorption sites was also noted.

In vitro incubation of pancreases

The procedure for the incubation of the 21 day old hamster pancreases was based on that described by Coore and Randle (1964) as follows:

1. The hamsters were killed by cervical dislocation and the pancreas from each was removed rapidly into a petri dish containing chilled (0-4^{o}C) Krebs bicarbonate buffer pH7.4, containing albumin (1mg/ml), sodium fumarate, sodium glutamate and sodium pyruvate (all at 5mM) and glucose (0.6mg/ml), previously gassed with a mixture of oxygen and carbon dioxide (95:5v/v) for one hour.
2. Each pancreas was dissected free from connective and adipose tissue, placed in a 10ml Ehrlenmeyer flask containing 2ml of the medium described above. The flasks were gassed for 30 seconds with the oxygen: carbon dioxide mixture and sealed with a rubber bung. Incubation was carried out at 37^{o}C for 30 minutes with shaking at 60 oscillations per minute. This initial incubation was for equibration of the tissue and the incubate was discarded.
3. Each pancreas was rinsed in fresh medium, transferred to a further 10 ml Ehlenmeyer flask containing 2 ml medium, gassed, sealed and incubated for 30 minutes.
4. This process was repeated for three further 30 minute incubation periods for which the incubation medium was: 1)basal medium containing also 2.4 mg/ml glucose; 2)basal medium; 3)basal medium containing also 2.4 mg/ml glucose and 0.66 mg/ml L-Leucine.
5. After removal of the tissue the incubate was stored immediately at - 20^{o}C for subsequent assay of the insulin content.
6. After the final incubation the pancreases were blotted and weighed.
7. The results were expressed as ng of insulin secreted per pancreas.

Statistical analysis of results

The data was treated as previously described in Chapter 2 (p.44) with the following additions:-
Analysis of variance was performed on the data as described by Bailey (1959).
Turkey's ω-procedure was used to compare overall treatment means (i.e either protein level or protein source) after detecting any influence by these factors using analysis of variance (ref.Steel and Torrie, 1960)

Results

Experiment 1

Stock Control Group

a) Maternal Data during gestation.

The mean weights and the standard error of the mean of the stock group are shown in Table 3.1 and graphically in Fig.3.1. The weights remained fairly constant between G0 and G8 then the period of weight gain began with the mean weight becoming significantly different from that at G0 at G12 ($P<0.05$) and the difference becoming greater until, at G15, the mean body weight had increased by 30%.

Food intake data is not reported between G0 and G4 because of the presence of the male during all or part of this period. Thereafter the food intakes are reported for 4 day periods i.e. G4-G8, G8-G12 and G12-G16 as well as for the whole period G4-G16 and also as metabolisable energy intake in kJ/animal/day. These data are shown in Table 3.1. Intakes during the three periods of gestation were similar.

b) Maternal data during lactation.

The mean weights, with the standard error of the mean are shown in Table 3.2 and displayed graphically in Fig.3.1. For the first week after birth (L0-L7) the lactating mothers lost weight at the rate of approximately 2g/d, then the weight loss decreased to approximately 1g/d for the remaining period to L21. The mean weight was significantly lower than that at L0 by L10($P<0.05$) and at L21 the mean weight was significantly lower than the weight at the start of

pregnancy (GO) ($P < 0.02$) the animals having lost 12% of their weight through the whole process of reproduction.

Food intakes were recorded through lactation between the days on which the animals were weighed and the data is shown in Table 3.2. Metabolisable energy intake (kJ/animal/day) is also shown. There was a gradual increase in food intake from the first period (L0-3) to the last (L14-21) but the difference was only significant between the first and last periods ($P < 0.05$). This could be due to increased milk production but must also, in part at least, be ascribed to the pups which were creep feeding at this age.

c) Pup Data

The data collected on the pups from mothers in the stock control group are recorded in Table 3.3.
The following indices of reproductive performance were calculated:-

1. Reproductive Index is defined as a ratio of the total weight of offspring surviving to 21 days to the number of animals brought to term. Therefore:

$$\text{Reproductive Index (RI)} = \frac{\text{total weight pups at L21}}{\text{no. animals brought to term}}$$

$$= 85.2$$

2. Neonatal Mortality is defined as the % mortality rate between L0 and L3 Therefore:

$$\text{Neonatal Mortality} = \frac{\text{no.pups at L0} - \text{no.pups at L3}}{\text{no.pups at L0}} \times 100$$

$$\text{Mean neonatal mortality} = 23.6 \pm \underset{(8)}{5.49}\%$$

3. Postnatal Mortality is defined as the % mortality rate between days L3 and L21. Therefore:

$$\text{Postnatal Mortality} = \frac{\text{no.pups at L3} - \text{no.pups at L21}}{\text{no.pups at L3}} \times 100$$

$$\text{Mean postnatal mortality} = 34.3 \pm \underset{(8)}{10.8}\%$$

4. Survival of pups $= \frac{\text{no.pups at L21}}{\text{no.pups at birth}} \times 100$

$$\text{Mean survival of pups} = 51 \pm \underset{(8)}{10.3}\%$$

Table 3:1 Mean weights and food intakes (± SEM) of eight hamsters given stock diet during gestation.

Day of gestation	Mean weight(g)	Food intake g/animal/d	Metabolisable energy intake kJ/animal/d)
G0	85.6 ± 3.6		
G1	86.0 ± 3.5		
G2	86.6 ± 2.9		
G3	88.1 ± 3.8		
G4	88.1 ± 3.8		
G5	87.1 ± 3.8	6.6 ± 0.8	73.1 ± 8.9
G6	87.1 ± 3.7		
G7	87.7 ± 3.5		
G8	88.4 ± 3.6		
G9	90.3 ± 3.9		
G10	93.0 ± 4.0	6.4 ± 0.5	70.9 ± 5.6
G11	96.1 ± 4.1		
G12	99.1 ± 4.0*		
G13	103.0 ± 3.9**		
G14	106.7 ± 4.3**	7.5 ± 0.5	83.1 ± 5.6
G15	110.4 ± 4.2***		
G16	93.3 ± 4.0		
Weight gain G0 - G15	24.9 ± 2.9		
Mean food/energy intake from G4 - G16		6.8 ± 0.5	75.7 ± 5.5

Significantly different from weight at G0:

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Figure 3.1 :Mean weights + SEM from mating through gestation and lactation of 8 animals given the stock diet.

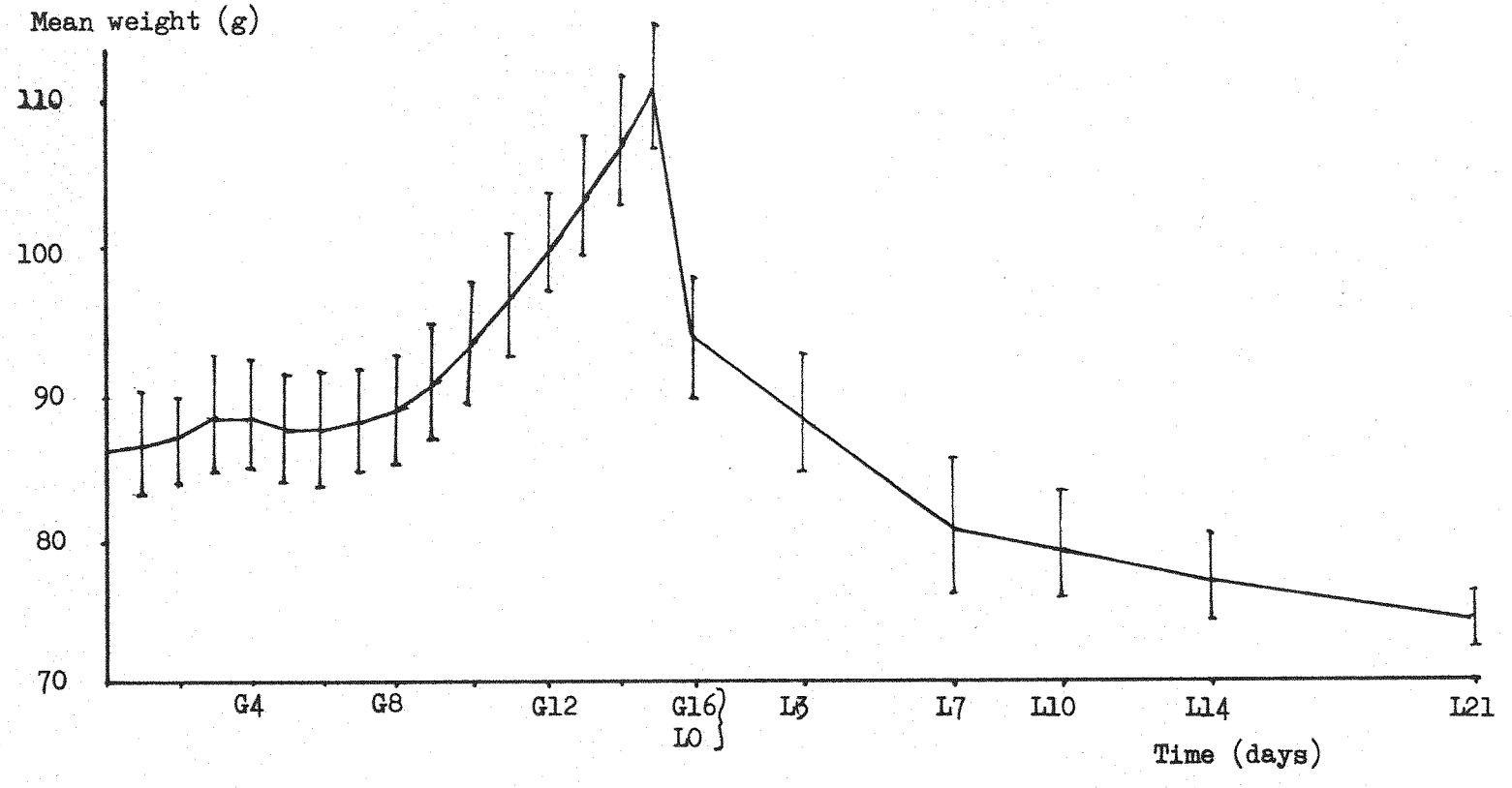


Table 3:2 Mean weights and food intakes (± SEM) of eight hamsters given stock diet during lactation.

Day of lactation	Weight (g)	Food intake (g/animal/d)	Metabolisable energy intake (kJ/animal/d)
L0	93.3 ± 4.0	8.9 ± 0.9	98.6 ± 10.0
L3	88.0 ± 4.0	9.4 ± 1.4	104.1 ± 15.5
L7	80.3 ± 4.7	10.7 ± 1.4	118.5 ± 15.5
L10	79.0 ± 3.6*	10.7 ± 1.0	118.5 ± 11.1
L14	76.9 ± 2.9**	14.6 ± 2.2*	161.8 ± 24.4*
L21	74.0 ± 2.2●***		

Weights: Significant differences from L0: *P < 0.05; **P < 0.01.
from G0: ●P < 0.05.

Food intake:
Significant differences from L0 - L3: *P < 0.05

Table 3:3 Data on litters born to mothers given stock diet during gestation and lactation (mean ± SEM (n))

Day of lactation	Total live weight of litter (g)	Live pups/litter	Mean pup weight (g)
L0	16.9 ± 1.3 (8)	6.75 ± 0.59 (8)	2.50 ± 0.02 (54)
L3	20.3 ± 2.9 (8)	4.88 ± 0.72 (8)	3.95 ± 0.09 (39)
L7	32.1 ± 5.9 (8)	4.38 ± 0.84 (8)	7.46 ± 0.08 (35)
L10	41.2 ± 8.1 (8)	4.13 ± 0.83 (8)	9.98 ± 0.14 (33)
L14	49.0 ± 8.4 (8)	3.80 ± 0.86 (8)	13.05 ± 0.46 (30)
L21	85.2 ±18.1 (8)	3.63 ± 0.86 (8)	23.50 ± 0.74 (29)

Number of animals brought to term : 8

$$\underline{\text{5. Survival of litters}} = \frac{\text{no. of litters at L21}}{\text{no. of animals brought to term}} \times 100$$

$$= 100\%$$

The data presented for the stock control group of this trial are considered to represent the normal reproductive behaviour of the Southampton colony when the animals are handled throughout the reproductive cycle and are used therefore as the reference point in the discussion of the performance of the groups offered experimental diets.

Groups offered diets containing Casein

a) Maternal data during gestation.

The mean weights of the animals throughout gestation are shown in Table 3.4. The group supplied with 15% reference protein (Diet 3) had similar weights to those of the stock group animals and, as in the case of the stock group, the mean weight became significantly greater than the weight at mating (G0) at G12 ($P < 0.02$). The animals in the group given Diet 2 remained at approximately constant weight throughout the first 15 days of gestation and were lighter than the stock group from G13 ($P < 0.05$) and the Diet 3 animals from G14 ($P < 0.05$). Their mean weight at G15 was 82% that of the stock group animals. The group of animals given 5% reference protein (Diet 1) experienced a weight loss through gestation becoming lighter than the Diet 3 animals at G8, the stock controls and their own weight at mating at G9 and lighter than the Diet 2 animals at G10. At G15 the Diet 1 animals were only 2/3 the weight of the stock control group. The weight gains from mating of all groups given diets containing casein are shown in Fig. 3.2 with the stock group included for comparison.

The food intake of the 3 experimental groups is shown in Table 3.5a. The only difference between the three 4-day periods considered was within the 5% reference protein group where the food eaten during the second period (G8-G12) was 40% less than that eaten during the first (G4-G8) ($P < 0.05$).

Metabolisable energy intakes are shown in Table 3.5b to allow a comparison with the stock group and between experimental groups. For the group offered the 15% reference protein diet (Diet 3) intakes were comparable to the stock group throughout. In the case of the groups supplied with the two lower levels of protein in the diets the energy intake during the two latter periods of gestation (G8-G12 and G12-G16) was less than that of the stock group and that of the Diet 3 group during the last period. The Diet 1 animals ate about 40% less than the stock animals from G8 to G12 which was also significantly less than the animals receiving Diets 2 and 3 during this period. The energy intake for the whole 12 day period was 76% and 66% of the stock intake for Diets 2 and 1 respectively, this was a significant reduction ($P < 0.01$)

b) Maternal Data during lactation.

The mean weights of the three groups are shown in Table 3.6 and the weight gains from mating with the stock group included in Fig. 3.2. The decreasing number of observations in the tables is due to the loss of complete litters. The mean weights of the high protein group were similar to those of the stock group throughout lactation. The Diet 2 group, although lighter than the stock group at L0 was similar to it thereafter, thus showing an increase in weight relative to the stock control group. None of the low protein pups survived to L3 so the only data on the mothers is at L0. At L0 the mean maternal weight of this group was significantly lower than that of the stock group and the other two experimental groups. After day 7 (Diet 2) or 10 (Diet 3) days of lactation the two higher protein groups were significantly lighter (15%) than their weight at mating. A similar reduction did not occur in the stock group until L21 (Table 3.2).

The food intake of the two groups with surviving pups is shown in Table 3.7a. There was no difference between any of

Table 3.4 Mean weights (g ± SEM) during gestation of groups given diets containing Casein or the stock diet

Diet	1	2	3	Stock
Number of animals	8	13	12	8
Day of gestation				
G0 (mated)	87.3 ± 2.3	86.3 ± 2.1	85.8 ± 2.6	85.6 ± 3.6
G1	86.1 ± 2.3	86.4 ± 2.3	85.6 ± 2.9	86.0 ± 3.6
G2	84.7 ± 2.3	86.5 ± 2.4	85.4 ± 3.0	86.6 ± 3.8
G3	83.7 ± 2.2	86.8 ± 2.6	86.0 ± 2.9	88.1 ± 3.8
G4	82.9 ± 2.2	87.3 ± 2.5	86.7 ± 2.7	88.1 ± 3.8
G5	82.6 ± 2.2	87.1 ± 2.5	87.5 ± 2.7	87.1 ± 3.8
G6	82.1 ± 2.4	87.6 ± 2.6	87.6 ± 2.4	87.1 ± 3.7
G7	81.4 ± 2.4	87.5 ± 2.8	88.1 ± 2.5	87.7 ± 3.7
G8	80.6 ± 2.4+	87.3 ± 2.8	89.2 ± 2.5	88.4 ± 3.6
G9	80.1 ± 2.4*	88.1 ± 2.8	91.1 ± 2.8	90.3 ± 3.9
G10	78.4 ± 2.5**	89.1 ± 3.0	92.7 ± 2.7	93.0 ± 4.0
G11	77.0 ± 2.4**	90.0 ± 3.0	93.5 ± 2.7	96.1 ± 4.1
G12	75.9 ± 2.3**	90.2 ± 3.2	95.0 ± 2.5*	99.1 ± 4.0*

Table 3.4 continued

Diet	1	2	3	Stock
Number of animals	8	13	12	8
Day of gestation				
G13	75.7 ± 2.3** ●●● ǂǂǂ	90.6 ± 3.61●	97.5 ± 2.4**	103.0 ± 3.9**
G14	74.7 ± 2.3** ●●● ǂǂǂ	90.1 ± 4.0● ǂ	100.9 ± 2.2***	106.7 ± 4.3**
G15	73.6 ± 2.0*** ●●● ǂǂǂ	90.2 ± 4.0●● ++	103.6 ± 2.2***	110.4 ± 4.2***
G16 (birth)	63.9 ± 2.1*** ●●● ǂǂǂ	78.4 ± 2.9* ●● ǂ	86.6 ± 2.3	93.3 ± 4.0
Weight gain G0 - G15	-13.7 ± 1.7●●● ǂǂǂ	3.9 ± 2.4●●● +++	17.7 ± 2.1	24.9 ± 2.9

Significant difference from weight at G0: *P < 0.05 **P < 0.01 ***P < 0.001
from stock group : ●P < 0.05 ●●P < 0.01 ●●●P < 0.001
from diet 3 group: +P < 0.05 ++P < 0.01 +++P < 0.001
from diet 2 group ǂP < 0.05 ǂǂP < 0.01 ǂǂǂP < 0.001

Figure 3.2 : Mean weight gains from mating through gestation and lactation of animals given diets containing casein. The stock group is also shown for comparison.

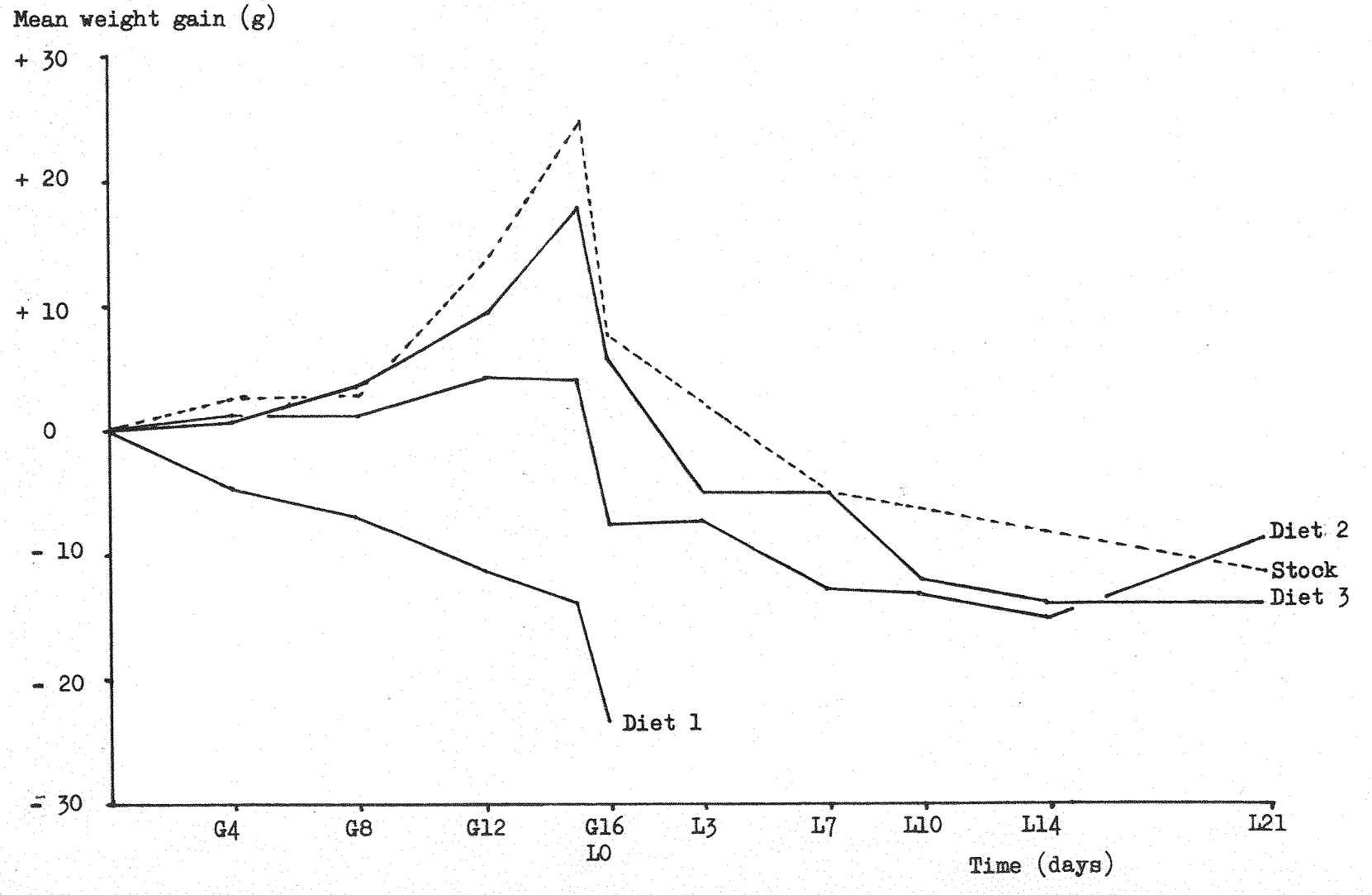


Table 3.5a Mean (± SEM) food intake (g/animal/d) during gestation of hamsters given diets containing Casein or the stock diet.

Diet	1	2	3	Stock
Number of animals	8	13	12	8
Period of gestation				
G4 - G8	4.3 ± 0.6[a]	4.0 ± 0.3	4.5 ± 0.5	6.6 ± 0.8
G8 - G12	2.6 ± 0.3[b]	3.5 ± 0.2	4.7 ± 0.6	6.4 ± 0.5
G12 - G16	3.1 ± 0.4[ab]	3.8 ± 0.2	5.2 ± 0.4	7.5 ± 0.5
G4 - G16	3.3 ± 0.3	3.8 ± 0.2	4.8 ± 0.4	6.8 ± 0.5

a,b, within dietary groups, values without common superscript are significantly different ($P < 0.05$)

Table 3.5b Mean (± SEM) metabolizable energy intake(kJ/animal/d) during gestation of hamsters given diets containing Casein or the stock diet.

Diet	1	2	3	Stock
Number of animals	8	13	12	8
Period of gestation				
G4 - G8	65.6 ± 9.2	61.0 ± 4.6	64.7 ± 7.2	73.1 ± 8.9
G8 - G12	39.7 ± 4.6●●●‡	53.4 ± 3.1●●	67.6 ± 8.6	70.9 ± 5.6
G12 - G16	47.3 ± 6.1++●●●	58.0 ± 4.1+●●●	74.8 ± 5.8	83.1 ± 5.6
G4 - G16	50.4 ± 4.6+●●	58.0 ± 3.1●●	69.0 ± 5.8	75.7 ± 5.5

Significant difference from stock group ●P < 0.05 ●●P < 0.01 ●●●P < 0.001
from diet 3 group +P < 0.05 ++P < 0.01 +++P < 0.001
from diet 2 group ‡P < 0.05 ‡‡P < 0.01 ‡‡‡P < 0.001

Table 3. 6 Maternal weights (g) during lactation of groups given diets containing Casein or the stock diet (mean ± SEM (n))

Day of lactation	Diet 1	2	3	Stock
L0	63.9 ± 2.1 (8)***●●●‡‡‡	78.4 ± 2.9 (13)*●●‡	86.6 ± 2.3 (12)	93.3 ± 4.0 (8)
L3	-	79.2 ± 3.7 (5)	81.0 ± 3.7 (6)	88.0 ± 4.0 (8)
L7	-	73.3 ± 3.4 (4)**	80.6 ± 4.0 (5)	80.3 ± 4.7 (8)
L10	-	72.7 ± 4.2 (3)*	74.0 ± 4.7 (5)*	79.0 ± 3.6 (8)
L14	-	71.3 ± 4.3 (3)**	72.0 ± 3.8 (3)*	76.9 ± 2.9 (8)
L21	-	77.5 ± 6.5 (2)	71.7 ± 1.9 (3)*	74.0 ± 2.2 (8)*

Significant differences from weight at GO *P < 0.05 **P< 0.01 ***P< 0.001
from stock group ●P < 0.05 ●●P < 0.01 ●●●P < 0.001
From diet 3group +P< 0.05 ++P < 0.01 +++P < 0.001
from diet 2group ‡P < 0.05 ‡‡P < 0.01 ‡‡‡P< 0.001

Table 3. 7a Food intake (g/animal/d) during lactation of groups given diets containing Casein or the stock diet (mean ± SEM (n))

Period of lactation	Diet 1	2	3	Stock
L0 - L3	-	4.9 ± 0.4 (5)	5.7 ± 0.9 (6)	8.9 ± 0.9(8)
L3 - L7	-	5. 0 ± 1.0 (4)	7.3 ± 0.6 (5)	9.4 ± 1.4(8)
L7 - L10	-	6.2 ± 2.1 (3)	6.9 ± 1.1 (3)	10.7 ± 1.4(8)
L10 - L14	-	6.7 ± 1.6 (3)	5.6 ± 1.5 (3)	10.7 ± 1.0(8)
L14 - L21	-	9.2 ± 0.5 (2)**	10.4 ± 1.9 (3)*	14.6 ± 2.2(8)*

Significant difference from L0 - L3 *P< 0.05 **P< 0.01

Table 3: 7b Metabolisable energy intake (kJ/animal/d) during lactation of groups given diets containing Casein or the stock diet (mean ± SEM (n))

Period of lactation	Diet 1	2	3	Stock
L0 - L3	-	74.8 ± 6.1 (5)	83.9 ±13.3 (6)	98.6 ± 10.0(8)
L3 - L7	-	76.3 ±15.3 (4)	107.4 ± 8.8 (5)	104.1 ± 15.5(8)
L7 - L10	-	94.6 ±32.1 (3)	101. 5 ±16.2 (3)	118.5 ± 15.5(8)
L10 - L14	-	102.2 ±24.4 (3)	82.4 ±22.1 (3)	118.5 ± 11.1(8)
L14 - L21	-	140.4 ± 7.7 (2)	153.0 ±28.0 (3)	161.8 ± 24.4(8)

Table 3.8 Data from litters born to mothers given diets containing casein during gestation and lactation (mean ± SEM (n))

	Day of lactation	Total live weight of litter (g)	Live pups/litter	Pup weight (g)
a.Diet 1	L0	2.9 ± 0.6 (7)●●●++	2.1 ± 0.4 (7)+●●●	1.36 ± 0.08 (15)●●●‡‡‡‡
	number of animals brought to term : 8			
b.Diet 2	L0	6.9 ± 1.6 (9)●●●	3.4 ± 0.8 (9)●●	2.01 ± 0.05 (31)+●●●
	L3	7.8 ± 2.4 (5)●	2.2 ± 0.5 (5)●	3.54 ± 0.29 (11)
	L7	14.3 ± 4.3 (4)	2.3 ± 0.5 (4)	6.30 ± 0.46 (9)●●●
	L10	24.0 ± 3.3 (3)++	2.7 ± 0.3 (3)+	9.00 ± 0.14 (8)●●
	L14	28.8 ± 6.6 (3)+	2.7 ± 0.3 (3)+	10.84 ± 0.84 (8)●
	L21	59.8 ± 8.8 (2)+	2.5 ± 0.5 (2)	23.90 ± 4.24 (5)
	number of animals brought to term : 13			
c.Diet 3	L0	10.0 ± 1.7 (9)●●	5.1 ± 0.7 (8)	2.16 ± 0.04 (2)●●●
	L3	12.0 ± 2.0 (7)	3.1 ± 0.4 (7)	3.80 ± 0.14 (22)
	L7	23.2 ± 4.4 (6)	3.3 ± 0.3 (6)	6.97 ± 0.43 (20)
	L10	37.6 ± 1.0 (4)	3.8 ± 0.3 (4)	10.07 ± 0.41 (15)
	L14	47.0 ± 3.6 (4)	3.8 ± 0.3 (4)	12.55 ± 0.88 (15)
	L21	93.5 ± 5.4 (4)	3.8 ± 0.3 (4)	24.96 ± 1.50 (15)

Number of animals brought to term : 12

Significantly different from stock group ●P < 0.05 ●●P < 0.01 ●●●P < 0.001 (see Table 3:3)
from Diet 3 group +P < 0.05 ++P < 0.01 +++P < 0.001
from diet 2 group ‡P < 0.05 ‡‡P < 0.01 ‡‡‡P < 0.001

the periods until L14-L21, by which time the pups would have been creep feeding. Table 3.7b shows the metabolisable energy intakes. In both experimental groups the energy intakes were similar to the stock control group and similar to each other for each period.

c) Pup data

The values for total weight of litter, live pups/litter and mean pup weight are shown in Table 3.8(a,b & c). Table 3.8a show that no pups from mothers on the lowest level of protein survived to L3 and that at birth all three parameters were significantly reduced when compared to the stock group and the Diet 3 group. The mean pup weight was also reduced by 30% even when compared to the Diet 2 group ($P < 0.001$). At the two higher levels of protein, although some of the values were lower than the stock group at birth and to a lesser extent at L3, after this time they were similar to the stock control group values with the exception of the mean weight of pups in the Diet 2 (10% reference protein) group where the reduction persisted. At birth the mean weight of the Diet 2 pups was also reduced (by 10%) when compared to the Diet 3 pups ($P < 0.02$). There were differences in the total live weight of litters between these two experimental groups towards the end of lactation. This reflected a difference in the litter sizes (live pups/litter), the mean pup weights being similar.

The indices of reproductive performance as described for the stock group are shown in Table 3.30a-g.

Groups offered diets containing fishmeal

a) Maternal data during gestation.

The mean weights of the animals are shown in Table 3.9 and the weight gains from mating in Fig. 3.3 with the stock group included for comparison. The mean weights of the pregnant animals did not change significantly from the weight at mating in any of the three groups during the first 15 days of gestation. Thus they became significantly lighter than the stock animals at G4 in the Diet 1 group ($P < 0.05$) and G11 in the other two groups ($P < 0.05$). The group given the diet

containing only 5% reference protein (Diet 1) actually lost weight throughout gestation and was 12% lighter than the other two experimental groups by G10. At G15 Diet 1 animals were 60% of the stock group weight and animals on Diets 2 and 3 were 80% of the stock mean weight.

The food intakes of the three groups measured in g/animal/d are shown in Table 3.10a and as metabolisable energy intake (kJ/animal/d) in Table 3.10b. During the period G8-G12 the animals offered Diets 1 and 3 consumed less than the stock group animals but not significantly less than those offered Diet 2. For the whole period (G4-G16) mean metabolisable energy intakes were similar in all 3 groups and about 85% of that of the stock control group. This decrease was only significant in the Diet 2 group.

b) Maternal data during lactation.

These data are shown in Tables 3.11 and 3.12 and the weight gains are shown in Fig. 3.3. The lack of data in this section reflects the poor reproductive performance in these three dietary groups.

c) Pup data

There is very little data (Tables 3.13a, b and c) on the offspring of mothers offered diets containing fishmeal as the protein source. At birth the mean weight of pups from animals on Diet 1 was reduced compared to the Diet 2 pups ($P < 0.001$) and Diet 2 pup weights were reduced compared to those of Diet 3 ($P < 0.05$). The mean weights of pups from all three experimental groups was reduced compared to the mean weight of the stock group pups ($P < 0.001$). The total litter weight was simlarly reduced in all groups compared to the stock litter weight at birth ($P < 0.001$)

The indices of reproductive performance, as described in the stock group result section, are shown in Tables 3.30a-g. No pups survived to weaning in any of the groups given this protein source thus the reproductive index is zero in all cases.

Table 3.9 Mean weights (g ± SEM) during gestation of groups given diets containing Fishmeal or the stock diet

Diet	1	2	3	Stock
Number of animals	9	12	12	8
Day of gestation				
G0 (mated)	82.9 ± 3.4	86.5 ± 2.3	85.1 ± 2.5	85.6 ± 3.6
G1	81.4 ± 3.4	86.1 ± 2.6	84.7 ± 2.5	86.0 ± 3.6
G2	79.6 ± 3.4	85.8 ± 2.7	84.3 ± 2.6	86.6 ± 3.3
G3	77.9 ± 3.6	85.3 ± 2.5	83.9 ± 2.7	88.1 ± 3.8
G4	76.3 ± 3.8●	85.1 ± 2.4	83.9 ± 2.8	88.1 ± 3.3
G5	76.5 ± 3.5	84.5 ± 2.4	84.1 ± 2.9	87.1 ± 3.8
G6	77.4 ± 3.5	84.9 ± 2.6	84.9 ± 2.9	87.1 ± 3.7
G7	76.9 ± 3.4●	84.3 ± 2.6	85.5 ± 2.8	87.7 ± 3.5
G8	76.1 ± 3.7●	84.1 ± 2.8	85.4 ± 2.7	88.4 ± 3.6
G9	75.4 ± 3.9●	84.3 ± 2.9	85.4 ± 2.6	90.3 ± 3.9
G10	74.6 ± 3.9●●	84.4 ± 2.7	84.9 ± 2.7	93.0 ± 4.0
G11	73.9 ± 4.0●●	84.5 ± 2.8●	85.4 ± 3.0●	96.1 ± 4.1
G12	73.9 ± 4.1●●●	84.7 ± 2.8●●	87.1 ± 2.9●	99.1 ± 4.0*

Table 3.9 continued

Diet	1	2	3	Stock
Number of animals	9	12	12	8
Day of gestation				
G13	73.9 ± 3.9 ŧŧŧ	84.7 ± 2.8 ●●	88.1 ± 2.7 ●●	103.0 ± 3.9 **
G14	73.4 ± 4.0 ŧŧŧ	84.3 ± 2.8 ●●●	88.6 ± 3.0 ●●	106.7 ± 4.3 **
G15	73.4 ± 3.9 ŧŧŧ	88.8 ± 2.5 ***	88.3 ± 3.2 ●●●	110.4 ± 4.2 ***
G16 (birth)	63.5 ± 2.8 *** ŧŧŧ	72.2 ± 1.8 *** ●●●	75.1 ± 3.0 * ●●	93.3 ± 4.0
Weight gain G0 - G15	-9.5 ± 1.6 ŧŧŧ	-3.2 ± 2.1 ŧ●●	3.2 ± 2.0 ●●●	24.9 ± 2.9

Significant difference from weight at G0: *P < 0.05 **P < 0.01 ***P < 0.001
from stock group : ●P < 0.05 ●●P < 0.01 ●●●P < 0.001
from diet 3 group: +P < 0.05 ++P < 0.01 +++P < 0.001
from diet 2 group ŧP < 0.05 ŧŧP < 0.01 ŧŧŧP < 0.001

Figure 3.3 : Mean weight gains from mating through gestation and lactation of animals given diets containing fishmeal. The stock group is also shown for comparison.

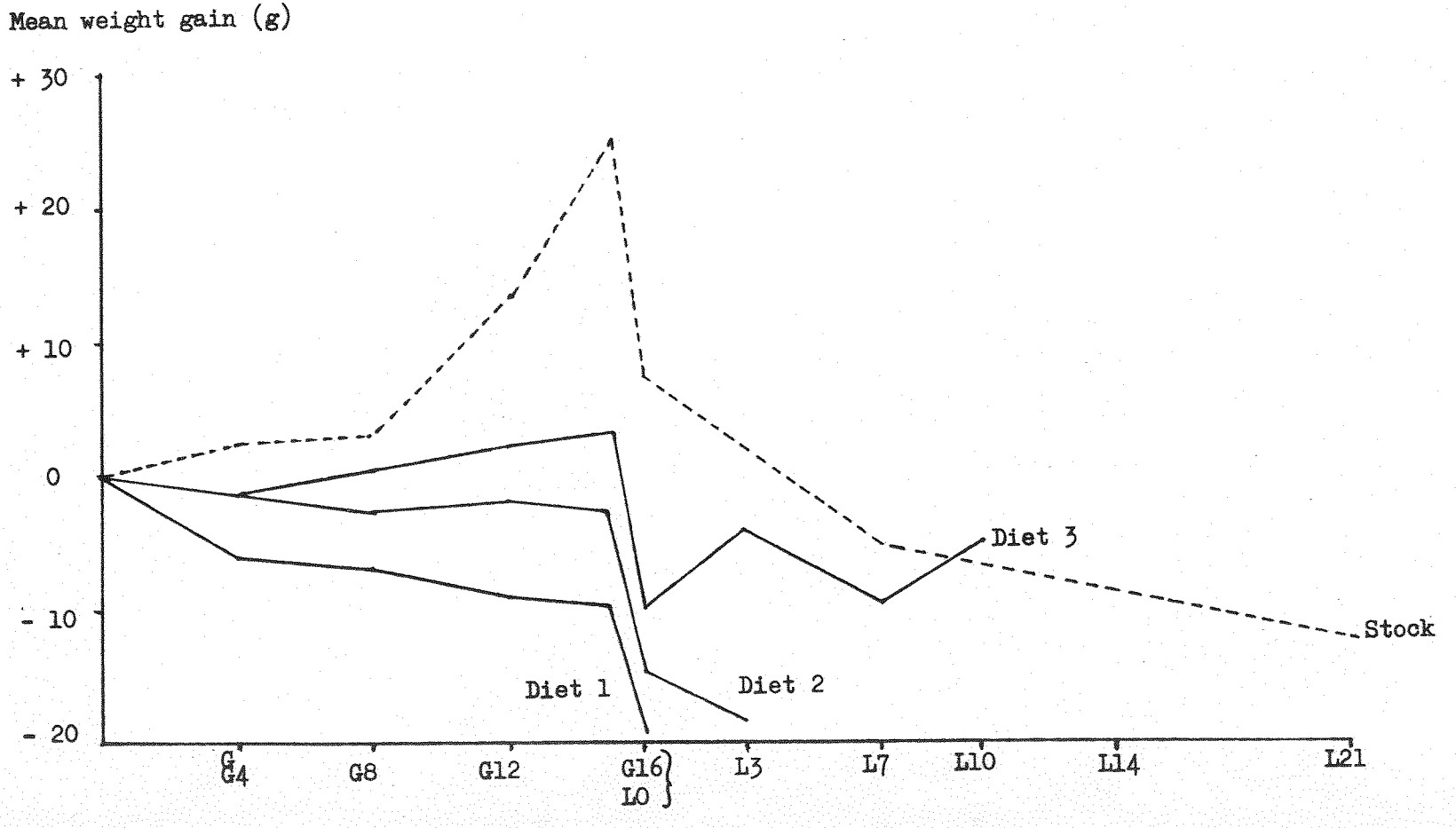


Table 3:10a Mean (± SEM) food intake (g/animal/d) during gestation of hamsters given diets containing Fishmeal or the stock diet.

Diet	1	2	3	Stock
Number of animals	9	12	12	8
Period of gestation				
G4 - G8	4.7 ± 0.6^{ab}	4.8 ± 0.4	4.8 ± 0.5^{ab}	6.6 ± 0.8
G8 - G12	3.7 ± 0.2^{a}	4.4 ± 0.4	3.8 ± 0.4^{a}	6.4 ± 0.5
G12 - G16	4.8 ± 0.4^{b}	4.5 ± 0.4	5.1 ± 0.4^{b}	7.5 ± 0.5
G4 - G16	4.4 ± 0.2	4.5 ± 0.2	4.7 ± 0.3	6.8 ± 0.5

a, b. within dietary groups, values without common superscript are significantly different ($P < 0.05$)

Table 3:10b Mean (± SEM) metabolizable energy intake(kJ/animal/d) during gestation of hamsters given giets containing Fishmeal or the stock diet.

Diet	1	2	3	Stock
Number of animals	9	12	12	8
Period of gestation				
G4 - G8	69.1 ± 8.8	68.0 ± 5.7	64.6 ± 6.7	73.1 ± 8.9
G8 - G12	54.4 ± 2.9●	62.4 ± 5.7	51.2 ± 5.4o	70.9 ± 5.6
G12 - G16	70.6 ± 5.9	63.8 ± 5.7●	68.6 ± 5.4	83.1 ± 5.6
G4 - G16	64.7 ± 2.9	63.8 ± 2.8●	63.4 ± 4.1	75.7 ± 5.5

Significant difference from stock group ●P< 0.05 ●●P< 0.01 ●●●P< 0.001
from diet 3 group +P< 0.05 ++P< 0.01 +++P< 0.001
from diet 2 group ‡P< 0.05 ‡‡P< 0.01 ‡‡‡P< 0.001

Table 3:11 Maternal weights (g) during lactation of groups given diets containing fishmeal or the stock diet (mean ± SEM (n))

	Diet			
Day of lactation	1	2	3	Stock
L0	63.5 ± 2.8(9)***●●●‡‡‡	72.2 ± 1.8(12)***●●●	75.1 ± 3.0(12)*●●	93.3 ± 4.0(8)
L3	-	68.5 ± 0.5(2)	81.0 ± 1.0(2)	88.0 ± 4.0(8)
L7	-	-	75.5 ± 3.5(2)	80.3 ± 4.7(8)
L10	-	-	80 (1)	79.0 ± 3.6(8)

Significantly different from weight at G0 *P< 0.05 ***P< 0.001
from stock group ●● P< 0.01 ●●●P< 0.001
from diet 3 group +P< 0.05
from diet 2 group ‡P< 0.05

Table 3:12a Food intake (g/animal/d) during lactation of groups given diets containing fishmeal or the stock diet (mean ± SEM (n))

	Diet			
	1	2	3	Stock
Period of lactation				
L0 - L3	-	2.7 ± 1.4 (2)	5.2 ± 0.5 (2)	8.9 ± 0.9 (8)
L3 - L7	-	-	6.3 ± 1.0 (2)	9.4 ± 1.4 (8)
L7 - L10	-	-	6.0 (1)	10.7 ± 1.4 (8)

Table 3:12b Metabolisable energy intake (kJ/animal/d) during lactation of groups given diets containing fishmeal or the stock diet (mean ± SEM (n))

	Diet			
	1	2	3	Stock
Period of lactation				
L0 - L3	-	38.3 ± 19.8 (2)●	70 0 ± 6.7 (2)	98.6 ± 10.0 (8)
L3 - L7	-	±	84.8 ±13.5 (2)	104.1 ± 15.5 (8)
L7 - L10	-	-	80.8 (1)	118.8 ± 15.5 (8)

Significantly different from stock group ●$P < 0.05$

Table 3:13 Data from litters born to mothers given diets containing fishmeal during gestation and lactation (mean ± SEM (n))

	Day of lactation	Total live weight of litter (g)	Live pups/litter	Pup weight (g)
a.Diet 1	L0	5.5 ± 0.5 (2)●●●	4.5 ± 0.5 (2)	1.40 ± 0.4 (8)●●●‡‡‡
	number of animals brought to term : 9			
b.Diet 2	L0	8.2 ± 1.0 (8)●●●	4.8 ± 0.7 (8)●	1.86 ± 0.05(38)+●●●
	L3	3.5 ± 1.5 (2)●●●	1.5 ± 0.5 (3)	2.33 ± 0.17(3)●●●
	number of animals brought to term : 12			
c.Diet 3	L0	6.9 ± 1.1 (8)●●●	4.1 ± 1.4 (8)	1.62 ± 0.10(33)●●●
	L3	6.8 ± 3.4 (3)●	2.0 ± 0.6 (3)●	3.40 ± 0.46(6)
	L7	14.8 ± 6.3 (2)	2.5 ± 0.5 (2)	5.92 ± 0.66(5)●●●
	L10	15.5 (1)	3.0 (1)	5.20 ± 0 (3)●●●
	number of animals brought to term : 12			

Significantly different from stock group ●P < 0.05 ●●●P < 0.001 (see table 3:3)
from Diet 3 group +P < 0.05
from Diet 2 group ‡‡‡P < 0.001

Groups offered diets containing mould

a) Maternal data during gestation

The mean weights of the pregnant females are shown in Table 3.14 and the weight gains from mating in Fig. 3.4. None of the groups became significantly heavier than their weight at G0 and therefore lost weight relative to the stock group. The only group which actually became lighter than its weight at mating was that given the diet containing only 5% reference protein. These animals lost approximately 10% of their body weight up to G15, at this time being significantly lighter than the Diet 2 and 3 animals as well as the stock group animals. At G15 animals on Diets 1, 2 and 3 weighed 70%, 80% and 84% respectively of the stock control group, all reductions being significant ($P<0.01$).

The food intake and energy intake of animals in these three dietary groups are shown in Table 3.15a and 15b. Throughout gestation the 15% reference protein group (Diet 3) had a lower metabolisable energy intake than the animals given stock diet, as did all three groups during the last period of gestation (G12-16). Considering the whole 12 day period (G4-16) mean energy intake decreased as the level of protein in the diet increased. The mean intakes were 78%, 72% amd 61% of that of the stock control group for Diets 1, 2 and 3 respectively.

b) Maternal data during lactation.

The weights of the lactating females are shown in Table 3.16. All the groups were lighter than the stock control at L0 but, in the 2 groups with surviving litters, the difference diminished i.e. their weight loss was less than that of the stock group. However these groups lost weight during the first two weeks of lactation to such an extent that the Diet 3 group was significantly lighter than its weight at G0 by L14 ($P< 0.05$). The weight losses are shown graphically in Fig. 3.4.

The food intake of the groups in g/animal/d (Table 3.17a) were constant throughout lactation and similar to the stock control group in terms of energy intake (kJ/animal/d)

Table 3.14 Mean weights (g ± SEM) during gestation of groups given diets containing Mould or the stock diet

Diet	1	2	3	Stock
Number of animals	8	13	11	8
Day of gestation				
G0 (mated)	83.0 ± 1.8	84.3 ± 2.6	84.6 ± 2.9	85.6 ± 3.6
G1	82.3 ± 1.8	83.4 ± 2.1	84.0 ± 2.9	86.0 ± 3.5
G2	81.1 ± 1.6	82.3 ± 2.2	82.4 ± 2.8	86.6 ± 3.8
G3	80.6 ± 1.6	81.9 ± 2.2	81.1 ± 2.7	88.1 ± 3.8
G4	80.0 ± 1.7	81.0 ± 2.4	79.9 ± 2.6	88.1 ± 3.8
G5	79.8 ± 1.5	79.7 ± 2.6	78.8 ± 2.8	87.1 ± 3.8
G6	79.6 ± 1.1	79.6 ± 2.8	78.0 ± 2.8	87.1 ± 3.7
G7	78.9 ± 1.3●	80.1 ± 3.0	77.6 ± 2.9●	87.7 ± 3.5
G8	78.5 ± 1.0●	81.1 ± 3.0	78.1 ± 3.0●	88.4 ± 3.6
G9	78.1 ± 0.6*●●	81.0 ± 3.3	79.5 ± 3.1●	90.3 ± 3.9
G10	78.0 ± 0.3*●●	81.5 ± 3.5●	80.9 ± 3.0●	93.0 ± 4.0
G11	78.0 ± 1.0*●●●	83.7 ± 3.7●	82.3 ± 2.9●	96.1 ± 4.1
G12	78.4 ± 1.1●●●	86.1 ± 3.7●	85.2 ± 2.9●	99.1 ± 4.0*

Table 3.14 continued

Diet	1	2	3	Stock
Number of animals	8	13	11	8
Day of gestation				
G13	78.3 ± 1.2●●●	87.2 ± 3.5●●	87.5 ± 3.0●●	103.0 ± 3.9**
G14	78.4 ± 1.0*●●●	88.6 ± 3.6●●	89.8 ± 2.8●●	106.7 ± 4.3**
G15	78.5 ± 1.0*●●●	89.3 ± 3.5●●	92.1 ± 2.6●●	110.4 ± 4.2***
G16 (birth)	68.9 ± 1.3***●●●	77.6 ± 3.5●●	79.8 ± 1.9●●	93.3 ± 4.0
Weight gain G0 - G15	-4.5 ± 1.9●●●	5.0 ± 2.6●●●	7.5 ± 1.8●●●	24.9 ± 2.9

Significant difference from weight at G0: *P< 0.05 **P< 0.01 ***P< 0.001
from stock group : ●P< 0.05 ●●P< 0.01 ●●●P< 0.001
from diet 3 group: +P< 0.05 ++P< 0.01 +++P< 0.001
from diet 2 group ∓P< 0.05 ∓∓P< 0.01 ∓∓∓P< 0.001

Figure 3.4 : Weight gains from mating through gestation and lactation of animals given diets containing mould. The stock group is included for comparison.

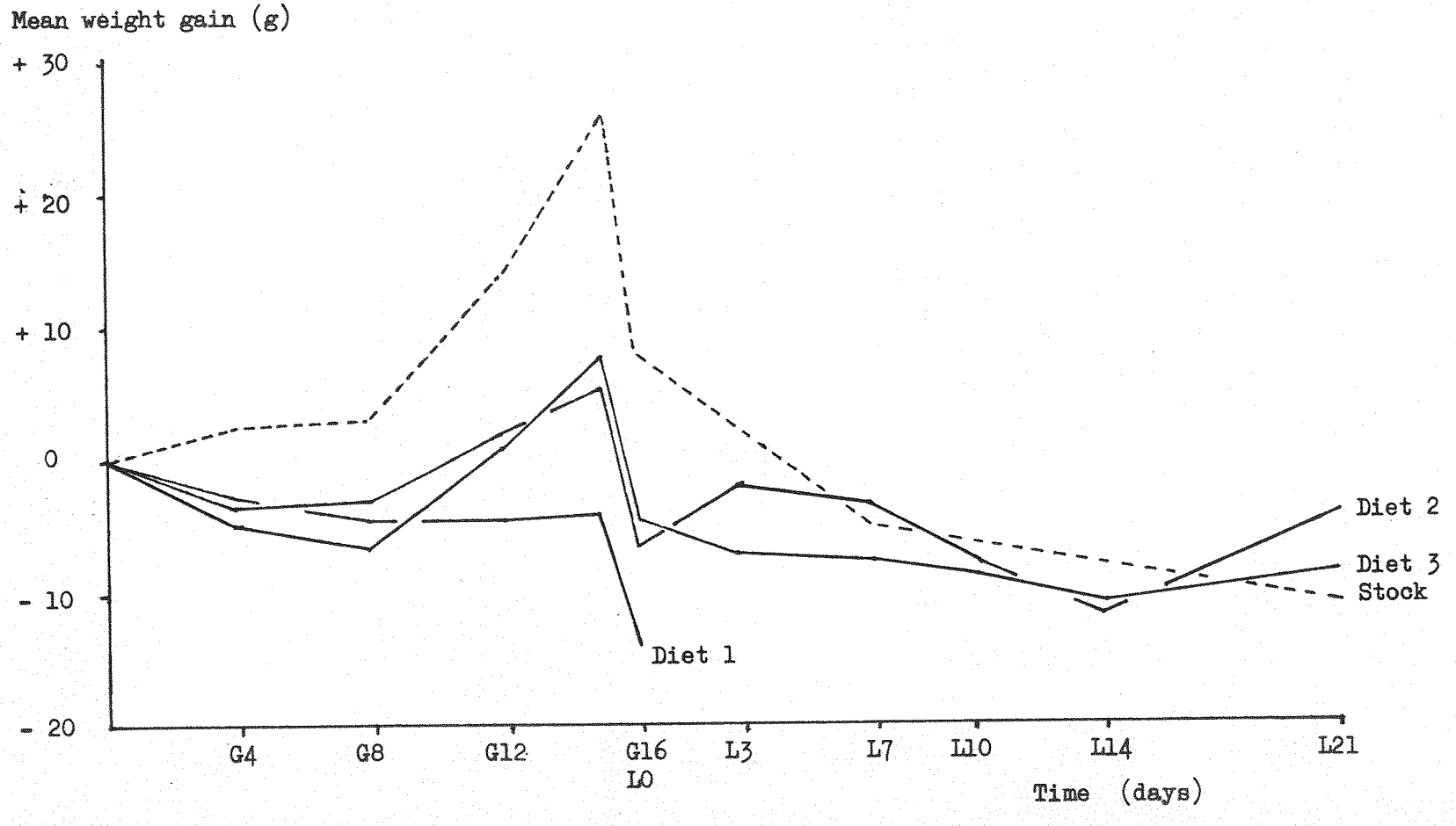


Table 3: 15a Mean (± SEM) food intake (g/animal/d) during gestation of hamsters given diets containing Mould or the stock diet.

Diet	1	2	3	Stock
Number of animals	8	13	11	8
Period of gestation				
G4 - G8	4.0 ± 0.4	3.6 ± 0.5	2.9 ± 0.3[a]	6.6 ± 0.8
G8 - G12	3.8 ± 0.3	4.1 ± 0.4	3.7 ± 0.4[a]	6.4 ± 0.5
G12 - G16	4.2 ± 0.3	4.3 ± 0.3	5.0 ± 0.3[b]	7.5 ± 0.5
G4 - G16	4.0 ± 0.2	4.0 ± 0.3	3.8 ± 0.3	6.8 ± 0.5

[a,b] within dietary groups, values without common superscript are significantly different ($P < 0.05$)

Table 3. 15b Mean (± SEM) metabolizable energy intake(kJ/animal/d) during gestation of hamsters given diets containing Mould or the stock diet.

Diet	1	2	3	Stock
Number of animals	8	13	11	8
Period of gestation				
G4 - G8	58.9 ± 6.0++	48.7 ± 7.2●	34.8 ± 4.0●●●	73.1 ± 8.9
G8 - G12	56.1 ± 4.9	55.5 ± 4.8	45.5 ± 4.3●●	70.9 ± 5.6
G12 - G16	61.2 ± 4.4●●	57.9 ± 4.1●●	60.4 ± 3.9●●	83.1 ± 5.6
G4 - G16	58.8 ± 2.9+	54.6 ± 4.1●●	46.4 ± 3.7●●●	75.7 ± 5.5

Significant difference from stock group ●P< 0.05 ●●P< 0.01 ●●●P < 0.001
from diet 3 group +P< 0.05 ++P< 0.01 +++P < 0.001
from diet 2 group ∓P< 0.05 ∓∓P< 0.01 ∓∓∓P< 0.001

Table 3.16 Maternal weights (g) during lactation of groups given diets containing Mould or the stock diet (mean ± SEM (n))

Day of lactation	Diet 1	2	3	Stock
L0	68.9 ± 1.3 (8)***●●●	77.6 ± 3.5 (13)●●	79.8 ± 2.0 (11)●●	93.3 ± 4.0 (8)
L3	-	82.0 ± 3.5 (4)	77.3 ± 1.9 (8)●	88.0 ± 4.0 (8)
L7	-	80.3 ± 2.8 (4)	76.3 ± 3.6 (6)	80.3 ± 4.7 (8)
L10	-	76.3 ± 3.7 (3)	76.0 ± 2.7 (6)	79.0 ± 3.6 (8)
L14	-	72.0 ± 4.5 (3)	73.0 ± 3.6 (6)*	76.9 ± 2.9 (8)
L21	-	79.3 ± 4.2 (4)	74.6 ± 3.7 (6)	74.0 ± 2.2 (8)*

Significant differences from weight at G0 *P< 0.05 ***P< 0.001
from stock group ●P< 0.05 ●●P< 0.01 ●●●P< 0.001

Table 3.17a Food intake (g/animal/d) during lactation of groups given diets containing Mould or the stock diet (mean ± SEM (n))

Period of lactation	Diet 1	2	3	Stock
L0 - L3	-	5.3 ± 0.4 (4)	7.0 ± 1.0 (8)	8.9 ± 0.9(8)
L3 - L7	-	6.8 ± 0.9 (4)	6.7 ± 0.7 (6)	9.4 ± 1.4(8)
L7 - L10	-	5.8 ± 1.3 (3)	9.6 ± 2.4 (6)	10.7 ± 1.4(8)
L10 - L14	-	8.2 ± 1.3 (3)	6.2 ± 0.5 (6)	10.7 ± 1.0(8)
L14 - L21	-	10.9 ± 2.8 (3)	8.6 ± 0.7 (6)	14.6 ± 2.2(8)*

Significant difference from L0 - L3 *$P < 0.05$

Table 3: 17b Metabolisable energy intake (kJ/animal/d) during lactation of groups given diets containing Mould or the stock diet (mean ± SEM (n))

Period of lactation	Diet 1	2	3	Stock
L0 - L3	-	72.3 ± 4.8 (4)	85.3 ± 12.2 (8)	98.6 ± 10.0(8)
L3 - L7	-	92.0 ± 12.5 (4)	81.9 ± 8.9 (6)	104.1 ± 15.5(8)
L7 - L10	-	79.0 ± 17.7 (3)	117.0 ± 29.3 (6)	118.5 ± 15.5(8)
L10 - L14	-	111.6 ± 17.7(3)++	75.7 ± 6.2 (6)●●	118.5 ± 11.1(8)
L14 - L21	-	148.4 ± 38.2 (3)	104.9 ± 8.5 (6)	161.8 ± 24.4(8)

Significantly different from Stock Group ●●$P < 0.01$
from Diet 3 Group ++$P < 0.01$

Table 3.18 Date from litters born to mothers given diets containing mould during gestation and lactation (mean ± SEM (n))

	Day of lactation	Total live weight of litter (g)	Live pups/litter	Pup weight (g)
a.Diet 1	L0	4.0 ± 1.1 (4)●●●	2.8 ± 0.8 (4)●●	1.46 ± 0.02 (11)●●●‡‡‡‡
	number of animals brought to term : 8			
a.Diet 2	L0	7.3 ± 1.1 (10)●●●	3.9 ± 0.6 (10)●●	1.86 ± 0.03 (39)●●●+++
	L3	6.0 ± 1.0 (5)●●●	2.2 ± 0.4 (5)●	2.77 ± 0.17 (11)●●●+++
	L7	13.0 ± 2.8 (5)●	2.2 ± 0.4 (5)	5.90 ± 0.42 (11)‡●●●
	L10	19.4 ± 3.2 (4)	2.3 ± 0.3 (4)	8.62 ± 0.51 (9)‡●●●
	L14	26.6 ± 3.4 (4)	2.3 ± 0.3 (4)	11.88 ± 0.56 (9)
	L21	38.4 ± 1.6 (4)	2.0 ± 0 (4)	19.20 ± 0.54 (8)●●
	number of animals brought to term : 13			
c.Diet 3	L0	8.7 ± 1.4 (10)●●●	4.1 ± 0.7 (10)●	2.14 ± 0.03 (41)●●●
	L3	6.6 ± 1.5 (9)●●●	1.9 ± 0.4 (9)●●	3.50 ± 0.14 (17)●●
	L7	15.2 ± 2.9 (7)●	2.1 ± 0.4 (7)●	7.11 ± 0.24 (15)
	L10	21.6 ± 3.8 (7)	2.1 ± 0.4 (7)	10.11 ± 0.32 (15)
	L14	26.4 ± 3.9 (7)●	2.0 ± 0.3 (7)	13.20 ± 0.74 (14)
	L21	41.3 ± 6.3 (7)●	1.9 ± 0.3 (7)	20.69 ± 1.31 (13)
	number of animals brought to term : 11			

Significantly different from stock group ●P<0.05 ●●P<0.01 ●●●P<0.001 (See Table 3:3)
from Diet 3 group +P<0.05 ++P<0.01 +++P<0.001
from Diet 2 group ‡P<0.05 ‡‡P<0.01 ‡‡‡P<0.001

(Table 3.17b) except for a reduction during one period in the Diet 3 group.

c) Pup data

The total weight of litters, live pups/litter and mean pup weight of the pups from the 3 groups throughout lactation are shown in Table 3.18a,b and c. The pups from the mothers on the lowest protein level had reduced values for all parameters at L0 and none survived to L3. At the two higher levels of protein the pups had reduced values for all parameters at days L0 and L3. The mean pup weight of the Diet 2 group was reduced throughout lactation but the other 2 parameters in this group were similar to those of the stock group during the latter part of lactation. In the high protein group the mean pup weight was comparable to that of the stock control group from L7 onwards but the total litter weight was somewhat reduced. This reflects a reduction in litter size compared to the stock group (although not significant after L7) in this group.

The indices of reproductive performance, as described for the stock group are shown in Table 3.30a - g.

Groups offered diets containing Soya Protein (sample 1)

a) Maternal data during gestation.

The maternal weights are shown in Table 3.19. The groups on the 2 higher levels of protein had weights similar to the stock control group throughout and were significantly heavier ($\simeq$ 10%) than their weight at G0 by G12. However the group on the lowest level of protein did not achieve a weight greater than that at G0 and therefore lost weight relative to the other 2 experimental groups, being significantly lighter (10%) by G3. At G15 the Diet 1 group was only 70% the weight of the stock control group, compared to 90% amd 95% (which were statistically similar to the stock weight) for animals on Diets 2 and 3 respectively. The weight gains from mating with the stock group included for comparison are shown in Fig. 3.5.

The food intakes in g/animal/d for the three periods

of gestation are shown in Table 3.20a. They were constant for each group throughout. The metabolisable energy intakes are shown in Table 3.20b. The only deviation from the stock group intake was in the Diet 1 group for the final period of gestation which had a reduced intake ($P<0.05$) but this was not a big enough reduction to result in a decreased energy intake for the whole period (G4 - G16) and all 3 groups were similar to the stock group in this respect.

b) Maternal data during lactation.

The weights of the lactating females are shown in Table 3.21. The weights of the animals were similar to the stock control weights throughout lactation in the case of the Diet 3 group and up to L14 in the Diet 2 group which became heavier relative to the stock group during the last week of lactation. The weight gains are shown in Fig. 3.5.

The food intake of the two groups with surviving litters are shown in Table 3.22a. After the first three days the high protein group increased its mean daily food intake. During the last week of lactation both groups had increased values compared to the first period of lacation which can be ascribed to the pups creep feeding, but may also be due to increased milk production. The energy intakes (Table 3.22b) were similar to the stock control group at the equivalent time of laction except in the Diet 3 group from L7 to L10 when it was increased ($P<0.05$). The energy intake of the Diet 2 group was generally lower than that of the Diet 3 group.

c) Pup data.

The pup data from the three dietary groups are shown in Table 3.23a, b and c. In the high protein group (Table 3.23c) the mean pup weight was reduced at various points along their growth curve relative to the stock group but were comparable to the stock pups by L21. The middle protein level had reduced total weight of litters and numbers of pups/litter, the mean pup weight being similar to that of the stock control group from L3 onwards. Mean pup weight in this group was lower than that of the Diet 3 group, perhaps reflecting

Table 3.19 Mean weights (g ± SEM) during gestation of groups given diets containing Soya protein (1) or the stock diet

Diet	1	2	3	Stock
Number of animals	10	13	12	8
Day of gestation				
G0 (mated)	83.1 ± 2.3	86.4 ± 1.9	86.0 ± 2.9	85.6 ± 3.6
G1	81.8 ± 2.1	87.0 ± 1.9	86.6 ± 3.0	86.0 ± 3.5
G2	79.6 ± 2.2	87.7 ± 1.9	87.1 ± 2.8	86.6 ± 3.8
G3	78.8 ± 2.4	87.8 ± 2.2	87.4 ± 2.7	88.1 ± 3.8
G4	78.0 ± 2.6	88.0 ± 2.3	88.4 ± 2.8	88.1 ± 3.8
G5	78.6 ± 2.6	88.0 ± 2.4	88.3 ± 2.8	87.1 ± 3.8
G6	78.9 ± 2.3	88.3 ± 2.4	88.5 ± 2.8	87.1 ± 3.7
G7	79.1 ± 2.4	88.7 ± 2.6	88.3 ± 2.8	87.7 ± 3.5
G8	79.4 ± 2.4	89.1 ± 2.8	88.5 ± 3.0	88.4 ± 3.8
G9	77.0 ± 2.8	89.7 ± 3.0	89.5 ± 3.2	90.3 ± 3.9
G10	77.0 ± 2.8	91.6 ± 2.9	91.1 ± 3.5	93.0 ± 4.0
G11	77.5 ± 3.2	93.4 ± 2.9	92.7 ± 4.0	96.1 ± 4.1
G12	78.8 ± 3.0	95.1 ± 2.8*	96.2 ± 3.4*	99.1 ± 4.0*

Table 3.19 continued

Diet	1	2	3	Stock
Number of animals	10	13	12	8
Day of gestation				
G13	80.0 ± 2.4	97.0 ± 2.8**	99.1 ± 3.4**	103.0 ± 3.9**
G14	79.4 ± 2.4	98.6 ± 2.7**	102.0 ± 3.4**	106.7 ± 4.3**
G15	80.3 ± 2.5	99.5 ± 2.7***	105.0 ± 3.4***	110.4 ± 4.2***
G16 (birth)	71.6 ± 2.0**	86.8 ± 2.2	90.4 ± 3.0	93.3 ± 4.0
Weight gain G0 - G15	-2.9 ± 2.0	13.1 ± 1.7oo	19.0 ± 3.2	24.9 ± 2.9

Significant difference from weight at G0: *P< 0.05 **P< 0.01 ***P< 0.001
from stock group : •P< 0.05 ••P< 0.01 •••P< 0.001
from diet 3 group: +P< 0.05 ++P< 0.01 +++P< 0.001
from diet 2 group : ǂP< 0.05 ǂǂP< 0.01 ǂǂǂP< 0.001

Figure 3.5 :Weight gains from mating through gestation and lactation of animals given diets containing soya protein (sample 1). The stock group is also shown for comparison.

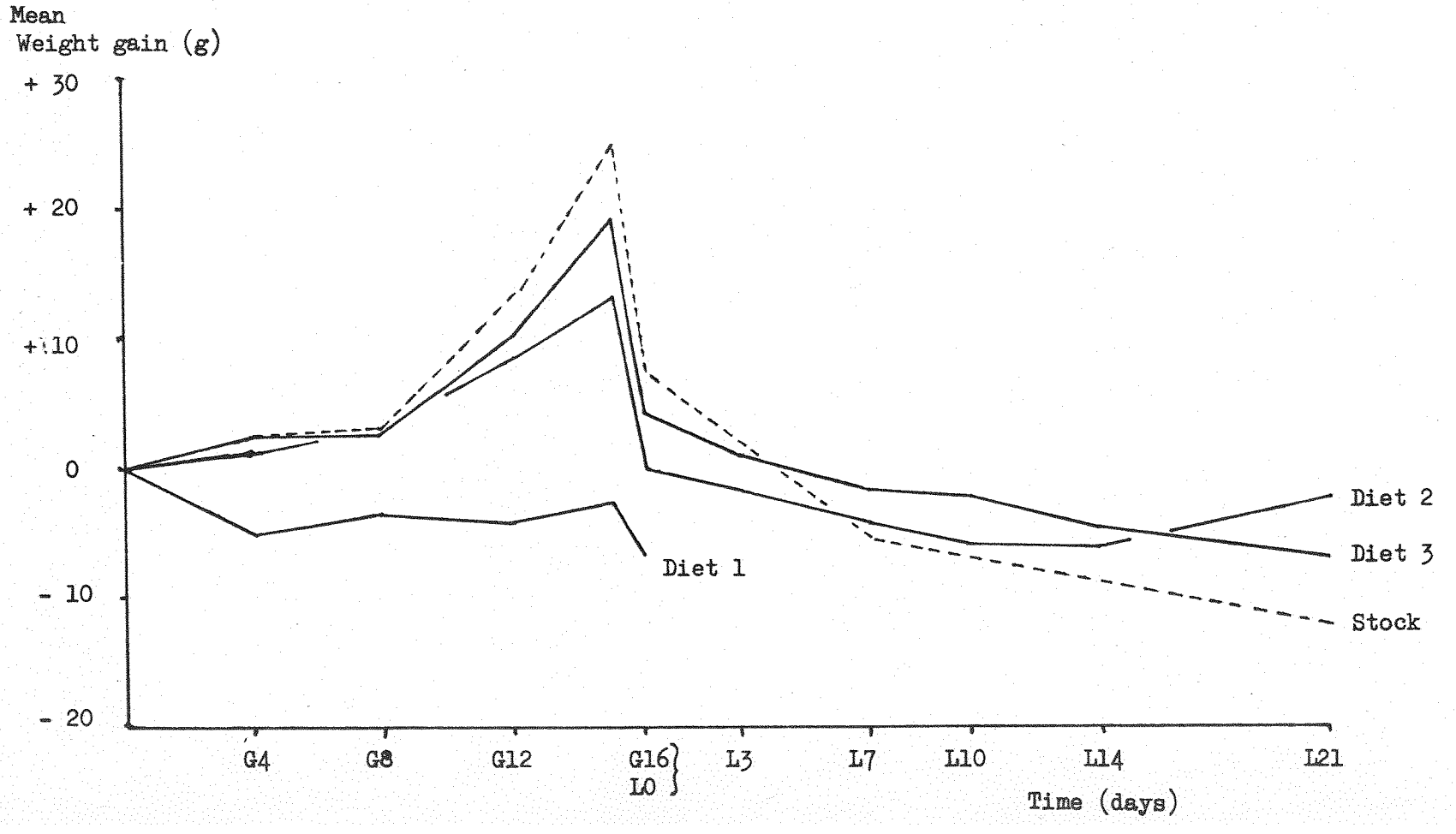


Table 3: 20a Mean (± SEM) food intake (g/animal/d) during gestation of hamsters given diets containing Soya protein (sample 1) or the stock diet.

Diet	1	2	3	Stock
Number of animals	10	13	12	8
Period of gestation				
G4 - G8	5.2 ± 0.4	5.7 ± 0.5	4.7 ± 0.4	6.6 ± 0.8
G8 - G12	4.2 ± 0.4	4.6 ± 0.3	4.9 ± 0.3	6.4 ± 0.5
G12 - G16	4.0 ± 0.5	5.4 ± 0.5	5.6 ± 0.3	7.5 ± 0.5
G4 - G16	4.4 ± 0.3	5.2 ± 0.3	5.0 ± 0.2	6.8 ± 0.5

Table 3.20b Mean (± SEM) metabolizable energy intake(kJ/animal/d) during gestation of hamsters given giets containing Soya Protein (sample 1) or the stock diet.

Diet	1	2	3	Stock
Number of animals	10	13	12	8
Period of gestation				
G4 - G8	80.3 ± 6.3	85.3 ± 7.4	70.6 ± 6.6	73.1 ± 8.9
G8 - G12	64.0 ± 6.6	68.6 ± 5.1	73.7 ± 5.0	70.9 ± 5.6
G12 - G16	62.0 ± 7.5+	81.3 ± 7.5	84.0 ± 4.7	83.1 ± 5.6
G4 - G16	67.6 ± 4.6	78.0 ± 4.5	70.6 ± 2.8	75.7 ± 5.5

Significant difference from stock group •$P < 0.05$
from diet 3 group +$P < 0.05$

Table 3. 21 Maternal weights (g) during lactation of groups given diets containing soya protein (sample 1) or the stock diet (mean ± SEM (n))

Day of lactation	Diet 1	2	3	Stock
L0	71.6 ± 2.0 (10)**●●●	86.8 ± 2.2 (13)	90.4 ± 3.0 (12)	93.3 ± 4.0 (8)
L3	-	84.8 ± 2.3 (9)	87.3 ± 3.0 (9)	88.0 ± 4.0 (8)
L7	-	82.1 ± 1.2 (9)	84.2 ± 3.1 (9)	80.3 ± 4.7 (8)
L10	-	80.8 ± 1.2 (9)*	83.7 ± 2.2 (9)	79.0 ± 3.6 (8)
L14	-	80.6 ± 1.2 (9)*	81.3 ± 2.8 (9)	76.9 ± 2.9 (8)
L21	-	84.1 ± 2.3 (9)●●	79.0 ± 2.4 (9)	74.0 ± 2.2 (8)

Significant differences from weight at GO: *P < 0.05 **P < 0.01
from stock group ●●P < 0.01 ●●●P < 0.001

Table 3.22a Food intake (g/animal/d) during lactation of groups given diets containing Soya protein (sample 1) or the stock diet (mean ± SEM (n))

Period of lactation	Diet 1	2	3	Stock
L0 - L3	-	6.0 ± 0.7 (9)	6.5 ± 0.8 (9)	8.9 ± 0.9(8)
L3 - L7	-	6.9 ± 0.5 (9)	8.7 ± 0.5 (9)*	9.4 ± 1.4(8)
L7 - L10	-	7.9 ± 0.8 (9)	10.5 ± 0.6 (9)***	10.7 ± 1.4(8)
L10 - L14	-	7.0 ± 0.8 (9)	8.6 ± 1.0 (9)	10.7 ± 1.0(8)
L14 - L21	-	9.0 ± 0.9 (9)*	13.1 ± 1.5 (9)**	14.6 ± 2.2(8)*

Significant difference from L0 - L3 *P< 0.05 **P< 0.01 ***P< 0.001

Table 3. 22b Metabolisable energy intake (kJ/animal/d) during lactation of groups given diets containing Soya protein (sample 1) or the stock diet (mean ± SEM (n))

Period of lactation	Diet 1	2	3	Stock
L0 - L3	-	90.3 ±10.0 (9)	99.9 ±12.2 (9)	98.6 ± 10.0(8)
L3 - L7	-	103.8 ± 7.4 (9)+	133.8 ± 8.3 (9)	104.1 ± 15.5(8)
L7 - L10	-	118.9 ±11.9 (9)+	161.5 ± 8.9 (9)●	118.5 ± 15.5(8)
L10 - L14	-	105.3 ±11.8 (9)	132.3 ±15.1 (9)	118.5 ± 11.1(8)
L14 - L21	-	135.4 ±14.1 (9)+	201.5 ±23.4 (9)	161.8 ± 24.4(8)

Significant difference from stock group : ●P< 0.05
from Diet 3 group : +P< 0.05

Table 3.23 Data from litters born to mothers given diets containing soya protein (sample 1) during gestation and lactation (mean ± SEM (n))

	Day of lactation	Total live weight of litter (g)	Live pups/litter	Pup weight (g)
a.Diet 1	L0	2.9 ± 0.9 (4)‡‡‡	1.5 ± 0.5 (4)‡‡‡●●	1.90 ± 0.14 (6)‡‡‡+++
	number of animals brought to term : 10			
b.Diet 2	L0	8.3 ± 1.0 (13)●●●	3.9 ± 0.5 (13)●●	2.12 ± 0.03 (51)●●●+++
	L3	8.0 ± 1.1 (11)●●●+++	2.2 ± 0.3 (11)●●+++	3.68 ± 0.15 (24)+
	L7	14.5 ± 1.9 (11)●●+++	2.1 ± 0.2 (11)●●+++	6.91 ± 0.34 (23)++
	L10	18.8 ± 3.0 (11)●●+++	1.9 ± 0.2 (11)●●+++	9.85 ± 0.49 (21)+
	L14	25.6 ± 4.1 (11)●+++	1.9 ± 0.2 (11)●+++	13.42 ± 0.64 (21)
	L21	42.4 ± 7.0 (11)●++	1.9 ± 0.2 (11)●++	21.19 ± 1.44 (21)+
	number of animals brought to term : 13			
c.Diet 3	L0	12.9 ± 1.5 (12)	5.3 ± 0.6 (12)	2.41 ± 0.02 (64)●●
	L3	17.2 ± 1.8 (11)	4.3 ± 0.4 (11)	4.03 ± 0.06 (47)
	L7	33.9 ± 3.1 (11)	4.2 ± 0.4 (11)	8.12 ± 0.22 (46)●
	L10	45.8 ± 4.3 (11)	4.2 ± 0.4 (11)	10.95 ± 0.22 (46)●●●
	L14	58.7 ± 5.9 (11)	4.1 ± 0.5 (11)	14.36 ± 0.41 (45)●
	L21	90.5 ± 15.3 (11)	3.6 ± 0.5 (11)	25.25 ± 0.89 (39)
	number of animals brought to term : 12			

Significantly different from stock group *●P < 0.05 ●●P < 0.01 ●●●P < 0.001
from diet 3 group +P < 0.05 ++P < 0.01 +++P < 0.001
from diet 2 group ‡P < 0.05 ‡‡P < 0.01 ‡‡‡P < 0.001

*(see Table 3:3)

the mothers reduced energy intake (Table 3.22b). The group supplied with the diet containing the lowest level of protein (Diet 1) showed reduced values in all three parameters at birth and no pups survived the neonatal period.

The indices of reproductive performance as described for the stock group are shown in Table 3.30a - g.

Groups offered diets containing Soya Protein (sample 2)

a) Maternal data during gestation

The mean weights of the group are shown in Table 3.24 and shown graphically as weight gain from mating with the stock group included for comparison in Fig. 3.6. The group on Diet 1 did not change its mean weight from that at G0 for the first 15 days and were 15% lighter than the stock controls at G11 ($P < 0.02$). This difference increased to 23% by G15 ($P \leq 0.001$). The group on the two higher levels of protein had mean weights which were not dissimilar to those of the stock control group throughout gestation, although the animals on Diet 3 increased their weight earlier than the stock controls and those on Diet 2 only increased their weight by 15% in 15 days as opposed to 22% in the stock control group.

The food intakes in g/animal/d are shown in Table 3.25a and as kJ/animal/d in Table 3.25b. The metabolisable energy intake of the group on Diet 1 was reduced during the second half of pregnancy compared to the stock group and the group on Diet 3, resulting in a reduced energy intake for the whole period (G4 - G16) compared to the 3 other groups. Energy intakes of the other two experimental groups were similar to the stock group.

b) Maternal data during lactation

The maternal data for these three groups of animals are shown in Tables3.26 (mean weights), 3.27a (food intake in g/animal/d) and 3.27b (metabolisable energy intake in kJ/animal/d). With the exception of a reduced mean weight at L0 in the Diet 1 group, there were no differences in weight

Table 3.24 Mean weights (g ± SEM) during gestation of groups given diets containing Soya protein(2) at the stock diet

Diet	1	2	3	Stock
Number of animals	13	12	13	8
Day of gestation				
G0 (mated)	85.3 ± 2.4	85.1 ± 3.3	88.8 ± 2.3	85.6 ± 3.6
G1	85.3 ± 2.4	85.8 ± 3.7	89.8 ± 2.4	86.0 ± 3.5
G2	85.3 ± 2.4	86.6 ± 3.9	90.9 ± 2.4	86.6 ± 3.8
G3	85.2 ± 2.4	87.3 ± 4.0	89.5 ± 3.0	88.1 ± 3.8
G4	85.4 ± 2.3	87.7 ± 4.2	92.3 ± 2.4	88.1 ± 3.8
G5	86.1 ± 2.2	87.8 ± 4.2	93.2 ± 2.4	87.1 ± 3.8
G6	86.7 ± 2.3	88.6 ± 4.3	93.9 ± 2.4	87.1 ± 3.7
G7	86.3 ± 2.4	89.0 ± 4.3	94.8 ± 3.4	87.7 ± 3.5
G8	85.9 ± 2.7+	89.6 ± 4.2	95.6 ± 2.4*	88.4 ± 3.6
G9	85.6 ± 2.9++	90.8 ± 4.3	97.4 ± 2.2*	90.3 ± 3.9
G10	84.5 ± 2.9+++	92.3 ± 4.2	99.3 ± 2.3**	93.0 ± 4.0
G11	83.5 ± 2.8●+++	94.8 ± 4.2	101.7 ± 2.3***	96.1 ± 4.1
G12	84.8 ± 2.9●●+++	97.1 ± 4.2*	104.4 ± 2.3***	99.1 ± 4.0*

Table 3. 24 continued

Diet	1	2	3	Stock
Number of animals	13	12	13	8
Day of gestation				
G13	85.0 ± 2.9 ●● +++ ‡	97.7 ± 4.1*	106.8 ± 2.6***	103.0 ± 3.9**
G14	84.9 ± 2.8 ●●● +++ ‡	97.6 ± 4.4*	108.0 ± 2.8***	106.7 ± 4.3**
G15	84.9 ± 2.8 ●●● +++ ‡	97.6 ± 4.6* +	109.7 ± 3.1***	110.4 ± 4.2***
G16 (birth)	71.4 ± 2.4 *** ●●● +++	80.7 ± 4.2	90.6 ± 2.7	93.3 ± 4.0
Weight gain G0 - G15	-0.4 ± 2.0 ●●● ‡‡‡	12.5 ± 2.6 ●●	21.1 ± 3.1	24.9 ± 2.9

Significant difference from weight at G0: *P < 0.05 **P < 0.01 ***P < 0.001
from stock group : ●P < 0.05 ●●P < 0.01 ●●●P < 0.001
from diet 3 group: +P < 0.05 ++P < 0.01 +++P < 0.001
from diet 2 group: ‡P < 0.05 ‡‡P < 0.01 ‡‡‡P < 0.001

Figure 3.6 : Maternal weight gains from mating through gestation and lactation of animals given diets containing soya protein (sample 2). The stock group is also shown for comparison.

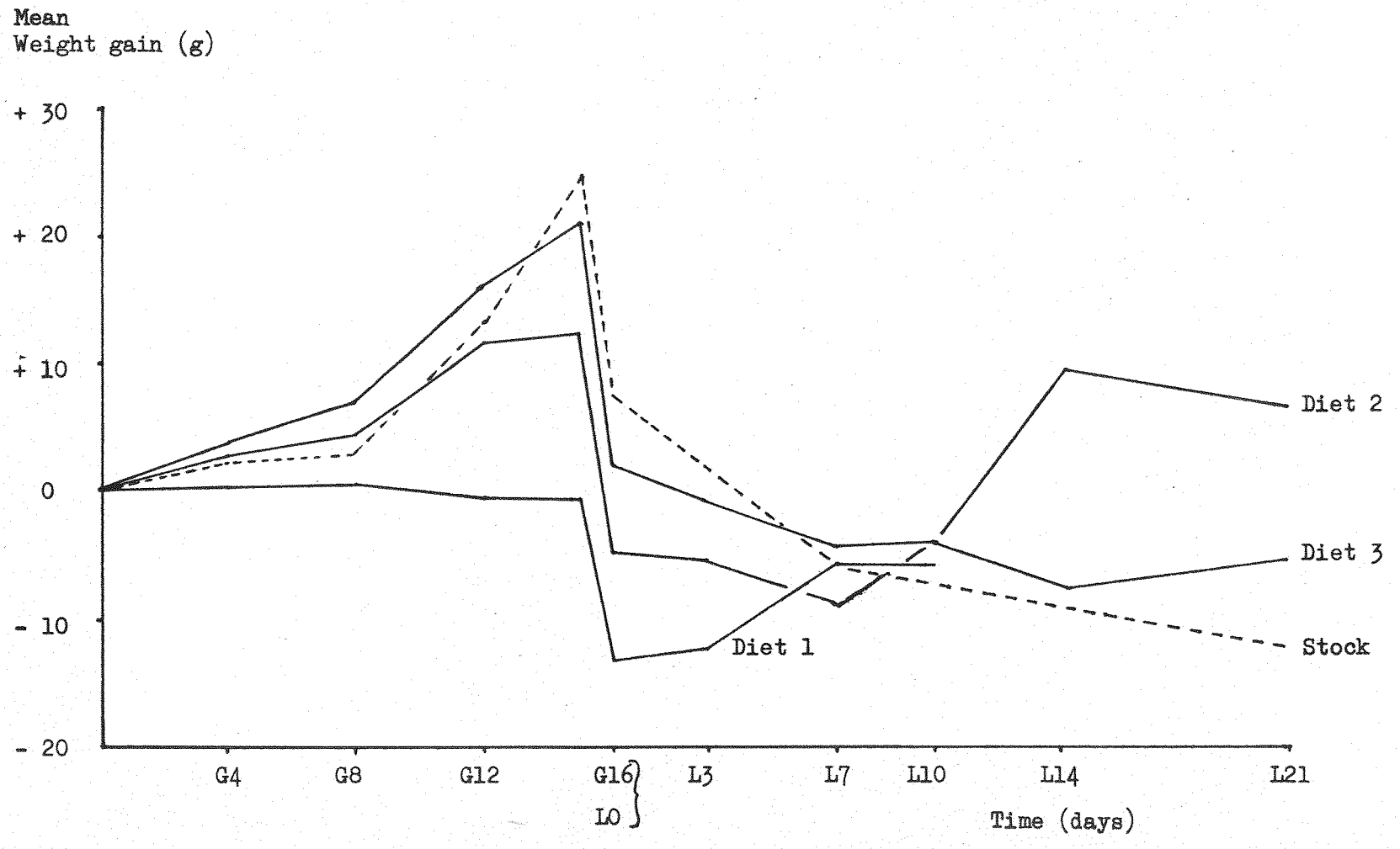


Table 3.25a Mean (± SEM) food intake (g/animal/d) during gestation of hamsters given diets containing Soya protein (sample 2) or the stock diet.

Diet	1	2	3	Stock
Number of animals	13	12	13	8
Period of gestation				
G4 - G8	4.6 ± 0.2 [a]	4.8 ± 0.4	5.2 ± 0.5	6.6 ± 0.8
G8 - G12	3.3 ± 0.3 [b]	4.5 ± 0.5	4.5 ± 0.3	6.4 ± 0.5
G12 - G16	3.8 ± 0.5 [ab]	5.1 ± 0.6	5.3 ± 0.4	7.5 ± 0.5
G4 - G16	3.9 ± 0.2	4.8 ± 0.3	5.0 ± 0.3	6.8 ± 0.5

a, b within dietary groups, values without common superscript are significantly different ($P < 0.05$)

Table 3.25b Mean (± SEM) metabolizable energy intake kJ/animal/d) during gestation of hamsters given giets containing Soya protein (sample 2) or the stock diet.

Diet	1	2	3	Stock
Number of animals	13	12	13	8
Period of gestation				
G4 - G8	70.8 ± 3.7	71.5 ± 6.5	77.8 ± 6.8	73.1 ± 8.9
G8 - G12	50.5 ± 4.5●+	67.0 ± 6.9	67.7 ± 5.1	70.9 ± 5.6
G12 - G16	59.2 ± 7.7●+	76.7 ± 8.4	79.9 ± 6.2	83.1 ± 5.6
G4 - G16	60.0 ± 3.1	72.1 ± 4.5	75.2 ± 4.5	75.7 ± 5.5

Significant difference from stock group ●$P < 0.05$
from diet 3 group +$P < 0.05$

Table 3. 26 Maternal weights (g) during lactation of groups given diets containing soya protein (sample 2) or the stock diet (mean ± SEM (n))

Day of lactation	Diet 1	2	3	Stock
L0	71.4 ± 2.4 (13)***●●●+++	80.7 ± 4.2 (12)	90.6 ± 2.7 (13)	93.3 ± 4.0 (8)
L3	73.0 ± 6.5 (3)+	80.0 ± 4.2 (4)	87.9 ± 2.3 (7)	88.0 ± 4.0 (8)
L7	80.0 (1)	76.3 ± 5.6 (4)	84.5 ± 3.1 (6)	80.3 ± 4.7 (8)
L10	80.0 (1)	81.7 ± 5.9 (3)	84.8 ± 0.6 (5)	79.0 ± 3.6 (8)
L14	-	95.0 (1)	81.0 ± 3.0 (3)	76.9 ± 2.9 (8)
L21	-	92.0 (1)	83.5 ± 0.5 (2)	74.0 ± 2.2 (8)*

Significant differences from weight at G0 ***P < 0.001
from stock group ●●●P < 0.001
from diet 3 group +P < 0.05 +++P < 0.001

Table 3. 27a Food intake (g/animal/d) during lactation of groups given diets containing Soya protein (sample 2) or the stock diet (mean ± SEM (n))

Period of lactation	Diet 1	2	3	Stock
L0 - L3	4.7 ± 1.9 (3)	4.2 ± 1.5 (4)	5.8 ± 1.2 (7)	8.9 ± 0.9(8)
L3 - L7	5.5 (1)	4.4 ± 0.5 (4)	6.5 ± 0.8 (6)	9.4 ± 1.4(8)
L7 - L10	5.3 (1)	4.2 ± 1.4 (3)	7.8 ± 0.9 (5)	10.7 ± 1.4(8)
L10 - L14	-	10.3 (1)	9.8 ± 0.9 (3)	10.7 ± 1.0(8)
L14 - L21	-	8.9 (1)	17.6 ± 4.5 (2)**	14.6 ± 2.2(8)*

Significant difference from period L0-L3 **P < 0.01

Table 3:27b Metabolisable energy intake (kJ/animal/d) during lactation of groups given diets containing Soya protein (sample 2) or the stock diet (mean ± SEM (n))

Period of lactation	Diet 1	2	3	Stock
L0 - L3	72.3 ± 29.3 (3)	62.3 ±22.6 (4)	87.4 ±18.1 (7)	98.6 ± 10.0(8)
L3 - L7	84.9 (1)	66.0 ± 7.8 (4)	97.8 ±11.3 (6)	104.1 ± 15.5(8)
L7 - L10	81.9 (1)	63.1 ±21.1 (3)	117.5 ±14.1 (5)	118.5 ± 15.5(8)
L10 - L14	-	155.1 (1)	147.6 ±13.1 (3)	118.5 ± 11.1(8)
L14 - L21	-	133.8 (1)	265.0 ±67.7 (2)	161.8 ± 24.4(8)

Table 3.28 Data from litters born to mothers given diets containing soya protein (sample 2) during gestation and lactation (mean ± SEM (n))

	Day of lactation	Total live weight of litter (g)	Live pups/litter	Pup weight (g)
a.Diet 1	L0	5.8 ± 1.1 (2)	3.4 ± 0.7 (10)	1.86 ± 0.06 (34)
	L3	2.8 ± 0.32 (2)	1.0 ± 0 (2)	2.8 ± 0.3 (2)
	L7	3.0 (1)	1.0 (1)	3.0 (1)
	L10	3.0 (1)	1.0 (1)	3.0 (1)
	number of animals brought to term : 13			
b.Diet 2	L0	9.7 ± 1.2 (9)	5.0 ± 0.3 (9)	1.95 ± 0.03 (45)
	L3	8.3 ± 1.4 (6)	2.5 ± 0.3 (6)	3.28 ± 0.13 (15)
	L7	12.6 ± 1.4 (5)	2.6 ± 0.2 (5)	4.85 ± 0.16 (13)
	L10	12.0 ± 2.9 (4)	2.5 ± 0.3 (4)	4.83 ± 0.42 (10)
	L14	30.0 (1)	3.0 (1)	10.0 ± 0 (3)
	L21	43.5 (1)	3.0 (1)	14.5 ± 0 (3)
	number of animals brougt to term : 12			

Table 3.28 continued

c.Diet 3 Day of lactation	Total live weight of litter (g)	Live pups/litter	Pup weight (g)
L0	13.6 ± 1.5 (12)	6.2 ± 0.6 (12)	2.21 ± 0.04 (74)●●●
L3	15.4 ± 3.1 (8)	3.5 ± 0.5 (8)	4.42 ± 0.18 (28)●
L7	22.0 ± 5.7 (7)	3.4 ± 0.7 (7)	6.44 ± 0.32 (24)●●●
L10	29.0 ±10.5 (5)	3.4 ± 1.1 (5)	8.55 ± 0.42 (17)●●●
L14	59.3 ± 6.2 (3)	5.0 ± 0.6 (3)	11.87 ± 0.04 (15)●
L21	107.0 ± 26.9 (3)	5.0 ± 0.6 (3)	21.42 ± 1.09 (15)

number of animals brought to term : 13

Significantly different from stock group* ●P < 0.05 ●●P < 0.01 ●●●P < 0.001
from Diet 3 group +P < 0.05 ++P < 0.01 +++P < 0.001
from Diet 2 group ∓P < 0.05

*(see Table 3:3)

from the stock control data. Similarly there were no significant differences from the stock group in energy intake during lactation.

c) Pup data

The data from the pups of the mothers fed the highest protein level (Diet 3) (Table 3.28c) show that the mean weight was reduced (except at L3) up to L10 compared to the stock group pup weight. The pups from the Diet 2 group (Table 3.28b) had a reduced mean weight throughout lactation and the total litter weight was similarly reduced. All three parameters were reduced in the data from the pups born to Diet 1 mothers (Table 3.28a)

The indices of reproductive performance as described for the stock group are shown in Table 3.30a - g.

Overall reproductive performance

Maternal weight gains and food intakes are summarised in Table 3.29.

The various indices of reproductive performance which were decribed earlier were calculated for all groups and are shown in Table 3.30 a - g.

Reproductive Index

The simplest way to compare the overall reproductive performance of the groups is by using the reproductive index (RI) (Table 3.30a). This takes into account the total weight of offspring at 21 days, which is a function of the number of pups surviving and their weight, and relates it to the number of animals brought to term. The value achieved by the stock groups was 85.2. The only experimental group to show a similar result was that supplied with 15% reference protein derived from soya protein (sample 1) which had a RI of 82.1. The reproductive indices of all other dietary groups were reduced, the next highest being those of the Diet 2 (10% reference protein) soya protein (sample 1) and Diet 3 casein groups which were approximately 33. In the Diet 3 mould and Diet 3 soya protein (sample 2) groups the RI was 25 and in the remaining groups it was very low. In all Diet 1

groups and all fishmeal groups no pups survived to 21 days of age thus the RI was zero.

Litter size (live) at birth and neonatal mortality

Data on litter size at birth, which has been presented earlier in the result sections for each protein source, is summarised in Table 3.30b for all dietary groups. Using the individual litter data, as opposed to the mean values, the 15 groups were compared by analysis of variance. There was a significant difference due to the level of protein in the diet ($P < 0.01$) but not to the protein source incorporated. The Tukey test (Steel and Torie '60) was used to compare the protein level means and the results of the tests are indicated in the table.

Neonatal mortality was defined as the number of deaths between birth and day 3 post partum calculated as a percentage of the number live at birth. There was a wide variation in the mean values of the 16 dietary groups from 25% for the groups on stock diet and Diet 3 soya protein (sample 1) up to 100% for all groups on Diets 1 (5% reference protein) with the exception of the group supplied with soya protein (sample 2) (Table 3.30c). Treatment of the individual litter data by analysis of variance shows a difference due to the level of reference protein in the diet ($P < 0.01$) and the protein source used ($P < 0.01$). The results of analysis using the Tukey test are shown in the table.

Litter size at day 3 and postnatal mortality

The mean litter size for experimental Diets 2 and 3 are summarised in Table 3.30d. The only Diet 1 group with any surviving pups was that given soya protein (sample 2) thus an analysis of variance was performed on just the 10 groups shown. The individual litter data was used in the comparison which shows a significant difference in litter size due to both the protein level ($P < 0.01$) and the protein source ($P < 0.05$) in the diet. The total mean values for the 2 levels and 5 sources were compared using the Tukey test and the differences are shown in the table.

Similarly for the postnatal mortality observations (Table 3:30e) a comparison was performed on Diets 2 and 3 of the 5 protein sources by the analysis of variance and between the source means by Tukey's test. There was no difference in the mortality, calculated as a percentage of live pups at L3, between L3 and L21 which was due to protein level in the maternal diet but there was considerable variation due to the protein source used, from 11% with soya protein (sample 1) up to 100% with fishmeal.

Survival of offspring

The data on survival of offspring has been evaluated in two ways. Table 3.30f shows the mean number of pups which survived per litter in each dietary group. The result of an analysis of variance of the individual litter data showed a difference in survival due both to the level of protein in the diet ($P < 0.01$) and also the source from which the protein was derived ($P < 0.01$). The results of comparing the means by Tukey's test are shown in the table. Table 3.30g shows the percentage of litters which were viable i.e. those which had pups surviving to weaning at 21 days of age. The rate of survival increased with the level of reference protein in the diet with a significant difference between the means of the Diet 1 and 3 groups ($P < 0.05$). There was also a difference due to the protein source where the range was from no viable litters when the mothers were fed diets containing fishmeal to 88.5% of the litters being viable when the major protein source was soya protein (sample 1). By considering the data in both these tables it can be seen that although the percentage of live pups at birth which survived to 21 days of age was similar to that in the stock group in at least 3 cases (soya protein (sample 1) Diets 2 and 3 and mould Diet 3) the number of viable litters produced was lower than the stock group in all experimental groups.

Table 3.29 Summary of mean maternal weight gain (G4-G15) and nutrient intake data (G4-G16) during gestation for all dietary groups

	Diet 1	2	3
Weight gain (g)			
Casein	-.9.3	2.8	16.8
Fishmeal	-.2.9	-1.3	4.6
Mould	-1.5	8.3	12.2
Soya protein (1)	3.5	11.5	16.6
Soya protein (2)	-0.5	9.9	17.3
Stock	22.3		
Metabolisable energy intake (kJ)			
Casein	610.4	689.6	828.4
Fishmeal	776.4	776.8	737.6
Mould	704.8	648.4	562.8
Soya protein (1)	825.2	940.8	913.2
Soya protein (2)	722.0	860.8	901.6
Stock	908.4		
Protein intake (g)			
Casein	2.24	5.06	9.50
Fishmeal	3.01	6.08	9.04
Mould	2.69	5.18	7.47
Soya protein (1)	3.06	6.97	10.09
Soys protein (2)	4.02	10.89	16.92
Stock	16.49		
Reference protein intake (g)			
Casein	2.08	4.70	8.81
Fishmeal	2.75	5.52	8.17
Mould	2.35	4.70	6.87
Soya protein (1)	2.63	6.15	8.94
Soya protein (2)	2.29	5.99	9.30
Stock	15.63		

Table 3.30a Reproductive index achieved with the experimental diets.

	Diet 1	2	3
Casein	0	9.2	31.2
Fishmeal	0	0	0
Mould	0	11.8	24.5
Soya protein (1)	0	35.8	82.1
Soya protein (2)	0	3.6	24.7
Stock group	85.2		

Table 3.30b Litter size (live) at birth

	Diet 1	2	3	Mean for all levels
Casein	2.14 (7)	3.44 (9)	5.13 (8)	3.62 (24)
Fishmeal	4.50 (2)	4.75 (8)	4.13 (8)	4.45 (18)
Mould	2.75 (4)	3.90 (10)	4.10 (9)	3.78 (23)
Soya protein (1)	1.50 (4)	3.92 (13)	5.30 (12)	4.16 (29)
Soya protein (2)	3.40 (10)	5.00 (9)	6.17 (12)	4.94 (31)
Mean for all protein sources	2.59(27)^{a}	4.16(49)^{b}	5.10(49)^{c}	

abc means without common superscripts are significantly different ($P < 0.01$)

Stock group 6.75 ± 0.59 (8)

Table 3.30c Neonatal (L0 - L3) mortality as % of live pups at L0

	Diet 1	Diet 2	Diet 3	Mean for all levels
Casein	100 (7)	66.1 (9)	48.0 (8)	69.95 (24)^{AB}
Fishmeal	100 (2)	93.3 (8)	77.0 (8)	86.78 (18)^{B}
Mould	100 (4)	76.1 (10)	40.7 (9)	66.39 (23)^{AB}
Soya protein (1)	100 (4)	52.1 (13)	26.3 (12)	48.03 (29)^{A}
Soya protein (2)	88 (10)	65.8 (9)	60.3 (12)	70.84 (31)^{B}
Mean for all sources	95.56(27)^{a}	68.81(49)^{b}	49.09(49)^{c}	

^{abc}Protein level means: values without common superscript are significantly different $P < 0.01$

^{AB}Protein source means: values without common superscript are significantly different $P < 0.05$

Stock group: 23.6 ± 5.49 (8)

Table 3.30d Litter size at L3

	Diet 2.	Diet 3	Mean for all levels
Casein	2.00 (5)	3.14 (7)	2.67 (12)^{AB}
Fishmeal	1.50 (2)	2.00 (3)	1.80 (5)^{AB}
Mould	2.10 (5)	2.00 (9)	2.07 (14)^{A}
Soya protein (1)	2.18 (11)	4.27 (11)	3.23 (22)^{B}
Soya protein (2)	2.67 (6)	3.50 (8)	3.14 (14)^{AB}
Mean for all sources	2.21 (29)^{a}	3.18 (38)^{b}	

^{ab}Protein level : values without common superscript are significantly different ($P < 0.01$)

^{AB}Protein source: values without common superscript are significantly different ($P < 0.05$)

Stock group: 4.88 ± 0.78 (8)

Table 3.30e Postnatal (L4 - L21) mortality as a % of live pups at L3

	Diet 1	2	3	Mean for all levels
Casein		60.0 (5)	46.4 (7)	52.1 (12)^{BC}
Fishmeal		100 (2)	100 (3)	100 (5)^{C}
Mould		33.2 (5)	33.3 (9)	33.3 (14)^{AB}
Soya protein (1)		6.8 (11)	15.4 (11)	11.1 (22)^{A}
Soya protein (2)	100	83.3 (6)	62.5 (8)	71.4 (14)^{BC}
Mean for all sources		42.79 (29)	41.95(38)	

^{ABC}Protein sources: values without common superscripts are significantly different ($P < 0.05$)

Stock group 34.3 $\pm$ 10.8 (8)

Table 3.30f Mean survival of pups to L21 as a % of those live at birth.

	Diet 1	2	3	Mean for all sources
Casein	0 (7)	17.8 (9)	35.6 (8)	18.54 (24)^{BC}
Fishmeal	0 (2)	0 (8)	0 (8)	0 (18)^{C}
Mould	0 (4)	18.7 (10)	46.4 (9)	26.30 (23)^{AB}
Soya protein (1)	0 (4)	44.6 (13)	61.4(12)	45.45 (29)^{A}
Soya protein (2)	0 (10)	6.7 (9)	20.5(12)	9.87 (31)^{BC}
Mean for all sources	0 (27)^{a}	20.14(49)^{b}	34.43(49)^{c}	

^{abc}Protein levels: values without common superscripts are significantly different ($P < 0.05$)

^{ABC}Protein sources: values without common superscripts are significantly different ($P < 0.05$)

Stock group 51.8 $\pm$ 10.3 (8)

Table 3.30g Survival of litters to L21 as a % of those produced.

	Diet 1	Diet 2	Diet 3	Mean of all levels
Casein	0	15	36	25.5^{B}
Fishmeal	0	0	0	0^{B}
Mould	0	31	64	47.5^{AB}
Soya protein (1)	0	85	92	88.5^{A}
Soya protein (2)	0	8	23	15.5^{B}
Mean for all sources	0^{a}	27.8^{ab}	42.4^{b}	

^{ab}Protein levels: values without common superscripts are significantly different ($P<0.05$)

^{AB}Protein sources: values without common superscripts are significantly different ($P<0.05$)

Stock group 100%

Table 3. 31 Data from the termination of pregnancies after 15 days of gestation in 4 females given stock diet (number of foetuses:n=38)

Number of implantation sites	10.25 ± 0.95
Number live foetuses	9.50 ± 1.19
Number resorptions	0.75 ± 0.48
Total resorptions	0/4
Mean length foetuses (mm)	35.8 ± 0.41
Mean weight foetuses (g)	1.557 ± 0.04
Length/weight ratio	23.44 ± 0.47
Foetal placenta weight (g)	0.227 ± 0.01
Foetus weight/placenta weight	5.69 ± 0.11

Table 3.32 Data from termination of pregnancies after 15 days of gestation in females given diets containing casein(mean ± SEM)

	Diet 1	Diet 2	Diet 3
No. animals (n)	3	4	5
no. implantation sites	11.67 ± 0.33	11.25 ± 0.75	10.20 ± 0.80
No. live foetuses	8.3 ± 1.5	10.0 ± 0.4	9.2 ± 0.9
No. resorptions	3.30 ± 1.20	1.25 ± 0.48	1.00 ± 0.32
Total resorptions	2/5	0/4	0/5
Total no. foetuses (n)	14	40	36
Foetus length (mm)	34.9 ± 0.65	35.1 ± 0.36	34.5 ± 0.43●
Foetus weight (g)	1.482 ± 0.08	1.491 ± 0.03	1.424 ± 0.04●
Length/weight ratio	24.21 ± 0.98	23.83 ± 0.34	24.85 ± 0.60
Foetal placenta weight (g)	0 176 ± 0.01●● ++ ‡‡‡	0.244 ± 0.01	0.233 ± 0.01
Foetus weight/ placenta weight	8.65 ± 0.58●●● +++ ‡‡‡	6.22 ± 0.17●	6.19 ± 0.16●

(see Table 3.31)

Significantly different from Stock group ●P< 0.05 ●●P< 0.01 ●●●P< 0.001

from Diet 3 group ++P< 0.01 +++P< 0.001

from Diet 2 group ‡‡‡P< 0.001

Table 3.33 Data from termination of pregnancies after 15 days of gestation in females given diets containing Fishmeal (mean ± SEM)

	Diet 1	Diet 2	Diet 3
No. animals (n)	3	4	4
no. implantation sites	10.33 ± 0.67	10.50 ± 1.03	9.00 ± 1.78
No. live foetuses	8.0 ± 0.7	9.3 ± 1.2	7.3 ± 1.7
No. resorptions	2.33 ± 1.45	1.25 ± 0.25	1.75 ± 0.75
Total resorptions	1/4	1/5	0/4
Total no. foetuses (n)	15	26	29
Foetus length (mm)	30.7 ± 0.81 ●●● ‡‡‡	33.2 ± 0.40 ●● +++	36.1 ± 0.49
Foetus weight (g)	1.053 ± 0.06 ●●● ‡‡‡	1.287 ± 0.03 ●●● +++	1.544 ± 0.04
Length/weight ratio	30.24 ± 1.34 ●●● +++ ‡‡‡	26.03 ± 0.39 ●●● +++	23.76 ± 0.45
Foetal placenta weight (g)	0.177 ± 0.01 ●● ‡‡‡	0.220 ± 0.01 ++	0.258 ± 0.01 ●
Foetus weight / placenta weight	6.11 ± 0.29	5.96 ± 0.21	6.03 ± 0.16

Significantly different from Stock group ● $P < 0.05$ ●● $P < 0.01$ ●●● $P < 0.001$ (see table 3.31)

from Diet 3 group ++ $P < 0.01$ +++ $P < 0.001$

from Diet 2 group ‡‡ $P < 0.01$ ‡‡‡ $P < 0.001$

Table 3.34 Data from termination of pregnancies after 15 days of gestation in females given diets containing Mould (mean ± SEM)

	Diet 1	Diet 2	Diet 3
No. animals (n)	5	4	6
no. implantation sites	11.0 ± 1.14	10.75 ± 1.32	9.33 ± 1.41
No. live foetuses	8.2 ± 1.4	9.8 ± 1.0	8.5 ± 1.3
No. resorptions	2.8 ± 1.46	1.0 ± 0.58	0.8 ± 0.31
Total resorptions	1/6	0/4	0/6
Total no. foetuses (n)	25	39	30
Foetus length (mm)	33.4 ± 0.34●●● ‡‡‡	34.7 ± 0.40+	36.1 ± 0.51
Foetus weight (g)	1.269 ± 0.03●●● ‡‡‡	1.489 ± 0.50	1.582 ± 0.05
Length/weight ratio	26.57 ± 0.44●●● ‡‡‡	23.83 ± 0.46	23.32 ± 0.55
Foetal placenta weight (g)	0.182 ± 0.01●● +++ ‡‡	0.232 ± 0.01	0.245 ± 0.01
Foetus weight/ placenta weight	7.24 + 0.37●●●	6.72 ± 0.30●●	6.57 + 0.22●●●

(see Table 3.31)

Significantly different from Stock group ●●P < 0.01 ●●●P < 0.001

from Diet 3 group ‡P < 0.05

from Diet 2 group ‡P < 0.05 ‡‡P < 0.01 ‡‡‡P < 0.001

Table 3.35 Data from termination of pregnancies after 15 days of gestation in females given diets containing soya protein (sample 1) (mean ± SEM)

	Diet 1	Diet 2	Diet 3
No. animals (n)	4	6	4
no. implantation sites	10.0 ± 1.15	11.3 ± 0.21	10.5 ± 0.50
No. live foetuses	7.5 ± 1.0	8.8 ± 0.5	8.3 ± 1.1
No. resorptions	2.5 ± 0.29●	2.5 ± 0.43●	2.25 ± 1.11
Total resorptions	1/5	0/6	0/4
Total no. foetuses (n)	24	36	32
Foetus length (mm)	31.9 ± 0.46●●● ‡‡‡	33.9 ± 0.50●●	33.8 ± 0.52●●
Foetus weight (g)	1.187 ± 0.04●●● ‡‡‡	1.352 ± 0.05●●	1.307 ± 0.04●●●
Length/weight ratio	27.03 ± 0.64●●●	25.82 ± 0.65●●	26.36 ± 0.58●●●
Foetal placenta weight (g)	0.163 ± 0.01 ●●● +++ ‡‡‡	0.212 ± 0.01	0.222 ± 0.01
Foetus weight/ placenta weight	7.42 ± 0.32●●● ‡‡‡	6.51 ± 0.23●● ‡	5.93 ± 0.11

(See table 3.31)

Significantly different from Stock group ●P< 0.05 ●●P < 0.01 ●●●P< 0.001

from Diet 3 group +P< 0.05 +++P< 0.001

from Diet 2 group ‡P< 0.05 ‡‡P < 0.01 ‡‡‡P< 0.001

Table 3.36 Data from termination of pregnancies after 15 days of gestation in females given diets containing soya protein (sample 2) (mean ± SEM)

	Diet 1	Diet 2	Diet 3
No. animals (n)	4	5	4
no. implantation sites	9.5 ± 1.44	10.2 ± 0.49	10.0 ± 0.41
No. live foetuses	7.8 ± 0.7	9.2 ± 0.7	9.3 ± 0.5
No. resorptions	1.78 ± 1.18	1.00 ± 0.32	0.75 ± 0.75
Total resorptions	1/5	0/5	0/4
Total no. foetuses (n)	25	34	37
Foetus length (mm)	33.6 ± 0.44 ●●● ‡‡‡	36.9 ± 0.5+	35.4 ± 0.36
Foetus weight (g)	1.338 ± 0.04 ●●● ‡‡‡	1.694 ± 0.08	1.542 ± 0.03
Length/weight ratio	25.75 ± 0.52 ●● ‡‡+	22.79 ± 0.68	23.13 ± 0.28
Foetal placenta weight (g)	0.228 ± 0.01 +++ ‡‡	0.276 ± 0.01 ●●●	0.304 ± 0.01 ●●●
Foetus weight/ placenta weight	5.97 ± 0.16 +++	6.26 ± 0.29 +++	5.14 ± 0.09 ●●●

Significantly different (See Table 3.31)

from Stock group ●●P < 0.01 ●●●P < 0.001

from diet 3 group +P < 0.05 ++P < 0.01 +++P < 0.001

from diet 2 group ‡‡P < 0.01 ‡‡‡P < 0.001

Results of terminating pregnancies after 15 days of gestation (day G15) (Experiment 2)

Females offered stock diet

The results from the females supplied with stock diet are shown in Table 3.31. There were no cases where the whole litter was being resorbed. The mean value of all parameters are considered to be the normal state of pregnancy after 15 days of gestation in our hamster colony and the effect of the experimental diets on these parameters will be described.

Females offered diets containing casein

The mean weight and length of the foetuses from the Diet 3 group mothers were reduced compared to the stock group ($P < 0.05$) (Table 3.32). The proportions of the foetuses were maintained, the length/weight ratio being similar to that of the stock group foetuses. The mean weight of the foetal placenta was reduced in the low protein group compared to the stock group and the other two experimental groups. Similarly, the foetus/placenta ratio was increased in this group compared to the other three groups. The ratio was also increased in the groups on Diets 2 and 3 compared to the stock group.

Females offered diets containing fishmeal.

The data on the 15 day old foetuses is shown in Table 3.33. They show a marked reduction in the weight ($P < 0.001$) and length ($P < 0.001$) of foetuses from mothers in the two lower protein groups compared with the stock group and Diet 3 group. Furthermore there was an increase in the lenght/weight ratio ($P < 0.001$) indicating small and thin pups in these groups. In the Diet 1 group there was a decrease in the mean weight of the foetal placenta compared to the other three groups producing an increase in the foetus weight/placenta weight ratio although this was not statistically significant.

Females offered diets containing mould

The foetal data for the mould groups is shown in Table 3.34. Most of the data were different from the stock

equivalents in the Diet 1 (5% reference protein) group, with the length and weight of the foetuses reduced, the weight of the foetal placenta reduced and the length/weight and the foetus/placenta ratios both increased. The foetus/placenta ratio was also increased in the two higher protein groups relative to the stock group. In all other respects the groups given Diets 2 and 3 produced similar results to the stock group.

Females offered Diets containing Soya Protein (sample 1)

The data collected when the pregnancies of animals supplied with diets containing soya protein (sample 1) were terminated are shown in Table 3.35. The lengths and weights of the foetuses in all groups were reduced compared to the stock group and the length/weight ratio was increased in all cases. The foetuses from Diet 1 animals were lighter and shorter than those from Diet 2 and 3 animals. The mean foetal placenta weight was reduced in the Diet 1 group compared to the other three groups and this caused an increase in the foetus weight/placenta weight ratio.

Females offered diets containing Soya Protein (sample 2)

The data from the termination of pregnancies of females in these three groups are shown in Table 3.36. The weights and lengths of the foetuses from the low protein females were reduced compared to the stock group and those of Diets 2 and 3, also the length/weight ratio was increased in the Diet 1 foetuses indicating small thin pups. The weight of the foetal placenta was increased in the 2 higher groups such that, in the Diet 3 group, the foetus weight/placenta weight ratio was decreased ($P<0.001$). Both these parameters in the Diet 1 group were similar to those of the stock group.

Size of litter at termination of pregnancy (G15)

The data concerning litter size at the termination of pregnancy for the 15 experimental groups were treated by the analysis of variance using the individual litter data. This was done in three parts, these being the total number of implantation sites, the number of live foetuses and the

number of resorption sites. There was found to be no difference in any instance due to the source of protein incorporated in the diet. The effects due to the level of reference protein in the diet are summarised below. The figures are the total means for each level including all sources of protein.

	Level of Reference Protein			
	5%	10%	15%	Statistical difference between levels.
Mean no.implantation sites.	10.47	10.83	9.78	NS
Mean no.live foetuses.	7.95	9.39	8.52	NS
No. of resorption sites.	2.53[a]	1.48[ab]	1.26[b]	$P < 0.05$
No. of observations.	19	23	23	

[ab]Comparison of mean by Tukey's test. Values without common superscript are significantly different $P < 0.05$

The analysis shows that there was a significant difference in the number of foetuses which died before the end of gestation which is due to the level of protein in the diet. As the level of protein increased the number of foetal deaths was reduced. Although there was no statistical evidence of an accompanying increase in the number of live foetuses (or decrease in the number of total sites) the difference in resportion cannot be ignored and the discrepancy must arise from insufficient data.

In vitro pancreas incubations.

The results of in vitro pancreas incubations are shown in Table 3.37. There are no data from Diet 1 groups or from groups given diets containing fishmeal because no offspring survived to 21 days of age. No sex differences were observed thus male and femala data have been combined. The only group which gave results consistently different from those of the stock group was the one containing offspring born to mothers given the diet containing the equivalent of 15% reference protein derived from casein. The basal secretion

Table 3.37 In vitro secretion of insulin by pancreases of 21 days old hamsters born to and reared by females given experimental diet or stock diet. Values are ng insulin secreted/pancreas (mean ± SEM).

Protein source.	n	Incubation medium: Basal	+ Glucose	+ Glucose + Leucine	Increment due to: Glucose;	Leucine
Diet 2 (10% reference protein).						
Casein	5	1.7 ± 0.1	22.9 ± 0.5	37.4 ± 8.1^{+}	21.1 ± 4.6	14.6 ± 7.0
Mould	8	2.7 ± 0.5	25.5 ± 4.4	42.1 ± 6.2	20.9 ± 5.0	18.1 ± 2.4
Soya protein (1)	21	2.7 ± 0.4	22.4 ± 3.5	43.6 ± 4.3	19.7 ± 3.3	21.2 ± 3.2
Soya protein (2)	3	1.7 ± 0.2	11.2 ± 5.1	33.3 ± 9.3	9.5 ± 5.0	22.2 ± 4.3
Diet 3 (15% reference protein)						
Casein	15	4.2 ± 0.9$^{\bullet}$	40.0 ± 4.9$^{\bullet\bullet\bullet}$	55.0 ± 2.4$^{\bullet\bullet\bullet}$	35.6 ± 4.6$^{\bullet\bullet\bullet}$	15.0 ± 2.2
Mould	13	2.0 ± 0.3	18.9 ± 4.9	41.7 ± 5.5	16.8 ± 4.8	22.9 ± 3.6$^{\bullet}$
Soya protein (1)	39	3.2 ± 0.7	25.9 ± 2.3	42.2 ± 2.8	22.5 ± 2.2	16.4 ± 1.8
Soya protein (2)	14	3.2 ± 5.4	24.4 ± 6.0	38.4 ± 6.7	14.7 ± 3.5	14.0 ± 3.3
Stock diet	29	2.3 ± 0.3	18.6 ± 3.0	34.2 ± 3.8	17.0 ± 2.9	15.6 ± 1.6

Significantly different from stock group: $\bullet P < 0.05$ $\bullet\bullet\bullet P < 0.001$

from Diet 3 group: $+P < 0.05$

of this group (4.2 ng insulin) was higher than that of the stock group (2.3 ng insulin), a difference which was significant ($P < 0.05$). Under the influence of glucose and glucose + leucine the secretion of the casein group was increased to 40.0 and 55.0 ng insulin for the 30 minute incubation periods and these values were considerably higher than the corresponding stock group results (18.6 ng and 34.2 ng insulin) ($P < 0.001$). The increase in secretion relative to the stock group was due to the presence of glucose, the increment due to leucine in this group being similar to all other groups. The pancreases of this group of offspring were not significantly heavier than those of the stock group (Table 3.38).

In general the behaviour of pancreases from all other dietary groups was similar to that of the stock control group. The pancreases of three of the four Diet 2 groups (casein excluded) were lighter than the Diet 3 counterparts but only those of the mould group were similarly lighter than those of the stock group. Thus the amount of insulin secreted was not a reflection of total pancreas weight.

Table 3.38 Mean (± SEM (n)) wet weights (mg) of pancreases of 21 day old hamsters born to and reared by females given experimental diets or the stock diet.

Dietary protein Source	Diet 2 (10% reference protein)	Diet 3 (15% reference protein)
Casein	65.7 ± 6.6 (5)	64.1 ± 6.1 (15)
Mould	42.3 ± 3.2 (8)•+	51.9 ± 2.9 (13)
Soya protein (1)	49.7 ± 2.7 (21)++	59.6 ± 2.3 (39)
Soya protein (2)	38.5 ± 1.9 (3)+	63.4 ± 5.1 (14)
Stock	56.1 ± 3.1 (29)	

Significantly different from stock group: •$P < 0.05$
from Diet 3 group: +$P < 0.05$ ++$P < 0.01$

Discussion

The ability of the hamsters to reproduce on the experimental diets must be assessed by the final outcome of pregnancy, that is the weight of the pups at the end of lactation and also their survival to this age. Diets containing the equivalent of 5% reference protein were totally inadequate for reproduction regardless of the source of the dietary protein. To identify the factors contributing to this poor overall performance all the stages of reproduction must be considered. The females were well nourished prior to the time they were paired with the males, but they could have eaten the experimental diets for three days before mating took place so it was possible that differences in the ovulation rates may have been produced. Also, since implantation occurs on the sixth day of gestation this event could also have been affected by the diet. No evidence was found for either possibility; in fact on the contrary, when the pregnancies were terminated on the 15th day of gestation the mean number of implantation sites in the uteruses of the females in each dietary group were not

statistically different. Kenney (1975) made a similar observation on the effect on implantation in rats of a low protein diet given from mating. Although the total number of implantation sites in the uteruses was similar in all dietary groups there was a difference in the number of resorptions which became evident when all the dietary groups were considered. As the level of dietary protein decreased so did the number of resorptions per litter increase. Resorptions of foetuses did not affect calculations of the survival of offspring since these were based on the number of live pups at birth but they did contribute to low values for reproductive index which were based on the number of animals brought to term. A further observation which should be noted regarding the number of total resportions was not taken into account in any of the calculations. In the 25 pregnancies terminated where the diet contained the equivalent of 5% reference protein 6 animals resorbed the entire litter. This compared unfavourably with 1 total resorption out of 24 pregnancies when the equivalant of 10% reference protein was supplied and no total resportion in 31 pregnancies when either 15% reference protein or the stock diet were given. Curtiss (1953)reported that with 5% protein (as casein) in the diet 52% of dams did not complete gestation. This observation was made on rats, thus a species difference is apparent. It is possible that hamsters having a gestation period of only 16 days, as opposed to approximately 22 days in rats, can complete gestation with less dietary protein simply because they have to use their body reserves to compensate for a shorter time period.

So even considering only animals still carrying live litters after 15 days of gestation there was a greater loss of foetuses in utero with low protein diets and, assuming this situation also exist in the pregnancies which were not terminated, this would have contributed to the difference in litter size at birth which was shown to be due to the level of reference protein in the diet, the live litter size decreasing with decreasing dietary protein. The difference in litter size at

birth, however, was not as great as that at the end of lactation, that is, the low protein diets had more severe effects during lactation than gestation. This observation cannot necessarily be interpreted as an indication that a higher level of dietary protein is necessary for lactation than gestation, as has been previously reported for rats (Blaxter, 1964) because to investigate this the dietary regimes would have to be imposed at the beginning of lactation so that at this time all groups were in similar nutritional states. It has been suggested that the foetus has a very high priority for the nutrients available and that the mother compensates for a poor diet by utilising her body reserves (Naismith, 1969; Hammond, 1944; Platt and Stewart, 1967, 1968). All the low protein groups in this trial had mean weights at the end of gestation significantly lower than those at mating and they were also lighter than the stock group and those receiving the same dietary protein at higher levels; thus they were in a nutritionally depleted state at the beginning of lactation. It has been shown in rats that the offspring of low protein (6 or 7% by weight in the diet) mothers which are suckled by high protein (18-24% by weight in the diet) mothers show a marked improvement in survival and conversely that the survival of offspring from well nourished dams suckled by low protein mothers is severely impaired (Venkatachalam and Ramanathan, 1967; Zeman, 1964). Hence it is very probable that here also if the low protein offspring had been suckled by well nourished mothers their survival would have been improved, but by extrapolating from the observations of these other workers it is unlikely that the effects of the prenatal malutrition could be totally overcome. However, in the parameters monitored, no changes were found in the pups or foetuses which were consistently associated with poor subsequent survival.

In the groups given diets containing fishmeal, mould and soya protein (sample 2) the animals given the low protein diets produced foetuses of reduced length and weight with

increased ~~weight/length~~ length/weight ratios compared to the stock group and the group given 15% reference protein derived from the same protein source. The fishmeal Diet 2 (10% reference protein) group had parameters similar to its low protein counterpart whereas those of the mould and soya protein (sample 2) Diet 2 groups were generally similar to the higher protein and stock groups. Within the casein group only the high protein females produced foetuses with a reduced mean weight. In this case the mean length was similarly reduced and the length/weight ratios of this and the other two casein groups were similar to that of the stock group. When the protein source was soya protein (sample 1) however all 3 groups of foetuses had reduced mean weights and lengths and also the length/weight ratio was increased compared to that of that of the stock group; so again the foetuses were not only lighter but thinner than those of the stock control group. Thus the increased length/weight ratio observed by Zeman (1967) in rat foetuses (indicating that the weight more than the length was affected by prenatal protein deprivation) was seen here also in some groups given lower levels of dietary protein but this factor did not seem to have any bearing on subsequent survival since pups from the mothers given Diet 2 and 3 containing soya protein (sample 1) showed similar survival rates to those from stock group mothers. The foetal data from the low protein casein group also seemed somewhat incongruous being the only low protein foetuses of "normal" size.

In all but the two experimental groups given the high protein diets containing soya protein (samples 1 and 2) there was a reduction in the total live birth weight of the litters. In the 5 groups given either the fishmeal, the 10% reference protein soya protein (sample 2) or the 15% reference protein casein diets, this reduction in litter weight reflected a reduced pup weight with the litter sizes in general being similar to that of the stock group . In all other groups the reduction in total litter weight was attributable to a reduct-

The mean pup weight at birth was in fact lower than that of the stock group in all groups given experimental diets. Thus the mean weights of all the pups at birth showed a different relationship to the stock group than those of the foetuses after 15 days of gestation in 7 out of the 15 experimental groups. The variation that developed during the last day of gestation was perhaps not surprising since the period of gestation in this species is so short that there are only 10 days for growth to take place after implantation.

No evidence was found to suggest that the length of gestation was affected by the level of protein in the diet. In a range of the dietary groups when the night of mating was determined by the appearance of a vaginal plug in the female the animals were allowed to come to term and deliver their young; birth always occurred during the 16th night after mating hence none of the foestuses at termination of pregnancies should be considered less mature than others.

It might be considered that a low foetus weight would be associated with a low placenta weight but this is not invariably so (McLaren, 1965; Tremblay, Sybulski and Maughan, 1965). If this were true the foetus/placenta ratio would be constant but in the experiment described here this was not so. The ratio was higher than that of the stock group in all groups given casein and mould diets and with the two lower levels of soya protein (sample 1). The group given the high level of soya protein (sample 2) had an increased placenta weight giving a reduced ratio compared to that of the stock group. The foetus/placenta ratio did not appear to have a direct bearing on the ultimate outcome of reproduction, i.e. survival of the offspring, since it was "normal" in all fishmeal groups and also the low protein group given soya protein (sample 2).

The time of greatest risk for any foetus is probably during the perinatal period - the 24h period from approximately 12h before to 12h after birth - although the perinatal mortality rate cannot be calcuated here directly, an estimate of the loss

may be made by extrapolating the observations made when pregnancies were terminated to give a predicted litter size at birth, then subtracting the observed litter size. The mean loss of foetuses in the groups given diets containing 5% and 10% of reference protein was 5·3 and 5·2 foetuses/litter respectively, whereas in the 15% reference protein groups it was 3·5 and in the group given stock diet, which has a reference protein content of 19%, it was only 2·7 foetuses/litter Thus the events during this period also must have contributed to the difference in live litter size after birth.

Therefore the loss of foetuses during gestation and the perinatal period contributed to the variations in reproductive index values achieved with the different experimental diets. These losses led to significantly different litter sizes after birth which, by considering all the dietary groups, was shown to be due to the level of reference protein supplied in the maternal diet but not influenced by the source of the dietary protein.

From the data on overall survival from birth to 21 days of age it is evident that there were major differences due to thesource of dietary protein as well as to the level incorporated in the diets. Most noteworthy is the observation that no offspring from mothers receiving diets containing fishmeal survived to this age.

Differences between performance of females given the different protein sources first became apparent during the neonatal period, which was defined as 0 to 3 days of age. Of the total number of pups alive after birth 95% of those in the low protein groups, 70% of the middle protein and 50% of those from the high protein groups died before day 3. This mortality rate, however, was not the same for all protein sources, being high in the fishmeal groups with the overall mean being 87% and low in the groups given soya protein (sample 1) with an overall mean of 48%. The high protein group given soya protein (sample 1) showed a mortality rate of 26% which was comparable with that of the stock group at 24%. This

variation in mortality rates during the neonatal period caused differences in the litter size at day 3 which depended on the source of dietary protein ($P < 0.05$) as well as the reference protein level used ($P < 0.01$).

The mortality rates during the postnatal period, defined as days 3 to 21, were shown to be dependant only on the source of protein in the diets. The difference in litter size at day 3 between all the 10% and 15% reference protein diets was maintained to day 21 with mean mortality rates of approximately 42% of the number of pups live at day 3 in both cases. This mortality was similar to that experienced by the stock group offspring. The variation due to the source of protein in the diets during this period was quite considerable with mean mortality rates ranging from 11% in the soya protein (sample 1) groups to 100% when fishmeal was the source of protein.

All the variations between the dietary groups from resorption in utero to postnatal deaths contributed to the differences observed when viewing the reproductive process as a whole. None of the groups showed 100% survival of litters as was the case with the stock control group but in terms of survival of pups the performance of groups given diets containing 15% reference protein from casein, mould and soya protein (sample 1) and 10% reference protein from soya protein (sample 1) was comparable with that of the stock group.

The differences in reproductive performance between the dietary groups must be ascribed to the differences in nutrient intake of the mothers from mating, all events prior to this time being equal.

It has been mentioned previously that the females given the low protein diets were lighter at the end of gestation than at mating. With three of the protein sources (mould, casein and soya protein (sample 2)) the maternal food intake was reduced so the weight loss could have been due to low energy as well as low protein intake but the energy intake of the other two

low protein groups was normal. It is perhaps surprising that females give birth to live offspring even when losing weight to a considerable extent, but a similar phenenomon has been reported in rates (Zeman, 1967).

Of greater interest here is the failure of females given diets containing fishmeal to produce any viable offspring. It is true that with the diets containing the two higher levels (10% amd 15%) of reference protein from fishmeal maternal weight gains were very poor, the mean weight after parturition in both cases being lower than that at mating. However the reason for the poor weight gain is not apparent, particularly in the high protein group where the energy intake was comparable to that of the stock group. It has previously been reported that white fishmeal in the diet of laying hens leads to reduced hatchability of the eggs (Martin, Chubb, Fox, Jennings and Morris, 1960). The reason for this affect is not clear but it has been postulated that during processing of the foodstuff the heat treatment causes breakdown of protein and the formation of toxic substances which are passed from the female to the developing foetus via its food supply (Coles, 1957). It is interesting that here also white fishmeal had adverse affects on reproduction. If the situation was analogous it might be expected that the effects would be seen during gestation and this could not be shown: It is possible however that development of the offspring was impaired in some way leading to subsequent mortality which occurred almost exclusively during the first week after birth. An alternative explanation is that the high level of calcium in the protein source affected metabobism in the females such that they were unable to increase their body weight - a factor which has been discussed in Chapter 2 as a possible cause of the poor growth response to diets containing fishmeal - thus leading to impaired performance during lactation.

In addition to the groups already discussed (those given diets containing the low level of proteins or fishmeal

as the protein source) differences in maternal weight gain compared to the stock group were also observed in other dietary groups. These were sometimes but not always accompanied by differernces in energy intake. In general, survival of the offapring was reasonably good in the groups where the females had increased their body weights sufficiently during the first 15 days of gestation to enable them to undergo a weight loss through lactation. In almost all cases pups in the experimental groups which survived to 21 days of age had mean weights similar to those of the stock group at this time.

The major observations of the work reported here can be summarised as follows:

1. Reproductive performance was severely impaired in hamsters given diets containing the equivalent of only 5% reference protein;
2. The level of reference protein supplied, but not the source, affected resorption of foetuses, perinatal mortality and thus live litter size at birth;
3. The source from which the dietary protein was derived, as well as the level of protein used, affected neonatal and postnatal mortality rates and thus overall survival of the foetuses;
4. Females given diets containing fishmeal showed very poor reproductive performance;
5. Variations in parameters measured in foetuses and pups did not relate consistently to survival. Poor survival of the offspring was associated with inadequate weight gain by the mother during gestation.

CHAPTER 4. SOME EFFECTS OF DIETARY SUCROSE IN THE NEW ZEALAND WHITE RABBIT

CHAPTER 4 : SOME EFFECTS OF DIETARY SUCROSE IN THE NEW ZEALAND WHITE RABBIT

Introduction

It is well known that changes in the diet can alter the pattern of growth, and that the earlier dietary aberration occurs, the greater are the effects. It has also been shown that dietary variations can result in impairment of the mechanism involved in glucose homeostasis, and thus carbohydrate metabolism.

The experiment described here was designed to induce by dietary means changes in carbohydrate metabolism, as indicated by glucose tolerance and insulin sensitivity, in the New Zealand White rabbit and to investigate relationships between these parameters, growth and food intake.

The experimental diet chosen contained 16% fat, 32% sucrose and 18% protein of the dry weight of the diet. These constituents supplied 36%, 32% and 20% of the total metabolisable energy respectively which simulates the high fat, high sucrose, high protein regimen typical of the UK diet (Dunnigan, Fyfe, McKiddie & Crosby, 1970). From this basis further diets were constructed containing either a moderate level of sucrose (8% by weight) or a moderately low level of protein (12% by weight) or both.

The dietary regimens were introduced at mating, thus it was expected, at least with the two lower protein groups that offspring of different weaning weights and growth patterns would be produced. In addition, a group offered the stock diet was included to act as a further control.

All offspring from both the high and moderate protein groups at each level of sucrose were to be weaned onto the respective high protein diet. However, due to poor survival of offspring from the females given high sucrose (32%) diets, the experimental

design was modified and the two remaining experimental groups-the offspring of females given moderate sucrose (8%) with high (18%)or moderate (12%) protein-were weaned onto the high protein, high sucrose diet and the experiment was continued with these two groups and the stock group. (The experimental work described in this chapter was performed in collaboration with Ms.J.S.Bryant under joint supervision Dr.M.R.Turner)

Methods and Materials

Animals and diets

Virgin female New Zealand White rabbits of similar weights and ages were mated and offered either a stock diet (RAG, Christopher Hill Ltd., Poole, Dorset) or one of four experimental diets. Two diets contained a high level of sucrose (32% of the dry weight of the diet) (S_{32}) with 18% (P_{18}) or 12% (P_{12}) protein. The other two contained a moderate level of sucrose (8%) (S_8) again with high or moderate protein. The four diets can thus be designated: $P_{18}S_{32}$; $P_{12}S_{32}$; $P_{18}S_8$; $P_{12}S_8$. The compostion of the diets is shown in Table 4.1. Survival of offspring of groups given the two high sucrose diets was poor, and the number at weaning were considered too low to continue with the experimental programme. (See Table 4.2). Offspring of the remaining two groups (moderate sucrose) were weaned on to the $P_{18}S_{32}$ diet and stock group offspring were weaned on to the stock diet at 7 weeks of age. A summary of the feeding programme is shown in Table 4.3.

At all times food was supplied ad libitum, and food intake of the offspring was measured by weight difference with correction for spillage at weekly intervals from 7 to 38 weeks of age. Body weight was also measured at weekly intervals.

The animals were housed individually from weaning in aluminium cages with slatted bottoms, to allow passage of faeces, urine and spilled food, at a room temperature of 22°C. Humidity was maintained by flooding the floor daily. Poor natural lighting was supplemented with artificial light for

Table 4.1a Composition of diets (g/kg dry weight)

	$P_{18}S_{32}$	$P_{18}S_{8}$	$P_{12}S_{32}$	$P_{12}S_{8}$
Soya protein ("Promine D")	179	179	114	114
Wheat bran	150	150	150	150
Corn oil	160	160	160	160
Sucrose	320	80	320	80
Silka floc	50	50	50	50
Maize starch	76	316	141	381
Ø [1]Vitamin mixture	10	10	10	10
Ø [2]Mineral mixture	47	47	47	47
Methionine	6	6	6	6
KCl	2	2	2	2
Calculated metabolizable energy (kJ/g)	15.5	15.5	15.5	15.8

Ø see Table 4:1b

Table 4.1b

Vitamin mixture (mg/kg diet)	
Biotin	0.26
Folic acid	2.64
Inositol	1.76
Nicotinic acid	52.8
Ca pantothenate	26.4
Pyridoxine HCl	8.8
Menaphthone K	3.52
Vit.B_{12}	0.04
Choline HCl	1760
Riboflavine	8.8
Thiamine HCl	3.52
Para amino benzoic acid	110
Vitamin A	5000 iu
Vitamin D	1000 iu
Vitamin E	77.5iu

Mineral mixture supplied		
g/Kg diet	CO_3	10.03
	PO_4	18.06
	Cl	4.19
	Ca	11.86
	Na	1.25
	K	7.39
mg/Kg diet	Mg	787
	I	13.9
	Cu	15.6
	Mn	107
	Zn	140
	Mo	1.78
	Cr	1
	Se	99.8
	SO_4	645
	Fe	176

Table 4.2 Survival of pups to weaning in four groups of rabbits given moderate or high sucrose in the diet.

	Maternal diet			
	moderate (8%) sucrose		high (32%) sucrose	
	$P_{18}S_8$	$P_{12}S_8$	$P_{18}S_{32}$	$P_{12}S_{32}$
Animals mated	5	6	5	6
Litter produced	5	6	3	5
Litters at weaning	4	5	1	4
Live pups/litter at birth	$6.6 \pm 0.9(5)$	$6.8 \pm 0.9(6)$	$3.0 \pm 0(3)$	$7.0 \pm 0.5(5)$
Live pups/litter at weaning	$4.0 \pm 0.4(4)$	$4.0 \pm 0.3(5)$	2.0 (1)	$2.0 \pm 0.4(4)$

Table 4. 3 Feeding programme of groups of offspring.

Group	Maternal diet Stock	Post weaning diet Stock
1		
2	18% protein, 8% sucrose (P_{18} S_8)	18% protein, 32% sucrose (P_{18} S_{32})
3	12% protein, 8% sucrose (P_{12} S_8)	18% protein, 32% sucrose (P_{18} S_{32})
4	18% protein, 32% sucrose (P_{18} S_{32})	) discontinued because of low
5	12% protein, 32% sucrose (P_{18} S_{32})	) numbers at weaning

12 hours a day.

Experimental programme

At six weeks of age all animals were subjected to an overnight (18 hours) fast. The following morning their tolerance to an intravenous glucose load and their sensitivity to insulin injected intravenously were measured. Insulin secretion after the glucose load was also monitored. This routine was repeated at 2 weekly intervals up to 18 weeks of age, then at 4 weekly intervals up to 38 weeks of age.

Intravenous glucose tolerance test (iv GTT)

A sterile aqueous solution of glucose (400 g/l) was injected into the peripheral ear vein, in a dose proportional to body weight (0.4g glucose/kg body weight). Blood samples were collected from the peripheral vein of the other ear before and at timed intervals from 4 to 27 minutes after the injection. The blood was kept in ice for 1 hour, then the serum collected by centrifugation. An aliquot of the serum was used for assaying glucose concentration and the remainder stored at -20°C to be assayed for insulin concentration.

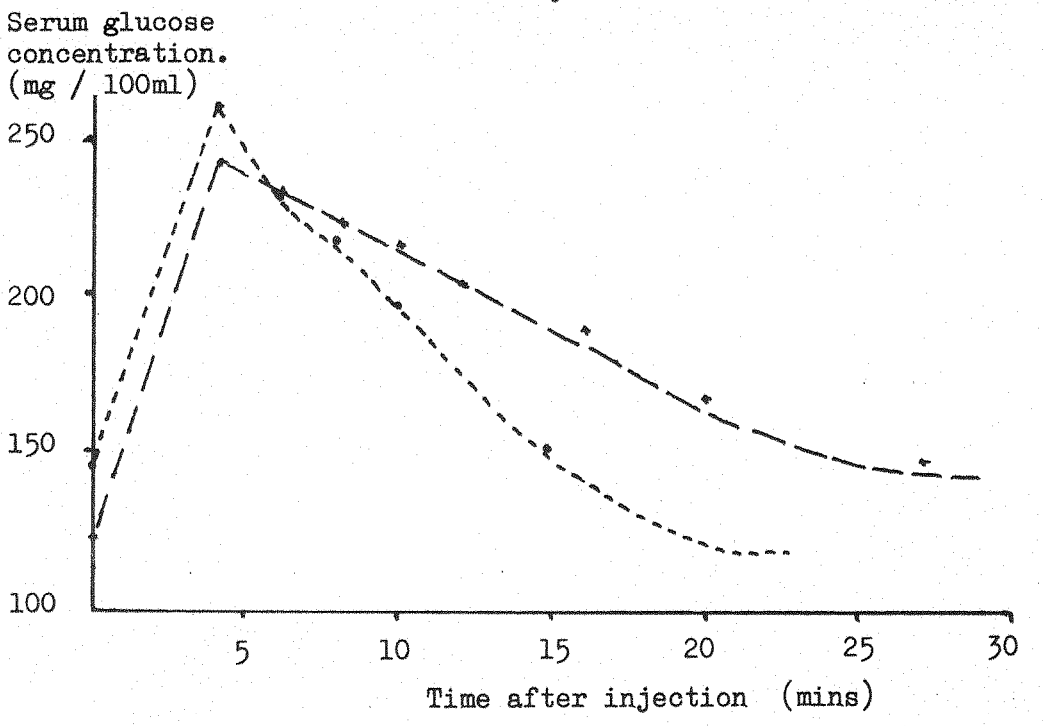
The glucose tolerance test was assessed in terms of the rate constant K_G which represents the percentage disappearance of glucose from the blood, and is calculated from the equation:

$$K_G = \frac{2.303\ (\log C_1 - C_2) \times 100}{t_2 - t_1}$$

where C_1 and C_2 are the glucose concentrations at times t_1 and t_2 after injection.

If glucose concentration is plotted against time on a semi-log plot, a straight line results, the slope of which x 100 represents K_G. A typical response is shown in Fig.4.1.

Figure 4.1 : Serum glucose concentration after injection of 1) glucose (— —) and 2) glucose + insulin (----) of a stock colony rabbit at 16 weeks of age.



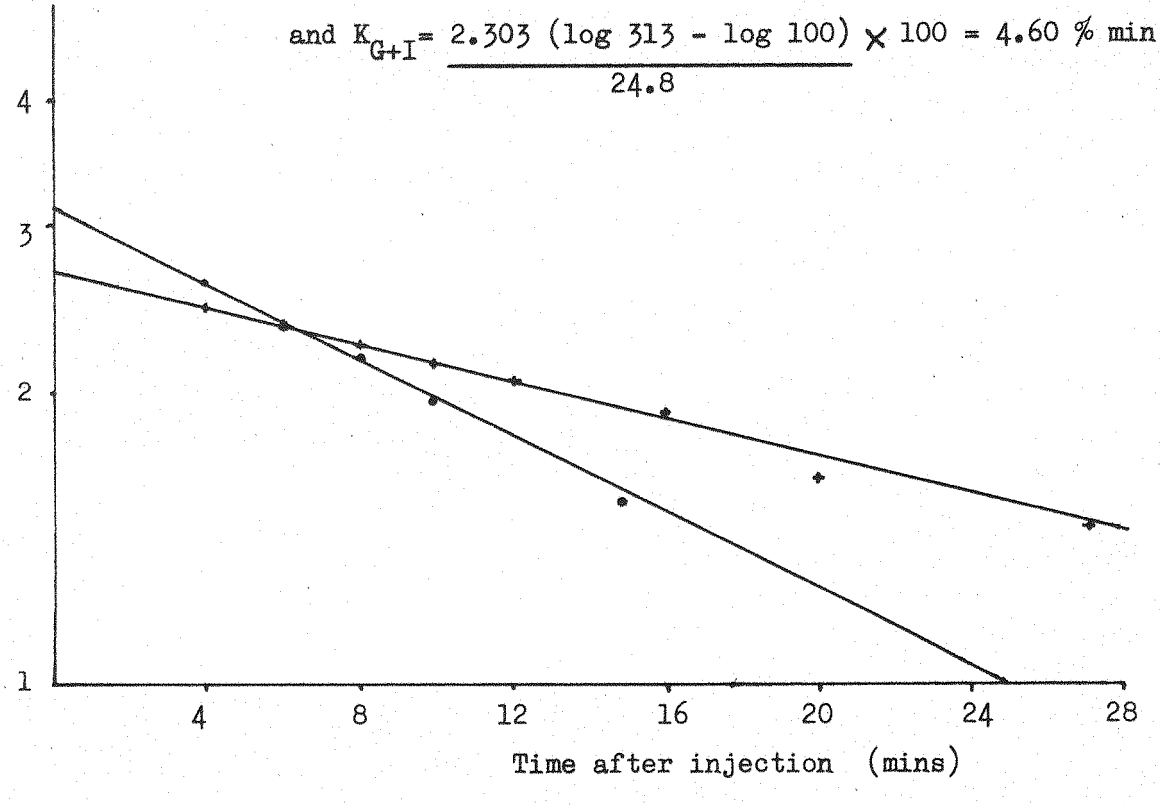
The same data is shown below on a semi - log plot.

using the equation :

$$K = \frac{2.303\ (\log C_1 - \log C_2)}{t_2 - t_1} \times 100 \quad \% \text{ min}^{-1} .$$

thus $K_G = \frac{2.303\ (\log 269 - \log 143)}{28} \times 100 = 2.26 \% \text{ min}^{-1}$

and $K_{G+I} = \frac{2.303\ (\log 313 - \log 100)}{24.8} \times 100 = 4.60 \% \text{ min}^{-1}$



Insulin sensitivity

This test was carried out immediately after the i.v. GTT so that the two parameters could be compared (Heard & Henry, 1969; Samols & Marks, 1965). Thirty minutes after the first injection a second was introduced into the same vein. The sterile solution again contained 400g glucose/l and in addition contained 100μU/l insulin. The animals were injected with 1ml/kg thus they again received 0.4g glucose and also 0.1μU insulin/kg body weight. Blood samples were collected from the other ear for a further 30 minutes, stored in ice and the serum collected as described above. Sensitivity to insulin was assessed in terms of the rate constant K_{G+I} which represents the percentage disappearance of blood glucose in the presence of the injected insulin (see Fig. 4.1).

Serum glucose

Glucose was measured by an automated method on a Technicon Autoanalyzer II (Clinical Method No.507-72E). The method is adapted from that of Trinder (1969) and employs the reactions:

(1) glucose + glucose oxidase + oxygen → gluconic acid + H_2O_2

(2) H_2O_2 + peroxidase + chromogen → coloured product.

Hence the glucose concentration in the samples can be determined colorimetrically.

Samples were prepared for the autoanalyzer by diluting serum 1:9 with distilled water.

Serum insulin

Insulin was determined by radio-immunoassay employing a double antibody based on the method of Hales & Randle (1963). This method was similar to that described fully in chapter 2(p.39) but separation of the bound from the free ^{125}I - insulin was achieved by centrifugation (as opposed to

filtration) as follows.

After the assay tubes had incubated for 18 hours at 4°C following the addition of ^{125}I - insulin the tubes were centrifruged in a "MSE Mistral 2L" centrifuge refrigerated at 4°C at a centrifugal force of 2000g for 20 minutes. The tubes were then inverted for 15 minutes to allow the supernatant to drain off. Any fluid persisting at the neck of the tube was blotted and the tubes were counted in a Beckman gamma counter as before.

With this modified method it was possible to use smaller assay tubes and glass facilitated the draining off process so the assay was set up in 50mm x 6.5mm glass test tubes.

Statistical treatment of data

Statistical analyses were performed as described in chapter 2 (p.44) with the following additions:

Regression coefficients were compared using the following formula:-

$$d = \frac{b_1 - b_2}{\sqrt{SE_1^2 + SE_2^2}}$$

where b_1 and b_2 are the regression coefficients of lines having the general formula $y = bx + C$ and SE_1 and SE_2 are the standard errors of b_1 and b_2 (ref. Bailey, 1959).

Results

The object of this experiment was to investigate relationships between body weight gain, food consumption and insulin status as indicated by insulin sensitivity, glucose tolerance and plasma insulin concentrations. The measurement of glucose tolerance and insulin sensitivity depends on giving the animal 2 injections, both cleanly delivered within 1-2 minutes. If this was not achieved for any animal all

its data at that age has been omitted, hence the variable number of observations for means within the groups.

Body weight

The mean body weights of animals in the 3 groups are shown in Table 4.4 and Figure 4.2. Almost throughout the trial the two groups weaned onto the high sucrose diet were significantly lighter than the stock group. Also the offspring of the mothers given the moderate protein diet (Group 3) were significantly lighter than the Group 2 (offspring of high protein mothers) animals. Group 2 animals grew at a comparable rate to the stock group with the growth curves being essentially parallel up to about twenty weeks of age when they experienced a more abrupt weight plateau. Group 3 animals, however, show a slower increase in body weight through the rapid growth phase (see Fig.4.2).

Food and energy intake

The mean food intakes (Table 4.5) are given as metabolisable energy intake in Table 4.6 and Figure 4.3 so that comparisons can be made between the two groups given experimental diet (P_{18} S_{32}) and the group given stock diet, which had a lower energy value. The metabolisable energy intake of animals in Group 3 (maternal diet P_{12} S_{8}) was less than that of the other two groups throughout the trial, but the intake of Group 2 animals (maternal diet P_{18} S_{8})was similar to that of the stock group except during the first week and the end period. However, when metabolisable energy intake was expressed per metabolic mass (ie kJ/kg $^{.75}$/d), as shown in Table 4.7 and Figure 4.4 neither of the groups given the high sucrose diet ate less than the stock group at any time.

Glucose tolerance (K_G)

The mean glucose tolerance values for the three groups at all ages are shown in Table 4.8 and Figure 4.5. In all

Table 4.4 Mean body Weights (g) of the 3 groups of rabbits from 6 - 38 weeks of age

Group	1	2	3
Maternal diet	Stock	$P_{18}S_{8}$	$P_{12}S_{8}$
Postweaning diet	Stock	$P_{18}S_{32}$	$P_{18}S_{32}$
Age (weeks)			
6	1050 ± 27 (3)	743 ± 58 (9)*	518 ± 11 (9)***++
8	1679 ± 61 (5)	1009 ± 68 (12)***	633 ± 36 (13)***+++
10	2056 ± 55 (6)	1294 ± 78 (13)***	798 ± 62 (9)***+++
12	2393 ± 71 (6)	1810 ±120 (8)**	987 ± 58 (11)***+++
14	2712 ± 66 (6)	2146 ±127 (7)**	1185 ± 66 (11)***+++
16	2953 ± 73 (6)	2339 ±120 (11)**	1433 ± 92 (8)***+++
18	3167 ± 111(2)	2735 ±109 (8)	1599 ± 80 (6)***+++
22	3416 ± 111(6)	3026 ±120 (6)*	1902 ±102 (6)***+++
26	3558 ± 100(6)	3194 ±118 (10)*	2173 ±138 (7)***+++
30	3736 ± 107(6)	3246 ±104 (11)**	2253 ±155 (6)***+++
34	3845 ± 118 (6)	3303 ±109 (10)**	2470 ±229 (4)***++
38	3949 ± 175 (6)	3489 ±137 (8)*	2532 ±241 (4)**+

Significant differences from stock group: *P< 0.05; **P<0.01; ***P<0.001.
from Group 2: +P<0.05; ++P<0.01; +++P<0.001.

Figure 4.2 : Mean body weight of three groups of rabbits from 6 to 38 weeks of age.

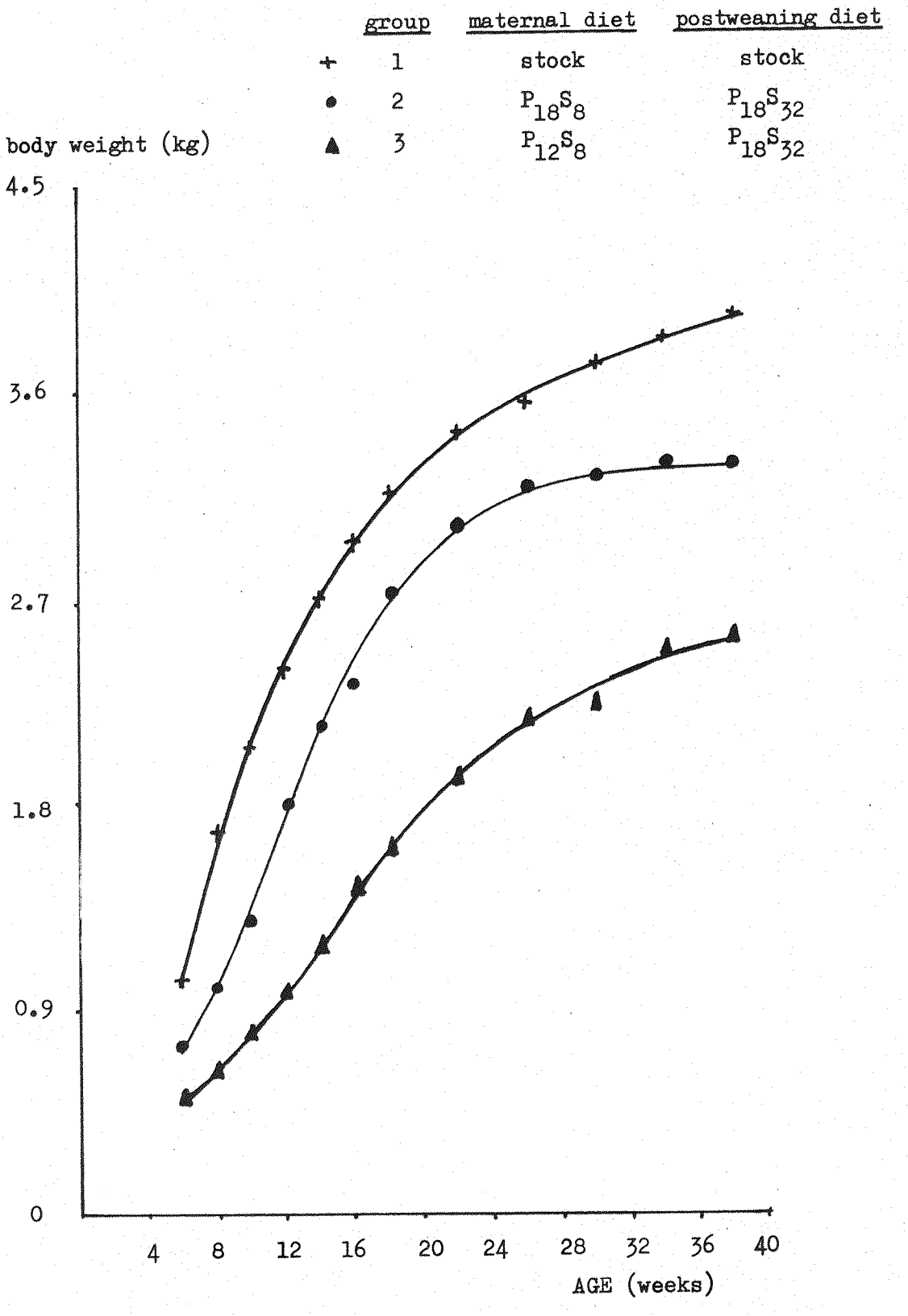


Table 4.5 Food Intake (g/animal/d) of three groups of rabbits from 7 to 38 weeks of age.

Group	1	2	3
Maternal diet	Stock	$P_{18}S_8$	$P_{12}S_8$
Postweaning diet	Stock	$P_{18}S_{32}$	$P_{18}S_{32}$
Age (weeks)			
7 - 8	116.9 ± 4.4 (5)	41.4 ± 6.4 (2)	32.9 ± 3.8 (13)
8 - 10	149.0 ± 6.5 (6)	72.0 ± 8.4 (13)	39.6 ± 3.1 (9)
10 - 12	136.2 ± 7.8 (6)	88.6 ± 5.6 (8)	49.5 ± 5.2 (11)
12 - 14	148.5 ± 7.7 (6)	105.4 ± 9.6 (7)	54.7 ± 3.2 (11)
14 - 16	164.8 ± 6.1 (6)	91.5 ± 6.9 (11)	59.1 ± 4.7 (7)
16 - 18	148.5 ±10.5 (2)	106.4 ± 7.3 (8)	57.3 ± 4.2 (6)
18 - 22	119.4 ± 5.9 (6)	101.7 ±12.3 (6)	63.7 ± 3.0 (6)
22 - 26	117.2 ± 8.1 (6)	81.4 ± 3.8 (10)	65.9 ± 4.2 (7)
26 - 30	124.7 ± 6.5 (6)	69.6 ± 4.7 (11)	58.3 ± 4.5 (6)
30 - 34	137.4 ± 5.6 (6)	71.7 ± 4.6 (10)	58.0 ± 8.9 (4)
34 - 38	132.1 ± 5.1 (5)	63.8 ± 5.6 (8)	63.0 ± 5.1 (4)

Table 4.6 ME Intake (kJ/animal/d) of the 3 groups of rabbits from 7 - 38 weeks of age

Group	1	2	3
Maternal diet	Stock	$P_{18}S_{8}$	$P_{12}S_{8}$
postweaning diet	Stock	$P_{18}S_{32}$	$P_{18}S_{32}$
Age (weeks)			
7 - 8	1169 ± 44 (5)	642 ± 99 (12)**	510 ± 59 (13)***
8 - 10	1490 ± 65 (6)	1116 ±130 (13)	614 ± 48 (9)***++
10 - 12	1362 ± 78 (6)	1373 ± 87 (8)	767 ± 81 (11)***+++
12 - 14	1485 ± 77 (6)	1634 ±149 (7)	848 ± 50 (11)***+++
14 - 16	1648 ± 61 (6)	1418 ±107 (11)	916 ± 73 (7)***++
16 - 18	1485 ± 105(2)	1649 ±113(8)	888 ± 65 (6)**+++
18 - 22	1194 ± 59 (6)	1576 ±191 (6)	987 ± 47 (6)*+
22 - 26	1172 ± 81 (6)	1262 ± 59 (10)	1021 ± 65 (7)+
26 - 30	1247 ± 65 (6)	1079 ± 73 (11)	904 ± 70 (6)**
30 - 34	1374 ± 56 (6)	1111 ± 71 (10)*	899 ±138 (4)**
34 - 38	1321 ± 51 (5)	989 ± 87 (8)*	977 ± 79 (4)**

Significant differences from stock group: *P< 0.05; **P< 0.01; ***P< 0.001.
from group2: +P< 0.05; ++P< 0.01; +++P< 0.001.

Figure 4.3 : Mean metabolisable energy intake of the three groups of N.Z.W. rabbits from 6 to 38 weeks of age.

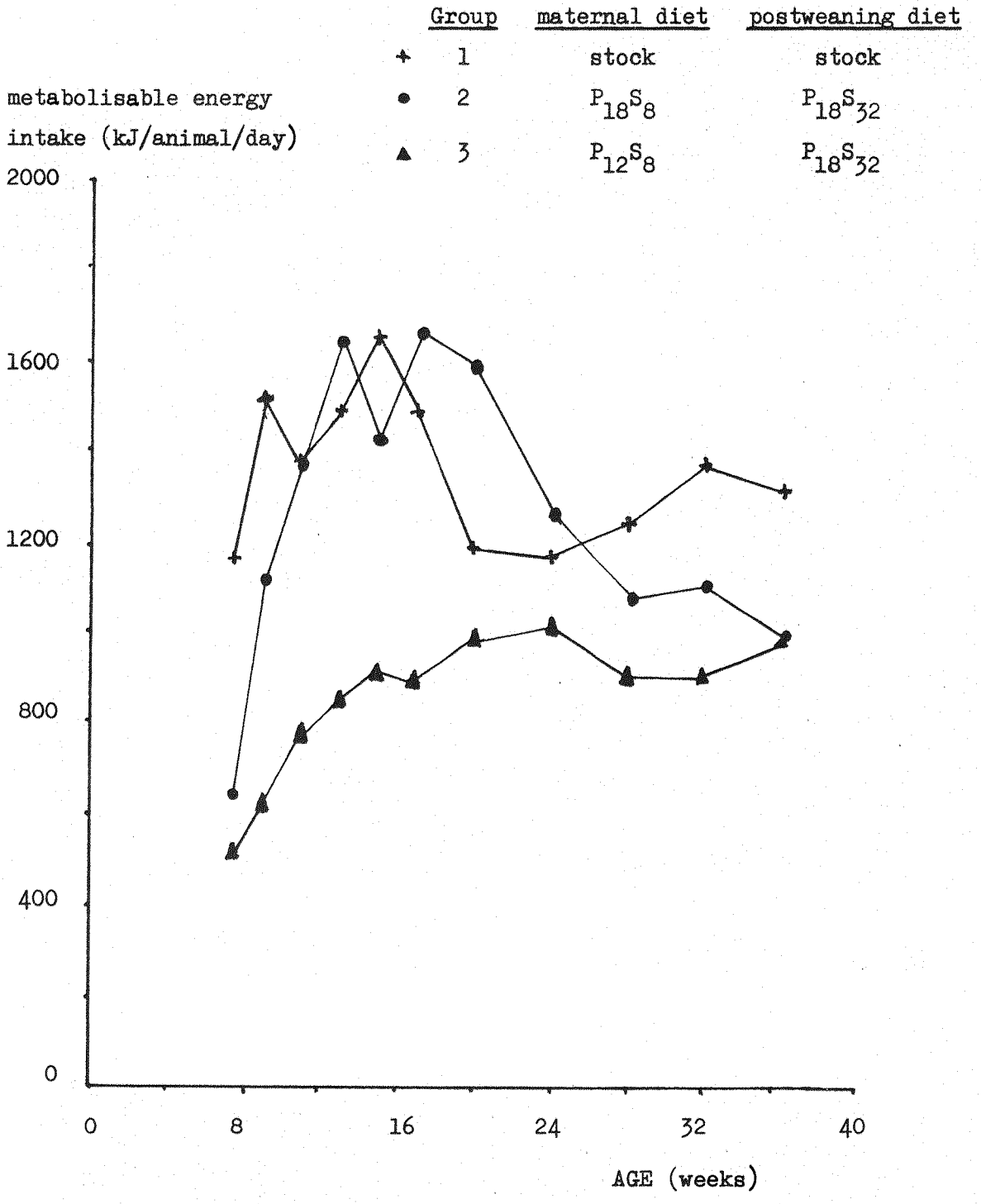
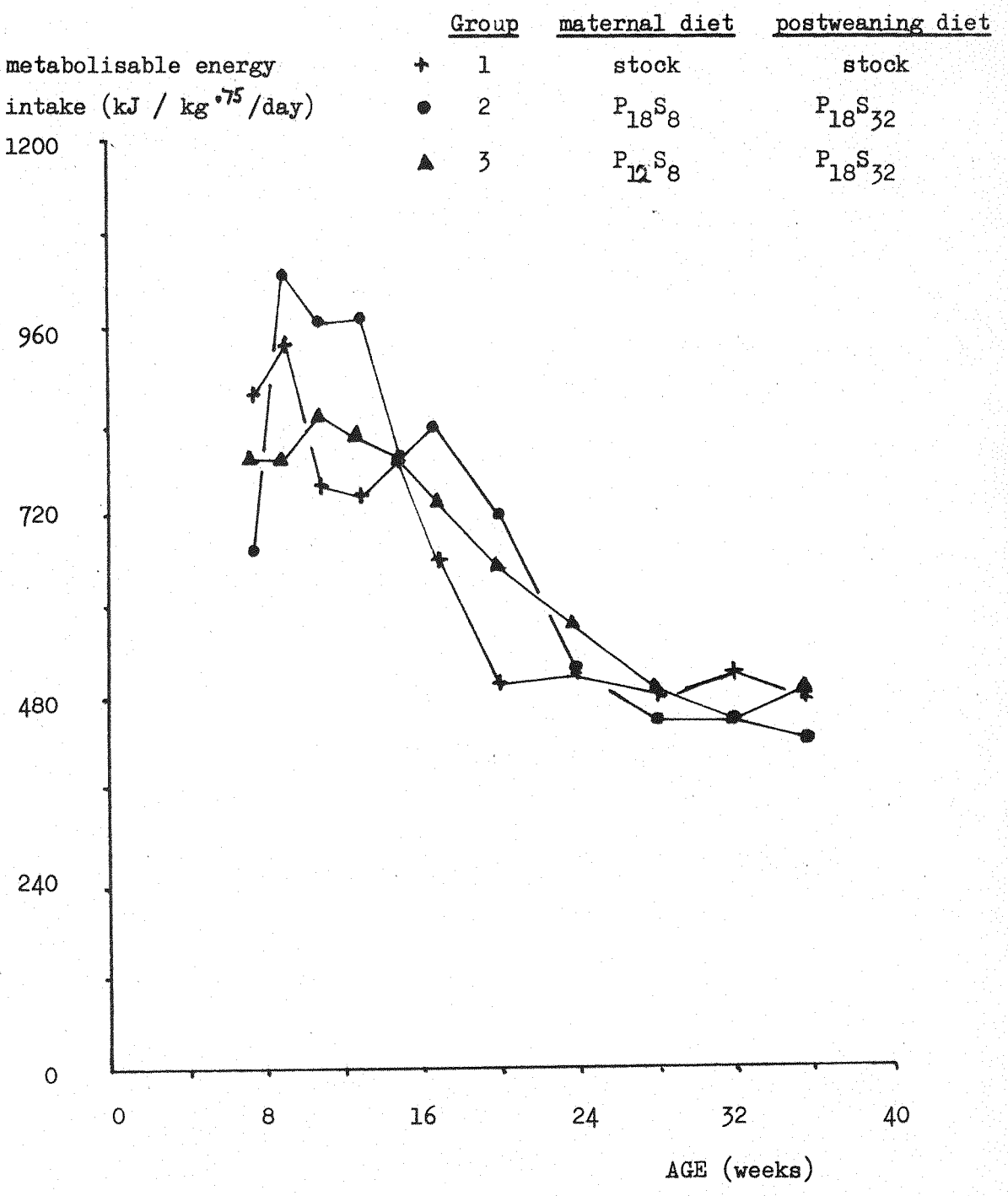


Table 4.7 ME intake (kJ/kg $^{.75}$/d) of the 3 groups of rabbits from 7 to 38 weeks of age.

Group	1	2	3
Maternal diet	Stock	$P_{18}S_{8}$	$P_{12}S_{8}$
Postweaning diet	Stock	$P_{18}S_{32}$	$P_{18}S_{32}$
Age (weeks)			
7 - 8	865 ± 19 (5)	672 ± 104 (12)	784 ± 90 (13)
8 - 10	931 ± 19 (6)	1031 ± 142 (13	785 ± 44 (9)
10 - 12	746 ± 30 (6)	969 ± 71 (8)*	837 ± 69 (11)
12 - 14	739 ± 27 (6)	974 ± 100 (7)	810 ± 52 (11)
14 - 16	784 ± 34 (6)	785 ± 43 (11)	776 ± 72 (7)
16 - 18	653 ± 40 (2)	833 ± 41 (8)	729 ± 48 (6)
18 - 22	493 ± 18 (6)	718 ± 76 (6)*	651 ± 23 (6)***
22 - 26	504 ± 21 (6)	520 ± 33 (10)	572 ± 25 (7)
26 - 30	477 ± 19 (6)	449 ± 30 (11)	491 ± 16 (6)
30 - 34	508 ± 14 (6)	454 ± 26 (10)	451 ± 44 (4)
34 - 38	476 ± 8 (5)	424 ± 41 (8)	494 ± 17 (4)

Significant differences from stock group: *$P < 0.05$; ***$P < 0.001$.

Figure 4.4 : Mean metabolisable energy intake of the three groups of N.Z.W. rabbits from 6 to 38 weeks of age.



groups the glucose tolerance was poor in the young animal and increased to a maximum at about 18 weeks of age. During this period when tolerance to glucose was developing, the offspring of the low protein mothers (Group 3) had mean glucose tolerance values of K_G= 1 to 1.9. Although not significant at all points, these values were generally below those of the stock animals (K_G=2 to 2.8) The mean values of Group 2 animals (K_G=1.3 to 2.2) were intermediate between those of Groups 1 and 3. After attaining a maximum value at 18 weeks of age, the tolerance to glucose in Groups 2 and 3 decreased to K_G=1 and 1.3 respectively, whereas that of the stock group was sustained at about K_G= 2.4. Thus it was found that there was a substantial decline in glucose tolerance after the peak values were reached in the groups of animals given the sucrose rich diet, whatever their previous protein status.

Insulin sensitivity (K_{G+I})

The mean insulin sensitivities of the three groups at each are are shown in Table 4.9 and Fig. 4.6 Mean values at each age were similar in all groups up to 14 weeks of age. Insulin sensitivity was poor in young animals (K_{G+I}=2.2 at 6 weeks) but increased to a maximum at 16 to 18 weeks of age when in group 1 K_{G+I}= 5.2, in group 2 K_{G+I}= 4.2 and in Group 3 K_{G+I}= 3.9. In all groups there was then a fall in insulin sensitivity which was exaggerated in the two groups of animals receiving the sucrose rich diet such that their insulin sensitivity was significantly less than that of the stock group towards the end of the trial.

There were similarities between the changes in glucose tolerance with age and the changes in insulin sensitivity with age and it was found that a strong correlation ($r \simeq 6$; $P < 0.001$) between these factors existed for all groups (see figure 4.7). The relationship between the two parameters as indicated by the slope of the regression line was significantly different ($P < 0.05$) in the stock group (slope = 0.26 ± 0.05) and in the experimental groups (slope = 0.39 ± 0.04) which were similar to each other and therefore

Table 4.8 Mean Glucose Tolerance (K_G) of the 3 groups of rabbits at different ages.

Group	1	2	3
Maternal diet	Stock	$P_{18}S_8$	$P_{12}S_8$
Postweaning diet	Stock	$P_{18}S_{32}$	$P_{18}S_{32}$
Age (weeks)			
6	2.01 ± 0.69(3)	1.28 ± 0.29(9)	1.00 ± 0.25(9)
8	2.07 ± 0.30(5)	1.60 ± 0.25(12)	1.08 ± 0.20(13)*
10	2.33 ± 0.38(6)	1.92 ± 0.31(13)	1.51 ± 0.31(9)
12	2.44 ± 0.32(6)	2.05 ± 0.28(8)	1.62 ± 0.19(11)*
14	2.56 ± 0.37(6)	2.13 ± 0.29(7)	1.73 ± 0.21(11)(*)
16	2.64 ± 0.38(6)	2.15 ± 0.18(11)	1.62 ± 0.11(7)*+
18	2.79 ± 1.11(2)	2.17 ± 0.31(8)	1.86 ± 0.32(6)
22	2.34 ± 0.31(6)	1.78 ± 0.32(6)	1.52 ± 0.39(6)
26	2.68 ± 0.40(6)	1.21 ± 0.20(10)**	1.65 ± 0.33(7)(*)
30	2.29 ± 0.19(6)	1.05 ± 0.23(11)**	1.21 ± 0.39(6)*
34	2.60 ± 33(6)	1.50 ± 0.27(10)*	1.70 ± 0.47(4)
38	2.38 ± 0.36(5)	1.01 ± 0.25(8)**	1.26 ± 0.43(4)(*)

Significant differences from stock group: *$P < 0.05$; **$P < 0.01$; (*)$P < 0.1 > 0.05$.
from group 2: +$P < 0.05$.

Figure 4.5 : Mean glucose tolerance of the three groups of rabbits from 6 to 38 weeks of age.

group		maternal diet	postweaning diet
1	+	stock	stock
2	●	$P_{18}S_8$	$P_{18}S_{32}$
3	▲	$P_{12}S_8$	$P_{18}S_{32}$

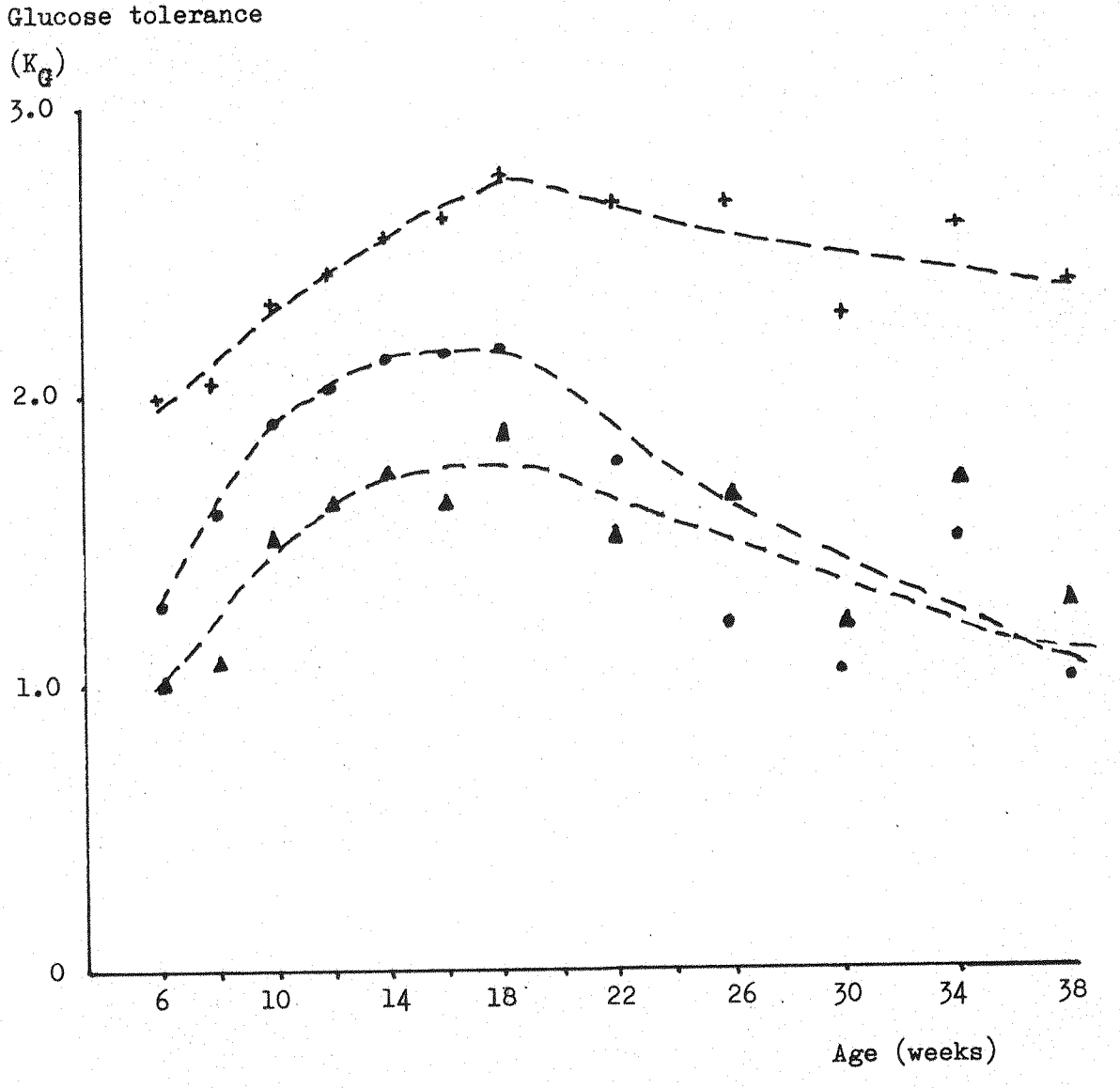


Table 4.9 Mean Insulin Sensitivity ($K_G + _I$) of the 3 groups of rabbits at different ages.

Group	1	2	3
Maternal diet	Stock	$P_{18}S_8$	$P_{12}S_8$
Postweaning diet	Stock	$P_{18}S_{32}$	$P_{18}S_{32}$
Age (weeks)			
6	2.09 ± 0.62(3)	2.37 ± 0.38(9)	2.09 ± 0.30(9)
8	3.13 ± 0.36(5)	3.02 ± 0.33(12)	2.71 ± 0.21(13)
10	3.60 ± 0.43(6)	3.60 ± 0.37(13)	3.52 ± 0.48(9)
12	3.67 ± 0.37(6)	3.60 ± 0.48(8)	3.54 ± 0.42(11)
14	4.94 ± 0.73(6)	3.39 ± 0.30(7)	3.28 ± 0.42(11)
16	5.20 ± 0.37(6)	4.22 ± 0.30(11)	3.63 ± 0.13(7)**
18	4.65 ± 0.11(2)	3.44 ± 0.31(8)	3.92 ± 0.46(6)
22	4.54 ± 0.58(6)	3.05 ± 0.21(6)*	2.55 ± 0.30(6)*
26	4.76 ± 0.70(6)	2.84 ± 0.35(10)*	3.09 ± 0.41(7)
30	4.03 ± 0.40(6)	2.80 ± 0.35(11)*	2.32 ± 0.39(6)*
34	4.34 ± 0.29(6)	2.74 ± 0.47(10)*	3.18 ± 0.15(4)*
38	4.07 ± 0.39(5)	2.16 ± 0.34(8)**	2.68 ± 0.45(4)

Significant differences from stock control group: *$P < 0.05$; **$P < 0.01$.
Differences between groups 2 and 3 were not significant at any age.

Figure 4.6 : Mean insulin sensitivity of the three groups of N.Z.W. rabbits from 6 to 38 weeks of age.

	group	maternal diet	postweaning diet
+	1	stock	stock
●	2	$P_{18}S_{8}$	$P_{18}S_{32}$
▲	3	$P_{12}S_{8}$	$P_{18}S_{32}$

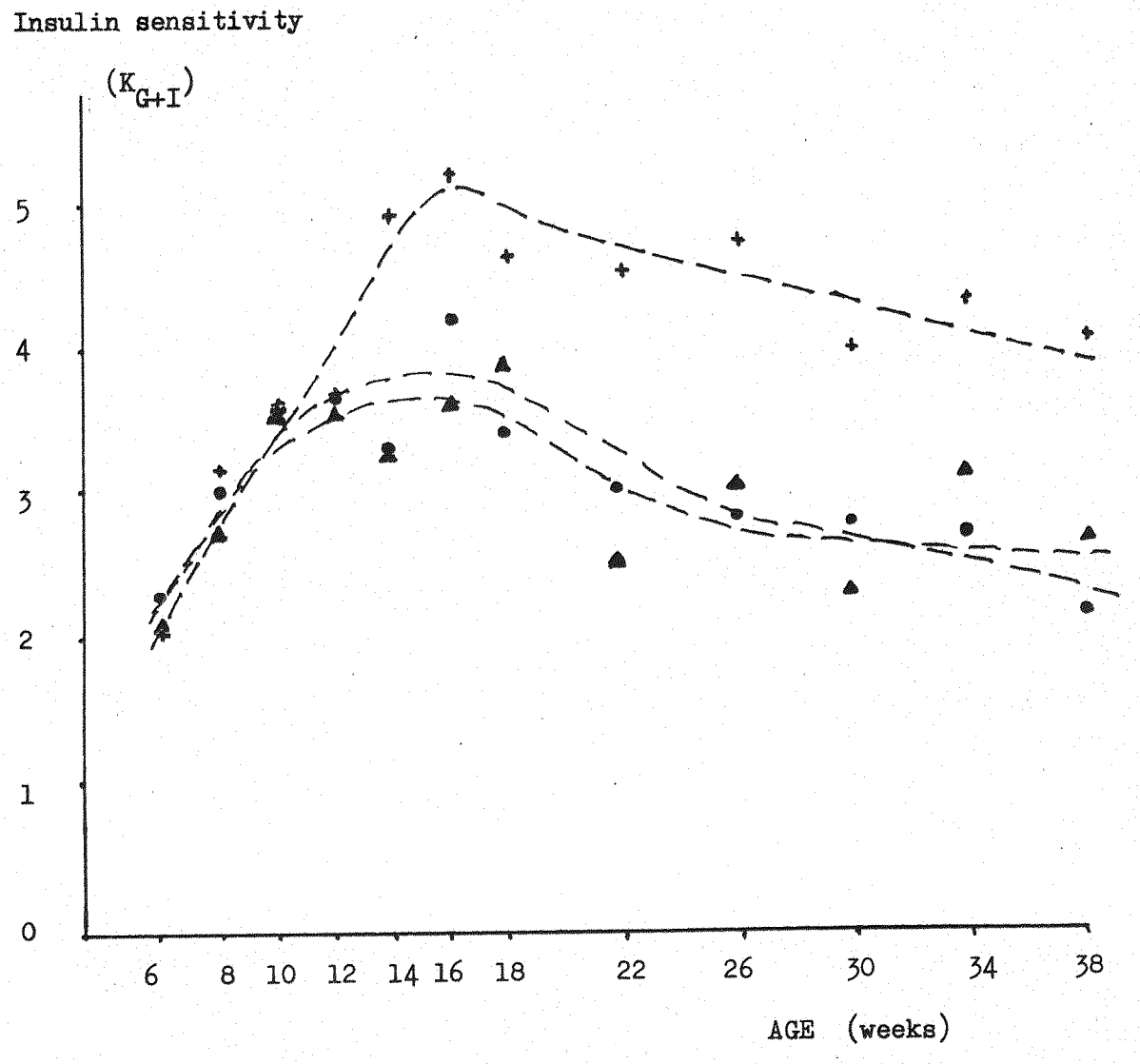


Figure 4.7 : Regression lines showing the relationship between glucose tolerance and insulin sensitivity for each of the 3 groups of N.Z.W. rabbits and also all the animals given experimental diet.

GROUP	Maternal diet	postweaning diet	Equation	Standard error of slope	n	R	P	Significant difference from stock group
1	stock	stock	$Y=1.34+0.2620X$	$\pm$ 0.0462	63	0.5874	<0.001	-
2	$P_{18}S_{8}$	$P_{18}S_{32}$	$Y=0.42+0.3899X$	$\pm$ 0.0473	113	0.6159	<0.001	NS ($P<0.1>0.05$)
3	$P_{12}S_{8}$	$P_{18}S_{32}$	$Y=0.23+0.3946X$	$\pm$ 0.0520	93	0.6223	<0.001	NS ($P<0.1>0.05$)
2+3			$Y=0.33+0.3932X$	$\pm$ 0.0353	206	0.6150	<0.001	$P<0.05$

where:

$Y = K_G$

and $X = K_{G+I}$

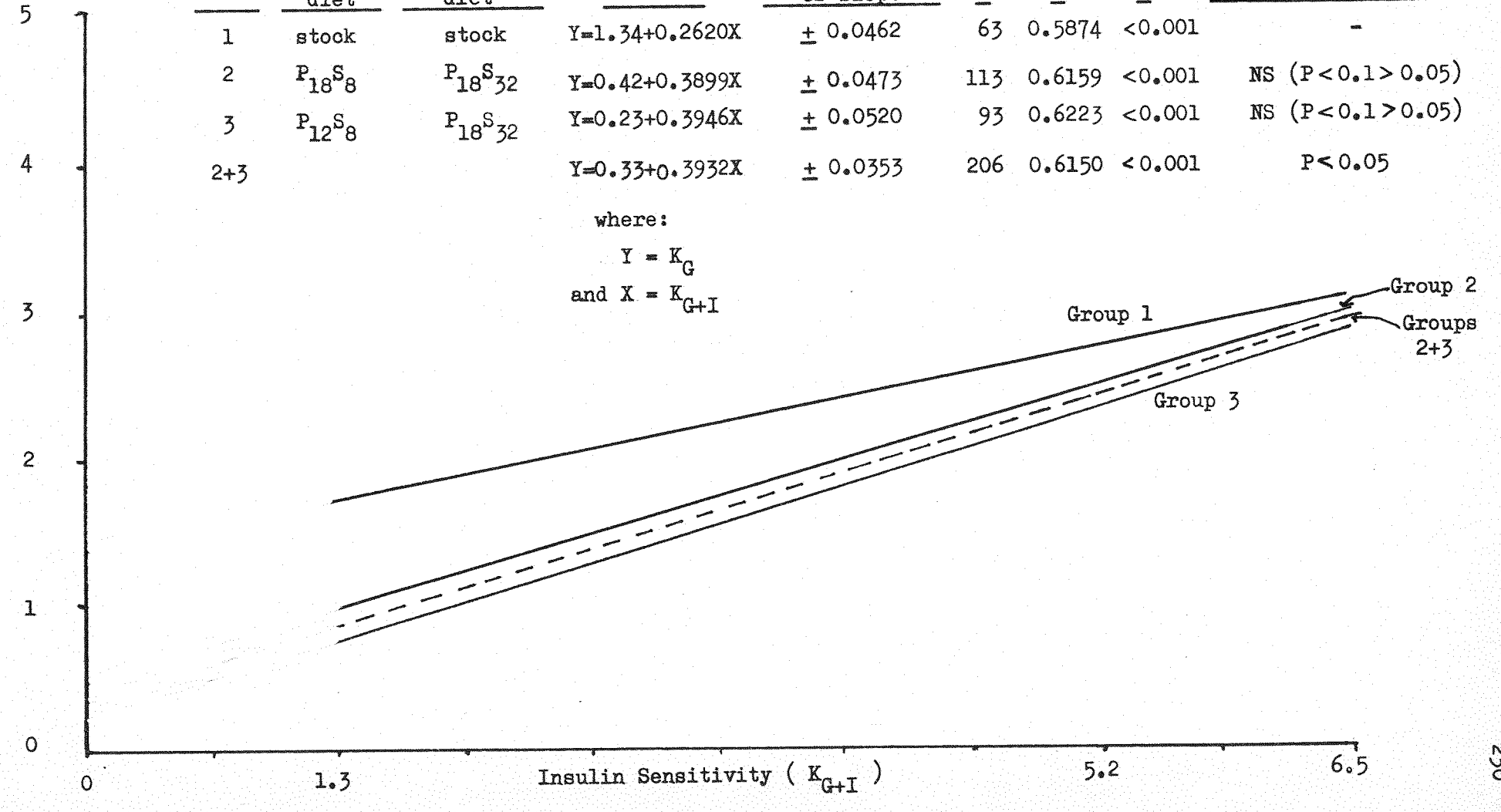


Table 4.10 Mean fasting Serum Glucose levels of the 3 groups of rabbits at different ages (m mole/l)

Group	1	2	3
Maternal diet	Stock	$P_{18}S_{8}$	$P_{12}S_{8}$
Postweaning diet	Stock	$P_{18}S_{32}$	$P_{18}S_{32}$
Age (weeks)			
6	7.03 ± 0.38 (3)	9.74 ± 0.55 (9)*	7.86 ± 0.34 (9)+
8	7.61 ± 0.12 (5)	8.57 ± 0.45 (12)	8.08 ± 0.26 (13)
10	7.22 ± 0.12 (6)	8.41 ± 0.40 (13)	9.00 ± 0.49 (9)*
12	7.90 ± 0.17 (6)	8.10 ± 0.26 (8)	7.96 ± 0.17 (11)
14	6.58 ± 0.17 (6)	8.09 ± 0.23 (7)***	7.77 ± 0.36 (11)*
16	6.61 ± 0.17 (6)	7.14 ± 0.18 (11)	7.55 ± 4.44 (7)
18	5.83 ± 0 (2)	7.33 ± 0.27 (8)*	7.01 ± 2.39 (6)*
22	6.58 ± 0.23 (6)	7.00 ± 0.23 (6)	6.85 ± 0.38 (6)
26	6.77 ± 0.28 (6)	7.52 ± 0.21 (10)*	6.61 ± 0.29 (7)+
30	6.09 ± 0.15 (6)	6.79 ± 0.23 (11)	6.89 ± 1.89 (6)**
34	5.81 ± 0.39 (6)	7.07 ± 1.89 (10)**	6.71 ± 0.23 (4)
38	5.82 ± 0.17 (6)	6.81 ± 0.32 (8)*	7.07 ± 2.11 (4)**

Significant difference from stock group: *$P < 0.05$. **$P < 0.01$, ***$P < 0.001$
from group 2: +$P < 0.05$

Table 4. 11 Maximun Serum Insulin levels within 12 minutes of glucose injection.

	Group 1	2	3
Maternal diet	Stock	$P_{18}S_{8}$	$P_{12}S_{8}$
Postweaning diet	Stock	$P_{18}S_{32}$	$P_{18}S_{32}$
Age in weeks			
6	40.4 ± 7.9 (3)	11.5 ± 7.3 (7)*	11.0 ± 4.1 (6)**
8	39.6 ± 10.4 (5)	11.7 ± 1.7 (12)***	13.5 ± 3.8 (11)***
10	46.1 ± 16.6 (5)	8.0 ± 1.5 (12)**	7.3 ± 2.3 (8)*
12	-	20.5 ± 4.2 (7)	9.8 ± 3.1 (10)
22	22.8 ± 4.6 (6)	36.0 ± 9.2 (5)	38.5 ± 2.9 (3)
26	51.9 ± 13.4 (6)	48.5 ±10.9 (8)	28.5 ±10.4 (5)
30	57.9 ± 9.8 (6)	31.8 ± 9.1 (11)	17.1 ± 7.1 (6)**
34	54.9 ± 8.2 (6)	26.1 ± 8.2 (10)*	14.5 ± 6.3 (4)**
38	36.5 ± 14.9 (5)	26.7 ± 10.8 (8)	10.8 ± 3.1 (4)

Significant differences from stock group *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$

combined. Thus despite overall similarities there were also differences in the changes with age between glucose tolerance and insulin sensitivity. Insulin sensitivity did not vary between the dietary groups during the rapid growth phase, whereas glucose tolerance did. This would suggest differences in insulin secretion at this time and indeed this was found to be the case.

Serum glucose levels

Mean fasting serum glucose levels are shown in Table 4.10. The two groups weaned onto the high sucrose diet (Groups 2 and 3) had generally similar levels that tended to be higher than those of the stock group, but the differences were not consistant.

Insulin secretion

These data are recorded in Table 4.11. The values show the maximum insulin secretion measured between 4 and 12 minutes after the glucose injection. Up to 10 weeks of age the insulin secretion of both groups given the high sucrose diets (Groups 2 and 3) was significantly lower than that of the stock group. Towards the end of the trial insulin secretion was again relatively poor in these two groups.

Unfortunately some of the frozen serum samples were destroyed during storage so there are no values from 14 to 18 weeks of age.However the data available suggest that after the poor response seen initially, in the two experimental groups, secretion did improve so that the responses in the three groups were not significantly different from one another at 22-26 weeks of age.

Discussion

It has previously been shown in pigs (Heard & Henry, 1969), dogs (Heard & Turner, 1967) and man (Baird & Farquhar, 1962) that glucose tolerance is poor in the young animal. During

there is a marked rise leading to a peak
the period of rapid growth/ in early adult life and thereafter a slow decline (Heard, Turner & Platt, 1964; Himsworth & Kerr, 1939). This normal pattern of development has been confirmed here in New Zealand White rabbits given stock diet ad libitum. The adult level was achieved by 4½ months of age when the animals' mean weight was just over 3 kg and the rate of growth had slowed. The adult level in the dog was achieved by 12 months of age which is a comparable period in the life span of this species. The mechanism for this age related change is not clear. It cannot be related to body weight since the initial increase with age, although at a lower level, was also seen in the groups of offspring which were of a lower weight throughout this development of glucose tolerance. It must therefore reflect some metabolic control system which is independant of these diet induced weight variations.

It is apparent that in rabbits reared on stock diet, variations of glucose tolerance with age mirror those of insulin sensitivity and further that a strong correlation exists between the two parameters. Coincident variations in circulating insulin were not observed thus it was tissue sensitivity to the hormone rather than the hormone itself which had the primary influence in determining tolerance to glucose in these animals. These findings are in agreement with those previously reported in man, pigs and dogs (Himsworth, 1940; Heard & Henry, 1969; Martin, Pearson & Stocks, 1968). Obviously this relationship cannot exist in conditions of absolute insulin deficiency such as usually occurs in juvenile onset diabetes, for example, but it has been shown to exist "for a wide range of nutritional conditions from one mammalian species to another" (Heard 1978).

In the two groups of rabbits which received diets containing a high level of sucrose parallelism between glucose tolerance and insulin sensitivity was also observed. However, when insulin sensitivity was poor, the K_G/K_{G+I} ratio was low relative to that of the stock group, and this resulted in a different slope for the regression line. An explanation

for this modified behaviour was found in the insulin secretory response to the glucose load. During the early period when insulin sensitivity was low but similar in all groups there was a tendency for glucose tolerance to be reduced in the experimental offspring compared to the stock group. During this time, insulin secretion following the glucose load was also low in these groups. Glucose tolerance was lower in offspring of low protein mothers than in those of high protein mothers, which suggests further impairment of insulin secretion. Although this is not borne out by comparisons of the maximum insulin secretion values differences may exist between the groups in total insulin secretion (i.e area under the curve) but this has not been measured. The reason for this poor insulin secretion must lie in the dietary regime imposed during gestation or lactation, but the specific cause is unknown.Low plasma insulin levels, both basal and in response to a glucose load, have been reported in children suffering from protein calorie malnutrition (Baig & Edozien, 1965; Lunn Whitehead Hay & Baker 1973), in pigs which were protein restricted (6% of the diet as protein) (Antinmo Baldijão, Pond & Barnes, 1976) and in malnourished rats given diets containing 0.5% lactalbumin (Young, Vilaire, Newberne & Wilson, 1973; Anthony & Faloona, 1974). It is possible that here also the effect in rabbits is one of malnutrition prior to weaning. The effect of a low protein diet during reproduction has been well documented and it was expected that the offspring of the mothers given the moderate protein (12%) level would be light at weaning, but the weaning weights of offspring of the mothers given 18% protein were also significantly lower than the stock group, which is a primary indicator of a poor nutritional regime. Levels of serum metabolite were not monitored in the pregnant or lactating females, nor was milk production. Perhaps these measurements would have clarified the situation. Thus lower insulin

secretion modified the extent, but not the nature of the changes in glucose tolerance with age, which essentially reflected changes in sensitivity to insulin.

After maximum levels of insulin sensitivity are attained it is part of the normal aging process for insulin sensitivity to decline gradually with increasing age (Silverstone, Brandfonbrener, Shock & Yiengst, 1957; Himsworth & Kerr, 1939). The rabbits given the normal stock diet followed this trend, but the animals on the high sucrose diet showed a greatly exaggerated decline in insulin sensitivity and also glucose tolerance. Himsworth (1940) showed that diets high in carbohydrate led to enhanced glucose tolerance whereas those in which the major energy source was fat led to impairment of glucose tolerance. However, it has become apparent that the nature of the dietary carbohydrate is of considerable importance when investigating this effect. Cohen (1967) replaced 60% of the fat in a typical "western" diet (in which fat supplied 36% of the total energy) with either bread or sucrose to provide respectively 60% and 50% of the total energy. After 5 weeks on the diets the glucose tolerance of the group of volunteers given the "high bread" diet was enhanced whereas that of the high sucrose group was slightly impaired. When the diets were interchanged again the new "high bread" group showed enhanced tolerance to glucose. Further, if rats are fed diets rich in sucrose (33% to 67% of the dry weight of the diet) glucose tolerance is impaired after 40 to 100 days, the time scale depending on the level of sucrose in the diet (Cohen & Teitelbaum, 1964). The serum of the sucrose fed rats in their study had reduced levels of insulin-like-activity (ILA) in response to a glucose load (measured by bioassay based on glucose uptake by hemidiaphragm muscle) compared to serum of control rats. The explanation offered is that because glucose absorbtion into the blood is more rapid following sucrose ingestion than starch

ingestion, it results in a much stronger stimulation of the insulin system and that if such stimulation is repeated—as when feeding sucrose rich diets—it may lead to impairment of the insulin secretory system (Cohen & Teitelbaum, 1964; Dohan & Lukens, 1947). This reasoning would explain the low insulin secretion in response to a glucose load which was observed here in the sucrose fed rabbits towards the end of the trial. On the other hand, it has been reported that sucrose feeding induced hyperinsulinaemia in 6 out of 19 human subjects (Szanto & Yudkin, 1969) and elsewhere that it had no effect on serum insulin levels in the human (Dunnigan, Fyfe, McKiddie & Crosbie, 1970). Normal serum insulin levels have been observed after feeding rats a diet containing 70% or 35% sucrose for 50 days (Vrána, Slabochová, Kazdová & Fábry, 1971) yet high levels resulted when a group of rats were fed a diet containing 68% sucrose for 24 days (Blasquez & Quijada, 1969). These conflicting data may result from the use of different methods of estimation (either biological assay of ILA or specific hormone measurement by radio immunoassay) from the different sucrose/carbohydrate ratio used and the length of time on diet, from species variation and, especially in the case of the human studies, from individual variation (Szanto & Yudkin 1969; Yudkin, Szanto & Kakkar, 1969). The results of investigations concerning the effect of a high sucrose diet on insulin sensitivity, however, are consistent. Tissue sensitivity to insulin has been assessed using in vitro techniques where glucose uptake by either adipose tissue (Blasquez & Quijada, 1968; Vrána, Slabochová, Kazdová & Fábry, 1971; Reiser & Hallfrisch, 1977) or diaphragm (Blasquez & Quijada, 1968) is monitored and in both cases the tissue sensitivity to insulin is reduced after feeding sucrose rich diets. In the work reported here insulin sensitivity has been measured in vivo and although, strictly speaking, there was no low sucrose control group, a reduction in

insulin sensitivity was observed after feeding a diet rich in sucrose compared to a stock diet.

It is evident therefore, that in determining the cause of impaired glucose tolerance an understanding of the mechanisms mediating the action of insulin at the cellular and subcellular level is essential, such that the effect of diet on these mechanisms can be elucidated.

It must be emphasized that the term "insulin sensitivity" has been used here solely in the context of glucose homeostasis. It would be interesting to know whether the other actions of insulin are similarly impaired by adverse dietary situations. Carbohydrate and protein metabolism are linked through the key hormones insulin, growth hormone and cortisol, which have a large controlling influence over both systems. For example, insulin and growth hormone exert a synergistic effect on growth (Manchester & Young, 1961; Knobil & Hotchkiss, 1964) although they have antagonistic actions in the control of carbohydrate metabolism (Weil, 1965) and release of both is related to blood glucose levels. Thus in addition to contributing as an energy source, which allows protein to be used as a source of nitrogen, dietary carbohydrate must influence protein metabolism through its effect on the endrocrine system. It follows that impairment of carbohydrate metabolism may also influence protein metabolism.

Diets with similar levels of protein (up to 18%) promote smaller weight gains when sucrose is substituted for carbohydrate sources such as corn starch and dextrin. This has variably been attributed to less efficient utilisation of protein for growth (Monson, Harper, Benton & Elvehjem, 1954) and lower food intakes (Spivey, Katayama, Yoshida & Harper, 1958). It has also been shown that this poor growth is accentuated if the protein level is decreased from 18 to 11% of the dry weight of the diet

and that accompanying this there is an increased impairment of glucose tolerance (Cohen & Teitelbaum, 1966). In the work reported here, it was found that there was a reduction in the growth rate of the experimental groups, particularly in the offspring of protein deficient mothers, which was not accompanied by a reduced energy intake when this was related to the size of the animal. In addition, this poor growth rate was accompanied by impaired glucose tolerance. However, in the absence of the moderate sucrose control group, discussion of the data in this respect is severely hampered. This would be an interesting area for further study.

The poor reproductive performance of the rabbits given the high sucrose diets from mating is inexplicable. Energy intake was not reduced during gestation or lactation in these animals. The data available indicates that there was some loss in utero, particularly in the high protein group. The majority of postnatal deaths occurred within one week of birth. the greatest loss being in the neonatal period. It is known that the association of diabetes and pregnancy carries an appreciable risk to the foetus and newborn and that strict control of maternal plasma glucose levels will result in decreased perinatal mortality (Pedersen & Brandstrup, 1956). Because the newborn of diabetic mothers often develop hypoglycaemia, which may be due to hyperinsulinism, it has been postulated that carbohydrate metabolism in utero may be abnormal and that this may contribute to the increased risk of death (Oakley, Beard & Turner, 1972). It is possible that the high level of sucrose in the maternal diet resulted in raised serum glucose levels and a reduced insulin sensitivity as occurs postnatally which could result in foetal hyperinsulinism - a situation comparable to that in diabetics.

The main observation from the work reported here can be summarised as follows:

1. In the New Zealand White rabbits, glucose tolerance is poor in the young animals and increases with age until the adult level is attained which is the normal pattern of development previously reported in other species;
2. The primary influence on glucose tolerance was tissue sensitivity to insulin. The relationship was modified in the animals given the high sucrose diet;
3. The poor glucose tolerance observed initially in the offspring of mothers given the experimental diets was probably due to poor insulin secretion since insulin sensitivity was similar to that of the stock group, but the loss of glucose tolerance subsequently was probably due to decreased tissue sensitivity to insulin;
4. The reproductive performance of rabbits given a diet containing 32% sucrose by weight during gestation and lactation was poor.

CHAPTER 5. FINAL DISCUSSION

CHAPTER 5 : FINAL DISCUSSION

The rabbit was used for the work involving glucose tolerance measurements mainly because it is a very convenient size. Being smaller than animals such as pigs and dogs, which have previously been used for this type of work, it is easier to house in large numbers yet it is still large enough for the repeated blood sampling necessitated by the experimental programme.

The pattern of development of glucose tolerance was found to occupy a similar time period in the life of this species as has been reported for other experimental models. Heard, (1978) in a recent review pointed out the differences between experimental animals and man in this respect. In man this peak value of tolerance is achieved at about 6 months of age whereas in other species studied this does not occur until after sexual ~~mating~~ maturity. He also noted that the normal K_G value for adult animals is about 5% per minute whereas that for man is about 2% per minute and discussed some possible biochemical consequences of this difference. The K_G value in the normal adult New Zealand White rabbit was found to be about 2.5% per minute, thus it is perhaps possible that the rabbit is a good animal model for studying the human condition.

The discussion of the observations made on the rabbit was mainly concerned with the effects of feeding a high sucrose diet after weaning. Previous work on rats and humans have shown that sucrose-induced impaired glucose tolerance can be reversed by dietary rehabilitation but the animals can not be completely recovered since re-impairment on the re-introduction of a high sucrose diet is a much quicker process. It is known that the earlier a dietary regime is imposed the more lasting are the effects, at least in terms of growth, so it is unfortunate in this respect that the females given the high sucrose diet from mating showed poor reproductive success because it would have been

interesting to compare the reversibility of effects seen in each group.

The impaired glucose tolerance and insulin secretion observed in malnourished children, which is the result of chronic deprivation, appears to be more difficult to reverse with rehabilitating diets even after catch-up growth had been observed (James and Coore, 1970; Cook, 1967). The results of measuring insulin secretion in vitro of the pancreases from offspring of hamsters given diets of different protein value reported in this thesis show no deviations from the stock control group - except in one instance when it was inexplicably increased. This could well have been because the pups which survived to weaning, which included none from the very low protein mothers, were not in fact malnourished, perhaps having competed successfully for a limited milk supply at the expense of their litter mates. In only one group where there were very few pups from just one litter was there any appreciable difference in mean body weight compared to the stock group.

In relation to the work presented in this thesis it would be an error not to include some mention of insulin status in obesity. That there is an association between obesity and maturity onset diabetes is well known (Keen, 1975). Obesity is accompanied by glucose intolerance which is a result of insulin insensitivity(Rabinowitz, 1968; Felig, Wahren, Hender and Brunden, 1973; Salans, Knittle and Hirsch, (1968) and these patients commonly show hyperinsulinism. It has been shown experimentally that the continuous ingestion of diets rich in sucrose can produce obesity (Blasquez and Quijada, 1969; Reiser and Hallfrisch, 1977). Indeed as early as 1916 Higgins concluded from studying respiratory quotients in man, that fructose, and hence sucrose, "shows a tendency to change into fat in the body" as compared with dextrose. It has since been shown with radioactive tracers in rats that the probable reason for this increased adiposity is that when fructose is present in

large amounts a much greater proportion follows the metabolic pathway to glycerol than that to pyruvate (Maruhama and MacDonald, 1970). Also, as mentioned previously, the inclusion of sucrose in diets instead of carbohydrates such as starch and dextrin, results in poorer weight gains. This is not a contradiction since the term obesity relates to the amount of fat in the body and not, as is often used for convenience, overall body weight. Whether or not the rabbits in the study reported here had increased fat in relation to their body weight is not known. Bierman Bagdade and Porte,(1968) suggested that the amount of body fat might show a correlation with fasting serum insulin levels. They had previously demonstrated a correlation between fasting serum insulin levels and per cent ideal body weight in healthy male volunteers (Bagdade, Bierman and Porte, 1967). A similar correlation is reported here in the hamsters, particularly the males, between fasting serum insulin levels and body weight at the termination of the growth trial.

The other aspect of the work reported in this thesis was to evaluate the response, in terms of growth and reproduction, to different levels of protein from various sources in the diet of the golden hamster. An attempt was made to equalise the utilisable protein levels in diets containing protein from different sources by supplying equivalent amounts of limiting amino acids. It might have been expected, therefore, that the response to diets containing equal amounts of protein would be similar regardless of the source. This was, however, found not to be the case in either the growth or the reproduction trials. Various explanations can be offered for these observations. Firstly the different constituents of the diets may have affected their palatability and thus food intake; this was apparently not the reason for the different results however because, at least with the growth trial, low weight gains were not a result of low energy intakes. Another possibility is that either the protein sources did not in fact supply amino acids

in the calculated amounts or the availability of certain amino acids may have been low. Utilization of amino acids present in relatively snall amounts can be affected by amino acid imbalance (Wethli, Morris and Shresta, 1975) and this could have existed particularly with soya protein (sample 2) where some amino acids were present in large amounts. However in the case of soya protein (sample 2) with regard to growth, performance was aimilar to that with the other soya protein source. Apart from the possibility of excess amino acids, some other factors toxic to the hamster may have been present in any of the protein sources. This possibility has been discussed in relation to both the poor growth and poor reproductive performance achieved in groups given diets containing fishmeal. Finally, the diets could have been deficient in some respect other than protein or energy but this is unlikely because all groups given experimental diets would have suffered to a similar extent.

A conclusion that can be drawn from the work presented here is that the observations support the principle that a mixed source of dietary protein is probably better than a single protein source.

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