EFFECT OF FEEDING RATS ON DIETS WITH DIFFERENT PROTEINS FOR ONE HOUR DAILY ON LIVEWEIGHT, FEED INTAKE, BODY COMPOSITION AND FREE AMINO ACIDS IN BLOOD

M. R. NAGY

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Dit proefschrift met stellingen van

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B.Sc. (Agric.) en M.Sc. (Dairying), Cairo University, geboren te Assiout, Egypte (V.A.R.), 13 september 1933, is goedgekeurd door de promotor, Dr. C. DEN HARTOG, hoogleraar in de leer van de voeding en de voedselbereiding.

De Rector Magnificus van de Landbouwhogeschool,
F. HELLINGA

Wageningen, 27 februari, 1970

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN
OP GEZAG VAN DE RECTOR MAGNIFICUS, DR. IR. F. HELLINGA,
HOOGLERAAR IN DE CULTUURTECHNIEK,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG, 20 MAART 1970 TE 16 UUR

DOOR

M. R. NAGY

THEOREMS

I

Attention should be turned to the influence of nutrients on intelligence in man rather than to their influence on bodyweight.

II

Although there has been great progress in knowledge of the requirements for various nutrients (FOOD & NUTR. BD., 1968), integrated information is still lacking on requirements for an optimum diet.

> FOOD & NUTR. BD. (1968). Recommended Dietary Allowances, Revised 1968. Natl. Acad. Sci. Pub.

III

In developing countries, faulty dietary patterns could in the long term be altered by effective education.

> BOSLEY, B. & HUENEMANN, R. L. (1968) J. Am. diet. Ass. 53: 99

IV

Suitable fermentation of food would improve the biological value of cereals and pulses in developing countries, in spite of the results of ANATHACHAR & DESIKACHAR (1962) and KHANDWALA et al. (1962).

> ANATHACHAR, T. K. & DESIKACHAR, H. S. R. (1962). J. scient. ind. Res. 21C: 191.
> KHANDWALA, P. K., AMBEGAOKAR, S. D., PATEL, S. M. & RADHAKRISHNA RAO, M. V. (1962). J. scient. ind. Res. 21C: 275.

V

Intake of protein-free diet is not recommendable as a routine test of protein quality in trials with protein and non-protein diets provided separately and in a restricted amount.

> THIS THESIS, p. 88.

VI

A strict limitation of egg consumption is not recommendable without taking into account the total available amounts of other nutrients in human diets.

VII

Use of antibiotics in feed of poultry and other animals should be restricted immediately to avoid hazards to the public health.

Wageningen, 20th March, 1970.
M. R. NAGY

VIII

Generally, addition of H_2O_2 to milk should be allowed in developing countries.

IX

Propionic acid should be allowed as a preservative in bread in the Netherlands

X

Part-time work for married women should be encouraged in the Netherlands, in spite of the general opposition of men to married women working mentioned by SCHELLEKENS-LIGTHART (1962).

SCHELLEKENS-LIGTHART, A. J. (1962). In 'De niet aanwezige huisvrouw' Hilversum, p. 131, Paul Brand N.V.

To Humanity

CONTENTS

1. GENERAL INTRODUCTION

Dietary proteins are important in biological functions. They are essential constituents of a good diet. They are required for the maintenance of body tissues and functions. They are also needed in growth, maturation, pregnancy, lactation, and recovery from injury and disease. Their functions are used directly or indirectly as indices for estimating their nutritive value, that is, their efficiency to satisfy the optimum requirements of the living organism. Differences in the nutritive value of proteins can be related logically, for instance, to differences in:

a. composition
b. requirements of the living organism
c. indices expressing the nutritive value
d. conditions under which an index was estimated.

Proteins differ more in composition than any other group of biologically active compounds. Genetical and environmental factors can affect the composition of proteins in the natural foods. Furthermore, different treatments of processed foods can affect the composition of proteins. Differences between species, ages and physiological states may or may not change the protein requirements. Indices determining specific functions of proteins may rank them in different manners from the indices measuring the overall biological value. The number of suggestions based on theoretical and experimental evidence for choosing criteria for the nutritive value of protein and the conditions under which it should be estimated is large. For detailed information on the literature evaluating the nutrititive value of protein, the reader is referred to ALBANESE (1959), FROST (1959), EL-SAMMAN (1961), LABIB (1962), ALLISON (1964) and MITCHELL (1964) who have extensively reviewed this subject. Briefly, however, one may say that each suggested method has limitation(s).

It has been established that the protein metabolism is considerably sensitive to energy content of the diet (MUNRO and NAISMITH, 1953; ROSENTHAL and ALLISON, 1956). Animals fed on a protein-free diet to appetite, separately from the protein (separate feeding), select a fixed ratio of protein-free diet (energy) to protein (ABRAHAM et al., 1961). This ratio is higher for the good quality protein than for the poor quality protein (POL and DEN HARTOG, 1966). When the protein is mixed with the other constituents and is available throughout the day (mixed feeding), animals have no opportunity to select their ratios of energy to protein consumed. Moreover, when fed to appetite by the system of mixed feeding, they do not eat equal amounts of diet containing proteins of different qualities. They usually consume more of the diet containing the good quality protein than of the one with the poor quality.

Therefore, it is important to understand the effect of protein types on rats fed to appetite on a protein-free diet which is given separately from the protein diet (separate feeding). Different protein types were used in this investigation to represent an animal protein (casein) and a plant protein from cereals (wheat

gluten), tubers (potato protein) or legumes (soya protein). In spite of the laborious and tedious work in the separate feeding system, this should provide information that food of natural origin eaten under normal conditions cannot yield.

Rats were chosen for experiment, because of:

a. their ready availability,

b. their common use in the evaluation of proteins and amino acids,

c. their convenient small size and relatively small feed intake and

d. their rapid growth, high fertility and short lifespan.

Generally, rats are used extensively for research in nutrition to obtain a better understanding of the function of foods and the effect of different factors. The information collected is useful in further investigation on rats and usually valuable in understanding human and animal nutrition.

The separate feeding system was used in three trails. Rats were fed on proteins for an hour and during the following 23 hours they had free access to a protein-free diet. In Trial 1, male rats were fed on an equal amount of nitrogen (N) from either casein or wheat gluten to eat it all in the experimental period (Expt. 1), which lasted 12 weeks after a training period. In Trial 2, the same amount of N from casein, potato protein, wheat gluten and soya protein was given daily to each male rat in the experimental period (Expt. 2), which lasted 16 weeks after a training period. In Trial 3, either casein or wheat gluten was offered to appetite to both sexes for the 20 weeks (Expt. 3) after weaning. Free access to the protein-free diet in the three experiments ensured that the energy intake was high enough to keep breakdown of tissue protein to a minimum. By providing the protein for only one hour each day, fasting levels of free amino acids in blood can be estimated. Four criteria during the relatively long trials were used to indicate and explain the overall biological values of proteins.

These criteria were:

a. liveweight, b. feed intake and c. body composition in the three trials and d. the free amino acids in the blood of rats in trials 2 and 3.

2. EXPERIMENTAL PROCEDURES

2.1. GENERAL

Before the beginning of the experimental periods in trials 1 and 2, weanling male rats were trained to eat daily 192 mg of nitrogen (N) in protein diets within an hour and protein-free diet to appetite in the other 23 hours. However, there was no need to train weanling male and female rats to eat the protein daily to appetite within an hour in Trial 3. The two periods in which weanling rats were trained in trials 1 and 2 are referred to as training periods 1 and 2, respectively. The periods following the training periods 1 and 2 and the weaning of rats in Trial 3 are denoted as experimental periods, i.e., Expt. 1, 2 and 3, respectively.

Unless otherwise stated, the following was used in each trial.

2.1.1. *Animals*

Albino rats of the Wistar strain, bred by the Netherlands Institute of Nutrition, were weaned at an average of 3.5., 3 and 4 weeks of age in trials 1, 2 and 3, respectively. Males were used exclusively in the three trials, whereas females were included in Trial 3. The weanling animals were housed in individual cages in trials 1 and 2, but in replicates of 15 rats per cage in Trial 3. Cages were screened with raised screen bottoms. Rats were kept in an air-conditioned room maintained at 21° to 23°C.

When a population of rats was divided into different groups, each group had as far as possible a similar mean initial weight and number of litter mates.

2.1.2. *Drinking water*

Fresh tap water was supplied constantly throughout the life of the rats.

2.1.3. *Feeding*

The weanling rats were fed on the protein for an hour and during the following 23 hours a protein-free diet was given to appetite in other clean feed containers in the three trials. Spilt and surplus feed was removed from the cages before each change in feed type. This separate system of feeding was not used, when the stock diet was given in a part of Training Period 1.

Commercial proteins containing more than 9.6% N were mixed with the protein-free diet up to this level in trials 1 and 2 (training and experimental periods), whereas commercial proteins were used without the previous adjustment of the N content with the protein-free diet in Trial 3. If the protein-free diet was mixed with the protein to adjust the protein content, it was about a quarter of the total mixture. Each rat was offered a restricted amount of 2 g protein diet containing 192 mg N in Expt. 1 and 2 for an hour a day, whereas the animals were given the protein to appetite for an hour a day in Expt. 3. The 2 g of protein diet offered in trials 1 and 2 were weighed and folded automatically into a piece of non-absorbant paper by the method of POL and DEN HARTOG (1966). The commercial casein was obtained from De Meijerij G. A., Veghel. The commercial

heated potato protein was bought from Avebe, Veendam. The commercial heated soya portein was received from N.V. W. Ruitenberg Czn., Amersfoort. The commercial heated wheat gluten was from Latenstein's Fabrieken N.V., Nijmegen.

The protein-free diet and vitamins were the same as those used by POL and DEN HARTOG (1966), except for Expt. 3 in which vitamins A, D and E were mixed with the protein-free diet to facilitate the handwork. Rovimix AD₃ type 325/65 30 mg and Rovimix E adsorbate 25 % 200 mg were added per kg protein-free diet in Expt. 3. These vitamins (Rovimix) were kindly supplied by F. Hoffman – La Roche and Co., Ltd. Basle, Switzerland.

The necessary proteins and protein-free diet were prepared at convenient intervals before use and kept in a deep-freezer at about –20°C to keep their nutritive value. When they were given to the rats, they had the same temperature as the air-conditioned room to prevent any possible effect of the low temperature of the diet on the feed consumption and health of the rats.

The stock diet, used in a part of Training Period 1, was made as described by DALDERUP et al. (1967) and was offered to appetite for the whole day, whenever used.

2.1.4. *Weighing*

The rats were weighed on a Berkel balance type E. H., capacity 0.5 to 3,000 g, at a fixed time of the day before offering the protein. The weight of animals was recorded weekly, except for the Training Period 1, when the intervals were less frequent.

The protein-free diet consumption was recorded by using the same balance, except for the feed consumed in Training Period 1 which was not estimated.

The amount of the 2 g protein diet (192 mg N) not eaten was recorded on a scale +, + + and + + + in Training Period 1, whereas it was weighed on a laboratory balance with an accuracy of ± 10 mg in Expt. 1, and in Trial 2. Protein intake in Expt. 3 was weighed on the Berkel balance and was recorded daily. When the stock diet was used, feed intake was not estimated.

The weight of spilt and surplus feed was taken into consideration, when calculating feed intake. If spilt or surplus feed had been moistened by urine or drinking water, it was dried in an oven before weighing, then compensated for the normal moisture content in the feed. This reduced the error which normally occurs in routine work, although it is time-consuming.

2.1.5. *Carcass analysis*

Carcass water (W), dry matter (DM), crude fat (F) and nitrogen (N) were estimated individually in trials 1 and 2 and on each 15 rats (replicate) as a whole at the end of Trial 3 by the methods of POL and DEN HARTOG (1966).

2.1.6. *Free amino acids in blood*

Free amino acids in blood were estimated only in the rats of trials 2 and 3. For hygiene, rats were moved to newly cleaned cages before blood sampling.

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Blood was collected from newborn rats in Trial 3 by decapitation, because it could not be taken from the tails. However, blood from the other rats in trials 2 and 3 was taken from their tails by the following procedure. The tails were warmed in water initially about 20°C higher than the rat's body temperature for about 5 min. They were immersed in absolute ethanol and then dried. As small as possible a piece from the end of the tail was cut with sterilized scissors, and the blood oozing out was sucked into a dried pipette that had previously been moistened with heparin solution. The total blood in a pooled sample was always 0.45 ml. The volume of blood taken from any rat in a pooled sample was as far as possible similar to the volume of blood taken from other rats in the same pooled sample. For example, if there were three rats in a pooled sample, 0.15 ml blood was sucked from each. Immediately after collection of blood the wounded tail was treated with 6% collodium.

The pooled sample was deproteinized by the method of FOLIN and WU (1919) and HADEN (1923). The pooled sample was mixed with 3 ml distilled water and allowed to stand for 10 min. Sulphuric acid $^2/_3$ normal concentration 0.5 ml was added and the tube was shaken for about 2 min. Thereafter, 0.25 ml 10% (W/V) sodium tungstate was added drop by drop with shaking, after standing for a minute another 0.25 ml was added. All contents were shaken thoroughly for one minute and after standing for 10 min. they were centrifuged at about 4,500 r.p.m. for about 10 min and filtered. About 1 ml toluene was added to the filtrate which was kept at about 4°C to await analysis. In trial 2, blood was analysed within a week of sampling, whereas in Trial 3 blood was analysed after the end of the trial.

The procedure of analyses was as follows:
Water and toluene were evaporated to dryness by heating a sample under vacuum in a waterbath. The residue was dissolved in 1 ml hydrochloric acid 0.1 normal concentration containing 0.1 μ mole norleucine and transferred quantitatively to a column of strongly acidic cation-exchange resin. Amino acids were estimated with a Technicon Auto-Analyzer, using the 21-hour procedure, by the methods of MOORE and STEIN (1954) and YEMM and COCKING (1955) adapted to the Technicon Auto-Analyzer (TECHNICON MONOGRAPH, No. 1, 1966).

2.1.7. Statistics

Standard errors (SE) are given with means in the results to represent the variability of observations. The coefficient of variation (CV) was used for the comparison of variations within two different groups of observations. Since the standard deviation (SD) and CV can be easily calculated from the SE given in the tables, these values were not tabulated. The significance of a difference between any two means was determined by Student's t test at a probability less than 0.05 ($P < 0.05$). The above calculations were not applied to the levels of free amino acids in blood and their special caculations are explained in Chapter 6. SD, SE, CV, and t test were calculated as described by SNEDECOR (1946).

2.2.1. *Training Period 1*

Ninety-two weanling rats were housed individually. The period of 47 days after weaning was used for training. During this period, rats were given for 9 days after weaning the first commercial heated potato protein containing 6.4% N manufactured from the new crop. This protein was not mixed with any amount of the protein-free diet due to its initial low N content. None of the rats consumed all the 2 g potato protein. The apparent ill-health of the animals as judged both from the decrease in weight and their appearance did not encourage continuation of this for a longer period. Consequently, the stock diet was given for the whole day for a further five days. After this, a second heated potato protein from the same bulk batch as that used by POL and DEN HARTOG (1966) was adjusted to 9.6% N and 2 g was offered for one hour daily per rat for 19 days. One rat died in this period after decreasing in weight for two weeks; its data were discarded. At the end of this period, 55% of the rats still did not consume 2 g of the second potato-protein diet. Nine rats were removed at random to be used in another investigation which was not related to this experiment. The remaining was divided into two groups. The first and second group were fed, respectively, on casein and potato protein containing 9.6%N in an amount of 2g for one hour each day per rat for a week. In the following week, the rats fed on potato protein were given the casein and vice versa. The animals were weighed at the beginning and days 7, 14, 21, 28, 33, and 47 of Training Period 1. The ability to consume 2 g (192 mg N) protein diet in one hour was observed in the training period.

2.2.2. *Experimental period (Expt. 1)*

The period of 12 weeks after Training Period 1 is referred to as the experimental period. The observation of the potato-protein consumption during the training period did not encourage the use of the second heated potato protein in Expt. 1. After Training Period 1, the rats which were about 10 weeks old, were divided into a casein group (Cn) and a wheat-gluten group (Gn). Sixteen rats, eight from each group were taken at random and sacrificed for individual carcass analysis. Cn and Gn received daily 2 g protein diet containing 9.6% N per rat. The data of a sick rat of Gn in Week 7 of Expt. 1 were discarded from the rats' results of Week 7 and following weeks. Seven rats of Gn were removed at random in Week 9 of Expt. 1 for another investigation not related to this one.

In Expt. 1, the weight was recorded weekly and the feed intake daily. The carcass was analysed in eight rats of each group (Cn and Gn) at the end of weeks 3 and 6 of Expt. 1; in eight rats of Cn and in four rats of Gn at the end of Week 9 of Expt. 1 and in the remaining ten rats of Cn and in the remaining five rats of Gn at the end of the experiment (Week 12).

2.3. TRIAL 2

2.3.1. *Training Period 2*

Fifty-one male rats were weaned and housed individually. Three rats were sacrificed for individual carcass analysis at weaning. The period of 4 weeks after weaning was used as a training period. The remaining 48 rats were given 2 g protein containing 9.6% N daily for one hour. The source of protein was changed every day so that each rat received casein, the third heated potato protein, heated wheat gluten and heated soya protein in each four days of the four weeks of the training period. These proteins were newly manufactured and their N content was higher than 9.6%. The rats were weighed weekly and feed intake was recorded daily.

2.3.2. *Experimental period (Expt. 2)*

The period of 16 weeks after Training Period 2 is referred to as the experimental period. After the training period, the rats were divided into a casein group (Cn), potato group (Pt), wheat-gluten group (Gn), soya group (Sy). Eight rats, two of each group, taken at random were sacrificed for individual carcass analysis. Casein, the third heated potato protein, heated wheat gluten and heated soya protein were given to Cn, Pt, Gn and Sy respectively. A restricted amount of 2 g protein diet containing 9.6% N was offered daily to each rat for one hour. Some rats did not eat all 2 g wheat-gluten or soya-protein diet. The quantity which was not eaten was given by a stomach tube after homogenization with water. On the day of sampling the blood from Sy, rats were given all 2 g soya protein by stomach tube, because they needed longer to consume it than the other proteins and some rats could not consume 2 g soya protein in one hour. In the experimental period, the liveweights were recorded weekly, feed intakes daily and the carcasses were analysed individually as follows:

12 rats (three of each group) at the end of Week 7 of Expt. 2 (about 14 weeks old).

12 rats (three of each group) at the end of Week 11 of Expt. 2 (about 18 weeks old).

16 rats (four of each group) at the end of Week 16 of Expt. 2 (about 23 weeks old).

Free amino acids in blood were estimated in Expt. 2 in pooled samples at three different times and ages. The 0.45 ml blood of a pooled sample were obtained from three rats fed on the same protein and the three times were:

First time   50 min before giving the protein
Second time A.   45 min after giving the casein, third potato protein and wheat gluten
Second time B.   30 min after gradually giving 2 g soya protein diet by stomach tube over a period of 15 min
Third time   60 min after the second time

The rats were about 7 weeks old when the experimental period began, so that ages at times of blood sampling in Trial 2 were about: 10, 14, and 18 weeks for Cn; 11, 15 and 19 weeks for Pt; 12, 16 and 20 weeks for Gn; 13, 17 and 21 weeks for Sy.

Blood samples for amino acid analysis from newborn rats and their mothers were taken shortly after birth, that is within a day. There were three pooled blood samples from the newborn rats and three pooled blood samples from their mothers. Each sample of mothers' blood was obtained from the tails of six animals which had borne 9 rats or more per mother on the same day. Each sample of the newborn rats was obtained by decapitation of two newborn rats from each mother (12 newborn rats). Mothers were bred on the stock diet. The number of rats in each litter was reduced to seven individuals after birth.

Ninety male weanling rats were divided into a casein group (Cn ♂) and a wheat gluten group (Gn ♂). Each group was triplicated. The groups of 15 rats were each housed in a large cage. The feed cup was fastened in the middle of the cage to give better opportunity to all rats in a replicate to consume the feedstuff at the same time. Sieves over the catch pans under the rat cages were used to facilitate separation of the faeces from the scattered protein-free diet. The positions of the cages of a certain replicate were random. The Cn and Gn were fed to appetite for one hour daily on commercial casein containing 12.82% N and commercial wheat gluten containing 12.78% N. No adjustment with the protein-free diet for the N content was needed. The protein was not offered on Day 1 of Expt. 3 (the day in which the rats were divided into groups), but the protein-free diet was given to obtain the fasting levels of the free amino acids in the blood before giving the protein on Day 2 of Expt. 3. Pooled blood samples for the analysis of free amino acids, representing a certain replicate, were taken before giving the commercial protein on Day 2 of Week 1 and Day 1 of weeks 9 and 21 of Expt. 3. After the blood samples were taken on Day 1 of Week 21 of Expt. 3, the rats were sacrificed in the afternoon without offering feed during that day. The carcasses were analysed for the replicate as a whole.

Ninety females were weaned two days after the males. They were treated in the same way as the 90 male weanling rats, except for being two days later in date than the males.

2.5. ABBREVIATIONS and SYMBOLS

For simplicity, abbreviations and symbols are used throughout this thesis. They are arranged alphabetically for each subdivision of this section.

2.5.1. *Rats' groups*

Abbreviations Explanations
Cn   rats fed on casein (casein group)
Gn   rats fed on wheat gluten (wheat-gluten group)
Pt   rats fed on potato protein (potato group)
Sy   rats fed on soya protein (soya group)

When age, sex and replicate of a group of rats are indicated with the previous, abbreviated, the method is as follows:

1. A subscript number after the abbreviation of a group shows the approximate age of the rats in weeks.
2. ♂ or ♀ after the abbreviation of a group illustrates that rats are male or female respectively.
3. Capital letter A, B or C after and over the abbreviation of a group means first, second or third replicate of a group, respectively.

For example, the symbol for male rats 24 weeks old fed on wheat gluten, of Replicate A of this group is simply $Gn_{24}\male^A$.

2.5.2. *Body composition*

Abbreviations	Explanations
DM	dry matter
F	crude fat (ether extract)
FDM%	fat in dry matter percentage
N	nitrogen
NDM%	nitrogen in dry matter percentage
N/W	nitrogen to water
W	water

2.5.3. *Abbreviations of scientific periodicals*

Most names of scientific periodicals are abbreviated as described by BROWN and STRATTON (1963).

2.5.4. *General abbreviations*

The common abbreviations and symbols in biology were taken mostly from the publication of CONFERENCE of BIOLOGICAL EDITORS COMMITTEE on FORM and STYLE (1964).

3. LIVEWEIGHT

3.1. INTRODUCTION

The liveweight of rats over a specific period illustrates the distance in weight achieved by growth if growth is considered as a form of motion. The gain in liveweight per unit time indicates rate of growth. These parameters are derived from the same observation by expressing them in different ways. The first liveweight, is integral of the second, gain in liveweight, whereas the second is differential of the first. Generally, the increments or losses in liveweight show the animal's state better at any particular time than the liveweight which depends so largely on the growth of the animal in all the foregoing intervals of time.

Increase in liveweight is only one feature of growth which is such a complex process that its precise definition is still debatable. However, it is a far easier and far more accurate to weigh rats than to measure dimensions such as length, width and girth, because these measurements have slight relative differences and much more sensitive and laborious techniques are necessary. The fur may hinder these measurements and measurements may be difficult, if the animal will not keep still.

Growth can be used to study protein quality. Gain in liveweight of growing animals under standard conditions is the simplest test of animal growth available. Differences from one age to the next in liveweight gain or loss between rats of similar initial weight have been used in these trials.

Most attempts to fit mathematical curves to animal growth data have not been completely successful. Due to the complexity of these mathematical systems, it is difficult to interpret them in a biologically meaningful manner. Moreover, recent data do not agree well with them. As liveweight, for example, exhibits differential growth rates for the different parts and tissues the measurements themselves are complex. Therefore, no mathematical curve has been fitted for growth data.

Rats seem to have no visible adolescent growth spurt as observed in the human being and other primates (TANNER, 1962) and they continue to grow for a proportionally longer period after sexual maturity. It should be remembered that although liveweight as criterion for growth is valuable, it must be particularized and not applied in a general sense. Growth in linear dimension can occure without increase in weight. So generalization about growth processes from liveweight alone can be sometimes misleading and hazardous.

3.2. RESULTS

(Liveweight)

Unweaned rats were not weighed to avoid disturbance which sometimes causes the mother to destroy her young. Anyway the liveweights before weaning were of no interest in this study.

10   *Meded. Landbouwhogeschool Wageningen 70-4 (1970)*

3.2.1. *Trial 1*

3.2.1.1. Training Period 1

The liveweights of rats during the training period are shown in Table 1. The rats varied widely in liveweights at weaning (30–64g). All rats were used, whatever their liveweight. The large number of rats in Training Period 1 allowed a better statistical analysis of the results. Decrease in liveweights of rats after a week of training on the first commercial potato protein, which contained 6.3% N and was given for 9 days, was 6.8 ± 0.3, range 1 to 14 g. After 5 days on stock diet, the rats regained these weight losses and gained 15.1 ± 0.5, range 1 to 24 g. On giving the second potato protein containing 9.6% N, the rats changed in weight by 2.6 ± 0.4, range –7 to 14 g during Week 1; 8.7 ± 0.3, range 0 to 17 g during Week 2 and 20.5 ± 0.7, range 1 to 32 g during the total period of 19 days. The rats fed on casein and potato protein in the last two weeks of Training Period 1 gained 46.0 ± 0.9, range 29 to 63 g during this period.

TABLE 1. Liveweight of male rats during Training Period 1

Training Period 1	No. of rats	Weight (g)	
		Range	Mean ± SE
Beginning (weaning)	92	30– 64	48 ± 0.9
Day 7	92	28– 55	41 ± 0.7
Day 14	92	45– 85	63 ± 1.0
Day 21	92	40– 90	66 ± 1.0
Day 28	92	43–100	74 ± 1.1
Day 33	92	55–111	84 ± 1.3
Day 47	83	99–158	131 ± 1.8

The rats could be divided into those that could and those that could not eat 192 mg N offered on days 21 and 33 of the training period (Table 2). Those that ate the given amount gained significantly more in the whole period than those that did not. There was no significant difference between the initial liveweights.

TABLE 2. Gain in liveweight of male rats in relation to a daily protein intake during Training Period 1

Protein intake[1]	No. of rats	Weight at weaning (g)	Gain in weight (g)
During Day 21			
2 g potato protein eaten by each rat	23	48 ± 1.7[2]	23.1 ± 1.0
Less than 2 g potato protein eaten by each rat	69	48 ± 1.1	15.9 ± 0.7
During Day 33			
2 g potato protein eaten by each rat	47	49 ± 1.3	42.0 ± 0.9
Less than 2 g potato protein eaten by each rat	45	48 ± 1.3	29.1 ± 1.3

[1] 2 g protein containing 9.6% N were given for 1 h daily. [2] Mean ± SE.

3.1.1.2. Experiment 1

The liveweights and gains in liveweight of the rats fed on 192 mg N from casein or wheat gluten daily for 1 h during the 12 weeks of Expt. 1 are given in Table 3. The large number of animals used facilitated the division into two similar groups. So the mean liveweights and SE of the Cn and Gn were similar at the beginning of Expt. 1. The weights of the rats in Week 1 decreased in 14 and 30 individuals, increased in 16 and 2 individuals and did not change in 4 and 1 individuals with an average decrease of 0.2 $\pm$ 0.7 and 4.1 $\pm$ 0.5 g in the individuals of Cn and Gn, respectively. The difference in this decrease between the two groups was significant. However, it did not result in a significant difference in their liveweights. This was due to the small difference which was masked by the variation within the groups. The weight of each rat in both groups increased in Week 2 and in the successive weeks, except for an ill rat which lost 15 g in weight during Week 7 of giving wheat gluten; its data were discarded from the results of this week and following weeks. Comparison of the average weight of the rats in one week with the next indicated no significant difference during any week for Gn. This is also applied to Cn from the beginning date and weeks 4, 6, 7, 8, 9, 10 and 11 to the next week. This was due to the relatively small gain in weight which was masked by the variation between individuals in their liveweights in addition to the decreasing number of animals in the groups with time due mainly to sacrificing.

The gains in weight varied from one week to another and their average shows rhytmic flactuations which were mostly similar in the two groups. The weight gain of Cn increased significantly in weeks 2, 3, 6, 8 and 11, decreased significantly in weeks 5, 7, 9 and 12, and did not differ significantly in weeks 4 and 10 from the previous week. The same trend was found in Gn, except that the weight gain in weeks 7, 8, 9, 10, 11 and 12 did not differ significantly from each other. Towards the end of Expt. 1, the SE increased with the decrease in number of rats.

The average weight of Cn was greater than that of Gn in each week of Expt. 1. The difference in weights between the two groups increased with age, except for the last week because the average gains in weight in that week in the two groups were equal. The difference in liveweights of the two groups did not attain a significant level in Week 1 but was significant in Week 2 and successive weeks. This is shown by the higher weekly gain in liveweight of Cn compared to Gn, except for the last week. The difference in the weekly gains in liveweight between the two groups was significant, except in weeks 7, 9, 10 and 12 of Expt. 1. The difference between the two groups in the gain in liveweight of each of the four 3-week intervals decreased with age. This difference was significant in the first three 3-week intervals and insignificant in the last 3-week interval.

3.1.2. *Trial 2*

3.1.2.1. Training Period 2

During Week 1, the weights of two rats decreased by 1 and 2 g, respectively, whereas each of the remaining 46 rats increased in weight. The decrease was less

TABLE 3. Effect of two protein types given in restricted amount [1] on the liveweight of male rats (Expt. 1)

	Number of rats		Weight (g)		Gain in liveweight (g)	
	Casein group (Cn)	Wheat-gluten group (Gn)	Casein group (Cn)	Wheat-gluten group (Gn)	Casein group (Cn)	Wheat-gluten group (Gn)
Beginning[2]	42	41	131 ± 2.7[3]	131 ± 2.6		
Week 1	34	33	131 ± 2.7	126 ± 2.3	-0.2 ± 0.7	-4.1 ± 0.5
Week 2	34	33	142 ± 2.6	129 ± 2.4	11.1 ± 0.4	2.4 ± 0.3
Week 3	34	33	159 ± 2.8	135 ± 2.5	17.3 ± 0.5	5.7 ± 0.3
Week 4	26	25	178 ± 3.2	143 ± 2.8	17.1 ± 0.7	6.4 ± 0.6
Week 5	26	25	184 ± 3.3	147 ± 2.8	6.6 ± 0.6	4.8 ± 0.4
Week 6	26	25	195 ± 3.4	153 ± 2.8	10.2 ± 0.5	5.9 ± 0.3
Week 7	18	16	203 ± 4.4	159 ± 3.3	7.3 ± 0.6	6.2 ± 0.4
Week 8	18	16	213 ± 4.6	164 ± 3.3	10.6 ± 0.7	5.2 ± 0.4
Week 9	18	9	219 ± 4.7	174 ± 4.5	6.2 ± 0.7	4.2 ± 0.6
Week 10	10	5	229 ± 5.4	180 ± 8.8	5.3 ± 1.1	3.5 ± 1.7
Week 11	10	5	237 ± 5.8	184 ± 9.5	8.0 ± 0.6	4.4 ± 1.2
Week 12	10	5	243 ± 6.1	190 ± 9.0	5.9 ± 0.8	5.9 ± 1.3
First 3 weeks	34	33			28.2 ± 0.8	4.0 ± 0.5
Second 3 weeks	26	25			34.0 ± 0.7	17.1 ± 0.6
Third 3 weeks	18	9			24.1 ± 1.1	15.6 ± 0.8
Fourth 3 weeks	10	5			19.2 ± 1.9	13.8 ± 1.7

[1] 2 g containing 9.6% N per rat for 1 h per day.
[2] Rats 10 weeks old on average.
[3] Mean ± SE.

than 5% of liveweight and can be considered negligible. In weeks 2, 3 and 4, each of the 48 rats increased in weight. The mean liveweight and gain in liveweight are presented in Table 4. The gain in weight increased significantly in Week 2, did not differ significantly in Week 3 and increased significantly in Week 4.

TABLE 4. Liveweights of male rats during Training Period 2

	No. of rats	Liveweight (g)	Gain in liveweight (g)
Beginning	48	38 ± 0.5[1]	
Week 1	48	41 ± 0.6	2.8 ± 0.2
Week 2	48	52 ± 0.6	10.8 ± 0.4
Week 3	48	62 ± 0.6	10.3 ± 0.3
Week 4	48	75 ± 0.7	12.8 ± 0.3

[1] Mean ± SE.

3.1.2.2. Experiment 2

Table 5 represents the liveweight and gain in liveweight of rats fed on casein alone, the third potato protein, wheat gluten and soya protein. None of the rats in the table decreased in weight from any week to the next. The weight of Cn was sometimes higher than that of Pt, but lower at other times. The difference between their liveweights was in any week less than 5% of their weights in that week. Their weekly gains in liveweight did not differ significantly in any week. The result was no significant difference in liveweights between Cn and Pt. These two groups can be considered similar in their liveweight. The average liveweight of Sy was greater than that of Gn in any week. The difference in liveweights of these two groups in weeks 2, 3, 4, 5, 6 or 7 of Expt. 2 exceeded 5% of their weights in the same week, whereas it did not in weeks 8 to 12. In Week 1, Gn and Sy gained nearly the same average weight and therefore showed no significant difference between their weights. In weeks 2 and 3, the gain in weight of Sy was significantly higher than that of Gn resulting in a significant difference between their weights. The gains in weight of these two groups did not differ significantly from each other in weeks 4, 5, 6 and 7 so that significant differences in liveweights remained constant from week to week. In Week 8, Gn gained significantly more weight than Sy, resulting in a decrease in the difference between their weights. So there was no significant difference between their bodyweights. The weekly gain in weight of Gn compared to Sy in weeks 9, 10, 11 and 12 fluctuated without significant differences and not resulting in any significant difference between their liveweights.

The liveweight of Cn and Pt in the first week and successive weeks was greater than that of Gn and Sy and the differences exceed 10% of their weights in the same week. The significantly higher gain in weight of Cn to that of Gn during weeks 1, 2, 4, 5, 6, 7, 9, 10 and 11 and their accumulation resulted in a significant difference in the weights of the two groups in every week of Expt. 2. The signifi-

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TABLE 5. Effect of four protein types given in restricted amount[1] on the liveweight of male rats (Expt. 2)

Experimental period	Number of rats	Liveweight (g)				Gain in liveweight (g)			
		Casein group (Cn)	Potato group (Pt)	Wheat-gluten group (Gn)	Soya group (Sy)	Casein group (Cn)	Potato group (Pt)	Wheat-gluten group (Gn)	Soya group (Sy)
Beginning[2]	10[3]	75 ± 1.1[4]	76 ± 1.7	75 ± 1.7	76 ± 0.9				
Week 1	10	96 ± 1.7	96 ± 2.1	80 ± 1.5	82 ± 1.2	20.4 ± 0.8	20.0 ± 0.8	4.7 ± 0.7	4.8 ± 0.8
Week 2	10	113 ± 1.8	113 ± 2.1	86 ± 1.8	91 ± 1.2	17.2 ± 0.9	17.2 ± 0.6	6.2 ± 0.7	9.7 ± 0.5
Week 3	10	127 ± 1.9	128 ± 2.6	93 ± 1.5	100 ± 0.8	14.0 ± 0.6	14.6 ± 0.6	7.1 ± 0.3	8.6 ± 0.5
Week 4	10	139 ± 2.5	140 ± 3.0	99 ± 1.6	107 ± 1.3	11.7 ± 0.8	12.2 ± 0.7	5.9 ± 0.7	7.0 ± 0.8
Week 5	7	152 ± 3.2	150 ± 3.7	105 ± 2.4	114 ± 1.7	12.0 ± 0.4	9.6 ± 1.4	6.9 ± 0.5	5.7 ± 1.0
Week 6	7	162 ± 3.3	160 ± 3.9	111 ± 2.6	120 ± 1.7	10.3 ± 0.8	10.2 ± 0.5	5.8 ± 0.4	6.4 ± 0.6
Week 7	7	173 ± 4.0	170 ± 4.0	117 ± 2.4	125 ± 1.7	10.6 ± 1.3	9.6 ± 0.4	6.4 ± 0.4	5.0 ± 0.6
Week 8	7	180 ± 5.7	179 ± 4.8	123 ± 2.4	128 ± 2.5	7.4 ± 2.0	8.8 ± 0.9	5.6 ± 0.3	3.3 ± 0.8
Week 9	4	191 ± 6.3	189 ± 5.8	129 ± 3.5	130 ± 3.3	10.8 ± 0.3	9.6 ± 1.1	4.4 ± 0.5	6.1 ± 1.0
Week 10	4	202 ± 6.4	196 ± 7.2	133 ± 3.2	134 ± 3.1	11.0 ± 1.9	7.1 ± 1.5	4.4 ± 0.6	4.1 ± 0.4
Week 11	4	209 ± 5.9	201 ± 8.3	138 ± 3.5	140 ± 3.2	6.5 ± 0.5	4.6 ± 1.4	4.5 ± 0.5	5.8 ± 0.8
Week 12	4	214 ± 6.5	207 ± 7.8	143 ± 3.9	144 ± 3.3	52. ± 1.4	6.2 ± 0.9	5.4 ± 0.7	3.5 ± 0.3

[1] 2 g containing 9.6% N per rat for an hour a day.
[2] Rats 7 weeks old on average.
[3] Rats were taken periodically from each group for blood and carcass analysis.
[4] Mean ± SE.

cantly higher gain in weight of Cn than of Sy in weeks 1, 2, 3, 4, 5, 6, 7, 9, and 10 and their accumulation resulted also in a significant difference in weights between the two groups in each week of Expt. 2. The significantly higher gain in weight of Pt than of Gn in weeks 1, 2, 3, 4, 6, 7, 8 and 9 and their accumulation resulted similarly in a significant difference in the weights of the two groups in every week of Expt. 2. The significantly higher weight gain of Pt than of Sy in weeks 1, 2, 3, 4, 6, 7, 8 and 12 and their accumulation resulted in a significant difference in the liveweights of these groups in every week of Expt. 2.

3.2.3. *Trial 3*

The liveweights and gains of male and female rats fed on casein or wheat gluten to appetite for an hour each day during five intervals of Expt. 3, each of 4 weeks, are listed in Table 6. The weekly changes in average liveweight and the average change in liveweight (gains and losses) are shown in Fig. 1. Above is plotted the liveweight attained at successive ages; below, the change in liveweight from one week to the next.

Females were on average 3 g heavier than males at weaning, even though from the same parents. The females were weaned two days later than the males. In Week 1 of Expt. 3 (Fig. 1), the liveweight of males fed on either casein or wheat gluten increased but of females decreased. From then until Week 19, the liveweight of both sexes fed on casein or wheat gluten increased. In the 20th and last week of the experiment, liveweight of the males fed on either of the two proteins still increased but of females decreased. The decrease in weight of the females in the last week of the experiment was associated with a decrease in intake of protein and protein-free diet without symptoms. The liveweight of the males was higher than of the females on the same protein each week of the experiment, except initially. The difference in liveweight between males and females increased with age throughout Expt. 3.

The liveweight of Cn was higher than that of Gn in both sexes. The difference between the liveweights of the two groups increased during the first five and six weeks for females and males, respectively. After this, the difference decreased. The difference in liveweights between rats fed on each of the proteins at the end of the experiment was 4% and 2% related to the bodyweight of rats fed on casein for males and females, respectively. This difference may be considered negligible.

From Table 6, the gain in weight in the first 4 weeks was significantly higher in Cn♂ than in Gn♂. So their liveweights differed significantly. The gains in bodyweight by Cn♂ did not differ significantly from those of Gn♂ in the second and third 4 weeks of the experiment and the differences in liveweights remained significant. In the fourth 4 weeks, Gn♂ gained significantly more weight than Cn♂. So the difference between their liveweights decreased and was not significant. The gain in weight of Cn♂ did not differ significantly from that of Gn♂ in the fifth 4 weeks. So their liveweights did not differ significantly either.

The gain in weight of Cn♀ in the first 4 weeks was significantly higher than that of Gn♀. So Their weights differed also significantly. In the second and

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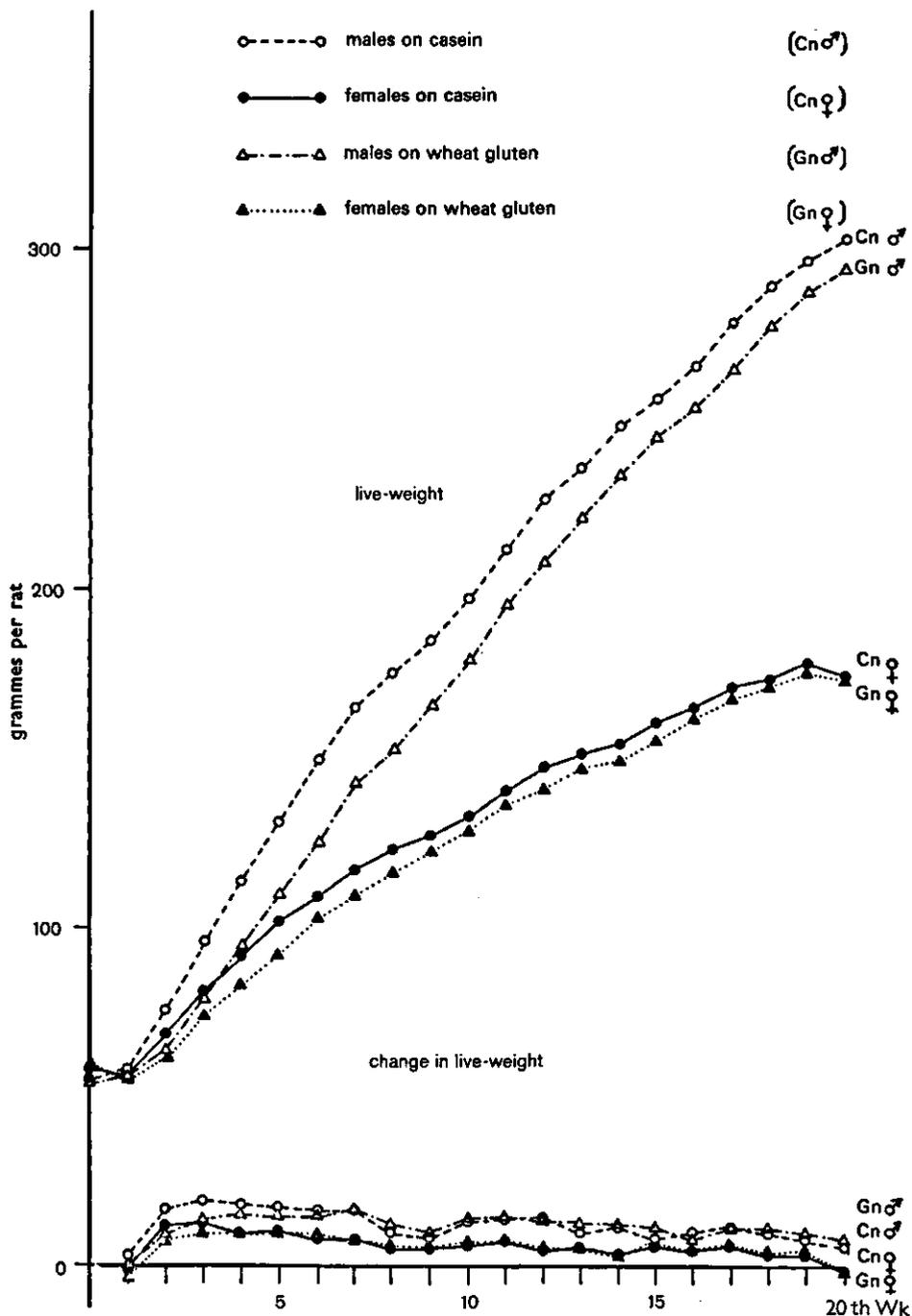
FIG. 1. Weekly liveweight and change in liveweight of rats fed on the protein and protein-free diet separately to appetite during Expt. 3.

TABLE 6. Effect of two protein types given to appetite[1] on the liveweight of rats (Expt. 3)

Experimental period	Liveweight (g)				Gain in liveweight (g)			
	Males		Females		Males		Females	
	Casein group (Cn♂)	Wheat-gluten group (Gn♂)	Casein group (Cn♀)	Wheat-gluten group (Gn♀)	Casein group (Cn♂)	Wheat-gluten group (Gn♂)	Casein group (Cn♀)	Wheat-gluten group (Gn♀)
Beginning[2]	55 ± 0.1[4]	55 ± 0.1	58 ± 0.1	59 ± 0.2				
First 4 weeks[3]	115 ± 1.3	95 ± 1.9	92 ± 2.2	83 ± 0.3	59.3 ± 1.3	39.8 ± 1.8	33.3 ± 2.1	24.3 ± 0.1
Second 4 weeks	176 ± 1.5	155 ± 3.3	123 ± 3.0	116 ± 0.3	61.1 ± 0.3	59.8 ± 1.4	31.0 ± 0.9	33.5 ± 0.1
Third 4 weeks[3]	226 ± 3.2	208 ± 2.2	146 ± 2.2	142 ± 0.8	50.6 ± 2.3	53.6 ± 2.0	23.0 ± 0.9	25.4 ± 0.5
Fourth 4 weeks	226 ± 3.4	254 ± 2.8	166 ± 3.2	162 ± 0.1	40.0 ± 0.1	45.8 ± 1.7	20.3 ± 1.0	20.0 ± 0.9
Fifth 4 weeks	303 ± 3.8	295 ± 5.1	179 ± 3.5	175 ± 0.4	37.1 ± 1.9	40.6 ± 1.3	11.4 ± 1.4	12.9 ± 0.5
Total of 20 weeks					248.2 ± 3.7	239.5 ± 5.1	119.1 ± 3.4	116.2 ± 0.4

[1] For one hour per day.
[2] Average age 4 weeks.
[3] About 0.03 ml blood taken at the beginning (per rat).
[4] Mean ± standard error of 3 replicates, each of 15 rats, divided by 15.

third 4 weeks, the gain in weight was higher in Gn♀ than that in Cn♀. Although this difference was not significant, it was near the significant level ($P < 0.1$). The difference between their liveweights decreased so much that it was no longer significant. In the fourth and fifth 4 weeks, the gain in weight of Cn♀ did not differ significantly from that of Gn♀. So their liveweights did not differ significantly from each other either. However, there was no significant difference in the total gain in liveweight of Cn and Gn of the same sex during the 20 weeks of Expt. 3 and, therefore, no significant difference was found between their weights at the end of the experiment.

3.3. DISCUSSION

(Liveweight)

The liveweight sums up the successive changes in weight which have occurred in all the preceding periods of the animal's life, whereas the gain in liveweight expresses the magnitude of a change in the animal's weight during a specific period of the lifespan.

Weaning weight of the rats in these three trails varied and the age range of rats at weaning was seven days. Variation in the weaning weight was influenced mainly by these age differences and by environmental and genetical factors. The mean liveweight of male rats weaned on average at 3.5, 3 and 4 weeks in trials 1, 2 and 3 was 48, 38 and 55 g, respectively. Therefore, rats weaned at slightly older ages had more liveweight than in rats weaned younger. In Trial 3, the female rats weighed 58 g when weaned two days later than the males which were 55 g, even though males are heavier in liveweight from birth than females (FARRIS, 1962) and onwards. The duration of rearing seemed to play a greater role in differences in liveweights at weaning in these trials than the condition of the mother, litter size, sex or heredity.

During the first week after weaning in these three trails, rats showed a decrease in liveweight or an increase which was the smallest weekly increment recorded in the test periods. The causitive factors which led to this inhibition of gain in liveweight are complex, but they may include disturbance by isolation, changing the cages, restlessness, reduced appetite change, to less nutritive food, change in intestinal flora and in metabolism.

Further test periods in the trails shows that gain in liveweight was erratic, being rapid in some instances and slow in others. Generally, liveweight gains were greatest early in the experiments. For example, in Expt. 1, maximum weekly gain for both groups occurred in the weeks 3 and 4, indicating, that the higher activity of the growth mechanism occurred in the early stages of the experimental period than in the latter ones. The liveweight curve for the trails is a characteristically curved, apart from the points of inflection. This indicates that the rate of growth, as measured by liveweight, changes with age and that as an animal grows older its growth rate decreases. The irregularities in the curve of the liveweight can be removed, if the data are treated statistically. In general, the steep part of the bodyweight curve expresses rapid growth, whereas the horizon-

tal one indicates the cessation of growth. The flatter portion of this curve was not completely achieved in these trails. The gain in liveweight in the three experiments did not follow exactly a smooth curve. This deviation may be due to heritable characteristics or to growth disturbances resulting from changes in food intake, bleeding, changing cages, changes in weather (thunder, atmospheric pressure), sexual maturity, unaccustomed noise or unnoticed illness. During Training Period 1, the effect of protein on gain was complicated by many factors. The first potato protein used for 9 days, containing 6.3% N was probably insufficient to replace the endogenous N excreted. The solanin content of the potato protein (KON, 1928) may also have influenced the weight loss. Furthermore, the weight loss may be related to the palatability of the food and reduced appetite rather than to any serious reduction in amino acids and their availability per unit potato nitrogen during the making of the protein concentrate. It seems that palatability resulting from concentration of the protein sometimes considerably influences the nutritive value determined by giving separately the protein concentrates.

During Training Period 1, rats on stock diet regained the weight loss and increased much more rapidly in weight than with the previous diet, because the stock diet has an acceptable flavour and a good nutritive value.

However rats fed on the second potato protein which contained 9.6% N and was from the same bulk batch as used by POL and DEN HARTOG (1966) gained weight contrary to the first potato protein. Alterations in the flavour of the second potato protein by mixing with some of the protein-free diet, higher nitrogen content, probable difference in solanin content and the older better trained rats may have improved the gain in liveweight. On receiving casein in Training Period 1, the rats consumed all the protein offered and increased in weight rapidly, indicating its acceptable flavour and good nutritive value. The gain in liveweight was higher on eating casein than the second potato protein. This was apparently due to the greater intake of casein than of the second potato protein. The increased intake perhaps could be due to differences in protein quality or in interfering substances.

The rats that ate 2 g protein within an hour in Training Period 1 (Table 2) were significantly heavier than those which did not. This was not due to a difference in the initial liveweights, because they were similar. However, a further division of the same rats in Table 2, made on the basis of their ability to eat up to 1.5 g protein in an hour, confirmed the conclusion. This conclusion agrees with that of OSBORNE and MENDEL (1919) who found that augmentation of protein intake in mixed feed increases weight gain. After weaning, the gain in liveweight during the first 4 weeks of trials 1 and 2 was 26 and 37 g, respectively. This indicates that the training method in Trial 2 was superior to that in Trial 1.

The weight of the rats at 4 weeks after weaning was 74 $\pm$ 1.1 g in Trial 1 and 75 $\pm$ 0.7 g in Trial 2 (end of Training Period 2). This similarity in liveweights indicates that the growth retardation at the beginning of Training Period 1 was without serious effect on the capacity of growth. The previous conclusion was

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confirmed by the fact that liveweight of Cn_{19} was 219 ± 4.7 and 215 ± 6.5 g in Expt. 1 and 2, respectively.

To compare the effect of protein type on the liveweight in Expt. 1 and 2, liveweights during 4-week intervals have been expressed as a percentage of those of Cn, which gives a first numerical index indicating the effect of protein type not only in the experiment but throughout life (Table 7). Similarly a second numerical index was calculated for the gain in liveweight (Table 7) and it would appear to indicate the previous effect better than the first one during a specific experimental period. Although the two indices were calculated from the same results (liveweights), the first index decreased as the experimental period was prolonged, whereas the second index mostly increased. This can be clarified by the fact that the first index was affected by the absolute difference in total gains which increased by prolonging the experimental period, whereas the second was affected by the relative difference in gain which decreased. The third numerical index can be the experimental t value and its P value (significance level). This index which was mentioned in the results of each trial is the most important one. The first and second numerical indices for the nutritive value of the protein cannot be completely compared in Expt. 1 and 2, because the liveweights and ages of the rats at the beginning of the two experiments were different. These indices ranked the wheat gluten after the casein in the two experiments, although their percentages were not indentical in Expt. 1 and 2 (Table 7). These indices (percentages) could be better used for comparison within an experiment. The difference in these percentages between Pt and Cn is not important, because t tests of their weights and gains were not significant. The decrease in growth rate with advancing age was one of the causes of the smaller gain in weight in Expt. 1 than in the preceding training period. The average gain in liveweight was 83 g during 47 days of Training Period 1 and 72 and 28 g during the first 49 days of Expt. 1 for Cn and Gn, respectively. After Week 1 of Expt. 1, though each rat (Table 3) increased in weight every week, this could not be detected by significant differences between mean liveweights of Gn from one week to the next. This applies also to Cn in weeks 4, 7, 8, 9, 10, 11 and 12 in Expt. 1. This was because the average increase in liveweight was too small in relation to the variation within the group to attain significance ($P<0.05$). Comparison of liveweights in alternate weeks or with longer intervals between records taken weekly in Expt. 1 should have shown significant differences, because the differences between means increased relatively more than variance. Gains in liveweight of the rats in Expt. 1 were not always significant between each consecutive week. When there was no significant difference in the weeks concerned, these were grouped. The periods, thus created, varied in duration and seemed to indicate a certain periodicity of growth. The greater decrease in liveweight of Gn than of Cn in the first week of Expt. 1 was influenced by a difference in protein quality and by the rats' lack of training to consume the wheat-gluten protein.

The length of the experiment (12 weeks) did not alter the ranking for casein and wheat gluten according to their nutritive value as measured by total weight

TABLE 7. Liveweights and total weight gains as percentages of those of rats fed on restricted amount of casein (Expt. 1 and 2)

	Liveweight (first index)[1]						Gain in liveweight every four weeks (second index)[1]					
	Casein group (Cn)		Potato group (Pt)	Soya group (Sy)	Wheat-gluten group (Gn)		Casein group (Cn)		Potato group (Pt)	Soya group (Sy)	Wheat-gluten group (Gn)	
	Expt. 1	Expt. 2	Expt. 2	Expt. 2	Expt. 2	Expt. 1	Expt. 1	Expt. 2	Expt. 2	Expt. 2	Expt. 2	Expt. 1
Week 4	100	100	101	77	71	80	100	100	101	49	38	23
Week 8	100	100	99	71	68	77	100	100	95	51	61	53
Week 12	100	100	97	67	67	78	100	100	82	58	56	71

[1] Calculated from tables 3 and 5.

gains or losses from similar initial liveweights at any period in Expt. 1 and 2. Casein was significantly more nutritive than wheat gluten. Prolongation of the experiment increased the scale units of ranking the proteins, because the difference in total weight gains between both groups increased continually till the end of Week 11 of Expt. 1 and 2, regardless of the variation within a group. The generally increased standard error with decreased number of rats seemed to mask the importance of the increased difference between liveweights of the two groups in Expt. 1 and 2. The weights of Cn in weeks 2 and 6 of Expt. 1 were approached by Gn in weeks 4 and 12, respectively. The higher nutritive value of casein than of wheat gluten is in accordance with ALLISON (1958), and TAGLE and DONOSO (1968). This is due to the better amino acid composition per unit N of casein than of wheat gluten for rat growth.

The liveweights and gains did not differ significantly between Cn and Pt in Expt. 2. This indicates a similar nutritive value for the casein and the third potato protein. GROOT (1942) mentioned in two experiments that there neither was a significant difference in weights between Cn and Pt when the proteins were given at a level of 8 % or 11 % protein to four to six male rats and two to four female rats for five to six weeks. The nutritive values of the three sources of potato protein used varied largely. JOSEPH et al. (1963) observed significant differences in the nutritive value of different sorts of potato flour.

The significantly higher weight and gain of Sy than of Gn in the first weeks of Expt. 2 illustrate the higher nutritive value of soya protein for recently weaned rats. This is in agreement with the data reported by HENRY (1965), representing the nutritive value of wheat gluten and soya protein in a testing period of 4 weeks for weaning rats. However, this superiority diminished later in Expt. 2. No significant difference in nutritive value between soya protein and wheat gluten was found, as judged in a long experimental period of 8–15 weeks in Expt. 2.

The significantly higher weights of Cn and Pt than of Gn and Sy in Expt. 2 show the better nutritive value of casein and the third potato protein than of the soya protein and wheat gluten at equal N intake with energy-yielding substances to appetite. In general agreement with the result of Expt. 2, ALLISON (1961) and DUTRA et al. (1967) showed that the nutritive value of casein exceeds that of soya protein.

Ranking of casein, potato protein, wheat gluten and soya protein for their nutritive values depended on the period of comparison within the experiment. In Expt. 2, casein and potato protein shared first rank and soya protein and wheat gluten were at 3rd and 4th, respectively in the first period of 4 weeks. However, the soya protein and wheat gluten shared third rank from Week 8 till the end of Expt. 2.

During Week 1 of Expt. 3, females in Cn and Gn lost 2 % and 4 % from their initial liveweight, respectively, whereas males in the two groups increased in liveweight. This decrease is negligible. However the earlier described trend suggests that the males adapted themselves better than females to adverse factors which inhibited growth in the first week after weaning. Furthermore Cn adapted better than Gn in Expt. 3 as in Expt. 1 and 2.

During the last week of Expt. 3, the liveweight of females on either casein or wheat gluten decreased by 1 %. This decrease is negligible but may be due to hearing the males squeak during blood sampling and to lack of males in nearby cages in the last two days of Expt. 3 which were associated with a decrease in feed intake without symptoms. Gain in liveweight was higher in the first eight weeks of Expt. 3 than in the later twelve, when growth is slowing.

The weight gain during the week after bleeding in Expt. 3 (Week 9) was less than that in the previous week. This decrease was greater in males than in females, because sexually mature females probably adapted themselves better than males to stress from bleeding.

During the first four weeks of Expt. 3, either sex gained more on casein than on Gn, but for the rest of the trial mostly less. The greatest difference between Cn and Gn was in Week 2 of Expt. 3, but the difference over the whole experiment was not significant, suggesting that casein and wheat gluten had a similar nutritive value as measured by gain in liveweight over a long period, if the two proteins were given to appetite and protein intakes were not considered. This is in accordance with CARPENTER and DUCKWORTH (1951) indicating that the inferiority of proteins could be offset by feeding at higher levels or when considered over a long period.

The superiority of casein (good quality) was demonstrated by the rapid growth, expressed by the gain in liveweight in Expt. 3. Rats seemed to adapt to deficiency of some amino acids in the wheat gluten, especially lysine, by increasing their protein intake in Expt. 3. So growth of rats fed on wheat gluten did not decrease relative to maximum liveweight, but growth rate decreased. This shows that growth is usually maintained with mildy adverse nutrition (inferior protein), when adapted rats complete the genetically determined growth curve, unless other environmental factors interfere. As maximum growth is essentially controlled by genetic and environmental factors, superior protein will expedite this maximum, whereas poor quality protein will delay it. If a. all the essential amino acids are present in the natural proteins, b. the experiment is long enough, c. no toxins interfere and d. the rats are weaned normally, mostly the difference in weight between groups should not be significant.

The total gain during Expt. 3 was about twice as much in males as in females given the same kind of protein (Table 6). Birthweight of males is higher than of females (King, 1915) and difference increases with age (SLONAKER, 1912). Although this may be due to males eating more than females in Trial 3, the sexual factors of growth are complex. Males grow faster than females, in spite of consuming equal amount of diet (KIM et al., 1952). Males are less active than females (HITCHCOCK, 1925). The weight of Cn♀ was higher than of Gn♂ until the Week 3 of the experiment (7 weeks of age), whereafter the reverse was true. At an average of 8 weeks, FARRIS (1962) reported that rats reached sexual maturity. Oestrogens inhibit growth, whereas the androgens stimulate growth (TANG, 1941; EMMENS and PARKES, 1947; LIGHT and TORNABEN, 1953). The sexual difference in liveweight (Fig. 1) is marked at 6 weeks of age (Week 2 of experiment) in accordance with the data of SPRAY and WIDDOWSON (1950).

Cn♂ in Expt. 3 grew more rapidly than in Expt. 1 and 2 as seen by comparing the gains in liveweight at the same age, mainly because each replicate of 15 rats was caged together and given protein to appetite in Expt. 3, whereas the rats were caged individually and offered a restricted amount of protein in Expt. 1 and 2. Gn♂ differed between the three experiments in the same way.

During the 12 weeks of Expt. 1 and 2 (restricted protein feeding) the differences between liveweights of males fed on casein and wheat gluten increased with age, whereas in Expt. 3 (protein to appetite) it decreased.

3.4. CONCLUSIONS
(Liveweight)

With the separate feeding, the type and quantity of protein affects the growth rate as expressed by weight gains. Therefore, the time that maximum liveweight is reached differs. Generaly, growth persists under the mildly adverse nutritional conditions of protein feeding (suboptimum amount and inferior protein).

Increased intake of protein resulted in a significant increase in the gained weight in Training Period 1 (Table 2). On casein and wheat gluten gains were greater when fed to appetite in Expt. 3 than when restricted in Expt. 1 and 2, because of increased intake of protein in Expt. 3.

The influence of the three sources of potato protein on liveweight varied remarkably. In Training Period 1 the first potato protein decreased weight of weanling rats, but in Training Period 1 the second potato protein which had been used also by Pol and Den Hartog (1966) increased it. The third potato protein in Trial 2 stimulated growth more than the second one in Training Period 1. This was associated with increased protein intake during Training Period 2. With the same amount of N, casein and the third potato protein in Expt. 2 accelerated gains, but soya protein and wheat gluten slowed gains. Initially Sy grew more rapidly but later grew less rapidly than Gn in Trial 2. In consequence, their liveweights were similar from Week 8 to the end of the trial. Data of Expt. 2 agree with those obtained in Expt. 1, indicating that casein stimulates growth, whereas the wheat gluten suppresses it.

If gain is accepted as criterion for the nutritive value of proteins, despite its limitations, it may show a different ranking for the proteins according to how they are compared. The difference in gains on the same batch of protein between Expt. 1 and 2 was clearly not due to protein quality; difference in age and initial liveweight had an influence. The similar total gains in liveweight of rats fed to appetite on casein and wheat gluten during Expt. 3 may be misleading, in that the amount of the two proteins eaten was remarkably different. Even if intake is discounted casein was significantly more nutritive than wheat gluten early in Expt. 3 as judged by weight gains. Although female rats in Expt. 3 were earlier in this than the males, the difference in the nutritive values of proteins was smaller for females. Duration of the experiment affects the nutritive ranking of soya and wheat gluten. With the same amount of N, soya proved significantly superior to wheat gluten from Week 2 to 7 of Expt. 2, but their nutritive values

were later similar (Week 8 to the end).

Since factors varied in the experiments, differences are to be expected on using a criterion to indicate the nutritive value. The nutritive value of different sources of potato protein varied widely and the best potato protein used in this study was the third one given in Expt. 2. Casein is superior for growth to wheat gluten. The nutritive values of casein and the third potato protein were similar and superior to soya and wheat gluten. Furthermore, the nutritive value of soya protein was higher than of wheat gluten in a short experiment and similarly in a longish one.

4. FEED INTAKE

4.1. INTRODUCTION

Feed intake, as commonly estimated in nutritional studies, provides some information on requirements. It is a component of equations for indices of food digestibility, and utilization. It may indicate sickness, dietary deficiency, imbalance or physiological state. It is a criterion of appetite, which affects the nutritive value of a diet constituent and vice versa. The nutritive value of a ration depends on such factors as quality, amount eaten, age and sex. Comparison of the intake which produces a desired fixed gain in liveweight or comparison of gains per unit intake has been used in evaluating nutritive value. In practical terms, recording feed intake needs more effort, time and money than recording liveweight.

Generally, animals on a good diet eat and grow more than animals on a poor one. The most usual method to correct for the difference in feed intake in evaluating the nutritive value of a diet is to calculate the efficiency of food conversion which is gain divided by intake. Similar is protein efficiency ratio (PER), i.e., gain per unit intake of protein. A low level of protein or inferior protein in the diet usually decreases intake (OSBORNE and MENDEL, 1915). It has been assumed that PER eliminates the factor feed intake and is superior to the criterion gain on its own. As gain correlates highly with intake, Hegsted and Worscester (1947) concluded that it is superfluous to measure feed intake for the calculation of PER in ranking the protein. This conclusion was confirmed by HEGSTED and HAFFENREFFER (1949) and Sherwood and Weldon (1953). However MORRISON and CAMPBELL (1960) noted that although PER is a function of growth, the coefficient of variation of gain was higher than that of PER calculated from the same result. Nevertheless, HEGSTED (1964) mentioned that this advantage can be easier obtained by weighing many rats per assay instead of weighing intake. JANSEN (1962) found that changes in the protein level affect the PER less than liveweight. DERSE (1960) reported that PER obtained with reference casein varied between 2.5 to 3.3 in different laboratories. CHAPMAN et al. (1959) and JANSEN (1962) lowered the coefficient of variation for test protein by correcting for PER to a reference casein, whereas DERSE (1960) did not.

Intake is influenced by genetic and environmental factors, which include species, strain, individuality, sex, age, activity, liveweight, livesize, health, feeding pattern, diet pattern, temperature and emotion. Generally, intake per day increases with liveweight, livesize and activity. Intake per unit liveweight is inversely correlated with liveweight. It is generally assumed to be correlated with the liveweight to the power of $^3/_4$ (active metabolic mass).

Intake per unit livesize is usually more constant than per unit liveweight. However, intake per unit active metabolic mass is probably more constant than intake per liveweight or size, if there was any accurate way of measuring it.

Differences in intake are generally attributed to appetite and palatability. As appetite is a desire (FOWLER and FOWLER, 1964), it may not be subject to

proper regulation. This is an oversimplication which covers a complex area of investigations to explain the highly complex mechanism controlling eating and the numerous related factors. Although sensations in the mouth are essential in selecting and identifying food, they have little or no effect on long-term regulalation of intake in normal individuals (EPSTEIN and TEITELBAUM, 1962). Variations in intake are not haphazard and intake is assumed to be regulated by biometric, short-term and long-term regulations (MAYER, 1953). The short-term mechanism is a day-to-day regulation within the large range of the biometric regulation. In the rat, the long-term regulation may function through a week or more to correct the short-term one. The short-term regulation may be partly a glucostatic mechanism in which a low effective level of glucose in the blood stimulates feeding and the long-term regulation by a lipostatic mechanism in which hypothalamus modifies the general level of intake and the activity of the animal in response to changes in body fat. Various regulatory mechanisms of intake have been proposed and are partly conflicting.

Adequate intake is mainly determined by the energy requirement for basal metabolism, physical activities or work and the specific dynamic effect of food. Decreasing the energy value of an adequate diet over a wide range by mixing it with kaolin or water (ADOLPH, 1947) increased intake to satisfy the animal's energy needs. This is limited by a maximum bulk of the diet which the animal can consume. Animals tend to balance energy intake and energy output by biochemical means and can store surplus energy consumed as fat. Generally animals choose food and nutrients from experience.

4.2. RESULTS
(Feed intake)

Tables 8, 9, 10 and 11 and Fig. 2 summarize the amounts of feed eaten by rats in this study. Daily or individual intake is sometimes mentioned in the text. When the fixed amount of protein was given, the change in intake of protein-free diet directly reflects the change in total feed intake (energy) and in the ratio of energy to protein. Therefore, the intake of protein-free diet was tabulated only in this case (Expt. 1 and 2).

Feed intake showed an irregular pattern for the daily or weakly change between animals as well as in individual animals. In general, the coefficient of variation of daily intakes of a rat in a period was greater than that of weekly ones. The same was true for the coefficient of variation in intakes between rats.

With increasing age, rats generally increased their intake and the increase was more rapid in the first periods than in the last periods of the three experiments. This can be clarified, for instance, by calculating total intake of every 3 weeks or longer in these experiments.

The significance of a difference between two values depends statistically on the difference in relation to the variance within two groups. The increase in the variance within groups contributed to some insignificant results obtained. The standard error increased in Expt. 1 and 2 with the decreased number of animals.

4.2.1. *Trial 1*

4.2.1.1. Training Period 1

After weaning, none of the rats ate all 2 g of the first potato protein offered daily for the first nine days. The mean daily intake was less than about 0.5 g. As the rats lost weight, this training regime was unsuccessful. The stock diet given to appetite for the next five days was eaten with good appetite and the rats increased in liveweight. The 2 g of the second potato protein offered on Day 15 of Training Period 1 was not all eaten by any rat on this or the next day. They were eaten by 5% of the rats on Day 17 and by 45% of the rats on Day 33 of the training period. At the end of the training period, rats on casein ate the 2 g within an hour, whereas 30% of the rats on potato protein did not. The ability to consume 2 g protein diet in some rats was not consistent. Some rats decreased intake in one day, then increased it on the next day. However, this variation diminished with increased time of training. The protein-free diet eaten in the training period was not weighed.

4.2.1.2. Experiment 1

Each rat of Cn consumed the 14 g protein diet offered weekly. The same was true for Gn except for three rats that ate on average 10 g protein diet from the 14 g offered in Week 1 of Expt. 1.

The intake of protein-free diet of Cn and Gn is shown in Table 8. Rats fed

TABLE 8. Effect of two protein types given in restricted amount[1] on intake of male rats (Expt. 1).

Experimental period	Number of rats		Protein-free diet in grams[3]	
	Casein group (Cn)	Wheat-gluten group (Gn)	Casein group (Cn)	Wheat-gluten group (Gn)
Week 1	34	33	73 ± 1.8[2]	75 ± 1.8
Week 2	34	33	75 ± 2.0	71 ± 2.0
Week 3	34	33	83 ± 2.1	72 ± 1.8
Week 4	26	25	88 ± 2.2	75 ± 1.9
Week 5	26	25	90 ± 1.8	75 ± 1.4
Week 6	26	25	95 ± 1.9	78 ± 1.8
Week 7	18	16	89 ± 3.1	73 ± 1.7
Week 8	18	16	98 ± 3.3	80 ± 2.3
Week 9	18	9	94 ± 3.1	81 ± 1.8
Week 10	10	5	99 ± 4.5	91 ± 5.0
Week 11	10	5	108 ± 5.3	95 ± 5.3
Week 12	10	5	106 ± 4.6	100 ± 5.1
First 3 weeks	34	33	231 ± 5.6	217 ± 5.3
Second 3 weeks	26	25	273 ± 5.6	228 ± 4.7
Third 3 weeks	18	9	281 ± 9.1	238 ± 6.1
Fourth 3 weeks	10	5	313 ± 12.6	287 ± 14.0

[1] Average weekly intake was 14 g protein containing 9.6% N.
[2] Mean ± SE.
[3] Offered daily to appetite for 23 hours.

on casein ate more protein-free diet than Gn except for Week 1 in which they ate less. The difference between the two groups in weeks 1 and 2 was small (3 % and 5 %) and not significant. However the change in Week 2 in Cn (+ 1.7 ± 0.96) and Gn (− 3.8 ± 0.98) differed significantly. The difference between the two groups in intake increased with age in such a way that weekly intake of Cn was significantly higher from weeks 3 to 9. Generally, this difference decreased and the standard error increased so that the intake of the two groups in weeks 10, 11 and 12 did not differ significantly. The weekly change in the intake of Cn was a significant increase in weeks 3 and 8 and that of Gn in Week 10, whereas the other weekly changes were not significant. Comparing the intake of protein-free diet in a three-week interval with the following one revealed a significant difference between the first interval and the following one in Cn, whereas their intake in the second or third interval and the following one did not differ significantly. In Gn, the difference was not significant between the first or the second interval and the next one, whereas it was significant between the third interval and the next interval.

4.2.2. *Trial 2*

4.2.2.1. Training Period 2

Each rat was given each of the 4 protein diets (2 g per day containing 9.6 % N) in turn in 4–day cycles during the training period. The amount not eaten by the 48 rats was 5 %, 13 %, 22 % and 33 % of the casein, potato protein, wheat gluten and heated soya protein, respectively, in the third 4 days of the training period. In this period, more than 5 % of the 2 g protein diet offered daily per rat was not eaten by 14, 39, 39 and 46 rats out of 48 on giving casein, potato protein, wheat gluten, soya protein, respectively. In the seventh (last) 4 days of the training period, the amount of protein diet not eaten was 2 %, 2 %, 5 % and 32 % of the amounts offered of the casein, potato protein, wheat gluten and soya protein, respectively. In this period, more than 5 % of the 2 g protein diet offered daily per rat was not eaten by one, six, eleven and forty rats out of 48 on giving casein, potato protein, wheat gluten and soya protein, respectively.

The average weekly intake of the 14 g protein diets, regardless of the kind of protein, and of the protein-free diet offered to appetite is shown in Table 9. In Week 2, each rat increased its weekly intake of protein diet, resulting in a

TABLE 9. Feed intake of male rats during Training Period 2.

Training period	Number of animals	Protein diet[1] (g)	Protein-free diet[2] (g)	N % in the two diets
Week 1	48	5.8 ± 0.1[3]	36 ± 0.3	1.3
Week 2	48	11.0 ± 0.2	38 ± 0.3	2.1
Week 3	48	11.7 ± 0.2	46 ± 0.9	1.9
Week 4	48	12.6 ± 0.2	48 ± 0.9	2.0

[1] 14 g protein (2 g per day for 1 hour) containing 9.6 % N offered weekly by the separate feeding system.
[2] Given to appetite for 23 hours after eating protein for 1 hour each day. [3] Mean ± SE.

significant increase. In that week, 33 rats increased their weekly intake of protein-free diet, whereas 15 rats decreased it. The decrease was more than 10% of the intake of the previous week in 4 rats. The average increase in intake of protein-free diet in Week 2 was significant. In Week 3, each rat increased its weekly intake of protein-free diet, resulting in a significant average increase. In that week, 38 rats increased their intake of protein, whereas 10 rats decreased it. This decrease was more than 10% of the previous intake in 1 of the 10 rats. However, the increase in protein intake in Week 3 was statistically insignificant. In Week 4, each rat increased its weekly protein intake, except one rat (3% decrease). This increase was significant. In that week, 38 rats increased their weekly intake of protein-free diet, whereas 10 rats decreased it. This decrease was more than 10% in 4 rats out of 10. The increase in total intake of protein-free diet during Week 4 did not differ significantly from that of Week 3. The largest increase in the mean intake of the protein diet and protein-free diet in the training period was in weeks 2 and 3, respectively. The relative increase in intake of protein diet in Week 2 was greater than that in the protein-free diet. Therefore, the N% in the total feed increased by a factor of 1.5 in Week 2. The values of this percentage were similar in weeks 2, 3 and 4, i.e., the change did not exceed 10% of these values.

4.2.2.2. Experiment 2

The weekly intake of protein diet of each rat was 14 g containing 9.6% N. The portion of the 14 g which an animal could not eat was given by stomach tube. This occurred with some rats of Gn only till Week 4 and of Sy till the end of the experiment.

TABLE 10. Effect of four protein types given in restricted amount[1] on the feed intake of male rats (Expt. 2).

Experimental period	No. of rats in each group	Protein-free diet[2] (g)			
		Casein group (Cn)	Potato group (Pt)	Wheat-gluten group (Gn)	Soya group (Sy)
Week 1	10	60 ± 2.3[3]	55 ± 2.2	42 ± 2.1	47 ± 1.5
Week 2	10	70 ± 2.5	68 ± 2.2	44 ± 2.5	53 ± 1.6
Week 3	10	75 ± 3.5	75 ± 2.6	46 ± 1.8	57 ± 2.0
Week 4	10	73 ± 3.0	77 ± 2.2	46 ± 2.3	57 ± 2.3
Week 5	7	82 ± 4.5	72 ± 2.4	50 ± 2.3	57 ± 2.3
Week 6	7	83 ± 4.7	78 ± 2.7	53 ± 2.2	64 ± 2.5
Week 7	7	85 ± 4.5	83 ± 2.5	54 ± 2.6	66 ± 2.6
Week 8	7	79 ± 5.5	85 ± 2.1	57 ± 3.1	67 ± 2.4
Week 9	4	80 ± 5.1	84 ± 3.6	60 ± 4.8	62 ± 7.5
Week 10	4	86 ± 7.7	80 ± 3.4	60 ± 3.1	65 ± 0.7
Week 11	4	88 ± 5.0	83 ± 3.8	60 ± 4.5	68 ± 1.3
Week 12	4	84 ± 4.1	88 ± 4.1	61 ± 4.9	68 ± 2.6

[1] 14 g protein in the week (2 g per day) containing 9.6% N.
[2] Given to appetite for 23 hours daily after a 1 hour interval of protein feeding. [3] Mean $\pm$ SE.

The weekly amounts of protein-free diet eaten by Cn, Pt, Gn and Sy are shown in Table 10. The rats in Table 10 were the same rats indicated in Table 5 for liveweight (p. 15). The intake of protein-free diet of Cn was higher than that of Pt in some weeks and lower in other weeks. The two groups did not differ significantly in their intakes of protein-free diet during any week of Expt. 2. The intake of protein-free diet of Sy was higher than that of Gn. This was significant in weeks 1–8 of the experiment and insignificant in later weeks. The intake of protein-free diet of Cn and Pt was more than that of Gn and Sy. This was significant every week except for Week 9 between Cn and Sy, because the standard error for Sy in that week was the highest for the group in that experiment.

4.2.3. *Trial 3*

The average weekly intake (protein diet, protein-free diet and total feed) of the weanling rats given casein (Cn) and wheat gluten (Gn) to appetite for an hour daily is shown in Fig. 2 (A and B). Table 11 represents the intake of these rats during each 4-week interval and the total period of 20 weeks.

The intake of protein-free diet was higher in Cn than in Gn of the same sex during each week of Expt. 3. Male rats cite more protein-free diet weekly than females fed on the same protein, but less in Gn during the first 3 weeks of Expt. 3. Weekly protein intake was less in Cn than in Gn of the same sex. Males ate more protein weekly than females on the same kind of protein. The weekly total intake of Cn exceeded that of Gn of the same sex. The weekly total intake of males was more than that of females on the same protein, but less in Gn during Week 1 of Expt. 3.

Differences in Table 11 between Cn and Gn of the same sex were tested for their significance. Differences in the intakes of protein-free diet of males offered either protein were significant. Differences in the total intakes of protein-free diet of females given either protein were significant in the first 4 weeks, second 4 weeks and total of 20 weeks, but insignificant in the third, fourth and fifth 4-week intervals. Differences in the intakes of protein diet of males given either protein were significant. Differences in the total intake of protein diet of females on either protein were significant during the first 4 weeks, second 4 weeks and total 20 weeks. Differences in the total feed intakes of males on either protein were significant during the first 4 weeks, second 4 weeks, fifth 4 weeks and total 20 weeks; and those of females were not significant during each 4-week interval and total 20 weeks.

Table 12 shows the effect of the second blood sampling in Expt. 3, which was taken on Day 1 of Week 9, on the feed intake of males and females on this day and in this week in comparison with the previous one. Experiment 3 began two days earlier for the males than the females. On the day of the second blood sampling of the males, the protein intake of males and females decreased. It was noteworthy that although there was no blood sampling from the females on that day, they also decreased their protein intake. The same was true on the day of the second blood sampling of the females. The percentage reduction was
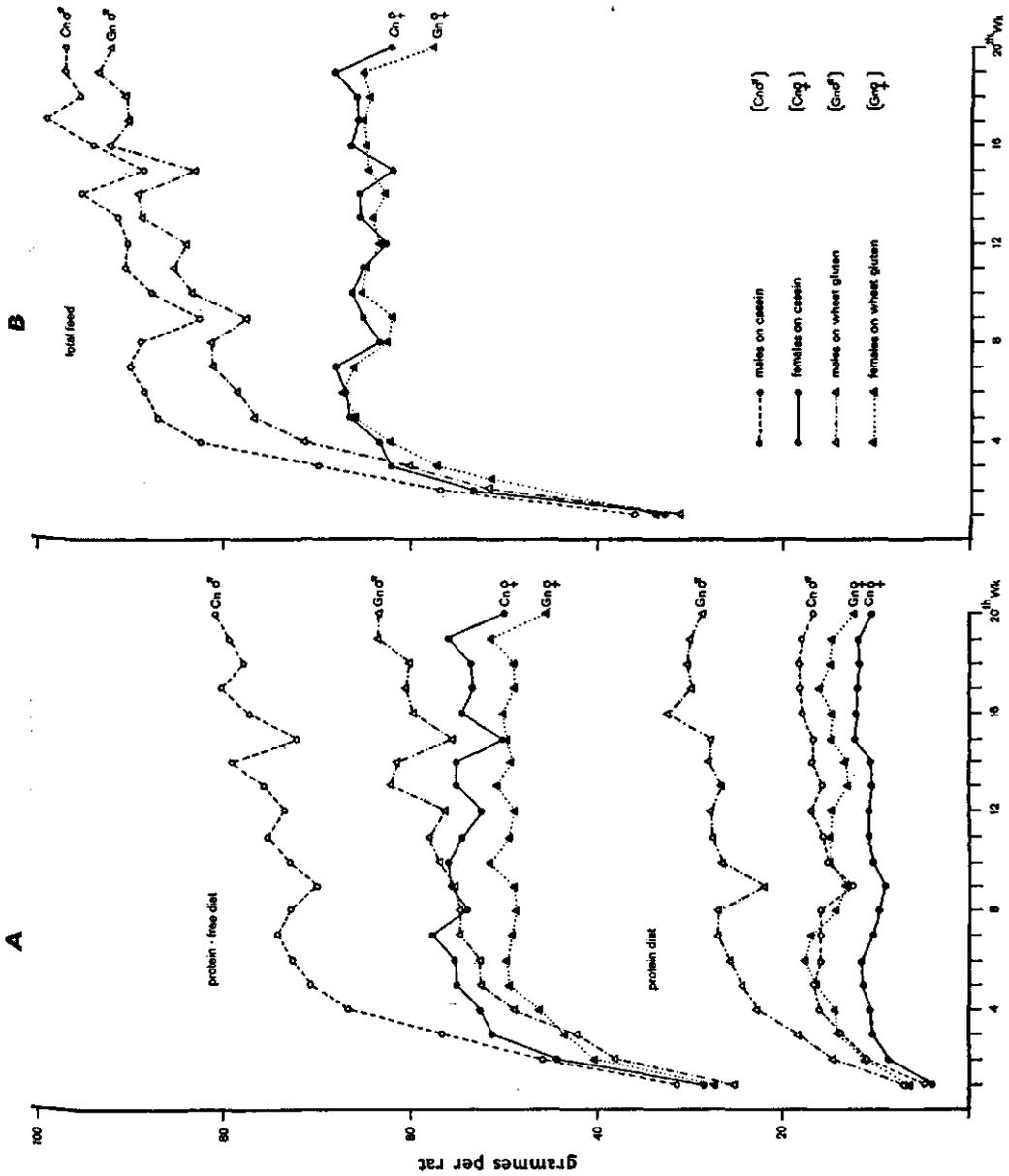
FIG. 2. Weekly feed intake of rats fed on the protein and protein-free diet separately to appetite during Expt. 3.

TABLE 11. Effect of two protein types given to appetite on the feed intake (Expt. 3).

| | Protein-free diet[1] | | | | Protein diet[2] | | | | Total feed | | | |
| | Male rats | | Female rats | | Male rats | | Female rats | | Male rats | | Female rats | |
	casein group (Cn♂)	wheat-gluten group (Gn♂)	casein group (Cn♀)	wheat-gluten group (Gn♀)	casein group (Cn♂)	wheat-gluten group (Gn♂)	casein group (Cn♀)	wheat-gluten group (Gn♀)	casein group (Cn♂)	wheat-gluten group (Gn♂)	casein group (Cn♀)	wheat-gluten group (Gn♀)
First 4 weeks[3]	201 ± 8	154 ± 3	177 ± 3	158 ± 4	46 ± 0	63 ± 1	34 ± 2	47 ± 1	247 ± 8	218 ± 4	211 ± 5	205 ± 4
Second 4 weeks	291 ± 5	215 ± 2	222 ± 2	197 ± 5	65 ± 1	104 ± 1	43 ± 2	66 ± 2	356 ± 4	319 ± 4	265 ± 3	262 ± 6
Third 4 weeks[3]	292 ± 4	227 ± 6	219 ± 8	199 ± 6	61 ± 3	104 ± 3	41 ± 3	59 ± 1	353 ± 7	331 ± 5	260 ± 5	257 ± 7
Fourth 4 weeks	303 ± 1	239 ± 6	215 ± 7	200 ± 10	67 ± 2	114 ± 2	46 ± 5	56 ± 2	370 ± 4	353 ± 8	260 ± 4	256 ± 10
Fifth 4 weeks	318 ± 1	248 ± 6	213 ± 11	195 ± 8	71 ± 3	119 ± 3	46 ± 4	58 ± 2	389 ± 3	367 ± 4	260 ± 7	254 ± 8
Total 20 weeks	1405 ± 6	1083 ± 22	1045 ± 20	948 ± 33	310 ± 7	505 ± 7	210 ± 17	286 ± 12	1714 ± 23	1588 ± 24	1256 ± 7	1234 ± 20

[1] Offered to appetite for 23 hours daily.
[2] Offered to appetite for 1 hour daily except for Day 1 of Expt. 3 and containing 12.8% N.
[3] About 0.03 ml blood per rat was taken at the beginning before protein feeding.
[4] Mean ±SE of 3 replicates divided by 15 to represent the intake per rat, all figures are rounded to the nearest gramme.

TABLE 12. Change in feed intake as percentage[1] in the day and week of the second blood sampling on protein to appetite (Expt. 3).

Dietary group	Male sampling time[2]				Female sampling time[3]			
	Protein		Protein-free diet		Protein		Protein-free diet	
	Day	Week	Day	Week	Day	Week	Day	Week
Male casein (Cn♂)	− 75%	− 21%	− 13%	− 4%	− 16%	−21%	+ 2%	− 4%
Female casein (Cn♀)	− 60%	− 8%	+ 9%	− 6%	− 74%	− 5%	− 22%	+ 3%
Male wheat gluten (Gn♂)	− 69%	− 19%	− 3%	+ 2%	− 13%	−19%	+ 8%	+ 2%
Female wheat gluten (Gn♀)	− 58%	− 15%	+ 18%	− 1%	− 55%	− 6%	− 31%	+ 0.5%

[1] (Feed intake of the day or week of sampling – the previous one) × 100/feed intake of the day or the week of sampling.
[2] Day 1 of Week 9 of the males in Expt. 3 corresponding in date with Day 6 of Week 8 of the females in Expt. 3.
[3] Day 1 of Week 9 of the females in Expt. 3 corresponding in date with Day 3 of Week 9 of the males in Expt. 3.

deminished by calculating the weekly intake, but not abolished. The percentage decrease in protein intake of either sex after sampling the blood was the highest which occurred from one day to the next in the experiment, whereas the change in the protein-free diet was in the range of the other daily variations observed without sampling the blood.

4.3. DISCUSSION
(Feed intake)

Generally, the amount of feed intake increased with advancing age in the three experiments due to increments in the required amounts of nutrients resulting from catabolic and anabolic processes.

The irregular daily or weekly increase in intake may be due, for instance, to periodicity and incidental factors. In general it was observed that intake tended to decrease during the weekends, which were characterized by a decrease in the surrounding sounds, days of changing the rats to clean cages, days of bleeding the rats, days of sickness, days in which damage stopped the air-conditioning system and so on.

The decrease in the average intakes of a group in any week of Expt. 1 and 2 did not exceed 10% of the intakes in the previous week, whereas the decrease of a group in Expt. 3 did in some weeks. The number of rats in each group of Expt. 3 was relatively large till the end of the experiment. Therefore, the highest weekly irregularity in feed intake of a group in Expt. 3 (Fig. 2) will be discussed, if the highest decrease exceeded 10% of the intake in the previous week. The highest decrease in protein eaten weekly by Cn♂ and Gn♂ throughout Expt. 3 was 21% and 19% in Week 9 of the experiment. Nevertheless, decreases in the protein-

free diet eaten weekly by Cn♂ and Gn♂ did not exceed 10% of the intake of protein-free diet in the previous week. It is possible that bleeding in Week 9 of Expt. 3 was a contributary cause in the remarkable decrease in the protein intake of Cn♂ and Gn♂ in that week. The highest decrease in the weekly protein intake of Cn♀ and Gn♀ throughout Expt. 3 was 13% and 17%, respectively, in Week 20 (last week). In addition the highest decrease in the protein-free diet eaten by Cn♀ and Gn♀ throughout Expt. 3 was 12% in the same week (Week 20). This remarkable decrease in the weekly intake of females in Week 20 of Expt. 3 was mainly caused by reduction in feed intake in the last two days of this week. This may have been due to the squeak of males on bleeding and to missing the accustomed sounds of males in these two days.

The amount of blood taken from each rat in Expt. 3 was about 0.03 ml. Since, the blood volume of the rat per 100 g liveweight is 6.7 ml (CARTLAND and KOCH, 1928) and slowly decreases with an increased liveweight (ORMOND and RIVERA-VALEZ, 1965), the blood taken would be about 1% or less of total blood volume. The rat can probably tolerate this amount. However, bleeding was followed by a reduction in protein intake of the rats from which samples of blood were taken or not. This shows that the effect seemed to be not only from bleeding, but also from disturbance by the squeak. Nevertheless, the change in the protein-free diet was in the range of variation from day to day which occurred without bleeding. The difference between the remarkable decrease in the protein intake and the slight change in the intake of protein-free diet may be due to the protein diet being given for a one-hour period after bleeding, whereas the protein-free diet for 23 hours. This supports the view that animals can better adjust their intake by compensation over a longer period than over a shorter period. This decreased protein intake may be an adaptation to decrease the effect of stress on the breakdown of protein, which increased probably after injury or disturbance. In Training Period 1, weaned rats ate too little of the first potato protein to promote growth. The cause of the seriously reduced appetite may be related to the protein diet, perhaps toxins in the potato protein. It is unlikely to be due to serious damage to the protein composition or inavailability of amino acids through processing. The stock diet was a good remedy for the previous low intake and compensated growth in Training Period 1 due to the good palatability and nutritive value. This supports the assumption that the previous reduction in the feed intake was not due to infection.

The second potato protein resulted in a better appetite than the first potato protein in Training Period 1. This may be due to virtual absence of the toxins, to improvement in the flavour of the second potato source by mixing with some of the protein-free diet, to the higher N content or to the rats being older and more trained. Intake of the second potato protein was less than that observed by POL and DEN HARTOG (1966). So training to eat 192 mg N of the second potato protein within an hour in Training Period 1 was not completely successful. Causes may have been storage of the second potato protein near room temperature for about a year, differences in training techniques and normal variation between trials.

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Training rats to eat the amount was easier with casein than with second potato protein, probably because of palatability.

Generally in Training Period 1, rats could eat more protein as they were trained and grew older.

In Training Period 2, the number of days before all the daily 2 g protein diet was eaten within the hour increased in the order casein, third potato protein, wheat gluten and soya protein. This order did not resemble the order of their expected nutritive value. The method of training in Expt. 2 was similar to that used by POL and DEN HARTOG (1966). During Training Period 2, rats were less successfully trained to eat 2 g protein diet within an hour than in the trial of POL and DEN HARTOG (1966), in which only 6 out of 72 rats did not eat 3 g of potato-protein or wheat-gluten diet by the end of 14 days of training. In Training Period 2, 1, 6, 11 and 40 rats out of 48 could not eat 95% of casein, third potato protein, wheat gluten and soya protein, respectively, during the 28 days of Training Period 2. The different durations may be due to differences in the proteins. They may also occur with one kind of protein, presumably through differences in the natural product manufactured and to a minor degree in a difference in the process of manufacturing. In Expt. 1 and 2, 90% of the rats could eat 2 g wheat-gluten diet within an hour at a similar age about 12 weeks regardless of differences in the previous training periods. Age may influence training. The ability to consume 2 g of the third potato-protein diet within an hour was better than that of the first and second potato-protein diet. This is possibly due to a difference in the quality of the potato-protein diets used in addition to the difference in methods of training.

Rats on superior proteins (casein or the third potato protein) significantly increased their weekly intake of energy earlier in the three experiments than rats on inferior proteins (wheat gluten or soya protein). This difference is mainly due to the difference in gains and consequently in liveweights.

The average intake of energy in each of the three experiments was significantly greater in rats on superior protein than in rats on inferior protein. The difference in gains and consequently in liveweights largely account for this result. As the proportion of inferior protein which can be used for building tissue protein is smaller than that of superior protein, more of the inferior protein is probably converted to energy. Consequently, the energy required with inferior protein is smaller for synthesis of new protein tissues and for all tissue activities than with superior protein. Therefore, the quality of the four proteins given throughout this study could be recognized from the energy intake per unit protein intake.

Rats on superior protein (Cn) to appetite ate less protein than rats of the same sex on the poor one (Gn) in Expt. 3. This shows the successful adaptation system of rats to compensate deficiency in composition of the wheat gluten by increasing their protein intake and probably using the extra amino acids as a source of energy.

In Expt. 3, female rats ate to appetite less of the same protein diet, protein-free diet and consequently total feed than males of the same age. The difference in intake between females fed on either proteins was lower than between males.

The sexual differences are mainly due to the higher gains in liveweight and consequently the higher liveweight of males than of females. However, total energy intake per unit liveweight of females was greater than of males. This agrees with BOZA et al. (1966) indicating that growth efficiency was significantly better for male rats than for females. LIGHT and TORNABEN (1953) showed that oestrogens stimulated feed intake and physical activity, whereas the androgens reduced intake and physical activity.

There are mathematical relationships between (a) the ratio of the protein-free diet: protein diet, (b) the reciprocal of this ratio and (c) the ratio of protein diet: total feed in any specific period of an experiment. The three ratios express, respectively, the same trend as (a) energy: protein ratio, (b) the reciprocal of this ratio and (c) the percentage of the protein in the feed chosen or the energy of protein to the total energy. The first ratio has the largest value of the three ratios. It is represented in Table 13 for Expt. 3. If this ratio expresses the same trend of optimum ratio of requirement, it suggests that optimum percentage of protein in a diet for growing females is less than that for males. This may be supported by that oestrogens decrease the growth of rats, but stimulate their physical activity, whereas the reverse holds for androgens (LIGHT and TORNABEN, 1953). The ratio was obviously higher in superior protein than in inferior one. This indicates that the optimum percentage of superior protein in a diet is less than in inferior one. The difference in this ratio due to the quality of protein and sex suggests that the protein quality and sex must be considered in calculating the requirement of the protein as a percentage of the total energy requirement. However, this ratio depends logically on physiological state of the animal.

TABLE 13. Effect of two types of proteins to appetite and sex on the ratio protein-free diet: protein diet (Expt. 3).

Experimental period	Male rats		Female rats	
	Casein group (Cn♂)	Wheat-gluten group (Gn♂)	Casein group (Cn♀)	Wheat-gluten group (Gn♀)
First 4 weeks	4.4	2.4	5.2	3.3
Second 4 weeks	4.5	2.1	5.1	3.0
Third 4 weeks	4.8	2.2	5.4	3.4
Fourth 4 weeks	4.5	2.1	4.7	3.5
Fifth 4 weeks	4.4	2.1	4.6	3.0
20 weeks	4.5	2.1	5.0	3.3

4.4. CONCLUSION

(Feed intake)

On restricted protein in Expt. 1, the ratio energy: protein was significantly higher on superior protein (casein) than on inferior one (wheat gluten). This was confirmed in Expt. 2. On restricted protein in Expt. 2, this ratio was also

significantly higher on superior proteins (casein and third potato protein) than on inferior proteins (wheat gluten and soya protein). The ratios energy: casein and potato protein were similar (no significant difference). On the other hand, the ratio of energy to soya protein was significantly higher than that to wheat gluten during the first stages of Expt. 2. Then these ratios were similar in the later stages without a significant difference. On protein to appetite, the ratio energy: protein was also higher for superior protein (casein) than for inferior (wheat gluten); in addition this ratio was higher for females than for males. This suggests that males require a higher percentage protein than females. Furthermore, the level of inferior protein required for both sexes is higher than of superior protein.

5. BODY COMPOSITION

5.1. INTRODUCTION

Gain divided by intake of feed or proximate constituent is usually used to evaluate feed efficiency. This criterion assumes that gain has a constant percentage of water, fat, protein and minerals. MITCHELL and CARMEN (1926) and KEYS and BROZEK (1953) indicated the necessity for the use of body analysis at the conclusion of careful studies designed to test efficiency. A disproportionate increase in one body constituent, such as fat, may be independent of gain. MILLER and BENDER (1955) reintroduced the use of carcass analysis in estimating the nutritive value of proteins. Since body constituents can vary independently of each other and, therefore, can occupy varying amounts of a total body mass, liveweight may reveal little. Carcass was, therefore, analysed in the recent study. If the bodily protein-again and gain in liveweight give different values for protein quality, protein increment is undoubtedly better. But, evaluation of protein quality by carcass analysis is laborious and in some instances impossible.

Water, fat and protein are the major body constituents and significant changes in their relative amounts can occur within the lifespan. Changes in rats were investigated by HATAI (1917), MOULTON (1923), CHANUTIN (1931), and SPRAY and WIDDOWSON (1950). In general, the percentages of fat and protein increases, and the percentage of water decreases with age. Of the three major constituents, fat is the most variable and to remove this variability from experimental data, fat-free composition is usually used. On this basis water and protein content in adult people and rats do not differ greatly, although the maximum fat content in the rat is about half that in man (SPRAY and WIDDOWSON, 1950). However, studies with laboratory animals hardly indicate quantitative relationships between body components in man. Water and protein expressed on the fat-free basis reach almost constant levels. MOULTON (1923) considered this as chemical maturity and found that it was reached at an age of 50 days in the rat. Logically, however, this noticeable constancy (compositional homoeostasis) can alter with factors such as genetics and diet. The concept of fat-free composition is biologically artificial, because a part of the body fat is essential for life. Lean composition considers this but the amount and classification of vital lipids needed for life under different conditions has not yet been precisely defined.

Generally, the ratio nitrogen: water (N :W) increases with age, since N percentage increases and the water percentage decreases with age. BENDER and MILLER (1953), DREYER (1957), and RAFALSKI and NOGAL (1966) showed the ratio N :W in rats of any age was almost constant. The calculated regressions which related this ratio to age varied in their constants. FORBES and YOHE (1955) stated that differences in the ratio N :W can be due to the different strains of rats. There is, however, some difference of opinion whether sex has any effect on the ratio N :W. DREYER (1957) found that female rats had a significantly higher ratio than male rats and, therefore, two regression equations for the correlation

of this ratio with the age of female and male rats were calculated separately. HENRY and TOOTHIL (1962) confirmed the existence of a sex difference in the ratio N:W. Nevertheless, BENDER and MILLER (1953) and RAFALSKI and NOGAL (1966) calculated one regression equation for both sexes, as they observed no sex difference in the ratio N:W. This relative constancy of the ratio N/W at any age is doubted by FORBES and YOHE (1955), WIDDOWSON and McCANCE (1963) and HEGGENESS (1965). The regression equation relating the ratio N:W to age was derived experimentally from results of rats covering a portion of the age range, but not all of it.

Moreover, the procedures of different investigators differ sufficiently to cause differences. The important point in investigating the existence of a close relationship between nitrogen and water is to calculate nitrogen content indirectly from water content. This is an easy, labour-saving and cheap method for protein evaluation recommended by BENDER and MILLER (1953). However, this is expected to be less accurate than the direct method of N estimation. FORBES and YOHE (1955) indicated that determining N content from water content could cause an error of 20 % in estimating the net protein content and it is considered safer to estimate N directly.

ABRAMS (1966) stated that no improvement was obtained in using liveweight instead of age in the indirect calculation of body N. RAFALSKI and NOGAL (1966) showed that the calculated N content of rats 26–45 days old from age, liveweight, body water or body dry matter (DM) did not differ significantly from estimating N content chemically. The difference between the estimated N and the calculated N from body dry matter had a greater coefficient of variation (3.6 %) than that (2 %) from liveweight or body water. They reported that a group of four rats was enough to calculate N. The above review emphasizes that before applying a formula for calculation of N, the relation between N and other constituents should be established for individual laboratories. Since this has not been investigated previously in the Netherlands Institute for Human Nutrition, it was necessary to estimate directly N content in the rats used for obtaining better information.

5.2. RESULTS
(Body composition)

5.2.1. *Trial 1*

Table 14 represents the body composition at different ages of rats fed on a restricted amount of casein and wheat gluten protein in Expt. 1. In Cn and Gn, the DM % of the rats sacrificed at any of the four stages 1, 2, 3 and 4 in Expt. 1 was significantly more than the initial value, whereas the DM % of rats fed on the same protein did not differ significantly between any two of the four stages.

There was no significant difference between the DM % of rats fed on casein and wheat gluten at the same stage.

In Cn and Gn, the initial value of F % was significantly lower than the value at any of the four successive stages, whereas F % of rats fed on the same pro-

TABLE 14. Effects of two protein types fed in restricted amount[1] on the body composition[2] (Expt. 1)

Age (approx.)	10	13		16		19		22	
Expt. stage	0 (beginning)	1 (Week 3)		2 (Week 6)		3 (Week 9)		4 (Week 12)	
Group		casein	wheat-gluten	casein	wheat-gluten	casein	wheat-gluten	casein	wheat-gluten
Numbers of rats	16	8	8	8	8	8	4	10	5
Dry matter (DM) %	32.6 ± 0.3	35.7 ± 0.5	35.6 ± 0.4	36.1 ± 0.6	36.0 ± 0.5	36.3 ± 0.7	35.9 ± 0.4	36.0 ± 0.5	36.0 ± 0.9
Crude fat (F) %	9.5 ± 0.3	12.7 ± 0.7	12.3 ± 0.5	12.7 ± 0.8	12.7 ± 0.6	12.5 ± 0.8	12.5 ± 0.5	12.6 ± 0.6	14.6 ± 1.3
Nitrogen (N) %	2.94 ± 0.02	3.01 ± 0.02	3.05 ± 0.02	3.11 ± 0.03	3.06 ± 0.02	3.14 ± 0.04	3.08 ± 0.05	3.13 ± 0.03	2.92 ± 0.07
Fat in the dry matter (FDM) %	29.1 ± 0.6	35.6 ± 1.0	34.3 ± 0.9	35.1 ± 1.6	35.3 ± 1.2	34.2 ± 1.6	34.8 ± 1.1	35.0 ± 1.4	39.7 ± 2.3
Nitrogen in the dry matter (NDM) %	9.03 ± 0.08	8.46 ± 0.15	8.58 ± 0.12	8.64 ± 0.20	8.52 ± 0.15	8.67 ± 0.24	8.57 ± 0.18	8.71 ± 0.17	8.02 ± 0.35
Nitrogen as per cent of water content (N/W %)	4.38 ± 0.04	4.68 ± 0.04	4.75 ± 0.04	4.88 ± 0.03	4.79 ± 0.03	4.91 ± 0.02	4.80 ± 0.08	4.89 ± 0.04	4.60 ± 0.06
Dry matter (g)	43.0 ± 1.8	55.4 ± 3.0	46.3 ± 2.9	70.2 ± 3.3	53.9 ± 2.6	77.7 ± 4.3	61.5 ± 2.0	87.6 ± 2.9	69.7 ± 4.4
Crude fat (g)	12.5 ± 0.7	19.9 ± 1.7	16.0 ± 1.3	24.9 ± 2.1	19.2 ± 1.4	27.0 ± 2.4	21.5 ± 1.4	30.8 ± 2.0	27.9 ± 3.1
Nitrogen (g)	3.89 ± 0.16	4.67 ± 0.20	3.95 ± 0.18	6.02 ± 0.16	4.57 ± 0.17	6.69 ± 0.25	5.26 ± 0.13	7.61 ± 0.17	5.55 ± 0.26

[1] 2 g protein containing 9.6 % N per rat for one hour daily.

[2] Mean ± SE.

tein did not differ significantly at any combination of two of the four stages.

There was no significant difference in F % of Cn and Gn at the same stage.

In Cn, N % was significantly less initially than at the four successive stages, and the N % at the first stage was significantly less than at the three successive stages, whereas the differences in N % between all other combinations of values were not significant. In Gn, the N % was significantly less initially than N % at the first, second and third stage, whereas the differences in N % between the other combinations were not significant.

The differences in N % between Cn and Gn were not significant at the first, second and third stages, whereas the N % of Cn at the fourth stage was significantly higher than that of Gn.

In Cn and Gn, FDM % was significantly less initially than at the four stages. In Cn, the differences in FDM % between any two of the four stages were not significant. In Gn, the difference in FDM % between the first and fourth stage was significant, whereas there were no significant differences between the rest of the combinations of four stages.

FDM % of Cn did not differ significantly from that of Gn in any stage.

In Cn, the NDM % was significantly more initially than at the first and second stage, whereas the differences in NDM % between any other combinations of the beginning and the four stages were not significant. In Gn, the NDM % was significantly more initially than at the four successive stages, whereas the difference in NDM % between any two of the four stages was not significant.

· There was no significant difference in the NDM % of Cn and Gn at the same stage.

In Cn, N/W % was significantly less initially than at the four successive stages. The N/W % of Cn in the first period was significantly less than that of the three successive stages, whereas there were no significant differences between the other combinations of the four stages. In Gn, N/W % was significantly less initially than at the four successive stages and N/W % at the first, second, third stage were significantly more than at the fourth stage, whereas there were no significant differences between N/W % of the other combinations of the four stages.

The N/W % of Cn was significantly more than that of Gn at the second and fourth stage, whereas the difference at the first and third stage was not significant.

The average amount of DM in Cn and Gn increased with age. In Cn, differences in DM (g) between the second and third stage and between the third and fourth stage were not significant, whereas those between the other combinations were significant, i.e., 0 + 1, 0 + 2, 0 + 3, 0 + 4, 1 + 2, 1 + 3, 1 + 4 and 2 + 4. In Gn, the differences in DM (g) between the initial and the first stage, between the first and second stage, between the second and third stage and between the third and fourth stage were not significant, whereas those between the other combinations were significant.

DM (g) in Cn was significantly more than in Gn at the same stage.

The average amount of F in Cn or Gn increased with age. In Cn the difference in F (g) between the beginning and any of the four stage, between the first

stage and the third or fourth stage were significant, whereas those between the first and second stage, between the second and two successive stages, and between the third and fourth stage were not signicant. In Gn, the differences in F (g) between the first and second stage, between the second and third stage, and between the third and fourth stage were not significant, whereas those between the other combinations were significant, i.e., between the beginning and the other stages, between the first stage and third or fourth stage and between the second and fourth stage.

The Cn contained on an average more F (g) than did Gn at the same age. The difference was not significant at the first, third and fourth stage, whereas the difference at the second stage was significant.

Average N (g) in Cn and Gn increased with age. In Cn, the differences in N (g) between the beginning and the four successive stages and between any two of the four stages were significant. In Gn, the differences in N (g) between the beginning and the first stage and between the third and fourth stage were not significant, whereas those between the other stages were.

Cn contained significantly more N (g) than Gn at the same stage.

The coefficient of variation as an indicator for the variance of the values tended to be slightly more in Cn and to increase slightly with age. Mostly, it did not differ greatly between Cn and Gn at the same age and between different ages in a group.

5.2.2. *Trial 2*

Tables 15.1–15.2 show body composition at different ages of rats fed on a restricted amount of casein, potato protein, wheat gluten and soya protein. Table 15.3 represents the average body composition of the 10 rats of each group during the experimental period.

DM of rats one month old rats was 30%. It increased significantly by 3 percentage units in rats two months old. It continued to increase significantly in the rats 3.5 months old which were sacrificed from Cn, Pt, Gn and Sy by 4, 4, 3 and 2% units respectively (compared with rats 2 months old). The increase in DM% of rats. 4.5 months old of Cn, Pt and Gn compared to rats 3.5 months old fed on the same protein was insignificant, whereas in Sy this increase was significant (3% units). DM% of rats 5.5 months old did not differ significantly from that of rats 4.5 months old of the same group. There was no significant difference in DM% of rats 5.5 months old and rats 3.5 months old of the same group except for Sy. In Sy, rats 5.5 months old had significantly more DM% than rats 3.5 months old.

At 3.5 months of age, DM% did not differ significantly between Cn, Pt and Gn. DM% of Sy 3.5 months old was 2 percentage units less than of rats on the other proteins at the same age (Cn, Pt and Gn). This difference in DM% between Sy and Pt was significant, whereas it was not significant between Sy and Cn or Gn. At 4.5 and 5.5 months of age, there were no significant differences in DM% between the four groups. Average DM% of ten rats in each group, sacrificed at the different stages, did not differ significantly between the four treat-

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TABLE 15.1. Effect of four proteins fed in restricted amount[1] for the first 7 experimental weeks on the body composition of male rats in Trial 2.

Experimental stage	At weaning	At end of training	Stage 1 at the end of Week 7 in Expt. 2			
Age (approximate)	1 month	2 months	3.5 months			
Dietary group			Casein (Cn)	Potato (Pt)	Wheat gluten (Gn)	Soya (Sy)
No. of rats sacrificed	3	8	3	3	3	3
Dry matter (DM) %	29.6 ± 0.6[2]	32.6 ± 0.3	36.7 ± 0.8	36.8 ± 0.3	36.0 ± 1.2	34.4 ± 0.3
Crude fat (F) %	9.3 ± 0.7	9.5 ± 0.5	13.6 ± 1.1	14.2 ± 0.9	13.4 ± 0.9	11.0 ± 1.1
Nitrogen (N) %	2.63 ± 0.03	2.87 ± 0.03	3.06 ± 0.01	2.97 ± 0.05	2.90 ± 0.02	3.00 ± 0.04
Fat/dry matter (FDM) %	31.4 ± 1.8	29.0 ± 1.3	37.1 ± 2.2	38.4 ± 1.9	37.1 ± 2.1	31.9 ± 2.9
Nitrogen/dry matter (NDM) %	8.88 ± 0.23	8.82 ± 0.13	8.33 ± 0.18	8.06 ± 0.20	8.06 ± 0.24	8.75 ± 0.20
Nitrogen/water (N/W) %	3.73 ± 0.01	4.26 ± 0.04	4.83 ± 0.04	4.69 ± 0.05	4.53 ± 0.07	4.57 ± 0.05
Initial liveweight g		72	76	75	78	76
Dry matter (DM) g	11.4 ± 1.6	23.6 ± 1.0	63.5 ± 2.7	61.9 ± 3.1	42.9 ± 1.2	40 ± 0.5
Crude fat (F) g	3.6 ± 0.3	6.9 ± 0.6	23.6 ± 2.4	23.8 ± 1.2	16.0 ± 0.4	13.0 ± 1.4
Nitrogen (N) g	1.0 ± 0.05	2.07 ± 0.08	5.29 ± 0.10	4.99 ± 0.28	3.45 ± 0.01	3.55 ± 0.04

[1] 2 g containing 9.6% N per rat for one hour daily.
[2] Mean ± SE.

TABLE 15.2. Effect of four proteins fed in restricted amount[1] for 11 and 16 weeks on body composition of male rats in Expt. 2.

Experimental stage Age (approximate)	Stage 2 (at Week 11 in Expt. 2) 4.5 months				Stage 3 (at Week 16 in Expt. 2) 5.5 months			
Dietary group	Casein (Cn)	Potato (Pt)	Wheat gluten (Gn)	Soya (Sy)	Casein (Cn)	Potato (Pt)	Wheat gluten (Gn)	Soya (Sy)
No. of rats	3	3	3	3	4	4	4	4
Dry matter (DM)%	38.4 ± 1.7[2]	37.6 ± 0.9	37.8 ± 1.0	37.4 ± 0.3	37.4 ± 0.5	38.9 ± 1.0	36.1 ± 1.0	37.1 ± 0.9
Crude fat (F)%	15.9 ± 2.1	14.4 ± 0.9	14.3 ± 0.9	13.3 ± 0.3	14.1 ± 0.4	15.8 ± 1.2	12.8 ± 1.0	14.1 ± 0.6
Nitrogen (N)%	2.90 ± 0.09	2.98 ± 0.06	2.89 ± 0.02	2.92 ± 0.01	3.06 ± 0.02	2.98 ± 0.02	2.82 ± 0.06	2.94 ± 0.18
Fat/dry matter (FDM) %	41.0 ± 4.2	38.3 ± 1.7	37.7 ± 1.1	35.6 ± 0.9	37.7 ± 0.9	40.5 ± 2.2	35.4 ± 1.4	38.0 ± 2.4
Nitrogen/dry matter (NDM) %	7.59 ± 0.54	7.94 ± 0.29	7.62 ± 0.10	7.81 ± 0.06	8.18 ± 0.15	7.65 ± 0.23	7.83 ± 0.35	7.91 ± 0.35
Nitrogen/water (N/W) %	4.71 ± 0.07	4.77 ± 0.05	4.65 ± 0.10	4.67 ± 0.03	4.89 ± 0.02	4.88 ± 0.17	4.41 ± 0.04	4.69 ± 0.34
Initial liveweight g	76 ± 2.3	79 ± 1.3	73 ± 1.5	77 ± 1.0	75 ± 3.2	74 ± 3.1	75 ± 2.9	74 ± 3.0
Dry matter g (DM)	77.2 ± 8.2	73.0 ± 2.0	51.2 ± 1.0	52.6 ± 2.3	88.1 ± 2.6	87.4 ± 5.3	57.0 ± 3.6	57.5 ± 2.1
Cruce fat g (F)	32.3 ± 6.4	28.0 ± 0.8	19.3 ± 0.9	18.8 ± 1.3	34.2 ± 1.4	35.8 ± 3.9	20.4 ± 2.5	21.7 ± 1.0
Nitrogen g (N)	5.76 ± 0.23	5.81 ± 0.36	3.91 ± 0.08	4.11 ± 0.14	7.41 ± 0.24	6.66 ± 0.28	4.43 ± 0.13	4.57 ± 0.35

[1] 2 g containing 9.6% N per rat for one hour daily.
[2] Mean ± SE

TABLE 15.3 Average effect of four proteins fed in restricted amount[1] on the body composition of 10 male rats sacrificed at about 3.5, 4.5 and 5.5 months of age (Expt. 2).

Dietary group No. of rats	Casein (Cn) 10	Potato (Pt) 10	Wheat gluten (Gn) 10	Soya (Sy) 10
Dry matter (DM) %	37.5 ± 0.5[2]	37.9 ± 0.5	36.6 ± 0.6	36.4 ± 0.6
Crude fat (F) %	14.5 ± 0.7	14.9 ± 0.6	13.4 ± 0.6	12.9 ± 0.6
Nitrogen (N) %	3.01 ± 0.03	2.97 ± 0.03	2.86 ± 0.02	2.96 ± 0.07
Fat/Dry matter (FDM) %	38.5 ± 1.4	39.3 ± 1.1	36.6 ± 1.1	35.4 ± 1.4
Nitrogen/dry matter (NDM) %	8.05 ± 0.19	7.86 ± 0.14	7.84 ± 0.16	8.13 ± 0.16
Nitrogen/water (N/W) %	4.82 ± 0.03	4.79 ± 0.07	4.52 ± 0.05	4.65 ± 0.12
Initial liveweight g	75 ± 1.1	76 ± 1.7	75 ± 1.7	76 ± 0.9
Dry matter (DM) g	78.5 ± 4.4	75.5 ± 4.2	51.0 ± 2.4	51.0 ± 2.6
Crude fat (F) g	30.5 ± 2.4	29.9 ± 2.0	18.7 ± 1.2	18.2 ± 0.9
Nitrogen (N) g	6.28 ± 0.32	5.91 ± 0.28	3.98 ± 0.15	4.13 ± 0.19

[1] 2 g containing 9.6% N per rat for one hour daily.
[2] Mean ± SE.

ments (Table 15.3).

F of rats one month old was 9%. This value did not change significantly from then until two months of age. It increased 4, 5, 4 and 2 units at 3.5 months of age in Cn, Pt, Gn and Sy, respectively. These increases were significant, except for Sy. The increase in F% from 3.5 to 4.5 months of age was insignificant but from 2 to 4.5 months was significant in all four groups. From 4.5 to. 5.5 months in a same group there was no significant difference. From 3.5 to 5.5 months the only significant difference was for Sy where F% increased by 3 units.

At 3.5, 4.5 or 5.5 months of age, there was no significant difference in F% between the four groups. Average F% for all three stages of sampling in the total of ten rats in each group differed significantly only between Pt and Sy (Pt being 2 percentage units higher than Sy).

N of rats one month old was 2.63%. It increased significantly 0.24 units in rats 2 months old. N% of rats fed on any protein did not differ significantly between 3.5, 4.5 and 5.5 months of age. These averages were higher than N% of rats 2 months old, except for Gn 5.5 months old and the increases were statistically significant in Cn 3.5 and 5.5 months old (0.19 units) and Sy 3.5 months old (0.13 units), whereas the other changes (the increases and decrease), were insignificant. N% of the total of ten rats fed on either casein or potato protein was significantly higher than of rats 2 months old (beginning of the experiment), whereas average N% of the rats of Gn and Sy did not differ significantly from the initial value.

At 3.5 months of age, Cn had significantly more N% (1% protein) than Gn, whereas there were no significant differences in N% in other combinations of the four groups. At 4.5 months of age, N% did not differ significantly between

groups. At 5.5 months, the same was true except for that of Cn which had significantly more (1.5% protein) than Gn. According to the average N% in the total of ten rats in a group at the three ages, the descending order of the four groups was Cn, Pt, Sy and Gn, respectively, and differences between these groups were not significant except between Cn and Gn.

The FDM of rats one month old was 31%. This value decreased in rats 2 months old but insignificantly. There were no significant differences in FDM% of any group between the three stages (3.5, 4.5 and 5.5 months of age). The FDM% in any of the four groups at any of the three stages was more than at weaning. This was significant for Cn 5.5 months old, Pt 4.5 and 5.5 months old and Gn 4.5 months old. Average FDM% in the total of ten rats of Cn, Pt or Gn was significantly more than that of the rats at weaning. Average FDM% of ten rats of Sy had a similar trend, but the difference was not significant.

At 3.5, 4.5 and 5.5 months of age, there were no significant difference in FDM% between groups. Average FDM% of ten rats in each group did not differ significantly between the four treatments, except that FDM% of Pt was significantly higher than of Sy.

The NDM in rats one month old was 8.9%. This value decreased in rats two months old but insignificantly. There were no significant differences in NDM% of any group between the three experimental stages, except for Sy at 3.5 and 4.5 months of age. NDM% of any of the four groups at any of the three stages was lower than that of rats two months old (weaning). These were significant for Cn at 5.5 months of age, Pt at 5.5 months of age and Gn at 4.5 months of age. Average NDM% of ten rats of Cn, Pt and Gn was significantly less than at the beginning of the experiment, except for that of Sy which had less, but the difference was not significant.

At 3.5, 4.5 and 5.5 months of age, there was no significant difference in NDM% between the four groups. Average NDM% of ten rats in each group did not differ significantly between the four treatments.

N/W% increased significantly during the training period. N/W% of any group at any of the three experimental stages was greater than at the beginning of the experiment. These increases were significant, except for Sy at 5.5 months of age. Average N/W% of ten rats of each group was significantly higher than during training. N/W% in any group at the three stages did not differ significantly from one stage to another except in Cn between 4.5 and 5.5 months of age.

At 3.5 months of age, there were no significant differences in N/W% between the four groups except for Cn which had significantly higher N/W% than Gn. At 4.5 months of age, there were no significant differences in N/W% between the four groups. At 5.5 months of age, there were no significant differences in N/W% between the four groups, except for Cn which was significantly higher than Gn. N/W% in the total of ten rats did not differ significantly in the four groups except for the casein and potato groups which was significantly higher than the wheat-gluten group.

DM, F and N (g) increased significantly in the training period. They were about double values at weaning. They continued to increase throughout the experi-

ment in the four groups. Although this increase from one age to the next was sometimes insignificant during the experiment, it was considered an important increase which was masked by a relatively high variability within the groups.

At 3.5, 4.5 and 5.5 months of age, there were no significant differences in DM, F and N (g) between Cn and Pt and between Gn and Sy, whereas those of Cn and Pt were significantly higher than those of Gn and Sy.

Gn_{14} (rats 14 weeks old fed on wheat gluten) had significantly less N%, N/W% and N (g) in Expt. 2 than Gn_{13} in Expt. 1. They did not differ significantly in N (g), when Gn was corrected for the average difference in liveweight, which was 10 g and not significant. Gn_{18} in Expt. 2 had less N%, N/W% and N (g) and more liveweight (6 g) than Gn_{13} sacrificed in Expt. 1. These differences between the two experiments were only significant for N%. The Gn_{23} sacrificed in Expt. 2 had less N%, N/W%, and N (g) and liveweight (32 g) than Gn_{22} in Expt. 1. These differences between the two experiments were only significant for N and liveweight. N%, N/W%, N (g) and liveweights of Cn_{14} sacrificed in Expt. 2. were between the values of Cn_{13} and Cn_{16} sacrificed in Expt. 1. Cn_{18} sacrificed in Expt. 2 had less N%, N/W% and N and more liveweight (5 g) than Cn_{16} sacrificed in Expt. 1. These differences between the two experiments were only significant for N% and N/W%. The Cn_{23} sacrificed in Expt. 2 had the same average weight (243 g) as Cn_{22} in Expt. 1. There were no significant differences in N%, N/W% and N (g) between these rats in the two experiments.

5.2.3. Trial 3

Table 16 shows average body composition of three replicates of male and female rats fed to appetite, on casein or wheat gluten for an hour a day.

In males, Cn had significantly more DM% (1.9 units) than Gn but in females, Cn had significantly less (1.4) than Gn. In Cn, males had significantly more DM % (1.7) than females but in Gn, males had significantly less (1.6) than females.

In males, F% of Cn was insignificantly higher (2.5) than of Gn, but in females, that of Cn was significantly less (2.4) than of Gn. In Cn, males had significantly higher F% (3.4) than females but in Gn, males had significantly less (1.5) than females.

There was no significant difference in N% between Cn♂ and Gn♂, between Cn♀ and Gn♀, between Cn♂ and Cn♀ and between Gn♂ and Gn♀.

In males, FDM% of Cn was insignificantly higher than of Gn. FDM% of Cn♀ was significantly lower than of Gn♀. In Cn, males had significantly higher FDM% than females but in Gn males had insignificantly lower FDM% than females.

There was no significant difference in NDM% between Cn♂ and Gn♂, between Cn♀ and Gn♀, between Cn♂ and Cn♀ and between Gn♂ and Gn♀. There was no significant difference in N/W% between Cn♂ and Gn♂, between Cn♀ Gn♀, between Cn♂ and Cn♀ and between Gn♂ and Gn♀.

In males, Cn had significantly more DM (g) than Gn but in females, Cn had insignificantly less than Gn. On casein or wheat gluten, males had significantly more DM (g) than females.

TABLE 16. Effect of two types of proteins given to appetite[1] on body composition of three replicates[2] for each sex (Expt. 3).

Sex	Male		Female	
Dietary group	Casein (Cn)	Wheat gluten (Gn)	Casein (Cn)	Wheat gluten (Gn)
Dry matter (DM) %	41.0 ± 0.5[3]	39.1 ± 0.4	39.3 ± 0.3	40.7 ± 0.1
Crude fat (F) %	19.1 ± 1.0	16.6 ± 0.4	15.7 ± 0.6	18.1 ± 0.3
Nitrogen (N) %	3.09 ± 0.03	3.10 ± 0.08	3.00 ± 0.06	29.9 ± 0.06
Crude fat in the dry matter (FDM) %	46.6 ± 1.4	42.5 ± 0.5	39.9 ± 1.2	44.5 ± 0.6
Nitrogen in the dry matter (NDM) %	7.56 ± 0.16	7.95 ± 0.27	7.64 ± 0.19	7.35 ± 0.13
Nitrogen to water (N/W) %	5.24 ± 0.01	5.08 ± 0.11	4.94 ± 0.08	5.04 ± 0.12
Dry matter (g) per replicate	1865 ± 43	1727 ± 47	1045 ± 14	1066 ± 7
Dry matter (g) per rat	124	115	70	71
Crude fat (g) per replicate	870 ± 53	733 ± 28	418 ± 16	475 ± 10
Crude fat (g) per rat	58	49	28	32
Nitrogen (g) per replicate	141 ± 1.3	137 ± 2.4	80 ± 2.3	78 ± 1.9
Nitrogen (g) per rat	9.4	9.1	5.3	5.2

[1] For an hour daily.
[2] 15 rats per replicate.
[3] Mean ± SE.

In males, F (g) of Cn was significantly higher than of Gn but in females, Cn had significantly less than Gn. Males had significantly more F(g) than females on the same protein.

Cn had slightly but insignificantly more N (g) than Gn (the same sex). Males had significantly more N (g) than females on the same protein.

A significant difference in W% was not always associated with a significant difference in N%, N/W%, quantity of W or DM of the same rats. Although rats of casein differed significantly in DM% from rats fed on wheat gluten and of the same sex, their liveweights did not. In spite of the significant difference in F% of females between the two proteins, there were no significant difference in N%.

5.3 DISCUSSION

(Body composition)

The methods of analysis do not yield the exact amounts of water (W), fat and protein in the body. Complete recovery of the entire weight of animals by determining chemical components is not expected. However the experimental errors are relatively small. W, even allowing for bound W, was not all the water in the body but a large majority of it under standard conditions in the three experiments. The materials extracted by ether were designated crude fat (F) in accordance with the AMERICAN ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (1965). The ether extract contains not only triglycerides but also other fatty materials. There is a small amount of fatty materials in cells which is not extracted by the method used. Furthermore, not all nitrogen (N) determined was protein N. The usual factor to convert N to 'crude protein' is 6.25 but the conversion factor varies between proteins. Factors of 5.7, 6.38 and 5.34–6.00 were reported for cereal proteins (TKACHUCK, 1966b), milk proteins (MCCANCE and WIDDOWSON, 1960) meat proteins (HARTOG, 1966), respectively. Therefore, N was reported as such in the tables and the term protein refers to crude protein in the ensuing discussion. Generally many ways of analysis may lead to the object; the best way will depend on the purpose of the study and the practical possibilities for the measurements. Although different chemical methods of estimation of a component may give different values, HATAI (1917) indicated that in view of size of individual variation such a difference can be neglected. The total W of the body was the largest single component on direct chemical analysis. W% was not reported in the result, as it can be easily derived by substracting DM% from 100. Hence, an increase in DM% means a decrease in W%. The coefficient of variation for W% is less than for DM%, as the standard deviation is the same for DM% and W%, and W% is greater than DM% in the rat. The sum of W, F and protein accounted for ± 95% of liveweight excluding salts which were not estimated.

The rat with most F% in a group at any time had least W%, N% or NDM%. This is not mainly due to the replacement of W or N by F. The calculated N% on a fat-free basis and N/W% for the rat of the highest F% were neither the

highest nor the lowest within the rats sacrificed from a group in a period. Consequently, this increase in F% is to a large extent due to the accumalation of water-free F in the body. Generally, the change in F% had a greater effect on DM% than that in N%, because changes in F% were mostly larger.

The individual variation in F% was mostly larger than that in N%, DM% or W%. This can be indicated by calculating the coefficients of variation of these constituents for the same rats. This would show that N%, DM% or W% varied in relatively narrow limits. Hence, N/W% would vary similarly, which agrees with the results of BRESSANI et al. (1965). The significant differences in N% and N/W% in the results of the experiments indicated that the relative constancy of these parameters was affected by the kind of protein fed in a restricted amount during the long period in Expt. 1 and 2.

It is usually assumed that rats sacrificed at a specific age should be similar in gross body composition to other rats, if they were sacrificed at that age. This assumption is substantially correct, if the number of animals is statistically enough. This number depends on variation of the constituent studied. Unfortunately, too few rats could be analysed on some occasions. The DM, F and N (g) in the rats increased with age. These increases were sometimes statistically insignificant. However, it is logically a real increase. Statistically the level of significance may be obtained by increasing the number of animals or the difference magnitude by prolonging the experimental period. Because the same rat could not be analysed at different ages, there were some discrepancies in the effect of age on body composition. Since rats sacrificed from the wheat-gluten group at the fourth stage of Expt. 1 had 2% more F than those at the third stage, a similar increase in DM% was expected, which was not actually observed. Statistically, the increases in F% (2%) and in DM% (0.1%) of the wheat-gluten group during the fourth period of Expt. 1 were insignificant. Generally, the increases in N% throughout the experiments were smaller than those of F%. Therefore NDM% decreased throughout the experiments. In general, the DM%, F%, N%, DM, W, F and N (g) increased with age, whereas the W% decreased. These effects of age on body composition were more marked earlier in life than later. These conclusions generally agree with those reported by others (HATAI, 1917; SPRAY and WIDDOWSON, 1950).

When the 45 observations of DM% in a group (3 replicates x15) were used instead of the three observations of Expt. 3 (the three averages of the three replicates) in t test, it resulted in a higher probability of significance. It seemed that if the number of F% observations in Expt. 3 was increased from 3 to 45, the difference in F% between Cn♂ and Gn♂ would achieve significance ($P < 0.05$). This might be unexpected for the differences of N% between Cn and Gn of the same sex in Expt. 3.

The data show that calculating N% from the estimated DM% or W% would not be completely successful and body composition cannot be completely predicted from the liveweight. However, body analysis did not yield any more useful information in ranking the proteins than that obtained from liveweight, except as confirmation.

On feeding a restricted amount of protein, data of Expt. 2 confirmed and extended the observations of Expt. 1, indicating that intake of the similar amounts of different N sources for a longish period resulted in significant differences in N%, N/W%, quantities of DM, F and N of the animals. Furthermore, F% of the total of potato group sacrificed in Expt. 2 was significantly more than that of soya group. This shows that the quality of protein can significantly affect body composition. Generally, the superior protein alters body composition more rapidly than the inferior one. These differences would decrease if the experiment was prolonged as long as the deficiency is not too severe. Generally, the effect of changes in the percentages of the estimated constituents on their amounts were too small compared with changes in liveweight.

In Expt. 1 and 2, the rats of the same group were from the same colony and were treated alike in the trials except in the training period and in age at the beginning of the experiments. Cn was more similar in composition in Expt. 1 and 2 than Gn, probably because Cn from 7 to 10 weeks old in the two experiments was more similar in the protein quality fed than Gn. The similarity of the experimental procedures in Expt. 1 and 2 would be expected to yield values more similar than those cited under other experimental details in the literature. However, a comparison of groups on the same protein in Expt. 1 and 2 revealed some significant differences in body composition. Hence comparison of these values with those in the literature would reveal more variation which mostly depends on the differences in the experimental procedures. Generally values of body composition reported by different investigators are highly various in strain, age, number of animals, feeding, method of analysis, statistical evaluation and other points in the experimental procedures to be judged for all the true causes of differences.

With casein to appetite, F% in males was significantly higher than in females but with wheat gluten significantly lower. This contradictory results were not associated with marked differences in the liveweight or total feed intake of Cn and Gn of the same sex. The higher F% in males than in females disagrees with the results of SPRAY and WIDDOWSON (1950). However, the data of BRESSANI et al. (1965) show that males exceeded the females in F% and the reverse according to the protein fed in their short experimental period and in general agreement with the results of Expt. 3. These data point out the well known relation between protein metabolism and the metabolism of energy-yielding substances which seemed to be affected not only by proteins, but also by sex. The mechanisms, whereby this results in rats, may include a diminished thyroid activity, contributing to the deposition of the extra fat and probably leading to changes in the metabolism through alternative enzymic pathways of intermediate metabolism. KAUNITZ (1958) reported that certain constituents in the diet may give a similar effect.

Sex has no significant effect on N/W%, when the protein was given to appetite. Furthermore, the average N/W% of males was higher than that of females fed on the same protein. This disagrees with the conclusions of DREYER (1957)

and HENRY and TOOTHILL (1962) but agrees with the findings of BENDER and MILLER (1953) and RAFALSKI and NOGAL (1966).

N (g) in Cn did not differ significantly from that in Gn of the same sex, when protein was to appetite, in spite of the difference in the protein quality. Animals adapted themselves to the difference in the protein quality by a greater intake of inferior protein than of superior. High intake of wheat gluten seemed to increase the amount of the limiting amino acids, and extra amino acids seemed to be converted to energy.

Giving the protein to appetite (Expt. 3) resulted in a significant difference between DM % of the males on casein and wheat gluten, whereas giving a restricted amount of protein (Expt. 1 and 2) did not. This can be attributed partly to F %. Difference in W % or N/W % between males on casein and wheat gluten attained the significant level on giving restricted amounts of protein, whereas it did not on protein to appetite. The same was true for N quantity. This is mainly clear from the significant difference in liveweights between males on restricted amounts of casein and wheat gluten, whereas liveweights of males fed on to appetite casein and wheat gluten were similar at the end of Expt. 3. In spite of the difference in the total energy intake, males on casein did not differ significantly from males on wheat gluten in their F %, when they ate the protein restricted or to appetite, partly because of the relatively large variation in F % of the rats.

Male rats fed on restricted amounts of protein (Expt. 1 and 2)had significantly lower DM %, F % and N/W % than males of similar age and given the same protein to appetite (Expt. 3). The N % of the male rats on a restricted amount of protein was less than that of the males of similar age and fed on the same protein to appetite. The difference in N % achieved the significance between feeding on restricted wheat gluten and to appetite, whereas it did not between feeding on casein restricted and to appetite. The quantities of DM, F and N in the males fed on restricted amounts of casein and wheat gluten were lower than those of the males of similar age and fed on the same protein to appetite, due to the lower liveweight and percentages of body constituents. This may indicate that giving protein to appetite enables the animal to attain the genetically determined properties sooner than giving the restricted amount of protein.

Mostly, the effect of dietary proteins on body composition (percentages) did not reveal significant differences. This shows that the body exhibits a remarkable compositional homoeostatis. Nevertheless, this constancy can be altered by dietary proteins. Alteration depends on the inferiority and feeding period.

5.4. CONCLUSIONS

(Body composition)

On feeding males on the same amount of N (192 mg daily), the quantities (DM, F and N) and the percentages of the body components (DM %, F %, N %, FDM %, NDM %, N/W %) differed significantly between the animals fed on superior proteins (casein and the third potato protein) and between those

on inferior proteins (wheat gluten and soya protein) in some cases. The significant differences between rats fed on superior and inferior proteins were as follows:

a. quantities of DM and N between the rats fed on casein and wheat gluten and sacrificed at any of the four stages of Expt. 1
b. quantities of F between the rats fed on casein and wheat gluten and sacrificed at the second stage of Expt. 1
c. N% between the rats fed on casein and wheat gluten and sacrificed in the fourth stage of Expt. 1
d. N/W% between the rats fed on casein and wheat gluten and sacrificed at the second or fourth stage of Expt. 1 (end)
e. N% and N/W% between rats fed on casein and wheat gluten and sacrificed at 3.5 months old, 5.5 months old or as a total (10 rats) in Expt. 2
f. DM% between rats 3.5 months old fed on potato protein and soya protein in Expt. 2
g. F% between the total of ten rats, sacrificed during the three stages, fed on potato protein and soya protein in Expt. 2
h. FDM% between the total of ten rats fed on potato protein and soya protein in Expt. 2
i. quantities of DM, F and N of any combination between the casein group or potato group and wheat gluten group or soya group for each subgroup sacrificed at any specific stage or the total of a group sacrificed at the three stages of Expt. 2.

These significant differences indicate that the superior protein can significantly increase not only the quantities of body components (DM, F and N), but also their proportions compared to the inferior protein. Furthermore, N% and N/W% are slightly, though significantly, affected by the quality of the protein given in a restricted amount for longish experiment.

Although giving the protein to appetite did not result in significant differences between the weights of casein and wheat gluten groups of the same sex at the end of Expt. 3, significant differences between their body constituents were found in some cases, i.e., DM%, F%, FDM%, quantities of DM and F, but not NDM%, N/W% and the quantities of N. These differences of male rats fed on either protein differed in some instances in their significance and direction (positive or negative difference) from the female rats fed on the same proteins.

In general, age influence was confirmed by a decrease in W% and an increase in N%, F% and quantities of DM, F, W and N.

6. FREE AMINO ACIDS IN BLOOD

6.1. INTRODUCTION

Free amino acids in blood are the building blocks of protein metabolism. They are derived mainly from dietary protein and breakdown of tissue protein and can be synthesized into cellular structures, broken down or absorbed while remaining chemically intact. The body contains less amino acids in free form than in protein-bound form. Concentrations of free amino acids in blood are less than in tissues and dynamic relationships exist between blood and tissue levels.

During the last decade free amino acids in blood have increased in significance because of improvements in methods of estimation. Values might indicate inborn errors in amino acid metabolism (HOLT and SNYDERMAN, 1964), protein adequacy of the diet (ALMQUIST, 1954; SNYDERMAN et al., 1968), amino acid requirements (MCLAUGHLAN, 1967) and amino acid availabilities (GUGGENHEIM et al., 1960; CHILDS and COMBS, 1964). WHITEHEAD and DEAN (1964) reported that values were reliable indicators of protein malnutrition before there are gross clinical symptoms. PEREIRA et al. (1968) suggested amino acid ratios as a parameter for assessing nutritive value of a dietary protein and nutritional status of a community. Many studies have shown that after animals eat a protein, limiting amino acids decrease in blood relative to other amino acids (LONGENECKER and HAUSE 1959; MORRISON et al., 1961; PUCHAL et al., 1962, HILL and OLSEN; 1963 and SWENDSEID et al., 1963). DEAN and SCOTT (1966) used free amino acids in chicken blood to detect deficiencies and excesses of dietary amino acids.

Generally, changes in levels of free amino acids in the systemic blood after protein meal depend upon the previous levels before protein-feeding, the amount and the composition of the protein, rate of the stomach emptying, the rate of release of free amino acids from the protein during digestion, the availability of amino acids, the rates of absorption of amino acids, the amino acid metabolism of absorbing cells, the role of the liver in regulating amino acid metabolism and the rates of removal of the absorbed amino acids from the blood.

Although free amino acids in blood have proved a reasonable criterion, results have been conflicting. Several factors can interfere and further investigations are still needed. In most studies large amounts of proteins have been used for short periods, whereas especially in developing countries people eat little protein. Probably such human diets are less deficient in one or more amino acids than experimental proteins or amino acids.

I studied free amino acids in blood at different stages in Expt. 2 and Trial 3. In Expt. 2, I estimated levels of free amino acids in blood before and after the rats had eaten 192 mg nitrogen from casein, potato protein, wheat gluten or soya protein. In Trial 3 where blood samples were taken from the same rats except at birth, only fasting values at about 4, 12 and 24 weeks of age were estimated before providing casein and wheat gluten to appetite for one hour daily. Information with respect to these concentrations of free amino acids in

blood may provide valuable indexes of metabolic interrelationships and clue to the reactions involved in protein nutrition.

6.2. RESULTS

(Free amino acids in blood)

6.2.1. *Experiment 2*

Fig. 3A.–6C. show the levels of free amino acids in blood of $Cn\male$ (Fig. 3), $Pt\male$ (Fig. 4), $Gn\male$ (Fig. 5) and $Sy\male$ (Fig. 6) at three ages after the protein deprivation for 23 hours, with protein-free diet to appetite (first sampling = fasting level), and 45 and 105 min. after giving the 192 mg N (second and third sampling). Fasting values in Pt_{15} are not available.

Values were usually higher after than before the protein meal and the highest percentage increases were generally observed in Cn_{10}.

Individual decreases in values at the second sampling were as follows:

a. Cn_{10}: none (Fig. 3A.). Cn_{14}: histidine (12%), arginine (5)%, glycine (13%), alanine (12%), serine (7%), and glutamic acid (4%) (Fig. 3B.). Cn_{18}: arginine (3%), taurine (12%), glycine (12%), alanine (7%), glutamic acid (5%) and serine (1%) (Fig. 3C.).

b. Pt_{11}: methionine (37%), histidine (23%), arginine (22%), valine (5%), total essential amino acids (4%), taurine (28%), glycine (19%), alanine (15 %), glutamic acid (15%), and serine (12%) (Fig. 4A.). Pt_{19}: threonine (2%), histidine (2%), serine (6%), alanine (5%), glutamic acid (2%) and glycine (1%) (Fig. 4C.).
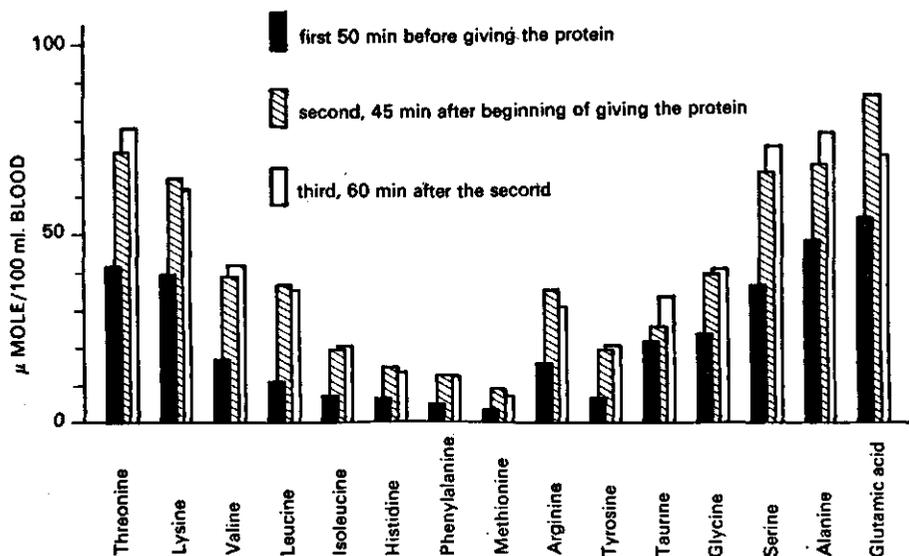
FIG. 3A. Levels of free amino acids in the blood of male rats 10 weeks old fed on caseine ($Cn_{10}\male$) before and after feeding 192 mg N. Rats were in Week 3 of Expt. 2.
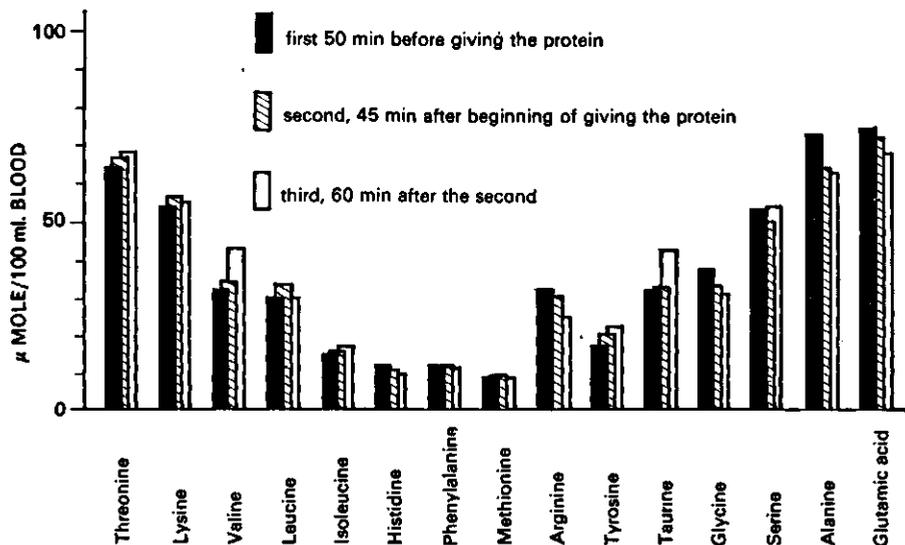
FIG. 3B. Levels of free amino acids in the blood of male rats 14 weeks old fed on casein ($Cn_{14}\male$) before and after feeding 192 mg N. Rats were in Week 7 of Expt. 2.
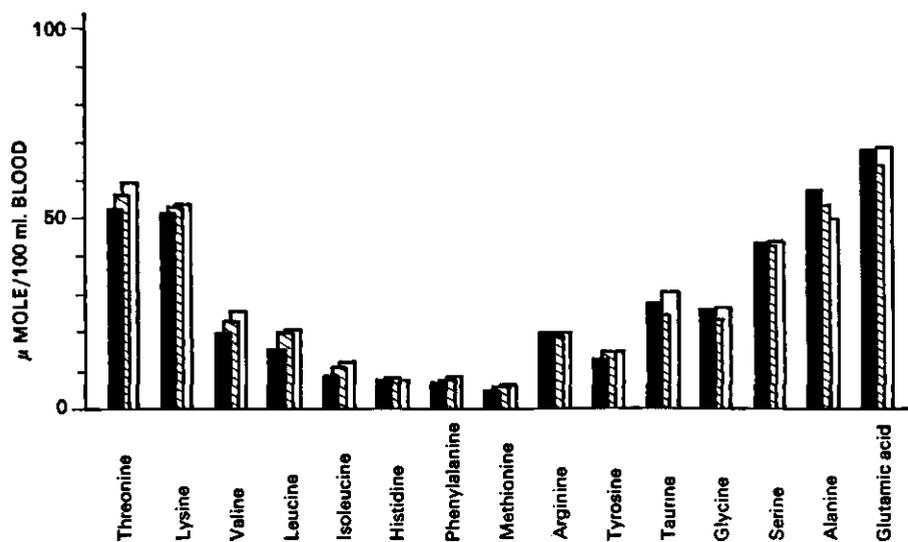
FIG. 3C. Levels of free amino acids in the blood of male rats 18 weeks old fed on casein (Cn_{18} $\male$) before and after feeding the 192 mg N. Rats were in Week 11 of Expt. 2. See Fig. 3B. for times of sampling.
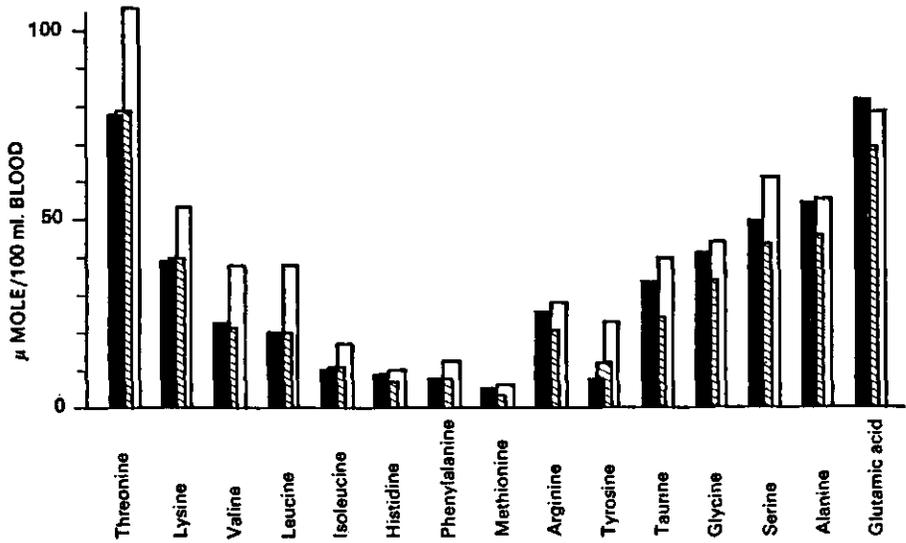
FIG. 4A. Levels of free amino acids in the blood of male rats 11 weeks old fed on potato protein (Pt$_{11}$♂) before and after feeding 192 mg N. Rats were in Week 4 of Expt. 2. See Fig. 3B. for times of sampling.
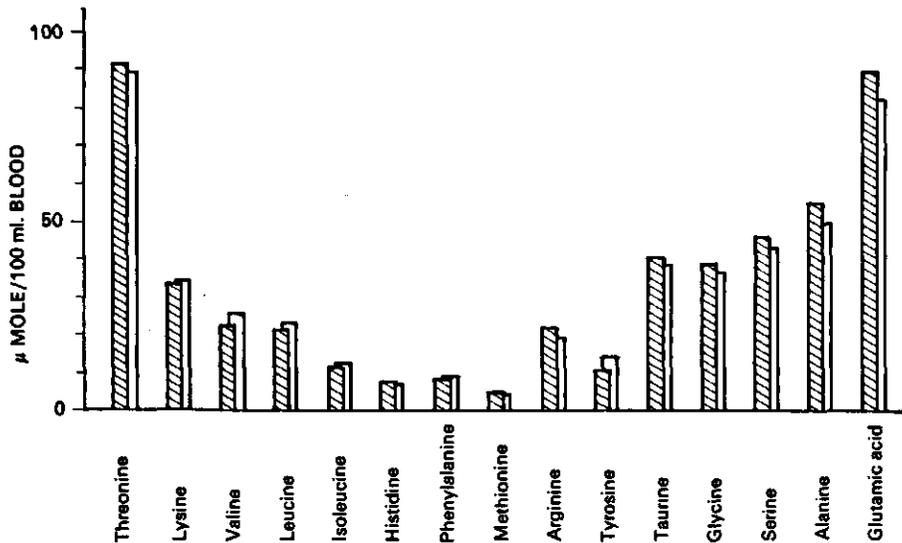
FIG. 4B. Levels of free amino acids in the blood of male rats 15 weeks old fed on potato protein (Pt$_{15}$♂) after feeding 192 mg N. Rats were in Week 8 of Expt. 2. See Fig. 3B. for times of sampling.
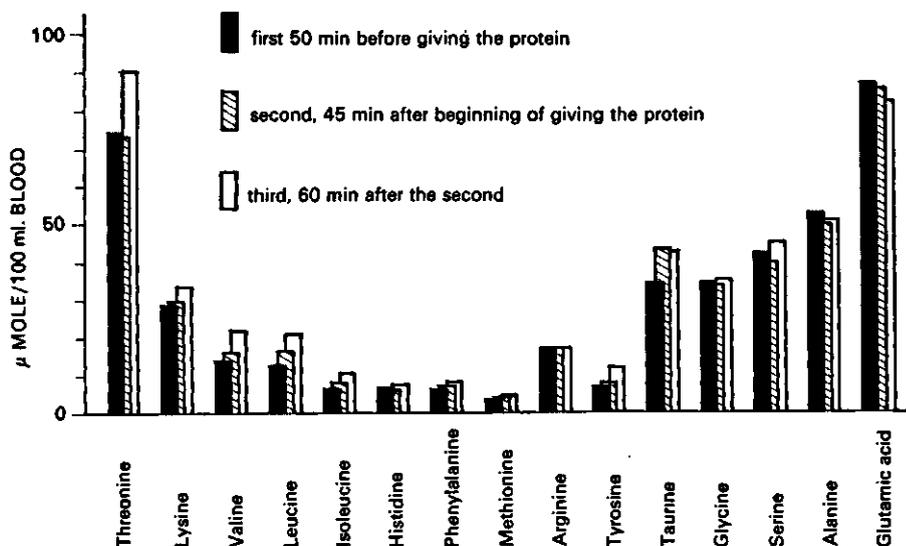
FIG. 4C. Levels of free amino acids in the blood of male rats 19 weeks old fed on potato protein ($Pt_{19}\delta$) before and after feeding 192 mg N. Rats were in Week 12 of Expt. 2.

c. Gn_{12}: arginine (22%), lysine (11%), histidine (9%), glutamic acid (11%), glycine (5%) and alanine (1%) (Fig. 5A.). Gn_{16}: arginine (3%), taurine (17%) and glycine (8%) (Fig. 5B.). Gn_{20}: nothing (Fig. 5C.).

d. Sy_{13}: methionine (40%), histidine (27%), arginine (11%), threonine (9%), total essential amino acids (5%), lysine (3%), glycine (30%), serine (21%), alanine (14%), and glutamic acid (14%) (Fig. 6A.). Sy_{17}: methionine (10%), glutamic acid (5%), glycine (4%) and alanine (2%) (Fig. 6B.). Sy_{21}: methionine (13%), glycine (12%), glutamic acid (8%) and serine (3%) (Fig. 6C.).

Most of the decreases in values at the second sampling were reversed at the third sampling. Exceptions to this were: in Cn_{14} arginine (18%), histidine (10 %), glutamic acid (6%), glycine (6%) and alanine (2%); in Cn_{18} alanine (7%); in Pt_{19} glutamic acid (4%); in Gn_{16} arginine (3%) and glycine (11%); in Sy_{17} methionine (36%) and glycine (5%). If an amino acid did not decrease at the second sampling, it sometimes did so at the third sampling in relation to fasting level: in Cn_{14} phenylalanine (3%); in Gn_{16} lysine (22%) and histidine (1%). Sometimes, an amino acid which decreased at the second sampling and increased at the third sampling did not attain the fasting level. These amino acids were glutamic acid in Pt_{11}, alanine in Pt_{19}, lysine in Gn_{12} and methionine in Sy_{21}.

Average values at the three ages increased in each group after the protein meal at the second or third sampling. Exceptions to this were:

a. in Cn none
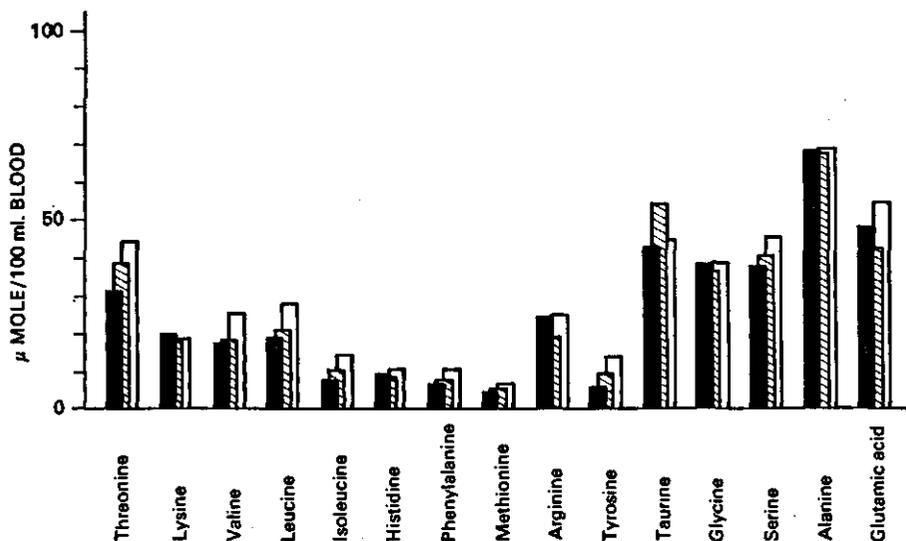
FIG. 5A. Levels of free amino acids in the blood of male rats 12 weeks old fed on wheat gluten (Gn$_{12}$♂) before and after feeding 192 mg N. Rats were in Week 5 of Expt. 2. See Fig. 4C. for times of sampling.
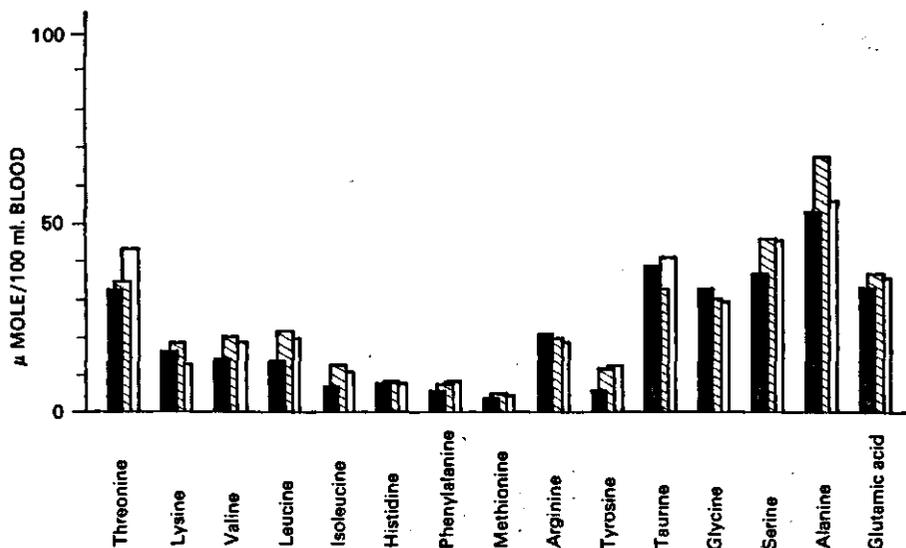


FIG. 5B. Levels of free amino acids in the blood of male rats 16 weeks old fed on wheat gluten (Gn$_{16}$♂) before and after feeding 192 mg N. Rats were in Week 9 of Expt. 2. See Fig. 4C. for times of sampling.
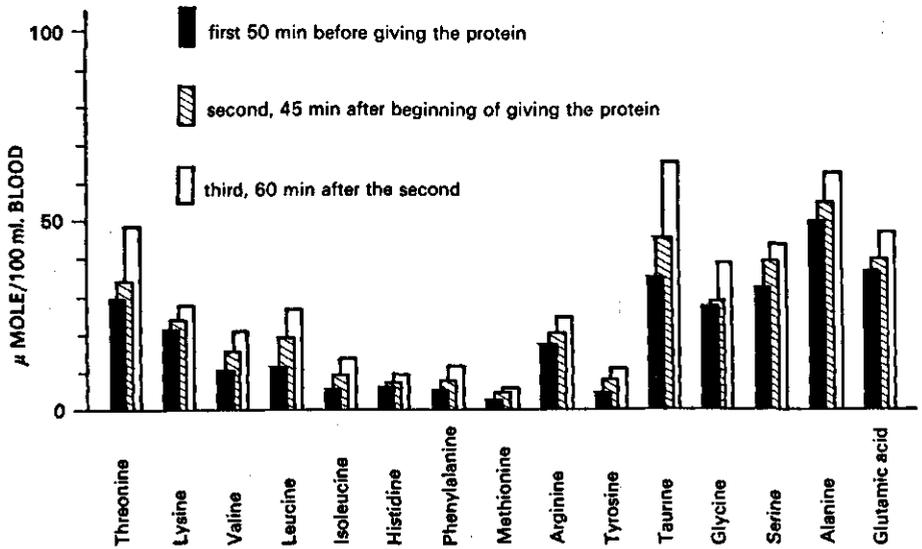
FIG. 5C. Levels of free amino acids in the blood of male rats 20 weeks old fed on wheat gluten (Gn$_{20}$♂) before and after feeding 192 mg N. Rats were in Week 13 of Expt. 2.
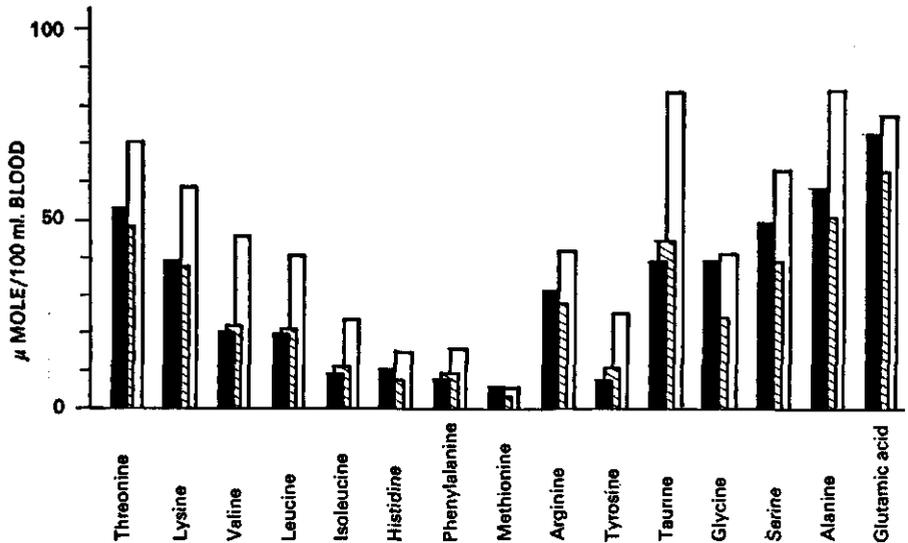
FIG. 6A. Levels of free amino acids in the blood of male rats 13 weeks old fed on soya protein (Sy$_{13}$♂) before and after feeding 192 mg N. Rats were in Week 6 of Expt. 2. See Fig. 5C. for times of sampling.
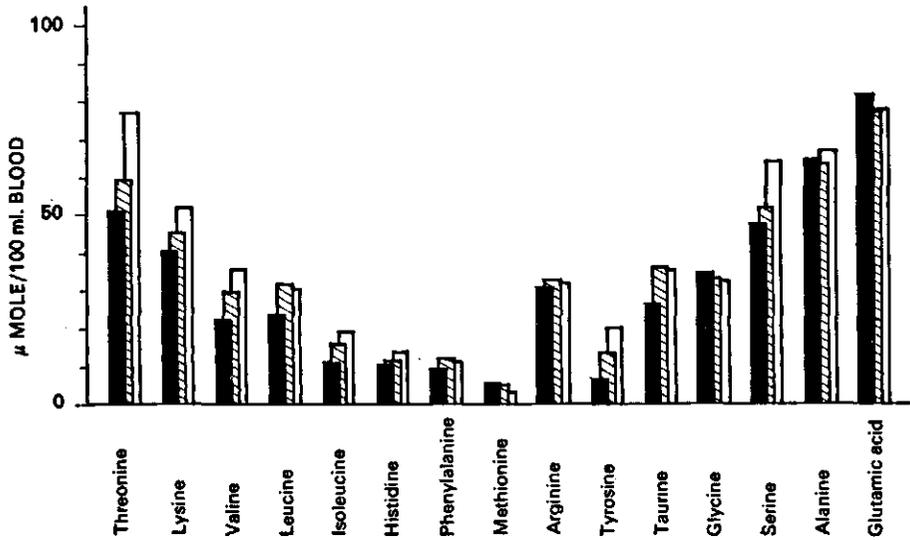
Fɪɢ. 6B. Levels of free amino acids in the blood of male rats 17 weeks old fed on soya protein (Sy₁₇♂) before and after feeding 192 mg N. Rats were in Week 10 of Expt. 2. See Fig. 5C. for times of sampling.
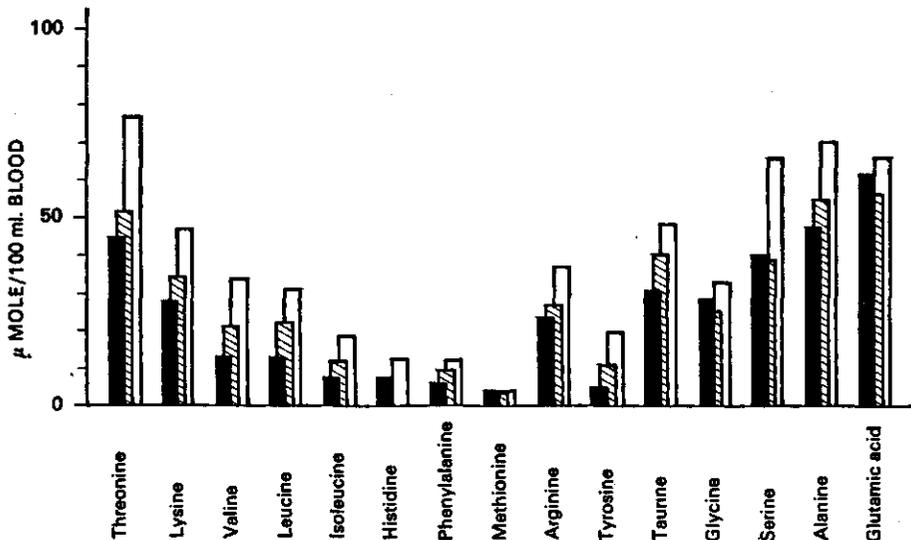
Fɪɢ. 6C. Levels of free amino acids in the blood of male rats 21 weeks old fed on soya protein (Sy₂₁♂) before and after feeding 192 mg N. Rats were in Week 14 of Expt. 2. See Fig. 5C. for times of sampling.

b. in Pt at the second sampling methionine (15%), histidine (14%), arginine (13%), threonine (< 0.5%), alanine (20%), glycine (11%), serine (9%), glutamic acid (8%) and taurine (< 0.5%) and at the third sampling alanine (14%), glutamic acid (4%) and glycine (3%)
c. in Gn at the second sampling arginine (5%)
d. in Sy at the second sampling methionine (23%), glycine (16%), glutamic acid (9%), serine (5%) and alanine (1%) and at the third sampling methionine (18%).

On average of about 22 changes after the protein meal, there was an increase in all amino acids: tyrosine (90%), isoleucine (65%), leucine (55%), valine (50%), phenylalanine (38%), taurine (28%), threonine (23%), lysine (20%), arginine (20%) methionine (18%), serine (18%), histidine (9%), alanine (5%), glycine (2%) and glutamic acid (2%).

The range in the levels of each amino acid in a group (three ages) at fasting (3 values mostly) or after protein-feeding (6 values mostly) overlapped with that of the other three groups. Exceptions were:
a. Threonine was higher in fasting Pt than in other fasting groups and less in fasting or protein-fed Gn than in any other group.
b. Lysine was less in Gn before or after the protein meal than in other groups.
c. Taurine was more in fasting Gn than in any other group.
d. Tyrosine differed between fasting Cn or Pt and Gn and between protein-fed Cn and Gn. The differences disappeared if tyrosine and phenylalanine were added together.
e. Serine differed between fasting Gn and Pt or Sy but total of serine and glycine was similar.
f. Glutamic acid was higher in fasting Pt and lower in fasting or protein-fed Gn than in the other three groups.

Among fasting rats, average values were highest for lysine, valine, isoleucine, leucine, methionine, tyrosine, serine and alanine in Cn; threonine, glycine and glutamic acid in Pt; taurine in Gn; phenylalanine, histidine and arginine in Sy. Among protein-fed rats, the same was found but average values of serine in Cn and isoleucine and alanine in Sy were higher than in the other three groups. Generally, levels of most amino acids were higher in Cn and lower in Gn than in other three groups.

The highest value for any amino acid was 91 μmole for glutamic acid in Pt_{15} at the second sampling and the lowest 3.0 μmole for methionine in Gn_{20} at the first sampling. The highest levels were usually glutamic acid followed by alanine and the lowest methionine. Of the essential amino acids, the highest levels were usually threonine followed by lysine and the lowest methionine. Average fasting levels were in the descending order glutamic acid, alanine, threonine, serine, lysine, taurine, glycine, arginine, valine, leucine, isoleucine, histidine, tyrosine, phenylalanine and methionine.

Fasting values were lower in Cn_{18} than in Cn_{14} or Cn_{10} and higher in Cn_{14} than in Cn_{10}. Average levels after protein-feeding were, however, lower in Cn_{18}

or Cn_{14} than in Cn_{10}, except for taurine (24%[1]), tyrosine (2%) and methionine (1%) in Cn_{14}. Average levels after protein-feeding were lower in Cn_{18} than in in Cn_{14}. Fasting levels were lower in Pt_{19} than in Pt_{11}, except for glutamic acid (6%) and taurine (3%). Average levels after protein-feeding were lower in Pt_{19} or Pt_{15} than in Pt_{11}, except for taurine (34%) and glutamic acid (13%) in Pt_{19} and taurine (24%), glutamic acid (16%) and alanine (3%) in Pt_{15}. Average levels after protein-feeding were lower in Pt_{19} than in Pt_{15}, except for taurine (8%). Fasting levels were lower in Gn_{20} or Gn_{16} than in Gn_{12}, except for lysine (8%) in Gn_{20} and threonine (4%) in Gn_{16}. Fasting levels were lower in Gn_{20} than in Gn_{16}, except for lysine (31%) and glutamic acid (11%). Average levels after protein-feeding were lower in Gn_{20} or Gn_{16} than in Gn_{12}, except for lysine (43%), taurine (13%), and phenylalanine (5%) in Gn_{20} and serine (7%) and tyrosine (<0.5%)) in Gn_{16}. Average levels after protein-feeding were lower in Gn_{20} than in Gn_{16}, except for tyrosine (20%), serine (10%), alanine (5%) and valine (3%). Fasting levels were lower in Sy_{21} than in Sy_{13}, but higher in Sy_{17} than in Sy_{13}, except for taurine (22%), tyrosine (5%), threonine(2%)and arginine (<0.5%) in Sy_{17}. In consequence, fasting levels in Sy_{21} were lower than in Sy_{17}, except for taurine (15%). Average levels after protein-feeding were lower in Sy_{17} and Sy_{21} than in Sy_{13}. Exceptions to these were threonine (14%), serine (14%), histidine (13%), glutamic acid (11%), isoleucine (3%), glycine (2%), leucine (1%) and lysine (<0.5%) in Sy_{17} and threonine (8%) and serine (2%) in Sy_{21}. The average levels after protein-feeding were lower in Sy_{21} than in Sy_{17}, except for taurine (23%). Usually amino acid levels before or after protein-feeding decreased with age but there were clear exceptions.

Each essential amino acid in Fig. 3–6 was recalculated as percentages of the total essential amino acids. Generally, the variations in essential amino acid levels after protein-feeding or at different ages were reduced by expressing them as percentages of the total.

TABLE 17. Average essential amino acids in Expt. 2 expressed as percentages of total essential amino acids

	Casein group (Cn)	Potato group (Pt)	Wheat-gluten group (Gn)	Soya group (Sy)
Threonine	26.2	39.3	22.5	26.2
Lysine	22.7	16.7	13.2	18.8
Valine	12.8	10.3	12.2	11.9
Leucine	10.8	9.7	13.5	11.7
Isoleucine	5.8	5.0	6.1	6.2
Histidine	4.2	3.5	5.9	4.9
Phenylalanine	4.0	3.8	5.3	4.5
Methionine	2.8	2.0	3.2	2.0
Arginine	10.5	9.5	14.2	13.9
Total essential amino acids	100%	100%	100%	100%

[1]. Values in parenthesis are the changes since the previous.

Therefore, Table 17 represents the average of these calculations for each group. The decreasing order, threonine, lysine, valine, leucine, isoleucine, histidine, phenylalanine and methionine was similar in the blood samples. The exceptions to this were as follow:

a. Lysine was less than valine and leucine in protein-fed Gn_{13} and Gn_{17}.
b. Valine was less than leucine in 8 out of 9 cases in Gn.
c. Isoleucine was less than histidine in 2 of the 3 samples in fasting Gn_{12}, Gn_{16}, Sy_{13} and Sy_{17}.
d. Histidine was usually less than phenylalanine in the four protein-fed groups.

Arginine was more than histidine and sometimes than lysine. Generally, arginine varied in order more than other essential amino acids. Ratios of threonine to other essential amino acids, ratios of valine to leucine, isoleucine or phenylalanine, ratios of leucine or isoleucine to phenylalanine and ratios of histidine to phenylalanine were higher in the blood samples than in the proteins.

6.2.2. *Trial 3*

Fig. 7A shows the free amino acid levels in blood of newborn rats and their mother. Fig. 7B–D shows average fasting levels of free amino acids in blood of replicates of male or female rats fed to appetite on casein or wheat gluten for one hour daily, when they were about 4, 12 and 24 weeks old in Trial 3. Unfortunately, certain blood samples were not available for analysis:

a. Sample C of the newborn rats and Sample C of their mothers (2 samples out of 6).
b. Eleven samples out of 12 taken at weaning; the remaining sample was $Cn_4♀^A$.
c. Two samples out of 12 taken in Week 8 at 12 weeks of age, i.e., $Cn_{12}♂^C$ and $Cn_{12}♀^C$.

6.2.2.1. Newborn rats and their mothers

Average respective values for histidine, glycine, valine, tyrosine, phenylalanine, methionine, leucine, isoleucine and serine levels in the two samples from newborn rats were 193, 112, 67, 55, 44, 33, 29, 26 and 1 % more than in their mothers, whereas glutamic acid, alanine, threonine, taurine, arginine and lysine levels were less (59, 32, 22, 19, 15 and 8 %). The trend in the difference between levels of amino acids in each sample of the newborn rats and their mothers resembled the average trend, except for lysine and serine. Lysine level of newborn rats was 28 % lower in the pooled Sample A and 12 % higher in the other Sample B with a lower average (8 %) compared with their mothers. Serine level of Sample A and B in new born rats was lower and higher, respectively, than in their mothers. The ratio eesential: non-essential free amino acids in blood was larger in newborn rats than in their mothers.
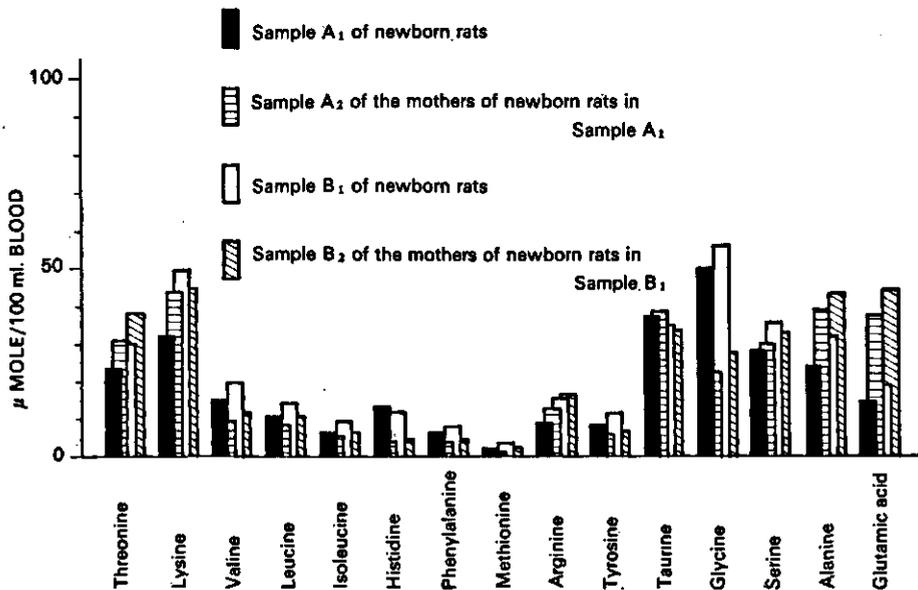
FIG. 7A. Levels of free amino acids in the blood of newborn rats and their mothers after delivery in Trial 3.
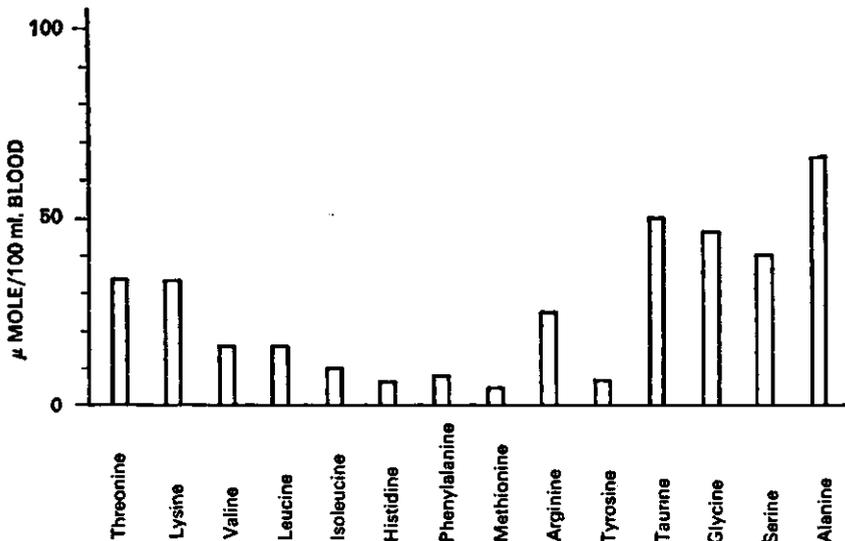

FIG. 7B. Fasting levels of free amino acids in the blood of female rats 4 weeks old after feeding a protein-free diet to appetite for the 23 hours following weaning of Replicate A which was given casein to appetite after sampling the blood. Rats (Cn₄♀^A) were at the beginning of Expt. 3.
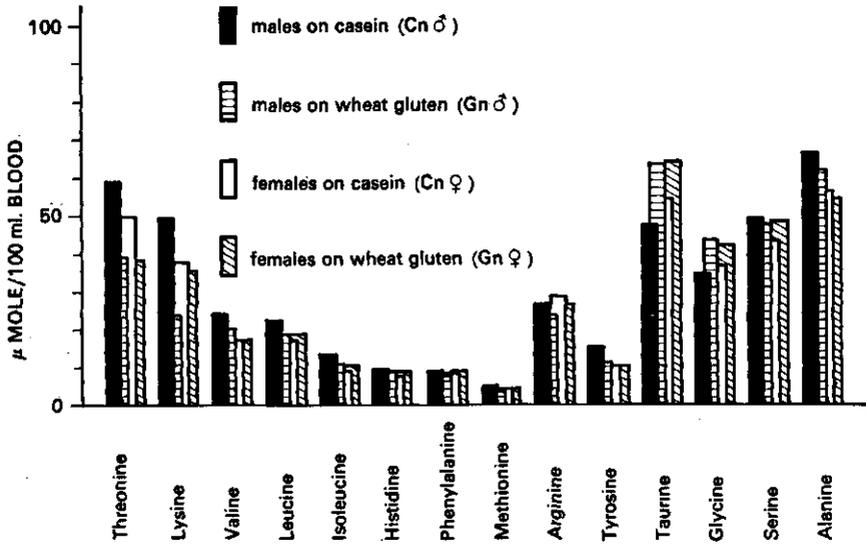
FIG. 7C. Average fasting levels of free amino acids in the blood of replicates of rats 12 weeks old fed on the protein to appetite for one hour daily. Rats were in Week 8 of Expt. 3.
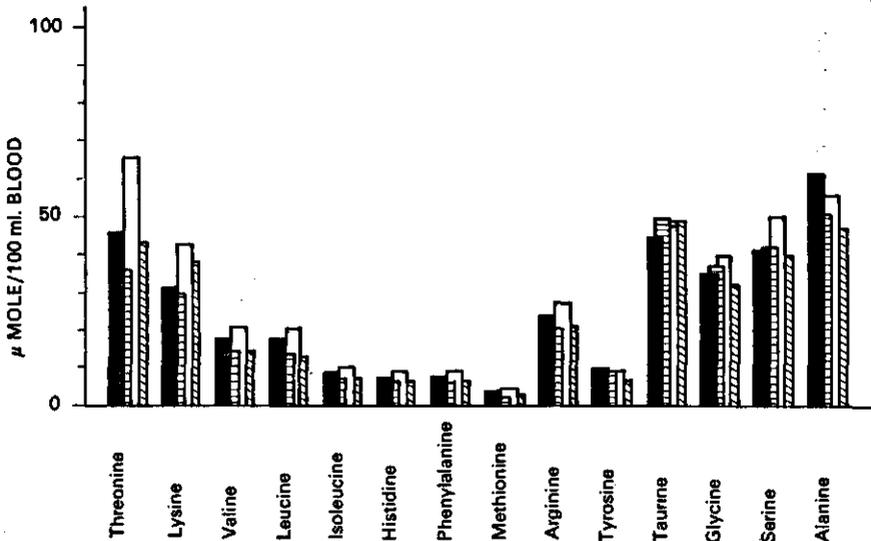
FIG. 7D. Average fasting levels of free amino acids in the blood of replicates of rats 24 weeks old fed on the protein to appetite for one hour daily. Rats were in Week 20 Expt. 3. See Fig. 7C. for dietary groups.

6.2.2.2. Differences in amino acid levels between rats on casein and wheat gluten to appetite (Expt. 3)

The range in values in replicates of Cn was compared with that of Gn of the same age and sex. Differences in any two ranges of Cn and Gn, which did not overlap between two groups were calculated as follows:

a. Protein difference (D_p) as an absolute value = the lowest value (μmoles/100 ml blood) of the higher range – the highest value of the lower range.

b. Percentage of protein difference ($D_p\%$) = $D_p \times 100$/lowest value of the higher range.

Values of $D_p\%$ were arranged in decreasing order and followed by the values of D_p between brackets.

I. Male rats twelve weeks old. The range in valine, leucine, isoleucine, histidine, phenylalanine, methionine, arginine, serine, alanine, total non-essential amino acids or total amino acids overlapped in replicates fed on the two proteins (casein and wheat gluten) to appetite. The lowest value of threonine, lysine, total essential amino acids or tyrosine in replicates fed on casein was higher than the highest value in repicates fed on wheat gluten, whereas the reverse was found for taurine and glycine. The value of $D_p\%$ was 45 % (22) for lysine, 18 % (11) for taurine, 16 % (8) for threonine, 13 % (28) for total essential amino acids, 1 % (1) for glycine and 1 % ($<$ 0.5) for tyrosine.

II. Female rats twelve weeks old. The range in lysine, valine, leucine, isoleucine, histidine, phenylalanine, methionine, arginine, total eesential amino acids, tyrosine, taurine, serine or total amino acids overlapped in replicates of $Cn_{12}♀$ and $Gn_{12}♀$. The lowest value of threonine in replicates fed on casein was higher than the highest in replicates fed on wheat gluten, whereas the reverse was found for taurine, glycine, and total non-essential amino acids. The value of $D_p\%$ was 15 % (8) for threonine, 7 % (15) for total non-essential amino acids, 5 % (2) for glycine and 2 % (1) for taurine.

III. Male rats twenty-four weeks old. The range in lysine, valine, leucine, isoleucine, histidine, phenylalanine, methionine, arginine, total essential amino acids, tyrosine, taurine, glycine, serine, alanine, total non-essential amino acids or total amino acids overlapped in replicates of $Cn_{24}♂$ and $Gn_{24}♂$. The lowest value of threonine in replicates fed on casein was higher than the highest in replicates fed on wheat gluten. The value of $D_p\%$ was 12 % (5) for threonine.

IV. Female rats twenty-four weeks old. The range in lysine, isoleucine, histidine, phenylalanine, methionine, tyrosine, taurine, glycine, serine, alanine or total non-essential amino acids overlapped in replicates of $Cn_{24}♀$ and $Gn_{24}♀$. The lowest value of threonine, valine, leucine, arginine, total essential amino acids or total amino acids in replicates fed on casein was higher than the highest value in replicates fed on wheat gluten. The value of $D_p\%$ was 18 % (11) for

threonine, 12% (23) for total essential amino acids, 12% (3) for arginine, 6% (1) for valine, 6% (1) for leucine and 4% (15) for total non-essential amino acids.

6.2.2.3. Sexual differences in amino acid levels (Expt. 3)

The range in levels of each amino acid in replicates of male rats was compared with that of female rats fed on the same protein to appetite and for the same age. Difference in any two ranges, which did not overlap in the two sexes were calculated in the same way as D_p and $D_p\%$, but were designated D_s and $D_s\%$, respectively, i.e., sexual difference absolute and as a percentage. Reported values of $D_s\%$ were arranged in decreasing order and followed by the values of D_s in parenthesis.

I. Rats twelve weeks old fed on casein (Cn_{12}). The range in methionine, arginine, taurine, glycine, serine or total non-essential amino acids overlapped in the two sexes. The range in threonine, lysine, valine, leucine, isoleucine, histidine, phenylalanine, total essential amino acids, tyrosine, alanine or total amino acids in the males was higher than in females. The value of $D_s\%$ was 24% (3) for isoleucine, 22% (3) for tyrosine, 19% (9) for lysine, 19% (4) for valine, 12% (26) for total essential amino acids, 11% (2) for leucine, 6% (23) for total amino acids, 6% (< 0.5) for histidine, 4% (2) for alanine, 4% (2) for threonine and 1% (< 0.5) for phenylalanine.

II. Rats twenty-four weeks old fed on casein (Cn_{24}). The range in valine, leucine, isoleucine, histidine, phenylalanine, methionine, arginine, total essential amino acids, tyrosine, taurine, glycine, serine, alanine, total non-essential amino acids or total amino acids in the blood overlapped in the two sexes. The range in lysine or threonine in females was higher than in males. The values of $D_s\%$ were 14% (8) and 7% (3), respectively, for lysine and threonine.

III. Rats twelve weeks old fed on wheat gluten (Gn_{12}). The range in threonine, valine, leucine, isoleucine, histidine, phenylalanine, methionine, arginine, total essential amino acids, tyrosine, taurine, glycine, serine, alanine, total-non-essential amino acids or total amino acids overlapped in the two sexes. The range in lysine in females was higher than in males. The value of $D_s\%$ was 23% (8) for lysine.

IV. Rats twenty-four weeks old fed on wheat gluten (Gn_{24}). The range in lysine, valine, leucine, isoleucine, histidine, phenylalanine, methionine, arginine, total essential amino acids, taurine, glycine, serine, alanine, total non-essential amino acids, or total amino acids overlapped in the two sexes. The range in threonine in blood of females was higher than in males, whereas the reverse was found for tyrosine. The value of $D_s\%$ was 8% (3) for threonine and 4% (< 0.5) for tyrosine.

6.2.2.4. Changes in amino acid levels of the same rats at different ages (Expt. 3)

The changes in levels of free amino acids in the blood with age were considered as absolute and relative values. Changes in amino acids at two ages of the same replicate were calculated according to these two formulae:

a. Age difference (D_a) as an absolute value = amino acid level in μmoles/100 ml blood – respective level at the preceding age

b. Percentage of age difference $(D_a\%)$ as a relative value $D_a \times 100$/highest level at the two ages

The calculations were carried out to two decimal places and the results were rounded to the first figure without decimals. The calculation of the results from the two formulae resulted in either increase or decrease with age. To show the importance of the changes in such levels with age, the results of a replicate were arranged in a descending order according to the value of $D_a\%$. When two values of $D_a\%$ for different amino acids were equal in a replicate, they were arranged in accordance with the absolute value D_a. The results of $D_a\%$ followed by that of D_a between parenthesis express:

a. changes from the beginning to the beginning of Week 9 of Expt. 3 (4–12 weeks of age) which include only $Cn\female^A$.

b. changes from the beginning of Week 9 to Week 21 of Expt. 3 (12–24 weeks of age) which include:

  I. two replicates of females on casein $(Cn\female^A$ and $Cn\female^B)$

 II. two replicates of males on casein $(Cn\male^A$ and $Cn\male^B)$

III. three replicates of females on wheat gluten $(Gn\female^A, Gn\female^B, Gn\female^C)$

IV. three replicates of males on wheat gluten $(Gn\male^A, Gn\male^B, Gn\male^C)$

The total levels of amino acids in $Cn\female^A$ at 12 weeks of age were higher than 4 weeks of age. According to $D_a\%$ and (D_a) values, increases with age were as follows: threonine 33% (17), tyrosine 29% (3), histidine 27% (2), arginine 19% (6), serine 14% (6), leucine 12% (2), valine 11% (2), lysine 5% (2), phenylalanine 5% (< 0.5), isoleucine 0% (0) and methionine 0% (0) but decreases as follows: glycine 15% (7), alanine 9% (6) and taurine 2% (1).

Total levels of amino acids in $Cn\female^A$ at 24 weeks of age were higher than at 12 weeks of age. From $D_a\%$ and (D_a) values, increases with age were as follows: threonine 35% (28), valine 27% (7), leucine 27% (7), serine 25% (15), lysine 25% (12), phenylalanine 25% (3), methionine 23% (1), isoleucine 20% (3), histidine 18% (2), tyrosine 17% (1), alanine 14% (10), taurine 13% (7), glycine 11% (8) and arginine 3% (1).

Total levels of amino acids in $Cn\female^B$ at 24 weeks of age were higher than at 12 weeks of age. From $D_a\%$ and (D_a), increases with age were as follows: histidine 20% (2), serine 17% (8), isoleucine 16% (2), methionine 14% (1), threonine 13% (8), phenylalanine 13% (1), valine 12% (3), glycine 10% (4), leucine 5% (3), lysine 4% (2) and tyrosine 1% (< 0.5). However, the decreases with age were taurine 34% (21), alanine 6% (3) and arginine 5% (1).

In $Cn\female$ (12–24 weeks of age) levels of amino acids, except for arginine and histidine increased more with age in Replicate A than in Replicate B. The greatest

differences in D_a in these two replicates in a descending order were taurine, threonine, lysine, serine and alanine. Levels of taurine, alanine and arginine in $Cn♀^A$ increased with age but decreased in $Cn♀^B$. The other free amino acids increased with age in Replicate A and B of $Cn♀$.

Total levels of amino acids in $Cn♂^A$ 12 weeks of age were higher than at 24 weeks of age. From $D_a\%$ and (D_a) values, the decreases with age were as follows: tyrosine 42% (7), isoleucine 40% (5), valine 37% (11), threonine 32% (21), methionine 30% (2), leucine 26% (6), phenylalanine 26% (3), histidine 25% (3), alanine 22% (15), arginine 21% (6), lysine 20% (10), serine 17% (9), taurine 14% (7) and glycine 7% (2).

Total levels of amino acids in $Cn♂^B$ at 12 weeks of age were higher than at 24 weeks of age. From $D_a\%$ and (D_a) values, the decreases with age were lysine 44% (22), isoleucine 43% (6), valine 32% (7), serine 30% (14), leucine 30% (6), tyrosine 29% (4), histidine 27% (2), threonine 19% (10), arginine 19% (5), phenylalanine 19% (2), methionine 19% (1), alanine 8% (5), taurine 8% (4) and glycine 4% (1).

In $Cn♂$ (12–24 weeks of age), levels of amino acids decreased with age in Replicate A and B. These decreases were greater in Replicate A than in B, except for the reverse of lysine, serine and isoleucine. The greatest differences of D_a values between these two replicates in a descending order were for lysine, threonine, alanine, serine and valine.

Total levels of amino acids in $Gn♀^A$ at 12 weeks of age were higher than at 24 weeks of age. From $D_a\%$ and (D_a) values, the decreases with age were tyrosine 33% (4), leucine 32% (7), isoleucine 32% (4), methionine 30% (2), arginine 28% (8), taurine 27% (18), phenylalanine 26% (2), histidine 24% (2), valine 22% (4), alanine 15% (9), serine 12% (6), glycine 2% (7) and threonine 1% (<0.5), but the increases lysine 11% (5).

The total levels of amino acids in $Gn♀^B$ at 12 weeks of age were higher than at 24 weeks of age. From $D_a\%$ and (D_a), decreases with age were tyrosine 33% (3), isoleucine 31% (3), histidine 29% (3), leucine 27% (5), glycine 23% (10), phenylalanine 22% (2), taurine 20% (13), methionine 19% (1), arginine 16% (4), valine 15% (3), alanine 14% (8), serine 14% (7), lysine 1% (<0.5); threonine increased by 26% (12).

Total levels of amino acids in $Gn♀^C$ at 12 weeks of age were higher than at the age of 24 weeks. From $D_a\%$ and (D_a) values, decreases with age were tyrosine 43% (4), glycine 37% (16), histidine 35% (3), leucine 32% (6), methionine 32% (1), phenylalanine 30% (3), serine 29% (14), taurine 26% (17), isoleucine 25% (2), valine 17% (3), alanine 15% (7) and arginine 9% (2); lysine and threonine increased by 4% (2) and 2% (1), respectively.

In $Gn♀$ (12–24 weeks old), total levels of amino acids increased with age in the three Replicates A, B and C. Threonine level decreased slightly in $Gn♀^A$, but increased markedly in $Gn♀^B$ and slightly in $Gn♀^C$. Lysine level increased markedly in $Gn♀^A$ and slightly in $Gn♀^B$; it decreased slightly in $Gn♀^C$. These three replicates tended to increase or decrease similarly for the other amino acids, but magnitude varied.

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Total levels of amino acids in Gn♂^{A} at 12 weeks of age were higher than at 24 weeks of age. From $D_a\%$ and (D_a), the decreases with age were

methionine 44% (2), isoleucine 32% (4), histidine 28% (3), valine 27% (6), tyrosine 27% (4), alanine 26% (19), leucine 23% (5), phenylalanine 22% (2), arginine 19% (5), taurine 18% (11), glycine 18% (9), serine 17% (10) and threonine 15% (7); lysine increased by 32% (11).

Total levels of amino acids in Gn♂^{B} at 12 weeks of age were higher than at 24 weeks of age. From $D_a\%$ and (D_a), the decreases with age were

methionine 30% (1), valine 29% (6), leucine 25% (5), isoleucine 25% (3), histidine 25% (2), taurine 24% (16), alanine 15% (9), phenylalanine 13% (1), arginine 11% (3), serine 10% (5), glycine 9% (4), threonine 7% (3) and tyrosine 2% (0); lysine increased by 16% (5).

Total levels of amino acids in Gn♂^{C} at 12 weeks of age were higher than at 24 weeks of age. From $D_a\%$ and (D_a), decreases with age were

histidine 40% (3), leucine 36% (6), isoleucine 35% (3), valine 33% (5), methionine 30% (1), taurine 25% (15), glycine 24% (9), phenylalanine 24% (2), tyrosine 16% (2), alanine 13% (6), serine 13% (5), arginine 12% (2), and threonine 1% (70); lysine increased by 8% (2).

In Gn♂ (12–24 weeks of age), each level of amino acids decreased with age in each replicate, except for lysine which increased.

6.2.2.5. Change in amino acid levels of the same and different rats with age (Trial 3)

I. From birth to weaning. Amino acid levels in the sample at weaning (Cn$_4$♀^{A}) were higher than the average levels in samples of newborn rats, except for lysine, valine, histidine, tyrosine and glycine, which were lower. However, levels of phenylalanine + tyrosine and serine + glycine at weaning also were higher than those at birth. Lysine, valine and tyrosine levels were within the range for newborn rats. The reverse trend was marked for histidine (52% lower at weaning than at birth).

II. From weaning to 12 weeks of age. Average of replicates for levels of each amino acid in Cn♂, Gn♂, Cn♀ and Gn♀ at 12 weeks of age was higher than that in the sample at weaning (Cn$_4$♀^{A}), except for lysine in Gn$_{12}$♂ (28% lower), isoleucine in Cn$_{12}$♀ (6%), methionine in Gn$_{12}$♂ (19%), in Cn$_{12}$♀ (5%) and in Gn$_{12}$♀ (11%), arginine in Gn$_{12}$♂ (2%), alanine in Gn$_{12}$♂ (6%), in Cn$_{12}$♀ (14%) and in Gn$_{12}$♀ (17%), taurine in Cn♂$_{12}$ (2%) and glycine + serine in Cn$_{12}$♂ (13%) and in Cn$_{12}$♀ (16%). Amino acid levels at weaning were within the range in levels of isoleucine in replicates of Cn$_{12}$♀, methionine in replicates of Cn$_{12}$♀ and of Gn$_{12}$♀, arginine in replicates of Gn$_{12}$♂, alanine in replicates of Gn$_{12}$♂, taurine in replicates of Cn$_{12}$♂ and glycine + serine in replicates of Cn$_{12}$♂. Therefore, there is a marked decrease (reverse trend) for lysine in Gn$_{12}$♂, methionine in Gn$_{12}$♂, alanine in Cn$_{12}$♀ and in Gn$_{12}$♀ and glycine + serine in Cn$_{12}$♀.

III. From 12 to 24 weeks of age. This has previously been described in details for the same replicate at different ages (6.2.2.4). Generally, most amino acid levels decreased in replicates of $Cn_{24}\male$, $Gn_{24}\male$ and $Gn_{24}\female$ at 24 weeks of age; the reverse was found in replicates of $Cn_{24}\female$. Within a replicate at 24 weeks of age there was a decrease in the level of taurine in $Cn_{24}\female^B$ and an increase in the level of threonine in $Gn_{24}\female$ and of lysine in $Gn_{24}\female^A$, $Gn_{24}\male^A$ and $Gn_{24}\male^B$. The ranges in these amino acids in replicates (same protein and sex) at 12 and 24 weeks old overlapped. These amino acids are still included in the remarked amino acids of reverse trend, because the variability of an amino acid in the same replicate is less than that between replicates.

IV. From weaning (4 weeks) to 24 weeks of age. As indicated, the change of amino acid levels in $Cn\male$, $Gn\male$ and $Gn\female$ usually differed in trend (increase then decrease) during the two ages of 4–12 and 12–24 weeks; however there was an increase in $Cn\female$ in both periods. Amino acid levels at weaning were within the respective ranges in the three replicates of $Cn_{24}\male$, except for threonine, histidine, tyrosine, phenylalanine + tyrosine, methionine and taurine. The value of methionine and taurine at weaning was higher than the highest level of each of these amino acids in the three replicates of $Cn_{24}\male$, whereas for threonine, histidine, phenylalanine, + tyrosine were lower than the lowest value. The level of lysine, isoleucine, phenylalanine, methionine, arginine, glycine, or alanine at weaning was higher than the highest level of each of these amino acids in replicates of $Gn\male_{24}$, whereas the reverse was found for threonine and tyrosine. Furthermore, level of valine, leucine, histidine, phenylalanine + tyrosine, taurine, serine or serine + glycine level was within the respective range of $Gn_{24}\male$. Each amino acid level at weaning ($Cn_4\female^A$) increased in the same rats at the end of Expt. 3 ($Cn_{24}\female^A$).

Moreover, amino acid levels at weaning were lower than the lower limits of the respective range in replicates of $Cn_{24}\female$, except for isoleucine, phenylalanine, phenylalanine + tyrosine, methionine, taurine, serine, glycine, serine + glycine and alanine levels which were within the range. Level of isoleucine, leucine phenylalanine, methionine, arginine, glycine, glycine + serine or alanine at weaning were above the upper limits of the range in replicates of $Gn_{24}\female$, whereas the reverse was found for lysine and threonine. In addition, valine, histidine, tyrosine, phenylalanine + tyrosine, taurine or serine at weaning was within the respective range in replicates of $Gn_{24}\female$. Generally, total essential amino acids in male and female rats fed on casein increased on average at the end of Expt. 3, whereas those on wheat gluten decreased or increased slightly.

6.3. DISCUSSION

(Free amino acids in the blood)

There were not enough data for ideal statistical analyses. Therefore, most differences were not conclusively assignable to proteins type, age, or variation within a treatment. In the literature (McLAUGHLAN et al., 1961; SWENDSEID et al.,

1963), such levels of a pooled sample from a group were compared with those of another group without statistical analysis, presumably, because amino acids are expensive and laborious to estimate. Furthermore, variations between group means are lower than between individuals. With the progress in analytical procedures, a cheaper, easier, more accurate and precise method of determination may become available to facilitate such investigations. Marked trends in the amino acids determined were observed and give a clue to the interrelation between the protein composition and metabolism. Unfortunately, amino acids were not estimated in the four proteins. It was assumed that their amino acid contents were similar to those reported by ELLINGER and BOYNE (1965) for casein and POIN and FAUCONNEAU (1966) for potato protein, wheat gluten and soya protein (Table 18). It is unlikely that any sample of the protein supplied would coincide with that cited in the literature. In fact, a wide scatter among individual published values from different laboratories has been demonstrated by EWART (1967). Nevertheless, TKACHUCK (1966a) found that amino acids were similar between major types of Canadian wheat.

The increases in most levels of free amino acids in the blood after eating the

TABLE 18. Amino acid composition of the proteins in mg/16gN and their recalculation in μmoles percentage of total essential amino acids.

	Casein[1]		Wheat gluten[2]		Potato protein[2]		Soya protein[2]	
	mg/16gN	μmoles %	mg/16gN	μmoles %	mg/16gN	μmoles %	mg/16gN	μmoles %
Threonine	4.4	11	2.6	9	4.1	11	4.0	10
Lysine	8.8	16	1.6	5	7.8	18	6.6	14
Valine	7.4	16	4.4	17	5.7	16	5.5	14
Leucine	10.0	20	7.0	23	7.1	18	7.9	18
Isoleucine	6.0	12	4.2	14	4.4	11	5.1	12
Total branched amino acids	23.4	48	15.6	54	17.2	45	18.5	44
Histidine	3.5	6	2.2	6	1.8	4	2.8	6
Phenylalanine	6.0	9	5.4	14	4.7	9	5.4	10
Methionine	3.3	6	1.4	4	1.3	3	1.3	3
Arginine	4.1	6	3.6	9	4.8	9	7.6	13
Total (EAA)	53.5	100	32.4	100	41.7	100	46.2	100
Tyrosine	6.6		3.5		2.6		4.0	
Glycine	2.2		3.2		4.0		4.4	
Serine	5.9		4.8		4.1		5.1	
Glycine + Serine	8.1		8.0		8.1		9.5	
Alanine	3.5		2.6		3.6		4.6	
Glutamic acid	23.6		37.6		10.7		18.6	
Total (NEAA)	41.8		51.7		25.0		36.7	
EAA/NEAA	1.3		0.6		1.7		1.3	

[1] From Ellinger and Boyne (1965).
[2] From Poin and Fauconneau (1966).

four proteins are in general accord with results of many investigators (RICHARDSON et al., 1953; DENTON and ELVEHJEM, 1954; LONGENECKER and HAUSE, 1959; GUGGENHEIM et al., 1960; MCLAUGHLAN et al., 1961; SWENDSEID et al., 1963). The generally higher increases in free amino acids in Cn_{10} than in others of Expt. 2 were due to the rather lower fasting levels or higher levels after feeding. Furthermore the rats were younger and the generally high essential amino acids in casein may also contribute.

Generally, levels of free amino acids in blood are controlled by the balance between their entry and exit from plasma. The remarkable decrease in one of the free essential amino acids in blood after a protein meal may refer to the limiting amino acids in proteins for growth. This accords with the assumption of LONGENECKER and HAUSE (1959) that essential amino acids are taken from the blood by tissues in proportion to the animal's needs. A true decrease must be more than experimental error. To consider a change in free amino acids after giving the protein as a marked decrease, it had to be more than double the coefficient of variation of the norleucine equivalent in standard runs of this amino acid (0.1 μmole/ml), except for methionine. For the latter, it was considered that it had to exceed fourfold its norleucine equivalent in the standard runs, because its peaks were relatively small on the recording paper. Table 19 represents the coefficients of variation of the amino acids and the values to be exceeded. These variations are in general agreement with the reproducibility of $\pm$ 3 to 7% reported by ALAM et al. (1966). Similarly, HAMILTON (1963) found that most ninhydrin-positive compounds can be estimated at 10^{-8} mole to within 5%. However, very large or small peaks of amino acids may result in a larger variation. The marked decreases in the free lysine levels in the blood of Gn_{12} and Gn_{16} after feeding wheat gluten probably indicate the well known fact that lysine is the first limiting amino acid in wheat gluten for growth (HOW and DOOLEY, 1963). Nevertheless, this was not observed in the later period in Gn_{20}. This may be partly because of adaptation. In addition that the rat's requirement for lysine as a percentage of protein decreases with age (ROA et al., 1959; and SMITH and JOHNSON, 1967). As free threonine, branched amino acids (valine, leucine

TABLE 19. Coefficients of variation (CV) of norleucine equivalent of standard runs of the amino acids in Experiment 2.

Amino acid	Coefficient of variation	Value to be exceeded[1]	Amino acids	Coefficient of variation	Value to be exceeded[1]
Threonine	4.6%	9.2%	Arginine	3.1%	6.2%
Lysine	4.5%	9.0%	Tyrosine	2.6%	5.2%
Valine	3.7%	7.4%	Taurine	8.6%	17.2%
Leucine	2.3%	4.6%	Glycine	4.4%	8.8%
Isoleucine	3.2%	6.4%	Serine	6.9%	13.8%
Histidine	2.4%	4.8%	Alanine	3.5%	7.0%
Phenylalanine	2.0%	4.0%	Glutamic acid	3.2%	6.4%
Methionine	2.0%	8.0%			

[1] Assumption in the discussion for marked change.

and isoleucine), phenylalanine and phenylalanine + tyrosine did not markedly decrease in the four groups after protein-feeding, these amino acids may not be seriously limited in the casein, potato protein, wheat gluten and soya protein, i.e., not the first limiting one. After protein-feeding, the markedly decreased levels of methionine in Pt_{11}, Sy_{13}, Sy_{17} and Sy_{21} probably show that the amount of sulphur amino acids is limiting in potato and soya proteins for the rat. In spite of the lower amount of sulphur amino acids in potato protein than in soya protein, Pt adapted themselves better to the methionine deficiency than Sy. This indicates that there are also other factors. These factors may be the rate of amino acid release during digestion, their availability, the proportion of the amino acids to each other in the protein in regard to the rat's requirements and interfering substances. Generally, the decreases in the sulphur amino acids could not easily be explained because of

a. the varied roles of methionine in intermediary metabolism
b. the sparing action between sulphur amino acids
c. the relatively large experimental error in regard to the other amino acids
d. a possible change in metabolic requirement either as such or as a methyl donor compound as suggested by SWENDSEID et al. (1968).

After protein-feeding, histidine and arginine levels decreased markedly in Pt_{11}, Gn_{12}, Sy_{13} and Cn_{14}. These two amino acids should not be limiting for growth in the four proteins. Such decreases are probably connected with metabolism interrelations rather than with a deficiency in histidine and arginine of the four proteins for growth. The decrease in arginine after protein-feeding may be partly due to its conversion in appreciable amounts in the intestinal cells to ornithine (FINCH and HIRD, 1960), to its involvement in the urea cycle and to its heavy contribution to the metabolic demands for non-essential N other than protein synthesis as such (KING, 1963). The decrease in histidine may also be influenced by a similar factor, because it is converted in appreciable amounts in the liver to carnosine which can be a source of histidine for organs (ELWYN, 1968). Although percentage glutamic acid in the proteins is the highest of the amino acids, its level decreased markedly in Pt_{11}, Gn_{12}, Sy_{13}, Sy_{21} and Cn_{14} after protein-feeding. Moreover, the percentage glutamic acid in the proteins fed in a suboptimal amount are more than that in the body proteins (POIN and FAUCONNEAU, 1966), except for potato proteins. These decreases may be partly due to

a. the lowest rate of absorption for glutamic acid among the amino acids (DELHUMEAU et al., 1962)
b. the transamination of glutamic acid with pyruvate in the intestine (FINCH and HIRD, 1960)
c. the large quantity of glutathione put out by the gut and liver which can serve for the transport of glutamic acid (ELWYN, 1968).

Glutamic acid is involved extensively in brain metabolism (QUASTEL and QUASTEL, 1961) and intestinal metabolism (SPENCER and KNOX, 1960). Generally, older animals adapted themselves better to this decrease. After protein-feeding, alanine decreased markedly in Pt_{11}, Sy_{13}, Cn_{14} and Cn_{18}. This may

be partly due to feeding suboptimal amount of N in relation to the rat's requirement. In addition the percentage of alanine in the proteins is lower than in the proteins of the rat's carcass (POIN and FAUCONNEAU, 1966). After eating casein, potato protein and soya protein, serine and glycine decreased markedly in some cases. In general, the percentage decrease in glycine levels was mostly more than in serine. This may be partly due to conversion of glycine to nucleic acid derivatives needed for growth and low conversion of threonine to glycine. In addition the amount of serine and especially glycine per 16 gN of the proteins fed in suboptimal amount was lower than that in the proteins of rat carcass (POIN and FAUCONNEAU, 1966). KING (1963) also showed that glycine in the rat's carcass is the highest of the amino acids. Generally, the decreases in non-essential amino acids in the blood are difficult to explain due to the nemerous factors affecting their levels, including the capacity of the body to synthesize them, and their interconvertibility. Nevertheless the decreases show that their rate of uptake in the cells was greater than their rate of input. This is important because non-essential amino acids may be regarded as physiologically essential and they exert sparing action on the non-essential amino acids. As indicated previously, factors decreasing their levels may include the suboptimal amount of N fed, and their lower rate of absorption in the intestine than for essential amino acids. Probably, rats make better use of non-essential amino acid at the later ages.

The decreases in free essential amino acid levels after protein-feeding were divided by the requirements of these amino acids for young rats reported by RAO et al. (1959). The results were arranged in a descending order in Table 20 to test whether they indicate the first or perhaps more limiting amino acids in the protein. The order in which an amino acid became limiting with respect to these results changed with time of sampling and age for the same protein. In general, the decreases in amino acid levels after feeding the protein was not completely successfull to predict the first limiting amino acid for growth during a relatively long period in Expt. 2. If the decreases in arginine and histidine (Table 20) were not considered, the first limiting amino acid would be methionine for Pt_{11}, Sy_{13}, Sy_{17} and Sy_{21} and lysine for Gn_{12} and Gn_{16}. These data show that decreases in the levels of free amino acids in blood as an indicator to the limiting amino acid in the protein must be interpreted with caution. The factors which probably interfered in Expt. 2 (and masked the unique amino acid composition of the given proteins) include the following:

a. availability of amino acids
b. the dynamic state of many body proteins which can contribute to the pool of free amino acids such as the admixture of the ingested protein with the endogenous protein in the intestinal tract (NASSET and JU, 1961; TWOMBLY and MEYER, 1961)
c. the amino acid metabolism of the absorbing cells
d. the special role of the liver in regulating amino acid metabolism
e. the comparative strength of the concentrative processes and the comparative rates of reactions yielding and utilizing the amino acids (CHRISTENSEN, 1963)

TABLE 20. Decreases in essential amino acid in relation to requirements by the growing rats[1] when male rats were fed on restricted amount of four proteins at different ages (Expt. 2).

Age approx. (week)	Protein fed	Sampling time	Descending order of the decrease[2]
10	casein	second	no decrease
10	casein	third	no decrease
		average	no decrease
14	casein	second	*arginine* and *histidine*
14	casein	third	*arginine, histidine* and phenylalanine
		average	*arginine, histidine* and phenylalanine
18	casein	second	arginine
18	casein	third	no decrease
		average	arginine
11	potato	second	*arginine, methionine, histidine* and valine
11	potato	third	no decrease
		average	*arginine, methionine* and *histidine*
19	potato	second	threonine and histidine
19	potato	third	no decrease
		average	no decrease
12	wheat gluten	second	*arginine, histidine* and *lysine*
12	wheat gluten	third	*lysine*
		average	*arginine* and lysine
16	wheat gluten	second	arginine
16	wheat gluten	third	*arginine, lysine* and histidine
		average	*arginine* and lysine
20	wheat gluten	second	no decrease
20	wheat gluten	third	no decrease
		average	no decrease
13	soya	second	*arginine, methionine, histidine, threonine* and lysine
13	soya	third	methionine
		average	*methionine*
17	soya	second	*methionine*
17	soya	third	*methionine*
		average	*methionine*
21	soya	second	*methionine*
21	soya	third	methionine
		average	*methionine*

[1] (Fasting level – the level after feeding the protein) / requirement of this amino acid for growing rats reported by Roa et al., (1959).
[2] Amino acids in italics decreased more than the value to be exceeded in Table 19.

f. hormonal effects (CHRISTENSEN, 1963)
g. the variation in fasting level which is assumed to approach steady-state levels of free amino acids in the blood.

After protein-feeding, the percentages of increase in tyrosine levels were the greatest of the amino acids. This may be partly due to the sufficient amount of phenylalanine + tyrosine in the four proteins, a rapid conversion of phenylala-

nine to tyrosine and a relatively slower metabolism rate of tyrosine. McMena-
my et al. (1962) showed that liver uptake of tyrosine was higher than liver me-
tabolism in the contrast to phenylalanine. The percentage increases in the
branched chain amino acids -isoleucine, leucine and valine- were among the
greatest of the amino acids, perhaps because they are among the most rapidly
absorbed amino acids (Delhumeau et al., 1962) and are slower metabolized by
the liver than other amino acids (Miller, 1962). The average percentage increas-
es (2 %) in glycine and glutamic acid were lowest of the amino acids after feeding
the proteins. This may be partly due to their low absorption rate, feeding sub-
optimal amount of N and their extensive involvement in the metabolism and
being the least absorbed amino acid by the intenstine (Robinson and Felber,
1964).

Surplus amino acids in natural proteins in relation to the requirements did
not usually result in distinctly higher levels of the free amino acids. This may
be partly so because the liver generally destroys faster the amino acids which are
provided in larger amounts in the proteins and the pathways of breakdown are
not easily saturated. Mostly, the direct relation between the levels of most in-
dividual free amino acids in the blood and their respective content in the pro-
teins was vague. Nevertheless, the data show that proteins affect amino acid
metabolism in some way.

The ratios of threonine to other essential amino acids, valine to leucine,
isoleucine or phenylalanine, leucine to phenylalanine, isoleucine to phenylala-
nine and histidine to phenylalanine were higher in blood than in the proteins.
Such data show that rats modify the ratio between essential amino acids of the
the proteins eaten in a tendency to maintain a relatively uniform mixture of
free amino acids in blood. This in general agrees with Guacci et al. (1963) and
Ganapathy and Nasset (1962). Comparison of the ratios of essential amino
acids in the four proteins with those subsequently found in the blood reveals
that essential amino acids are drawn from the blood at different rates. This may
be partly due to differences in the requirement for the synthesized proteins
or differences in degradation rates. It seemed that the differences in breakdown
play the main role with advancing age. Generally, as the animals grow older,
the differences in rates of degradation increase in importance. As threonine was
probably the lowest of the amino acids drawn from the essential ones, it had,
as expected, the highest levels between the free essential amino acids in the
blood.

Ratios of phenylalanine: tyrosine in the fasting levels were lower on feeding
192 mg N from superior protein (0.5–1.0) than from inferior protein. The avera-
ge of their ratios in Expt. 2 were 0.6, 0.9, 1.3 and 1.3 in Cn, Pt, Gn and Sy, re-
spectively. Furthermore, there was a tendency for this ratio to be related rough-
ly to the gains in liveweight between the four groups. When liveweight of the
rats did not differ significantly in Expt. 3, the ratio in Cn was similar to that in
Gn of the same sex and age. The average of ratios phenylalanine: tyrosine in
Expt. 3 was 1.2 in $Cn_4 ♀^A$; 0.6 in $Cn_{12}♂$ against 0.7 in $Gn_{12}♂$, 0.8 in $Cn_{12}♀$
against 0.8 in $Gn_{12}♀$; 0.8 in $Cn_{24}♂$ against 0.7 in $Gn_{24}♂$ and 0.9 in $Cn_{24}♀$ against

1.0 in Gn_{24}♀. The data are in general agreement with the result of WHITEHEAD and DEAN (1964), indicating a lower ratio in healthy children well fed compared to children with protein malnutrition. Nevertheless, it is clear that this ratio varied with sex, age, protein and individuality. There was a tendency for this ratio to be higher in females than in males and at 24 weeks of age than at 12 weeks in Expt. 3.

The increasing order of ratio glutamic acid: alanine in the proteins (3.0–14.5) is potato protein, soya protein, casein and wheat gluten, whereas the ratio in blood (0.4–1.7) was Gn, Cn, Sy and Pt. This shows that the ratio in proteins was not reflected in the free amino acids in blood. This conclusion is in general agreement with that of PERAINO and HARPER (1963).

At fasting in Expt. 2, average ratio of essential: non-essential amino acids was higher on feeding superior protein (0.8 in Cn or Pt) than that on feeding inferior protein (0.6 in Gn or Sy). This accords generally with the results of SWENDSEID et al. (1963) for the influence of casein and wheat gluten on this ratio. This ratio increased mostly after providing the four proteins. This may be partly because the non-essential amino acids are absorbed more slowly as a group than the essential ones (ADIDI et al., 1967). However, the difference in this ratio was not proportional to the difference in growth rate.

Free lysine levels in the blood before and after protein-feeding were the lowest in Gn among the four groups fed on a restricted amount of protein during Expt. 2. The average fasting level of free lysine in blood, the most expected limiting amino acid in the wheat gluten was also lower in Gn than in Cn of the same sex at 12 and 24 weeks on protein to appetite in Expt. 3. However, the ranges of free lysine levels overlapped in the two groups (Cn and Gn), except for male rats 12 weeks of age in Expt. 3. The overlapping ranges in two groups in Expt. 3 were concomitant with no significant difference in weights between Cn and Gn in Expt. 3 and vice versa. In general, levels of free lysine in blood slightly reflected the low content of lysine (mg/16N) in wheat gluten. This agrees with the low values in species such as chicken, dog, rat and man, when different proteins limiting in lysine were fed (RICHARDSON et al., 1953; DENTON and EL-VEHJEM, 1954; LONGENECKER and HAUSE, 1959; GRAY et al., 1960; MCLAUGH-LAN et al., 1961; MORRISON et al., 1961; ALBANASE and ORTO, 1963; LONGE-NECKER, 1963; HILL and OLSEN, 1963; SWENDSEID et al., 1963; MCLAUGHLAN, 1964). The relationship between low levels of free lysine in blood and its low content in wheat gluten was markedly due to the large difference in lysine content (g/16N) between wheat gluten and the other proteins, being among the highest essential amino acids in blood and probably the low masking effect of other factors such as availability and metabolism interrelations. Furthermore, MEISTER (1965) reported that lysine does not participitate appreciably in reversible transamination or deamination reactions (metabolicably inert) as compared to most of the other amino acids.

Free threonine levels in Gn were lowest of the four groups before and after feeding a restricted amount of protein in Expt. 2. This may be partly related to the low amount of threonine in wheat gluten among the four proteins. On casein

and wheat gluten to appetite in Expt. 3, the fasting levels of free threonine were markedly lower in Gn than in Cn of the same age (12 and 24 weeks old) and sex, in spite of the similar amount of threonine eaten from the two proteins. This indicates that metabolism interrelations are the main cause and probably related to the too low lysine content in wheat gluten. Fasting levels of free threonine in Pt were the highest of the four groups in Expt. 2. Furthermore, levels of free threonine in Pt after feeding-protein were mostly highest in Expt. 2. As casein contains more threonine than potato protein (POIN and FAUCON-NEAU, 1966), metabolic interrelations are probably the main cause. This probably shows that free threonine levels in the blood was affected more by the ratio of amino acids in the proteins than by threonine levels in the proteins. GRAY et al. (1960), MORRISON et al. (1961) and SANAHUJA and HARPER (1963) found also that plasma threonine levels were unrelated to the amount in the diet. However, threonine does not participate in the general exchange of amino acid nitrogen and fifth to a third of dietary threonine is cleaved to glycine and acetate (MEISTER, 1965). Before and after protein-feeding, levels of free valine, leucine, isoleucine, histidine, phenylalanine, methionine and arginine in the blood overlapped in the four groups of Expt. 2. This also applied to the fasting levels of rats fed on casein and wheat gluten to appetite in Expt. 3. This masked effect of these amino acids eaten on free amino acids in the blood can be caused by many factors including metabolic interrelations of amino acids, competitions for renal tubular reabsorption or other causes already discussed. Feeding on 192 mg N of potato protein and of wheat gluten which contain low and high amounts of glutamic acid, respectively, resulted in a reverse relation for free glutamic acid in blood, i.e., high and low levels in Pt and Gn, respectively. Furthermore levels of glutamic acid in Gn before and after protein-feeding were lowest of the four groups. This may be partly because glutamic acid as glutamine was more needed for degradations of amino acids of wheat gluten which were not used for protein synthesis than those in the potato protein. Although potato protein clearly contains less glutamic acid than the other proteins, the fasting levels of glutamic acid in Pt was the highest of the groups in Expt. 2. This shows also the importance of the metabolism interrelations in regard to the composition of the proteins. Fasting levels of free taurine in Gn were highest of the four groups in Expt. 2 due to a probably high conversion.

Although there are rough relationships between the levels of free amino acids in blood in some cases and their respective content in the protein source, it is clearly not accurately reflected in the fasting or fed levels and patterns of the free amino acids in blood.

Data of Expt. 3 suggest that an interaction of protein type, age and sex affects the metabolism of amino acids in some way. Levels of lysine, the first limiting amino acid in wheat gluten, were markedly higher in $Gn_{12}♀$ than in $Gn_{12}♂$. At 24 weeks of age, levels of threonine, the second limiting amino acid in wheat gluten (HOW and DOOLEY, 1963), in these rats were also markedly higher in females than in males. This may indicate that females adapt themselves better to deficiency of lysine and threonine in wheat gluten than males.

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Differences in average levels of individual free amino acids between males and females changed the trend (positive or negative) either by advancing age or feeding other protein, except for arginine, tyrosine and alanine. The markedly higher levels of most amino acids in $Cn\male$ than in $Cn\female$ at 12 weeks of age overlapped in the two sexes or were reversed at 24 weeks of age. This is because most of the amino acids in $Cn_{12}\female$ increased in the last 12 weeks of Expt. 3, whereas they decreased in males. In consequence, the average of amino acids in Cn_{12} showed the same effect, except for arginine, taurine and glycine. These exceptions are also interesting, because the pattern of arginine (HILL and OLSEN, 1963), cystine (SWENDSEID et al., 1963) and glycine in casein is probably lower than requirements. In Gn_{12}, males had higher average levels of free amino acids in the blood than females, except for lysine (markedly), leucine, phenylalanine, arginine, methionine, total essential amino acids, taurine and serine. At 24 weeks of age, the exceptions were lysine, threonine (markedly), methionine, arginine and total essential amino acids. The results probably indicate that metabolism of amino acids is affected by sex in some way. This in general agrees with the conclusion of OEPEN and OEPEN (1965) of the existence of sex-specific differences in the concentration of serum amino acids. In addition, age and protein types can enhance or mask the sexual effect with other factors.

With age, most free amino acid levels in blood tended to decrease during Expt. 2 (10–21 weeks old) in male rats fed on a restricted amount of casein, potato protein, wheat gluten and soya protein. This tendency was also confirmed in Expt. 3, when the rats ate casein and wheat gluten to appetite and blood samples were taken from the same rats at 12 and 24 weeks of age. Furthermore, the ratio of protein consumed to appetite to liveweight also decreased from 12 to 24 weeks of age in Trial 3 as in Trial 2. Before 12 weeks of age in Trial 3, most levels of amino acids generally increased from birth to weaning at 4 weeks of age and from the beginning to Week 8 of Expt. 3 (4–12 weeks of age as shown in $Cn_{12}\male$, $Gn_{12}\male$, $Cn_{12}\female$ and $Gn_{12}\female$ and after this age decreased from Week 8 to 20 of Expt. 3 (12–24 weeks old) except for $Cn_{20}\female$. In general, these decreases suggest that growing animals tend to maintain high levels of circulating free amino acids in blood which have a curve-shape with advancing age. These highly homoeostatic levels decreased as growth begins to cease and they indicate probably a kind of ageing processes. This decrease in general agrees with the statement of ALBANASE (1959) that children have higher fasting levels of plasma amino nitrogen than adults. With advancing age, some levels of amino acids in a pooled sample (replicate) dit not change unlike most amino acids. Such reverse change in a replicate at two ages is considered marked, if it exceedes the values in Table 19. Similarly, levels at two ages which do not overlap with the levels compared in other rats are also considered marked. These markedly reverse trends with age were as follows:

a. Decrease in histidine level from birth to weaning (Trial 3).
b. Decrease in levels of lysine, and methionine in Gn, glycine + serine in $Cn\female$, and alanine in $Cn\female$ and in $Gn\male$ from weaning to 12 weeks old (Expt. 3).
c. Decrease in level of taurine in $Cn\female^{B}$ and increase in the level of threonine in

Gn♀[B] and of lysine in Gn♂[A] and in Gn♀[B] from 12 to 24 weeks of age (Expt. 3).
e. Decrease in methionine and taurine in Cn♂ and increase in lysine and threo-
nine levels in Gn♀ from weaning to the end of Expt. 3.

The marked decrease in histidine from birth to weaning may be due to con-
version of histidine to other compounds such as carnosine and β-alanyl- histi-
dine. The marked decrease in lysine in Gn♂ and others at 12 weeks of age in
Expt. 3 can be related partly to lysine deficiency in wheat gluten and the lower
amount of wheat gluten eaten per g gain in liveweight of males (1.69) compared
with females (1.96). Since, it is well known that wheat gluten is not deficient in
methionine, the marked decrease of methionine levels in Gn_{12}♂ may be related
to the marked increase in taurine levels. The marked decrease in alanine levels
of females in Week 8 of Expt. 3 (Cn_{12}♀ and Gn_{12}♀) suggest a sexual difference in
the metabolism of amino acids. This is also observed in primary results during
the recent investigation. Serine level was added to glycine level and similarly
tyrosine to phenylalanine, because of interconversion of glycine and serine and
the conversion of phenylalanine to tyrosine are metabolic phenomena of con-
siderable significance in mammalian tissue. The marked decrease in glycine +
serine level in Cn_{12}♀ was mainly due to decrease in glycine level. This may be
partly due to glycine more required in growth to form components such as nuc-
leic acid derivatives and to the lower amount of glycine eaten to appetite from
casein (SWENDSEID et al., 1963) than from wheat gluten. The marked decrease in
taurine in Cn_{24}♀[B] may be due to the deficiency of cystine in casein and the ten-
dency of the animal to maintain methionine higher. The marked increase in the
level of threonine in Gn_{24}♀ may be an adaptation to deficiency of threonine in
wheat gluten. The marked increase in lysine in Gn_{24}♀[A], Gn_{24}♂[A] and Gn_{24}♂[B]
may be also a similar adaptation or due to the decreased requirement of lysine.
The amino acid levels at weaning were never lower than the lower limits of
ranges in Cn_{24}♂ and Cn_{24}♀ in Expt. 3, except for taurine in Cn_{24}♂, whereas 7
amino acids out of 14 determined had a reverse trend in Gn_{24}♂ and Gn_{24}♀. This
low level of taurine may be partly due to the deficiency of cystine in casein and
consequently the formation of taurine. The lower seven amino acids cannot be
related to their deficiency in wheat gluten, because the amount of most of them
which was eaten to appetite was more in wheat gluten than in casein. Further-
more, the levels of limiting amino acids, lysine and threonine, in wheat gluten
were higher in Gn_{24}♀ than the value of the rats at weaning (Cn_4♀[A]). This means
that metabolic factors masked the composition of the proteins. The marked
exceptions which were found in some cases with advancing age in Expt. 3 may
indicate in general an adaptation in the metabolism for correcting deficiency and
excesses of amino acids in the protein. Furthermore, the inconsistency of the
result also may be partly due to the degree of adaptation.

In general, wheat gluten tended to reduce average levels of most amino acids
in blood, when the protein was supplied in restricted amount or to appetite.
This was more in 12 weeks old male rats in Expt. 3 than in females and the rever-
se later (24 weeks old).

Generally, fasting levels of free amino acids in blood did not relate well with

increased protein intake. This in general agrees with those found by ISOBE et al. (1964) in man. Nevertheless, the too high intake of the inferior protein (wheat gluten) in Expt. 3 compared with Expt. 2 for a longish period was reflected slightly by an increase in some levels. Level of lysine, threonine, isoleucine, phenylalanine, tyrosine, taurine or serine was lower in fasting Gn_{12} in Expt. 2 than in replicates of Gn_{12} in Expt. 3. However, levels of methionine showed a reversed trend and the levels of the other six amino acids overlapped in rats with free access or restricted intake of wheat gluten. Similarly, fasting levels of amino acids in $Gn_{20}\male$ in Expt. 2 compared to those in Gn_{24} in Expt. 3 led to the same conclusion. On the other hand, fasting levels of amino acid in Cn in Expt. 2 and 3 reveal contradictory tendencies due to the high levels in Cn_{14} of Expt. 2.

The ratio of threonine to lysine in the female rat after parturition was less than one (on average 0.8), whereas this ratio in the virgin female rat was more than one, except for $Gn_{12}\female^B$ which was one. This difference may be due to pregnancy. It is well known that metabolic balance of nutrients in some aspects is changed in pregnancy. Nevertheless, it is also probably due to the difference in feeding. The pregnant rats were allowed free access to protein the whole day, whereas non-pregnant rats were fed on protein for one hour daily. This may elevate threonine levels in the blood of protein-starved rats more than lysine levels. The threonine is particularly resistant to deamination and accumulates in blood, when amounts of amino acids are released through tissue breakdown during protein starvation as reported by CHARKEY et al. (1953).

Generally, most amino acid levels in blood of female rats post partum were lower than those of non-pregnant rats. This in general agrees with the conclusion of SOUPART (1959), OEPEN and OEPEN (1965) and BJÖRNESJÖ (1968). The tendency for low values in pregnancy may be partly due to failure to compensate for the normal gestational increase in plasma volume (CATON et al., 1949), hyperamino-acidurea (WALLRAFF et al., 1950) alteration in nitrogen metabolism (MACY and HUNSCHER 1934), active transport of amino acids through the placenta against a concentration gradient and the increased cellular uptake of amino acids (BJÖRNESJÖ, 1968) in response to pregnancy.

Most free essential amino acids in blood were higher in the newborn rats than in their mothers. This in general accords with the results obtained in guinea-pig and rabbit (CHRISTENSEN and STREICHER, 1948) in man (BJÖRNESJÖ, 1968) and in sheep (HUGGETT and SLATER, 1966) indicating generally greater but occasionally lower levels of individual free amino acids. The difference in the levels of free amino acid of the newborn rats and their mothers is caused by many factors. Such factors may include the previous effect of pregnancy, the selective concentration capacity of the placenta (HAGERMAN and VILLEE, 1960) and difference in the enzymic activity between newborn rats and their mothers (MATHEWS and PARTINGTON, 1967). MORROW et al. (1967) showed that liver homogenates from foetal rats had less catabolic activity (per mg N per hour) for free hydroxyproline and proline than from adult rats.

To solve a problem is to create new problems; new knowledge immediatelly reveals new areas of ignorance.

6.4. CONCLUSIONS

(Free amino acids in blood)

In most instances, higher levels of free amino acids were recorded after eating proteins than during fasting, i.e., on the protein-free diet. Generally, the percentage increases in tyrosine or the branched amino acids (isoleucine, leucine and valine) were highest of amino acids, whereas those of glycine and glutamic acid were lowest.

Detection of limiting amino acid(s) in the proteins -casein, potato protein, wheat gluten and soya protein-from the levels of the free amino acids in blood before and after eating proteins was not completely successful. Free arginine and histidine levels decreased to a great extent in some instances after eating casein, potato protein, wheat gluten and soya protein in spite of being probably, not the most limiting amino acids in these four proteins. This probably indicates metabolism interrelations. Care must be taken, therefore, in the interpretation of levels of free amino acids in the blood as an index for limiting amino acids, i.e,, they may not reflect the exact limiting order of the dietary amino acids Nevertheless, the data show that wheat gluten is deficient in lysine and soya protein is more deficient than potato protein in methionine.

Markedly, fasting levels of free threonine and glutamic acid in blood of $Pt\male$ and taurine in $Gn\male$ were the highest values of the four groups on feeding 192 N in Expt. 2. Moreover, levels of free threonine, lysine and glutamic acid in blood of $Gn\male$ before and after feeding on the proteins were the lowest values. When fed on casein and wheat gluten to appetite (Expt. 3), free lysine and threonine were mostly lower in blood of male and female Gn than in Cn. Although there were sometimes rough relationships between the levels of free amino acids in blood and their contents in dietary proteins, rats modify the ratio between essential amino acids of dietary proteins to maintain almost constant ratio between free essential amino acids in blood. Generally, variations in the levels of free essential amino acids in blood due to feeding, ages, individuals or amount of protein were reduced on expressing them in percentages of the total essential amino acids.

At 12 weeks of age in Expt. 3, levels of lysine were markedly higher in blood of $Gn\female$ than of $Gn\male$. At 24 weeks of age, level of free threonine were also markedly higher in blood of $Gn\female$ than of $Gn\male$. This suggests that females adapt themselves better for the deficiency of lysine and threonine in wheat gluten than males.

With age, most free amino acids in the blood, though not the same, increased from birth to weaning at about 4 weeks old, from beginning to Week 8 of Expt. 3 (4–12 weeks old) as shown in $Cn_{12}\male$, $Gn_{12}\male$, $Cn_{12}\female$ and $Gn_{12}\female$, then decreased from Week 8 to 20 of Expt. 3 (12–24 weeks old) except for $Cn_{24}\female$. Furthermore, most free amino acids in blood of male rats also decreased in many cases in Expt. 2 with age from 10–21 weeks, in spite of the difference in the amount and type of the protein in Expt. 2 and Expt. 3. It is noteworthy that a markedly reversed trend (decrease) was found in level of free histidine from birth to weaning. Furthermore, levels of free lysine in blood of rats on deficient diet in

lysine increased with age, whereas most free essential amino acids decreased. Genereally, intake of wheat gluten compared to casein tended to reduce the average levels of most free individual amino acids in the blood, when restricted or fed to appetite. Most fasting levels of free amino acids in the blood of Gn were lower on restricted amount of wheat gluten (Expt. 2) than on free access to the protein (Expt. 3).

Most essential amino acid in the blood of newborn rats were higher than their mothers. Furthermore, rats post partum had lower levels of free amino acids in the blood than virgin rats.

Data of the experiments show that alteration in levels of free amino acids in blood is affected by the interaction of protein type, age and sex in addition to other factors such as pregnancy and metabolic interrelationships.

7. GENERAL DISCUSSION AND CONCLUSIONS

The liveweight, feed intake, body composition and free amino acids in blood indicates that significant differences resulted from protein types in the experiments. The effect of protein on animals is a parameter of the nutritive value of dietary proteins. Such experiments with growing animals and tests on the whole animal are most common. The weight of rats under the experimental conditions classified the proteins into two major categories: superior proteins and inferior ones. Superior proteins were casein and the third potato protein and inferior proteins wheat gluten or soya protein. The liveweight of rats given equal a-mounts of protein ranked them in the same order as the classical test of biological value. Nevertheless, this criterion ranked the casein and wheat gluten in the same order on feeding of the two proteins to appetite after a long-term experiment (Expt. 3). Correcting for an equal protein intake on the free access to protein also ranked these two proteins in the expected order. Energy intake and liveweight ranked the proteins in the same order. However, recording feed intake is more laborious than recording liveweight. The nitrogen content of the rats ranked the proteins in the expected order as the parameters liveweight and feed intake, but it is of course a more direct index of protein quality than the other indices. Determination of nitrogen, however, is more time-consuming and thus expensive than weighing. Indeed the nutritive value of dietary proteins in the experiments correlated nicely with the three criteria of gain in liveweight, energy intake and gain in body nitrogen. The determinations of nutritive value were complicated in the long-term experiments regardless of the criteria used since the difference between animals changed widely in most cases. The gain in liveweight, feed intake and nitrogen as indices sum up changes in individual body tissues and they suffer from the experimental error and difficulties which are inherent in these determinations. The free amino acids in blood did not rank the proteins fed in the expected order. They are not a perfect screening method for protein status of a population under the experimental conditions. Various factors affect the concentration of free amino acids in blood. Their levels are, however, sometimes very useful and provide a better understanding of protein nutrition.

In the three experiments, there was a regular relationship between rate of growth and feed intake. The relationship between weight gain and nitrogen intake is curvilinear, possibly semilogarithmic but essentially linear at low nitrogen intakes (ALLISON, 1964). The nitrogen intake required for growth varied with dietary protein types, sex and age. To reach about twice the initial liveweight (75 g) in Expt. 2, rats on casein, potato protein, wheat gluten and soya protein ate about 7, 7, 16, 16 g of nitrogen, respectively. The amounts of total nitrogen which resulted in a 5.5-fold increase in weight of males (55 g) at the end of Expt. 3 were 40 and 65 g from casein and wheat gluten, respectively. In Expt. 3, females consumed freely 27 and 37 g N from casein and wheat gluten to gain less than half the total weight gained by males.

From logical point of view, poor intake means poor diet. Nevertheless, feed intake by itself tells mostly nothing about the nature of inferiority or superiority of proteins. Weight gain can be related to the protein intake as an index of the nutritive value of proteins, namely the protein or nitrogen efficiency ratio. This index can be defined as the liveweight gain relative to a unit weight (gramme) of protein or nitrogen consumed. The data in the three experiments show clearly that the higher nutritive value of protein (casein and third potato protein against the wheat gluten and soya protein), the greater the protein or nitrogen efficiency ratio. These ratios decreased in general with advancing age since the gain in liveweight diminished and the protein intake increased. The changes in the protein or nitrogen efficiency ratio with age or different nitrogen intakes of the same protein were greater for the superior protein than for inferior protein. The value of protein or nitrogen efficiency ratio was not characteristic of a protein and varied under different conditions. Such ratios are not always additive for mixed proteins and they may differ between species and with physiological state. Therefore, these values in the three experiments must be interpreted cautiously due to differences in procedures.

The total gain in liveweight in Expt. 1 was equivalent to retention of 3.6 and 1.3 g of nitrogen from casein and wheat gluten, respectively. This means that about 25% and 9% of the same nitrogen intake of casein and wheat gluten were retained in the rat for growth during the 12 weeks of Expt. 1. Similarly, 25% and 21%, 11% and 12% of the same nitrogen intake of casein, potato protein, wheat gluten and soya protein, respectively, were retained by the rats for growth during the 16 weeks of Expt. 2. During the 20 weeks of Expt. 3, 20% and 14% of the free nitrogen casein were retained in males and females, respectively, for growth, whereas nitrogen gained from wheat gluten were 12% and 10% for males and females, respectively. The data show that nitrogen retention is less in females than in males and higher for superior protein than inferior one. The difference between total nitrogen intake and gain in body nitrogen could represent the nitrogen needed from the different protein types for maintenance together with some loss through excretion. This reveals that superior protein is also better in nutritive value for maintenance than inferior one. Furthermore, the females need proportionally more of total protein for maintenance (per gramme liveweight) than males.

Gain in liveweight generally correlated well with gain in body nitrogen. However, the differences of actual nitrogen gained between proteins used were larger than those calculated from gain in liveweight only. This is mainly because the nitrogen percentages of the gains in liveweight are not exactly the same.

The units in which results are expressed vary with the parameter. Hence for all, casein is made 100 to facilitate comparison (Table 21). Table 21 shows that the criteria of nitrogen gain on restricted protein resulted generally in a larger difference between proteins than the other criteria (energy intake and weight gain), whereas the three criteria were similar with free access to protein.

Free amino acids levels in blood under the experimental condition were not a parameter of overall biologic value and probably indicate specific functions.

TABLE 21. Total of weight gains, energy intakes and nitrogen gains compared with those of rats on casein as 100.

Expt.	Protein feeding[1]	Criterion	Casein group (Cn) ♂	Casein group (Cn) ♀	Potato group (Pt) ♂	Wheat-gluten group (Gn) ♂	Wheat-gluten group (Gn) ♀	Soya group (Sy) ♂
1	restricted[2]	energy intake	100	–	–	88	–	–
1	restricted	weight gain	100	–	–	53	–	–
1	restricted	nitrogen gain	100	–	–	36	–	–
2	restricted	energy intake	100	–	98	67	–	77
2	restricted	weight gain	100	–	94	49	–	49
2	restricted	nitrogen gain	100	–	86	44	–	47
3	to appetite	energy intake	100	100	–	93	98	–
3	to appetite	weight gain	100	100	–	96	97	–
3	to appetite	nitrogen gain	100	100	–	97	98	–

[1] For an hour daily.
[2] 192 mg N daily.

The data show that it is very difficult to express the nutritive values in a single universally applicable figure for each protein. Results in one experiment may not necessarily allow deductions to be made for the other experiment. No single criterion of the nutritive value of protein with one method of feed assignment is superior to all others.

Rats had great recuperative power, provided the adverse conditions are not carried too far or continued too long. On feeding inferior protein to appetite, the rats slowed up in growth until the genetical growth curve is reached or approached once more, and subsequently followed. Female rats adapted themselves better to inferior protein than males perhaps because the two x-chromosomes provide better regulatory forces than one x and the small y chromosome.

In general, it is not practical to use changes in concentrations of free amino acids in the systemic blood as a measure of protein quality. This in general accords with the finding of GOLDBERG and GUGGENHEIM (1962). That an abnormally low level of a particular amino acid in the plasma indicates a deficiency of that amino acid in dietary protein (LONGENECKER and HAUSE, 1959; RICHARSON et al., 1953; ALMQUIST, 1954), is sometimes not correct. This agrees with the conclusion of CHRISTENSEIN (1963) and MORRISON et al. (1961). Alteration in the levels of free amino acids after feeding was not completely successful index of the order of limiting amino acids in the proteins. Fasting levels for amino acids probably did not reach a static minimum. However, the base line must to some degree level or stabilize amino acid nutrition and approximates to steady states between entry and exit in the blood. To explain that every amino acid follows a particular pathway is not practical and profitable. Free amino acids in the blood tend towards uniformity and stability in normal body states. The changes in free amino acids suggested the existence of homoeostatic mechanisms through several types of control phenomena. Increases in free amino acids in the blood did not parallel the amino acid composition of the diet and this is in

accordance with FRAME (1958). The relation between amino acid composition of proteins and free amino acids in blood is not direct or simple. The studies show interrelations of quality, amount of protein, sex, and age with levels of free amino acids in blood. These levels sometimes responded to an inadequate intake of the corresponding amino acids in the proteins. Growth and free amino acid levels do not correlate well, as MCLAUGHLAN (1964) also concluded.

8. SUMMARY

8.1. ENGLISH

Three experiments were carried out to obtain more knowledge about the use of feeding protein diet separately and protein-free diet to estimate the biological value of proteins.

Proteins were given in three experiments for one hour daily and free access to protein-free diet for the following 23-hour. In Expt. 1, casein and wheat gluten were eaten daily in an amount of 192 mg nitrogen (N) per male rat for 12 weeks after a training period. In Expt. 2, these two proteins in addition to potato protein and soya protein were given daily in an amount of 192 mg N per male rat for 16 weeks after a training period. In Expt. 3, casein and wheat gluten were given to appetite for three replicates of weanling male or female rats for 20 weeks. Liveweight, feed intake and carcass analysis of water, fat and N were recorded in the three experiments. Levels of free amino acids in blood (FAA) before and after the protein meal were estimated at different intervals in Expt. 2, and fasting levels (before giving the protein) of the same rats at different ages were determined in Expt. 3. Furthermore, FAA of some newborn rats and their mothers (Trial 3) on the day of birth were estimated.

As judged from the effect of the protein types on different parameters, casein and the third potato protein ranked alike in their nutritive value. They were superior in their nutritive value to wheat gluten and soya protein (heated). The wheat gluten was inferior in nutritive value to the soya protein in a relatively short experimental period, but similar in a relatively long experimental period.

On an equal amount of N, rats on inferior proteins increased in weight more slowly than rats on superior proteins. Prolongation of the experiment with restricted protein resulted in a similar total gain in weight, when the difference in the protein quality was not too large (wheat gluten and soya protein). Furthermore, rats on proteins with large difference in quality also gained similar total liveweight, when the proteins (casein and wheat gluten) were offered to appetite for a relatively long experiment (Expt. 3).

The ratio of energy to protein was higher on giving superior protein to appetite or in restricted amount than on inferior protein. When the protein was to appetite, rats seemed to adapt themselves to the deficiency of amino acids (AA) in wheat gluten as judged from the similar liveweight of the casein and wheat-gluten group of the same sex in Expt. 3 by increasing the protein intake.

On restricted protein, the percentage and quantities of the component of the rats' bodies determined did not differ significantly between casein and potato groups and between wheat-gluten and soya group, whereas there were significant differences between the casein or potato group and wheat-gluten or soya group. These significant differences indicate that superior protein can increase rapidly not only quantities of body components but also some of their proportions. The ratio of N to water and N% are slightly, though significantly, affected by the quality of protein in a restricted amount over a relatively long period

(Expt. 1 and 2). However this ratio and percentage varied generally in relatively narrow limits in the three experiments. Although there were no significant differences between the weights of the casein and wheat-gluten group of the same sex at the end of Expt. 3, significant differences between their body constituents were found in some cases but not between the amount or percentage N. In general, age influence was confirmed to be a decrease in water percentage and an increase in nitrogen percentage, in fat percentage and in the quantities of the body components.

In most instances, higher levels of FAA were recorded after eating proteins than during fasting on the protein-free diet. Generally, the percentage increases in tyrosine or the branched amino acids (isoleucine, leucine and valine) were highest of FAA, whereas those of glycine and glutamic acid were lowest.

Detection of limiting amino acid(s) in the protein – casein, potato protein, wheat gluten and soya protein – from the levels of FAA before and after eating proteins was not completely successful. Free arginine and histidine levels decreased to a great extent in some instances after eating casein, potato protein, wheat gluten and soya protein in spite of being probably, not the most limiting AA in these four proteins. This probably indicates metabolism interrelations. Care must be taken, therefore, in the interpretation of levels of FAA as an index for limiting AA, i.e., they may not reflect the exact limiting order of the dietary AA. Nevertheless, the data show that wheat gluten is deficient in lysine and soya protein is more deficient than potato protein in methionine.

Markedly, fasting levels of free threonine and glutamic acid in blood of potato group and taurine in blood of wheat-gluten group were the highest values of the four groups on feeding 192 N in Expt. 2. Moreover, levels of free threonine, lysine and glutamic acid in blood of wheat-gluten group before and after feeding on the proteins were the lowest values. When fed on casein and wheat gluten to appetite (Expt. 3), free lysine and threonine were mostly lower in blood of males and females of wheat-gluten group than of casein group. Although there were sometimes rough relationships between the levels of FAA and AA contents in dietary proteins, rats modify the ratio between essential AA of dietary proteins to maintain almost constant ratio between essential FAA. Generally, variations in the levels of essential FAA due to feeding, ages, individuals or amount of protein were reduced on expressing them in percentages of the total essential amino acids.

At 12 weeks of age in Expt. 3, free lysine levels in blood of wheat-gluten group were markedly higher in females than in males. At 24 weeks of age, levels of free threonine in blood of wheat-gluten group were also markedly higher in females than in males. This suggests that females adapt themselves better for the deficiency of lysine and threonine in wheat-gluten diet than males.

With age, most FAA, though not the same, increased from birth to weaning at about 4 weeks old, from beginning to Week 8 of Expt. 3 (4–12 weeks old) as shown in males and females of casein and wheat-gluten group at 12 weeks of age, then decreased from Week 8 to 20 of Expt. 3 (12–24 weeks old) except for females of casein group at 24 weeks of age. Furthermore, most FAA of

male rats also decreased in many cases in Expt. 2 with age from 10–21 weeks, in spite of the difference in the amount and type of the protein in Expt. 2 and Expt. 3. It is noteworthy that a markedly reversed trend (decrease) was found in level of free histidine from birth to weaning. Furthermore, levels of free lysine in blood of rats on deficient diet in lysine increased with age, whereas most free essential FAA decreased. Generally, intake of wheat gluten compared to casein tended to reduce the average levels of most individual FAA, when restricted or fed to appetite. Most fasting levels of FAA of wheat-gluten group were lower on restricted amount of gluten (Expt. 2) than on free access to the protein (Expt. 3).

Most essential FAA of newborn rats were higher than their mothers. Furthermore, rats post partum had a lower level of FAA than virgin rats.

Data of the experiments show that alteration in levels of FAA is affected by the interaction of protein type, age and sex in addition to other factors such as pregnancy and metabolic interrelationships.

· 8.2. SAMENVATTING

Om de voedingswaarde te bepalen van verschillende soorten eiwitten werden drie uitvoerige experimenten met albinoratten uitgevoerd, waarbij de eiwitten en het eiwitvrije voer afzonderlijk verstrekt werden.

In deze experimenten werden de eiwitten elke dag gedurende 1 uur verstrekt; de overige 23 uur hadden de dieren ad libitum de beschikking over het eiwitvrije voer. In experiment 1 werden, na een trainingsperiode, caseïne en tarwegluten aan mannelijke ratten gedurende 12 weken dagelijks gegeven in een hoeveelheid van 192 mg stikstof per dag. In experiment 2 werden, eveneens na een trainings-periode, caseïne, (verhit) tarwegluten, (verhit) aardappeleiwit en (verhit) soja-eiwit dagelijks gegeven aan mannelijke ratten gedurende 15 weken in een hoe-veelheid van 192 mg stikstof per dag. In experiment 3 werden gedurende 20 weken caseïne en tarwegluten ad libitum gegeven aan drie groepen gespeende mannelijke en vrouwelijke ratten. In de drie experimenten werden de voedsel-consumpties, de lichaamsgewichten, en de water-, vet- en stikstofgehaltes van de dieren bepaald. In experiment 2 werden met verschillende tussenpozen de vrije aminozuren in het bloed vóór en nà de eiwittoediening op verschillende leeftijden van de dieren bepaald. Voorts werden in experiment 3 de gehaltes van vrije aminozuren in het bloed van sommige pasgeboren ratten en van hun moe-ders op de dag van de geboorte bepaald.

Volgens de gestelde criteria (groei, voedselconsumptie en lichaamscomponen-ten) bleek dat caseïne en aardappeleiwit (van de derde partij) een gelijke voe-dingswaarde hadden. Hun voedingswaarde was groter dan die van tarwegluten en sojaeiwit. De voedingswaarde van tarwegluten was kleiner dan die van soja-eiwit in een betrekkelijk korte proefperiode, maar gelijk in een langere proef-periode.

Bij een gelijke stikstofopname groeiden ratten op de eiwitten van hogere biologische waarde aanvankelijk sneller dan op eiwitten van lagere waarde. Deze groeiverschillen verdwenen in de loop van de tijd, mits het verschil in biologische waarde niet te groot was zoals bij tarwegluten en sojaeiwit. Bij ad libitum-toediening van eiwitten met grote kwaliteitsverschillen (caseïne en tar-wegluten) over een betrekkelijk lange periode werd geen significant verschil in lichaamsgewicht waargenomen (experiment 2).

De verhouding energie:eiwit, zowel bij ad libitum- als bij beperkte eiwit-voedering, was groter wanneer de dieren eiwit van hogere biologische waarde kregen dan wanneer zij eiwit van lagere waarde ontvingen.

Wanneer het eiwit ad libitum verstrekt werd, konden de ratten zich aanpassen aan het aminozuurtekort in tarwegluten door hun eiwitopname te vergroten; dit blijkt uit de gelijke gemiddelde lichaamsgewichten van de dieren van hetzelf-de geslacht op caseïne en tarwegluten in experiment 3.

Bij beperkte eiwitvoedering waren er tussen de groepen op caseïne en aard-appeleiwit- en tussen de groepen op tarwegluten en sojaeiwit geen significante verschillen in hoeveelheden en gehaltes aan water, vet en stikstof van de karkas-

sen. De verschillen tussen de caseïne (of aardappeleiwit)-groep en de tarweglu-
ten (of sojaeiwit)-groep waren echter significant. Deze verschillen wijzen erop
dat eiwit van goede kwaliteit niet alleen een snelle toeneming van de hoeveelhe-
den van deze lichaamscomponenten kan veroorzaken, maar ook van enkele van
hun verhoudingen. De verhouding stikstof : water en het stikstofpercentage wer-
den significant beïnvloed door de kwaliteit van het eiwit, wanneer dit eiwit in be-
perkte hoeveelheid verstrekt werd gedurende een betrekkelijk lange periode
(experimenten 1 en 2).

Deze verhouding en dit percentage varieerden in de drie experimenten in het
algemeen binnen nauwe grenzen. Hoewel er geen significante verschillen in ge-
wicht waren tussen de groepen op caseïne en tarwegluten van hetzelfde geslacht
aan het einde van experiment 3, waren er in enkele gevallen wel significante
verschillen in hun lichaamssamenstelling, maar niet in stikstofgehalte of hoe-
veelheid. Bij toenemende leeftijd werd een afneming in het percentage water,
een toeneming in de percentages stikstof en vet en ook een toeneming van de
hoeveelheden van deze componenten waargenomen.

In de meeste gevallen werden na het eten van eiwitten hogere gehaltes aan
vrije aminozuren waargenomen dan gedurende de periode waarin de ratten
slechts eiwitvrij voer kregen. In het algemeen waren de verhogingen van de
percentages tyrosine of van de aminozuren met vertakte ketens (isoleucine, leu-
cine en valine) het hoogst en die van glycine en glutaminezuur het laagst.

Vaststelling van het beperkende aminozuur of aminozuren in de gevoerde
eiwitten (caseïne, aardappeleiwit, tarwegluten en sojaeiwit) door een vergelijk
van de gehaltes van de vrije aminozuren in het bloed vóór en nà het eten van de
eiwitten is niet mogelijk gebleken. In sommige gevallen daalden de gehaltes aan
vrij arginine en histidine aanzienlijk na het eten van caseïne, aardappeleiwit,
tarwegluten en sojaeiwit, ondanks het feit dat deze aminozuren niet de meest
beperkende aminozuren van deze eiwitsoorten zijn. Dit wijst waarschijnlijk op
een onderling verband in de stofwisseling. Er is daarom reden tot voorzichtig-
heid bij de interpretatie van de gehaltes aan vrije aminozuren in het bloed als
aanwijzing van beperkende aminozuren. De gegevens tonen echter aan dat
tarwegluten een tekort heeft aan lysine. Sojaeiwit heeft een groter gebrek aan
methionine dan aardappeleiwit.

Op eiwitvrij voer waren de gehaltes aan vrije threonine en glutaminezuur in
het bloed van mannelijke ratten van de aardappeleiwitgroep, en de taurinege-
haltes in het bloed van mannelijke dieren van de tarweglutengroep de hoogste
van de vier groepen, die dagelijks 192 mg stikstof kregen in experiment 2. De
gehaltes aan vrij threonine, lysine en glutaminezuur in het bloed van manne-
lijke ratten van de tarweglutengroep waren voor en na toediening van de eiwitten
het laagst. Wanneer de eiwitten ad libitum verstrekt werden (experiment 3),
waren de gehaltes aan vrije lysine en threonine in het bloed van mannelijke en
vrouwelijke ratten op tarwegluten lager dan bij die op caseïne.

Hoewel er soms enige overeenkomst was tussen de gehaltes aan vrije amino-
zuren en de aminozuurgehaltes van de verstrekte eiwitten, passen ratten de
verhouding tussen essentiële aminozuren van de toegediende eiwitten aan om

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een vrijwel constante verhouding tussen de aminozuren in het bloed te handhaven.

In het algemeen werden variaties in de gehaltes aan essentiële aminozuren tengevolge van de voeding, leeftijd, individuele verschillen of de hoeveelheid verstrekt eiwit gereduceerd door ze uit te drukken in percentages van de totale essentiële aminozuren.

Op een leeftijd van 12 weken waren in experiment 3 de gehaltes aan vrije lysine in het bloed van ratten, die tarwegluten ad libitum kregen, aanzienlijk hoger bij de vrouwelijke dan bij de mannelijke dieren. Op een leeftijd van 24 weken waren bij de ratten, die tarwegluten ad libitum kregen de threoninegehaltes in het bloed van de vrouwelijke aanzienlijk hoger dan in dat van de mannelijke dieren. Dit wijst er op dat vrouwelijke ratten zich beter aanpassen aan een lysine- en threoninetekort in tarwegluten dan mannelijke ratten.

De gehaltes aan de meeste vrije aminozuren in het bloed, hoewel niet steeds dezelfde, namen toe met de leeftijd: vanaf de geboorte tot een leeftijd van 4 weken, vanaf het begin tot de 8e week in experiment 3 (4–12 weken oud), en namen dan van de 8e tot de 20e week van experiment 3 af (12–24 weken oud), behalve bij de dieren (♀) van 24 weken op caseïne. Verder namen de vrije aminozuurgehaltes in het bloed van mannelijke ratten ook af in vele gevallen in experiment 2 met de leeftijd van 10–21 weken, ondanks het verschil in hoeveelheid en soort van eiwit in experiment 2 en experiment 3.

Het is opmerkelijk dat een omgekeerde neiging (afname) gevonden werd in het histidinegehalte vanaf de geboorte tot aan het spenen.

Verder namen de gehaltes aan vrije lysine in het bloed van ratten op een dieet met een lysinetekort toe met de leeftijd, terwijl de gehaltes aan de meeste vrije essentiële aminozuren afnamen. In het algemeen verminderde de toediening van tarwegluten de gemiddelde gehaltes aan vrije individuele aminozuren in het bloed, zowel wanneer het in beperkte mate, als wanneer het ad libitum verstrekt werd.

De meeste gehaltes aan vrije aminozuren in het bloed van ratten op eiwitvrij voer waren lager wanneer de hoeveelheid tarwegluten beperkt was dan bij ad libitum verstrekking (experiment 3).

De gehaltes aan de meeste essentiële vrije aminozuren in het bloed van pasgeboren ratten waren hoger dan in het bloed van hun moeders. Verder hadden ratten post partum meestal lagere gehaltes aan vrije aminozuren in het bloed dan maagdelijke ratten.

Gegevens van de experimenten tonen aan dat veranderingen in de gehaltes aan vrije aminozuren beïnvloed worden door wisselwerkingen tussen de soort eiwit, de leeftijd en het geslacht, naast andere factoren als zwangerschap en stofwisselingsrelaties.

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