Long-term effects of feeding rats on casein and gluten diets of the same protein value

MARÍA ANGÉLICA TAGLE AND GONZALO DONOSO
Laboratory of Nutrition, School of Public Health and Chair of Biochemistry, School of Agriculture, University of Chile, Santiago de Chile

SUMMARY

Long-term experiments were conducted with rats fed casein and gluten diets at two levels of protein value (4 and 7 NDpCals%), in an attempt to show whether diets of the same protein value, obtained with proteins widely different in quality, can be considered nutritionally equivalent. No significant differences were found for carcass and liver composition, serum albumin and globulin, activities of glutamic-pyruvic, glutamic-oxalacetic aminotransferases and alkaline phosphatase in the liver of the rats fed casein and gluten diet at the two levels studied. However, significant differences could be shown for growth response, hematocrit and weight of kidneys between rats fed casein and gluten diets at both levels. Oxygen consumption was significantly higher for animals fed casein diet than for those fed gluten at the higher level of utilizable protein. The weight of the liver was significantly higher for the animals that received gluten at the low level of protein value. The performances of the rats on diets of the same NDpCals% over 37 days (4%) and 133 days (%), differs in some respects that seem nutritionally important.

World resources for the production of foodstuffs of good protein quality seem limited and costly to develop, especially where they are most urgently needed. This has resulted in a search for new protein for human consumption. Unfortunately, many of the most promising — because of their potentially inexhaustible supply and low cost — are of poor biological quality (1-3). It therefore becomes important to de-

1 This study was supported by funds from Comisión de Investigación Científica, Facultad de Medicina, Universidad de Chile, project Nº 2-4509-3 (66-30).
Received: 24-4-1967

termine whether protein needs can be met in the same way by means of a diet that contains a low amount of good quality protein or a larger amount of a lesser quality.

According to Plett, Miller and Payne (4), diets are of equal protein value when they have the same Net Dietary protein Calories per cent total Calories (NDpCals%). This value is obtained multiplying the Net Protein Utilization operative (NPUop), determined experimentally by feeding the diet to weanling rats for ten days, by the protein concentration of the diet, expressed in protein calories percent total calories (P%), thus,

$$\text{NDpCals\%} = \frac{\text{NPUop} \times \text{P\%}}{100}$$

There is no information to show if long-term feeding of diets with the same protein value (as NDpCals%) obtained with different proteins produces the same effects. This is clearly important if diets of the same protein value are to be considered nutritionally equivalent (4, 5).

We have compared the effects of feeding casein and gluten diets of 4 NDpCals%, level reported as sufficient to maintain body weight (4), and 7 NDpCals%, level which allows good growth in long term with rats (6).

EXPERIMENTAL

Diets. 4 NDpCals% level. Casein diet (in grams): casein[2], 72; cornstarch, 428; vegetable fat, 150; vitamin and mineral mixture (6), 350. The calorie value of this diet calculated from chemical analysis using Atwater's factors was 4.2 kcal/g. Casein supplied 5.8% of the total calories of the diet. Gluten diet: gluten[2], 146; cornstarch, 354; vegetable fat, 150; vitamin and mineral mixture (6), 350. The calorie value of this diet calculated from chemical analysis was 4.2 kcal/g. Gluten supplied 13.0% of the total calories of the diet. 7 NDpCals% level. Casein and gluten diets as described previously (6). Diets and drinking water were offered *ad libitum.* Fat soluble vitamins were given orally every other day, according to the quantities

2 Nutritional Biochemicals Co., Cleveland, Ohio, U.S.A.

used by Kodicek and Carpenter in a long-term experiment (7).

Animals. 4 NDpCals% level. 43 male weanling albino rats 21 day-old, with a mean weight of 33 g, were divided in three groups: 10 that were killed at the beginning of the experimental period (initial group), 14 that received the casein and 19, the gluten diet for a period of 37 days (58 days of age). 7 NDpCals% level. 20 male weanling rats, 22 day-old, with a mean weight of 30 g, were divided in two groups: 10 that received the casein and 10, the gluten diet for a period of 133 days (155 days of age).

During the trials the animals were caged in groups of three to four individuals, weighed once a week and the food intake was measured. The room temperature was maintained at 28°C.

Procedures

Water, nitrogen and fat content of the rat carcass and liver were individually determined by chemical analysis. The composition of the tissue gained was calculated as the difference between the individual final values and the mean carcass composition of the initial group. Body N/H_2O ratio was calculated dividing body nitrogen (g/100 g wet weight) by body water (g/100 g) and multiplying by 1000. Oxygen consumption was estimated for each rat in a closed circuit calorimeter. Microhematocrit, according to ICNND (8), serum albumin and globulin, by the method of Lowry (9), glutamic-pyruvic and glutamic-oxalacetic aminotransferases, and alkaline phosphatase in the liver as described by Reitman and Frankel (10) and Roe and Whitmore (11), using a 10% homogenate of this organ in cold water. Liver and kidneys were weighed and histological examination of these organs was carried out.

The number of animals, their age and days on the experimental diets at which the determinations were performed are given in the tables under Results.

RESULTS

General appearance. The animals fed the low level looked miserable, showed lassitude and seemed emaciated from the second week onwards. Their coat was thin, and when hair was

plucked it did not grow again, leaving bald patches till the end of the experimental period (animals of the same age fed on a stock diet regained plucked hair in 8 to 12 days). Their extremities were cyanotic and the rats showed a tendency to hide away from light. All these features were very much the same in the animals fed both proteins.

The rats fed the 7% level of utilizable protein locked lively, their coat was shiny and their behaviour was similar to that of those on stock diet. No diarrhoea was shown by the animals on the gluten diet (53.5 g gluten per hundred g of diet).

Growth curves of the rats fed casein and gluten diets of 4 and 7 NDpCals% are shown in figure 1. A clear difference can be seen, as should be expected, in the growth rate at the two levels of utilizable protein. At the 4% level the weights of the rats on the gluten diet were statistically different from those exhibited by the animals on the casein diet, since the 14th day of feeding (casein, 43.3 $\pm$ 1.2 kg; gluten, 36.6 $\pm$ 0.9 g; $p < 0.001$). The animals fed the 7% level only showed statistically different growth from the 93rd day onwards (casein), 224.7 $\pm$ 3.0 g; gluten, 249.4 $\pm$ 6.4 g; $p < 0.01$).

Histology. The histology of liver and kidney in the animals fed on the 4% level can be considered normal. However, in a few liver preparations of three of the animals on the gluten diet, small inflammatory foci appeared, formed by accumulation of mononuclear cells and some neutrophiles. No cellular necrosis or fibrosis was found. There was no fatty infiltration, but two animals on the casein diet exhibited some cellular vacuolization, possibly fatty in origin. The kidneys indicated no alteration in the tubules, glomeruli, vasa or interstitial tissue. No differences could be found between the rats fed casein or gluten.

Liver and kidney of the animals fed the 7% level looked normal. No fibrosis, cellular infiltration, vacuolization or necrosis was found in any liver preparation. In some of the renal slices examined, the height of the cells in the distal tubules seemed less than normal, but the distribution was not in relation to the protein the animal had received.

Table 1 shows carcass and liver composition, serum albumin and globulin of the animals fed the casein and the gluten diets. No statistical difference was found for any of these values for groups fed the same utilizable protein level.

Oxygen consumption, hematocrit, weights of kidneys and liver and the activities of glutamic-pyruvic, glutamic-oxalacetic aminotransferases and alkaline phosphatase in this organ are given in table 2.

Body weight and body nitrogen, the calorie and nitrogen intake of the animals on the different diets are given in table 3. Figure 2 shows the body N/H_2O ratio for rats fed casein and gluten at both levels of protein values and compares with what can be considered normal for our colony, i. e. rats of the same age fed on stock diet.

Figure 3 shows the composition of the tissue gained during the feeding trial, expresed as protein to water and fat to water increments per 100 weight gained.

DISCUSSION

Our purpose was to study the behaviour of rats on diets of the same protein value, obtained with casein or gluten, in long-term feeding experiments at 4 and 7 NDpCals%. It would have been of added interest to compare the performances of the animals on the high with those on the low level of utilizable protein. Unfortunately, this was not possible because the rats on the 4 NDpCals% diets had so considerably lost their vitality that it was decided to end the experiment after only 37 days.

Miller and Payne have reported that the weight increments of rats fed diets of the same NDpCals% obtained by varying the concentration of calories per gram of diet (without changing the nature of the protein), in a 35 day experiment with weanling rats is similar (12). There is no information on the long-term weight increase produced by diets of the same content in utilizable protein obtained with different proteins. Presumably the weight gain should be similar (12). Our results evidence that the growth response is different: rats fed casein or gluten at the 7 NDpCals% level showed the same weight increments until the 93rd day, but highly significant differences in favour of gluten were found from then onwards (Figure 1). It should be indicated that the total calorie intake of the rats receiving casein or gluten was very similar. Furthermore, it was the rats on the gluten diet those that showed the smaller intake. The rats fed the low level of utilizable

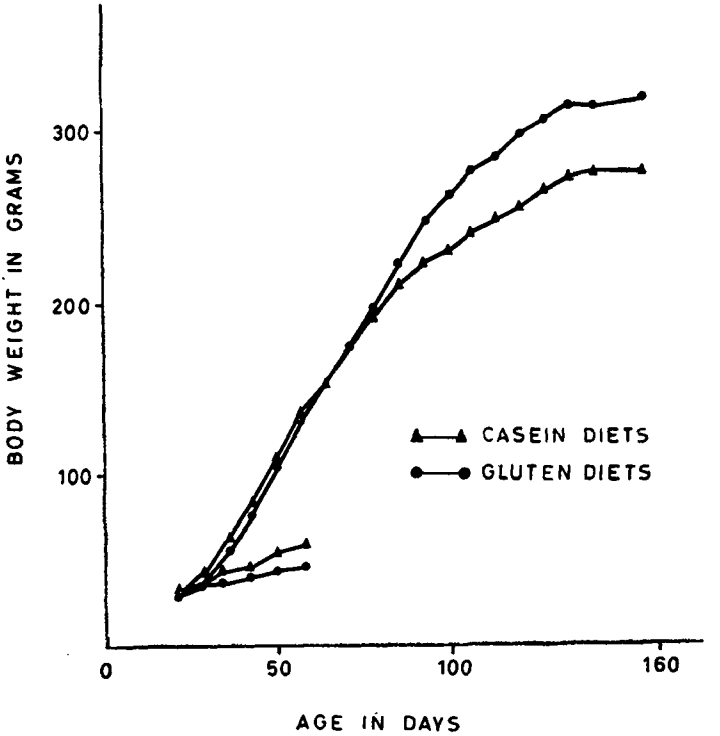

Figure 1.—Growth curves of rats fed casein and gluten diets of 4 and 7 NDpCals%.

protein exhibited significant differences from the 14th day onwards, those on the casein diet having the higher increment (Figure 1 and Table 3), but also a higher calorie intake (Table 3).

A value of 4 NDpCals% has been given as sufficient to maintain body nitrogen (4, 13), although this figure has been contended as too high by Njaa (14), but the experimental evidence from which these values have been derived was obtained from young rats in short-term trials.

In our laboratry adult female rat have been found to maintain weight on the casein diet of 4 NDpCals% in three weeks experiments[3]. However, the weanling rats used in the present trial increased their body weight with both the casein and the

3 Grunwald, J. Eficiencia de la ingesta proteica en la rata preñada. Efecto del valor proteico de la dieta. Tesis 1966. Escuela de Agronomía, Universidad de Chile.

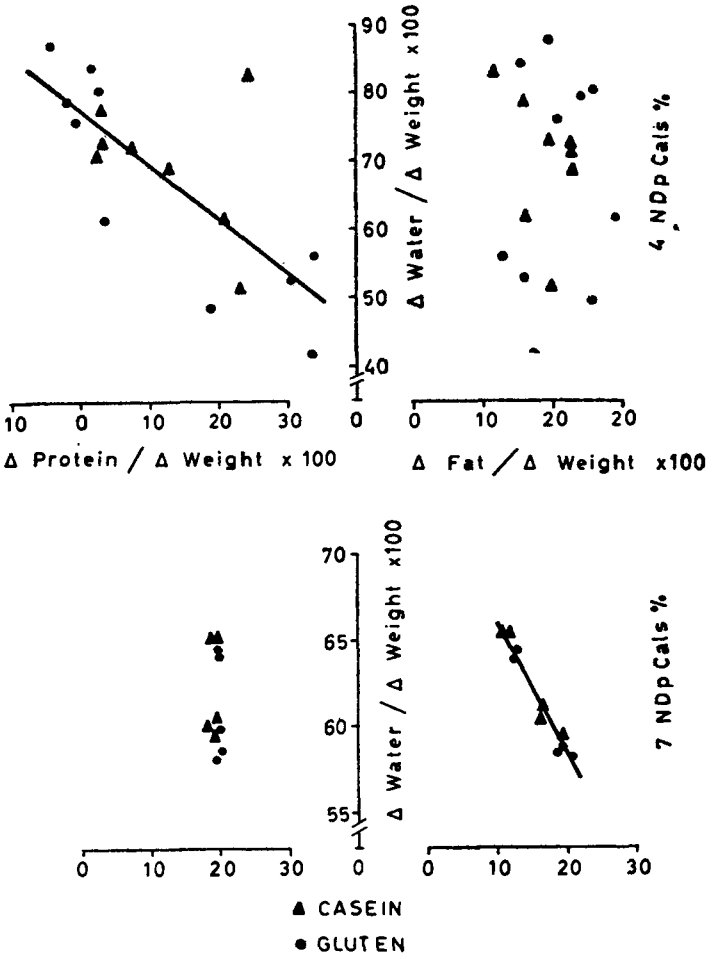

Figure 3.—Protein to water and fat to water increments (per 100 g of gained weight) in rats fed gluten and casein diets of 4 and 7 NDpCals%.

casein and the gluten diet fed at the low level showed a wide variability of this ratio (Figure 2).

In the rats on the 4% diets the gain in water is negatively correlated with the gain in protein, when expressed as a percentage of the total body weight increment ($r = -0.769$; $p < 0.001$) (Figure 3). No such correlation exists with fat. Figure 3 is also indicative of the wide range in the composition of the tissue gained. Rats fed the high level of utilizable pro-

tein showed: very little variation in the composition of the tissue gained, no correlation between the water and protein increments, but a good correlation ($r = -0.983$; $p < 0.001$) between water and fat (Figure 3), as should be expected.

The liver composition did not present significant difference at either level studied (Table 1). It should be stressed, however, that no fatty infiltration was found at the low level and that the fat content can be considered within normal limits (20). Fatty infiltration of the liver of rats fed low levels of protein in the diets has been reported by Waterlow (19), and the increase in the fat content of this organ in infant protein malnutrition is a constant feature of this syndrome (15).

The weight of the liver of rats on the 4 NDpCals% diets showed a highly significant difference in favour of the group fed the gluten diet (Table 2), but at the high level of utilizable protein no such differences exists between the rats fed casein and gluten.

The gluten diets contain about two and four times the concentration of protein than the casein diets. However, remarkably similar activities were found for the enzymes assayed in the liver (Table 3) within the same level of utilizable protein. It has been reported that the increase in concentration of a given protein in the diet induces higher values for aminotransferase activities, especially for the glutamic-pyruvic (21). Our results suggest that the activity of these enzymes is more related to the protein value of the diet than to the concentration of protein "per se".

With the due reserves, because of the difference in age and length of the dietary treatment, the animals on the low level of utilizable protein showed considerably less activity for the enzymes involved in protein metabolism. However, the alkaline phosphatase activity was higher in younger animals than in the older, as been described (22). Protein malnutrition in the child has been reported to lower the alkaline phosphatase activity of the serum (20, 23), but to values still higher than those for the normal adult. A statistically significant difference can be shown to exist in favour of the rats on the casein diet, when activity of alkaline phosphatase is referred to 1 gram liver (casein: 2.85 ± 0.25; gluten: 1.98 ± 0.17; $p < 0.01$), but this difference is cancelled when expressed per hundred

TABLE 1

CARCASS AND LIVER COMPOSITION, AND SERUM PROTEINS IN RATS FED CASEIN AND GLUTEN DIETS OF 4 NDpCals% (58 DAYS) AND 7 NDpCals% (155 DAYS)

DIET		BODY COMPOSITION			LIVER COMPOSITION			SERUM PROTEINS	
		Water	Protein g/100 g	Fat	Water	Protein g/100 g	Fat	Albumin g/100 ml	Globulin g/100 ml
4 N D p Cals %	casein mean ± e. m.	71.9 ± 1.4	13.8 ± 1.5	10.9 ± 0.9	73.9 ± 1.1	12.4 ± 0.5	5.7 ± 1.6	3.8 ± 0.2	3.8 ± 0.3
	n	8	8	8	8	8	8	7	7
	gluten mean ± e. m.	71.5 ± 1.5	14.6 ± 1.4	9.7 ± 0.6	73.4 ± 0.5	12.0 ± 0.6	4.2 ± 0.9	3.7 ± 0.3	3.8 ± 1.2
	n	10	10	10	10	10	10	11	11
7 N D p Cals %	casein mean ± e. m.	63.5 ± 1.1	18.9 ± 0.2	13.6 ± 1.5	68.3 ± 0.5	21.0 ± 0.8	3.7 ± 0.5	3.6 ± 0.1	4.4 ± 0.2
	n	5	5	5	5	5	5	10	10
	gluten mean ± e. m.	61.9 ± 1.4	19.6 ± 0.1	15.4 ± 1.6	67.9 ± 0.7	20.7 ± 1.1	4.3 ± 0.7	3.4 ± 0.1	4.3 ± 0.1
	n	5	5	5	5	5	5	10	10

No statistical difference was found for any of these values for groups fed the same protein value.

TABLE 2

OXYGEN CONSUMPTION, HEMATOCRIT, WEIGHT OF KIDNEY AND LIVER, ACTIVITIES OF GLUTAMIC-PYRUVIC, GLUTAMIC-OXALACETIC AMINOTRANSFERASES AND ALKALINE PHOSPHATASE IN LIVER OF RATS FED CASEIN AND GLUTEN DIETS OF 4 AND 7 NDpCals%

DIET		Oxygen consumption ml/hr/cm²	Hematocrit %	Kidney g/100 g rat	Liver g/100 g rat	GP amino-transferase mg pyruvic/30' per 100 g rat	GO amino-transferase mg pyruvic/60' per 100 g rat	Alkaline phosphatase mg P/60' per 100 g rat
4 N D p Cals %	casein							
	mean ± e. m.	0.61 ± 0.07	42.2 ± 1.0	0.92 ± 0.02	3.73 ± 0.13	18.4 ± 2.8	60.0 ± 10.0	11.0 ± 1.0
	n	9	14	7	8	8	8	8
	gluten							
	mean ± e. m.	0.83 ± 0.14	37.6 ± 0.7	1.19 ± 0.04	4.87 ± 0.15	15.4 ± 2.5	60.1 ± 5.0	10.3 ± 0.4
	n	11	17	10	10	8	10	9
	t	—	3.8	6.7	5.7	—	—	—
	p <	—	0.001	0.001	0.001	—	—	—
	age (days)	54	58	58	58	58	58	58
7 N D p Cals %	casein							
	mean ± e. m.	1.37 ± 0.07	44.0 ± 0.7	0.54 ± 0.03	3.48 ± 0.13	148.6 ± 16.8	147.2 ± 10.4	2.86 ± 0.2
	n	10	9	5	5	5	5	5
	gluten							
	mean ± e. m.	1.02 ± 0.06	48.0 ± 0.8	0.65 ± 0.02	3.39 ± 0.18	146.7 ± 17.0	143.9 ± 12.7	2.24 ± 0.7
	n	10	10	5	5	5	5	5
	t	3.8	4.0	3.0	—			
	p <	0.001	0.001	0.02				
	age (days)	130	155	155	155	155	155	155

t and p values were obtained comparing the figures of the animals fed on casein with those fed on gluten at the same protein level.

TABLE 3

WEIGHT, BODY NITROGEN, CALORIE AND NITROGEN INTAKE IN RATS FED CASEIN AND GLUTEN DIETS OF 4 AND 7 NDpCals%

Age (days)	Diet	Body weight (g)	Body nitrogen (g)	Nitrogen intake (g)	Calorie intake (Cals)
21	—	33.2	0.85	—	—
58	casein	60.1	1.33	1.88	829.4
58	gluten	47.7	1.11	3.54	616.4
22	—	30.0	0.77	—	—
155	casein	277.2	8.44	23.42	6472.0
155	gluten	318.8	10.07	93.60	6292.0

grams of rat, because of the larger liver of the animals that received gluten.

The weight of the kidney was significantly higher in the animals fed gluten than in those fed casein. Weight increase of this organ has been reported in relation with high protein content of the diet (24). The mechanism by which this increase is produced is obscure, but it can be compared to the so called "compensatory hypertrophy" after unilateral nephrectomy (25). It is remarkable that although gluten and casein diets contain the same amount of utilizable protein (i. e. usable for synthetic purposes), there is a preferential synthesis of kidney protein with the gluten diets. The osmotic work that must be effected by the animals on the gluten diets is, of course, greater than on the casein diets because of the larger excretion of urea.

Serum proteins did not show significant differences with the casein or gluten diets. The values for serum albumin at the 4% level of utilizable protein are of the same order of magnitude than those of the rats that received the 7% diets. Low serum albumin have been reported consistently as a late event in established protein malnutrition in the child (26), and experimentally in pigs and rats (27, 28). However, it has been shown that in the marasmic type of malnutrition the levels of serum albumin are close to normal and are associated with a decreased degradation of this serum protein, possibly adaptative in nature (29).

The well known experiments of Whipple and associates (30) showed that proteins differ significantly in their capacity of replenishing the hemoglobin content of blood. In our experiments, hematocrit values differ at each level of utilizable protein, but the difference is not related with the protein in the diet, i. e. at the 7% level gluten produces higher values than casein, whilst the reverse is true at the 4% level (Table 2). Higher hematocrit was consistently associated with higher weight increments at both levels of utilizable protein.

Oxygen consumption in rats fed both diets at the 7 NDpCals% level fall in the range considered normal for the rat (31-33), but the values found for the animals on the casein diet are significantly higher than those on the gluten diet (Table 2). At the 4% level no significant difference could be shown, but the values were considerably lower than those given in the literature as normal for rats of comparable age (32). Lower oxygen consumption has been reported in infants suffering from protein-caloric malnutrition (34). At both levels of utilizable protein greater weight increments were associated with lower oxygen consumption (Tables 2 and 3).

The true NPU of the casein and gluten diets, calculated according to the formula proposed by the authors in a previous paper (6), agree well with those obtained experimentally by the method of Miller and Bender (19) in short-term feeding trials with weanling rats: casein, 76 and 74 in long-term trials at 4 and 7 NDpCals%, versus 73 and 71 in short-term trials; gluten, 32 and 21 in long-term trials at 4 and 7 NDpCals%, versus 32 and 20 in short-term experiments. The protein values, calculated with the long-term true NPU are, therefore, very similar to those obtained with the short-term determinations.

The mineral mixture used supplied less copper, manganese and zinc than the requirements for the rat, according to the National Research Council (publication 990), but the animals were kept in galvanized cages which would make adequate (NRC) the level of zinc supplied in our diets. The amounts of copper and manganese in the mineral mixture, although low, were considerably higher than those of the widely used USP XII salt mixture. These considerations seem important because the protein preparations used could supply different amounts of the above mentioned micronutrients.

Our results show that the performance of rats on diets of the same protein value —as NDpCals%— obtained with proteins that differ widely in quality, is not the same in some respects that seem nutritionally important, both at the 4 and 7 NDpCals% level.

RESUMEN

Efectos de la alimentación prolongada de la rata con dietas de caseína y de gluten de igual valor proteico

Se alimentaron ratas con dietas de caseína y de gluten, a los niveles de 4 y 7% de Calorías Proteicas Netas, por períodos prolongados, con el fin de constatar si dietas de un mismo valor, obtenido con proteínas de muy diferente calidad, pueden ser consideradas nutricionalmente equivalentes.

No hubo diferencias estadísticamente significativas en cuanto a composición corporal y hepática, albúminas y globulinas séricas, entre los animales estudiados. Tampoco las hubo en la actividad hepática de aminotranferasas glutámico-pirúvica y glutámico-oxalacética, ni en la de fosfatasa alcalina, referida a cien gramos de rata.

A ambos porcentajes de proteína utilizable en la dieta hubo diferencias significativas para crecimiento, hematocrito y peso de los riñones, entre las ratas alimentadas con caseína y las con gluten.

Al nivel de 7% el consumo de oxígeno fue significativamente mayor para los animales almentados con caseína que para los con gluten.

Al nivel de 4%, el peso del hígado fue significativamente más alto para las ratas que recibieron gluten que para las con caseína.

El comportamiento de los animales sometidos a dietas de caseína y gluten del mismo valor proteico, durante 37 días (4%) y durante 133 días (7%), es diferente en varios aspectos nutricionalmente importantes.

BIBLIOGRAPHY

(1) Lubnitz, J. A.—The protein quality, digestibility and composition of algae Chlorella 71105. J. Food Sci., 26:229, 1963.

(2) Anonimous.—Algae as a source of human food. Nutr. Rev., 17:238, 1959.

(3) Champagnat, A., C. Vernet, B. Lainé & J. Filosa.—Biosynthesis of protein concentrates from petroleum. Nature, 197 (Nº 4862):13, 1963.

(4) Platt, B. S., D. S. Miller & P. R. Payne.—Protein values of human food. In: "Recent Advances in Clinical Nutrition", ed. J. F. Brock, J. and A. Churchil Ltd., London, p. 351, 1961.

(5) Hryniewiecki, L.—Nuclear protein in the liver in protein-calorie deficiency. Biochem. J., 95:238, 1965.

(6) Tagle, M. A. & G. Donoso—Net protein utilization determined in short and long-term experiments with rats. J. Nutrition, 87:173, 1965.

(7) Kodicek, K. E. & K. J. Carpenter.—Experimental anemias in the rat. Blood, 5:22, 1950.

(8) Interdepartmental Committee on Nutrition for National Defense

(ICNND). Manual for Nutrition Surveys. U. S. Government Printing Office, Washington 25, D. C., p. 68, 1957.

(9) Lowry, O. H., N. J. Rosebrough, A. L. Farr & R. J. Randall.—Protein measurement with the Folin phenol reagent. J. Biol Chem., 193: 265, 1951.

(10) Reitman, S. & S. Frankel.—A colorimetric method for the determination of serum glutamic-oxalacetic and glutamic-pyruvic transaminases. Amer. J. Clin. Path., 28:56, 1957.

(11) Roe, J. H. & E. R. Whitmore.—Clinic-pathologic application of serum phosphatase determinations with special reference to lesions of bones. Am. J. Clin. Path., 8:233, 1938.

(12) Miller, D. S. & P. R. Payne.—Problems in the prediction of protein value of diets. The influence of protein concentration. Brit. J. Nutr., 15:11, 1961.

(13) Miller, D. S. & P. R. Payne.—A theory of protein metabolism. J. Theoret. Biol., 5:1, 1963.

(14) Njaa, L. R.—A note on the method of Miller and Payne for prediction of protein value. Brit. J. Nutr., 16:185, 1962.

(15) Monckeberg, F. & G. Donoso.—Epidemiología de la desnutrición infantil. Nutr. Bromatol. Toxicol., 4:39, 1965.

(16) Mc Cance, R. A.—Severe undernutrition in growing and adult animals. I. Production and general effects. Brit. J. Nutr., 14: 59, 1960.

(17) Cabak, V., J. W. T. Dickerson & E. M. Widdowson.—Response of young rats to deprivation of proteins or of calories. Brit. J. Nutr., 17:601, 1963.

(18) Dckerson, J. W. T. & R. A. Mc Cance.—Severe undernutrition in growing and adult animals. 3. Avian skeletal muscle. Brit. J. Nutr., 14:331, 1960.

(19) Miller, D. S. & A. E. Bender.—The determination of the net utilization of proteins by a shortened method. Brit. J. Nutr., 9:382, 1955.

(20) Waterlow, J. C.—Biochemical aspects of protein malnutrition. In: "Protein malnutrition", ed. J. C. Waterlow. University Press, Cambridge, p. 1, 1955.

(21) Rosen, F., N. R. Roberts & C. A. Nichol.—Glucocortocosteroids and transaminase activity. I. Increased activity of glutamic-pyruvic transaminase in four conditions associated with gluconeogenesis. J. Biol. Chem., 234:476, 1959.

(22) Barrows, C. H. & L. M. Reeder.—Effect of age on protein synthesis in rats. J. Geront., 16:321, 1961.

(23) Tagle, M. A., J. Hormazábal, I. Soto, D. Ballester & G. Donoso.—Características del Síndrome Pluricarencial Infantil (SPCI) en Chile. Fosfatasa alcalina, calcio y fósforo séricos. Nutr. Bromatol. Toxicol., 1:145, 1962.

(24) Walter, F. & T. Addis.—Organ work and organ weight. J. Expt. Med., 69:467, 1939.

(25) Jerusalem, C.—Studies on compensatory kidney hypertrophy. I. Weight increase and water content of the remaining following unilateral nephrectomy in rats. Anat. Anz., 114:86, 1964.

(26) Waterlow, J. D.—The assessment of marginal protein malnutrition. Proc. Nutr. Soc., 22:66, 1963.

(27) Friend, C. J., C. R. C. Heard, B. S. Platt, R. J. C. Stewart & M. R. Turner.—The effect of dietary protein deficiency on transport of vitamin A in the blood and its storage in the liver. Brit. J. Nutr., 15:231, 1961.

(28) Vakr, U. K., O. A. Roels & M. Trout.—Storage and transport of Vitamin A in relation to protein instake. Brit. J. Nutr., 18:217, 1964.

(29) Donoso, G., O. Brunser & F. Monckeberg.—Metabolism of serum albumin in marasmic infants. J. Pediatrics, 67:306, 1965.

(30) Robschneit G. Robbins, F. S. & G. H. Whipple.—Dietary effects on anemia plus hypoproteinemia in dogs. I. Some proteins further the production of hemoglobin and other plasma protein production. J. Exp. Med., 39:339, 1949.

(31) Farris, E. J. & J. Q. Griffith.—"The rat in laboratory investigation." J. B. Lippincott Company, Philadelphia, 1949.

(32) Ermatova, D. U. K.—Characteristics of energy expenditure in the rat at different ages. Bjull. eksp. Biol. Med., 3:25, 1965.

(33) Heusner, A. & M. L. Hamelin.—Relation entre le poids et la consommation d'oxygene. I. Etudes chez le rat. C. R. Soc. Biol., 157:376, 1963.

(34) Monckeberg, F., F. Beas, I. Horwitz, A. Dabancens & M. González. Oxygen consumption in infant malnutrition. Pediatrics, 33:554, 1964.

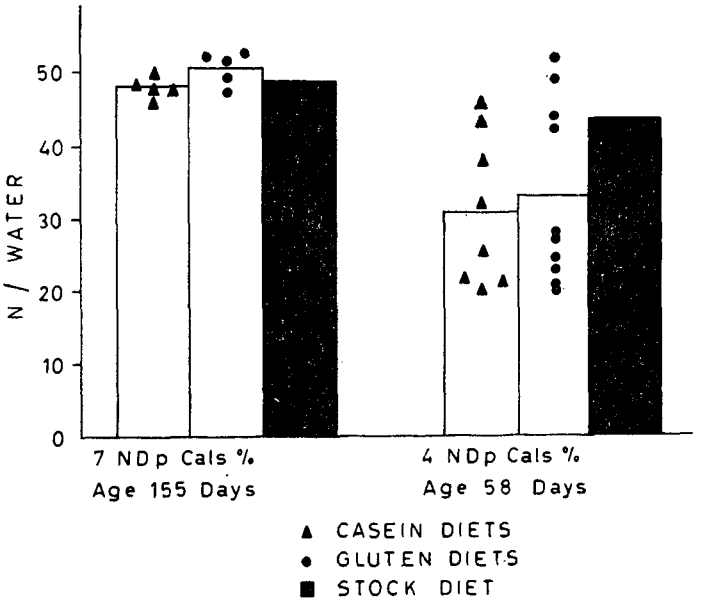

Figure 2.—Nitrogen to water ratio in rats fed casein and gluten diets of 4 and 7 NDpCals%. Comparison with rats of the same age fed on stock diet.

gluten diets. Nevertheless, the calculated composition of the tissue gained in several of the rats examined is abnormal, i. e. in some there was no nitrogen increment, others even showed a loss, the weight increase being accountable by water and fat. Indeed, the animals presented a higher hydration than rats of our colony of a comparable age on stock diet[4]. This was especially true in some individuals where the high water content is reminiscent of the edematous type of malnutrition in the child-kwashiorkor-produced by weaning to diets of low protein value (15). Similar results have been obtained by long-term feeding of low protein diets in the piglet, rat and cockerel (16-18).

Carcass nitrogen to water ratios have been used to calculate body nitrogen in the evaluation of protein quality according to the method of Miller and Bender (19). Rats fed the high level of utilizable protein showed remarkably constant N/H_2O ratios, which were very similar to those of rats of comparable age fed on stock diet[4]. On the other hand, the animals on the

4 Yáñez, E. and G. Donoso, 1963. Variación de la composición corporal de la rata en función de la edad. Primer Congreso de Nutrición, Bromatología y Toxicología. Santiago de Chile.