To the Graduate Council:

I am submitting herewith a thesis written by Mary A. Setlow entitled "Reproductive and Growth Performance of Athymic Mice on Three Dietary Levels of Crude Protein." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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Karl M. Barth, Major Professor

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REPRODUCTIVE AND GROWTH PERFORMANCE OF ATHYMIC MICE ON THREE DIETARY LEVELS OF CRUDE PROTEIN

A Thesis

Presented for the

Master of Science

Degree

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Mary A. Setlow

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ABSTRACT

A six-month experiment was conducted to study the reproductive response of 42 nu/+ female mice and 21 nu/nu thymus transplanted male mice of BALB/c background to three dietary protein levels. The mice were housed as breeding trios (one male and two females), and seven trios each were assigned to one of three rations—12, 18, or 24% crude protein. Data were collected on reproductive performance, weight of pups, hematological parameters and organ weights. Analysis of variance showed no significant differences among females with respect to reproductive performance. No significant differences were found in hematological parameters or organ weights when expressed as a percentage of bodyweight except among the nu/nu transplanted males, where the 24% protein males had significantly higher ($P < .10$) spleen weights. The pup weights showed no differences at birth but at weeks 1, 2, and 3 the 18 and 24% pup weights were higher ($P < .05$) than the 12% pup weights. Gain for weeks 1 and 2 and total gain were significantly higher in the 18 and 24% pups, while gain for week 3 was significantly ($P < .025$) higher in the 12% pups.

Three rations differing in crude protein levels, 12, 18, and 24%, were fed to 128 nude females, 103 nude males, 126 nu/+ females and 102 nu/+ males for one month (28 days) from 21 days of age (weaning) to 49 days of age. The weanling mice were maintained on the same level of dietary crude protein that had been fed to their parents. The mice were weighed every four days and food consumption was calculated for four-day

periods. At the end of 28 days, the mice were sacrificed, their organs weighed, and serum protein levels recorded.

The nu/+ female mice grew best on the 24% protein ration, while the nu/+ male mice grew best on the 18% protein ration. The nude mice, many of which succumbed to wasting disease (thought to be mouse hepatitis virus), gained best on the 12% ration (females) and on the 18% ration (males). These figures were obtained from averages which included those mice which ceased to grow, lost weight and became moribund. When these mice were removed from the data, the nude females grew best on 24% protein while the nude males still grew best on the 18% protein. The incidence of wasting disease, death, and necrotic liver lesions was lowest in the 12% protein nude females (5.6%). Twelve percent nude males had the next lowest incidence (23.8%) followed by 18% nude males (38.1%), 24% nude females (41.0%), 18% nude females (48.1%) and 24% nude males (51.2%).

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CHAPTER I

INTRODUCTION

The discovery of the mutant mouse gene nu (nude) and the subsequent finding that the pleomorphic effects of this gene included athymia as well as hairlessness have been the basis for much research in immunology and oncology. The susceptibility of these immunologically incompetent animals to disease and infection has made them difficult to breed, raise and house in a conventional manner. This fact, coupled with an increasing interest in their use as an animal model in research, has necessitated studies directed towards the improvement of all aspects of their husbandry.

That optimum growth, survival, and reproductive performance are dependent upon optimal nutrition is a well-known, though often-overlooked, fact. With any research animal, the ideal situation would be to define the optimum diets for growth, maintenance and reproduction. With this idea in mind, two studies were conducted in an effort to measure the reproductive and growth performances of nude mice on three dietary levels of crude protein.[1]

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CHAPTER II

LITERATURE REVIEW

I. Athymic Mice

Discovery of the Nu Gene and the Pleomorphic Effects of the Gene

Flanagan (1966) reported the discovery of a new mutant gene "nu" in an albino strain of mice. The homozygous recessive (nu/nu) animal was hairless, lighter in weight than the heterozygous (nu/+) animals, and had a shortened lifespan due to wasting disease and liver disease. The lack of haircoat was attributed to failure of the hair follicle to penetrate the epidermis and imperfect keratinization of the hair shaft. Pantelouris (1968) first reported the absence of a thymus in the mutant and reported lowered peripheral blood leucocyte counts. Fukuda et al. (1974) found a bilobed thymus rudiment in one to seven day old nude mice and a small number of lymphoid cells in close vicinity of the thymic rudiment. Holub et al. (1975) described a dysgenetic thymus.

Ohsawa et al. (1974) reported intact adrenal, thyroidal and gonadal functions in nudes. These researchers reported lower body weights in nudes during suckling, while the postweaning growth of nudes approached the values of BALB/c+/+ controls under specific pathogen-free conditions. Deutsch et al. (1974) reported that the immunodeficiency associated with the nude mice was not due to an adenosine deminase deficiency. The nude mice were shown to have significantly lower hematocrits and hemoglobin (g/100 ml) and higher spleen weights than

the nu/+ control animals (Bamberger, et al., 1977). Transplantation of thymic tissue into the nude mouse restored normal immune function (Pritchard, et al., 1973).

Disease Problems

Many researchers have reported liver lesions, wasting disease, early mortality, subcutaneous abscesses and parasite infections in nudes (Flanagan, 1966; Pantelouris, 1968; Jutila and Reed, 1972). Sebesteny (1974) associated wasting disease in nude mice with a neurotropic variant of mouse hepatitis virus which he identified using electron microscopy and serological testing. Tamura et al. (1976) isolated the hepatitis virus from a wasting nude mouse of BALB/c background with multiple focal necrosis of the liver. The isolated virus was capable of reproducing disease in heterozygous mice only when accompanied by cortisone injections but consistently reproduced the disease in nude mice. A great variation in the survival time and susceptibility was noted among nudes.

Special Husbandry Practices

The disease problems associated with nude mice have necessitated the use of special procedures in the husbandry of these animals. Conventional colonies of nudes have shorter lifespans than those nudes raised in specific pathogen-free environments. Germfree (Caeserean derived) nude mouse colonies have been the most successful in lengthening lifespans (Hetherington and Hegen, 1975; Eaton, et al., 1975).

Special mating systems have been devised to produce maximal numbers of nude mice. Nude males mated to nude females and nude females mated

to nu/+ males result in 100% mortality (Eaton et al., 1975). A successful breeding system is one that mates a nu/+ female to a nude male (transplanted with thymic tissue) to produce 50% nude mice and 50% nu/+ mice (Hetherington and Hegen, 1975).

II. Protein Nutrition

Only in recent years have the nutritional requirements for the laboratory mouse been defined. The assumption has erroneously been made in the past that the mouse had the same nutritional requirements as the rat. Bauer (1943) first reported the ability of the mouse to synthesize arginine in contrast to the rat. Recent work in the field of mouse nutrition has produced data elucidating the amino acid requirements of laboratory mice (John and Bell, 1976) and recommendations for dietary levels of crude protein in open-formula rations on several strains of mice (Hoag and Dickie, 1962; Bell, 1972; Knapka, et al., 1977). Knapka, et al. (1974) published standards for an open-formula ration found to be supportive of growth and reproduction in three strains of laboratory mice. Differences in response to dietary regimes of the various inbred strains havebeen well documented (Hoag and Dickie, 1962; Knapka, et al., 1974; Fenton and Carr, 1951).

Reproduction and Protein Requirements

Vandenbergh et al. (1972) fed 8, 16, and 24% crude—protein diets to albino mice. The maximum production of litters was maintained on the 24% protein diet. Littersize and weight of the young at birth were similar among the three diets. This was in contrast to the work by

Hoag and Dickie (1960) who showed the best reproductive performance based on birth totals and weights, weaning totals and weights, and littersize at birth and at weaning on a diet containing 17% protein with 11.6% fat in two inbred strains of mice.

These same workers (Hoag and Dickie, 1962) showed large differences among strains in response to protein-fat interaction. Knapka et al. (1977) showed a significant fat × protein interaction with neither nutrient having a significant effect singly. Bell (1972) showed 17% protein to be as effective as 19 or 21% protein for reproduction.

Growth and Protein Requirements

Growth definition. Growth was defined as protein accretion by Eisen (1974) and the use of the measurement of growth by increases in weight was supported. Miller (1970) has suggested that weight is not the ideal measure of growth because it does not exclude fat deposition. He also demonstrated a direct relationship between weight gain and protein intake in the rat beginning with a normal concentration and decreasing the concentration. Bergen (1974) has pointed out that protein and fat accretion occur simultaneously during early growth and that protein accretion becomes negligible in later growth.

Sex and strain differences. Sex and strain differences affect the rate of growth. Male mice have a more rapid post-weaning growth rate (Timon, 1968) while female mice reach their asymptotic weight at an earlier age (Eisen, 1974). Henderson and Titus (1968) plotted growth curves for germfree and conventional mice. The body weights of the

germfree mice were greater at all ages (up to 72 days of age) than those of the conventional mice. The female mice were lighter than the males after 48 days in both the conventional and germfree animals.

Organ weights. Doljanski (1960) reported that high protein diets elicited increase in liver proteins as well as large increases in liver weight and liver cell mitosis in rats. Pregnancy increased the relative liver weight and liver protein content. Priestly and Robertson (1973) showed that the water content of liver, spleen and heart in mice stayed at a constant percentage and that the protein percentage went up slightly with increasing age. Absolute amounts of all components of the organs except for DNA appeared to either increase or decrease in proportion to weight. Stein et al. (1976) stated that most nitrogen loss on protein or calorie deficient diets was from muscles. These workers also reported that protein synthesis decreased with low calorie intake, whereas nitrogen deficiency had little effect on protein systhesis although it did increase protein catabolism in the liver.

Preweaning environment influence on postweaning growth. Rutledge et al. (1972) reported that, up to the age of seven weeks, growth was influenced more by maternal effects than by genetic differences. Monteiro and Falconer (1966) had reported previously that the maternal influence on postweaning growth declined after four and one-half weeks. These workers termed the reduction of the environmental component of variance as compensatory growth, and claimed that the maternal effects were the most important natural source of variation in the environment of the

mouse. Mixing of litters to attain a constant cage density resulted in decreased weight gains between 21 and 42 days of age and decreased adult body weights at 63 days of age as compared to housing litters in separate cages (Doolittle et al., 1976).

Protein requirements. There are varying recommendations in the literature for the optimum dietary protein content for growth in different strains of mice. Bell (1972) in the NRC Standards states that 17% crude protein is adequate for growth. Goodrick (1973) reported that mice maintained throughout growth on low protein diets (4% casein) had lower body temperatures and higher oxygen consumption rates than mice maintained throughout growth on adequate protein diets (26% casein). The mice fed the lower protein level had higher food intake per gram of body weight and still maintained a modest level of growth. Knapka, et al. (1977) showed a significant decrease in the mean weanling weight of pups from four strains when the dietary crude protein increased from 18 to 24%. These workers showed a significant strain × protein interaction.

III. Effects of Dietary Protein on Disease Susceptibility

Using 5, 8, and 20% casein diets Schaedler and Dubos (1959) showed that young mice maintained on these diets for 1-6 weeks had differences in susceptibility to bacterial infections. The 5 and 8% casein-fed animals were more susceptible than the 15 or 20% casein-fed animals. Feeding inbalanced proteins also had an adverse effect on susceptibility

to infection. No correlation was shown between weight gain and resistance to bacterial infections. The effects were pronounced even when the mice were placed on the diets at the time of inoculation.

In contrast to the usual effects of dietary deficiency which render animals insusceptible to viral infection, Ruebner and Bramhal (1959) found that the mortality rate in Swiss Webster mice infected with mouse hepatitis virus on a high protein diet (26% casein) was one-half the mortality rate of the mice on a low protein diet (8% casein). These workers hypothesized that the high protein diet was protective against hepato-toxic agents. In direct contrast to this work, Schneider (1960) reported that the effects of nutritional factors on resistance are on survival time and not on frequency of survival. The reduction of protein leads to an increase in heat output per calorie ingested, and this increased basal metabolic rate may be related to the changes in survival time or disease susceptibility (Schneider, 1960).

II. Introduction

It has been demonstrated repeatedly by many different researchers (Fenton and Carr, 1954; Hoag and Dickie, 1960; Knapka et al., 1977) that inbred strains of mice have different dietary requirements for optimum reproduction and growth, and that these requirements differ among the strains. Although this fact is well documented in the literature, the same ration is routinely fed to mice of all strains and in all phases of growth, maintenance or reproduction.

Because of increasing interest in the mouse mutant gene nu and due to expense involved in the production of nude mice, there is a need to breed and raise these animals more efficiently. Therefore, this experiment was conducted to study the effect of rations containing three dietary levels of protein on the reproductive performance of male nude mice and their heterozygous female siblings. This effort was designed as a beginning to define optimal diets for reproduction of breeders and preweaning growth of nude mouse pups.

III. Materials and Methods

Diet Formulation

The three diets, calculated to contain 12, 18, and 24% crude protein, were formulated by Zeigler Brothers, Inc.[2] according to the specifications shown in Table 1. The rations were patterned after those shown by Knapka et al. (1974) to be adequate for reproduction in three

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[2]Zeigler Brothers, Incorporated, Gardner, Pennsylvania.

TABLE 1

RATION COMPOSITION

Ingredient (%)	Crude Protein Concentration (%) 12	18	24
Fish meal (60% protein)	5.00	8.50	15.00
Soybean oil meal (50% protein)	2.50	7.00	12.00
Alfalfa meal (17% protein)	4.00	5.00	5.00
Corn gluten meal	0.50	1.50	3.00
Ground corn	43.50	29.00	20.00
Ground winter wheat	15.75	21.75	18.00
Wheat middlings	5.00	10.00	10.00
Brewers dried yeast	0.50	2.00	2.00
Corn starch	17.50	10.75	12.00
Soybean oil	1.25	1.25	1.00
Salt	0.75	0.50	0.50
Dicalcium phosphate	3.00	2.00	1.25
Ground limestone	0.50	0.50	---
Premixes (mineral and vitamin)	0.25	0.25	0.25
Total	100.00	100.00	100.00

strains of laboratory mice and conformed to NRC standards (Bell, 1962). The main sources of protein in the three rations were fish meal, soybean oil meal, alfalfa meal, wheat middlings, Brewers dried yeast, ground winter wheat and ground corn. Soybean oil meal, soybean oil and fish meal were the major sources of fat in all three diets. Corn starch was used to supply additional calories to make the three rations approximately isocaloric. The fat and fiber percentages of the three rations were approximately equal (Table 2), although the fiber content of the 12% ration was somewhat lower than that of the 18 and 24% rations due to the large amount of corn starch in that ration.

TABLE 2

FAT AND FIBER CONTENT OF THE RATIONS

Constiuent (%)	Ration Protein Percentage		
	12	18	24
Fat	3.84	3.94	4.04
Fiber	3.17	3.98	3.85

The amounts and ratios of essential amino acids as calculated from feed composition tables (Cramption and Harris, 1969) were adequate even in the 12% ration (John and Bell, 1976). The amino acid content present in the three rations is shown in Table 3. The 12% ration contained approximately 50% and the 18% ration approximately 75% the amounts of the essential amino acids found in the 24% ration. No attempt was made to account for possible amino acid or protein destruction which may have occurred during the autoclaving process. Mineral and vitamin fortification (calculated total content and the concentration present in the

TABLE 3

AMINO ACID CONTENT OF RATIONS (%)

Amino Acid	12% Protein	(% of 24% Ration)	18% Protein	(% of 24% Ration)	24% Protein
Arginine	.635	(44.6)	1.01	(71.0)	1.423
Cystine	.196	(55.5)	.274	(77.6)	.353
Glycine	.651	(48.2)	.988	(73.1)	1.351
Histidine	.309	(52.1)	.444	(74.9)	.593
Isoleucine	.596	(43.7)	.965	(70.7)	1.365
Leucine	1.105	(53.4)	1.545	(74.6)	2.070
Lysine	.586	(41.6)	.982	(69.8)	1.407
Methionine	.276	(52.6)	.379	(72.2)	.525
Phenylalanine	.061	(50.6)	.892	(75.1)	1.187
Threonine	.472	(47.2)	.732	(73.2)	1.00
Tryptophane	.144	(50.5)	.218	(76.5)	.285
Tyrosine	.422	(53.1)	.606	(76.1)	.796
Valine	.623	(47.5)	.959	(73.2)	1.311

fortified ration before autoclaving) is presented in Tables 4 and 5. Allowances were made for the destruction of Vitamin A, thiamin, and Vitamin K during the sterilization process.

Preparation and Feeding of Diets

The diets were autoclaved at 121°C for 15 minutes at 15 psi in a steam autoclave.[3] The rations were fed in pelleted form ad libitum.

Mice

The mice used in the reproduction experiment were obtained from the nude mouse colony at the University of Tennessee Memorial Research Center[4] and were in the fifth generation of inbreeding (brother × sister matings). The mice were of BALB/c background onto which the nude gene had been bred. As the colony was very small, it was necessary to acquire the mice used in this experiment over a period of time. To avoid seasonal effects, a breeding trio (one nude male and two of his heterozygous female siblings) was weaned and assigned to each of the three treatment groups on the same day. Over a period of several weeks, seven breeding trios were assigned to each dietary group.

To increase the longevity of the male nude mouse, he was transplanted at weaning with the thymus from a male heterozygous littermate sibling (McCann and Sussdorf, 1974). The recipient mice were anesthetized with methoxyfluorane[5] during the surgical procedure. The donor mouse was

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[3]American Sterilizer, Erie, Pennsylvania.

[4]University of Tennessee Memorial Research Center, Knoxville, Tennessee.

[5]Metafane, Pitman-Moore, Inc., Washington Crossing, N. J.

TABLE 4

MINERAL FORTIFICATION/100 LBS RATION, TOTAL/100 LBS RATION AND CONCENTRATION IN 12, 18 AND 24% PROTEIN RATIONS[1]

	12%			18%			24%		
	Fortification	Total	Concentration	Fortification	Total	Concentration	Fortification	Total	Concentration
Calcium	---	1.275 lbs	1.275%	---	1.248 lbs	1.248%	---	1.24 lbs	1.24%
Phosphorus	---	.989 lbs	.989%	---	1.005 lbs	1.005%	---	1.08 lbs	1.08%
Cobalt	20.0 mg	29.6 mg	.65 PPM	20 mg	32.2 mg	.71 PPM	20 mg	30.5 mg	.67 PPM
Copper	470 mg	731 mg	16.1 PPM	380 mg	746 mg	16.4 PPM	325 mg	743 mg	16.3 PPM
Iron	---	18,130 mg	3.99 PPM	---	15,284 mg	337 PPM	---	12,909 mg	284 PPM
Magnesium	---	.165 lbs	.165%	---	.181 lbs	.181%	---	.161 lbs	.161%
Manganese	3,450 mg	4731 mg	104 PPM	3,000 mg	4,635 mg	102 PPM	3,000 mg	4,556 mg	100 PPM
Potassium	180 g	.828 lbs	.828%	100 g	.833 lbs	.833%	90 g	.886 lbs	.886%
Sodium	---	.371 lbs	.371%	---	.328 lbs	.328%	---	.389 lbs	.389%
Zinc	1,000 mg	2,276 mg	50.1 PPM	500 mg	2,267 mg	49.9 PPM	250 mg	2,287 mg	50.4 PPM
Iodine	70 mg	80.1 mg	1.76 PPM	65 mg	79.6 mg	1.7 PPM	60 mg	82 mg	1.8 PPM

[1]Ingredients prior to autoclaving.

TABLE 5

VITAMIN FORTIFICATION/100 LBS RATION, TOTAL/100 LBS RATION AND CONCENTRATION IN 12, 18 AND 24% PROTEIN RATIONS

	12%			18%			24%		
	Fortification	Total	Concentration	Fortification	Total	Concentration	Fortification	Total	Concentration
Vitamin A[1]	776,000 IU	1,362,764 IU	30.0 IU/g	660,00 IU	1,360,343 IU	30.0 IU/g	645,000 IU	1,363,340 IU	30.0 IU/g
Biotin	10 mg	12.75 mg	.28 PPM	5 mg	10 mg	.22 PPM	5 mg	12 mg	.26 PPM
Choline	58 g	91,439 mg	2,014 PPM	40 g	90,766 mg	2,000 PPM	27 g	91,384 mg	2,012 PPM
B_{12}	650 mcg	1,200 mcg	12 mcg/lb	300 mcg	1,235 mcg	12 mcg/lb	---	1,650 mcg	16.5 mcg/lb
Folic acid	160 mg	186 mg	4.1 PPM	120 mg	186 mg	4.1 PPM	100 mg	180 mg	3.9 PPM
Niacin	2,350 mg	3,714 mg	81.8 PPM	1,600 mg	3,692 mg	81.3 PPM	1,550 mg	3,714 mg	81.8 PPM
Pantothenic acid	700 mg	978 mg	21.5 PPM	550 mg	972 mg	21.4 PPM	500 mg	958 mg	21.1 PPM
Pyridoxine	150 mg	468 mg	10.3 PPM	50 mg	457 mg	10.1 PPM	---	576 mg	12.6 PPM
Riboflavin	300 mg	380 mg	8.4 PPM	250 mg	381 mg	8.4 PPM	250 mg	397 mg	8.7 PPM
Thiamin[2]	2.1 g	2,275 mg	50.1 PPM	2 g	2,264 mg	49.8 PPM	2 g	2,244 mg	49.4 PPM
Vitamin E	1,000 mg	1,619 mg	35.7 PPM	900 mg	1,629 mg	35.8 PPM	900 mg	1,626 mg	35.8 PPM
Vitamin D	180,000 IU	180,908 IU	4.0 IU/g	180,000 IU	181,135 IU	4.0 IU/g	180,000 IU	181,135 IU	4.0 IU/g
Vitamin K[3]	700 mg	700 mg	15.4 PPM	700 mg	700 mg	15.4 PPM	700 mg	700 mg	15.4 PPM

[1]Amount present was double the requirement (Knapka et al., 1974) to allow for destruction during autoclaving.

[2]Amount present was seven times the requirement (Knapka et al., 1974) to allow for destruction during autoclaving.

[3]Amount present was five times the requirement (Knapka et al., 1974) to allow for destruction during autoclaving.

sacrificed and his thymus asceptically removed and placed in sterile physiological saline solution while a small axillary incision was made in the recipient mouse. The thymus was then implanted subcutaneously and a two-suture closure was made. The transplanted mice were placed in clean cages with their two sisters to comprise the breeding trio. Sutures were removed after five days.

Husbandry

Breeding trios were changed to clean cages with clean bedding[6] twice each week. Fresh chlorinated water (15-20 ppm chlorine) and clean water bottles were supplied twice weekly. Food was supplied ad libitum. As the females became pregnant, paper towels were provided as nesting materials. Each breeding cage was checked daily for new litters and upon birth of a litter, the date of birth, number of pups in the litter, and the litter weight were recorded. The litters were weighed and counted every seven days until weaning at 21 days of age. At weaning, the sex and genotype of the mice and their individual weights were recorded.

Termination of Experiment

The experiment was terminated when the food was used up or before this time, if the mice failed to reproduce. All breeding animals were sacrificed, organ weights determined and blood parameters measured. Serum protein was measured in g/100 ml of blood using a temperature

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[6]Ab-sorb-dri® Michael Woods Products, Inc., Garfield, New Jersey.

compensated TS Meter.[7] Hemoglobin was measured in g/100 ml of blood using an Hb-Meter.[8] All blood samples were drawn from the retroorbital plexus into micro-capillary tubes, using heparinized tubes for the hematocrit readings[9, 10] and nonheparinized tubes for the serum protein and Hb readings. Organs weighing less than one-half gram were weighed on a torsion balance,[11] those weighing more than one gram were weighed on a trip balance.[12]

Liver, kidney, thyroid, and ovary or testicle tissues were taken for three males and three females on the 12% protein diet, three females and two males on the 18% protein diet, and two females and one male on the 24% protein diet. The tissues were fixed in 10% buffered formalin, embedded in parafin, sectioned at 6μ thickness and stained with hemotoxylin and eosin for histological examination.

Statistical Analysis

The dependent variables were divided into three categories for analysis. The first category was the breeding female data which included

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[7]TS Meter, American Optical Corporation, Scientific Instrument Division, Buffalo, New York.

[8]Hb-Meter, American Optical Corporation, Buffalo, New York.

[9]International (IEC) Micro-Capillary Centrifuge, Model M.B., Needham Hts., Mass.

[10]International (IEC) Micro-Capillary Reader, Needham Hts., Mass.

[11]Precision Torque Balance, Vereenigde Draadfabrieken Nijmegen, Holland.

[12]Harvard Trip Balance, Ohaus Scale Corporation, Union, New Jersey.

the number of breeding months each female was on treatment, the age at birth of first litter, the average litter size, the number of litters born per month, the number of pups born per month, the number of mice weaned per month, the percent of the total weaned that were nude, the number dead at birth, the number dead at weaning and the number of whole litters lost. The second category consisted of the individual pup data from birth to weaning which was obtained by dividing the total litter weight or gain by the number of pups in the litter. The third category consisted of blood parameters and organ weights as a percentage of body weight for both male and female breeders. A one-way analysis of variance was performed to assess the effect of ration protein level on performance and physiological parameters. When significant F values were obtained from the analysis of variance, Student-Newman-Keuls test was used for mean separation and the Studentized range values were used to assign significance to the means (Sokal and Rohlf, 1969).

IV. Results and Discussion

Breeding Female Data

There were no statistically significant differences due to the ration protein level for any of the parameters used to evaluate the reproductive performance of the breeding females (Table 6). The average litter size, number of litters born per month, number of pups born per month and number of pups weaned per month tended to be somewhat lower for the 12% protein females. This lack of significance may have been due partly to the small sample size (14 females per treatment).

TABLE 6

BREEDING FEMALE DATA[1]

Variable	Probability of F[2]	12% Protein	18% Protein	24% Protein
No. of breeding months	NS[3]	6.08	6.14	6.27
Age at birth of first litter, days	NS	82.29	79.31	77.00
Average litter size	NS	7.38	8.46	8.16
No. litters born/month	NS	.489	.559	.540
No. pups born/month	NS	3.51	4.71	4.27
No. pups weaned/month	NS	2.63	3.82	3.19
% weaned of those born	NS	77.22	80.72	76.51
% nudes weaned of total weaned	NS	43.69	42.27	45.27
No. dead at birth	NS	1.57	2.14	1.47
No. dead at weaning	NS	1.43	2.71	3.50
No. whole litters lost	NS	.286	.21	.21

[1]All figures averaged for the 14 females on each treatment.

[2]Probability of the F ratio (treatment mean square/error mean square).

[3]Not significant, $P > 0.25$.

The 12% crude protein level is considered inadequate for optimum reproduction in most strains of laboratory mice (Geottsch, 1960; Hoag and Dickie, 1962).

Individual Pup Data from Birth to Weaning

There were no significant differences among treatments with respect to average pup weight at birth (Table 7). All other parameters were significantly affected by ration protein level (ranging in significance from the .025 to the .001 level of probability). The 12% protein pups were consistently lower in weight at 1, 2, and 3 weeks of age than either the 18 or the 24% protein pups. No differences were observed between the 18 and the 24% protein pups with respect to weight at those ages. Average gain per pup during weeks 1 and 2 was significantly lower for the 12% pups and similar for the 18 and 24% pups. The 12% pups gained significantly more during week 3 than either the 18 or 24% pups. The average total gain per pup was significantly higher for the 18 and 24% pups than for the 12% pups. Litter weight at weaning was shown to be an adequate measure of milk production by Nagai (1971) and Jara-Almonte and White (1972). Lower weaning weight could have resulted because either the amount of milk or the quality of the milk produced by mice fed the 12% protein diet was less supportive of growth than the milk produced by those fed the 18 or 24% protein diets.

Blood Parameters and Organ Weights as a Percentage of Bodyweight for Male and Female Breeders

Effects of ration protein level on blood parameters and organ weights (expressed as a percentage of bodyweight) for the male and female

TABLE 7

INDIVIDUAL PUP DATA FROM BIRTH TO WEANING

Variable	Probability of F[1]		12% Protein	18% Protein	24% Protein
Average weight/pup at birth	NS[3]	---	1.46	1.49	1.53
Average weight/pup at week 1	P < .05	.05	4.22[a]	4.73[b]	4.73[b]
Average weight/pup at week 2	P < .001	.01	6.42[a]	7.94[b]	8.02[b]
Average weight/pup at week 3	P < .025	.01	8.75[a]	9.74[b]	10.02[b]
Average gain/pup for week 1	P < .05	.01	2.76[a]	3.23[b]	3.20[b]
Average gain/pup for week 2	P < .001	.01	2.20[a]	3.20[b]	3.29[b]
Average gain/pup for week 3	P < .025	.05	2.33[b]	1.80[a]	1.99[a]
Average total gain/pup	P < .025	.01	7.31[a]	8.23[b]	8.48[b]

[1]Probability of the F ratio (treatment mean square/error mean square).

[2]Probabilities of the Studentized range.

[3]Not significant, P > 0.25.

[a], [b]Values within a row not sharing a common superscript are significantly different.

breeders are shown in Table 8. Variation in ration protein level did not significantly affect any of the parameters studied for the female breeders. The male animals showed a significant difference in spleen weight as a percentage of bodyweight ($P < .10$) with the 24% protein nude males having the heavier spleens, while spleen weights of 12 and 18% protein nude males were similar. All other parameters measured for the nude male breeders were not affected by treatment. Although no macroscopic or microscopic lesions were found in the samples taken, the possibility remains that spleen enlargement in the 24% protein male nude mice was associated with a disease process.

TABLE 8

BLOOD PARAMETERS AND ORGAN WEIGHTS AS PERCENTAGE OF BODYWEIGHT FOR MALE AND FEMALE BREEDERS

	Males (Nu/Nu)					Females (Nu/+)				
Parameter	Probability of F[1]	α[2]	12% Protein	18% Protein	24% Protein	Probability of F	α[2]	12% Protein	18% Protein	24% Protein
Right kidney % body weight	NS[3]	---	.916	.866	.870	NS	---	.68	.66	.63
Spleen % body weight	P < .10	.05	.355[a]	.378[a]	.650[b]	NS	---	.396	.366	.400
Liver % body weight	NS	---	5.71	5.45	5.18	NS	---	6.08	5.73	5.98
Hematocrit	NS	---	47.0	46.25	44.86	NS	---	45.81	47.50	47.77
Hemoglobin (g/100 ml)	NS	---	14.50	15.50	13.58	NS	---	15.25	15.33	16.13
Serum protein (g/100 ml)	NS	---	5.60	5.62	5.54	NS	---	5.34	5.49	5.50

[1]Probability of the F ratio (treatment mean square/error mean square).

[2]Probabilities of the Studentized range. [3]Not significant P > 0.25.

[a], [b]Values within a row not sharing a common superscript are significantly different.

CHAPTER IV

GROWTH OF NU/NU AND NU/+ MICE FED THREE LEVELS OF CRUDE PROTEIN

I. Summary

Three rations containing 12, 18, and 24% crude protein levels were fed to 128 nude females, 103 nude males, 126 nu/+ females and 102 nu/+ males for one month (28 days) following weaning at 21 days of age. The weanling mice were maintained on the same level of dietary crude protein that had been fed to their parents. The mice were weighed every four days and food consumption was calculated by four-day periods. At the end of the 28-day feeding trial, the mice were sacrificed, their organs weighed, and serum protein levels recorded.

The nu/+ female mice grew faster on the 24% protein ration than on the 12 and 18% ration, while the nu/+ male mice grew best on the 18% protein ration. Nude mice, many of which succumbed to wasting disease (thought to be mouse hepatitis virus), gained best on the 12% ration (females) and on the 18% ration (males). Mice which ceased to grow, lost weight and became moribund were included when calculating the mean. When these mice were removed from the data, the nude females grew best during the postweaning period on 24% protein while the male nudes still grew best on the 18% protein. The incidence of wasting disease, death, and necrotic liver lesions was lowest in the 12% protein nude females (5.6%). The 12% nude males had the next lowest incidence (23.8%)

followed by 18% nude males (38.1%), 24% nude females (41.0%), 18% nude females (48.1%) and 24% nude males (51.2%).

II. Introduction

The production of nude mice for experimental purposes in conventional and specific pathogen free environments requires special husbandry practices (Eaton et al., 1975; Hetherington and Hegen, 1975). These mice are athymic and are plagued by viral, bacterial and parasitic infections which cause few problems in immunologically competent mice. This immunological incompetence results in a high incidence of mortality in the nude mouse (Tamara, et al., 1977; Flanagan, 1966). The nutritional requirements of this mouse mutant may differ from those of other mice. In order to investigate this possibility, a growth experiment was conducted to determine the effect of rations containing 12, 18 and 24% crude protein on the performance of weanling nu/nu and nu/+ mice during a four-week postweaning period.

III. Materials and Methods

Diet Formulation

The three diets used in this growth experiment were the same as those used in the reproduction experiment detailed in Chapter III, pages 11, 12, 13, 15 and 16.

Preparation and Feeding of Diets

The diets were autoclaved prior to feeding in an American Sterilizer autoclave. After autoclaving, the pelleted food was crushed to a fine

powder and placed in glass jars covered with a small mesh hardware cloth with an opening .5 cm × .5 cm cut in the center of the screen. These feeders were designed to allow the mice easy access to the food, but to cut down on wasting and scattering of food in order to obtain more accurate feed consumption data. As the level of food in the jar dropped, the screen level dropped also so that it always rested on the food and the animals were never without food.

Mice

At 21 days of age (weaning age) the mice were removed from the breeding cages used in the reproduction experiment as they became available over a seven month period of time. The weanling mice were fed the same level of crude protein during the postweaning studies that was fed to their parents.

Husbandry

The mice were separated according to sex and genotype and housed in groups of two to eight. Feed jars were filled with 50 grams of feed. Every four days the mice were weighed, the remaining food in the feed container was weighed, and the jars were refilled with 50 grams of fresh food. The cages were changed and fresh chlorinated water supplied twice weekly as detailed in Chapter III, page 17. Deaths and abnormalities were recorded as they occurred, and the nude mice which died while on experiment were posted and checked for macroscopic liver lesions.

Termination of Experiment

At the end of the 28-day postweaning growth period, the experimental mice were sacrificed. A final weight and feed consumption was recorded, serum protein was measured and liver, spleen and kidney weights were recorded. Any macroscopic pathology was recorded. Macroscopic detection of lesions ranging from small white foci to large areas of pitted, irregular surfaces was the criterion used to classify the livers as diseased. Representative samples of organs from both moribund and healthy, nu/nu and nu/+ mice were fixed in 10% buffered formalin, embedded in parafin, sectioned 6μ thick, and stained with hemotoxylin and eosin for histological examination.

Statistical Analysis

Three groups of dependent variables were used to evaluate the performance of the nu/nu and the nu/+ mice on the three protein levels. The first group included average food consumption in grams per mouse per day and feed efficiency (grams of feed/grams of gain). The second group included the variables initial weight at 21 days of age, gain by 4-day periods, and total gain for the entire 28-day period. The third group of parameters included spleen weight as a percentage of body bodyweight, liver weight as a percentage of bodyweight, kidney weight as a percentage of bodyweight plus serum protein in grams per 100 ml of blood.

A one-way analysis of variance was performed on these variables with the percentage protein in the ration as the independent variable holding sex and genotype constant. A second one-way analysis of

variance was performed on the same variables using the sex and genotype of the mice as the independent variable, holding the percent protein in the diet as constant. When significant F ratios were obtained from the analysis of variance, the Student-Newman-Keuls test was used for mean separation and significance levels were assigned to the means (Sokal and Rohlf, 1969).

IV. Results

Performance of Nude Female Mice

Initial weight and gain during the first four periods were similar for mice fed the three rations (Table 9). Gain during the last three 4-day periods and total gain for the entire 29-day period were significantly higher in nude females fed the 12% CP ration. Spleen, liver and kidney weight, expressed as a percentage of body weight, and serum protein level were significantly lower in the 12% mice than in either the 18 or 24% mice. The 18 and 24% mice had similar spleen and liver weights but the kidney weight of the 24% group was significantly higher than that of the 18% group.

Performance of Nu/+ Female Mice

Gain of 12% mice was significantly lower than that of the 24% mice during the first three postweaning periods and higher than the gain of 24% mice in the next three periods and not different in the final period (Table 10). The weight gain of the 18% mice during the seven four-day periods was erratic. Total gain was lowest in 12% mice, highest in the 18% mice and intermediate in the 24% mice. No ration differences were

TABLE 9

EFFECTS OF PROTEIN LEVEL ON FOOD CONSUMPTION, GAIN, ORGAN WEIGHTS AND SERUM PROTEIN IN NU/NU FEMALE MICE

Variable	Probability of F[1]	DF[2]	α[3]	Crude Protein (%) 12	18	24
Average food consumption (g/day/mouse)	P < .025	24	.05	3.25[b]	2.65[a]	3.15[b]
Feed efficiency (G feed/g gain)	P < .001	97	.01	9.94[b]	8.38[a]	12.12[c]
Initial weight (g)	NS[4]	124	---	6.55	6.87	6.9
Gain (g) days 21-25	NS	119	---	1.40	1.51	1.38
Gain (g) days 25-29	NS	115	---	1.32	1.15	1.45
Gain (g) days 29-33	NS	113	---	2.24	2.38	2.35
Gain (g) days 33-37	NS	114	---	1.44	1.67	1.36
Gain (g) days 37-41	P < .025	107	.01	1.17[b]	.54[a]	.55[a]
Gain (g) days 41-45	P < .05	105	.05	.88[b]	.07[a]	.30[a]
Gain (g) days 45-49	P < .05	94	.05	.97[b]	.41[a]	.11[a]
Total gain (g)	P < .025	97	.05	9.38[b]	8.38[a]	8.46[a]
Spleen percentage bodyweight	P < .001	88	.01	.56[a]	.85[b]	.89[b]
Liver percentage bodyweight	P < .1	88	.05	5.67[a]	6.16[b]	6.14[b]
Kidney percentage bodyweight	P < .001	88	.01	.67[a]	.76[b]	.82[c]
Serum protein (g/100 ml)	P < .10	91	.05	4.86[a]	5.09[b]	5.04[b]

[1]Probability of the F ratio (treatment mean square/error mean square).

[2]Degrees of freedom.

[3]Probabilities of the Studentized range.

[4]Not significant, P > 0.25.

a, b, [c]Values within a line not sharing a common superscript are significantly different.

TABLE 10

EFFECTS OF PROTEIN LEVEL ON FOOD CONSUMPTION, GAIN, ORGAN WEIGHTS AND SERUM PROTEIN IN NU/+ FEMALE MICE

Variable	Probability of F[1]	DF[2]	α[3]	Crude Protein (%) 12	18	24
Average food consumption (g/day/mouse)	P < .10	18	.05	3.55[b]	3.1[a]	3.4[b]
Feed efficiency (g feed/g gain)	P < .001	122	.01	12.3[c]	10.1[a]	11.3[b]
Initial weight (g)	P < .001	125	.01	9.8[a]	11.0[b]	11.9[c]
Gain (g) days 21-25	P < .025	125	.01	1.4[a]	1.5[a]	1.9[b]
Gain (g) days 25-29	P < .10	125	.01	1.9[a]	1.9[a]	2.2[b]
Gain (g) days 29-33	P < .001	125	.05	1.5[a]	2.5[c]	2.2[b]
Gain (g) days 33-37	P < .001	125	.05	1.4[b]	1.8[c]	1.1[a]
Gain (g) days 37-41	P < .001	125	.01	.84[c]	.1[a]	.5[b]
Gain (g) days 41-45	P < .05	125	.05	.8[b]	.7[b]	.4[a]
Gain (g) days 45-49	NS[4]	125	---	.3	.2	.1
Total gain (g)	P < .025	125	.05	8.01[a]	8.7[b]	8.5[b]
Spleen percentage bodyweight	NS	124	---	.46	.46	.5
Liver percentage bodyweight	P < .001	125	.05	4.7[a]	4.9[b]	5.3[c]
Kidney percentage bodyweight	P < .001	123	.01	.6[a]	.7[b]	.7[b]
Serum protein (g/100 ml)	P < .001	122	.01	5.18[a]	5.4[b]	5.6[c]

[1]Probability of the F ratio (treatment mean square/error mean square).

[2]Degrees of freedom.

[3]Probabilities of the Studentized range.

[4]Not significant, P > 0.25.

a, b, [c]Values within a line not sharing a common superscript are significantly different.

observed in spleen weight as a percentage of bodyweight. Liver weight and serum protein level increased significantly as the protein level increased from 12 to 18 to 24%. Kidney weight was significantly lower in 12% mice.

Performance of Nude Male Mice

Initial weight of the 12 and 24% groups was significantly lower than in the 18% group (Table 11). During the first three periods, 12% mice gained less than the 18% mice and less than or equal to the 24% mice. During the next two growth periods the 12% mice gained more than the 24% mice and more than or equal to the 18% mice. In the final period, gain was similar among treatments. Total gain was significantly lower in the 12 and 24% groups than in the 18% group. Weight was significantly higher in the 24% protein group. Serum protein was significantly higher in the 18% mice.

Performance of Nu/+ Male Mice

Initial weight of the 12% protein group of mice was significantly lower than that of the other groups (Table 12). Total gain was lowest in the 12% protein mice and highest in 18% protein mice. Gain during the seven growth periods was erratic, but the 12% protein mice tended to gain less than the 18 or the 24% mice. Spleen weights of mice fed the three rations were not significantly different. Liver as a percentage of bodyweight was significantly higher in the 24% group. Kidney weight was lowest on 12% protein, intermediate on 18% protein and highest on 24% protein.

TABLE 11

EFFECTS OF PROTEIN LEVEL ON FOOD CONSUMPTION, GAIN, ORGAN WEIGHTS AND SERUM PROTEIN IN NU/NU MALE MICE

Variable	Probability of F[1]	DF[2]	α[3]	Crude Protein (%) 12	18	24
Average food consumption (g/day/mouse)	NS[4]	21	---	3.17	3.19	3.11
Feed efficiency (g feed/g gain)	NS	85	---	9.42	8.86	9.81
Initial weight (g)	P < .005	99	.01	6.36[a]	7.70[b]	6.70[a]
Gain (g) days 21-25	P < .005	89	.01	1.35[a]	2.06[c]	1.71[b]
Gain (g) days 25-29	P < .005	88	.01	1.50[a]	2.22[b]	1.35[a]
Gain (g) days 29-33	P < .10	88	.05	2.18[a]	2.95[b]	2.18[a]
Gain (g) days 33-37	P < .01	84	.05	1.12[a]	1.56[a]	2.29[b]
Gain (g) days 37-41	P < .025	83	.05	1.75[b]	.82[a]	1.04[a]
Gain (g) days 41-45	P < .025	81	.05	1.22[b]	1.04[b]	.62[a]
Gain (g) days 45-49	NS	58	---	.33	-.26	-.005
Total Gain (g)	P < .025	79	.05	10.0[a]	11.50[b]	10.22[a]
Spleen percentage bodyweight	NS	71	---	.584	.75	.72
Liver percentage bodyweight	NS	71	---	5.76	6.10	6.10
Kidney percentage bodyweight	P < .10	71	.05	.83[a]	.86[a]	.93[b]
Serum protein (g/100 ml)	P < .001	70	.01	4.87[a]	5.28[b]	5.0[a]

[1]Probability of the F ratio (treatment mean square/error mean square).

[2]Degrees of freedom.

[3]Probabilities of the Studentized range.

[4]Not significant, P > 0.25.

[a], [b], [c]Values within a line not sharing a common superscript are significantly different.

TABLE 12

EFFECTS OF PROTEIN LEVEL ON FOOD CONSUMPTION, GAIN, ORGAN WEIGHTS AND SERUM PROTEIN IN NU/+ MALE MICE

Variable	Probability of F[1]	DF[2]	α[3]	Crude Protein (%) 12	18	24
Average food consumption (g/day/mouse)	NS	16	---	4.26	4.16	4.01
Feed efficiency (g feed/g gain)	P < .001	99	.01	10.8^{c}	8.4^{a}	9.1^{b}
Initial weight (g)	P < .10	100	.05	10.77^{a}	11.5^{b}	11.9^{b}
Gain (g) days 21-25	P < .001	100	.01	1.5^{a}	2.7^{b}	2.7^{b}
Gain (g) days 25-29	P < .001	100	.05	2.1^{b}	1.9^{a}	2.8^{c}
Gain (g) days 29-33	P < .005	98	.01	1.1^{a}	2.9^{b}	3.5^{c}
Gain (g) days 33-37	P < .001	99	.01	1.5^{a}	2.7^{b}	1.3^{a}
Gain (g) days 37-41	P < .001	99	.01	1.4^{c}	$.97^{b}$	$.6^{a}$
Gain (g) days 41-45	P < .025	99	.05	1.7^{c}	1.3^{b}	$.8^{a}$
Gain (g) days 45-49	P < .10	99	.05	$.98^{a}$	1.2^{b}	$.7^{a}$
Total gain (g)	P < .001	99	.01	10.93^{a}	13.7^{c}	12.28^{b}
Spleen percentage bodyweight	NS	87	---	.35	.34	.34
Liver percentage bodyweight	P < .025	87	.05	4.82^{a}	5.04^{a}	5.3^{b}
Kidney percentage bodyweight	P < .005	86	.05	$.73^{a}$	$.78^{b}$	$.8^{c}$
Serum protein (g/100 ml)	P < .10	84	.05	5.37^{a}	5.6^{b}	5.6^{b}

[1]Probability of the F ratio (treatment mean square/error mean square).

[2]Degrees of freedom.

[3]Probabilities of the Studentized range.

[4]Not significant, P > 0.25.

a, b, [c]Values within a line not sharing a common superscript are significantly different.

Performance of Four Sex-genotype Combinations

Males tended to be heavier and gain faster than females regardless of genotype (Tables 13, 14 and 15) and nu/+ animals tended to be heavier and gain faster than nu/nu animals regardless of sex. The exception was the nude females which tended to weigh less at weaning but gained similarly to the nu/+ females.

Spleen weight as a percentage of bodyweight was higher in nu/+ females than nu/+ males on 24% protein diet. Nudes had significantly heavier livers than nu/+ mice but no differences were observed due to sex. Kidney weight as a percentage of bodyweight on the 12% protein diet was highest in nude males, nu/+ males next, nu/nu females next and nu/+females last. Similar trends were observed in the 18% protein mice. Kidney weight of nude males fed the 24% ration was higher than those of mice fed the other two rations. Serum protein levels were significantly lower in the nudes than the heterozygotes with the exception of the nude males on the 18% diet which had a lower serum pertein value but not significantly lower than the nu/+ males.

Growth Curves

Nu/+ females. Bodyweights, starting with those obtained at 21 days of age, and for each successive 4-day period until 49 days of age, were plotted for nu/+ and nu/nu females as shown in Figure 1. The curves for the nu/+ female mice show that the 24% protein ration resulted in heaviest final bodyweights at 48 days of age. The 12% ration resulted in the lowest final bodyweights. The initial weights (21 day weights)

TABLE 13

EFFECTS OF GENOTYPE AND SEX ON GROWTH PARAMETERS WITH 12% PROTEIN DIET

Parameter	Probability of F[1]	DF[2]		Sex and Genotype			
				Male Nu/nu	Female Nu/nu	Male Nu/+	Female Nu/+
Average food consumption (g/day/mouse)	P < .01	21	.01	3.17[a]	3.25[a]	4.26[b]	3.55[a]
Feed efficiency (g feed/g gain)	P < .001	113	.01	9.92[a]	9.94[a]	10.8[a]	12.3[b]
Initial Weight (g)	P < .001	118	.01	6.36[a]	6.55[a]	10.77[c]	9.8[b]
Gain (g) days 21-25	NS[4]	117	---	1.35	1.40	1.5	1.4
Gain (g) days 25-29	P < .001	117	.001	1.50[a]	1.32[a]	2.1[b]	1.9[b]
Gain (g) days 29-33	P < .005	117	.005	2.18[b]	2.24[b]	1.1[a]	1.5[a]
Gain (g) days 33-37	NS	115	---	1.12	1.44	1.5	1.4
Gain (g) days 37-41	P < .001	116	.001	1.75[c]	1.17[b]	1.4[b]	.84[a]
Gain (g) days 41-45	P < .005	116	.005	1.22[a]	.88[a]	1.7[b]	.8[a]
Gain (g) days 45-49	P < .001	115	.001	.33[a]	.97[b]	.98[b]	.3[a]
Total Gain (g)	P < .001	114	.01	10.0[c]	9.38[b]	10.93[d]	8.01[a]

TABLE 13 (Continued)

Parameter	Probability of F[1]		DF[2]	α[3]	Sex and Genotype: Male Nu/nu	Female Nu/nu	Male Nu/+	Female Nu/+
Spleen percentage bodyweight	P	.005	110	.01	$.584^{c}$	$.56^{c}$	$.35^{a}$	$.46^{b}$
Liver percentage bodyweight	P	.001	110	.01	5.76^{b}	5.67^{b}	4.82^{a}	4.7^{a}
Kidney percentage bodyweight	P	.001	109	.01	$.83^{d}$	$.67^{b}$	$.73^{c}$	$.6^{a}$
Serum protein (g/100 ml)	P	.001	106	.01	4.87^{a}	4.86^{a}	5.37^{b}	5.18^{b}

[1]Probability of the F ratio (treatment mean square/error mean square).

[2]Degrees of freedom. [3]Probabilities of the Studentized range.

[4]Not significant, P > 0.25.

a, b, c, d Values within a line not sharing a common superscript are significantly different.

TABLE 14

EFFECTS OF GENOTYPE AND SEX ON GROWTH PARAMETERS WITH 18% PROTEIN DIET

Parameter	Probability of F[1]	DF[2]	α[3]	Sex and Genotype: Male Nu/nu	Female Nu/nu	Male Nu/+	Female Nu/+
Average food consumption (g/day/mouse)	P < .001	33	.01	3.19[a]	2.65[a]	4.16[b]	3.1[a]
Feed efficiency (g feed/g gain)	P < .01	170	.01	8.86[a]	8.38[a]	8.4[a]	10.1[b]
Initial weight (g)	P < .001	188	.01	7.70[b]	6.87[a]	11.5[c]	11.0[c]
Gain (g) days 21-25	P < .001	184	.01	2.06[b]	1.51[a]	2.7[c]	1.5[a]
Gain (g) days 25-29	P < .001	185	.01	2.22[b]	1.15[a]	1.9[b]	1.9[b]
Gain (g) days 29-33	P < .10	184	.05	2.95[b]	2.38[a]	2.9[b]	2.5[a]
Gain (g) days 33-37	P < .001	188	.01	1.56[a]	1.67[a]	2.7[b]	1.8[a]
Gain (g) days 37-41	P < .001	180	.01	.82[b]	.54[b]	.97[b]	.1[a]
Gain (g) days 41-45	P < .001	177	.01	1.04[b]	.07[a]	1.3[b]	.7[b]
Gain (g) days 45-49	P < .001	155	.01	.26[a]	.41[a]	1.2[b]	.2[a]
Total Gain (g)	P < .001	172	.01	11.50[b]	8.38[a]	13.7[b]	8.7[a]

TABLE 14 (Continued)

Parameter	Probability of F[1]	DF[2]	α[3]	Sex and Genotype			
				Male Nu/nu	Female Nu/nu	Male Nu/+	Female Nu/+
Spleen percentage bodyweight	P < .001	154	.01	.75[b]	.85[b]	.34[a]	.46[a]
Liver percentage bodyweight	P < .001	155	.01	6.10[b]	6.16[b]	5.04[a]	4.9[a]
Kidney percentage bodyweight	P < .001	154	.01	.86[c]	.76[b]	.78[b]	.7[a]
Serum protein (g/100 ml)	P < .001	159	.01	5.28[b]	5.09[a]	5.6[c]	5.4[b]

[1]Probability of the F ratio (treatment mean square/error mean square).

[2]Degrees of freedom. [3]Probabilities of the Studentized range.

a, b, [c]Values within a line not sharing a common superscript are significantly different.

TABLE 15

EFFECT OF GENOTYPE AND SEX ON GROWTH PARAMETERS WITH 24% PROTEIN DIET

Parameter	Probability of F[1]	DF[2]	α[3]	Sex and Genotype			
				Male Nu/nu	Female Nu/nu	Male Nu/+	Female Nu/+
Average food consumption (g/day/mouse)	P < .10	25	.05	3.11^{a}	3.15^{a}	4.01^{b}	3.4^{a}
Feed efficiency (g feed/g gain)	P < .025	120	.05	$9.81^{a,b}$	12.12^{b}	9.1^{a}	11.3^{b}
Initial weight (g)	P < .001	142	.01	6.70^{a}	6.9^{a}	11.9^{b}	11.9^{a}
Gain (g) days 21-25	P < .001	131	.01	$1.71^{a,b}$	1.38^{a}	2.7^{b}	1.9^{a}
Gain (g) days 25-29	P < .001	126	.01	1.35^{a}	1.45^{a}	2.8^{c}	2.2^{b}
Gain (g) days 29-33	P < .001	123	.01	2.18^{a}	2.35^{a}	3.5^{b}	2.2^{a}
Gain (g) days 33-37	P < .001	119	.01	2.29^{b}	1.36^{a}	1.3^{a}	1.1^{a}
Gain (g) days 37-41	NS[4]	119	---	1.04	.35	.6	.5
Gain (g) days 41-45	NS	117	---	.62	.30	.8	.4
Gain (g) days 45-49	P < .05	106	.05	0^{a}	$.11^{a}$	$.7^{b}$	$.1^{a}$
Total gain (g)	P < .001	114	.01	10.22^{b}	8.46^{a}	12.28^{c}	8.5^{a}

TABLE 15 (Continued)

Parameter	Probability of F[1]	DF[2]	α[3]	Sex and Genotype: Male Nu/nu	Female Nu/nu	Male Nu/+	Female Nu/+
Spleen percentage bodyweight	P < .001	106	.01	.72[c]	.89[d]	.34[a]	.5[b]
Liver percentage bodyweight	P < .001	106	.01	6.10[b]	6.14[b]	5.3[a]	5.3[a]
Kidney percentage bodyweight	P < .001	105	.01	.93[b]	.82[a]	.8[a]	.7[a]
Serum protein (g/100 ml)	P < .001	102	.01	5.0[a]	5.04[a]	5.6[b]	5.6[b]

[1]Probability of the F ratio (treatment mean square/error mean square).

[2]Degrees of freedom. [3]Probabilities of the Studentized range.

[4]Not significant, P > 0.25.

a, b, c, d Values within a line not sharing a common superscript are significantly different.

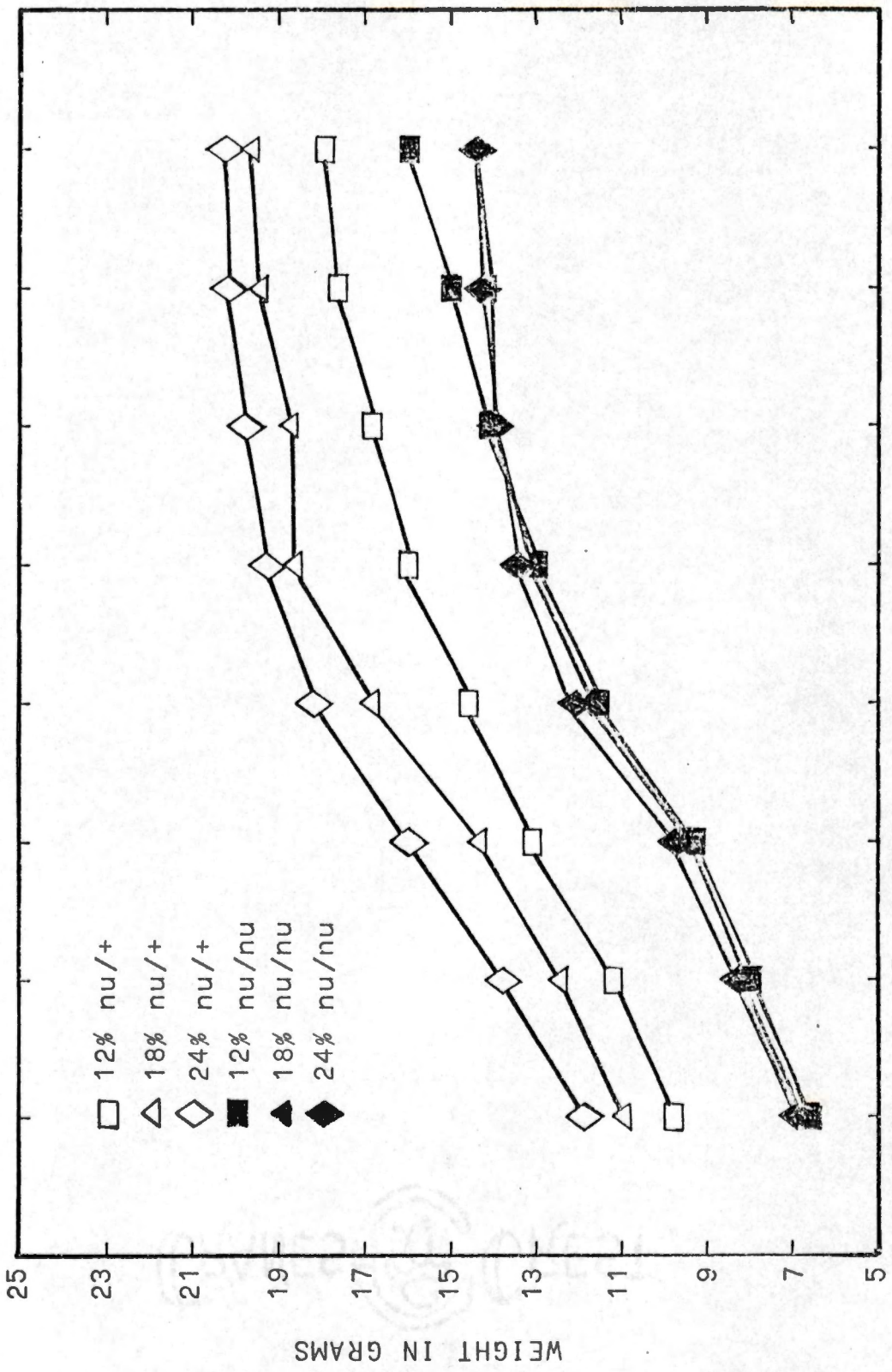

Figure 1. Weight gain curves for female Nu/+ and Nu/nu mice (moribund animals and those with liver lesions included).

of these animals were significantly different ($P < .001$) with the 12% group having the lowest initial weight and the 24% group having the highest initial weight.

Nu/nu females. The same data was also plotted for the nu/nu females and is also shown in Figure 1. Crude protein level had little effect on these females up to day 41, after which the nude females showed the best gains on the 12% protein ration. Final weights of the 12 and 24% protein groups were approximately equal. The initial weights of these mice were not significantly different at day 21. This graph included data from those nude female mice which developed wasting disease and lost weight during the experimental period, or which showed liver lesions at post-mortem examination.

When the moribund mice were removed from the data, the curves changed (Figure 2). These curves show that for the female nudes which did not manifest symptoms of disease, the 24% protein ration resulted in the highest gains in bodyweight with the 18% protein and 12% protein giving about the same results.

Nu/+ males. Bodyweight changes at four-day intervals for the nu/+ male mice are presented in Figure 3. The 18 and 24% protein treatments produced greater weight gains than those produced on the 12% protein treatment. The initial weights of the 12% protein mice were significantly ($P < .10$) lower than the initial weights of the 18 or the 24% protein mice.

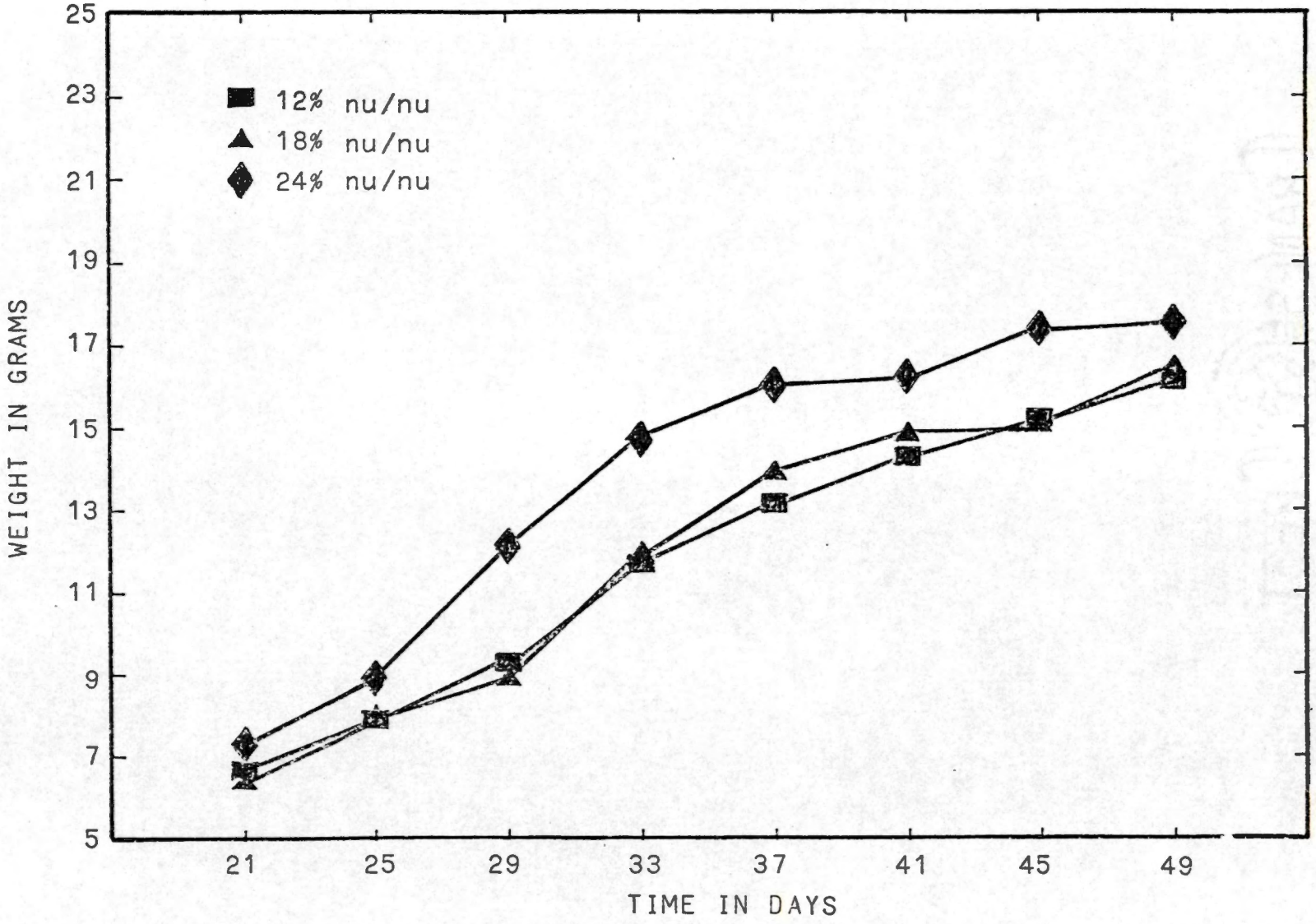

Figure 2. Weight gain curve for nu/nu females (moribund animals and those with liver lesions excluded).

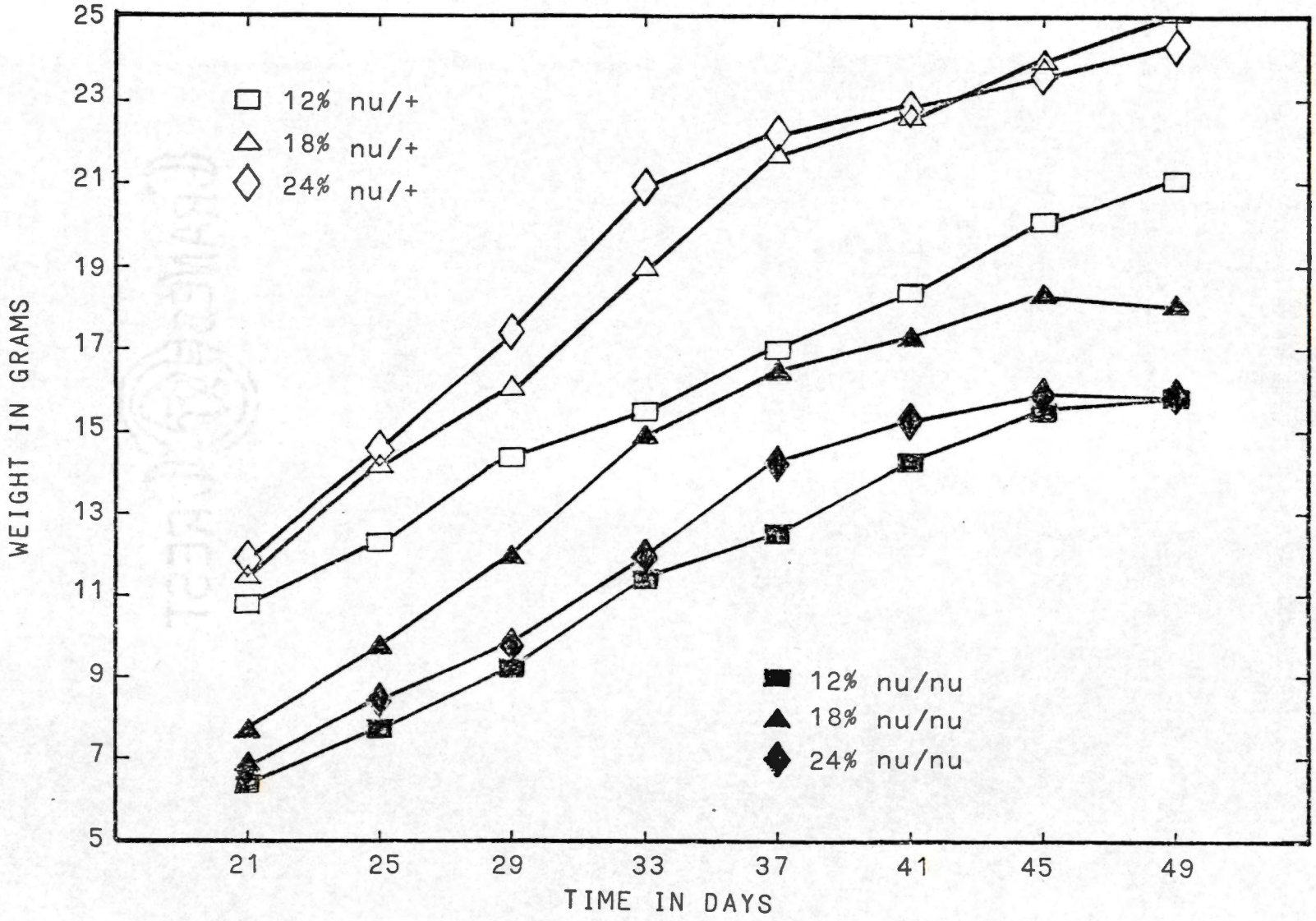

Figure 3. Weight gain curves for male Nu/+ and Nu/nu (moribund animals and those with liver lesions included).

Nu/nu males. The growth curves for the nude male mice, including those which showed liver lesions at post-mortem examination, are shown in Figure 3. The highest gains were achieved in the 18% protein ration. Gain of mice fed the 24, and 12% rations were similar. Initial weights of all three treatment groups were significantly different. The 12% protein group had the lowest initial body weight, the 24% protein group was intermediate and the 18% protein group was the heaviest.

When the moribund mice or those which exhibited liver lesions were removed from the data of nude male mice, estimated growth rate changed (Figure 4). The 18% protein ration still supported the greatest weight gains and the difference between the 24 and 12% groups was increased.

Histological Examinations

Tissue samples of moribund nude mice, those exhibiting wasting syndrome and macroscopic liver lesions, were examined for microscopic pathological lesions. No liver lesions (either macroscopic or microscopic) were found in nu/+ mice. The liver lesions of the nude mice were characterized by multifocal necrotic hepatitis ranging from acute to chronic.

Incidence of Liver Disease among Treatments

The incidence and distribution of liver disease among the three treatment and four genotype-sex groups is shown in Table 16. The nude mice fed the 12% protein ration had the lowest incidence of death and

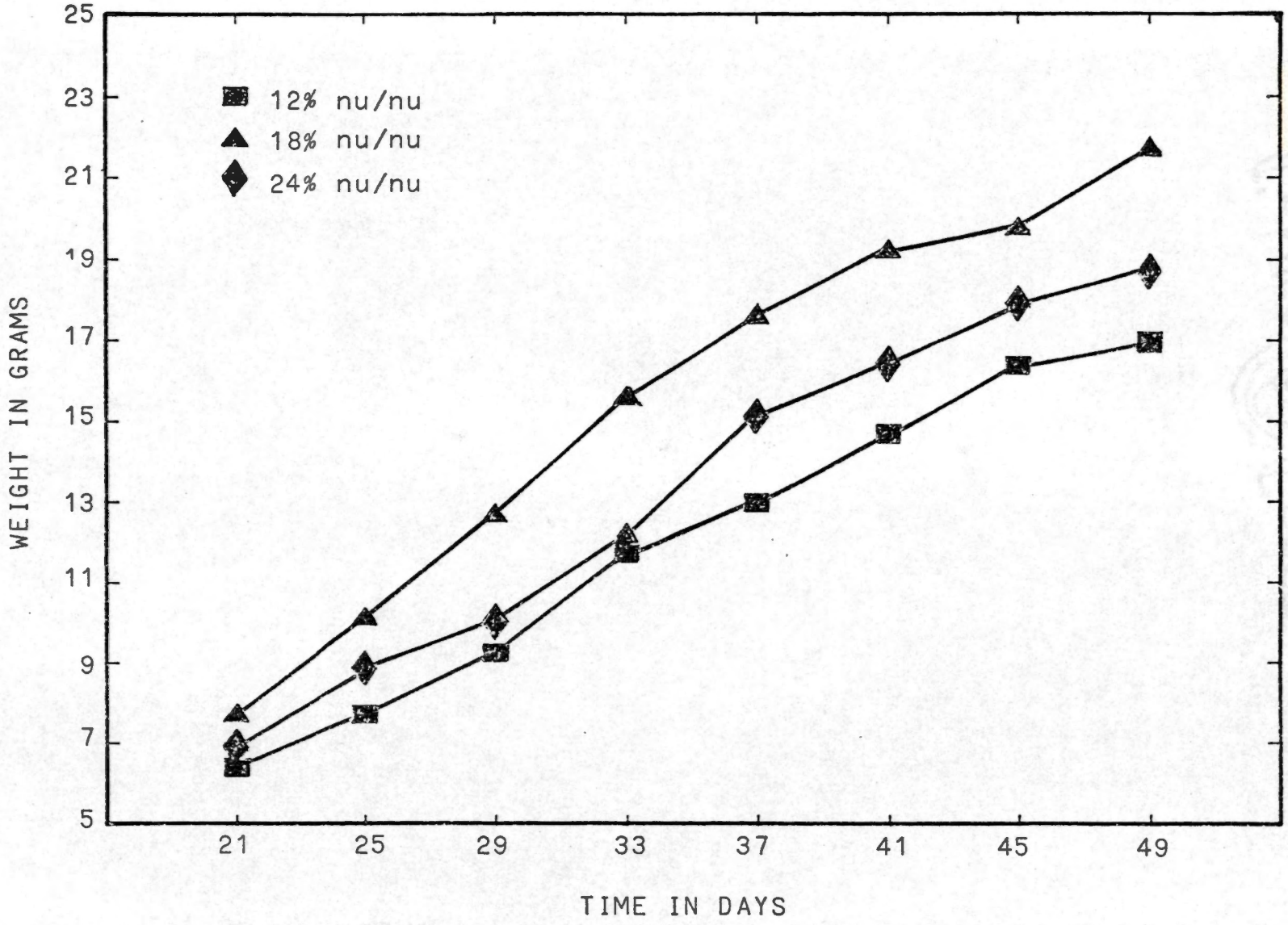

Figure 4. Weight gain curve for Nu/nu males (moribund animals and those with liver lesions excluded).

TABLE 16

THE RELATIONSHIP OF LIVER DISEASE AND DEATH TO DIETARY PROTEIN LEVEL

% Protein	Sex and Genotype	Number of Mice	Average Number/Cage	% Dead (Number)	% Diseased Livers (Number)	Total % Dead or with Diseased Livers (Number)
12%	Nu/+ female	43	6.1	0	0	0
	Nu/+ male	22	5.5	0	0	0
	Nu/nu female	35	4.5	0	5.6 (2)	5.6 (2)
	Nu/nu male	21	3.5	14.3 (3)	9.5 (2)	23.8 (5)
18%	Nu/+ female	51	6.4	0	0	0
	Nu/+ male	47	5.2	0	0	0
	Nu/nu female	52	5.2	23.1 (12)	25.0 (13)	48.1 (25)
	Nu/nu male	42	4.2	4.8 (2)	23.3 (14)	38.1 (16)
24%	Nu/+ female	34	5.8	0	0	0
	Nu/+ male	34	5.7	0	0	0
	Nu/nu female	39	4.1	33.3 (13)	7.7 (3)	41.0 (16)
	Nu/nu male	39	4.4	28.2 (11)	23.1 (9)	51.2 (20)

diseased livers, with the female nude mice having the lower percentage (5.6) of the two sexes. The 18% protein ration resulted in 48.1% combined deaths and diseased livers in nude females and 38.1% in nude males. The 24% protein ration resulted in 41.0% incidence in nude females and 51.2% in nude males. When deaths or diseased livers were found at necropsy within a cage, most of the mice in that cage tended to have diseased livers (Table 17).

V. Discussion

Mouse rations containing higher crude protein levels, when all other nutrients were adequate, supported higher weight gains according to Fenton and Carr (1951), who used casein as the sole source of protein. These investigators also pointed out differences in response of different strains of mice to the same diet. Bell (1972) stated that 17% protein was just as effective for growth as 19 or 21% protein levels. However, Knapka et al. (1977) found that growth rate decreased as crude protein level was increased from 18 to 24% in four strains of inbred mice. In the present study, heterozygous males responded to the various protein levels in a manner similar to that reported by Bell (1972) and Knapka et al. (1977). When protein levels were increased from 13% to 17 or 18%, weight gains increased. At higher protein levels, either no additional growth response or a decrease was observed. Results from female heterozygotes in the present study are in contrast to the aforementioned results, since each increment in dietary CP percentage resulted in an additional growth response. The cause for this differential response of the two sexes is not readily apparent.

TABLE 17

DISTRIBUTION OF HEALTHY AND SICK NUDE MICE BY SEX-RATION PROTEIN GROUPS

Protein Level	Sex	% of Cages with 100% of Mice with Healthy Livers	% of Cages with 100% of the mice with Diseased Livers	Total of 100% Cages
12%	Female	87.5	0	87.5
	Male	83.3	16.7	100.0
18%	Female	33.3	44.4	77.7
	Male	50.0	40.0	90.0
24%	Female	42.86	28.57	71.43
	Male	42.86	42.86	85.71

The growth responses to the various protein levels by nude mice were more difficult to interpret if the weights of all mice (moribund and those which died during the experiment) were included (Figures 1 and 3, pages 42 and 45). However, when the data from moribund and those with macroscopic liver lesions at sacrifice were eliminated, response to protein levels by nude mice was lower but of similar relationship to responses of the heterozygotes of the same sex. Both the nudes and the nu/+ females grew better on 24% protein while both the nude and nu/+ males grew better on 18% protein.

When evaluating results of this experiment, it must be considered that the mice used in the growth experiment were fed the same level of protein that their parents had been fed. It has been demonstrated that preweaning environment affects postweaning growth (Rutledge, et al., 197]; Jara-Almonte and White, 1973). Litter size in the 12% protein mice was smaller than that in the 18 and 24% groups. Smaller litters generally result in heavier pups at weaning when rations are the same. Standardization of litter size should have increased the differences between the 12% and the 18 and 24% groups rather than decrease the differences. It is possible that the preweaning period adversely affected the weanling 12% mice and that they did not recover from the effects during the four-week growth period. The effects of the dietary protein level on growth in the preweaning period cannot be separated from the effects of the dietary protein level on growth in the postweaning period.

The food consumption data, and consequently the feed efficiency data, were not accurate enough to be considered as representative of

the response to treatment. The nude mice huddled together in their food containers more frequently than the nu/+ mice, perhaps due to their greater heat requirements. The female mice seemed to scatter more food and contaminate their food more frequently than the males of both genotypes. The nu/+ mice were more active and wasted and scattered more food than the nudes. The varying number of mice per cage also affected the amount of activity and wasting of food which occurred.

Plata and Murphy (1972) reported that male BALB/wm mice (derived from BALB/c mice) had larger kidneys than females. This finding is supported in this research by the data from both the nude and nu/+ mice. Liver weights increased consistently with increased protein levels which is in agreement with Doljanski's (1960) work with rats. Also in the present research spleen weights were consistently higher in the nude mice than in the nu/+ mice. Data reported by Bamberger et al. (1977) are in agreement with this result. Serum protein levels tended to increase with increasing dietary protein levels. Waterlow (1960) reported decreases in plasma protein level with protein malnutrition, the change being due to the albumin fraction rather than the globulin fraction. Wang, et al. (1976) reported that the ratio of essential to nonessential amino acids in the plasma dropped markedly in protein malnutrition in rats.

The incidence of macroscopic liver lesions and deaths was more frequent in the nude mice of both sexes fed the 18 and 24% protein diets than in mice fed the 12% protein diet. The liver lesions found

in this colony have been attributed to mouse hepatitis virus.[12] Large spleen weights accompany the presence of the liver lesions and have been reported in nude mice (Bamberger et al., 1977). Since the livers of all experimental mice were not examined microscopically, there may have been a much higher incidence of disease than is reported in the present study. This finding is of importance, as the 12% protein diet appeared to either decrease the severity of the disease or to delay the time of onset of symptoms of the disease.

Regarding optimum dietary protein levels for growing nude mice, conflicting results were obtained depending on the criterion of evaluation. If weight gains of the apparently healthy mice of both sexes is the criterion, one of the two higher protein levels is clearly superior. However, the 12% protein level was clearly superior when the sum of survival rate and incidence of disease (Table 16, page 48) is used as the criterion of evaluation. Considering production of nude mice for use in research it would appear that improved survival of healthy animals is more important, even though they might grow at a slower rate.

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[12]Personal communication, Dr. E. A. Machado, University of Tennessee Memorial Research Center.

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