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Dietary proteins on reproductive performance in three consecutive generations of rats

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SUMMARY. The cumulative effects of the long-term consumption of the Regional Basic Diet (RBD) of Northeast Brazil on gestational and lactational performances were assessed in three consecutive generations of *Sprague Dawley* rats (n=1,334). The animals were distributed into three groups: RBD (8% predominantly vegetable-based proteins), Control (8% casein) and Standard (22% casein). Primiparous fertilized rats aged 120 days old and their offsprings made up generation 1. Consecutive generations were obtained by mating products from previous generations. Statistical differences between groups and generations were analysed by the method of Kruskal-Wallis. In comparison with their respective controls, data for RBD-fed groups were as follows: decreased conception rates, gradual declines in gestational body weight gains and reduction in the mean number of youngs per litter; the weight loss of lactating rats aggravated in consecutive generations; suckling rats had lower values for birthweight and weight gain, higher values for death rates (including soon after weaning) and severe immaturity at weaning (21 days). This dietary experimental model proved to be valid in terms of providing information for further studies about the relationship between quality and quantity of dietary proteins in order to allow decisisons on supplementary feeding programmes for people now most in need.

Key words: Quantity and quality of dietary proteins, reproductive perfomance, generations.

RESUMO. Efeitos cumulativos de proteínas dietéticas sobre a reproduçao em três gerações de ratos. Foram avaliados, em três gerações sucessivas de ratos *Sprague-Dawley* (n=1.334), os efeitos cumulativos do consumo prolongado da Dieta Básica Regional (DBR) do Nordeste brasileiro sobre a gestação e a lactação. Os animais foram distribuídos em três grupos dietéticos: Grupo DBR (8% de proteínas predominantemente vegetais), Grupo Controle (8% de caseína) e Grupo Padrão (22% de caseína). Ratas primíparas, fertilizadas, de 120 dias de idade e suas proles constituíram a geração 1. As gerações seguintes foram obtidas mediante o acasalamento das gerações anteriores. As diferenças estatísticas entre grupos e gerações foram analisadas através do método de Kruskal-Wallis. Quando comparadas a seus respectivos controles, as ratas DBR apresentavam baixa fertilidade, declínio gradual do ganho em peso corporal durante a gestação e menor número médio de filhotes por ninhada; entre as lactantes, a perda de peso corporal se acentuava de uma geração para outra; os lactentes tinham valores mais baixos de peso ao nascer e de ganho em peso, mortalidade mais elevada (inclusive logo após o desmame) e imaturidade acentuada ao desmame (aos 21 dias de vida). Através deste modelo experimental ratificou-se a importante relação entre quantidade e qualidade das proteínas dietéticas, obtendo-se informações que poderão ser utilizadas pelos planejadores e implementadores de programa de suplementação alimentar destinados às comunidades carentes.

Palavras-chaves: Quantidade e qualidade das proteínas dietéticas, reprodução, gerações.

INTRODUCTION

An adequate nutritional condition is known to be directly dependent upon the consumption of diets containing all the nutrition factors in proper amounts to meet specific requirements of the individual in all stages of life (1).

In Brazil, large population groups are suffering from some degree of malnutrition. The main reason for this is the low purchasing power of families (2). A condition of poverty seems to perpetuate and nutritional disorders pass from one generation to the next.

Experiments using only one generation of subjects showed that the host may be affected in a more detrimental manner as a consequence of malnutrition being imposed upon the organism during pregnancy, lactation, adolescence and senescence (3- 9)

In spite of controversial opinions, the foetus has been considered as a perfect parasite during some stages of the intrauterine life (10). To maintain desirable lactational performance protein-depleted rats mobilize their tissue reserves (11). A decreased milk production reflects a reduced protein metabolism in the mammary gland (12). Severe dietary restrictions during lactation account for significant alterations in milk composition (13).

In 1973, Stewart, Preece and Sheppard (14) showed the detrimental effects of a deficient diet on the behavior, nervous system and other body systems in 10 generations of growing rats, pregnant and lactating females.

Sriwastava et al (15), using different dietary models of malnutrition, demonstrated that food restriction imposed to one generation affected negatively the development of the next progenies.

Brain deficits were found to be present in ten generations of animals from mothers to which malnutriton had been imposed *in utero.* F3 generation had learning deficits (16). Similar data were obtained by Cowley and Griesel (17) demonstrating that alterations aggravated in consecutive generations.

The body weight loss between conception and the first weighing after parturition was lower in protein-deficient females than in energy-depleted ones. In protein-restricted rats, however, foetal absorption occurred and all pups of the second generation died during the first five days after birth (18).

Similar data were obtained by Mc Leod, Goldrick and White (19) who used different experimental models.

Resnick and Morgane (20) found lower values for the weight in the first generation of pregnant rats fed the 8% casein diet; in consecutive generations weights for the body and brain of their progenies were lower than in the F1. In the postweaning period, excessive leaneness was still present in such a manner that animals aged 30 days old had the same body weight of 11 day old-animals.

Zamenhof and van Marthens (21), in six generations of rats to which malnutrition had been imposed during pregnancy and after weaning, showed that body and brain weights of neonates were low and that the number of non-fertilized females decreased in consecutive generations.

Malnutrition imposed to three generations of rats during foetal and lactation periods affected subsequent food intake, sexual receptivity, the number of youngs per litter and milk production. The free access to diets after weaning did not appear to reverse the effects of food restriction before weaning (22).

In four generations of rats, Pires Leal (23) demonstrated that dietary protein restrictions, in terms of quantity and quality, affected birth and death rates, as well as the weight curve, until adult life; these effects passed from one generation to the next.

The deficiency of other dietary macronutrients (the alpha-linolenic acid, for example) decreased fertility rates and postnatal development and increased perinatal mortality in three consecutive generations of rats (24).

Most of the studies have used semisynthetic diets for laboratory animals to reproduce clinical, biochemical and pathological features seen in malnourished human subjects (14,20,22,25).

To investigate the effects of dietary restrictions on reproduction in three consecutive generations of rats, however, the dietary model of choice needs to be representative of diets as similar as possible to meals of human populations from economically deprived environments in their cultural and social context. Then, based on data from food consumption surveys (3,4) performed by the Section of Nutrition and Public Health, Department of Nutrition, Federal University of Pernambuco, a dietary model was formulated to contain, in the same proportions as actually eaten, the four major staples ingested usually by low-income groups in Northeast Brazil. This diet, referred as Regional Basic Diet (RBD), when given to growing animals, induces alterations resembling a protein-energy malnutrition syndrome which is highly prevalent in the region (3,4).

This study was designed to assess the effects of the long-term consumption of RBD on reproduction in three consecutive generations of rats.

MATERIAL AND METHODS

A total of 1,334 male and female *Sprague Dawley* rats from the Colony of the Department of Nutrition, Federal University of Pernambuco (Recife, Brazil), was used.

Rats were maintained at 21°-23°C and with 14-h light/10-h dark cycles. Humidity was 65%.

The experimental diet, the Regional Basic Diet (RBD), was prepared with the four foods most frequently consumed by the low-income populations from the Mata area of the State of Pernambuco, Northeast Brazil: beans (*Phaseolus vulgaris*), sweet potatoes (*Ipomea batatas*), cassava flour (*Manihot esculenta*), dried and salted meat (Table 1).

Beans and meat were cooked in boiled water for 2 hours; sweet potatoes were cooked in boiled water for 30 minutes.

Foods were dried in a forced air over (FANEM model) at 60-70°C, for 48-60h, ground (Flour Grind Mill-type D) and sieved (a 60 wire-mesh sieve).

Cassava flour was added to the mixture and the proportions of all ingredients used in the preparation of RBD were the same of the meals consumed by the population.

The contents of mineral salts and vitamins of RBD were estimated using previously described procedures (3,4).

The standard diet (S) contained 22% casein and was balanced to meet the nutritional requirements of the rat (Table 1).

The control diet (C) was formulated to contain 8% casein at expenses of carbohydrate increases (Table 1).

The animals had free access to diets and water.

The chemical compositon of diets is described in Table 1.

Initially, 65 primiparous rats aged 120 days old and fed Labina (Ralston Purina do Brasil, Ltda.), from the Colony of the Department of Nutrition, were mated. After fertilization, which was detected by the presence of sperma in vaginal smears, the rats were individually housed in plastic cages and received their respective diets in accordance with the following feeding schedule: 25 were fed RBD, 13 were fed the control diet and 20 were fed the standard diet. Then, pregnant females and their litters formed generation 1 (F1). Generations 2 (F2) and 3 (F3) were obtained from progenies produced by mating animals of the first and second filial generations, respectively.

Gestational body weights were measured weekly. The evolution and time of pregnancy were examined every day. At parturition, the number of newborns and stillborns, birthweight and general health conditions of progenies were recorded.

During lactation, body weights of mothers and pups were

TABLE 1
Chemical composition of diets

	g/100g	Proteins	Carbohydrates	Fats	Vitamins		Mineral salts	Fibers	kcal	NDpCal %
					Water soluble	Fat soluble				
Standard diet (22% casein)*										
Commercial casein	27.08	22.00	-	-				-	88.00	
Corn starch	57.12	0.58	50.44	0.12			0.09		205.16	
Vegetable oil	7.50			7.50					67.50	
Water soluble vitamins	1.00	-	-	-	1.00		-		-	
Fat soluble vitamins	1.00	-	-	1.00		1.00			9.00	
Mineral salts	4.00						4.00			
Methionine	0.30									
Filter paper	2.00							2.00	-	
Total	100.00	22.58	50.44	8.62	1.00	1.00	4.09	2.00	369.66	16.8
Control diet (8% casein)										
Commercial casein	9.85	8.00	-	-				-	32.00	
Corn starch	74.50	0.76	65.49	0.15			0.12		266.35	
Vegetable oil	7.50			7.50					67.50	
Water soluble vitamins	1.00	-	-	-	1.00		-		-	
Fat soluble vitamins	1.00	-	-	1.00		1.00			9.00	
Mineral salts	4.00						4.00			
Methionine	0.15									
Filter paper	2.00							2.00		
Total	100.00	8.76	65.49	8.65	1.00	1.00	4.12	2.00	374.85	7.5
Experimental Diet (Regional Basic Diet - RBD*										
Beans	18.34	3.99	10.66	0.24			0.57	1.09	60.76	
Cassava flour	64.81	0.84	48.59	0.12			0.43	5.64	198.80	
Deffated salted dried meat	3.74	2.74	-	0.06			0.06	-	11.50	
Salted dried meat fat	0.35			0,35					3.15	
Sweet potatoes	12.76	0.30	9.99	0.03			0.20	0.48	41.43	
Total	100.00	7.87	69.24	0.80			1.26	7.21	315.64	5.98

* Source of data: reference (3)

measured. Offsprings were uniformly distributed (6-8 youngs per litter). Lactational performance, mortality rates and the time for the eyes opening were recorded.

The non-parametric test of Kruskal-Wallis was used for statistical comparisons between groups in terms of body weight (pregnant, lactating and suckling rats), weight gain (pregnants) and weight losses (lactating rats) (26). Differences among groups were statistically significant when $p < 0.05$.

RESULTS

Gestational and lactational performances of RBD-fed rats are shown in Table 2.

In generations F1, F2 and F3, 89% 100% and 82% of RBD rats and 76%, 89% and 100% of control dams were fertilized. All rats had a full-term pregnancy and gestation length was about 21 days.

In F1-RBD and F3-RBD, as well as in F2-C and F3-C, pregnants the weight gain was lower than in the standard group. When RBD generations were compared to each other, the weight gain was lower in F1-RBD. Among control generations values were lower in F3-C pregnants. In F3-RBD pregnants the weight gain was higher than in the respective control generation (F3-C).

The number of newborns in the three generations on the three diets was as follows: 176, 193 and 134 (F1, F2 and F3-RBD, respectively), 128, 184 and 173 (F1, F2 and F3 controls, respectively) and 183 in the reference standard. The mean number of pups per litter, execept in F1-RBD. varied (range, 9.1 to 10.8). Stillborns totalized 30%, 32% and 34% (F1, F2 and F3-RBD, respectively), 34%, 46% and 43% (F1, F2 and F3-C, respectively) and 10% (standard group). The number of devoured pups was higher in F2-RBD and F1-C: 11% and 12%, respectively).

Birthweight was significantly lower in all RBD and control generations than in the reference standard.

The opening for the eyes in all RBD pups was remarkably delayed (when the animal was 18 days old).

In terms of lactational performance, only 76%, 85% and 93% of RBD females (generations F1, F2 and F3, respectively)

TABLE 2
Gestational and lactational performances in rats fed the Regional Basic Diet (RBD) over three consecutive generations compared with control (8% casein) generations and the reference standard (22% casein)

	RBD generations			CONTROL generations			STANDARD
	F1	F2	F3	F1	F2	F3	S
PREGNANCY							
Number of matings	28	27	17	17	19	19	20
Pregnant rats (%)	89	100	82	76	89	100	100
Weight gain, (g) $\bar{x} \pm$ se	52,5±4,74a	94,8±4,55b	79,0±6,44ab	89,7±6,01b	80,9±7,0a	62,4±5,06ade	102,5±6,05
	(25)	(20)	(14)	(13)	(17)	(19)	(20)
Number of births	176	193	134	128	184	173	183
Mean of pups per litter	7,0	9,6	9,5	9,8	10,8	9,1	9,2
Stilborn (%)	30	32	34	34	46	43	10
Devoured (%)	6	11	-	12	6	6	3
Birth weight $\bar{x} \pm$ se	5,2± 0,09a	4,9±0,11a	5,3±0,17a	5,3±0,22a	5,6±0,14ac	5,3±0,09a	6,0±0,10
	(25)	(20)	(13)	(13)	(17)	(19)	(20)
LACTATION							
Lactating rats %	76	85	93	77	53	84	100
Weight loss (g) $\bar{x} \pm$ se	35,5±5,78a	40,1±7,04a	48,1±3,93a	44,4±12,70a	48,9±8,40a	35,3±6,22ad	19,7±2,5
	(19)	(17)	(13)	(10)	(9)	(17)	(20)
Weaning pups (%)	58	49	66	48	34	44	75
Body weight at weaning (g) $\bar{x} \pm$ se	24,7±1,44a	16,8±0,70ab	18,3±0,87ab	26,2±1,16a	28,5±1,15ac	23,1±1,70adef	45,0±0,91
	(19)	(17)	(13)	(10)	(9)	(13)	(20)
Mortality rates (two days after weaning) (%)	36	69	45	-	-	-	-

a significantly different from the standard
b significantly different from F1 - RBD e significantly different from F1 - C
c significantly different from F2 - RBD f significantly different from F2 - C
d significantly different from F3 - RBD

$\bar{x}$ ±se = standard error of the mean ( ) = sample size

and 77%, 53% and 84% of control rats (F1, F2 and F3-C, respectively) were able to suckle from parturition to weaning (on day 21). All mothers of the standard group suckled their youngs until weaning.

Weight losses in RBD and control lactating females were higher than in the standard group. In F3-RBD losses were significantly higher than in the respective control generation.

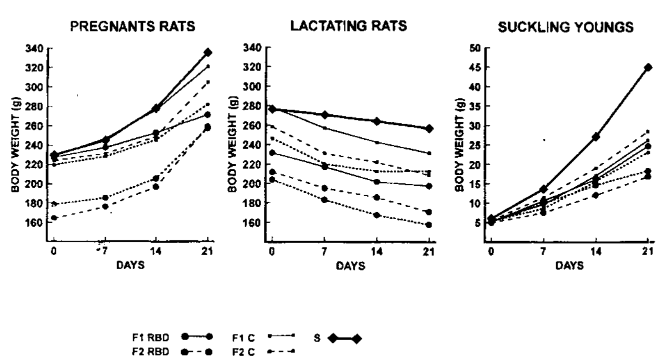
The number of weaning rats was as follows: 58%, 49% and 66% in RBD generations (F1, F2 and F3, respectively), 48%, 34% and 44% in control generations (F1, F2 and F3, respectively) and 75% in the standard group.

At weaning, body weights of all RBD and control generations were significantly lower than in the standard group. In F2 and F3-RBD generations values were significantly lower than in their respective controls. When control generations were compared to each other, body weights were lower in F3-C progenies.

Mortality rates were high in all RBD progenies in the first two days after weaning: 36%, 69% and 45% (F1, F2 and F3 generations, respectively).

Body weights and the weight curve profile of experimental, control and standard groups are shown in Tables 3, 4 and 5, and in Figure 1.

FIGURE 1
Body weight of three consecutive generations of Sprague Dawley rats



The weight curve in F1-RBD pregnants was significantly lower than in their control and standard from the 14th day of pregnancy, remaining reduced until delivery in generations 2 and 3, to which malnutrition had been imposed since fertilization of generation 1.

Curves showing the weight of control pregnants, which superposed upon the curve of the reference standard in generation 1, deviated significantly from the standard in generation 2 from the 14th day and in F3 generation since the beginning of pregnancy (Table 3, Figure 1).

The weight curve profile and body weights of RBD lactating rats differed from their respective control generations and the reference standard. In generation 1, the curve distribution of control lactating dams was similar to that of the standard, but in generations 2 and 3 a significant decline was detected (Table 4, Figure 1).

Curves showing the weight of the body in the three generations of RBD and control suckling rats deviated significantly from the standard reference. In generation F1, the weight curve of RBD group superposed upon that of the respective control, but it declined in F2-RBD generation. At the end of the lactational period (21 days), however, in F3-RBD generations curves differed from those of controls. When RBD generations were compared to each other the weight curve of suckling youngs was higher in generation 1 than in generation 2. Among controls, curves were significantly smaller in generation 3 from the 7 th day of lactation (Table 5, Figure 1).

TABLE 3

Body weight in pregnant rats fed the Regional Basic Diet (RBD) over three consecutive generations compared with control (8% casein) generations and the reference standard (22% casein)

Gestation	RBD generations						CONTROL generations						STANDARD	
(days)	F1		F2		F3		F1		F2		F3			
	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
0	227,41 (25)	2,72	164,29ab (20)	2,26	178,46abc (14)	2,14	229,13 (13)	2,74	224,06c (17)	3,22	219,54ad (19)	3,26	229,64 (20)	3.39
7	237.52 (25)	2,88	176,35ab (20)	2,71	185,58abc (14)	3,53	247,12 (13)	3,24	231,35ce (17)	3,34	228,23ade (19)	3.32	244,98 (20)	5,03
14	252,57a (25)	4,02	196,91ab (20)	3,45	205,45ab (14)	3,11	276,28b (13)	5,56	248.83ace (17)	5,03	245,40ade (19)	5,02	278.14 (20)	5,42
21	271,42a (25)	5.58	259,05a (20)	5,06	257,50a (14)	6,82	320,95b (13)	6,50	304,95ace (17)	8,39	281,98ade (19)	4,98	335.62 (20)	6,87

a significantly different from the standard
b significantly different from F1 - RBD e significantly different from F1 - C
c significantly different from F2 - RBD
d significantly different from F3 - RBD
se = standard error of the mean ( ) = sample size

TABLE 4

Body weight in lactating rats fed the Regional Basic Diet (RBD) over three consecutive generations compared with control (8% casein) generations and the reference standard (22% casein)

Lactaction	RBD generations						CONTROL generations						STANDARD	
(days)	F1		F2		F3		F1		F2		F3			
	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
0	231,55a (25)	5,07	211,90ab (21)	6,82	203,91ab (14)	5,24	278,04b (13)	7,02	258,29c (17)	6.94	246,37ade (19)	0,51	276,23 (20)	8.62
7	217,09a (19)	5.86	195,21ab (19)	6,57	183,00ab (13)	4,03	257,00b (10)	6,32	230,92ae (13)	6,40	219,97ade (18)	6.75	270.67 (20)	8.65
14	201,59a (19)	5,69	185,56ab (18)	6,47	167,32abc (13)	2,99	242,39b (10)	7,86	222,05ac (12)	6.54	212,46ade (17)	6,99	263,95 (20)	7.68
21	197,54a (19)	5,68	170,84ab (17)	6,15	157,66ab (13)	3,42	231,29b (10)	5,56	208,87ac (9)	9,23	212,64ad (17)	6,46	256.57 (20)	7.98

a significantly different from the standard
b significantly different from F1 - RBD e significantly different from F1 - C
c significantly different from F2 - RBD
d significantly different from F3 - RBD
se = standard error of the mean ( ) = sample size

TABLE 5
Body weight in suckling rats from mothers fed the Regional Basic Diet (RBD) over three consecutive generations compared with control (8% casein) generations and the reference standard (22% casein)

Lactaction (days)	RBD generations F1 mean	se	F2 mean	se	F3 mean	se	CONTROL generations F1 mean	se	F2 mean	se	F3 mean	se	STANDARD mean	se
0	5,17a (25)	0,09	4,94a (20)	0,11	5,32a (13)	0,17	5,28a (13)	0,22	5,56ac (17)	0,14	5,34a (19)	0,09	5,99 (20)	0,10
7	10,58a (19)	0,52	7,53ab (19)	0,24	9,87ac (13)	0,59	9,64a (10)	0,63	11,48ace (12)	0,48	8,60af (19)	0,53	13,66 (20)	0,51
14	16,13a (19)	0,72	12,08ab (18)	0,41	14,68ac (13)	0,94	17,15a (10)	0,87	19,13a (12)	0,96	15,64acf (17)	0,94	27,16 (20)	0,65
21	24,68a (19)	1,44	16,81ab (17)	0,70	18,34ab (13)	0,87	26,16a (10)	1,16	28,47ac (9)	1,15	23,13adef (13)	1,70	44,98 (20)	0,91

a significantly different from the standard
b significantly different from F1 - RBD e significantly different from F1 - C
c significantly different from F2 - RBD
d significantly different from F3 - RBD
se = standard error of the mean ( ) = sample size

DISCUSSION

Early malnutrition has been shown to affect subsequent gestational and lactational performances, as well as the development of the litter (3,4,6,8,19,20,27,30).

Some effects can not be reversed and seem to aggravate from one generation to the next.

Dietary protein composition and level have been correlated with conception rates (21,22). This was found to be evident in chronically malnourished female rats (F3-RBD) exposed to protein restriction since fertilization of generation 1.

The curve profile showing the weight of the body in F1-RBD prègnants is indicative that low-protein diets containing poor quality proteins, as RBD, given to dams from fertilization appear to induce earlier reductions in body weight than low-protein diets with good quality proteins. In F1-RBD rats the weight loss was šeen from the 14th day of pregnancy, but losses only became detectable in the second generation of controls (F2-C) from the 14th day of pregnancy.

It is noteworthy that weight deficits seemed to aggravate over consecutive generations (F2-RBD and F3-RBD) to which malnutriton had been imposed since fertilizaton of generation 1 (F1-RBD). Curves showing the weight of the body deviated from their respective control generations notedly from the standard group (22% protein-fed pregnants). At lactation, growth deceleration, which was negligible in the reference standard, aggravated in consecutive generations of control dams and particularly of RBD mothers which presented an already noticeable decrease in body weight at the beginning of lactation. Results were similar to those reported by others (20,27,28).

Kanarek et al.6, however, on studying diets containing varying protein contents, showed that after the first week of lactation mothers fed 25% casein increased in weight, whereas in the 6% and 8% casein-fed mothers growth deceleration persisted.

Irrespective of the nutrition values of dietary proteins, lactating dams fed successively on a low-protein diet not able to suckle their progenies from parturition to weaning (21 days). In the present paper the weight distribution curves in the three generations of suckling youngs from RBD and control mothers deviated significantly from the reference standards.

The number of newborns per litter seemed unaffected by the low content of a high quality protein, which does not agre with findings from Stewart and Sheppard (29) and Turner (30) who detected smaller litters from mothers given the 8% casein diet.

The smaller litter sizes in the F1-RBD mothers suggest possible implications for reproduction due to abrupt changes in dietary protein composition.

Values for birthweight in youngs from RBD and 8% casein fed mothers were significantly lower when compared to the reference standard. Results were similar to those described previously (3). However, Resnik and Morgane (8) and Turner (30) failed to show differences between animals on 8% and 25% casein diets. On the other hand, Kanarek et al. (5) demonstrated that dietary influences (6%, 8% and 25% casein) only become detectable from the 6th day after birth.

Another point of interest was the evident immaturity of pups from RBD dams, characterized by disparity between the large head and the average size body, reeling unsteady gait and body weight deficits (only half of the weight in the 22% casein fed rats). In addition, rats fed the 8% casein diet had more fully develop locomotive apparatus than their pairs in the three generations, but bearing no comparison with the reference standars.

The results suggest that dietary protein composition and level affect the reproductive performance of rats.

It follows that when diets are poor in protein quality and content fertilization and gestational body weight gains are lowered, body weight losses aggravate during lactation, mortality rates are significantly high among suckling and weaning rats, physical immaturity is evident at weaning and body weight deficits aggravate over consecutive generations.

When diets provide low contents of a high quality protein the weight distribution curves of pregnant and lactating subjects deviate significantly from of the reference standard over generations, the weight distribution curves of suckling rats differ from the standards, immaturity is less evident at weaning.

On the other hand, a well-balanced diet, containing proper amounts (22%) of a high quality protein and planned to meet specific requirements of the individual, enables an animal to grow, mature an reproduce in a normal manner.

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