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EFFECTS OF FEEDING LOW PROTEIN DIET WITH AND WITHOUT LEUCINE SUPPLEMENTATION ON PROTEIN STATUS OF LACTATING FEMALES AND THEIR PUPS

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Abstract : Female rats were fed low protein diet (10% casein) either as such or supplemented with 3% leucine during pregnancy and lactation. Changes in litter size and the survival rate, growth and protein status of the pups were noted. The milk yield and hepatic and mammary gland protein status of the mothers were also studied. Feeding low protein diet reduced litter size, increased their mortality and resulted in poor growth of the pups. It also resulted in poor hepatic and mammary gland protein status of the mothers, as well as reduced their milk yield. On adding 3% leucine to 10% casein in the diet, the changes observed in the low protein group, did not alter in any manner.

Key words : low protein diet; leucine supplementation; pregnancy and lactation; milk yield; protein status

INTRODUCTION

Leucine supplementation of the diet does not produce a uniform effect in different experimental conditions. When weanling rats were fed a diet containing 9% casein the addition of 3% leucine reduced growth. However, when the basal diet contained 10% casein, no such growth depression was observed with the addition of 3% leucine (5). Similarly, dietary restriction was found to be less harmful to adult rats than dietary restriction with leucine supplementation (2, 12). When diet containing 20% casein was fed *ad lib* to the female rats during pregnancy and lactation, addition of 3% leucine not only improved their nursing performance and mammary gland development but also showed protein anabolic effects in their young ones (3, 8). In the present study it was proposed to feed female rats during pregnancy and lactation with a diet containing 10% casein, with and without leucine supplementation, and observe their liver and mammary gland protein status, and the growth and protein status of their litters.

METHODS

Female albino rats weighing between 150-200 g were selected for this study and maintained at a room temperature of 25±1°C. Food and water were given *ad lib* and the animals were divided into the following three groups:

Group I : (Control group) received the control diet which contained 20% casein, the composition of which was the same as reported earlier (12).

Group II : (Low protein group) received low protein diet which contained 10% casein instead of 20% present in the control diet.

Group III : (Experimental group) was given low protein leucine-supplemented diet, which contained 3% leucine and 10% casein.

All the experimental animals were fed on their different dietary regimes from two weeks prior to mating, upto 15th day of lactation. The average food

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intake of animals in different groups was about the same.

After mating all sperm positive rats were separated and housed individually. After birth the litter size for each mother was adjusted to 7 pups per litter, by adding or removing the pups within the same group.

The weights of the mother and the pups were recorded on every alternate day till they were sacrificed. All young ones from a particular litter were sacrificed either at birth, or on day 15 after birth. Livers from all the members of the litter sacrificed at birth were pooled and homogenized for the subsequent analysis of protein and nucleic acids. The livers of pups sacrificed on day 15, were also pooled and similarly treated. The mothers of these pups were also sacrificed after their lactation for 15 days. From them in addition to their livers, mammary glands were also dissected out.

All the tissues taken from different groups of pups and mothers, so pooled, were then homogenised in normal saline and the concentration of protein and nucleic acids were estimated from these homogenates. Protein was estimated by the method of Lowry et al (9). Nucleic acids extraction and estimations were carried out by the method of Schneider (11).

During lactating periods the milk yield of the mothers was calculated by the test weighing method (4).

All the data were analysed statistically by students' t-test.

RESULTS

Mothers from the control group (normal diet) and low protein diet fed group produced mean litter sizes of 12.00±0.09 and 10.00±1.00 pups, respectively. However, the mean value for the low protein diet group was not significantly different when compared with the mean value for the control group ($p>0.05$). All the pups from mothers of the control group survived till day 15, when they were sacrificed. However, pups from mothers of the low protein diet group showed higher mortality, and by day 7, only 70% of these pups survived. By day 15, only 40% of the pups from this group had survived, when they were sacrificed. In the experimental group (mothers fed low protein diet with additional

TABLE I : Pup's weight and liver contents of protein, RNA and DNA.
(Values are mean ± SE for 5-6 rats in each group)

Parameters studied	*Control group*	*Low protein group*	*Low protein with excess leucine (Experimental group)*
Body weight of pups (g)			
— At birth	5.40 ± 0.47	5.10 ± 0.07*	4.90 ± 0.06*
— On day 15	18.00 ± 1.48	13.60 ± 0.66*	11.30 ± 0.71*
Liver weight (g)			
— At birth	0.15 ± 0.01	0.11 ± 0.01*	0.11 ± 0.01*
— On day 15	0.54 ± 0.04	0.39 ± 0.03*	0.36 ± 0.03*
Protein (mg)			
— At birth	19.80 ± 1.04	16.30 ± 2.31*	15.10 ± 1.44*
— On day 15	96.30 ± 5.81	56.20 ± 3.77*	50.80 ± 4.00*
RNA (mg)			
— At birth	3.49 ± 0.28	2.86 ± 0.13*	2.50 ± 0.20*
— On day 15	12.40 ± 0.97	7.40 ± 0.66*	6.50 ± 0.59*
DNA (mg)			
— At birth	0.80 ± 0.04	0.67 ± 0.04*	0.66 ± 0.04*
— On day 15	2.94 ± 0.26	1.96 ± 0.18*	1.82 ± 0.16*

*Difference statistically significant when compared with the control group ($P<0.05$).

TABLE II : Milk yield, maternal liver and mammary gland weights and their protein, RNA ,and DNA contents on day 15 of lactation. (values are mean ± SE for 5-6 rats in each group)

Parameters Studied	*Control group*	*Low protein group*	*Low protein with excess leucine (Experimental group)*
Milk yield (g)	20.60 ± 2.40	11.30 ± 1.01*	10.00 ± 1.03*
Liver			
— weight (g)	8.20 ± 0.22	7.00 ± 0.20*	6.80 ± 0.20*
— Protein (g)	1.64 ± 0.09	1.15 ± 0.09*	1.02 ± 0.08*
— RNA (mg)	98.00 ± 3.21	70.20 ± 2.45*	68.60 ± 2.11*
— DNA (mg)	20.80 ± 1.83	15.10 ± 1.00*	15.00 ± 1.07*
Mammary gland			
— Weight (g)	4.16 ± 0.29	3.06 ± 0.21*	2.84 ± 0.20*
— Protein (mg)	380 ± 40.87	210 ± 20.36*	200 ±12.39*
—RNA (mg)	43.40 ± 3.46	29.30 ± 2.60*	20.10 ± 2.20*
—DNA (mg)	12.20 ± 1.00	9.00 ± 0.30*	8.50 ± 0.20*

*Difference statistically significant when compared with the control group ($p<0.05$)

leucine), the mean litter size was 8.60±0.80 pups. Out of these only 60% of the pups survived by day 7 and 30% by day 15 when these were sacrificed. The litter sizes and mortality rates were not significantly different in the two groups of low protein diet and low protein plus leucine diet ($P > 0.05$).

As seen from Table I, both at the time of birth and day 15 after birth a significant reduction in body and liver weights and liver protein, RNA and DNA contents was observed in the pups from mothers fed low protein diet when compared with pups from mothers of the control group ($P<0.05$). In mothers also similar reductions in their milk yield, as well as their liver and mammary gland weights and their protein, RNA and DNA contents, was observed in low protein diet fed group as compared with the control group ($p<0.05$) (Table II).

However, the changes in various parameters studied, in the mothers fed low protein diet and low protein plus leucine diet, and their pups, showed no significant difference ($P>0.05$) (Table I and II).

DISCUSSION

The results of the present study demonstrate that *ad lib* feeding of low protein diet during gestation and lactation did not alter the litter size significantly when compared with the control group. All the pups from the control group survived till day 15 after birth while the pups from the low protein diet group showed increased mortality with advancing lactation. Other similar studies have also shown that protein restriction throughout the nursing period results in very high or 100% mortality of the young ones (6, 13).

The pups born of mothers fed low protein diet showed significantly decreased body and liver weights and liver protein, RNA and DNA contents, both at birth as well as on day 15 after birth. It is known that both the quality and the quantity of dietary protein in nursing rats influences the growth of their offsprings (1, 7, 10).

Milk yield of mothers was also found to be significantly reduced on day 15 of lactation, in low protein fed group as compared with the control group. Moreover, feeding low protein diet also altered the liver and mammary gland protein status of the mothers. Further, it was noted that the protein status of liver and mammary glands of low protein fed mothers showed similar changes, and the reduction in the protein status of the mammary gland was highly correlated with their milk yield and growth of their pups.

On feeding low protein plus leucine diet, the changes observed both in the mothers and their pups were similar to those observed in animals given only low protein diet, showing thereby that supplementation of 3% leucine in a diet containing low protein did not result in any additional adverse effects. This effect is different of what is observed in adult rats on *ad lib* feeding of 3% leucine with 9% casein (5), or on feeding 3% leucine with 20% casein in restricted quantities (2, 12). It thus appears that the metabolic effects of leucine supplementation depend upon the nature of dietary schedule as well as the physiological state of the animal. In the present study a diet containing 3% leucine with 10% casein was fed *ad lib* during pregnancy and lactation and their effects were observed in the female rats and their pups, while in the previous studies (1, 5, 12) the effects of leucine supplementation were observed in nonpregnant adult rats fed either low protein diet (9% casein) or diet containing sufficient protein (20% casein) but in restricted quantities.

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