Fish-Oil High-Fat Diet Intake of Dams after Day 5 of Pregnancy and during Lactation Guards against Excessive Fat Consumption of Their Weaning Pups

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Summary To investigate the influence of parental fat intake on preferential fat intake by pups after weaning, two groups of dams in study 1 were fed either a low-fat diet (LFD) or a lard high-fat diet (HFD) and those in study 2 were fed either a LFD or a fish-oil HFD after day 5 of pregnancy and during lactation. In study 1, when pups were placed on a self-selection regimen of the LFD and the lard HFD within the first week after weaning, the ratio of the lard HFD intake [lard HFD intake (g)/total intake (g)] by pups of both groups was about 70%. Although pups nursed by dams fed the lard HFD continued to eat the same ratio of the lard HFD, the ratio for pups nursed by dams fed the LFD gradually decreased to 20% in week 3 after weaning. In study 2, when pups were placed on a self-selection regimen of the LFD and the fish-oil HFD after weaning, the ratio of the fish-oil HFD intake in both groups of pups nursed by dams fed the LFD and the fish-oil HFD was about 20% for 3 wk after weaning. In studies 1 and 2, although no significant difference in dietary intake or body weight of dams and pups was observed among all groups through the experimental period, perirenal fat tissue weight of dams fed the lard HFD was higher than that of dams fed the LFD. These findings indicate that (1) fat preference of weaning pups nursed by dams fed the lard HFD is higher than that of weaning pups nursed by dams fed the LFD, and (2) intake of dam’s fish-oil HFD diet guards against pups’ intake of excessive fat.

Key Words prenatal nutrition, self-selection, preferable fat intake, fish-oil HFD

An adequate intake of polyunsaturated fatty acids during pregnancy and lactation is important for optimal fetal and postnatal development. However, neither the role nor the requirements for individual polyunsaturated fatty acids are yet established (1). The National Nutrition Survey in Japan revealed that recently, young people in Japan have come to prefer a Western-style diet to a Japanese-style diet, and now more than 25% of the calories consumed by young people are provided by fat (2). Specifically, animal products such as meat, fish, milk, milk products and eggs contribute more than half of the total fat in the Japanese diet. Moreover the fish consumption of young people decreased and meats were found to provide the major source of animal fats. Although young people in Japan have increased the fat (especially animal fat) content of their diets, it is clear that greater consumption of fats is associated with higher caloric intake as well as increased body weight, body fat and adipose tissue depositions (3, 4). Indeed, physiological homeostasis is maintained through complex nutrient metabolic pathways regulated by hormones and the central nervous system (5, 6). Although the physiological factors contributing to fat diet selection are unknown, animal fats in the diet are a key environmental component influencing the development of obesity in animals and humans (7). It has also been recognized that the amount and kind of fats ingested is related to high blood cholesterol levels, hyperlipidemia and risks of atherosclerotic vascular disease, coronary heart disease, and obesity (8).

The large variations that exist in food choice, especially for fat preference, can be linked to animal strain, age, environment, genetic background or prenatal nutrition, diet history, and modifications of energy expenditure such as exercise and food deprivation (9). Although determinants of food choice are still a matter of conjecture, it is likely that rats select macronutrients according to their need. There have been many studies of macronutrient (carbohydrate, fat and protein) consumption by rats placed in a self-selection regimen from weaning to maturity (7, 9–17). In these studies, vegetable oil and lard were used as dietary fats. Therefore, inherited preference for fish-oil is unknown, which is of interest since fish-oil does not stimulate a higher caloric intake along with increased body weight, total body fat and fat tissue depositions (18). The purpose of the study is to compare the preference for lard high-fat diet (HFD) with fish-oil HFD with resulting consequences to fat intake and fat tissue deposition.

We are particularly interested in examining whether fat-feeding of dams during pregnancy and lactation had any effect on the food choice of their pups after weaning. We reported previously that three groups of dams were fed either a low-fat diet (LFD) containing 3.5% soy
bean oil (Table 1), a HFD containing 7% soybean oil and 14% lard or a two-choice diet of the LFD and the HFD during pregnancy and lactation, and after weaning their pups were placed on a self-selection regimen of the LFD and the HFD (19). When pups were placed on the self-selection regimen, although the ratio of the HFD intake [HFD intake (g)/total intake (g)] by pups of dams fed the two-choice diet was about 60, that by pups of dams fed either the LFD or the HFD was over 80%. In a prior paper, we indicated that the HFD, which contains higher metabolizable energy than the LFD, which maintains higher metabolizable energy than the LFD,

was calculated. We also examined body weight, fat tissue weight and plasma lipid concentrations of the dams and their pups, and discussed their relation to diet regimen and observed dietary preferences.

**MATERIALS AND METHODS**

**Materials.** Lard, soybean oil and dietary components were obtained from Oriental Yeast Co. (Tokyo, Japan). Fish-oil (sardine oil) was purchased from Nihon-Yushi Co. (Tokyo, Japan). To avoid the oxidation of lipids, fish-oil was divided into several portions, sealed in air-free bags and stored at 0˚C until use. Triglyceride E-test Wako, cholesterol E-test Wako and other chemicals were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

**Diets.** The composition of powdered diets shown in Table 1 was based on AIN-93G (21). Basically, to avoid essential fatty acid deficiency, the amount of soybean oil in LFD was reduced to 1/2 that in the AIN-93G and replaced by cornstarch. Lard HFD and fish-oil HFD were prepared by adding lard (14%) and fish-oil (14%) to the AIN-93G, respectively, and substituting cornstarch. Furthermore, cellulose was added to the diets to maintain the same metabolizable energy concentration as the LFD. Therefore, the LFD, the lard HFD and the fish-oil HFD each supplied 3.5 kcal/g of diet. To avoid oxidation of lipids, the fish-oil HFD was freshly prepared every day.

**Animals.** Sperm-positive pregnant rats of Sprague-Dawley strain (10 wk old) were commercially obtained from Japan Clea (Tokyo, Japan) on day 5 of pregnancy (vagina plug was confirmed on day 0 of pregnancy). They were housed individually in plastic cages with paper chip (ALPHA-DrITM, Shepherd Specialty Papers, Inc., Michigan) bedding. They were maintained in a room kept at a constant temperature (23 ± 1˚C) and illuminated with a 12-h light/12-h dark cycle. They were given free access to food and distilled water. Dams and pups were weighed, and food intake was measured every day to determine daily intake during the experimental period.

**Experimental design.** This study consists of studies 1 and 2. In study 1, eight pregnant rats on day 5 of pregnancy were divided into two equal groups. One group was fed the LFD and the other group the lard HFD during the experimental period (pregnancy and lactation). Within 24 h of birth, litters were measured for anogenital distance and culled into 10 pups (5 male and 5 female pups) each and nursed by their dams. On days 10 and 20 after birth, two suckling pups each from the litters of dams were killed by decapitation and their blood collected. Perirenal fat tissues were removed from each 20 d-old suckling pup and weighed. After weaning (at 20 d of age), the remaining pups were housed individually and provided with both the LFD and the lard HFD, placed in separate cups. Dietary ingestion of both the LFD and the lard HFD as well as body weight were measured. The remaining pups were sacrificed on day 41 after birth and dams were sacrificed immediately after weaning their pups. They were anesthetized and

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Table 1. Composition of the experimental diets.1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Low-fat diet</th>
<th>High-fat diet</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Lard</td>
<td>Fish-oil</td>
</tr>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>16.6</td>
</tr>
<tr>
<td>t-Cystine</td>
<td>0.4</td>
<td>0.28</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>56.45</td>
<td>31.6</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Lard</td>
<td>—</td>
<td>11.6</td>
</tr>
<tr>
<td>Fish-oil</td>
<td>—</td>
<td>11.6</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>22.1</td>
</tr>
<tr>
<td>Mineral mixture2</td>
<td>3.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Vitamin mixture2</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.25</td>
<td>0.22</td>
</tr>
<tr>
<td>tert-Butylhydroquinone</td>
<td>0.0014</td>
<td>0.0014</td>
</tr>
</tbody>
</table>

1 Composition for all ingredients is given in grams per 100 g of diet.
2 Mineral mixture and vitamin mixtures were based on the AIN-93G formulation (21).
sacrificed by cardiac puncture and their blood was collected with heparinized syringes. After centrifugation (3,000 rpm / 30 min), plasma was removed. Perirenal fat tissues were obtained from dams and pups and weighed. The collected samples were frozen immediately and stored at 20˚C.

Experimental design for study 2 was as same as that for study 1 except we used the fish-oil HFD instead of the lard HFD.

All procedures accorded with the guidelines for Seiitoku University.

Methods of analysis. Triglycerides and total cholesterol concentrations were measured using test kits (triglyceride: triglyceride E-test Wako, total cholesterol: cholesterol E-test Wako).

Statistical analysis. Values are presented as individual group means±SD. Repeated-measures analysis of variance (ANOVA) was used to evaluate the effects of preference group and time on food intakes. Differences in mean values between groups were tested by Scheffe’s multirange test. Student’s t test was used for all pairwise comparisons. Differences were considered significant at p<0.05.

## RESULTS

### Food intake, body and perirenal fat tissue weight and plasma lipid concentration in dams of studies 1 and 2

In study 1, total food intake of dams fed the LFD and lard HFD during pregnancy and lactation was 1,259±109 and 1,272±94 g, respectively and in study 2, that of dams fed the LFD and fish-oil HFD was 1,309±145 and 1,289±57 g, respectively. Therefore, no significant difference in food intake of dams was observed between the groups fed the LFD and that fed the lard HFD or the fish-oil HFD, during pregnancy and lactation. In study 1, although no significant difference in body weight was observed between the two groups, perirenal fat tissue weight of dams fed the lard HFD was higher than that of dams fed the LFD at the end of lactation (Table 2). Although plasma triglyceride concentrations of dams fed the lard HFD was significantly higher than that of dams fed the LFD, no significant difference in total-cholesterol concentration was observed between the two groups. In study 2, no significant difference in body or perirenal fat tissue weight, or plasma lipid concentrations was observed between the groups of dams fed the LFD and the fish-oil HFD.

In study 1, male and female litter size in the group of dams fed the LFD was 7.1±1.8 and 7.5±0.9 g, respec-
Preferential Fat Intake of Pups after Weaning

In study 1, litter weight in the group of dams fed the LFD and the lard HFD was 95.1±8.9 and 96.1±12.1 g, respectively, and body weight/dam was 6.5±0.3 g (male: 6.5±0.3 g, female: 6.5±0.2 g) and 6.9±0.5 g (male: 7.0±0.5 g, female: 6.8±0.5 g), respectively. Within 24 h of birth, litters were weighed. In study 1, litter weight in the group of dams fed the LFD and the lard HFD was 95.1±8.9 and 96.1±12.1 g, respectively, and body weight/dam was 6.5±0.3 g (male: 6.5±0.3 g, female: 6.5±0.2 g) and 6.9±0.5 g (male: 7.0±0.5 g, female: 6.8±0.5 g), respectively. In study 2, litter weight in the group of dams fed the LFD and the fish-oil HFD was 92.5±10.2 and 91.8±14.6 g, respectively, and body weight/dam was 6.7±0.4 g (male: 6.7±0.3 g, female: 6.6±0.4 g) and 6.3±0.5 g (male: 6.3±0.6 g, female: 6.3±0.5 g), respectively. Therefore, in both studies 1 and 2, these maternal diets did not affect pregnancy outcomes such as litter size, ratio of female vs. male pups, litter weight or weight/dam.

Body and perirenal fat tissue weight and plasma lipid concentration in suckling pups nursed by dams fed the LFD and HFD in studies 1 and 2

In study 1, on day 20 after birth, the body weight of male and female pups nursed by dams fed the LFD was 55.3±3.1 and 54.9±3.5 g, respectively, and that of male and female pups nursed by dams fed the lard HFD was 60.5±3.3 and 59.5±3.0 g, respectively. In study 2, on day 20 after birth, the body weight of male and female pups nursed by dams fed the LFD was 55.5±3.3 and 56.2±2.8 g, respectively, and that of male and female pups nursed by dams fed the fish-oil HFD was 57.8±2.9 and 58.0±1.9 g, respectively. Therefore, in studies 1 and 2, no significant difference in body weight of suckling pups was observed between the male and female pups.

In both studies 1 and 2, no significant difference in body or perirenal fat tissue weight of suckling pups was observed between the group nursed by dams fed the LFD and that nursed by dams fed the lard HFD or fish-oil HFD, respectively (Table 2). In study 1, although on day 10 after birth, no significant difference in plasma triglyceride concentration was observed between suckling pups nursed by dams fed the LFD and the lard HFD, on day 20 after birth, the concentration of suckling pups nursed by dams fed the lard HFD was significantly higher than that of pups nursed by dams fed the LFD. On day 10 and 20 after birth, no significant difference in plasma total-cholesterol concentration was observed between suckling pups in the two groups. In study 2, on day 10 after birth, no significant difference in plasma triglyceride or total-cholesterol concentration was observed between suckling pups nursed by dams fed the LFD and the fish-oil HFD. On day 20 after birth, although the plasma triglyceride concentration of suckling pups nursed by dams fed the fish-oil HFD was significantly higher than that of pups nursed by dams fed the LFD, no significant difference in plasma total-cholesterol concentration was observed between suckling pups in the two groups.
Self-selection of LFD and HFD by weaning pups nursed by dams fed LFD or lard HFD (study 1) and LFD or fish-oil HFD (study 2).

Although no significant difference in growth was observed between male and female suckling pups, it is known that growth of male pups is significantly larger than that of female pups after weaning (19, 20). Therefore, we showed male and female data separately in Fig. 1 and Table 3. Immediately after weaning, all pups were placed on a self-selection regimen of the LFD and HFD for 3 wk. In study 1, for both males and females, pups of both groups preferred the lard HFD within the first week after weaning and no significant difference in the ratio of the lard HFD intake to the total intake was observed between the two groups (Fig. 1, Study 1). However, for both males and females, although the ratio of pups nursed by dams fed the LFD was gradually decreased during the 2nd and the 3rd weeks after weaning, pups nursed by dams fed the lard HFD continued to have a large amount of the lard HFD. Therefore, on week 3 after birth, the ratio of the lard HFD intake for the pups nursed by dams fed the LFD was significantly lower than that for pups nursed by dams fed the lard HFD.

In study 2, for both males and females, both groups of pups nursed by dams fed the LFD and fish-oil HFD did not show any preference for the fish-oil HFD over the LFD after weaning. For both males and females, no significant difference in the ratio of the fish-oil HFD intake was observed between the pups nursed by dams fed the LFD and the fish-oil HFD for 3 wk after weaning.

Body and perirenal fat tissue weight and plasma lipid concentration in weaning pups nursed by dams fed the LFD and the lard HFD or fish-oil HFD

On day 41 after birth, the body weight of male pups in studies 1 and 2 was significantly higher than that of female pups (Table 3). In study 1, in both male and female pups, although no significant difference in body weight was observed between pups nursed by dams fed the LFD and the lard HFD, the perirenal fat tissue weight of the pups nursed by dams fed the lard HFD was significantly higher than that of pups nursed by dams fed the LFD. Plasma triglyceride concentration in male and female pups nursed by dams fed the lard HFD was significantly higher than that of pups nursed by dams fed the LFD, while in both male and female pups, no significant difference in total-cholesterol concentration was observed between the pups nursed by dams fed the LFD and the lard HFD.

In study 2, in both male and female pups, no significant difference in body or perirenal fat tissue weight or total-cholesterol concentration was observed between the pups nursed by dams fed the LFD and the fish-oil HFD. However, the plasma triglyceride concentration of male and female pups fed the fish-oil diet was significantly higher than that of pups fed the LFD.

**DISCUSSION**

One major finding of this study was that, although both groups of pups nursed by dams fed the LFD and the lard HFD preferred the lard HFD within the first week after weaning, both groups of pups nursed by dams fed the LFD and the fish-oil HFD showed no preference for the fish-oil HFD (Fig. 1). In our previous
paper, we reported the results of an experiment in which pups were nursed by dams fed either the lard HFD without added cellulose (4.6 kcal/g) or the LFD (3.5 kcal/g) during pregnancy and lactation (19, 22). When they were placed on a self-selection regimen, the ratio of the lard HFD intake for pups of both groups was over 80%. However, the ratio of the HFD intake for pups nursed by dams fed the two-choice diet was lower than both groups of pups nursed by dams fed the LFD or the HFD (19). From these results, we concluded that the pups of both groups ate a large amount of the lard HFD due to their preference for a high-energy diet.

However, in study 1 of this study, pups nursed by dams fed the lard HFD which was adjusted to maintain the same energy concentration as the LFD continued to eat a large amount of the HFD (fat energy ratio: 35%). On the other hand, 3 wk after weaning, the ratio of the HFD intake by pups nursed by dams fed the LFD decreased to about 20% of intake (fat energy ratio: 17%). Thus, if the LFD and the HFD have the same energy concentration, fat-feeding during pregnancy and lactation could affect pups’ preferences for fat. However, in study 2, the ratio of the fish-oil HFD intake for both groups of pups nursed by dams fed the fish-oil HFD and the LFD was about 20% during the self-selection period. Therefore, it is obvious that the lard HFD has food properties preferable to the fish-oil HFD, because pups do not prefer the fish-oil HFD in any case.

Keen et al. reported that protein, carbohydrate and fat concentration in rat milk was 8.55–12.05, 2.46–3.72 and 10.91–17.54%, respectively and no clear changes were observed in the patterns of change in ingestion of these macronutrients during lactation (23). From these values, the fat-energy ratio of rat milk was calculated to be about 70%. Del Prado et al. reported that the fat-energy ratio of milk from rats fed a LFD (25 g/kg) and a HFD (200 g/kg) was about 74 and 76%, respectively (24). It thus appeared that in study 1, both groups of pups fed high-fat milk during infancy self-selected a large amount of the lard HFD immediately after weaning. Nevertheless, the ratio of fish-oil HFD intake in both groups of pups nursed by dams fed the LFD and the fish-oil HFD was lower than that of the lard HFD.

However, one major finding of this study was that in study 1, although both male and female pups nursed by dams fed the lard HFD continued to eat about a 35% fat-energy ratio diet after weaning, the fat-energy ratio for pups nursed by dams fed the LFD had decreased from 35 to 17% of total energy intake in week 3 after weaning, and also the fat-energy ratio for both groups of pups nursed by dams fed the LFD and the fish-oil HFD was about 17% during the self-selection period. The amount of fat intake was calculated to be 6.3 g/100 g diet. When we compared the amount of fat intake (6.3 g/100 g) during the self-selection period with the fat content of AIN-93G, the pups nursed by dams fed the LFD self-selected the LFD and the lard HFD adequately in week 3 after weaning and both groups of pups in study 2 also self-selected the LFD and the fish-oil HFD adequately after weaning.

In our previous study, we also reported that the ratio of the fish-oil HFD intake of adult rats after being fed the fish-oil HFD during their growth period was also about 20% (6.3 g/100 g) (18). Therefore, we concluded that although the fish-oil HFD was less preferable to the lard HFD, the fish-oil HFD had the property of guarding against fat overfeeding.

On the other hand, the pups of dams fed the lard HFD continued to eat a large amount of the HFD after weaning, and their preference for it was clearly strong. It was thus found that fat-feeding of dams during pregnancy and lactation could affect their pups’ preference for the lard HFD. One reason for this finding appeared to be that the pups were nibbling their dam’s diet with their dams before they were weaned and continued to eat the lard HFD after weaning. In this fashion, nibbling might have affected the pups’ dietary preferences. Therefore, parental and early postnatal experience may predispose infants to favorably respond to food that has become familiar to them. However, physiological factors possibly contributing to HFD and LFD selection and the ratio of consumption of both diets remain unknown.

We found that the fish-oil HFD proved to be less preferable to the lard HFD. Fish-oil contains n-3 long-chain polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The difference in fatty acid composition between lard and fish-oil is relevant to the physical qualities of lipids, such as their melting point, viscosity, emulsification and plasticity (25). The physical qualities of lipids is one of the factors influencing food preference. Furthermore, n-3 PUFA is major modulator of many genes and affects the expression of several key proteins implicated in lipid metabolism and energy utilization (26). The involvement of n-3 PUFA in the lipid metabolism affects the difference in plasma triglyceride concentration and perirenal fat tissue weight of dams fed the lard HFD and the fish-oil HFD (Table 2). Further study is needed to clarify the mechanism by which n-3 PUFA is involved in lipid metabolism. Parrish et al. have shown that fat consumption influences fat tissue mass and the fat tissue mass is smaller in rats fed fish-oil than in rats fed lard (27, 28). Karotkova et al. have shown that the maternal deficiency of PUFA in rats affects the serum leptin levels of their offspring and alters milk leptin concentration. Leptin is an adipose tissue-derived hormone that regulates food intake and energy expenditure (29). The difference of PUFA composition in fish-oil and lard might affect preference of the lard HFD and the fish-oil HFD due to the involvement of n-3 PUFA in the lipid metabolism.

Since the composition of fatty acid in milk is related to maternal diet, fish-oil intake results in rising concentrations of n-3 PUFA in milk (30, 31). It has been reported that maternal diet supplementation with linseed oil (n-3 PUFA-enriched) increased the concentration of n-3 PUFA in dams as well as in their milk. In addition, the white adipose tissue of the suckling pups, also showed an increase compared to the pups of moth-
ers who had soybean oil (28). However, body weight, body length and inguinal fat pad weight of the pups receiving the linseed oil diet were significantly lower during the suckling period compared with the soybean oil diet-fed pups. The pups fed the linseed oil diet had a deficiency in n-6 PUFAs, because the content of n-6 fatty acids in the linseed oil diet was much lower compared with the soybean oil diet. In order to avoid essential fatty acid deficiency, in this study, we used a lard HFD and a fish-oil HFD prepared by adding lard and fish-oil to AIN-93G (21), respectively (Table 1). Therefore, no significant differences in growth, perirenal fat tissue weight or plasma lipid concentration were observed between the suckling pups nursed by dams fed the LFD, the lard HFD or the fish-oil HFD. However, on day 20 after birth, the plasma triglyceride concentration of pups nursed by dams fed the LFD was lower than that of pups nursed by dams fed the lard HFD or the fish-oil HFD (Table 2).

However, due to the feeding of high-fat, low-carbohydrate rat’s milk (23, 24), the plasma lipid concentration of suckling pups on day 10 after birth was higher than that of their dams (Table 2). On day 20 after birth, the plasma lipid concentration of suckling pups showed a decrease when the pups had nibbled their dam’s diet and this decrease continued during infancy (Tables 2 and 3). It appeared that the fat level of the diets which weaning pups self-selected was lower than that of their mother’s milk, since the serum lipid level is easily affected by dietary fat level.

Cellulose addition was used to adjust the energy content in this study, and cellulose probably induced loss of appetite and a corresponding decrease in food intake. Nevertheless, the pups of dams fed the lard HFD continued to eat large amounts of lard HFD when they were placed on a self-selection regimen, and their preference for the lard HFD was clearly strong. However, the preference for the fish-oil HFD was not strong. It was thus found that fat-feeding during pregnancy and lactation could affect pup preference for fat. Although the importance of early experience in the determination of likes and dislikes in humans has been disputed, we suggest that prenatal and early postnatal experience in young infants causes them to favorably respond to foods that have become familiar to them. The present findings suggest pups prefer the lard HFD to the low-fat diet, and that this contributes to greater consumption of it compared to the LFD. Further study is needed to clarify the mechanism by which culture-specific fat preferences are initiated early in life, and whether such preferences remain as infants mature to adults. It would also be useful to consider whether they pass on their food habits to the next generation. We found in the present study that fat-feeding during pregnancy and lactation affected the food choice of pups after weaning.

REFERENCES


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