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Effects of Feeding Fat During Pregnancy and Lactation on Growth Performance, Milk Composition and Very Low Density Lipoprotein Composition in Rats

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ABSTRACT

The effects of dietary fat during pregnancy and lactation on growth performance of pups, milk composition and very low density lipoprotein composition in rats were studied. A total of 33 dams were used in this study and each litter was adjusted to 8 pups per dam. The dams were fed on high fat (150 g fat/kg diet, HF), medium fat (75 g fat/kg of diet, MF) and low fat (2.5 g fat/kg diet, LF) diets. The body weights of dams increased during pregnancy and decreased after pregnancy. The HF pups had a higher body weight and higher weight gain than those of LF pups. The amount of feed intake of HF dams was significantly higher than LF and MF dams. The HF dams had significantly higher milk fat and water concentrations than LF dams. The milk protein was not significantly different among the treatment groups. All dams showed hypertriacylglycerolaemia in their very low density lipoprotein (VLDL) in late pregnancy. The VLDL-protein concentrations increased during the first week after parturition. The HF dams showed a greater response to the dietary fat than that of LF and MF dams. The findings suggest that addition of fat in the diet during pregnancy and lactation may improve the milk quality through modifying the composition of VLDL contents, leading to better growth of pups.

INTRODUCTION

The mammary gland is the sole organ for providing nutrients for nursing animals. Understanding the physiology of the mammary gland is important for maximizing growth performance of nursing animals during the preweaning period. It has been demonstrated that first week of growth performance affects future growth performance (Loh, Dodds & Lean, 1998) and the post weaning growth performance, however, mainly depends on preweaning growth (McConnell, Eargle & Walsort, 1987). The preweaning growth solely depends on the milk produced by the dams. Composition of colostrum and milk can affect the type of growth that occurs in the neonate. For example, colostrum and milk fats primarily are utilised by the newborn mammal for the deposition of body fat. Milk composition can be altered by diet to some degree. Lactose and milk protein concentrations generally are not subjected to major changes by modifying diet. However, milk fat can be altered by dietary fat level. Jackson *et al*. (1995) reported that feeding supplemental maize oil during the last two weeks of gestation and during lactation had a significant impact on the colostral and milk fat contents. Therefore, there is a need to understand

better about fat inclusion in the maternal diet as the nutritional value of milk produced during pregnancy and lactation may be important for improving preweaning growth and development of mammals.

The effects of genetic potential for milk production and dietary energy on neonatal growth, maternal weight and maternal body composition can vary widely even with a species (Pettigrew *et al*., 1993; Pond, 1986). However, maternal hypertriglyceridemia is one of the physiological changes that occur at late gestation in both human (Knopp, Montes & Warth, 1978; Potter and Nestel, 1979; Fåhraeus, Larson-Cohn & Walleutin, 1985; Knopp *et al*., 1986) and experimental animals (Jones, 1976; Argiles & Herrera, 1981). Very low density lipoprotein (VLDL) plays a pivotal role in supplying triacylglycerol (TG) for milk fat production and it has greater significance in large litter size per farrowed species such as the rat and pig (Herrera *et al*., 1988; Wright *et al*., 1995). Milk fats are derived from *de novo* synthesis within the mammary gland from lipids of dietary origin or lipids mobilised from adipose tissue. The fat composition of milk is highly variable and depends on the lipid composition of the maternal diet. Increment concentrations of fats added in the diets of rats during lactation have been shown to decrease (Beare *et al*., 1961) or increase (Grigor & Warren, 1980) or showing no effect (Burnol *et al*., 1987 and Green, Dohner & Green, 1981) on the milk lipid concentration. These inconsistent results might be due to the variation in the experimental designs. Some studies provided the fat with different concentrations, ranging from 10 to 60 g fat/100g diet. The timing of fat inclusion in the diet differed among the studies, some included for a short period after parturition, while others did throughout pregnancy and the lactation period. Furthermore, the types of fat included in the diets differed; some used corn oil, tuna oil, lard or tallow. None of the studies mentioned above was designed to use palm oil in the maternal diets. Additionally, palm oil is always available and cheaper than other types of edible oils.

The objectives of this study were to determine the effects of different levels of palm oil inclusion in the maternal diet on the concentrations of nutrients in the milk and the growth performance of the litters from rat dams.

MATERIALS AND METHODS

Animals and diets

Thirty-three female Sprague-Dawley rats weighing 188-200g at 12 weeks of age were used in this study. They were housed individually in plastic cages in a temperature-controlled room (26±2ºC) with a 12-h light dark cycle. They were randomly assigned to three numerically equal groups, each of 11 dams: Low Fat (LF) (2.5g fat/100g diet), Medium Fat (MF) (7.5g fat/100g diet) and High Fat (HF) (15g fat/100g diet). All diets had the same energy density and supplied 15.20kJ of digestible energy per gram of dry diet. All compositions of the different diets are presented in Table 1. Fat contributed 3%, 22% and 40% of total energy to the LF, MF and HF diets, respectively. Rats had free access to diet and water. All the rats were adapted to the respective diets for a week before mating.

Body weight and feed intake were recorded every week for 7 weeks: 3 weeks of pregnancy and 4 weeks of lactation. The animals weighing 200–280g at 14 weeks of age were mated. Day 1 of pregnancy was indicated by the day on which sperm was identified in vaginal smears, whereas day 1 of lactation was designated on the day of parturition. Litters were weighed and adjusted to 8 pups per dam. No sex differentiation was done. Litter were weighed weekly throughout the lactation period.

Milk collection and analysis

Milk samples were collected on days 5, 10 and 15 of lactation from 11 rats of **Table 1.** Composition of experimental diets each dietary group. The litters were separated from their dams for a period of 4 hours before milking. Milk composition can be affected if there is a longer period of separation (Keen *et al*., 1980). Milking was done manually from all teats after intraperitoneal injection of oxytocin (4UI) under moderate pentobarbital anaesthesia (35mg/kg BW). All the milk samples were kept at –20^{o}C until further analysis.

Milk protein concentration was determined by the method of Lowry *et al*. (1951). Lipid concentrations were estimated as described by Brigham, Sakanashi & Rasmunseen (1992).

Table 1. Composition of experimental diets

Ingredients	Low fat	Medium fat	High fat
		g/kg of diet	
Soybean meal	220	220	220
Glucose	450	375	300
Corn	212	237	237
Palm oil	25	75	150
DL-methionine	3	3	3
Mineral mix[1]	28	28	28
Vitamin mix[2]	12	12	12
Wheat bran	50	50	50
Proximate analyses:			
Crude protein	10.35	10.35	10.34
Fat	2.12	5.27	9.65
Ash	3.08	4.02	4.52
Calculated DE, kJ/g	14.45	14.45	14.45

[1] Minerals (per kg of diet): $CaHPO_4$, 15g; K_2HPO_4, 2.5g; KCl, 5g; NaCl, 5g; $MgCl_2$, 2.5g; Fe_2O_3, 2.5mg; $MnSO_4$, 125mg; $CuSO_4.7H_2O$, 0.2mg; $ZnSO_4.7H_2O$, 100mg; KIO_3, 0.4mg.

[2] Vitamins (per kg of diet): thiamin, 20mg; riboflavin, 15mg; pyridoxin, 10mg; nicotinamide, 100mg; calcium pathothenate, 70mg; folic acid, 5mg; biotin, 0.3mg; cyanocobalamin, 0.05mg; retinyl palmitate, 1.5mg; DL-α-tocopheryl acetate, 125mg; cholecalciferol, 0.15mg; menadione, 1.5mg; ascorbic acid, 50mg; myo-inositol, 100mg; choline, 1.36g.

Blood collection

Blood samples were collected from tail vein on days 0, 7 and 14 of pregnancy and days 7 and 14 of lactation period. All the rats were handled gently and carefully. Very low density lipoprotein TG concentrations were determined as previously described (Tan *et al*., 2000; Loh *et al*., 2002).

Statistical analysis

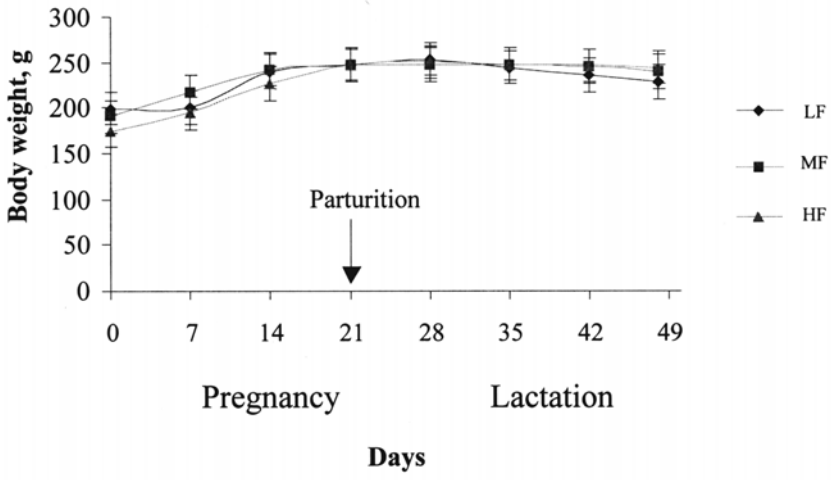
The results are presented as mean and its standard error of difference (s.e.d.). Differences between groups were analyzed by Student's t test for independent samples (Minitab, 1995). Differences of $p<0.05$ were considered significant.

RESULTS

The average body weights from various treatment groups of dams increased steadily during pregnancy and decreased after parturition (Figure 1). However no differences ($p>0.05$) were observed between groups throughout pregnancy and lactation.

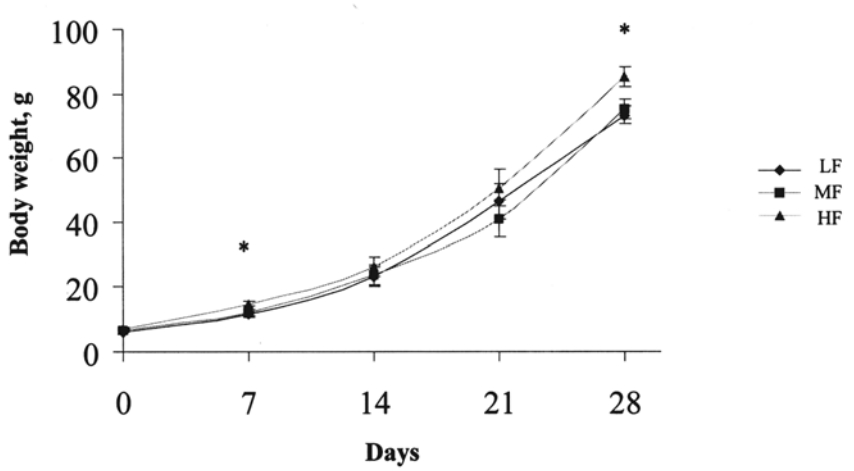
Body weights for HF pups were significantly higher ($p<0.05$) than the LF pups on days 7 and 29 of lactation (Figure 2). However, there was no significant different ($p>0.05$) for body weight between LF and MF. The pups of HF had significantly higher ($p<0.05$) weight gain than those of LF and MF pups (weight gain for LF, MF and HF pups were 67.22 g, 68.76 g and 78.31 g, respectively).

The data presented in Table 2 summarizes the weekly feed intake of different treatment groups of dams. During pregnancy and lactation, weekly feed intake increased progressively for all the rats. Clear differences ($p<0.05$) in the feed intake were observed with the highest feed consumption for HF dams and lower intakes for the LF and MF dams during pregnancy. One week before weaning, dams from HF had a higher ($p<0.05$) feed intake than the other groups of rats.



Error bars show s.e. values.

Figure 1. Body weight during pregnancy and lactation of dams fed low fat (LF), a medium fat (MF) and a high fat (HF) diets



* indicates significant difference at p<0.05; error bars show s.e. values.

Figure 2. Body weights of pups of dams fed with a low fat (LF), medium fat (MF) and high fat (HF) diets

Table 2. Weekly feed intake of dams fed a low fat (LF), medium fat (MF) and high fat (HF) diets

	Dietary groups[1]	LF	MF	HF	s.e.d.
Days of Pregnancy	7	79.77ab	78.77b	85.02a	2.55
	14	82.92c	87.40b	95.02a	0.72
	21	128.63b	127.77b	139.62a	1.23
Days of lactation	7	147.32b	148.23b	154.38a	1.35
	14	169.02a	166.27a	167.28a	1.53
	21	183.38b	185.70b	191.72a	1.43
	28	192.32b	194.30b	198.50a	1.32

[1] LF: low fat (25 g fat/kg of diet); MF: medium fat (75 g fat/kg of diet) and HF: high fat (150 g fat/kg of diet)
a,b and c within each row, means with different alphabet are significantly different (p<0.05).

Table 3 shows the results of the milk composition of dams during lactation. Milk fat was significantly higher (p<0.05) for HF rats than for LF rats throughout the lactation. There were no differences (p>0.05) between HF and MF, and LF and MF dams. The milk protein concentrations were not significantly different (p>0.05) among the treatment groups.

Analysis of the composition of VLDL obtained from dams throughout their pregnancy showed a progressive increase in the TG concentration measured (Figure 3, expressed as µg/ml of plasma) in the 3 weeks prior to parturition. After parturition, the VLDL-TG concentrations decreased progressively for all the treatment groups. The VLDL-TG concentrations were significantly higher (p<0.05) for HF rats than for LF rats. However, no difference was observed (p>0.05) between LF and MF rats. In contrast, VLDL-protein concentrations (Figure 4) varied very little

among the treatment groups throughout pregnancy and lactation. However, the VLDL-protein concentrations increased for all the dams from different treatment groups during the first week of lactation. The VLDL-protein concentration of HF rats was significantly higher ($p<0.05$) than for LF rats.

Table 3. Fat and protein (g/L) concentrations of milk from dams fed a low fat (LF), medium fat (MF) and high fat (HF) diets

	Dietary groups[1]	LF	MF	HF	s.e.d.
Fat	Day 5	1.41a	1.68a	1.92a	0.33
	Day 10	1.38b	1.47ab	1.74a	0.18
	Day 15	1.44b	1.74ab	1.86a	0.18
Protein	Day 5	1.20	1.24	1.28	0.32
	Day 10	1.12	1.24	1.24	0.36
	Day 15	1.12	1.28	1.28	0.40

[1] LF: low fat (25 g fat/kg of diet); MF: medium fat (75 g fat/kg of diet) and HF: high fat (150 g fat/kg of diet)
a,b within each row, means with different alphabets are significantly different ($p<0.05$).

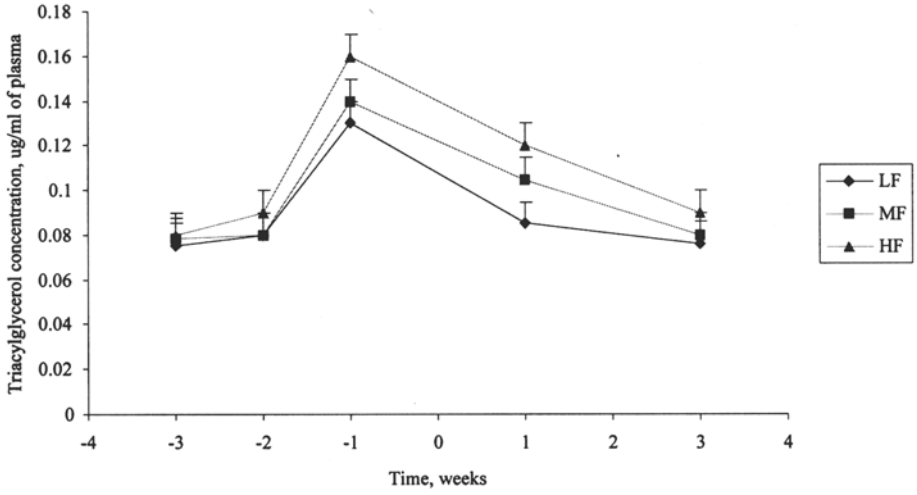


Figure 3. The concentrations of very low density lipoprotein triacylglycerol in the plasma of pregnant and lactating dams. The time scale is expressed in weeks prior to (–) or following (+) parturition (zero week).

DISCUSSION

The greater feed intake observed in rats fed HF diet may be associated with a better palatability of the diet than other diets (MF and LF). The body weight of dams from different treatment groups showed a similar increment in weight gain. The results show similar trends to those reported elsewhere for rats (Rolls *et al*., 1984; Del Prado, Delgado & Vallalpanda, 1997) and for

other species (Montelongo *et al*., 1992). This is important for the pregnant dams to prepare themselves for the lactating period. It has been shown that feed intake of dams is correlated with the growth of their pups (Rolls *et al*., 1984). The growth of HF pups was better than those of LF and MF pups. This faster growth may be associated with a better quality of milk produced from their dams. This explanation could be supported by the results of higher milk fat concentration in HF milk compared to the LF and MF milk. Brandorff (1980) reported that a high fat diet changes the fatty acid composition of rat milk by increasing the long-chain fatty acid content at the expense of medium chain fatty acids. The medium fatty acids are more readily utilised for energy and less deposited into adipose tissue triacylglycerols (Bray, Lee & Bray, 1980). The findings of improvement in the growth of pups from dams fed a high fat diet are inconsistent (Grigor and Warren, 1980; Green *et al*., 1981; Rolls *et al*., 1984; Del Prado *et al*., 1997). Grigor and Warren (1980) fed lactating rats a diet containing peanut oil (a mixture of oleic and linoleic acids). That diet resulted in a better growth rate in pups from dams fed a diet containing peanut oil than control rats fed with a commercial diet. In contrast, pups from lactating dams fed a diet containing coconut oil (490g lauric acid/kg oil) had a similar growth rate with the pups from dams fed with a commercial diet. Experiments in lactating dams fed a fat-added diet showed a better weight gain of the litter relative to that of controls fed a commercial diet only (Green *et al*., 1981). The inconsistent results among these studies might be due to differences in fat types used and only one concentration was used in a specific study. This did not allow for the effects of fat in the diets of dams to be seen clearly. However, in the present study only palm oil and three different concentrations were used.

The LF dams lost more weight than MF and HF dams during lactation. These results indicate LF dams had a more deficit energy balance, which could be explained by the limited energy intake and high energy utilization in milk production (Del Prado *et al*., 1997).

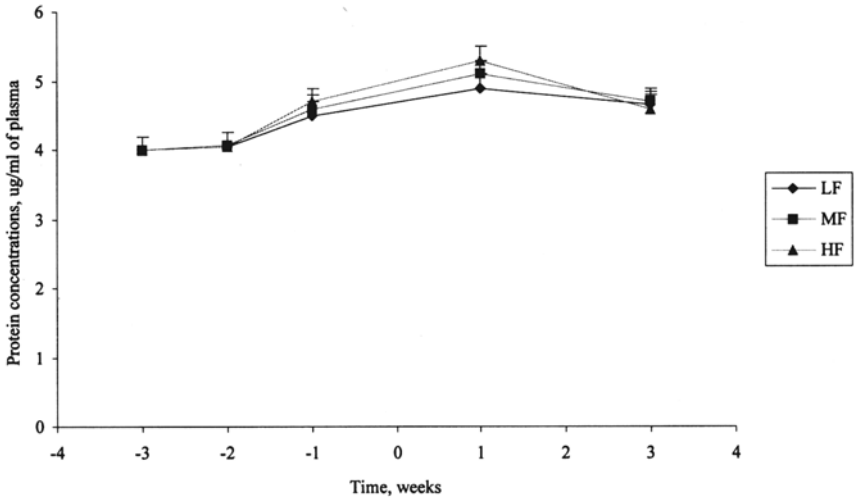


Figure 4. The concentrations of very low density lipoprotein protein in the plasma of pregnant and lactating dams. The time scale is expressed in weeks prior to (–) or following (+) parturition (zero week).

Milk fat concentrations response to high fat diet

The results herein suggest that the composition of milk could be modified by dietary fat. Del Prado *et al.* (1997) showed that dams fed on a high lipid diet during pregnancy and lactation had a higher milk lipid concentration and daily milk volume and lipid production. In a study on pigs by Shurson *et al*. (1986), addition of 10% dried fat to the diets of sow during the late gestation and lactation periods resulted in heavier litters and heavier average piglet weights at 21 days compared to control pigs. There was a 13% increase in estimated milk yield and the higher fat concentration of milk consumed by the nursing sow pigs supplemented fat diet.

The composition of TG in VLDL increased progressively prior to parturition, and reducing to the values obtained prior to mating. The results show similar trends to those reported elsewhere for human (Herrera *et al*., 1987; Montelongo *et al*., 1992; Knopp *et al*., 1986) and for pigs (Reese *et al*., 1984; Wright *et al*., 1995). In mammals, it has been shown that adipose triacylglycerol is mobilised during late pregnancy; the liberated fatty acids are incorporated into very low density lipoprotein by the liver and secreted into the blood stream (Herrera *et al*., 1992; Montelongo *et al*., 1992; Wright *et al*., 1995). The results in this study corroborated these findings by showing hypertriacylglycerolaemia in late-pregnant rats. However, the responses were greater in those rats fed with HF diet than those with LF and MF diets. The measurement of VLDL-protein indicates the number of VLDL particles in the plasma (Wright *et al*., 1995) due to the presence of apo-B which is the largest of the apolipoproteins and is the major protein constituent of the lipoprotein (Davis, 1991). The concentration of VLDL-protein increased in the plasma for all the treatment groups one week after parturition. The results are therefore an indication that plasma contains a higher number of VLDL during the first week of lactation.

During lactation, there was a corresponding fall in VLDL-TG and a rise in the plasma concentration of VLDL-protein. These results suggest that the VLDL number in the plasma increases in response to the increased requirement for milk fat synthesis by the mammary gland. The HF dams showed a better response to the dietary fat than LF and MF dams. The relationship between higher fat concentrations in the milk of rats fed HF diet is congruent with the high body weight of pups. Body weight of pups before weaning depends solely on the milk produced from the dams. Therefore, we can conclude that the higher milk fat production could be achieved by greater extraction of lipid from plasma VLDL through provision of a high fat diet during pregnancy and lactation.

In conclusion, the rats fed with high fat diet during pregnancy and lactation develop higher VLDL-TG in the plasma during late gestation and higher VLDL-protein immediately after parturition and produce higher concentration of fat in the milk. These results imply that dietary fat has an important role in altering milk quality through greater movement of plasma lipids, particularly VLDL into mammary cells. Furthermore, this improves the growth of pups.

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