Maternal protein reserves and their influence on lactational performance in rats

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To determine the contribution of tissue protein reserves to lactational performance, multiparous female Sprague-Dawley rats were mated, caged individually and offered a diet high in protein (215 g crude protein (N × 6·25; CP)/kg dry matter (DM);H) *ad lib.* until day 12 of gestation. Subsequently half the rats continued to receive diet H while the remainder were offered a diet low in protein (65 g CP/kg DM;L) until parturition. This treatment aimed to produce a difference in carcass protein at parturition. On day 1 of lactation females were allocated to either diet H or a low-protein diet (90 g CP/kg DM;L_2) offered until day 13 of lactation, giving four lactation treatment groups HH, HL_2, LH and LL_2. Groups of females were slaughtered on days 2 and 12 of gestation and days 1 and 13 of lactation and carcass and major organs were analysed. Weight gain of standardized litters was used as an indicator of lactational performance. Maternal carcass protein contents at parturition were 43·5 (SE 1·2) and 38·7 (SE 0·8) g ($P < 0·01$) for diets H and L respectively. During lactation there was little change in carcass protein content of HH rats while LH rats appeared to replenish their depleted reserves. Food intake or lactational performance did not differ between these two groups. HL_2 and LL_2 rats lost carcass protein with HL_2 rats losing more than LL_2 rats ($P < 0·05$). Intake and lactational performance were reduced compared with that on diet H ($P < 0·05$) but for the first 6 d of lactation were both greater ($P < 0·05$) for diet HL_2 than for diet LL_2. All four groups showed a considerable loss of body fat during lactation which was not affected by diet. The ability of HL_2 rats to catabolize more protein and consume more food allowed them to sustain a greater lactational performance. Previous maternal protein depletion had no influence on lactational performance as long as an adequate supply of dietary protein was provided.

Lactation: Protein mobilization: Feed intake: Rat

The concept of mammals having a store of protein in tissues that is capable of being depleted in times of stress and thereby contributing to the free amino acid pools of the body has been well documented for rats (Allison & Wannemacher, 1965), chicks (Fisher *et al.* 1964) and cattle (Paquay *et al.* 1972; Biddle *et al.* 1975). The body's major protein reserve is reported to be found in skeletal muscle (Swick & Benevenga, 1977) and can represent approximately 250 g/kg body protein (Allison & Wannemacher 1965; Botts *et al.* 1979).

Lactation imposes an enormous demand on a mother's protein and energy supplies and although there is a concomitant elevation of food intake, the use of body fat stored during gestation has been shown to make an important contribution to the additional energy cost of lactation in rats (Naismith *et al.* 1982), humans (Butte *et al.* 1984) and cattle (Bauman & Currie, 1980) especially during early lactation. Maternal protein reserves may also be

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catabolized to supply amino acids for milk protein synthesis and gluconeogenesis in rats (Naismith *et al.* 1982; Sainz *et al.* 1986; Naismith & Robinson, 1987), humans (Motil *et al.* 1989) and cattle (Belyea *et al.* 1978; Trigg & Topps, 1981; Wilson *et al.* 1988).

Although it has been well established that the degree of fatness at parturition influences subsequent responses to nutrition during lactation (Garnsworthy, 1988), the situation regarding the influence tissue protein repletion has on lactation remains uncertain.

The utilization of tissue protein reserves during lactation has been suggested to be important in allowing lactating sows to sustain lactational performance under conditions of dietary protein inadequacy (Mahan & Mangan, 1975). The extent to which these reserves could influence lactational performance was also thought to depend on the size of the available protein reserve at parturition, although no quantitative measurements of tissue protein reserves were made. Friggens (1990) has also suggested that in rats the ability to sustain lactation on marginal diets depends on the extent of maternal tissue reserves and the capacity of females to mobilize such reserves. Therefore, in many studies of tissue mobilization during lactation using rats as a model, the unstated assumptions that either the degree of repletion of tissue reserves at parturition is not important to subsequent changes during lactation or that the reserves are fully replete (Naismith *et al.* 1982; Sainz *et al.* 1986) are clearly not tenable.

The objective of the current study was to investigate the influence that the maternal protein reserves and the extent of their depletion have on lactational performance in rats offered an adequate or inadequate dietary protein supply.

MATERIALS AND METHODS

The current experimental protocol was designed to establish at parturition two groups of female rats that had distinct differences in the size of their maternal protein mass and, thus, protein reserve, which will be described as either 'full' or 'depleted'. In this experiment (Expt 1) only four (treatment H) and three (treatment L) rats were slaughtered at parturition for body composition analysis. This analysis identified maternal protein masses of 44·6 (SE 1·7) and 39·3 (SE 1·7) g for H and L respectively which, by convention, were not significantly different ($P = 0{\cdot}08$). As the purpose of the present work was to examine the impact of differences in maternal protein mass on lactational performance, it was felt necessary to consolidate these measurements of initial protein mass by amalgamating them with those from a complimentary experiment (Expt 2) in which rats of the same type and from the same source were treated similarly during gestation and culled immediately after parturition on day 1 of lactation. This second study involved the same experimental protocol but was designed to investigate changes in tissue protein metabolism during lactation. Dams used in this complimentary experiment (Expt 2) and offered treatments H and L during gestation had initial body weights of 300·7 (SE 2·7) and 303·9 (SE 3·4) g respectively. The body-weight changes during gestation in Expt 2 were qualitatively similar to those reported for Expt 1 and, following parturition, the body weight of these females were 312·7 (SE 4·0) and 307·2 (SE 5·8) g, whilst the carcass composition analysis of dams culled on day 1 of lactation established their body protein masses to be 42·3 (SE 1·6) and 38·6 (SE 0·9) g for H and L respectively. Between the two experiments there was no significant difference in the relationship between carcass protein content and maternal body weight (from regression analysis).

Experimental design

Multiparous female Sprague-Dawley rats (Harlan Olac Ltd., Shaws Farm, Bicester, Oxon.) were caged individually in a room regulated at 22° and humidity from 40–60 % with a light

period from 08.00–20.00 hours. At the appropriate time, females were placed individually in a wire-bottomed cage with a proven male breeder. The morning on which mating was confirmed, through the presence of vaginal plugs, was designated day 1 of gestation and the females were returned to solid-bottomed plastic cages for the remainder of the experiment.

Following mating the females were offered a high-protein diet (215 g crude protein (N × 6·25; CP)/kg dry matter (DM);H) (Table 1) *ad lib.* until day 12 of gestation. Subsequently half the females continued to receive diet H while the remainder were offered a low-protein diet (65 g CP/kg DM;L) *ad lib.* until parturition. Groups of females in Expt 1 (*n* 4) were selected at random for slaughter on days 2 and 12 of gestation and immediately following parturition. Their carcasses were analysed for DM, protein, ash and fat (see pp. 15–16). Litters from females slaughtered following parturition were also used for carcass analysis.

Dietary treatments described here for lactation relate to Expt 1 animals only. On day 1 of lactation females were allocated factorially to either diet H or a low-protein diet (90 g CP/kg DM; L_2) which were offered *ad lib.* for the rest of the experiment. This allocation produced four groups of females (HH, HL_2, LH, and LL_2; the first letter representing dietary treatment from day 12 of gestation and the second letter representing the lactation diet) that reached day 13 of lactation, at which point females and litters were slaughtered and analysed (see pp. 15–16).

All diets were formulated to provide 21 MJ gross energy (GE)/kg DM with a constant carbohydrate:fat value of 2·3:1. Litters were standardized to twelve pups on day 1 of lactation and litter weights were measured daily. Dam body weights and feed intakes were recorded daily throughout the experiment. All females were given free access to drinking water.

Carcass analysis

Dams were killed by decapitation and the liver, mammary gland, gastrointestinal tract (empty), viscera and carcass were dissected from all animals and analysed for dry matter, protein, ash and fat. The DM content was designated as the constant weight achieved following freeze-drying. Protein was calculated as Kjeldahl N × 6·25. Ash content was estimated following combustion at 550° for 24 h. Fat was estimated from the GE/kg DM of each carcass using the equation:

$$\text{carcass fat (g/kg DM)} = (\text{GE} - 23{\cdot}6 \times \text{CP}/1000)/39{\cdot}6/1000,$$

where 23·6 and 39·6 represent the GE contents (MJ/kg) of CP and fat respectively (McDonald *et al.* 1988). GE was estimated using a Gallenkamp bomb calorimeter.

The carcass composition of females slaughtered on day 13 of lactation was estimated for days 2 and 12 of gestation and day 1 of lactation by regression *v.* body weight and composition of females offered similar dietary treatments slaughtered at each point. Regression equations produced for day 1 of lactation utilized data for females slaughtered in the two parallel experiments.

Statistical analysis

For the statistical treatment of results two-way analysis of variance and one-way analysis of variance were used, and where appropriate by the calculation of least significant differences, *t* tests were used to compare between means of the four lactation treatment groups.

Table 1. *Diet formulation (g/kg dry matter (DM))*

Diet...	High (H)	Low (L)	Low (L_2)
Casein*	215	65	90
Maize oil	191	236	239
Starch-sucrose†	444	549	531
Vitamin mix‡	50	50	50
Mineral mix‡	100	100	100
Diet analysis:			
Protein (g CP/kg DM)	214·8	67·7	90·9
GE (MJ/kg DM)	21·3	21·2	21·4
Emulsifier (lecithin) (g/kg fresh wt)	2	2	2
Antioxidant (butylated hydroxytoluene) (g/kg fresh wt)	0·01	0·01	0·01

CP, crude protein (N × 6·25); GE, gross energy.
* Casein supplemented with DL-methionine (990 + 10 g/kg respectively).
† Starch and sucrose mixture (2:1, w/w).
‡ Vitamin and mineral mixes formulated to meet NRC (1978) requirements.

RESULTS

Feed intake and body-weight changes during gestation

The mean initial body weights of the four treatment groups HH, HL_2, LH and LL_2 on day 1 of gestation were 321·3, 316·3, 325·7 and 322·3 (SD 5·1) g respectively. Feeding of diet L during the second half of gestation reduced the body-weight gains of pregnant females compared with those receiving the diet H ($P < 0·001$), although there was no significant difference in feed intakes (Table 2).

Treatment L from day 12 of gestation had no significant effect on litter size but did significantly reduce the mean pup birth weight ($P < 0·01$).

Main effects of gestation and lactation dietary treatments on dam feed intake, body-weight and tissue changes

The lactation results for the four treatment groups HH, LH, HL_2 and LL_2 are shown in Table 3. Feeding diet L_2 in lactation reduced food intake throughout lactation, increased body weight and tissue protein losses significantly ($P < 0·001$) but had no significant effect on body fat loss. Feeding diet L in gestation resulted in a significant reduction in food intake only during the first half of lactation. There was a significant ($P < 0·05$) interaction between gestation and lactation treatments on food intake during days 1–6 of lactation. For groups HH and LH feed intakes rose throughout the period of lactation.

Maternal protein rose from 49·6 (SE 2·8) to 53·1 (SE 2·2) g between days 2 and 12 of gestation. Thereafter, dams that continued to receive diet H reduced their body protein to 43·5 (SE 1·2) g by day 1 of lactation. This reduction was significantly greater in those females offered diet L ($P < 0·01$) and body protein was reduced to 38·7 (SE 0·8) g. This confirms that the gestation treatment L was of sufficient severity to deplete protein reserves significantly in such females.

Subsequent changes in carcass protein content during lactation were not only related to the lactation diet offered but also to the initial state of carcass protein reserves (i.e. gestational treatment; Table 3), as estimated from regression *v.* live weight (see above), with feeding diet L_2 during lactation significantly ($P < 0·001$) reducing maternal protein mass. Groups HL_2 and LL_2 reduced their carcass protein contents to 35·2 (SE 0·8) and 35·3

Table 2. *Maternal body weight gain, feed intake and pup birth weight of rats offered high (H)- and low (L)-protein diets in sequence during gestation*

Dietary sequence† ...	HH (*n* 14)	HL (*n* 12)	SD
Dam wt gain‡ (g) days 1–22	39·6	13·9	19·5***
Feed intake (g DM):			
days 1–11	185·7	192·6	$20·5^{NS}$
days 12–22	204·4	185·3	$29·8^{NS}$
Litter size (pups/litter)	14·5	12·7	$2·7^{NS}$
Mean pup birth wt (g)	6·4	5·6	0·7**

DM, dry matter; NS, Not significant.
** $P < 0·01$, *** $P < 0·001$.
† For details of diets and dietary treatments, see Table 1 and p. 15.
‡ Dam wt gain following parturition.

Table 3. *Feed intake, body-weight loss and carcass composition change during lactation of rats offered either high (H)- or low (L)-protein diets during gestation and then H or low (L_2)-protein diet during lactation*

Dietary sequence from day 12 of gestation to day 13 of lactation...	HH	LH	HL_2	LL_2		Diet effect		
n...	6	4	4	5	SD	Gestation	Lactation	Gestation × lactation
Feed intake (g DM/12 d)	391·6	390·7	164·4	129·6	131·2	—	***	—
Day 1–6 (g DM)	142·9	140·7	88·7	45·3	46·4	*	***	*
Day 7–12 (g DM)	248·7	249·9	75·7	84·2	89·8	—	***	—
Dam wt change (g/12 d)	−11·6	10·1	−109·1	−85·7	52·2	*	***	—
Dam gains (g) of:								
Carcass protein‡	−1·6	1·9	−10·3	−5·8	5·2	*	***	—
Carcass fat‡	−15·5	−16·4	−20·5	−18·9	5·4	—	—	—

DM, dry matter.
* $P < 0·05$, *** $P < 0·001$.
† For details of diets and dietary treatments, see Table 1 and p. 15.
‡ Dam carcass composition changes adjusted for initial composition on day 1 of lactation using regression equations derived from data for females slaughtered in the present experiment and Expt 2:
Protein: H = 20·9 + 0·0692 body wt (*n* 8, r^2 0·54, $P < 0·05$),
L = 18·2 + 0·0677 body wt (*n* 7, r^2 0·77, $P < 0·05$),
Fat: H = −39·8 + 0·192 body wt (*n* 8, r^2 0·86, $P < 0·01$),
L = −16·3 + 0·131 body wt (*n* 7, r^2 0·86, $P < 0·01$).

(SE 1·6) g respectively, while during the same period group HH maintained their carcass protein content and that of LH increased from 40·3 (SE 1·6) to 42·6 (SE 2·2) g. Changes in the carcass protein content for the four lactation treatment groups are shown in Fig. 1.

In all animals there was considerable storage of fat in the carcass during gestation between day 12 and parturition (Fig. 2). This accumulation of adipose stores was not affected by diet offered during the second half of pregnancy, and the carcass fat contents at parturition were 22·8 (SE 2·5) and 23·5 (SE 1·5) g for dams receiving diets H or L respectively. During lactation the four treatment groups all showed a considerable loss of carcass fat; this loss was not significantly affected by gestational or lactational dietary treatment (Table 3, Fig. 2).

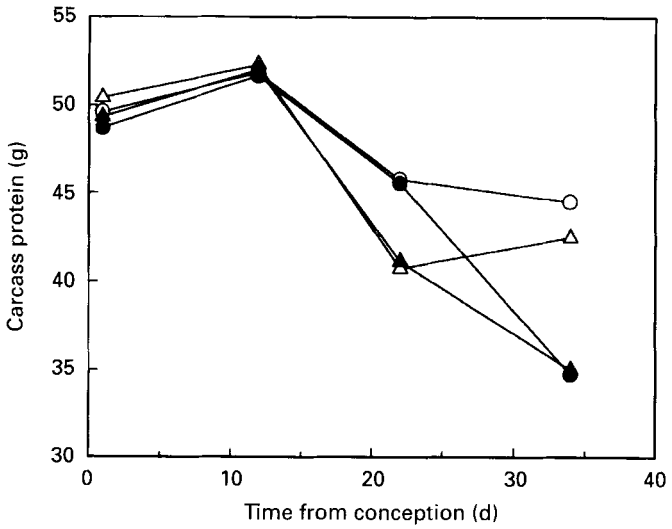

Fig. 1. The change in carcass protein from day 2 of gestation to day 12 of lactation in groups of rats offered either a high (H)- or a low (L)-protein diet from day 12 of gestation followed by either H or a low (L_2)-protein diet during lactation: (○), HH (*n* 6); (●), HL_2 (*n* 4); (△), LH (*n* 4); (▲), LL_2 (*n* 5). All rats gave birth on day 22. For details of diets and dietary treatments, see Table 1 and p. 15.

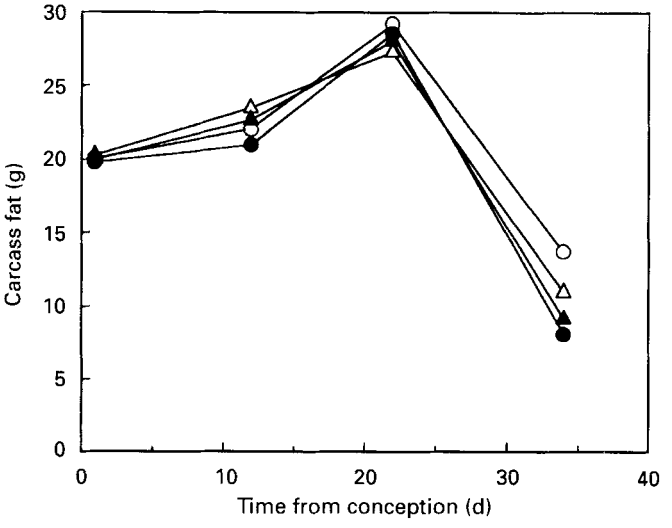

Fig. 2. The change in carcass fat from day 2 of gestation to day 12 of lactation in groups of rats offered either a high (H)- or a low (L)-protein diet from day 12 of gestation followed by either H or a low (L_2)-protein diet during lactation: (○), HH (*n* 6); (●), HL_2 (*n* 4); (△), LH (*n* 4); (▲), LL_2 (*n* 5). All rats gave birth on day 22. For details of diets and dietary treatments, see Table 1 and p. 15.

There was also considerable accumulation of fat in the abdominal stores during gestation, rising from 11·9 (SE 0·6) g on day 1 of gestation to 19·6 (SE 2·3) and 21·1 (SE 2·3) g for dams receiving diets H or L respectively and culled on day 1 of lactation. Also, the estimated (Table 3) loss of fat from the abdominal stores during lactation by the four treatment groups was not significantly affected by gestation or lactation dietary treatment, being 12·8, 16·3, 15·9 and 20·2 g for HH, LH, HL_2 and LL_2 respectively.

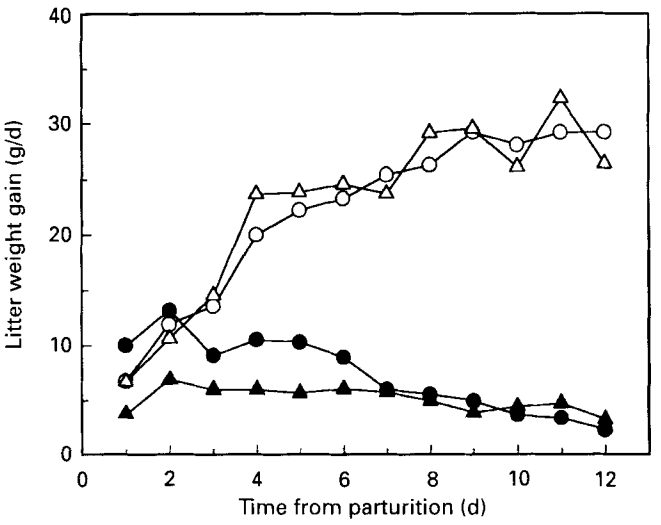

Fig. 3. Mean growth rates of standard litters of twelve pups for groups of rats offered either a high (H)- or a low (L)-protein diet for the last half of gestation followed by H or a low (L_2)-protein diet during lactation: (○), HH (*n* 6); (●), HL_2 (*n* 4); (△), LH (*n* 4); (▲), LL_2 (*n* 5). For details of diets and dietary treatments, see Table 1 and p. 15.

Litter weight gain and composition changes

Lactational performance, as represented by litter weight gain, was significantly greater in females which received diet H in lactation ($P < 0{\cdot}001$) and in general followed the pattern shown for maternal dietary intakes (Table 4), but was not significantly influenced by gestation treatment. Whilst weight gain increased with age in the litters of 'high-protein' mothers, it tended to reduce in litters from 'low-protein' mothers (Fig. 3). Although lactational performance was significantly impaired by feeding diet L_2, the capacity of group HL_2 to mobilize greater quantities of carcass protein and consume more food than group LL_2 allowed them to maintain a significantly ($P < 0{\cdot}05$) higher lactational performance during the first 6 d of lactation than LL_2. However, group HL_2 were unable to maintain this increased performance during the second half of lactation, at which time their litter weight gain was less than 50% of that in the first half. Group LL_2 showed very little difference in performance between the first and second half of lactation even though their food intakes almost doubled.

The significantly greater litter weight gain during lactation supported by diet H was also reflected in a significantly greater litter gain in protein and fat compared with the two low-protein groups (Table 4). The greater lactational performance shown by HL_2 during lactation (significant during days 1–6) compared with LL_2 is reflected in the significantly greater litter protein gain, although there was no significant difference in their fat gains (Table 4).

Effect of gestational dietary treatment on maternal organ weight and composition on day 1 of lactation

The maternal organ weights of females killed on day 1 of lactation are shown in Table 5. Dietary treatment L from day 12 of gestation had generally no significant effect on either the weights of the major organs (liver, mammary gland and gastrointestinal tract) or their composition on day 1 of lactation. Only liver protein content was significantly reduced by treatment L during the second half of gestation.

Table 4. *The effect of gestation (high (H)- and low (L)-protein) and lactation (H and low (L_2)-protein) dietary treatments on litter-weight gain and change in litter composition during lactation in female rats*

Dietary sequence from day 12 of gestation to day 13 of lactation...	HH	LH	HL_2	LL_2		Diet effect		
n...	6	4	4	5	SD	Gestation	Lactation	Gestation × lactation
Weight gain (g/12 d)	264·9	270·7	87·5	61·1	102·4	—	***	—
Days 1–6	97·8	104·3	62·0	34·3	32·0	—	***	*
Days 7–13	167·1	166·6	25·5	26·8	73·3	—	***	—
Gains of (g/12 d):								
Protein‡	38·7	37·9	14·6	10·0	13·9	—	***	—
Fat‡	40·5	42·6	12·0	7·3	17·7	—	***	—

* $P < 0{\cdot}05$, *** $P < 0{\cdot}001$.
† For details of diets and dietary treatments, see Table 1 and p. 15.
‡ Litter protein and fat gains adjusted for initial composition on day 1 of lactation.

Table 5. *Maternal organ weights and composition on day 1 of lactation of female rats offered a high (H)- or low (L)-protein diet from day 12 of gestation*

Diet offered from day 12 of gestation†...	H	L	SD
Liver:			
Wet wt (g)	16·40	13·02	2·68
Dry wt (g)	4·12	3·32	0·64
Protein (g)	2·97	2·22*	0·50
Fat (g)	0·67	0·72	0·22
Ash (g)	0·18	0·15	0·17
Mammary gland:			
Wet wt (g)	24·99	21·08	4·48
Dry wt (g)	14·76	12·25	2·66
Protein (g)	2·10	1·81	0·58
Fat (g)	12·09	10·10	2·15
Ash (g)	0·16	0·15	0·03
GI tract:			
Wet wt (g)	8·78	8·12	0·93
Dry wt (g)	2·86	2·52	0·59
Protein (g)	0·99	0·95	0·06
Fat (g)	1·67	1·39	0·54
Ash (g)	0·08	0·07	0·00

GI, gastrointestinal.
* $P < 0{\cdot}05$.
† For details of diets and dietary treatments, see Table 1 and p. 15.

Effect of gestational and lactational dietary treatments on maternal organ weight and composition during lactation

The main effects of the gestation and lactation dietary treatments on maternal organ weights and composition on day 13 of lactation are shown in Table 6. Lactation treatment, but not gestation treatment, had a significant effect ($P < 0{\cdot}001$) on all measures reported in Table 6.

Table 6. *Maternal organ weights and composition on day 13 of lactation of the four lactation treatment groups (high (H)- or low (L and L_2)-protein diets) of female rats*

Dietary sequence from day 12 of gestation to day 13 of lactation†...	HH	LH	HL_2	LL_2		Diet effect		
n...	6	4	4	5	SD	Gestation	Lactation	Gestation × lactation
Liver:								
Wet wt (g)	22·67	22·61	14·06	13·47	5·12	—	***	—
Dry wt (g)	6·49	6·33	3·81	3·61	1·54	—	***	—
Protein (g)	3·85	3·76	2·32	1·94	0·95	—	***	—
Fat (g)	1·70	1·61	0·78	0·95	0·48	—	***	—
Ash (g)	0·23	0·26	0·15	0·13	0·06	—	***	—
Mammary gland:								
Wet wt (g)	25·04	28·00	12·38	11·84	7·63	—	***	—
Dry wt (g)	9·24	8·88	4·31	4·72	2·57	—	***	—
Protein (g)	4·12	4·34	1·83	1·60	1·35	—	***	—
Fat (g)	4·22	3·74	2·17	2·84	1·09	—	***	—
Ash (g)	0·41	0·45	0·16	0·14	0·15	—	***	—
GI tract:								
Wet wt (g)	11·54	12·02	6·90	6·53	2·85	—	***	—
Dry wt (g)	2·94	3·02	1·60	1·62	0·80	—	***	—
Protein (g)	1·56	1·62	0·88	0·79	0·41	—	***	—
Fat (g)	1·07	1·12	0·53	0·66	0·41	—	***	—
Ash (g)	0·15	0·15	0·09	0·07	0·04	—	***	—

*** $P < 0·001$.

† For details of diets and treatment groups, see Table 1 and p. 15.

Feeding diet H during lactation increased the size and composition of the major organs analysed by day 13 of lactation when compared with sizes following parturition (Table 5). Both the liver and gastrointestinal tracts showed considerable increases in their weights (wet and dry) and protein contents, while liver fat content increased as gastrointestinal tract fat declined. The mammary gland did not increase in size to the same extent as the liver and gastrointestinal tract but it did show considerable changes in composition. As milk production had increased between day 1 and 13 of lactation, the dry matter content of the gland was reduced along with mammary fat content while protein content was more than doubled.

DISCUSSION

The biphasic nature of protein metabolism during gestation (Naismith & Morgan, 1976; Naismith & Emery, 1988) dictates that there is storage of protein in maternal reserves during the first half of pregnancy (anabolic phase) to be utilized in support of the development of the feto-placental unit during the second half (catabolic phase). The changes in carcass protein content of the four treatment groups HH, HL_2, LH and LL_2 represented in Fig. 1 support this view of changes in tissue protein masses during gestation.

In order to establish differences in the level of maternal protein reserve before lactation we aimed to amplify the catabolism of maternal protein during the second phase by feeding a low protein diet from day 12 onwards. Zartarian *et al.* (1980) had previously reported a significant effect of feeding a 75 g protein/kg diet during the second phase on the loss of weight and protein of skeletal muscles in rats.

In our first experiment the gestational treatments resulted in mean values of maternal protein mass which were different but not significantly so by convention ($P = 0·08$). As it

was central to the thesis we were exploring that we had confidence that the gestational treatments established real differences in maternal protein mass at parturition we incorporated data from a second experiment, involving the same gestation treatments, into this study. By combining the data for females slaughtered on day 1 of lactation from two parallel experiments we have shown that our gestational dietary treatments did produce a significant depletion in maternal carcass protein content before lactation ($P < 0{\cdot}01$; 11%). This significant 11% difference in maternal protein reserves on day 1 of lactation might have been greater if the females on diet H could have limited the mobilization of their carcass protein during the second phase of gestation, but this was not the case even though protein intake was high. It is possible that intakes of diet H during this phase could have been increased if the energy density of the diet had been lower.

However, during lactation group LL_2 were still capable of losing tissue protein (5·8 g). It appears, therefore, that such dams were able to prevent too great a depletion of maternal reserves during their gestational malnutrition with consequential effects on foetal growth. The significant reduction in foetal birth weight with low-protein feeding in gestation supports the view that there is a limit to which foetal parasitism can prevent foetal growth restriction during gestational maternal malnutrition (Anderson *et al.* 1980).

Lactation imposes enormous demands on the body's metabolism and to ensure that lactation proceeds successfully there are co-ordinated adaptations in metabolism (homeorhesis) that partition available nutrients towards the mammary gland and away from tissues which are not essential to lactation (Bauman *et al.* 1980). Along with an elevation of feed intake, physiological changes include hypertrophy of liver, intestines, heart and mammary gland (Williamson, 1980). At the same time in well-nourished animals there is an expansion of cardiac output and an increase in blood flow to these tissues (Chatwin *et al.* 1969). Thus, lactation is associated not only with an increase in mammary size but also in other organs involved in supplying nutrients for milk biosynthesis.

In the present study, feeding diet L from day 12 of gestation until parturition generally had no significant effect on organ weight including the mammary gland, although in each case the mean weight was usually less (not significant) for diet L compared with diet H. This contrasts with a previous study in which dietary protein and energy restriction from day 5 of gestation resulted in a considerable reduction in mammary size by day 21 (Rosso *et al.* 1981).

By day 13 of lactation feeding diet H promoted hypertrophy of the major organs associated with lactation, particularly the liver and intestines, in line with earlier observations (Williamson, 1980). Although the mammary gland did not show such a marked increase in size it did undergo a distinct change in composition, with its dry weight and fat content declining while its protein content was considerably increased.

Feeding diet L_2 during lactation prevented any such organ hypertrophy and on day 13 of lactation the liver, mammary gland and gastrointestinal tract weights of groups HL_2 and LL_2 were significantly lower than the two high-protein groups. Blood flow to the mammary gland could also be expected to have been considerably reduced under such dietary conditions (Sakanashi *et al.* 1987). Such a restriction of organ growth in rats has also been reported under conditions of protein–energy restriction (Sakanashi *et al.* 1987) and reductions of protein quality and quantity (Sampson *et al.* 1986). These reductions in organ size associated with feeding diet L_2 during lactation reflect the low rates of food consumption achieved by rats offered these diets. Thus, the hypertrophy observed in females offered diet H was probably a function of food consumption rather than an inevitable consequence of the state of lactation although, from the pup growth data, intakes of diet L_2 were also associated with impaired lactation.

The comparable feed intakes and lactational performances of the groups offered

lactation diet H occurred while group LH were attempting to gain weight and replenish protein reserves. These results show that diet H can be sufficient in allowing litter weight gain not to be hampered by a depletion of tissue reserves which had occurred before lactation, and agrees with work by Mahan & Mangan (1975) involving first litter sows.

In earlier studies Sainz *et al.* (1986) proposed that even females offered a high protein diet will catabolize maternal protein in support of lactation and that this may be influenced by the litter size and dam maturity. The non-significant carcass protein loss of group HH in the present study, with a litter size of twelve pups, does not support this proposition. However, it is plausible that in such females tissue reserves are catabolized during the initial stages of lactation before being replenished later on. The measurements of carcass composition made here were insufficiently frequent to check this possibility. We suggest, however, that from our evidence there is no obligatory loss of maternal protein during lactation.

The control of tissue protein mobilization during lactation obviously involves changes in the relative rates of tissue protein degradation and synthesis. From limited work in lactating sheep (Bryant & Smith, 1982; Vincent & Lindsay, 1985) and rats (Sainz *et al.* 1984) it seems that a rise in degradation is primarily responsible for muscle protein losses during lactation. Subsequent work of our own (Pine *et al.* 1992) would confirm this.

While in the present study the net catabolism of maternal protein reserves during lactation depended on the lactation diet, the loss of body fat deposited during gestation seemed to occur independently of the gestation and lactation diets. While the diets used here were isoenergetic there were considerable differences in the energy intake during lactation, being 8·34 and 3·52 MJ GE/12 d for groups HH and HL_2 respectively, and this was reflected in significant differences in the lactational performance as measured by pup growth. That the rate of net maternal fat loss under these circumstances was similar suggests that fat was being lost from the bodies of these rats at a rate that was close to maximal. A similar loss of fat reserves during lactation is shown by genetically-obese rats under conditions of cafeteria feeding (Van Duijuenvoorde & Rolls, 1985).

Whether such high rates of fat loss would have been seen if the rats had not been allowed to increase the size of their adipose stores during gestation may be doubtful. Loss of fat during lactation is not obligatory, as thin females can compensate for their lack of fatness by enhancing food intake whilst sustaining equally copious lactation in comparison with fatter contempories (Garnsworthy, 1988). The mass of fat in the body (largely determined by previous nutrition), current nutrition, physiological status and genotype will all play a part in determining both the rate at which fat is lost from the maternal body and the total amount that can be lost. Whilst the suggestion by Naismith *et al.* (1982) that the catabolism of body stores is under hormonal rather than dietary control is sustained by the results presented here, it is perhaps more appropriate to say that the maximum rate at which these reserves can be lost will be subject to hormonal control. The amount of fat which could be lost at that rate would logically depend on the mass of fat that was present and that will largely be a reflection of previous nutrition. This active partitioning of milk fat precursors would be favoured by the hypoinsulinaemia (Williamson, 1980) and high levels of prolactin (Vernon, 1989) that are associated with lactation in rodents. Whilst this hypoinsulinaemia and reduced adipocyte responsiveness (Burnol *et al.* 1987) favours the release of fat from the adipose tissue following a shift in the balance of lipogenesis (Williamson, 1980) and lipolysis (Smith & Walsh, 1976), the enhanced mammary gland insulin responsiveness (Burnol *et al.* 1987) would allow the anabolic processes associated with lactation to be maintained. Reciprocal changes in the adipocyte and mammary gland lipoprotein lipase (*EC* 3.1.1.34) activity (Hamosh *et al.* 1970; Mendelson *et al.* 1977) further alters the utilization of circulating lipid and, while there is evidence to suggest that prolactin may be

involved (Zinder *et al.* 1974), its action may be indirect and require a functioning mammary gland (Flint *et al.* 1981).

Feeding a diet inadequate in protein quantity or quality during lactation has been associated with a suppression of feed intake and a reduction in lactational performance in rats (Naismith *et al.* 1982; Jansen & Hunsaker, 1986; Friggens, 1990) and also in pigs (Mahan & Mangan, 1975), even though lactating females attempt to support lactation through the catabolism of tissue protein reserves. The results of this current study are in agreement with these previous observations. The situation during lactation presents an interesting contrast to that which can be seen during growth, where animals (for example, pigs: Kyriazakis *et al.* 1990) offered highly digestible diets which have a low concentration of protein will attempt to maintain their dietary protein intake by increasing food consumption over that which is seen for similar diets of higher protein content. The consequence of such an action would be, of course, an increase in the intake of energy-yielding nutrients, as well as protein, which would possibly be intolerable in rats that are already mobilizing body fat.

During the second half of gestation feeding diet L had no significant effect on feed intake. This contrasts markedly with the low intakes achieved in lactation by rats offered diet L_2. An important difference between growing or pregnant rats and lactating rats in their response to a food of a low protein:energy ratio is likely to be in the manner in which energy-yielding nutrients are used for fat storage. During both growth and gestation storage of surplus energy-yielding nutrients as fat is both possible and, at least in gestation, even desirable. In lactation where body fat, as here, is being mobilized even when dietary energy intake is high (groups HH and LH), an animal offered a low-protein–high-energy feed perhaps fails to eat adequately (in terms of protein) because the balance of protein and energy-yielding nutrients which results could create a metabolic embarrassment when associated with the release of fat from the body.

During the first 6 d of lactation females of group HL_2 had significantly greater intakes and litter weight gains than group LL_2, whilst being able to mobilize significantly more tissue protein. This mobilization of protein, alongside fat, would have alleviated the imbalance between protein and energy-yielding nutrients which resulted from the combination of diet composition and tissue mobilization. In rats that were protein-depleted at parturition (after receiving diet L during gestation) there was still some tissue protein loss during lactation when diet L_2 was offered. However, the extent of this loss was constrained by what appeared to be the lower limit of maternal protein mass. Shields *et al.* (1985) have also reported that during early lactation losses of body protein from first-litter sows were significantly reduced by the feeding of a low-protein diet (50 g/kg) during gestation.

When the gestation diet had an adequate protein content and maternal protein mass at parturition was relatively high (gestation diet H), subsequent feeding of diet L_2 during lactation had a less severe affect on pup growth than when the gestation diet was also low in protein (diet L). Thus, it appears that the mobilization of maternal protein during lactation was capable of acting as a buffer against dietary protein inadequacy, at least for a while. The stage of lactation at which pup growth of group HL_2 dropped considerably and came to reflect directly diet composition and intake (Fig. 3) was possibly the point at which the readily-labile tissue reserve approached a minimum.

The improved lactational performance of group HL_2 during the first 6 d of lactation was also reflected in a significant alteration in litter composition. The HL_2 litter gained more protein but not fat than LL_2 between days 1 and 13 of lactation. The greater capacity to catabolize labile protein reserves by group HL_2 appeared to allow, therefore, a significantly improved pup growth, both through the extra dietary protein consumed during the first 6 d of lactation (4 g) as well as the use of residual labile protein. This greater lactational

performance and improved litter protein gain may not just be the result of alterations in milk yield but also milk composition (or both), although no measurements of milk composition were made.

The carcass protein contents on day 13 of lactation (Fig. 1) were possibly reached before this point and these females could be approaching the limit of their protein reserves. From the patterns of litter growth (Fig. 2) it might be reasonable to suggest that the support of litter growth by the mobilization of maternal protein reserves was exhausted by day 6 or 7 of lactation in dams of group HL_2. If day 6–7 was the point at which the bulk of tissue labile protein reserves are expended and, thus, its impact on lactation was curtailed, the balance of tissue protein metabolism would need to be adjusted to prevent further mobilization of tissue protein. The controlling factors involved in such a mechanism remain to be elucidated. It is of interest to note the similar carcass protein contents of the HL_2 and LL_2 females on day 13 of lactation which could represent the limit to which tissue reserves could be catabolized (Glore & Layman, 1985). In these animals this was approximately 72% of the carcass protein on day 2 of gestation and suggests that during the whole period of reproduction these females lost approximately 28% of carcass protein, close to the 25% suggested by Allison & Wannemacher (1965).

The results of the current study confirm the findings of Naismith and co-workers (Naismith *et al.* 1982; Naismith & Robinson, 1987) that although lean tissue can be catabolized during lactation in response to dietary protein restriction, the supply of endogenous protein is insufficient to allow lactation to continue at the level of similar females receiving an adequate protein supply, at least not beyond the first few days of lactation. We also confirm that the mobilization of endogenous adipose stores tends to suppress the intake of a low-protein diet. However, we have extended these findings by presenting evidence that suggests that assumptions concerning the extent of protein reserve repletion at parturition can under- or overestimate the ability of a female to respond to inadequate dietary protein during lactation. These results also confirm that females can actively regulate the loss of protein from carcass reserves during gestation and lactation.

In summary we conclude that the utilization of maternal protein reserves during lactation can improve lactational performance under conditions of dietary protein inadequacy when intake is suppressed by the loss of maternal adipose stores. However, this influence is constrained by the extent of the maternal reserves available and the capacity of females to mobilize such reserves. The depletion of protein reserves before lactation does not inhibit lactational performance when an adequate supply of dietary protein is provided. In act a more efficient use of the dietary protein could occur as females attempt to replenish depleted reserves while maintaining lactational performance.

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