Interactions between major nutrients in the diet and the lactational performance of rats

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The effect on lactational performance of replacing feed carbohydrate with fat at two different protein levels was studied. Lactating Sprague–Dawley rats with a standardized litter size of thirteen pups were allocated one of eight feeds containing either 300 or 150 g protein/kg organic matter (OM) and ranging in fat content from 100 to 550 g/kg OM from day 2 until day 14 of lactation. Daily food intake, live-weight gains, and changes in body composition of both dams and litters were measured. Feeds of low protein content resulted in a significant decline ($P < 0.001$) in lactational performance despite a significant increase ($P < 0.001$) in maternal protein mobilization. Maternal lipid mobilization was not significantly affected by feed composition. Litter lipid gain was significantly increased ($P < 0.05$) as fat replaced carbohydrate in the high-protein feeds, due to an increase in maternal energy intake. In contrast, lactational performance was severely depressed ($P < 0.001$) as fat replaced carbohydrate in the low-protein feeds. This interaction between feed components on lactational performance was in accordance with the hypothesis that the heat production of lactating rats is maximal and, hence, constraining intake.

Lactation: Feed composition: Rat

Lactational performance is sensitive to dietary protein concentration in all species which have been studied (e.g. in rats, Nelson & Evans, 1958; pigs, Mahon & Grifo, 1975; cattle, Ørskov *et al.* 1977; humans, Forsum & Lonnerdal, 1980). In rats the available evidence suggests that lactating and non-lactating rats differ in their response to variation in feed protein content. In response to a dilution of feed protein content non-lactating rats show an increased intake (Musten *et al.* 1974), whereas lactating rats have been found to decrease their intake (Naismith *et al.* 1982; Grigor *et al.* 1987). The lactating rats did not seem able to compensate for a decline in feed protein content, despite a critical deficit in protein supply (Naismith *et al.* 1982). This suggests that unacceptable metabolic consequences arise from the excess intake of non-limiting feed components and that consequently the food intake of lactating rats is constrained.

In both these studies (Naismith *et al.* 1982; Grigor *et al.* 1987) differences in protein content were achieved by substitution of carbohydrate for protein; there were no other changes in feed composition. Thus, concomitant increases in the energy:protein ratio and in the carbohydrate content of the feeds occurred as the protein content was decreased. Any constraint arising from those components which were not different between the two feeds, such as bulk content, would be expected to result in similar intakes of the two feeds and not in a depression of intake on the low-protein feed. The dams on the low-protein feeds had a lower energy intake than those on the high-protein feed and were mobilizing relatively large amounts of body lipid. Consequently the reduction in food intake cannot be readily ascribed to a constrained energy intake resulting from the increase in energy:protein ratio of the feed. By inference it would seem that the increase in
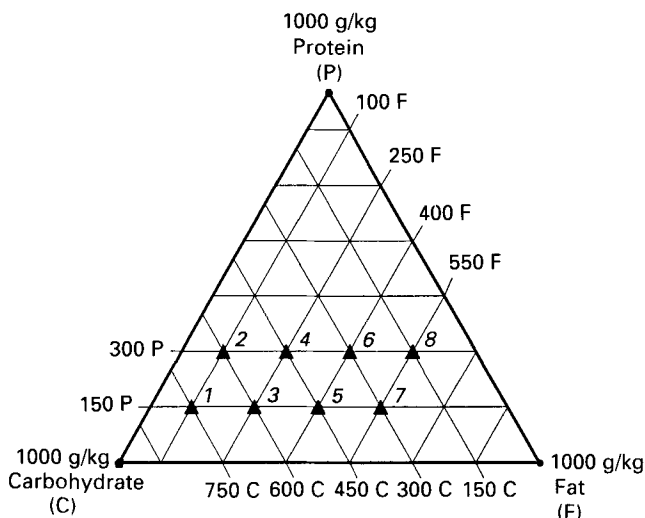
Fig. 1. The composition of the eight experimental feeds (g/kg organic matter) within a triangular representation of nutrient mixtures consisting of three components; protein, carbohydrate, and fat. Lines of equal nutrient content run parallel to that side of the triangle which does not form part of the apex of 1000 g/kg content for a given nutrient.

carbohydrate content of the feed, either in absolute terms or per unit energy, had an influence on food intake.

There is some evidence which indicates that the carbohydrate content of the feed may influence lactational performance. Contrary to the previous experiments, Sainz *et al.* (1986) did not find a decrease in the food intake of lactating rats as feed protein content decreased, indeed there was a small, but non-significant, increase in intake. In their experiment a constant fat:carbohydrate value was maintained as the protein content of the feeds decreased; the proportion of the non-protein energy arising from carbohydrate remained constant. This work suggests that the proportion of the non-protein energy arising from carbohydrate may influence intake and subsequent lactational performance. A decrease in the proportion of the non-protein energy arising from carbohydrate, at equal protein and energy intakes, has been found to enhance lactational performance (Maynard & Rasmussen, 1942). Further, in this work the high-carbohydrate feed was associated with a lower *ad lib.* intake than the low-carbohdyrate feed (rats were pair-fed). The enhanced lactational performance was presumably due to the greater efficiency of milk-fat production from fat rather than carbohydrate (Chudy & Schiemann, 1969).

The purpose of the present work was to explore in more detail the extent to which the lactational performance of rats is influenced by the carbohydrate:fat ratio of the feed and to investigate the influence of the carbohydrate:fat ratio on the lactational response of rats to changes in feed protein content. A graphical representation of nutrient mixtures (Fig. 1) was employed in the design of the present experiment; this has been described in detail by Parks (1982).

On the high-protein feeds (nos. 2, 4, 6, and 8; Fig. 1) it was expected that a decrease in carbohydrate content would result in improved lactational performance. On the feeds of low protein content (nos. 1, 3, 5, and 7; Fig. 1) the same expectation was held for those feeds whose carbohydrate content was sufficient to meet requirements for lactose production. For those feeds of both low protein and limiting carbohydrate content further decline in carbohydrate content was expected to cause a depression in lactational

performance. However, no clear indication of the minimal feed carbohydrate content for adequate lactation in rats was available from the literature.

METHODS

Animals and management. Forty-seven mature lactating Sprague–Dawley rats (third parity) were allocated on day 2 of lactation to one of eight feeding treatments (Table 1) or to an initial cull (*n* 5). Pups were cross-fostered on day 1 of lactation to give a standardized litter size of thirteen pups. The smell of novel pups was masked by contact with bedding material from the cage of the recipient dam. New pups were introduced into the centre of the litter whilst the dam was absent from the cage. Fecundity, pup mortality, and general reproductive data have been previously reported (Friggens, 1991). Dams and their litters were housed in solid floor plastic cages with shredded plastic for nesting material and cat litter to absorb urine. Room temperature was maintained at 24·5 (SD 0·6)° and natural lighting conditions prevailed. The experiment was carried out in the last 2 weeks of May.

Feeds, feeding and measurements. Experimental feeds were designed to encompass a wide range of carbohydrate:fat ratios at two different protein levels (Fig. 1). Feeds consisted of casein and DL-methionine (99:1, w/w), groundnut oil (including an antioxidant; 1·5 g/kg Rendox, Kemin Europa Ltd), starch and sugar (2:1, w/w), an emulsifier (Montane/ Montanox 80; Honeywell and Stein, Leatherhead) and a mineral–vitamin supplement designed to provide double the recommended requirements (National Research Council, 1978); feed compositions are given in Table 1. Food and water were offered *ad lib.* until day 14 of lactation, when dams and litters were culled. In order to reduce spillage the feeds were offered as a mash of a consistency just too soft to prevent removal of lumps from the feed container. To ensure that a change in the form of feed did not influence intake dams were fed on a mashed feed throughout pregnancy (protein, carbohydrate and fat at 300, 450 and 250 g/kg organic matter (OM) respectively). Feed containers consisted of 120 ml wide-necked Beatsen jars (neck diameter 47 mm) inserted in a tight-fitting plastic base (150 × 150 mm) to prevent the jars being tipped over. There was no visible spillage of feed from these containers and no allowance was made for spillage in the food intake measurements. As the feeds contained no fibre a plastic chew ring was given to each rat to allow natural tooth trimming. During the experimental period the weights of food offered and refused, maternal live weight, and litter live weight were measured daily.

Daily feed refusals were collected fresh, weighed, and stored under trichloroethane for subsequent chemical analysis. Unfortunately, this solvent did not prevent the refusals from fermenting and growing mould. Dry matter (DM) and composition values for the refusals were, therefore, lost. Consequently the composition and DM of the food refusals were assumed to be the same as those of the offered food, so ignoring evaporative losses. Given the magnitude of the differences in feed intake between the treatments, for comparative purposes, the slight overestimation of feed intake resulting from this assumption is negligible.

Dams were culled by intraperitoneal injection of pentobarbitone (200 mg) and litters were culled by inhalation of diethyl ether. Immediately after culling the gut contents of the dams and the stomach contents of the pups were removed by sequential squeezing of the gastrointestinal tract. Carcasses were then analysed for water (freeze-drying), protein (Kjeldahl method using a Tecator 1030 analyser), lipid (light petroleum (b.p. 40–60°) extraction for 5 h), ash (500° for 5 h), and gross energy contents (Gallenkamp adiabatic bomb calorimeter). Due to a problem in the analysis of lipid by diethyl ether extraction (attributed to emulsifier residues in the carcass), lipid values were calculated by difference from the measured gross energy and the energy as protein (23·9 kJ/g).

Table 1. *The composition of the experimental feeds* (g/kg dry matter (DM)), unless otherwise stated)*

Feed no.	Casein (10 g methionine/kg)	Starch + sugar	Groundnut oil	CP (g/kg OM)	GE (MJ/kg OM)	No. of rats
1	136	643	85	149	19·9	6
2	279	523	85	299	21·0	6
3	136	507	221	148	23·3	5
4	279	367	221	296	24·5	4
5	136	371	357	145	26·8	6
6	280	231	356	302	28·0	5
7	137	235	492	148	30·3	5
8	280	94	492	295	31·5	5

CP, measured crude protein = $N \times 6·407$; GE, gross energy calculated from measured ingredient gross energies; OM, organic matter.

* All feeds had a mineral content of 100 g/kg DM and a vitamin content of 40 g/kg DM. The vitamin carrier was maize meal (CP 113; oil 57; carbohydrate 768 g/kg DM).

Results were analysed by one- and two-way analyses of variance in conjunction with Levenes test for homogeneity of variance using Genstat software (Rothamstead Experimental Station, Harpenden, Herts.). Initial maternal live weight was used as a covariate in the analyses of maternal live weight and carcass gains. The use of the covariate was justified by a significant reduction in the residual variance. Dams and their litters on feeds nos. 5 and 7 were culled earlier than day 14 on account of an unexpectedly severe depression in litter growth. The average cull dates for feeds nos. 5 and 7 were days 11·9 and 10·5 of lactation respectively, by this time the litters had ceased to gain weight. For comparison with the other rats it was assumed that the weight of these dams and litters on day 14 of lactation would have been the same as their weight when culled. Similarly, their 12 d cumulative food intakes were calculated by extrapolation from their previous daily intakes (see Friggens, 1991).

RESULTS

The results are presented in three sections: the first two sections describe the effects of substituting carbohydrate for fat in the feeds of high protein content and in the feeds of low protein content respectively. These two sections have been sub-divided into effects on litter growth, maternal body reserves, and food intake. The third section makes comparisons between the two feed protein contents. Statistical significances quoted in the first two sections refer to comparisons between feeds of constant protein content. Statistical significances quoted in the third section refer to comparisons made between all feeds. *F* values for the different comparisons are given in Table 2. Average values of initial live weight and body composition for dams and litters are presented in Table 3. The mean values of litter performance, maternal weight change, and food intake of the group on feed no. 7 were heavily influenced by one deviant individual which was excluded from the values.

The high-protein feeds (300 g/kg OM; feeds nos. 2, 4, 6 and 8)

Litter growth. Litter gains of live weight, body protein, and body fat are presented in Figs. 2 and 3. On the high-protein feeds all the litters grew quickly and litter live-weight gain was not significantly affected by decreasing the carbohydrate content from 600 to 150 g/kg OM with a concomitant increase in fat content. As expected, litter lipid gains were significantly enhanced ($P < 0.05$) by successive reductions in feed carbohydrate content from 600 to

Table 2. *Comparison of the effects of feed composition on maternal intake and maternal and litter live-weight gains of rats**

(Values are the standard errors of the difference between means (SED) and the variance ratios (F) from the two-way analysis of variance comparing all feeds, and from the one-way analyses of variance for feeds of the same protein content. Maternal gains have been analysed using maternal live weight on day 2 of lactation as a covariate)

	Two-way analysis of variance for all feeds: F					One-way analysis of variance: F					
						High-protein feeds			Low-protein feeds		
	SED (max−min)†	Protein	Fat	Interaction	Covariate	SED	Fat	Covariate	SED	Fat	Covariate
df‡		1/33	3/33	3/33	1/32		3/16	1/15		3/16	1/16
Maternal intake of:											
DM	38·91	126·65	40·73	11·68	—	31·6	8·80	—	41·9	36·73	—
Protein	7·36	577·97	32·48	3·16	—	8·54	8·80	—	5·66	36·73	—
Fat	8·70	314·07	44·75	65·32	—	9·65	75·22	—	7·23	22·48	—
Carbohydrate	21·42	8·68	112·03	4·27	—	11·87	142·71	—	25·83	45·84	—
Gross energy	0·81	198·41	15·07	20·14	—	0·74	0·55	—	0·82	31·21	—
Maternal gains of:											
Live wt	12·63	119·93	9·20	12·48	12·95	11·95	0·71	6·46	12·76	18·54	6·59
Protein	2·39	108·50	11·11	4·37	9·78	2·09	2·17	3·17	2·45	11·97	7·78
Fat	5·90	0·72	1·50	0·88	0·79	5·67	2·17	0·65	5·95	0·37	0·19
Ash	0·41	1·86	2·96	1·94	21·16	0·30	2·32	20·29	0·47	2·59	7·17
Gross energy	0·24	10·38	0·78	1·51	2·31	0·23	1·42	1·28	0·25	0·91	1·05
Litter gains of:											
Live wt	26·46	282·57	12·26	10·63	—	29·6	0·21	—	21·67	30·69	—
Protein	3·53	312·27	17·48	5·64	—	4·31	1·15	—	2·43	41·32	—
Fat	5·65	146·83	1·28	12·35	—	6·81	4·57	—	4·01	12·54	—
Ash	0·63	268·18	14·98	9·85	—	0·75	0·31	—	0·45	44·13	—
Gross energy	0·29	192·81	2·79	10·41	—	0·36	1·79	—	0·20	19·66	—

* For details of experimental feeds, see pp. 61–62 and Table 1.

† SED for the comparison of the maximum and minimum number of replicates.

‡ df are presented as (treatment df)/(residual df). For analyses which used the covariate, the residual df for all comparisons is as given for the covariate.

Table 3. *Average values of live weight, body crude protein* ($N \times 6.25$) *and diethyl ether-extracted body fat mass of the dams and litters at the start of the experimental period*

(Mean values with their standard errors)

	Maternal		Litter	
	Mean	SEM	Mean	SEM
Live wt (g)	374·9	5·3	111·0	1·6
Crude protein (g DM)	68·0	0·9	11·7	0·2
Diethyl extract (g DM)	62·5	1·3	4·9	0·4

DM, dry matter.

Body composition was derived by regression from the initial cull data relating live weight and natural litter size to body composition (Friggens, 1991).
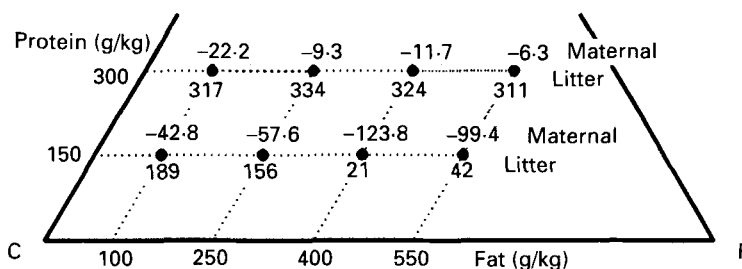
Fig. 2. The effects of feed composition on maternal and litter live-weight gains (g/12 d) in rats. Standard errors of differences between means were 12·6 and 26·5 g/12 d for maternal and litter gains respectively. For details of experimental feeds, see pp. 61–62 and Table 1.

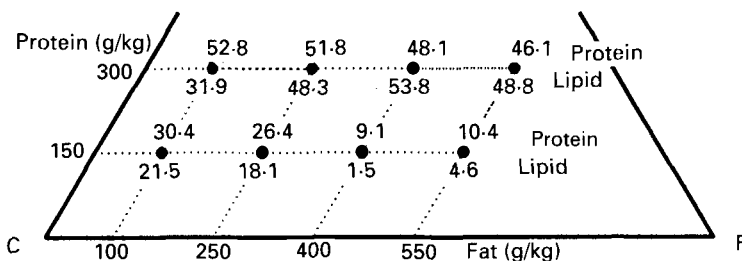Fig. 3. The effects of feed composition on litter gains of body protein and fat (g/12 d) in rats. Standard errors of differences between means were 3·5 and 5·7 g/12 d for body protein and fat respectively. For details of experimental feeds, see pp. 61–62 and Table 1.

300 g/kg OM. However, at the lowest feed carbohydrate content (feed no. 8; 150 g carbohydrate/kg OM) litter lipid gain was less (not significant (NS)) than that on feed no. 6 (300 g carbohydrate/kg OM); this was not expected. Litter protein gains were not significantly affected by the carbohydrate–fat content of the high-protein feeds, but there was a trend (NS) for litter protein gain to decline with decreasing carbohydrate content.

Maternal body reserves. On all high-protein feeds dams lost live weight, lipid and energy between days 2 and 14 of lactation. Changes in maternal live weight and body protein (Figs. 2 and 4) were not significantly affected by the carbohydrate–fat content of the high-protein
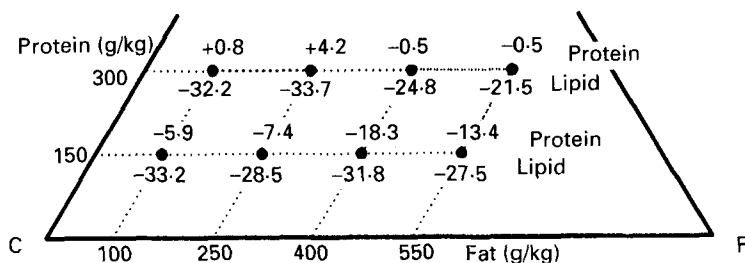
Fig. 4. The effect of feed composition on maternal gains of body protein and fat (g/12 d). Standard errors of differences between means were 2·4 and 5·9 g/12 d for body protein and fat respectively. For details of experimental feeds, see pp. 61–62 and Table 1.
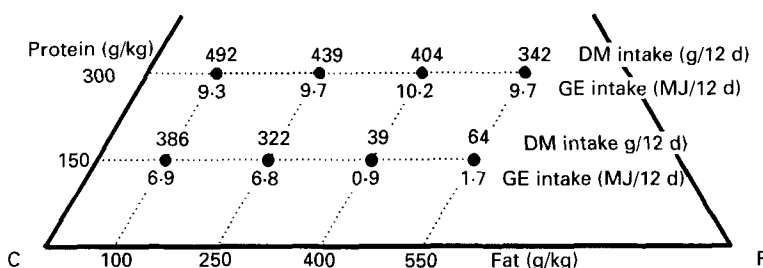

Fig. 5. The effect of feed composition on maternal dry matter (DM) and gross energy (GE) intakes. Standard errors of differences between means 39 g/12 d and 0·8 MJ/12 d for DM and GE intakes. For details of experimental feeds, see pp. 61–62 and Table 1.

feeds (feeds nos. 2, 4, 6 and 8). The average value across the four feeds were − 12 g live weight and + 1·0 g body protein. Maternal body lipid losses were reduced by decreasing feed carbohydrate–increasing fat content, but not significantly.

Food intake. As the feed carbohydrate content of the high-protein feeds decreased, food intake (Fig. 5) and consequently protein intake also decreased ($P < 0.01$). Despite this decline in food intake there was a non-significant increase in energy intake (Fig. 5) as fat replaced carbohydrate, because of the increasing energy content of the feeds. At the lowest feed carbohydrate content (feed no. 8; 150 g carbohydrate/kg OM) this increased energy intake was curbed to a level similar to feed no. 4 (600 g carbohydrate/kg OM). The energy intake data complement the results on litter lipid gain and maternal lipid loss. As the carbohydrate content of the feed decreased from 600 to 300 g/kg OM, energy intake increased from 9·3 to 10·2 MJ/12 d. This was accompanied by increased litter lipid gains and decreased maternal lipid losses.

The low-protein feeds (150 g/kg OM; feeds nos. 1, 3, 5 and 7)

Litter growth. In general, performance on the low-protein feeds for both dams and litters was much inferior to that on high-protein feeds. As the carbohydrate content of the low-protein feeds decreased and fat content increased there was a dramatic decline in litter growth ($P < 0.001$; Figs. 2 and 3). As expected, the highly significant effect of decreasing feed carbohydrate–increasing fat content was not uniform across the range of feed carbohydrate contents offered.

At the high-carbohydrate–low-fat end of the range, defined as feeds nos. 1 and 3, the average litter live-weight gain was 173 g/12 d. Contrary to expectations the drop of 150 g/kg OM in carbohydrate content between feeds nos. 1 and 3 resulted in non-
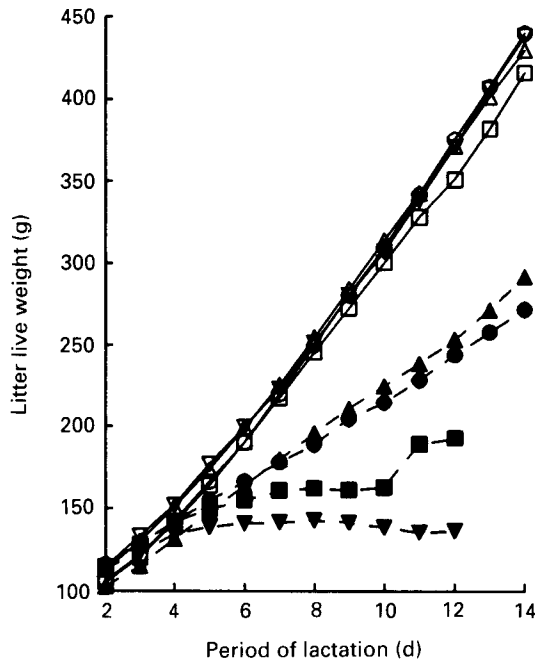
Fig. 6. The interaction between feed protein and carbohydrate–fat content on litter live-weight gain of rats. Feed no: (▲), 1; (△), 2; (●), 3; (○), 4; (▼), 5; (▽), 6; (■), 7; (□), 8. For details of experimental feeds, see pp. 61–62 and Table 1.

significant decreases in litter live weight, protein and lipid gains of 33, 4·0 and 3·4 g/12 d respectively. The subsequent step down in feed carbohydrate content and, hence, step up in fat content from feed no. 3 to feed no. 5 had a catastrophic effect on lactation. The differences between feeds nos. 3 and 5 values for litter live-weight, protein and lipid gains were -135, $-17·2$ and $-16·6$ g/12 d respectively ($P < 0·001$). At the low-carbohydrate end of the range, defined as feeds nos. 5 and 7, the average litter live-weight gain was 31 g/12 d. The effect of the final step down in carbohydrate content from feed no. 5 to feed no. 7 was a small non-significant increase in litter performance; a decrease had been expected.

Maternal body reserves. Changes in the maternal body stores (Fig. 4) were in accordance with the effects seen on litter growth. All dams on the low-protein feeds (feeds nos. 1, 3, 5 and 7) lost live weight, body lipid, and body protein. At the high-carbohydrate end of the range (feeds nos. 1 and 3) average maternal live weight and body protein losses were 50 and 6·6 g/12 d. At the low-carbohydrate end of the range (feeds nos. 5 and 7) average losses of maternal live weight and body protein were 112 and 15·9 g/12 d respectively. The difference between the high- and low-carbohydrate feeds for both live weight and body protein losses was highly significant ($P < 0·001$). There was no significant effect of changing feed carbohydrate–fat content on maternal body lipid losses. The average body lipid loss was 30·3 g/12 d.

Food intake. Food intake values for the low-protein feeds are presented in Fig. 5. Food intake was expected to increase as fat replaced carbohydrate in the carbohydrate-rich low-protein feeds (nos. 1 and 3). Instead there was a non-significant decline in food intake. As the carbohydrate content of the feed decreased from 600 to 450 g/kg OM (and fat content increased) there was a massive decline ($P < 0·001$) in food intake. Average intakes for the
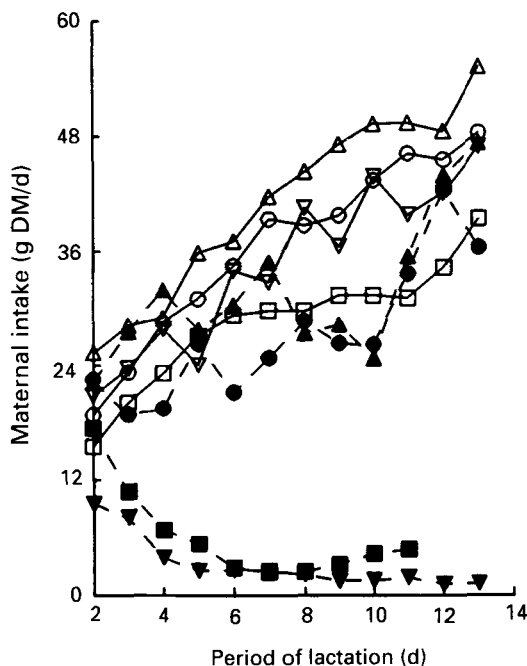
Fig. 7. The interaction between feed protein and carbohydrate–fat content on average daily maternal food intake of rats. Feed no: (▲), 1; (△), 2; (●), 3; (○), 4; (▲), 5; (▽), 6; (■), 7; (□), 8. For details of experimental feeds, see pp. 61–62 and Table 1.

high and low feed carbohydrate contents (feeds no. 1, 3 and 5, 7) were 353·7 and 60·7 g/12 d respectively. Despite the increasing energy density of the feed as feed carbohydrate was replaced by fat, energy intake fell ($P < 0.001$) as the carbohydrate content decreased (Fig. 6).

There was no significant difference in food intake between feed nos. 5 and 7. This was contrary to expectation for feeds which severely limited lactational performance.

Comparison between protein levels

The effect of a decrease in feed protein content from 300 to 150 g/kg OM was, as expected, a depression in energy and protein intakes which resulted in poorer litter growth and litter carcass gains, and greater mobilization of maternal body protein ($P < 0.001$). Maternal body lipid mobilization was not significantly affected by feed composition.

There was a massive and highly significant interaction between the effect of feed protein content and the effect of feed carbohydrate–fat content on lactational performance (Figs. 6 and 7) except for maternal lipid loss. The interaction was such that the difference between the two protein levels in lactational performance was amplified as carbohydrate was replaced by fat in the feed.

DISCUSSION

High-protein feeds. At a high fixed level of feed protein content (300 g/kg OM) the only significant effect on litter growth of replacing feed carbohydrate by feed fat was an increase in lipid gain. These results consolidate the finding that the glucogenic requirements of lactation can be adequately met by feeds of very low carbohydrate content (Steingrimsdottir *et al.* 1980) or even no carbohydrate (Follis & Straight, 1943) provided that the protein content of the feed is high.

As feed fat replaced carbohydrate and the protein:energy ratio of the feed declined there was a significant decrease in maternal protein intake ($P < 0.01$). There was an associated increase in litter lipid gain ($P < 0.05$) and in maternal energy intake (NS), indicative of an energy surplus. However, all the dams on high-protein feeds mobilized large amounts of body lipid, a seemingly counter-productive strategy in a situation of energy surplus. An obligatory body lipid loss, due to physiological state change during lactation, could explain this, but fails to account fully for the observed lipid losses since the body fat content (260 g/kg DM) resulting from lactational lipid loss was considerably lower than the body lipid content of non-lactating rats kept on similar feeds (370 g/kg DM; derived from Friggens, 1991).

A more satisfactory explanation for the observed effects arises from the alternative view that the increase in litter lipid gain resulted from the amelioration of an energy deficit rather than from an increasing energy excess. This explanation is based on the assumption that the dams' capacity to dispose of heat was constraining maternal food intake, as follows. It has been shown with growing rats on low-protein feeds that capacity to dispose of heat can constrain intake, and facilitating heat loss results in improved growth (Andik *et al.* 1963). Further, there is evidence to suggest that in rats the heat production during lactation is of the same magnitude as the capacity for heat loss. Brody *et al.* (1938) found the total heat production of lactating rats to be 4·6 kJ/g live weight$^{0.73}$ per d at an environmental temperature of 28°. In non-lactating rats (250 g live weight) heat losses of 4·6 kJ/g live weight$^{0.73}$ per d would occur at an environmental temperature of 42° (derived from Kirmiz, 1962); rats kept at 45° for 2 h died. Roberts & Coward (1985) found that the thermoneutral range for lactating rats was between 8 and 20° and that maternal heat production was depressed as environmental temperature rose above 20°, suggesting that in the present experiment such a constraint may apply. Consideration of other possible constraints on intake failed to suggest an alternative to heat production. For example, if feed bulk was the constraint then, as fat replaced carbohydrate in the feed, intake would have remained constant until the increase in energy content of the feed permitted energy requirements to be met, further increase in the fat content of the feed would prompt a decline in intake accompanied by zero body lipid mobilization. This was not the case; moreover, these feeds contained no fibrous material. Analogous arguments can be applied to other feed components, such as feed energy content (discussed previously), indicating that no single feed component was constraining intake.

Given a constraining heat production, the observed increase in energy intake as fat replaced carbohydrate in the feed can be explained. Forbes *et al.* (1946a, b) have shown in non-lactating rats that the heat increment per unit feed energy decreases as fat replaces carbohydrate in that feed, at equal protein intakes (summarized by Swift & Black, 1949). Thus, in order to reach the same food-derived heat production more feed energy could be ingested as feed carbohydrate content decreased. It should, however, be noted that the final step down in carbohydrate content from feed no. 6 to feed no. 8 did not cause an increase in energy intake. Under conditions of maximal heat production body lipid mobilization is also justified. Production of milk from body reserves is more efficient than from food (Noblet & Etienne, 1987); body lipid, therefore, represents an energy source for milk production which carries a low heat production penalty. The conclusion that substitution of feed fat for carbohydrate is beneficial to lactational performance is in agreement with a number of previous studies (Maynard & Rasmussen, 1942; Loosli *et al.* 1944; Nelson & Evans, 1947; Steingrimsdottir *et al.* 1980).

Low-protein feeds. Clearly the dams on the low-protein feed had a shortage of protein; maternal protein losses were several times greater than on the equivalent high-protein feed. Consequently, litter growth was severely depressed. The food intake of dams on the low-

protein feeds was also depressed. These findings are in agreement with previous work in this area (Mueller & Cox, 1946; Nelson & Evans, 1958; Drori & Folman, 1973; Naismith *et al.* 1982; Grigor *et al.* 1987).

The current study explored the effects of substituting feed fat for carbohydrate in low-protein feeds on lactational performance over a much wider range (750–300 g carbohydrate/kg OM) than previous studies. As fat replaced carbohydrate in the low-protein feeds lactational performance declined. However, this effect was not linear across the range; there was a dramatic fall ($P < 0.001$) in performance as carbohydrate content decreased from 600 to 450 g/kg OM (feeds nos. 3–5). The severity and rapidity of the decline (Figs. 6 and 7) in lactational performance suggested that a chronic deficiency may have occurred. It had been expected that decreasing feed carbohydrate content to low levels in feeds of low protein content would result in a deficit of glucogenic nutrients and a further impairment of lactational performance. However, the effect of feed nos. 5 and 7 cannot be ascribed to their low content of glucogenic nutrients alone. Calculation of the protein balance of the dams on the high-protein feed no. 8 (see Friggens, 1991) indicates that there was relatively little surplus protein available for gluconeogenesis. Both the absolute carbohydrate content and the calculated glucogenic content of feed no. 8 were approximately half those of feed no. 7 (assuming no surplus protein on feed no. 7), yet the dams on feed no. 8 had a greatly superior lactational performance (Fig. 2). The fat content of feed no. 8 was the same as that of feed no. 7; thus, the depression in performance cannot be attributed to the high fat content or to any associated effects such as the possibility of mineral loss due to insoluble mineral soap formation. Indeed, as a precaution feed mineral and vitamin contents were double those recommended by the National Research Council (1978). All other possible nutrient deficiencies can be discounted by equivalent arguments.

Intakes as low as those on feed nos. 5 and 7 could also arise from feeds that were either toxic to the rats or resulted in loss of appetite, or both. Given the design of the experiment there is no convincing evidence for these feeds being toxic. All eight feeds were made using the same batch of ingredients, including an antioxidant, which were mixed and stored (frozen) in the same manner at the same time. The ingredients used in feed nos. 5 and 7 were palatable for lactating rats when mixed in other proportions (all other feeds). It is, therefore, extremely difficult to envisage a particular combination of these ingredients being so unpalatable as to make maternal weight losses of 30% and negative litter weight gain preferable to eating the food. Indeed it is questionable whether the concept of palatability can be applied to a situation where not eating jeopardizes litter survival.

A far more plausible hypothesis is that these feeds resulted in a metabolic disorder associated with appetite loss by the dams. The circumstances suggest that a ketotic state may have been induced. Feed nos. 5 and 7 were of low glucogenic content and had the highest energy content per unit protein. These dams attempted to maintain milk production in spite of negligible food intakes by massive use of body reserves, greater than the dams on all the other feeds. This is a situation similar to that found in high-producing dairy cows in early lactation; relatively short of glucose, and mobilizing large amounts of body lipid. In this situation cows are most prone to ketosis (Hibbitt, 1979). It is, therefore, reasonable to suggest that the lactating dams offered diet nos. 5 and 7 became ketotic. Ketosis results in depressed milk production and loss of appetite; in extreme cases the animal ceases to produce milk (Schultz, 1979). Unfortunately no measurement of blood metabolite levels were made so this explanation remains unsubstantiated.

Whilst providing a statisfactory explanation for the extent of the lactational failure which occurred on high-fat–low-protein feeds, the previous argument does not address the cause of the food intake depression on low-protein feeds which precipitated the lactational collapse. Maternal mobilization of both protein and lipid indicate that neither feed protein

nor energy contents were the constraint which forced down intake of the low-protein feeds. This also applies to those relevant studies which reported maternal body composition (Naismith et al. 1982; Grigor et al. 1987). The simplest viable explanation for the depression in food intake relies again on the assumption that the capacity of lactating dams to dispose of heat was constraining food intake. This hypothesis accounted for the effects of changing the carbohydrate–fat content of the high-protein feeds. In relation to the high-protein feeds, the low-protein feeds were all of a lower protein:energy ratio. Thus, the low-protein feeds required the disposal of a greater amount of energy per unit protein than the high-protein feeds. Clearly the associated heat production would also have been greater and, hence, the intake which resulted in maximal heat production would have been lower.

Comparison between protein levels. Accepting the hypothesis that heat production was the controlling influence on intake, it was expected that substitution of fat for carbohydrate in the low-protein feeds would benefit lactational performance on those feeds which were not carbohydrate limited. As discussed previously, there is no evidence that the carbohydrate content *per se* of any of the feeds was limiting, yet as fat replaced carbohydrate in the low-protein feeds lactational performance declined. This was contrary to expectations and contrary to the effect of substituting fat for carbohydrate in the high-protein feeds. This interaction between feed components on lactational performance can be explained as follows.

For a lactating animal constrained by its capacity to lose heat, substitution of fat for carbohydrate carries the benefit of a decrease in heat production (4·1 kJ/g substituted; Chudy & Schiemann, 1969) due to an improvement in the efficiency of milk fat synthesis. This permits a higher energy intake at the same, maximal, heat production. However, the substitution of feed fat for carbohydrate also carries the potential penalty of an increased feed energy content (23·1 kJ/g substituted), such that the increase in energy intake will be derived from a decreased food intake. Therefore, the substitution of feed fat for carbohydrate will only benefit lactational performance when the resultant decrease in food intake does not cause a shortage in the supply of dietary protein or carbohydrate for milk production. This would appear to have been the case on the high-protein feeds. However, on the low-protein feeds milk production was protein limited so the decreased food intake arising from the substitution of fat for carbohydrate further exacerbated the dietary protein deficit causing further depression in lactational performance. Clearly the effect of substituting fat for carbohydrate in the feed of lactating rats is subject to an interaction with the feed protein content.

In conclusion, the present experiment has shown that: (1) at constant protein content, the carbohydrate–fat content of the feed affects lactational performance of rats as measured by net pup growth and maternal body composition changes; (2) at constant carbohydrate or fat content, the feed protein content affects lactational performance; (3) there is an interaction between feed protein, carbohydrate and fat contents, which results in massive depression of lactational perfomance at low protein, low carbohydrate contents. Further, the discussion has proposed that: (4) the collapse of lactation at low protein, low carbohydrate content may have been the result of ketosis in the dams; (5) the effects observed can be explained by the hypothesis that heat production is maximal in these lactating dams and constrains food intake.

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