Journal of Animal and Feed Sciences, 13, 2004, 647–664

Effects of substitution of dietary protein with carbohydrate on lactation performance in the mink (*Mustela vison*)*

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(Received 2 April 2004; revised version 26 July 2004; accepted 25 October 2004)

ABSTRACT

Thirty mink dams nursing litters of 6 kits were assigned to one of 3 dietary treatments to investigate the effects of changing the protein:carbohydrate ratio on nutrient utilization, heat production, milk production and kit growth. Three diets were formulated to contain 65:3 (LC), 48:15 (MC) or 34:33 (HC) % of the metabolizable energy (ME) from protein and carbohydrate, respectively. The diets were fed *ad libitum* for 4 weeks from parturition. Twelve dams were held in an intensive care unit and subjected to balance and respiration experiments and the kits were injected with deuterium oxide to measure water kinetics and milk production. Eighteen dams were kept under normal farm conditions and feed intake of dams and weight gain of the kits were determined. Milk samples were collected from the dams. Metabolizable energy intake was not affected by dietary treatment. Carbohydrates were efficiently utilized with a digestibility coefficient of 84% in dams fed the HC diet. Dams fed the HC diet had a lower ($P<0.05$) percentage weight loss, lower ($P<0.05$) total heat production (HE), lower ($P<0.05$) protein oxidation (OXP), lower ($P<0.05$) water intake and a lower ($P<0.05$) nitrogen (N) excretion than dams fed the LC diet. Milk production, and thereby

* Part of the Nordic Joint Committee for Agricultural Research Project No. 100 "Stable isotopes in comparative studies of milk production and energy requirement in pigs, mink and foxes", supported by The Danish Veterinary and Agricultural Research Council, Grant No. 9701275
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liveweights of the kits 4 weeks post partum, was higher ($P<0.05$) in dams fed the HC diet than in dams fed the LC diet. In conclusion, lactating mink dams are able to utilize digestible carbohydrates with positive effects on lactation performance and reduced nitrogen excretion.

KEY WORDS: mink, carbohydrate supply, nutrient oxidation, milk yield, pre-weaning kit growth, nitrogen excretion

INTRODUCTION

In the Scandinavian countries, recommendations for supply of protein, fat and carbohydrates for mink dams during the lactation period are presently a minimum of 40% of metabolizable energy (ME) from protein, 40-50% of ME from fat, and a maximum of 20% of ME from carbohydrates (Hansen et al., 1991). However, dietary protein supply is usually considerably higher than the minimum recommended, and a conventional Danish lactation diet typically consists of 55:33:12% of ME derived from protein:fat:carbohydrates, respectively.

During lactation, glucose is an important intermediate in the metabolism as precursor in lactose synthesis, and an adequate supply is essential for milk secretion (Annison et al., 1968). Børsting and Damgaard (1995) found that lactating mink fed a diet with 12% of ME derived from carbohydrates had to provide about 75% of their glucose requirement by gluconeogenesis. However, although the mink has a well-developed capacity for gluconeogenesis (Sørensen et al., 1995), lactation places very high energy demands on the female mink, and usually voluntary feed intake of the dams is insufficient to meet nutrient demands for maintenance and milk production. Weight losses in the order of 25% during a 6-week lactation period are common (Tauson, 1988; Hansen and Berg, 1998), and in extreme cases mobilization of body reserves can result in emaciation and death (Clausen et al., 1992).

The digestive tract of the mink is very simple (Szymeczko and Skrede, 1990) with a low α-amylase enzyme activity (Elnif et al., 1988) and microbial activity (Williams et al., 1998). Therefore, to ensure high digestibility, the dietary carbohydrate supply has to be either monosaccharides or gelatinised starch. However, previous experiments have shown that the mink has maintained a high capacity for utilization of digestible carbohydrates, since also the glycolytic capacity is large (Sørensen et al., 1995; Fink et al., 2002a,b).

The objective of the present experiment was to test our hypothesis that replacement of dietary protein by carbohydrates has beneficial effects in reducing nutritional stress on the dam, increasing milk production during the first 4 weeks of lactation, increasing kit growth, and reducing nitrogen emission from the mink industry.

MATERIAL AND METHODS

Animals and feeding

Thirty, two-year-old mink dams of the standard brown genotype (Nes et al., 1987) were allocated into 3 groups at parturition. When necessary, kits were cross-fostered within 48 h after birth to obtain six kits per litter. The dams had free access to drinking water and were fed *ad libitum* from parturition until the fourth week of lactation on diets with, on average, 34% (range 32-37) of ME from fat and ratio (% of ME) between protein:carbohydrates ranging from [low carbohydrates (LC): 65:3, medium carbohydrates (MC): 48:15 to high carbohydrates (HC): 34:33]. The diets were mixed on a single occasion and weighed out into plastic bags and immediately frozen. Feed was taken out of the freezer the day before use and thawed overnight. Feed samples were collected daily and pooled for each week, and stored at -18°C until analysis. Dietary composition and chemical analyses of the diets are given in Table 1, and analysed amino acid composition in Table 2.

Balance and respiration experiments

Twelve of the dams (n=4 per dietary treatment) were transferred from the experimental farm to the laboratory for adaptation approximately one week prior to expected delivery. In the laboratory, the dams were maintained under natural daylight conditions (May, 55°N 12°E) in individual metabolism cages designed as described by Jørgensen and Glem-Hansen (1973), equipped with nest boxes and devices for feeding and drinking water supply and quantitative collection of feed residues, faeces and urine. The experiment was divided into four consecutive one-week balance periods, which started on May 4 and continued until June 1. Recording of drinking water intake and quantitative collection of feed residues and excreta took place once daily between 8.30 and 12.00 a.m., and the pooled amount collected from each period was stored at -18°C until analysis. The collection procedures have been described in detail by Wamberg et al. (1996). Each balance period included a 22-h respiration experiment by means of indirect calorimetry in an open-air circulation system. In the respiration chamber, temperature and relative humidity were 15-18°C and 65-75%, respectively. Air from the chambers was analysed every third min for concentrations of O_2 and CO_2. Oxygen was analysed by a paramagnetic analyser (Magnos 4G, Hartmann and Braun, Frankfurt, Germany), and CO_2 was analysed using the infrared principle (Uras, Hartmann and Braun, Frankfurt, Germany). Measurements of milk intake of the kits at weekly intervals by the water isotope dilution technique were performed as described in detail by Fink et al. (2001). The animals were weighed at weekly intervals.

Milking procedure

The remaining 18 dams (n=6 per dietary treatment) were kept under normal farm conditions and daily feed intake, and weekly liveweights of kits and dams were recorded. Milk samples representing each group and lactation week were collected from the dams by separating the dam from her kits for approximately one hour, after which the dam was anaesthetised [0.4 ml Xylazin (Narcoxyl®), 10 mg/ml + 0.2 ml ketamin (Ketaminol®) 50 mg/ml]. The anaesthetised dam received an intramuscular injection of 0.1 ml oxytocin (10 IU/ml, Leo Vet, Ballerup, Denmark) and all milk glands were milked and emptied as much as possible by intermittent suction using a small rubber tubing connected to a water vacuum pump. The milk samples were stored at -18°C until analysis.

Ethical approval

The experimental procedures complied with Danish National Legislation and the guidelines approved by the Member States of the Council of Europe for protection of vertebrate animals (Anonymous, 1986).

Analytical procedures, feed, excreta and milk

Diets, feed residues, faeces and milk were analysed for: dry matter (DM) by evaporation to constant weight at 105°C, ash by combustion at 525°C for 6 h, nitrogen (N) by the micro-Kjeldahl technique using the Tecator-Kjeltec system 1030 (Tecator AB, Höganäs, Sweden), and crude protein (CP) was calculated as N×6.25 (diets and faeces) and N×6.38 (milk). Fat in diets and faeces was determined by petroleum ether extraction after HCl hydrolysis, and gross energy (GE) by use of an adiabatic bomb calorimeter. In milk samples, fat was analysed by a method slightly modified from Baverstock et al. (1976) as described by Tauson et al. (2004), and GE was calculated by use of the data for milk composition and the energy factors (in kJ/g): protein (23.9), fat (39.8) and carbohydrates (17.6). In all samples, contents of carbohydrates (CHO) were calculated by difference (CHO = DM – ash – CP – fat). The DM in urine was taken to be 10% (Neil, 1988), and energy in urine (UE) was estimated from the N content in urine (UN) by the following regression equation: UE, kJ = 27.1×UN, g - 0.8 (Tauson, unpublished data). Amino acids in the diets were determined by oxidation with a performic acid/phenol mixture and hydrolysed with HCl (6 N) for 23-h. The hydrolysate was adjusted to pH 2.20. The amino acids were separated by ion exchange chromatography and determined by reaction with ninhydrin using photometric detection at 570 nm (440 nm for proline) (EC, 1998).

Calculations, balance, respiration and milk energy data

Metabolizable energy (ME) was calculated as GE - energy in faeces (FE) - UE. The N balance was calculated as the difference between daily N intake in feed and N output in faeces, urine and milk. Heat production (HE) was calculated from O_2 consumption, CO_2 production and UN according to the formula by Brouwer (1965):

$$HE, kJ = (16.18 \times l, O_2) + (5.02 \times l, CO_2) - (5.99 \times g, UN)$$

Retained energy (RE) was calculated as ME - HE. If RQnp (non-protein respiratory quotient) was higher than 0.707, net oxidation of fat (OXF), carbohydrates (OXCHO) and protein (OXP) was calculated from measurements of gas exchange and excretion of UN in accordance with the methods described and validated for pigs by Chwalibog et al. (1992). However, in cases where RQnp<0.707 there was no net oxidation of carbohydrates (OXCHO=0), whereas the amount of oxidized fat included both net fat oxidation and energy cost of fat mobilization (Chwalibog, personal communication). The respiration experiments were performed with dam and litter, and since dam and kits are huddling, results relating to metabolic weight ($kg^{0.75}$) consist of the total metabolic weight of dam and litter. Nutrient and energy output in milk (LE) was calculated based on the estimated milk production, the chemical composition and the DM content of the milk, e.g., energy output in milk (LE).

Statistical analyses

Statistical analyses on animal liveweights, feed and drinking water consumption, energy metabolism data, milk intake, milk production and chemical composition of milk were carried out by means of the MIXED procedure in SAS (Littell et al., 1996) by the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_k + \alpha\beta_{ik} + \varepsilon_{ijk}$$

where Y_{ijk} is the Y_{ijk} th observation, μ the general mean, α_i the fixed effect of treatment (diet: LC, MC or HC), β_k the fixed effect of time (weeks one to four of lactation), $\alpha\beta_{ik}$ the interaction effects between treatment and week, and ε_{ijk} the residual error.

Lactation week was used as a repeated measure and the autoregressive order 1 (AR (1)) covariance structure was fitted (Littell et al., 1996). Animal liveweights showed no differences between animals housed on the farm or in the laboratory, therefore results on liveweights were pooled (females n=30, kits n=180). Results are presented as least squares means (LS-means), and effects were considered significant at $P<0.05$.

RESULTS

The fat contents in % of ME were very similar and thus the protein was replaced by carbohydrates as planned (Table 1). However, the ME content increased from 15.7 kJ/kg DM in the LC diet to 17.2 kJ/kg DM in the HC diet (Table 1).

TABLE 1

Dietary composition and chemical analysis (g/kg) of the three experimental diets [low carbohydrate (LC), medium carbohydrate (MC) and high carbohydrate (HC)], and calculated metabolizable energy (ME)

Protein:fat:carbohydrates, % of ME	Dietary treatment		
	LC	MC	HC
Dietary composition, g/kg			
cod offal	500	350	250
cod, whole	100	100	100
fish meal	80	60	0
chicken, whole[1]	200	250	300
barley and wheat (heat treated; 1:1)	20	40	80
steamed rolled oats	0	20	40
potato mash powder	5	25	40
rapeseed oil	0	10	20
vitamin/mineral mixture[2]	2.3	2.3	2.3
water	95	145	170
Chemical analyses, g/kg DM			
dry matter (DM)	289	316	342
ash	154	119	82
crude protein	666	537	378
fat	130	165	151
carbohydrates	50	179	389
Gross energy, MJ/kg DM	22.0	22.6	21.9
ME MJ/kg DM[3]	15.66	16.90	17.24

[1] chicken prepared for human consumption, i.e. without head, feet, skin and entrails

[2] containing mg/kg: α-tocopherol 21840, thiamine 10000, riboflavin 4800, pyridoxine 3200, D-pantothenic acid 3200, nicotinic acid 8000, betain anhydrate 33600, folic acid 240, biotin 80, cyanocobalamin 16, paraaminobenzoic acid 800, Fe 19712, Zn 12560, Mn 6237, Cu 1025; retinol 2800 i.e./g, cholecalciferol 280 i.e./g

[3] calculated with use of individual coefficients of digestibility for the diets, the amount of digestible nutrient/kg diet and metabolizable energy coefficients (18.4, 39.8 and 17.6 kJ/g digested protein, fat and carbohydrate, respectively; Hansen et al., 1991)

The content of amino acids (g/kg DM) increased with increased protein content of the diets (Table 2). However, when calculated as g amino acids per 16 g N, the total amount of amino acids decreased with increasing dietary protein, being 76.5, 67.0 and 65.9 g per 16 g N in the HC, MC and LC diet, respectively (Table 2).

TABLE 2

Amino acid composition of the experimental diets [low carbohydrate (LC), medium carbohydrate (MC) and high carbohydrate (HC)]

Amino acids	g/kg DM			g/16 g N		
	LC	MC	HC	LC	MC	HC
Essential						
lysine	26.9	24.1	16.9	4.42	4.93	4.87
phenylalanine	18.7	15.8	11.4	3.09	3.24	3.29
methionine	12.1	10.9	6.7	2.00	2.22	1.93
histidine	11.2	9.6	8.1	1.85	1.95	2.34
valine	22.6	19.1	13.7	3.71	3.90	3.95
isoleucine	19.9	17.2	12.5	3.28	3.51	3.61
leucine	34.1	28.4	20.5	5.61	5.81	5.92
threonine	17.8	14.6	10.4	2.93	2.97	3.00
arginine	30.3	23.3	18.9	5.00	4.75	5.45
all essential	193.6	162.9	119.0	31.89	33.29	34.36
Non-essential						
cystine	3.2	3.3	2.7	0.52	0.67	0.79
glycine	26.7	19.0	13.8	4.40	3.88	3.98
aspartate	36.0	34.6	25.0	5.93	7.06	7.22
alanine	24.9	16.6	14.8	4.10	3.39	4.28
tyrosine	13.6	11.0	9.1	2.24	2.26	2.64
glutamate	55.9	45.2	50.8	9.21	9.23	11.66
proline	23.6	18.2	16.6	3.88	3.73	4.80
serine	22.7	17.2	13.2	3.73	3.50	3.80
all non-essential	206.6	165.0	146.1	34.01	33.72	39.17

Feed and drinking water consumption

Feed intake increased ($P<0.001$) as lactation progressed in all dams. Measured in grams per day, dams fed the LC diet consumed approximately 20% more ($P<0.01$) per day than dams fed the HC diet, the difference increasing for each lactation week (Table 3). The average carbohydrates intakes were 5, 14 and 32 g per day for dams fed the LC, MC and HC diets, respectively. However, the metabolizable energy intake was not significantly affected by dietary treatment (Table 3). The average digestibility coefficients were 82% (range 81-83%) and 97% (range 97-98%) for protein and fat, respectively. For carbohydrates the digestibility coefficients were 81 and 84% in dams fed the MC and HC diet, respectively, and 57% in dams fed the LC diet. Feed intake provided the main water supply in all dams. However, drinking water consumption was affected ($P<0.01$) by lactation week and dietary treatment, dams fed the LC diet

consuming approximately 43% more drinking water per day during weeks one to four of lactation than dams fed the HC diet (Table 3).

TABLE 3

Effect of dietary treatment [low carbohydrate (LC), medium carbohydrate (MC) and high carbohydrate (HC)] on feed and drinking water consumption (n=4 per treatment group) and liveweight change (n=10 per treatment group) of mink dams in weeks one to four post partum (LS means)

Indices	Diet			Lactation week				RR*	P-value; effect of		
	LC	MC	HC	1	2	3	4		diet	week	D×W
Feed intake, g/day	306[a]	247[b]	244[b]	194	238	296	336	33.9	0.01	<0.001	0.58
Feed intake, KJ ME/day	1481	1371	1453	1048	1284	1595	1813	5.8	0.52	<0.001	0.87
Drinking water, g/day	88[a]	65[a]	50[b]	62	57	65	87	23.0	0.09	<0.001	0.02
Liveweight change, %	-10[a]	-3[b]	-1[b]								

* RR: square root of residuals

[a,b] values that share no common superscript differ significantly (P<0.05); effect of diet

Nitrogen metabolism

The N intake increased as lactation progressed (P<0.001) in all dams, and with increased protein content of the diets (P<0.05), the N intake being higher (P<0.01) in dams fed the LC diet than in dams fed the MC and HC diets (Table 4). Consequently, the total N excretion increased (P<0.001) as lactation progressed, but with the N excretion *via* faeces and urine being higher (P<0.01) in dams fed the LC diet than in dams fed the MC and HC diets. The N output in milk was not affected (P>0.05) by dietary treatment, but there was a tendency for lower excretion in HC dams, this difference being significant (P<0.01) in the fourth week of lactation. The resulting N balance was not affected by dietary treatment, and was not significantly different from 0 in any group. However, it decreased significantly (P<0.001) from week 1 post partum, reaching a negative value in week 4 (Table 4). Because the N balance was 0, the efficiency of utilization of digested N (DN) for milk production could be calculated: it increased significantly (P<0.001) with decreasing dietary protein supply from 31% for LC dams to 44% for HC dams. Also the effect of lactation week was significant (P<0.05) and the efficiency of utilization DN was moderately increased from 32% in lactation week 1 to 38% in the following weeks (Table 4).

TABLE 4

Effect of dietary treatment [low carbohydrate (LC), medium carbohydrate (MC) and high carbohydrate (HC)] on nitrogen (N) intake, N excretion and N balance, g/day and utilization of digested nitrogen (DN) for milk production and retention, % of mink dams (n=4 per treatment group) during weeks one to four of lactation

Indices	Diet			Lactation week				RR*	*P*-value; effect of		
	LC	MC	HC	1	2	3	4		Diet	Week	D×W
Dietary N	9.3[a]	6.6[b]	5.0[c]	5.1	6.4	7.7	8.7	0.96	<0.001	<0.001	0.07
Faecal N	1.6[a]	1.2[b]	0.9[c]	0.9	1.1	1.3	1.6	0.16	<0.001	<0.001	0.06
Digested N	7.7[a]	5.4[b]	4.1[c]	4.2	5.2	6.3	7.1	0.85	<0.001	<0.001	0.08
Urinary N	5.2[a]	3.4[b]	2.3[c]	2.7	3.3	4.0	4.5	0.59	<0.001	<0.001	0.08
N in milk	2.2[a]	2.0[a]	1.8[b]	1.1	1.5	2.1	3.4	0.28	0.09	<0.001	<0.001
Total N output	9.0[a]	6.6[b]	5.0[c]	5.0	6.0	7.4	9.4	0.80	<0.001	<0.001	0.03
N balance	0.3	0	0	0.4	0.4	0	-0.8	0.45	0.64	<0.001	0.33
Utilization of DN for milk	30.9[a]	34.7[a]	43.6[b]	32.4	38.1	37.5	37.7	4.1	<0.001	0.02	0.06

* RR: square root of residuals

[a,b,c] values that share no common superscript differ significantly (P<0.05); effect of diet

TABLE 5

Effect of dietary treatment [low carbohydrate (LC), medium carbohydrate (MC) and high carbohydrate (HC)] and week of lactation on daily metabolizable energy (ME) intake, heat production (HE), energy output in milk (LE) and retained energy (RE) in mink dams in relation to metabolic liveweight ($kg^{0.75}$), and oxidation of protein (OXP), fat (OXF) and carbohydrate (OXCHO) in relation to total HE

Indices	Diet			Lactation week				RR*	P-value; effect of		
	LC	MC	HC	1	2	3	4		diet	week	D×W
ME, kJ	1324	1282	1383	958	1193	1498	1669	180	0.56	<0.001	0.99
HE, kJ	676[a]	644[a]	546[b]	670	616	626	576	102	0.02	0.40	0.41
LE, kJ	780	833	856	325	622	854	1491	147	0.72	<0.001	0.10
RE, kJ	-92[a]	-165[a]	-31[b]	72	-37	41	-390	117	0.03	<0.001	0.26
OXP, % of HE	42[a]	31[b]	21[c]	29	32	32	32	5.1	<0.001	0.62	0.48
OXF, % of HE	56[a]	66[ac]	73[bc]	68	64	62	65	12.7	0.03	0.74	0.36
OXCHO, % of HE	2	3	6	3	4	6	3	10.3	0.74	0.82	0.35

* RR: square root of residuals

[a,b,c] values that share no common superscript differ significantly (P<0.05); effect of diet

Respiration experiments

Heat production, calculated in relation to metabolic liveweight of dam with litter (kJ/kg$^{0.75}$), was relatively constant during the lactation period, but was significantly ($P<0.05$) higher in LC dams (676 kJ/kg$^{0.75}$) than in dams fed the HC diet (546 kJ/kg$^{0.75}$), the difference being approximately 19% (Table 5). The oxidation of protein (OXP) in relation to total HE was about twice as high ($P<0.001$) in dams fed the LC diet than in dams fed the HC diet, whereas the opposite was the case for the oxidation of fat (OXF) ($P<0.05$), OXF contributing more than 50% to the total HE in all diets. Oxidation of carbohydrates (OXCHO) in relation to total HE was not affected ($P>0.05$) by dietary treatment, or lactation week, and values were generally low (Table 5). The energy output in milk (LE) increased ($P<0.001$) during the lactation period, but the LE in relation to metabolic liveweight of the dams was not significantly different between dietary treatments (Table 5). Average values for retained energy (RE) for all 4 measurement weeks were negative, and most negative for MC dams (Table 5). During lactation week 4, LE was close to (LC and HC) or even slightly higher (MC) than the ME intake, which was reflected in clearly negative RE values, suggesting mobilization in the order of 10 g fat per day.

Dam liveweights

The absolute liveweights of the dams in weeks 1, 2, 3 and 4 post partum did not differ. However, the individual weight loss from week one to week four post partum, was significantly ($P<0.001$) higher in dams fed the LC diet, losing 126 g or 10% compared with 34 (3%) and 13 g (1%) in dams fed the MC and HC diets, respectively (Table 3).

Milk production

Daily milk intake of the kits, and thereby the daily milk production, increased significantly in all treatment groups as lactation progressed ($P<0.001$). The daily milk intake was highest in kits nursed by dams fed the HC diet, and during weeks three and four post partum the milk production was significantly higher ($P<0.05$) in dams fed the HC diet than in dams fed the MC and LC diets (Table 6).

Chemical composition of milk

The DM, fat and gross energy (GE) contents of the milk increased ($P<0.05$) as lactation progressed, but were not affected ($P>0.05$) by dietary treatment (Table 6). There was a tendency for decreasing protein content with decreasing dietary

TABLE 6

Effect of dietary treatment [low carbohydrate (LC), medium carbohydrate (MC) and high carbohydrate (HC)] on milk production and chemical composition of the milk of mink dams weeks one to four post partum, and liveweights of kits (n=180) nursed by these dams

Indices	Diet			Lactation week				RR*	P-value; effect of		
	LC	MC	HC	1	2	3	4		diet	Week	D×W
Milk yield, g/day	170[a]	154[a]	198[b]	106	148	199	244	22.6	0.04	<0.001	0.84
Milk dry matter (DM), %	21.7	24.1	20.9	18.3	21.8	21.3	27.4	3.4	0.19	0.02	0.59
Protein, g/kg DM	259	240	190	278	228	197	216	52	0.08	0.17	0.07
Fat, g/kg DM	309	340	315	253	315	313	406	47	0.40	0.02	0.63
Carbohydrates, g/kg DM	245[a]	270[a]	312[b]	244	302	302	253	41	0.02	0.11	0.83
Gross energy, MJ/kg DM	22.8	24.0	22.6	20.8	23.1	22.8	25.9	0.8	0.17	0.02	0.85
Milk intake, g/kit, day	28.1[ac]	25.3[c]	32.9[b]	16.4	24.2	32.9	41.7	6.3	<0.001	<0.001	0.98
Kit liveweight, g	97[a]	100[a]	104[b]	35	72	120	174	17.7	0.04	<0.001	<0.001

* RR: square root of residuals

[a,b,c] values that share no common superscript differ significantly ($P<0.05$); effect of diet

protein supply, and in the fourth week of lactation the difference between dams fed the HC and LC diets was significant ($P<0.05$). The carbohydrates concentration in milk was higher ($P<0.05$) in dams fed the HC diet compared with dams fed the LC diet (Table 6).

Kit liveweights

Growth of the kits was affected ($P<0.05$) by dietary treatment of the dams, HC kits being the heaviest (Table 6). At four weeks post partum, individual body weights of kits nursed by dams fed the HC diet were 6 and 10% higher ($P<0.001$) than those of kits nursed by dams fed the MC and LC diets, respectively.

DISCUSSION

The experimental diets, in this study, were not designed with the purpose of determining the requirements of individual amino acids, but the performance results indicate that the amino acid requirements for lactation and growth of the suckling kits were sustained even by the HC diet. However, the HC diet had a relatively better protein quality than the other diets measured in terms of contents of essential amino acids and total amino acid content per 16 g N. This was caused by the higher content of chicken and lower content of cod offal.

The digestibility of the nutrients was high and almost the same in all 3 experimental diets, except for a lower carbohydrates digestibility in dams fed the LC diet, probably being an artefact caused by the low carbohydrates intake of only 5 g/day. However, the high carbohydrates digestibility coefficients in dams fed the MC and HC diet, confirm that mink is able to metabolize high amounts of heat-treated carbohydrates, as previously found by Sørensen et al. (1995) and Fink et al. (2002a,b). The ME consumption was not significantly affected by dietary treatment. Monogastric animals are, within certain limits, able to regulate their feed intake independent of dietary energy concentration (pigs: Cole et al., 1967, 1969; hens: de Groot, 1972). Previous experiments in mink have shown that lactating dams are able to compensate for decreased dietary energy concentration by an increased feed intake when dietary energy concentration ranged from 15.4 to 19.2 MJ ME/kg DM (Tauson, 1988). The diets used in this experiment were in the range where compensation could be expected, and the animals adjusted their food intake to the different dietary energy densities.

Diets with high protein content impose great demands on the animals' water supply, because excess N is excreted in urine after deamination. In the present experiment, dams fed the LC diet had a significantly higher drinking water consumption and excretion of urinary water than dams fed the HC diet,

corresponding to results by Neil (1988). Although mink have a great capacity to concentrate their urine (Valtonen et al., 1982), this ability is reduced during lactation, and lactating animals do not regulate the amount of water secreted in milk (Olsson, 1986). Therefore, lactating dams fed high protein diets may be vulnerable to shortages of water supply.

Nitrogen balance studies can be used to indicate if the protein supply meets the requirement, but in carnivores they often overestimate N retention (mink: Skrede, 1978; ferret: Jarosz and Barabasz, 1988; cat: Zentek et al., 1998), mainly because of incomplete recovery of urinary N as demonstrated by Tauson et al. (1997b). However, the presented N balance data are based on very careful collection routines, and thus are as reliable as possible without direct determination of N recovery (Tauson et al., 1997a). The negative N balances recorded during the fourth week of lactation might indicate that some mobilization of body protein occurred, but this was not supported by the protein oxidation data, since they were not affected by stage of lactation.

The HE was calculated from the O_2 consumption and CO_2 production from the dam with litter, because separation of mother and young during the 22-h measurement period is impossible. However, age of the kits and litter size were equal, and hence differences in HE and substrate oxidation could be equally related to the dam in all treatment groups. The significantly higher HE per kg metabolic weight found in dams fed the LC diet compared with dams fed the HC diet, demonstrated clearly that when the protein supply is above the requirement, excess protein is deaminated and used as an energy source and excess N is excreted in urine by an energetically costly process. Furthermore, among animals fed the LC diet, protein oxidation contributed with 42% to the total HE, whereas it declined to 21% of HE when the protein supply was reduced to 33% of ME (HC). However, this level is higher than the about 13% of HE reported for lactating sows (Theil, 2002) and indicates, most likely, that also the HC diet sustained the protein requirement of the lactating dams.

It is suggested that evolutionary pressures have resulted in deletion or modulation of the activities of different key enzymes involved in protein and carbohydrates metabolism, which may explain the high protein requirement of mink. In another strict carnivore, the cat, the activities of some key transaminating and gluconeogenic enzymes did not change noticeably in response to changes in dietary protein supply (Rogers et al., 1977), but recent results suggest that the cat is capable of metabolic flexibility: hence net protein oxidation of cats fed diets with 35.3 vs 51.9% protein energy was significantly higher among cats on the highest protein supply (Russell et al., 2002). Furthermore, data from whole-body protein turnover studies on cats given more extreme dietary protein supplies (20 vs 70% protein energy) demonstrated that flux, protein synthesis and protein breakdown were all significantly lower in cats given the lowest protein supply (Russell et al.,

2003). Corresponding data are almost lacking for the mink but the few available results indicate that the mink has a high activity of hepatic gluconeogenic enzymes (Sørensen et al., 1995) as well as a high glycolytic capacity (Fink et al., 2002a,b), and the present results concur with the concept of ability to regulate protein oxidation rate.

Individual kit body weights four weeks post partum were 27% higher in kits nursed by dams fed the HC diet than in kits nursed by dams fed the LC diet, irrespective of housing conditions. This further confirms that dams fed the HC diet had a higher milk production than dams fed the LC diet. The higher body weights of kits nursed by dams fed the low fat:carbohydrates ratio (33:33) compared with dams fed the high ratio (32:3), were in contrast to results by Skrede (1981), who found improved kit growth performance already twenty-one days post partum, indicating positive effects on milk production by increased fat:carbohydrates ratio from 32:24 to 50:6. The diverging results can probably be explained by a relatively constant fat content and substitution of dietary protein with carbohydrates in our experiment, hence a substitution with limited effect on dietary energy density, whereas Skrede (1981), by increasing the dietary fat content, simultaneously increased the energy density of the diet. However, variation in growth rate may not depend solely on the volume of milk consumed by the kits. Alterations in milk composition may also modulate the differences in volume intake, and differences may occur in the efficiency of nutrient utilization (Fiorotto et al., 1991).

During the four weeks of lactation the DM and fat content of the milk increased from approximately 18 to 27%, and 5 to 11%, respectively, unaffected by dietary treatment, corresponding to previous results in mink (Olesen et al., 1992; Fink et al., 2001). Mink is a rapidly growing species, and it is believed that the suckling mink has a higher protein requirement than that of most mammalian species. In the present experiment, the protein contents of milk in the fourth week of lactation were approximately 11.0, 9.4 and 6.5% in dams fed the LC, MC and HC diets, respectively, and thereby significantly lower in dams fed the HC diet compared with dams fed the LC diet. Olesen et al. (1992) found protein contents in mink milk of 8-10%, which are in accordance with most other carnivores (Widdowson, 1965; Oftedal, 1984). This indicates that 6.5% milk protein may be low for a carnivore, however, kits from these dams had the highest body weight four weeks post partum. The fact that the carbohydrates content of milk was highest in dams fed the HC diet and that the carbohydrates oxidation was low in all dams, may indicate that the dietary carbohydrates fraction was used mainly in the synthesis of milk. However, in all dams the carbohydrates fraction calculated by difference was several times higher than the lactose content of 1% found in mink milk by Olesen et al. (1992), indicating as Conant (1962) claimed, that lactose is not the dominant carbohydrates in mink milk. However, a detailed profile of the carbohydrates fraction of mink milk has yet to be established.

CONCLUSIONS

In conclusion, dams fed the high carbohydrates diet had a lower weight loss, lower heat production, lower protein oxidation and lower water intake and total N excretion, together with higher milk production in weeks three and four of lactation, resulting in a higher liveweight of the kits at four weeks post partum, than dams fed the low carbohydrates diet. Thus, the results suggest that there is a considerable potential to reduce the protein content in diets for lactating mink with positive effects on animal performance and a reduced nitrogen excretion.

ACKNOWLEDGEMENTS

The authors wish to thank Merethe Stubgaard, Ebba de Neergaard Harrison, Susanne Holbæk and Boye Pedersen for skilled technical assistance throughout the experiment, Liv Torunn Mydland for performing the amino acids analysis and Kirsten Bislev Hansen for collection of the milk samples.

REFERENCES

Annison E.F., Linzell J.L., West C.E., 1968. Mammary and whole animal metabolism of glucose and fatty acids in fasting lactating goats. J. Physiol. 197, 445-459

Anonymous, 1986. European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes. Council of Europe, Strasbourg, European Treaty Series No. 123

Baverstock P.R., Spencer L., Pollard C., 1976. Water balance of small lactating rodents. II. Concentration and composition of milk of females on ad libitum and restricted water intakes. Comp. Biochem. Physiol. Pt. A 53, 47-52

Børsting C.F., Damgaard B.M., 1995. The intermediate glucose metabolism in the nursing period of the mink. NJF-Seminar, Gothenburg, NJF-Utredning/Rapport No. 106, pp. 104-111

Brouwer E., 1965. Report of sub-committee on constants and factors. In: Proceedings of the 3rd Symposium on Energy Metabolism. EAAP Publication No. 11, pp. 441-443

Chwalibog A., Jakobsen K., Henckel S., Thorbek G., 1992. Estimation of quantitative oxidation and fat retention from carbohydrate, protein and fat in growing pigs. J. Anim. Physiol. Anim. Nutr. 68, 123-135

Clausen T.N., Olesen C.R., Hansen O., Wamberg S., 1992. Nursing sickness in lactating mink (Mustela vison). I. Epidemiological and pathological observations. Can. J. Vet. Res. 56, 89-94

Cole D.J.A., Clent E.G., Luscombe J.R., 1969. Single cereal diets for bacon pigs. 1. The effects of diets based on barley, wheat, maize meal, flaked maize or sorghum on performance and carcass characteristics. Anim. Prod. 11, 325-335

Cole D.J.A., Duckworth J.E., Holmes W., 1967. Factors affecting voluntary feed intake in pigs. The effect of digestible energy content of the diet on the intake of castrated male pigs housed in holding pens and in the metabolism crates. Anim. Prod. 9, 141-148

Conant R.A., 1962. A milking technique and the composition of mink milk. Amer. J. Vet. Res. 23, 1104-1106

de Groot G., 1972. A marginal income and cost analysis of the effect of nutrient density on the performance of White Leghorn hens in battery cages. Brit. Poultry Sci. 13, 503-520

EC, 1998. European Communities, Commission Directive 98/64/EC 3 September 1998 Establishing Community Methods of Analysis for Determination of Amino-Acids, Crude Oils and Fats, and Olaquindox in Feedstuffs and Amending. Directive 71/393/EEC

Elnif J., Hansen N.E., Mortensen K., Sørensen H., 1988. Production of digestive enzymes in mink kits. In: B.D. Murphy, D.B. Hunter (Editors). Biology, Pathology and Genetics of Fur Bearing Animals. Proceedings of the IV International Congress in Fur Animals Production. Ontario, pp. 320-326

Fink R., Børsting C.F., Damgaard B.M., 2002a. Glucose homeostasis and regulation in lactating mink (Mustela vison): Effects of dietary protein, fat and carbohydrate supply. Acta Agr. Scand., Sect. A, Anim. Sci. 52, 102-111

Fink R., Børsting C.F., Damgaard B.M., 2002b. Glucose metabolism and regulation in lactating mink (Mustela vison) - Effects of low dietary protein supply. Arch. Anim. Nutr. 56, 155-166

Fink R., Tauson A.-H., Hansen K.B., Wamberg S., Kristensen N.B., 2001. Milk production in mink (Mustela vison) - Effect of litter size. Arch. Anim. Nutr. 55, 221-242

Fiorotto M.L., Burrin D.G., Perez M., Reeds P.J., 1991. Intake and use of milk nutrients by rat pups suckled in small, medium, or large litters. Amer. J. Physiol.-Regul. Integr. C 29, R1104-R1113

Hansen B.K., Berg P., 1998. Mink dam weight changes during the lactation period. I. Genetic and environmental effects. Acta Agr. Scand., Sect. A, Anim. Sci. 48, 49-57

Hansen N.E., Finne L., Skrede A., Tauson A-H. (Editors), 1991. Energy Supply for Mink and Foxes (in Danish). NJF-Utredning/Rapport no. 63. DSR Forlag, Landbohøjskolen, København, pp. 59

Jarosz S., Barabasz B., 1988. Effects of various levels of dietary protein and energy on nitrogen retention in pregnant fitch. In: Biology, Pathology and Genetics of Fur Bearing Animals. Proceedings of the IV International Scientific Congress in Fur Animal Production. Ontario, pp. 377-381

Jørgensen G., Glem-Hansen N., 1973. A cage designed for metabolism and nitrogen balance trials with mink. Acta Agr. Scand. 23, 3-5

Littell R.C., Milliken G.A., Stroup W.W., Wolfinger R.D., 1996. SAS® System for Mixed Models, Cary, NC, SAS Institute Inc., pp. 633

Neil M., 1988. Effects of dietary energetic composition and water content on water turnover in mink. Swed. J. Agr. Res. 18, 135-140

Nes N., Einarsson E.J., Lohi O. (Editors), 1987. Beautiful Fur Animals - and Their Colour Genetics. Scientifur. Hillerød, pp. 271

Oftedal O.T., 1984. Lactation in the dog: milk composition and intake by puppies. J. Nutr. 114, 803-812

Olesen C.R., Clausen T.N., Wamberg S., 1992. Compositional changes in mink (Mustela vison) milk during lactation. Norw. J. Agr. Sci., Suppl. 9, 308-314

Olsson K., 1986. Control of water balance during gestation and lactation (in Swedish). Svensk Veterinärtidning 38, 706-709

Rogers Q.R., Morris J.G., Freedland R.A., 1977. Lack of hepatic enzymatic adaptation to low and high levels of dietary protein in the adult cat. Enzyme 22, 348-356

Russell K., Murgatroyd P.R., Batt R.M., 2002. Net protein oxidation is adapted to dietary protein intake in domestic cats (*Felis silvestris catus*). J. Nutr. 132, 456-460

Russell K., Lobley G.E., Millward D.J., 2003. Whole-body protein turnover of a carnivore, *Felis silvestris catus*. Brit. J. Nutr. 89, 29-37

Skrede A., 1978. Utilization of fish and animal byproducts in mink nutrition. I. Effect of source and level of protein on nitrogen balance, postweaning growth and characteristics of winter fur quality. Acta Agr. Scand. 28, 106-129

Skrede A., 1981. Changing fat:carbohydrate supply to mink. I. Effect on reproduction, growth, survival rate and chemical body composition of the kits (in Norwegian). Meld. Norg. LandbrHøgsk. 60 (16), 2-18

Szymeczko R., Skrede A., 1990. Protein digestion in mink. Acta Agr. Scand. 40, 189-200

Sørensen P.G., Petersen I.M., Sand O., 1995. Activities of carbohydrate and amino acid metabolizing enzymes from liver of mink (Mustela vison) and preliminary observations on steady state kinetics of the enzymes. Comp. Biochem. Physiol. Pt. B 112, 59-64

Tauson A.-H., 1988. Varied energy concentration in mink diets. II. Effect on kit growth performance, female weight changes and water turnover in the lactation period. Acta Agr. Scand. 38, 231-242

Tauson A.-H., Elnif J., Wamberg S., 1997a. Nitrogen balance in adult female mink (*Mustela vison*) in response to normal feeding and short-term fasting. Brit. J. Nutr. 78, 83-96

Tauson A.-H., Fink R., Chwalibog A., 1997b. Can gas exchange measurements be used for calculation of nutrient oxidation in mink (Mustela vison) exposed to short-term changes in energy supply? Z. Ernährungswiss. 36, 317-320

Tauson A.-H., Fink R., Hansen K.B., Hansen N.E., Chwalibog A., 2004. Utilisation of milk energy for suckling mink kits. Arch. Anim. Nutr. 58, 181-194

Theil P.K., 2002. Energy and protein metabolism in pregnant and lactating sows. PhD. Thesis, Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Copenhagen, pp. 141

Valtonen M., Mälelä J., Eriksson L., 1982. Concentration capacity of the kidneys in healthy and plasmacytocsis mink. In: Proceedings of the 14th Nordic Veterinary Congress. Copenhagen, pp. 372-373

Wamberg S., Tauson A.-H., Elnif J., 1996. Effects of feeding and short-term fasting on water and electrolyte turnover in female mink (*Mustela vison*). Brit. J. Nutr. 76, 711-725

Widdowson E.M., 1965. Food, growth and development in the suckling period. In: O. Graham-Jones (Editor). Canine and Feline Nutritional Requirements. Pergamon Press, Oxford, pp. 9-17

Williams C., Elnif J., Buddington R.K., 1998. The gastrointestinal bacteria of mink (*Mustela vison* L): Influence of age and diet. Acta Agr. Scand. 39, 473-482

Zentek J., Dekeyzer A., Mischke R., 1998. Influence of dietary protein quality on nitrogen balance and some blood parameters in cats. J. Anim. Physiol. Nutr. 80, 63-66

STRESZCZENIE

Wpływ zastąpienia białka diety węglowodanami na produkcję mleka u norek (*Mustela vison)*

Trzydzieści norek odchowujących mioty po 6 norcząt podzielono na 3 grupy doświadczalne celem zbadania wpływu zmiany stosunku białko:węglowodany w diecie na wykorzystanie składników pokarmowych, produkcję ciepła, produkcję mleka i wzrost norcząt. Przygotowano trzy diety, w których stosunek energii metabolicznej (ME) pochodzącej z białka do ME węglowodanów był następujący: 65:3 (LC), 48:15 (MC) oraz 34:33 (HC)%. Diety podawano do woli przez 4 tygodnie od porodu. Dwanaście norek utrzymywano w kontrolowanych warunkach laboratoryjnych i przeprowadzano na nich badania bilansowe i respiracyjne; ich norczętom podano w formie injekcji tlenek ciężkiej wody dla pomiaru kinetyki wody i produkcji mleka. Osiemnaście norek utrzymywano w normalnych warunkach fermowych, oznaczając pobranie paszy przez matki oraz przyrosty norcząt. Od norek pobierano próby mleka.

Pobranie energii metabolicznej nie zależało od diety. Węglowodany były dobrze wykorzystywane; u norek otrzymujących dietę HC współczynnik strawności wyniósł 84%. Procentowy ubytek masy ciała u norek otrzymujących dietę MC był mniejszy ($P<0,05$), niższa ($P<0,05$) była u nich całkowita produkcja ciepła (HE), mniejsze ($P<0,05$) utlenienie białka (OXP), pobierały mniej ($P<0,05$) wody oraz wydalały mniej ($P<0,05$) azotu (N) niż norki otrzymujące dietę LC. Produkcja mleka, a więc i przyrosty norcząt w ciągu 4 tygodni od urodzenia były większe ($P<0,05$) u norek żywionych dietą HC niż otrzymujących dietę LC.

W podsumowaniu stwierdzono, że norki w okresie laktacji są zdolne do wykorzystania strawnych węglowodanów, czego efektem jest dobra produkcja mleka oraz zmniejszenie wydalania azotu.