Z Ernährungswiss 36: 317–320 (1997)

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Can gas exchange measurements be used for calculation of nutrient oxidation in mink (*Mustela vison*) exposed to short-term changes in energy supply?

Können Respirationsmessungen für die Berechnung der Nährstoffoxidation bei Nerzen (*Mustela vison*) bei Kurzzeitänderungen der Energieversorgung eingesetzt werden?

Summary Nutrient oxidation was calculated from gas exchange measurements for 6 control and 12 flush fed female mink, measured in six consecutive, one week periods. The energy supply to controls and flushed animals in periods 1 and 6 was ca 850 kJ ME/day, and during restriction and flush feeding, it was ca 450 kJ ME/day and ca 1300 kJ ME/day, respectively. Over the total experimental period the energy intake was similar in both groups, but it differed significantly between periods in the flushed group. Protein, fat, and carbohydrate oxidation averaged 39, 38, and 21 %, of the total heat production (HP), respectively in the control group. During restriction, protein oxidation was ca 35% of HP in flushed animals, then increasing to 55% of HP during the first period of refeeding. High values for fat oxidation were recorded during restriction because of fat mobilization and values were low when feed supply was ample. It was concluded that the calculation method was a good indicative method, but some short-comings were discussed.

Zusammenfassung Bei Nerzen (6 Kontrolltiere (Gruppe I) und 12 reichlich gefütterten weiblichen Tieren (Gruppe II)) wurde die Nährstoffoxidation aus Respirationsmessungen berechnet. Die Gaswechselmessungen erfolgten in 6 aufeinanderfolgenden einwöchigen Versuchsperioden.

Die Energieversorgung für die Gruppe I und II betrug in der Periode 1 und 6 ca. 850 kJ ME/d, während der Restriktion und der Fütterung auf hohem Niveau betrug sie 450 bzw. 1300 kJ ME/d. Während der gesamten Versuchsperiode war die Energieaufnahme für beide Gruppen vergleichbar. Es gab aber signifikante Unterschiede zwischen den Perioden bei der Gruppe II. Die Mittelwerte der Protein-, Kohlenhydrat- und Fettoxidation betrugen 39%, 38% und 21 % von der totalen Wärmeproduktion (WP) in der Gruppe I. Während der Restriktion betrug die Proteinoxidation bei der Gruppe II ca. 35% der WP. Während der ersten Periode der Fütterung auf hohem Niveau wuchs dieser Wert auf 55%. Während der Restriktion wurde eine hohe Fettoxidation gemessen, weil die Fettmobilisierung anstieg. Die Fettoxidation war niedrig, wenn die Futterzufuhr reichlich war. Es wird daraus geschlußfolgert, daß die Berechnungsmethode geeignete Aussagen liefert. Einige Nachteile der Methode werden diskutiert.

Key words Indirect calorimetry – substrate oxidation – feeding level – fat mobilization

Schlüsselwörter Indirekte Kalorimetrie – Substratoxidation – Fütterungsniveau – Fettmobilisierung

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Introduction

The mink (*Mustela vison)* is a strict carnivore with one annual reproductive season, and annual cycles of changes in feed intake and live weight. Fat accretion occurs during the autumn, and body weights then decrease during the winter and spring (5). Mink diets are mainly based on fresh frozen by-products from the fishing industry and abbatoirs and have a high protein and fat content as compared with diets for other species.

The breeding season is short, extending from early until late March in the Northern hemisphere. Body condition is of utmost importance for reproductive performance, and conditioning by nutritional flushing, performed as a two week period of restricted feeding followed by a two week period of *ad libitum* feeding, starting 3-5 day prior to the start of the breeding season, improves reproductive performance (8). During the winter and during conditioning, periods of low feed intake occur, in response to which changes in body weight are rapid. Moreover, the pattern of energy intake and heat production of pregnant and lactating female mink has revealed that they are in negative energy balance and hence mobilizing reserves from the body during parts of these demanding physiological stages (10). Therefore, the relative importance of the metabolic fuels, provided by the feed or mobilized from the body, for supporting the energy requirement during reproduction, is of interest in this species. The use of gas exchange measurements could provide a useful tool for estimation of the nutrient oxidation processes. Here we have used data from an experiment into the effects of nutritional flushing on energy metabolism and some metabolic parameters (4) for evaluation of the reliability of this method for calculation of nutrient oxidation when animals were exposed to short-term changes in energy supply.

Material and methods

The experiment was carried out with a total of 18 yearling female mink in six consecutive, one week balance periods, each including a 22 h respiration experiment by means of indirect calorimetry in an open-air circuit system. The animals were 6 controls, which were fed as to remain in energy balance, and 12 females flush fed according to the model described in (8) and as outlined in Table 1. The chemical composition of the diet was 31.5% dry matter (DM), and in % of DM, crude protein (CP; Kjeldahl N x 6.25) 56.0, fat (after HCl hydrolysis) 14.7, carbohydrate (CHO; calculated by difference) 18.3, and gross energy (GE; adiabatic bomb calorimeter) 22.3 MJ/kg DM.

Oxidation of protein (OXP), fat (OXF), and carbohydrate (OXCHO) were calculated by use of gas exchange measurements (RQ_{np} 0.7-1.0) and urinary nitrogen (UN) according to (1, 2).

Statistical analyses were carried out according to the GLM-procedure in Statistical Analysis System (6). The dependent variables were analyzed according to a model comprising the fixed effects of treatment group and period the interaction between treatment group and period and random animal within treatment group. When evaluating effects of treatment group, animal within treatment group was used as error term. Results are given as least squares means according to the described model.

Table 1 Experimental animals and design of the experiment

	Control	Flushing			
	Ad lib	*Ad lib*	*Restricted*	*Flushing*	*Ad lib*
Period	1-6	1	2-3	4-5	6
No. of animals	6	12			
Feed supply, g/d	200	200	100	300	200
ME, kJ/d (ca)	850	850	450	1300	850

Results

The daily intake of metabolizable energy (ME) over the total experimental period was not significantly different between groups, and the control group had a constant intake averaging 720 kJ ME/$kg^{0.75}$. In the flushing group, however, intake was significantly affected by period. It was similar to that of the contol group in period 1, and during restriction the animals consumed the total allowance. When given *ad libitum* supply the intake was very high in period 4, and then leveling off in period 5, and finally reached a level below that of the control group in period 6 (Table 2).

Similarly, when heat production (HE) was calculated over the total experimental period, it was not affected by treatment group (785 vs. 750 kJ/$kg^{0.75}$), but fluctuated in response to ME intake in the flushing group, values during refeeding being significantly higher than during restriction (Table 2).

On average the control group was in a slightly negative energy balance, whereas the flushing group exhibited retained energy (RE) values ranging from -220 to 330 kJ/$kg^{0.75}$. This was reflected by body weights decreasing during restriction, and the animals regaining weight in the flushing period (Table 2).

Table 2 Main energy metabolism parameters

	Control Ad lib	*Flushing*						*P*-value; group effect
		Ad lib	*Restricted*		*Flushing*		*Ad lib*	
Period	1-6	1	2	3	4	5	6	
ME, kJ/kg$^{0.75}$	717	779^{a}	447^{b}	486^{b}	1088^{c}	764^{a}	707^{a}	0.88
HE, kJ/kg$^{0.75}$	785	720^{ab}	682^{a}	679^{a}	783^{bc}	816^{c}	822^{c}	0.39
RE, kJ/kg$^{0.75}$	-83	77^{a}	-223^{b}	-180^{be}	330^{c}	-33^{d}	-98de	0.08
Weight, kg$^{0.75}$	1.011	1.027^{a}	1.006^{a}	0.948^{b}	0.970^{b}	1.019^{a}	1.009^{a}	0.08

$^{a\text{-}e}$ Flushing group values that share no common superscript differ significantly ($p < 0.05$)

Nutrient oxidation was fairly stable throughout the experiment in the control group with OXP/HE 39% (range 35-42), OXF/HE 38% (range 34-41) and OXCHO/HE 21% (range 16-25). In flush fed animals OXP/HE generally was high when ME intake was high and decreased significantly from 50% in period 1 to 33 and 35% during restriction in periods 2 and 3. During the first period of refeeding (period 4) the highest value of 55% was recorded, after which it decreased to 44% period 5) and 41% (Period 6) as ME intake leveled off (Fig. 1).

Conversely, OXF/HE was high during restriction (50 and 46% in periods 2 and 3), then decreasing to 29% during refeeding in period 4. OXCHO/HE averaged 17% (range 13-22), and was not affected by feed supply. However, when calculated in absolute values it turned out that OXCHO exceeded the energy from digested CHO (DCHO) in both the control and the flushing group (Table 3).

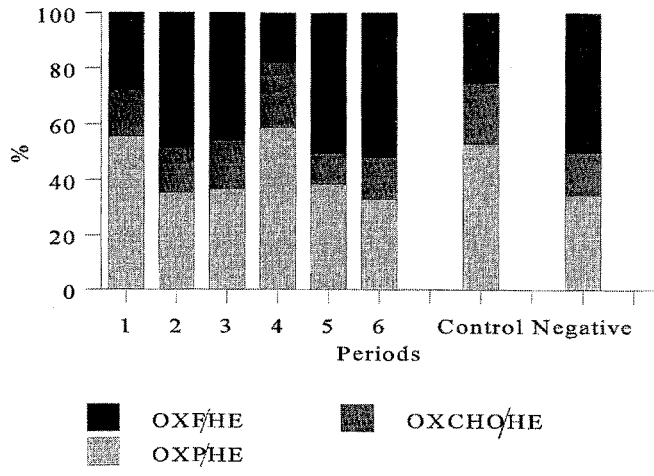

Fig. 1 Heat from oxidation of protein (OXP/HE), fat (OXF/HE) and carbohydrate (OXCHO/HE) in relation to total heat production (HE) for the flushing group in the 6 balance periods and as average for the whole experiment for the control groups

Discussion

Earlier calculations of nutrient oxidation with use of gas exchange measurements on monogastric animals (1, 2, 11, 12) have been made under conditions of a stable level of energy supply. The present results, however, show that this method for calculations of nutrient oxidation can provide useful indications of the relative importance of each nutrient as a metabolic fuel also under conditions of a varied energy supply.

There are, however, some obstacles that must be taken into consideration. First, OXP does not reflect the true protein oxidation, but the amount of deaminated amino acids. For carnivores, which are fed diets supplying protein above the requirement, a substantial amount of the dietary protein will be used as an energy source either by direct oxidation or as a substrate in gluconeogenesis, and these pathways cannot be separated by our present method of calculation. The OXP/HE values found here demonstrated the importance of protein as energy source by reaching levels of 55% when feed supply was abundant and remaining above 30% also during restriction. This should be compared with a level of about 15% which is usually found in pigs (1, 2). Also previous data

Table 3 Intake of digestible nutrients and absolute values for nutrient oxidation data

	Control *Ad lib*	Flushing			
		Ad lib	*Restricted*	*Flushing*	*Ad lib*
DP, g	18.7	21.0	12.4	23.0	19.3
OXP, g	17.1	19.7	11.6	21.3	18.2
DF, g	5.6	6.2	3.7	7.1	5.7
OXF, g	7.9	4.8	8.1	6.9	8.8
DCHO, g	6.1	6.6	3.4	7.1	5.6
OXCHO, g	9.7	9.4	6.1	6.9	7.4

on mink have demonstrated that OXP/HE is strongly related to ME intake, and levels below 30% have not yet been found even during physiologically demanding situations such as late gestation or lactatation (9, 11, 12).

Secondly, UN is usually considerably underestimated in balance studies with carnivores (3, 13) owing to incomplete urine collection. This implies that despite high values for OXP/HE they may still be underestimated since it is reasonable to assume that the urinary recovery was about 80% with the collection routines applied in this study (13).

Our estimates of absolute values for OXCHO were higher than DCHO values. This discrepancy is probably explained by accumulated analytical errors on the CHO fraction. Since this fraction is quantitatively small in mink diets and was calculated by difference in this investigation, even small analytical inaccuracies on other fractions can result in errors of significant importance for the determination of the CHO contents in diets and faeces. Moreover, since UN has a relatively large importance in the function used for calculation of OXCHO (1, 2), underestimated UN values will be a further complication. The relevance of the results of the OXCHO calculations might also be doubted to some extent since it cannot be assumed that gluconeogenesis has been minimal, which is necessary for indirect calorimetry to provide consistent glucose oxidation rates (7).

Therefore, for quantitative calculations on mink data this method can be considered too coarse a tool, but it still has its value as an indicative method. The response to changes in energy supply showed that protein is the relatively most important energetic fuel for the mink when feed supply meets requirements, and that fat, despite a high dietary level, plays a less dominant role under such conditions, whereas the reverse is true when feed supply is limited. High OXF/HE values and decreasing body weights clearly demonstrated that a considerable part of the energy requirement was supplied by mobilization of fat from the body. The mink could therefore provide a useful experimental model for demonstrating both the importance of the relative amount of each nutrient provided by the diet and the velocity of the response to changes in energy supply.

Because of the short-comings of the present method for calculations, a future experimental approach ought to focus on measurement of the true protein oxidation, preferably by use of labeled amino acids in breath test experiments and to combine data achieved by that technique with measurements of gas exchange.

Acknowledgment This study was financially supported by the Danish Agricultural and Veterinary Research Council, grant no. 9400435.

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