**Determination of endogenous fat loss and true total tract digestibility of fat in mink (*Neovison vison*)**

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**Abstract:** Endogenous fat loss (EFL) is a part of the faecal fat content and will therefore contribute to reductions in apparent total tract digestibility (ATTD). The objective of this study was to determine the EFL and to calculate true total tract digestibility (TTTD) of fat in mink (*Neovison vison*) using soybean oil-based diets with different fat levels. The ATTD of macronutrients and energy was also evaluated. Four diets with 6.30, 13.9, 22.0 and 34.0% of fat in dry matter (DM) basis were used in a digestibility assay. Sixteen adult male mink were distributed in a complete randomized design. The fat ATTD values in the diets were 90.8, 95.9, 96.9 and 97.8%, respectively. The apparent total digestible fat [g/kg DM intake] was linearly related to dietary fat intake [g/kg DM] as follows: y = 0.9926x − 5.09 (*r*2= 0.99). The TTTD of fat was estimated from the slope of the regression equation, and the intercept value was considered the EFL [g/kg DM intake]. From the results, EFL was determined to be 5.09 g/kg DM intake and the TTTD of soybean oil was determined to be 99.3%. Therefore, in practice, TTTD values will have negligible impact in feed formulation as they are close to ATTD values with the dietary fat levels normally used for mink. However, when comparing fat ATTD values in diets with very different fat levels for mink and other non-ruminant animals, the effect of EFL must be considered.

**Keywords:** carnivores; estimative; metabolism; regression

**1. Introduction**

In animal nutrition research, endogenous losses of protein and amino acids are more important and of main concern than those from fat and fatty acids (FA) since these losses quantitatively are higher. Digestive enzymes and the presence of sloughed cells from the digestive tract in the feaces account for a greater difference between apparent and true protein digestibility. Endogenous fat in the digestive tract originate mainly from bile and from cells shedding from the mucosa of the digestive tract (Nutrition Reviews 1955; Clement 1975). The impact of the endogenous losses on apparent total tract digestibility (ATTD) of both protein and fat will be dependent on the dietary level, in low-protein or low-fat diets, the endogenous losses will account for a larger proportion of the total protein and fat in the faeces.

Endogenous losses can be measured by two methods. One method to determine protein and fat endogenous losses is therefore to give the animal a protein-free or a fat-free diet and measure the content of the nutrient in the faeces, which represents the endogenous loss. The other common method used is to offer graded dietary levels of the evaluated nutrient and then perform a regression to estimate the endogenous losses.

The ATTD of fat is high in mink, 70 – 96% and FA profile dependent (Austreng et al. 1979; Rouvinen 1990). Several studies, in different species, have reported lower ATTD for saturated FA compared to unsaturated FA (Rouvinen et al. 1988; Rouvinen 1990; Pontieri 2008; Rostagno et al. 2011; Marx et al. 2015). However, the excretion of endogenous fat is not adjusted in ATTD fat calculations. Therefore, endogenous fat loss (EFL) in interspecies comparisons is interesting to have knowledge on, as well as for establishing a standard dietary fat level in studies determining fat digestibility.

Previous studies evaluating the EFL reported values of 8.0 g/kg dry matter (DM) intake in mink (Rimeslåtten and Jørgensen, (1971); referred in Austreng et al. 1979) and 7.9 g/kg DM intake in dogs (Marx et al. 2017). We wanted to confirm this result since mink is applied as model for digestibility in dogs and other monogastric species.

**2. Materials and Methods**

The experiment was conducted at the Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway. The use of live animals at the department was authorised by the Norwegian Animal Research Authority.

***2.1. Animals and experimental design***

Sixteen healthy adult (1.5 to 3 years of age) male mink weighing 2.5 – 3.0 kg, were assigned in a complete randomized design assay to test four diets with four replications each. The study was conducted in two experimental periods: first for adaptation to the diets and cages (d 1 to 3), followed by the total faecal collection period (d 4 to 7) and precise feed intake registration. The animals were housed indoors in metabolic cages designed for faeces and urine collection and separation.

***2.2. Dietary treatments***

Four semi purified diets with increasing levels of fat (6.30, 13.9, 22.0 and 34.0% of fat on DM basis) were used to evaluate the ATTD of macronutrients and energy (Table 1). Diets were prepared by adding all ingredients to a bakery mixer and adding water up to the desired consistency. The mink were offered, once a day, about 65 g of feed [DM basis] corresponding to 1.23, 1.33, 1.45, 1.57 MJ/d of gross energy (GE), for the four diets, respectively from the order given in Table 1.

**Table 1.** Dietary treatments formulation [as-fed basis] and chemical composition.

|  |  |
| --- | --- |
|  | Fat level [%] |
|  | 6.30 | 13.9 | 22.0 | 34.0 |
| Ingredients [%] |  |  |  |  |
| Soybean oil | - | 2.40 | 5.30 | 9.00 |
| Fishmeal | 15.5 | 19.0 | 21.3 | 24.0 |
| Precooked corn starch | 8.40 | 7.90 | 6.20 | 4.00 |
| Cellulose powder | 1.90 | 2.40 | 2.70 | 3.00 |
| Vitamins/mineral Premix\* | 0.01 | 0.01 | 0.01 | 0.01 |
| Water  | 74.2 | 68.3 | 64.5 | 60.0 |
| Total | 100 | 100 | 100 | 100 |
| Chemical Composition† |  |  |  |  |
| Ash [%] | 9.80 | 9.40 | 9.50 | 9.30 |
| Crude protein [%] | 43.5 | 43.8 | 43.0 | 42.9 |
| Ether extract [%] | 6.30 | 13.9 | 22.0 | 34.0 |
| Carbohydrate¶ [%] | 40.4 | 32.9 | 25.5 | 13.8 |
| Starch [%] | 26.9 | 20.4 | 14.7 | 8.90 |
| Gross energy [MJ/kg] | 18.9 | 20.5 | 22.3 | 24.2 |
| Metabolizable energy [MJ/kg]ǂ | 14.5 | 16.2 | 17.9 | 20.4 |
| % from Crude protein | 43.9 | 39.7 | 35.3 | 30.8 |
| % from Ether extract | 15.4 | 30.6 | 43.8 | 59.3 |
| % from Carbohydrate | 40.7 | 29.8 | 20.9 | 9.9 |

\*Provided per kg diet: vitamin A, 200 IU; vitamin D3, 20 IU; vitamin E, 5 mg; vitamin B1, 1.5 mg; vitamin B2, 300 µg; vitamin B6, 300 µg; vitamin B12, 2 µg; pantothenic acid, 333 µg; niacin, 500 µg; biotin, 3 µg; folic acid, 30 µg; iron, 2 mg; copper, 125 µg; manganese, 750 µg; zinc, 1 mg; iodine, 6 µg; selenium, 10 µg; cobalt, 6 µg; †Values in dry basis; ¶Carbohydrate calculated by difference as follows: Carbohydrate [%] = Dry matter [%] – (Crude protein [%] + Ether extract [%] + Ash [%]);ǂMetabolizable energy estimative adapted from AAFCO (2008), % crude protein · 0.01465 + % ether extract · 0.03556 + % carbohydrate · 0.01465.

***2.3. Sample collection***

Total feces collection was performed twice a day and stored at –20oC. At the end of the trial the total fecal outputs from each mink were weighed and mixed and dried at 55oC in a forced-air oven for 72 h according to the AOAC International (AOAC 1995) followed by grinding in a Wiley mill using a 1-mm screen.

***2.4. Chemical analyses***

Diets and faeces were analysed for DM (method 934.01), ash, crude protein (CP) (method 954.01), ether extract using acid hydrolysis (EE) (method 954.02) according to the AOAC International (AOAC 1995), while the analysis of starch followed the procedure described by McCleary et al. (1984). Dietary and faecal GE were determined by using isoperibolic bomb calorimetry (model C2000 basic; IKA Werke GmbH & Co. KG, Staufen, Germany).

***2.5. Calculations***

The following equation was used to determine ATTD of macronutrients and energy:

ATTD [%] = {(Intake [g/d] – Faecal output [g/d]) / Intake [g/d]} · 100%.

The carbohydrate content was calculated by difference as follows:

Carbohydrate [%] = DM [%] – (CP [%] + EE [%] + Ash [%]).

The EFL was estimated by the association between the apparent total digested fat [g/kg DM intake] and the dietary fat intake [g/kg DM]; the true digestibility of fat was estimated from the slope of the regression equation, and the EFL [g/kg DM intake] was the intercept value.

***2.6. Statistical Analysis***

Differences in the ATTD of macronutrients and energy, between dietary treatments were tested for significance using ANOVA and the GLM procedure (Statgraphics Plus for Windows 4.1; <http://www.statgraphics.com/>). The means were compared using the Student-Newman-Keuls (SNK) test at 5% probability. Also, the ATTD of macronutrients and energy were estimated according to dietary fat level by polynomial regression equations and the EFL was estimated by linear regression using the “Advanced Regression” module of Statgraphics Plus for Windows 4.1 software.

**3. Results and discussion**

The mink is a strict carnivore and often responds to dietary changes decreasing feed intake. This sensitivity to dietary changes is probably related to palatability and is most evident for ingredients of vegetable origin (Skrede and Cheeke 2005). The mean intake (g/d) of macronutrients and energy in the total faecal collection period is shown in Table 2. The DM intake was similar for all diets (*p* > 0.05), but individual variations were observed. The CP and GE intakes also were similar for all diets (*p* > 0.05), however, as designed, the fat intake increased (*p* < 0.01) as the carbohydrate and starch intakes decreased (*p* < 0.01) according to dietary contents.

**Table 2.** Mean daily intake of macronutrients [g/d] and energy [MJ/d] of adult mink fed diets with increasing levels of soybean oil.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Fat level [%] | SEM\* | *p*-Value |
| 6.30 | 13.9 | 22.0 | 34.0 |
|  Dry matter | 54.7 | 48.2 | 41.0 | 54.0 | 3.51 | 0.53 |
|  Crude protein | 23.8 | 21.1 | 17.6 | 23.2 | 1.53 | 0.52 |
|  Ether extract | 3.39c | 6.72bc | 9.02b | 18.4a | 1.54 | <0.01 |
|  Carbohydrate† | 22.1a | 15.9ab | 10.4b | 7.48b | 1.75 | <0.01 |
|  Starch | 15.3a | 10.2b | 6.22b | 4.95b | 1.22 | <0.01 |
|  Gross Energy | 1.07 | 1.03 | 0.94 | 1.34 | 0.08 | 0.35 |

\*SEM, standard error of the mean; a,bMeans within a row with different superscripts differ significantly (*p* < 0.05); †Carbohydrate calculated by difference as follows: Carbohydrate [%] = Dry matter [%] – (Crude protein [%] + Ether extract [%] + Ash [%]).

The ATTD of macronutrients and energy was measured, values are shown in Table 3. The ATTD of DM and CP was similar for all diets (*p* > 0.05). However, the ATTD of fat, carbohydrate, starch and energy were different among diets, whereas the ATTD of fat and energy increased quadratically with fat inclusion level from 90.8% to 97.8% and 81.9% to 86.7%, respectively (*p* < 0.01). The ATTD of carbohydrate and starch decreased linearly with fat inclusion level from 69.8% to 29.2% and 97.2% to 95.9%, respectively (*p* < 0.01). The polynomial regression equations for ATTD of fat, energy, carbohydrates and starch according to dietary fat level are shown as a footnote at Table 3.

**Table 3.** Apparent total tract digestibility (ATTD) of macronutrients and energy of adult mink fed diets with increasing levels of soybean oil. Polynomial regression equations of ATTD of macronutrients and energy showed as footnotes.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Fat level [%] | SEM\* | *p*-Value |
| 6.30 | 13.9 | 22.0 | 34.0 |
|  Dry matter | 76.3 | 77.6 | 76.4 | 78.1 | 0.31 | 0.09 |
|  Crude protein | 87.2 | 87.8 | 86.3 | 86.7 | 0.26 | 0.17 |
|  Ether extract | 91.4c | 95.9b | 96.9ab | 97.8a | 0.63 | < 0.01 |
|  Carbohydrate† | 69.8a | 65.2b | 55.8c | 29.2d | 4.24 | < 0.01 |
|  Starch | 97.2a | 97.0a | 96.4b | 95.9c | 0.15 | < 0.01 |
|  Energy | 81.9c | 83.8b | 84.4b | 86.7a | 0.48 | < 0.01 |

\*SEM, standard error of the mean; a,bMeans within a row with different superscripts differ significantly (*p* < 0.05); †Carbohydrate calculated by difference as follows: Carbohydrate [%] = Dry matter [%] – (Crude protein [%] + Ether extract [%] + Ash [%]); ATTD Ether extract [%] = 87.6774 + 0.071601 · fat level – 0.000124848 · fat level2 (*r*2 = 0.88, SE = 0.92, *p* < 0.01); ATTD Carbohydrate [%] = 68.8084 + 0.0411609 · fat level – 0.000463224 · fat level2 (*r*2 = 0.99, SE = 2.03, *p* < 0.01); ATTD Starch [%] = 97.5752 – 0.00500761 · fat level (*r*2 = 0.78, SE = 0.28, *p* < 0.01); ATTD Energy [%] = 81.1416 + 0.0162296 · fat level (*r*2 = 0.82, SE = 0.81, *p* < 0.01).

The ATTD of carbohydrate decreased upon dietary fat increase as a result of the starch:cellulose ratio of the carbohydrates decreased in the higher fat diets. Cellulose is not digested by mink, while precooked starch is highly digestible as shown in Table 3. The high ATTD of starch, above 95% for all diets, shows that despite the mink being a strict carnivore, the animals were capable to digest, almost completely, this important polysaccharide. The inclusion of cellulose was necessary to adjust diets consistency.

The ATTD of fat for all diets were above 90% and shows that soybean oil is a highly digestible fat source for mink diets. The regression of total tract apparently digested fat [g/kg DM] on the dietary fat intake [g/kg DM intake]; y = 0.9926x − 5.09 (*r*2 = 0.99); is shown in Figure 1. The true digestibility of fat was estimated from the slope of the regression equation, and the intercept value was considered the EFL [g/kg DM intake]. The EFL obtained in this study was in the same magnitude shown by Rimeslåtten and Jørgensen, (1971) (referred in Austreng et al. 1979) in mink and Marx et al. (2017) in dogs, with both EFL values around 8.0 g/kg DM intake. In pigs, Jørgensen et al. (1993) determined EFL as 4.7 g/kg DM intake at distal ileum and 4.4 g/kg DM intake at faeces. Kil et al. (2010) evaluating different forms of fats (extracted or intact) to pigs, found EFL from 3.77 to 12.08 g/kg of DM intake depending on the dietary fat source, respectively.

**Figure 1.** Regression of total tract apparently digested fat on the dietary fat intake is represented as follows: y = 0.9926x − 5.09 (*r*2 = 0.99).

Kendall (1984) determined EFL in cats to be 150 mg · kg BW-1· d-1 and 242 mg · kg BW-1· d-1 in dogs, while Marx et al. (2017) determined 155 mg · kg BW-1· d-1 in dogs. The EFL [mg · kg BW-1· d-1] in 2.5 – 3.0 kg male mink, estimated by our data range was from 100 – 120 mg/d, lower than values found for dogs and cats, probably due to the anatomical differences regarding the large intestine of these animals. The mink has a short digestive tract, lack a caecum and exhibit minimal hindgut microbial activity (Skrede 1979).

The TTTD of fat calculated using the correction factor found in the present study, shown very similar values; 98.9%, 99.5%, 99.1%, 99.2%, respectively for the dietary treatments. In practice, TTTD fat values will have little impact in feed formulation as they are close to apparent values with the fat levels normally used for mink. The low-fat diet in the present study showed about 8% difference between apparent and true fat digestibility. However, the energy distribution from protein, fat and carbohydrates of this diet (Table 1) is not usually seen in practical diets for mink. As carnivorous, protein and fat-based diets, with very low carbohydrate content, are commonly applicable in mink farms.

**4. Conclusions**

The EFL in mink is 5.09 g/kg DM intake, corresponding to 100 – 120 mg/d for a 2.5 – 3.0 kg adult male mink. The TTTD of soybean oil was determined to be 99.3%. In practice, use of true fat digestibility values will have negligible impact in feed formulation as they are close to apparent values with the fat levels normally used for mink. However, when comparing fat ATTD values in diets with very different fat level for mink and other monogastric animals, the effect of EFL must be considered.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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