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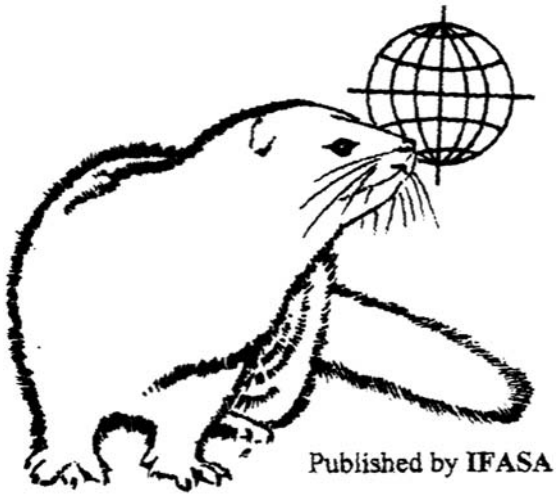

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III: Nutrition

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III – 1 RP

A systematic approach to sustainable fur farming with special reference to feed and feeding

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Introduction

Sustainability and sustainable development are terms that are often used. They were first used by Robert Silvers in 1974 and widely brought into publicity by Bruntlandt Commission in 1987 and later refined and developed more in the United Nations Agenda 21 (Rio de Janeiro 1992). According to Bruntland Commission sustainability means 'meeting the needs of the present without compromising the ability of future generations to meet their own'. More practically sustainability is understood as wise use of natural resources.

Sustainability in fur farming is the key for modern and future fur farming. Sustainability is necessary, not only in reference to new (European) legislation, but also to produce a well-balanced and societal justified fur product. Sustainable fur business means a financially healthy business that is accepted by the society and therefore fulfils the demands of the current and future society.

Sustainable fur production is necessary if we want to maintain fur production in the fur-producing countries. Production must also be both economically and culturally sustainable. If fur breeders cannot accept production conditions and therefore stop producing, it does not matter whether or not production is sustainable in other respects. In that case, production will move to countries where it is sustainable according to other criteria and conditions. Balanced sustainability in all its aspects must be the basis for fur production in the future.

Sustainability in fur animal feeding – fact or false?

Traditionally fur animal farming has been considered sustainable, mainly connected to the type of feed it uses. The feed that mink and foxes consume can not be eaten by other species. Mainly it is made of by-products or even waste that elsewhere would cause significant costs to their producer to dispose it of. Fur industry recycles millions of kilograms of mostly organic phosphorus back into use as fertilizer and thus saves non-renewable phosphorus sources.

What is the cost of recycling, in terms of using non-renewable energy? Would it be more sustainable not to recycle than recycle and what alternatives do we have? All efforts should be evaluated for considering sustainability. Sustainability is a very complex concept where all affects - there is no such concept as absolute sustainability in modern society. Some activities are only more sustainable than others. As mentioned before the use of slaughter by-products contributes to sustainable fur farming. As an example, in 2003 approximately 500 million poultry are slaughtered in the Netherlands, this is equivalent to 690 million kilogram of poultry by-products. To produce a mink skin approximately 35 kg of feed is needed. The Dutch fur animal feed consists, on average during the year, of 65% poultry slaughter by-products, 25% fish by-products and 10% of carbohydrates and premix. Producing 3 million mink skins is then equivalent to the use of 73.5 million kg poultry by-products. Rendering these by-products will cost 5 eurocent per kilogram. Producing mink feed will cost approximately 13 eurocent on energy, logistics and depreciation. Summarizing, for net 8 eurocent per kilogram, a product that is not to be used for human consumption or other animal feed is valorised to a useful feed product. Otherwise approximately 37 million euro has to be spent for rendering these poultry by-products. So mink eating poultry by-products contributes to sustainability of the poultry and the fur business!

There is not necessarily a conflict between an economically and an ecologically sustainable fur production.

General Strategy

How could sustainability be enhanced in fur animal feeding? The answer is 'by strengthening the basic concept, use of by-products, but in a more sustainable way'. Two principles arise above others - saving nutrients, saving energy, enhancing health, well-being and production efficiency. In addition to saving non-renewable mineral sources (e.g. phosphorus) saving nutrients means reduced feeding costs and is therefore also economically sustainable; lower price per kg of feed and lower costs needed to return nutrients (manure) back into circulation. The fact that also saving energy is economically sustainable, gives a good direction and support to the future developments of feeding of fur animals; less use of non-renewable natural energy sources (oil, even electricity) is a core thought of sustainability, together with high efficiency.

Remarkable results in sustainability, even before the idea was introduced, have already been achieved. The first principle, saving nutrients, has long been in the minds of both farmers and researchers. Whereas fur animal feed from this point of view is much more sustainable than it was forty years ago, direction has been right, not much attention was paid to phosphorus, a key element in environmental protection until water protection laws were set in the early 1990's.

Reduced feed consumption per produced area of skin improves the sustainability of fur production. The economy will be improved and the use of energy from production to delivery at the cage of the animal as well as release to the environment in the form of manure and evaporation will be reduced. The reduced impact on the environment will lead to improved acceptance by society.

The large variation in feed requirement at different times of the year is well known. A large spread in the period of birth needs a high degree of individual feeding that puts a very big responsibility to the person in charge with the feeding management. The spread in the peak of birth-date can go up to 10 days. Especially at an advanced stage of lactation the needs of the different farms will differ a lot. Farms with an early birth peak are demanding for a higher energy content opposite to farms that still need the lower energy level in the feed to support the lactation in females with the younger kits and the kits just starting to eat. The broad variation of needs makes it almost necessary to produce two kinds of feed in this period, but the extra labour and energy involved in this goes straight against sustainability. But also other approaches can be useful to provide sustainable solutions. As an example it can be mentioned dividing the animals into weight groups in order to obtain a high whelping result via an easier and more uniform winter condition or in the lactation period the date of birth and the litter size or the daily feed requirement are registered and fed in intervals of , for example, 100 gram. Both methods are used to ensure optimal feeding. The latter feeding method makes it possible to conduct health control and select dams with desirable traits. These initiatives have not resulted in direct selection for improved feed efficiency. Over the years, selection for increased body size and more adapted animals may have increased feed efficiency indirectly. Research results confirm that and show a strong correlation between weight gain and feed efficiency.

When fur farming, in terms of feed manufacturing, grew to industrial size, development work was directed to effectively handling of fish. Big catches required freezing capacity that in economical sense was not in relation to the benefit – a fast and cheap preservation. Fish silage first, and slaughterhouse by-product silage just recently have become economical and more sustainable replacements for freezing, thus saving a lot of energy.

Sustainability, can also be improved by paying specifically attention to the different periods during the production cycle of fur. During late winter and early spring a special attention to animal health and economics is made. Taking care of a perfect animal health and welfare in combination with a good body condition is a base for the start of the mating period and provides the best chance of birth of viable kits. From breeding until nursing precision feeding is necessary to maintain optimal growth with minimum mortality of kits and females. Actually, nowadays a discrepancy between the needs of the females in different lactation stages and the growing kits in reference to the composition of the feed is occurring. Nowadays, the demands for the breeding females are very high, good pelt quality, good size, high fertility and the capacity to nurse many kits. All these mainly commercial demands have to go parallel with an excellent health and a good well-being. If welfare and health are not guaranteed, sustainability is out of the question. Development of the mink feed in combination with good health management is one of the measures taken to fulfil these high demands. In comparison to 20 years ago, a clear adjustment of feed energy levels has occurred. In that time a variation of 1150 to 1400 kcal/kg in feed energy was normal , while at present

a variation through the whole season of 950 to 1700 kcal is the standard in order to provide the mink with optimal feed.
Using the broader variation in feed energy helps to fine-tuning the animal body condition and to supply the animals demands to a larger volume of feed during wintertime in order to secure the welfare. A broader variation in energy can also have a positive effect on the environment, a higher percentage of fat in the feed during summer and autumn provides the farmer a change to perform precision feeding what leads to a lesser amount of manure and decreases the volume and weight leading to a lower logistic pressure to transport the feed from factory to farmer. Still new efforts have to be taken to examine the possibility to reduce the transport of 70% water in the feed.

Energy efficient feeding process
Undoubtedly research towards more sustainable use of nutrients needs to be continued, there is still a lot to be done, but even more remarkable results in sustainability can be achieved by saving energy during the feeding process. Even though dry feed system is not utilizable in large scale 'yet', next step towards higher energy efficiency in feed manufacturing and delivery should be taken without delays. The feed delivery system has not changed almost at all in thirty years. Economical crises have forced it to be changed – number of feed centres has fallen below half what it was. However, many feed centres still work below half capacity and high moisture content fur animal feed is transported to the farms, daily, even in winter. Use of energy, from the receiving raw materials to the distribution of feed on cage wire mesh, is a major challenge in developing sustainability in fur animal feeding. Even though many people think that fur animal feeding deserves a sustainability label already now, many also think that it shouldn't be granted to it for the wasting of energy.

Area ecological model
At present the trend is towards larger scale and efficiency in animal production units, in all animal husbandries. The more animal production concentrates and the larger production units become, the more outspoken becomes also the challenges to manage with environmental and sustainable targets. While use of energy in core production decreases, per unit of product, other costs may increase. One solution to keep up with sustainability is to utilize a model that evaluates nutrient flow on certain geographical area. Production units are placed in such a way that nutrient circulation is optimal and cost effective (to farmer, environment, society, future). The future will show if sustainability will be achieved only in large scale plants – or will new technology provide room also to 'medium size farms'? Much depends on cost structure of fur animal production. Will the policy enhances sustainability in all its aspects (economical, ecological, social and cultural)?
Society's demands for agriculture to reduce its impact on the environment are increasing, especially for productions situated in remoted areas where the environment is often sensitive. Many fur farms are located in such areas. If we are not able to comply with the increased requirements, our production conditions will deteriorate and it will be more difficult to increase existing farm sizes and establish new farms.

Use of by-products stays, use of energy decreases
Recycling is a cornerstone in fur farming and will be an important viewpoint in further development of sustainability in fur animal feeding. Future fur animals will be fed with feedstuffs that are still derived from by-products but that are preserved and stabilized with less use of non-renewable energy sources. Feed stability on one hand but especially storage conditions on farms will be improved which implies that feed is delivered fuel efficiently, year round. Requirements of animals will be better taken care for; bulk feed suitable for all fur animal species and developmental stages won't anymore be the most economical solution. Good storage stability of the feed provides possibilities to manufacture and deliver different feeds e.g. during same week or month. All this imply a higher degree of sustainability in fur animal feeding process.

Animal Health
To control health and production of fur animals at such a specialized farm different actions should occur, including analysis of production disturbances, creating awareness of the farmer, planning, monitoring and corrective measures. These events result in a program, used by the farmer that fits into good mink husbandry practices. To obtain this a systematic approach have to be followed. In the Netherlands the system of hazard analysis critical control points (HACCP) is followed (Urlings, 1999). Weak points in the production cycle of mink are clarified and a general preventive management system is designed and adapted to

individual farms and farmers, nutrition and feeding practices plays a paramount role in these health and welfare management systems.
Health management is realised through vaccination and good farm management. Generally, under farm management is understood good housing, adjust to the specific period and demands, clear breeding systems, but primarily a feeding management that secures health and welbeing.

Present and future possibilities
The conditions for moving fur production into a more sustainable direction are now being created. Through research knowledge is gathered about feed efficiency and its correlation to other characteristics as weight gain, feed consumption and behaviour. It has been shown that feed efficiency measured in the growth period has a relatively high heritability. Moreover, feeding data from practical farms and experiences have been collected. Technical development and the increased focus on the feed consumption have led to new feeding technology being developed. It will make it easy to register, to feed the required amount of feed, to collect and to analyse feeding data. A system called "Individual feeding" is being developed in Denmark. More than 35.000 females and 200.000 mink kits are now on this system on Danish farms.
Individual feeding can be used most of the year. In the winter and spring, a large variation in the feed requirement is seen. The feeding method will contribute to winter condition, flushing and feeding during pregnancy in order to keep the animals in a optmal health shape. It is expected that some farms will attain a better whelping result. During lactation and the early growth period the feeding method will make it easier to satisfy the large variation in feed requirements caused by differences in birth date, litter size, whelp size, sex ratio in the litter, and the ability of the mother to take care of herself and her kits. In the growth period "Individual feeding" will provid a system to feed more precisely and thereby cover the actual feed requirement. A more precise feeding will allow for full expression of the growth potential, thereby increasing the average size of the produced skins, and lead to better selection of new breeding animals, resulting in a higher production level. It is expected that more precise feeding will reduce feed wastage.
Year round individual feeding puts new demands on the farmer. Habits must be changed. In the future, farmers will not merely be satisfied with a high feed intake during the growth period, but will demand knowledge of the resulting production level. Besides the previously mentioned advantages, the farmer will have a better overview of the farm and, thereby, better health control year around. An abnormal feed intake will be easier to register. The system will make it easier to allow different people feed the animals, giving the farmer greater flexibility. The feeding machine and the feed storage conditions must be in good order to ensure delivery of the correct amount of feed as well as palatable feed. Last, but not least, "Individual feeding" requires that the feeding kitchen is able to deliver a homogeneous feed, which is easy to deliver at the cage and which gives a stable feed intake.
The conditions for a more sustainable fur production in all its aspects are present. Many people seem to be interested, but it requires a coordinated effort and a far-reaching strategy in the various fur-producing countries and, preferably, coordination between the countries. We have a common goal. If we work together and make use of the available competences we can achieve much more.

III – 2 RP

Influence of using enzymatic preparations: α-amylase, β-glucanase and xylanase on nutrient digestibility in polar foxes (*Alopex lagopus*)

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Abstract
The aim of the study was to determine the influence of enzymatic preparations: Bio-Feed Alpha® (active enzymes: α-amylase and β-glucanase), which increase starch hydrolysis and Bio-Feed Wheat® (active enzyme: xylanase), which increases hydrolyzed fractions of fiber from cellular walls, on nutrient digestibility in polar foxes. The enzymatic preparations used in this experiment were added in proportions 200 or 400 mg of each per 1 kg of fresh food for experimental animal groups; 21% of cooked grain was used in the animals' diet. Slightly better results observed in experimental groups were not statistically significant.

Introduction

As typical carnivorous fur animals the polar foxes need meat components in their diet. However, opposite to mink they tolerate higher level of cereal products (20-40% of diet), which are the main source of carbohydrates (Jarosz, 1993). Despite of good digestibility of carbohydrates, foxes do not have any enzymes to hydrolyze fractions of fiber (Slawon, 1997). In carnivorous, as opposed to herbivorous animals, low activity in the gastro–intestinal tract of amylolitic and cellulitic enzymes produced by microorganisms is the main reason why fiber is not degraded (Jarosz, 1996). The fiber level of about 1-2 % of dry matter beneficially influences the apparent digestibility of nutrients (Szymeczko et al., 1996). Fiber doses with more than 3% of dry matter decrease nutrient digestibility (Slawon, 1997). Using enzymatic preparations added to grain components helps degrade fiber fractions from cellular walls and improves digestibility of nutrients inside the cells. They thus help improve animal production and reduce expensive production costs (Krzeminski et al., 1995). Enzymatic preparations are mainly used in poultry and swine feeding with positive effects. Many experiments have been carried out on these species (Flemming et al., 1994; Frankiewicz et al., 1999; Kaoma et al., 1998). In fur animal production, the final product is fur and the quality of it depends on feeding. Using preparations helping nutrient digestion can therefore indirectly influence fur quality of foxes.

The mixture of 3 enzymes: α-amylase, β-glucanase and xylanase were used in this experiment. α-amylase and β-glucanase are active enzymes, which added to barley increase starch hydrolysis. Xylanase, if added to wheat, increases hydrolyzed fractions of fiber from cellular walls. α-amylase and β-glucanase are active enzymes in Bio-Feed Alpha®; xylanaze is active enzyme in Bio-Feed Wheat ®. NovoNordisk produces both enzyme preparations.

The aim of the study was to determine the influence two levels (200 mg and 400 mg per 1 kg of fresh food) of the above mentioned enzymatic preparations on nutrient digestibility indices in polar foxes.

Material and Methods

Experiment was carried out on farm, located 200 km south from Warsaw in Poland, in the period from August 15th to December 10th in two seasons: 2000 and 2001. The study spanned over two periods: growing and maturing of fur coat. In both seasons young polar foxes (male), after weaning, were divided into 2 groups: control (6 cubs) and experimental (6 cubs). Daily diet consist in 79% of meat (poultry offal, contains bone and poultry offal, soft, muscle meat, animal fat) and 21% cooked grain (64 % wheat, 34 % barley). The chemical analyze of used diets and theirs energy value are presented in table 1. Enzymatic preparations Bio-Feed Alpha® and Bio-Feed Wheat® were added to the experimental diet 200 mg each per 1 kg of fresh food (200 g per tone) in 2000 and 400 mg each per 1 kg of fresh food (400 g per tone) in 2001. The fecal for estimating digestibility were collected from each animal by 7 days, in the beginning of September (growing period) and middle of November (furring period), in both seasons. For statistical analyses, a 2-sample t-test was used to test differences between control and experimental groups, separately for each season.

Table 1 Composition and Metabolizable Energy (ME) value (in %) of diets used during both experimental periods

Parameter	Season 2000		Season 2001	
	16.07-15.09 (growing)	16.09-15.12 (fur maturing)	16.07-15.09 (growing)	16.09-15.12 (fur maturing)
Chemical composition, %				
dry matter	30.61	33.98	35.34	38.01
crude protein	10.49	12.97	11.16	12.70
crude fat	6.52	9.73	15.02	13.66
nitrogen-free extract	8.95	6.31	6.21	6.37
crude fibre	0.65	0.50	0.81	0.62
ash	3.98	4.46	2.12	4.65
Energy value, MJ/kg				
Protein	1.97	2.43	2.09	2.38
Fat	2.53	3.78	5.84	5.31
Nitrogen-free extract	1.53	1.08	1.06	1.09
Total	6.03	7.29	8.99	8.78
ME derived from, %				
Protein	32.66	33.33	23.24	27.10
Fat	41.97	51.85	64.97	60.47
Nitrogen-free extract	25.37	14.82	11.79	12.43

Results and Discussion

Chemical composition and energetic value of diets used in the study are presented in table 1. Energy share derived from protein, fat and nitrogen-free extract in diets, was compared with norms for polar foxes presented by Slawon, 1997. Diet used during the growing period in 2000 was well balanced; only the % of ME from protein (32.66%) was below the level of recommendations (35-40% ME). According to experiments carried out by Rimeslatten (after Slawon, 1997), a level of 25% ME from protein is adequate for young polar foxes, which are older than 16 weeks. In younger foxes, age 14-16 weeks, low protein can cause reducing growth, but after this period low level of protein does not influence body weight gain or fur quality. The amount of ME deriving from nitrogen-free extract (14.82%) was lower than in norms (30-40% ME). In 2001 season during growth period the share of ME derived from protein was 23.24%. It was a result of the addition of animal fat, which increased the share of ME from crude fat (64.97%). The share of ME from crude fat was even higher than recommended by the Norwegian norms (up to 60% of ME from fat, Heggset, 2000). During fur maturing period the share of ME derived from fat was lower (60.47%) and share of EM from protein was above minimum level (27.10%). According to Slawon, 1997, by using high fat content and reducing the amount of energy from protein and polysaccharides during the last period before pelting, it is possible to improve the growth of foxes.

Digestibility indices of feeding components were much higher in the experimental group during growing period in both seasons (table 2). Young foxes seem to be very sensitive to the addition of feed supplements during that period. During the fur maturity period no differences were found in digestibility (table 3), although the digestibility indices seemed to be numerically slightly higher in the experimental group.

The addition of enzymatic preparations: α-amylase and β-glucanase (Bio-Feed Alpha®) and xylanase (Bio-Feed Wheat®) at 2 levels: 200 mg/kg and 400 mg/kg fresh food, improved digestibility of diet nutrient during the growing period but did not influence digestibility during the furring period. This is probably due to the low level of fiber (under 1.00% of crude fiber) as result of low share of grain used in diet on the farm (21%).

Table 2 Digestibility of diet nutrients by polar foxes during the growing period (means±sd)

Digestibility %	Season 2000			Season 2001		
	C (N=6)	E (N=6)	P-value	C (N=6)	E (N=6)	P-value
Protein	85.49 ± 5.00	91.10 ± 3.44	0.000***	85.55 ± 4.85	90.70 ± 2.87	0.000***
Fat	97.53 ± 1.01	97.27 ± 0.71	0.000***	97.73 ± 0.95	98.16 ± 0.78	0.038*
Fiber	69.29 ± 9.71	71.06 ±11.21	0.442	67.83 ± 11.32	79.02 ± 5.43	0.000***
Nitrogen-free extract	84.95 ± 4.54	89.68 ± 4.15	0.000***	84.74 ± 5.38	89.780 ± 3.35	0.000***

C – control group
E – experimental group
N – number of animals in group

Table 3 Digestibility of diet nutrients by polar foxes during the furring period (means±sd)

Digestibility %	Season 2000			Season 2001		
	C (N=6)	E (N=6)	P-value	C (N=6)	E (N=6)	P-value
Protein	91.64 ± 3.24	92.22 ± 2.11	0.334	86.55 ± 5.20	87.30 ± 5.56	0.557
Fat	99.10 ± 0.42	99.20 ± 0.34	0.256	98.37 ± 0.82	98.04 ± 0.99	0.128
Fiber	64.05 ± 11.45	60.91 ± 14.56	0.275	63.78 ± 18.50	66.35 ± 9.35	0.459
Nitrogen-free extract	86.41 ± 5.73	86.23 ± 3.84	0.865	78.47 ± 8.23	80.29 ± 9.29	0.382

C – control group
E – experimental group
N – number of animals in group

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III – 3 RP

Different ratio between n-6 and n-3 fatty acids in diets for lactating mink (*Mustela vison*) dams – effect on milk and kit tissue fatty acid composition

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Abstract

Three groups of female mink were fed experimental diets from mating until weaning of the kits. The experimental diets were supplemented with sunflower oil, rapeseed oil or fish oil as main fat sources in order to achieve different ratios between n-6 and n-3 fatty acids (2.3:1, high (H), 1.5:1, medium (M) and 0.2:1, low (L)). Brain from newborn kits and brain, liver and adipose tissue (obtained from the inguinal region) from 28 days old kits were sampled. Milk samples were taken from females on day 2 and 28 pp. Fatty acid composition of feed, milk and tissues were analysed. Fatty acid composition of milk, liver and adipose tissue reflected dietary treatment, recorded as higher levels of total n-6 fatty acids in the H group and a higher level of n-3 fatty acids in the L group. Fatty acid composition in kit brain tissue was also affected and showed the same pattern as seen in milk and tissues. Docosahexaenoic acid (DHA) and arachidonic acid (AA) were detected in larger amounts in brain tissue than in other tissues. From this study it was concluded that mink milk and body tissues was affected by the maternal dietary fat source and ratio between the fatty acid series. It was also seen that mink brain tissue had high contents of AA and DHA compared to the other organs and milk.

Introduction

Polyunsaturated fatty acids (PUFA) like arachidonic acid (AA, C20: 4 n-6), eicosapentaeonic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C20:6 n-3) play important roles in the development of the infant brain and retina (Innis, 1991). Linoleic acid (LA, C18: 2 n-6) and AA are members of the n-6 series, because the first double bond is located at the 6th carbon atom from the methyl end. Linolenic acid (ALA, C18: 3 n-3), EPA and DHA belong to the n-3 fatty acid series, because the location of the first double bond is at the 3rd carbon atom from the methyl end. LA and ALA are essential to mammals, because they lack the capability of inserting af double bond beyond the 9th carbon atom from the carboxylic acid end. DHA and AA are formed by elongation and desaturation from ALA acid and LA respectively. The two fatty acids series use the same enzyme systems in desaturation and elongation. This may lead to a competition between the two fatty acid series, and means that high levels of one fatty acid series may block for the modification of the other series and thereby lower the content of one of the fatty acid series in the organism.(Innis, 1991). Fish oil feeding with high levels of n-3 fatty acids to mice was shown to inhibit Δ-6 desaturase activity followed by a decrease in the levels of AA in the tissues (Raz et al., 1997). Low AA content in the foetus may have negative effects on growth and development (Carlson et al., 1991) and this may be a consequence of a low n-6:n-3 ratio.

The long chain PUFA are very important for the development of the foetus, and therefore the foetus has to be supplied from maternal circulation through the placenta. A preferential uptake of long chain PUFA over non-essential fatty acids in the human placenta was reported, and this reflects higher requirements for PUFA than for non-essential fatty acids in the foetus (Dutta-Roy, 2000). Studies with pregnant women have shown positive relations between the maternal plasma phospholipid concentrations of AA, ALA, LA and DHA and the concentrations of these fatty acids in the foetal phospholipids (Elias & Innis, 2001). The selectivity for PUFA may also be regulated at the cellular level

through a selective oxidation of fatty acids and a selective incorporation of the fatty acids into placental phospholipids (Herrera, 2002). There are, however studies that show that the foetus is capable of converting ALA and LA to DHA and AA, but the efficiency of this process is not known (Dutta-Roy, 2000; Green & Yavin, 1993).

It has been documented that deficiency of n-3 polyunsaturated fatty acids in the brain and retina may lead to functional disturbances like impaired learning abilities and visional function (Bourre et al., 1989). Accumulation of DHA in the developing brain and retina is dependent on the dietary intake of n-3 fatty acids in form of the precursor ALA and also its long chain derivatives like EPA and DHA. The supplementation of dietary n-3 fatty acids from fish oils to pregnant and lactating rats have been shown to alter the fatty acid composition of foetal and neonatal rat brain (Yonekubo et al., 1993). Guesnet et al. (1997) have shown that DHA in the brain increases when the content of ALA in the maternal diet increases.

The aim of the present study was to determine the effect of different ratios between the n-6 and n-3 fatty acids in the diet for pregnant and lactating mink on the deposition of fatty acids in foetal and postnatal tissues of the kits It was expected that dietary fat source and ratios between the fatty acids would affect the fatty acid patterns of the milk and body tissues.

Materials and Methods

Seventy-five Scanbrown female mink were divided into 3 groups. The groups comprised of 50% yearlings and 50% adult females. The experiment was carried out from 25. February until weaning of the kits at 8 weeks of age. The animals were housed individually in conventional farm cages with wooden nest boxes. The females were mated from 3. March. They were tried for a second mating 9 days after the first mating. The experimental diets were composed as shown in Table 1. In order to provide the planned ratios between n-6 and n-3 fatty acids at 12.4:1 (high, H), 4,1:1 (medium, M) and 0.25:1 (low, L) the basal diet was supplemented with sunflower oil, rapeseed oil or fish oil.

Table 1 Diet composition (%), and results from chemical analysis of the diets and calculated fatty acid content.

	High (H)	Medium (M)	Low (L)
Fish offal < 3% fat	72.05	72.05	72.05
Wheat, popped 90%<0.5mm	4.20	4.20	4.20
Barley, popped 90%<0.5mm	4.20	4.20	4.20
Haemoglobin meal	3.00	3.00	3.00
Potato protein	2.00	2.00	2.00
Maize gluten meal	3.30	3.30	3.30
Sunflower oil	3.30	3.00	-
Rapeseed oil	2.00	1.00	-
Fish oil	-	1.30	5.30
Water	5.70	5.70	5.70
Vitamin and mineral premix[1]	0.25	0.25	0.25
No. of chemical analyses	5	5	5
Dry matter (DM)	33.0±1.7	33.7±1.8	33.7±1.7
Ash, % of DM	10.5±2.9	8.6±3.3	8.1±2.8
Crude protein, % of DM	49.0±1.6	49.1±1.0	49.3±1.7
Crude fat, % of DM	18.9±2.1	18.6±1.9	18.9±2.3
CHO, % of DM	21.6±2.5	23.7±2.1	23.7±2.4
ME, MJ/kg DM	17.05	17.01	16.90
Vitamin E mg/kg DM	60	60	60

[1] Contains kg $^{-1}$: Vitamins. A: 28,00,500 IE, D_3: 280000 IE E: 24021 IE B_1: 10002 mg, B_2: 4801 mg, B_6: 3201 mg, B_{12}: 16008 mg, calcium-pantothenate: 3207 mg, biotin: 80 mg, folic acid: 241 mg, niacin: 8002 mg, Minerals Fe: 19712 mg, Cu: 1025 mg, Zn: 12561, Mn: 6238 mg.

Milk samples were collected from 5 females in each group on day 2 pp. and on day 28 pp. The same females were used at both samplings. The milk samples were stored at –20°C until analysis. One newborn kit from each of the litters, of females who were used for milk samplings, was sacrificed by decapitation and their brains were removed. On day 28 pp. five kits from each group were killed by an overdose of sodium pentobarbital 20% (Skanderborg Pharmacy) and decapitated. Brain, liver and adipose tissue (obtained from the inguinal region) were removed and stored in plastic bags at -20°C until analysis. Samples of feed, milk, liver, brain and adipose tissue were lyophilised prior to fat extraction. The fat was extracted by using CO_2 supercritical fluid extraction with ethanol as modifier (Speed SE, Applied Separations) except for brain tissue, which was transesterified directly because of very small amounts. Fatty acid profiles of the diets, milk, liver, brain and adipose tissue were determined by gas chromatography (GC-17A Shimadzu, Kyoto, Japan) of the fatty acid methyl esters (FAME). The FAME was prepared by transesterification by sodium hydroxide and boron triflouride (both in methanol) according to a method modified after Morrison and Smith (1964).

Data from fatty acid composition in milk, brain, liver and adipose tissue was analysed in procedure MIXED in SAS (Littell et al, 1996) after the following general model:

$$Y_i = \mu_i + \eta(female_i) + \kappa_i$$

Where Y_i was the measured response variable fatty acid composition in milk, brain, liver and adipose tissue; at the i'th observation, μ_i was the general mean describes as:

$\mu_i = \beta(group)$

and $\eta(female_i)$ is the random effect of female and κ_i is the residual error.

Results and Discussion

The analyses of fatty acids in the diets showed that the ratios between the n-6 and n-3 fatty acids in the diets were 2.3:1; 1.5:1 and 0.2:1 in the H, M and L groups, respectively, which differs considerably from the planned ratios (Table 2). Thereby the difference in ratios between the H and M diets was rather small.

The dietary fat content is analysed by two different methods. The super critical fluid extraction gives a higher fat content (Table 2), than the traditional soxhlet extraction (Table 1). The explanation to this may be that super critical fluid extraction (with ethanol as modifier) extracts a higher amount of amphiphilic lipids than the traditional method.

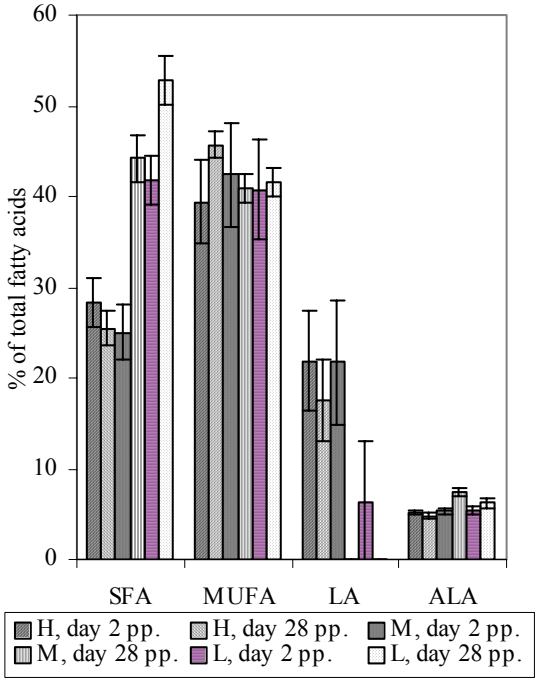
The fatty acid composition of the milk from the L group was characterized by a higher content of SFA, total n-3 PUFA and a lower content of n-6 PUFA compared to the H group (Figure 1). Similar findings from studies with mink were reported by Wamberg et al. (1992) and also in other species dietary fatty acid composition is reflected in the fatty acid pattern of the milk (Yonekubo et al., 1993).

Table 2 Results from analysis of fat content by Super critical fluid extraction (% of DM), fatty acid composition and n-6 and n-3 ratios of the maternal diets (% of total fatty acids). Values are LS-means ± SE (n=3). SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

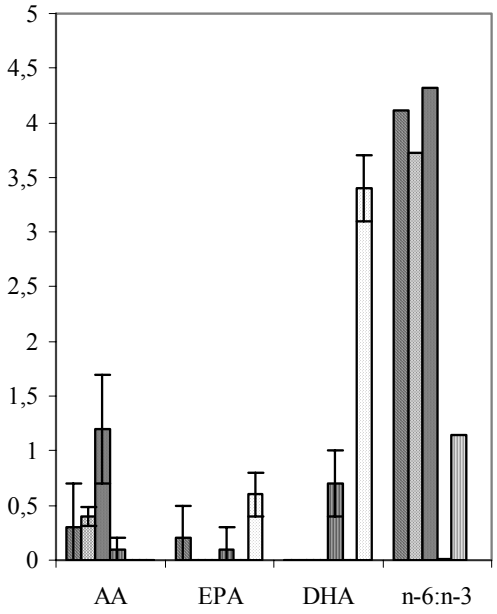
	Dietary treatment		
	High (H)	Medium (M)	Low (L)
Fat % of DM	20.0	19.4	27.9
Total SFA	15.3±0.9	22.3±0.9	34.3±0.9
Total MUFA	53.5±2.5	48.3±2.5	37.6±2.5
Total n-6 PUFA	15.6±3.7	12.9±3.7	3.1±3.7
Total n-3 PUFA	7.0±0.6	8.8±0.6	15.4±0.6
Other	8,6	7,7	9,6
n-6:n-3	2.3:1	1.5:1	0.2:1

Figure 1 A: SFA, MUFA, LA and ALA in mink milk from day 2 and 28 pp. in the H, M and L group (n=5)
1 B: AA, EPA, DHA and n-6:n-3 in mink milk from day 2 and 28 pp. in the H, M and L groups (n=5 per group)

1. A



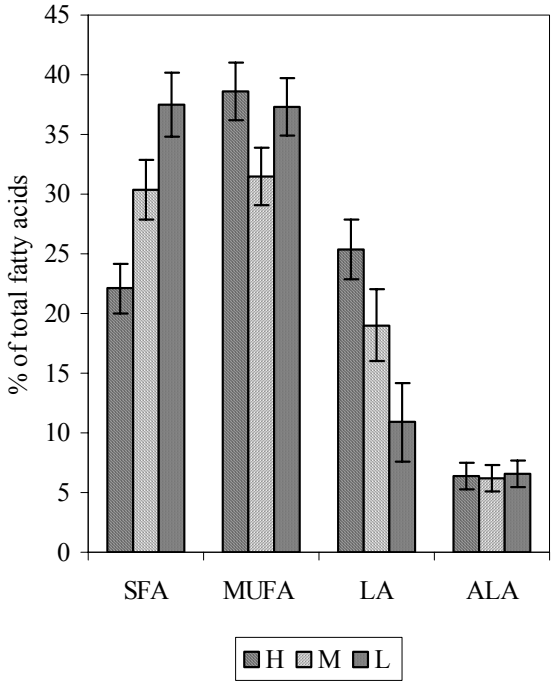
1. B



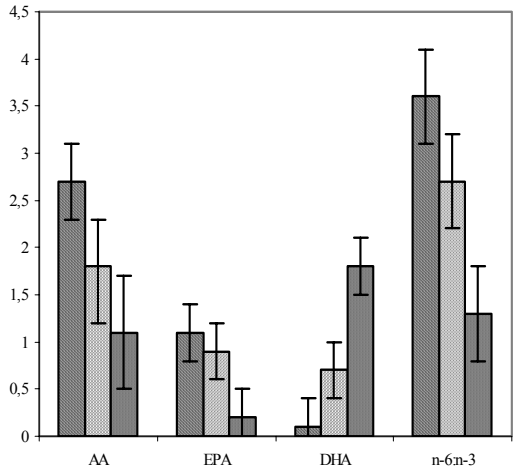
At 28 days of age, the liver fatty acid composition of the kits in the L group was characterized by higher levels of SFA and lower levels of total n-6 PUFA than the other two groups (Figure 2). There were lower levels of both AA and LA in the L group compared to the H and M groups, resembling the fatty acid patterns of the milk. Total n-3 PUFA was not affected by treatment, but the levels of DHA showed significantly higher values in the L group than in the other two groups.

Figure 2 A: SFA, MUFA, LA and ALA in total liver tissue from 28-day-old kits in the H, M and L groups. (n=5 per group).
2 B: AA, EPA, DHA and n-6:n-3 in total liver tissue from 28-day-old kits in the H, M and L groups. (n=5 per group).

2 A



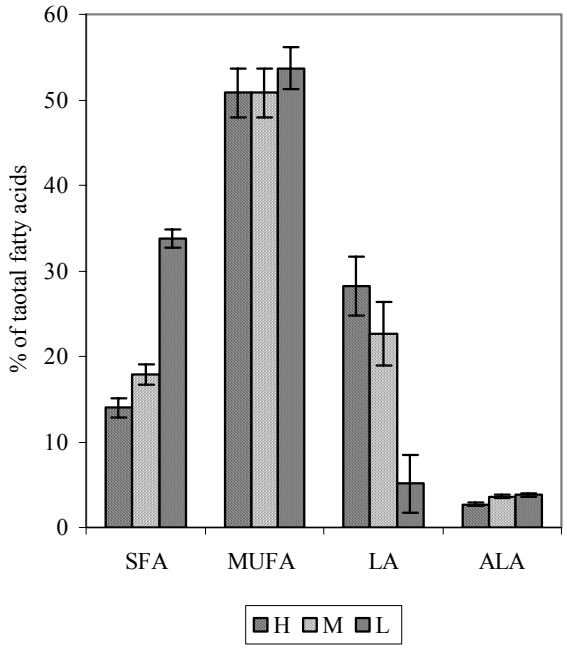
2 B



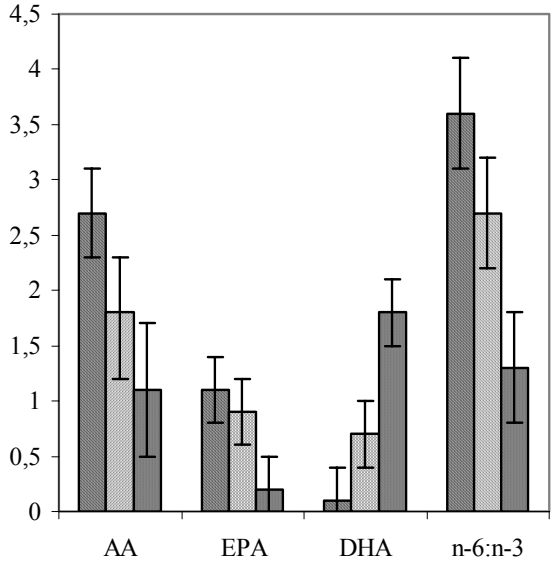
Similar to the milk and liver tissue, the amount of total SFA in the adipose tissue (inguinal region) increased as the ratio between the n-6 and n-3 fatty acids in the diets decreased (Figure 3). The total n-6 fatty acids in the adipose tissue decreased with decreasing dietary ratio, mainly due to a decrease in LA, which was the dominating n-6 PUFA in the adipose tissue. The levels of AA in the adipose tissue were also affected by treatment with higher levels in the H group compared to the M and L groups. The n-3 PUFA were significantly lower in the H group compared to the other groups. Bjergegaard et al. (2003) also reported that incorporation of fatty acids into the adipose tissues of mink kits was highly dependent on the fatty acid composition of the maternal diet.

Figure 3 A: SFA, MUFA, LA and ALA in adipose tissue (inguinal region) from 28-day old kits in the H, M and L groups.
3 B: AA, EPA, DHA and n-6:n-3 in adipose tissue (inguinal region) from 28-day old kits in the H, M and L groups.

3 A



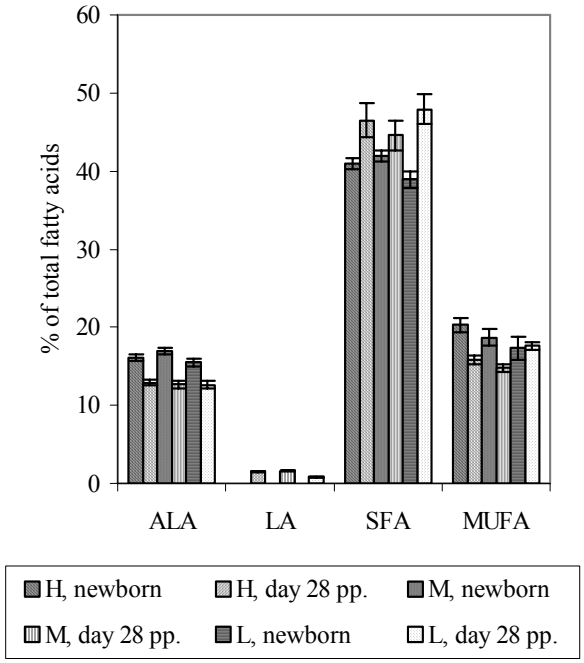
3 B



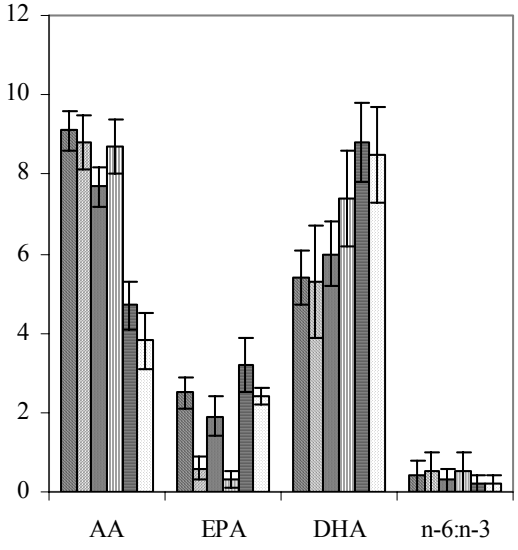
The contents of n-6 and n-3 fatty acids in the brain tissue from the newborn kits were also affected by maternal diet. In the L group the content of total n-6 fatty acids was lower than in the H group, caused by a lower content of AA, because LA was not detectable in the brain tissue from the newborn kits in any of the groups (Figure 4). In brain tissue from the 28 days old kits total n-6 PUFA were significantly lower in the L group compared to the two other groups (P<0.001), caused by a lower content of both LA and AA. The content of n-3 fatty acids was, on the contrary, not significantly higher in the L group compared to the H and M groups. Guesnet et al. (1997) found that the most dominating PUFA in the brain tissue in rats were AA and DHA and Hamosh (1997) reported higher levels of AA compared to DHA in the foetal ferret brain. Also in the present study higher levels of AA than of DHA were found in the brain from newborn kits except from the L group where the opposite was found. This indicates that the level of fish oil added in this group was too high to support normal deposition of AA in the brain during pregnancy. The high level of n-3 PUFA may have suppressed the deposition of AA into the tissues and this may have lead to impairment of the brain function. Similar findings were reported by Yonekubo et al. (1993), who found higher levels of DHA in the brain from rats suckling mothers fed a fish-oil diet compared to rats suckling mothers fed a diet without fish oil.

Figure 4 A: ALA, LA, SFA and MUFA in total brain tissue from newborn and 28-day old kits in the H, M and L groups.
4 B: AA, EPA, DHA and n-6:n-3 in total brain tissue from newborn and 28-day old kits in the H, M and L groups.

4 A



4 B



In conclusion, this study showed that the fatty acid composition of maternal diet affects fatty acid composition in milk and kit tissues. Further research is needed to find the importance of the fatty acid deposition in mink fetal tissues.

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III – 4 RP

Physico-chemical properties of different carbohydrate sources in the gut of mink

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Abstract
The carbohydrate sources (CHO) in mink feed may act as controllers of feed intake and satiety. The chemical composition and physico-chemical properties of CHO may change the properties not only of the feed but also the gastrointestinal environment. Studies in other monogastrics have shown that viscosity and water binding capacity (WBC) influence feed intake, rate of passage, enzymatic activity, rate and extent of digestion of nutrients. We studied the in vivo physico-chemical properties of 4 different diets with the CHO comprising either expanded wheat, expanded barley, rolled oats or gelatinized maize starch + sugar beet pulp. The adult male mink were offered 1.26 MJ (300 kcal) metabolisable energy (ME) once daily for 4 days followed by 1.05 MJ (250 kcal) ME for 6-8 days. The rate of feed intake was registered on day 2, 6, and 10. On day 12-14 the mink were euthanized three hours after feeding. The gastrointestinal tract was removed for allometric measurements of the gut, and the contents collected quantitatively for estimation of dry matter content, viscosity, and WBC. The diet containing sugar beet pulp increased WBC of the gut contents, but otherwise the diets showed no or only marginal differences in the gastrointestinal environment. In all groups, there were large variations in the amount and rate of feed consumption with no effects of diet or eating pattern between different days. Higher feed intakes on the day of euthanasia may have been obtained if the mink were fed a meal after overnight fasting although this would have reflected practical farming conditions to a lesser extent.

Introduction

The use of carbohydrate sources in mink feed in practise is often limited to processed cereals like wheat and barley although alternatives have been tested for many years, often with the conclusion that they can be used only in limited amounts (Glem-Hansen & Joergensen, 1978). However, due to environmental and health and welfare considerations renewed interest has been taken in the use of higher proportions of carbohydrate and use of alternative carbohydrate sources in different phases of mink production. It was recently shown that using barley hulls as an energy diluting compound of mink feed increased the time spent on eating and reduced the frequency of stereotypies in mink dams in the winter period without negative effects on production (Hansen et al., 2003). Similar observations have been made previously with pregnant sows fed a diet based on sugar beet pulp (Danielsen & Vestergaard, 2001).

In mink, on the other hand, dietary fibre (DF) is known to have a negative impact on the apparent digestibility of particularly nitrogen (Møller, 1985). Previous studies have also demonstrated a negative impact on mineral balance in mink fed increasing levels of DF from beech, sugar beet pulp and wheat bran due to increased faecal output (Hansen et al., 1985). Broiler chickens are particularly sensitive to variation in DF content and composition, which appears to be associated to a strong influence on the viscosity of the gastrointestinal contents (Svihus & Gullord, 2002). If similar effects are observed in mink gastrointestinal contents, variations in DF content and composition could have negative consequences during the growing period. The current study was performed in order to get preliminary information on the effects of various carbohydrate sources on the physico-chemical properties in the gut of mink.

Materials and methods

Diets

Initially 6 different carbohydrate sources were included in the study; expanded wheat (EW), expanded barley (EB), rolled oats (RO), sugar beet pulp (SBP), pure sugar beet pectin (PP), and pure cellulose (PC). The sources were chosen to cover variation among cereals, SBP as an alternative CHO sources, and PP and PC as pure fibre sources representing contrasting properties of SBP. In the cereal based diets, all CHO came from one cereal

source only. The diets containing high DF ingredients were made to match the dietary fibre (DF) content of a conventional mink feed with the carbohydrates coming from a 50:50 mixture of expanded wheat and barley by supplementation with gelatinised maize starch. Thus the total DF/soluble DF content of the diets were aimed at 4.8/0.9, 7.4/1.8, 4.1/1.8, 6.0/2.5, 6.0/5.8, and 6.0/0.0 % of dry matter (DM), respectively. However, due to low feed intake of the PP and PC diets in the first 4 days, these treatments were excluded in the remainder of the study.
Composition of the 4 experimental diets used in the study is shown in Table 1.

Table 1. Ingredients and energy content of experimental diets.

	EW	EB	RO	SBP
Ingredients before water addition				
Expanded wheat	18.1			
Expanded barley		18.1		
Rolled oats			19.30	
Dried sugar beet pulp				4.40
Maize starch				11.31
Fish meal	2.2	2.2	1.9	4.6
Soy bean oil	3.9	3.9	3.0	3.9
Other ingredients*	75.8	75.8	75.8	75.8
Metabolisable energy, MJ/kg DM§	19.5	18.7	20.9	19.9
Energy distribution	29:50:22	29:50:21	27:55:18	26:55:19

**All diets contained in percentage: Fish offals (<3% fat), 7.7; Industrial fish (5-8% fat), 18.9; Cooked poultry offal, 28.0; Fish silage, 15.0; Haemoglobin, 2.0; Maize gluten, 2.0, 3.9; Lard, 1.9, and Vitamin-mineral premix, 0.3.*
§ Based on chemical analyses and digestibility values presented in Table 4.

Animals and feeding
The animals were kept in cages designed for the measurement of feed intake and faecal output (Jørgensen & Glem-Hansen, 1973) for a total of 12-14 days. Eight adult males per treatment group were offered 1.26 MJ (300 kcal) ME once daily for 4 days followed by 1.05 MJ (250 kcal) ME for 6-8 days. The amount of ingested feed at 4, 8, 10, 12, 14, and 24 hours after the feeding was registered on day 2, 6, and 10. On day 6-10 faeces and feed residues were collected quantitatively and stored at –20ºC until further analysis in order to calculate digestibility of the diets. On day 12-14 the mink were euthanized by an overdose of pentobarbital-Sodium three hours after feeding. The GI tract was removed and separated by ligatures for quantitative collection of contents from the stomach, small intestine, and large intestine. The empty stomach, small intestine, and large intestine were weighed, and the length of the small and large intestine.

Analyses
Viscosity of extracts of the diluted carbohydrate sources (2 g + 8 ml 0.9% NaCl + 0.02% w/w NaN_3 solution) and undiluted diets was determined after incubation at 39ºC for 1 hour followed by centrifugation at 11,400 x g at 4ºC for 20 min. Extract viscosity of digesta was determined by centrifugation immediate after collection. The supernatant was withdrawn and the viscosity (mPa·s) was determined in a Brookfield DV-II cone/plate viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA; USA) maintained at 39ºC and a shear rate of 6-60 s^{-1}. Absolute viscosity values are presented at shear rate 45 s^{-1}.
Water binding capacity (WBC) of the undiluted and diluted feeds and carbohydrate sources (2 g + 8 ml 0.9% NaCl + 0.02% w/w NaN_3 solution) was measured as the amount of water withheld per gram of DM after incubation for 20 h at 39ºC and centrifugation at 4,000 x g at 4ºC for 20 min and drying of the sediment. WBC of digesta was determined immediately after collection by centrifugation at 11,400 x g for 20 min at 4 ºC, followed by freeze drying of the sediment.
Swelling capacity of the carbohydrate sources (200 mg) and the diluted diets (1 g) in 0.9% NaCl + 0.02% w/w NaN_3 solution was analysed as the volume (ml) occupied by sample in a 10 ml measuring cylinder after 1 hour at 39°C, and calculated per g of DM.
The feeds and faeces were freeze-dried prior to further analysis. Ash was determined according to AOAC (2000), protein was determined as N x 6.25 by the Dumas method (Hansen, 1989), and HCl- fat according to Stoldt (1952). Without prior extraction

of low-molecular weight sugars, starch was determined essentially as described by Knudsen & Hessov (1995), and DF, including total (T-NSP) and soluble non-starch polysaccharides (S-NSP), according to Knudsen (1997). Duplicate analyses were performed on all samples.

Calculations and statistical analyses

Crude carbohydrates (CHO) were calculated as DM – (crude protein + crude fat + ash). Digestibility of N, fat, and CHO was calculated as the percentage of consumed component not excreted in faeces.

For each separate day percentage of feed eaten was calculated with the feed source as a fixed effect, and time after feeding as fixed repeated measurements using PROC MIXED in SAS software. Response parameters obtained from the day of euthanasia were calculated with the feed source as a fixed effect, and day of euthanasia as a random effect using PROC MIXED with 'GROUP' option to account for any differences in variance between dietary treatments. Data calculated from the period of quantitative collection (digestibility) were analysed using the same model but without the random effect. Statistical evaluation of viscosity was performed on logarithmized values, and data presented are geometric means with the corresponding 95 % confidence limits.

Results and Discussion

Physico-chemical properties of carbohydrate sources, diets and gastrointestinal contents

The swelling capacity of the cereals, (wheat, barley, and oats) were similar (7.8-8.1 ml/g DM), whereas the swelling capacity of sugar beet pulp and maize starch were 11.3 and 20.3 ml/g DM, respectively. This was reflected in the diets (Table 2), although absolute differences between diets were smaller. Similarly, expanded wheat and barley had a WBC of 2.9 g water/g DM, whereas the WBC of rolled oats was only 1.7 water/g DM. Sugar beet pulp and maize starch, on the other hand, had higher WBCs (5.0 and 3.7 g/g, respectively). Also these differences were reflected in the diets (Table 2). The extract viscosities of the carbohydrate sources and the diets did not show the same pattern. The batch of expanded barley used in the present study had a very high extract viscosity (111 mPa·s), whereas the viscosity of expanded wheat and sugar beet pulp were very low (2.6 and 1.4 mPa·s, respectively), and autoclaved maize starch had a viscosity of 4.4 mPa·s. Rolled oats had a viscosity of 21.6 mPa·s, but in spite of this, diet RO had an extract viscosity equivalent to diet EW, whereas the viscosity of EB was only 2.5 times the viscosity of diet EW (Table 2).

Table 2. Chemical composition and physico-chemical properties of diets after water addition.

	EW	EB	RO	SBP
Water added (%)	18.7	21.0	13.2	32.1
DM (%)	39.6	37.1	43.1	33.4
Chemical composition, % of DM				
Crude CHO	32.6	32.7	27.3	29.9
Starch	24.1	21.5	23.8	21.3
DF	7.8	12.0	6.3	10.9
T-NSP	4.9	6.7	4.2	6.2
S-NSP	1.5	2.1	2.5	2.7
Klason lignin	2.8	5.3	2.0	4.6
Physico-chemical properties				
Viscosity undiluted, mPa·s	2.9	7.2	2.8	4.1
Swelling diluted, ml/g DM	4.7	5.2	4.9	7.5
WBC undiluted, g water/g DM	2.3	2.6	1.8	3.6
WBC diluted, g water/g DM	1.2	1.2	1.0	1.5

The differences in the physico-chemical properties of the carbohydrates sources and the corresponding diets were only partially reflected in the properties in the gut (Table 3). The WBC of the contents of the stomach and the small and large intestines was significantly higher with the SBP diet than the other diets, whereas the WBC in the stomach of the RO fed mink was numerically, but not significantly, lower. No differences in extract viscosity of the stomach contents were seen, and in the small intestine significantly lower viscosities were measured in mink fed the EB and SBP diets than mink fed the EW diet. These differences could not be explained by differences in the properties of the diet. Some dietary fibre sources may influence the osmotic pressure in the gut by binding water. Negative consequences of this may be increased excretion of Na and K ions (Hansen et al., 1985). Diet-related differences in DM content of stomach contents could have been induced either by increased water intake or differences in secretory

response. However, in the present study we did not observe any significant differences in the DM percentage (Table 3). Due to the limited amount of material collected, this value could not be estimated for the small intestinal contents, but we did not observe any differences in DM content of the faeces collected during 5 days either (Table 4). On the other hand, the generally lower digestibility of DM, organic matter, protein, and particularly ash with the EB and SBP diets indicates that the fibre sources of these diets increase endogenous secretion and reduce the digestibility of other dietary components as previously demonstrated in mink as well as other carnivores (Fekete et al., 2001). In other monogastric animals DF is fermented to varying extent, leading to increased faecal excretion of microbial protein (Eggum, 1992). Although very easily fermentable CHO sources like oligofructose have been shown to increase the density of anaerobic and decrease the number of aerobic bacteria (Williams et al, 1998), fermentation of DF is unlike to add significantly to excreted protein (Børsting et al. 1995) due to a very short rate of food passage, and a generally lower microbial activity bacteria in mink compared to other monogastric animals (Williams et al, 1998).

Feed intake

No differences were seen between the dietary treatments in the rate of feed intake, or eating patterns between the different days (data not shown). However, intra- and inter-individual variations were very big, and on each day 2-5 mink out of 8 per treatment group had ingested less than 95 % of the offered meal within 24 hours. On average, 28, 49, 59, 65, 69 and 83 % of the ration was eaten at 4, 8, 10, 12, 14, 24 h after feeding. Corresponding to this, the mink had consumed 18-32 % of a full ration 3 hours after feeding on the day they were euthanised. The variable feed intake (ranging from 0 to 59 % of the ration offered in the morning) also resulted in vary variable amounts collected from the GIT (2.5-78.7 g). Even from mink who ate nothing or less than 10 g of the diet offered, we observed digesta in the GIT (2.5-38.9 g). Presumable the mink had eaten feed from the previous day just prior to changing feed in the morning. Having fasted the mink overnight would probably have reduced the variability among individuals. However, this strategy would have made results of the study less relevant to practical farming conditions. The relatively low content of DF used in the present experiment might explain why we did not find differences in eating pattern as observed in previous studies with fibre as energy diluting ingredients (Hejlesen & Sandbøl, 2003).

Table 3. Characteristics of gastrointestinal contents.

	EW		EB		RO		SBP		P-value
WBC stomach, g water/g DM	1.57[a]	*0.12*	1.67[a]	*0.16*	1.40[a]	*0.20*	2.39[b]	*0.24*	0.048
WBC SI, g water/g DM	2.79[a]	*0.15*	2.76[a]	*0.28*	3.07[a]	*0.17*	3.72[b]	*0.23*	0.007
Viscosity stomach*, mPa·s	1.3	*(0.9-1.8)*	1.2	*(1.0-1.4)*	1.3	*(1.1-1.6)*	1.6	*(0.9-2.9)*	0.303
Viscosity SI*, mPa·s	4.4[a]	*(3.2-6.0)*	2.5[b]	*(1.8-3.4)*	3.2[ab]	*(2.0-5.1)*	2.6[b]	*(1.7-4.0)*	0.043
Total amount of digesta, g	34.0	*9.4*	47.4	*9.5*	45.9	*8.8*	25.0	*7.6*	0.146
Amount in stomach, g	23.0	*8.0*	35.1	*7.4*	33.7	*7.1*	13.9	*5.1*	0.067
Amount in SI, g	6.9	*1.8*	8.1	*1.8*	8.3	*1.7*	8.0	*2.1*	0.878
Amount in LI, g	4.0	*1.4*	4.2	*1.1*	4.0	*0.8*	3.2	*1.0*	0.861

Values in italics are standard error of means. Values in the same row with different superscripts are significantly different p<0.05. Values in brackets are 95 % confidence intervals obtained from logarithmised values. Values are geometric means. SI, small intestine. LI, large intestine.*

Table 4. Feed intake, faecal output and digestibility of diets.

	EW		EB		RO		SBP		P-value
Intake and output									
Feed intake, g	474[b]	*20*	525[ab]	*34*	432[b]	*32*	620[a]	*47*	0.028
Intake, %	81.3	*3.5*	86.0	*5.6*	79.2	*6.0*	89.0	*6.7*	0.643
Intake DM, g	187.9	*8.1*	194.5	*12.8*	186.1	*14.0*	206.8	*15.5*	0.714
Faeces excreted, g	134.2[bc]	*9.5*	178.3[a]	*12.0*	120.6[c]	*12.5*	162.2[ab]	*12.1*	0.017
Excreted DM, g	37.8[bc]	*2.0*	46.7[a]	*2.9*	33.5[c]	*2.7*	45.7[ab]	*3.3*	0.015
% DM, faeces	28.4	*0.5*	27.1	*2.5*	28.3	*0.9*	29.1	*3.1*	0.956
Digestibility, %									
DM	81.3[b]	*0.6*	77.8[d]	*0.3*	83.4[a]	*0.6*	79.5[c]	*0.3*	
OM	84.2[b]	*0.6*	81.1[d]	*0.3*	85.9[a]	*0.5*	82.8[c]	*0.3*	<0.001
Ash	35.7[a]	*1.9*	28.3[b]	*1.1*	38.9[a]	*2.4*	27.2[b]	*0.7*	0.001
N	83.2[a]	*0.6*	80.4[b]	*0.4*	82.9[a]	*0.6*	80.2[b]	*0.4*	<0.001
Fat	96.2[a]	*0.2*	95.4[b]	*0.1*	95.6[ab]	*0.5*	96.3[a]	*0.2*	0.004
Crude carbohydrates	75.9[b]	*0.9*	70.9[d]	*0.6*	79.4[a]	*0.6*	73.0[c]	*0.6*	<0.001
Starch	98.0[ab]	*0.9*	99.2[b]	*0.0*	99.3[a]	*0.0*	99.4[a]	*0.0*	<0.001

Values in italics are standard error of means. Values in the same row with different superscripts are significantly different p<0.05.

Conclusions

In the present study we observed only minor differences in the physico-chemical properties of the gut contents from mink fed different carbohydrate sources. In practical mink feeds, carbohydrate sources are diluted with other protein- and fat rich sources and water, so differences between cereal sources and different batches of cereal are presumably obscured. However, using higher amounts of fibre rich carbohydrate sources than the SBP diet of the present study is expected to induce differences in physico-chemical properties and consequently effects on eating pattern.

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III – 5 RP

Effect of lactic acid bacteria and β-glucanase treatments on the nutritive value of barley for growing blue fox

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Abstract
The effect of lactic acid bacteria or β-glucanase supplements on the chemical composition of mixtures of barley - water or barley –slaughter by-product-water was studied in a laboratory-scale experiment. The effects of cooking, lactic acid bacteria or β-glucanase supplementation on the digestibility of barley diets were evaluated using 20 growing male blue foxes. Barley fed to the foxes was either untreated (control), cooked, lactic acid fermented or supplemented with ß-glucanase enzyme. In the laboratory experiment, the content of total ß-glucans decreased in all barley-water and barley-water-slaughter by-product mixtures during 48 hours. The degradation of total ß-glucans was greatest for the barley-water mixture supplemented with ß-glucanase. In the digestibility experiment, total tract digestibility of carbohydrates and starch were higher for the cooked barley diet (carbohydrates: 63.7% and starch: 72.4%) than for the untreated (carbohydrates: 33.1% and starch: 45.7%), fermented (carbohydrates: 33.7% and starch: 45.0%) or ß-glucanase supplemented barley diets (carbohydrates: 34.0% and starch 43.8%) ($p<0.05$). No differences in the digestibility of carbohydrates or starch were observed between the untreated, fermented or enzyme supplemented barley diets.

Introduction
Barley is the main feed crop in Finland. Unfortunately it has some anti-nutritional properties containing substantial amounts of soluble fibre, mixed-liked β-glucans, which are indigestible for non-ruminants. β-glucans may cause viscous digesta, reduced diet digestibility and cause sticky droppings especially for poultry. Anti-nutritional effects of barley can be reduced by β-glucanase enzyme or treatments such as lactic acid fermentation (Skrede et al. 2001, 2003). Feed enzymes have improved digestibility of diets containing cereals both in mink and dogs (Børsting et al. 1995, Twomey et al. 2003). In blue fox nutrition there is limited experience with β-glucanase enzyme supplementation.
In previous investigations with growing blue foxes, digestibility of carbohydrates in raw barley has been 5-10% lower than that in cooked or heat-treated barley (Kiiskinen et. al. 1988). However, in blue fox growth experiments, high amounts of raw barley have been used successfully (Valaja et al. 2003).
The purpose of this investigation was to evaluate the effects of different chemical and physical treatments on the nutritive value of barley for the growing blue fox. In a laboratory study, the effects of lactic acid fermentation or ß-glucanase supplementation on the chemical composition of barley were studied. In a digestibility study, the effects of cooking, lactic acid fermentation or ß-glucanase supplementation on the digestibility of barley diets by growing blue foxes were studied.

Material and Methods
Laboratory experiment
The effect of lactic acid bacteria or β-glucanase supplementation on the chemical composition of barley-water (1:1.33) and barley-slaughter by-product-water (1:1.66:1.33) mixtures was studied in a laboratory-scale experiment. Barley was ground before mixing using a 2.0 mm sieve. The slaughter by-product was formic acid preserved. The barley-water and barley-slaughter by-product-water mixtures were made as such or supplemented with lactic acid bacteria (Valio AIV Biostart, Valio Ltd; 2 x 10^6 cfu/g mixture) or β-glucanase enzyme (Avizyme 1110, Danisco Nutrition Ltd; 0.45 ml/kg mixture). The mixtures were stored in a 0.5 l plastic bucket with a lid for 24 or 48 hours at room temperature. Three replicates per treatment and time point were used in the study. The mixtures were analysed for β-glucans.

Digestibility experiment
A digestibility experiment was conducted with 20 growing male blue foxes (*Alopex lagopus*) (3.5-4.5 month of age). The animals were housed

individually in digestibility cages throughout the trial. They were allotted according to their initial weight to five blocks of four animals each. The experimental design was incomplete latin-square with four diets and three periods. Each 10-day period contained 7 days of adjustment and 3 days of total collection of faeces. Weighing of the animals was performed at the beginning and the termination of the experiment, as well as at the start and end of each collection period.

The four experimental diets contained either untreated barley (control), or cooked, lactic acid fermented or ß-glucanase enzyme supplemented barley as a source of cereal. The cooked barley was mixed with water (30% barley and 70% water) and cooked for 20 min. Before fermentation the barley was mixed with water (46% barley and 64% water). Lactic acid bacteria product (Valio AIV Biostart,) was mixed with water (1.677 g/2.5 l) and added to the barley-water mixture (555 ml/kg). Fermentation lasted for 24 hours at room temperature. ß-glucanase enzyme (Avizyme 1110) was added to the mixed feed (0.45 ml/kg feed) and the feed was stored overnight in a refrigerator. The diets consisted of the experimental barley (about 50% of diet dry matter), preserved slaughter by-products, fish meal, rape seed oil, methionine and vitamins and minerals (Table 1). The experimental feeds were made all at once and stored frozen pending consumption. Animals were fed once daily at 0900 according to diet dry matter content. Average daily allowance was increased from 772 to 859 g/day during the experiment.

The feeds and faecal samples were analysed for proximal composition, starch, neutral detergent fibre (NDF) and gross energy. Digestibility coefficients were calculated by total collection of faeces. Experimental data were subjected to analysis of variance using GLM procedure of SAS using the following statistical model: $y_{ijkl} = \mu + b_i + b(a)_{ij} + p_k + d_l + (b \times d)_{il} + e_{ijkl}$, where y_{ijkl} is the dependent variable, μ the overall mean, b_i the square effect, $b(a)_{ij}$ the effect of the animal within the square, p_k the effect of period, d_l the effect of the diet, and e_{ijkl} normally distributed random variable.

Results and Discussion

The laboratory experiment revealed that content of total ß-glucans in the barley decreased linearly during the 48-hour incubation (Figure 1) As expected the degradation of the total ß-glucans was the greatest in the barley-water mixture supplemented with the ß-glucanase enzyme, where the amount of the total ß-glucans after 48 hours

Table 1. Dietary ingredients (%) and chemical composition of the diets (g/kg DM).

Treatment	Control dried barley	Cooked barley	Lactic acid fermented barley	ß-glucanase supplemented barley
Ingredient, %				
Acid-treated slaughter by-product	32.00	32.00	32.00	32.00
Barley	21.36	64.48	45.40	21.36
Fish meal	1.00	1.00	1.00	1.00
Vitamins and minerals	0.43	0.43	0.43	0.43
Rape seed oil	2.00	2.00	2.00	2.00
Methionine	0.10	0.10	0.10	0.10
Water	43.12	0.00	19.08	43.12
Composition				
ME, MJ/kg DM	13.95	13.95	13.95	13.95
Dry matter, g/kg	392.6	306.7	380.9	398.2
Crude protein	232.2	240.1	241.8	234.9
Ether extract	177.4	179.4	184.6	175.8
Crude carbohydrates	530.6	517.4	508.5	527.9
Starch	348.0	123.4	332.5	328.6
Total ß-glucans	18.9	21.4	12.7	16.1
Lactic acid	1.73	1.83	11.58	1.61

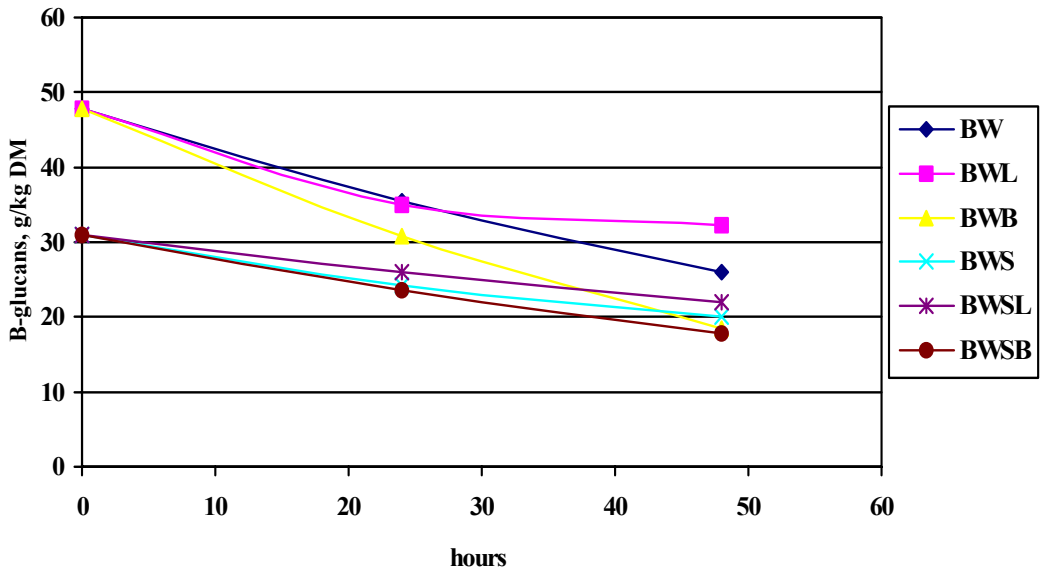
decreased 55% from that in the beginning. The average decrease in the other treatments was between 30-45% after 48 hours. This may be explained by activation of intrinsic ß-glucanase enzymes and lactic acid bacteria by the moisture content to break down soluble fibre components also in barley-water and barley-water-slaugher house by-product mixtures. In earlier studies, fermentation of barley and wheat has been shown to reduce the content of all ß-glucan fractions, whereas lactic acid bacteria preferred the degradation of soluble fraction (Skrede et al. 2001, 2003).

Contrary to what was expected based on the laboratory experiment, the content of the total ß-glucans in barley diets decreased only slightly with the ß-glucanase supplementation (Table 1). Lactic acid fermentation produced lactic acid very efficiently in the diet with fermented barley. Cooking of the barley seemed to degrade starch to sugar components, which resulted in a much lower content of starch in the diet with cooked barley.

All the foxes ate their diets willingly and no refusals were observed. Large differences between the barley treatments were found in the digestibility of carbohydrates (Table 2). The digestibility of starch and crude carbohydrates were clearly the highest in the cooked barley diet ($p<0.05$). Difference in the digestibility of starch varied from 26.7 to 28.8 %-units between the cooked barley and the other treatments. Kiiskinen et al. (1988) also obtained similar differences in the digestibility between cooked and untreated barley diets. There may be several reasons for the low carbohydrate digestibility in the untreated barley diets. Rapid transit time of feed though intestine and insufficient amylase activity may limit digestion of starch. Low starch digestibility in raw barley may also be due to the inability to amylase to penetrate to the starch granules when they are not disrupted.

The digestibility of carbohydrates in the untreated, fermented and ß-glucanase supplemented barley diets were similar. In contrast to our results, Skrede et al. (2001) observed that fermentation of barley clearly improved the digestibility of starch and total carbohydrates in mink compared to untreated barley diets. There are no results so far on the effects of fibre degrading enzymes on the digestibility of barley in blue fox. However, in mink, enzyme treatment has been shown to increase carbohydrate digestibility in whole wheat over that in raw or even boiled wheat (Børsting et al. 1995). In dogs, an enzyme product containing β-glucanase improved digestibility of energy and ether extract of diets containing 51% of barley (Twomey et al.

Figure 1. Effect of different physical and chemical treatments on content of total ß-glucans in barley



(Treatments: BW: Barley+water, BWL: Barley+water+lactic acid bacteria, BWB: Barley+water+ß-glucanase enzyme, BWS: Barley+water+slaughter by-product, BWSL: Barley+water+slaughter by-product+lactic acid bacteria, BWSB: Barley+water+slaughter by-product+ß-glucanase enzyme).

2003). In our experiment, the conditions for the β-glucanase enzyme were not optimal, since the feeds were frozen. According to Børsting et al. (1995) exogenous enzymes are likely inactive in frozen or cold conditions.

The metabolizable energy (ME) content of the barley diets calculated based on the digestibility results revealed great differences between the cooked barley and the other treatments (Table 2). These differences were likely caused by incomplete carbohydrate digestibility. Practical growth experiments have shown that blue foxes fed diets with high content of raw barley grew as well as those fed diets with low content of barley (Valaja et al. 2003). In our current experiment, the daily gain of the foxes fed the cooked barley was clearly higher than that of foxes fed the other diets ($p<0.05$). In practice, the foxes may increase their feed intake when fed diets with a low dietary ME-value.

In conclusion, cooking clearly improved digestibility of carbohydrates in barley whereas lactic acid fermentation or β-glucanase supplementation had no effect on carbohydrate digestibility.

Table 2. Effect of different treatments on the digestibility of barley diets (%) (LS-means presented).

Treatment	Control, dried barley	Cooked barley	Lactic acid fermented barley	ß-glucanase supplemented barley	SEM
Organic matter	52.4[b]	70.9[a]	55.2[b]	53.7[b]	0.74
Crude protein	69.1[b]	73.8[a]	73.9[a]	71.0[b]	0.69
Ether extract	88.2	88.1	90.0	89.8	0.59
Crude carbohydrates	33.1[b]	63.7[a]	33.7[b]	34.0[b]	1.12
Starch	45.7[b]	72.4[a]	45.0[b]	43.8[b]	1.20
NDF	20.0[a]	22.7[a]	13.2[b]	21.8[a]	1.57
ME, MJ/kg DM	12.1	15.1	12.8	12.4	
Daily weight gain, g	36.2[b]	69.7[a]	38.7[b]	45.7[b]	3.66

[a,b,c] Means within the same row with the same superscript do not differ significantly ($p>0.05$). SEM=standard error of the means.NDF=neutral detergent fibre.

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III – 6 RP

Ideal Protein for Mink (Mustela vison) in the Growing and Furring Periods.

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Abstract
The investigation aimed at establishing the optimal protein requirement for mink in the growing and the furring periods. Based on the present amino acid norm for mink and cat and the amino acid composition of whole mink, an ideal protein was constructed.
Two trials of each 5 groups of 120 males were carried out. Each male was housed with a female. A basal feed containing 32:55:13 % of the metabolizable energy (ME) from protein, fat and carbohydrate, was composed. Animals were weighed monthly and dead animals authopsied. At pelting livers were sampled and the pelts were graded.
In trial 1 the diets contained 32, 28, 24, 20 and 16 % of ME from protein. The lower levels of protein were achieved by substituting the protein fraction with pregelatinized maize starch and fat. The diets had identical amino acid profiles and almost identical energy contents. Based on earlier results from the furring season, Methionine Hydroxy Analog (MHA) was used instead of dl-methionine. The diets were fed from July to pelting. The longest skins ($p<0.0001$) were found in the groups with 24, 28 and 32 % of ME from protein. And the best pelt quality in the groups with 28 and 32 % of ME from protein.
In trial 2 all groups received a diet containing 22 % of ME from protein from July to September. From September to pelting the diets contained 30, 26, 22, 18 and 14 % of ME from protein. There was no difference in skin length and only the group with 14 % of ME from protein had significantly lower pelt quality ($p<0.0006$) as compared to the other groups.
It is concluded, that with the used amino acid profile (including MHA) the optimal skin length is achieved already at 24 % of ME from protein and the optimal pelt quality from 28 % of ME from protein during the whole period. For the furring period, it seems that the requirement may be as low as 18% of ME from protein. In a parallel trial MHA showed inferior results in the growing period and we can not exclude that the effects found, are mere methionine responses.

Introduction
Animals utilize protein for such different purposes, as maintenance, growth (including feathers and fur), reproduction and milk production. The ideal amino acid profiles of the protein used for these different purposes, are distinctly different (i.e. Fuller et al., 1989 and Rodehutscord et al., 1997). Consequently, the ideal amino acid profile changes during an animals growth period, as the maintenance requirement increases and that for growth decreases. For fur bearing animals this is further influenced by the onset of winter fur growth.
It has been known for many years, that the amino acid profile of milk or whole body, is a good basis for the determination of the ideal amino acid profile, i.e. Kim & Lall (2000) and Rollin et al., (2003).
A comparison, of the amino acid profile, of the present norm for mink (N) (Børsting & Clausen, 1996 and Børsting, 1998) in the growing-furring period, with that of mink body and pelt (M) (Glem-Hansen & Hansen, 1981 and Chavez, 1980) reveals a

Table 1. Amino acid profile (relative to lysine) of mink norm (N), mink body+pelt (M), catnorm (C) and "Ideal Protein" (IP)

	N [1)]	M [2)]	C [3)]	IP
Arg	115	119	125	115
Cys	22	67	44	39
Met	59	32	50	47
M+C	81	99	94	86
His	56	38	38	40
Ile	96	54	63	65
Leu	185	122	150	150
Val	130	78	75	80
Lys	100	100	100	100
Phe	107	62	50	65
Tyr	67	56	56	65
Thr	70	74	88	70
Trp	22	22	19	22

[1)] Børsting & Clausen, (1996) and Børsting, (1998); [2)] modified from Glem-Hansen & Hansen, (1981) and Chavez, (1980); [3)] NRC, (1986).

considerable difference for certain amino acids, especially for those where only a so called maximum norm has been established.
Mink are often compared with cats and the amino acid profile of the cat norm (C) (NRC, 1996) is relatively close to either that of the mink norm or that of the mink body plus fur.
An ideal protein (IP) was constructed, based on the present mink norm, the amino acid profile of mink body plus fur and the present cat norm (Table 1).
Two trials were carried out to establish the optimal protein requirement of this ideal protein in respectively the growing and the furring periods.
Generally the trials, which formed the basis of the present amino acid norms for mink, suggested that a level of protein lower than 25% of ME was not supporting the need of the mink in the growing period. For most of these low-protein-diets it was characteristic, that they had a sub-content of one or more amino acids compared to the norms later specified. Rasmussen & Børsting (2000) proved that a low content of protein (21% of ME from protein) influenced the pelt quality negatively, when this was given as feed from the age of 22 weeks, and furthermore there was a tendency of declining growth the sooner it was given in the growing period. By way of comparison a feed with 34% of ME from protein was used. This had an excess of 10% of the sulphur-containing amino acids and from 20 to 90% of the rest of the essential amino acids compared to the norms at present. The diet with 21% of ME from protein was only containing 70% of the sulphur-containing amino acids and from 75 to 106% of the rest of the essential amino acids compared to the present norms.
Apart from the sulphur-containing amino acids, the present norms are the same for both the growing and the furring periods.
Estimating the need of protein on the present norm for essential amino acids plus a corresponding amount of protein from non-essential amino acids, you will find, that the protein need of the mink ought to be in the order of 20% of ME from protein. The corresponding need for cats is estimated to be in the order of 16% of ME from protein (Andersen et al., 1980) considering dry feed with 19.7 MJ ME/kg. Smalley et al. (1985) concluded that the need was higher in terms of protein/kg feed. They used a feed with 21.0 MJ ME/kg and correcting for this and the digestibility of the feed, that has been used, their results will end up to be between 13.0 and 18.6 and their recommendations between 13.8 and 17.0% of ME from protein with 19.7 MJ ME/kg Corresponding to this, Burger et al. (1984) found that the need of maintenance for cats was covered at 12% of ME from protein.
Based on the norms at present for mink during the growing period, the composition of the mink body and the present amino acids profile for cats, trials are carried out in order to shed light on the optimum protein-level in the growing and furring periods respectively.

Materials and Methods

A trial with 120 Scanbrown males in each group was carried out. Each male was housed with a female kit in a two row open house with 6 cages per section and free access to drinking water and nesting box. The animals were pelted from the 10th to the 12th of November.

Feed

The feed was compounded with a so-called ideal protein based on the present mink norm and the amino acid profile of mink body plus fur and the present cat norm.
The decline of the protein was achieved with a proportional decline of all protein-sources and they were regulated with maize starch. The decline of specific protein-sources which led to removal of fat, was adjusted by adding fat with a corresponding fatty acid profile. This should eliminate a possible influence from the composition of the fat. The feeds were analysed at the analytical laboratory of Danish Fur Animal Feed according to official EU methods and the ME content calculated using the following values: 18.8 kJ/g of digestible Crude Protein, 39.8 kJ/ g of digestible Crude Fat and 17.6 kJ/ g of digestibleCarbohydrate (Calculated by difference).

Table 2. From the middle of September until furring we use levels from 30 to 14% from protein and from 15 to 31% from carbohydrate respectively.

<table>
<tr><td colspan="2">Energy Distribution</td></tr>
<tr><td>9th July
- 15th of September</td><td>15th of September
- Pelting</td></tr>
<tr><td colspan="2">32:55:13</td></tr>
<tr><td colspan="2">28:55:17</td></tr>
<tr><td colspan="2">24:55:21</td></tr>
<tr><td colspan="2">20:55:25</td></tr>
<tr><td colspan="2">16:55:29</td></tr>
<tr><td rowspan="5">22:55:23</td><td>30:55:15</td></tr>
<tr><td>26:55:19</td></tr>
<tr><td>22:55:23</td></tr>
<tr><td>18:55:27</td></tr>
<tr><td>14:55:31</td></tr>
</table>

During the period from early July until the middle of September we used feed, where the ME from protein made up between 32 and 16% with a decline of 4 % units for each group. ME from carbohydrate correspondingly increased from 13 to 29% and ME from fat was kept constant on 55%.
From the middle of September until pelting we used levels from 30 to 14% from protein and from 15 to 31% from carbohydrate respectively. The trial setup is shown in table 2, the feed composition in tables 3 & 4 and the calculated amino acid content in table 5. At 20% of ME from protein the norm for essential amino acids was fulfilled compared to the "Ideal Protein", but compared to the present norms, some of the amino acids were not fulfilled until 24 or 28% of ME from protein. Essential amino acid : total amino acid ratio was 0.54.

Statistics

Statistical analyses of data were carried out by means of the SAS data recording and processing system (SAS, 1988). Differences in body weight gain and skin length were compared using a one-way analysis of variance (GLM procedure). Pelt quality, pelt colour and clarity were tested using a non-parametric test (GENMOD). Skin length was used as a covariate when testing pelt quality. Silkiness and wool quality were tested using the Chi-Square test.

Table 3. Composition for the growing-furring-period-trial with protein requirement for mink kits, in % of diet.

ME from protein, %	32	28	24	20	16
Fish Offal	5.7	5.1	4.5	3.8	3.1
Poultry Offal	27.2	24.2	21.2	18.1	14.8
Slaughter House Offal	35	31.2	27.3	23.3	19.1
Popped Barley	4.0	3.5	3.1	2.6	2.2
Popped Wheat	4.0	3.5	3.1	2.6	2.2
Maize Starch		3.4	7.0	10.7	14.7
Feather Meal	1.8	1.6	1.4	1.2	1.0
Blood Meal	0.5	0.4	0.4	0.3	0.3
Peas	6.0	5.3	4.7	4.0	3.3
Potato Protein	3.0	2.7	2.3	2.0	1.6
Maize Gluten	3.9	3.48	3.05	2.60	2.1
Protao	0.9	0.76	0.66	0.57	0.5
Soya Bean Oil	5.0	5.63	6.36	7.12	7.9
Lard	2.5	2.8	3.2	3.6	4.0
MHA, Methionine Value *	0.54	0.48	0.42	0.36	0.29
Tryptophan	0.062	0.055	0.048	0.041	0.034
Lysine	0.039	0.035	0.030	0.026	0.021
Threonine	0.081	0.072	0.063	0.054	0.044
Vitamins/Minerals	0.25	0.25	0.25	0.25	0.25
Water		5.5	11.0	16.8	22.8
Analytical Composition					
Energy:					
MJ / kg	9.16	9.42	9.84	10.64	11.21
MJ / kg DM	19.9	20.2	20.6	20.9	21.4
Dry Matter, %	46	46.6	47.8	50.9	52.4
Energy Distribution	32:54:14	29:53:18	25:53:22	20:52:28	18:52:30
Ash, %	1.7	1.7	1.5	1.4	1.2

The feed with 32% ME from protein was used as basic blend. From this one the other blends are mixed by adding fat and maize starch.

***NB!** We used Methionine-Hydroxy-Analog instead of methionine, to avoid a toxic effect when adding too much methionine, % addition: 0.304 – 0.271 – 0.237 – 0.202 – 0.165 – 0.21.*

Table 4. Composition for the furring-period-trial with protein requirement for mink kits, in % of diet.

ME from protein, %	30	26	22	18	14
Fish Offal	5.4	4.8	4.1	3.5	2.8
Poultry Offal	25.7	22.8	19.7	16.5	13.12
Slaughter House Offal	33.0	29.3	25.3	21.2	16.9
Popped Barley	3.7	3.3	2.9	2.4	1.9
Popped Wheat	3.7	3.3	2.9	2.4	1.9
Maize Starch	1.7	5.2	8.9	12.7	16.7
Feather Meal	1.7	1.5	1.3	1.1	0.8
Blood Meal	0.4	0.4	0.3	0.3	0.2
Peas	5.7	5.0	4.3	3.6	2.9
Potato Protein	2.8	2.5	2.2	1.8	1.45
Maize Gluten	3.7	3.3	2.8	2.4	1.9
Protao	0.8	0.7	0.6	0.5	0.4
Soya Bean Oil	5.3	6.0	6.7	7.5	8.3
Lard	2.6	3.0	3.4	3.8	4.2
MHA, Methionine Value *	0.51	0.45	0.39	0.33	0.26
Tryptophan	0.058	0.052	0.045	0.038	0.030
Lysine	0.037	0.033	0.028	0.024	0.019
Threonine	0.076	0.068	0.059	0.049	0.039
Vitamins/Minerals	0.25	0.25	0.25	0.25	0.25
Water	2.8	8.2	13.9	19.8	26.0
Analytical Composition					
Energy:					
MJ / kg	9.10	9.49	10.13	10.36	10.64
MJ / kg DM	19.6	20.4	21.0	21.3	21.7
Dry Matter, %	46.5	46.5	48.3	48.7	49.2
Energy Distribution	29:52:19	26:53:21	22:54:24	19:53:28	16:53:31
Ash, %	1.7	1.5	1.4	1.3	1.4

The feed with 32% ME from protein was used as basic blend. From this one the other blends are mixed by adding fat and maize starch.

***NB!** We used Methionine-Hydroxy-Analog instead of methionine, to avoid a toxic effect when adding too much methionine, % addition: 0.304 – 0.271 – 0.237 – 0.202 – 0.165 – 0.21.*

Table 5. Estimated content of amino acids in gram/MJ during the growing period, compared to an Ideal Protein (IP) and the present norm.

						22						
ME from protein, %	32	28	24	20	16	→ 30	→ 26	→ 22	→ 18	→ 14	IP	Present Norm
Met incl, MHA*	1.09	0.92	0.80	0.67	0.54	1.00	0.88	0.75	0.59	0.46	0.67	0.67
Met	0.59	0.52	0.44	0.37	0.30	0.54	0.49	0.41	0.33	0.26	0.67	0.67
Cys	0.40	0.35	0.30	0.25	0.20	0.38	0.33	0.28	0.23	0.18	0.25	0.25
Lys	1.80	1.59	1.34	1.13	0.92	1.72	1.46	1.26	1.00	0.80	1.13	1.13
Thr	1.26	1.13	0.96	0.80	0.63	1.17	1.05	0.88	0.71	0.54	0.80	0.80
Trp	0.40	0.35	0.30	0.25	0.20	0.37	0.32	0.27	0.23	0.18	0.25	0.25
His	0.75	0.63	0.54	0.46	0.37	0.67	0.59	0.50	0.41	0.33	0.46	0.63
Phe	1.55	1.38	1.17	0.96	0.80	1.46	1.26	1.09	0.88	0.67	0.92	1.21
Tyr	1.21	1.05	0.92	0.75	0.59	1.13	0.96	0.84	0.67	0.54	0.75	0.75
Leu	2.93	2.55	2.22	1.84	1.46	2.76	2.39	2.01	1.63	1.30	1.67	2.09
Ile	1.34	1.17	1.00	0.84	0.67	1.26	1.09	0.92	0.75	0.59	0.71	1.09
Val	1.80	1.55	1.34	1.13	0.88	1.67	1.46	1.21	1.00	0.80	0.92	1.46
Arg	2.09	1.80	1.55	1.30	1.05	1.97	1.67	1.42	1.17	0.92	1.30	1.30
Gly	2.01	1.76	1.51	1.26	1.00	1.88	1.63	1.38	1.13	0.88		
Ala	1.93	1.67	1.42	1.21	0.96	1.80	1.55	1.30	1.09	0.84		
Ser	1.59	1.38	1.17	1.00	0.80	1.46	1.30	1.09	0.88	0.71		
Asp	2.43	2.09	1.80	1.51	1.21	2.26	1.97	1.67	1.34	1.05		
Glu	4.35	3.81	3.26	2.72	2.18	4.06	3.52	2.97	2.43	1.88		
Pro	2.01	1.76	1.51	1.26	1.00	1.88	1.63	1.38	1.13	0.88		

** Estimated content of Met after addition of Methionine-Hydroxy-Analog.*

Results

Due to the way of diluting the protein content of the diets, amino acids were only analyzed in three of the diets. The calculated contents of digestible amino acids/MJ based on these results, showed that the results are within the acceptable deviations due to analytical error. The results are not shown.

The was no statistical significant difference in the initial weights between any of the groups. The calculated weight gains are shown in table 6.

Discussion

In the first series, where the ME from protein ranged from 32 to 18% during the whole period from early July until pelting, we noticed a better growth and longer skins when the ME from protein was in the area of 25-32%. A smaller content of protein caused less growth and shorter skins (Tables 6 & 7). The quality of the skin culminated at 29-32% of ME from protein, and there was most silky and good pelts at 25-32% of ME from protein (Tables 7 & 8). The norm of essential amino acids compared to the "ideal protein" was fulfilled at 20% of ME from protein (Table 5). Compared to the present norm this was not the case for all amino acids before we reached 25 or 29% of ME from protein (His - Ile - Leu .- Val - Phe - Tyr). The reason for these low results of production at 20% of ME from protein, might be the fact that either the proposed IP is too low for some of the amino acids or the total content of protein was too low. Anyhow at 18% of ME from protein the content of amino acids was below the present norm of the first 6 essential amino acids (Arg - Cys - Met - Lys - Thr - Trp).

When ME from protein was 18-20%, there was an increased number of dead kits authopsied with fatty liver (Table 9). However, at the time of pelting there was no difference in neither the Hepato-Somatic-Index, nor the fat content of the livers in the different groups (Table 10).

In the second test series with 22% of ME from protein in all groups until the 15th of September and then variations from 16-29% until pelting, there was no difference in their growth during the whole period, but every single group had less growth from early July till 13th of August compared to the corresponding groups in the first test series (Table 6). We have not been able to find a reason why, but there must be a systematical difference between the 2 series. The length of the furs in the second trial series were not different and only the group, that had

Table 6. Weight gains for male kits, fed with different levels of ME from protein respectively during the growing-furring period or the furring period

ME from Protein, %	Weight gain, g														
	9th July - 13th Aug.			13th Aug. - 4th Sept.			4th Sept. - 25th Sept.			25th Sept. - Pelting			9th July - Pelting		
32	820 (107)	BC	BC	407 (99)	AB	A	436 (118)	A	A	254 (138)	AB		1920 (299)	A	A
29	858 (135)	A	A	416 (136)	A	A	428 (117)	AB	A	222 (180)	AB		1927 (340)	A	A
25	853 (146)	AB	AB	369 (118)	C	B	409 (134)	ABC	A	266 (178)	A		1913 (360)	A	A
20	805 (158)	CD	C	358 (121)	C	B	435 (131)	A	A	209 (179)	BC		1799 (361)	B	B
18	679 (159)	F	D	310 (152)	D	C	345 (169)	D	B	234 (235)	AB		1581 (400)	E	C
22 → 29 from Sep.	778 (147)	DE		383 (131)	BC		378 (138)	CD	BC	255 (151)	AB	A	1803 (309)	BC	
22 → 26 from Sep.	769 (144)	E		384 (109)	BC		420 (124)	AB	A	218 (172)	BC	AB	1800 (310)	BCD	
22 → 22 from Sep.	779 (153)	DE		389 (119)	ABC		401 (122)	BC	AB	218 (183)	B	A	1799 (363)	BCD	
22 → 19 from Sep.	773 (122)	DE		376 (115)	BC		427 (132)	AB	A	137 (251)	D	C	1714 (324)	D	
22 → 16 from Sep.	792 (135)	CDE		405 (113)	AB		352 (157)	D	C	167 (180)	CD	BC	1725 (302)	CD	
P-values:															
All		***			***			***			***			***	
32 – 16			***			***			***			NS			***
22 → 29 - 16			NS			NS			***			***			NS

The groups in the second series started on a diet with 22 % of ME from protein and the feed was changed over a period from 12th – 15th of September.
*The bracketed figures are the standard deviations. Different letters in a column indicate a statistically significant difference. NS indicates no difference. ***: P<0.001.*

Skin length and fur quality are shown in table 7, and number of silky furs plus wool quality in table 8.

Table 7. Length, quality, colour and clarity of the pelts from the male kits, fed with different levels of ME from protein respectively during the growing-furring period or the furring period

ME from Protein, %	Number of furs	Length, cm			Quality, 1-12 *			Colour, 1-5 #			Clarity 1-5 ¤		
32	107	86.1 (4.5)	AB	AB	7.5 (2.3)	A	A	3.2 (1.0)			2.9 (1.0)	CDE	
29	109	86.5 (4.2)	A	AB	7.2 (2.2)	AB	AB	2.9 (1.1)			2.8 (1.0)	DEF	
25	105	86.6 (4.8)	A	A	6.9 (2.5)	BC	B	3.1 (1.0)			2.9 (1.0)	CDEF	
20	105	85.3 (4.2)	BC	B	6.4 (2.1)	DE	C	2.9 (1.0)			2.8 (1.1)	EF	
18	104	82.6 (5.8)	D	C	5.6 (2.3)	F	D	3.2 (1.0)			2.6 (1.0)	F	
22 → 29 from Sep.	103	84.9 (4.2)	BC		7.1 (1.9)	BC	A	3.0 (0.9)			3.1 (0.9)	BC	B
22 → 26 from Sep.	103	85.0 (4.3)	BC		6.6 (2.3)	CD	A	2.9 (1.0)			3.0 (1.0)	BCDE	B
22 → 22 from Sep.	104	85.5 (5.0)	BC		6.9 (2.2)	BCD	A	2.9 (1.0)			3.2 (1.0)	AB	AB
22 → 19 from Sep.	103	84.7 (4.3)	C		6.7 (1.9)	CD	A	2.9 (1.0)			3.1 (0.9)	BC	B
22 → 16 from Sep.	96	84.6 (4.4)	C		5.9 (2.2)	EF	B	3.0 (0.9)			3.4 (0.9)	A	A
P-values:													
All			***			***			NS			***	
32 – 16				***			***			NS			NS
22 → 29 - 16				NS			***			NS			**

**12 is best, # 5 is darkest, ¤ 5 is most reddish*
*The bracketed figures are the standard deviations. Different letters in a column indicate a statistically significant dif - ference. NS indicates no difference. ***: P<0.001 and **: P<0.05.*

16% of OE from protein had a poorer fur quality than the other groups (Table 7). There were very few silky and only few furs with good wool in all the groups compared to the first test series (Table 8). This indicates that 22% of ME from protein in the first part of the growing period is too low to achieve a fine quality of the fur, probably because the initial development of the winter fur is beginning as early as July-August. Furthermore it seems that variations in content of protein during the last part of the period does not have the same importance as it had in the first test series, as only the quality of the fur at 16% of ME from protein differed from the others.

When the share of ME from protein was 16%, there was an increased number of dead kits authopsied with fatty liver (Table 9). However, at the time of pelting there was no difference in neither the Hepato-Somatic-Index, nor the fat content of the livers in this group as compared to all of the groups in series one (Table 10).

Table 8. The frequency of silky, flat and full furs of the male kits.

ME from Protein, %	Furs, n	Silky, %	Wool, %		
			Flat	Normal	Good
32	107	11.2	10.2	74.8	15.0
29	109	13.8	14.7	74.3	11.0
25	105	11.4	8.6	77.1	14.3
20	105	8.6	12.4	81.9	5.7
18	104	2.9	16.4	77.9	5.8
22 → 29 from Sep.	103	6.8	13.6	76.7	9.7
22 → 26 from Sep.	103	1.9	7.8	83.5	8.7
22 → 22 from Sep.	104	4.8	17.3	77.9	4.8
22 → 19 from Sep.	103	3.9	12.6	84.5	2.9
22 → 16 from Sep.	96	4.2	16.7	81.3	2.1
P-values:					
All		**	**		
32 – 16		NS	**		
22 → 29 - 16		NS	NS		

*The bracketed figures are the standard deviations. Different letters in a column indicate a statistically significant difference. NS indicates no difference. **: P≤0.05*

Mortalities are reported in table 9.

Table 9. The frequency of dead kits (males and females).

ME from Protein, %	Dead kits, n	
	Total	With fatty liver
32	4	0
29	0	0
25	3	0
20	5	2
18	4	3
22 → 29 from Sep.	1	0
22 → 26 from Sep.	0	0
22 → 22 from Sep.	1	0
22 → 19 from Sep.	0	0
22 → 16 from Sep.	9	4

The results from the liver analysis are shown in table 10.

Table 10.Hepato-Somatic-Index (liver weight in % of body weight), liver dry matter (DM) and calculated liver fat (%) (liver fat, % = 1,15 * liver DM – 24,9)*.

ME from protein, %	HSI, %	Liver DM, %	Liver fat, %
32	2,33 (0,20)	31,9 (2,8)	11,8 (3,2)
28	2,39 (0,32)	30,6 (1,4)	10,4 (1,6)
24	2,23 (0,18)	31,2 (2,0)	11,1 (2,3)
20	2,28 (0,23)	31,6 (2,0)	11,5 (2,3)
16	2,34 (0,27)	30,5 (3,0)	10,2 (3,4)
22 → 14 from Sep.	2,45 (0,28)	31,9 (2,6)	11,9 (3,0)
P-value	NS	NS	NS

** Calculation according to Clausen & Sandbol (2004). The bracketed figures are the standard deviations. NS indicates no difference.*

The results above indicate, that the optimal protein content with an "ideal protein" is in the range of 25-32% of ME. As a consequence, at the lower end of this range, one or more of the amino acids with so-called max. norms might have been first limiting.
Based on an earlier trial in the furring period (Sandbol et al., 2003a) we used MHA instead of dl-Methionine, to avoid any risks associated with excess methionine. However results from Sandbol et al. (2003a & 2003b) indicate, that the requirement for methionine *per se*, may be 20% below the recent norm, and our conclusion (Sandbol et al., 2003a) as to the utilisation of MHA may be wrong. A resent trial (Sandbol et al., 2004) indicates that mink are not or only partially able to utilize MHA in the growing period. If this holds, the response found in the first series may be a response to methionine *per se*.

Conclusion

Considering that Methionine-Hydroxy-Analog is utilized by the mink, the optimal protein content for growth with an "ideal protein" is about 26% of Metabolizable Energy from protein and one or more of the Amino Acid's with present max. norm have been first limiting. Considering that Methionine-Hydroxy-Analog is not, or only partially utilized by the mink, the result found in the first series may be a response to methionine *per se*. Further investigations are required to clarify the mink's ability to

utilize Methionine-Hydroxy-Analog. The requirement for ideal protein in the furring period seems to be well below the present levels used in practical feeding.

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Effect of feeding intensity on body condition and glycemic control in mink *Mustela vison*

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Abstract
Thirty kits from five litters, one male and one female from each, were allocated to three feeding regimes: 80, 100 or 120% of the recommended dietary allowance (RDA) of ME. The mink were weighed (BW), scored for body condition (BCS, scale 1-5, 3=ideal), and sampled for blood and urine monthly from mid-August (start) to mid-December (end). In December, 6/10 mink in the 100%RDA group scored 3, while in the 80%RDA group 7/10 mink received a BCS 2 (thin), and in the 120%RDA group 8/10 mink had a BCS 4-5 (heavy-obese)($P<0.001$). The final blood glucose levels of all mink in the 120%RDA group were higher (6.59mmol l^{-1}) in comparison to the 80%RDA (5.21mmol l^{-1}) and the 100%RDA groups (4.95mmol l^{-1})(SEM=0.37, P#0.01). The males in the 120%RDA group showed hyperinsulinemia (2.06ng ml^{-1}, SEM=0.164, P=0.043) in comparison to the rest of the mink (range 1.17-1.51ng ml^{-1}). No glucosuria was detected. The development of obesity appears to be associated with elevated blood glucose concentrations and hyperinsulinemia in the mink suggesting insulin resistance.

Introduction
In the mink *Mustela vison*, the relationship between obesity and glycemic control has not been characterized. The species shows significant fluctuation in body weight in response to seasonal changes in the nutritional and hormonal status, being the slimmest during summer and the heaviest during winter (Korhonen & Niemelä, 1998, Tauson & Forsberg, 2002). In other carnivore species, such as dogs and cats, obesity has been shown to result in poor glycemic control (Hand et al., 2000). It increases the levels of non-esterified fatty acids in blood circulation, which in turn decrease glucose metabolism in other tissues leading to hyperglycemia (Frayn, 2001). This may eventually result in the development of insulin resistance (Frayn, 2001). According to Plotnick and Greco (1995), there is a positive relationship between obesity and blood glucose levels in dogs. Hyperglycemia and the presence of glucose in the urine indicates that blood glucose concentration has exceeded the renal absorptive threshold and that there is potential for development of more serious disorders (Hoenig & Ferguson, 1989). It has recently been proposed that in the mink the history of obesity and the associated development of insulin resistance may be a key predisposing factor to the later development of nursing sickness (Rouvinen-Watt, 2003), the etiology of which is strongly linked to poor glycemic control. The objectives of this research were to study the effect of feeding intensity on body weight, body condition, feed intake, and selected blood and urine parameters in juvenile male and female mink, and to evaluate the impact of obesity on glycemic control.

Materials and Methods
Experimental design, weighing and body condition scoring
Thirty (30) mink kits from five litters, 3 male and 3 female kits in each, were selected for this research. One male and one female mink from each litter were allocated to three different feeding intensity regimes (Table 1) and fed at 80%, 100% or 120% of the recommended dietary allowance (RDA) of metabolizable energy (ME) (NRC, 1982). The experiment lasted from mid-August until mid-December. The early growth diet fed during August contained 17.4 MJ of ME with an energy distribution

between fat, protein and carbohydrates (CP:CF:CHO) 34:44:22. From September until December a diet containing 19.2 MJ of ME (CP:CF:CHO 32:53:15) was fed. The diet included 40% of cod and haddock racks, 25% of chicken necks and backs, 5% liver, 5-10% eggs, 1-2% corn gluten meal, 10-14% extruded wheat, 1-4% fat supplement (herring oil, canola oil, poultry grease) and a vitamin-mineral premix. The mink were housed individually in a conventional two-row shed with a nest-box attached to a rearing cage and adequate bedding material provided. Freshly mixed feed was provided daily according to the designated feeding intensity regime. The amount of feed dry matter consumed daily per mink was measured once a month over a three-day period. The mink were weighed to the nearest 0.1g at the beginning of the project and every four weeks thereafter using a catching cage. The mink were manually caught and restrained in compliance with standard animal management practices (CCAC, 1993). Prior to weighing the mink were scored for body condition using a five-point scale, where Body Condition Score (BCS) 1 = very thin, 2 = thin, 3 = ideal, 4 = heavy, and 5 = obese (Rouvinen-Watt & Armstrong, 2002).

Blood and urine collection and analysis

Urine samples were collected at the beginning of the experiment, in September and in December by placing the mink into a metabolism cage until urine was voided. Each sample was analysed using a DiaScreen 1G urine glucose test strip (MEDgenesis). A blood sample was then taken by toenail clipping and analysed using an Accu-Chek Compact blood glucose monitor (Roche Diagnostics). The blood and urine samples were collected post-prandially and were obtained within 1-2 hours. The exact time from feeding was not measured except at the end of the experiment. The blood and urine were collected over a three-day period, with 12 mink per day being used, including an equal number of males and females from each test group. At the end of the experiment in December, the blood glucose was measured post-prandially prior to anaesthesia, from a sample collected from a clipped toenail, as described above. The time from feeding to anaesthesia was on average 102± 26 min. The mink were anaesthetized using 0.17 ml kg^{-1} BW of Rompun® (xylazine 20 mg ml^{-1}) and 0.09 ml kg^{-1} BW of Ketalean® (ketamine hydrochloride 100 mg ml^{-1}). Blood samples were obtained by cardiac puncture for serum clinical-chemistry (7mL Vacutainer® tubes) and haematology (5mL Vacutainer® EDTA tubes). The mink were then euthanized with an intracardiac injection of Euthanyl® (pentobarbitol 240 mg ml^{-1}, 0.44ml kg^{-1} BW) for subsequent organ and tissue sampling. The blood samples were analysed for haematology and clinical chemistry at the Veterinary Services Laboratory, Nova Scotia Department of Agriculture and Fisheries, Truro, NS. The insulin, triacylglycerol and free fatty acid analyses were carried out in the Obesity and Diabetes Lab at the Atlantic Veterinary College, Charlottetown, PEI. The insulin radioimmunoassay utilized rat insulin as standard; serial dilution of mink serum generated a curve parallel to the rat insulin standard curve, indicating similar cross-reactivity with the antiserum. The antisera was guinea-pig anti-insulin, a kind gift of R. A. Pederson, Vancouver, Canada. Triacylglycerol and free fatty acids were determined using commercially available kits (Diagnostic Chemicals, Charlottetown, Canada and Roche, Laval, Canada, respectively).

Statistical analysis

The MIXED procedure in SAS was used to examine the effects of the sex, treatment, and month and their interactions on body weight, energy consumption, and blood and urine parameters of the mink (Littell et al., 1998). In the model, the litter of origin was used as a random statement and the month as a repeated statement. Where significant effects were detected ($P < 0.05$), the lsmeans± SEM values were tested using the PDiff test. The frequency distribution of the body condition scores in the different treatment groups was evaluated using the Fisher's exact test.

Results and Discussion

Body weight and energy consumption

In August, at the start of the experiment, the body weights of the mink in the different feeding regimes did not differ. The males (1904 g) were on average significantly heavier than the females (1130 g, SEM=73.0, $P < 0.001$). In September, the males in the 80%RDA group differed from the males in the 100%RDA ($P = 0.024$) and the 120%RDA ($P = 0.002$) groups. In October, all male feeding regime groups differed from each other (Figure 1) ($P < 0.05$), whereas in November also the female groups showed

significant differences with the exception of the 100%RDA and 120%RDA groups. At the end of the experiment in December, all groups differed from each other in BW except the 100%RDA and 120%RDA female groups (females: 80%RDA 1144g; 100%RDA 1395g; 120%RDA 1502g; males: 80%RDA 1440g; 100%RDA 2377g; 120%RDA 2755g; SEM=104, P#0.05). The daily energy intake of the mink (Figure 2) differed significantly between some of the feeding regimes already in August, measured one week after the start of the experiment. In September, all male feeding regime groups differed in their energy intake (P#0.021), whereas the females did not show significant differences. In October, all male and female feeding regime groups were significantly different from each other in their diet energy consumption (P<0.05). In November, the male 120%RDA group differed from the other two male groups (P<0.05), whereas the females no longer showed differences among the feeding regimes.

Figure 1.
Body weight of juvenile male and female mink in different feeding regime groups based on Recommended Dietary Allowance. The males are presented with closed and the females with open symbols. The symbols used are as follows: circle 80%RDA, triangle 100%RDA, and square 120%RDA. Presented are lsmeans∀SEM. Planned comparisons: August all groups P>0.05; September males: 80%RDA-100%RDA P=0.02 80%RDA-120%RDA P=0.002; October males: all groups P<0.05; November females: 80%RDA-100%RDA P=0.013, 80%RDA-120%RDA P<0.001, males: all groups P#0.004; December females: 80%RDA-100%RDA P=0.052, 80%RDA-120%RDA P=0.006; males: all groups P<0.01.

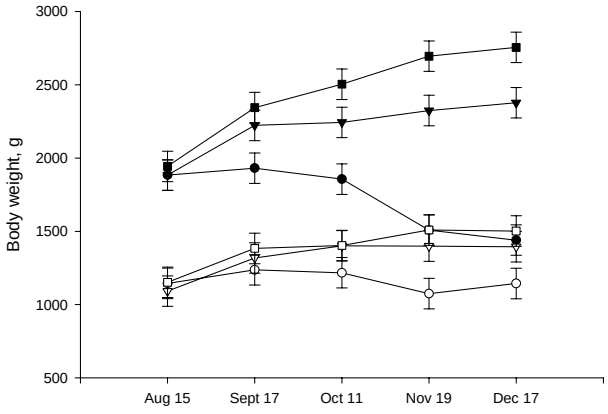
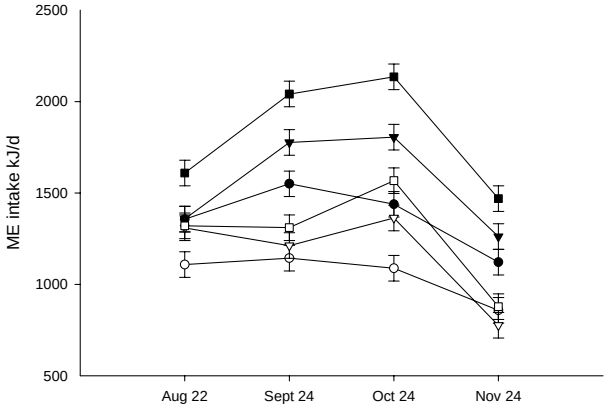


Figure 2.
Daily metabolizable energy intake of juvenile male and female mink in different feeding regime groups based on Recommended Dietary Allowance. The males are presented with closed and the females with open symbols. The symbols used are as follows: circle 80%RDA, triangle 100%RDA, and square 120%RDA. Presented are lsmeans∀SEM. Planned comparisons: August females: 80%RDA-100%RDA P=0.040, 80%RDA-120%RDA P=0.030, males 80%RDA-120%RDA P=0.010, 100%RDA-120%RDA P=0.011; September males: all groups P#0.021; October females: all groups P<0.05, males: all groups P<0.001; November males: 80%RDA-120%RDA P<0.001, 100%RDA-120%RDA P=0.033.



As expected, increasing the daily energy intake resulted in heavier body weights in both juvenile male and female mink, whereas restricted feeding reduced weight gain. The mink is very responsive to changes in energy supply and has a propensity for seasonal fatness (Tauson & Forsberg, 2002). Short-term variations are seen in mink body condition particularly during winter when cold weather induces reduced feed intake and diminished locomotor activity (Korhonen & Niemelä, 1993). It is likely that the colder weather during the latter part of the current experiment had more of an impact on the feed intake and subsequently body weight maintenance of the female mink resulting in an overall less gain in body condition. This may be also related to the more pronounced heat loss in the females due to their smaller body size. The only exception to this were the male mink in the 80%RDA regime, where rapid loss in body weight was apparent from October to December. It is to be noted, although no detailed behavioural observations were carried out, that these

males were also physically very active and were often observed running back and forth in the pen when approached by humans. It is interesting to note that the males in this group also had the highest levels of blood urea nitrogen (17.94 mmol l^{-1}) and the lowest levels of calcium (2.21mmol l^{-1}) in comparison to all other mink (BUN: range 9.99-12.11 mmol l^{-1}, SEM=1.21, Ca: range 2.35-2.45 mmol l^{-1}, SEM=0.045)($P<0.05$).

In the end of the experiment, when organ and tissue samples were taken, it was notable that the 80%RDA males had hardly any subcutaneous body fat and the internal fat depots present had the appearance of brown adipose tissue. This may suggest cold acclimation due to reduced body insulation. The 100%RDA and the 120%RDA regime males, on the other hand, had well developed body fat depots, and the latter exhibited excessive visceral adiposity. The observed differences in the females were much less apparent and were thus in line with the less extreme differences in feed consumption and body weight accumulation (Figures 1 and 2). Similar findings have been reported earlier by Korhonen and Niemelä (1998) where *ad libitum* and restricted feeding of male mink resulted in more pronounced differences among test groups in comparison to those seen in the females.

Body condition score

At the start of the experiment, no differences existed in the body condition among the mink in the different feeding intensity groups (P=0.668), however, the males tended to be scored heavy more often (8/15), while all the females scored ideal (P=0.002). In October, 9/10 mink in the 80%RDA group and 7/10 in the 100%RDA group received a BCS 3 (ideal), while in the 120%RDA group 7/10 animals scored 4-5 (heavy-obese) (P=0.040). In December, in the 100%RDA group 6/10 mink scored 3, while in the 80%RDA group 7/10 mink received a BCS 2 (thin), and in the 120%RDA group 8/10 mink had a BCS 4-5 ($P<0.001$).

Glycemic control

There were no differences in the blood glucose values between the treatment groups at the start of the experiment. The average values for the males and females were found to be 5.33 and 5.08 mmol l^{-1} (SEM=0.30). Throughout the experiment, the only statistically significant effect was that of the experimental treatment (P=0.013). In October, the blood glucose values measured were higher in the females in the 120%RDA group (6.04 mmol l^{-1}) compared to those in the 100%RDA group (4.48 mmol l^{-1}; SEM 0.52, P=0.039) but not different from the 80%RDA group (4.84 mmol l^{-1}; P=0.110). The male groups did not differ from each other at this time point. The final blood glucose levels measured in December (Figure 3) of all mink in the 120%RDA group were on average higher (6.59 mmol l^{-1}) in comparison to the 80%RDA (5.21 mmol l^{-1}) and the 100%RDA feeding intensity groups (4.95 mmol l^{-1}) (SEM=0.37, P#0.01). There was no significant sex effect at this time. None of the mink exhibited glucosuria during the experiment. In December, the males in the 120%RDA feeding regime group showed pronounced hyperinsulinemia (2.06 ng ml^{-1}, SEM=0.164, P#0.046) in comparison to the rest of the mink (range 1.17-1.51 ng ml^{-1})(Figure 4). There was no effect of the feeding regime on the post-prandial free fatty acid values (males 388.1 μmol ml^{-1}, females 372.4 μmol ml^{-1}, SEM= 38.3), but a trend among the regime groups in the triacylglycerol concentrations (males: 80%RDA 40.2 μmol ml^{-1}, 100%RDA 60.3 μmol ml^{-1}, 120%RDA 65.7 μmol ml^{-1}, females: 80%RDA 40.9 μmol ml^{-1}, 100%RDA 80.2 μmol ml^{-1}, 120%RDA 34.5 μmol ml^{-1}SEM=10.5) (P=0.052).

Since the juvenile female mink achieve their adult body size earlier (Hansen et al., 1991), it is likely that the autumnal body fat deposition may have interfered with glucose disposal in the female mink earlier than what was observed in the males, as shown in the 120%RDA group in October. Based on the documented higher activities of glucose-6-phosphatase and pyruvate kinase in the female mink (S□rensen et al., 1995), they may also show more pronounced gluconeogenesis and glycolysis. This may result in higher blood glucose levels, which may be further augmented by handling stress (Clausen et al., 1999). Later in the fall, the females gained relatively less body condition following the 100%RDA and the 120%RDA feeding regimes in comparison to the males in the respective regimes.

Figure 3.
Final blood glucose concentrations of juvenile male and female mink in the different feeding regime groups based on Recommended Dietary Allowance (RDA). The males are presented with black and the females with white bars. Presented are lsmeans∀SEM. Planned comparisons: males: 80%RDA-120%RDA P=0.015, 100%RDA-120%RDA P=0.008; females: 100%RDA-120%RDA P=0.099.

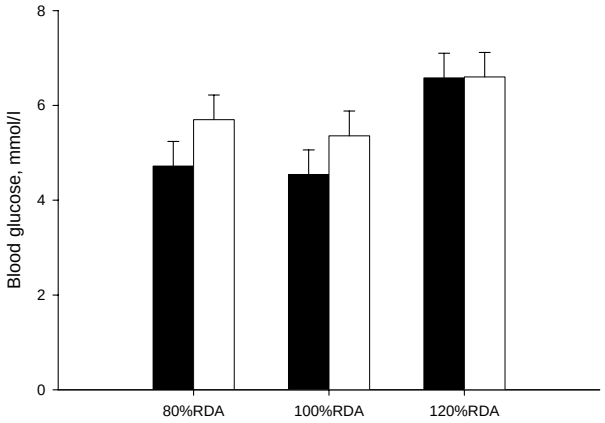
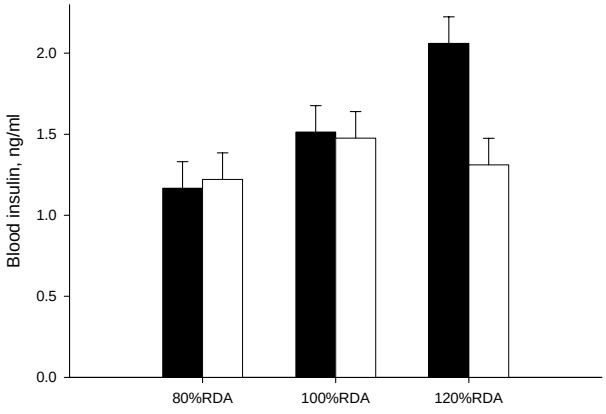


Figure 4.
Final blood insulin concentrations of juvenile male and female mink in the different feeding regime groups based on Recommended Dietary Allowance (RDA). The males are presented with black and the females with white bars. Presented are lsmeans∀SEM. Planned comparisons: males 120%RDA – other groups P<0.05.



Therefore, although the overfeeding and associated heavy-obese body condition did result in hyperglycemia in the females also, it did not cause abnormally high insulin levels as was observed in the heavy-obese males. Based on body composition analyses using tritium-labeled water, juvenile male mink have more lean body mass than female mink in August and in November (Boudreau, 2004). This is likely to result in better clearance of glucose from the blood stream by the muscle tissue and may therefore delay the development of hyperglycemia in response to autumnal fattening. However, later in the fall the males, due to being better able to cope with the cold weather due to their larger body size, may not lose much body fat, and may then show more pronounced signs of compromised glycemic regulation. This is perhaps further compromised by the cold weather inducing longer periods of physical inactivity.

The adipose tissue is an active endocrine and secretory organ that performs a vital role of buffering fluxes of fatty acids in the circulation. When this buffering capacity has been exceeded, such as in obesity, deposition of fat in other tissues interferes with insulin-mediated glucose disposal and leads to insulin resistance (Frayn, 2001). It is important to note that the increased availability of non-esterified fatty acids is also a potent stimulus for hepatic glucose production (Frayn 2001). It has recently been suggested that uncontrollable gluconeogenesis during lactation may cause hyperglycemia (Fink & Børsting, 2002), a key clinical finding in females suffering from nursing sickness (Wamberg et al., 1992). In the current study, contrary to what was expected, the post-prandial triacylglycerol or free fatty acid concentrations did not differ between the treatment groups. Therefore this mechanism may not explain the development of insulin resistance in the mink. Most recently, Dandona et al. (2004) have proposed that the link between obesity, insulin resistance and diabetes is the inflammatory response. Obesity (chronic overnutrition) and hyperglycemia are both classified as pro-inflammatory states, which via inflammatory mechanisms would result in the inhibition of insulin signaling and the development of insulin resistance (Dandona et al. 2004). It is evident from the results of this study that the macro nutrient supply and the body condition of the mink significantly impact glycemic regulation. The hyperinsulinemia observed in the male mink also suggests that autumnal fattening may lead

to the development of insulin resistance. This in turn may be a key predisposing factor to the later development of nursing sickness, the etiology of which is strongly linked to poor glycemic control.

Conclusions

Increasing daily energy intake by a higher feeding intensity resulted in heavier body weights in juvenile male and female mink, whereas restricted feeding resulted in lower weight gain (females) or loss of body condition (males). Chronic overnutrition and the development of obesity during the fall was associated with elevated blood glucose concentrations in both male and female mink and hyperinsulinemia in the males suggesting insulin resistance. It is surmised that autumnal fattening may be a significant predisposing factor to the development of nursing sickness, the etiology of which is strongly linked to impaired blood sugar regulation.

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The effect of *ad libitum* and restricted feeding on growth curves and growth rate curves in mink selection lines

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Abstract
The effect of *ad libitum* feeding (AL), restricted feeding (RF) and farm feeding (FF) on growth curves and growth rate curves is studied in lines of mink destined for selection for high November weight. The results show that the growth curves can be described by a fourth degree polynomial specific to line and sex and significant differences are found between the AL-line and the RF-line ($P<0.0001$ for both November weight and overall growth rate). Body weight and growth rate are reduced in the RF-line compared to the AL-line. This suggests a moderate to low genetic correlation between growth on the two feeding regimes that is the basis for a differentiated response to selection.

Introduction
Selection for growth on restricted feeding is assumed to improve feed efficiency as found in mice (Hetzel & Nicholas, 1986; McPhee & Trappett, 1987; Urrutia & Hayes, 1988). Technological progress in management of feeding has made recording of feed allowance possible in mink production and made both *ad libitum* and restricted feeding possible. Thus, the use of restricted feeding to improve feed efficiency can be studied in mink as well. The way of presenting the problem is one of genotype-environment interaction.
The effect of the restricted feeding has to be surveyed regularly during the growth period to secure a current reduction in growth. Successive observations of weights from an animal are correlated. A way of dealing with records from a growth curve is to analyze data using a random regression model (Andersen & Pedersen, 1996). This study reports the growth patterns in lines of mink on *ad libitum* feeding (AL), restricted feeding (RF) and farm feeding (FF) in a selection experiment in the first generation before selection is performed. Weight is modelled as a function of age and a function for growth rate is obtained as first derivative. Furthermore, the average curves are estimated for growth and growth rate.

Materials and methods
Animals: The animals used for the present feeding experiment are the base population in a selection experiment. Three lines were established for the selection experiment. Line FF is a control line. Line AL and RF are lines selected for high November weight on *ad libitum* and restricted feeding, respectively. All three lines were established by crossing two previous scanbrown mink selection lines. The one line was selected exclusively for high November weight, while in the other line, male mink were selected for high November weight and female mink were selected for high litter size. Line FF, AL and RF were constructed to be as genetically similar as possible. Each line is maintained by 100 female mink. For the feeding experiment recordings from 381, 363 and 366 animals from line FF, AL and RF, respectively are used in the data analysis.

Feeding: The animals were set out and weighed across three days in late June and the test feeding was commenced within a week and at the same day for all lines. A standard feed kitchen diet was used. A detailed description of the feeding procedure is given by Møller *et al*. (2004). Line FF was farm fed according to normal farm procedure. Line Al was fed *ad libitum* and line RF was kept under a restrictive feeding regime and fed 90% of the amount of feed offered to the control line. *Ad libitum* feeding in line AL was obtained by individual feeding at cage level using a computerized feeding machine regulated by a Palm Pilot. The feeding machine was used for feeding line FF and RF as well.

Weights: Individual weights were recorded in all lines at three weeks intervals from the time the animals

were set out in pairs (from 26 June to 1 July) until pelting. Eight weights were recorded for each animal.

Models: In the analysis of growth curves it has to be considered that observations from an animal are correlated. Thus, a random regression model with orthogonal Legendre polynomials was used. The model assumes that the growth curve for each animal is described by a fourth degree polynomial and random animal deviations following a second degree polynomial. The model is:

$$W_{ia} = .(a_{ls}+A_i)+(c_{ls}+C_i)*lgc_1+(g_{ls}+G_i)*lgc_2+h_{ls}*lgc_3+ j_{ls}*lgc_4+e_{ia} \quad (1)$$

W_{ia} is the weight of animal i at age a. In the model a_{ls}, c_{ls}, g_{ls},, h_{ls} *and* j_{ls} are fixed effect parameters of line and sex. A_i, C_i and G_i describe random regression parameters, describing permanent animal effects, while the deviation from the individual curve is modelled by e_{ia}. (A_i, C_i, G_i) is multivariate normally distributed N(0,V) and the e_{ia} are independent N(0,σ^2). lgc_1 - lgc_4 are normalized Legendre covariates. A set of covariates is obtained for each age.
The average curves are estimated as:

$$\text{Average}(W_a) = \hat{a}_{ls}+\hat{c}_{ls}*lgc_1+\hat{g}_{ls}*lgc_2+\hat{h}_{ls}*lgc_3+\hat{j}_{ls}*lgc_4 \quad (2)$$

$\hat{a}_{ls}$, ,$\hat{c}_{ls}$, $\hat{g}_{ls}$, $\hat{h}_{ls}$, and $\hat{j}_{ls}$ are the estimates of the parameters.

A function for growth rate is obtained as the first derivative of the function for weight. Thus the model for growth rate of animal i at age a is:

$$GR_{ia} = (c_{ls}+C_i)*lgc_1'+(g_{ls}+G_i)*lgc_2'+h_{ls}*lgc_3'+j_{ls}*lgc_4'. \quad (3)$$

lgc_1' -lgc_4' are the derivatives of lgc_1 - lgc_4. The average growth rate curves are estimated as:

$$\text{Average}(GR_a) = \hat{c}_{ls}*lgc_1'+\hat{g}_{ls}*lgc_2'+\hat{h}_{ls}*lgc_3'+\hat{j}_{ls}*lgc_4' \quad (4)$$

Results

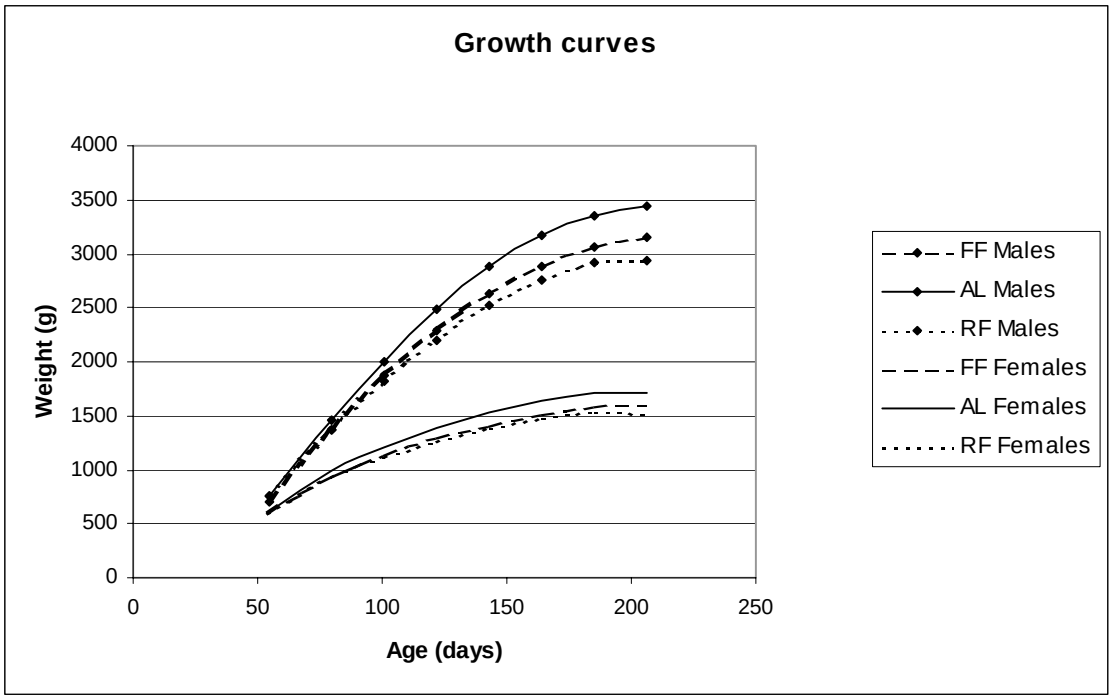
The estimates of the parameters obtained from analysis of model 1 have no biological interpretation but serve in fitting the growth curves. The estimates of the parameters are given in Table 1. The growth curves are shown in Fig. 1 and the growth rate curves are shown in Fig. 2.

Growth: The weight of the females was less than the weight of males at the start of the test (P<0.0001) and a significant difference between line-sex groups was found at all times of weight recordings (P<0.0001). Comparison of lines within sex showed a significant difference between the AL-males and the RF-males already at the first weighing on test at an age of about 84 days (P=0.01). The difference between the respective females at the same time was also large but not significant (P=0.05). Thus, the effect on growth

Table 1. Fixed effect parameters (±S.E.) for growth curves estimated from the random regression model. Feeding regimes are: farm fed (FF), *ad libitum* (AL) and restricted feeding (RF).

Feeding regime	FF		AL		RF	
Parameter	Males	Females	Males	Females	Males	Females
	2319± 15	1290± 15	2522± 16	1385± 15	2224± 15	1258± 15
c	1102± 11	435± 11	1222± 11	484± 11	996± 11	401± 11
g	-269± 5	-139± 5	-296± 5	-159± 5	-268± 5	-152± 5
h	-7± 3	15± 3	-29± 3	9± 3	-22± 3	4± 3
j	6± 3	-21± 3	13± 4	-15± 4	-11± 4	-28± 4

Figure 1. Growth curves from 55 to 206 days of age in the line on farm feeding (FF), *ad libitum* feeding (AL) and restricted feeding (RF).



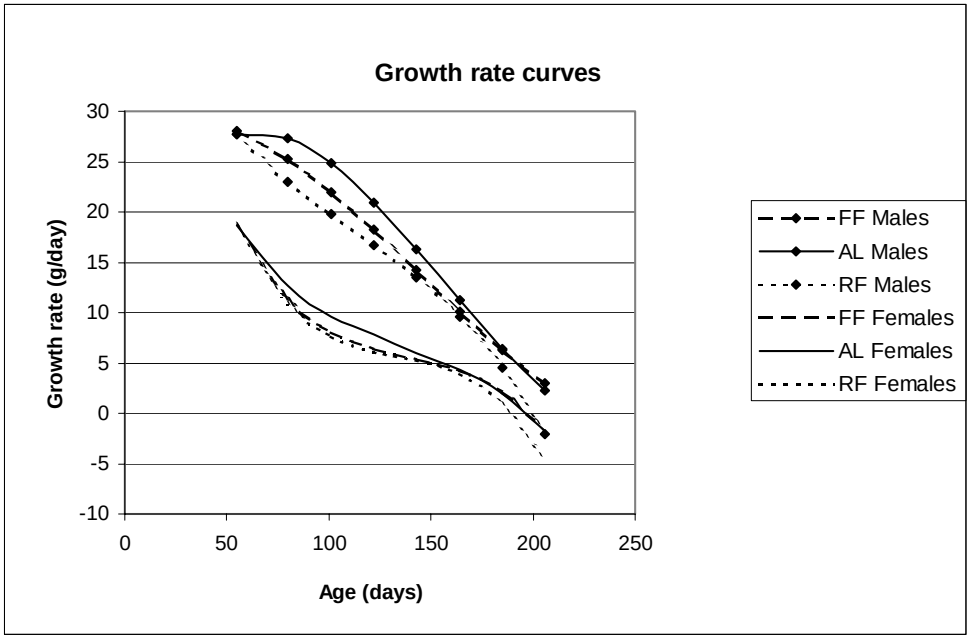
was obtained immediately after start of the test feeding. At the following recordings all differences between lines within sex were significantly different with one exception. FF females and RF females never diverged significantly (P>0.08).

Growth rate: An overall significant difference between line-sex groups was also found for growth rate in all periods (P<0.0001). The overall growth rate was considerably smaller in females than in males (P<0.001) and from 50 to 100 days of age the decrease in growth rate was larger in females (Fig. 2). Until around 105 days of age, comparison showed large and often significant differences between lines except for the difference between the FF females and the RF females. In the following periods the difference between these two lines was only significantly different for both males and females in the last part of the growth period (P<0.0001 for males and P=0.008 for females). This difference was probably obtained due to an intentionally larger feed restriction with the purpose of an increased reduction in growth in the RF line. Comparison of line AL and RF showed that growth rate generally was significantly different for both males and females (P<0.04 for both males and females). However, between 148 and 168 days of age the restriction of line RF was not sufficient to create a significant difference between these lines (P=0.18 for males and P=0.57 for females).

Discussion and conclusion

Results from selection experiments have shown that growth on a restricted diet or a diet with a suboptimal feed composition compared with growth on *ad libitum* feeding has a rather different genetic basis (Hetzel & Nicholas, 1986; McPhee & Trappett, 1987; Nielsen & Andersen, 1987; Urrutia & Hayes, 1988). This requires that the restricted feeding causes a reduction in growth. The results of the present feeding experiment show significant differences in weight development and growth rate during the growth period between the AL and RF line. Thus the results suggest that the two feeding regimes creates a basis for a differentiated response to selection.

Figure 2. Growth rate from 55 to 206 days of age in the line on farm feeding (FF), *ad libitum* feeding (AL) and restricted feeding (RF).



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III – 9 P

Sodium bisulfate as a mink food preservative

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Introduction

For half a century, phosphoric acid (75% feed grade) has been employed in mink ranch diets throughout the world wide fur industry as a feed "on the wire" preservative and as an effective urinary acidifier prophylactic medicinal program for minimizing the formation of struvite urinary calculi in mink and fox diets at the 2%, Leoschke, 1956; and 2.5%, Leoschke, 1996; levels on a dehydrated basis.

In recent years, there has been public concern in the United States and Europe about phosphate pollution of the environment. Sodium bisulfate is an alternative mink feed and urine acidifier which has been shown to be safe and effective for modern mink nutrition in field observations in the United States and Canada in recent years.

It is of interest to note that sodium bisulfate has been shown to be a useful urine acidifier for the prevention of struvite calculi in cats for a number of years, Kneuven, 2000. It has also been used in Europe as a silage preservative for cattle feed for many years.

Materials and Methods

The pKa (the chemists' mathematical basis for designating the relative acidity of a specific acid) of phosphoric acid, 2.16 and bisulfate anion, 1.99 are very similar and thus their employment as a mink feed preservative at equal levels of acid concentration should in theory yield about the same final pH of the feed as noted in experimental data involving a fish slurry provided by Jones-Hamilton, Co., Walbridge, Ohio. The product marketed by this company contains about 93% sodium bisulfate and 7% disodium sulfate and thus contains about 75% bisulfate anion similar to the level of phosphoric acid provided in commercial phosphoric acid (75% feed grade).

Results

All factors considered, it does appear that in terms of field observations of United States mink ranchers employing sodium bisulfate that this acid resource is not quite as effective as a mink feedstuff preservation chemical or as an acidifier for mink feed "on the wire" as phosphoric acid. Rancher observations of Zimbal, 1998; and Brown, 2000; have indicated that sodium bisulfate was not as effective as phosphoric acid (75% feed grade) for the preservation of raw egg product at room temperature. In the case of the Walter Brown Mink Ranch, the employment of 3% phosphoric acid (75% feed grade) with his raw egg resource achieved a raw egg mixture with a pH of 3.5 and zero bacteria population after 3-5 days storage.

The employment of sodium bisulfate at a level of 2.0% yielded a raw egg mixture pH of 3.6 but a reduced effect on lowering bacterial populations. Observations of Durant, 1999; on the urinary pH of the mink fed a redi-mix program with 0.8% phosphoric acid (75% feed grade) (equivalent to 2.0% phosphoric acid dehydrated feed basis) or 0.9% sodium bisulfate indicated a higher mink urinary pH with the sodium bisulfate supplementation relative to that with the phosphoric acid addition. It is of interest that Durant employed sodium bisulfate at levels as high as 2.5% (dehydrated basis) in the ready-mix program without any observations of negative effects on feed consumption.

Experimental studies reported by Jones-Hamilton Co. with cats on a pellet program indicated that 0.9% sodium bisulfate yielded an average urinary pH of 6.4 – a urinary pH considered to be effective in minimizing struvite calculi formation in cats and mink. This urinary pH data is similar to studies with mink at the National Research Ranch with sodium bisulfate at a 1% level (dehydrated basis), Michels, 2002. It is of interest to note that the field studies in North America in 2002 involved the employment of sodium bisulfate at the level of 1.5%, dehydrated basis.

Discussion

It is apparent that sodium bisulfate is a useful alternative to phosphoric acid (1) as a mink feed preservative "on the wire" and (2) as an effective urinary acidifier for the prevention of the formation of struvite calculi in the mink – made to those levels

of sodium bisulfate required for the top performance of the mink.

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III – 10 RP

The "boiling" phenomenon in formic acid preserved poultry by-products

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Abstract
Formic acid preserved poultry by-products has become an important ingredient in Norwegian fur animal feed. Occasionally this semi-liquid product tends to overrun tanks during transport owing to gas coming from the mass. This so called "boiling" phenomenon causes practical problems cleaning up and sometimes destruction of the product. This study showed that *Clostridia* may be responsible for the significant gas production and that hydrogen gas production derived by *Clostridium* bacteria play an important role in the over-running. The findings suggest that formic acid preserved poultry by-products should be handled with caution, since it may represent a health risk related to the exposure to pathogenic *Clostridia* and secondly, because of the occurrence of hydrogen, which is highly explosive. The use of additives as sodium bisulphite (0,5 %), which may have an inhibitory effect on gas generation can be a future solution to the "boiling" phenomena.

Introduction

In Norway, poultry by-products (viscera, heads, feet) used as ingredient in fur animal feed are preserved with formic acid at pH 3.5-3.8 to prevent bacterial growth.. The preserved product is semi-liquid (app. 25 °C) and is transported in tank trucks to the fur animal feed producers. Occasionally, the mass expands and runs over the tanks during transport and may continue running over after being loaded to storage tanks. Typical signs of this so called "boiling" phenomena, are bubbles and foaming similar to boiling, indicating the occurrence of gas production the mass. The over-running may last for 1-3 days and it appears to terminate by it self. Similar problems with overrunning have been reported with fish silage preserved with formic acid. The economic cost of this phenomenon is related to extra clean-up work and in some cases loss due to destruction of the product.

The main objective of the present experiment was to identify causes for the so-called "boiling" cases, i.e. gas –production during storage and transport of acid stabilised poultry by-products. This was obtained by carrying out small scale laboratory storage trials on freshly preserved raw material in combination with volumetric measurements of gas production, chemical gas analysis and microbial analysis. In addition trials were carried out to test the inhibitory effect of gas-production by a sodium bisulphite, which in earlier studies has shown to have a possible inhibitory effect on gas-production.

Material and methods

Samples

Measurement experiments were performed on raw material from different batches from the slaughter-waste plant at Prior Sør, Rakkestad, Norway. Samples of grinded (20 mm hole plate) poultry by-products were taken before and after addition of formic acid (2-3 %) from the slaughterhouse. Acid was added just a few minutes after slaughter. In addition, acid preserved samples were taken, which had been kept for a few days on the storage tank at the plant. The acid preserved samples represented both stable and unstable samples with regard to extent of gas production.

Chemical contents and hygienic quality characteristic

Protein, fat, ash content, pH, total volatile nitrogen, were determinedin unpreserved and preserved samples. In addition, analysis of organic volatile

compounds using gas-chromatography mass-spectrometry was performed on selected samples. Hydrogen analysis was performed by using a gas chromatography (HP 6890, Supelco, 80/100 mesh mole sieve 5A, 3 m, 1/8") in combination with a thermal conductivity detector.

Microbiological analysis

The samples were characterised by analysing the microflora under both aerobic and anaerobic conditions. Total viable count, coliform bacteria and fungi were determined using standard methods at Norwegian Fur Breeders' Association, Laboratory Division. For determination of *Clostridia* blood agar, TSC-medium and boiled meat broth growth media were applied. Catalase test was used to confirm the presence of *Clostridia* like bacteria. In addition, DNA fingerprinting analysis using RAPD techniques was applied on a few samples for the confirmation of *Clostridia* strains. Also microscopy was used for identification of bacteria colonies based on morphology.

Measurement of gas-production

Three Samples (450 g) of formic acid preserved by-products were transferred to 500 ml flasks where silicone tubing were connected through the sealing and led into a water bath and into a water-filled graded burette for volumetric measurement of water displacement during gas production. Corresponding samples (n=3) (450 g) were packed in plastic bags and vacuumated. The samples were stored at room temperature (21°C) for seven days. About 50 g samples were heat shocked (80°C) to kill living microorganismes and to analyse for sporogenous bacteria in the material afterwards. The same experiment with vacuumated bags was repeated including also acid preserved samples added 0.5 % sodium bisulphite.

Statistical treatment

Because of the preliminary character of this study, only average values with standard deviations are presented.

Results and discussion

Chemical contents, hygienic quality characteristic and microbiological analyses

The pH of the starting material varied from 3.3-3.8. Chemical composition data revealed that the by-products had somewhat higher dry matter content before than after acid preservation (Table 1). This difference is probably due to the fact that the fresh samples were taken just after grinding, while the acids preserved were taken from a larger mixed volume. However, the most likely reason is a dilution effect from adding formic acid. Typically, dry matter content is about 30 %, and protein and fat account for approximately 40-50 % each. Ash content is often 2-4 % (10-15 % of DM). High ash levels may challenge the buffer capacity of formic acid by releasing Ca, and by that make the pH increase above 4.0, which is a critical level to stay below to avoid bacterial growth. The TVN values were low, showing that the protein degradation had been moderate. However, the hygienic quality of the fresh, unpreserved product revealed considerable bacterial counts, up to log

Table 1. Dry matter, protein, fat, ash, total volatile N, total bacterial count, coliform bacteria and fungus in poultry by-products before and acid preservation (n=6). Standard deviation in parentheses

	Fresh	Formic acid preserved
Dry matter (%)	31.4 (2.2)	27.4 (2.2)
Protein (%)	14.2 (1.3)	12.1 (1.1)
Fat (%)	13.0 (2.8)	10.9 (1.7)
Ash (%)	3.2 (0.8)	2.2 (0.3)
TVN (mg/100g)	8.7 (3.4)	7.5 (0.8)
PH	6.62 (0.19)	3.60 (0.22)
Total viable count (logN)	6.5 (6.4)	3.2 (3.2)
Coli bacteria (logN)	5.3 (5.1)	<1
Fungus (logN)	3.7 (3.8)	<1

6.84 (average log 6.5), but the variation between samples were large. The efficiency of formic acid preservation was clear. Adding 2-3 % formic acid (85%) improved the hygienic quality by killing of microorganisms to a considerable extent. Coli bacteria and fungus were brought to a very low level. During the storage experiments, growth of yeast could be observed by microscopy on the surface of the flask samples, due to the presence of air, and *Clostridium* like bacteria further down in the mass. No yeast growth could be detected under the anaerobic conditions prevailing in the bag samples. Microbial analysis of both stable and unstable freshly acid preserved raw material before storage at room temperature revealed the presence of only *Clostridia* under anaerobic conditions, which was indicated by microscopy and catalyse test. Colony morphology suggested the presence of *Clostridium perfringens* and possibly *Clostridium tetani*. *Clostridium perfringens* was positively confirmed by the RAPD method, but the second strain could not be confirmed uniquely. The occurrence of *Clostridia* is in agreement with previous findings in poultry meat (Kaldhusdal et al. 2001). During the storage experiment, an increase in pH could be observed up to 4.5 over one week. The pH and water activity are crucial growth limiting factors for *Clostridia*, having an optimum around pH 7, and DM preference up to 30 % (McDonald, 1981). Water activity in our case around the limit, but at the acidic conditions used, it is little likely that these bacteria should be able to grow. However, the raw material represents a rather heterogeneous mass, with possibility for local pockets with favourable conditions that may allow the growth of *Clostridia*. On the other hand, growth of some *Clostridia* strains may occur down to pH 4.1 (McDonald, 1981), which is at pH levels found during our storage experiments.
Experience from practice indicates that Norwegian produced poultry by-products might have higher levels of *Clostridia* than in by-products from Denmark and Finland. The reason for this difference is not clear, but it could be owing to different feeding regimes in the production of poultry.

Measurement of gas production
Incubation of the formic acid preserved poultry by-products in flasks and vacuum bags produced considerable amounts of gas. In the bags one could see clearly bubbles coming out of the product. By repeated experiments on different batches, total gas production rates under anaerobic conditions varied from 500 up to 9000 ml/kg/day.

Figure 1. Gas volume produced from formic acid preserved poultry by-products in flasks during seven days at room temperature.

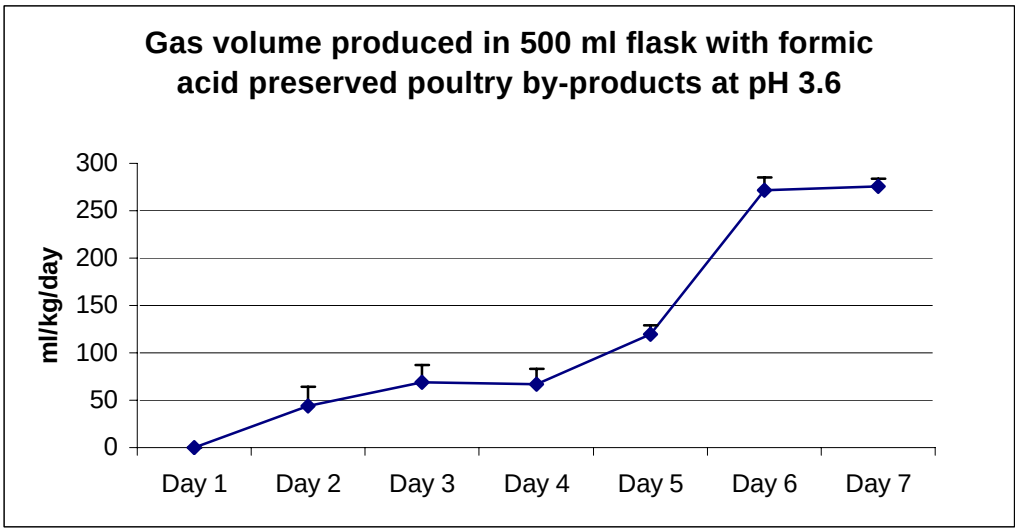
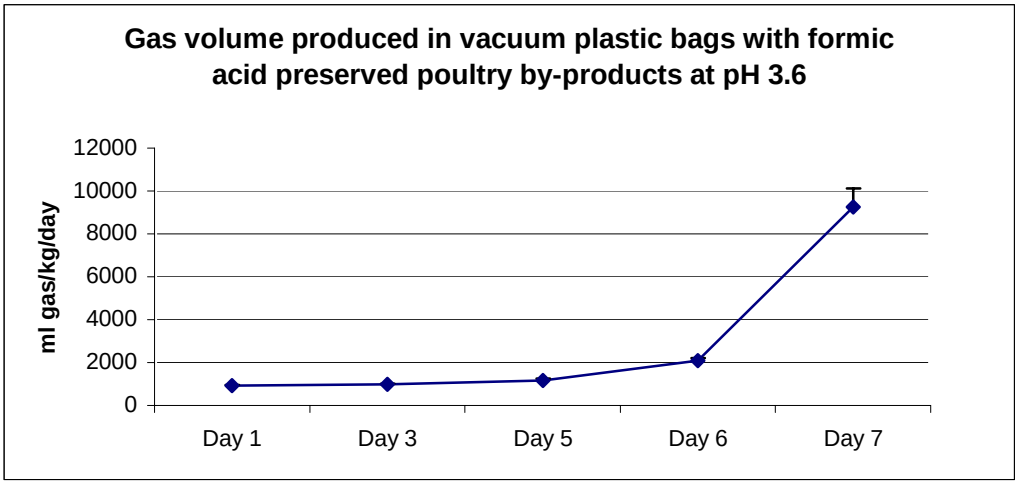


Figure 2. Gas volume produced from formic acid preserved poultry by-products in vaccum plastic bags during seven days at room temperature



In aerobic conditions the rates were 10- 30 times lower compared with the anaerobic conditions, varying from 50-250 ml/kg/day. The rates increased exponentially with time resembling a bacterial growth curve as shown for two cases (Figure 1 and 2). Extrapolation of the observed gas production rates under anaerobic conditions to tank load dimensions of real industrial conditions, are in agreement with the expansion volumes observed in practice.

Analyses of hydrogen gas and volatile compounds

Gas from the flask contained small concentrations of hydrogen gas after four days, but during the last three days the hydrogen concentration in the flasks increased 3-4 times. In the anaerobic bags the hydrogen concentrations were even 2-3 times higher than in the flasks after 7 days seven. Since *Clostridia* may consume formate as substrate under anaerobic conditions, producing hydrogen and carbon dioxide, this is the most likely explanation for the detected hydrogen. After heat-shocking the samples, still a considerable hydrogen production was detected, indicating germination and regrowth of *Clostridia.* Results from the GC/MS analysis showed that the major compounds, besides from formate, were dominated by typical volatile secondary metabolites, generated by *Clostridia*, which is another evidence for the dominance of these strains in the sample. In contrast to our result, Urlings et al. (1988) found that *Lactobacilli*, *Enterobacteriaceae* and *Mesohilic aerobic bacteria* were considerably more dominating than *Clostridia* in a mink feed mainly based on poultry by-products. However, in their study no addition of organic acids that could have had bactercidal effect was applied. In our experiment, addition of formic acid had probably changed the growth and survival conditions in favour of *Clostridia*.

The inhibitory effect of sodium bisulphite

Repeated storage experiments with and without addition of sodium bisulphite indicated no consistent effect (results not presented). There was a tendency that in cases of low rates of gas production there could not be demonstrated any effect by adding this salt. However, at substantially higher rates of gas production, there was a positive effect by adding sodium bisulphite, suggesting an inhibitory effect on gas production. The reason for the findings at low gas production rates may be due to the fact that these were cases which could not be considered similar to the "boiling" phenomena. Urlings et al. (1988) observed reverse effects on bacterial growth when adding 0.1 % sodium

metabisulphite to poultry by-products. The reason for this difference to our study is probably that we used a higher concentration (0.5 %) of sodium bisulphite.
Sodium bisulphite is commonly used for preservation in fish silage in Denmark and for preservation of poultry by-products in The Netherlands. This year's practical experience from Norway, indicates that sodium bisulphite (0.2 %) may have effect against "boiling" of formic acid preserved poultry by-products, but more information is provided. One negative factor to be aware of when using feed ingredients preserved with sodium bisulphite is that it destroys thiamine as discussed by Jensen & Jørgensen (1975). Thiamine deficiency problems is, however, unlikely since thiamine supplementation to fur animal feed is much higher than the requirement.

Conclusions

Results from this experiment, show strong evidence that *Clostridia* may be responsible for the significant gas production occurring in acid preserved poultry waste. Accordingly, it may be concluded that hydrogen gas production derived by *Clostridium* bacteria play an important role in the over-running, the so called "boiling" phenomena, of acid preserved poultry slaughter by-products. The findings suggest that poultry slaughter by-products should be handled with care, since it may represent a health risk during treatment and transportation related to the exposure to pathogenic *Clostridia* and secondly, because of the occurrence of significant amounts of hydrogen, which is highly explosive. The use of additives as sodium bisulphite, which may have an inhibitory effect on gas generation can be a future solution to the "boiling" phenomena encountered in formate preserved poultry by-products.

Acknowledgements

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III – 12 P

The importance of protein for young minks fed with dry food

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Abstract
The aim of this research is to determine the main parameters of nitrous exchange in young pastel minks.
The mink were fed only with dry full ration granulated feed. The results of 4 balance experiments held in June, September and November (48 minks) were used. Calculations were made using the method of extrapolation by the equation of regression y= bx + a. Endogen urinal nitrate turned out to be 1,0-1,1 per kg of metabolic mass ($W^{0,75}$). The level of faecal metabolic nitrate was 0,258 g in 100 g of eaten dry food.
It was determined that to support zero balance of nitrate, young minks need 30,2 g of protein per kg of live weight in July and 13,0-14,0 g during September to November.

Introduction
It was decided to feed animals according to the norms of exchange energy and digestive protein to increase productivity and economical effictiveness of mink breeding.
This norms had been worked out and used successfully in minks fed with wet fodder.
For rational full ration granulated feed usage the norms have to be defined more precisely.
Earlier we had detected the norms of nitrogen that have to be lost in young minks with full dry ration in period from July till November (Kulikov, 2001).
Endogen nitrogen that is being excreted with urine in all periods 1,0 – 1,1 g per 1 kg. of exchanging weight ($W^{0,75}$). The exchanging nitrogen of faeces is 0,258 g per 100 g eaten dry fodder.
The norms of exchanging energy (EE) and final live weight for young minks of different ages (June - November) were calculated (Kulikov, 2002).
Considerable scientific and practical interest for us is in researching of young minks digestive protein need.

Materials and Methods
The experiments were held in V. Afanasiev research Institute of Fur Bearing Animals and Rabbits "Rodniki", Moscow region, Russia.
From July until slaughtering isolated young minks were fed Only with dry full ration granulated mixed fodder and had free access to water. Granules maintained fish flower, extruded grains, melted oil, stern barm, dry milk, sunflower oil cake, bone flower, mineral and vitamin additions in different ratio.
On 100 kKl EE digestive g.: protein – 9,00; fat – 4,35; carbohydrate – 4,70. Periodically mails were put in special cages that allow calculate amount of eaten food, excreted faeces and urine. Preliminary adapting period lasted for 3 days and registration for 4 days. Analyses of eaten food, excreted faeces and urine let us calculate a nutrient balance and ability to be digested it in minks.
The level of nitrogen sediment in the minks body in different periods of growth was calculated from practically accepted nitrogen and excreted with faeces and urine.
According to the linear regression equation y = bx + a it was calculated the dependence between sedimented (x) and eaten(y) nitrogen.
During the extrapolation to the zero mark of nitrogen sedimentation in the body(x) the equation shows needed nitrogen amount (protein = Nx6,25) to supply zero balance(taking into account exchanging and endogenous nitrogen).

Results of the research
In all it was studied nitrogen balance in 40 minks with the age of 3, 5 and 7 months.
Nitrogen balance data were statistically worked up. According to the results it was calculated the connection between sedimented and eaten(digested) nitrogen amount (Table 1).

Table 1 Season variation of nitrogen sedimentation in young minks

Indexes	Growing period		
	July	September	November
N of minks	12	9	19
Live weight, g	1032± 35	1460± 59	1875± 35
X, sedimented nitrogen g/animal/day	0,933± 0,382	1,902± 0,442	0,861 ± 0,089
У,eaten nitrogen g/animal/day	5,567± 0,445	5,596± 10,661	5,653± 0,311
Coefficient "в"	0,682	1,355	1,575
Coefficient "a"' g/animal/day	4,989	3,019	4,298
Correlation coefficient, r	0,586	0,906	0,4 –51
P<	0,05	0,001	0,05

We have got following data: July y = 0,682x + 4,989; September y = 1,355x + 3,019; November y = 1,575x + 4,298. Correlation coefficient is true (P<0,001 – 0,05).

In experiment animals got various amount of food with different chemical composition, in general actually eaten nitrogen is 5,60 – 5,67 g/head/day.

However it's usage for sedimentation differs a lot: 16,5 % in July; 34,0% in September and 15,2% in November. Thus on 1 g of sedimented nitrogen minks need 5,67; 4,37 and 5,87 g digestive nitrogen per a head per day in July, September and November.

Estimated digestive protein (N x 6,25) for zero balance is 30g in July, 13 – 14 in September – November (on 1 kg of life weight) with indispensable optimal fat-carbohydrate ratio and EE entry.

It is known that protein assimilation depends on its quality and biological value.

When there's no growth of young minks we detect nitrogen loss (endogenous and exchanging), besides the body uses it for energy (to compensate lack of carbohydrate and oil energy).

Thus, the connection between life weight growth and eaten protein lets us to count its 'the life needed' amount and growth.

Experimental data are presented in table 2, where x – a day growth, y – eaten digestive protein per head a day. During the extrapolation to the growth zero mark: the coefficient 'a' is a life needed protein (including exchanging and endogenous), 'b'- g of protein for 1 g of growth.

Equation of the regression: For July y = 0,421x + 22,4; For September y = 0,513x + 30,0; For November y = 0,499x + 25,2.

In November minks had finished growth and moulting, it reflexes in lower digestive protein need for life support (per 1 kg exchange weight).

Then we used approximate dynamics of young minks life weight on the beginning and the end of month (Pereldik N.Sh. at al., 1987) to calculate the minks' need in digestive protein according to the planned life weight to the 1st November. The example is in table 3.

Some growth of the need in September in comparison with the recommended by Pereldik N.Sh. at al. (1987) (on a 100 kKl of exchange energy 8 –9 g. of digestive protein) connected with the loss of biological value of dry protein for minks.

In September the growth of muscles stops but the moulting and winter fur forming is going on. It increases the need in qualitative protein.

In August and October the minks need may be defined with the methods of interpolation and previous and next months.

Thus, the norms of minks need in digestive protein are being proposed with the accounting of its' life weight and the period of growing. The norms may be used as a base for mixed fodder ration calculation for minks.

Table 2 Calculation of the young minks need in digestive protein for life and growth.

Data	Growth period		
	July	September	November
N of minks	12	12	18
Average live weight (W), g	1032± 35	1476± 85	1907+ 45
Exchange weight, kg ($W^{0,73}$)	1,023	1,329	1,602
x, growth, g/animal/day	11,9± 5,4	-4,6± 2,8	4,6+ 2,2
y, eaten protein g/animal/day	27,5± 2,7	27,6± 2,8	28,1± 1,5
Coefficient 'в'	0,421	0,513	0,499
Coefficient 'a' g/animal/day	22,4	30,0	25,8
per 1 kg live weight	21,7	20,3	13,5
Per 1 kg exchanging energy ($W^{0,73}$)	21,9	22,6	16,1

Table 3 Calculation of need in digestive protein in young minks final live weight 2,3 on 1.11.

Data	Growth period					
	01.07	31.07	01.09	30.09	1.11	30.11
Planed live weight, kg (W)	0,81	1.35	1,80	2,21	2,30	2,23
Exchanging weight ($W^{0,73}$), kg	0,85	1.24	1,54	1,78	1,84	1,80
Average day growth, g	17,7		13,6		-2,3	
Life needed protein, g/animal/day	18,6	27,2	34,8	40,2	29,6	29,0
Protein for growth, g	7,45		6,98		-1,15	
General protein need, g/animal/day	26,1 — 34,7		41,8 — 47,2		28,8 — 27,9	
Per 100 kKl of exchange energy, g	9,1		10,9		7,7	
in % from exchanging energy	41,0		49,0		34,7	
According to Pereldik N.Sh. at al. (1987)						
Protein need, g per animal/day	27,6 — 31,1		37,2 — 41,9		28,4 — 32,0	

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III – 13 P

Nourishing qualities of APK concentrate for minks' cubs

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Abstract
The exchange of expensive protein in traditional feed composition by the protein of mixed feed, reduces substantially the feeding costs for mink. This work shows the results of a balance experiment on the use of nourishing substances APK concentrate, which was fed to minks kits of the colour type demi-buff. APK concentrate is a homogenous mix of crumbled feed of both animal and vegetable origin. Protein of APK consists of protein of animal origin at the rate of about 80%. Based on biochemical analysis, 100 g of APK concentrate contains, (g): dry substance - 90,2; organic substance - 85,3; raw protein - 27,3; raw fat - 2,5; carbohydrates - 55,5; including raw cellulose - 10,0; ashes - 4,5; gross energy - 1706 KJ. In the balance experiments coefficients of digestion of nourishing substances of APK concentrates in mink kits (%): dry substance - 66,0; organic substance - 67,0; protein - 68,4; fat - 70,1; carbohydrates - 62,4; energy - 85,8.APK concentrate contains per 100 g digestible nourishing substances (g): protein - 18,7; fat - 1,8; carbohydrates - 34,6; metabolic energy - 1016 KJ.

Introduction
Nowadays in Russian mink selection there are high expenses spent for feeding. They reach 70% from the cost of skin production.
One of the ways of reducing the price of animals' feedings is the replacement of a part of deficit and expensive animal protein in the diet with a protein of mixed fodder - concentrates.
In domestic fur farming it is recommend to use dry forages with up to 30 % of protein. It is mostly fish flour or unconventional forages. But a fish flour is quite an expensive product (G.S.Taranov, 1979), and unconventional forages have low nutritional value (N.E.Kulikov, 1999).
For reducing the price of minks' feedings and preservations of their high productivity there were developed a compounding and the technology of a mixed fodder - concentrate APK production.
The APK concentrate represents a homogeneous multicomponent mix of the crushed fodder of animal and vegetable origin, granulated and crushed. In structure of protein of APKconcentrate 80 % of a protein is of animal origin.
In the work the results of studying of nutritional value of APK concentrate for young growth of minksare represented.

Materials and Methods
Amino acid structure of APK concentrate was determined with the use of amino acid analyzer « Eppendorf-biotronics LC-3000 ».
Nutritional value for young growth of minks of APK concentrate was studied in balance experiment Kladovshikov, Samkov, 1975).
Experimental animals were put in the special exchange cages allowing separately to collect excrements and urine and providing absence of forages losses. The preliminary period for habituation took 3 days, registration - 4 days.
In experimental and control group there were 4 males of wild type brown mink in each.
On the tabulated data the daily diet of each animal contained 300 kcal
(1257 MJ) of exchange energy (for I control group - 260 g in crude weight; for II experimental - 180 g).
In the day of preparation of feed mixes there were taken their average tests for the definition of a chemical compound. In the beginning and the end of the experiment for all animals there was determined alive weight to 10g.
During the preliminary and registration periods minks daily at the same time received the defrozen portions of feed mixes. Excrements and urine were collected 3-4 times a day, preserved with 10 % of solution of hydrochloric acid (HCl). After the registration period there was defined weight of excrements and volume of urine, allocated by each animal. Up to the analysis urine was kept in a refrigerator, and average test of excrements were dried up to constant weight at 65°. The analysis of chemical compound of APK concentrate, feed mixes and minks' excrements were carried out in biochemical laboratory with the use of standard methods (Lukashik, Tashilin, 1965).

Results and discussion

According to the results of amino acids analysis, 100 g of APK concentrate contains, mg %: triptofan - 420; lizin-1950; histidin - 570; arginin-1280; treonin-1430; cistin-320; valin-1290; metionin-540; isoleycin-1230; leycin-1230; fenilalanin-1230.

The results of chemical compound of feed mixes APK concentrate are represented in table 1.

The results of balance experiment for each group of minks are represented in table 2.

According to the majority of investigated parameters there was marked statistically authentic difference between groups: at a zero gain of live weight in control group less excrements were allocated, in experimental group the maintenance of water in excrements is more upon identical allocation of water within urine, so the ways of allocation of water from the organism are redistributed: minks of II experimental group allocate the largest part of water (and waste products of a metabolism) with excrements, when in calculations on 100 g of dry substance of the consumed forage allocation of water in both groups was identical.

It indicates specific action of a APK concentrate on water balance which is more intense in group П.

By results of the analysis of excrement samples from each experimental mink there were calculated factors of the digestion of nutrients from diets (tab. 3).

Table 1 Chemical compound of provenders

Indexes	g/100g dry substance		
	1- control	II – experiment	APK concentrate
Dry substance	31,90	42,70	90,20
Organic substance	79,60	81,60	85,30
Grude protein	31,00	26,00	27,30
Grude fat	15,20	11,40	2,54
Carbohydrates	33,40	44,20	55,50
Including raw cellulose	1,62	6,57	9,97
Ash	13,81	11,14	4,5
Gross energy kcal|MJ	458,0/1919,0	437,7/1834,0	407,3/1706,6

Table 2 Water balance in experimental minks

Treatment	Groups		The degree of validity P <
	I-control	II-experiment	
Increase of live weight	0,0±1,0	-9,3±3,6	no
Food eaten, g/day	225,0	151,9	-
The amount of water in food	68,0	57,3	-
Excrements allocated, g/day	76,3±4,4	150,2±2,2	0,001
Dry substance in excrements, %	34,6±1,6	23,8±0,1	0,001
Water allocated, ml/day	120,6±3,7	173,7±10,5	0,01
Including within urine, ml/day	77,3±7,4	59,3±9,3	not
Including within excrements, ml/day	50,1±4,0	114,4±1,6	0,001
Water allocated on 100 g of dry substance, ml/day	180,8±5,6	168,8±10,7	no

The introduction of APK concentrate has seriously enlarged the digestion of dry substance and minerals, but brings down the digestion of organics, lipids, carbohydrates and energy. The digestion of protein practically has not changed. It is connected, probably, with its high (about 50 % from dry substance) inclusion in a diet.

The balance of nitrogen in minks was studied with the purpose of studying influence of APK concentrate on the animals' albuminous (tab. 4).

Minks of experimental group digested more nitrogen, than of control group. Experimental minks had authentically more nitrogen. Precipitation of nitrogen testifies to it in relative units (%), both to accepted, and to the digested nitrogen. The precipitation of nitrogen in percentage to digested characterizes quality of protein. It is called « seen biological value of a protein » (P. Mc. Donald at all. 1970).

True biological value of protein (IBC) was counted by formula:

$$IBC = \frac{(AKo + AMe + KPA + (PA - AK - AM}{PA - AK + AKo} \times 100\ \%$$

Where: AK - nitrogen of excrements; AM - nitrogen of urine, PA - precipitated nitrogen, AKo - exchange nitrogen of excrements, AMe - endohene nitrogen of urine, KPA - skin losses of nitrogen by hair, epithelium, perspiration (it is difficultly taken into account).

Biological value of protein of a experimental and control groups' diet is submitted in table 5.

The tested biological value of protein in a diet with APK concentrate appeared to be authentically above, than in a typical control diet. Such parameters as productive nitrogen, use of pure fiber and truly precipitated nitrogen also were higher. It testifies to the greater adequacy of protein of diet with APK concentrate to the requirements of young minks.

Knowing the rate of digestion of nutrients of the basic ration, the digestion of nutrients of the APK concentrate have been calculated with the use of differential method (tab. 6).

Thus, as a result of the work, there was established the nutritional value of APK concentrate which should be used at drawing up of diets of feeding for young.

Table 3 Digestion of nutrients from diets,%

Indexes	Groups		The degree of validity, P<
	I – control (main ration)	II-experiment (main ration+APK)	
Dry substance	60,8±0,8	65,3±0,6	0,01
Organic substance	71,0±0,9	67,7±0,7	0,05
Protein	64,6±1,7	67,8±1,7	not
Fat	94,0±0,3	89,7±0,7	0,01
Ash	7,2±2,5	53,6±2,6	0,001
Carbohydrates	66,0±0,8	61,8±0,4	0,01
Energy	74,3±0,9	70,8±0,8	0,05

Table 4 The balance of nitrogen in minks

Indexes	Groups		The degree of validity, P<
	I - control	II-experiment	
Eaten, g/day	3,31±0,01	4,29±0,03	0,001
Allokated with excrements, g/ day	1,17±0,06	1,38±0,07	not
Allocated with urine,g/ day	2,29±0,16	1,31±0,17	0,01
Digested,g/ day	2,14±0,06	2,91±0,09	0,001
Precipitated, g/ day	-0,15±0,19	1,6±0,20	0,001
Precipitated to eaten,%	-4,6±5,7	37,2±4,5	0,01
Precipitated to digested,%	-7,5±8,6	54,8±6,4	0,01

Table 5 Biological value of protein in minks

Indexes	Groups		The degree of validity, P<
	I - control	II-experiment	
The exchange nitrogen of excrements,g	0,17	0,26	-
The endohene nitrogen of urine,g	1,78±0,10	1,67±0,19	not
Productiv nitrogen,g	1,97±0,06	2,64±0,09	0,05
Realy digested nitrogen,g	2,31±0,06	3,17±0,09	0,001
Real biological value of protein,%	77,0±8,2	110,5±9,1	0,05
Truly digestability of protein,%	69,50±1,60	73,25±1,65	not
Use of pure protein,%	53,7±6,4	81,1±7,5	0,05
Use of nitrogen,%	2,67±0,32	3,38±0,31	not

Table 6 Digestabili of nourishing substances of APK cobcentrate

Treatment	Dry substance,g	Organic substance,g	Protein, g	Fat,g	Carbohyd-rates,g	Energy, Kcal/MJ
Factors of digestability, %	66,0	67,0	68,4	70,1	62,4	85,8
Presence digestible substances,%			18,7	1,78	34,6	242,6/1016,5

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III – 14 RP

Individual *ad libitum* feeding of male + female pairs of mink kits during the growth period increases weight gain and feed efficiency

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Abstract
Most farmed mink in Denmark are fed close to the average *ad libitum* intake during the growth period, based on feed leftovers at farm, shed or row level. Variation in voluntary feed intake between male + female pairs is ignored apart from the distribution of feed leftovers to cages without feed left over from the day before. Technological development has facilitated individual feeding and thus the possibility for true *ad libitum* feeding of mink. The variation in voluntary feed intake was studied in 174 male + female pairs of Scanbrown mink kits during 16 weeks from 11 weeks of age in July to 26 weeks of age in November. The feed allowance was adjusted Tuesday and Friday based on feed leftovers registered Monday + Tuesday and Thursday + Friday. The average feed intake was 44.3 kg per pair of kits equivalent to 395 g per day. The average weight gain was 2490 g per male + female pair. The average feed efficiency (g gain/kg feed) was 56 g/kg and in general, the feed efficiency increased with weight gain. The average difference between the lower and upper quartile of feed efficiency was 29% equal to an estimated difference in feed consumption of 15 kg for both quartiles to reach the average weight gain of 2.5 kg during the 16 weeks of growth. Compared to the normal feeding practice, individual *ad libitum* feeding provides the opportunity to utilise the full potential of the mink kits for growth and feed efficiency, and thereby for effective selection for these traits.

Introduction

Most farmed mink in Denmark are fed close to the average *ad libitum* intake during the growth period. The regulation of the daily feed allowance is based on feed leftovers from the day before at farm, shed or row level. Variation in voluntary feed intake between individual male + female pairs is to a large extent ignored apart from the distribution of feed leftovers to cages without feed left over. During the last decades mink farmers have selected for body weight in order to maximise their economic outcome. At the same time, the amount of feed and thus the main cost per pelt produced has increased accordingly, (e.g. from 35.5 kg in 1995 (Møller, 1998) + to 38.1 kg in 2003 (Møller, 2004)). Although the possibility to increase the feed efficiency has been documented (Berg & Lohi, 1992; Sørensen, 2002), the potential for reducing the feed cost per pelt produced has not been applicable in practice. Technological development of hand held computers (PDAs) has made individual feeding on each cage possible e.g. based on barcodes or other types of identification, and thus facilitated true *ad libitum* feeding of mink.
In a four-year experiment, the possibility of increasing the body size as well as the feed efficiency under different feeding strategies is investigated. Three lines of male+female pairs are fed either according to normal farm practice, restricted (10% below farm practice) or *ad libitum*. The present paper describes the results in terms of weight gain, voluntary feed intake and feed leftovers of the first growth season, with special emphasis on the *ad libitum* fed line.

Material and Methods

Animals: The mink in the present feeding experiment are the base population in a selection experiment established by crossing two Scanbrown lines previously selected for high November weight, and for high weight as well as litter size (Nielsen et al., 2004). Three selection lines with different feeding strategies: Farm Feeding (FF), *Ad Libitum* (AL) and Restricted Feeding (RF) were established in December 2002 each with 100 females. The feeding strategies were applied to 198, 192 and 192 male + female pairs of kits from line FF, AL and RF, respectively, in the period from weaning in July to live animal grading in November.

Feeding: The animals were separated in male + female pairs and weighed within three days in late June and the test feeding was commenced in early July on the same day for all three lines. The mink were fed a commercial standard diet delivered daily from the local feed kitchen. The feed allowance was regulated by feed leftovers registered at 9 a.m. 2 hours before the daily feeding. The leftovers were graded as no feed left, Grade 1: less than 5 square inches left (what will be eaten before next feeding) and Grade 2: more than 5 square inches left. Leftovers were registered Monday + Tuesday and Thursday + Friday and regulated Tuesday and Friday. The control line FF was fed the same amount on each cage and feed leftovers were fed to cages with no feed left, according to normal Danish farm practice. Occasional excessive feed leftovers were collected and weighed. The feed allowance to line FF was regulated in the following manner:

- Grade 2 leftovers on less than 33% of the cages both days: The allowance was increased by 20 g per cage per day.
- Grade 2 leftovers on more than 66% of the cages both days: The allowance was reduced by 20 g per cage per day.

Line RF was kept under a restrictive feeding regime and fed 90% of the amount of feed offered to the control line FF. Occasional feed leftovers were collected and weighed.

Line AL was fed *ad libitum* at cage level and feed leftovers were collected before feeding and weighed each day. The feed allowance was regulated in the following manner:

- No leftovers one or both days: The allowance was increased by 20 g per cage per day.
- Grade 2 leftovers both days: The allowance was reduced by 20 g per cage per day.

An overview of the regulation of the feed allowance in each line is given in Table 1.

The feed allowance at cage level in all three lines was controlled by a computerised feeding machine regulated by a Palm Pilot (the Farm Pilot used in the breeding programme from Copenhagen Fur Center). By reading a bar code on each cage the Individual Feeding programme developed by "Tved Maskinbyg" pumped out the pre-programmed daily ration on each cage.

Data: The kits were weighed individually every three weeks from separation to live animal grading in November. Eight weights were recorded for each animal. The feed allowance was recorded as the amount of feed registered by the Farm Pilot. The Farm Pilot feeding was measured to give on average 8.4 g or 2.2% more feed than programmed. If programmed to feed 380 g the feed allowance was measured to 388.4±26.9 g. However, if the actual amount of feed was more than 20 g above or below the pre-programmed amount, the actual amount was registered by the Farm Pilot and used in the calculations.

Feed leftovers on each cage in each line were observed most weeks on Monday, Tuesday, Thursday and Friday and a total of 82 observation days were obtained. The total feed consumption for each cage was calculated as the total feed allowance in the period minus the weight of collected feed leftovers in the period attributed to each cage relative to the number of feed leftovers observed. For the feeding experiment, recordings from 16 weeks (week 30 to 45, 11 to 26 weeks post partum) were used for data analysis from 184, 174 and 178 pairs of kits in line FF, AL and RF, respectively.

Statistics: The average weekly feed consumption in each line was calculated as the average feed allowance minus the weekly average of leftovers per cage (male + female pairs of kits). The average weight gain and standard deviation from 11 to 26 weeks of age was calculated for male + female pairs of kits in each line. The feed efficiency was calculated as the weight gain in g divided by the feed consumption in kg for each pair of kits. The average feed efficiency and standard deviation from 11 to 26 weeks of age was calculated for each line.

Table 1. Feed regulation of male + female pairs of kits from 11 to 26 weeks of age in line FF on farm feeding, line AL on *ad libitum* feeding and line RF on restricted feeding.

Line	N	Feed leftovers registered	Observation	Feed allowance
AL	174	Mon+Tue and Thu+Fri	no leftover one or both days > 5 " leftover both days	increased 20 g decreased 20 g
FF	184	Mon+Tue and Thu+Fri	> 5 " leftover on < 33% of the cages > 5 " leftover on > 66% of the cages	increased 20 g decreased 20 g
RF	178		none	90% of line FF

Results and Discussion

The three different strategies for regulating the feed allowance succeeded as planned in the sense that the average feed allowance to line AL and RF was 10 % above and below the feed allowance for line FF, respectively, almost every week from 8 to 29 weeks post partum. On average 4.7 kg of feed leftovers were collected per cage equal to 42 g daily in line AL while excess of feed very rarely had to be collected from line FF or RF. When these feed leftovers were subtracted, the weekly feed consumption in line AL was only a few % higher than in line FF (Fig 1). The kits in the three lines responded to the different feeding regimes by significant differences in weight gain (Fig 2).

Figure 1. Average daily feed consumption by male+female pairs of kits from 8 to 29 week of age in line FF on farm feeding, line AL on *ad libitum* feeding and line RF on restricted feeding

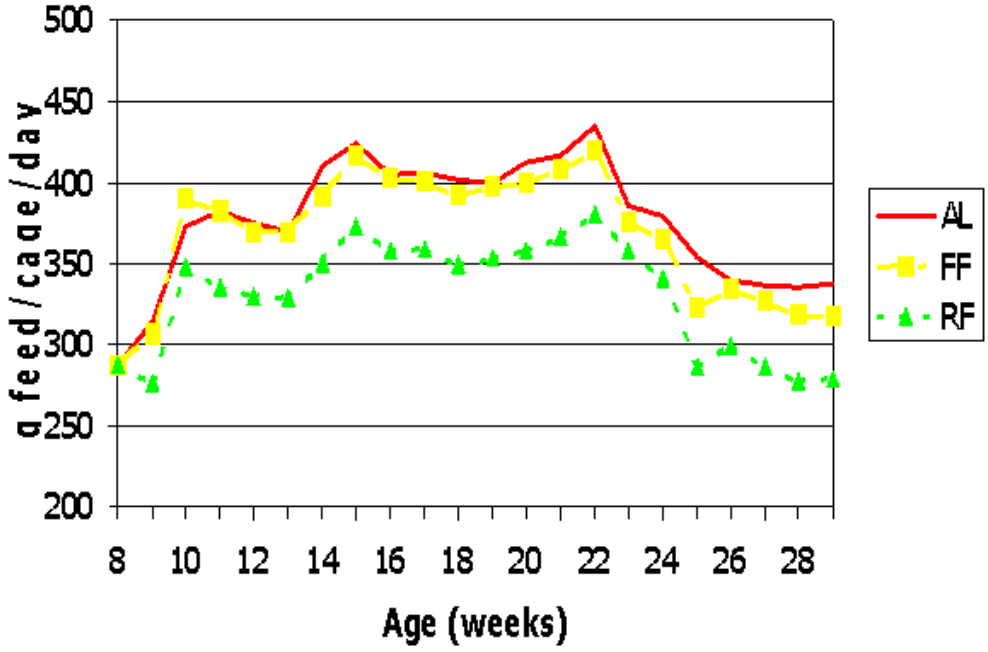
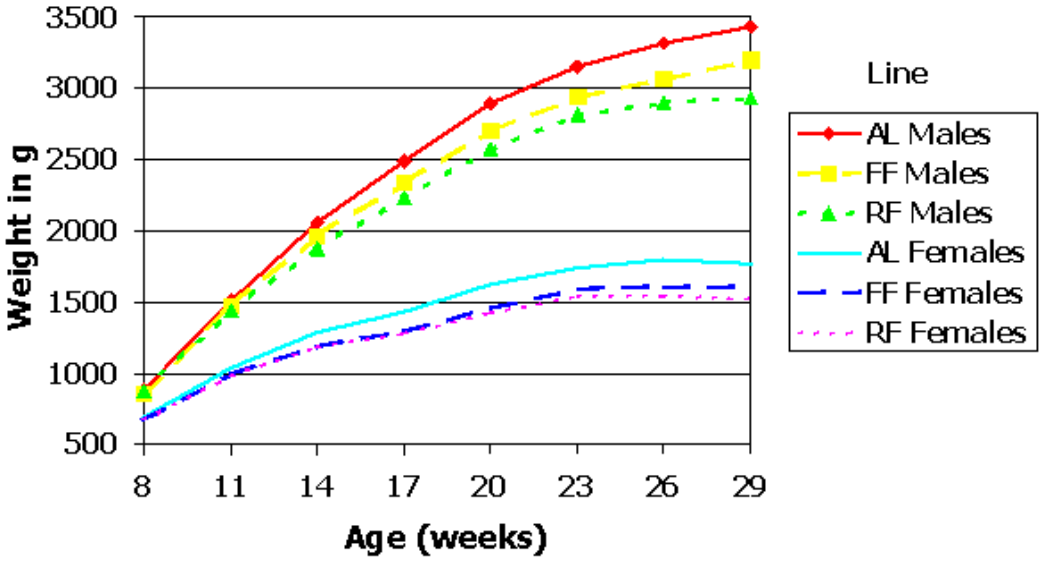


Figure 2. Weight development of kits from 8 to 29 weeks of age in line FF on farm feeding, line AL on *ad libitum* feeding and line RF on restricted feeding



The average feed consumption in all lines from 11 to 26 weeks post partum was 42.06 kg per male+female pair of kits. This resulted in a weight gain of 2239 g and thus an average feed efficiency of 53.13 g of gain per kg feed consumed. The individual *ad libitum* feeding resulted in the highest weight gain, feed consumption and feed efficiency. The restricted feed allowance in line RF reduced the weight gain but at the same time increased the feed efficiency compared to line FF (Table 2).

The application of individual ad libitum feeding in line AL increased the weight gain by 276 g (or 12%) by use of only 1.1 kg (or 2.6%) of extra feed, compared to line FF resulting in a very high average efficiency of 244 g of gain per kg extra feed consumed, due to the individual *ad libitum* feeding. An explanation for this can be that the more appropriate distribution of almost the same amount of feed gave the mink in the AL line the opportunity to utilize their potential for growth better than the mink in the FF line. This further implies that under Farm Feeding practice, pairs of kits with low appetite are fed *ad libitum*, pairs of kits with average appetite are fed close to *ad libitum* while pairs of kits with large appetite are fed a restricted amount of feed, and the distribution of feed leftovers does not compensate the pairs of kits with large appetite.

The feed efficiency ranged from 24 to 83 g gain/kg feed for individual male+female pairs of kits and in all three lines there was a substantial variation in feed efficiency, as the coefficient of variation was 18% in line FF, 13% in line AL and 13% in line RF. The larger variation in line FF may be due to how the farm feeding is experienced by the mink, depending on their appetite as suggested above. The smaller variation in line AL and RF may then be explained by the fact that all the mink in these lines experience either *ad libitum* or restricted feeding, respectively. This explanation is supported by the fact that grade 2) leftovers were observed on average 31 ± 15, 37 ± 5 and 6 ± 7 times in line FF, AL and RF, respectively, out of 82 observation days. In line FF and RF the same cages had grade 2) leftovers most days while grade 2) leftovers were more randomly distributed among cages in line AL, due to the strategy for regulation of the feed allowance.

In the *ad libitum* fed line the average feed efficiency in the best 25% of the line was 65.56 g gain/kg feed consumed compared to 47.38 in the lowest 25%. This difference of 28% in feed efficiency between the upper and lower quartile was equal to an estimated difference in feed consumption of 14.6 kg (38.0 kg in the upper quartile and 52.6 kg in the lower quartile) if the mink pairs from each quartile should reach the average weight gain of 2490 g in the *ad libitum* fed line. The average weight gain and feed consumption in the upper and lower feed efficiency quartiles, as well as in the middle 50% of line AL is given in Table 3.

The feed efficiency in line AL was highly correlated to weight gain (r=0.91) and moderately correlated to feed consumption (r=0.37). The high correlation between feed efficiency and gain confirms the results by Berg & Lohi (1992) measured at group level and by Sørensen (2002) measured over four weeks in July.

Table 2. Average weight gain, feed consumption and feed efficiency of male + female pairs of kits from 11 to 26 weeks post partum in / of age in line FF on farm feeding, line AL on *ad libitum* feeding and line RF on restricted feeding.

Line	N	Weight gain, g	Feed consumption, kg	Feed efficiency, g/kg
AL	174	2490 ± 438	44.26 ± 3.50	56.05 ± 7.3
FF	184	2214 ± 411	43.13 ± 0.22	51.31 ± 9.4
RF	178	2020 ± 253	38.76 ± 0.56	52.13 ± 6.6

Table 3. Average weight gain, feed consumption and feed efficiency of male + female pairs of kits from 11 to 26 weeks of age in the upper and lower feed efficiency quartiles as well as in the median 50 % of line AL on *ad libitum* feeding.

Quartile of line AL	N	Weight gain, g	Feed consumption, kg	Feed efficiency, g/kg
75%	43	3008 ± 350	45.82 ± 3.44	65.56 ± 4.6
50% Median	87	2464 ± 246	44.17 ± 3.39	55.74 ± 2.7
25%	44	2035 ± 222	42.91 ± 3.23	47.38 ± 3.3

However both authors found a negative correlation between feed consumption and feed efficiency. The large variation in feed consumption in line AL that is only moderately correlated to feed efficiency indicates that it should be possible to increase the feed efficiency without increasing the feed consumption. A relatively high heritability for feed efficiency in July of 0.30 has been calculated by Sørensen (2002). If the same is true for the entire growth period feed efficiency can be rapidly improved by selection, when the practical management tools for registration of the feed consumption and controlling the feed allowance are available.

The large variation in weight gain has for many years been exploited in selection programmes while selection for feed efficiency has not been performed due to lack of management and feeding technology. Despite the positive genetic correlation between weight gain and feed efficiency (Sørensen, 2002), selection for weight under farm feeding conditions may not be an effective way to improve feed efficiency due to different feeding regimes experienced by the mink. Individual ad libitum feeding during the growth period provides a homogenous environment allowing the full expression of the growth potential, and may thereby facilitate improvement of feed efficiency in mink production.

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III – 15 RP

Effects of dietary methyl donors on health status in blue fox (*Alopex lagopus*) vixens given a low protein diet during body fat mobilisation

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Abstract

The feeding trial was carried out on 60 blue fox vixens before the breeding season (age six months at the beginning of the trial). The aim of the present study was to find out to which extent low dietary protein supplemented with methyl donors affects fat and liver metabolism and body weight reduction in the period November-March. Treatments were: control (blue fox feed, 20% protein from ME), Alimet® (control feed with supplemental methionine hydroxy analogue), betaine (control feed with supplemental betaine), choline (control feed with supplemental choline), methionine (control feed with supplemental DL-methionine) and positive control (commercial blue fox feed, 35 % protein from ME). Body weight loss, blood parameters, liver histology, enzyme activities, and cortisol:creatinine ratio of urine were measured. In conclusion, the experiment showed that methyl donors had no effect on health status on blue fox vixens given a low protein diet during the body fat mobilisation period. Generally, the low dietary protein level was reflected in some health parameters, but no animals revealed symptoms of malnutrition. The results demonstrated that the blue fox have extreme abilities to maintain health on a suboptimal diet during body fat mobilisation.

Introduction

Farmed blue foxes are typically very obese before the onset of the breeding season. First year breeders often have higher body weights than older females. In practice, high body weights have been shown to be negative for the reproduction result in blue fox. Therefore they must reduce body weight during November to March to be prepared for the mating season. This probably causes stress to animals and affects on liver metabolism. In the liver many metabolic reactions take place, such as detoxifying functions, secretion of bile etc. Animals in negative energy balance may have fatty liver (*hepatic lipidosis*). In chronic cases chirrosis of the liver can ensue. Thus, blue fox vixens for breeding should be selected earlier in the autumn than at present to avoid excessive obesity during the breeding season.

Choline, betaine and methionine have different functions in the metabolism, except they can all act as methyl donors, needed in fat metabolism and to prevent fatty liver. While choline and betaine are classified as vitamins/precursors, methionine is an amino acid. Fatty liver is common finding in fur animals at post mortem investigations. Fatty liver may be the primary or secondary cause of death. Fatty liver appears mostly in the spring among blue fox vixens.

The aims of the present study were: to find out to which extent low protein level in feed supplemented with methyl donors affect fat and liver metabolism during rapid dieting and weight declining period in winter.

Material and Methods

Experimental design and management

The experiment was carried out at the fur animal research station of Kannus, MTT Agrifood Research Finland. The experiment was conducted with 60 blue fox vixens. Vixens were six months old and average body weight was 13.4 kg (±0.9 kg SD) at the beginning of the trial. It lasted for 120 days, from November 11th 2002 to March 10th 2003. For the first four weeks the animals were fed with high-energy commercial blue fox feed (8.0 MJ/kg feed).

After December 14th (average body weight 14.5 kg ± 0.1 SD) the animals were fed with experimental diets to cause weight loss until the end of the trial (energy allowance 1.14 MJ day^{-1}). The animals were maintained according to common farming practices in wire mesh cages, one animal per cage. In the trial experimental animals were blocked according to litter, and one full-sib pairs were assigned to separate experimental groups (6 x 10 animals).

Treatments:

1. Control: blue fox feed, 20% protein from ME
2. Alimet®: control feed with methionine hydroxy analogue
3. Betaine: control feed with supplemental betaine
4. Choline: control feed with supplemental choline
5. Methionine: control feed with supplemental DL-methionine
6. Positive control: commercial blue fox feed, 35 % protein from ME

After December 14th the diets 1-5 contained Baltic herring, chicken slaughterhouse by-products, cooked and dehydrated barley, rape seed oil, molasses, oat hull meal and FPF-vitamin mix without methionine. Protein from ME was sub optimal in feeds 1-5 in order to maximize the use of proteins and methyl groups. The diets were identical and isocaloric, except for the methyl donor. Betaine, methionine, choline and methionine hydroxy analogue were added to yield the number of methyl groups in positive control. The positive control was formulated on the basis of the recommendation of the Finnish Fur Breeders' Association with added methionine (total methionine in feed 8 g/kg DM) . The experimental diets were produced in one batch and stored frozen until use. Compositions and chemical analyses of the diets are shown in Tables 1 and 2.

Recordings, chemical and statistical analyses

The animals had free access to water and were reared singly outdoors in conventional pelter cages in a two-row shed. The animals were weighed every second week during the experiment. Food consumption was measured daily. At the end of the trial blood samples were collected before the euthanasia. Blood ALT, AST, creatinine kinase, glucose, HCT, HGB, chol, total prot, RBC, triglyserides, WBG, LDL, HDL, uric acid,

Table 1. Composition of the diets 1-5 (Control, Alimet®, Betaine, Choline, Methionine) and Positive Control

Ingredients, %	Diets 1-5	Positive Control
Baltic herring	10.2	25.0
Chicken slaughterhouse offal	17.4	20.0
Barley, cooked dehydrated	20.5	7.0
Protein mix		8.0
Oat hull meal	3.1	3.0
Molasses	2.0	2.0
Rape seed oil	2.0	0.5
FPF-vitamin mix	1.5	1.5
Potassium sorbate	0.03	0.03
Vitaquine	0.1	0.1
Water	43.0	32.9

Table 2. Chemical analyses of the diets 1-5 (Control, Alimet®, Betaine, Choline, Methionine) and Positive Control

	Diets 1-5	Positive Control
Dry matter (DM), g/kg	350	355
Ash, g/kg	17	22
Crude protein, g/kg	67	121
Fat, g/kg	62	65
Carbohydrate, g/kg	204	146
MJ kg DM	15.8	16.7
Metabolisable energy (ME):		
Protein, %	20.6	34.7
Fat, %	40.4	39.3
Carbohydrate, %	39.0	26.0

ammonia, glycerol, bilirubin and creatine were analysed.

Animals were slaughtered by electrocution. The liver and adrenal glands were weighed. Liver dry matter and fat% were analysed and hepatocyte vacuolisation was evaluated. The enzyme activities of liver and kidney were determined

spectrophotometrically. Urine cortisol and creatinine were analysed at the beginning and in the end of the experimental period and the cortisol:creatinine ratio was calculated. The data was statistically analysed by the GLM procedure of SAS.

Results

Average body weight loss was approximately 29% during period November-March. Food consumption and body weight loss were not different between the experimental groups at any weight recording (Figure 1). Liver weight, liver dry matter and liver fat% were not different between groups (Table 3). Hepatocyte vacuolization was significantly lower in the positive control group compared to all the other groups (Table 3).

Blood parameters were not significantly different between groups except ALT, chol and prot (Table 4). ALT and cholesterol were significantly lower in the positive control group in comparison to the choline group. ALT was higher in choline group compared to betaine group. Plasma total protein was significantly higher in the positive control group compared to the betaine and control group.

Figure 1. Weight gain of the blue fox vixens

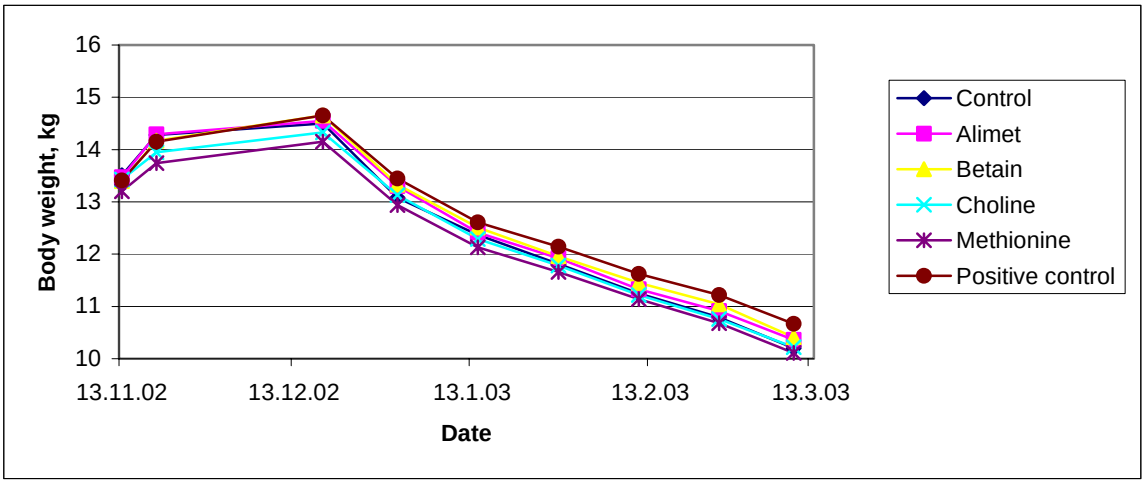

Table 3. Liver weight, liver fat-%, liver dry matter, fat% in dry matter and hepatocyte vacuolisation by arbitrary scale of 0-4 (0=nil, 1=very mild, 2=mild, 3=moderate, 4=moderately severe), (mean±SE).

	Control	Alimet®	Betaine	Choline	Methionine	Positive control	P*
Liver, g	171.0 ± 11.9	161.0 ± 5.7	170.6 ± 7.0	170.4 ± 11.8	163.5 ± 7.3	150.6 ± 4.7	NS
Liver fat %	6.2 ± 0.9	5.1 ± 0.3	6.6 ± 1.0	6.3 ± 0.9	5.1 ± 0.6	3.9 ± 0.2	NS
Liver DM	32.0 ± 0.7	31.5 ± 0.4	32.4 ± 0.6	31.9 ± 0.7	31.4 ± 0.4	31.5 ± 0.3	NS
Fat % in DM	18.9 ± 2.4	16.2 ± 0.9	19.8 ± 2.6	19.2 ± 2.4	16.2 ± 1.5	12.5 ± 0.6	NS
Hepatocyte vacuolisation	2.50 ± 0.4^{b}	2.20 ± 0.2^{b}	2.22 ± 0.3^{b}	2.10 ± 0.4^{b}	2.20 ± 0.3^{b}	0.30 ± 0.2^{a}	<0.001

** NS = not significant, P < 0.05 = significant difference between groups*

Table 4. Blood parameters of the blue fox vixens (mean ±SE)

	Control	Alimet®	Betaine	Choline	Methionine	Positive control	P*
ALT U/l	$113.9^{ab} \pm 22$	$128.5^{ab} \pm 14$	$103.6^{a} \pm 18$	$178.9^{bc} \pm 18$	$141.2^{ab} \pm 19$	$97.3^{a} \pm 14$	<0.05
AST U/l	31.8 ± 1.1	26.5 ± 2.2	26.0 ± 1.3	33.4 ± 4.2	29.00 ± 1.8	27.3 ± 2.1	NS
Creatine kinase U/l	150.3 ± 28	133.0 ± 16	79.7 ± 5.0	129.3 ± 29	122.0 ±10	103.8 ± 9	NS
Glucose mmol/l	6.90 ± 0.2	7.65 ± 0.4	7.16 ± 0.5	6.77 ± 0.3	6.79 ± 0.3	7.29 ± 0.6	NS
HCT %	51.9 ± 1.1	53.3 ± 0.9	52.1 ± 1.2	54.8 ± 1.0	52.5 ± 1.0	52.1 ± 1.3	NS
HGB g/l	163.3 ± 0.3	165.1 ± 0.2	162.1 ± 0.2	168.8 ± 0.2	164 ± 0.3	168.7 ± 0.2	NS
Chol mmol/l	$5.54^{ab} \pm 0.2$	$5.65^{ab} \pm 0.1$	$5.64^{ab} \pm 0.3$	$5.84^{b} \pm 0.2$	$5.69^{ab} \pm 0.2$	$4.87^{a} \pm 0.3$	<0.05
Total prot g/l	$54.7^{a} \pm 0.8$	$58.6^{ab} \pm 1.0$	$56.4^{a} \pm 0.8$	$58.0^{ab} \pm 1.0$	$57.4^{ab} \pm 0.6$	$60.6^{b} \pm 1.3$	<0.05
$RBC10^{12}/l$	8.97 ± 0.1	9.02 ± 0.1	9.01 ± 0.1	9.09 ± 0.1	9.01 ± 0.1	8.67 ± 0.3	NS
TG mmol/l (triglycerides)	0.69 ± 0.1	0.70 ± 0.1	0.38 ± 0.0	0.72 ± 0.1	0.59 ± 0.0	0.73 ± 0.1	NS
WBC $10^{9}/l$	6.34 ± 0.4	5.91 ± 0.6	6.90 ± 0.9	6.99 ± 0.8	7.88 ± 0.8	6.69 ± 0.5	NS
LDL mmol/l	0.24 ± 0.0	0.23 ± 0.0	0.32 ± 0.1	0.25 ± 0.0	0.24 ± 0.0	0.23 ± 0.0	NS
HDL mmol/l	4.76 ± 0.3	4.53 ± 0.3	4.86 ± 0.3	4.67 ± 0.2	4.97 ± 0.3	4.16 ± 0.3	NS
Uric acid µmol/l	23.6 ± 4.3	28.0 ± 4.1	25.5 ± 3.5	32.2 ± 4.4	18.9 ± 2.9	32.4 ± 5.3	NS
Ammonia µmol/l	229.7 ± 4.9	233.5 ± 4.9	236.3 ± 7.3	221.6 ± 12.5	231.3 ± 9.4	227.0 ± 7.1	NS
Glycerol µmol/l	484.2 ± 60	476.4 ± 51	431.3 ± 40	471.0 ± 40	443.3 ± 43	584.0 ± 44	NS
Bilirubin µmol/l	4.01 ± 0.3	4.93 ± 0.4	4.14 ± 0.4	5.33 ± 0.4	4.27 ± 0.4	4.66 ± 0.6	NS
Creatinine µmol/l	85.7 ± 7.6	77.8 ± 6.7	84.0 ± 7.4	89.2 ± 8.6	67.1 ± 8.6	82.4 ± 7.2	NS

** NS = not significant, P < 0.05 = significant difference between groups*

Liver glycogen content was significantly lower in the positive control group in comparison to the choline group (Table 5). Kidney lipase activity was significantly higher in the positive control than in the Alimet®, betaine or methionine groups. Liver glucose-6-phosphatase activity was higher in the positive control group than in the Alimet®, betaine, choline and methionine groups. Kidney glycogen, liver and kidney phosphorylase, liver and kidney lipase and kidney glucose-6-phosphatase activities were equal in all groups. Weights of adrenal glands and cortisol:creatinine ratio in all groups was equal.

Table 5. Enzyme activities of the livers and kidneys of the blue fox vixens (mean ±SE)

	Control	Alimet®	Betaine	Choline	Methionine	Positive control	P*
Liver glycogen mg/g	32.8 ± 3.9[ab]	33.7 ± 4.6[ab]	30.1 ± 4.4[ab]	37.2 ± 5.9[a]	31.2 ± 4.0[ab]	17.3 ± 3.1[b]	<0.05
Kidney glycogen mg/g	1.43 ± 0.14	1.87 ± 0.38	1.28 ± 0.15	1.64 ± 0.33	1.43 ± 0.20	2.17 ± 0.61	NS
Liver phosphorylase µg P/mg/h	54.1 ± 4.2	49.1 ± 3.8	47.6 ± 4.0	47.1 ± 3.2	48.0 ± 4.4	48.4 ± 4.0	NS
Kidney phosphorylase µg P/mg/h	3.73 ± 0.18	2.80 ± 0.15	3.58 ± 0.80	3.18 ± 0.25	3.79 ± 0.38	3.70 ± 0.20	NS
Liver lipase µg 2-naphthol/mg/h	25.6 ± 2.1	23.4 ± 2.4	24.6 ± 2.5	29.6 ± 3.0	23.1 ± 2.8	26.6 ± 2.2	NS
Kidney lipase µg 2-naphthol/mg/h	27.9 ± 2.5[ab]	19.5± 1.6[a]	20.0 ± 1.5[a]	23.7 ± 3.4[ab]	16.9 ± 1.8[a]	34.8 ± 6.0[b]	<0.05
Liver glucose-6-phosphatase µg P/mg/h	16.6 ± 2.3[ab]	15.3 ± 1.0[a]	14.7 ± 2.1[a]	14.7± 1.2[a]	15.6± 2.6[a]	24.1 ± 2.2[b]	<0.05
Kidney glucose-6-phosphatase µg P/mg/h	9.23 ± 1.38	7.77 ± 0.49	7.91 ± 0.54	8.75 ± 0.54	7.87 ± 0.66	8.09 ± 0.44	NS

** NS = not significant, P < 0.05 = significant difference between groups*

Discussion

Weight loss was similar in all groups as it was expected because of the same average energy consumption in the groups. All parameters except ALT were not different in the control, Alimet®, betaine, choline and methionine groups. These supplementation levels of methyl donors with sub optimal protein level did not affect liver metabolism with these weight loss levels. The positive control group seemed to be the healthiest in most measured parameters. There were no signs of fatty liver in the positive control group and only few indications in the other groups. Probably the amino acid content was better in the positive control group and, as a consequence, their plasma ALT and AST activities were at the lowest observed level indicating a very low probability of any liver damage due to weight loss. The diet of the control group contained 35 % protein from ME and the other diets approximately 20% protein from ME.

Normally the liver contains 2-4 % fat of the total weight. In studies of Juokslahti et al. (1978) fatty livers contained more than 50 % fat of total weight. Mink liver contains more fat than liver from blue fox (Ahlstrøm & Skrede, 1997). In Sweden mink livers contained 4.3-6.7 % fat at pelting (Alden et al. 1997). In our study liver fat contents of the blue fox vixens were 3.9-6.6 %, indicating quite good state of liver. However the positive control groups' liver hepatocyte vacuolisation was almost nonexistent in contrast to mild or moderate changes in liver hepatocytes of other groups.

The blood parameters did not reveal that the animals suffered from severe malnutrition from the low protein level. The activities of liver and kidney enzymes taking part in the carbohydrate or lipid metabolism were almost at the same level in animals receiving low protein as for those in the positive control. Thus blood glucose and lipid levels were maintained almost at the same level at the different feeding regimes.

Weight loss didn't affect the general well being of the blue fox vixens before the breeding season. Cortisol:creatinine ratios of the urine and the adrenal gland mass were similar in all groups. Long-term stress is thought to cause an increase in adrenal gland mass weight (Selye, H. 1950). The urine cortisol:creatinine ratio indicates stress level and is assumed to be more reliable way to measure adrenal function than straightforward blood cortisol values (Beerda, M. et al. 1996). Urinary cortisol was expressed as the cortsol:creatinine ratio to correct for variation in the dilution of urine (Lasley, B.L. et al. 1991, Novak et al. 1989).

The experiment ended just before the mating season started. It is possible that the experimental feeding of the low protein diets could have given more pronounced effects during coming mating season even if the dietary protein level had been increased.

The positive control feed (35% protein of ME) was formulated on the basis of current recommendation (38% protein of ME) from the Finnish Fur Breeders' Association. Animals maintained good health in the positive control group. Therefore the recommended feed and a weight loss of approximately 29 % in

three months could be safely introduced. Optimum protein level could be lower than recommended 38% protein of ME.

In conclusion, the experiment showed that methyl donors had no effect on health status on blue fox vixens given a low protein diet during the body fat mobilisation period (November-March). Generally, the low dietary protein level was reflected in some health parameters, but no animals revealed symptoms of malnutrition. The results demonstrated that the blue fox have extreme abilities to maintain health on a suboptimal diet during body fat mobilisation.

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III – 16 RP

Some biochemical parameters in serum of mink fed high energy feedstuff with antioxidant and preservative supplement*

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Abstract
The objective of the investigations was to determine a variability range of reference values of the chosen biochemical parameters in the serum of minks at varied levels of energy feeding. At the some time the antioxidant and preservative supplement at different rate was used. The studies were performed at the mink farm „C" situated in Poland. Blood was collected from heart puncture from mink yearlings twice in December. In serum of animals the enzyme activity of aspartic aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), a level of urea, creatinine, glucose, bilirubin, and uric acid were determined by the spectrophotometric method with monotests of Cormay. The obtained results showed substantial activity increase of ALT, AST in D_2 group and LDH in both experimental groups. The other parameters were found within the ranges presented by other authors.

Introduction
The widespread introduction of new feeding methods of carnivorous fur animals by the leading countries needs complex assessment of their impact on animal health and performance. The new feeding methods consist in an increase of dietary energy through increased content of energy from fat, that as a consequence induces higher reproduction indices as well as skin of the optimum quality parameters. It may however, lead to enhanced disturbances of metabolism at the cellular level, unnoticeable over the short animal life. This fact has been mentioned in few scientific papers and breeders` observations (Sławoń,1986 and Winnicka,1997). The feedstuff including fish offal, poultry wastes and slaughterhouse offal requires the use of preservatives and antioxidants that are not indifferent for animal health. The present authors` earlier investigations indicate the pathological changes in the intrinsic organs of polar foxes caused by, among others feeding with feedstuff of increased energy value.

Material and Methods
The investigations were performed at the "C" farm situated in the south eastern part of Poland. Stock of the basic pack comprised 500 females. The yearlings for pelting. Blood was collected by heart puncture from 60 minks "scan brown" fine brown variety. The 3 treetment groups were selected:K – kontrol, experimental D_1 and D_2 , the same number of males and females esch. Comparing the groups, each litter was divided randomly into three parts, regareling sex. It allowed to compare the genetic material among the groups. In blood serum of the animals examined activity of aspartic aminotransferase (AST), alanine selected for the experiment were meant aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and the level of urea, creatinine, glucose, bilirubin and uric acid.
The animals were fed properly balanced feedstuff. The feed composition was based on Cod, sprat x, poultry waste, horses stomach, meat - bone meal, animal fat, wheal meal – crude, bran, potatos boiled, water. It was also supplemented with pure fat animal. The doses were worked out with a computer program aid. The feedstuff energy value of 1 kg are enclosed at Table 1. The feed administred at all the groups contained vitamin-mineral premix GuyoFox at amount 0,10 kg/t feed (Garrido et al., 1996). The D_1 group was supplied with the antioxidant additive Rendox 0,02% of ready feed mass and preservative (sodium pyrosulphite) 0,28% ready feed mass.

Table 1. The quantitative composition of feed supplied to minks and energy value of 1 kg.

	01.08. - 15.09.			16.09. - 10.10.			11.10 - pelting		
	THE ENERGY VALUE (Kcal/kg)								
	K	**D_1^***	**D_2**	**K**	**D_1^***	**D_2**	**K**	**D_1^***	**D_2**
	1700	1700	1700	1670	1670	1820	1690	1690	1900
	% EM								
PROTEIN	35.1	35.1	35.1	33.3	33.3	33.3	33.0	33.0	31.0
FAT	52.4	52.4	52.4	52.5	52.5	53.9	52.2	52.2	55.2
CARBO-HYDRATES (CH_2O)	12.5	12.5	12.5	14.2	14.2	12.8	14.8	14.8	14.8

* - *This group was supplied with SODIUM PYROSULPHITE and RENDOX*

Both preparations were added to meat-fish materials kept in cold storage (Brandt, 1989, Kopczewski et al., 2000 and Slawoń, 1986) . Throughout the experimental period feed was provided ad libitum, with permanent water access.

About 2 weeks prior to the pelting, in D_2 group feed caloricity was reduced by 150 Kcal. owing to the animal anwillingness to have it.

In July and October, immediately after the feed was prepared 10 samples were taken at random from the mixer in order to perform the bacteriologic and microbiologic examinations. The laboratory examinations were performed according to the obligatory regulations PN-R-64791, PN-75/R-64787, PN-74A-74016 (Kopczewski et al., 2000; Kopczewski et al. 2001). The obtained results were analysed statistically computing arithmetic mean and standard deviation. Significance of differences between the means at 5% error risk of inference was verified with Student`s t-test (Microsoft Excel NT).

Results and Discussion

There have not been recorded any Salmonella rods in the examined feed, yet a greet contribution of poultry and poultry waste to animal feeding is hazardous for feed polution with various serotypes. Escherichia coli rods appeared to be isolate most frequently, its growth was obtined as mean numerous in 60% of the samples. There was also detected mean numerous growth of microbes Proteus g. in 80% of the studied samples and numerous growth of fungol colnies and moulds in all the samples.

The mean ALAT activity ranged from 120.28 to 224.80 U/l. This enzyme activity in serum increases not only at cell necrobiosis but at their damage as well (Garrido et al., 1996) . Alike, the AST activity exceeded the reference value oscillating from 95.66 to 133.49 U/l (Garrido et al., 1996; Heggset et al.,1999; Heggset 2000; Winnicka 1997) , whereas the ALP enzyme activity ranged from 60.20 U/l to 96.73 U/l. The results are presented in Table 2.

Elevated activity of this enzyme in serum may be connected with its discharge impairment through the bile ducts due to their occlusion, yet it is well known that an increase in ALP activity is more reliable for the evaluation of bone profile disturbances, vitamin D deficit and neoplastic processes (Winnicka 1997) . Liver is the main organ of the cholesterol and apolipoprotein biosynthesis. The cholesterol level - mean from three collections - in blood serum showed levels ranging from 5.82-6.21 nmol/l, yet contained within the limits (Garrido et al. 1996; Kopczewski et al. 2000; Sławoń 1986; Winnicka1997) .

Table. 2. The biochemical parameters in serum of mink (n=60).Means ($\bar{x}$) and Standard deviation (SD)

Collec-tion		Bilirubin Mmol/l	Glucose mmol/l	Urea mmol/l	Uric acid mmol/l	Creatinine mmol/l	Cholesterol mmol/l	AP U/l	LDH U/l	AST U/l	ALT U/l
D_1	$\bar{x}$	3.67	6.39	5.95	0.21	69.46	5.82	74.39	1093.10	133.49	120.28
	SD	0.89	1.47	1.05	0.06	8.98	1.34	9.88	238.23	53.12	31.26
D_2	$\bar{x}$	2.05	7.71	6.10	0.21	56.03	6.21	96.73	883.27	132.3	224.80
	SD	0.29	1.95	2.61	0.03	3.93	1.98	17.77	425.53	28.30	80.30
K	$\bar{x}$	2.28	10.85	11.23	0.20	50.53	5.98	60.20	432.77	95.67	120.57
	SD	0.99	2.74	0.85	0.02	21.15	2.23	3.84	55.61	27.62	41.89

Alike, a mean bilirubin level in serum varied from 2.05-3.66 umol/l within the standard values and mean glucose level that reached 6,39-10,85 nmol/l at the standard up to 8,0 nmol/l (Winnicka1997).
Evaluating the renal profile, a urea level was detected, which is a final metabolite of protein metabolism in organism. Its concentration in blood serum depends on protein supply in a dietary unit, endogenic protein breakdown or excretory activity of kidneys. It is assumed that only at a glomerular filtration drop by over 50% there is recorded an increase of urea concentration in serum
(Winnicka1997) . A urea level was 5.94-11.23 nmol/l and was contained within the standards
(Sławoń 1986; Winnicka1997) . Creatinine present in serum is a metabolite of the skeletal muscles. It is discharged through the kidneys and useful for its excretory performance assessment
(Winnicka 1997) . The changes of a creatinine level are of a regular character with the lowest level recorded for 50.53 umol/l and the highest 69.46 umol/l not surpassing the reference values (Brandt, 1989; Winnicka 1997) . A uric acid level was very balanced and reached 0.203 mmol/l – 0.213 mmol/l being contained within the reference values (Brandt, 1989; Winnicka 1997) . Hypouricaemia may be conditioned by the enhanced excretion of uric acid through the kidneys, impaired reabsorption of this metabolite through the renal tubules as well as inborn deficiency of xanthine oxidase or an increase of its inhibitor level in organism (Brandt, 1989; Winnicka, 1997) . Summing up the results of the researches it may be stated that in the minks fed high energy feed there was detected the substantial growth of the AST and ALT activity. The rest parameters were contained within the values presented by the other authors (Brandt, 1989; Sławoń et al. 2000; Winnicka, 1997) .

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III – 17 RP

Correlation between liver fat and dry matter in mink (*Mustela vison*)

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Abstract
In our investigations on the protein requirement of mink in the growing period, we have often observed an increased fat content in the liver, when we reduced the protein / increased the fat content in the feed.
A chemical analysis of the fat content in the liver is relatively slow and expensive. We have used a semiquantitative test submerging liver samples into water and copper sulphate solutions with different specific gravities. On the basis of buoyancy in these liquids, liver samples were classified as containing > 34 % fat, 25 – 34 % fat, 13 – 25 % fat, or less than 13 % fat. The method is cheap but rather inaccurate. Furthermore the liquid cannot be used for more than a few liver samples before it has to bee replaced.
The dry matter content of fat is almost 100 percent, so we decided to determine a correlation between liver fat and liver dry matter. We took out liver samples from mink dying during October 2003. The livers were analysed for crude fat and dry matter content.
The results showed a very fine correlation between the dry matter and the fat content of the livers:
Liver fat, in percent = 1.15 * liver dry matter – 24.9 ($R^2 = 0.97$)
It is concluded that this method can bee used for a quick, cheap and acceptably precise evaluation of liver fat content.

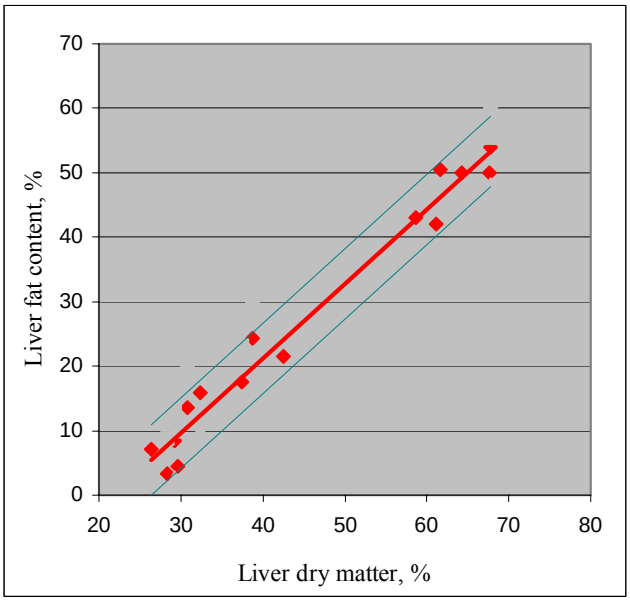
Introduction
The liver function is highly influenced by the feed. Investigating the consequences of different feed compositions on growing mink kits, often makes it interesting to see if there are any fat infiltration in the livers. In our investigations on the protein requirement of mink in the growing period, we have often observed an increased fat content in the liver, when we reduced the protein / increased the fat content in the feed (Damgaard et al, 1994; Damgaard et al, 1998a; Damgaard et al, 1998b).
A chemical analysis of the fat content in the liver is slow and expensive. In order to screen the livers from many animals, we have searched for a fast and cheap method to determine liver fat content.
A semiquantitative test described by Herdt (1992), has been used for some years at the Research Center (Clausen, 1992; Damgaard et al, 1994; Damgaard et al, 1998a; Damgaard et al, 1998b). Liver samples were submerged into water and copper sulphate solutions with different specific gravities (1.000, 1.025 or 1.055). On the basis of buoyancy in these liquids, liver samples were classified as containing > 34 % fat, 25 – 34 % fat, 13 – 25 % fat, or less than 13 % fat. The method is cheap but rather inaccurate. Further the liquid cannot be used for more then a few liver samples before it has to bee replaced.
The dry matter content of fat is almost 100 percent. In this investigation we analysed dry matter and crude fat content of the livers and estimated the correlation between these two variables.

Material and methods
To the investigation we used liver samples from mink dying during October 2003. A total of 15 livers were chosen from their macroscopic appearance. 9 livers had a normal size and colour, 6 livers were very enlarged and yellow. From all livers we took out two equal samples from the same liver lobuli. One sample was analysed for crude fat (Stoldt fat, EU(98/64EØF)) and dry matter (104 ºC in 4 hours, EU(71/393/EØF)) content, at the Danish Fur Breeders Laboratory, and one sample was analysed for dry matter at the Research Center. At the Center we divided the liver sample into two parts each 2 - 4 grams and dried those at two different temperatures of respectively 80 ºC and 110 ºC. For practical reasons a drying time of 26 hours was chosen, as the samples were taken out in the morning and we then had the results the following day. The liver samples were mashed with a fork, and placed in small tin foil cubs before drying.

Results and discussion
Dry matter analyzed at the laboratory and at the Research Center was very equal. The best correlation was found between laboratory dry matter

and dry matter determined at the Research Center at 110 ºC for 26 hours (correlation coefficient 0.998, $p<0.0001$). Relationship between liver dry matter and crude fat content is shown in Figure 1.

Figure 1
Dry matter (percent) and the corresponding fat content (percent) of 15 mink livers. Regression line (y = 1,1523x - 24,903; R^2 = 0,973) with 95 percent confidence interval is shown.



The analyzed livers split up into two groups, one group with high dry matter above 58 percent and fat above 43 percent, and one group with dry matter below 43 percent and crude fat below 22 percent. The macroscopic appearance of the livers corresponded very well to the crude fat content; all the livers in the high fat group were big and yellow. A calculation based on an earlier investigation (Clausen, 1992) also showed a good relationship between liver fat and dry matter.

Conclusion

The results showed a very fine correlation between the dry matter and the crude fat content of the livers: Liver fat (percent) = 1.15 * liver dry matter (percent) – 24.9 (R^2 = 0.97)
It is concluded that this method can bee used for a quick, cheap and acceptably precise evaluation of liver fat content.

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III – 18 RP

Effects of feeding strategy on behaviour, physiological parameters and feed residues in mink females

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Abstract
The effects of three feeding strategies were investigated in groups of 60 female mink from August to March. The three feeding strategies were: *ad libitum* feeding with a conventional diet from October to February, *ad libitum* feeding but with a substantial diet (high content of barley) from December to February, and restricted feeding with a conventional diet from October to February. The body weight was registered approximately every second week. Behavioural observations were made using focal sampling before and after feeding in December, January, February and March. Physiological parameters were measured in November, February and March. Feed residues were recorded individually on a two level scale (yes, no) each morning. The statistical methods included the proc mixed and proc genmod procedures in the SAS System. The results showed that restricted feeding increased stereotypic behaviour. It was demonstrated that it was possible to reduce the body weight of mink by feeding them a low energy feed without increasing the incidence of stereotypies. The feeding strategy had limited effects on physiological parameters. The interaction between feeding strategy, behaviour and occurrence of feed residues was demonstrated.

Introduction
In the wild and in production systems, mink increase their body weight from the summer period to the period of molting in October/November (Valtonen et al., 1995). The females that are to be used for breeding are selected in November among these often fairly fat females, and they are to be slimmed in order to best prepare them for flushing immediately before the mating season.
Restrictive feeding during the winter period results in an increase in the level of activity and, in particular, in the incidence of stereotypies (Bildsøe et al., 1991; Hansen et al., 2002; Houbak & Møller, 2000).

The purpose of this study was to examine the effects of traditional and alternative feeding strategies on the females' weight and behaviour during wintertime on physiological parameters and on the probability of the occurrence of feed residues in the morning.

Materials and Methods
The study included 180 female mink (*Mustela vison*) of the colour-type 'wildmink' divided into three groups. The study was carried out from August to March. During the wintertime from December 22 to February 16 two groups (groups ADL and RE) were fed a conventional wet mink diet (dry matter (DM): 32.6%, metabolisable energy (ME): 5.16 MJ kg^{-1} wet diet (15.9 MJ kg^{-1} DM), distribution of ME with the following ratios of protein, fat, carbohydrate: 56% : 34% : 10%), and the third group (group SUB) was fed a substantial diet (DM: 35.2%, ME: 4.71 MJ kg^{-1} wet diet (13.4 MJ kg^{-1} DM), distribution of ME with the following ratios of protein, fat, carbohydrate: 49% : 25% : 26%). During the rest of the experimental period all three groups were fed conventional wet mink diet.
From October 17 to February 16 the three experimental groups were fed according to the following feeding strategies:

- Group ADL: ad libitum feeding – conventional wet mink diet
- Group SUB: ad libitum feeding – December 22 to February 16 substantial otherwise conventional wet mink diet
- Group RE: restrictive feeding – conventional wet mink diet

Each morning feed residues were recorded individually on a two level scale (yes, no). The females were weighed approximately every second week. Behavioural observations were made using focal sampling before and after feeding on December 14 and 28, January 24, February 14 and 28, March 7 and 28. The observer was placed 1 m

away from each cage section consisting of six cages. After 1 min waiting, the incidence and duration of certain behavioural patterns were recorded for 1 min. The first round of observations started 90 min before feeding and the second round started 15 min after feeding. On the basis of the behavioural observations from December 14 to February 14, the females were classified as stereotypic females (St-females) or as non-stereotypic females (Non-St-females).

Blood samples from 20 females per experimental group were collected in November, February, and March.

The packed cell volume (PCV) was determined in whole blood. The concentrations of urea, free fatty acids (FFA), and insulin were determined in plasma.

Statistical analyses were performed by means of the Statistical Analysis System (SAS) software (SAS Institute Inc., 1996). Effects and differences according to treatment, behaviour and time period were estimated using the MIXED and the GENMOD procedures in SAS.

Results and discussion

The planned approximated ad libitum feeding of the ADL and SUB groups was successful in that between 30% and 70% of the females left feed. As regards the RE group, the restrictive feeding strategy was successful and approximately 10 % of the females left feed. (Table 1)

The probability of the occurrence of feed residues was lower for the group fed restrictively (group RE) than for the two groups fed ad libitum (ADL and SUB groups) during the experimental period (Table 1, P=0.008). In the period from December 22 to February 16 the SUB group was fed a substantial diet and the ADL group a conventional diet but this difference in feed composition did not result in any differences in the probability of the occurrence of feed residues between the two groups (Table 1). Stereotypic females (St-females) had a lower probability of the occurrence of feed residues than non-stereotypic females (Non-St-females) in all experimental groups (Table 1, P=0.008). The probability of the occurrence of feed residues was lower in the period from October to December than in the period from December to February (Table 1, P<0.001).

On the basis of the behavioural classification 27% of the females in the groups fed ad libitum were classified as stereotypic females, while 53 % of the females fed restrictively were classified as stereotypic females.

In this experiment, the weight of stereotypic females was lower than the weight of non-stereotypic females. In the SUB group, the change from a conventional diet to a low energy diet resulted in a weight loss in all females independent of stereotypic classification.

Table 1.Probability of the occurrence of feed residues in stereotypic (St-females) and non-stereotypic (Non-St-females) females in the experimental groups ADL, SUB, and RE (*n*=60 per experimental group) in the periods from October 17 to December 22 and December 22 to February 16. Values are least square means and SE.

Experimental group	Period 1 October 17 – December 22		Period 2 December 22 - February 16		P-value Effects of:
	St-females	Non-St-females	St-females	Non-St-females	
ADL	35.1 % ± 12.2 % B	49.4 % ± 12.2 % A	42.3 % ± 13.1 % B	57.0 % ± 13.0 % A	Exp.group: <0.001 Stereotypy: <0.001 Exp.* Ste.: 0.008 Period: <0.001
SUB	35.5 % ± 11.1 % B	49.4 % ± 18.4 % A	42.7 % ± 10.5 % B	56.9 % ± 17.9 % A	
RE	4.3 % ± 18.4 % D	19.1 % ± 18.5 % C	5.8 % ± 19.7 % D	24.2 % ± 19.3 % C	

Mean values in the same period marked with different letters are significantly different (P<0.05).

Table 2.Packed cell volume (PCV) in blood and plasma concentrations of urea, free fatty acids (FFA) and insulin in stereotypic (St-females) and non-stereotypic (Non-St-females) females (*n*=60) in November, February and March. Values are least square means and SE.

Parameter/ Stereotypy	November 23-24	February 15-16	March 1-2	March 8-9	P-value Effect of:
PCV, %					Exp. group: 0.51
St-females	57.9 ± 0.99	55.5 ± 0.48	58.1 ± 0.53	55.2 ± 0.57	Stereotypy: 0.03
Non-St-females	58.3 ± 0.84	57.7 ± 0.42	59.0 ± 0.47	56.7 ± 0.49	Date: <0.001
Urea, mmol L^{-1}					Exp. group: 0.11
St-females	4.3 ± 0.24	6.3 ± 0.54	4.3 ± 0.28	6.4 ± 0.38	Stereotypy: 0.45
Non-St-females	4.2 ± 0.20	6.9 ± 0.45	4.5 ± 0.24	6.5 ± 0.33	Date: <0.001
FFA, mEqv L^{-1}					Exp. group: 0.54
St-females	0.48 ± 0.03	0.41 ± 0.03	0.51 ± 0.03	0.43 ± 0.03	Stereotypy: 0.32
Non-St-females	0.47 ± 0.03	0.46 ± 0.03	0.49 ± 0.02	0.51 ± 0.02	Date: 0.04
Insulin, mU L^{-1}					Exp. group: 0.18
St-females	14.3 ± 1.2	12.7 ± 1.2	8.7 ± 1.1	15.2 ± 1.1	Stereotypy: <0.001
Non-St-females	16.2 ± 1.0	17.3 ± 1.0	14.1 ± 1.0	20.3 ± 1.0	Date: <0.001

The PCV value in the blood and the plasma concentrations of urea, FFA and insulin were not affected by the feeding strategies (Table 2, P>0.05). Earlier investigations in mink females during the winter and reproduction periods have shown that mink females can maintain nutrient homeostasis within a wide variation in feed composition and feeding strategy (Børsting et al., 1998, Damgaard et al., 2003).

The blood PCV value was lower for St-females than for Non-St-females (Table 2, P=0.03), which may be correlated to the activity level and the fluid balance of the females. The PCV value was highest on March 1-2 at the end of the period with restrictive energy supply included in flushing (P<0.001). The activity level of the females did not affect the plasma concentrations of urea and FFA (Table 2, P>0.05). During the winter the plasma concentration of urea was lowest on March 1-2 which may be a result of a low energy supply and thereby a low protein metabolism in the beginning of March (Table 2, P<0.001). The plasma concentration of FFA was highest on March 1-2, which may be a result of the mobilisation of fat deposits at a time with low energy supply. The plasma concentration of insulin was lower for St-females than for Non-St-females (Table 2, P<0.001), and the concentration increased during the flushing period from February 15-16 to March 8-9 (Table 2, P<0.001). In earlier investigations in mink females, restrictive feeding followed by ad libitum feeding resulted in a significant increase in the concentration of insulin (Fink & Tauson, 1998; Tauson et al., 2000), which corresponds to the present results.

Conclusion

Restrictive feeding increased stereotypic behaviour. It was demonstrated that it was possible to reduce the body weight of mink females by feeding them a low energy diet without increasing the incidence of stereotypies. The probability of the occurrence of feed residues was affected by feeding strategy and stereotypic behaviour. The different feeding strategies had limited effects on metabolic and hormonal parameters. The metabolic parameters (FFA and urea) and the plasma concentration of insulin were influenced by the daily energy supply. The activity level of the females influenced the blood PCV value and the plasma concentration of insulin.

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III- 19 RP

Regulation of lipid and glucose metabolism in the mink (*Mustela vison*) sequence analysis and development of molecular probes

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Abstract
Nursing sickness is an example of a metabolic disorder, which develops during the high energy demands of lactation and is characterized by a disruption in glucose homeostasis. We are examining the basic molecular mechanisms governing lipid and glucose metabolism in order to develop a better understanding of the regulation and relationship of body fat and blood sugar levels. Presently this research is focused specifically on the influences of obesity and dietary omega-3 fatty acid enrichment on glucose uptake by peripheral tissue and the deposition and mobilization of body fat in the mink. In the process of examining the molecular mechanisms governing lipid and glucose metabolism in the mink, we have developed laboratory procedures for the isolation and characterization of mink liver, skeletal muscle and adipose tissue mRNA. We will be evaluating changes in the gene expression of key enzymes and regulatory proteins of fat and glucose metabolism in response to dietary fatty acid composition and obesity. For the development of molecular probes for this work we have isolated and partially sequenced the mink acetyl-CoA carboxylase (ACC) using complementary DNA, which was prepared from reverse-transcribed mRNA. This sequence is highly conserved with respect to other mammalian species including human. Using similar methodology we have also partially sequenced the mink glucose transporter 4 (Glut4). In this paper we report these DNA sequences, which are the first for these enzymes in mink.

Introduction

Nursing sickness in the mink is a classic example of a metabolic disorder, which develops when the demands for lactation require extensive mobilization of body energy reserves. The animal enters a strongly negative energy balance and death usually follows soon after the first clinical signs (Clausen et al., 1992). The increasing age of the dam, followed by litter size and weight loss are major determinants for the development of nursing sickness. Although the ranch level epidemiology and the clinical pathology of nursing sickness are well documented, the etiology of this metabolic disorder remains unclear. The clinical symptoms of high blood glucose and insulin levels are commonly seen in the affected dams (Wamberg et al., 1992a), and it has been suggested that the development of the disorder is linked to disruption in glucose homeostasis (Børsting & Gade, 2000). Most recently it has been proposed (Rouvinen-Watt, 2003) that the underlying cause of mink nursing sickness may be acquired insulin resistance with obesity, omega-3 fatty acid deficiency or high protein oxidation rate as the key contributing factors.

Being an obligate carnivore, glucose homeostasis in the mink is directly dependent on protein and amino acid nutrition (Børsting & Gade, 2000). However, carbohydrate and protein metabolism are also linked to that of lipids (Frayn, 2001), which has received little consideration in the pathology of nursing sickness (Rouvinen-Watt, 2003). Long chain polyunsaturated fatty acids have been shown to regulate the expression of a variety of genes including major lipogenic enzymes (Clarke & Jump, 1994) such as fatty acid synthetase and acetyl-CoA carboxylase. In this context it is now well established that long chain polyunsaturated fatty acids can act as transcriptional activators of lipid metabolism (Tontonoz et al., 1995). More recently long chain omega-3 polyunsaturated fatty acids have been shown to attenuate the down-regulation of glucose transporter 4 mRNA which occurred when rats were fed high fat diets (Takahashi & Ide, 2000) indicating an involvement in glucose metabolism. Furthermore, these scientists were able to demonstrate in rats that dietary fish oil reduces blood glucose levels, improves glucose tolerance and increases insulin-stimulated glucose transport and metabolism in fat cells.

Very little is known about the molecular regulation of energy metabolism in carnivores. Using molecular

probes our objective is to examine the basic molecular mechanisms governing lipid and glucose metabolism in order to develop a better understanding of the regulation of blood sugar levels in the mink.

Material and Methods

RNA Isolation

Total RNA was extracted from mink (*Mustela vison)* adipose tissue using guanidinium isothiocyanate according to Chomczynski & Sacchi (1987) with the following modifications. A 1:1 mixture of water-saturated phenol and chloroform/isoamyl alcohol (24:1) was used for all phenol extractions. Shaking during the extractions was conducted for a minimum of 10 minutes and extractions were repeated until the interface was clear. The mink used in the study were of the standard black genotype.

Primer Selection

Forward and reverse primers for ACC were 5'-AGCACGCCAGGTTCTTATTG - 3' and 5' - GTGGTTGAGGTTGGAGGAGA - 3', respectively and were based on human ACC cDNA sequence. Forward and reverse primers for Glut4 were 5' - ATGTGTGGCTGTCGGATC - 3' and 5' - GAAGGTGAAGATGAAGAAG - 3', respectively and were based on canine cDNA sequence.

Reverse Transcription - Polymerase Chain Reaction

First strand cDNA was synthesized using 2 µg total RNA, oligo(dT) primers and MMLV reverse transcriptase following the manufacturer's protocol (RETROscript, Ambion). Reverse transcription product (2-5 µl) was used as template for PCR amplification using Taq DNA polymerase, the primers described above and following the protocol provided with the RETROscript kit (Ambion). The ACC and Glut4 PCR products were gel purified, eluted and sequenced.

Results

Reverse transcription-polymerase chain reaction (RT-PCR) of RNA from mink adipose tissue using the ACC primers corresponding to nucleotides 3240-3259 and nucleotides 3605-3624 of human ACC1 mRNA (Mao, 2003) resulted in a cDNA fragment of approximately 385 base pairs. Sequence analysis was obtained for 308 base pairs (Figure 1) of the RT-PCR product, corresponding to nucleotides 3291-3598 of the human ACC1 cDNA. This sequence has been submitted to GenBank (Rouvinen-Watt & Glover, 2004a). The nucleotide sequence obtained shows greatest similarity to that of human (93%) followed closely by rat (92%), mouse (91%) and sheep (91%). The derived amino acid sequence of the mink ACC cDNA fragment (Rouvinen-Watt & Glover, 2004b) is given in Figure 2. All differences in nucleotide sequence among the five species compared were at positions of degeneracy in the genetic code and thus did not result in changes in amino acid sequence.

Reverse transcription-polymerase chain reaction (RT-PCR) of RNA from mink adipose tissue using the Glut4 primer pair corresponding to nucleotides 61-78 and nucleotides 447-465 of canine Glut4 mRNA (Christophe, 1999) resulted in a cDNA fragment of approximately 405 base pairs. Sequence analysis was obtained for 314 base pairs (Figure 3) of the RT-PCR product, corresponding to nucleotides 133-436 of the canine Glut4 cDNA. The nucleotide sequence obtained shows greatest similarity to that of horse (90%) followed closely by cow (89%), dog (88%) and human (88%). The derived amino acid sequence of the mink Glut4 cDNA fragment is given in Figure 4. Unlike the mink ACC cDNA fragment, nucleotide substitutions between the five species compared were not silent, resulting in differences between the amino acid sequences. There are seven amino acid differences between the mink and dog sequences but only three differences between mink and horse, cow or human. Two amino acid positions (15 and 28) contained phenylalanine, which appears unique to mink among these species comparisons. The Glut4 mink cDNA fragment will be sequenced again to confirm the nucleotide substitutions and derived amino acid sequence differences.

Discussion

Acetyl-CoA carboxylase catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, the donor of the two carbon units in the synthesis of long chain fatty acids. Activity of acetyl-CoA carboxylase is the rate- limiting step for *de novo* fatty acid synthesis in all organisms (Kim, 1997). Regulation of enzyme activity has been demonstrated at many levels including posttranslational short-term control via allosteric interactions with metabolites and reversible phosphorylation activated by hormones (Kim et al., 1989) and long-term control at the transcriptional level in response to various nutrients and hormones (Mao & Seyfert, 2002, Barber et al., 2001). It

is typically highly expressed in lipogenic tissues such as liver, adipose and lactating mammary gland and its regulation has been extensively studied in the rat, ovine, bovine and recently human tissue (Mao et al., 2003). In contrast to other species, *de novo* synthesis of fatty acids in mink mammary is thought to be minimal (Wamberg et al., 1992b). Tissue specific expression of ACC has been shown to be controlled at the transcriptional level by different promoters (Mao & Seyfert 2002). Very little is known about the structure and regulation of expression of the mink ACC gene in different tissues.

Insulin stimulates glucose uptake in fat and muscle by mobilizing a specific transporter known as glucose transporter 4 (Glut4), which enables glucose to be transported across the plasma membrane and into the cell. Glut4 is sequestered intracellularly in the absence of insulin, and is redistributed to the plasma membrane within minutes of insulin stimulation. This insulin sensitive, facilitated transport is critical for the control of blood glucose levels. The mechanisms that control Glut4 sequestration have not been fully discerned and are the subject of current research (Bogan et al., 2003; Lalioti et al., 2002). Although Glut4 is responsive to glucose, Glut4 gene expression also changes in response to dietary fat content and composition (Takahashi & Ide, 2000). As such Glut4 may represent a link between lipid and glucose metabolism. The relationship between dietary fat and glucose tolerance is being extensively investigated in the context of obesity and the development of diabetes in humans. Similar work in a carnivore such as the mink has not been conducted.

Both the ACC and Glut4 cDNA sequence data presented here represent the only such sequence available for the mink. The ACC and Glut4 RT-PCR products we have developed have been tested for their efficacy in ribonuclease protection assays and we will be using these products to study the transcriptional regulation of these enzymes. The goal of this work is to offer another dimension to on-going research concerning lipid and glucose metabolism in the mink and in particular the relationship of dietary fatty acid source to production related disorders, such as nursing sickness.

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Figure 1.Comparison of mink acetyl-CoA carboxylase (ACC) cDNA nucleotide sequence with sequence of human (Mao, 2003), mouse (Strausberg, 2003), rat (Lopez-Casillas & Kim, 1988) and sheep (Barber, 1994). Letters in bold denote differences between the species. Sequence alignment obtained from Blastn (NCBI).

```
  1   taaccaagta gagtccatct tcctttcagc aattgacatg tacggacatc mink
      taaccaagta gagtctatct tcctatcagc tattgacatg tatggacatc human
      taaccaagta gagtctatct tcctatcagc cattgacatg tatggacacc mouse
      taaccaagta gagtccatct tcttatcagc catcgacatg tatggacacc rat
      caaccaagtc gagtctatct tcctgtccgc cattgacatg tacggacatc sheep

 51   agttttgcat tgagaactta cagaaactca tcttgtctga aacgtctatt mink
      aattttgcat tgagaactg cagaaactca tcctatcaga aacatctatt human
      agttttgcat tgagaactg cagaaactca tcctctcgga aacatctatt mouse
      agttttgcat tgagaactg cagaaactca tcctatcaga aacatctatt rat
      agttctgcat cgagaactg cagaaactca tcttgtccga aacgtcgatt sheep

101   tttgacgtcc taccaaactt cttctaccac agcaaccagg tagtgaggat mink
      tttgatgtcc taccaaactt cttctatcac agcaaccaag tagtgaggat human
      ttcgatgtcc tcccaaactt ttttaccac agcaaccagg tggtgaggat mouse
      ttcgatgtcc tcccaaactt ttttaccac agcaaccagg tggtgaggat rat
      tttgatgtcc tgccaaactt cttctatcac agcaaccagg tcgtgaggat sheep

151   ggcagctctg gaggtttatg ttcgaagggc ttatattgcc tatgaactta mink
      ggcagctctg gaggtgtatg ttcgaagggc ttatattgcc tatgaactta human
      ggcagctctg gaggtgtatg ttcgaagggc ttacattgcc tatgaactca mouse
      ggcggctctg gaggtatatg ttcgaagagc ttatatcgcc tatgagctca rat
      ggcagctctg gaggtgtatg ttcgaagggc ttatatcgcc tatgaactta sheep

201   acagtgtaca gcatcgccag cttaaggaca acacctgtgt ggtggaattt mink
      acagcgtaca acaccgccag cttaaggaca acacctgtgt ggtggaattc human
      acagcgtaca acaccgccag cttaaggaca acacctgtgt ggtggaattt mouse
      acagtgtaca gcatcgccag cttaaggaca acacctgtgt ggtagaattt rat
      atagcgtaca acaccggcag ctgaaggaca acacctgcgt ggtggaattc sheep

251   cagttcatgc tgcccacatc tcatccaaac agagggaaca tccccacgct mink
      cagttcatgc tgcccacatc tcatccaaac agagggaaca tccctacgct human
      cagttcatgc tgcccacatc ccatccaaac agagggaaca tccccacgct mouse
      cagttcatgc tgcccacatc tcatccaaac agagggaaca tccccacgct rat
      cagttcatgc tgcccacatc acatccaaac agagggaaca tccccacgct sheep

301   aaacagaa                                                mink
      aaacagaa                                                human
      aaacagaa                                                mouse
      aaacagaa                                                rat
      aaacagaa                                                sheep
```

Figure 2.Comparison of inferred protein amino acid sequence of mink acetyl-CoA carboxylase (ACC) with that of human, mouse, rat and sheep. Sequence references are given in Figure 1. Sequence alignment obtained from Blastp (NCBI).

```
 1    NQVESIFLSAIDMYGHQFCIENLQKLILSETSIFDVLPNFFYHSNQVVRMA          mink
      NQVESIFLSAIDMYGHQFCIENLQKLILSETSIFDVLPNFFYHSNQVVRMA          human
      NQVESIFLSAIDMYGHQFCIENLQKLILSETSIFDVLPNFFYHSNQVVRMA          mouse
      NQVESIFLSAIDMYGHQFCIENLQKLILSETSIFDVLPNFFYHSNQVVRMA          rat
      NQVESIFLSAIDMYGHQFCIENLQKLILSETSIFDVLPNFFYHSNQVVRMA          sheep

52    ALEVYVRRAYIAYELNSVQHRQLKDNTCVVEFQFMLPTSHPNRGNIPTLNR          mink
      ALEVYVRRAYIAYELNSVQHRQLKDNTCVVEFQFMLPTSHPNRGNIPTLNR          human
      ALEVYVRRAYIAYELNSVQHRQLKDNTCVVEFQFMLPTSHPNRGNIPTLNR          mouse
      ALEVYVRRAYIAYELNSVQHRQLKDNTCVVEFQFMLPTSHPNRGNIPTLNR          rat
      ALEVYVRRAYIAYELNSVQHRQLKDNTCVVEFQFMLPTSHPNRGNIPTLNR          sheep
```

Figure 3.Comparison of mink glucose transporter 4 (Glut4) cDNA nucleotide sequence with sequence of horse (Jose-Cunilleras et al., 2004), cow (Kang et al., 2003), dog (Christophe, 1999) and human (Fukumoto et al., 1989). Letters in bold denote differences between the species. Sequence alignment obtained from Blastn (NCBI).

```
  1   ctgggcctgg caggaatgtg tggctgcgcc atcctgatga cttttgcgct      mink
      ctgggcctgg cggggaatgt tggctgtgcc atcttgatga ctgtggccct horse
      ctgggcctgg caggcatgtg tggctgcgcc atcttgatga ctgtggctct cow
      ctgggcctgg caggaatgtg tggctgtgcc atcttgatga ccatagccct dog
      ctgggcctgg cgggcatgtg tggctgtgcc atcctgatga ctgtggctct human

 51   gcttctgctg gagcgtgttc ctgccatgag cttcgtctcc atcgtggcca mink
      gcttctgctg gagcgagttc cagccatgag ctatgtctcc atcgtggcca horse
      gcttctgctg gagcgggttc cagccatgag ctatgtctcc attgtggcca cow
      gcttctgctg gagcggcttc cagccatgag ctacgtctcc atcgtggcca dog
      gctcctgctg gagcgagttc cagccatgag ctacgtctcc attgtggcca human

101   tctttggctt tgtggcattc tttgagatcg gccccggccc catcccctgg mink
      tctttggctt tgtggcattc tttgagattg gcccтggccc catcccctgg horse
      tctttggctt cgtggccttc tttgaaattg gcccтggccc catcccctgg cow
      tctttggctt tgtggccttc tttgagattg gcccaggccc cattccctgg dog
      tctttggctt cgtggcattt tttgagattg gcccтggccc cattcctтgg human

151   ttcattgtgg ctgaactgtt cagccagggc ccccggccag cggccatggc      mink
      ttcatcgtgg ctgagctctt cagccaggga ccccgcccgg cagccatggc horse
      ttcatcgtgg ccgagctctt cagccaggga ccccgcccag cggccatggc cow
      ttcatcgtgg ccgagctgtt cagccagggc ccccgcccag ccgccatggc dog
      ttcatcgtgg ccgagctctt cagccaggga ccccgcccgg cagccatggc human

201   tgtggccggc ttctccaact ggacgtgcaa cttcatcatt ggcatgggtt      mink
      tgtggctggc ttctccaact ggacgtgcaa cttcatcatt ggcatgggct horse
      agtggctggg ttctccaact ggacatgcaa cttcatcatc ggcatgggtt cow
      cgtggctggc ttctgcaact ggacaagcaa cttcatcatt ggcatgggtt dog
      tgtggctggt ttctccaact ggacgagcaa cttcatcatt ggcatgggtt human

251   tccagtatgt ggcggaggct atggggccct acgtcttcct tctcttcgcc      mink
      tccagtatgt cgcggatgct atgggtccct acgtcttcct tctatttgcg horse
      tccagtatgt ggcggatgct atgggtccct acgtctttct tctattcgcg cow
      tccagtatat cgcggangcc atggggccct atgtcttcct tctgttcgcg dog
      tccagtatgt tgcggaggct atggggccct acgtcttcct tctatttgcg human

301   gtcctcctgc ttgg                                          mink
      gtcctcctgc ttgg                                          horse
      gtcctcctgc ttgg                                          cow
      gttctcctgc tcgc                                          dog
      gtcctcctgc tggg                                          human
```

Figure 4.Comparison of inferred protein amino acid sequence of mink glucose transporter 4 (Glut4) with that of cow, horse, human and dog. Sequence references are given in Figure 3. Letters in bold denote differences between the species. Sequence alignment obtained from Blastp (NCBI).

```
  1   LGLAGMCGCAILMTFALLLLERVPAMSFVSIVAIFGFVAFFEIGPGPIPWFIVAELFSQG mink
      LGLAGMCGCAILMTVALLLLERVPAMSYVSIVAIFGFVAFFEIGPGPIPWFIVAELFSQG cow
      LGLAGMCGCAILMTVALLLLERVPAMSYVSIVAIFGFVAFFEIGPGPIPWFIVAELFSQG horse
      LGLAGMCGCAILMTVALLLLERVPAMSYVSIVAIFGFVAFFEIGPGPIPWFIVAELFSQG human
      LGLAGMCGCAILMTIALLLLERLPAMSYVSIVAIFGFVAFFEIGPGPIPWFIVAELFSQG dog

 61   PRPAAMAVAGFSNWTCNFIIGMGFQYVAEAMGPYVFLLFAVLLL                  mink
      PRPAAMAVAGFSNWTCNFIIGMGFQYVADAMGPYVFLLFAVLLL                  cow
      PRPAAMAVAGFSNWTCNFIIGMGFQYVADAMGPYVFLLFAVLLL                  horse
      PRPAAMAVAGFSNWTSNFIIGMGFQYVAEAMGPYVFLLFAVLLL                  human
      PRPAAMAVAGFCNWTSNFIIGMGFQYIAXAMGPYVFLLFAVLLL                  dog
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III – 20 RP

The effect of protein level on N-balance in adult mink (*Mustela vison*)

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Abstract

Diets containing 14.9, 19.0 and 26.7 % of metabolizable energy (ME) from protein and identical amino acid profile were fed ad lib. to adult male mink for 11 days. The average voluntary energy intake decreased (343, 306 and 261 kcal/day/animal, $p<0.0001$) as dietary energy content from protein increased. Daily energy requirement for maintaining constant weight was measured to 171 kcal ME/$kg^{0.75}$ at a temperature of 9.4 °C.

In the last 4 days, nitrogen (N) intake and N excretion (collection of urine and faeces) was measured and the N-balance calculated. N-balance was positive (0.08-0.24 g/day, NS) where as weight change was negative (÷1.6 - ÷8.3 g/day, NS). Regardless of dietary treatment the urinary N excretion declined linearly as digested N decreased. The conflicting positive N-balance and negative weight change was assumed reflecting an incomplete recovery of urinary nitrogen. If weight loss was regarded as either muscle or fat, the average urinary N-recovery was calculated to 86.3% and 92.9% respectively.

Oxidation of protein (OXP) per a calculated total heat production (HE) increased (11%, 16% and 19%, $p<0.05$) as dietary ME from protein increased.

Introduction

Basically the need for nutrients is a requirement for maintenance and a requirement dependant on a production (growth, foetus, milk, hair etc.). As nutrients, this is also the case for amino acids. The current dietary norm for 5 essential amino acids to mink in the growing-furring period were established in 1996 (Børsting & Clausen, 1996) and revised as well as including the 6th essential amino acid in 1998 (Børsting, 1998). In 2002 a minimum dietary content of all amino acids to mink in the winter- and reproduction periods were proposed (Hejlesen & Clausen, 2002). The results of these experiments cover periods which are quite different in regards to the production and are therefore a requirement for both maintenance and production. As the requirement for different kinds of production expectably is different, it would be opportune to know the requirement for maintenance. In the literature there have not been found any attempt to establish the dietary requirement of amino acids for maintenance to mink.

The requirement for amino acids for maintenance is a requirement for a continuous supply to the amino acid pool. Being a carnivore mink has a huge ability of gluconeogenesis, and therefore the importance of protein as energy source has been stressed (Chwalibog et al., 1998). On the other hand – the mink has a large glycolytic capacity (Fink, 2001).

The purpose of the experiment presented was to measure the N-balance in adult male mink fed low and decreasing levels of protein, with a constant level of fat and thereby a increasing level of carbohydrate. It was hypothesized that regardless of diet - a difference between nitrogen digested and nitrogen excreted in urine would reflect the accuracy in urinary nitrogen collection.

It was further-more the intention to evaluate 3 protein levels (with the same amino acid profile) in relation to the requirement of protein for maintenance by calculating the requirement of metabolizable energy for maintaining constant weight and by calculating N-balance. And finally to calculate oxidation of protein relative to total heat production.

Material and Method

The experiment was carried out with 5 adult male mink of the colour type Scanbrown, per diet. Three diets were composed to supply 14, 20 and 26% of metabolizable energy (ME) from protein and 55% from fat (table 1). The amino acid profile (Sandbøl et al. 2004) was the same in all 3 diets (amino acids relative to lysine (%): ala 107, arg 115, asp 133, cys 22, glu 241, gly 111, his 41, ile 74, leu 163, met 59, phe 85, pro 111, ser 89, thr 70, trp 22, tyr 67 and val 100). The animals were housed in metabolic cages designed for separate collection of faeces and urine (modified after Jørgensen, 1973). The temperature in the stable was 9.4 °C (8-11 °C) and relative

humidity 81% (65-83%). The experimental period was 11 days (16th to 27th of February 2004), with a 7 day preliminary period and a 4 day collection period (faeces and urine). The animals were fed 400 kcal per day ($07^{\underline{30}}$). Each day the feed consumption was measured (corrected for dry matter in left-overs), and in the collection period faeces was collected daily and stored at ÷18 °C until analysis (dry matter, crude ash, crude protein and crude fat). Urine was collected in bottles (500 ml) fitted under the funnels (collection period). The bottles were added 5% v/v H_2SO_4 (approx 60 ml) to keep pH low, and urine in the feeding tunnel was absorbed with N-free filtration paper and put in the storage bottle. Every day the collected urine (including drinking water wastage) was filled in bottles (2000 ml) which were stored at ÷18 °C until analysis (crude protein, pH). Animal weight change was recorded both in the preliminary and the collection periods.

Table 1. Composition of the diets used in the N-balance experiment with adult male mink.

Protein (% of ME)	**14.9%**	**19.0%**	**26.7%**
Industrial fish	2.8	3.8	4.8
Poultry offal	13.1	18.1	22.8
Slaughter offal	16.9	23.3	29.3
Barley/Wheat	3.8	5.3	6.6
Corn starch	16.7	10.7	5.2
Feather meal	0.8	1.2	1.5
Haemoglobin	0.2	0.3	0.4
Peas	2.9	4.0	5.0
Potato protein	1.5	2.0	2.5
Corn gluten	1.9	2.6	3.3
Protao	0.4	0.6	0.7
Soya bean oil	8.3	7.1	6.0
Lard	4.2	3.6	3.0
Methionine (DL)	0.3	0.4	0.5
Tryptophane	0.03	0.04	0.05
Lysine	0.02	0.03	0.03
Threonine	0.04	0.05	0.07
Vit. & Min.	0.3	0.3	0.3
Water	26.0	16.8	8.2
Analysis			
Dry matter, %	38.9	38.0	38.9
Kcal/100 g (wet)	201.6	193.9	188.0
ME from: Protein Fat Carbohydrate	 14.9 54.0 31.1	 19.0 53.8 27.2	 26.7 52.0 21.3

By measuring the apparent digestibility of crude protein, crude fat and crude carbohydrate the dietary content of ME was calculated per diet. Crude carbohydrate was calculated as dry matter ÷ content of crude ash, crude protein and crude fat.
When calculating the metabolic weight ($kg^{0.75}$), the absolute weight was calculated as average of the start and the final weight.
Quantitative oxidation of protein (OXP) was calculated by the formula

OXP, Kcal = (Urine N, g * 6.25 * 18.42)/4.1855, (Chwalibog 1992).

Results

For the entire experiment, the voluntary energy intake (ME) was significantly reduced as ME from protein increased resulting in a non-significant increase in weight loss (table 2).

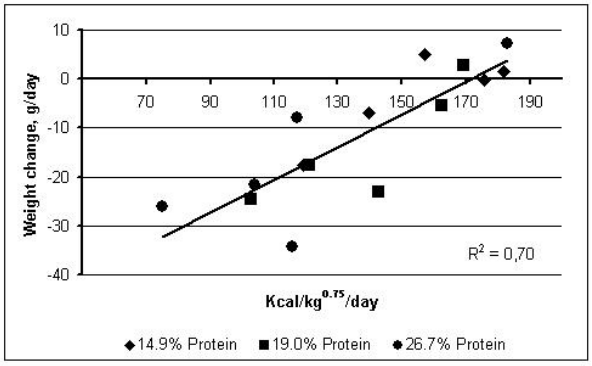
Table 2. Metabolic weight [1)] and daily energy ingested and weight change in the 11 day experimental period in the N-balance experiment with adult male mink.

Protein (% of ME)	**14.9**	**19.0**	**26.7**	**p<**
Metabolic weight, $kg^{0.75}$	2.22	2.19	2.19	NS
Kcal ingested	343[a]	306[b]	261[c]	0.0001
Weight change, g	-3.6	-13.6	-16.5	NS

1) Metabolic weight calculated on the average of initial and final weight of the period.

The required metabolic energy for maintenance was 167, 177 and 169 kcal ME/$kg^{0.75}$ with a mean of all three groups of 171.4 kcal ME/$kg^{0.75}$ (R^2=0.703) (Figure 1).

Figure 1. Energy requirement (regardless of diet) for maintaining constant weight was 171 kcal ME/kg$^{0.75}$ per day (at 9.4 °C) (R^2=0.7) in the N-balance experiment with adult male mink.



In the collection period one animal was excluded from the analysis because it played with the drinking water nipple, which made analysis of N in urine impossible.

The pH in collected urine was 0.75 (± 0.14) which should prevent loss of volatile nitrogen.

The measured digestibility of crude protein (N) varied between 78.4 and 79.8 (NS), crude fat varied between 95.2 and 96.3 (NS) and crude carbohydrate increased from 74.5 to 84.9 with decreasing content of ME from crude protein. This was caused by the addition of the higher digestible starch when percentage of ME from crude protein was reduced.

Also in the collection period the voluntary energy intake (ME) decreased as the percentage of ME from protein increased, however the difference was not statistically significant (table 3). There was a slightly negative weight change in all three groups (NS). With the increasing ME from protein the digested N increased ($p<0.02$), but the degree of increment was reduced by the lower intake of feed. Urinary nitrogen increased ($p<0.005$) with increasing N content in the diets. The calculated nitrogen balance was positive with no difference (NS) between diets.

Discussion

Average daily voluntary intake of ME for the 11 day experimental period decreased as dietary protein content increased. This was also reported by Greaves & Scott (1960) for ad lib. fed adult cats on different dietary protein content. But it is in contrast to findings by Glem-Hansen & Chwalibog (1978). The reason to the reduced energy intake has not been identified, but it is unlikely to be an effect of palatability as the inclusion of the highly palatable ingredients (industrial fish, poultry offal and slaughter offal) increased with increasing dietary content of protein.

Table 3. Metabolic weight [1] and daily weight change, energy intake and Nitrogen parameters in the 4 day collection period in the N-balance experiment with adult male mink.

Protein (% of ME)	**14.9**	**19.0**	**26.7**	**p<**
Metabolic weight, kg$^{0.75}$	2.21	2.17	2.14	NS
Weight Change, g	-4.4	-1.6	-8.3	NS
Kcal ingested	355	308	290	NS
Ingested N, g	2.4^{a}	2.6^{ab}	3.5^{b}	0.0230
Faecal N, g	0.5	0.6	0.7	NS
Digested N, g	1.88^{a}	2.07^{ab}	2.75^{b}	0.02
Urinary N, g	1.64^{a}	1.99^{ab}	2.60^{b}	0.01
N-Balance, g	0.24	0.08	0.15	NS
Recovery of urinary N, % (% of Digested N)	87.8	96.5	95.1	NS
Recovery of urinary N, % (Weight change=protein)	80.6	96.3	84.1	NS
OXP/HE[2], %	11^{a}	16^{ab}	19^{b}	0.05

1) Metabolic weight calculated on the average of initial and final weight of the period.
2) Assuming weight change is fat.

Energy requirement for maintenance

By expressing weight change (g/day) as a function of digested energy per metabolic weight (kcal ME/kg$^{0.75}$) the energy requirement for maintenance was calculated to 171 kcal ME/kg$^{0.75}$ (R^2=0.703) (figure 1). This is above the 147.8 kcal ME/kg$^{0.734}$ found by Harper et al. (1978) however without reporting ambient temperature. Glem-Hansen & Chwalibog (1978) reported the requirement to 143 kcal ME/kg$^{0.75}$ at 20 °C, and a further requirement of 3.7 kcal ME/kg$^{0.75}$ per 1 °C decrease. Using this temperature dependant requirement results in a energy requirement for maintenance in this experiment corresponding to 132 kcal ME/kg$^{0.75}$ at 20 °C.

Even though there was only a minor discrepancy to the results by Glem-Hansen and Chwalibog (1978) they measured it as energy accretion per unit of energy digested. By expressing energy requirement as weight change per unit of energy digested it is assumed that the relative body composition (protein, fat and glycogen) remains constant. Further more there is a potential risk of a bias when weighing the animals. As the animals have unrestricted access to feed and drinking water it is unknown whether the animals just ate, drank or defecated at the time of weighing.

Recovery of urine N

The N-balance (digested N ÷ urinary N) was positive for all three groups (0.08 – 0.24 g/day) (table 3). Calculating with 25% protein in "muscle" gain it corresponds to a "muscle" gain of 2.0 – 6.1 g per day.

Even though mink has the ability to reduce oxidation of protein when protein supply is reduced (Chwalibog et al., 1998; Fink, 2001 & 2003; Tauson, 2000) protein accretion is unlikely when weight change is negative.

So, even with the above mentioned reservation to weight change, a muscle gain is in conflict to the measured negative weight change.

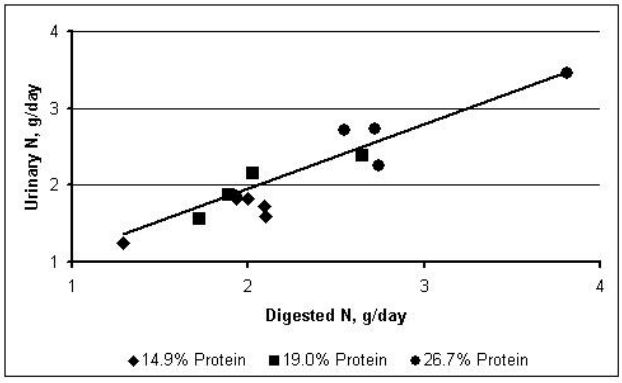
The reason to the discrepancy is presumably a recovery rate of urinary nitrogen below 100%. Using precollected urine (not stabilized with acid) Elnif (1992) simulated urination and measured recovery of nitrogen of 62% to 71%. A incomplete recovery (72%) in urine was also demonstrated by Wamberg et al. (1996) with female mink. Probably because of the female anatomy and behaviour when excreting they too found 6% of the urinary excretion in the collected faeces.

Assuming the weight change was fat, the recovery of urinary nitrogen (100 * Urinary N/Digested N) in this experiment was 88% to 97% whereas it was 81% to 96% if the weight change was regarded as protein (table 3). These recovery rates are higher than reported by Elnif (1992) and Wamberg et al. (1996). This could be an effect of collecting wastage water and urine in the same bottle. Wastage water might function as a continuously partly washing of the collection funnels. The possible negative effect is however that the dilution of the urine can have a magnitude which jeopardizes the determination of urinary nitrogen.

N-balance

For animals at maintenance the urinary N excretion decrease linearly when digested N decreases as long as the digested quantity of N meets the requirement of the animal. If it doesn't, N-balance is negative i.e. urinary excretion exceed digested quantity. The mean N-balance for the animals fed 14.9 percent of ME from protein was not negative or lower than for the other two groups. And when urinary N excretion is plottet against digested N (figure 2) individual data for animals fed 14.9% of ME from protein fits well with the regression line for animals fed 19.0 and 26.7% of ME from protein.

Figure 2. Urinary N excretion declines as digested N decreases. Individual results for 14.9 % of ME from protein fits well (and are not above) the regression line for 19.0 and 26.7 % of ME from protein in the N-balance experiment with adult male mink.



This indicates that 14.9 % of ME from protein and the used amino acid profile did fulfil the animals requirement for maintenance.

OXP, % of HE

Assuming the weight loos is fat (contributing 9.5 kcal heat energy/g) and weight gain is fat (contributing 2.4 kcal heat/g (20% heat production in fat synthesis from fat and carbohydrate) and assuming total heat production is equal to digested energy corrected for contribution from oxidation or synthesis of fat the heat production from protein oxidation would account for 11.3%, 16.2% and 19.3 % of the total heat production in the 3 different diets (table 3).

It has been shown, that mink has the ability to reduce oxidation of protein if protein supply is reduced either by reducing the dietary protein content (Fink, 2001 & 2003 and Tauson, 2000) or by reducing the dietary quantity (Chwalibog et al., 1998).

Even though the heat production is calculated, the data presented here strongly suggest, that the oxidation of protein can be reduced to less than found by Chwalibog (1998) for non productive females, Tauson (2000) and Fink (2001 & 2003) for lactation females. Their higher measured oxidation of protein (% of HE) is most likely a result of diets containing more of the ME as protein. A low supply of protein does however require a sufficient supply of carbohydrates to maintain glucose homeostasis (Fink, 2001).

Conclusion

The recovery of urinary nitrogen was calculated to a mean of 92.9% if weight change was regarded as fat, and 86.3 % if weight change was regarded as protein.

Heat energy from oxidation of protein in % of total heat production was reduced when the protein content of the diet was lowered from 26.7% to 19.0% and 14.9 % of ME.

The data indicated that 14.9 % of ME from protein (and the used amino acid profile) did fulfil the animals protein requirement for maintenance, corresponding to 0.85 g digestible $N/kg^{0.75}$ per day.

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III – 21 RP

Bacterial protein produced on natural gas as a protein source in dry diets for the growing-furring blue fox

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Abstract
Bacterial protein meal (BPM) grown on natural gas as the carbon and energy source, and ammonia as the nitrogen source, was evaluated with respect to digestibility, feed intake, growth and fur characteristics in the growing-furring blue fox (*Alopex lagopus*). The BPM was produced by continuous aerobe bacterial fermentation, using methanotrophic bacteria (*Methylococcus capsulatus* Bath, *Alcaligenes acidovorans*, *Bacillus brevis* and *Bacillus firmus*), and contained approximately 70% crude protein and 10% lipids. Four extruded dry diets containing 0, 4, 8 and 12% BPM, replacing fishmeal, soybean meal and meat meal in a 3:1:1 ratio on a crude protein basis, were fed to groups of 20 weaned blue fox cubs (10 males and 10 females) from August 8 to December 5. The highest level of BPM corresponded to 30% of total dietary crude protein. Digestibility studies showed no significant effect of increasing levels of BPM on digestibility of crude protein, fat or carbohydrate. The growth experiment was carried out without health problems and there was no mortality. Body weights and weight gain were not significantly different among the diets, but there was a tendency ($P<0.10$) towards increased body weight gain with increasing level of BPM. Feed conversion appeared to be slighly improved with increasing dietary inclusion of BPM. Skin size and fur characteristics were not significantly affected by dietary treatment. It is concluded that bacterial protein meal produced from natural gas-utilising bacteria seems to be a suitable alternative protein source for growing-furring blue foxes.

Introduction

The idea of using microorganisms as sources of protein in animal nutrition is not a new one. Single cell protein sources (bacteria, yeast, algae or fungi) have high protein content and the potential yield per unit area may be very high. Thus the rapid growth rate and high protein content are well known advantages of bacteria for protein production (Roth, 1980; Stringer, 1982).

Recently, a commercial scale production plant with an annual capacity of 10 000 metric tons of bacterial protein meal, has been built at Tjeldbergodden, Norway. The production of bacterial protein meal (BPM) is founded on continuous aerobe fermentation using natural gas as the carbon and energy source, and ammonia as the nitrogen source for protein synthesis. Briefly, the BPM consists of the killed and spray-dried biomass of four different naturally occurring bacteria, *Methylococcus capsulatus* (Bath), *Alcaligenes acidovorans*, *Bacillus brevis*, and *Bacillus firmus* (Skrede et al., 1998). The BPM is a reddish/brownish meal containing approximately 96% dry matter, 70% crude protein and 10% fat (Skrede et al., 1998). The average apparent amino acid digestibility of BPM is about 80% in several animal species, including mink, Atlantic salmon, pigs and young chicks, typically with high digestibilities of lysine and arginine, and rather low digestibility of cysteine (Skrede et al., 1998). Recent studies have shown that BPM is suitable as a major protein source in diets for weanling and slaughter pigs, broiler chicken and Atlantic salmon (Øverland et al., 2001; Skrede et al., 2003; Berge et al., 2004; Storebakken et al., 2004).

The potential of BPM as an ingredient of diets for foxes has not been extensively investigated. In the present study, experimentally produced BPM gradually replaced fishmeal, soybean meal and meat meal in dry diets for blue foxes during the growing-furring period. The aims were to study if this replacement influenced nutrient digestibility, feed intake, growth, survival, or fur characteristics.

Material and methods

The study was carried out at the Department of Animal and Aquacultural Sciences, The Agricultural University of Norway, Ås, Norway.

Four extruded dry diets, containing 0% BPM (BPM0) as a control diet, 4% BPM (BPM4), 8%

BPM (BPM8) or 12% BPM (BPM12), were used. The diets were formulated to contain equal levels of crude protein, fat and carbohydrate, and to cover requirements for methionine + cysteine and other essential amino acids for foxes. The highest level of BPM corresponded to 30% of total dietary crude protein. Increasing levels of BPM was balanced by a reduction in fishmeal, meat meal, and soybean meal (SBM) protein, approximately at a 3:1:1 ratio. The chemical composition and amino acid profile of the protein sources are given in Table 1.The BPM ("BioProtein") was supplied by Norferm AS, Stavanger, Norway. Other dietary ingredients were obtained from commercial suppliers.

Table 1. Proximate composition (g kg^{-1}) and amino acid composition (g/16g N) of the protein sources (BPM= bacterial protein meal; SBM= soybean meal).

	BPM	Fish-meal[a]	SBM[b]	Meat meal[c]
Dry matter	958	927	911	952
Crude protein (Nx6.25)	697	717	484	604
Crude fat	102	96	15	94
Ash	80	117	67	198
Amino acids				
Cysteine[1]	0.61	0.97	1.51	0.50
Methionine	2.91	2.96	1.26	1.99
Aspartic acid	8.88	9.56	11.47	7.69
Threonine	4.65	4.25	3.92	3.62
Serine	3.69	4.30	5.82	3.85
Glutamic acid	10.57	12.91	20.16	13.53
Proline	4.18	4.10	5.75	7.59
Glycine	5.16	5.81	4.65	11.87
Alanine	7.15	6.06	4.83	8.55
Valine	6.01	5.29	5.51	4.88
Isoleucine	4.69	4.77	5.18	3.73
Leucine	7.72	7.86	8.24	6.83
Tyrosine	3.71	3.25	4.09	2.99
Phenylalanine	4.28	4.13	5.36	3.93
Histidine	2.33	2.56	2.92	2.51
Lysine	5.93	7.99	5.74	6.59
Arginine	6.31	6.07	7.45	6.73
Tryptophan	2.08	0.93	1.13	*1.10

[1]*Cysteine and cystine.* Table value.*
[a]*Norseamink, Norsildmel, Bergen, Norway.*
[b]*Denofa AS, Fredrikstad, Norway.*
[c]*Norsk Protein, Hamar, Norway*

Table 2. Ingredient composition (g kg^{-1}) and chemical content of extruded blue fox diets containing from 0 – 12 % of bacterial protein meal (BPM).

Diet	BPM0	BPM4	BPM8	BPM12
Bacterial protein meal	0	40	80	120
Fish meal[a]	155	135	115	95
Soybean meal[b]	114	104	94	84
Meat meal[c]	104	94	84	74
Carbohydrate mix[d]	436	436	436	436
Beet pulp	40	40	40	40
Soybean oil	148	148	148	148
Vit./min. mix[e]	3	3	3	3
Calculated content (g kg^{1})				
Dry matter	920	921	922	923
Crude protein	270	272	274	276
Crude fat	191	190	190	189
Ash	56	54	53	51
Total carbohydrate	403	405	405	407
Analysed content (g kg^{-1})				
Dry matter	939	933	917	933
Crude protein (Nx6.25)	274	270	275	278
Crude fat	191	203	185	203
Ash	68	65	62	65
Total carbohydrate	406	395	395	387

[a]*Norseamink, Norsildmel, Bergen, Norway.*
[b]*Denofa AS, Fredrikstad, Norway*
[c]*Norsk Protein, Hamar, Norway.* [d]*FK-Carbo, Felleskjøpet Øst Vest, Oslo, Norway.* [e] *Norsk Mineralnæring, Hensmoen, 3516 Hønefoss, Norway, containing per kg: Fe 2000 mg, Cu 125 mg, Mn 750 mg, Zn 1000 mg, vit. A 200 000 IE, vit. D_3 20 000 IE, vit. E 50 000 mg, vit. B_1 15 000 mg, vit. B_2 3000 mg, vit. B_6 3000 mg, vit. B_{12} 20 mg, Calcium-d-pantothenate 3 000 mg, niacin 5000 mg, folic acid 300 mg, and biotin 30 mg.*

The diets were produced at Centre for Feed Technology, Ås, Norway. The ingredient composition and contents of the experimental diets are shown in Table 2. Each batch was mixed in a Dinnisen twin shaft high-speed mixer, conditioned in a Milltenz singler shaft pre-conditioner (501S, Millband Technology LTD, Auckland, New Zealand), and extruded at defined conditions using a twin-screw Bühler extruder (EX-50/134 L 90 kW, Uzwil, Switzerland). The diets were dried to approximately 93% dry matter in a Milltenz counterflow dryer (VC010 Gas, Millband

Technology Ltd, Auckland, New Zealand before soybean oil was added using a Dinnisen vacuum coater (Oscar Menger, Sevenum, Holland). Prior to bagging the diets were cooled in a Münch counter flow cooler (Münch-Edelstahl GMBH, Hilden, Germany).

A digestibility experiment was carried out from October 27th to November 10th, using three male blue foxes per dietary treatment. The animals were kept at approximately normal hours of day length and a temperature of about 16 °C. The individual cages were provided with devices for controlled feeding and separate collection of feces and urine. The experimental period consisted of a 3-day preliminary period and a 4-day fecal collection period. Individual samples of feces were bulked, homogenized and freeze dried pending analysis.

In the main experiment, each diet was given to 20 weaned blue fox cubs, 10 males and 10 females, with an initial body weight of approximately 2.8 kg. The animals were allocated to four groups according to body weight and genotype. The groups were randomly assigned to treatment. The experiment started August 8th, when the animals were approximately two months old, and terminated December 5th. The animals were kept under conventional farm conditions, using two animals of the same sex in each cage. The dry diets were added water in proportion 1/3 feed to 2/3 water. The wet mix was allowed to swell to a dough consistency for 14-15 h before feeding on boards once a day. Ad libitum feeding was adopted. The animals were given free access to water through a semi-automatic watering system.

The animals were weighed individually at the start of the experiment, and at 4-week intervals thereafter. Feed consumption was recorded on a group basis as feed offered minus feed rejected. Evaluation of fur characteristics was carried out on dried and undressed skins by experienced graders.

Analyses of dry matter, crude protein (Kjeldahl-N x 6.25), crude fat (HCl/ethyl ether extract) and ash, were carried out by the Laboratory of Analytical Chemistry, Agricultural University of Norway, using the methods of AOAC (1980). Crude carbohydrate was determined using difference calculation. Apparent values of total tract digestibility were determined as the average of three individual determinations.

Statistical analysis was carried out using the GLM procedure of SAS (1990). The level of significance was set at $P<0.05$.

Results and discussion

Digestibility experiment

All diets were well eaten by the animals and there were minor differences in fecal consistency. There were no significant differences between diets in digestibility of crude protein, fat or carbohydrate (Table 3). Linear regression analysis showed no significant effect of increasing levels of BPM on nutrient digestibility.

Table 3. Average digestibility of main nutrients (%) in experiment with diets containing from 0 to 12 % of bacterial protein meal (BPM) for blue foxes. Standard deviations in parentheses.

Diet	BPM0	BPM4	BPM8	BPM12
Crude protein	94.5 (1.5)	92.4 (1.5)	92.8 (0.7)	93.0 (1.6)
Crude fat	96.4 (0.5)	96.2 (0.5)	95.9 (0.6)	96.7 (0.5)
Crude carbohydrate	68.6 (5.1)	62.9 (2.2)	64.9 (4.7)	69.3 (3.6)

No significant differences were found.

Production experiment

All animals accepted the diets well throughout the entire experimental period. Increasing dietary levels of BPM appeared to have no effect on palatability. No signs of any health problems were seen during the experiment and there was no mortality.

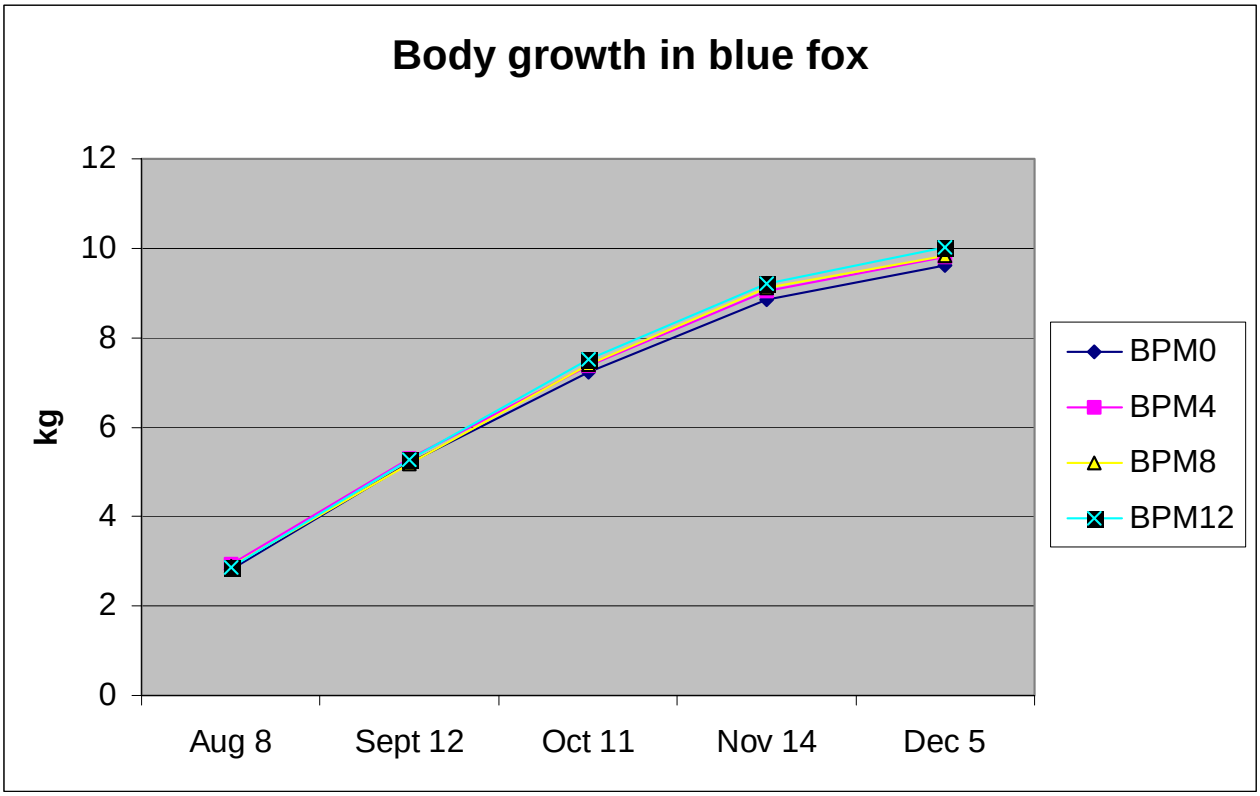
Data on feed consumption are shown in Table 4, and growth data are presented in Table 4 and Figure 1. The animals revealed generally satisfactory weight gain, considering the genetic potential of the blue fox strain used in the present experiment. There was a tendency ($P>0.05$) towards enhanced body gain with increasing levels of BPM. This slight increase in growth was achieved without increase in feed consumption, indicating an improvement of feed conversion due to the feeding of BPM (Table 4). Similar results have been obtained in studies with broiler chicken (Skrede et al. 2003). A possible mechanism affecting feed conversion may be related to the amino acid profile of BPM, especially the high level of tryptohan (Table 1). Tryptophan is a precursor of the neurotransmitter serotonin, which indirectly may influence energy requirement by modulating animal behavior as shown in studies with silver foxes (Rouvinen et al., 1999).

Table 4. Overall body weight gain, feed consumption, and feed conversion from August 8th to December 5th in blue foxes fed diets containg from 0 to 12 % of bacterial protein meal (BPM). Standard deviation in parentheses.

Diet	BPM0	BPM4	BPM8	BPM12
Overall gain (kg)	6.79 (1.18)	6.87 (1.36)	6.95 (1.03)	7.16 (1.31)
Average dry feed consumption per animal (g/day)	295.8	294.6	295.2	293.8
Feed conversion rate (kg feed/kg body gain)	5.18	5.10	5.05	4.88

No significant differences in body weight gain were found.

Figure 1. Body growth in blue foxes fed diets containg from 0 to 12 % of bacterial protein meal (BPM).



Skin size, measured by length and weight, and the evaluation of fur quality characteristics showed no significant differences between treatment groups (Table 5). Thus, fur quality was not influenced by dietary inclusion of BPM. However, this should be confirmed in future experiments with a larger group size than used in the present study.

It was concluded from the present study that bacterial protein meal produced from natural gas-utilising bacteria seems to be a suitable alternative protein source for the growing-furring blue fox.

Acknowledgement

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Table 5. Average skin length and weight, and fur characteristics in blue foxes fed diets containg from 0 to 12 % of bacterial protein meal (BPM). The figures represent 50% males and 50 % females. Standard deviation in parentheses

Diet	BPM0	BPM4	BPM8	BPM12
Skin length (cm)	109.0 (6.1)	109.0 (4.4)	109.8 (3.8)	109.2 (5.7)
Skin weight (g)	636.1 (80.6)	624.5 (75.5)	674.2 (65.5)	664.1 (96.4)
Colour[1]	5.5 (1.2)	5.7 (1.5)	5.4 (1.2)	5.8 (1.1)
Cover[2]	5.6 (1.2)	5.7 (1.0)	5.3 (1.0)	5.2 (1.2)
Hair quality[2]	5.3 (1.2)	5.1 (1.2)	5.3 (1.5)	4.8 (1.3)
Hair density[2]	5.2 (1.3)	5.2 (1.2)	5.7 (1.2)	5.3 (1.4)
Texture[2]	5.3 (1.4)	5.0 (1.4)	5.3 (1.0)	5.0 (1.1)
Overall Impression[2]	4.9 (1.1)	4.8 (1.0)	5.4 (1.0)	5.0 (1.3)

[1]Grading points from 1 (dark) to 10 (pale). [2]Grading points from 1 (poorest) to 10 (best).
No significant differences were found.

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