Original research article

Effects of dietary protein level on nutrients digestibility and reproductive performance of female mink (*Neovison vison*) during gestation

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A B S T R A C T

The objective of this study was to determine whether nutrient digestibility and reproductive performance of pregnant mink (*Neovison vison*) were affected by different dietary protein levels. One hundred and twenty female mink were randomly assigned to four groups, receiving diets of fresh material with different protein levels. The dietary protein levels, expressed as percentage of dry matter (DM), were 32, 36, 40 and 44% respectively. These values corresponded to average 320, 360, 400 and 440 g protein/kg DM, respectively. Results were as follows. All of crude protein digestibility, nitrogen (N) intake, N retention increased along with dietary protein level increasing. Low protein level (32%) significantly reduced the above indicators ($P < 0.05$). DM digestibility and ether extract digestibility were not affected by dietary protein level. Results of mated females, barren females, kids per litter, live born kids per mated female, birth survival rate, and birth weight showed that mink achieved optimal reproductive performance when dietary protein level was 36%. In conclusion, dietary protein was anticipated to significantly influence some nutrients' utilization. Adopting the appropriate dietary protein level allow better reproduction performance. The most preferable reproductive performance was achieved when diet contained 275.5 g digestible protein per kg DM for female mink in gestation.

1. Introduction

Mink are widely farmed in China as economic carnivorous animals with a higher demand for protein than other domestic animals. Protein constitutes the most expensive part of mink feed. Consequently, possibilities to decrease the level of dietary protein in order to provide inexpensive feed but still support animal performance and health have been the subject of research for several years (Nutrition, 1982; Skrede, 1978a, 1978b; Työppönen et al., 1986). Furthermore, there has been a general wish to minimize the emission of nitration via urine and feces to the environment (Bikker et al., 2010).

Reproduction of mink has been intensively studied in many research projects. Dietary protein deficiency was associated with reduced body weight of the offspring (Vesterdorf et al., 2012). A diet with low dietary protein content, which is 14% of metabolizable energy (ME) during late gestation, affected reproductive performance (Matthiesen et al., 2010). And the progression in breeding result is poor when compared with other mammals like sows or cows. Research has been conducted to demonstrate the effect of different feeds on reproductive capacity (Crum et al., 1993; Dahlman et al., 2002; Fink and Børsting, 2002; Fink et al., 2006; Matthiesen et al., 2010; Skrede, 1978b; Travis and Schaible, 1961). However, no specific information was found about the protein

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requirement of female mink during gestation. Thus, it is necessary to carry out the study on dietary nutrient levels on female mink in breeding season.

This present study investigates whether different dietary protein levels affect reproductive performance of female mink during gestation. The specific aims were to investigate the effect of gestational protein supply on feed intake, nutrients digestibility, nitrogen (N) metabolism and reproductive performance.

2. Materials and methods

The experiment was carried out at the Fur Animal Breeding Base of Institute of Special Economic Animal and Plant Science, Chinese Academy of Agricultural Sciences (44.02°N, 126.15°E) in the northeast of China. The animals used in this work were managed according to the requirements of the national Experimental Animals Protection Law, and with bioethics and biosecurity committee approval.

2.1. Animals, diets and management

A total of 120 two-year-old female mink (*Neovison vison*) of the standard black genotype were housed in roofed standard sheds with open sides. The experimental period extended from 14 d before mating until parturition. All animals were weighed at start of experiment and distributed randomly into four experimental groups from A to D with dietary protein levels of 32, 36, 40 and 44%, respectively. These levels corresponded to average 320, 360, 400 and 440 g protein/kg dry matter (DM), respectively. Decreasing dietary protein levels (in the experiment from 317.9 to 453.9 g/kg DM) from groups A to D were compensated by increasing the level of dietary carbohydrates (from 413.8 to 246.7 g/kg DM). Ether extract (EE) content (g/kg DM) in the diets remained constant within the range from 166.5 to 167.0 g/kg DM, so the diets were isocaloric. Throughout the experimental period, mink were fed the experimental diets twice a day ad libitum at 0800 and 1600 (Beijing time), and drinking water was taken freely by mink. The ingredients of the four diets were listed in Table 1, chemical composition in Table 2, and amino acid content in Table 3.

2.2. Mating and nitrogen balance experiment

The female mink were mated starting from 5 March, 2010. After about 25 d, eight pregnant mink were randomly selected from each group and housed in individual cages used for digestibility measurements and N-balance experiments.

Table 1
Ingredient of the experimental diets (air-dry basis, %).

Item	Group A	Group B	Group C	Group D
Extruded corn	53.34	48.36	43.74	39.46
Yellow croaker	12.02	16.49	20.49	24.16
Poultry offal	5.00	5.00	5.00	5.00
Heated eggs	7.00	7.00	7.00	7.00
Lard	16.15	16.65	17.27	17.88
OX liver	5.00	5.00	5.00	5.00
Salt	0.50	0.50	0.50	0.50
Premix[1]	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00

Groups A to D denoted dietary protein levels, 32, 36, 40 and 44%, respectively.
[1] Premix provided per kilogram diet: Vitamin A 1,000,000 IU; Vitamin D 3,200,000 IU; Vitamin E 6,000 IU; Vitamin B_1 600 mg; Vitamin B_2 800 mg; Vitamin B_6 300 mg; Vitamin B_{12} 10 mg; Vitamin K_3,100 mg; Vitamin C 40,000 mg; Nicotinic acid 4,000 mg; Pantothenic acid 1,200 mg; Alkaloid 20 mg; Folic acid 80 mg; Choline 30,000 mg; Fe 8,200 mg; Cu 800 mg; Mn 1,200 mg; Zn 5,200 mg; I 50 mg; Se 20 mg; Co 50 mg.

Table 2
Nutrient composition of the experimental diets, % of DM.

Item	Group A	Group B	Group C	Group D
Ash	6.08	8.99	9.43	11.24
CP	31.79	36.53	41.39	45.39
EE	16.65	16.67	16.69	16.77
CC	45.48	37.81	32.49	26.67
Ca	2.49	2.48	2.44	2.40
TP	1.32	1.28	1.30	1.33
ME, MJ/kg	13.27	13.29	13.38	13.40
P:F:C	34:47:19	38:46:16	42:44:14	46:42:12

Groups A to D denoted dietary protein levels, 32, 36, 40 and 44%, respectively. DM = dry matter; CP = crude protein; EE = ether extract; CC = crude carbohydrate; TP = total phosphorus; ME = metabolizable energy; P:F:C = energy distribution of protein, fat and carbohydrate.
Values of CP, EE, Ca and TP were measured and other values were calculated.

Table 3
Amino acids content of the experimental diets, % of DM.

Item	Group A	Group B	Group C	Group D
Aspartic acid	1.34	1.54	1.73	1.89
Threonine	0.89	1.01	1.12	1.22
Serine	1.09	1.21	1.32	1.45
Glutamic acid	3.25	3.65	4.01	4.34
Glycine	1.12	1.31	1.48	1.63
Alanine	1.28	1.44	1.58	1.71
Valine	1.13	1.26	1.37	1.48
Methionine	0.72	0.81	0. 90	0.98
Isoleucine	0.78	0.88	0.98	1.06
Leucine	1.74	1.92	2.07	2.21
Tyrosine	0.65	0.73	0.81	0.88
Phenylalanine	1.00	1.11	1.21	1.30
Lysine	1.30	1.53	1.73	1.91
Histidine	0.47	0.51	0.56	0.60
Arginine	1.07	1.23	1.37	1.50
Proline	0.59	0.64	0.69	0.73

Groups A to D denoted dietary protein levels, 32, 36, 40 and 44%, respectively.

The experiment consisted of a three-day collecting and recording period carried out on the eight pregnant mink from respective treatment groups. The animals were kept in metabolism cages constructed for separate collection of feces and urine. Feed residues, feces and urine were quantitatively collected, weighed and recorded daily and stored at $-20\ °C$ until the end of the balance period. To avoid ammonia evaporation from the urine, 20 mL sulfuric acid (5% solution) was added to the urine collection bottles, and the urine collection trays were sprayed with citric acid (20% solution) daily. In the N-balance calculations, retained N was determined as ingested N- (fecal N + urinary N).

2.3. Chemical analysis

Wet samples of diets and feces were analyzed for DM and N (AOAC and Helrich, 1990). The freeze dried samples of diets and feces were analyzed for crude protein (CP) and EE. Urine and citric acid rinse were analyzed for N content.

DM, ash, CP (Kjeldahl-N × 6.25) and EE after acid hydrolysis were determined according to standard procedures (AOAC and Helrich, 1990). Crude carbohydrate was calculated as the difference by subtracting ash, CP and EE from the DM content. Amino acids in diets were analyzed by amino acid analyzer (L-8900, HITACHI, Japan), as described by Ma et al. (2010). The apparent digestibility (AD) coefficient of nutrients was calculated as follows: AD = (a − b)/a × 100, where "a" is nutrient intake from feed, and "b" is nutrient excretion in feces. The calculation of ME content and the proportional composition of ME were based on the digestibility coefficients achieved and the following values of ME:

protein 18.8 MJ/kg, EE 39.8 MJ/kg and carbohydrate 17.6 MJ/kg (Clauss et al., 2010).

2.4. Reproductive performance evaluation

Mated female mink, barren female mink, kids per litter, and live born kids per mated female were recorded or calculated. Mink kids were weighted at birth. These were used as indexes to determine the reproductive performance of dams (Korhonen et al., 2002; Shaw et al., 1997; Tauson and Aldén, 1984; Vesterdorf et al., 2012).

2.5. Statistical analysis

The statistical analyses of data were carried out using the Chi-square statistics and one-way ANOVA procedure of SAS (version 8.2, SAS Institute, Inc., Cary, NC, USA). Data were represented as mean ± SD. Values of P less than 0.05 were considered statistically significant and those of P less than 0.01 were considered statistically highly significant.

3. Results

3.1. Feed intake and nutrients digestibility

Effects of different dietary protein levels on performance and nutrient digestibility are showed in Table 4. DM intake reached the peak in group B and was significantly higher than group D ($P < 0.05$). No significant difference was observed among the four groups in DM output and DM digestibility ($P > 0.05$). CP digestibility linearly increased with increasing dietary protein levels. Group D had a significantly higher CP digestibility than group A ($P < 0.05$). Results also showed protein supply had no effect on EE digestibility in gestation ($P > 0.05$).

3.2. Nitrogen-balance

Effects of different dietary protein levels on N-balance are presented in Table 5. Dietary protein caused highly significant higher N intake ($P < 0.01$), while fecal and urine N were not affected ($P > 0.05$).

3.3. Reproductive performance

Data of reproductive performance is presented in Table 6. Assessed by using chi-square statistics, the numbers of mated females and barren females were not affected by dietary protein levels, but reduced number of barren females was observed in group B ($P > 0.05$). None of kids per litter, live born kids per mated

Table 4
Effect of dietary protein levels on feed intake and nutrients digestibility of female mink during gestation.

Item	Group A	Group B	Group C	Group D
DM intake, g/d	61.40 ± 2.52[ab]	63.24 ± 2.55[a]	59.93 ± 2.67[ab]	57.92 ± 8.79[b]
DM output, g/d	13.66 ± 1.85	14.63 ± 1.57	14.60 ± 2.52	12.85 ± 3.47
DM, %	77.78 ± 2.62	76.90 ± 2.65	75.66 ± 3.93	78.17 ± 4.34
CP, %	75.35 ± 4.35[Bb]	76.64 ± 2.65[ABb]	78.03 ± 5.29[ABab]	81.62 ± 3.58[Aa]
EE, %	95.13 ± 1.64	95.91 ± 2.47	96.11 ± 1.55	95.20 ± 1.92

Groups A to D denoted dietary protein levels, 32, 36, 40 and 44%, respectively.
DM = dry matter; CP = crude protein; EE = ether extract.
[a, b]Means in the same row with different lowercase of superscripts were significantly different at $P < 0.05$.
[A, B]Means in the same row with different capital letters of superscripts were greatly and significantly different at $P < 0.01$.

Table 5
Effect of dietary protein levels on nitrogen metabolism balance of female mink during gestation.

Item, g/d	Group A	Group B	Group C	Group D
N intake	3.12 ± 0.13[C]	3.70 ± 0.15[B]	3.97 ± 0.18[AB]	4.25 ± 0.15[A]
Fecal N	0.77 ± 0.05	0.86 ± 0.07	0.87 ± 0.07	0.79 ± 0.05
Urine N	1.54 ± 0.12	1.53 ± 0.12	1.68 ± 0.14	1.72 ± 0.16
N retention	0.81 ± 0.08[Bb]	1.30 ± 0.08[ABab]	1.41 ± 0.11[ABa]	1.74 ± 0.13[Aa]

Groups A to D denoted dietary protein levels, 32, 36, 40 and 44%, respectively.
N = nitrogen.
[a, b]Means in the same row with different lowercase of superscripts were significantly different at $P < 0.05$.
[A, B]Means in the same row with different capital letters of superscripts were greatly and significantly different at $P < 0.01$.

female, birth survival rate or birth weight was significantly affected by dietary protein level ($P > 0.05$), but the birth survival rate of group A was significantly decreased ($P < 0.05$), and most of these indexes reached the highest in group B.

4. Discussion

Present studies suggested that protein intake may play a part in feed intake regulation, and high protein diets caused a depression in feed intake (Harper et al., 1970; Musten et al., 1974; Scharrer et al., 1970). In this study, DM intake increased initially, reaching the highest in the 36% dietary protein group and then decreased, indicating mink are regulating the feed consumption so as to balance their gains of protein (Mayntz et al., 2009). Less DM intake in the higher protein diet group was probably due to the higher energetic costs of using amino acids as glucose precursors, as demonstrated by Chwalibog et al. (2004).

The apparent digestibility of DM was highly dependent on the dietary protein level. The lower the dietary protein level, the lower was the DM digestibility (Dahlman et al., 2002). Our results showed that dietary protein level had no significant effect on DM digestibility, probably because energy densities of all diets were within the range where mink could be expected to regulate their intake.

As strict carnivores, mink have a higher demand for protein than other domestic animals. Previous studies found that decreasing dietary protein level leads to the decreasing values of apparent digestibility (Skrede, 1979; Szymeczko and Skrede, 1990). Our study demonstrated that the apparent protein digestibility decreased in group A with the lowest protein content, but not significantly affected at higher protein levels. During the breeding season, pregnant mink need protein not only for growth of the gravid uterus, but also for reserving protein as mobilizable amino acids available to meet the increased N requirement of early lactation, the

Table 6
Effect of dietary protein levels on reproductive performance of female mink during gestation.

Item	Group A	Group B	Group C	Group D
Mated females, %	100.00	100.00	100.00	100.00
Barren females, %	29.57	11.11	25.00	39.29
KPL	5.4 ± 1.88	6.38 ± 2.02	5.52 ± 2.44	5.00 ± 3.26
LBKPMF	4.5 ± 2.33	6.04 ± 1.97	5.10 ± 2.41	4.65 ± 3.31
Birth survival rate, %	81.35 ± 12.40[a]	94.41 ± 10.03[b]	92.05 ± 13.39[b]	91.18 ± 16.43[b]
Birth weight, g	11.33 ± 1.63	11.67 ± 1.72	11.29 ± 1.21	12.08 ± 2.26

Groups A to D denoted dietary protein levels, 32, 36, 40 and 44%, respectively.
KPL = number of kids per litter; LBKPMF = number of live born kids per mated female.
[a, b]Means in the same row with different lowercase of superscripts were significantly different at $P < 0.05$.

development of the mammary gland, and a likely increase in protein requirement for the hypertrophy of visceral tissues, including gut and liver, in late pregnancy (Fell et al., 1972; Robinson et al., 1978) and in maternal tissue. In adequately nourished dams fed high protein diets (groups with dietary protein levels of 36, 40 and 44%), part of the protein digested for fetal growth could possibly be sustained by mobilization of a labile protein reserve that has accreted during early gestation (Tauson et al., 2004).

According to the present research, N excretion in fecal and urine were not significantly affected by protein provision during gestation, probably due to endogenous N secretion from the digestive tract. A previous study on pigs demonstrated that reduced dietary protein would lead to an enhanced relative amount of endogenous N secretion (Le Bellego and Noblet, 2002), which could explain the stable N excretion among groups. In the present study, CP intake had a significant effect on N retention as determined by collection of urine and feces during pregnancy, as previously found in mink (Matthiesen et al., 2010; Zhang et al., 2013). Although N retention increased along with protein level, one cannot make conclusion on the protein requirement of pregnant mink until the reproductive performance is investigated.

Our data showed mink of all groups were successfully mated. The group with 36% dietary protein showed the lowest number of barren females, and significantly higher number of kids per litter, the number of live born kids per mated female, and birth survival rate. The fact that low protein diets impair the reproductive performance has been demonstrated by extensive studies (Bellinger et al., 2006; Galler and Tonkiss, 1991; Matthiesen et al., 2010; Pinheiro et al., 2008; Sherman et al., 1999). Hansen (1974) found that low levels of protein (25% of ME from protein) throughout gestation resulted in a tendency for suboptimal reproductive performance. A diet with lower dietary protein content resulted in 15% barrenness, 30.6% fewer kids per mated female and a lower number of live kids born per litter, and F1-generation kids with protein restriction during fetal life had a lower birth weight than F1-generation kids with adequate protein (Matthiesen et al., 2010).

Studies had shown that increasing dietary protein level increased protein oxidation rates and metabolic rates, which leaded to an acute increase in reactive oxygen species (ROS) generation (Fink and Børsting, 2002; Mohanty et al., 2002; Rouvinen-Watt, 2003). Agarwal et al. (2005) reported that, ROS, playing a role with regard to the female reproductive tract, can affect oocyte maturation and follicle development, fertilization and implantation, the development of the fetus, and pregnancy itself by causing abortions, birthing complications and defects in the offspring. Thus, excessive protein intake may stimulate ROS generation in high protein level groups, leading to poorer reproductive performance.

Therefore, it could be concluded that birth rate of female mink could be adversely affected by both deficiency and excess of protein, and 36% dietary protein level (275.5 g digestible protein per kg DM) met the protein requirement of pregnant mink in this investigation.

5. Conclusions

Our findings suggested that dietary protein level during gestation significantly affected the nutrients digestibility and N-balance of mink. Optimal reproductive performance was observed with the dietary protein levels up to 36% (275.5 g digestible protein per kg DM), which we recommend to be adopted in mink farming business.

Conflict of interest statement

The authors declare that they have no competing interests.

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