DIETARY PROTEIN LEVELS AFFECT THE NURSING PERFORMANCE AND SERUM BIOCHEMICAL PROFILE OF LACTATING MINK (*Neovison vison*)

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ABSTRACT

This study was aimed to evaluate effects of dietary protein levels on the nursing performance of lactating mink (*Neovison vison*). A total of 120 pregnant mink were randomly assigned to 4 dietary treatment groups with protein levels of 32% (P32, control), 36% (P36), 40% (P40) and 44% (P44) (dry matter basis, corresponding to 29%, 32%, 36% and 39% of ME), and fed *ad libitum* for 8 weeks from late gestation to the end of lactation. Results showed that daily weight gain of kits increased linearly with the increasing dietary protein levels although the difference was not significant. Similar trend was noted for the survival rate of kits at weaning. Dams fed diets with higher protein levels showed less body weight loss during lactation. Improved metabolic status was demonstrated by the changed metabolic profiles as indicated blood urea nitrogen level (P<0.05), total protein (P<0.05), glutamate pyruvate transaminase activity and blood glucose content (P<0.05). In conclusion, we found that dietary protein intake significantly affects the nursing performance of female mink. We suggest that an estimated dietary protein level of 44% (39% of ME) could be used as a guidance to achieve the optimal performance of lactating dams.

Key words: dietary protein; female mink; lactation; nursing performance.

INTRODUCTION

Mink (*Neovison vison*) are widely raised in fur industry and they have only one breeding season per year. In the northern hemisphere, mink mate during the month of March, which lasts for approximately 20-25 days both in farm and in the wild. After an average of 51 day-pregnancy parturition occurs, which is followed by lactation of 45-60 d (Hammer *et al*. 2017; Kiik *et al*. 2017).

Lactation is one of the most challenging physiological stages of mink's life cycle. As an obligate carnivore, mink require for diets with high in protein content. In particular, protein deficiency may significantly undermine the reproductive performance of mink. It is known that nutritional restriction during fetal development leads to permanent damage to physiology and metabolism of animals in adult life (Mcmillen and Robinson 2005). The negative effect of protein malnutrition was reported by and Vesterdorf *et al*. (2012) in mink dams of late gestation and lactation. Although protein requirement during lactation is high, excess intake can also lead to obesity, which can deleteriously impact mink productivity (Korhonen and Niemelä 1998). Studies have shown that increasing dietary protein intake results in increased metabolic rate and protein oxidation rate, leading to elevated oxidative stress (Fink and Børsting

2002; Rouvinen-Watt 2003). Such metabolic disorder often leads to the development of nursing sickness, which is responsible for most of the mortalities in lactating mink dams (Clausen *et al*. 1992). However, current recommendations of dietary protein level for the lactating mink are inadequate, and systematical evaluation of dietary protein requirement of female mink in lactation has been largely neglected (Skrede 1978; Glem-Hansen 1979; NRC 1982; Fink *et al*. 2002; Hynes and Rouvinen-Watt 2007). Thus, with the purpose for better estimating the protein requirement of lactating mink, this study was carried out to investigate effects of dietary protein levels on the nursing performance of lactating mink by measuring growth rate of the offspring, change of the dam's body weight and serum biochemical and metabolic parameters.

MATERIALS AND METHODS

Animals and management: The experiment was carried out at the Fur Animal Breeding Base of Institute of Special Animal and Plant Science, Chinese Academy of Agricultural Sciences (44.02° N, 126.15° E) in the northeast of China, from March 2012 to May 2012, with the experimental protocol being reviewed and approved by the Animal Care Committee of the Institute. All procedures followed the Laws, Regulations, and

Guidelines for Animal Research in China (Ogden *et al*. 2016) and were abided by the Welfare of Animals Kept for Fur Production of European Commission. 120 standard black genotype female mink (*Neovison vison*) of two-year-old were housed and managed as previously described (Jiang *et al*. 2015). The experimental period started from 14 day before parturition (March 2012) and end at weaning (May 2012). All animals were weighed prior to experiment and randomly distributed to four experimental groups employing the completely randomized design (CRD), and subjected to diets with protein levels of 32% (P32, control group), 36% (P36), 40% (P40) and 44% (P44), corresponding to an average of 320, 360, 400 and 440 g protein/kg dry matter (DM), respectively. Dietary carbohydrates (in the experiment from 413,8 to 246,7 g/kg DM) were adopted to compensate the reduced protein levels (from 317.9 to 453g/kg DM) from group P32 to P44. To achieve isocaloric diets among groups, content of ether extract (EE) (g/kg DM) remained constant (from 166,5 to 167,0 g/kg DM). The ingredients and chemical composition of diets were listed in Table 1, and amino acid content in Table 2.

Blood sampling and measurement of metabolic parameters: After weaning (45 days after birth), blood samples from these dams were collected for the determination of serum biochemical and metabolic parameters. Serum was immediately isolated by centrifugation at 2,500g at 4°Cfor 5 min and kept frozen at -80 C for the determination of biochemical indicators. The blood urea nitrogen level (BUN), total protein content (TP), glutamate pyruvate transaminase activity (GPT), and blood glucose content (GLU) were measured using Hitachi 7020 automatic biochemistry analyzer (Hitachi High Technologies, Inc., Ibaraki, Japan) following kits' manufactural instructions (Nanjing Jiancheng Biochemical Corporation, Nanjing, China).

Chemical analysis: Chemicals analysis and ME calculation were performed as previously described (Jiang *et al*. 2015). Amino acids in diets were estimated on amino acid analyzer (L-8900, HITACHI, Japan), as described by Ma *et al*. (2010).

Nursing performance evaluation: All animals were inspected twice daily. Weights of individual nursing females and kits were recorded at regular intervals using an electronic scale, on the day after delivery or birth (= day 1), at days 9, 18, 27, 36, and on the day before weaning (= day 45). The average number of live-born kits per dam was considered as litter size. Nursing performance of dams were determined by parameters/indexes including mated female mink, live-born kids per mated female, kits per litter, kits weight and barren female mink (Tauson and Aldén 1984; Shaw *et al*. 1997; Korhonen *et al*. 2002).

Statistical analysis: Data were analyzed as previously described (Jiang *et al*. 2015). Briefly, the data were analyzed with one-way ANOVA according to CRD design using SAS (version 8.2, SAS Institute, Inc., Cary, NC, USA). Mean ± SD are shown. $P < 0.05$ stands for statistical significance and $P < 0.01$ for highly statistical significance.

RESULTS

Kit growth dynamics and survival rate: No significant difference was observed regarding average litter size at birth and birth weight as compared to the control group (P32), indicating minimal effect of fetal life on the growth and mortality of the kits at birth. In contrast, dams fed the P44 exhibited much higher daily weight gain of kits and better growth performance throughout the lactation as compared to other groups, in particular to the control group. Similar tendency was also found on the survival rate of kits at weaning (Table 3, Fig.1).

Dams weight loss: After parturition, 8 female mink farrowing 5 to 9 kits were selected from each group for the measurement of growth dynamics of kits and body weight loss of the dams. Fig.2 shows the live weight change of dams measured within 45 days after delivery and before weaning. While all animals showed decreased total body mass during the course of lactation, dams fed with high protein diet (P44) had significant less weight loss in comparison with dams fed with reduced protein diet (P36) ($P< 0.05$) (Table 4).

Serum parameters: To assess the potential effect of dietary protein levels on the health of dams, blood was collected at the end of lactation after overnight fasting for biomedical parameters measurement. Results indicated that BUN, TP and GLU increased steadily from control group to P44 with statistical significance (Table 5, $P<0.05$). GPT did not show differences among treatments, but notable elevation along with the increasing protein levels was observed, similar to results of other parameters.

DISCUSSION

Protein provision exerts significant effect on growth performance, nutrients digestibility, protein oxidation and fat metabolism of mink at various developmental stages (Matthiesen *et al*. 2012; Zhang *et al*. 2012; Zhang *et al*. 2013; Matthiesen *et al*. 2014). However, the impact of dietary protein on dams in lactation has been so far largely neglected in mink research. The present study focused on understanding the response of dams to different levels of dietary protein intake during lactation, with an eventual goal of improving nursing performance of the lactating mink.

Table 1. Composition and nutrient levels of the experimental diets (air-dry basis, %).

Items	P32	P36	P40	P44
Ingredients				
Extruded corn	33.82	29.45	24.70	19.02
Yellow croaker	26.87	30.75	35.05	40.32
Poultry offal	5.00	5.00	5.00	5.00
Boiled Eggs	7.00	7.00	7.00	7.00
Lard	20.81	21.30	21.75	22.16
Ox Liver	5.00	5.00	5.00	5.00
Salt	0.50	0.50	0.50	0.50
Vitamin/mineral premix[1]	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
Nutrient levels[2]				
Ash, % of DM	5.12	6.99	7.43	9.24
CP, % of DM	32.28	36.33	40.94	44.68
EE, % of DM	24.87	25.15	24.76	24.72
Ca, % of DM	2.86	2.89	2.85	2.84
TP, % of DM	1.92	1.89	1.96	1.83
ME (MJ/kg)	18.37	18.59	18.85	18.90
CP:Fat:CC ratio (% of ME)	29:54:17	32:54:14	35:53:12	39:51:10

Notes: [1] The premix contained per kg: Vitamin A 1000 000 IU; Vitamin D 3200 000 IU; Vitamin E 6000 IU; Vitamin B1 600 mg; VB2 800 mg; Vitamin B6 300 mg; Vitamin B12 10 mg; Vitamin K3 100 mg; Vitamin C 40000 mg; Nicotinic acid 4000 mg; Pantothenic acid 1200 mg; Biotin 20 mg; Folic acid 80 mg; Choline 30000 mg; Fe 8200 mg; Cu 800 mg; Mn 1200 mg; Zn 5200 mg; I 50 mg; Se 20 mg; Co 50 mg. [2] Values of CP, EE, Ca and TP were measured, and others were calculated values. CP = crude protein; DM = dry matter; EE = ether extract; CC = crude carbohydrate; TP = total phosphorus; ME = metabolizable energy.

Table 2. Amino acids content of experimental diets (% DM).

Items	P32	P36	P40	P44
Aspartic acid	2.02	2.19	2.39	2.63
Threonine	1.29	1.40	1.52	1.66
Serine	1.49	1.59	1.71	1.85
Glutamic acid	4.56	4.91	5.30	5.77
Glycine	1.75	1.92	2.10	2.32
Alanine	1.80	1.94	2.10	2.29
Valine	1.56	1.67	1.79	1.94
Methionine	1.04	1.12	1.21	1.32
Isoleucine	1.13	1.22	1.32	1.45
Leucine	2.31	2.46	2.62	2.83
Tyrosine	0.92	1.00	1.08	1.17
Phenylalanine	1.36	1.46	1.56	1.69
Lysine	2.05	2.24	2.46	2.72
Histidine	0.63	0.67	0.71	0.77
Arginine	1.59	1.72	1.87	2.05
Proline	0.76	0.80	0.85	0.91

Table 3. The effect of dietary protein levels on mink litter size and birth weight, daily weight gain and survival rate through weaning.

Items	P32	P36	P40	P44
Litter size at birth	6.63±1.69	6.88±1.64	5.88±1.46	6.25±1.58
Birth weight (g)	11.77±1.16	11.36±1.77	11.91±2.58	10.76±1.50
Daily weight gain (g)	7.00±0.29	7.33±1.28	7.28±1.37	7.95±1.23
Weaning survival rate (%)	84.46±11.10	85.84±12.60	86.83±12.97	87.50±14.77

**Table 4. The effect of dietary protein levels on weaning weight loss of mink dams.**

Items	P32	P36	P40	P44
Weaning weight loss	376.66±35.80[ab]	443.78±39.71[a]	381.56±37.61[ab]	319.94±35.62[b]
Weaning weight loss rate (%)	21.92±1.36[ab]	24.54±1.90[a]	20.85±1.27[ab]	17.32±1.77[b]

Notes: Means with different lowercase of superscripts were significantly different at $P < 0.05$, and with different capital letters of superscripts were highly significantly different at $P < 0.01$.

Table 5. Effect of dietary protein levels on serum biochemical endpoints in female mink at the end of lactation.

Items	P32	P36	P40	P44
BUN, mmol/L	5.04±0.64[b]	5.19±0.61[b]	6.04±1.11[ab]	7.18±1.154[a]
TP, g/L	66.63±5.18[Bc]	68.27±5.48[Bbc]	74.91±5.89[ABab]	80.39±6.61[Aa]
GPT, U/L	117.13±11.06	127.49±17.45	135.88±18.61	145.77±14.77
GLU, mmol/L	4.75±0.38[Bb]	5.03±0.50[Bb]	5.75±0.24[ABab]	6.53±0.51[Aa]

Notes: Means with different lowercase of superscripts were significantly different at $P < 0.05$, and with different capital letters of superscripts were highly significantly different at $P < 0.01$.
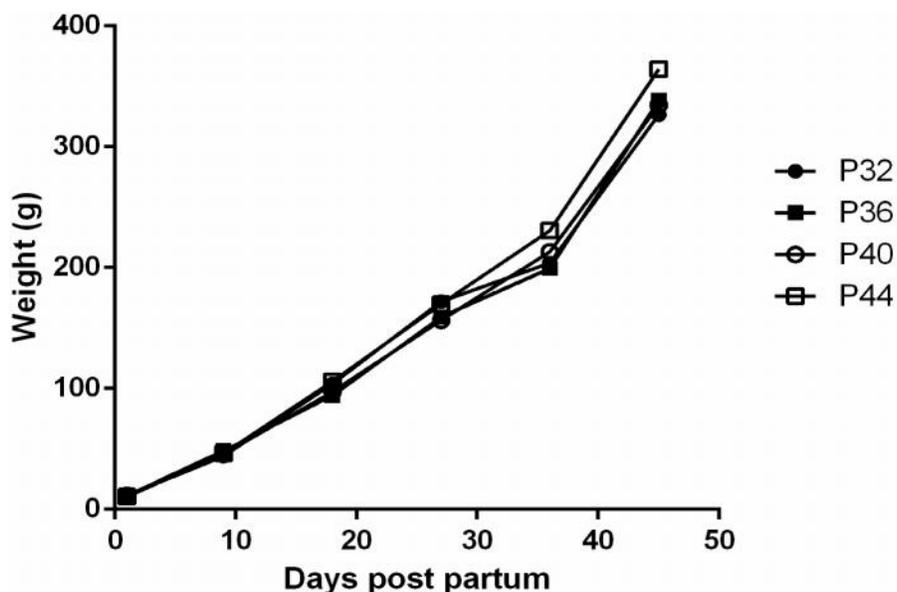
Fig 1. Growth curve of individual kits form all groups in lactation period.
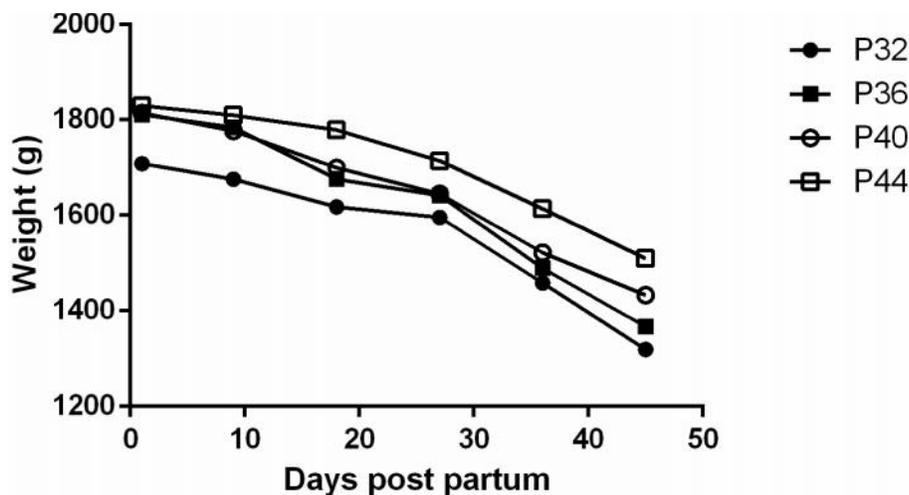
Fig 2. Live weight changes in mink dams of different groups during lactation period.

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Previous studies indicated that diet with adequate protein content significantly increased body weight of the kitten for up to 21 days of age (Matthiesen *et al*. 2010). In contrast, dams fed with inadequate protein supply were associated with increased loss of kits up to weaning (Vesterdorf *et al*. 2012). It is well known that for the first 24 to 25 days of life after birth, mink kits are totally dependent on mothers' milk for nourishment as they are born immature (Wamberg and Tauson 1998). Thus, meeting nutrient requirement of mink dams for maintenance and milk production are keys to the healthy growth of kits and decreasing their mortality. Other factors affecting kit mortality and growth include genetic causes, environmental factors and litter size (Rouvinen-Watt 2003; Schou and Malmkvist 2017; Karimi *et al*. 2018). In the present study, despite the variations in litter size within groups (with 12%-20% mink carrying 8-9 kitten), no significance was observed among treatments. These results are in line with findings by Damgaard *et al*. (2000) showing that decreasing protein supply from 61% to 39% of ME had no negative affect on body weight of the kits. In contrast, inadequate voluntary protein intake of dams could lead to decreased daily milk production of dams in those groups (Fink *et al*. 2001) and higher mortality of kit. Therefore, our study implies that adequate protein supply servers as an important factor for kitten survival in mink females with both average and high litter size during lactation.

Despite the lack of detailed knowledge of how body weight loss affects post-weaning performance of mink, available information from experimental studies on other animals, could be of referential values. For instance, it was reported in lactating sows that body weight loss and/or catabolic status may have detrimental effect on the interval period of weaning-to-service (Sterning *et al*. 1989; Zak *et al*. 1997; Zak *et al*. 1998), litter size (King and Williams 1984a; Kirkwood *et al*. 1987; Yang *et al*. 1989) as well as ovulation rate (Hughes and Pearce 1989; Zak *et al*. 1997). Moreover, body weight loss was found to be correlated negatively with the energy status in cows (Staples *et al*. 1990). In lactating mink, early research had shown that protein supply below the current recommendations increased dam weight loss (Fink *et al*. 2004). From parturition to 4 weeks postpartum, the loss of body weight (calculated in % of individual live weight), was lower in dams fed with medium protein (45% of ME) and low protein (29% of ME) diets than in others fed with high protein (63% of ME) (Fink *et al*. 2006). Similarly, our observations of reduced body weight loss in the group with 39% of ME were in agreement with previous findings in sows (King and Williams 1984b). Dietary requirement for nutrient standard in lactating mink varies depending on multiple factors including feed composition, nursed kits' number and lactation length. Our study was conducted under no-choice conditions, and since the mink were able to balance the macronutrients intake to maintain a constant energy intake, it is possible that the dams, when fed on diets of a low proportion of protein, chose to avoid overconsuming carbohydrate and/or lipids to gain limiting protein, as demonstrated by Mayntz *et al*. (2009). Therefore, the unbalanced feed might have failed to meet nutrient requirements for milk production and maintenance (Tauson 1996), consequently leading to impaired kit growth and weight loss of the dams.

Body reserves and profile changes of protein metabolism are affected by nutrients levels. Liver, as the primary source of serum protein, is known for its vigorous protein metabolism. Hence, hepatic protein metabolic status could be reflected by the serum TP content as a result of dietary treatments. Meanwhile, serum concentration of BUN can be used as an indicator of amino acid utilization and metabolism, due to its nature as a main end product of protein metabolism. In the current study, significantly decreased TP and BUN were observed in group P32 and P36, suggesting low-protein diets may induce metabolic disorders by affecting hepatic protein metabolism after lactation.

GPT is one of the enzymes mainly located in hepatic cells and plays a critical role in the intermediary metabolism of amino acids and glucose. It has been reported that nutrient composition of a diet could influence the outcome of liver enzymes (Ryan *et al*. 2007).Our study showed an increasing trend in GPT activity from groups P32 to P44 with increasing protein content, which is in line with the early observation by Muramatsu and Ashida (1962), who noted GPT activities increased linearly in rats fed with diets with increasing casein content (10,25,40 and 60 %). This could be explained by the possibility that high protein diet could increase gluconeogenesis by enhancing activities of enzymes involved in glucagon secretion, as demonstrated in rats(Eisenstein and Strack 1971; Eisenstein *et al*. 1974). Numerous studies have linked dietary protein intake with glucose homeostasis. The branched-chain amino acids support metabolic processes by serving as precursors for glutamine and alanine generation (Wahren *et al*. 1974; Ruderman 1975) and by modulating insulin-signaling pathway(Patti *et al*. 1998; Xu *et al*. 1998; Anthony *et al*. 2001). Studies have shown that indicated amino acids are the primary carbon source in hepatic gluconeogenesis (Jungas *et al*. 1992).Meanwhile, hepatic endogenous glucose production is a critical factor in blood glucose maintenance (Pascual *et al*. 1997; Katz and Tayek 1998). Enhancement of blood glucose utilization is especially critical during body weight loss to maintain body mass(Ludwig 2000). Layman *et al*. (2003) outlined that diets containing elevated protein and decreased CHO/protein ratio helped stabilize blood glucose level during weight loss via amino acids-glucose metabolism interaction. Therefore, the low-protein diets in this study might have led to reduced blood glucose level in dams,

resulting in more weight loss. Furthermore, the positively correlated GLU level and GPT activity also suggests that with increasing dietary protein, the nutrient metabolism could be regulated to promote gluconeogenesis and amino acid degradation, in order to provide energy and blood glucose for overall being of metabolism.

Conclusion: In summary, our study shows that dietary protein influences nursing performance of female mink by affecting both growth rate of the kits and body weight loss of the dams. Signs of metabolic disorder, as indicated by serum biochemical parameters, are found in dams fed with low-protein diets. Thus, dietary protein content appears to be of crucial importance as a nutritional metabolic factor in modulating the nursing performance of dams. Based on our findings in this study, higher protein level (39% of ME) could be used as dietary guidance to achieve optimal performance for lactating dams.

Conflict of interest statement: The authors have no financial conflicts of interest.

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