Effects of dietary kelp (*Ascophylum nodosum*) supplementation on survival rate and reproductive performance of mink challenged with Aleutian mink disease virus

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Abstract: Infection with Aleutian mink disease virus (AMDV) has negative effects on reproductive performance and survival rate of American mink (*Neovison vison*). The objectives of this study were to assess the effects of kelp (*Ascophylum nodosum*) supplementation on survival, growth rate, and reproductive performance of mink challenged with AMDV. AMDV-free female black mink (*n* = 75) were intranasally inoculated with a local AMDV strain. Mink were fed a commercial pellet supplemented with 1.5% or 0.75% kelp or were kept as controls (received no kelp) for 451 d. Body weight and rectal temperature were recorded on days 0, 31, 56, 99, 155, 366, and 451 post inoculation (PI). Annual mortality rates were 13.6%, 20.0%, and 31.8% for mink fed 1.5%, 0.75%, or 0.0% kelp, respectively (*P* = 0.29). Mink which were fed 1.5% kelp had a significantly (*P* < 0.01) greater daily weight loss during breeding and post-breeding periods (days 155–366 PI), and outperformed (*P* < 0.01) the other groups in regard to litter sizes at birth and weaning. Differences among treatments were not significant for the number of females mated, or whelped of those exposed to males, kit survival from birth to weaning, or rectal temperature. It was concluded that 1.5% kelp supplementation had beneficial effects on survival rate of adult mink and litter size.

Key words: Aleutian mink disease virus, American mink, *Ascophylum nodosum*, body weight, reproductive performance, survival rate.

Résumé : L’infection par le virus de la maladie des visons aléoutiens (AMDV — « Aleutian mink disease virus ») a des effets négatifs sur la performance de reproduction et le taux de survie du vison d’Amérique (*Neovison vison*). Les objectifs de cette étude étaient d’évaluer les effets des suppléments de varech (*Ascophylum nodosum*) sur la survie, le taux de croissance, et la performance de reproduction des visons inoculés à l’AMDV. Les visons noir femelles exemptes d’AMDV (*n* = 75) ont été inoculées de façon intranasale par une souche locale d’AMDV. Les visons ont reçu une diète commerciale de granulés avec des suppléments de varech de 1,5 % ou 0,75 % ou ont été gardés comme témoins (n’ont pas reçu de varech) pendant 451 j. Le poids corporel et la température rectale ont été enregistrés aux jours 0, 31, 56, 99, 155, 366, et 451 post inoculation (PI). Les taux annuels de mortalité étaient de 13,6 %, 20,0 %, et 31,8 % chez les visons ayant reçu 1,5 %, 0,75 % ou 0,0 % de varech, respectivement (*P* = 0.29). Les visons qui ont reçu 1,5 % de varech avaient une perte de poids quotidienne significative (*P* < 0.01) pendant les périodes de reproduction et de post-reproduction (jours 155–366 PI), et ont eu une meilleure performance (*P* < 0.01) que les autres groupes en matière de taille de portée à la naissance et au sevrage. Les différences parmi les traitements n’étaient pas significatives par rapport au nombre de femelles accouplées ou ayant mise bas parmi celles exposées aux mâles, au taux de survie des visonneaux de la naissance au sevrage, ou à la température rectale. Il a été conclu que les suppléments de varech de 1,5 % avaient des effets bénéfiques sur la survie des visons adultes et sur la taille de portée. [Traduit par la Rédaction]

Mots-clés : virus de la maladie des visons aléoutiens, vison d’Amérique, *Ascophylum nodosum*, poids corporel, performance de reproduction, taux de survie.
Introduction

Ascophyllum nodosum, a brown seaweed that grows in abundance along the shores of North Atlantic (Allen et al. 2001), has been fed to livestock for many decades (Evans and Critchley 2013). Reports on chemical composition and nutritional properties of various seaweed species have been extensively reviewed in recent years (O’Sullivan et al. 2010; Pangestuti and Kim 2011; Wijesekara et al. 2011; Evans and Critchley 2013; Ngo and Kim 2013; Collins et al. 2016; Pérez et al. 2016), concluding that seaweeds contain an array of natural bioactive compounds and trace minerals with antioxidant, antibacterial, antiviral, and anti-inflammatory properties. Although there are inconsistencies in the results of various experiments, the consensus of those reviews is that dietary use of seaweed and their extracts have positive effects on health status of humans and animals. Phaeophyta (brown seaweeds) are particularly rich sources of polysaccharides (alginate, fucoidan, and laminarin) and polyphenols (phenolic acids, flavonoids, stilbenes, and lignans) (O’Sullivan et al. 2010; Kim et al. 2014; Collins et al. 2016). Among Phaeophyta, A. nodosum, including Tasco®, the proprietary product marketed by Acadian Seaplant (http://www.acadianseaplants.com/), has been studied most extensively (reviewed by Evans and Critchley 2013; Luthuli et al. 2019).

In vivo and in vitro studies have shown that dietary use of seaweed extracts, particularly polysaccharides, increase the abundance of beneficial bacteria and reduce levels of pathogenic bacteria in the gut, modulate the immune system, and increase beneficial volatile fatty acids in livestock (O’Sullivan et al. 2010; Sweeney and O’Doherty 2016). Similarly, feeding whole seaweed caused favorable changes in the abundance of beneficial and pathogenic bacteria in the digestive tract of pigs (Dierick et al. 2009), layer hens (Kulshreshtha et al. 2014), and rats (Liu et al. 2015); increased the concentration of short-chain fatty acids in hens (Kulshreshtha et al. 2014) and rats (Liu et al. 2015); increased villi height and crypt depth of the small intestine in chickens (Kulshreshtha et al. 2014); and increased the depths of the colonic crypt, mucosa, externa muscularis, and colonic total wall in rats (Liu et al. 2015). On the contrary, Michiels et al. (2012) found no positive effect of A. nodosum supplementation on gut microbial profile, plasma oxidative status, or gut tissue morphology in piglets fed a balanced diet.

The effects of A. nodosum supplementation on mink infected with Aleutian mink disease virus (AMDV) has not been studied but could be of great interest to mink producers in countries where AMDV positive mink are not culled. AMDV causes Aleutian disease (AD), resulting in serious health problems for mink in most mink-producing countries. The AD is an immune-complex-mediated syndrome characterized by persistent antiviral antibody production, hypergammaglobulinemia, plasmacytosis, and progressive renal disease (reviewed by Bloom et al. 1994) and negatively affects reproductive success and kit survival (Alexandersen 1986; Hansen and Lund 1988; Broll and Alexandersen 1996; Reichert and Kostro 2014; Andersson et al. 2017). There is no cure nor effective vaccine against AD (Aasted et al. 1998; Castelruiz et al. 2005; Liu et al. 2018) and the virus cannot be easily destroyed by heating or composting (Hussain et al. 2014). Testing of mink for antibodies against AMDV and removal of seropositive animals has not been effective in permanent virus eradication (Themudo et al. 2011; Farid et al. 2012).

Macrophages and cytokines, especially interleukin-6 (IL-6), have been implicated in the pathogenesis of AD (Bloom et al. 1994). Because pigments and sulfated polysaccharides in seaweeds have anti-inflammatory activities by modulating macrophage functions and by production of anti-inflammatory cytokines, such as IL-6 (reviewed by Pangestuti and Kim 2011; Wijesekara et al. 2011; Sweeney and O’Doherty 2016), it seems logical to assume that seaweeds could alleviate the harmful effects of AMDV infection. In addition, animals of various species which were under stressful conditions, such as those challenged with bacteria or viruses, exposed to heat or experienced long-haul transportation showed stronger positive responses to seaweed supplementation than those which were not stressed and fed balanced diets, possibly because stress causes immune suppression, resulting in higher susceptibility to infection by pathogens (reviewed by Allen et al. 2001 and Evans and Critchley 2013), making AMDV infection an attractive case for testing the effects of seaweed supplementation to mink. The objectives of this study were to assess the effects of A. nodosum supplementation on survival, weight gain, and reproductive performance of mink inoculated with AMDV.

Materials and Methods

Statement of animal care

Mink were managed according to the industry standards (NFACC 2013) and sampling protocols were performed according to the standards of the Canadian Council for Animal Care (http://www.ccac.ca) after approval by the Institutional Animal Care and Use Committee. Prior to inoculation and weighing, animals were anesthetized by intramuscular injection of ketamine hydrochloride (Ketalean, Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada) and xylazine hydrochloride (Rompun 2%, Bayer Animal Health, Mississauga, ON, Canada) at the dose of 10 and 2 mg kg−1 live weight, respectively.

Source of seaweed

Ascophyllum nodosum (kelp) was hand harvested by Tidal Organics (http://tidalorganics.com/) from small skiffs using a cutter rake. It was harvested from June through October in south-western Nova Scotia’s coastal region.
from Lunenburg to Pubnico, mechanically dried, and particle sizes reduced with a hammer mill to 14 mesh.

**Animal management and experimental design**

A total of 75 five-month-old female black American mink (*Neovison vison*) were purchased in September 2013 from an AMDV-free farm in Nova Scotia, Canada. Animals were transferred to the Aleutian Disease Research Center (ADRC), which is a building designed to minimize the chance of viral introduction or escape. Animals were individually kept in 61.0 cm × 30.5 cm × 20.3 cm wire-meshed cages with a wooden nest box containing aspen shavings. Cages were separated by a solid plastic sheet to prevent direct physical contact. Animals had free access to water via nipples connected to water-lines which were heated during the winter. Three days after arrival at ADRC, animals were anesthetized and intranasally inoculated with 60 μL of a 10% (v/v) passage 2 of a local strain of AMDV prepared from the spleens of mink harvested 10 d post inoculation (PI) and stored at −80 °C, as previously described (Farid et al. 2015). All mink were infected by AMDV on day 56 PI (unpublished data).

The diet gradually changed from the wet feed used on the farm of origin to a commercial dry pellet (National Feeds Inc., Maria Stern, OH, USA) with kelp supplementation of 1.5% (T1.5), 0.75% (T0.75), or 0% (T0, control) of the feed. The pellet’s nutritional composition changed based on the production cycles of the mink. To enhance the attachment of the kelp to the dry pellets, a combination of 96.0% pellet, 2.5% flour, and 1.5% kelp was thoroughly mixed to make the T1.5 diet. The composition of T0.75 diet was 96.75% pellet, 2.5% wheat flour, and 0.75% kelp, and no flour was added to the control diet. Animals had free access to the feed which was added to feeders daily. Some kelp particles settled out in feeders, which could not be measured. One row of cages was divided into two blocks of three sections of 12 or 13 cages each, and the three treatments were assigned at random within each block. On PI days 0 (before inoculation, 9 Sep. 2013), 31 (10 Oct.), 56 (5 Nov.), 99 (17 Dec.), 155 (11 Feb. 2014), 366 (9 Sep.), and 451 (4 Dec.), animals were sedated, and their body weights were recorded. Rectal temperature was measured by a digital thermometer on days 31, 56, 99, and 155 PI.

**Breeding**

Females which survived until breeding season (n = 62) were exposed to three different AMDV-free males up to six times between 2 Mar. and 20 Mar. 2014. Females whelped between 26 Apr. and 9 May, and the number of dead and live kits was recorded at birth and on day 7 postpartum. Kits were weaned on 28 June at 50–64 d of age, were fed the control diet (0% kelp) after weaning, and their body weights were recorded on 30 Oct. 2014, at an average age of 180 d.

![Average body weight of mink supplemented with different percentages of kelp over time.](image)
The likelihood ratio $\chi^2$ test was applied to compare treatments for the number of females mated and whelped.

### Results

#### Survival rate

Mortalities during the first 21 d PI were 3, 1, and 3 in T1.5, T0.75, and T0, respectively, which could not have been the result of AMDV infection and were excluded from the analysis of mortality data. To confirm that AD was not the cause of death, these animals were necropsied and none showed signs of AD in the liver, spleen, or kidneys. Four of the dead mink had suffered from fatty liver. Mortalities from 1 Oct. 2013 to 1 Oct. 2014 were 13.6%, 20.0%, and 31.8% in mink fed T1.5, T0.75, and T0 diets, respectively (Table 1), but the differences among treatments for cumulative mortality at the termination of the experiment were not significant ($\chi^2_{(2, df)} = 2.47, P = 0.29$). There was no mortality from 1 Oct. 2014 until the termination of the experiment on 4 Dec. 2014. The means and standard errors of survival times measured by LIFETEST for uncensored data were 401.7 ± 12.3, 377.9 ± 19.7, and 351.8 ± 26.2 d PI for T1.5, T0.75, and T0, respectively, and the differences were not significant ($\chi^2_{(2, df)} = 2.50, P = 0.28$).

#### Body weight and average daily gain

The MIXED model analysis revealed that treatment effect on body weight was not significant from days 0 to 155 but approached significance ($P = 0.08$) from day 155 to 451. Mink which were fed 1.5% kelp tended to have lower average body weight than the other two groups (Table 2). Regressions of body weight on the first segment of sampling dates were quadratic and for the second period were linear. The regression equations of body weight on the first segment were $\log Y = 1196.29 + 26.03X + (1.6135 + 0.340)X^2$ for days 0–155 and $(1315.53 + 38.50)X – (0.6595 ± 0.07649)X^2$ for days 155–451, where $X$ is days from the analysis of mortality data. To confirm that AD was not the cause of death, these animals were necropsied and none showed signs of AD in the liver, spleen, or kidneys. Four of the dead mink had suffered from fatty liver. Mortalities from 1 Oct. 2013 to 1 Oct. 2014 were 13.6%, 20.0%, and 31.8% in mink fed T1.5, T0.75, and T0 diets, respectively (Table 1), but the differences among treatments for cumulative mortality at the termination of the experiment were not significant ($\chi^2_{(2, df)} = 2.47, P = 0.29$). There was no mortality from 1 Oct. 2014 until the termination of the experiment on 4 Dec. 2014. The means and standard errors of survival times measured by LIFETEST for uncensored data were 401.7 ± 12.3, 377.9 ± 19.7, and 351.8 ± 26.2 d PI for T1.5, T0.75, and T0, respectively, and the differences were not significant ($\chi^2_{(2, df)} = 2.50, P = 0.28$).

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number of days PI. Differences among treatments for average daily gain from days 0 to 155 PI, 366 to 451 PI, and during the entire period (days 0 to 451) were not significant, but mink on T1.5 diet lost almost twice as much weight as those on T0.75 and T0 diets from days 155 to 366 PI ($P < 0.01$) (Table 2).

Reproductive performance

Mink which were fed 1.5% kelp outperformed the other two groups for the percentages of females mated of those exposed to males, whelped of those mated, and whelped of those exposed to males, but the differences were not significant (Table 3). Mink supplemented with 1.5% kelp significantly outperformed the other two groups for the total number of kits born (dead or alive), kits born alive, kits alive on day 7 postpartum, and kits weaned per females whelped, per females mated, and per females exposed to males (Table 4), but differences between mink on T0.75 and T0 were not significant for any of the measurements. Kelp supplementation did not have any effect on mortality of live-born kits to day 7 postpartum or until weaning (Table 4).

Rectal temperature

The MIXED model analysis showed that the effects of the treatment and treatment by sampling day interaction on rectal temperature were not significant, but changes in rectal temperature over time, although small, were significant ($P < 0.01$). Mean rectal temperature was the highest on day 31 PI and was significantly different from those on other sampling dates (Table 5). Rectal temperature decreased by almost 1 °C to its lowest levels on days 56 and 99 PI, which were not statistically different, followed by a minor increase on day 155 PI.

Body weight of progeny at 180 d of age

The dam’s diet had negligible effects on body weight of progeny at 180 d of age ($P = 0.41$), and male kits were significantly heavier than females at this age (Table 5). The interaction between maternal diets and sex of the kits was not significant.

### Table 3. Effect of kelp supplementation on fertility of female mink.

| Measurement                                      | 1.5% kelp | 0.75% kelp | Control | $\chi^2_{(2 df)}$ |
|--------------------------------------------------|-----------|------------|---------|------------------|
| Number of females exposed to males               | 22        | 22         | 18      | 0.64 (0.73)      |
| Females mated of females exposed                 | 86.4%     | 77.3%      | 83.3%   |                  |
| Females whelped of females mated                 | 89.5%     | 70.6%      | 80.0%   | 2.09 (0.35)      |
| Females whelped of females exposed               | 77.3%     | 54.5%      | 66.7%   | 2.57 (0.27)      |

Note: $P$ values are in brackets.

### Table 4. Means and standard deviations of number of kits born and weaned of mink supplemented with different levels of kelp and the results of Kruskal–Wallis tests.

| Measurement                                      | 61.5% kelp | 0.75% kelp | Control | $\chi^2_{(2 df)}$ |
|--------------------------------------------------|------------|------------|---------|------------------|
| Number of females whelped                        | 19         | 17         | 15      |                  |
| Per female whelped                               |            |            |         |                  |
| Total kits born                                  | 6.82 ± 1.74a | 4.17 ± 2.21b | 2.75 ± 1.29b | 20.3 (<0.01)   |
| Kits born alive                                  | 6.71 ± 1.79a | 3.25 ± 2.41b | 2.67 ± 1.23b | 21.0 (<0.01)   |
| Kits alive on day 7                               | 5.94 ± 1.75a | 3.08 ± 2.57b | 1.82 ± 1.44b | 18.7 (<0.01)   |
| Kits weaned                                      | 4.59 ± 2.26a | 2.42 ± 2.43b | 1.67 ± 1.28b | 11.4 (<0.01)   |
| Per female mated                                 |            |            |         |                  |
| Total kits born                                  | 6.11 ± 2.71a | 2.94 ± 2.68b | 2.20 ± 1.61b | 16.6 (<0.01)   |
| Kits born alive                                  | 6.00 ± 2.71a | 2.29 ± 2.52b | 2.13 ± 1.55b | 18.1 (<0.01)   |
| Kits alive on day 7                               | 5.32 ± 2.49a | 2.18 ± 2.58b | 1.53 ± 1.51b | 16.8 (<0.01)   |
| Kits weaned                                      | 4.11 ± 2.58a | 1.71 ± 2.31b | 1.33 ± 1.39b | 11.8 (<0.01)   |
| Per female exposed to males                      |            |            |         |                  |
| Total kits born                                  | 5.27 ± 3.29a | 2.27 ± 2.66b | 1.83 ± 1.69b | 12.8 (<0.01)   |
| Kits born alive                                  | 5.18 ± 3.27a | 1.77 ± 2.41b | 1.78 ± 1.63b | 14.6 (<0.01)   |
| Kits alive on day 7                               | 4.59 ± 2.97a | 1.68 ± 2.44b | 1.28 ± 1.48b | 14.1 (<0.01)   |
| Kits weaned                                      | 3.55 ± 2.79a | 1.32 ± 2.14b | 1.11 ± 1.36b | 10.9 (<0.01)   |
| Percent kit mortality                            |            |            |         |                  |
| Dead by day 7 per born alive                     | 8.4 ± 20.3 | 15.0 ± 33.7 | 25.7 ± 38.5 | 1.97 (0.37)     |
| Dead by weaning per born alive                   | 32.2 ± 30.6 | 38.7 ± 45.0 | 34.0 ± 43.0 | 0.19 (0.91)     |

Note: Treatment means within a row followed by different lowercase letters differ significantly at the $P < 0.017$ when tested by the Mann–Whitney U test. $P$ values are in brackets.
Discussion

The two novel aspects of this study were the long duration of kelp supplementation to mink (451 d) and measuring the effects of kelp supplementation on reproductive performance of mink challenged with AMDV. To our knowledge, no trial with seaweed longer than 100 d has previously been conducted, and there are no studies on the effects of seaweed supplementation on reproductive performance of animals of any species challenged with any pathogen. Whole A. nodosum meal was tested in this study because it is abundantly available in Nova Scotia at a relatively low cost. Seaweed meals contain a large array of bioactive compounds and trace minerals with unknown effects on the digestive and immune systems of animals and could have different effects than any specific seaweed extract on enzymatic digestion (Chater et al. 2016) and animal performance. The downfall of feeding whole seaweed meal is the variation amongst different batches caused by the location and season of harvest and other climatic and processing conditions, which are reported for A. nodosum (Apostolidis et al. 2011; Kim et al. 2014; Tabassum et al. 2016) and other species of seaweed (Rioux et al. 2009).

The rather high annual mortality rate of the control group was likely because the virus isolate used in this study was moderately pathogenic (Farid and Hussain 2019). The observation that mink which were fed 1.5% and 0.75% kelp had 18.2% and 11.0% lower mortality and lived 49.9 and 23.9 d longer than the control group, although statistically nonsignificant because of the small number of observations, has important economic implications for mink farms infected with AMDV. The significantly greater weight loss of the mink fed 1.5% kelp from day 155 (February) to 366 PI (September), which encompassed the pregnancy and lactation periods, was likely the result of significantly larger litter size of this group. Mink with large litters had high nutritional requirements, but the high mineral content (Rupérez, 2002; Rioux et al. 2009; Evans and Critchley 2013), or the taste of the kelp might have caused reduced feed consumption.

The results of studies on diet supplementation with seaweed or seaweed extracts on weight gain and feed intake of farm animals have been variable. Pigs that were exposed to porcine reproductive and respiratory syndrome virus supplemented with various amounts of Tasco® during a 5 wk nursery period showed significantly higher weight gain, higher feed intake, and better feed conversion than the controls (Allen et al. 2001).

Table 5. Least-squares means ± standard errors of rectal temperature of adult experimental mink and body weight of their progeny for mink fed different levels of kelp.

| Parameters females | Rectal temperature (°C) | Parameters progeny | Body weight (g) |
|--------------------|-------------------------|-------------------|-----------------|
| Kelp (%)           |                         | Number            | Body weight     |
| 1.5                | 39.58 ± 0.11            | 76                | 1547 ± 20       |
| 0.75               | 39.37 ± 0.11            | 28                | 1587 ± 32       |
| 0.0                | 39.34 ± 0.12            | 20                | 1593 ± 38       |
| Days PI            |                         | Sex               |                 |
| 31                 | 40.32 ± 0.08a           | Female            | 67              | 1263 ± 23a       |
| 56                 | 39.01 ± 0.13b           | Male              | 57              | 1889 ± 24b       |
| 99                 | 38.92 ± 0.15b           |                   |                 |
| 155                | 39.48 ± 0.10c           |                   |                 |

Note: Means within a column followed by different lowercase letters differ significantly at the P < 0.05. PI, post inoculation.
The effects of seaweed supplementation were not significant on body weight or feed intake of piglets in a 28 d trial (Michielset al. 2012), or of lambs after 74 d (Samara et al. 2013), on body weight of layer hens after 30 d (Kulshreshtha et al. 2014), broilers after 21 d (Abudabos et al. 2013), goats after 84 d (Yates et al. 2010), or rats after 21 d (Liu et al. 2015). Supplementing finishing lamb diet with 1% or 2% of A. nodosum for 7, 14, or 28 d did not affect feed intake or weight gain (Bach et al. 2008). Supplementing the diet of rabbits with 1% green seaweed (Ulva lactuca) meal did not affect body weight in two experiments, but 2% supplementation significantly decreased body weight (Okab et al. 2013).

Seaweed extract supplementation significantly decreased average daily gain of growing–finishing pigs in a 61 d feeding trial with no effect on feed intake. The negative effect of seaweed on weight gain was attributed to the high levels of phenolic compounds, chelated metals, or alginates (Gardiner et al. 2008). In contrast, seaweed extracts did not have a significant effect on weight gain of weaned piglets after feeding for 28 d (Sweeney et al. 2012) or 40 d (O’Shea et al. 2014), whereas it significantly improved growth rate of piglets after weaning in 25 d (O’Doherty et al. 2010) and 32 d (Heim et al. 2014) feeding trials, respectively. It may be concluded from the current and previous studies that dietary supplementation of seaweed at the rate of 1.5% of dry matter intake has negligible effect on weight gain in growing–finishing animals, and more information is needed on the effect of seaweed supplementation on feed consumption and weight gain when animals’ nutrient requirements are high.

Maternal supplementation with kelp did not have any effect on body weight of their progeny at 180 d of age (pelting time). Body weight was recorded at this age because maternal supplementation with a seaweed extract from day 83 of gestation and throughout the lactation period was shown to enhance the immune response of weaned pigs challenged with Escherichia coli (Heim et al. 2014). Dietary supplementation of pregnant sows with seaweed extracts had positive effects on health and growth rate of piglets before and after weaning, including increased serum immunoglobulin G concentrations, favorable changes in the gut’s microflora, enhanced immune function, and improved villous architecture of small intestine (reviewed by Sweeney and O’Doherty 2016).

The negative effects of AMDV infection on reproductive success and kit survival have been widely reported (Padgett et al. 1967; Alexandersen 1986; Hansen and Lund 1988; Broll and Alexandersen 1996; Andersson et al. 2017), and the magnitude of the effect of infection is determined by the genotype of the mink and the strain of the virus (Hadlow et al. 1983). In a previous study, the incidence of females which did not whelp of those exposed to males was 11.8%, 12.4%, and 2.5% in two AMDV-infected and one AMDV-free farm in Poland, respectively, and the corresponding values for the number of kits born alive were 3.4, 3.1, and 5.8, and for the number of kits weaned were 2.2, 2.1, and 4.9 (Reichert and Kostro 2014), showing substantial negative effects of AMDV infection on reproduction. The measures of female fertility and litter size in the control group in the current study could be considered the estimates of reproduction of black mink in Nova Scotia infected with a moderately pathogenic local AMDV isolate. AMDV infection in the current study resulted in a low percentage of females whelping of those exposed to males in the control group (66.7%), which is lower than 31% and 14% barren females on two AMDV-infected farms in Sweden (Andersson et al. 2017), and 11.8% and 12.4% barren females on two AMDV-infected farms in Poland (Reichert and Kostro 2014).

The most noteworthy result of the current study was the observation that all the 13 measures of reproduction (female fertility, number of kits born, and weaned) were higher in mink fed 1.5% kelp than the controls and those fed 0.75% kelp, although only the measures of litter size were statistically significant. The small number of kits born alive per female whelped in the controls (2.67) was considerably smaller than the numbers of live-born kits per female mated and per female whelped (5.71 and 6.29, respectively) in wild-type AMDV-infected mink in Argentina (Martino and Villar 1990). The average number of kits born alive per female whelped in mink fed 1.5% kelp (6.71) was 4.04 kits (151%) greater than that in the control group. This estimate is in the upper range of the reported values for uninfected mink, namely 5.8 and 5.28 in two studies in Poland (Socha and Markiewicz 2002; Reichert and Kostro 2014), 5.68, 7.17, and 7.46 live kits 24 h postpartum on three Danish farms (Thirstrup et al 2014b), 6.75 and 5.0 kits within 48 h after birth in brown and black mink, respectively, in Denmark (Thirstrup et al. 2014b), 5.0 and 5.67 kits in two studies in Denmark (Malmkvist et al. 1997; Hansen et al. 2010), 5.77 in Nova Scotia (Karimi et al. 2018), and 5.6 in Sweden (Elofson et al. 1989). The number of kits weaned per female whelped for mink fed 1.5% kelp was greater than in the controls by 174.8% and was marginally smaller than 4.8 to 5.12 for uninfected mink in previous studies (Socha and Markiewicz 2002; Hansen et al. 2010; Reichert and Kostro 2014; Karimi et al. 2018). The reason for the low reproductive performance of mink supplemented with 0.75% kelp is not clear. The findings somewhat agree with the report that green seaweed (Ulva lactuca) supplementation at 2% of dry matter intake decreased conception rate but increased litter size in female rabbits, but supplementation at 1% level had no effect on these parameters (Okab et al. 2013). Contrary to the result of the current study, dietary supplementation of pregnant sows with brown seaweed extracts from day 83 of gestation had no effect on the number of live-born piglets or piglet birth weight (Heim et al. 2014).
In the present study, pre-weaning mortality rates in mink fed the three diets were higher than 25.8% kit mortality from birth to weaning at 4 wk of age on two AMDV-infected farms in Argentina (Martino and Villar 1990). Pre-weaning mortality rates were also higher than those of uninfected mink, which ranged between 11.3% and 27.4% (Schneider and Hunter 1993; Malmkvist et al. 1997; Hansen et al. 2010; Farid et al. 2018; Karimi et al. 2018). The lack of any effect of 1.5% kelp on pre-weaning survival was probably partly the result of significantly greater litter size in this group which negated the possible positive effects of kelp supplementation. It is also possible that beneficial effects of kelp were not passed into milk, and the amount of solid feed consumed by kits before weaning was too low to influence kit health and survival. The small litter size, low female fertility, and low survival rate of kits in the control group were likely because the mink used in the current study have never been exposed to the virus and thus suffered more than those which had a history of exposure to AMDV.

The negligible effect of kelp supplementation on rectal temperature in the current study contradicts the results of previous studies that Tasco® slightly increased rectal temperature of piglets infected with the porcine reproductive and respiratory syndrome virus compared with the controls after a 5 wk suckling period (Allen et al. 2001) and significantly increased rectal temperature of goats exposed to high environmental temperatures (Yates et al. 2010). On the contrary, body temperature of lambs which were fed 2% A. nodosum extract were lower than that for controls when exposed to stress conditions (heat and transportation) (Archer et al. 2008).

Conclusions

The results of this long-term feeding experiment showed that 1.5% kelp supplementation improved litter size and tended to increase survival rate of adult mink. Feed supplementation with 1.5% kelp showed a significantly higher weight loss compared with the control group during the pregnancy and lactation periods when the nutrient requirements of female mink were high. Further research is needed to validate the positive effects of 1.5% kelp supplementation.

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