



RESEARCH ARTICLE

Blood biochemical Parameters in Male American Mink (*Neovison vison*) before and During the Breeding Season

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ABSTRACT

This study was aimed at selected blood biochemical parameters in the male American mink before (September-November) and during the breeding (January-March) season. Blood from 143 Black and Sapphire male mink at one year age was collected. The plasma was assayed for the concentration of glucose, total protein, cholesterol, HDL and LDL fractions, triacylglycerides (TG), and the activity of ALT and AST. Concentrations of glucose, protein, total and HDL/LDL cholesterol, and AST activity were generally slightly higher during the breeding season than during the non-breeding season, but remained within the reference range. In the case of ALT activity and TG concentration, the relations were reversed. The parameters studied in the Sapphire mink showed greater variation, both in- and out of the season. In Black and Sapphire males of the American mink, the studied parameters revealed slightly higher values during the breeding season than the non-breeding season. This will vary depending on the color variety. The decrease in TG concentration during the breeding season may indicate an increased energy demand due to ambient temperature falls, and/or may be a sign of increased energy consumption associated with physical exertion during mating. Consideration should be given to the nutrition of male mink during the breeding season.

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INTRODUCTION

Hematologic and biochemical blood parameters reflect the physiological state of an animal at the time of blood sampling. Several factors affect these parameters, including age and sex of the animal, gestational status, stress, the season of the year, etc (Hunter, 1996; Oruc *et al.*, 2012; Pimpimol *et al.*, 2012; Gul *et al.*, 2013; Khan *et al.*, 2013; Qureshi *et al.*, 2013). Hematological and biochemical blood parameters are also routinely used to identify non-physiological and pathological conditions. In research involving mink, however, these tests are rarely used, as the animals are difficult to tame, and drawing a proper blood sample is difficult without sedation or anesthesia. Consequently, cost of blood collection and laboratory analysis may exceed the value of the pet. Nevertheless, blood biochemical parameters represent a great diagnostic tool for many important mink diseases - such as Aleutian disease or viral enteritis; and help in

finding toxic agents, or may reveal symptoms of improper management, such as overstocking, poor nutrition etc (Valdovska and Pilmane, 2011). The importance of blood biochemical parameters of mink requires establishing their standard reference ranges (Song *et al.*, 2012).

The literature provides much information on hematological and biochemical blood parameters in mink of both sexes and of different color varieties, with blood taken using various methods and on various dates (Miknienė *et al.*, 2010; Damgaard *et al.*, 2012; Harrington *et al.*, 2012; Rui *et al.*, 2012). It is well known that low density lipoprotein (LDL) enables transport of free and etherified cholesterol in the blood serum of both humans and animals. In male mink, the determination of cholesterol with LDL and HDL density is of special importance, since these substances are essential for testosterone formation. Testosterone again plays a significant role in the physiology of the testis. Triacylglycerides are also of great importance. In mice,

serum triacylglycerides decrease can be induced by testosterone deficiency (Senmaru *et al.*, 2013). Of all the classes of lipids, these play the key role as an energy depot for the organism and, with cholesterol, will form the basis of fat metabolism (Neumeister *et al.*, 2001).

There are no comparative reports on biochemical blood parameters in male mink before and during the breeding season. The aim of this study was to determine selected biochemical blood parameters in male Black and Sapphire mink, before and during the breeding season. This study focused on blood levels of protein, glucose, triacylglycerides, cholesterol with lipoproteins HDL and LDL, as well as on the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

MATERIALS AND METHODS

Animals and blood collection: The tests were performed on a total of 143 male mink, representing two color varieties, Black (n=68) and Sapphire (n=75), which were kept in a breeding facility in the north-western part of Poland. None of the examined animals had any clinical symptoms of disease throughout the experimental period. The experiment was divided into two subperiods: the non-breeding season (September-November) and the breeding season (January-March). Throughout the experiment, the animals were kept under the same conditions and supplied with the same feeding. The diet consisted of a standard mixture for mink based on fish. Blood samples were taken without the use of sedatives or anesthetics. After brief fixation of the animal by an expert worker, the blood was taken by claw-cutting (Nova Scotia Department of Agriculture, 2007). The blood was collected directly into heparinized capillary tubes. Blood collections took place between 9.00 am and 11.00 am, before feeding. The blood was centrifuged on the same day, within 1.5 to 3 hours after the start of the samplings, and the plasma was subsequently placed in Eppendorf tubes, and stored frozen at -20°C until time of analysis (20 to 90 days).

Biochemical analyzes: After thawing, plasma concentrations of the following biochemical parameters were determined: glucose, total protein, triacylglycerides, total cholesterol, high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol. The activity of alanine aminotransferase (ALT) and asparagine aminotransferase (AST) was also determined. The individual blood parameters were determined using the Cobas Mira autoanalyzer (Roche). For the determination of glucose, cholesterol and triacylglyceride concentrations, as well as ALT and AST activity, we used kits from Applied Biosystems; for protein determination, kits of Pointe Scientific were used.

Statistical analysis: The statistical analysis of data was performed using the package Statistica PL v.9.0 (StatSoft Polska). Effects of season and color groups were determined using a two-way ANOVA. Following testing, the normality of distribution, comparisons of the means of the respective parameters was performed with the Tukey's test.

RESULTS

Biochemical blood parameters including glucose, protein, cholesterol, HDL and LDL concentrations, and AST activity in two color varieties were in general slightly higher during the reproductive season than the off-season values, and only in protein (66.6 ± 0.40 vs 63.3 ± 1.40 g/l), as well as the HDL and LDL content (3.31 ± 0.10 vs 2.66 ± 0.17 mmol/l and 1.75 ± 0.14 vs 1.22 ± 0.13 mmol/l, respectively), were the differences significant ($P \leq 0.05$). The ALT activity and triacylglycerides concentration were higher outside of the breeding season than in the season, only the latter parameter showing a large and significant difference (2.82 ± 0.21 vs 1.28 ± 0.07 mmol/l, $P \leq 0.01$); in ALT activity the difference was non-significant (94.1 ± 4.27 vs 89.1 ± 3.24 U/l). Sapphire mink differed more than the Black, since the cholesterol and HDL concentration in the previous color was significantly greater within the reproductive season (Table 1). In contrast, only a significantly lower ($P \leq 0.01$) triacylglycerides concentration was detected in Black mink during the reproductive season (Table 1). A significant difference ($P \leq 0.01$) between the males of either color was found in ALT activity within the reproductive season and cholesterol concentration in the non-breeding season (Table 1).

DISCUSSION

When considering blood biochemical parameters, it is taken into account that certain postprandial values may differ from those of the fasting period (Barrows and Parks, 2006, Klein 2010). The cited authors either fail to provide the information about the time of blood sampling or whether blood was taken from postprandial or fasting animals. Hunter (1996) specify the reference value for glucose in male mink in the range of 5.9 to 8.0 mmol/l. Hurrington *et al.* (2012) observed a larger range in free-living, non-native American mink, namely 7.6-15.6 mmol/l; however, the samplings took place in April and May and the reported levels pertained to animals of either sex. The glucose content in the present study ranged from 6.89 to 7.35 mmol/l, depending on the season and color of the coat. Rui *et al.* (2012) found lower concentrations in male Black mink, i.e. 5.41 mmol/l. Slightly higher glucose concentrations were determined by Rouvinen-Watt *et al.* (2010), in fasting animals, and Miknienė *et al.* (2010), respectively 8.96 mmol/l and 9.14 mmol/l; the later report, however, does not specify the time of blood collection. The time between blood collection and centrifugation is mentioned as an influential factor when comparing glucose values. This factor can sometimes be of importance, since glucose concentration in whole blood in a non-centrifuged and stored at room temperature samples decreases by about 5-12 mg/dl (about 0.3 to 0.7 mmol/l) per hour (Overfield *et al.*, 1972). Studies by Frank *et al.* (2012) revealed that the decline in human serum glucose concentration stored at room temperature for 4 hours was lower than 5%, as compared with the level measured directly on sample collection.). This time span was different with the cited authors, or was not specified at all. In the present study, the interval between blood collection and plasma recovery was 1.5-3 hours, thus the resulting values could be lower by 0.34 to a maximum of 2.1

Table 1: Blood biochemical parameters (mean±SE) of male mink during and out of the breeding season

Parameters	Units	Breeding Season		Non-Breeding Season	
		Black	Sapphire	Black	Sapphire
Glucose	mmol/l	7.41±0.20 (49)	7.37±0.32 (39)	7.05±0.35 (49)	7.35±0.81 (39)
Protein	g/l	65.9±0.5 (49)	67.2±0.5 (54)	62.3±3.0 (49)	64.1±1.0 (54)
ALT	U/l	102.9±3.1 ^A (51)	76.6±4.9 ^B (56)	93.1±3.9 ^{AB} (51)	95.0±7.4 ^{AB} (56)
AST	U/l	96.0±4.0 (51)	91.8±3.4 (56)	88.8±5.5 (51)	88.6±6.7 (56)
TG	mmol/l	1.39±0.16 ^A (38)	1.20±0.05 ^A (48)	3.26±0.45 ^B (14)	2.50±0.30 ^{AB} (19)
Chol	mmol/l	5.44±0.13 ^{AB} (34)	5.56±0.15 ^A (33)	5.75 ±0.55 ^A (13)	4.61±0.17 ^B (19)
HDL	mmol/l	3.24±0.14 ^{AB} (35)	3.37±0.15 ^A (36)	3.05±0.29 ^{AB} (9)	2.44±0.21 ^B (16)
LDL	mmol/l	1.55±0.13 (31)	1.93±0.22 (31)	1.36±0.07 (8)	1.18±0.20 (17)

Values marked by the different letters in a row differ at $P \leq 0.01$; TG= triacylglycerides; Chol=cholesterol; Values in parenthesis indicate number of samples tested.

mmol/l than the actual values immediately after blood collection. Glucose concentration was relatively balanced between animals of both color varieties, and between the seasons.

Protein concentrations determined in this study match those established by Hunter (1996)-5.94 g/dl (59.4 g/l). Slaska *et al.* (2003) reported, on the other hand, higher values: 72.1 g/l and 67.6 g/l, respectively, in male Brown and Standard mink. Rouvinen-Watt *et al.* (2010) and Miknienè *et al.* (2010) also reported higher protein concentrations (corresponding to 68 g/l and 74.2 g/l). In wild mink, this parameter ranged from 56 to 72 g/l (Harrington *et al.*, 2012). An influence of the color variation cannot be excluded. In the present study, the only significant difference found in blood biochemical parameters between the shades of color was in ALT activity and cholesterol concentration. Slaska *et al.* (2003) reported that the color variety significantly influenced the concentration of protein.

The ALT activity remained within the range reported by Hunter (1996), from 14.7 to 128.5 U/l, and differed from the values by Rouvinen-Watt *et al.* (2010), who reported 135 U/l and Rui *et al.* (2012), who reports 237.71 U/l in 6 month old male of Black American mink. According to Valdovska and Pilmane (2011), who studied Aleutian disease virus-positive Dark Brown mink though without clinical signs of any disease, the ALT level ranged from 87.4 to 220.6 U/l. On the other hand, Harrington *et al.* (2012) reported the range 52 to 912 U/l for wild mink. The relatively wide range of variation of the values presented above is certainly multifactorial. The retention of samples in a frozen state can be named as one of the factors. Cray *et al.* (2009) studied biochemical parameters in rat serum and found that even at a storage time of 90 days at -20°C a decrease in ALT activity was up to 10%. However, storage of samples exceeding 360 days at -20°C resulted in a decline of over 50% of this activity. In the present study, this factor should play a minor role, since the samples were stored for up to maximum 90 days.

The AST activity in the reproductive season was a little higher than out of the season. This parameter was similar in either color, both before and during the season, and was higher than the reference value for male Brown mink (53.3 to 60.7 U/l) given by Hunter (1996). Rui *et al.* (2012) observed in Black male mink the level 229.01 U/l, which is more than twice higher compared to that presented in this article. In turn, Harrington *et al.* (2012), in blood collected in April and May (non-breeding season) from wild American mink, found AST activity in the range 56-652 U/l.

Cholesterol concentration ranged from 4.61 to 5.75 mmol/l, which is similar to that reported by Harrington *et al.* (2012) for wild forms, 3.2-6.8 mmol/l, or Rouvinen-Watt *et al.* (2010), 5.0 mmol/l. Higher values were presented by Rui *et al.* (2012) for males of the Black type of mink (8.32 mmol/l) and Slaska *et al.* (2003) - Brown mink at 391.6 mg/dL (10.18 mmol/l) and Standard 342 mg/dL (8.89 mmol/l) - which differed considerably from the values obtained in this study. The latter authors also found a significant difference between colors in the total cholesterol and HDL level. The results of this study partly confirm those findings, i.e. only for cholesterol concentration during the non-breeding season.

TG concentration - both within the breeding season and out of it-was much higher compared to data of Rouvinen-Watt *et al.* (2010) for Standard mink, 0.97 mmol/l. Rui *et al.* (2012) reported slightly higher values for males of the Black type, 1.35 mmol/l. The significant decrease in TG concentration in the breeding season in each color can either be a result of ambient temperature drop and, consequently, a greater demand of energy. On the other hand, probably also the energy consumption increases in the breeding season as a result of energy expenditure for mating. Through the above two factors, the triacylglycerides deposits are more heavily loaded as an energy source. This, however, requires further comparative studies, including the females.

Conclusion: In male Black and Sapphire mink, glucose, protein, cholesterol, HDL and LDL concentrations and AST activity were generally slightly higher level during the reproductive season as compared to the non-breeding season. Also the parameters in each color variety (Black and Sapphire) were slightly higher during the breeding season than in the non-breeding period; however, their levels will also vary depending on the color type. The decrease in TG concentration in the breeding season, on the one hand, indicates an increased energy demand due to lower outdoor temperatures. On the other hand, its low level may be due to increased consumption compensating for energy expenditure during mating.

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