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The Level of Selected Hormones in Peripheral Blood in Female Polar Foxes (*Alopex lagopus* L.) in Relation to Age

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The objective of the study was to determine the concentration profile of selected hormones in the blood serum of blue polar fox vixens at various ages during the non-mating period, three months after lactation. The investigation was performed on 50 clinically healthy female polar foxes derived from a domestic reproductive farm. The animals were divided into 5 age groups (n=10) ranging from the 1 to 5 years of life. In the blood serum the contents of insulin, triiodothyronine (total and free), thyroxin (total and free), leptin and ghrelin (total and active) were determined. No significant, female age-dependent differences were found in the contents of insulin, total and free triiodothyronine and total ghrelin in the blood serum. Significant ($P<0.05$) differences were observed in the concentration of total and free thyroxin (the highest in the blood of 4-year old females) as well as leptin and active ghrelin (the lowest and the highest content in 3-year old vixens). However, no distinct, female age-dependent tendencies for change in the content of these hormones in the blood serum were observed.

Key words: Polar fox, age, hormones.

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It is widely believed that hormones play a key role in the regulation of physiological processes and adaptation of organisms to the environment. In the case of carnivorous fur animals whose ancestors were predators, the main factors that synchronise many biological processes are daylight length and changes in the activity of the pineal gland and in the pituitary-adrenocortical system (VALTONEN 1989; KOLESNIKOVA 1996). Polar foxes, apart from the cyclic nature of processes associated with reproduction, are also characterised by a strong seasonality of their basic metabolic rate (BMR), manifested in the reduction of the BMR in autumn and winter (about 15 to 30%) following its maximum rate in the summer period (BERESTOV & KOZHEVNIKOVA 1989; FUGLEI & ØRITSLAND 1999). In adult foxes, seasonal changes in the basic metabolic rate correspond with annual differences in their body weight and the degree of body fattening. Tissue and visceral fat accumulation attains a maximum in November and December when fat reserves reach 20% of the body weight. These reserves are released rapidly from March to May and the lowest levels of fat are recorded in early summer months (6% of body weight) (PRESTRUD & NILSSEN 1992). This is associated with the "switching over" from summer to winter metabolism in foxes (FUGLEI *et al*. 2000; KOZHEVNIKOVA *et al.* 2000; FUGLEI *et al*. 2004). Seasonal changes in hormone activities regulating reproduction and metabolism in adult polar foxes are well documented. There is also information concerning the activity of the hormonal system in fox pups during the first months of life in which intensive pro-

cesses of organogenesis, tissue differentiation and development of thermoregulatory mechanisms take place (VALTONEN 1989; PRESTRUD & NILSSEN 1992; SIROTKINA & TYUTYUNNIK 1998; RENDAKOV *et al*. 2003). On the other hand there is a lack of information in the available literature about the profile of hormone concentrations in the blood of adult foxes depending on their age and consecutive reproductive season.

The aim of this study was to determine quantities of selected hormones in the peripheral blood in female blue polar foxes in the non-mating period in relation to age.

Material and Methods

Experimental animals

The experiment was carried out in September 2006 on 50 adult, clinically healthy females of the blue fox (*Alopex lagopus* L.) derived from the domestic reproductive farm in Łachowo near Szubin. All of the vixens were in the non-mating period, three months after lactation. The number of puppies raised by each female during the last reproductive season was similar (from 7 to 10). The experimental females were divided into 5 age groups (n=10): one-year (group I), two-year (group II), three-year (group III), four-year (group IV) and five-year-old females (group V). The animals were reared in standard farm conditions, fed once a day and had *ad libitum* access to water. Diets used on the farm for feeding reproductive blue foxes during this breeding period were based on poultry and fish offals, meat-and-bone meal, extruded cereals and rapeseed oil supplemented with a mineral-vitamin mixture. The content of metabolisable energy (ME) in 1 kg feed was 7100 kJ. The dietary ME distribution from protein, fat and carbohydrates was 29, 54 and 17%. The metabolisable energy content from fat was higher than the recommended level given in the country feeding standards (JAROSZ 1994) but was in agreement with Scandinavian feeding standards (HANSEN 1992).

Chemical analysis

Blood samples for analysis were collected from the cephalic vein (*vena cephalica*) in the morning (8.00-10.00) before feeding the animals. Prior to analysis, the centrifuged samples of blood serum were stored at a temperature of -20^{o}C. The level of the following hormones was determined: insulin, triiodothyronine (T_3) total and free, thyroxin (T_4) total and free, leptin and ghrelin total and active. Hormones in the serum were analyzed radioimmunologically using the insulin RIA kit, ghrelin (total and active) – RIA kits, multi-species leptin – RIA kit obtained from Linco Res., (USA), and with RIA-gnost T_3, RIA-gnost FT_3, RIA-gnost T_4, RIA-gnost FT_4 obtained from CIS Bio International (France).

Statistical analysis

The obtained results were analysed statistically with one way analysis of variance (ANOVA). The post hoc Duncan test was applied. The significance of differences was set at $P<0.05$.

Results

Table 1 presents the content of insulin, thyroxin and triiodothyronine, leptin and ghrelin determined in the blood of experimental female blue polar foxes. There were no significant differences in the content of insulin in the blood of the animals. The level of this hormone fluctuated from 9.51 to 12.27 uU ml^{-1}. Moreover, also the content of total and free T_3 in the blood serum of females did not differ significantly between the examined groups. However, significant differences ($P<0.05$) were determined in the total and free thyroxin. The lowest level of this hormone was found in the blood serum of one- and five-year-old vixens (respectively: T_4 total – 24.98 and 33.31 ng ml^{-1}; T_4 free – 6.77 and 6.74 pg ml^{-1}), while the highest content of total (52.35 ng ml^{-1}) and free (10.39 pg ml^{-1}) thyroxin was determined in four-year-old females ($P<0.05$). The lowest concentration of leptin was determined in the blood of three-year-old vixens. The mean level of this hormone in the experimental females from group III amounted to 1.16 ng ml^{-1} and was significantly lower ($P<0.05$) in comparison to the level of leptin determined in the serum of one-, two- and five-year-old vixens. The analysis of the total ghrelin content did not demonstrate statistically significant differences between age groups. The content of this hormone in the blood of the experimental females ranged from 1275 pg ml^{-1} (group V) to 1496 pg ml^{-1} (group III). On the other hand, significant ($P<0.05$), age-dependent differences were found in the concentration of active ghrelin. The highest and similar levels of this hormone were observed in two- and three-year-old animals (51.91 and 52.00 pg ml^{-1}, respectively), while the lowest were noted in the group of one-year-old females (32.04 pg ml^{-1}).

Table 1

Hormone content in the blood serum of polar fox females ($\bar{x}$ ± SD)

Hormone	Group				
	I	II	III	IV	V
Insulin, uU ml^{-1}	9.51 ± 2.09	12.27 ± 4.78	9.51 ± 5.52	9.52 ± 4.67	11.30 ± 2.98
T_3 total, ng ml^{-1}	0.38 ± 0.04	0.41 ± 0.04	0.36 ± 0.06	0.40 ± 0.05	0.38 ± 0.06
T_3 free, pg ml^{-1}	1.10 ± 0,28	1.49 ± 0.29	1.35 ± 0.39	1.46 ± 0.27	1.07 ± 0.26
T_4 total, ng ml^{-1}	24.98^{a} ± 7.15	38.60^{b} ± 13.66	36.17^{a,b} ± 12.78	52.35^{c} ± 19.54	33.31^{a,b} ± 6.85
T_4 free, pg ml^{-1}	6.77^{a}± 1.39	8.42^{a,b} ± 2.32	8.66^{a,b} ± 3.56	10.39^{b} ± 2.82	6.74^{a} ± 2.08
Leptin, ng ml^{-1}	1.49^{a} ± 0.16	1.56^{a} ± 0.37	1.16^{b} ± 0.24	1.35^{a,b} ± 0.24	1.58^{a} ± 0.38
Ghrelin total, pg ml^{-1}	1450 ± 331	1295 ± 208	1496 ± 205	1351 ± 317	1275 ± 117
Ghrelin active, pg ml^{-1}	32.04^{a} ± 9.16	51.91^{b} ± 12.55	52.00^{b} ± 15.91	38.71^{a} ± 5.66	41.52^{a,b} ± 17.15

a,b,c – means in the rows with different letters differ significantly (P<0.05).

Discussion

Despite of the domestication process, the life of polar foxes is regulated by seasonality. The strict rhythm of biological cycles such as reproduction, moulting and metabolism is reflected in a number of physiological parameters in this species and provides a basis in the farm breeding of carnivorous fur animals (BERESTOV & KOZHEVNIKOVA 1989). However, there is no information in the available literature on physiological indices, including hormone contents, in the blood of farmed polar foxes depending on age and consecutive reproductive season. In the present study carried out on polar vixens of 1 to 5 years of age, it was found that the concentration of insulin, total and free triiodothyronine and total ghrelin in blood serum did not differ significantly among groups. However, significant (P<0.05), age-dependent differences were recorded in the concentrations of total and free thyroxin, leptin and active ghrelin.

Insulin, as an anabolic hormone, exhibits a strong antilipolytic activity. Its level in the blood of carnivorous fur animals is associated with the state of nutrition and seasonal changes in basic metabolism. Investigations carried out on wild raccoon dogs and farm minks showed that high insulin activity, observed in the autumn, is associated with the accumulation of energy saved in the form of intra-abdominal fat and glycogen for the winter period, while fasting is a factor reducing the concentration of this hormone in the blood serum (ASIKAINEN *et al*. 2004; MUSTONEN *et al.* 2005). The impact of age on insulin concentration in the blood is observed in young, growing animals in which anabolic processes prevail, whereas aging processes are associated with reduced insulin secretion and intensification of catabolic processes (ŚLEBODZIŃSKI 1979; PERRY 1999). It should be mentioned that polar foxes attain somatic maturity before the age of 12 months. They reach a lifespan of 10 years but are used for reproductive purposes for no more than 4 to 6 (BERESTOV & KOZHEVNIKOVA 1989). According to earlier studies, the best reproduction results are achieved in polar vixens at an age of 2 to 5 years, afterwards the number of puppies delivered and reared by females decreases significantly (STANISŁAWSKA & BERNACKA 1984). Therefore, in our study the growth and somatic development of the experimental vixens was complete and the animals were in the period of full reproductive capacity. It should be noted that the content of insulin in the blood serum of the blue polar vixens of all the examined age groups was within the range of reference values determined for mature dogs (WINNICKA 2004).

Despite the significant differences found in the present study in the concentrations of total and free thyroxin in the blood of the experimental females, no significant, age-dependent changes in the concentrations of the total and free triiodothyronine were recorded. It is known that triiodothyronine is a metabolically active form of thyroid hormone. Therefore, the results obtained in the experiment suggest that during the non-mating period, metabolic rate in polar vixens from 1 to 5 years of age remains at an equal level. Also earlier investigations carried out on adult, farm mink females (from 2 to 5 years of age) did not show significant, age-dependent differences in the concentration of total T_3 (KASPRZAK *et al*. 1993). Complex investigations on the activity of the thyroid gland in polar foxes revealed that the highest total T_3 and T_4 levels in the blood were determined in 1 to 2-month old puppies (2.28-2.93 nmol l^{-1} and 47.60-100.80

nmol l^{-1}, respectively) and, later on, the concentration of these hormones, mainly triiodothyronine, declined significantly. In 4- to 5-month-old fox cubs, the content of the total T_3 reaches the average level of 1.90 nmol l^{-1} (SIROTKINA & TYUTYUNNIK 1998; RENDAKOV *et al.* 2003). Also investigations conducted on dogs showed that the amount of thyroid hormones in the blood of cubs remains at an elevated level in comparison with adult dogs and decreases significantly in old animals (PALAZZOLO & QUADRI 1987; GONZALEZ & QUADRI 1988; REIMERS *et al.* 1990). In adult foxes, the concentration of thyroid hormones, changing in relation to the phase of the reproductive cycle, is associated with the moulting process and state of nutrition. Depending on the species (common foxes or blue foxes) as well as the season of the year, the total thyroxin content in the blood of adult foxes ranges widely (from 14 to 50 nmol l^{-1}). It is the lowest during the winter months (metabolic depression) and then it increases in spring (mating season) and at the beginning of autumn (moulting season) (VALTONEN 1989; SIROTKINA & TYUTYUNNIK 1998; FUGLEI *et al*. 2000). The results obtained in the present study correspond with the values quoted earlier.

Leptin, synthesised and secreted primarily by adipocytes, is a key hormone in the regulation of energy balance. Leptin administered to *ob/ob* mice (which genetically can not produce leptin) decreases feed intake, body weight and fattening (PELLEYMOUNTER *et al*. 1995). It is well known that in humans and rodents the concentration of this hormone in blood is positively correlated with body fat content (MAFFEI *et al.* 1995; RYAN & ELAHI 1996). Moreover, relationships were also demonstrated between the status of other hormones involved in metabolism such as thyroid hormones, insulin, glycocorticoids or estrogens and leptin concentration in the blood of humans and rodents (KIEFFER *et al.* 1996; SHIMIZU *et al.* 1997; DIEKMAN *et al*. 1998; FRIED *et al.* 2000). In experiments carried out on carnivorous fur animals, both wild and farm kept, changes in leptin concentrations in the blood of foxes, minks and raccoon dogs were investigated taking into consideration seasonal changes and endocrine adaptation of organisms to a periodical lack of food (NIEMINEN *et al*. 2002; RYÖKKYNEN *et al*. 2003; ASIKAINEN *et al.* 2004; FUGLEI *et al*. 2004; MUSTONEN *et al.* 2005). However, no information was found in the literature about the impact of age of fur animals on the level of this hormone in blood. In the present study, a similar leptin concentration in the female blood serum, with the exception of vixens from group III, was found. A significantly ($P<0.05$) lower concentration of this hormone in the 3-year old females is difficult to explain on the basis of the available results. It could be attributed to the lower fattening of the experimental vixens from group III. In studies carried out on dogs of different breeds and in different conditions, ISHIOKA *et al.* (2007) demonstrated that the content of leptin in the blood of obese dogs could be more than 4 times higher in comparison with dogs maintained in optimal conditions. On the other hand, they did not find a significant correlation between the age of mature dogs and the concentration of leptin in blood, independently of the degree of obesity. Also in the case of young (less than 1 year old) and adult (over 1 year old) wild raccoon dogs, no effect of age on the level of this hormone in blood was determined (ASIKAINEN *et al.* 2004). It should be noted that in the present study the leptin content in the blood serum of experimental vixens of all age groups was lower in comparison with that found in the blood of adult, wild polar foxes (approx. 2.00-2.25 ng ml^{-1}) and raccoon dogs (2.12-3.00 ng ml^{-1}) in the spring – late autumn period and adult dogs (3.00-12.80 ng ml^{-1}) (ASIKAINEN *et al.* 2004; FUGLEI *et al.* 2004; ISHIOKA *et al.* 2007).

Ghrelin, a peptide hormone produced primarily by the secretory cells of the stomach and intestinal mucosa, increases food intake and body weight, stimulates the secretion of growth hormone and reduces fat utilisation. Both in humans and rodents, ghrelin concentration in the blood plasma increases rapidly during fasting and declines after refeeding and in obesity (TSCHÖP *et al.* 2000; LEE *et al.* 2002; YILDIZ *et al.* 2004; GILG & LUTZ 2006). By stimulating appetite, ghrelin contributes to maintaining energetic homeostasis of the organism and, in wild-living animals, it can increase the chances for survival in conditions of periodical food shortages. It was found that the level of plasma ghrelin in polar foxes (FUGLEI *et al*. 2004) and raccoon dogs (NIEMINEN *et al.* 2002) exhibits seasonal variability which is connected with seasonal changes in appetite, body weight and basic metabolism of carnivorous fur animals (BERESTOV & KOZHEVNIKOVA 1989). On the other hand, no significant correlation was found between the age of raccoon dogs (< i > 12 months of life) and ghrelin concentration in blood (ASIKAINEN *et al.* 2004). LEE *et al*. (2002) demonstrated that in rats, ghrelin synthesis increased progressively in the first 2-3 weeks of life and reached a relatively stable level at the end of the rearing period. However, considerable variability of the ghrelin concentration in blood was demonstrated without a distinct age-related trend in an experiment carried out on immature dogs (YOKOYAMA *et al*. 2005). In adults, the concentration of this hormone in blood depends on food availability and the type of food. It was found that high-fat diets decrease considerably ghrelin concentrations, while low protein sup-

ply intensifies its secretion (LEE *et al.* 2002). In our experiment the highest level of total and active ghrelin was determined in the blood serum of 3-year old females. The lowest leptin concentration was recorded in the same animals. The recorded differences were not caused by the type of diet (standard feeding conditions on the farm) or different time of blood sample collection. This may indicate a poorer condition and lower level of fattening of the three-year old females. The level of total ghrelin determined in our investigations was slightly lower in comparison with the values determined in August in raccoon dogs (2.20-2.70 ng ml^{-1}) and mink (1.67-1.98 ng ml^{-1}) and comparable with values reported for rats (1.04-6.54 ng ml^{-1}) (LEE *et al.* 2002; NIEMINEN *et al.* 2002; RYÖKKYNEN *et al.* 2003).

In conclusion, the studies carried out on adult, clinically healthy blue polar fox females during the non-mating period did not reveal significant, age-dependent differences in the content of insulin, total and free triiodothyronine as well as total ghrelin in the blood serum. Significant differences were determined in the concentration of total and free thyroxin, leptin and active ghrelin. However, no distinct, female age-dependent tendencies for change in the content of these hormones in the blood serum were observed.

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