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Biochemical blood parameters in platinum fox females and males in ontogenesis

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ABSTRACT

Currently, veterinarians pay much attention to the diagnostic examination of animals, including animals kept on fur farms. Blood is the main material used for such examinations. Changes in its composition allows veterinary practitioners to identify disorders in various systems and organs of animals, as well as to assess metabolism in animals. Results of biochemical tests of serum samples from platinum fox males and females of different age groups and comparative assessment thereof are presented. The levels of aspartate aminotransferase (U/L), alanine aminotransferase (U/L), alkaline phosphatase (U/L), total protein (g/L), albumin (g/L), urea (mmol/L), creatinine (μmol/L), α-amylase (U/L), cholesterol (μmol/L) were determined. Aspartate aminotransferase levels in females at the age of 6 months were lower by 69% than that ones in males. Increase in aspartate aminotransferase by the age of 6 months helps animals to accumulate body weight before winter. Sexual differences in the alkaline phosphatase levels were detected in 1.5-month-old kits: alkaline phosphatase levels were higher by 21.05% in males than in females. By the age of 6 months, the alkaline phosphatase levels decreased in both males and females. The decline in alkaline phosphatase level with the age is associated with participation of this enzyme in the development of animal skeleton during ontogenesis. From the age of 4 months, the growth and development of the skeleton slows down, and by the age of 6 months the animals gain the size and body weight of adult animals. Urea and creatinine levels in foxes of both sexes increased during their growth, but remained within the reference limits. Changes in urea levels in blood can be caused by feeding excessively high-protein or excessively low-protein diets. The total protein content in sera from 4 month-old males and females decreased by 32.51 and 43.24%, respectively, compared with that one in sera from animals at the age of 1.5 months, and increased at the age of 6 months up to the level observed at the age of 4 months. According to the literature, rather rapid stabilization of protein metabolism is a biological feature of many mammals born in spring, their growth rate is accelerated and, in general, the maturity phase is shortened.

Keywords: platinum foxes, alkaline phosphatase, cholesterol, amylase, urea, creatinine, albumins, aspartate aminotransferase, alanine aminotransferase

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Биохимические показатели крови у самок и самцов лисиц платинового окраса в онтогенезе

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РЕЗЮМЕ

В настоящее время ветеринарные специалисты уделяют много внимания диагностическому обследованию животных, в том числе в пушном звероводстве. Основным материалом для таких обследований является кровь. Изменение ее состава позволяет врачам-практикам выявить нарушения в работе различных систем и органов животных, а также оценить метаболизм. В представленных материалах отражены результаты биохимических исследований сыворотки крови самок и самцов лисиц платинового цветового типа разных возрастных групп, проведена их сравнительная характеристика. Определяли

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уровень аспартатаминотрансферазы (Е/л), аланинаминотрансферазы (Е/л), щелочной фосфатазы (Е/л), общего белка (г/л), альбумина (г/л), мочевины (ммоль/л), креатинина (мкмоль/л), α -амилазы (Е/л), холестерина (мкмоль/л). Показатели аспартатаминотрансферазы у самок в возрасте 6 мес. были ниже, чем у самцов, на 69%. Увеличение аспартатаминотрансферазы к 6-месячному возрасту способствует накоплению массы тела в период подготовки зверей к зиме. У 1,5-месячных щенков выявлены половые различия в уровне активности щелочной фосфатазы: у самцов данный показатель выше, чем у самок, на 21,05%. К 6-месячному возрасту уровень щелочной фосфатазы понижался как у самцов, так и у самок. Снижение активности щелочной фосфатазы с возрастом животных обусловлено участием фермента в формировании скелета в процессе онтогенетического развития. С 4-месячного возраста рост и развитие скелета замедляется, а к 6 мес. звери приобретают размеры и массу тела взрослых животных. Показатели мочевины и креатинина у лисиц обоих полов в процессе роста животных увеличивались, но оставались в пределах референтных границ. Изменение количества мочевины в крови может наблюдаться при потреблении корма со слишком малым или чрезмерно большим количеством белка. Содержание общего белка в сыворотке крови у самцов и самок в возрасте 4 мес. снизилось на 32,51 и 43,24% соответственно по сравнению со значениями в 1,5 мес., а в возрасте 6 мес. показатели снова поднялись до уровня 4 мес. По литературным данным, относительно быстрая стабилизация белкового обмена является биологической особенностью, характерной для многих млекопитающих, рожденных весной, у них ускорен темп роста и в общем сокращена фаза достижения зрелости.

Ключевые слова: лисицы платинового окраса, щелочная фосфатаза, холестерин, амилаза, мочевина, креатинин, альбумины, аспартатаминотрансфераза, аланинаминотрансфераза

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INTRODUCTION

The analysis of biochemical blood parameters allows us to identify changes in animal and human organisms, as well as to assess the specificity of changes characteristic of a certain pathology.

Despite numerous and long-term studies, blood composition remains understudied, especially its changes depending on the sex and age of fur animals. This could be accounted for different blood sample collection procedures and different methods used for blood testing [1].

Analysis of scientific and methodological literature shows that the main publications were made 40–60 years ago, and during this, albeit seemingly short, time, there have been quite noticeable changes: adaptation of fur animals to cage rearing conditions has continued, the fur animal dietary pattern has deteriorated resulting in blood composition changes. Therefore, even the reference values should be reviewed every 10–15 years. The transaminase levels change first [2]. It should be noted that different methods use different units of blood parameter measurement that hampers comparison of results from tests carried out in different years, especially with a difference of 40–60 years. Fur animal blood tests are described mainly in V. A. Berestov's monographs published more than half a century ago [3, 4]. These monographs are rare and it is difficult to find them in libraries. Therefore, V. A. Berestov's monograph on fur animal clinical biochemistry was republished 20 years ago, but it contained old data without their clarifications and changes [5].

Biochemical reactions in the body are closely interrelated, metabolic reactions are highly coordinated. Taking into account various biological functions of blood, study of blood biochemical parameters in ontogenesis is of current importance. So, the study was aimed at testing of sera from platinum foxes for biochemical parameters in ontogenesis.

MATERIAL AND METHODS

Platinum foxes kept on ООО "Vyatka" fur animal breeding farm located in the Kirov Oblast were used for testing. Blood samples were collected from 10 fox females and 10 fox males at the age of 1.5; 4 and 6 months. The foxes were fed a diet generally used on this fur farm.

The tests were carried out at the Veterinary Laboratory of the Professor Zhitkov Federal State Budgetary Russian Research Institute of Game Management and Fur Farming. Blood samples for biochemical tests were collected from the lateral subcutaneous vein of the lower part of a fox leg into a special tube containing a clot activator before morning feeding of the foxes. Then, sera prepared by centrifugation at 2,000 rpm for 15 minutes. The levels of aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L), alkaline phosphatase (U/L), total protein (g/L), albumin (g/L), urea (mmol/L), creatinine (μ mol/L), α -amylase (U/L), cholesterol (μ mol/L) were determined using the semi-automatic BiochimSA analyzer (USA) and commercial test-kits.

The results were processed using the licensed MS Excel (Office 2019) IBM SPSS Statistics 26 software.

Mann – Whitney U-test, non-parametric statistical test, was used for each group to assess the homogeneity of the groups and the reliability of the differences in means between the groups. Differences between the compared groups were considered statistically significant at $p < 0.05$. The data were summarized in terms of mean (M), standard error of the mean (m) [6].

The works were performed in compliance with the international principles laid down in the Declaration of Helsinki for Animals (Declaration of Helsinki, 2013), Directive 2010/63/EC of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes as well as in accordance with the Rules for experimental animal handling (Annex to Order of the Ministry of Health of the USSR No. 755 of 12 August 1977) and methodological guidelines for conducting scientific and field experiments on feeding of fur animals [7, 8, 9].

RESULTS AND DISCUSSION

Biochemical tests of sera for AST and α -amylase showed that there was no difference in AST and α -amylase levels between fox females and fox males at the ages of 1.5, 4 and 6 months. AST levels in fox females at the age of 6 months increased by 5.25% as compared to that ones in fox females at the age of 1.5 months ($p \leq 0.05$). In fox males, AST levels decreased by 13.68% by the age of 6 months ($p \leq 0.05$).

ALT levels in fox females determined at the age of 1.5 months were 37.28% higher than in fox females at the age of 4 months ($p \leq 0.05$). In fox males at the age of 1.5 months ALT level was 37.57% higher than in fox males at the age of 4 months ($p \leq 0.05$). AST/ALT ratio, known as De Ritis ratio, varies from 0.9 to 1.73 U/L in healthy animals [10]. The AST/ALT ratio in foxes of different sexes at the age of 1.5 months was the same, and in fox females at the age of 4 months was 31.58% higher

than in fox females at the age of 1.5 months, but at the age of 6 months the AST/ALT ratio became similar to that one at the age of 1.5 months (Table 1).

The AST/ALT ratio increased by 53.13% ($p < 0.05$) in fox males at the age of 6 months, as compared to that one in fox males at the age of 4 months. Berezina Yu. A. et al. studied ALT and AST activities in silver-black foxes in post-natal ontogenesis. The ALT level increased more than AST level in foxes throughout the studied period of the fox life. It should be noted that the enzyme activity in fox females was lower than in fox males. ALT and AST levels in blood increased during fox ontogenesis, and the highest AST and ALT levels were detected in adult foxes. Thus, ALT level increased by 27% ($p < 0.01$) and by 34% ($p < 0.05$) in adult fox females and adult fox males, respectively, as compared to that ones in 2 months-old kits as well as AST level increased by 16 and 26% ($p < 0.01$), respectively, according to literature data [11]. The AST level became 12.04% lower in platinum fox males at the age of 6 months than in the animals at the age of 1.5 months that was not reliable. The maximum transaminase content observed in fur animals in autumn (6 months of age), during the preparation for the cold season, contributed to an active increase in body weight in animals that also correlated to the results of other studies [12, 13]. ALT (cytoplasmic enzyme) activity increases in case of mild hepatocyte injury, and AST (mitochondrial enzyme) activity increases in case of significant hepatocyte injury [14, 15].

Tests for alkaline phosphatase showed that the alkaline phosphatase level was 33.65% higher in fox females at the age of 1.5 months than in animals at the age of 4 months and 2 times higher than in foxes at the age of 6 months ($p \leq 0.05$). The alkaline phosphatase activity decreases with age of animals, since the enzyme is involved in bone calcification reducing with the age. Fur animals grow rapidly, so they acquire the size and weight of adult animals by the age of 6 months [10, 16]. The age

Table 1
Biochemical blood parameters in platinum fox females and fox males during postnatal ontogenesis

Parameters	Age of fox females and fox males					
	1.5 months		4 months		6 months	
	♀	♂	♀	♂	♀	♂
AST, U/L	^A 39.18 ± 2.00	38.80 ± 3.05**	37.73 ± 1.06	34.50 ± 0.74	^A 41.24 ± 1.97*	34.13 ± 1.35
ALT, U/L	68.16 ± 2.53*	^B 73.64 ± 11.17**	49.65 ± 2.23	^B 53.53 ± 2.90	69.98 ± 8.47	70.78 ± 7.91
De Ritis ratio (AST/ALT), U/L	0.57	0.52	0.75	0.64	0.58	0.98
Alkaline phosphatase, U/L	^A 112.78 ± 7.55*	^C 136.52 ± 8.42***	^A 74.83 ± 7.45	^C 100.70 ± 6.53***	^A 55.94 ± 2.93	^C 64.65 ± 2.89
Urea, mmol/L	4.52 ± 0.16	4.04 ± 0.64	3.93 ± 0.40	3.85 ± 0.53	5.38 ± 0.59	5.80 ± 0.42**
Creatinine, μ mol/L	38.94 ± 4.17	47.74 ± 5.44	68.40 ± 1.47**	77.65 ± 3.57*	77.14 ± 1.26*	62.85 ± 7.68
Total protein, g/L	88.71 ± 3.36*	83.72 ± 2.88**	50.35 ± 2.26	56.50 ± 2.82	83.24 ± 3.14	84.55 ± 13.13
Albumin, g/L	43.00 ± 1.59	42.34 ± 0.64**	39.05 ± 1.33	39.05 ± 0.81	38.32 ± 1.43	42.98 ± 2.10
Cholesterol, μ mol/L	7.21 ± 0.47	5.84 ± 0.18	6.67 ± 0.77	6.06 ± 0.09	6.00 ± 0.41	5.98 ± 0.63
α -amylase, U/L	534.38 ± 43.59	569.42 ± 48.59	563.25 ± 24.12	651.83 ± 36.52	619.38 ± 18.79	600.80 ± 35.47

* $p \leq 0.05$ – between ♀ at the age of 1.5 and 4 months; ** $p \leq 0.05$ – between ♂ at the age of 1.5 and 4 months;

^A * $p \leq 0.05$ – between ♀ at the age of 1.5 and 6 months; ^B ** $p \leq 0.05$ – between ♂ at the age of 1.5 and 6 months;

^C *** $p \leq 0.05$ – between ♀ and ♂ at the age of 4 months.

of the animals is directly proportional to the alkaline phosphatase level. Sex differences in the alkaline phosphatase level were found in 1.5-month-old kits. It was higher by 21.05% ($p \leq 0.05$) in fox males than in fox females. By the age of 6 months, the alkaline phosphatase level became similar in fox females and fox males.

Urea level increased by 19.03 and 43.56% ($p \leq 0.05$) in fox females and fox males, respectively, during their growth but remained within the reference limits (Table 2). Urea is synthesized in the liver, travels with blood to the kidneys, then, it is filtered through the vascular glomerulus and excreted in the urine. Urea plays an osmotically active role in the body. Urea accumulation facilitates edema development in parenchymal organs (lungs, liver, kidneys, pancreas, spleen, thyroid gland), central nervous system, myocardium, subcutaneous tissue. Tests for urea are important since it contributes to about half of the total residual nitrogen as liver converts ammonia into urea, being the final product for protein metabolism. It should also be noted that nitrogen deficiency significantly increases urea reabsorption in the kidneys. Changes in urea levels (decrease or increase) in animal blood can be caused by feeding excessively high-protein or excessively low-protein diets. It is known that the urea level in dogs fed with dry food is about 1.7 times lower than in dogs fed with canned meat [17, 18].

Tests for creatinine showed that its level increased by 75.65% ($p \leq 0.05$) and by 62.65% ($p \leq 0.05$) in fox females and in fox males at the age of 4 months, respectively, as well as by 98.1% ($p \leq 0.05$) and by 31.65% ($p \leq 0.05$) in fox females and in fox males at the age of 6 months, respectively, as compared to creatinine levels in 1.5-month-old animals. Serum creatinine is the most widely used functional biomarker of kidney function. Its concentration is quite stable and mainly depends on the total muscle mass. Creatine is synthesized in the liver from guanidine acetic acid, released into the blood stream and travels to the muscle cells where it is phosphorylated and transformed into phosphocreatine to be taken up to produce the energy required

for muscle contractions. Dehydrated creatinine, being a non-threshold substance, is excreted in the urine. The creatinine level in the blood mainly correlates with the muscle mass and the kidney excretory ability. In case of chronic renal failure, an increase in creatinine level in the blood is accompanied by an increase in the concentration of other residual nitrogen components and, first of all, urea. A similar pattern is observed in case of urinary tract blockage [19, 20].

Protein including its fractions (albumin and globulins of several types) at a certain quantitative and structural ratio is one of the major components of the blood. Proteins play an important role in maintaining the colloidal osmotic pressure of the blood plasma. The circulating blood volume remains constant and the formed elements remains suspended owing to the protein ability to drag and retain water. In platinum fur animals the total protein content in sera from 4-month-old fox males and fox females were by 32.51 and 43.24 % ($p \leq 0.05$) lower, respectively, as compared with that one in sera from animals at the age of 1.5 months, and increased at the age of 6 months up to the level observed at the age of 4 months. According to some researchers [21], protein metabolism in mammals born in the spring season is relatively faster stabilized, that is a biological feature of such animals. Such animals grow faster and reach their maturity within a shortened period.

Tests for albumin levels showed no significant difference in both fox females and fox males of different age groups. Albumin is a homogeneous plasma protein containing a small amount of carbohydrates. Albumen is the most important fraction and amounts for more than 40–60% of the total serum protein. In domestic animals, albumin accounts for 35 to 50% of the total serum protein. Albumin is the main protein maintaining intravascular colloidal osmotic pressure, that prevents plasma from leaving capillaries [14, 18, 19]. In our case, albumin concentration in both fox females and fox males practically remained unchanged (Table 1) showing no statistical difference, the albumin levels were within the reference

Table 2
Reference limits for biochemical blood parameters in platinum fox females and fox males in ontogenesis

Parameters	Age of fox females and males					
	1.5 months		4 months		6 months	
	♀ min/max	♂ min/max	♀ min/max	♂ min/max	♀ min/max	♂ min/max
AST, U/L	31.50/47.00	28.80/45.30	35.00/39.70	33.50/36.70	36.00/45.30	30.90/36.40
ALT, U/L	60.20/78.80	36.10/106.20	44.10/53.60	47.40/61.30	42.80/84.20	57.60/90.10
Alkaline phosphatase, U/L	80.90/135.40	109.80/160.00	59.60/94.70	8.70/112.50	49.60/65.50	60.10/72.70
Urea, mmol/L	4.30/5.30	3.30/5.30	2.80/4.60	3.00/5.20	3.90/7.40	4.70/6.50
Creatinine, μ mol/L	29.90/54.00	34.80/63.10	64.00/70.10	70.50/86.30	73.70/81.30	46.60/76.80
Total protein, g/L	72.30/93.90	76.20/93.50	47.10/56.70	51.90/63.80	75.30/92.30	54.80/118.30
Albumin, g/L	38.10/48.00	40.60/44.00	36.60/42.20	37.70/41.10	33.80/41.40	36.90/46.00
Cholesterol, μ mol/L	5.49/8.95	5.44/6.46	5.31/8.39	5.85/6.22	5.00/7.10	5.10/7.80
α -amylase, U/L	417.00/717.40	459.30/703.00	521.30/631.80	554.90/731.70	571.70/672.50	522.20/680.80

limits (Table 2). Changes in albumin concentration are observed during fasting, chronic gastroenteritis, when protein digestion and absorption are impaired, as well as chronic liver diseases (hepatitis, hepatodystrophy, cirrhosis) [17].

Tests for cholesterol showed that its level decreased by 7.49 and 16.78% in fox females at the age of 4 and 6 months, respectively, as compared to that one in fox females at the age of 1.5 months. Cholesterol is the most important structural component of cell membranes. It participates in cell permeability regulation and protects red blood cells from hemolytic toxins. Cholesterol is used for synthesis of steroid hormones, vitamin D₃, and bile acids. Cholesterol is synthesized in all cells of the body but cholesterol released in the blood stream is synthesized in hepatocytes and small intestine cells. The liver plays a key role in cholesterol synthesis and cholesterol catabolism. Changes in cholesterol levels are characteristic of such diseases and pathologies as hepatic disease (hepatitis, bile duct obstruction), nephrotic syndrome, hypothyroidism, chronic pancreatitis, obesity, vitamin deficiency [20, 22, 23].

CONCLUSIONS

Thus, tests of fox female and fox male blood for biochemical parameters in ontogenesis showed the following:

1. Alkaline phosphatase levels (U/L) were higher by 21.05% in fox males than in fox females starting from the age of 1.5 months. By the age of 6 months, the alkaline phosphatase levels decreased in both fox males and fox females. The decline in alkaline phosphatase level with the age is accounted for its participation in the animal skeleton development during ontogenesis. From the age of 4 months the skeleton growth and development slow down and by the age of 6 months the animals gain the size and body weight of adult animals.

2. Urea and creatinine levels in foxes of both sexes increased during the growth of animals, but remained within the reference limits.

3. The total protein content in sera from 4-month-old fox males and fox females decreased by 32.51 and 43.24%, respectively, as compared to that one in sera from animals at the age of 1.5 months. According to V. A. Afanasyev and N. Sh. Pereldik [21], rather rapid protein metabolism stabilization is a biological feature of many mammals born in spring; such animals grow faster and reach their maturity within a shortened period.

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