O.I. Sebezhkoet al /J. Pharm. Sci. & Res. Vol. 9(9), 2017, 1530-1535

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Influence of anthropogenic pollution on interior parameters, accumulation of heavy metals in organs and tissues, and the resistance to disorders in the yak population in the Republic of Tyva

O.I. Sebezhko[1], V.L. Petukhov[1], R.B. Chysyma[2], E.E. Kuzmina[2], N.I. Shishin[1], O.S. Korotkevich[1], T.V. Konovalova[1], *K.N. Narozhnykh[1], A.I. Zheltikov[1], V.G. Marenkov[1], A.G. Nezavitin[1], L.V. Osadchuk[1,3]

[1]*Federal State Budgetary Educational Institution of Higher Education «Novosibirsk State Agrarian University» 630039, Novosibirsk, Dobrolubova str. 160, Russia*
[2]*Tyvinian Research Institute of Agriculture, 667005, Kyzyl, Bukhtueva street, 4, Russia*
[3]*Federal Research Center «Institute of Cytology and Genetics of the Siberian Branch of the RAS» 630090, Novosibirsk, Ac. Lavrentieva, 10, Russia.*

Abstract

The study where conducted in the contaminated (I) and conditionally clean (II) zones of the Republic of Tyva.
Hematological, biochemical, cytogenetic indices, incidence and accumulation of cadmium, lead and zinc in organs and tissues were studied in adult yaks in these zones. The aim of the research was to study the influence of anthropogenic pollution on a complex of interior parameters and the yak incidence of a disease.
In different zones the animals had differences in some parameters of protein and mineral metabolism and an increased content of cadmium in muscle tissue, heart, liver, kidneys, lungs and spleen in the animals located in contaminated areas. Animals from zone I experienced an increased incidence of polyploidy (2.2%) and chromosomal aberrations (16.2%). The pollution of the environment caused an increased incidence of a disease (15 to 27%) and prenatal mortality in yaks (24.0%).

Keywords: anthropogenic pollution, biochemical, chemical, cytogenetic indices, yaks.

INTRODUCTION

Anthropogenic pollution of the environment has been of a great concern in recent decades in all countries, since area of the territories affected by man-made impact under the influence of polyfactorial pollution continues to increase continuously [1-5]. The growth of environmental problems in many regions occurs against the background of the disappearance of some wild species and a decrease in the number of breeds and populations of farm animals and their productivity [6].

An important problem is the provision of people with environmentally safe livestock products due to the possible entry of toxicants into food chains into the human body [7-12].

Many authors emphasize that, depending on the nature and the degree of habitat animal pollution, the concentration of ecotoxic ants in animals and livestock production is usually several times higher than their content in soil and forage and exceeds the permissible limits [13-15].

A rare anthropogenic impact is temporary contamination of territories with fuel from separated parts of space launch vehicles. This is one of the environmental problems in the cultivation of yaks in the territory of the Tyva Republic in the Russian Federation. The western regions of this republic were the site of the missiles detachable components fall. The main constituents of the propellant are high-viscosity asymmetric dimethylhydrazine (heptyl) and an oxidizer of nitrogen tetroxide. Heptyl refers to the substances of the first toxicity group, it has a multiple damaging effect on the body. It is perfectly soluble in water, highly volatile, can migrate, is able to accumulate in soil, plants and therefore has several ways of getting into the body. Nitric tetroxide is a substance of the first hazard class, forming toxic complexes of nitrites and nitrates in the body [16].

Level contamination of the territories with rocket fuel components is made by the final products of its decomposition - formaldehyde, ammonium and nitrate nitrogen.

Yak population assessment based on a set of interior indicators will allow their physiological and adaptive capabilities, as well as the state of health in anthropogenic pollution to be analyzed [17-20].

Homeostasis maintenance, which ensures the body's resistance to specific environmental conditions, is carried out, as it is known, by physiological mechanisms, among which metabolism takes a leading place [21-24]. Biochemical parameters monitoring in animals at early stages will allow dysfunctions of protein, lipid, carbohydrate, micro- and macro elements exchange to be revealed [25, 26].

The aim of the work was to study the anthropogenic pollution influence on the heavy metals content in soil, water, feed, organs and tissues of yaks, their hematological, biochemical, cytogenetic status and incidence of disease.

MATERIALS AND METHODS

The study was conducted in 2015-2016 in the yak farm in the western zone of the Republic Tyva (Russian Federation). Blood, kidney, liver, spleen, lung and heart samples were taken in adult yaks in the environmentally polluted (I) and conditionally clean (II) zones. Ecologically unfavorable zone has been contaminated with rocket fuel from parts separated fall launch vehicles since 1974. All animals were in free all-year-round pasture.

Vacuum tubes with anticoagulant K2EDTA were used carrying out hematology; the activator of a clot (SiO2) formation for reception was used carrying out biochemistry.

A complex of standard hematological parameters was studied using the automatic hematological analyzer PCE 90Vet (USA): WBC (White Blood Cells) – the number of leukocytes; RBC (Red Blood Cells) – the number of red blood cells; HGB (Hemoglobin) – level of hemoglobin; PLT (Platelet) – the number of platelets. And also estimates the rate of erythrocyte sedimentation (ESR) according to Panchenkov.

The concentration of biochemical indices was studied on a semi-automatic biochemical analyzer Photometer 5010 (Germany). The level of total protein, albumin, glucose, cholesterol, triglycerides, HDL, urea, uric acid, calcium, magnesium, chlorine, iron, phosphorus inorganic, potassium, creatinine, bilirubin and its fractions.

The albumin-globulin ratio, De Ritis coefficient and atherogenicity were calculated. Activity was ALT, AST, alkaline phosphatase (alkaline phosphatas), total acid and prostatic acid phosphatase (PAP), lactic dehydrogenase (LD), Gamma-glutamiltranspeptidase (GGT), creatinekinase (CK), α-amylase.

Measurements were carried out to the procedures provided with the standard set reagents company "VectorBest" (Koltsovo, NSO, Russia) and “Olvex-Diagnosticum” (Russia, St. Petersburg,)

In the laboratory of the Federal State Institution GSAS Tyvinskaya, 105 soil samples were examined in accordance with GOST 17.4.02.84 «Soils», 50 – water, 105 – fodder. Samples of food were selected in accordance with GOST 27262-87 “Forages of vegetable origin». Heavy metals are determined on an atomic-absorption spectrophotometer AAS-3 according to the method for determining toxic elements in food products. Samples for studying the microelement composition of organs and tissues were prepared by the method of dry ashing.

The mean arithmetic and the mean error were calculated for each parameters studied. For all indicators, the normal distribution test was carried out using the Shapiro-Wilk test (W). The reliability of the differences between the groups, the distribution in which corresponded to the normal, was assessed using the Student’s test. To compare the indicators, the distribution of which did not correspond to the normal one, were carried out using the Mann-Whitney U test.

The statistical processing of data was carried out using the Statistica, and the Gnumeric 1.12.9 program was also used.

RESULTS AND DISCUSSION

Monitoring indicators that characterize the physiological status and health of yaks will always be relevant, since the issues of biodiversity conservation, selection for increasing productivity, resistance to disease and productive longevity have traditionally been given considerable attention [27, 28].

For most hematological aspects, statistically significant differences were not revealed in yaks cultivated in favorable and unfavorable zones [29].

The conducted researches established that the quantitative characteristics of peripheral blood in all yaks varied within physiological norm: the number of leukocytes, erythrocytes, platelets and hemoglobin level. However, the calculated hematological parameters deviated from physiological values. Characterizing the erythrocyte unit of the hemogram, one can note the following: the content of erythrocytes in yaks from the favorable zone was at the lower limit of the norm (3.80 - 6.10x10^{12}/ l) (Table 1). The mean volume of erythrocyte (MCV) in yaks was below the values normally found in adult animals (normal 80-100 fl). MCV allows you to talk about the amount of water contained in the red blood cell: under conditions of hypertonic dehydration, the average volume of erythrocytes decreases, and at numbers below 80 fl it is estimated as microcytosis.

Analyzing the indicator of heterogeneity of RDW erythrocyte size, it can be said that in the yak group in the contaminated zone this indicator exceeded the optimal values, whereas in the group of animals from the favorable zone, it did not exceed the recommended limits. The norm of the width of distribution of erythrocytes is 11.5-14.5%.

Differences ($p>0.01$) were established for the rate of erythrocyte sedimentation rate. The value of ESR in yaks from the unfavorable was 4.2 times higher (0.73±0.27 mm/h) than in animals from the contaminated zone (0.18±0.01 mm/h).

In the group of animals raised in a conditionally clean zone, the total protein content and albumin concentration were significantly higher (Table 2). The phenotypic variability of albumin levels was higher.

In zone II, the level of immunoglobulin Ig G was 1.3 times lower than 15.0±4.0 mg/ml, than in an ecologically safe zone (18.9±5.0 mg / ml).

Biochemical blood counts and many serum enzymes can be indicators that reflect environmental problems and the accumulation of toxic substances in the body of animals (Table 3).

Evaluation of biochemical parameters of blood serum showed that in animals from contaminated and conditionally pure zones, the activity of enzymes fluctuated within normal biological limits. It was found that the activity of asparagine aminotransferase was 2.1 times higher in yaks of an ecologically clean zone than in animals from a contaminated zone, which, in combination with a higher level of total protein and albumins, indicates activation of the protein synthesis function.

Table 1. Hematology status of yaks from different ecological zones of the Republic of Tyva

Name	Zone I		Zone II	
	Mean±SE	Range	Mean± SE	Range
Leukocytes (WBC), $x10^9$/l	11.29±1.14	4.17-20.52	9.93±0.41	7.52-12.41
Erythrocytes (RBC), $x10^{12}$/l	5.16±0.78	3.54-7.98	3.87±0.70	3.0-7.74
Hemoglobin (HGB), g/l	167.7±8.8	86.0-226.0	157.7±3.4	133.0-178.0

Table 2. Indicators of protein metabolism in yaks from different ecological zones of the Republic of Tyva

Name	Zone I		Zone II	
	Mean±SE	Range	Mean±SE	Range
Total protein, g/l	81.3±2.8[a]	63 – 103	93.6±3.0	76 – 113
Albumins, g/l	55.1±3.5[a]	41 – 87	70.2±3.1	51 – 86
Globulins, g/l	28.0±3.0	4 – 43	23.9±3.4	5.0– 45.0
Urea, mmol/l	4.06±0.12	3.58 – 5.23	3.84±0.07	3.58 – 4.83
Creatinine, mcmol/l	129.9±16.4	31.9 – 147.8	141.6±2.6	120.2 –148.2

a – Significant differences between zones ($p<0.01$)

Table 3. Serum enzyme activity

Name	Zone I		Zone II	
	Mean±SE	Range	Mean±SE	Range
ALT, mmol/lh	37.5±4.5	9.5 – 67.5	49.0±5.6	5.6 – 99.1
ACT, mmol/lh	31.3±3.8[a]	0.42 – 52.0	64.8±6.5	20.9 – 104.1
De Ritis ratio	1.02±0.19	0.02 – 2.83	1.35±0.14	0.48 –2.54
Lactate dehydrogenase, u/l	264.4±48.4	123.0 –762.1	678.7±141.3	93.0 –1757.0
Alkaline phosphatase, u/l	58.2±20.3	4.0 – 133.0	103.5±69.0	5.0 –788.0
Acid phosphatase total, u/l	28.4±8.7	3.8 – 110.2	11.6±2.0	0.005 –28.2
Acid phosphatase, prostatic, u/l	6.75±2.06	0.01 – 29.8	2.68±0.89	0.001 – 9.18
Amylase, u/l	8.6±1.77	1.0 – 28.0	9.2±2.67	2.0 – 43
Gammaglutamylpeptidase, u/l	5.16±1.14	0.26 – 16.7	11.71±3.56	0.63 - 48

a – Significant differences between zones ($p<0.01$)

Table 4. Indicators of mineral metabolism

Name	Zone I		Zone II	
	Mean±SE	Range	Mean±SE	Range
Calcium, mmol/l	4.44±0.6	0.6 –8.3	6.31±0.99	3.5– 16.2
Phosphorus, mmol/l	3.75±0.49	0.3– 7.9	3.87±0.56	0.19– 6.93
Ca/P, relation	1.66±0.46	0.34– 7.67	9.97±5,79	0.66–85.26
Magnesium, mmol/l	1.23±0.16	0.19–2.45	1.22±0.13	0.7–2.4
Chlorine, mmol/l	105.5±5.6[a]	66.0 – 143.1	158.1±13.64	69.7–316.2
Iron, mcmol/l	8.8±1.4[a]	0 –19	17.66±3.74	1– 60

a – Significant differences between zones ($p<0.01$)

An analysis of the mineral composition of the serum of yaks showed (Table 4) a decrease in the concentration of iron (2.1 times) and chlorine (1.5 times) in the contaminated zone compared with the net zone was observed.

All yaks have a sufficiently high level of glucose in the blood serum, which probably reflects the intensity of the ongoing metabolism of carbohydrates (Table 5).

In the contaminated zone of the Republic of Tyva, there was increased cadmium content in the muscles, heart, liver, kidneys, lungs and spleen (Table 6).

In the contaminated area in the myocardium and lungs, the concentration of both cadmium and lead has increased. The ratio of this element in organs and tissues in different ecological zones was significantly different. In zone I in the heart, the zinc content was 2.4 times higher, 1.6 times in the lungs and 1.3 times in the spleen.

Thus, the pollution of the territory of the Republic of Tyva influenced the level of accumulation and the ratio of heavy metals in the organs and tissues of yaks.

Anthropogenic pollution caused an increase in the frequency of polyploidy, chromosomal aberrations and a decrease in the frequency of diploidy (Table 7).

A similar pattern was observed in cattle of black and motley breed. In an ecologically unfavorable zone, the frequency of rupture of chromosomes increased by 1.5 times, and chromosome fragments by 2.7 times (4.1±0.4%) [30].

In the dysfunctional zone, the incidence of the cardiovascular system, liver, gastrointestinal tract, hoof deformation and perinatal mortality increases several fold in yaks (Table 8).

In a conditionally clean zone, perinatal mortality was 4.0, while in yaks from the disadvantaged zone this figure was 6 times higher.

Repeated studies have established an increase in the concentration of degradation products of rocket fuel, in particular formaldehyde in the territory of the Republic of Tyva (Zone 1). There is also an increased level of nitrites, nitrates, mercury, and lead. For example, the level of cadmium in the soil was 0.40±0.06 mg/kg, which exceeded the maximum permissible concentrations (MPC) by 1.3 times, and in some areas the CD content reached 0.89-0.95 mg/kg. In the Republic of Tyva, chestnut soils are quite common, formed in conditions of poor vegetation and dry climate. In pastures with this type of soils in contaminated areas, the lead concentration reached 950 mg/kg (with a maximum permissible concentration of 32 mg/kg) and the zinc content was 61 mg/kg (MPC 55 mg/kg). In grain for feed, the Cd level was 1.3 times higher (0.4±0.04). In addition, natural reservoirs were contaminated in these areas. In water, the cadmium level exceeded the MPC by a factor of 2 (0.002 mg/kg). Concentration of lead exceeded the MPC by 1.3 times and was 0.04±0.01 mg / kg. According to the data of the Ministry of Health, the incidence of the adult yak population and adolescents was higher by 27 and 65% respectively in the unfavorable zone.

The average annual temperature in the Republic of Tyva is from - 3.3 to 6.3^0 C. In valleys in winter, the temperature can drop to -55 -60^0 C. The frost-free period ranges from 60 to 125 days per year. In July, the maximum temperature can rise to +36-39^0 C. The annual amount of precipitation in the valleys is in the range of 150-300 mm, and in the mountains - 500-800 mm. Mountain soils in the Republic are 82%, among which taiga, tundra, chestnut trees prevail. On the plains, the soils of the steppe and desert-steppe group are distributed (chestnut, light chestnut and brown semi desert). Yak livestock in Tyva is based on year-round pasture content. Preservation of grass in winter is 60-80%.

In zone I and II hematological, biochemical and mineral status of yaks was studied.

The parameters of protein metabolism characterize the multifaceted importance of protein molecules in metabolic processes in animals; reflect the adequacy of feeding to physiological needs [12]. It is the amount of total protein in the blood serum and the ratio of the fractions of serum proteins that are associated with the productivity of the animals [31].

Due to the fact that the level of enzymes, their activity are genetic, enzymatic monitoring of animals in different zones can assess the contribution of environmental and climatic conditions to the level of directivity, intensity of metabolic processes, biochemical adaptation and fitness of animals [32].

Key enzymes that characterize metabolism in livestock and are involved in the formation of productive traits include transamination enzymes - alanine aminotransferase (ALT) and asparagine aminotransferase (AST), oxidative peroxidase and catalase, phosphorylation - acid and alkaline phosphatase, hydrolysis - alpha-amylase. Enzyme activity in the blood serum always reflects the balance of the rate of enzyme synthesis inside cells and their subsequent release [33]. It is shown that in the zone of anthropogenic pollution in the blood serum the content of total protein, albumins and aspartate aminotranspherase-T decreased.

Table 5. Biochemical indicators in yaks from different ecological zones of the Republic of Tyva

Name	Zone I		Zone II	
	Mean±SE	Range	Mean±SE	Range
Glucose, mmol/l	37.33±3.54	13.0 – 64.4	28.13±4.81	5.0–71.0
Cholesterol, mmol/l	2.44±0.44	2.25 – 2.93	2.6±0.21	1.3–5.3
Triglycerides, mmol/l	0.18±0.05	0.06 – 0.5	0.12±0.02	0.05–0.37
HDL, mmol/l	3.36±0.71	9.71– 6.93	2.97±0.43	1.13–6.6
Total bilirubin, mcmol/l	13.87±3.4	0.01– 44.8	15.77±2.55	2.8– 41.4
Conjugated bilirubin mcmol/l	8.29±2.54	0.01– 30.6	14.36±2.67	2.8– 37.7
Unconjugated bilirubin, mcmol/l	5.33±1.96	0.01– 23.3	1.97±0.67	0.01–7.2

Table 6. Accumulation of heavy metals in different ecological zones, mg/kg

Organ, tissue	Zone I			Zone II		
	Cd	Pb	Zn	Cd	Pb	Zn
Muscle	0.38±0.02[a]	0.62±0.03	86.0±3.6	0.12±0.03	0.58±0.04	114.8±7.1
Heart	0.30±0.03[a]	0.53±0.06	85.6±4.6	0.06±0.003	0.31±0.02	36.3±1.1
Liver	0.40±0.01[a]	0.061±0.004	104.5±3.5	0.029±0.005	0.07±0.006	107.4±15
Kidneys	0.71±0.02[a]	0.59±0.03	78.5±8.2	0.35±0.01	0.76±0.04	74.3±2.5
Spleen	0.38±0.02[a]	0.85±0.11	96.0±3.2	0.13±0.007	0.73±0.04	73.3±4.0
Lungs	0.43±0.04	1.20±0.12	87.4±3.5	0.15±0.004	0.73±0.03	55±2.0

a – Significant differences between zones ($p<0.05$)

Table 7. Somatic chromosomal instability in different ecological zones of the Republic of Tyva

Mutation type	Number of metaphases	Zone I	Zone II
Polyploidy	5800	2.20±0.22[b]	0.50±0.20
Aneuploidy	3400	0.82±0.28	0.49±0.08
Aberrations	3400	16.2±0.4[b]	11.0±1.2
Diploidy	3400	87.0±0.7[a]	91.9±1.6

a – Significant differences between zones ($p<0.05$)
b – Significant differences between zones ($p<0.001$)

Table 8. Incidence and perinatal mortality of yaks

Name	Zone I(n=190)	Zone II (n=150)
Diseases of the cardiovascular system	27.4±3.2[b]	2.6±1.3
Diseases of the liver	14.7±2.6[a]	6.0±1.9
Disturbance of the gastrointestinal tract	16.3±2.7[b]	4.0±1.6
Deformation of hooves	15.8±2.6[b]	2.0±1.1
Osteolysis of caudal vertebrae	15.8±2.6[b]	3.3±1.5
Perinatal Mortality Chat	24.0±3.1[b]	4.0±1.6

a – Significant differences between zones ($p<0.05$)
b – Significant differences between zones ($p<0.001$)

Particular attention should be paid to micro- and macro elements, which are involved in the regulation of basic physiological processes. Calcium and phosphorus are the most important components of living organisms. Calcium in the body performs two main functions: passive (as part of supporting tissues) and active (in cells and tissues to support vital processes) [34]. In zone I, a decrease in the concentration of iron and chlorine was observed. Pollution of the environment causes a significant reduction in the resistance of yaks to diseases.

In many organs and tissues there was a significant increase in heavy metals such as lead, cadmium and zinc. A similar pattern has been established in other animal species [35-37]. Pollution of the environment causes a wide range of changes in the interior performance of animals. In the yaks in zone I, the frequency of polyploidy and chromosomal aberrations increased. Similar chromosomal changes were found in cattle at different periods of ontogenesis. In healthy calves and calves with abnormalities in the contaminated zone, the frequency of chromosomal aberrations was 2 and 3 times higher, respectively, than in animals from an ecologically safe area [38].

CONCLUSION

The majority of hematological and biochemical indicators in yaks both in the contaminated zone and in the clean zone were within the limits of reference values, which indicates a wide adaptation of animals to the conditions of anthropogenic pollution.

Yaks from the ecologically unfavorable zone had a lower content of mineral exchange indicators: iron and chlorine in 2.1 and 1.5 times, respectively. The content of cadmium in muscle tissue, heart, liver, kidneys, lungs and spleen was significantly higher in the zone of anthropogenic pollution.

Yaks from the conditional-favorable zone were characterized by active processes of protein biosynthesis, which is expressed in a higher level of total protein, albumin and ALT activity.

In the zone of anthropogenic pollution in animals, the frequency of polyploidy, chromosome aberrations increases and diploidy decreases.

In the ecologically unfavorable zone of the Republic of Tyva, yaks experienced an increased incidence of cardiovascular disease, impaired activity of the gastrointestinal tract, liver diseases, hoof deformation and an increase in prenatal mortality.

Thus, in the Republic of Tyva the yak population in the pollution zone has a large spectrum of changes, especially in the accumulation of heavy metals in organs and tissues, an increase in the frequency of somatic chromosomal instability and a decrease in resistance to diseases.

ACKNOWLEDGEMENT

This research was supported by Russian Science Foundation (project № 15-16-30003).

REFERENCES

1. Chysyma, R.B., Petukhov, V.L., Kuzmina, E.E., et al., *Journal de Physiqe*. 2003, *107*, 297-299.
2. Chysyma, R.B., Bakhtina, Ye.Ye., Petukhov, V.L., et al., *Journal de Physiqe*. 2003, *107*, 301-302.
3. Kuzmina, E.E., Chysyma, R.B., Korotkevich, O.S., *Zootechny*. 2007, *10*. 25-26.
4. Kuzmina, E.E., Chysyma, R.B., Korotkevich, O.S., *Siberian Herald of Agricultural Science*. 2008, *2*, 72-77.
5. Petukhov, V.L., Gorb, T.S., Petukhov, I.V., et al., *Journal De Physique*. 2003, *107*, 1065-1066.
6. Petukhov, V.L., Tikhonov, V.N., Zheltikov, A.I., et al., *Gene and gene pool of Siberian Northern breed and Siberian Black and White breed group pigs*, NSAU, Novosibirsk 2012.
7. Korotkevich, O.S., Petukhov, V.L., Sebezhko, O.I., et al., *Russian Agricultural Sciences*, 2014, *40(3)*, 195-197.
8. Marmuleva, N.I., BarinovE.Ya., Petukhov, V.L., *Journal De Physique*. 2003, *107*, 827-829.
9. Narozhnyh, K.N., Efanova, Y.V., Petukhov, V.L., et al., *E3S Web of Conferences*. 2013, 15003(1). DOI: https://doi.org/10.1051/e3sconf/20130115003.
10. Petukhov, V.L., Korotkevich, O.S., Zhetilkov, A.I., Petukhova, T.V., *Patent for invention RUS* 2426119 24.03.2010.
11. Petukhova, T.V., *E3S Web of Conferences*. 2013, 15003(1) DOI:http:// dx.doi.org/10.1051/e3sconf/20130115002.
12. Solovjeva, V.M., Khakhaev, V.G., Chysyma, R.B. Kuzmina, E.E., *Siberian Herald of Agricultural Science*. 2003, *3*, 142-144.
13. Ludu, B.M., Kan-ool, B.K., Siberian Herald of Agricultural Science. 2016, *2*, 57-60.
14. Petukhov, V.L., Syso, A.I., Narozhnykh, K.N., et al., *RJPBCS*. 2016, *7(4),* 2458-2464.
15. Xiao-Yun, S., Agricultural Sciences in China. 2009, 8(8), 1000-1003.
16. Chysyma, R.B., Ludu, B.M., Kuzmina, E.E., *Siberian Herald of Agricultural Science*. 2015, *6,* 65-70.
17. Schaller, G.B., Liu, W., *Biological Conservation*. 1996, *76(1)*, 1-8.
18. Kuang, L.D., Zheng, Y.C., Lin, Y.Q., et al., *Biochem. Genet*. 2010, *48*, 418–427.
19. Lin, Y.Q., Wang, G.S., Feng, J., et al., *Livestock Science.* 2011, *135(1),* 93-97.
20. Zheng, Y.C., Zhao, X.B., Zhou, J., et al., *Sci. China Life Sci*. 2008, *51*, 431–434.
21. Barsila, S., Kreuzer, M., Devkota, et al., *Livestock Science*. 2014, *169*, 125–136.
22. Ding, X.Z, Liang, C.N, Guo X., et al., *Livestock Science. 2014, 162*, 233-239.
23. Ding, L., Chen, J., Long, R., et al., *Animal Feed Science and Technology*, 2015, *209*, 30-39.
24. Minqiang Wang, Pingli Li, Jiyie Li, *Livestock Science*. 2008, *116(3*), 203-208.
25. Bai, W.L., Yin, R.H., Zheng, Y.C., et al., *Livest. Sci*. 2010, *129*, 122–128.
26. Lin, Z., *Asian-Aust. Anim. Sci*. 2007, *20(9),* 1333-1437.
27. Ludu, B.M., Kan-ool, B.K., Siberian Herald of Agricultural Science. 2015, 6, 71-75.
28. Sarkar, M., Das B.C., Bora B.D., et al., *General and Comparative Endocrinology*. 2007, *154(3)*, 85–90.
29. Wu, X.Y., Ding, X.Z., Chu, M., et al., *Journal of Integrative Agriculture*. 2015, *14(4)*, 741-748.
30. Nezavitin, A.G., Petukhov, V.L., Vlasenko, A.N. et al., *Problems of agricultural ecology*. Nauka Siberian publishing firm RAS, Novosibirsk 2000.
31. Amano, T., Yamado, W., Nabika, T., Zhang, X.L., *Rep. Soc. Res. Nativ. Livest*. 1990, *13*, 1–11.
32. Guosheng Wang, Xingbo Zhao, Juming Zhong, et al., *Int. J. Mol. Sci*. 2013, *14,* 11994-12003.
33. Zongping L., Qinbing Z., Li, H., *Veterinary Research Communications*. 1995, *19*, 473-473.
34. Veranyan, O.A., *Issues of Nutrition*. 1987, *1*, 54-57. [in Russian].
35. Miranda, M., Lopez-Alonso, M., Castillo, C., *Environ Int*. 2005, *31*, 548-548.
36. Narozhnykh, K.N., Konovalova, T.V., Petukhov, V.L., et al., *International Journal of Advanced Biotechnology and Research*. 2016, *77(4)*, 1758-1764.
37. Zaiko O., Petukhov V., Konovalova T., et al., *17th International Conference of Heavy Metals in the Environment*. 2014, 76.
38. Kulikova, S.G., Petukhov, V.L, Ernst, L.R., *Russian Agricultural Science*. 1998, *24(9),* 37-40.