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EFFECT OF DEUTERIUM WATER ON BLOOD VALUES AND DIGESTIBILITY OF NUTRIENTS OF RHESUS MACAQUE

Abstract. The use of water with a modified isotopic composition in the diet of humans and animals, for example, with a reduced deuterium content, affects the rate of biological processes. In this regard, the targeted formation of the isotopic D / H gradient in the body can be used to increase its adaptive properties to external influences. Primates have a high anatomical and physiological similarity with humans, therefore, are an indispensable model for reproducing various pathological and toxic conditions in humans. The aim of the study was to study the effect of water with a low deuterium content on the hematological and biochemical blood parameters of rhesus monkeys and on the digestibility of nutrients of the diet. In the control group, primates consumed water with a natural regional content of deuterium equal to 150 mg / l, and in the experimental group - with reduced to 50 mg/l. The duration of the experiment was 35 days. The material for the study was venous blood, serum and feces of primates. Blood was drawn from monkeys before the use of deuterium depleted water and after the completion of the experiment. All blood samples (2.5-3.0 ml) were taken from the cubital or femoral veins of animals on an empty stomach and stabilized with a heparin solution. Laboratory studies of animal blood were performed on an automatic hematological analyzer (Beckman Coulter, USA) of the CoulterAcT 5diffCP brand. In order to study the effect of water with a reduced deuterium content on hematological parameters, we determined the number of red blood cells, white blood cells, platelets, hemoglobin concentration, hematocrit, the average volume of red blood cells, red blood cell anisocytosis, and erythrocyte sedimentation rate (ESR).

The metabolism trial was divided into two periods. Preliminary - to exclude the influence of previous feeding and accustoming primates to cage conditions of keeping. Experimental - conducting a thorough accounting of consumed feed, excreted excrement. Feces were collected daily at the same time (morning and evening), weighed and ground in a mortar. At each collection, 50% of the homogenized mass was taken for analysis. The collected portions were stored in the refrigerator. After the end of the experimental (accounting) period in the collected feces, the initial moisture was determined by drying at 60-70 °C to constant weight. The resulting air-dry mass was thoroughly ground and analyzed.

It has been found that the introduction of water with a reduced deuterium content into the diet of primates has a positive effect on feed conversion rate and blood biochemical parameters.

Key words: digestibility, feed, blood, additives, primates, rhesus macaques, water with a low deuterium content, hematological and biochemical blood value.

Introduction. Among the wide variety of water molecules that exist, deuterium or heavy water molecules are of particular importance. Deuterium is an isotope of hydrogen with a molecular mass of 2 and has one proton and one neutron in the nucleus of an atom. For living organisms, the ratio of light water with a molecular weight of 18 and heavy water with a molecular mass of 20 is important. It leads to their differences in physical properties. The natural ratio of light and heavy water in the oceans was disrupted after nuclear testing, when spent heavy water (used as a neutron moderator) was pumped deep underground, and it spread to all water bodies. In addition, deuterium accumulates in the surface layers of water bodies due to the loss of lighter protium during dissociation into the surrounding space [1].

The use of water with a modified isotopic composition in the diet of humans and animals, for example, with reduced deuterium content, affects the rate of biological processes. Moreover, in living organisms, protective systems counteract changes occurring in the internal isotope environment, striving to achieve a natural isotope ratio [2,3,4].

At present, there are a sufficient number of experiments in which the effects of various ratios of deuterium and protium (D / H) on the functional activity of living systems [5,6,7], as well as hypotheses that explain the results of studies [8,9,10], are studied. However, the mechanisms of action of ultralow concentrations of D water, affecting the metabolism, have been less studied and are presented only for lipids [11], serotonin [12] and deoxyribonucleic acid [13]. But, despite this, there is no data on the effect of deuterium-depleted water on metabolic processes in the body under conditions of body pre-adaptation to a lower level of deuterium compared to the natural level. In this case, the influence of isotope exchange reactions on biochemical processes and the state of the body remains poorly studied due to the low rate of behaviour of these reactions under physiological conditions, which is due to the phenomenon of constancy of the isotopic composition of the substance in the natural environment. Therefore, the targeted formation of the isotopic D / H gradient in the body can be used to increase its adaptive properties to external influences [14].

Given the above, the aim of the study was to study the effect of water with a reduced deuterium content on hematological, biochemical blood parameters of primates and on the digestibility of nutrients in the diets of male rhesus macaques.

Materials and research methods. Water with a reduced composition of deuterium was obtained at a facility created at Kuban State University. The mineral composition of water with a reduced content of deuterium (50 mg / l) and ordinary fresh water (150 mg / l), which the animals received, was identical. The daily water intake by animals of all groups during the experiment averaged 1200 ml per head.

All animals received a complete granular feed. In the control group, primates consumed water with a natural regional content of deuterium equal to 150 mg / l, and in the experimental group - with reduced to 50 mg / l. The duration of the experiment was 35 days. The experimental design is presented in table 1.

Table 1 – The scheme of the experiments, n=5

Groups	Feeding conditions
	Macaca mulatta at the age of 7- 15.
Control	CF* + water with a natural (regional) concentration of deuterium
Trial	CF + water with reduced concentration of deuterium
*CF- complete feed.	

Complete granular compound feed for the experiment was made at the production site of the FSBSI "Research Institute of Medical Primatology". In the feed used 21.4% of energy nutrition accounted for wheat. The share of soybean meal in the structure of feed was 17.42%. Sunflower meal amounted to 13.83%. A significant part of the diet's energy - 14.39%, is skimmed milk powder. The diet is balanced in energy by the introduction of sunflower oil, which amounted to 0.8%. The remaining 32.16% of the energy came from corn gluten - 11.24%, corn - 13.35%, egg powder - 3.3% and sugar - 4.27%.

The material for the study was venous blood, blood serum and feces of primates. Blood was drawn from monkeys prior to the use of deuterium depleted water and 35 days after the completion of the experiment. All blood samples (2.5-3.0 ml) were taken from the ulnar or femoral veins of animals on an empty stomach and stabilized with a heparin solution. Laboratory studies of animal blood were performed on an automatic hematological analyzer (Beckman Coulter, USA) of the Coulter AcT 5diff CP brand. In order to study the effect of water with a reduced deuterium content on hematological parameters, we determined the number of red blood cells, white blood cells, platelets, hemoglobin concentration, hematocrit, the average volume of red blood cells, red blood cell anisocytosis, and erythrocyte sedimentation rate (ESR). ESR was determined by the Panchenkov method.

Blood serum was obtained according to a standard method. Venous blood without anticoagulants in a centrifuge glass tube was settled at room temperature (+15 - + 20ºC) until a clot formed. A thin glass rod was passed along the inner walls of the tube (in a circle) to separate the clot from the walls of the tube and

centrifuged for 10 minutes (1000-1500 g). The resulting serum was poured into disposable plastic tubes with screw caps. Using standard kits of the High Technology Inc company, the biochemical parameters were determined on a BioChem SA semi-automatic analyzer: total protein, glucose, total bilirubin, calcium, phosphorus.

The metabolism trial, on determining the digestibility of the nutrients of the diet, was divided into two periods: preparatory and experimental, which lasted 5 days. The purpose of the preparatory period was to eliminate the influence of previous feeding and to teach primates to the conditions of cage keeping.

Feces were collected daily at the same time (morning and evening), weighed and ground in a mortar. At each collection, 50% of the homogenized mass was taken for analysis. The collected portions were stored in the refrigerator. After the end of the experimental (registration) period, the initial moisture was determined by drying the collected feces at 60-70 ° C to constant weight. The resulting air-dry mass was thoroughly ground and analysed.

Experiments on animal were carried out in accordance with the requirements of the order of the Ministry of Health of the Russian Federation No. 267 of June 19, 2003, "On the approval of laboratory practice rules", orders of the Ministry of Health of the USSR No. 742 of November 13, 1984 "On the approval of the rules of work using experimental animals" and No. 48 dated January 23, 1985 "On the control of work using experimental animals," the ethical standards set forth in the Laboratory Practice Rules (GLP), the Helsinki Declaration (2000) and the European Community Directives 86 / 609EEC.

The results were statistically processed using the Microsoft Excel 2010 computer program. The differences were considered significant at a level of statistical significance $p < 0.05$.

Results and discussion. Blood test is of great diagnostic value. The most important role in the animal's body is performed by blood corpuscles. The main part of the blood corpuscles are red blood cells. Having a large specific surface area, red blood cells can adsorb numerous organic and mineral substances and transport them to tissues. According to the results of our study at the beginning of the experiment (table 2), the number of red blood cells in all the studied groups is within a slight increase relative to the physiological norm (5-6.2 million in 1 mm^3), this pattern can be associated with a significant increase in the ambient temperature by the moment the experiment begins.

Table 2 – Hematological blood values (X±Sx)

Values	Reference values	Groups			
		beginning of the experiment		end of the experiment	
		control	trial	control	trial
Leukocytes, $\times 10^9$/l	5.5-13	9.8 ±1.65	11.26±0.89	10.82±1.99	11.32±0.20
Erythrocytes $\times 10^{12}$/l	5.0-6.2	6.30±0.05	6.76±0.32	6.43±0.17	6.40±0.27
Hemoglobin, g/l	110-145	143.40±1.40	145.20±5.04	145.00±0.50	140.40±4.51
Hematocrit, l/l	0.26-0.45	0.43±0.01	0.44±0.02	0.43±0.01	0.42±0.01
Average volume of erythrocyte, fl	52-97	67.80±0.42	65.40±2.33	68.20±0.42	66.00±2.12
Average content of hemoglobin in erythrocyte, pg	18-33	22.76±0.29	21.60±0.89	22.84±0.30	21.94±0.92
Erythrocyte anisocytosis, %	11-16	12.98±0.20	13.18±0.31	13.22±0.29	13.40±0.20
Platelets, $\times 10^9$/l	200-400	308.40±18.15	357.20±47.30	316.00±25.62	266.00±18.13
Average platelet volume, fl	6-10	9.74±0.19	9.04±0.52	9.94±0.33	10.12±0.64
ESR, mm / h	0.5-5.0	0.89±0.10	1.10±0.27	0.91±0.12	2.60±0.78

At the final stage, the number of red blood cells became closer to the reference values and indicates the effectiveness of the use of D-water in the experimental group, which is reflected in an increase in resistance. The main function of erythrocyte is respiratory, inextricably linked to the properties of the hemoglobin protein contained in them. Therefore, an important indicator is the level of hemoglobin, which depends on the content of protein, iron, copper and cobalt in the diet, as well as on the functioning of the liver and blood-forming organs. In our experiment, the results of the studies indicate that the level of hemoglobin in the blood before setting up the experiment is within the physiological norm, but there is a

slight increase in hemoglobin (HGB) in the control group after the experiment is completed. This may be due to the above physiological increase in the level of erythrocytes in the blood.

The level of leukocytes in the blood is of great importance. The overall functioning of the immune system directly depends on the concentration of these white blood bodies in the blood. And this means that the level of leukocytes in the blood can also indicate the degree of protection of the body as a whole. In our experiment, the level of leukocytes was within normal limits. But at the end of the experiment there was a slight increase in the experimental group by 4.62% compared with the control. This phenomenon may have a natural physiological character, since this slight increase is within the physiological norm. An increase in white blood cell count is observed with increasing ambient temperature. This type of increase is reversible; in this case leukocytosis is able to independently return to the normal range of values.

Platelets take an active part in blood coagulation and nonspecific defense reactions of the body. At the beginning of our experiment, the platelet count in animals was within the range of reference indicators (200-400 billion / liter), but before the experiment, in the experimental group there was a slight increase in the values in comparison with the control variant by 48.8 billion/ l. The results of blood tests at the end of the experiment showed that in the experimental and control groups, as a result of the use of D-water, the platelet count was within the reference values.

Erythrocyte sedimentation rate (ESR) depends on the size, volume of red blood cells, their number, hemoglobin concentration in the red blood cells, viscosity and other factors. In connection with the optimal picture presented by the above indicators (table 2), ESR is within normal limits (0.5-5.0 mm / h). A slight increase in the values of hemoglobin, erythrocytes and leukocytes, and the level of hematocrit in the blood of monkeys of the experimental groups within the upper limits of physiological norms may indicate that water with a reduced concentration of deuterium stimulates erythropoiesis and leukopoiesis, without changing the stability of hematopoiesis and constancy in the composition and total amount of peripheral blood.

Biochemical blood parameters have a certain value with internal non-communicable diseases, intoxications, but to a greater extent reflect the level of feeding and metabolic processes. In this regard, biochemical indicators cannot provide answers to all questions, but with the correct understanding of physiological changes, they become a solid basis for decision-making on the development of scientifically based norms of feeding (table 3).

Table 3 – Biochemical parameters of blood serum (X±Sx)

Values	Groups			
	beginning of the experiment		end of the experiment	
	control	trial	control	trial
Glucose (mmol / l)	4.43±0.44	5.39±0.90	3.32±0.38	4.58±0.45
Bilirubin (μmol / l)	6.99±2.74	5.92±2.11	6.98±1.47	4.79±2.21
Phosphorus (mmol /l)	1,17±0,19	1,29±0,10	0,61±0,12	0,48±0,13
Calcium (mmol / l)	2,56±0,06	2,41±0,11	2,01±0,17	2,38±0,06
Protein (g / l)	89,31±8,40	89,76±6,12	80,12±1,66	101,72±5,48

Blood glucose was determined to monitor the state of carbohydrate metabolism. At the beginning of the experiment, the glucose level was within the physiological norm. As with hematological parameters, there is a slight increase in the experimental group. But at the end of the experiment, after applying deuterium-depleted water, the glucose level in the experimental group approached the parameters of the control variant with a slight deviation upward by 1.26 mmol / l.

Bilirubin is a bile pigment formed from hemoglobin due to the breakdown of rythrocytes in the liver cells. In our experiment, the level of bilirubin at the end of the experiment decreases by 2.19 mmol / l, which indicates a positive effect of D-water on primate liver cells, thereby optimizing the rate of hemoglobin breakdown.

The level of phosphorus in the blood of primates of the experimental group at the beginning of the experiment was slightly higher than the control option, but was within the reference values. By the end of the experiment, the values in the experimental group decreased to the control level.

The level of calcium in the serum depends on the content of calcium, phosphorus and vitamin D in the diet, the state of the hormonal system, and the gastrointestinal tract. In our experiment, the level of calcium at the beginning and end of the experiment was within the normal range. But in the experimental group, the level of calcium is lower by 0.37 mmol / l.

Analysis of protein metabolism showed that in the experimental group there were deviations from the values in the control upward by 21.60 g / l of total protein levels, which indicates a positive effect of deuterium water on the body of primates.

Daily accounting of animal feed eaten and analysis of their chemical composition made it possible to establish the amount of nutrients consumed per day. A calculation of feces and its chemical composition made it possible to determine the amount of digested nutrients and establish the digestibility coefficient (table 4).

Table 4 – Digestibility of nutrients, % (X±Sx)

Indicators	Groups	
	control	trial
Crude protein	27.34±1.04	50.67±1.31
Crude fat	20.09±0.98	20.92±1.53
Crude fiber	16.88±1.01	18.00±1.81*
Crude ash	49.58±0.88	49.27±2.01
Crude NFE	54.05±1.12	57.37±0.97*
Calcium	18.37±1.94	53.92±2.11
Phosphorus	21.79±2.01	30.51±1.99
*p<0.05.		

The digestibility of crude protein in the experimental group is 85.33% higher than this indicator in the control group. A slight increase in digestibility is observed in the experimental group in raw fat by 4.13%. Within such limits, there is a significant increase in the digestibility of fiber by 6.63%. The digestibility rate of NFE in the experiment was significantly higher by 6.14%. But the highest assimilation, as can be seen from the table, is observed for inorganic substances Ca and P.

Conclusions. The use of depleted deuterium water in the diets of primates does not lead to a deviation of the hematological indices of rhesus monkeys relative to reference indices, and, as the studies confirm, it has a positive effect on feed conversion and biochemical values of blood.

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ҚАН КӨРСЕТКІШТЕРІНЕ ДЕЙТЕРИЙ СУЫНЫҢ ӘСЕРІ ЖӘНЕ МАКАК-РЕЗУСТАРДЫҢ ҚОРЕКТІК ЗАТТАРЫНЫҢ ҚОРЫТЫЛУЫ

Аннотация. Адам мен жануарлардың рационында модификацияланған изотоптық құрамы бар, мысалы, дейтерийдің азайған құрамы бар суды пайдалану биологиялық процестердің жылдамдығына әсер етеді. Осыған байланысты, ағзадағы Краснодар облыс аралық мал д рігерлік зертхана изотоптық D/H градиенттің мақсатты қалыптасуы оның сыртқы әсерлерге бейімделу қасиеттерін арттыру үшін пайдаланылуы мүмкін. Приматтар адаммен жоғары анатомиялық-физиологиялық ұқсастыққа ие, сондықтан адамның әртүрлі патологиялық және уытты жағдайларын ойнату үшін таптырмас үлгі болып табылады. Зерттеудің мақсаты – құрамында дейтерий бар судың макак-резус қанының гематологиялық және биохимиялық көрсеткіштеріне және рационның қоректік заттарының қорытылуына әсерін зерттеу. Бақылау тобында приматтар 150 мг/л тең табиғи аймақтық дейтерий бар суды, ал тәжірибелік топта 50 мг/л дейін төмендетілген суды тұтынды. Эксперимент ұзақтығы 35 тәулікті құрады. Зерттеу үшін материал көктамырлық қан, қан сарысуы және

примат нәжісі болды. Маймылдан ішетін дейтерий суын қолдану басталғанга дейін және тәжірибе аяқталғаннан кейін қан алынды.Қанның барлық үлгілері (2.5-3.0 мл) шынтақ немесе сан тамырынан алынган және гепарин ерітіндісімен тұрақтандырылган. Жануарлардың қандарының зертханалық зерттеулерін «Beckman Coulter», USA фирманың CoulterAcT 5diffCP маркасының автоматты гематологиялық анализаторында жүргізілді. Дейтерий мөлшері төмен судың гематологиялық көрсеткіштерге әсерін зерттеу мақсатында біз эритроциттер, лейкоциттер, тромбоциттер санын, гемоглобин концентрациясын, гематокрит, эритроциттердің орташа көлемін, эритроциттер анизоцитозын, эритроциттер шөгу жылдамдыгын анықтадық.

Баланстық тәжірибе екі кезеңге бөлінді. Дайындық кезеңі – алдыңгы азықтандырудың әсерін болдырмау және жасушалық ұстау жагдайларына приматтарды үйрету үшін. Тәжірибелік кезеңі – тұтынылган жемді, бөлінген экскременттерді мұқият есепке алу. Нәжіс күн сайын бір уақытта (таңертең және кешке) жиналып, өлшеп, Ұсақталган. Әрбір жинақта талдауга гомогенизацияланган массаның 50 % алынды. Жиналган порциялар тоңазытқышта сақталды.Тәжірибелік (есептік) кезең аяқталганнан кейін жиналған нәжісте бастапқы ылгалды 60-70 °С кезінде тұрақты массага дейін кептіру арқылы анықтады. Алынган ауа-құргақ массаны Мұқият Ұсақтап, талдауга жіберілді.

Құрамында дейтерий азайган судыприматтардың рационына енгізу жемнің конверсиясына және қанның биохимиялық көрсеткіштеріне оң әсер ететіні анықталды.

Түйін сөздер: қорытылу, жем, қан, қоспалар, приматтар, макак-резустар, дейтерий мөлшері төмен су, қанның гематологиялық және биохимиялық көрсеткіштері.

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ВЛИЯНИЕ ДЕЙТЕРИЕВОЙ ВОДЫ НА ПОКАЗАТЕЛИ КРОВИ И ПЕРЕВАРИМОСТЬ ПИТАТЕЛЬНЫХ ВЕЩЕСТВ МАКАК-РЕЗУСОВ

Аннотация. Использование в рационе человека и животных воды с модифицированным изотопным составом, например, со сниженным содержанием дейтерия, влияет на скорость биологических процессов. В связи с этим, целенаправленное формирование изотопного D/H градиента в организме может быть использовано для повышения его адаптационных свойств к внешним воздействиям. Приматы обладают высоким анатомо-физиологическим сходством с человеком, поэтому являются незаменимой моделью для воспроизведения различных патологических и токсических состояний человека. Целью исследования являлось изучение влияния воды с пониженным содержанием дейтерия на гематологические и биохимиические показатели крови макак-резусов и на переваримость питательных веществ рациона. В контрольной группе приматы потребляли воду с естественным региональным содержанием дейтерия, равном 150 мг/л, а в опытной группе – с пониженным до 50 мг/л. Длительность эксперимента составила 35 суток. Материалом для исследования служила венозная кровь, сыворотка крови и кал приматов. Кровь у обезьян брали до начала применения обеднённой дейтериевой воды и после завершения опыта. Все образцы крови (2,5-3,0 мл) были взяты из локтевой либо из бедренной вены животных натощак и стабилизированы раствором гепарина. Лабораторные исследования крови животных проводили на автоматическом гематологическом анализаторе фирмы «Beckman Coulter», USA марки CoulterAcT 5diffCP. С целью изучения влияния воды с пониженным содержанием дейтерия на гематологические показатели нами определялись количество эритроцитов, лейкоцитов, тромбоцитов, концентрация гемоглобина, гематокрит, средний объём эритроцитов, анизоцитоз эритроцитов, скорость оседания эритроцитов (СОЭ).

Балансовый опыт разделяли на два периода. Подготовительный – для исключения влияния предшествующего кормления и приучения приматов к условиям клеточного содержания. Опытный – для проведения тщательного учёта потреблённого корма, выделенных экскрементов. Кал собирали ежедневно в одно и то же время (утром и вечером), взвешивали и растирали в ступке. При каждом сборе на анализ брали 50% гомогенизированной массы. Собранные порции хранили в холодильнике. После окончания опытного (учётного) периода в собранном кале определяли первоначальную влагу высушиванием при 60-70°С до постоянной массы. Полученную воздушно-сухую массу тщательно размалывали и передавали на анализ.

Установлено, что введение в рацион приматов воды со сниженным содержанием дейтерия положительно влияет на конверсию корма и биохимические показатели крови.

Ключевые слова: переваримость, корма, кровь, добавки, приматы, макаки резусы, вода с пониженным содержанием дейтерия, гематологические и биохимические показатели крови.

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