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THE XANTHINE OXIDASE AND ITS ASSOCIATED ACTIVITIES IN THE OVINE MILK AND LIVER: DISTINCTIVE IN IMPACT OF *IN VIVO* MOLYBDENUM

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ABSTRACT
Xanthine oxidase is molybdenum and iron-containing flavoprotein, catalyzing the final oxidation stage of purines and oxidative transformation of pterins and some aliphatic and aromatic aldehydes. Despite the importance of this enzyme, the distribution of xanthine oxidase in traditional household animal's milk and tissues is unknown. Formerly, we have found most of the xanthine oxidase molecules in animal milk are inactive because of a lack of molybdenum. Ovine milk was processed by inserting *in vivo* molybdenum (tungsten) into drinking water. We gave opposite dates in the presence of tungsten too. Heating the milk of animals at 80 °C for 5 minutes in the presence of molybdenum and cysteine led to a sharp increase of xanthine oxidase and its associated – nitrate reductase and nitrite reductase activities. The change of xanthine oxidase and its associated activities were examined by spectrophotometry after treatment. It was established that metal ions added in drinking water for animals have an impact on enzyme activities. The activity is formed in the ovine liver even in the absence of exogenous molybdenum in drinking water. The associated activities of liver enzymes in the presence of molybdenum in drinking water had slightly increased. Tungsten-containing water led to the loss of all activities of liver xanthine oxidase. It is proposed that the liver contains a special protein involving in the incorporation of molybdenum (or tungsten) into xanthine oxidase molecule, however, the milk or mammary gland compounds lack this protein.

Keywords: tungsten; reductase; protein; heat treatment; ovine milk

INTRODUCTION
Xanthine oxidase (XO) is the enzyme that is responsible for the synthesis of uric acid in mammalian. Xanthine oxidase is widespread in mammalian tissues and is a major component of the membrane of milk fat globules that surround the fat globules in milk. Uric acid is the final major product of the metabolism of nitrogen-containing compounds in animals, and it functions as an antioxidant to reduce oxidative stress **(Matata and Elahi, 2007)**. Purines and other substrates react with xanthine oxidase at the site containing molybdenum, and the electron acceptors react at the FAD site **(Harrison, 2002; Schwarz, 2005)**. The protein part of the enzyme is rich in cysteine and contains 60 – 62 free sulfhydryl (-SH) groups. In the structure XO, some centers represent the 2Fe-2S complex **(Harrison, 2006)**. Molybdenum (Mo) is in the body of animals, and its concentrations were varying depending on tissue type. Molybdenum is an essential cofactor of animal molybdenum-containing enzymes (Mo-enzymes) such as xanthine oxidase, aldehyde oxidase, sulfite oxidase, and recently discovered mitochondrial amidexime-reducing protein (mARC) **(Hille et al., 2011; Santamaria-Araujo et al., 2012; Carroll et al., 2017)**. Two S-bonds connect it with the side chain of the cofactor molecule **(Bryan et al., 2009)**.

It was found earlier that homogenous xanthine oxidase purified from cow's milk reduces the nitrate (NO_3^-) to nitrite (NO_2^-) **(Alikulov, Lvov and Kretovich, 1980)**. However, it was unclear in what substances nitrite reduces. Later, scientists had established that the xanthine oxidase isolated from the liver of the cow reduces nitrite under anaerobic conditions and converts it into nitric oxide (NO) **(Millar et al., 1998)**.

At present, *in vivo* studies of milk xanthine oxidase in sheep liver have not been performed. But attempts to study milk xanthine oxidase activity in the liver for comparative analysis can be considered using the example of other animals. Research by **Grebennikova et al. (2013)** defines molybdenum as an important enzyme for the development of plant growth. The lack of molybdenum leads to a decrease in xanthine oxidase activity. The artful addition of molybdenum to camel and mare milk helped to remove nitrates and nitrites from it. According to research by **Ozturk et al. (2019)** on the effect of heat treatment on xanthine oxidase in cow's milk, it can be concluded that the higher the temperature, the faster the inactivation of

xanthine oxidase occurs, in contrast to raw milk, where high activity was not observed. By **Sharma et al. (2016)**, Everett compared the xanthine oxidase activity of bovine milk treated with an impulsive electric field and an increase in temperature. The denaturation of xanthine oxidase was less after electric field treatment compared to heat treatment. Thus, an increase in temperature promotes the decontamination of milk and an increase in xanthine oxidase in all cases.

Thus, xanthine oxidase, contained in the tissue and liquids in the animal body, is a unique tool for decontamination of nitrates and nitrites and the formation of important substances for the body nitric oxide. This study aimed to determine the effect of heat treatment of milk on the activity and biological functions of xanthine oxidase in sheep liver *in vivo* **(Sharma et al., 2016)**. To accomplish this goal, it is necessary to perform the following tasks: conducting an experiment on the activity of milk xanthine oxidase in the liver using the example of 3 control groups and determining their results, pointing out the differences, and conducting an experiment of xanthine oxidase activity during freezing and heating milk. Concluding what factors influence the increase in milk xanthine oxidase activity in the liver of sheep. Consequently, the data for the study on nitrates and nitrites reduction activity mechanisms has both scientific and practical value. So, the knowledge base will increase by research ovine liver and milk xanthine oxidase in this area.

Scientific Hypothesis

Xanthine oxidase, contained in the tissue and liquids in the animal body is a unique tool not only for decontamination of nitrates and nitrites but also for the formation of important substances for the body – nitric oxide. Consequently, the data for the study on nitrates and nitrites reduction activity mechanisms has both scientific and practical value. So, the knowledge base will increase by research ovine liver and milk xanthine oxidase in this area.

MATERIAL AND METHODOLOGY

Samples

Ovine milk was obtained from healthy six sheep in the mid-lactation period (from May to June) based on a farm in the Almaty region (Kazakhstan). Liver tissues were obtained from six non-lactating animals.

Chemicals

The experiments carried out using molybdenum (M = 241.95), sodium tungstate (M = 329.9), L-cysteine (M = 157.6), sulfanilamide (M = 172.21) from Sigma-Aldrich Chemical Co., N-(1-naphthyl)-ethylenediamindigidro-chloride (M = 259.18) from AppliChem (Germany).

Instruments

Preparation of the milk for the detection of the various enzymatic activities. Before treatment in the milk of domestic animals added 10 µM ethylenediaminetetraacetic acid (EDTA) to bind heavy metals. For boiling fresh milk is poured into the narrow conical tubes in a volume of 2 mL. Then, for further determination of the enzymatic activity the tubes are placed in the water bath at 35 °C temperature and kept for 10 min, then using the special reagents different activities of XO are determined.

Laboratory Methods

It is known that the smallest concentration of molybdenum in water can prevent the inclusion of tungsten in xanthine oxidase. Therefore, tungsten is given in high concentrations. 100 g liver slices of slaughtered cattle were also frozen at -20 °C. The frozen samples were defrosted after 30 days. The xanthine oxidase (XO), nitrate reductase (NR), and nitrite reductase (NiR) activities were simultaneously determined in milk and liver samples. The liver homogenate was obtained by carefully rubbing the mixture in a porcelain mortar. Samples of liver were mixed in a 1:5 ratio with a cold 0.1 M sodium phosphate buffer containing 10 µm EDTA and 10 µm phenylmethylsulfonyl fluoride for protease inhibition. The supernatant was obtained by centrifugation of liver homogenate and milk aliquot at 15000g for 20 minutes **(Harrison, 2002)**.

Sodium phosphate buffer solution was added to milk to a final concentration of 100 mM, pH 6.5, 10 µM EDTA solution, 2 mM sodium molybdate (Na_2MoO_4), or sodium tungstate (Na_2WO_4), and 2 mM cysteine solution. Then, the milk and liver of the supernatant are heated at 80 °C for 5 minutes. After cooling to determine the enzyme activity, 100 mL aliquots of milk and liver supernatant were used. Each activity was determined in triplicate (n = 3, *SD* ±) **(Godber et al., 2000)**.

To determine the steps carried out, the xanthine oxidase activity is described in the developed method **(Alikulov, Bespaev and Yakupbaev, 1999)**. Activation of the milk and liver samples using metal ions and thiols.

The sodium phosphate buffer solution was added to the milk in final concentrations of 100 mM, and pH is 6.5; 10 µm EDTA solution, 2 mM sodium molybdenum (Na_2MoO_4) or sodium tungstate (Na_2WO_4), and 2 mM cysteine solution. The liver supernatant has the same concentration of sodium molybdenum or tungstate and cysteine solutions. After the milk aliquot and liver, the supernatant warmed up at a temperature of 80 °C for 5 min. After cooling, 100 µL aliquots of milk and liver supernatant were used to determine enzymatic activities. The determination of each activity was carried out in three repetitions.

Nitrate reductase activity (NR) was determined by the formation of nitrites, and nitrite reductase activity (NiR) – by the disappearance of nitrites in the reaction mixture after incubation. The reaction mixture in the volume of 500 mL (do not take into account milk or liver supernatant) contained 20 mM sodium nitrate ($NaNO_3$) or 80 µM sodium nitrite ($NaNO_2$), 20 mM methyl- or benzyl viologen reduced by dithionite ($Na_2S_2O_4$). The mixture was incubated at 37 °C for 15 minutes. Then to the reaction mixture, 500 mL of sulfanilamide solution and 500 mL of N-(1-naphthyl)-etilendiamine (NEDA) was added. These reagents were prepared in the following steps: 6 g of sulfanilamide was dissolved in 1 liter of 20% HCl; 1.23 g NEDA – in 1 L of water distilled by milliQ. At pH 2-2.5, sulfanilamide nitrite forms a red diazonium compound.

Sample preparation

The live weight of animals at the beginning of the experiments was 40 to 45 kg. Animals were fed with freshly

Table 1 Dynamics of changes in the associated activities of XO of fresh ovine milk obtained daily during the month.

Days of milking	Activities					
	XO activity*		NO_3-reductase activity**		NO_2-reductase activity***	
	Milk + Mo	Milk + Mo +heating	Milk + Mo	Milk + Mo + heating	Milk + Mo	Milk + Mo + heating
0	>0.2	~3.2	~1.4	136.8 ±13.7	~2.3	243.7 ±28.3
4	>0.2	~3.2	~1.4	136.7 ±20.1	~2.3	243.7 ±24.9
8	>0.2	~3.0	~1.4	136.3 ±18.6	~2.2	243.1 ±19.8
12	>0.2	~2.8	~1.3	132.6 ±12.3	~2.0	240.2 ±22.3
16	>0.2	~2.8	~1.3	132.5 ±14.8	~1.8	240.2 ±27.6
20	>0.2	~2.8	~1.2	130.7 ±21.6	~1.8	238.3 ±21.4
24	>0.2	~2.6	~1.1	128.8 ±23.5	~1.8	236.4 ±18.7
28	>0.2	~2.6	~1.1	128.6 ±18.9	~1.7	236.4 ±21.8
32	>0.2	~2.6	~1.0	128.4 ±10.3	~1.7	236.2 ±22.4

Note: XO-activity*: nanomoles of uric acid formed/100 µL milk/min; NR** activity: nanomoles of NO_2- formed/100 µL milk/min); NiR*** activity: nanomoles of NO_2- disappeared/100 µL milk/min.

Table 2 Influence of exogenous Mo on the dynamics of changes in the associated activities of sheep milk.

Days milking	Activities						Mo*
	XO activity		NO_3-reductase		NO_2-reductase activity		
	Milk + Mo	Milk + Mo + heating	Milk + Mo	Milk + Mo + heating	Milk + Mo	Milk + Mo + heating	
0	>0.2	3.2 ±0.4	~1.4	136.8 ±24.6	~2.3	243.7 ±41.6	>2
4	>0.2	3.2 ±0.3	~1.4	136.8 ±22.4	~2.3	243.7 ±42.3	>2
8	~0.3	3.2 ±0.4	~1.5	142.7 ±28.6	~2.5	249.7 ±43.7	12.6 ±2.1
12	~0.4	3.4 ±0.5	~1.5	149.8 ±19.4	~2.6	249.5 ±51.6	42.7 ±7.2
16	~0.4	3.4 ±0.4	~1.6	152.9 ±12.6	~2.5	252.3 ±32.4	48.5 ±6.3
20	~0.4	3.2 ±0.5	~1.5	150.7 ±13.2	~2.5	252.2 ±28.6	51.3 ±8.4
24	~0.4	3.2 ±0.3	~1.4	148.9 ±24.3	~2.4	248.5 ±35.4	51.3 ±7.8
28	~0.4	3.0 ±0.3	~1.4	148.6 ±13.8	~2.3	246.7 ±28.3	51.4 ±9.4
32	~0.4	2.9 ±0.4	~1.3	148.3 ±12.8	~2.2	246.8 ±32.6	51.5 ±11.3

Note: *molybdenum content in milk in nanograms in milliliter.

cut green plants (Adlib feeding). For the experiment, three control groups were defined. To study exogenous molybdenum, ammonium molybdate $(NH_4)_2MoO_4$ was added to the drinking water of animals per 10 kg of animal weight. During the reporting period, the animals were watered twice – the first time, the volume of water with molybdenum was three liters, the second time, they gave the same portion, but without molybdenum. Animals were weighed weekly for correcting the dose of molybdenum. The second pair of animals was fed similarly to the first group, but they were given tungsten water once a day – one liter of such water contained 100 mg of sodium tungstate. Control third group animals were fed daily with freshly cut grass. They drank natural water without the addition of molybdenum or tungsten. Once a day, sheep were milked. Every four days, 100 mL portions of fresh milk were immediately frozen at a temperature of -20 °C. Thus, 18 samples of each pair of sheep (about 1.8 L) were frozen (5.4 L were obtained from six animals) **(NIIP, 2017)**.

A parallel experiment was conducted to study the effect of molybdenum or tungsten on sheep liver xanthine oxidase activity. Since the slaughter of six non-lactating animals was planned, one pair of animals was kept as a control, i.e., they were fed with green grass, freshly cut in a certain field watered with natural water for 30 days before the slaughter. The second pair of animals received the same plant food and drank the same water, but containing 10 mg.mL^{-1} of ammonium molybdenum. Instead of molybdenum, the third pair received 10 times the tungsten concentration in the form of 100 mg.mL^{-1} of sodium tungstate compared to molybdenum.

Statistical Analysis

The absorbance obtained color solution was measured photometrically at a wavelength of 548 nm in spectrophotometer ("Specol-2000", Germany). The calibration curve is constructed by using increasing concentrations of nitrite ($NaNO_2$) colored with sulfanilamide and NEDA. The advantage of the photometric method is its high sensitivity. The amount of molybdenum was determined using the method developed by our research group. The heat treatment of molybdenum led to determine the total amount of molybdenum **(Alikulov, Mukhamejanova and Kultaeva, 2017)**. The statistical analysis data were produced by Microsoft Excel.

RESULTS AND DISCUSSION

According to the results, sheep that were drunk with molybdenum-contain water for a month accumulated molybdenum in their milk. The concentration of which reached a maximum (51 nanograms.mL^{-1}) on the 20th day. Just such an amount of molybdenum in milk did not lead to the demonstration of all associated XO activities after heat treatment in the presence of cysteine (but without exogenous molybdenum). In this case, only exogenous

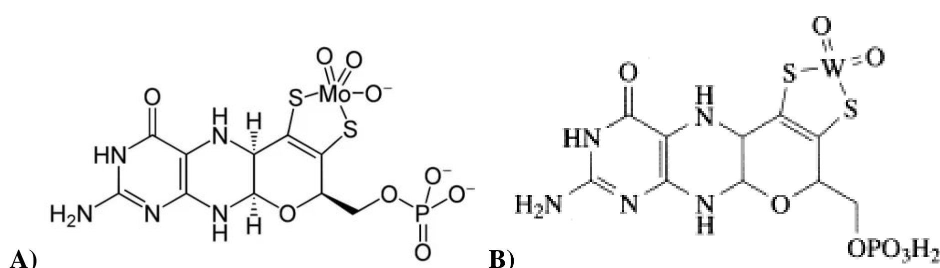

Figure 1 The structure of the molybdenum-factor and its relation to the molybdenum (A) and tungsten (B) atoms in the active center of xanthine oxidas.

Table 3 Influence of tungsten on the dynamics of the changes in the associated activities of sheep milk.

Days of milking	Activities					
	XO activity		NO_3-reductase		NO_2-reductase activity	
	Milk + Mo	Milk + Mo + t°	Milk + Mo	Milk + Mo + t°	Milk + Mo	Milk + Mo + t°
0	0.0	3.2 ±0.4	0.0	136.7 ±21.6	0.0	243.6 ±23.6
4	0.0	3.2 ±0.3	0.0	136.8 ±22.2	0.0	243.5 ±20.3
8	0.0	3.1 ±0.4	0.0	135.5 ±24.7	0.0	242.3 ±22.3
12	0.0	3.1 ±0.5	0.0	135.5 ±19.1	0.0	240.4 ±28.2
16	0.0	2.9 ±0.4	0.0	134.7 ±15.8	0.0	239.3 ±31.4
20	0.0	2.9 ±0.5	0.0	134.6 ±21.6	0.0	238.7 ±27.5
24	0.0	2.8 ±0.4	0.0	131.6 ±15.2	0.0	237.5 ±26.3
28	0.0	2.8 ±0.4	0.0	130.4 ±17.3	0.0	237.8 ±23.2
32	0.0	2.7 ±0.4	0.0	129.3 ±20.3	0.0	236.6 ±21.9

Table 4 Effect of molybdenum and tungsten on associated liver xanthine oxidase activity.

Variants	Activities					
	XO activity		NO_3-reductase activity		NO_2-reductase activity	
	Control	+ M	Control	+ Mo	Control	+ Mo
Control	2.9 ±0.3	3.5 ±0.4	118.7 ±14.6	132.7 ±18.6	197.6 ±25.9	218.6 ±23.6
+ molybdenum	3.2 ±0.3	3.3 ±0.4	130.9 ±16.3	132.9 ±21.6	217.6 ±22.3	219.6 ±20.3
+ tungsten	0.0	3.1 ±0.2	0.0	128.9 ±25.4	0.0	216.3 ±24.2

molybdenum activated XO activity after heat treatment in the presence of cysteine (Table 1).

It can be assumed that before embedding molybdenum in the active center (or before binding to the MPT in the active center), newly synthesized XO molecules are involved in the formation of the inner membrane of the milk fat globule (MFGM) **(Cebo et al., 2012; Saadaoui et al., 2013; Mendel, 2013)**. XO is located in the inner MFGM, as indicated in the research **(Pisanu et al., 2011; Lu et al., 2005)**. It is no longer available for molybdenum *in vivo*.

However, in the heat treatment of ovine milk in the presence of molybdenum and cysteine, XO was activated and showed high associated activity (Table 2), comparable to the version of molybdenum-containing water. Cysteine only slightly increased these liver XO activities of sheep. The steady presence of tungsten for a month in drinking water leads to the inactivation of liver xanthine oxidase and its associated activities, i.e., inactive molecules of this enzyme are formed. As it is known, tungsten, as a chemical analog of molybdenum, is widely used to identify new molybdenum enzymes **(Harrison, 2002; Schwarz, 2005; Sharma et al., 2016)**. Tungsten is very close to molybdenum in atomic and ionic radius, as well as chemical properties. Therefore, in the absence of molybdenum, tungsten is easily embedded in the active center of enzymes, instead of molybdenum **(Brondino et al., 2006)** (Figure 1).

In the active center of the enzymes, one molybdenum atom binds to two sulfhydryl groups of the cofactor. However, the relationship between the cofactor and molybdenum is not strong, i.e., when a cofactor is isolated from an apoenzyme or when an enzyme molecule is denatured, molybdenum is easily separated from the cofactor. Moreover, the cofactor is extremely sensitive to oxygen since it is instantly inactivated in the air (two SH-groups oxidize to form disulfide-S-S). As a result, the presence of thiols in experiments with a cofactor is necessary. This is most likely a non-physiological effect of the "mass action" of molybdenum *in vivo*. This is most likely a non-physiological effect of the "mass action" of molybdenum *in vivo*. Consequently, the presence of excess oxygen does not need in molybdenum or a chemical analog – tungsten for the synthesis of XO in milk and its inclusion in the MFGM. The results of this experiment are presented in Table 3.

The results show that natural drinking water contains enough molybdenum for the normal xanthine oxidase activity, the normal xanthine oxidase activity in the liver. Increasing the content of molybdenum in drinking water only slightly increased the liver xanthine oxidase-associated activity. As can be seen from Table 4, liver XO shows high associated activity without heat treatment and warming up at 80 °C in the presence of molybdenum. Determination of the associated activities of xanthine oxidase (NR and NiR

Figure 2 Kumys, a fermented dairy product traditionally made from mare's milk.

activity) in fresh milk and after storage at -20 °C showed that storing frozen milk preserves the activities of the milk.

Results show that in fresh sheep milk obtained during the month, none of the associated XO activities were detected as one of the associated XO activities.

However, after the heat treatment of milk at 80 °C for 5 minutes (in Table 4 shown as + Mo + to) in the presence of exogenous molybdenum and cysteine, all associated activities of this enzyme appear (Table 4). Among other natural and artificial thiols, glutathione and mercaptoethanol were active in restoring these activities by exogenous molybdenum. The absence of molybdenum or cysteine during heat treatment did not lead to the appearance of these activities.

The results presented in the table show that the associated XO activity up to the 10th day in milk. It slightly increased first and decreased then (Tables 1, 2, and 4). There is an assumption that the relatively high activity of XO at the beginning of lactation is associated with the anti-pathogenic property of this enzyme. However, our results show that milk XO does not contain molybdenum. Accordingly, XO is inactive. Perhaps there is another explanation that the superoxide-producing center does not contain molybdenum but contains FAD (**Godber et al., 2000; Maia et al., 2007**). The results show that in fresh sheep milk, xanthine oxidase does not contain molybdenum. XO, located in the fat globule micelles (MFGM), is not available for exogenous molybdenum. During heat treatment at 80 °C, the milk globules are destroyed, then the molecule is denatured. As a result, the access of molybdenum to the MPT (molybdopterin or metal-binding Pterinene-1.2-diThiolate) – containing active center increases. Our and other numerous studies have shown that MPT is extremely sensitive to oxygen. Therefore, the presence of antioxidant-cysteine protects sulfhydryl groups of MPT from oxygen. Cysteine forming temporary disulfide bonds with MPT protects it from oxidation. The active MPT is easily displaced by cysteine and associated with him in the XO active site from the active MPT in exogenous molybdenum (**Hille et al., 2011; Santamaria-Araujo et al., 2012; Carroll et al., 2017**).

An experiment was conducted to determine the effect of elevated dietary Mo and duration of feeding on its concentrations in internal organs (Figure 2). Forty lambs were fed diets containing different concentrations of added Mo as sodium molybdate for 14 or 28 days. The concentration of Mo increased linearly in internal organs as dietary Mo increased. Liver Mo concentration increased more rapidly at 14 days than at 28 days, but other organ Mo increased to a greater extent in lambs fed 28 days (**Pott et al., 1999**). The molybdenum absorption in the gastrointestinal tract depends on its chemical nature. Molybdenum and its compounds penetrate directly to the gastrointestinal tract. For instance, water-soluble molybdate, thiomolybdate, and oxothiomolybdate are absorbed from 75 % to 90 % in the gastrointestinal tract and after that completely excreted in the form of molybdenum, mainly by the urine. The minimum toxic concentrations of molybdenum in drinking water for calves varied between 10 and 50 ppm (**National Toxicological program, 1997**).

The defined protein includes metal ions in molybdopterin (molybdcofactor) at relatively low concentrations of molybdenum concentrations in liver tissues. For example, the bacterial MogA (or animal protein – gefirin) protein exhibits an affinity for molybdenum. It acts as a molybdenum chelatase, which includes molybdenum in the molecule of the molybdenum cofactor (**Liu et al., 2000; Harrison, 2002; Harrison, 2006; Dutta et al., 2013**). This protein likewise incorporates this tungsten into the molecule of xanthine oxidase at high concentrations of it. There is no

protein in the milk or the mammary gland. Therefore heat treatment at 80 °C of milk in the presence of exogenous molybdenum and cysteine led to activate xanthine oxidase in sheep milk **(Dyusembayev et al., 2016)**; liver xanthine oxidase shows high associated activity without heat treatment, and absence of exogenous molybdenum in drinking water shows normal associated activities in sheep liver than in milk **(Nine et al., 1971; Johnson et al., 1974; Raina et al., 2015)**; there are distinctive in the effect of *in vivo* molybdenum in sheep milk and liver **(Kincaid, 1980; de Araújo et al., 2017)**.

CONCLUSION

The paper analyzes the effect of molybdenum and tungsten on the activity of conversions of xanthine oxidase in milk and liver *in vivo*. Xanthine oxidase is present in almost all tissues of the animal body. It has the highest specific activity in the liver, and milk serves as an object for its excretion. Determined that the activity of xanthine oxidase directly depends on the content of molybdenum in the body. It is important to note that xanthine oxidase activity largely depends on the intake of exogenous molybdenum into the body. One way to study the *in vivo* effect of exogenous molybdenum on the activity of xanthine oxidase is by adding a salt of the metal in the drinking water of domestic animals.

It was determined that the xanthine oxidase of the milk of the sheep is inactive in its natural state. However, heat treatment of milk in the presence of exogenous sodium molybdate and thiols (cysteine) led to the appearance of associated xanthine oxidase activities. Because at high temperatures, the enzyme molecule is partially denatured and, as a result, the active site is available for exogenous molybdenum and cysteine. Natural drinking water contains enough molybdenum for normal xanthine oxidase activity, oxidase activity in the liver without thermal treatment. Tungsten replaces molybdenum in the active center of the enzyme, which leads to its irreversible inactivation.

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Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This research was approved by the Ethics Committee of the Astana Medical University following the legislation of the Republic of Kazakhstan (Project identification code: 1253/GF4, Date of approval: April 17, 2020. Ethics Committee of the Astana Medical University).

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