

# MOLYBDENUM SUPPLEMENTATION OF FAT-TAILED EWES DIETS IN AN ARID REGION

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➤Supporting Information

**ABSTRACT:** The study aimed to establish the normal molybdenum requirements for open ewes of meat-and-fat breeds in arid climatic conditions. The study was carried out in the production conditions of the Buddha farm of the Republic of Kalmykia (Russia). The norm has been established based on a detailed study of the molybdenum content in organs and tissues and the degree of its absorption from the diet, considering the endogenous losses. It has been established that the norm of molybdenum is 4.5 mg per head per day, 2.8 mg per 1 kg of dry matter of the diet and 0.80 mg per 1 kg of live weight of a single ewe. Recommendations have been made to solve the problem of molybdenum deficiency in the diet of open ewes of meat-and-fat breeds in arid climatic conditions of Republic of Kalmykia.

**Key Words:** Open ewes, Norm, Element, Concentration, Kalmyk breed

**RESEARCH ARTICLE**  
 PII: S222877012200021-12  
 Received: February 15, 2022  
 Revised: May 21, 2022  
 Accepted: May 28, 2022

## INTRODUCTION

Among the factors determining the usefulness of feeding ewes, microelements, including molybdenum, occupy a significant place. The significance of molybdenum can be judged by the fact that it is an integral part of such enzymes as xanthine oxidase, aldehyde oxidase, and sulfoxidase (Kalnitskii, 1978; Novotny, 2011; Novotny and Peterson, 2018). This trace element is essential for plants, animals and micro-organisms especially those involved in the bacterial digestibility of fiber in the ruminant pancreas (Ellis et al., 1958; Kalnitskii, 1978; Novotny and Peterson, 2018). Molybdenum, like other elements, takes an active part in the metabolism in the body of animals and thereby has a significant impact on their productivity (Moskalev 1985; Ellis et al., 1958).

On the other hand, high intake of molybdenum into the body of animals, especially cattle and sheep, leads to metabolic disorders, severe toxicosis, accompanied by diarrhea, anemia, paleness of the hair, and a decrease in body weight (Merkur'eva 1970 and Russell et al., 2001), because induce secondary copper deficiency in these animals (Novotny and Peterson, 2018, Stepanova et al., 2020). A very high intake of molybdenum in rodents also led to kidney and liver histological damages, reproductive failure, renal failure, anemia, bone deformities and even growth retardation (Russell et al., 2001; Novotny, 2011).

Therefore, it is very important to control the intake of this element into the body of animals, considering its content in the diet feed. A review of the literature shows that the issues of molybdenum content in the diet have been studied in more detail on the example of dairy cows and sheep of the meat-and-wool and wool-and-meat breeds and molybdenum still has not been included in the mandatory controlled indicators that should be taken into account when compiling diets. As for the open ewes of the meat-and-fat breeds in the conditions of the arid zone of their breeding, there is currently no information regarding their requirement for this element. Considering these circumstances, the purpose of this study was to investigate the molybdenum metabolism in open ewes, and developing standards for the requirements of this element for the Kalmyk fat-tailed breed in the conditions of the arid zone.

This work was performed to A) determine the molybdenum content in the organs, tissues, and contents of the gastrointestinal tract of ewes during the period of their being in an open state; B) determine the degree of absorption of this element from the diet; and C) calculate the daily intake requirement and establish the norm of molybdenum for fat-tailed open ewes by the factorial method.

## MATERIALS AND METHODS

This study was carried out in the production conditions of the Buddha farm at the Republic of Kalmykia (Russia) on ewes of the Kalmyk fat-tailed breed after the weaning of the lambs and before mating, 3 heads of each period with a live

weight of 55-57 kg. During the experiment, the animals were kept in individual cages, feeding was carried out according to the recommended standards of the Russian Academy of Agricultural Sciences (RASKhN) (Kalashnikov et al. 2003), considering the chemical composition of local feeds.

The composition of the diet of open ewes included grass of herb-bunchgrass pasture (3.5 kg), alfalfa hay (0.3 kg), barley (0.1 kg), table salt (11 g), copper sulfate (29 mg), zinc sulfate (124 mg), manganese sulfate (58 mg) and cobalt chloride (1.8 mg). The feed contained 3.193 mg of molybdenum.

To study the molybdenum content in organs, tissues, and in the whole body, on the day of the end of each balance experiment, 3 heads of sheep were slaughtered after the weaning of the lambs and before mating. At that time, the mass of organs, tissues, and contents of the digestive tract of sheep was determined. In those samples, the concentration of molybdenum was determined on an atomic absorption spectrometer.

The factorial method was used to calculate the requirement of molybdenum in open ewes:

1. Based on the data on the concentration of molybdenum in organs and tissues and the contents of the digestive tract, the total content of this element in the body was determined;
2. The amount of molybdenum that is deposited during the idle period of ewes and per day was determined;
3. Endogenous molybdenum losses were determined with feces according to Bell et al. (1964); Udris and Neiland (1976), and the losses with urine were calculated directly;
4. The daily deposition of molybdenum in the body of ewes and endogenous losses with feces and urine were summed up and based on this, the true daily total requirement for this element was established;
5. According to the results of balance experiments, considering endogenous losses in feces, the true digestibility of molybdenum from diets was revealed as a percentage using the following formula:

$$D = \frac{I - (E - En)}{I} \times 100$$

Where: D is the true digestibility of the element (%); I is the intake of the element with the diet (mg); E is the excretion of an element with feces (mg); En is the endogenous excretion of an element with feces (mg);

6. The established total true requirement was divided by the percentage of true absorption and, as a result, the recommended amount of the element that should be contained in the diet was obtained.

### Statistical analysis

The digital material of the experiments was processed biometrically according to E.K. Merkureva's method (Merkureva 1970) on a computer using the program "Statistics" version -2.6. The results obtained were studied and compared by the group method. The difference in mean scores between groups was considered significant at the level of probability (P=0.05) determined by Student's t-test.

### Ethical approval

The work was carried out in accordance with the passport of scientific directions of the Department of Animal Science named after Professor S.A. Lapshin National Research Mordovian State University named after N.P. Ogaryov on the topic "Optimization of feeding of highly productive animals and poultry using digital technologies in the development of resource-saving technologies for the production of livestock and poultry products", Ogaryova, protocol No. 5 of 08/20/2018).

## RESULTS AND DISCUSSION

The results of the study showed that the Kalmyk fat-tailed open ewes fed dietary molybdenum of 2.86-2.82 mg, daily (Table 1). Out of this amount of the element, 60.14% or 1.72 mg per day was absorbed by open ewes after the weaning of the lambs (Table 1). During the idle period, the absolute value of molybdenum absorption decreased by 0.08 mg, and the relative value decreased by 1.99%. Molybdenum excretion in fat-tailed open ewes mainly occurred through the gastrointestinal tract and in small amounts (0.24-0.22 mg/day) through the kidneys.

The main mass of endogenous molybdenum was excreted from the body in the urine by open ewes of the Kalmyk fat-tailed breed. It should be noted that the total loss of this element during the idle period of fat-tailed ewes did not change significantly and remained at the level of 1.50-1.52 mg, and the deposition in the body decreased slightly from 1.36 to 1.30 mg. In our studies, it was found that the idle period of ewes had a significant effect on the distribution and concentration of molybdenum in their tissues and organs (Table 2). Thus, the concentration of this element in the blood of ewes after the weaning of the lambs was 1.68 mg/kg, and by the mating period, it increased by 2 times reaching up to 3.40 mg/kg (P<0.001). Due to an increase in the concentration of molybdenum, its total amount in the blood also increased by 2.3 times or from 5.77 mg to 13.20 mg (P<0.05).

Of all the tissues of open ewes, bone tissue (81.81-81.769 mg/kg) and skin with wool (15.194-25.721 mg/kg of raw tissue) had the highest concentration of molybdenum (P<0.05). At the same time, it should be noted that if the concentration of molybdenum during the idle period in the bone tissue remains at the same level (Bell et al., 1964), then in the skin with wool, it increases by 1.7 times (P<0.001) as it follows from the current study (Table 2). Then the tissues can be arranged in descending order by molybdenum content in the following way: nerve tissue, muscle tissue, peri-renal adipose tissue, internal adipose tissue. The smallest amount of molybdenum (0.145-0.100 mg/kg) is contained in the fat

tail. By the mating period of fat-tailed ewes the concentration of the element in the tongue increased by 19.8% ( $P>0.05$ ), by 2.5 times in the udder ( $P>0.001$ ) and in the uterus, on the contrary, it decreased by 5.7% ( $P>0.05$ ).

The total molybdenum content in the muscle tissue increased by 1.4 times by the mating period of ewes ( $P<0.001$ ), while in the bone tissue it increases by 6.2% ( $P<0.001$ ), in the skin with wool by 1.7 times ( $P<0.001$ ), in the internal fat by 2 times ( $P<0.05$ ), in the perirenal fat by 1.6 times ( $P>0.05$ ), and in the brain by 1.2 times ( $P<0.05$ ). At the same time, it should be noted that during the idle period, the absolute molybdenum content in the fat and udder did not change significantly, in the tongue it decreased by 26.6% ( $P>0.05$ ), and in the uterus, on the contrary, it increased by 2.4% ( $P>0.05$ ).

Conducted studies have also shown that molybdenum is unevenly distributed in the internal organs of open ewes of the meat-and-fat breed. It was found that out of the internal organs the highest concentration of this element was contained in the liver. The liver contains from 25.128 to 28.853 mg of the element/kg of raw tissue, which indicates the active participation of molybdenum in the metabolic processes of the body of open ewes. At the same time, the total amount of molybdenum in this organ also increases.

Of the other internal organs, the spleen also has a high concentration of this element (10.678-43.460 mg/kg). The concentration of molybdenum in it is 3.9 and 17.3 times higher than in the heart, 6.2 and 25.4 times higher than in the lungs, and 10 and 32.5 times higher than in the kidneys. As for the total amount of the element, the internal organs can be arranged in descending order as follows: liver (20.00-22.50 mg), spleen (0.903-4.204 mg), lungs (0.885-0.973 mg), heart (0.619-0.624 mg), and kidneys (0.102-0.135 mg). The absolute amount of the element in the heart during the idle period of the ewes does not change significantly. In the lungs, it increases by 9.9%. In the liver, the dynamics of this indicator is also 12.5% mg. The absolute amount of molybdenum in the kidneys of ewes during the study period increased by 32.3%, and in the spleen by 4.6 times (Table 2). Our studies have also shown that the amount of this element in the walls of the digestive tract of meat-and-fat open ewes is also subject to fluctuations. Of all the departments of the stomach, the manifold walls had a higher concentration of molybdenum (25.494-37.760 mg/kg), and the concentration in the abomasum walls was low (1.547-1.910 mg/kg). It should be noted that during the idle period, the concentration of the element in the manifold increases by 1.5 times ( $p < 0.05$ ), and in abomasum by 1.2 times ( $P<0.05$ ). The remaining sections of the gastrointestinal tract of open ewes can be arranged in the descending order by molybdenum content: large intestine (13.811-17.676 mg/kg), rumen (5.499-6.692 mg/kg), small intestine (2.857-6.393 mg/kg), honeycomb stomach (2.016-3.051 mg/kg) and abomasum (1.547-1.910 mg/kg).

In all parts of the gastrointestinal tract of ewes, the absolute amount of molybdenum increased during the idle period, and its maximum content was observed in the large intestine (16.015 - 18.550 mg) and the walls of the rumen (5.596-8.130 mg). Low content of this element was observed in the honeycomb stomach (0.312-0.433 mg) and abomasum (0.729-0.767 mg) ( $P>0.05$ ). Our studies also showed that the accumulation of molybdenum in the contents of the digestive tract of fat-tailed open ewes has differences. Thus, the concentration of this element during the studied period increases by 1.5 times in the chyme of the rumen ( $P<0.05$ ), by 5.4% in the chyme of the honeycomb stomach ( $P>0.05$ ), by 10.8% in the chyme of the manifold ( $P<0.05$ ), by 5.4% in the chyme of the honeycomb stomach ( $P>0.05$ ), by 22.7% in the chyme of the abomasum ( $P<0.05$ ), by 5.4% in the chyme of the honeycomb stomach ( $P<0.05$ ), by 2.3 times in the chyme of the small intestine ( $P<0.001$ ) and by 2.8 times in the chyme of the large intestine ( $P<0.01$ ).

Of the total absolute amount of molybdenum contained in the chyme, the main share (74.90-77.90%) falls on the rumen, 10.2-13.0% on the chyme of the large intestine, and 8.4-10.0% on the chyme of the small intestine. The absolute molybdenum content in the body of ewes after the weaning of the lambs was 805.12 mg, and by the mating period, it was increased by 25.4%, and reached up to 1010.32 mg.

As a result of our study, it was found that the absolute deposition of molybdenum in the body of ewes during the idle period increased from 805.12 to 1010.32 mg or by 1.25 times, i.e. during this period, 205.12 mg of molybdenum was deposited in their body. At the same time, the daily deposition of the element equals 2.28 mg (Table 3). Calculations also showed that the true molybdenum requirement for open ewes of the meat-and-fat breed with a live weight of 55-57 kg equaled 2.62 mg per 1 head per day to ensure their normal functioning and obtain high gains (Table 3).

**Table 1 - The absorption of molybdenum from the diet by open ewes, mg**

Indicators	Ewes after the weaning of the lambs	Ewes before mating
Taken with food and water, mg	2.86±0.02	2.82±0.01
Excreted with feces total, mg	1.26±0.03	1.30±0.02
incl. endogenous losses, mg	0.12±0.01	0.12±0.01
Visible absorption, mg	1.60±0.02	1.52±0.01
True absorption, mg	1.72±0.01	1.64±0.01
True absorption, %	60.14±0.53	58.15±0.07
Excreted in urine, mg	0.24±0.01	0.22±0.01
Total excreted, mg	1.50±0.02	1.52±0.01
Retained in the body, mg	1.36±0.01	1.30±0.01
Percentage of the amount taken	47.55±0.50	46.10±0.11

**Table 2 - The molybdenum content in the tissues and organs of open ewes, mg**

Indicators	Concentration, mg/kg		The total amount, mg	
	Idle periods		Idle periods	
	Ewes after the weaning of the lambs	Ewes before mating	Ewes after the weaning of the lambs	Ewes before mating
Blood	1.68±0.19	3.40±0.14*	5.77±0.29	13.20±0.07***
Muscle tissue	4.83±0.06	6.305±0.12	80.10±0.67	116.55±0.04***
Bone tissue	81.81±3.34	81.769±2.89	529.80±2.12	563.00±2.51
Leather with the wool coat	15.194±0.22	25.721±0.44***	94.13±0.94	162.00±1.73***
Internal fat	0.428±0.01	0.654±0.03	0.218±0.03	0.443±0.02*
Pararenal fat	1.512±0.12	0.928±0.01	0.119±0.02	0.190±0.03
Fat tail	0.145±0.03	0.100±0.05	0.55±0.03	0.530±0.01
Brain	5.960±0.20	6.923±0.07	0.803±0.03	0.955±0.03*
Tongue	3.398±0.37	4.070±0.06*	0.365±0.02	0.268±0.03
Heart	2.738±0.25	2.504±0.10	0.619±0.06	0.624±0.03
Lungs	1.704±0.04	1.708±0.03	0.885±0.05	0.973±0.02
Liver	25.128±1.75	28.853±0.42	20.00±0.80	22.50±0.16
Kidneys	1.065±0.02	1.338±0.09	0.102±0.02	0.135±0.02
Spleen	10.678±0.51	43.460±1.66	0.903±0.02	4.204±0.08
Uterus	5.142±0.17	4.854±0.13	0.592±0.02	0.606±0.03
Udder	1.185±0.08	3.00±0.19***	0.258±0.03	0.268±0.03
Rumen	5.499±0.18	6.692±0.04	5.596±0.08	8.130±0.03
Honeycomb stomach	2.016±0.06	3.051±0.07	0.312±0.03	0.433±0.03
Manifold	25.494±1.06	37.760±1.30* <sup>b</sup>	4.318±0.02	7.160±0.03
Abomasum	1.547±0.07	1.910±0.02* <sup>a</sup>	0.755±0.03	0.907±0.03
Small intestine	2.857±0.11	6.393±0.09	3.702±0.04	7.990±0.04
Large intestine	13.811±0.18	17.676±0.32	16.015±0.01	18.550±0.07
Contents of the rumen	5.277±0.14	10.323±1.14*	30.560±0.03	60.42±0.06
Contents of the honeycomb stomach	0.764±0.04	0.807±0.02	0.277±0.03	0.266±0.03
Contents of the manifold	3.956±0.38	4.383±0.10	0.776±0.03	0.950±0.04
Contents of the abomasum	0.811±0.03	0.995±0.04	0.275±0.02	0.313±0.05
Contents of the small intestine	4.083±0.08	9.567±0.12***	3.305±0.04	8.13±0.03
Contents of the large intestine	7.589±0.23	21.310±1.68**	4.015±0.05	10.52±0.04
Total			805.12	1,010.32

\* P<0.05; \*\* P<0.01; \*\*\* P<0.001; <sup>a</sup> the lowest result; <sup>b</sup> the highest.

**Table 3 - The daily molybdenum requirement for open ewes and the molybdenum norm in diets, (mg)**

Indicators	Ewes after the weaning of the lambs	Ewes before mating
Total molybdenum content in the body	805.12	1010.32
Total deposition of molybdenum in the body during the period	-	205.12
Daily deposition of molybdenum	-	2.28
Endogenous losses	with feces	0.12
	with urine	0.24
	total	0.36
True daily molybdenum requirement	-	2.62
True digestibility from the diet, %	-	58.15
The actual daily norm in the diet	per 1 head	-
	per 1 kg of dry matter of the diet	-
	per 1 kg of live weight	-

The studies on the ewes of the Kalmyk fat-tailed breed in the arid zone conditions were carried out for the first time. The limited studies have been conducted on effect of dietary molybdenum in sheep (Bampidis et al., 2019); therefore, the information about the rationing of molybdenum for ewes of the meat-fat direction of productivity was not found among the scientific studies published to the moment. It makes it impossible to give a comparative assessment of the obtained results.

## CONCLUSION

Thus, based on the data on the molybdenum concentration in the organs and tissues of open ewes of meat-and-fat breeds, it can be concluded that the content of this element increases both, the molybdenum concentration in the organs

and tissues, due to an increase in the mass of organs and tissues, and an increase in the concentration of elements in them, and is also determined by the duration of the idle period of ewes. Based on data on the molybdenum daily deposition in the body, its content in organs and tissues, the degree of absorption from the diet, and endogenous losses with feces and urine, we have calculated the molybdenum requirement for open ewes of the meat-and-fat breed in arid climatic conditions. Since only 58.6% of this element is absorbed from the diets, open ewes should receive 4.50 mg of molybdenum per 1 head per day in the daily diet. There is 2.80 mg per 1 kg of dry matter of the diet, and 0.80 mg of molybdenum per 1 kg of live weight.

## DECLARATIONS

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### Author's contribution

D. Sh. Gayirbegov performed conceptualization, methodology, formal analysis, validation, writing original, review, statistics and editing; D. B. Mandzhiev performed methodology, review, editing, and validation; and T. B. Tyurbeev performed conceptualization, review and editing.

### Competing interests

The authors declare that they have no competing interests.

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