



colloquium-journal

ISSN 2520-6990

Międzynarodowe czasopismo naukowe

Economics
Jurisprudence
Biological sciences
Veterinary sciences
Agricultural sciences
Physical education and sport

№18(70) 2020

Część 2



colloquium-journal

ISSN 2520-6990

ISSN 2520-2480

Colloquium-journal №18 (70), 2020

Część 2

(Warszawa, Polska)

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[DOI: 10.24411/2520-6990-2020-12011](https://doi.org/10.24411/2520-6990-2020-12011)

INFLUENCE OF METISEVIT AND LIPOINTERSIL ON MORPHOLOGICAL INDICES OF BULL BLOOD UNDER CADMIUM LOADING

The article deals with the results of researches of the influence of cadmium load on the organism of young cattle. The purpose of the research was to study the influence of metisevit and lipointersil on the morphological indices of bull blood under cadmium loading. According to the morphological analysis of the quantitative and qualitative composition of blood, it is possible to judge quite objectively the functional state of the hematopoietic system of the organism of young cattle. The research was conducted on the basis of a farm in the village of Ivanivtsi, Zhydachiv district, Lviv region, on 15 bulls, six months of age, Ukrainian Black-Spotted dairy breed, which were formed into 3 groups, 5 animals in each: control and two experimental. The bulls of the control group were fed cadmium chloride compound feed at a dose of 0.04 mg/kg of body weight for 30 days. The bulls of the first experimental group were fed cadmium chloride at a dose of 0.04 mg/kg of body weight for 30 days and fed a dietary supplement "Metisevit" at a dose of 0.36 g/kg of feed. The bulls of the second experimental group were fed cadmium chloride at a dose of 0.04 mg/kg of body weight for 30 days and fed a dietary supplement "Metisevit" in the above dose. On the first and seventh days of the experiment, bulls of this experimental group were injected intramuscularly with liposomal preparation "Lipointersil" at a dose of 5 ml per animal. It was found that bulls feeding with cadmium chloride contributed to reduce the level of hemoglobin, red blood cell count and increase methemoglobin content. It was found that the average content of hemoglobin in one erythrocyte of bulls blood of the experimental group on the 5th and 10th day was increased by 11.4 and 13.9%. The results of the study revealed a decrease in hematocrit in bulls of the control group for 10, 15 and 20 days of the experiment. A probable increase in the number of leukocytes in the blood of bulls of the experimental group was found on the 15th day of the experiment. "Metisevit" feed supplement and liposomal drug "Lipointersil" under cadmium load in bulls contributed to the normalization of blood morphological indices. It should be noted that the additional use of the drug "Lipointersil" contributed to a better normalization of these indicators than feeding only the feed additive "Metisevit".

Keywords: toxicology, Cadmium, bulls, blood, erythrocytes, leukocytes, hemoglobin, methemoglobin, feed additive, liposomal preparation.

Introduction

Cadmium is one of the most toxic heavy metals. It belongs to the second class of danger - highly dangerous substances. Like many other heavy metals, cadmium has a pronounced tendency to accumulate in the organism: the period of its half-withdrawal is 10-35 years. By the age of 50, its total amount in the human organism can reach 30-50 mg. Cadmium in the organism accumulates in the kidneys (30-60% of the total number) and liver (20-25%). Cadmium is also found in the pancreas, spleen, tubular bones, other organs and tissues [1-3].

Due to the intensive emissions of industrial enterprises, cadmium pollution is constantly increasing. As a result, it also increases the contamination of soils and food products grown on them [4].

The results of many experimental studies indicate that in mammals organism cadmium has a toxic influence on a number of organs and systems, including the cardiovascular, sexual, excretory, respiratory, hematopoietic system, musculoskeletal system [5, 6]. The most dangerous influence are carcinogenic and mutagenic

effects of this element. It is known that under conditions of intoxication of animals organism with cadmium compounds there is anemia, suppression of the functional state of the immune system and other disorders in hematopoietic processes [3, 7].

The purpose of the study was to investigate the influence of metisevit and lipointersil on the morphological indices of bull blood under cadmium loading.

Material and research methods

The research was conducted on the basis of a farm economy in the village of Ivanivtsi, Zhydachiv district, Lviv region, on 15 bulls of Black-Spotted breed, 6 months old, of which 3 groups of 5 animals were formed in each: control and two experimental groups. The bulls of the control group were fed cadmium chloride compound feed at a dose of 0.04 mg/kg of body weight for 30 days. The bulls of the first experimental group were fed cadmium chloride at a dose of 0.04 mg/kg of body weight for 30 days and fed a dietary supplement "Metisevit" at a dose of 0.36 g/kg of feed. Feed additive "Metisevit" was developed at the Department of Pharmacology and Toxicology of Stepan Ghzyskyj Lviv National University of Veterinary Medicine and

Biotechnologies, which contains vitamin E, selenium and methyphene.

The bulls of the second experimental group were fed cadmium chloride at a dose of 0.04 mg/kg of body weight for 30 days and fed a dietary supplement "Metisevit" in the above dose. On the first and seventh days of the experiment, bulls of this experimental group were injected intramuscularly with liposomal preparation "Lipointersil" at a dose of 5 ml per animal. The composition of the liposomal preparation "Lipointersil" includes interferon and milk thistle.

To conduct research, the rules required for zootechnical experiments on the selection and maintenance of animals-analogues in groups, harvesting technology, use and accounting of feed consumed were followed. The ration of the animals was balanced in terms of nutrients and minerals, which provided their need for basic nutrients.

Keeping, feeding, care and all manipulations of animals were carried out in accordance with the European Convention "For the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (Strasbourg, 1986) and "The General Ethical Principles of Animal Experimentation", adopted by the First National Congress on Bioethics (Kyiv, 2001). The experiments were carried out in accordance with the principles of humanity set out in the European Community Directive.

Under the experimental cadmium load, blood from bulls was collected on 1, 5, 10, 15, 20 and 30 days after feeding the above-mentioned toxicant.

The number of erythrocytes and leukocytes was counted on the Goryaev system of the counting chamber according to the generally accepted method. The level of hemoglobin in the blood was determined by the cyanhemoglobin method using FEKM by the method of G.V. Derviza and A.G. Vorobjov. The concentration of methemoglobin was determined by I.F. Boyarchuk et al. The value of hematocrit was determined by centrifugation of blood in micropipettes at 3000 rpm. By the values of the number of erythrocytes, hemoglobin level and hematocrit, according to the appropriate formulas, by mathematical calculations, were calculated the following values of red blood indices: the average volume of one erythrocyte (MCV), the average mass of hemoglobin in the erythrocyte (MCH) [8].

The analysis of research results was performed using the software package Statistica 6.0. The probability of differences was assessed by Student's t-test. The results of the mean values were considered statistically significant at * - $P < 0,05$, ** - $P < 0,001$ (ANOVA).

Results and their discussion

When using the feed additive "Metisevit" and the preparation "Lipointersil" it was found that in the blood of bulls of the second and third experimental groups under cadmium loading, the hemoglobin content was increased throughout the experiment. However, it should be noted that with the use of metisevit in the blood of the second experimental group of bulls, the hemoglobin content at 15, 20 and 30 days was increased by 9.1, 22.4 and 19%, respectively, relative to the control group of animals.

Table-1

The influence of metisevit and lipointersil on the content of hemoglobin in the blood of bulls on chronic cadmium toxicosis; ($M \pm m$, $n = 5$)

| Blood test time (day) | Hemoglobin (g/l) | | |
|-----------------------|-------------------|--------------------------|--|
| | Groups of animals | | |
| | Control | Experimental 1 Metisevit | Experimental 2 Metisevit +Lipointersil |
| Initial values | 104,4±1,99 | 103,5±2,31 | 103,5±2,31 |
| 1 st day | 105,0±1,87 | 104,1±2,24 | 103,9±2,85 |
| 5 th day | 116,6±1,60 | 110,8±1,98 | 111,5±3,11 |
| 10 th day | 126,1±1,86 | 118,0±2,33 | 120,2±1,57 |
| 15 th day | 98,9±1,86 | 107,9±2,10* | 113,7±3,06** |
| 20 th day | 84,5±1,60 | 103,4±3,10* | 112,4±1,90** |
| 30 th day | 86,7±1,58 | 103,2±2,45* | 113,2±1,98** |

Concomitant use of metisevit and lipointersil in bulls of the third experimental group contributed to a more likely increase in hemoglobin than the use of metisevit alone. It was found that the content of hemoglobin at 15 and 20 days of the experiment in the blood of the third experimental group was increased by 15 and 33% relative to control values. On the 30th day of the experiment, the content of hemoglobin in the blood of this experimental group ranged from 113.2±1.98 g/l, which was 30.6% higher than the control group of animals.

It is set up that the development of chronic cadmium toxicosis in animals is accompanied by an increased content of methemoglobin. The experimental preparations "Metisevit" and "Lipointersil" contributed to the reduction of the studied indicator throughout the experiment. Thus, in the blood of animals of the second experimental group, the level of methemoglobin ranged from 4.0±0.059 - 4.4±0.074%, while in the third experimental group of animals this figure was slightly lower, and accordingly ranged from 3.8±0.064 - 3.9±0.090%.

The influence of metisevit and lipointersil on the content of methemoglobin in the blood of bulls on chronic cadmium toxicosis; (M±m, n=5)

| Blood test time (day) | Methemoglobin (%) | | |
|-----------------------|-------------------|--------------------------|--|
| | Groups of animals | | |
| | Control | Experimental 1 Metisevit | Experimental 2 Metisevit +Lipointersil |
| Initial values | 4,1±0,081 | 3,9±0,067 | 4,0±0,093 |
| 1 st day | 4,2±0,075 | 4,0±0,059 | 3,8±0,064 |
| 5 th day | 4,3±0,095 | 4,0±0,090 | 3,9±0,072 |
| 10 th day | 4,8±0,089 | 4,1±0,064* | 3,9±0,090* |
| 15 th day | 5,2±0,099 | 4,1±0,080* | 3,8±0,056* |
| 20 th day | 5,0±0,061 | 4,4±0,074* | 3,9±0,083* |
| 30 th day | 4,8±0,092 | 4,2±0,081 | 3,8±0,078* |

In the research of the influence of metisevit and lipointersil on the average hemoglobin content in one erythrocyte of bull blood in chronic cadmium toxicosis, it was found that this indices at 15 and 20 days of the

experiment was higher by 5.5 and 5.4% relative to the control group. It is known that this indicator indicates the saturation of erythrocytes with hemoglobin.

Table-3

The influence of metisevit and lipointersil on the average hemoglobin content in one erythrocyte of bull blood on chronic cadmium toxicosis; (M ± m, n = 5)

| Blood test time (day) | The average content of hemoglobin in the erythrocyte (pg) | | |
|-----------------------|---|--------------------------|---|
| | Groups of animals | | |
| | Control | Experimental 1 Metisevit | Experimental 2 Metisevit + Lipointersil |
| Initial values | 14,81±0,30 | 14,68±0,24 | 14,68±0,32 |
| 1 st day | 14,85±0,32 | 14,66±0,35 | 14,47±0,28 |
| 5 th day | 16,01±0,35 | 15,41±0,29 | 15,46±0,30 |
| 10 th day | 16,83±0,29 | 16,32±0,47 | 16,49±0,32 |
| 15 th day | 14,38±0,50 | 15,17±0,42 | 15,68±0,34 |
| 20 th day | 13,76±0,46 | 14,50±0,33 | 15,54±0,42* |
| 30 th day | 13,18±0,70 | 14,58±0,34* | 15,72±0,41* |

In the third experimental group of animals on the 10th day of the experiment the highest average content of hemoglobin in one erythrocyte of blood of bulls was found out. In the future, we note a decrease in this indicator, however, when compared with the control group, the average hemoglobin content in one erythrocyte at 15 and 20 days of the experiment increased by 9.0 and 12.9%, respectively. On the 30th day of the experiment we note an increase in this indicator to 15.72±0.41 pg, while in the control group the average hemoglobin content in one erythrocyte was 13.18 ± 0.70 pg.

It has been set up that the number of erythrocytes in the blood of bulls decreases during cadmium load-

ing. On the 15th day of the experiment we note the lowest number of erythrocytes in the blood of animals of the control group. The use of preparations "Metisevit" and "Lipointersil" contributed to a probable increase in the number of erythrocytes in the second and third experimental groups starting with 15 days of the experiment. The number of erythrocytes in the blood of the second experimental group in this period increased by 3.3%, and in the third - by 5.4%, respectively. When feeding metisevit to bulls of the second experimental group on the 20th day of the experiment, the number of erythrocytes increased by 16.1%, and on the 30th day of the experiment - by 7.6%, respectively.

Table-4

The influence of metisevit and lipointersil on the number of erythrocytes in the blood of bulls on chronic cadmium toxicosis; (M±m, n=5)

| Blood test time (day) | Erythrocytes (T/l) | | |
|-----------------------|--------------------|--------------------------|--|
| | Groups of animals | | |
| | Control | Experimental 1 Metisevit | Experimental 2 Metisevit +Lipointersil |
| Initial values | 7,05±0,25 | 7,05±0,20 | 7,05±0,20 |
| 1 st day | 7,07±0,20 | 7,10±0,22 | 7,18±0,23 |
| 5 th day | 7,28±0,19 | 7,19±0,18 | 7,21±0,22 |
| 10 th day | 7,49±0,43 | 7,23±0,29 | 7,29±0,20 |
| 15 th day | 6,88±0,29 | 7,11±0,26* | 7,25±0,27* |
| 20 th day | 6,14±0,35 | 7,13±0,27** | 7,23±0,29** |
| 30 th day | 6,58±0,45 | 7,08±0,33* | 7,20±0,34** |

In the blood of the third experimental group of bulls, which in addition to feeding with metisevit were used liposomal preparation "Lipointersil", was found an increase in the number of erythrocytes on 20th and 30th days of the experiment by 17.8 and 9.4%, respectively, relative to the control group of animals.

When determining the average volume of one erythrocyte in bulls of the second experimental group,

its increase was found throughout the experiment. Thus, at 10th and 15th days of the experiment, this figure was higher than the control by 6.9 and 3.7%, respectively. On the 20th day of the experiment, we note a slight increase in the average volume of one erythrocyte in the blood of bulls of the second experimental group compared to the previous day.

Table-5

The influence of metisevit and lipointersil on the average volume of one erythrocyte in the blood of bulls on chronic cadmium toxicosis; (M±m, n=5)

| Blood test time (day) | The average volume of one erythrocyte (µm ³) | | |
|-----------------------|--|--------------------------|--|
| | Groups of animals | | |
| | Control | Experimental 1 Metisevit | Experimental 2 Metisevit +Lipointersil |
| Initial values | 46,81±1,15 | 45,39±1,23 | 45,39±1,20 |
| 1 st day | 46,68±1,45 | 46,48±1,25 | 47,16±1,34 |
| 5 th day | 46,70±1,56 | 47,29±1,63 | 47,16±1,65 |
| 10 th day | 41,39±2,01 | 44,26±1,59 | 48,0±1,84* |
| 15 th day | 40,70±1,85 | 42,19±1,62 | 45,52±1,70* |
| 20 th day | 47,23±1,60 | 43,47±1,75* | 47,02±1,45 |
| 30 th day | 47,11±1,30 | 45,19±1,51 | 47,22±1,47 |

In the blood of bulls of the third experimental group, the normalization of the investigated indicator was set up throughout the experiment. The highest average volume of one erythrocyte was in the blood of bulls which along with feeding metisevit were used liposomal preparation "Lipointersil". It was found that on the 10th and 15th day of the experiment, this figure increased by 16 and 11.8% relative to the control group.

Under cadmium loading in bulls of the control group, a decrease in hematocrit to 0.28±0.009 l/l was found. When using experimental preparations to bulls under cadmium load, an increase in hematocrit was found, where, accordingly, it fluctuated within physiological values.

Table-6

The influence of metisevit and lipointersil on hematocrit of bull blood on chronic cadmium toxicosis; (M±m, n=5)

| Blood test time (day) | Hematocrit (l/l) | | |
|-----------------------|-------------------|--------------------------|--|
| | Groups of animals | | |
| | Control | Experimental 1 Metisevit | Experimental 2 Metisevit +Lipointersil |
| Initial values | 0,33±0,010 | 0,32±0,014 | 0,32±0,012 |
| 1 st day | 0,33±0,011 | 0,33±0,012 | 0,34±0,010 |
| 5 th day | 0,34±0,015 | 0,34±0,014 | 0,34±0,012 |
| 10 th day | 0,31±0,014 | 0,32±0,009 | 0,35±0,011* |
| 15 th day | 0,28±0,009 | 0,30±0,012 | 0,33±0,014* |
| 20 th day | 0,29±0,014 | 0,31±0,011 | 0,34±0,012* |
| 30 th day | 0,31±0,013 | 0,32±0,016 | 0,34±0,013 |

On the 20th day of the experiment, the level of hematocrit in bulls of the third experimental group was probably increased by 17.2% relative to the control group.

The influence of metisevit and lipointersil on the number of leukocytes in the blood of bulls in chronic cadmium toxicosis is shown in table 7. It was found that on the 5th day of the experiment after the use of metisevit in experimental calves, the number of leukocytes in their blood was decreased by 2.9%, and on the 10th day - by 4.6%. On the 15th day of the experiment, the animals of the second experimental group showed a slight

increase in the number of leukocytes in their blood compared to the previous day, but compared with the control group, this figure was lower by 4.8%. On the 20th day of the experiment in the blood of the second experimental group of animals the number of leukocytes was 7.67±0.25 G/l, while in the control group - 7.97±0.30 G/l. On the 30th day of the experiment, the number of leukocytes in the blood of this experimental group was decreased by 3.5% relative to the control group of bulls.

The influence of metisevit and lipointersil on the number of leukocytes in the blood of bulls on chronic cadmium toxicosis; (M±m, n=5)

| Blood test time (day) | Leukocytes (G/l) | | |
|-----------------------|-------------------|--------------------------|--|
| | Groups of animals | | |
| | Control | Experimental 1 Metisevit | Experimental 2 Metisevit +Lipointersil |
| Initial values | 7,29±0,19 | 7,31±0,23 | 7,32±0,21 |
| 1 st day | 7,31±0,21 | 7,31±0,17 | 7,33±0,18 |
| 5 th day | 7,64±0,56 | 7,42±0,23 | 7,36±0,20 |
| 10 th day | 7,81±0,20 | 7,45±0,31 | 7,38±0,33 |
| 15 th day | 8,21±0,21 | 7,82±0,27 | 7,40±0,19** |
| 20 th day | 7,97±0,30 | 7,67±0,25 | 7,37±0,21* |
| 30 th day | 7,74±0,35 | 7,47±0,30* | 7,34±0,22* |

When using the liposomal preparation "Lipointersil" under cadmium loading and feeding metisevit was found a decrease in the number of leukocytes in the blood of the third experimental group on the 5th day of the experiment by 3.7%. On the 10th day of the experiment, the number of leukocytes in the blood of the experimental group of bulls which were used lipointersil was decreased by 5.5%, and on the 15th day of the experiment - by 9.9% relative to the control group. On 20th and 30th days of the experiment, the number of leukocytes in the blood of the third experimental group fluctuated within physiological values.

Conclusions

Metisevit feed supplement and liposomal preparation "Lipointersil" under cadmium load in bulls contributed to the normalization of blood morphological indices. It should be noted that the additional use of the preparation "Lipointersil" contributed to a better normalization of these indicators than feeding only the feed additive "Metisevit".

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Colloquium-journal №18(70), 2020

Część 2

(Warszawa, Polska)

ISSN 2520-6990

ISSN 2520-2480

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Umowa z RSCI nr 118-03 / 2017 z dnia 14.03.2017.

Redaktor naczelny - **Paweł Nowak, Ewa Kowalczyk**

«Colloquium-journal»

Wydrukowano w «Chocimska 24, 00-001 Warszawa, Poland»

Format 60 × 90/8. Nakład 500 egzemplarzy.

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