The Toxic Effects of Low Molecular Weight Components of Cow Colostrums: The Short-Term and Long-Term Effects

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Abstract: *Background*: Low-molecular components of colostrum (LMWCC) have a pronounced biological activity. We investigated the effect of different doses of LMWCC (0.01, 0.1, 1 and 5 g/100 g of body weight) on the behavior, dynamics of growth, acute and chronic toxicity, as well as the relative weight of the liver, spleen and kidneys of the experimental animals.

Methods: We used 100 3-month male rats in the experiment. All animals were divided into 5 groups of 20 animals in each group, LMWCC was administered *per os*, and the control group received sterile water.

Results: LMWCC revealed no pirogenic effect in a wide diapason of doses (0,01-5 g/100 g of animal mass). Immediately after the LMWCC administration the short-time decrease in the motion activity was observed. The low doses of LMWCC induced diarrhea in 10-20 % of of animals, the super-large dose – in 75% of animals. The acute toxicity of LMWCC (death of 15 % of animals) was detected only in super-large dose of LMWCC and it was accompanied by pronounced diarrhea. LMWCC influenced the mass of liver, spleen and kidney. 60 days after LMWCC administration the mass of liver was restored to control weight, but the mass of spleen and kidney was not restored.

Conclusion: LMWCCdo not have chronic toxicity and can be attributed to non-toxic compounds with a possible side effect - a violation of the digestive system.

Keywords: Toxicity, Colostrum, Diarrhea.

1. INTRODUCTION

Colostrum is a unique biologically active product that is formed in the mammary gland of mammals in the first few days (up to 5 days) after the beginning of lactation [1, 2].

It can be assumed that reproduction of colostrum is an important evolutionary "acquisition", what promotes the resistance of newly born mammals to aggressive action of environmental factors. Moreover, at early stages of the postnatal development, the epigenetic memory and metabolic memory are formed and remained throughout the ontogenesis, and the colostrum components may influence their formation [3, 4].

It is known that a large number of biologically active compounds enter into the composition of colostrum that affects the functions of the immune system [5, 6]; it removes inflammatory reactions [7-9]; it is used even in the treatment of a number of serious pathologies [10-12].

At the present time, a transfer factor has been isolated from the colostrum, which shows an expressed biological activity [13, 14]. However, its isolation and purification is a labor-intensive task and high-priced.

Along with this, we have isolated a complex of relatively low-molecular components of the colostrum, which eliminates the manifestations of the liver fibrosis [15] and has an immunomodulatory and antioxidant effect [16, 17].

Taking into consideration the high biological value, colostrum components can be used in the development of functional food products [18]. At the same time, the researchers face a number of complex problems when using colostrum as a raw material o receive biologically active compounds: the composition of colostrum changes hour after hour after the calving [19, 20]; the received colostrum is not stored, and it continues to change due to the multicomponent composition; it is technologically difficult to process, as it has a lot of fat and protein and even whole cells.

Most of the existing technologies for the colostrum processing are based on the isolation of separate colostrum components with their subsequent modification [15-17].

We previously isolated the relatively low-molecular components of the colostrum (LMWCC) and determined their biological activity [21, 22].

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One of the main and defining stages in the research of functional food products is the determination of the effect of different doses on a number of vital physiological indexes. Along with this, the problem of dose dependence has become more acute in recent years not only in relation to xenobiotics but also to food products, in connection with the described effects of the action of small and ultra-small doses [23, 25].

In this regard, the effect of large doses of LMWCC is of the utmost interest. There is a belief that traditional products of biological origin should not be toxic and their research is not given due attention as a rule. Moreover, the research of the dose dependence of the physiological effects of LMWCC is important not only for the establishment of therapeutic and preventive doses, but also in the research of the mechanisms of their action.

In this work we investigated the effect of different doses of LMWCC: from small (0.01 g/100 g of body weight), potentially therapeutic (0.1 g/100 g of body weight), large (1 g/100 g of body weight) and "super-large" (5 g/100 g of body weight), on the behavior and general condition (presence of diarrhea, body weight and body temperature), as well as the manifestation of toxicity in experimental animals).

2. MATERIALS AND METHODS

Experimental Models

The experiments were conducted on 3-month mature males of the *Wistar* line. Animals were kept in standard vivarium conditions - they always received food and water at the same time of a day (9-11 a.m.) *ad libitum*. All manipulations (administration of LMWCC, weighing, temperature measurements) were always conducted at the same time of a day from 9:00 to 11:00 local time.

LMWCC was obtained from colostrum only of the first milk yield after the parturation, as it was described earlier [15]. In this purpose all lipid components were eliminated by three consequent separations. The proteins were eliminated by centrifugation at 6000 g during 10 min at room temperature and consequent membrane gel-filtration (0,65→0,45→0,22 μm). The protein contents of colostrums was assessed by electrophoresis in polyacrilamide gel. LMWCC included proteins of molecular mass less than 25-30 kD [15]. A suspension of freeze-dried LMWCC was dissolved on sterile distilled water before the administration and it was administered *per os* at the same time of the day from 9:00 to 11:00 local time. The control group of animals was administered the sterile distilled water *per os*. The experiments were conducted on 100 intact animals, i.e. there were 20 animals in each experimental group that received different doses of LMWCC - 0.01; 0.1; 1 and 5 g/100 g of body weight.

Methods for Determining the Physiological Indexes

The motion activity was determined on the basis of expert assessments according to a points system where the usual physiological activity of animals was taken for 5 points, and inactivity - 1 point. The assessment of motion activity was carried out by three independent experts and the obtained data were averaged.

The presence of diarrhea was determined visually, and rectal body temperature was measured with a special thermometer for laboratory animals (USA), body weight and organ weight were determined by weight method.

The weight of the liver, spleen and kidneys was determined at 30 and 60 days after the first administration of LMWCC, while animals were immersed in ether anesthesia and all manipulations with animals were carried out in compliance with the accepted bioethical rules [25].

The obtained results were processed statistically using the "Excel" program and the differences between the control and experimental groups were considered reliable at $p \leq 0.05$.

3. RESULTS

The Influence of Different Doses of LMWCC on some Physiological Indexes of the Control Group of Animals after a Single Administration per os

The administration of even a small dose of LMWCC, 0.01 g/100 g of body, induced diarrhea in 10–20% of the experimental animals and was accompanied by a loss of motion activity at 1-5 hours after the administration, and the motion activity was restored and did not differ from the control group at 10 hours after the administration (Figure **1A**). A 10-fold increase of LMWCC dose was accompanied by the same suppression of animal activity as at a dose of 0.01 g/100 g of body, the only difference being that they were restored more slowly (Figure **1A**).

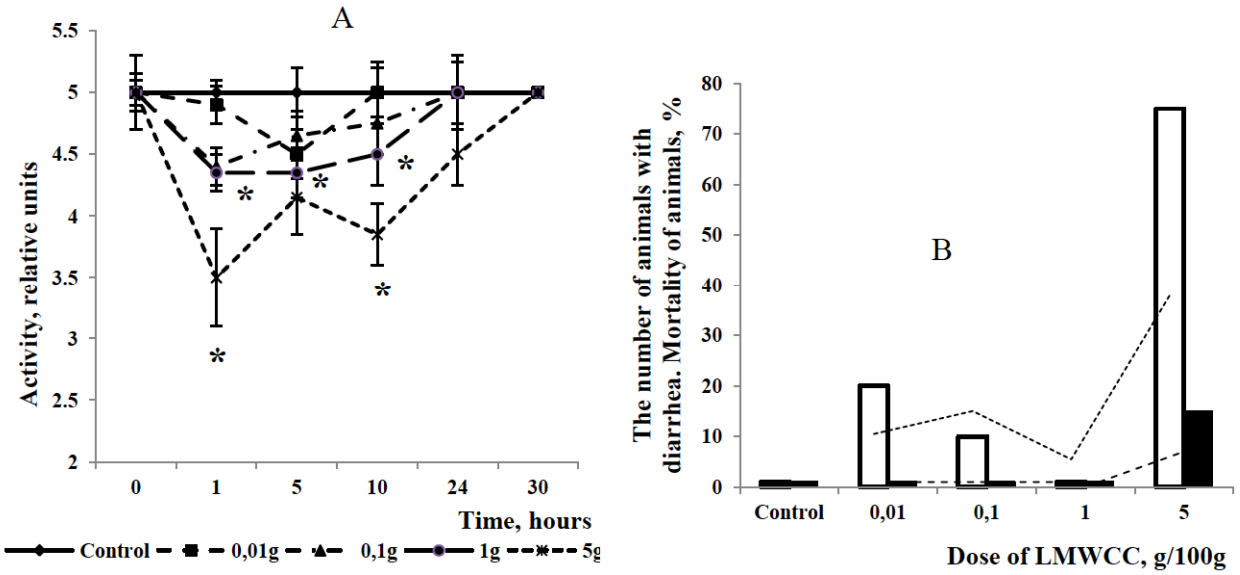

Figure 1: The motion activity of the experimental animals of the control group, and the animals that were administered LMWCC per os on a single occasion at doses: 0.01 g/100 g; 0.1 g/100 g; 1 g/100 g and 5 g/100 g at 1, 5 and 10 hours after the administration (**A**). The number of animals in percentage who had diarrhea (□) and it was registered death (■) at 10 hours after the administration of different doses of LMWCC (**B**). * – p < 0,05 in comparison with the control.

A 10-fold increase in the dose, that is up to 1 g/100 g of body weight, suppressed the motion activity to a greater extent (Figure **1A**), which was restored only at 24 hours after a single administration.

It should be noted that the dose of 1 g per 100 g of body, if it is converted to an adult human with an average weight of 70 kg, would be 700 grams of colostrum in a single dose, which is an excessively large dose for the drug or food additive. If the dose of LMWCC was increased to 5 g per 100 g of body weight, which would be 3.5 kg for an adult human (that is a "super-large" dose), it strongly suppressed the activity of animals that did not restore even at 24 hours after the administration (Figure **1B**).

Consequently, a single administration of LMWCC to experimental animals in a wide range of doses - from 0.01 to 5 g per 100 g of body weight, was accompanied by dose- and time-dependent suppression of motion activity, which was especially expressedat a dose of 5 g per 100 g of body weight.

The loss of motion activity after the administration of LMWCC in large doses could be due to their effect on the function of the digestive and other systems of the body. Indeed, at 10 hours after the administration of even a small dose (0.01 g per 100 g), 20% of the animals showed mild diarrhea, and it was absentat 24 hours, i.e. they completely recovered. However, an increase of LMWCC dose up to 0.1/100 g caused diarrhea only in 10% of animals, and at a dose of 1 g/100 it was not detected (Figure **1B**), i.e. there was a linear decrease of the effect in this range. After the administration of a "super-large" dose of LMWCC (5 g/100 g), diarrhea was detected in 75% of the animals and they recovered slowly (Figure **1B**).

Moreover, if diarrhea passed quickly at doses of LMWCC 0.01-1 g per 100 g of body weight, and it was absent in all animalsat 24 hours after the administration of LMWCC, it was accompanied by 15% of the animal death in the group that received 5 g per 100 g of body weight (Figure **1B**). Animal death was not observed in other groups (Figure **1B**).

Consequently, the components of LMWCC do not have a toxic effect and they influence on the function of the digestive system at large doses, as a result, the motion activity is suppressed in healthy animals and a part of the animals died at a very large dose (5 g per 100 g of body weight), i.e. super-large doses can show the toxicity, which is associated with a violation of the function of digestion and intoxication, and, as a result, cause the death of animals.

The Influence of Different Doses of LMWCC on some Physiological Indexesin Animals in a Chronic Experiment

As it is known, the change of body weight is an integral index of metabolic activity and it correlates well with the changes in body weight at chronic pathologies. The increase of body weight is not a constant and

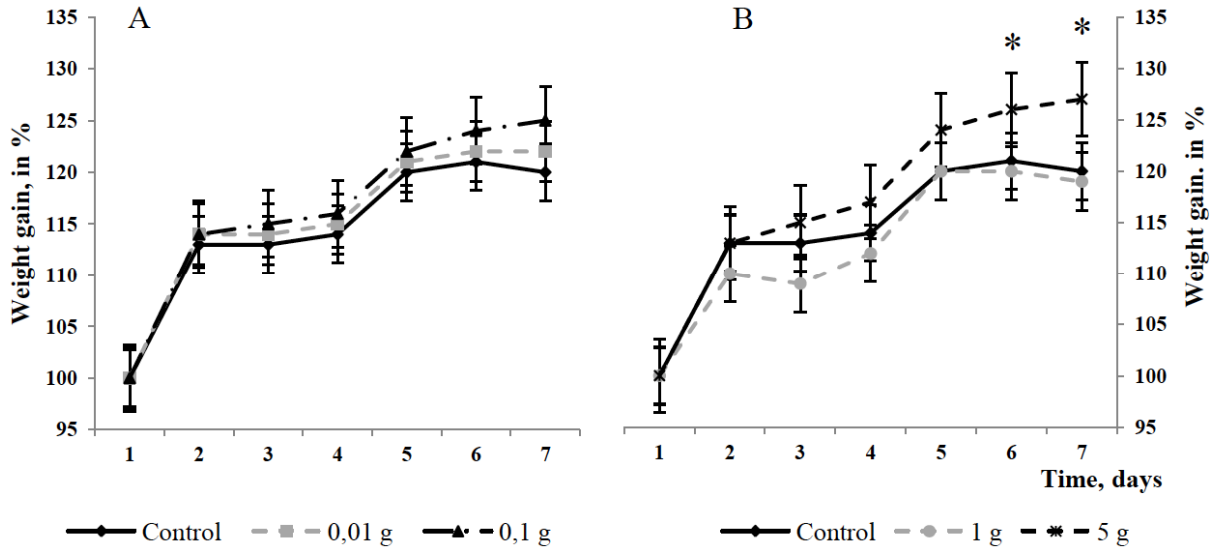

Figure 2: Weight gain as the percentage of initial which is taken as 100%. For the experimental groups: control, received distilled water at a dose of 0.5 ml for 7 days; animals received LMWCC at a dose of 0.01 g per 100 g daily for 7 days; animals received LMWCC at a dose of 0.1 g per 100 g daily for 7 days (**A**); and control, received distilled water at a dose of 0.5 ml for 7 days; animals received LMWCC at a dose of 1 g per 100 g on a single occasion, animals received LMWCC at a dose of 5 g per 100 g on a single occasion (**B**).

depends on feeding conditions, seasonality, physical activity and number of other factors [26, 27].

The control group of the 3-month animals, which were also influenced (weighing, administration of water *per os*, temperature measurement) was characterized by nonlinear growth (Figure **2A**). The non-linearity of body weight growth can be explained by the manifestation of stress reactions caused by the manipulations with animals.

The daily administrations of LMWCC at a small dose of 0.01 g/100 g of body weight did not influence on the change of body weight for 7 days (Figure **2**). An increase of the dose of LMWCC up to 0.1 g/100 g of body weight was accompanied by a slight stimulation of the animals' growth, which manifested itself on the 5^{th}-7^{th} day (Figure **2**).

Since the repeated daily administration of doses of 1 and 5 g/100 g of body weight is technically impossible because of the large volume, they were administered on a single occasion. The single administrations of dose of 1 g/100 g of body weight 2–3 days after LMWCC administration the animals growth rate decreased, but later these animals not differed from control group in growth rate. did not influence on the growth rate, and a dose of 5 g/100 g of body weight stimulated the increase of body weight to the greatest extent after a single administration at 3-7 days in comparison with the small doses, the animals also exceeded the control group by 16-17 g (Figure **2**). Therefore, different doses of LMWCC had a different effect on the growth rate of experimental animals: a daily dose of 0.01 g / 100 for 7 days did not affect the body weight of the animals, repeated doses of 0.1 g / 100 were accompanied by an increase in the body weight of animals by 5- 7 days, a single dose of 1 g / 100 was accompanied by a delay in body weight growth for 2-3 days, and later these animals did not differ from control ones, a single dose of 5 g / 100 was accompanied by stimulation of the growth of animals.

Consequently, LMWCC did not inhibit or stimulate body weight growth during 7 days of the observations, and there was no direct dependence between the dose and effect.

However, the body weight in the control group of animals increased by 33% from the initial group at 30 days after the LMWCC administration, and it increased by 53% from the initial mass at 60 days after the LMWCC administration (Table **1**).

The body weight of the animals that received the small dose (0.01 g/100 g of weight) of LMWCC did not differ from the control animals at 30 and 60 days, and those who received a dose of 0.1 g/100 g of weight exceeded the control ones at 30 days but later their body weight did not change (Table **1**).

At the same time, at a dose of LMWCC 1 g/100 g of weight the animals were behind the the control group on body mass on 18%, and they did not differ with

Table 1: Body weight of the experimental animals as percentage of the initial at 30 and 60 days after the administration of different doses of low-molecular components of the colostrums *per os*

Body weight (in grams)					
Terms after the administration of LMWCC, days	**Dose of LMWCC, g/100 g of the mass**				
	Control (0)	**0.01**	**0.1**	**1**	**5**
30	133.8 ±6.9	129.7 ±4.9	156.9 ±6.9	120.5 ±4.7	135.7 ±3.9
60	153.4 ±10.6	149.9 ±8.3	154.3 ±8.0	141.7±10.0	180.6*±15.6

control group at 30 days and exceeded the control group at 60 days at a dose of 5 g/100 g of the weight (Table **1**).

Therefore, the aftereffect of LMWCC depended on the dose. Thus, at a dose of 0.01 g / 100 g after 30 and 60 days, the body weight of the animals did not differ from the control ones, at a dose of 0.1 g / 100 g, the animals exceeded the controls after 30 days and did not differ from the controls after 60 days; at a dose of 1 g / 100 g, the animals lagged behind the control in both 30 and 60 days, and at a dose of 5 g / 100 g did not differ from the control after 30 days and exceeded the control after 60 days. Consequently, the components of LMWCC did not inhibit the growth of body weight and accelerated the growth of the animals after the cut-off of the administration, which is especially expressed at the doses of 0.1 and 5 g per 100 g of body. There is no direct connection between the intensity of body weight growth and the dose of LMWCC.

The Liver, Spleen and Kidney Mass Variation

It is demonstrated that the relative weight of the liver changes during the ontogenesis [28], at the expressed food loads [29], and various pathologies [30]. The change in the mass of organs is based on their hypertrophy (due to the proliferation or polyploidization), due to the apoptotic processes.

The administration of small doses of LMWCC to the experimental animals resulted in a slight decrease (by 15%) of the relative weight of the liver at 30 days after the last administration of LMWCC (Table **2**).

An increase of the dose of LMWCC up to 0.1 g per 100 g was accompanied by a decrease of the relative mass of this organ by 27% in comparison with the control, and an increase of the dose up to 1 g per 100 g was accompanied by a decrease even by 33% at 30 days after the administration of LMWCC (Table **2**).

A super-large dose of LMWCC (5 g per 100 g) resulted in a less expressed decrease of the relative mass of the liver in comparison with a dose of 1 g/100 g of weight. Such a non-linear nature of the change of the liver mass can be explained by the possible hypertrophy of the liver with such a large dose of LMWCC.

The relative weight of the liver was restored at 60 days after the last administration of LMWCC (Table **2**). Thus, in the group of animals receiving a dose of 0.01 g/100 g of weight, it did not differ from the control

Table 2: The change of the relative weight of the organs in percentage relative to the body weight at 30 and 60 days after the start of the first administration of low-molecular components of the colostrum

Doses of LMWCC, g/100 g of the mass	**Liver**		**Spleen**		**Kidneys**	
	30 days	**60 days**	**30 days**	**60 days**	**30 days**	**60 days**
Control	4.92 ± 0.28	4.92 ± 0.28	0.72 ± 0.05	0.72 ± 0.05	0.82 ± 0.10	0.82 ± 0.10
0.01	4.20 ± 0.09	4.86 ± 0.34	0.35* ± 0.01	0.41* ± 0.01	0.74 ± 0.02	0.72 ± 0.03
0.1	3.61* ± 0.11	4.66 ± 0.19	0.36* ± 0.02	0.32* ± 0.03	0.75 ± 0.04	0.65* ± 0.03
1	3.32* ± 0.06	4.51 ± 0.13	0.45* ± 0.03	0.44* ± 0.04	0.76 ± 0.03	0.67* ± 0.03
5	3.78* ± 0.11	4.51 ± 0.23	0.45* ± 0.05	0.41* ± 0.01	0.76 ± 0.03	0.67* ± 0.05

group, and at a dose of 0.1 g/100 g of mass it was less only by 6%, at large doses of 1 and 5 g/100 g of mass it was less only by 9% (Table **2**).

Consequently, the administration of LMWCC was accompanied by a decrease of the relative weight of the liver, while there was no direct dependence between a decrease of the relative weight of the organ and the dose of LMWCC, and the relative weight of the liver was restored to the control values at 60 days after the administration.

The spleen belongs to the immunocompetent organs and the change of its relative mass may reflect the participation of the immune system in the formation of a response to the action of xenobiotics. It turned out that its weight decreased by 52% at the administration of 0.01 and 0.1 g/100 g to the healthy animals, and only by 38% at doses of 1 and 5 g/100 g of weight in comparison with the control at 30 days after the last administration of LMWCC (Table **2**).

It had lower mass by 44% and 56% at doses of 0.01 and 0.1 g/100 g, respectively, in comparison with the control group of animals, and by 39% at doses of 1 and 5 g/100 g of the mass at 60 days after the completion of LMWCC administration (Table **2**).

Consequently, the spleen mass decreased after the administration of LMWCC and was not restored to initial values even at 60 days after the administration of LMWCC.

The relative mass of the kidneys was slightly smaller in comparison with the control animals (8-10%) at 30 days after the administration of all the studied doses of LMWCC, and even by 20% at doses of 0.1-5 g/100 g of the mass at 60 days after the administration (Table **2**).

Consequently, the repeated daily administrations of LMWCC for 7 days were accompanied by a decrease of the relative mass of the liver, spleen and kidneys. Those changes did not have the directly dependence from the dose and terms after the completion of the administration of LMWCC.

Body temperature remained unchanged after the repeated administration of LMWCC for 7 days (Table **3**). Consequently, LMWCC does not possess pyrogenicity even at super-large doses.

4. DISCUSSION

It was shown previously that LMWCC in doses of 0.1 g/100 g of a body weight have immunotropic and antioxidant properties and restore a number of physiological parameters in animals with Cu-induced liver fibrosis [15, 16].

In this work we showed that the administration of large and especially super-large doses of LMWCC (5 g/100 g) was accompanied by the manifestation of a complex of physiological responses. Thus, the small doses (0.01 g/100 g) led to a short-term inhibition of animal activity and an increase of doses led to a prolonged persistent inhibition of activity. The inhibition of motion activity correlates with the animals' aversion to food and water intake in some cases. This behavioral pattern is also due to changes in the overall metabolism. The change in metabolism and, as a consequence, the behavior of animals was due to the induction of diarrhea. As it is known, diarrhea is a symptom complex caused by various infectious and non-infectious agents [31]. Diarrhea is a clinical manifestation of impaired absorption of water and electrolytes in the intestines and disorders of intestinal motility [32].

The danger of diarrhea lies in the fact that it can lead to dehydration of the body, malabsorption, weight loss and death in the case of acute form.

Table 3: Rectal body temperature in animals of different experimental groups from 1st to 5th days after a single administration of LMWCC in different doses

The dynamics of body temperature changes (in ºC)					
Doses of LMWCC, g/100 g of the mass	**1**	**2**	**3**	**4**	**5**
Control	36.24 ± 0.15	36.42 ± 0.66	36.44 ± 0.55	36.57 ± 0.47	36.71 ± 0.24
0.01	37.00 ± 0.39	36.7 ± 0.42	37.03 ± 0.57	37.12 ± 0.63	37.24 ± 0.51
0.1	36.95 ± 0.63	37.33 ± 0.56	37.46 ± 0.54	37.59 ± 0.55	37.55 ± 0.45
1	36.79 ± 0.50	36.33 ± 0.52	36.63 ± 0.63	36.74 ± 0.15	36.61 ± 0.74
5	36.86 ± 0.40	36.59 ± 0.77	36.82 ± 0.64	36.48 ± 0.32	36.65 ± 0.81

It is known that the reception of milk is accompanied by induction and increase of diarrhea in some cases [33].

The results of this work showed a complex dependence between the dose of LMWCC and the manifestation of diarrhea. Thus, in the range of doses 0.01-1 g/100 g, the inverse dose dependence was observed (Figure **1**), and in the range of doses 1-5 g/100 the direct dependence was observed (Figure **1**). The presence of such a complex dose dependence points at the several aspects of the LMWCC effect on the body: 1) LMWCC contains various biologically active compounds; 2) the threshold concentrations that cause the biological effects will change at different doses of LMWCC, and the biological response is determined by the ratio between the components that exceed the threshold concentrations (by threshold concentration we imply the concentration at which a biological response can be recorded); 3) the causes and consequences of diarrhea may be different depending on a dose. Thus, if the cause of diarrhea can be a dysbacteriosis at a small dose of 0.01 g/100 g of animal weight (a rapid response of the intestinal microbiota even to small doses of LMWCC), then not only changes in the microbiota, but also the normalization of the water-electrolyte metabolism, which reduces the manifestations of diarrhea, can be present with an increase of LMWCC dose. At the same time, the intestinal motility was disrupted, along with the described symptoms, which greatly increased diarrhea that was accompanied by dehydration and death of some animals at super-large doses (5 g/100 g).

Therefore, different doses of LMWCC, can form a variety of response patterns for a multicomponent colostrum mixture, which leads to different manifestations of changes in the functions of the gastrointestinal tract.

Along with this, those animals that successfully tolerated diarrhea increased body weight actively and those animals that received a large dose of LMWCC increased it most of all. This shows the presence of components in the LMWCC that accelerate the overall metabolism.

It should be noted that these metabolic changes could persist for a long time. Thus, an increase in the body weight of animals that received large doses of LMWCC was manifested 60 days after the last injection of LMWCC.

We believe that such long-term effects in the change in body weight and mass of the spleen and partially of the kidneys even after a single administration may be due to the fact that the components of the colostrum induce long-lasting cycles. Of course, the detailed characteristics of the temporal response to biologically active compounds of natural origin require special studies, but this deserves special attention.

Reducing the mass of the liver, spleen and kidney after the administration of large doses of LMWCC can also be associated with various causes, for the elucidation of which special investigations are required. However, the data already obtained indicate significant changes in the functional characteristics of these vital organs after receiving large doses of LMWCC. These functional changes persisted long enough for more than 2 months for the spleen and kidneys after a single injection of LMWCC.

The results show that even the natural products with high biological activity can deeply reorganize metabolism and require careful examination.

The super-large dose (5 g / 100 g of weight) causes diarrhea in 75%, which is accompanied by the death of 15% of animals due to dehydration and intoxication. Also, this dose caused a decrease in the mass of the liver, spleen and kidneys, which slowly (at least 2 months) were restored. LMWCC do not have chronic toxicity and can be attributed to non-toxic compounds with a possible side effect - a violation of the digestive system.

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