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# INFLUENCE OF COMPLEX MICROBIAL PREPARATION ON PRODUCTIVITY AND CLINICAL HAEMATOLOGICAL STATUS OF RABBITS KITS

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***Abstract***

*The research aimed to study the influence of a bioactive compex microbial product, obtained from yeast biomass from beer and wine industry wastes and residual cyanobacterial biomass (spirulina) after production of medicinal remedies, on the productive parameters, health and clinical haematological status of recently weaned rabbit kits. The experiment was carried out on 3 batches of 14 kits from weaning to about 80 days of age. The rabbits in two experimental groups were supplemented with proposed preparation at doses of 3.0 and 9.0 g per 1 kg of concentrated feed per day. The use of the complex microbial preparation in the daily ration has a beneficial effect on the rabbit kits, evidenced by the general health status of the animals, reduction in the number of leukocytes and lymphocytes in the blood and the increase in the number of segmented neutrophils.*

***Key words****: clinical haematological status, haematopoiesis, microbial preparation, productive parameters, rabbits.*

## INTRODUCTION

In recent years, rabbit farming (cuniculture) has emerged as a relatively new sub-branch of the livestock sector, which contributes essentially to the implementation of one of the most difficult tasks of the national economy - ensuring the population with products of animal origin. In addition, rabbit meat is a food product with extraordinary nutritional qualities, that is in high demand by the people, as well as is recommended to the sick persons. (Macari, 2019; Macari et al., 2017). The phenomenon of vertiginous development of rabbit farming, including Republic of Moldova, might be explained by the physiological and metabolic properties of rabbit regarding growth rate, reproduction, especially high prolificacy, accessibility of maintenance and exploitation.
However, it is widely accepted that the animal husbandry, particularly of rabbits - scarry animals, under intensive conditions can be influenced by the impact of stressors, considered unavoidable on modern livestock farms, which can negatively impact the growth process. In this context, the researchers in the fields of biology, biotechnology, veterinary medicine, animal husbandry are studying possibilities to develop and test biologically active products that are safe to animals, humans and the environment (Galip & Seyidoolu, 2012; Rotaru, 2016).
The essence of the present research was to find more knowledge and to evaluate the impact of a biologically active microbial preparation complex (CMP-4) on some parameters of clinical and haematological status, health and productivity in recently weaned young rabbits.

## MATERIALS AND METHODS

The applied research, focused on testing (CMP-4), was carried out at the rabbit farm "Mațencu Dmitrii" from the village of Braviceni, district Orhei, Republic of Moldova, on recently weaned young rabbits. The study was conducted on 3 batches of rabbit kits. The following Table 1 demonstrates the principle of the experiment.

Table 1. The scheme of CMP-4 administration to rabbit kits

| Batch | Number of rabbit kits | Route of administration | Dose of administration, g/kg feed supplement | Mode of administration |
|---|---|---|---|---|
| CB | 14 | - | - | |
| EB 1 | 14 | peroral | 3.0 | daily |
| EB 2 | 14 | | 9.0 | |

The object of the research was a natural complex microbial preparation (CMP-4) that contains biologically active compounds obtained from *Saccharomyces* yeast biomass from wine and beer production and *Spirulina (Arthrospira*) *platensis* biomass.

CMP-4 presents a combination of 6 biologically active extracts, 2 [β-glucan (LB-GL)] and aminoacid protein (LB-AAP) from yeast of beer production (Beşliu et al., 2022; Chiselita et al., 2022), 2 [β-glucan (SRM-GL)] and aminoacid protein (SRM-AAP) from yeast of wine production (Chiselita et al., 2023) and 2 [peptidoglycan (PPGE) and polysaccharide (PS)] from cyanobacterial biomass.

CMP-4 is obtained by the mixing of PPGE with LB-GL and SRM-GL, the gradually introducing of LB-AAP, SRM-AAP and PS in this mixture and drying at +45-50˚C for 24-48 hours. CMP-4 is a solid, dark green, spirulina-smelling liquid product with 97.08 ± 0.23% DW. and moisture of 2.92 ± 0.23%, which containins not less than 550 mg/g PPEG, 100 mg/g LB-GL, 100 mg/g SRM-GL, 100 mg/g LB-AAP, 30 mg/g SRM-AAP and 25 mg/g PS.

Yeast extracts were obtained from yeast (*Saccharomyces*) from the wastes of the production of beer and red Merlot wine. PPGE was obtained in the same technological flow from dry cyanobacterial biomass, residual after obtaining of some peptide extracts and represents the insoluble sediment, remaining after the extraction of pigments, lipids and proteoglycans, which was dried in an oven at a temperature of 50 ± 5°C to a constant mass with 7-8% moisture and grounded to a powder.

Due to its complex composition, CMP-4 contains a broad spectrum of biologically active substances - proteins, including essential and immunoreactive amino acids, β-glucans, sulphated polysaccharides, pigments, especially anthocyanins and β-carotene, macro-, microelements. It possesses a total antioxidant activity of 39.8 ± 0.15% inhibition (ABTS) and antioxidant enzymes catalase (CAT) of 813.85 ± 42.66 mmol/min/mg protein and superoxide dismutase (SOD) of 92.71 ± 2.86 U/mg protein.

CMP-4 was introduced into the basic feed using the granulator, intended for the production of dense granules. Thus, 3 type of granulated feeds were produced for rabbit kits. The feed intended for the control batch (Control batch CB) contained only basic feed, and the feed intended for the experimental batch 1 (EB 1) and the experimental batch 2 (EB 2) additionally contained CMP-4 at a concentration of 3.0 and 9.0 g per 1 kg (0.3 and 0.9%/V), respectively. The feeds were administrated to the respective batch from weaning until slaughter (Table 1).

During the experiment the young rabbit kits were continuously monitored and, periodically, body mass was measured by individual weighing. Body temperature, heart and respiratory rate per minute were determined for 5 rabbits from each batch at the start of the study, and, subsequently, throughout the period of investigation.

For laboratory tests, blood samples were taken from rabbits in two stages: at the beginning of the study, until the administration of the tested remedy, from five rabbits randomly selected, and at the end of the study, at about 80 days of age, already from 5 animals from each group. It is also important to mention that during the handling of the rabbit kits, attention was also paid to their behaviour and possible adverse reactions following the use of CMP-4.

Animals used in study of the same breed, age, body mass were housed in the same shelter, at the similar hygienic parameters. The haematological constants, determined in the blood of rabbit kits, were: Haemoglobin concentration- HgB, g/l; Total erythrocyte count- RBC, 1012/l; Haematocrit content - HCT, % - indicator representing erythrocyte volume fraction; MCV, fl - mean corpuscular volume; MCH, pg - mean erythrocyte haemoglobin; MCHC, g/l - mean erythrocyte haemoglobin concentration; total leucocyte count - WBC, 109/l. Blood samples were read on a haematological analyser: Myndrey 500. Determined marker parameters of leukocyte formula were analysed by microscopic examination with immersion objective of blood smear stained by the usual method - Romanovsch.

Statistical evaluation of clinical and haematological indices was performed using the parametric t-Student criterion with $P<0.05$.

## RESULTS AND DISCUSSIONS

The obtained results demonstrated that the administration of CMP-4 to rabbit kits for a period of about 40 days did not cause any changes or deviations from the normal physiological state of the animals, which was confirmed by the physiological and metabolic tests, bio productive and haematological indices of the rabbits. The body temperature is the basic parameter of the animals' health status that is easy to monitor. The value of this clinical parameter at the beginning of the experiment was 39.51 ± 0.10°C in rabbits from the control batch (CB), +39.50 ± 0.06°C in rabbits from the experimental batch1 (EB 1) and 39.53 ± 0.06°C in rabbits from the experimental batch2 (EB 2). The values of baseline respiratory rate of rabbits of all three groups at the beginning of the study were within the range of 87.39-87.51 breaths/min and averaged heart rate - 157.08-158.54 beats/min. These results, indicate on the high animal health status in all batches on the onset of research.

The changes of haematological indices at intact rabbit kits from CB, EB 1 and EB 2 is demonstrated in Table 2.

At the end of the study red blood cell count (RBC) demonstrated a trend to increase in EB 2 by about 3% compared to the control and by 10.7% compared to the values recorded in EB 1. Haemoglobin and hematocrit levels showed lower values in EB 1 experimental batch (Table 2).

The mean corpuscular volume (MCV) demonstrated an increasing trend at rabbit kits from the control batch, constituting an increase of 3.1% compared to baseline values. Towards the end of the experiment, the tested product manifested a beneficial effect, characterized by a decrease of the MCV value by 2.0-4.8%, compared to the control (Table 2).

Table 2. Indices of haematological parameters in rabbit kits under the influence of CMP-4 (M ± m) at the start and the end of experiment

| Parameter (the end of experiment) | Baseline values | Batch of animals | | |
|---|---|---|---|---|
| | | CB | EB 1 | EB 2 |
| RBC, $10^{12}$/l | 5.35±0.26 | 5.43±0.20 | 5.05±0.45 | 5.59±0.23 |
| HgB, g/l | 112.00±2.56 | 116.80±1.52 | 108.60±6.99 | 115.40±2.26 |
| HCT,% | 34.76±0.93 | 36.26±0.36 | 33.00±2.13 | 35.64±1.12 |
| MCV, fl | 65.12 ±1.69 | 67.14±2.42 | 65.78±1.74 | 63.90±1.47 |
| MCH, pg | 21.04±0.62 | 22.06±0.72 | 21.60±0.59 | 20.66±0.45 |
| MCHC, g/l | 323.00±1.70 | 323.00±2.32 | 328.80±0.89 | 327.00±7.15 |

Similar results were also recorded at rabbit kits, treated with the injectable remedy BioR (Macari, et al., 2017), as well as in rabbit kits, whose feed was supplemented with the product - ZooBioR (Macari, 2019). The possibility of decreasing of the MCV value in rabbit blood by the utilization of different biologically active products has been reported by some researchers (Ewuola et al., 2012; Ojokuku et al. 2011). Similar changes, of the investigated parameter were found in rabbits raised in a non-polluted area, compared to those raised in intensely polluted area (Kashapova, 2007).

It was established that mean corpuscular haemoglobin (MCH) values in the experimental groups were with 2.1-6.3% lower than the control, while the mean corpuscular haemoglobin concentration (MCHC) parameter values were with 1.3-1.8% higher than the control values, an undeniably beneficial phenomenon, revealing enhanced haematopoiesis in rabbit kits from both experimental groups (Table 2). A high level of MCHC was also found in rabbits receiving other bioactive remedies (Ojokuku et al., 2011; Galip & Seyidoolu, 2012; Macari, 2019). Thus, our results indicated that CMP-4 has positive impact on hematopoietic function and nonspecific resistance in rabbit kits.

The role of evaluation of the action of test product at the cellular level through the determination of the absolute number of leukocytes and the highlighting of certain aspects in the leukocyte formula cannot be underestimated. The dynamics of leukocytes and the basic components of the leukocyte formula in rabbit kits under the influence of CMP-4 are presented in Table 3.

Leukocyte count (WBC) towards the end of the experiment has demonstrated an increasing trend in rabbit kits from all batches compared to baseline values. However, at the end of the study in rabbit kits from CB the WBC value in the blood was 12.94 ± 2.19 x $10^9$/l, when in EB 1 and EB 2 this value has decreased to 10.21-11.27 x $10^9$/l, which represent a reduction with 12.9-21.1% (Table 3). The decrease in the total number of white blood cells in both experimental groups can be considered beneficial, as it testifies to reduction of the negative impact of stress, especially technological stress, persistent in modern rabbit breeding and farming and, also, to the anti-stress and adaptogenic properties of the tested product. Similar blood leukocyte dynamics have been reported by other authors which have tested various bioactive remedies on animals (Pistol et al., 2021; Curca et al., 2014).

Rabbit lymphocytes from CB (control) and EB 1 group exhibited an increasing trend compared to baseline values, with a maximum lymphocyte count of 63.40 ± 8.00% in CB rabbits, which was 15.7% more than baseline. At the same time, the values of this haematological indicator of rabbits from EB 1 and EB 2, which received different doses of CMP-4, are lower compared to the values recorded in CB group, the decrease being 5.0-14.5% (Table 3). Similar results of blood lymphocyte reduction in rabbit kits blood treated with another bioactive remedy obtained from *Spirulina platensis* were reported (Macari, 2019). Thus, the decrease in blood lymphocytes in EB 1 and EB 2 groups and the dynamics of other haematological indices, highlight the increase of non-specific resistance in rabbit kits blood under the impact of CMP-4 product.

Table 3. Dynamics of leukocytes and basic components of the leukocyte formula in rabbit kits at the administration of CMP-4 (M ± m)

| Parameter(the end of the experiment) | Baseline values | Batch of animals | | |
|---|---|---|---|---|
| | | CB | EB 1 | EB 2 |
| WBC, $10^9$/l | 8.89±0.89 | 12.94±2.19 | 11.27±2.00 | 10.21±0.79 |
| Limfocyte, % | 54.80±0.57 | 63.40±8.00 | 60.20±10.92 | 54.20±0.84 |
| Nonsegmented neutrophils,% | 3.60±0.91 | 3.20±0.67 | 2.60±0.70 | 3.80±0.89 |
| Segmented neutrophils,% | 33.00±4.87 | 29.00±2.83 | 33.20±3.40 | 36.60±4.34 |
| Eosinophil, % | 0.60±0.27 | 1.00±0.00 | 0.80±0.22 | 0.80±0.22 |
| Monocyte,% | 8.00±1.77 | 3.40±1.41 | 3.20±1.14 | 4.60±1.74 |

Unsegmented neutrophils did not manifest essential changes, remaining at a practically constant level throughout the study in animals from all study groups (Table 3).

Segmented neutrophil values in CB group of rabbits towards the end of the experiment has demonstrated a weak decreasing trend compared to baseline values. At the same time, the values of these indices in EB 1 and EB 2 groups were higher by up to 14.5 and 26.2% respectively compared to the values determined in CB group (Table 3). Similar effect was established at the administration of medicinal preparations obtained from spirulina, a study in which the haematological analysed index was with 6.9-27.1% higher compared to the values of the control group (Macari V., 2019). Supplementation of the feed ration of EB 1 and EB 2 rabbits with CMP-4 did not induce at the end of the study a significant difference in the number of eosinophils in the blood of rabbit kits compared to the CB control (Table 3). This effect can be considered beneficial as it highlights the harmlessness of CMP-4 to the rabbit kits.

Blood monocyte levels decreased with age of rabbits from all groups trained in the experiment compared to baseline values. Thus, in CB group the number of monocytes decreased by 2.4, in EB 1 by 2.5 and in EB 2 by 1.7 times compared to baseline values. At the same time, the number of monocytes in EB 2 rabbits averaged 4.60±1.74%, which was with 35.3% more than CB and 43.8% more than EB 1 groups. Based on the obtained results, it can be mention that CMP-4, administered daily with the feed during the period of intensive rearing and fattening, was well tolerated by rabbits, demonstrated beneficial effect on haematopoiesis and non-specific resistance of the animals.

The investigation of zootechnical parameters has an important role in clarifying of the effect of CMP-4 at the rabbit organism. The dynamics of body weight indices in rabbit kits under the influence of CMP-4 are presented in Table 4.

The results demonstrated that CMP-4 had a beneficial effect on the growth and development of rabbit kits in EB 1 and EB 2. Thus, at the age of 50 days, the body weight of CB rabbits was in average 1243.94 ± 11.13 g with an addition of 384.7 g during 10 days of experiment. During the same period the weight gain of EB 1 and EB 2 rabbits was 435.0 and 420.0 g respectively and body weight was 1294.18 ± 6.36 and 1279.96 ± 16.48 g respectively, which was with 13.1 ($p<0.001$) and 9.2% more than CB values (Table 4). It must be mentioned that in the condition of stress for the animals, 10 days after weaning, the body mass of EB 1 rabbits fed with the minimum dose of CMP-4 - 3 g/kg feed, was with 3.6% higher compared to the mass of EB 2 rabbits fed with CMP-4 - 9 g/kg feed (Table 4).

At 70 days of life the growth rate in rabbits remained practically similar to previous periods. Finally, at 83 days of age, the body weight of CB rabbits was 2834.75 ± 28.26 g and that of EB 1 and EB 2 was significantly higher ($p<0.001$) by 114.4-154.1 g or 4.0-5.4% compared to the baseline (Table 4).

The indicator - total gain/period/rabbit, shows an increasing trend in EB 1 and EB 2 group. Thus, if in CB rabbit group the total gain during the experimental period was 1975.55 g, then in EB 1 and EB 2 group, whose feed was supplemented with CMP-4, it was 114.4-153.4 g or 5.8-7.8% higher than the reference value and constitutes 2089.93 and 2128.92 g, respect-tively. It can be mentioned that the total gain of rabbits in EB 2 was also with 1.9% higher than the values recorded in EB 1 (Table 4).

Table 4. Dynamics of body weight indices in rabbit kits under the influence of CMP-4 (M ± m)

| Parameter | Batch of animals | | |
|---|---|---|---|
| | CB | EB 1 | EB 2 |
| Number of animals/baseline, heads | 14 | 14 | 14 |
| Body weight - baseline experiment (40 days), g | 859.20±4.66 | 859.22±3.93 | 859.96±3.23 |
| Body weight - 50 days, g | 1243.94±11.13 | 1294.18±6.36*** | 1279.96±16.48 |
| Body weight - 60 days, g | 1553.82±14.39 | 1675.21±15.51*** | 1687.66±9.77*** |
| Body weight – 70 days, g | 1937.75±7.62 | 2067.49±14.78*** | 2080.60±8.47*** |
| Body weight - 83 days, g | 2834.75±28.26 | 2949.15±10.84*** | 2988.88±18.84*** |
| Total gain/period/rabbit - 43 days, g | 1975.55 | 2089.93 | 2128.92 |
| The average daily/period gain (43 days), g | 45.94 | 48.60 | 49.51 |
| Viability, % | 100 | 100.0 | 100.0 |

**Note:** ** - P<0.01; *** - P<0.001

The average daily/period gain in CB rabbits was 45.94 g, and in EB 1 and EB 2 group was 48.60 and 49.51 g respectively, which was with 5.8% and 7.8% more. Amany et al. (2017) in a study conducted on rabbits found that the highest non-significant weight gain was associated with feed supplementation with dried taro (*Colocasia esculanta*) waste at (0.0, 7.5, 15 and 22.5%) and in combination with dried yeast (*Saccharomyces cerevisiae*, 0.5%) at the age of 6-14 weeks. Supplementation of rabbit feed with biologically active remedies, was studied by Macari et al. (2019). The researchers investigated the effect of ZooBioR, a spirulina product, on productivity of rabbit kits. The rations were supplemented with 2.5, 5.0 and 10.0 mg/kg feed. There was a significant effect on animal performance. The weight of a rabbit at 80 days of age in the experimental groups was 114.3-264.3 g, or with 3.9-8.5% higher than the control group ($P<0.01$, for experimental group 2, ZooBioR dose of 5.0 mg/kg fodder).

The obtained results indicated that the administration of the complex biologically active microbial preparation CMP-4 in the daily feed ration of rabbit kits at the rate of 3 or 9 g per 1.0 kg of feed (0.3 or 0.9%/V) for 43 days has increased the productivity (body weight) of animals, has had an anti-stress and hematopoietic stimulating effect, confirmed by haematological blood indices.

## CONCLUSIONS

Rabbit farming is a relatively new branch of animal husbandry, and rabbit breeding and exploitation can be successfully carried out under modern animal husbandry conditions, with the application of advanced technologies, which, providing benefits, undeniably also generate stressors with negative impact on the health and welfare of the animals. As a result, the development and testing of new remedies with anti-stress and bio stimulatory properties is becoming a key priority of modern science. In this context, complex undertaken studies have highlighted a new biologically active, environmentally friendly remedy of microbial origin. The elaborated complex has had beneficial effect on the bioproductive parameters of rabbit kits, also, possessed anti-stress and haematopoiesis-stimulating effect in animals, which was confirmed by zootechnical and haematological indices.

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