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THE CURATIVE EFFICIENCY OF THE CMP-3 IN AVIAN CLOSTRIDIOSIS

Mariana CARAMAN[1], Natalia CHISELIȚA[2], Oleg CHISELIȚA[2], Igor PETCU[1], Oleg MASHNER[1]

[1]Scientific and Practical Institute of Biotechnologies in Zootechny and Veterinary Medicine, Anenii Noi Region, Maximovca Village, Republic of Moldova
[2]Technical University of Moldova, Institute of Microbiology and Biotechnology, Chisinau, 1 Academiei Street, Republic of Moldova

Corresponding author email: mcaraman994@gmail.com

Abstract

The aim of the work was to study the curative-prophylactic efficiency of the complex biologically active microbial preparation and its effect on some growth and development indicators of chickens. The preparation was included in a proportion of 40 g/kg feed, in the composition of the daily ration of the chickens in the experimental group, starting from the first day of life. At the age of 17 days, an infection with Clostridium perfringens was registered in the chickens from both groups, which was treated with the Bromex preparation. As a result of the daily administration in the ration of the complex biologically active microbial preparation, the viability of 100% of the chickens in the experimental group was ensured compared to 72% in the control group; decreasing the titer of pathogenic bacteria E. coli, Enterococcus spp., Clostridium spp. by 6.5-10.6%, in the gastrointestinal tract; increasing the titer of beneficial microorganisms Bifidobacterium spp., Bacillus spp. and yeast fungi by 3.6-15.4%; the increase in the body weight of the chickens at the end of the experiment by 25.5% compared to the control.

***Key words**: chicken, complex microbial preparation, avian clostridiosis.*

INTRODUCTION

Avian clostridiosis (anaerobic enterotoxemia, necrotic enteritis) is an acute anaerobic infection caused by *Clostridium perfringens*, types A, C and D (Hutu et al., 2006; Parent, 2008).
C. perfringens is part of the obligate microflora of the large intestine and can be isolated from both sick and healthy birds (Cooper & Songer, 2009; Karim & Khaled, 2013). The bacteria are excreted by the birds in droppings and can be isolated from the walls and ventilators of the bird shelter, from workers ' boots, from feed and litter (Greenham et al., 1987; Craven, 2000; 2001; Parent, 2008; Hafez, 2011).
For many decades, avian clostridiosis was considered a sporadic disease with a minor economic impact.
Currently, it is one of the emerging diseases that threaten poultry farms, due to low productivity, increased mortality, as well as the risk of contamination of products of avian origin intended for human consumption (Hafez, 2011).
The disease occurs in chickens older than 15 days, more frequently in those increased on the ground. The evolution of the disease is favored by nutritional factors (undernutrition, overnutrition), various components of the ration, such as fish meal, wheat or barley flour, high cellulose content or change in the fodder ration; environmental factors (humidity, cold, excessive heat); some diseases with a digestive localization (some bacterioses, viruses or parasitoses) or changing chemotherapeutic preparations (Hutu et al., 2006; Parent, 2008).
Over several decades the monitoring of avian clostridiosis and, in generally, bacterial diseases in poultry youth was carried out by using antimicrobials in water or feed (Dibner & Richards, 2005). But, the intensive use of antibacterial preparations have increased the resistance of pathogens to them (Parent, 2008).
Contemporary approaches to the treatment of avian clostridiosis tend to limit the use of antibiotics in poultry farming and a complete ban on some antibacterial preparations. For these reasons, the identification of some phytogenic and microbial additives, organic

acids, probiotics, prebiotics etc. with immunomudalatory and antibacterial activity (Caly et al., 2015), which could completely replace the use of antibiotics for the control of *C. perfringens* and associated diseases remains paramount for specialists in the field (Thanissery et al., 2010; Agunos et al., 2017; Blake et al., 2021).

The purpose of the work was to study the efficiency of the complex biologically active microbial preparation on growth and development indicators of chickens and curative-prophylactic efficiency in preventing and combating some pathologies of bacterial origin that frequently affect chickens in the first weeks of life.

MATERIALS AND METHODS

As objects of research served: 50 chickens of the breed Lohmann brown 1 day old and the complex microbial preparation (CMP-3).

CMP-3, is a natural microbial complex containing biologically active compounds, derived from *Spirulina (Arthrospira) platensis* biomass and *Saccharomyces* yeast biomass, is a combination of three biologically active extracts, two cyanobacterial: lipid extract (LE), peptidoglycan extract (PPGE) and a mannoprotein extract RSM-MP obtained from yeast biomass from sediments from the production of red *Merlot* wine (Beşliu et al., 2022).

LE and PPGE are obtained in the same technological flow with two other products from dry cyanobacterial biomass, residual after obtaining some peptide extracts. LE is obtained by extraction with 96% ethyl alcohol, biomass: extractant ratio 1:2 v/v, at a temperature of 60˚C for 1 hour with periodic stirring from the remaining biomass after the extraction of pigments.

PPGE represents the insoluble sediment, remaining after the extraction of pigments, LE and proteoglycan extract, which is dried in an oven at a temperature of 50 ± 5˚C to a constant mass with 7-8% moisture and ground to powder.

CMP-3 is obtained by gradually imbibing of LE and RSM-MP in PPGE and drying at 50 ± 5˚C for 24-48 hours. CMP-3 is a dark green powder with 97.47 ± 0.12% d.w. and moisture of 2.53 ± 0.12%, containing not less than 800 mg/g PPGE, 100 mg/g RSM-MP, 70 mg/g LE. Due to its complex composition, CMP-3 contains a wide spectrum of biologically active substances - amino acids and proteins, lipids, including phospholipids and diglycerides, polysaccharides, including mannoproteins and sulfated polysaccharides, pigments, especially anthocyanins and β-carotene, macro-, microelements and possesses a total antioxidant activity of 35.2 ± 0.9% inhibition (ABTS) and of the antioxidant enzymes catalase (CAT) of 826.91 ± 3.04 mmol/min./mg protein and SOD of 71.66 ± 1.64 U/mg protein.

RSM-MP, which is a part of CMP-3, possesses significant antioxidant properties, CAT (catalase) and SOD (superoxid dismutase) activities and nutritional value, which is evidenced by the content of essential and immunoactive amino acids, the presence of macro-, microelements and anthocyanins (Besliu et al., 2022; Chiselita et al., 2023).

Microalgae and cyanobacteria represent a natural source rich in compounds with biological activity which could be used as functional ingredients (Maddiboyina B. et al., 2023). Their valuable biochemical composition, high quality proteins, balanced profiles of fatty acids, vitamins, antioxidants, minerals and their useful properties can be applied in the formulation of new products (Spolaore et al., 2006).

The chickens selected for the experiment had the average veight 36.10 ± 2.00 g, body development and were free clinicall healty.

During the research, the following technological parameters were respected: density of placement of chickens at $1m^2$ of surface; light and temperature regime according to age; nutrition regime according to age; water at discretion.

The experiment took place under the conditions of the laboratory Methods of Disease Control and Prophylaxis of the Scientific and Practical Institute of Biotechnologies in Zootechny and Veterinary Medicine of the Republic of Moldova.

The scheme of the experiment is presented in Table 1.

Table 1. Scheme of the experiment

Specification	Control batch	Experimental batch
Biological material	1 day old chickens	1 day old chickens
Number of chickens	25	25
Duration of the experiment, days	28	
CMP-3 inclusion dose, %	-	4

The chickens were fed with combined fodder balanced in proteins, vitamins, minerals and amino acids in accordance with age requirements.

In the composition of the combined feed, intended for feeding chickens from the experimental batch, in the basic feed, 4% of the quantity of fodder yeast was substituted by the same amount of CMP-3 (1 kg basic feed: 40 g CMP-3).

The feeds were administered to the chickens of the corresponding batches, daily for 28 days.

The composition of the feeds used in the experiment and their nutritional value is shown in Table 2.

Table 2. Composition (%) of the combined feed used in the experiment and its nutritional value

Components, %	Combined feed	
	Control batch	Experimental batch
Maize	54	54
Soy meal	22	22
Wheat	13	13
Feed yeast	5	1
Sunflower meal	3	3
CMP-3	0	4
Premix	2	2
Fodder calcium	1	1
Metabolic energy, kcal/kg feed	2703	2698
Crude protein, %	22.1	21.9

The clinical condition of the chickens from both groups was monitored daily by assessing the general condition, behavior, consumption of food and water, consistency of fecal masses, appearance of plumage and cloacal region.

Bacteriological study of feces and sensitivity of *C. perfringens* to antimicrobian preparations was performed according to the usual methods (Licker et al., 2019; Carp-Cărare et al, 2014).

For microbiological investigations were used: culture media such as Nutrient Agar, HiCrome *E. coli* Agar, HiCrome Bacillus Agar Base, HiCrome Endo Agar, HiCrome Kligler Iron Agar, HiCrome Streptococcus Lactis Differential Agar, Anaerobic Agar, MRS Agar, HiCrome Candida Differential Agar, HiCrome Sabouraud Dextrose Agar; putties for staining smears according to the Gram method etc.

The dynamics of the body mass of the chickens from both groups was assessed by individual weighing.

The therapeutic efficacy of CMP-3 on *C. perfringens* was established based on the clinical status of the chickens, bacteriological results of the droppings, recovery dynamics and weight gain.

RESULTS AND DISCUSSIONS

In poultry factories, for several decades, the administration of antibacterial preparations has been practiced, for the prophylaxis of potential disease outbreaks (Starciuc et al., 2018), due to the intensive breeding system limited space for exploration of chickens, closed sheds etc. (Kumar & Patyal, 2020).

In the experiment carried out, in the first days of the chickens' life, no antimicrobial preparations were administered because the goal was to determine the curative-prophylactic efficiency of the CMP-3 preparation in the case of the evolution of bacterial pathologies (colibacillosis, salmonellosis, clostridosis etc.) specific for this age period.

Initially, at the age of one day, the chickens from both groups weighed 36.10 ± 2.00 g, were apathetic, consumed little feed and water. This condition was determined by transport stress. Already the next day the condition of the chickens improved.

In the age period 1-16 days, a positive dynamic of weight gain was observed in the chickens from the experimental batch, favored by the consumption of the combined feed with the addition of CMP-3. Thus, on the 15th day of the experiment, the body weight of the chickens in the experimental batch exceeded that of the control batch by 18.21%.

At the age of 17 days, an infection with *C. perfringens* was registered in the chickens from both groups. To determine the path of infection of the chickens with the pathogen, the water used in their daily consumption, the feed and the dust in the room were examined bacteriologically. The cause of infection was determined to be compound feed containing 3.2×10^7 CFU/g *C. perfringens*.

For these reasons, both contaminated feeds were excluded from the chicks' feed and substituted with microbiologically qualitative equivalent feeds, which were then administered to the corresponding groups throughout the duration of the experiment.

Clarke (2007) mentions that trypsin inhibitors commonly found in soybean meal provide an ideal environment for the development of *C. perfringens*. Timbermont et al. (2011) described the risk of developing necrotic enteritis in chickens fed cereals with a high digestible fiber content, such as oats, barley, rye and wheat. At the same time, the incidence of necrotic enteritis in chickens fed with protein-rich rations is ten times more frequent compared to those fed corn-based rations (Cooper & Songer, 2009).

In the food rations used in the experiment, soybean meal constituted 22%, being a valuable source of protein and energy necessary for the growth and development of chickens. Thus, there is a high probability that this component favored the increase in the amount of the pathogen in the combined feed used in our experiment.

According to the results of the specialists in the field, the prevention of avian necrotic enteritis consists in controlling the risk factors, one of which is the protein-rich ration (Parent, 2008; Hafez, 2011).

Another predisposing factor of avian clostridiosis is invasion by coccidia (Avi et al., 2023; Rodgers et al., 2015; Timbermont et al., 2011). In young birds, coccidiosis frequently precedes cases of clostridiosis (Timbermont et al., 2011).

In the experiment carried out, the invasion with coccidia was not observed, neither in the chickens from the control batch, nor from the experimental one. The extent of invasion with *Eimeria* spp. oocysts was 8% in both batches, and the intensity of invasion was 0-2 oocysts in the field of view of the microscope.

In the control batch, clinically, the disease manifested itself with diarrhea, dehydration, greenish-white feces with a fetid odor, dyspnea, drowsiness, ataxic gait. The same clinical signs in chickens are described by Cooper et al. (2013).

On the 3rd day of manifestation of signs of disease were detected 4 corpses, and on the 4th-5th day another 3 corpses. Morbidity was 100%, mortality - 28 %. Thus, 7 chickens died in the control batch, and the remaining ones lost significantly in weight compared to the chickens in the experimental batch.

According to Uzal et al. (2014), *C. perfringens* strains can produce up to 17 different toxins, and necrotic enteritis induced by *C. perfringens* in chickens leads to sudden death, with mortality rates of up to 50% (Lee et al., 2011).

In the experimental batch, clinical, the disease manifested itself with apathy, 9 chickens presented inappetence and diarrhea. Morbidity constituted 36 %, mortality - 0%.

Hutu et al. (2006) describes avian clostridiosis in chickens over 15 days old, with very rapid evolution (1-5 days), or chronic (7-30 days), with the following symptoms: inappetence, drowsiness, cyanotic crest, hemorrhagic diarrhea, or feces frothy, and in chronic forms, diarrhea with greenish-white feces and fetid smell.

Hafez (2011) specifies that infections with *C. perfringens* in poultry can cause several clinical manifestations and lesions include necrotic enteritis, necrotic dermatitis, cholangiohepatitis as well as gizzard erosion. However, subclinical infection can take place too.

At the necropsy examination of some corpses, catarrhal-hemorrhagic enteritis was found, and in others, intestines with thickened walls and gas bubbles. No pathological changes were observed in the internal organs, although some authors describe *C. perfringens* is also associated with fibrosing hepatitis (Craven, 2000).

Hutu et al. (2006) mentions that at the necropsy examination, the main lesions are found in the anterior portion of the intestine, which presents catarrhal-hemorrhagic and necrotic enteritis; dilated intestine; ulcers covered with fibrin; peritonitis.

Clostridiosis of chickens from both groups was treated with bromex preparation containing 200 mg/mL enrofloxacin and 15 mg/mL bromhexine HCl. Enrofloxacin is a chemotherapeutic with a broad spectrum of antimicrobial action, part of the last generation of synthetic quinolones, with a notable bactericidal activity on Gram positive and Gram negative bacteria. Compared to the rest

of the quinolone group, enrofloxacin has less toxicity.
C. perfringens showed a sensitivity zone to bromex of 26 mm, to enrofloxacin of 20 mm and to amoxicillin of 18 mm.
Bromex was administered in water, 0.3 ml per litre, for 5 consecutive days.
The clinical condition of the chickens in the experimental batch improved on the 3rd day of treatment, and those in the control batch on the 7th day. The chickens became active, began to consume the feed, droppings became consistent, their color normalized.
Chickens in the experimental batch went through the infection more easily, survived completely and the recovery period was faster.
In the control batch the recovery of the chickens was uneven and more later.
As a result of the study of the microbiological composition of the droppings of the chickens from both groups, an increase in the titer of the total number of germs (NTG) by 0.3 log CFU/g (3.5%), the titer of the beneficial microorganisms *Bifidobacterium* spp. (P<0.001), *Bacillus* spp. (P<0.05) and yeast fungi was recorded by 0.3-1.5 log CFU/g and the decrease by 0.6-1.0 log CFU/g of the titer of pathogenic and conditionally pathogenic microorganisms *E. coli* (P<0.001), *Enterococcus* spp. (P<0.05), *Clostridium* spp., in the gastrointestinal tract of chickens from the experimental group, compared to the control group (Table 3).

Table 3. Titer of strains of microorganisms in chicken droppings from the control and experimental batch at the end of the experiment, log UFC/g

Indicators	Control batch	Experimental batch
NTG	9.8±0.04	10.1±0.05
E. coli	9.5±0.04	8.5±0.07***
Enterococcus spp.	9.3±0.23	8.3±0.05*
Clostridium spp.	9.3±0.30	8.7±0.02
Lactobacillus spp.	9.8±0.15	9.6±0.07
Bifidobacterium spp.	9.2±0.04	10.7±0.07***
Bacillus spp.	9.5±0.07	9.8±0.04*
Fungi	9.4±0.06	9.9±0.02**

Note: * - P<0.05; ** - P<0.01;*** - P<0.001

The titer of beneficial microorganisms in the gastrointestinal tract of chickens from the experimental batch increased by 3.6-15.4% compared to the control, and that of pathogenic and conditionally pathogenic ones decreased by 6.5-10.6% (Figure 1).

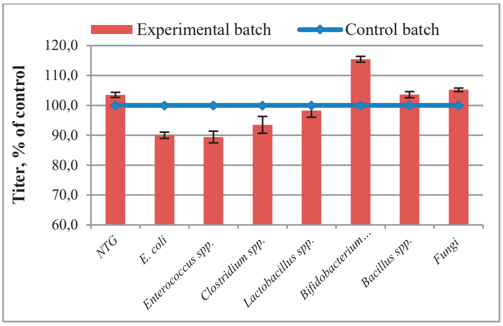

Figure 1. The effect of experimental feed with CMP-3 on the titer of different microorganisms in the gastrointestinal tract of chickens

The results of this study are in agreement with those reported by Craven (2000), who observed a reduction in the number of *C. perfringens* in the intestinal contents of chickens and proved that the numbers of *C. perfringens* detected in the fecal droppings of birds generally reflected the numbers found in the ileum.
In recent research conducted on rats, Li et al. (2019) demonstrated that *S. platensis* benefits health by inhibiting chronic inflammation and intestinal dysbiosis. Modulation of intestinal permeability occurs due to high molecular weight lipids and polysaccharides in *S. platensis*. The lipids have been shown to contain high levels of polyunsaturated fatty acids, including linolenic acid which is a precursor of arachidonic acid and several kinds of sterols (Bensehaila et al., 2015). High molecular weight polysaccharides with multiple biological activities, including anti-cancer, anti-oxidant, immunomodulatory, hypolipidemic and hypoglycemic, anti-thrombotic, anti-viral, regulation of the gut microbiota properties, and other biological activities (Kwak et al., 2015; Xiaopeng et al. 2023; Yadavalli et al., 2023).
One of the main conditions for obtaining maximum productivity from chickens raised in an industrial system is to ensure adequate, balanced feed, in which the main role is played by energy value and protein content (Starciuc et al., 2018).
The energy and protein content of the combined feeds administered to the chickens in the control batch was, respectively, 2703 kcal/kg and 22.1%, and in the experimental batch, respectively, 2698 kcal/kg and 21.9% (Table 2). So, the nutritional value of the feed

was sufficient to ensure the physiological needs of the chickens.

During the experiment chickens from the control batch consumed 9.69% less feed (Table 4) compared to those from the experimental batch, because during the period of infection with *C. perfringens* they showed inappetence, which negatively influenced not only feed consumption, but also the weight gain.

Table 4. Combined feed consumption, g/day

Period, weeks	Control batch	Experimental batch
1	14.86	16.33
2	14.51	19.71
3	21.72	20.40
4	36.85	40.00
Average	21.98	24.11

It is important to note that CMP-3, added in the amount of 4% in the chickens ration, did not negatively influence the smell and taste of the feed, but significantly favored the weight gain of the chickens.

The chickens from the experimental batch at 28 days had a body mass of 238.40 g, weight corresponding to the age, and those from the control batch registered only 190.00 g, which is 13.64% less than the minimum norm and 25.47% (P<0.001) less compared to the chickens from the experimental group (Table 5).

Table 5. Body mass of the chickens during the experiment, g

Age, days	Control batch	Experimental batch
8	58.70±0.11	58.84±0.35
15	62.62±0.10	74.02±0.04***
28	190.00±6.18	238.40±14.20***

Note: ***- P<0.001

It is known that in birds that have been ill with clostridiosis, the intestinal villi remain atrophied, and this subsequently affects productivity and feed conversion (Avi et al., 2023). Because of this, the chickens in the control group at the age of 28 days had a body mass below the age limit.

The results obtained by us indicate that the administration of CMP-3 in the daily ration of chickens, in a proportion of 4%, had a beneficial effect on the body by stimulating the beneficial microflora in the gastrointestinal tract of chickens and inhibiting the pathogenic and conditionally pathogenic one, strengthening the immune system, the chickens suffering a milder form of the disease, which allowed the mortality of the chickens to be reduced to 0% and the faster recovery after the disease, by returning to the normal body weight, characteristic of the age.

Analogous results were obtained by Hashim et al. (2018), Santovito et al. (2019), Chiselita et al. (2023) who established the effectiveness of different mannoprotein preparations from yeasts, in maintaining performance, health of chickens and reducing mortality, caused by *C. perfringens* infections.

Finally, we can mention that thanks to its antioxidant activity and its natural and complex composition, which combines biologically active substances of cyanobacterial and yeast nature, CMP-3 strengthens the immune system of young poultry, improves the health of the intestine by stimulating the growth of beneficial bacteria and inhibiting the development of pathogens, including clostridia, increasing the resistance of the chickens' body, which allows to reduce the negative impact of the infection. At the same time, the post-infection recovery of the chickens is faster, as evidenced by their weight gain.

CONCLUSIONS

The use of CMP-3 in the feed of chickens ensured the survival of all chickens in the experimental batch, compared to 72% in the control group; decreasing the titer of pathogenic bacteria *E. coli*, *Enterococcus* spp., *Clostridium* spp. by 0.6-1.0 log CFU/g, increasing the titer of beneficial microorganisms *Bifidobacterium* spp., *Bacillus* spp. and yeast fungi by 0.3-1.5 log CFU/g, compared to the control in the gastrointestinal tract of chickens; the increase in the body weight of the chickens from the experimental batch at the end of the experiment by 25.47% (P<0.001) compared to the chickens from the control batch.

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