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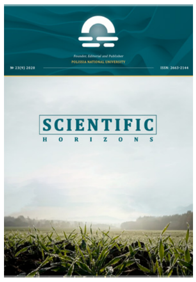

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Determination of the effect of the enzyme and probiotic complex on animal productivity

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Abstract. During weaning, rabbits experience production and feed stress, which results in a decrease in body resistance. Young animals in the transition period often have gastrointestinal disorders and significant weight loss. Probiotic strains of microorganisms and enzymes are a safe alternative for stimulating growth and

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supporting rabbit immunity. The purpose of the study was to determine the effect of additives to the basic diet on the haematological status, biochemical and productive parameters of rabbits. Methods used: zootechnical, haematological, biochemical, statistical. In the experimental groups where enzymes with probiotics were used, an increase in live weight was observed on the 30th day of the experiment: in the first experimental group by 4.18%, in the second – by 9.69%, in the third – by 18.72%. At the time of reaching the slaughter age, the live weight of rabbits increased by 5.47%, in the second by 11.0%, and in the third by 12.6%. The average daily increase in the groups where the complex supplement was used was higher, respectively, by: 6.64%; 12.58%, 32.86%. During the entire period of the experiment, gastrointestinal diseases were observed in three control rabbits that received treatment. Feed conversion was better in the first experimental group by 90.73 %, in the second – by 76.58 %, in the third – by 82.92%, compared to the control. In the group where three probiotic strains were used, an increase in red blood cells by 68.19% ($P<0.05$), haemoglobin by 28.44%, and white blood cells by 72.17% was recorded. In rabbits of the experimental groups, lipid metabolism improved against the background of cholesterol reduction in the first by 25.3%, the second – by 36.6%, and the third – by 39.4%. Protein and albumin absorption increased in the experimental groups: in the first by 15.33%, in the second – by 24.04%, in the third – by 28.57%. Mineral metabolism improved in animals of the experimental groups due to an increase in the calcium content in the first by 10.1%, the second – by 8.7%, and the third – by 17.9%. The practical significance of this study is to increase productivity and reduce gastrointestinal disorders by applying enzymes and probiotic strains of bacteria to the main diet

Keywords: alternative growth stimulants; live weight gain; feed conversion; morbidity; rabbit metabolism

INTRODUCTION

In countries where the economy has been disrupted, one of the most important problems is people's nutrition. Rabbits are herbivores that are not demanding on nutrition, can consume waste from processing plant products. In addition, rabbits reproduce and grow quickly, and can be kept in a limited area in large numbers. Rabbit meat is characterised by its high nutritional content (Cullere & Dalle Zotte, 2018) and dietary properties. To increase the productivity of rabbits, it is necessary to use safe growth stimulants. The relevance of the subject matter lies in the use of enzymes and probiotic strains of microorganisms to improve rabbit metabolism.

Research by D. Ye *et al.* (2023) showed that changes in cold and warm seasons negatively affect intestinal microflora, immunity, and productivity. Researchers have proposed strategies to mitigate the effects of off-season changes, but have not provided a way to avoid this problem. To maintain immunity in calves and prevent dysbiosis, V.A. Gryshchenko *et al.* (2023) used dietary supplements for basic nutrition. As a result of the experiment, the epithelium of the empty intestinal mucosa was restored in calves. The studies have not clarified the effect of the supplement on the microflora of the gastrointestinal tract.

An experiment by Z. Wang *et al.* (2023) on avoidance of coprophagy in rabbits demonstrated a negative impact on performance. The level of total cholesterol and triglycerides decreased, and the villi of the caecum were destroyed. The results of the study show the importance of constant restoration of microflora in the gastrointestinal tract. Z. Li *et al.* (2022) analysed the composition and amount of microflora and cytokines in the contents of the caecum. Researchers have concluded that coprophagy is an analogue of probiotic therapy and plays an important role in the intestinal immunity of rabbits.

Research by K.A. Alayande *et al.* (2020) confirm that probiotics are a safe alternative to antibiotics to increase animal productivity. The collected information provides the basis for further testing of probiotic strains for use by farm animals. Results obtained in the paper by M.I. Hossain *et al.* (2017) have shown the ability of probiotics to synthesise hydrolytic enzymes to kill bacterial toxins. Therefore, it is necessary to search for the most effective probiotic communities for raising animals. S. Saha *et al.* (2023) showed that the addition of probiotics to rabbits' diets helped increase carcass weight, improve colour, muscle fibre structure, and taste. Studies do not provide information on the properties of probiotic strains, their dosage, administration method and mechanism of use.

The results obtained by P. Cheng *et al.* (2018) show differences in the composition of the gut microbiome and different levels of cellulose fibre digestion in pigs of different breeds of Lantang and Duroc. The paper does not consider the impact of different conditions of keeping and feeding animals. R. Du *et al.* (2018) found that the bacteria of the family *Butyricimonas* produce butyrate in the course of their life, which aids digestion in calves. There is a need to investigate what other microorganisms have a positive effect on the metabolism and final live weight of animals. Experiment by A. Daskalova *et al.* (2023) proved that an increase in the bacterial content of *Lactobacillus* and *Bifidobacterium* in the gut microbiome of broiler chickens contributes to increased productivity and feed conversion. Researchers have found that dietary supplements based on phytobiotics have a positive effect on changes in the microbial composition and metabolism of animals and can be used as an alternative to antibiotics.

To increase the productivity of rabbits, Z. Li *et al*. (2023) tested the addition of a probiotic strain *Clostridium butyricum* to the diet. The results obtained showed a positive effect of live weight gain due to an increase in lipid synthesis in the liver. *The purpose of the study* was to investigate the effect of supplements with different compositions on metabolism and live weight gain in rabbits.

MATERIALS AND METHODS

The rabbits were kept for 36 days in accordance with animal welfare requirements. The experiment was conducted in November-December 2023 in the vivarium of the Faculty of Veterinary Medicine of Sumy National Agrarian University. The object of the study was rabbits (Californian breed) aged 14 days in the amount of 10 units in each group. Variants of samples of experimental enzyme and probiotic complexes (EPC) were provided by Kronos Agro. The first experimental group was fed with enzyme-based EPC (xylanase, phytase, cellulase) and probiotic strains of microorganisms of the bacterium *Enterococcus spp*. For the second experimental group, EPC was based on enzymes and *Lactobacillus spp*. The enzyme and probiotic complex for Group 3 of the study consisted of enzymes and three probiotic strains of bacteria. EPC presented in powder form was given to rabbits with feed at the rate of 250 g per 1 tonne of feed, starting from the age of 14 days. The experimental design is shown in Table 1.

Table 1*. Experiment design*

Groups	Characteristics of groups, dosage
experimental 1	EPC: enzymes + *Enterococcus spp.*
experimental 2	EPC: enzymes + *Lactobacillus spp.*
experimental 3	EPC: enzymes + *Enterococcus spp., Lactobacillus spp., B. coagulans*
control	Feed without additives

Source: *compiled by the authors*

During the studies, rabbits did not receive any drugs other than the above.

Research of productive indicators in rabbits. The live weight of rabbits was studied by weighing on the 30th and 60th days of the experiment. Feed conversion, absolute and average daily growth, and rabbit survivability were calculated.

Determination of haematological parameters of blood. On day 60, rabbit blood tests were determined: red blood cells, white blood cells, haemoglobin, lymphocytes, monocytes, neutrophils, eosinophils using an automatic analyser (Shenzhen Mindray BC3000 plus, China).

Investigation of the metabolic status of rabbits. Metabolites were determined on the 60th day of in rabbit blood serum: enzyme activity: aspartate aminotransferase (SOP-BP-09-2017), alanine aminotransferase and alkaline phosphatase (SOP-BP-08-2017), total protein, including albumin and globulin (SOP-BP-02-2017), total cholesterol (SOP-BP-07-2017), direct and indirect bilirubin, total calcium (sop-BP-05-2017), glucose.

Study of the composition of the rabbit gastrointestinal microbiome. Faecal masses were taken from rabbits of three experimental and control groups at the end of the experiment on 60th day. Faecal samples were examined by bacteriological methods to determine the quantitative composition of intestinal microorganisms in animals. The number of bacterial species was determined: *Coprococcus sp., Ruminococcaceae sp., Akkermansia sp., Lachnospiraceae sp., Clostridium sp., Subdoligranulum sp., Firmicutes sp., Bacteroidetes sp., Cyanobacteria sp., Verrucomicrobia sp., Proteobacteria sp., Tenericutes sp.* Faecal samples were diluted 1: 10 and inoculated on nutrient selective media. The media used were from Farmaktiv LLC (Ukraine) and Himedia Laboratories Prv. Limited (India). The species affiliation of microbial cultures and their number were determined using the R-biopharm test system, namely RIDA ® COUNT, RIDA CHECK. LumitesterPD-20; LuciPacPen, RIDASCREENVerotoxin, RIDASCREENSETA, B, C, D, (ENISO 16140), RIDASCREENCampylobacter, SureFoodBAC.

Investigation of antagonistic properties of enzyme and probiotic complex. The agar well diffusion method was used. The size of the demarcation zone in mm around the different composition of the enzyme and probiotic complex was visually determined on Petri dishes with the corresponding microorganism, as previously mentioned, for three experimental variants and a control (Garkavenko *et al*., 2021). Each dish with meat-peptone agar was inoculated with the appropriate EPC and left for a day in a thermostat for incubation at 37°C. The size of the demarcation zone in mm around the wells with experimental EPC was determined.

Statistical analysis. For statistical analysis of the results, the Fischer-Student method (Mosteller & Fisher, 1948) was used, and data from control and experimental groups were compared with a probability of more than 95% ($p < 0.05$). The studies were conducted in accordance with DSTU EN ISO/IEC 17025:2019 (2019), in compliance with the Rules of Bioethics and Humane Treatment of Vertebrates 2010/63/EU (Hartung, 2010) of the European Convention (1986) and Law of Ukraine No. 249 (2012).

RESULTS

Results of the study of productive indicators in rabbits. The first stage of the study was to determine the productivity of rabbits when growing with the use of enzyme and probiotic complex (Table 2).

***Table 2**. Comparative data on rabbit growth (M ± m, n = 10)*

Indicators	Control	Research groups		
		1	2	3
Number of animals, units	10		10	
Initial live weight (14 days), kg	0.25 ± 0.08	0.25 ± 0.05	0.25 ± 0.13	0.26 ± 0.07
Live weight (30 days), kg	0.59 ± 0.06	0.62 ± 0.11	0.65 ± 0.09	0.71 ± 0.12
% to control	-	4.18	9.69	18.72
Live weight (60 days), kg	1.28 ± 0.02	1.35 ± 0.23	1.41 ± 0.14	1.62 ± 0.32*
% to control	-	5.47	10.15	61.60
Absolute gain, kg	1.03 ± 0.36	1.10 ± 0.38	1.16 ± 0.27	1.37 ± 0.56
Average daily gain, g	28.62 ± 1.34	30.53 ± 1.50	32.20 ± 2.53	38.03 ± 2.10*
% to control	-	6.64	12.58	32.86
Morbidity, units	3	-	-	-
Feed consumption, kg	13.02	13.61	13.52	12.15
Feed consumption per 1 kg of live weight gain, kg % to control	2.05 100.00	1.86 90.73	1.70 82.92	1.57 76.58

***Note:** *P < 0.05 – relative to the control*
***Source:** compiled by the authors*

During the experiment, the live weight of one rabbit in 30 days was increased by 4.18% in the first experimental group, in the second – by 9.69%, in the third – by 18.72%, compared to the control group. On the 60th day of the study, the live weight of rabbits in the first experimental group was 5.47% higher, in the second – 11.0%, and in the third – 12.6% ($p < 0.05$), respectively, compared to the control. The average daily increase by group was higher, respectively, by: 6.64%; 12.58%, 32.86% ($P < 0.05$) compared to the control.

The morbidity for the entire period of the experiment was three animals in the control group. Gastrointestinal disorders were observed in rabbits, so three animals received timely treatment. The animals were isolated from the main group and treated with antimicrobial agents. The feed intake of rabbits was higher in the control group. Feed consumption was as follows: in the first experimental group – 90.73%, in the second – 76.58%, in the third – 82.92%, compared to control. The study shows that the average daily gain of rabbits was higher in the third experimental group, where enzymes with three probiotics were added to the feed of rabbits. However, feed consumption per 1 kg of live weight gain was lower in the third experimental group.

Results of haematological parameters of blood. Changes in haematological status in rabbits were determined during the experiment (Table 3).

***Table 3**. Haematological status of rabbits, (m ± m, n = 10)*

Indicators	Experimental group 1	Experimental group 2	Experimental group 3	Control	Reference values of laboratory
Red blood cells, 10^6/ml	5.85±0.12	5.78 ± 0.18	7.72 ± 0.10*	4.59 ± 0.25	5.3-7.1
White blood cells, 10^3/ml	6.90±0.15	8.73 ± 0.23	10.52 ± 0.24*	6.11 ± 0.13	5.1-10.7
Haemoglobin, g/dl	10.91±0.19	11.75 ± 0.20	12.78 ± 0.25*	9.95 ± 0.24	9.8-14.0
Lymphocytes, 10^3/ml	3.84±0.11	4.42 ± 0.05	5.83 ± 0.06	4.73 ± 0.09	3.3-6.5
Monocytes, 10^3/ml	0.39±0.02	0.36 ± 0.04	0.28 ± 0.01	0.44 ± 0.03	0-0.5
Neutrophils, 10^3/ml	1.31±0.04	2.14 ± 0.06	1.82 ± 0.07	1.64 ± 0.09	1.5-2.3
Eosinophils, 10^3/ml	0.04±0.05	0.03 ± 0.05	0.02 ± 0.01	0.02 ± 0.01	0-0.05

***Note:** *P < 0.05 – relative to the control*
***Source:** compiled by the authors*

A significant increase in red blood cells at the end of the experiment was recorded in the third experimental group by 68.19% ($P < 0.05$), compared to the control group. However, the haemoglobin content in the blood was also higher in rabbits of this experimental group by 28.44% ($P < 0.05$). The use of three probiotics in rabbits of the third group had a positive effect on immunity in animals. Thus, the absolute number of white blood cells on the 60th day of the study was significantly ($P < 0.05$) higher in rabbits of the third experimental group by 72.17%, compared to the control group. The first and second experimental groups also showed an increase in red blood cells and haemoglobin compared to the control group, but the difference was not reliable. Studies have shown that the use of an enzyme and probiotic complex in rabbits as part of the basic diet has a positive effect on immunity and erythrocytopoiesis.

Results of the study of the metabolic status of rabbits. During the experiment, the level of metabolism in the blood of experimental animals was established (Table 4).

***Table 4**. Biochemical analysis of blood serum, (M ± m, n = 10)*

Indicators	Experimental group 1	Experimental group 2	Experimental group 3	Control	Reference values of laboratory
AST, IU/l	64.92 ± 3.15	54.80 ± 5.65	72.8 ± 3.15	92.22 ± 3.55	20-120
ALT, IU/l	45.62 ± 2.67	48.53 ± 3.26	46.00 ± 4.52	47.45 ± 3.36	25-120
GGT, IU/l	6.61 ± 0.23	7.14 ± 0.35	6.51 ± 0.24	6.73 ± 0.42	0-7
Alkaline phosphatase, IU/l	6.23 ± 0.56	5.04 ± 0.20	5.22 ± 0.17	7.03 ± 0.30	0-8.6
Total bilirubin, μmol/l	5.56 ± 0.2	4.65 ± 1.15	5.39 ± 1.22	5.84 ± 0.32	0-6.8
■ Direct, μmol/l	1.43 ± 0.15	1.23 ± 0.83	1.24 ± 0.56	1.71 ± 0.16	0-1.7
■ Indirect, μmol/l	4.13 ± 0.35	3.42 ± 0.42	4.15 ± 0.81	4.13 ± 0.66	0-5.12
Creatinine, μmol/l	65.12 ± 3.45	59.04 ± 4.38	56.22 ± 4.26	78.34 ± 3.34	50-100
Urea, mmol/l	3.52 ± 0.22	3.74 ± 0.35	2.50 ± 0.20	4.21 ± 0.15	2.18-5.5
Total cholesterol, g/l	0.53 ± 0.05	0.45 ± 0.08	0.43 ± 0.05*	0.71 ± ±0.20	0-0.75
Total protein, g/l	57.72 ± 4.25	65.10 ± 0.52*	74.43 ± 4.16*	55.40 ± 5.81	54-82
Albumins, g/l	33.50 ± 2.72	35.60 ± 2.01*	36.9 ± 3.52*	28.72 ± 2.67	25-37
Globulin, g/l	26.85 ± 2.23	27.76 ± 1.34	32.31 ± 2.18	25.30 ± 2.34	23-50
Calcium, mmol/l	3.14 ± 0.23	3.10 ± 0.56	3.36 ± 0.32*	2.85 ± 0.12	2.4-4.2

Note: **P < 0.05 – relative to the control*
Source: *compiled by the authors*

The conducted studies show that the activity of enzymes (aspartate aminotransphenase, alanine aminotransferase, gamma-glutamate transferase and alkaline phosphatase) was within the physiological norm in animals of the experimental and control groups. The activity of the enzyme aspartate aminotransferase was studied to determine the toxic effect of the enzyme and probiotic complex on the rabbit myocardium. Studies have shown that the level of AST in the first experimental group was significantly lower by 29.60%, in the second – by 40.57%, in the third – by 21.01%, compared to the control. The content of the enzyme alanine aminotransferase in the blood demonstrates the effect of experimental EPC on the liver and kidneys of animals. ALT activity in the first experimental group was less by 3.85% and the third – by 3.05%, more in the second – by 2.27%.

An increase in the activity of the enzyme gamma-glutamate transferase indicates damage to haepatocytes and bile ducts. According to the results of the study, it was found that GGT activity in the first experimental group was less by 1.78%, in the second – by 6.09%, in the third – by 3.26%, compared to the control. Alkaline phosphatase activity was also determined in the blood serum of rabbits to find out the effect of EPC on lipid metabolism. It was found that the activity of the alkaline phosphatase enzyme in the first experimental group was less by 11.38%, in the second – by 28.30%, in the third – by 25.74%, compared to the control.

The content of total bilirubin in the blood was interrelated with the activity of the enzymes ALT, GGT, and alkaline phosphatase. As a result of the conducted studies, it was found that total bilirubin, including direct and indirect, did not have a significant difference between the groups. The level of creatinine and urea in the blood serum corresponded to the reference indicators in rabbits of the experimental and control groups, which indicates the absence of toxic effects of EPC on the body of rabbits. The blood creatinine content of rabbits in the first experimental group was lower by 16.87%, in the second – by 24.30%, in the third – by 28.24%, compared to the control. The level of urea was lower in the blood serum of rabbits of the first experimental group by 16.38%, in the second – by 11.16%, in the third – by 40.61%.

A decrease in cholesterol levels was observed in rabbits of the first experimental group by 25.3%, the second – by 36.6%, the third – by 39.4% ($p < 0.05$), compared to the control group. There was also a significant increase in the level of total protein in animals of the experimental groups: in the first by 4.18%, in the second – by 17.5%, in the third – by 34.35% ($P < 0.05$). A positive result can also be considered an increase in albumin in the first experimental group by 15.33%, in the second – by 24.04%, in the third – by 28.57%, compared to the control. The level of globulins in the blood serum of rabbits of the experimental and control groups was within the normal range, which indicates the absence of a negative reaction to EPC from the immune system.

In rabbits of the first experimental group, the globulin content was higher by 6.26%, in the second – by 9.72%, in the third – by 27.70%, compared to the control.

An important indicator of metabolism is the level of calcium, which was higher in the blood of experimental animals by 10.1%, the second – by 8.7%, the third – by 17.9% ($p < 0.05$), compared to the control. The results obtained showed that the use of the enzyme and probiotic complex affected the biochemical and haematological parameters in rabbits. Addition of EPC to the feed also had a positive effect on the increase in live weight of rabbits.

Results of the study of the composition of the microbiome of the gastrointestinal tract of rabbits. Studies were conducted to determine the effect of the enzyme and probiotic complex on the microflora of the gastrointestinal tract of rabbits. It is known that a violation of the normal composition of the microbiome can lead to dysbiosis. As a result, diarrhaea, impaired food absorption, weight loss, intoxication, and death of the animal occur. The results obtained will affect the final version of the EPC composition, which will be proposed for use in rabbit breeding (Fig. 1).

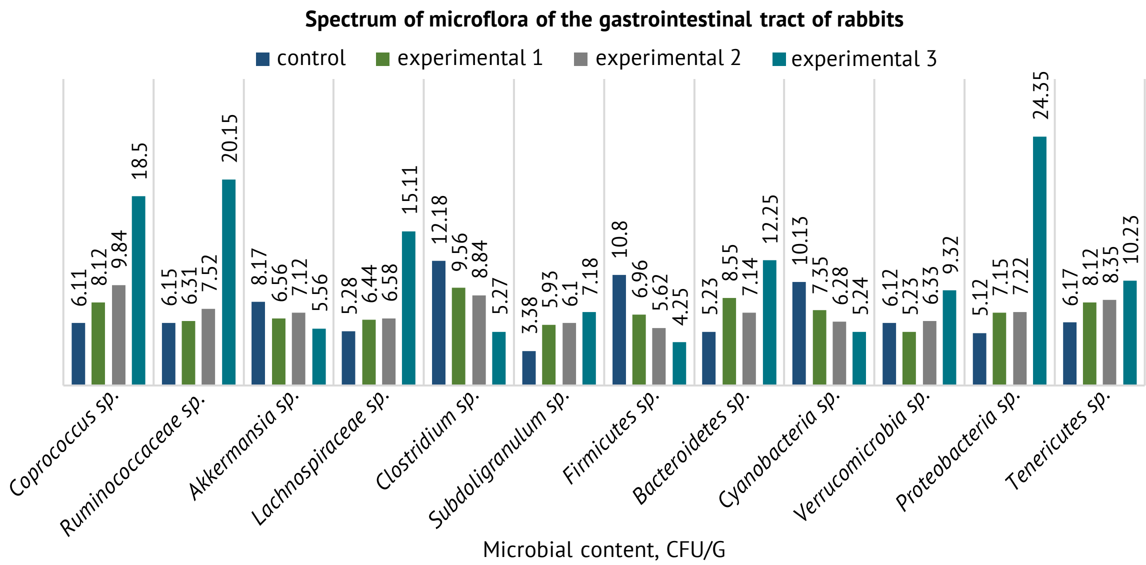

***Figure 1**. Spectrum of microflora of the gastrointestinal tract of rabbits when using an enzyme and probiotic supplement*

***Source:** compiled by the authors*

According to the results of the study, it was established that the content of *Coprococcus sp.* was higher in the first experimental group by 32.89 %, in the second experimental group – by 61.04%, in the third experimental group – by 202.78%, compared to the control. The content of *Ruminococcaceae sp.* in the first experimental group was 2.60% higher, in the second – 22.27%, in the third – 227.64%. Number of bacteria of the species *Akkermansia sp.* was less in the first experimental group by 19.70%, in the second – by 12.85%, in the third – by 30.84%, compared to the control group. The content of bacteria of the species *Lachnospiraceae sp.* in rabbits of the first experimental group was 21.97% higher, in the second – 24.62 %, in the third – 186.17%. The content of *Clostridium sp.* tended to decrease compared to the control in the first experimental group by 21.51%, in the second – by 27.42%, in the third – by 56.73%. The content of *Subdoligranulum sp.* in the intestine did not differ significantly between experimental and control animals and ranged from 3.38-7.18 CFU/g of faecal mass. However, the content of *Firmicutes sp.* was lower in the first experimental group by 35.55%, in the second – by 47.96%, in the third – by 60.64%, compared to the control. The addition of an enzyme and probiotic complex does not directly change the composition of the rabbit gut microbiome, but it does affect the amount of bacteria of the same species.

The content of *Bacteroidetes sp.* in the first experimental group was 63.47% higher, in the second – by 36.52%, in the third – by 134.23%. The level of *Cyanobacteria sp.* was less in rabbits of the first experimental group by 27.44%, in the second – by 38.00%, in the third – by 48.27% on the 60th day of research. The content of *Verrucomicrobia sp.* was not affected by the addition of EPC. So in the first experimental group, *Verrucomicrobia sp.* was less by 14.54%, in the second and third –more by 3.43-52.28%, respectively. The content of *Proteobacteria sp.* in the first experimental group exceeded the indicators of rabbits in the control group by 39.64%, in the second – by 41.01%, in the third – by 375.58%. The level of *Tenericutes sp.* was higher by 31.60% in the first experimental group, in the second – by 35.33%, in the third – by 65.80%, compared to the control. Thus, the same types of microorganisms were isolated in all animals of the experimental and control groups, but in different quantities. The study proves that the composition of the gut microbiome affects the productivity and metabolism of rabbits.

Investigation of antagonistic properties of enzyme and probiotic complex. The antagonistic activity of EPC was determined against microorganisms that were isolated from the faecal masses of rabbits. Isolates of microorganisms that are not pathogens were selected, but an increase in their content can lead to diseases in rabbits in the form of metabolic disorders and gastrointestinal disorders (Table 5).

Table 5*. Antagonistic properties of EPC, (M ± m), n = 10*

Microbial cultures	Enzyme and probiotic complex		
	experiment 1 (enzymes + *Enterococcus spp.*)	experiment 2 (enzymes + *Lactobacillus spp.*)	experiment 3 (enzymes + *Enterococcus spp., Lactobacillus spp., B. coagulans*)
	Size of the growth retardation zone, mm		
Coprococcus sp.	3.34 ± 0.04	6.50 ± 0.07	6.45 ± 0.15
Firmicutes sp.	9.25 ± 0.04	11.15 ± 0.02	26.33 ± 1.22*
Clostridium sp.	3.45 ± 0.03	5.87 ± 0.12	25.38 ± 1.18*
Cyanobacteria sp.	4.76 ± 0.15	5.50 ± 0.10	5.49 ± 0.20
Subdoligranulum sp.	3.45 ± 0.06	5.36 ± 0.08	8.89 ± 0.12
Akkermansia sp.	8.36 ± 0.08	9.15 ± 0.15	22.50 ± 1.45*

Note: **P* < 0.05 – relative to experiment 1*
Source: *compiled by the authors*

Based on the results obtained, it was found that in Petri dishes with the bacterium *Coprococcus sp.* in the second experiment, the growth retardation zone was 4.61 % larger, in the third – by 3.11%, compared to the first experiment. That is, the growth retardation zone in all EPC prototypes was almost the same. Demarcation zone in Petri dishes with *Firmicutes sp.* it was higher in the second experiment – by 20.54% and in the third – by 184.65% (P < 0.05). In the second experiment with *Clostridium sp.* growth retardation zone, it was an increase of 29.85%, in the third – by 635.65% (P < 0.05). In the dishes with *Cyanobacteria sp.*, the growth retardation zone was almost the same in all experiments and ranged from 4.76 to 5.49 CFU/g. Demarcation zone with bacteria *Subdoligranulum sp.* in the second group was 55.36% more, in the third – by 157.68%, compared to the first experiment. A significant proportion of the rabbit gut microbiome is made up of the species shown in Table 5, but an increase in any bacteria at the expense of other beneficial microflora has a negative impact on gut health and performance. Thus, the demarcation zone of *Akkermansia sp.* growth retardation in the second experiment was 9.45% larger and 169.13% larger in the third experiment (P < 0.05) compared to the first experiment.

Notably, in all experiments with EPC, despite the difference in the constituent components, antagonistic properties were manifested. The maximum antagonistic effect was achieved in the third experiment, where the EPC included three probiotic strains: *Enterococcus spp., Lactobacillus spp., B. coagulans*. The conducted studies have proven that the use of an enzyme and probiotic complex is aimed at improving metabolism, haematological parameters, restoring gastrointestinal microflora, and increasing the live weight of rabbits.

DISCUSSION

The use of an enzyme and probiotic complex for rabbits had a positive effect on the increase in live weight at days 30 and 60. This result is associated with better absorption of nutrients through the use of enzymes. Probiotic strains of bacteria also help maintain microflora in the gastrointestinal tract. In rabbits of the experimental groups, a decrease in feed intake was observed against the background of an increase in live weight gain, compared with the control.

Research by M.M.Y. Elghandour *et al.* (2020) proved positive impact of *Saccharomyces cerevisiae* on the gut microbiota and an increase in the number of white blood cells. The study by O. Shkromada *et al.* (2022) found that adding *B. megaterium* to the diet of cows reduced the inflammatory process in the udder and increased protein absorption. A study of the haematological status of rabbits showed a significant increase in red blood cells, haemoglobin, and white blood cells in the study group, which used enzymes and three strains of probiotics. According to L. Kadja *et al.* (2021), when using three probiotic strains, a similar result was obtained to increase the productivity of rabbits and improve haematological parameters.

Gastrointestinal disorders were observed in only 3 animals of the control group during the entire study period. The result shows a positive effect on the immunity of rabbits and intestinal microflora. K. Amoah *et al.* (2019) and S.S. Xing *et al.* (2019) found that the use of probiotic strains as a supplement to the main diet of animals reduces gastrointestinal diseases in young animals. In addition, the study by C. Maltecca *et al.* (2020) confirmed that the composition of the intestinal microflora is an important indicator that affects animal feed intake and metabolism. Consequently, the relationship between the composition of the gut microbiome and the final weight of rabbits can be traced.

The study of the biochemical composition of the blood serum showed the activity of enzymes (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) within the physiological norm, indicating no toxic effects on the body. The positive effect of EPC was manifested in lowering cholesterol levels in all research groups. An increase in the level of total protein and albumins was observed in all experimental groups compared to the control group. A.A. Abdel-Wareth *et al.* (2021) have shown that the use of probiotics in rabbits together with fenugreek seeds contributed to protein absorption and increased carcass weight.

Mineral metabolism in animals is also a very important indicator. Studies have shown that in the experimental groups where the enzyme and probiotic complex was used, the level of calcium was higher compared to the control. Microbiological studies have established that the amount of beneficial microflora: *Coprococcus sp., Ruminococcaceae sp., Lachnospiraceae sp., Cyanobacteria sp., Proteobacteria sp.* in the gastrointestinal tract of rabbits, was most common in the third experimental group, where rabbits received enzymes and *Enterococcus spp., Lactobacillus spp., B. coagulans.*

Research by A.J. Wolf and D.M. Underhill (2018) proved that the content of microorganisms *Coprococcus, Ruminococcus* and *Lachnospiraceae* in the gastrointestinal tract of rabbits, have a direct correlation with the productivity and growth of rabbits of meat breeds. These results are confirmed by S. Dabbou *et al.* (2020) who reported that a sufficient content of *Ruminococcus* microorganisms in the gut of rabbits has a positive effect on their resistance. On the contrary the number of bacteria *Akkermansia sp.* in the intestinal contents were significantly lower in rabbits of the experimental groups. A. Bohatko and M. Utechenko (2024) found that an elevated content of *Akkermansia sp.* in the gastrointestinal tract negatively affects the final weight of rabbits.

S.Y. Chen *et al.* (2019) proved that the types of *Cyanobacteria, Firmicutes, Bacteroidetes, Verrucomicrobia, Proteobacteria* are the most dominant bacteria in the rabbit microbiome, regardless of breed. In addition, M. Velasco-Galilea *et al.* (2020) showed that incomplete feeding and the use of antibiotics directly affect the content of microflora in the gastrointestinal tract. The studies revealed an increase in the content of *Bacteroidetes sp.* in the faeces of rabbits treated with the enzyme and probiotic complex, which coincides with the results obtained by N. Grosu and L. Caisîn (2020). In research groups, the level of *Firmicutes sp.* was lower than in the animals of the control group, which proves the positive effect of EPC on rabbit metabolism. This is confirmed by the results of the study by K.A.O. Gandy *et al.* (2019), which shows that an increased content of the bacterium *Firmicutes sp.* is responsible for obesity and lipid metabolism disorders.

The relative number of the main types of bacteria did not differ between different study groups of rabbits, which were added to the main diet of an enzyme and probiotic complex based on enzymes and probiotic microorganisms. This can be explained by changes in the quantitative composition of microorganisms that make up the microbiome of the rabbit gastrointestinal tract, since they are influenced by nutritional factors, which is confirmed by Z. Wu *et al.* (2019). According to the results of the experiment, the positive effect of the enzyme and probiotic complex on haematological parameters, metabolism, microflora of the gastrointestinal tract, and productivity of rabbits.

CONCLUSIONS

Studies have established an increase in the live weight of one rabbit unit in 30 days in the experimental groups: in the first – by 4.18%, in the second – by 9.69%, in the third – by 18.72%. At the end of the experiment, the live weight of rabbits in the first experimental group was 5.47% higher, in the second – 11.0%, and in the third – 12.6%. The average daily growth by group was increased by 6.64%; 12.58%, 32.86%, respectively, compared to the control. Feed conversion compared to the control group was 90.73% in the first experimental group, 76.58% in the second group, and 82.92% in the third group.

The use of an enzyme and probiotic complex with three probiotics in rabbits contributed to a likely increase in the content of red blood cells by 68.19 %, white blood cells by 72.17%, and haemoglobin by 28.44% ($P < 0.05$). The use of the enzyme and probiotic complex in rabbits showed no negative effect on metabolites. Enzyme activity, total bilirubin, creatinine, and urea within the reference level in experimental and control animals. There was an improvement in the lipid profile due to a decrease in cholesterol levels in the first experimental group by 25.3%, the second – by 36.6%, the third – by 39.4% ($P < 0.05$). Protein uptake increased in the experimental groups: in the first by 4.18%, in the second – by 17.5%, in the third – by 34.35% ($P < 0.05$) and albumin by 15.33%, 24.04%, and 28.57%, respectively. The level of calcium in the blood of rabbits in the experimental groups was correspondingly higher by 10.1%, 8.7%, and 17.9% ($P < 0.05$) compared to the control group.

It was found that the use of an enzyme and probiotic complex in rabbits does not change the composition of the microbiome, but it affects the quantitative content of various types of bacteria. Thus, the use of three probiotics in the diet significantly increases the content of species: *Coprococcus sp., Ruminococcaceae sp., Lachnospiraceae sp., Bacteroidetes sp., Proteobacteria sp.* and *Tenericutes sp.* The antagonistic properties of the enzyme and probiotic complex, which includes three probiotics, showed the maximum effect on *Firmicutes sp., Clostridium sp. Akkermansia sp.* The prospect of further research is to investigate the effect of the enzyme and probiotic complex on rabbits in production conditions.

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CONFLICT OF INTEREST

None.

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Визначення впливу ферментно-пробіотичного комплексу на продуктивність тварин

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Анотація. Під час відлучення від кролематки відбувається виробничий та кормовий стрес у кроленят, в результаті чого знижується резистентність організму. Молодняк у перехідний період часто має розлади шлунково-кишкового тракту та значну втрату ваги. Пробіотичні штами мікроорганізмів та ферменти є безпечною альтернативою для стимуляції росту та підтримки імунітету кроликів. Метою дослідження було визначити вплив добавок до основного раціону на гематологічний статус, біохімічні та продуктивні показники кролів. Використані методи: зоотехнічний, гематологічний, біохімічний, статистичний. В дослідних групах, де застосовували ферменти з пробіотиками, спостерігали збільшення живої ваги на 30 добу експерименту: у першій дослідній групі на 4,18 %, у другій – на 9,69 %, у третій – на 18,72 %. На момент досягнення забійного віку у кролів жива вага збільшилась на 5,47 %, у другій на – 11,0 %, та у третій на – 12,6 %. Середньодобовий приріст у групах, де використовували комплексну добавку, був більше відповідно на: 6,64 %; 12,58 %, 32,86 %. За весь період проведення експерименту захворювання шлунково-кишкового тракту спостерігали у трьох кролів контрольної групи, які отримали лікування. Конверсія корму була краща у першій дослідній групі на 90,73 %, у другій – на 76,58 %, у третій – на 82,92 %, порівняно до контролю. У групі, де застосовували три пробіотичні штами, зафіксовано збільшення еритроцитів на 68,19 % ($P<0,05$), гемоглобіну на 28,44 %, лейкоцитів на 72,17 %. У кролів дослідних груп відбулось покращення ліпідного обміну на фоні зниження холестерину у першій на 25,3 %, другої – на 36,6 %, третьої – на 39,4 %. Збільшилось засвоєння білку та альбуміну у дослідних групах: у першій на 15,33 %, у другій – на 24,04 %, у третій – на 28,57 %. Покращився мінеральний обмін у тварин дослідних груп за рахунок підвищення вмісту кальцію у першій на 10,1 %, другої – на 8,7 %, третьої – на 17,9 %. Практичною цінністю роботи є збільшення продуктивності та зниження шлунково-кишкових розладів за рахунок застосування ферментів та пробіотичних штамів бактерій до основного раціону

Ключові слова: альтернативні стимулятори росту; приріст живої маси; конверсія корму; захворюваність; метаболізм кролів