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Application of herbs and propolis in rabbits with chronic diarrhea

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Abstract: The aim of this study was to determine the effect of ethanolic extract of propolis (EEP) and an herbal mixture (*Rumex crispus*, *Potentilla anserina*, and *Polygonum aviculare*) on diarrhea course, hematological and biochemical parameters, and acid-base balance of rabbits. Twenty-four meat rabbits (Hyplus) were divided into four groups of eight animals. The rabbits were randomly divided into the following groups: HR – healthy rabbits, DR – rabbits with diarrhea symptoms, H – treated with 10% herbal extract, and EEP – treated with 10% ethanolic extract of propolis at 4 mL/L. The extracts were supplemented for 10 days into the drinking water. Hematological and biochemical blood parameters were examined, as well as the parameters of acid-base balance and antioxidant status. All the rabbits were subjected to clinical examination and their stool samples were tested for the presence of enteropathogenic *E. coli*. The used amounts of herbal mixture or ethanolic extract of propolis added to drinking water for rabbits with chronic diarrhea caused a decrease in the duration of diarrhea, concurrently improving feed intake and final body weight. Both the ethanolic extract of propolis and the herbal extract resulted in normalization of the acid-base balance and hematological parameters. The ethanolic extract of propolis has also shown explicit hepatoprotective effects.

Key words: Rabbits, diarrhea, propolis, herbal extract, blood parameters

1. Introduction

Diarrhea and intestinal inflammation caused by bacterial imbalance in the gastrointestinal tract are the major causes of collapses in the farm breeding of meat rabbits. Adult animals' deaths in the basic herd reach 3.4% (1), while the death incidences in growers range from 4.03% to 17.12% (2). These diseases are also the cause of high economic losses. The most important pathogens responsible for diarrhea in rabbits include enteropathogenic strains of *Escherichia coli* (EPEC), also referred to as rabbit-specific enteropathogenic *E. coli* (REPEC). These strains are characterized by the *eaeA* gene, responsible for producing intimin (3). This is an adhesive protein of the pathogenic strain that enables strict adhesion of bacterial cells to enterocytes. Concurrently, it causes changes in the cytoskeleton and enterocyte functions, which is a direct cause of diarrhea (3). Additionally, the presence of specific genes responsible for production of fimbriae related to adhesion to enterocytes of rabbit intestines of a sequence very similar to bundle-forming pili (4) as well as the *rpeA* gene (3) was noted in REPEC strains. Morphological changes occurring during infection with REPEC are indistinguishable from those induced in humans by EPEC (5) and are referred to as attaching and effacing (A/E) lesions. Nutritional mistakes may be a significant factor predisposing to diarrhea. Feed with a high content of nonfiber carbohydrates can promote bacterial infections when given before the development of the kits' digestive functions is completed. The risk of the outbreak of such diseases is enhanced by unstable intestinal flora.

Due to the ban on antibiotic growth stimulators in animal nutrition in the European Union, the interest in alternate therapeutic/preventive agents, such as herbal preparations (6,7) and bee products (8), has increased. Preventive or therapeutic application of herbs has been known for many years; however, the identification of active substances has been possible only recently. Active substances contained in herbs include alkaloids, glycosides, saponins, bitter compounds, tannins, aromatic compounds, essential oils and terpenes, plant fats, gluconins, mucilages, and phytoncides (9,10). Due to the presence of numerous biological compounds, propolis also exhibits a range of profitable properties, e.g., antibacterial, antiviral, antifungal, and antiprotozoal (11,12). Plants with antibacterial and antiinflammatory properties include *Potentilla anserina*, *Rumex crispus*,

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and *Polygonum aviculare* (13,14). *Rumex crispus* showed antimicrobial activity against *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *Candida albicans* (13). The antibacterial activity of *Polygonum aviculare* against both gram-positive and gram-negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds (14). Both propolis and some herbs contribute to intestinal microflora stabilization and thus reduce the susceptibility to infections with enteropathogens and/or diarrhea. Moreover, propolis application enhanced immunological resistance in the case of rabbits vaccinated against *Pasteurella multocida* (15). Health status improvement obtained in rabbits with diarrhea symptoms after an application of propolis or herbal extract would find practical applications.

The aim of this study was to determine an effect of the ethanolic extract of propolis (EEP) and an herbal mixture (*Rumex crispus*, *Potentilla anserina*, and *Polygonum aviculare*) on diarrhea course, hematological and biochemical parameters, and acid-base balance of rabbits.

2. Material and methods

2.1. Animals and experimental design

Hybrid conventional meat rabbits (Hyplus breed), aged between 60 and 70 days, were used in this experiment. The experiment included the rabbits from a farm on which chronic diarrhea symptoms were identified based on clinical observations. During the experiment, the animals stayed at the vivarium of the Department of Epizootiology with Clinic of Birds and Exotic Animals of the Wroclaw University of Environmental and Life Sciences, Poland.

The rabbits were divided into 4 groups (n = 6), with 2 control groups (HR – healthy rabbits, DR – rabbits with diarrhea symptoms) and 2 experimental group with diarrhea (HD – group treated with 10% herbal extract, EEPD – rabbits treated with 10% EEP at 4 ml/L). The rabbits were fed different additives for 10 days. All animals were subjected to clinical examination each day. Rabbits with diarrhea presented with loose, dark brown stools and abdominal distention from fluids and gas.

The amounts and methods of preparation of the herbal extract and EEP were determined in a previous in vivo study (16). The components of the herbal extract were *Rumex crispus* (inflorescence), *Potentilla anserina* (herb), and *Polygonum aviculare* (herb) in a weight ratio of 1:1:1 (herb manufacturer: Herbapol, Poland). Herbal extracts were obtained by heating at a temperature of ca. 80 °C for 10 min. During the extraction of the herbs no chemical solvents were used. After 20 min of extraction at room temperature the mixture was filtered and was ready for use in the experiments.

All the animals were fed a commercial diet (pellets, De Heus, Poland) for growing rabbits (containing 15.8% crude protein, 5.2% crude fat, and 16.50% crude fiber) during the entire experiment with free access to water (control groups and the groups receiving propolis or herbal extract in addition to water).

The animals were housed in vivarium with two animals per cage (60 × 40 × 35 cm, l × h × w) at a temperature of 20 ± 2 °C and a 12:12 light:dark cycle (lights on at 0600 hours), with a minimum of 0.4 m^3 air changes per hour until the 70th day of age. Animals were observed daily to record their health statuses, checking for the presence of diarrhea, depression, sneezing, and coughing. Rabbits were weighed in the morning before feeding at the beginning of the experiment and after that every second day until the end of the experiment. All experiments were performed in accordance with the protocol approved by the Animal Experimentation Committee of the 2nd Local Ethical Committee for Experiments on Animals in Wroclaw, Poland (no. 156/2010).

2.2. Microbiology

All animals were subjected to microbiological examination on the first day of the experiment. The stool samples of all rabbits were tested for the presence of EPEC using the polymerase chain reaction (PCR) methods described below. Moreover, the stool samples were examined for the presence of *Salmonella* spp.

2.2.1. *E. coli* isolation and identification

Rectal swabs were cultured on MacConkey agar (Oxoid Ltd., UK). After an overnight incubation at 37 °C, suspected *E. coli* colonies were subjected to biochemical identification. Isolated strains were kept at –80 °C in Microbank Storage Boxes (Pro-Lab Diagnostics, Canada). Five strains from each animal were used for PCR analysis.

2.2.2. PCR testing

Microbial DNA was obtained by resuspending strains incubated on Mueller-Hinton broth (Oxoid Ltd.) in 500 µL of ultrapure water and boiling at 100 °C for 10 min. A PCR test that detects gene virulence factors of EPEC was preformed using the methods described by Hassan and Al-Azeem (17) based on sequences complementary to *eaeA* and *bfpB*. Multiplex PCRs were conducted in a total volume of 25 µL with 5 µL of DNA templates, 2.5 µL of 10X polymerase buffer, 5 µL of 25 mM $MgCl_2$, 1 U of Dream Taq DNA polymerase (Fermentas, Lithuania), and primers at a concentration of 0.5 µmol each. All the primers were synthesized by Oligo (Warsaw, Poland). The procedure of PCR amplification included 1 cycle at 95 °C (10 min) and 30 cycles at 90 °C (30 s each), 55 °C (45 s), and 72 °C (90 s), followed by 1 cycle at 72 °C (10 min). All the reactions were performed in a thermal cycler (Bio-Rad, UK). The amplification products were resolved in 2% agarose gel and stained with Midori Green (Nippon Genetics, Japan).

2.2.3. Serotyping of EPEC

All strains positive for the *eaeA* gene in PCR were typed with commercially available somatic antigen (antigen O) antisera with latex agglutination for EPEC (Coli Lateks EPEC, Biomed, Poland).

2.3. Blood analyses

Blood samples from all animals were collected from the central artery of the ear at the beginning and the end of the experiment, after 10 days of treatment. Blood was collected into sterile test tubes for serum (5 mL, Sarsted, Poland), into tubes with anticoagulant EDTA-K3 (0.5 mL, Sarsted, Poland), and into 200-µL heparinized capillaries (Microcaps, IDEXX, USA) for arterial blood gas examinations (analyses were made immediately after blood collection). The blood samples for serum were centrifuged for 15 min at 3000 × *g* at room temperature 2 h after sample collection, and the serum samples were frozen (–20 °C) until use. Hematology was studied within 2 h after blood collection.

Hematological parameter analysis was performed using an ABC Vet analyzer (Horiba ABX, France), taking into account parameters such as red blood cells (RBCs), white blood cells (WBCs), platelets (PLTs), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Acid-base parameters were determined using a VetStat analyzer (IDEXX, USA). The laboratory analyses of blood serum were done using a Pentra 400 biochemical analyzer (Horiba ABX, France). The following parameters were estimated: glucose by the oxidase method, lactic acid (LA) by the colorimetric method, aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) enzyme activities by the kinetic method, total bilirubin concentration by the colorimetric method, total cholesterol (TC) by enzymatic methods, and total antioxidant capacity (TAC) by the colorimetric method. All biochemical tests were performed using reagents from Horiba ABX (France), and the TAC was determined using a reagent kit from Randox (UK).

2.4. Statistical analysis

The results were subjected to statistical analysis with Statistica ver. 10. All variables were tested for normality using the Shapiro–Wilk test. Differences among treatment means were tested for significance using post hoc comparisons accomplished via Duncan's multiple range test. Effects were considered significant at a probability of $P < 0.05$. All data are reported as mean ± standard error.

3. Results

The lowest body weight during the study was observed in the HR control group of healthy rabbits (Table 1). Administration of the preparations caused improved final body weight. In that period, the lowest weight was noted in the control rabbits with diarrhea (the DR group). Higher fodder and water intake was observed in group EEPD compared to DR. The applied preparations reduced the duration of chronic diarrhea symptoms.

Two control rabbits (HR group) turned out to be colonized with EPEC (*eaeA*$^+$, *bfp*$^-$) belonging to serotype O26 (Table 2). The presence of EPEC strains belonging to serotypes O128 or/and O26 was noted in most of the rabbits with diarrhea (groups DR, HD, and EEPD). None of the rabbits were colonized with *Salmonella* spp.

The lowest WBC count value at the first day of the experiment was noted in the case of the HR control group and the highest values were seen in rabbits with diarrhea symptoms (Table 3). High dynamics of WBC changes were observed during the experiment, and this resulted in a decreased leukocyte count in all the groups receiving

Table 1. Growth, feed intake, water intake, and health status of rabbits (means ± standard deviations).

Item	Group[1]			
	HR	DR	HD	EEPD
Initial body weight (g)	2015 ± 56^a	1720 ± 41^b	1740 ± 34^b	1750 ± 28^b
Final body weight (g)[2]	2324 ± 39^{ac}	1940 ± 57^b	2103 ± 62^{bc}	2085 ± 71^{bc}
Feed intake (g/day)	158 ± 18^{ac}	116 ± 14^b	130 ± 28^{bc}	135 ± 24^{bc}
Water intake (mL/day)	291 ± 22^a	235 ± 29^b	272 ± 12^b	264 ± 34^b
Symptoms of chronic diarrhea (days)	0.0	5.2 ± 0.7^a	3.4 ± 0.3^b	4.1 ± 0.4^b

[1]HR – Healthy control rabbits, DR – control rabbits with diarrhea symptoms, HD – group treated with 10% herbal extract, EEPD – group treated with 10% ethanolic extract of propolis.
[2]Period from the 60th day to the 70th day of age.
a, b, c - Differences between the groups were significant at $P < 0.05$.

Table 2. Number of samples positive for enteropathogenic strains of *E. coli* (EPEC; serogroups O128 and O26) in the rabbits.

Item	Serogroups		
	O128	O26	O128 and O26
HR	0	2	0
DR	3	4	2
HD	4	5	3
EEPD	3	5	1

[1]HR – Healthy control rabbits, DR – control rabbits with diarrhea symptoms, HD – group treated with 10% herbal extract, EEPD – group treated with 10% ethanolic extract of propolis.

the preparations (HD and EEPD) at the final day of the experiment. Significant decrease ($P < 0.05$) in Hct value was also found in all groups of rabbits with diarrhea. Moreover, a decrease in MCV was noted in the DR group after 10 days of the experiment, and an increase in that parameter was seen in groups HD and EEPD ($P < 0.05$).

On the first day of the experiment, blood pH in rabbits with chronic diarrhea (groups DR, HD, and EEPD) was significantly ($P < 0.05$) lower compared to the healthy animals (group HR). On the last day of the experiment, reduced pH was only found in the rabbits with diarrhea that were not given the preparations (pH 7.36). Base excess (BE) on the first day of the experiment was significantly lower ($P < 0.05$) in rabbits with diarrhea compared to the control group. On the last day of the experiment, BE had a positive value, while the lowest value of BE (0.6 mmol/L) was noted in group DR. Normocapnia was observed in all the animals in both examinations (Table 4). Significant increase ($P < 0.05$) in metabolic component (HCO_3^- and BE) was observed after 10 days of propolis supplementation. Similar tendencies were noted in the group supplemented with herbal extract. Despite lack of the treatment, group DR demonstrated a pH increase as a result of respiratory compensation.

Mean values of biochemical parameters in blood serum are presented in Table 5. At the first day of the experiment, a higher concentration of total protein (TP) was noted in groups DR, HD, and EEPD compared to the HR control group. Supplementation with herbs or EEP caused a decrease in TP value in blood serum. An elevated value was still observed in group DR. Lower concentration of albumins was noted in all the groups with

Table 3. The values of selected hematological variables in the blood of the rabbits (means ± standard deviations).

Item		Group[1]			
		HR	DR	HD	EEPD
WBCs ($\times 10^9$/L)	Before[2]	8.18 ± 2.73[a]	11.57 ± 2.02[*b]	11.63 ± 3.73[*b]	10.92 ± 2.57[*b]
	After[2]	7.07 ± 0.95[a]	9.37 ± 1.69[*b]	6.83 ± 1.21[**]	9.63 ± 4.46[*b]
RBCs ($\times 10^{12}$/L)	Before	5.59 ± 0.13[*]	6.69 ± 1.13[*]	6.40 ± 0.91[*]	6.28 ± 1.14[*]
	After	6.10 ± 0.27[*a]	4.97 ± 0.11[*b]	5.49 ± 0.90[*]	5.17 ± 0.99[*]
Hb (mmol/L)	Before	7.24 ± 0.28	8.40 ± 1.47[*]	7.68 ± 0.50	7.37 ± 1.11
	After	7.38 ± 0.18[a]	5.70 ± 0.26[*b]	7.03 ± 0.89[b]	6.67 ± 1.40
Hct (L/L)	Before	0.38 ± 0.01	0.43 ± 0.08[*]	0.40 ± 0.08[*]	0.41 ± 0.11[*]
	After	0.39 ± 0.01[a]	0.31 ± 0.02[*b]	0.36 ± 0.05[*]	0.34 ± 0.05[*c]
MCV (fL)	Before	67.40 ± 2.07[*a]	64.33 ± 1.53[*]	62.50 ± 5.61[*b]	62.00 ± 5.90[*b]
	After	64.67 ± 1.63[*]	62.00 ± 2.00[*a]	66.67 ± 4.08[*b]	66.17 ± 4.49[*b]
MCH (fmol)	Before	1.29± 0.04	1.25 ± 0.02	1.21 ± 0.10	1.18 ± 0.10
	After	1.21± 0.05	1.19 ± 0.05	1.29 ± 0.07	1.30 ± 0.10
MCHC (mmol/L)	Before	19.14 ± 0.11	19.23 ± 0.51	19.37 ± 0.47	19.02 ± 0.42
	After	18.72 ± 0.43	19.07 ± 0.57	19.30 ± 0.24	19.58 ± 0.38

[1]HR – Healthy control rabbits, DR – control rabbits with diarrhea symptoms, HD – group treated with 10% herbal extract, EEPD – group treated with 10% ethanolic extract of propolis.
[2]Before and after treatment.
a, b, c - Differences between the groups were significant at $P < 0.05$.
*Differences between samplings before and after treatment were significant at $P < 0.05$.

Table 4. Values of acid-base balance parameters in rabbit blood (means ± standard deviations).

Item		Group*			
		HR	DR	HD	EEPD
pH	Before[2]	7.47 ± 0.05[a]	7.32 ± 0.04[*b]	7.34 ± 0.07[*]	7.35 ± 0.05[*]
	After[2]	7.48 ± 0.04[a]	7.36 ± 0.05[*b]	7.47 ± 0.09[*]	7.46 ± 0.08[*]
pCO_2^- (kPa)	Before	5.20 ± 0.50	5.52 ± 0.53	5.30 ± 0.70[*]	5.40 ± 0.31[*]
	After	5.40 ± 0.60	5.45 ± 0.69	5.74 ± 0.46[*]	5.68 ± 0.33[*]
HCO_3^- (mmol/L)	Before	21.60 ± 2.40	18.60 ± 2.40	17.90 ± 8.40[*]	19.10 ± 2.20[*]
	After	23.10 ± 3.90	20.15 ± 3.90	23.50 ± 4.00[**]	24.38 ± 1.05[*]
BE (mmol/L)	Before	0.20 ± 1.60[*a]	–3.29 ± 1.62[*b]	–4.30 ± 1.50[*b]	–3.40 ± 1.87[*b]
	After	1.60 ±2.00[*]	0.60 ± 0.87[*]	0.98 ± 0.18[*]	2.30 ± 0.19[*]
TCO_2 (kPa)	Before	22.60 ± 2.50	20.60 ± 2.50	19.60 ± 3.57[*]	20.10 ± 2.24[*]
	After	24.10 ± 3.90	21.70 ± 3.92	24.60 ± 2.10[*]	25.48 ± 1.17[*]

[1]HR – Healthy control rabbits, DR – control rabbits with diarrhea symptoms, HD – group treated with 10% herbal extract, EEPD – group treated with 10% ethanolic extract of propolis.
[2]Before and after treatment.
a, b - Differences between the groups were significant at $P < 0.05$.
*Differences between samplings before and after treatment were significant at $P < 0.05$.

Table 5. Values of biochemical blood parameters in rabbit blood and total antioxidant capacity (TAC) (means ± standard deviations).

Item HR		Group*			
		DR	HD	EEPD	
TP (g/L)	Before[2]	55.02 ± 4.57[a]	63.33 ± 3.00[b]	68.65 ± 4.88[b]	69.08 ± 6.04[b]
	After[2]	56.37 ± 3.08	65.53 ± 3.99	56.70 ± 3.93[*]	53.55 ± 5.34[*]
Albumin (g/L)	Before	36.15 ± 1.85[a]	33.55 ± 1.20	32.23 ± 2.27[b]	33.87 ± 1.80[b]
	After	37.05 ± 1.57[a]	33.40 ± 0.83	32.98 ± 1.55[b]	32.82 ± 1.79[b]
AST (U/L)	Before	28.50 ± 4.23[a]	42.00 ± 7.56[b]	44.88 ± 5.48[*b]	51.67 ± 8.32[*b]
	After	32.48 ± 11.68[a]	51.70 ± 12.45[b]	34.87 ± 14.21[*]	34.37 ± 5.52[*]
GGT (U/L)	Before	6.17 ± 3.49[a]	18.50 ± 2.12[*b]	19.13 ± 4.64[*b]	18.78 ± 2.01[*b]
	After	7.83 ± 3.13[a]	12.75 ± 4.03[*]	14.30 ± 1.93[*b]	10.67 ± 3.88[*]
Bilirubin (µmol/L)	Before	3.58 ± 1.15[a]	4.20 ± 0.30[b]	4.89 ± 0.71[a]	4.81 ± 0.53[a]
	After	3.10 ± 0.59	3.18 ± 2.27	4.17 ± 0.67	3.42 ± 0.63
Glucose (mmol/L)	Before	7.21 ± 0.43	8.69 ± 1.28[*]	5.03 ± 0.60	6.98 ± 1.20
	After	7.00 ± 2.14	5.41 ± 1.70[*]	7.21 ± 0.62	7.46 ± 1.66
LA (mmol/L)	Before	2.00 ± 1.56[a]	5.37 ± 1.03[*b]	5.19 ± 3.24[*b]	4.93 ± 2.28[*b]
	After	1.83 ± 4.55	2.61 ± 2.38[*]	2.88 ± 1.71[*]	2.07 ± 3.89[*]
TC (mmol/L)	Before	2.10 ± 0.42	2.80 ± 0.75	2.32 ± 0.61	2.53 ± 0.66
	After	2.08 ± 0.24	2.71 ± 0.31	1.92 ± 0.50	2.25 ± 0.63
TAC (mmol/L)	Before	1.60 ± 0.13	1.59 ± 0.19	1.56 ± 0.21	1.61 ± 0.16
	After	1.59 ± 0.34	1.55 ± 0.38	1.66 ± 0.19	1.63 ± 0.13

[1]HR – Healthy control rabbits, DR – control rabbits with diarrhea symptoms, HD – group treated with 10% herbal extract, EEPD – group treated with 10% ethanolic extract of propolis.
[2]Before and after treatment.
a, b - Differences between the groups were significant at $P < 0.05$.
*Differences between samplings before and after treatment were significant at $P < 0.05$.

diarrhea compared to the HR group. This tendency was still observed after 10 days of the experiment. Higher AST activity at the beginning of the experiment was noted in groups DR, HD, and EEPD. EEP administration caused a decrease ($P < 0.05$) in AST activity. Similar changes were observed in group HD. An increase in AST activity was noted in group DR. Increased GGT activity was subject to a decrease in the rabbits with diarrhea after EEP application. Less significant decrease in GGT activity was observed after 10 days of herbal application (group HD). A decrease ($P < 0.05$) in glucose content was noted in the DR control group, while an increase in that parameter value was observed in both experimental groups. A decrease in LA content during the experimental period was found in the groups supplemented with EEP or herbs (Table 5). The supplemented EEPD and HD groups demonstrated TAC increase after 10 days of the experiment. A decrease ($P < 0.05$) in TAC value was noted in the rabbits with diarrhea that were not subject to treatment. These changes were not significant.

4. Discussion

A variety of biologically active components contained in plants affect their wide range of activity in animal organisms. Nutritional studies conducted so far (18,19) point toward a significant influence of biologically active compounds on metabolic changes and immunological status of animals. Propolis also demonstrates a range of profitable biological features, especially antiseptic ones (12,15,20).

The present study showed that the tested preparations have beneficial effects, in particular on the shortening of the duration of diarrhea. They also allow to obtain higher body weight gains in sick animals as compared to healthy animals with diarrhea fed a standard fodder. Beneficial effects of herbs on production parameters, quality of animal products, and animal health were found using them as an additive for rabbits (19) and other animal species (21,22). Similar results were also obtained in the case of propolis (23,24).

In this study, EPEC belonging to two serotypes was isolated. Both strains are frequently isolated from diarrhea cases in weaned rabbits, which differ in pathogenicity. Serogroup O26 was recorded as a very virulent strain for weaned rabbits with high mortality, whereas O128 is less virulent (5,17). Previous in vitro studies have shown inhibitory action of EEP on EPEC strains isolated from rabbits (16). Some papers suggest that propolis is characterized by good antibacterial properties with respect to gram-positive bacteria strains (e.g., *S. aureus*), but is poorer in regards to gram-negative ones (20). The antibacterial activity of propolis is also affected by its origin and composition (25). Earlier in vitro studies indicated reduction in growth of EPEC strains after using EEP. The mixture of propolis and herbal extracts also showed bacteriostatic properties, while there were no inhibitory properties of an examined herbal extract in regards to *E. coli* (16). Regardless of the results obtained in vitro, the results of studies on rabbits show that the use of the herbal mixture has a significant impact on faster recovery. Moreover, some herbs included in the mixture have a curative effect in certain bacterial infections.

Common silverweed (*Potentilla anserina*) has been used in traditional medicine in the treatment of inflammatory conditions; for infections by bacteria, fungi, and viruses; or in the treatment of diarrhea (26). Common knotgrass (*Polygonum aviculare*) has, among others, a positive effect on intestinal and antibacterial activity (27). This study showed that administration of the given formulations can accelerate recovery on the basis of shorter duration of diarrhea, hematological parameters, and acid-base homeostasis. Additionally, curly dock (*Rumex crispus*) is a rich source of crude protein (22.25%) and minerals (Zn 0.199 mg/100 g, Mg 2.725 mg/100 g) (28).

At the beginning of the experiment, compensated metabolic acidosis was found in rabbits with chronic diarrhea. This type of disorder occurs in rabbits suffering from diarrhea, but it can also be the result of reduced food intake. Similar changes were observed by Kiwull-Schöne et al. (29) in rabbits kept without food and hence energy. It should be noted that ill rabbits initially reduce their food intake. In the group of rabbits with diarrhea the average feed consumption was the lowest, while in the groups with the addition of herbs or propolis, the initial decrease of food intake was compensated and average feed intake was the highest in these groups.

The use of preparations resulted in shortening of the duration of chronic diarrhea symptoms. This condition also resulted in an improvement of blood hematological parameters. In the group receiving EEP and herbs, Hct was reduced. Results of erythrograms revealed a significant ($P < 0.05$) decrease in RBCs. Furthermore, the initial value of WBCs decreased ($P < 0.01$) in groups HD and EEPD. In other studies, regarding the protective effect of EEP used in rabbits in combination with vaccination against *Pasteurella multocida*, there were no significant changes in the values of Hb, MCH, and MCHC (10). The study showed the normalization of the hematological parameters. On the final day of the study, the values of WBCs, RBCs, and Hct were similar to these noted in growing commercial hybrid rabbits (30).

Use of the investigated formulations in treatment has revealed that herbs do not have a negative impact on the basic biochemical blood parameters. Propolis, however, showed a hepatoprotective effect, as shown by the significant decrease in AST and GGT activity. Similar

results (activity of AST) were found in the research of Nassar et al. (8). Moreover, Nader et al. (12) suggested that EEP has a protective effect against atherosclerosis development in rabbits fed with a high-cholesterol diet. However, in the present study, there was no significant effect of the supplementation of the examined additives on blood serum cholesterol. It should be noted that this effect has been reported only in rabbits with prolonged pituitary hypertension that orally received a mixture made from an herbal preparation (27). The reduced concentration of TP in groups HD and EEPD at the end of the experiment was the result of dehydration. Examined biochemical parameters in the supplemented groups at the end of the experiment were within reference value ranges provided for commercial hybrids rabbits (30). The hematological and biochemical data observed in commercial hybrids are similar to those described in laboratory rabbits. Generally, differences in hematological and biochemical values could be caused by nutritional and environmental conditions typical for each industrial farm.

In conclusion, it should be noted that the application of an herbal mixture or EEP to drinking water for rabbits with chronic diarrhea resulted in accelerated recovery (reducing the duration of diarrhea), improved feed intake, and improved final body weight. Both the EEP and the herbal extract resulted in normalization of acid-base homeostasis and hematological parameters. EEP has shown an explicit hepatoprotective effect. These results may imply that the preparations have a favorable impact on the welfare and production of rabbits.

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