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GRAPEFRUIT EXTRACT IN STABILIZATION OF BACTERIAL FLORA IN POULTRY ALIMENTARY TRACT

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ABSTRACT

Grapefruit biologically active substances are found not only in its pulp, but also in its seed vessel (*flavedo*) and seeds. These complex substances exert a strong antioxidant and impeding influence on free radicals processes. We observed the stabilization effect of grapefruit extract on the microorganisms isolated from faecal samples of chicken. In this study we used of grapefruit extract dilutions - 0.1 per cent, 0.2 per cent, and 0.4 per cent. The grapefruit extract dilutions were added to water given to chickens. The reduction of the number of bacterial cells was observed as early as 30 min of incubation for *Staphylococcus aureus* 209 P. and *Escherichia coli* with the 0.4 per cent grapefruit extract. The reduction of the colonies of *Pseudomonas aeruginosa* with the 0.1 per cent, 0.2 per cent and 0.4 per cent grapefruit extract was observed after the second hour of incubation. In chicken we observed the decresing tendency in the number of bacterial colonies in all the groups of strains *Proteus vulgaris* and *Enterobacter cloaceae.* Additional microbiological tests for the presence of *Salmonella spp.* proved negative.

Key words: grapefruit, microorganisms, bacterial, chicken.

INTRODUCTION

Citrus paradisi (Mac Fayden) is the Latin name for the grapefruit which probably originated in Jamaica and is a hybrid of *C. grandis*. The grapefruit has a yellowish or yellow-red and juicy pulp with a distinctive sour-bitter flavour. The fruit has not only been enjoyed for its palatable qualities, but its medical features were already known to ancient Greeks due to Pliny, the botanist. Theophrastus, the Greek philosopher, thus wrote in 310 BC: "*The flesh of this fruit is used as a cure in poisoning, it can also refresh the breath...*" [13]. However, not earlier than 1980, Jacob Harich, the American immunologist, described the properties of the grapefruit which encouraged intensive phytochemical and pharmacological studies and its possible application to agriculture, breeding as well as to cosmetic, food and pharmaceutical industries [14, 15].

Grapefruit biologically active substances are found not only in its pulp, but also in its seed vessel (*flavedo*) and seeds [4, 10]. These complex substances exert a strong antioxidant and impeding influence on free radicals processes. Apart from a high content of bioflavonoids, flavones, and flavanoles, there also occur glycosides in the form of narginin, neohelperidins and cumarin derivatives [4, 5, 6]. In addition, the grapefruit contains mono- and sesquiterpenes, as well as polysaccharides (peptides, cellulose), mineral salts and vitamins (B_1, C, PP) [3]. The compounds described are both in the grapefruit itself as well as in its extract produced from the fruit pulp in a biothermical fermentation process.

Extensive data on the application of grapefruit extract in people was shown in many experiments whose focus was on the treatment for bacterial and mycotic infections. The experiments showed its high killing abilities for bacteria, fungi and even viruses. Yet there is a lack of reports on grapefruit extract in preventive and infection therapies in domestic animals. Therefore we decided to undertake research on possible applications of grapefruit extract to reduce and stabilize bacterial flora in the alimentary tract in poultry.

MATERIAL AND METHODS

The evaluation of the effect of grapefruit extract on the microorganisms isolated from chicken faecal samples was carried out *in vitro* on three bacteria species, namely *Escherichia coli,* a hemolythic strain, *Pseudomonas aeruginosa* and *Staphylococcus aureus* strain 209P (control). The strains were inoculated five times into enriched broth to receive a pure culture. First the bacterial suspension density was determined with the McFarland scale (bioMerieux, France), followed by a range of dilutions of the original suspension in order to obtain these densities: 10^3, 10^4, 10^6, and 10^8 bacteria per 1 ml. Simultaneously, a range of dilutions of 60 per cent grapefruit extract were made ready at hand; 0.1 per cent, 0.2 per cent, and 0.4 per cent. Then to each of these solutions bacterial suspension of the densities specified above was added in the proportion 1:1. The prepared mixture (0.1 ml) was transferred with an aseptic glass pipette onto a petri dish with enriched agar. The mixture was evenly streaked on its surface with an aseptic glass rod. It was then incubated for 3 hours, yet the bacterial colony was assessed after 30 minutes, at 1, 2 and 3 hour of incubation. Simultaneously, onto Petri dish was inoculated a mixture of the suspension of each bacterial strain and sterile placebo (glycerol of palmetinian oil).

Grapefruit extract dilutions - 0.1 per cent, 0.2 per cent, and 0.4 per cent, were added to water given to chickens (n=12). The chickens were divided into four groups. Group 1 drank 0.1 per cent water dilution of the extract, group 2 - 0.2 per cent water dilution, group 3 - 0.4 per cent water dilution and group 4- control - pure water. Each group was given the same amount of dilutions or water, ie. 800 ml per day. As the chickens were reluctant to drink water with the 0.2 and 0.4 per cent grapefruit extract, for the sake of the coming studies new dilutions were prepared which were more acceptable to the birds. The solution to begin with was 0.1 per cent (group 1), then 0.05 per cent - group 2 and 0.025 per cent - group 3. The screening remained unchanged.

Chicken observations were complemented with microbiological tests of chicken faecal samples to determine the reduction of the bacterial flora in the alimentary tract. The test material was collected at observation hour 3, 24, and 96. Twenty minutes after it had been collected, the material was inoculated onto the blood-agar, McConkay's and Sabouraud's media. At specified time intervals, the culture was examined for the intensification of microbial growth as a) +++ - big growth (over 400 cells), b) ++ - average growth (20-400 cells), c) + - single cell growth (up to 20 cells). In order to exclude the rods of *Salmonella spp.,* the faecal samples were inoculated into peptonic water. After 24 hour incubation, it was further cultured onto the Rappaport-Vassiliadis medium and selenine broth with cystin, which was followed by another 24-hour incubation. Finally, the material was inoculated onto a medium of brilliant green with cystin and McConkay's.

RESULTS

The results of microbiological tests which were carried out *in vitro* have been given in tables 1 and 2. In the control group the strain density was increasing with incubation time (Tab. 1). At 3 hour of incubation the number of colonies of *Pseudomonas aeruginosa* and *Escherichia coli* was innumerable, while the number of colonies of the standard strain for all the densities was 20-fold higher than the original colony number. In the chickens drinking the extract, the growth was either curbed or hindered in all the strains under study. The reduction of the number of bacterial cells was observed as early as 30 min of incubation for *Staphylococcus aureus* 209 P. and *Escherichia coli* with the 0.4 per cent grapefruit extract. The reduction of the colonies of *Pseudomonas aeruginosa* with the 0.1 per cent, 0.2 per cent and 0.4 per cent grapefruit extract was observed after the second hour of incubation. Hindering the growth of the colonies of *Staphylococcus aureus* 209 P and *Escherichia coli* was significant. At 3 h of incubation was observed a profound limitation in the strain of *Pseudomonas aeruginosa* in all the concentrations of grapefruit extract (Tab. 2)

Table 1. The concetrations of bacteria *in vitro* on some microorganisms not treated with grapefruit extract

Hours (h)	*Staphylococcus aureus* 209P				*Pseudomonas aeruginosa*				*Eschericha coli* (haemolitic strain)			
	Concentrations of bacteria				Concentrations of bacteria				Concentrations of bacteria			
	10^3	10^4	10^6	10^8	10^3	10^4	10^6	10^8	10^3	10^4	10^6	10^8
0	2	8	8	22	n (*	100	1276	n (*	13	5	4	2
½	10	14	60	60	n (*	358	n (*	n (*	25 180	30	11	30
1	40	20	191	156	n (*	n (*	n (*	n (*	77	35	13	60
2	40	32	200	268	n (*	n (*	n (*	n (*	180	700	25	120
3	40	43	224	370	n (*	n (*	n (*	n (*	n (*	n (*	101	830

Explanations: n (* - innumerable

Table 2. The concetrations of bacteria *in vitro* on some microorganisms treated with grapefruit extract

Concentrations of bacteria	Bacterial colonies								
	Staphylococcus aureus 209P			*Pseudomonas aeruginosa*			*Eschericha coli* (haemolitic strain)		
	concentrations of grapefruit extract			concentrations of grapefruit extract			concentrations of grapefruit extract		
	0.1%	0.2%	0.4%	0.1%	0.2%	0.4%	0.1%	0.2%	0.4%
after ½ hours									
10^3	n (*	n (*	0	3	2	0	0	2	0
10^4	4	0	32	10	10	0	40	1	0
10^6	0	1	0	86	0	0	50	3	0
10^8	0	n (*	0	180	39	4	46	1	0
after 1 hours									
10^3	3	0	0	3	23	0	0	5	0
10^4	1	0	45	0	2	0	3	0	0
10^6	0	6	0	140	0	0	30	20	0
10^8	0	100	0	220	0	0	140	0	0
after2 hours									
10^3	0	0	0	0	0	0	0	0	0
10^4	5	0	1	0	0	0	6	0	0
10^6	0	0	0	70	0	0	8	0	0
10^8	0	0	0	120	0	0	30	0	0
after 3 hours									
10^3	0	0	0	0	0	0	0	0	0
10^4	0	0	0	0	0	0	2	0	0
10^6	0	0	0	14	0	0	0	0	0
10^8	0	0	0	70	0	0	35	0	0

Tab. 3 shows feed and water intake by the chickens which were administered various concentrations of grapefruit extract during 20 days. With this data, one can say that water intake was similar in all the groups (4.14 - 4.29 litre per chicken). The data analysis of feed intake, when compared with body increase in particular groups, was the most effective in group 1, receiving 0.1 per cent solution of the extract. In groups 2 and 3 as well as the control group, the feed intake was higher, which made the mean intake per 1 kg of body weight increase higher. Tab. 4 presents mean body weight in all groups of chickens during the time of observations. The 24-hour measurements of body weight reveal that after 5 days of observations, the highest body weight increases were observed in group 1 and the control group. Up to 20 day of observation, the highest dynamics of body weight increases was seen in the group receiving 0.1 per cent and 0.05 per cent solution of grapefruit extract.

Table 3. Feed and water intake during 20-day observation of chickens which were administered various grapefruit extract solutions

Concentrations of grapefruit extract (%)	Water intake (l per chicken)	Feed intake (g per chicken)	Body weight per unit of mass of feed (b.g.w./1000g feed)
Group 1 0.1 %	4.15	1970	1430
Group 2 0.05 %	4.29	1960	1434
Group 3 0.025 %	4.20	1960	1540
Group 4 (control)	4.14	1900	1476

Table 4. Mean body weight and gain of body weight in chickens which were administered various grapefruit extract solutions during 20-day observation

Concentrations of grapefruit extract (%)	Mean body weight (g)				Gain of body weight (g)		
	day "0"	after 5 days	after 12 days	after 20 days	after 5 days	after 12 days	after 20 days
Group 1 0.1 %	551 ± 105.13	774 ± 122.27	1286 ± 241.05	1928 ± 291.45	223	512	1377
Group 2 0.05 %	575 ± 110.28	771 ± 177.28	1388 ± 220.27	1941 ± 368.69	196	617	1366
Group 3 0.025 %	571 ± 81.68	771 ± 135.09	1306 ± 144.38	1843.5 ± 197.33	200	558	1272
Group 4 (control)	527 ± 80.85	734 ± 143.5	1227 ± 228.06	1814 ± 291.2	207	493	1287

Microbiological tests of faecal samples are presented in Tab. 5. Bacterial flora in the alimentary tract did not manifest any significant fluctuations in groups 1, 2 and 3. The number of colonies of *Escherichia coli* was reduced in group 2, and in groups 1 and 3 remained on the same level throughout the observation time. The decresing tendency in the number of bacterial colonies was observed in all the groups of strains *Proteus vulgaris* and *Enterobacter cloaceae.* Additional microbiological tests for the presence of *Salmonella spp.* proved negative.

Table 5. Results of microbiological tests of chicken faeces samples which were administered various grapefruit extract solutions

Examination	Groups	*Eschericha coli*	*Proteus vulgaris*	*Proteus mirabilis*	*Enterobacter cloaceae*	*Enterobacter faecalis*
day "0"	Grupa 1	++	+			
	Grupa 2	+++	+	++		
	Grupa 3	+	+		+	
	Grupa 4	++				
after 3 hours	Grupa 1	+ - +++	+	+	+ - ++	++
	Grupa 2	+ - ++	+	+		+ - ++
	Grupa 3	+ - +++		+	+ - ++	
	Grupa 4	+ - +++		+ - ++		
after 24 hours	Grupa 1	4 kolonie - ++		1 kolonia - +		++
	Grupa 2	b/w - ++		+		
	Grupa 3	b/w - ++		3 kolonie	1 kolonia	
	Grupa 4	+ - +++	+	+	+ - ++	++
after 96 hours	Grupa 1	++		+		
	Grupa 2	1 kolonia				
	Grupa 3	++		+	1 kolonia	
	Grupa 4	++		+++		+

Explanations: +++ - big growth in bacterial colony, ++ - average bacterial growth, + - single bacterial colony , b/w - no growth

DISCUSSION

Natural bacterial flora in the alimentary tract is a stronghold against pathogenic microorganisms like *Salmonella spp,* or against the outnumbering of bacteria which can be pathogenic under certain conditions (*Clostridium spp.*) and yeast-like fungi (*Candida*). Antibiotic theraphy frequently results in originating resistant strains which makes the treatment ineffective and helps these bacteria propagate. Besides, administering medications of wide spectrum may evoke systemic mycoses mostly caused by *Candida* fungi. That is why physicians' interest in phytotherapy is growing, including grapefruit extract whose germicidal, fungicidal and virucidal effect has been confirmed by both *in vivo* and *in vitro* tests. The extract disturbs the intracellular respiratory processes, impairs cell membrane as well as impeding aminoacids synthesis leading to cell inactivation and its death [2, 4, 10, 12, 13]. Such an effect can be observed for ponciretin impeding the activity of urease which eventually hinders the growth of *Helicobacter pylori* [1]. In anaerobic microorganisms, grapefruit extract has proven highly effective in the case of Gram-negative strains impeding their growth by 87% (*Bacterioides spp., Prevotella spp, Porphyromonas spp.*) with MIC (minimum inhibitory concentration) not exceeding 1.2 mg/ml. In the case of Gram-positive strains (*Peptostreptococcus spp., Actinomyces spp, Propionibacterium spp.*), a 33 per cent concentration of the extract hindered bacterial growth by 100 per cent (MIC ranging <0.07-2.5 mg/ml) [6]. A similarly high sensitivity and low MIC values showed fungi of *Candida spp.* species isolated from stoma, vagina, urethra, faeces, for which mean MIC value on the Sabouraud medium was 3.75 µg/L, with mean MIC for a standard strain *Candida abbicans* 10231 ATCC 12.5 µg/L [9]. The growth in yeast-like fungi isolated from infections in the stoma was also inhibited by grapefruit extract in 30 per cent with the MIC below 50 µg/ml. The remaining strains had the MIC values - 200 µg/ml. Similarly, *Candida albicans* dominating in stoma infections in children, was impeded by 47 per cent by the extract and in the case of other strains - *C. krusei* and *C. tropicalis* - by 50 and 60 per cent, respectively [7, 8].

The results of our own observations are similar to those by other researchers. No negative effect of grapefruit extract has been observed on the feed and water intake. In microbiological tests *in vitro* hindering of the growth in bacterial strains was observed and in the examinations *in vivo* - flora normalisation in the alimentary tract. These observations lead to the conclusion that grapefruit extract with its array of properties can be an alternative or complementary therapy in the alimentary tracts diseases caused by bacteria.

CONCLUSIONS

1. Was obserwed *in vitro* the reduction of the colonies of bacterial with the 0.1 per cent, 0.2 per cent and 0.4 per cent grapefruit extract during incubation.
2. During *in vivo* observation was obserwed the highest dynamics of body weight increases in the group receiving 0.1 per cent and 0.05 per cent solution of grapefruit extract.
3. In microbiological tests of faecal samples we obserwed reduction the number of colonies of *Escherichia coli* in group 2, and in groups 1 and 3 remained on the same level throughout the observation time. The decresing tendency was observed in all the groups of strains *Proteus vulgaris* and *Enterobacter cloaceae*.

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