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LABORATORY STUDY TO DETERMINE THE EFFECT OF A PROBIOTIC MIXTURE ON CHICKEN-BROILER[1]

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Abstract: The experiment was performed in two groups of broiler chickens. The birds from the experimental group were treated with the combination of 3% lactic bacteria, 1% baker's yeasts and 0.7% citric acid, added to food. It was found out that the treatment resulted in shifting the microbial balance in avian gastrointestinal tract in favour of Gram-positive bacteria (77-80%) while in control birds Gram negative organisms prevailed (90%). Both the volume and the weight of viscera of experimental chickens, obtained following slaughtering was by 20-60% higher compared to controls. Furthermore, 75% of treated birds reached a slaughtering weight (1800 g) for 42 days with an average daily weight gain of 57 g and expenditure of 2.3 kg fodder per 1 kg weight gain, whereas the body weight of control birds was by 26.5% lower than the standard one, the fodder expenditure was 3.1 kg per 1 kg weight gain and the average daily gain was 42 g. The mortality in controls was 13% while in treated birds there were no lethal cases. The price of one kilogramme body weight in experimental birds was by 0.15 $ lower compared to controls.

Key words: chickens, probiotics, organic acids, micro flora.

Introduction

It is known that the disorders in the composition of normal gastrointestinal micro flora in animals could result in dysbacteriosis caused by E.coli and coliform bacteria followed by various pathologies. The diet of skim milk powder, soy bean meal or fish meal has a high acid binding or buffer capacity. This fact, together with an excessive intestinal pH, allows pathogenic Gram-negative bacteria such as *E. coli and coliforms bacteria* to colonize the digestive tract causing inflammation

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and digestive disorders, so the gut absorbs fewer nutrients (*Blanchard and Wright 1999; Geboes 1999; Anonymous 1999). Van Kol (1999)* reported that in stressed birds, the amount of *E. coli* in gastrointestinal tract increased as well as intestinal pH, thus decreasing Gram-positive micro flora and producing a dysbacteriosis of Gram-negative pathogenic bacteria that colonize intestines, cause inflammation of intestinal mucosa, decreasing the absorption of nutrients and stunting the growth of birds. *Shane (1999)* suggested that there were several causes responsible for the slow growth rate and the low weight gain in broiler chickens. *Edelson (2002*) reported that in some instances, the continuous administration of high nutritive doses of antibiotics or the use of subtherapeutic doses of antibiotics was followed by dysbacteriosis and infection with *Proteus spp., Pseudomonas spp., Aspergillus spp., Candida albicans* etc.

Karadjov et al. (1984) showed that deep manner litter contains 10^9 E.coli per gramm and serves for reinfection in chickens with this bacterium. The same authors emphasised that the aerosol with E.coli more than 300000/m^3 cause microbial stress and enhance the outbreak of colibacteriosis in chickens. *Karadjov and Kaloyanov (1978*) found out that the fodder contaminated with E.coli 078 and 026 causes a colibacterioses in birds with 2,9% mortality. According to *Gross W.B. and C.H.Domermuth (1979*) there are probably many pathogenic E.coli strains which do not belong to known serotypes and a serological identification will not distinguish infecting organisms from faecal contaminants of birds.

During last years, numerous studies aiming to normalize intestinal micro flora composition and to preserve the animal gastrointestinal tract from colonizing with pathogenic organisms have been performed. *Boycheva (1988*) found out high inhibitory activity of L.bulgaricus and Str.thermophylus on enteropathogenic E.coli in vitro and described a significant decrease in morbidity and the death rate in newborn pigs, treated with lactobacilli and enterococci or "Anticolin"-product. *Kondareva (1993*) gave evidence for the presence of high inhibitory activity of lactobacilli and enterococci isolated from birds on the E.coli. *Pal (1990)* recommended the supplementation of avian fodder with lactic bacteria and yeasts in cases of stress. *Ohhira(2000)* has reported that Lactic bacteria inhibit both putrefying and ammonifying bacteria in human intestines, decreasing the intoxication of the organisms with polyamines (cadaverin, putrescin) and ammonia moreover, an immuno stimulating effect of *Lactobacillus spp*. manifested by increased

phagocyte activity and production of immunoglobulins in blood, was shown. *Luzkanov (2000)* has communicated that "Avigard" (probiotic of Bayer Company) shows very good preventive effect on an artificial infection with S.enteritidis in chickens without any carrier of salmonella but well demonstrated protection of guts barrier. According to *Hooge D. (2003)* direct-fed microbials contribute micro-organisms like Bacillus, Lactobacillus, Streptococcus, bacteroides, yeasts and moulds with the goal of increasing intestinal microbial load and, by competitive exclusion, decreasing pathogenic bacteria populations in gut and an enhance the growth of broilers. *Dallout R.A. et al. (2003)* found out that the probiotic bacteria have a positive impact on the local immune response and increased the birds' resistance to Eimeria acervulina which was demonstrated by reduced oocyst shedding.

The data of *Blanchard and Wright (1999)* demonstrate that the acid environment (pH 3.5-4.0) favourizes the development of lactobacilli and inhibits the replication of *E. coli, Salmonella spp.* and other Gram-negative bacteria. The combination of lactic, propionic and formic acid is said to kill *E. coli* and *Salmonella spp*. organisms *(Hansen 1999)*. The studies of *Ivanov (2001a, 2001b)* and *Luc (2002)* established that organic acids decreased pH and create unfavourable conditions for vegetation of many Gram-negative organisms. Lactic acid reduces the count of Gram negative and increased that of Gram-positive organisms in animal digestive tract (*Geboes 1999*), improves the health and the weight gain of animals (*Adams 1999*). According to *Puyalto M. and J.Mesia (2002*) acid-type additives are especially useful in young animal feeds because they are often effective against disease-causing microbes in the gut such as Salmonella spp. and E.coli as well.

The review of literature and the current problems of poultry breeding (anti-bio resistant strains of pathogenic bacteria, contaminated poultry meat and eggs with *Salmonella*, *Listeria*, *E.coli* etc., residues of antibiotics in poultry products, stunting and runting problems in broiler stocks, immunosuppression, etc.) motivates the necessity of additional studies and attempts for regulation and optimization of micro flora balance in avian gut. The aim of the present study was to study the influence of a combination of organic acids and probiotics upon the health condition and the weight gain in broiler chickens.

Material and methods

Laboratory experiment

It was performed to elucidate the influence of probiotics and organic acids upon the growth of E.coli 078, 026 isolated form dead birds and on growth and health of chicken broilers.

The nutrient media, used for isolation, were Müller-Hinton agar, ordinary agar, blood agar (indicator for haemolytic strains) and MacConkey's agar (indicator for *E. coli, Salmonella spp., Proteus spp., Citrobacter spp., Pseudomonas spp.*).

The activities of oxidase and catalase of grown colonies was determined. The Krigler medium was used for detection of H_2S-producing, glucose- and lactase-utilizing strains. Preparations were stained according to Gram for determination of microbial shape (cocci, rods), Gram-positive and Gram-negative isolates.

The oxidase test was done with Bactident, Oxidase test strips (Merck, 64271 Darmstadt, Germany, 04071 JUL 02). The positive reaction as manifested by a blue-violet coloration of the test strip.

The catalase test was done with 3% hydrogen peroxide upon a glass slide. The catalase producing strains were evidenced by the presence of oxygen bubbles in the peroxide-microbial colony mixture.

Probiotics

A pure culture of *Lactobacillus bulgaricus* (Silo Guard, Denver, Colorado) and *Streptococcus thermophilus* (Temp Lac, Denver, Colorado) in hydrolyzed milk and extruded baker's yeasts were used. The influence of probiotics upon pathogenic bacteria was assessed in a suspension experiment (*Boycheva, 1988*) and by the disk diffusion method (*Giraffa et al., 1994*).

Acidifiers

The influence of organic acids upon gastrointestinal micro flora was evaluated using crystalline citric acid (molecular weight 144, solubility 1:0.6; tricarboxylic/ tribasic; price 1.5 $/kg, Himsnab, Bulgaria). The citric acid was added to the fodder in concentration 0.7% daily until the end of the experiment.

Determination of pH

pH was determined using Precision Digital pH-meter type OP-208 (VEB Feinchemie Sebnitz, Germany) and indicator strips for pH within the range 4.5–10.0 (LaChema, Brno, Czech Republic).

Determination of microbial cell counts

The total counts of microbial cells of *E.coli* in one gram of intestinal content, faeces and fodder was determined via tenfold dilutions (from 10^{-1} to 10^{-7}) and inoculation of 0.1 ml of each dilution in Petri's dishes with MacConkey's agar. The cultures were incubated at 37 ^{o}C for 24 hours and the number of grown colonies was determined. The differentiation of micro organisms was done visually (shape, size and colour of colonies, motility), biochemically (utilization of sugars, alcohols, production of indole, ammonia, hydrogen sulphide, catalase, oxidase, urease, change in cultural pH), serologically (O- H-agglutination with mono- and group-specific sera). The yeasts were cultivated on Saburo agar for 72 hrs at 22^{o}C (*Bergey, 1986*).

Test microorganisms

The in *vitro* efficacy of both organic acids and the probiotic was evaluated using *E. coli* 078,026 and *S. enteritidis*, isolated from dead chickens and *S. aureus, L. monocytogenes, Proteus spp.* and *Pseudomonas spp.* strains (*Ivanov, 2001*).

Fodder

The fodder that was used contained(has been consisted) 35% of corn meal, 32% wheat meal, 9% sunflower meal, 12% soybean meal, 4% fish meal, 4% powder of skim milk, 1,1% dicalcium phosphate with a pH of 7,5. For in vitro experiments there have been prepared 5 (five) probes: #1 contained 50g fodder+70ml water+1ml E.coli ($3x10^{8}$pfu), #2 consisted 50g fodder+70ml water+1ml E.coli ($3x10^{8}$pfu)+10ml L.bulgaricus and Str.thermophylus in hydrolyzed milk, #3 was with 50g fodder+70ml water+1ml E.coli ($3x10^{8}$pfu)+1g extruded baker's yeasts, #4 was with 50g fodder+70ml water+1ml E.coli ($3x10^{8}$pfu)+0,7g citric acid, #5 was with 50g fodder+70ml water+1ml E.coli ($3x10^{8}$pfu)+0,7g citric acid+10ml L.bulgaricus and Str.thermophylus in hydrolyzed milk+1g extruded baker's yeasts. All of these probes were incubated at

37°C for 6 hours. After that the number of E.coli in each of these probes was measured.

Experimental animals

The influence of probiotics and organic acids upon the chickens was determined in 60 one-day-old chickens from the French Chaiver mini-bro hybrid, divided into two equal groups:
First group (control) was supplemented with 1 ml 24-hour *E. coli* broth culture at a dose of 10^8 CFU in fodder per bird, morning and evening, for 6 weeks.

Second group (experimental) received 3% probiotics (*L. bulgaricus* plus *Str. thermophillus*) in fodder plus 10^8CFU *E. coli* per bird plus 1% baker's yeasts and 0.7% citric acid for 42 days. The litter was treated with 4% citric acid as well.

Each 7 days, samples from the crop and intestinal content (colon) were obtained for determination of the ration between Gram-negative (especially E.coli) and Gram-positive (rod and cocci) organisms. At days 20 and 42 after the beginning of the treatment, the body weight of broilers was measured.

The data were statistically processes using the Student's t-test (*Sepetliev, 1980*).

Results and Discussion

In our studies using the agar-diffusion method and the suspension technique, we found out that lactic acid bacteria (Lactobacillus bulgaricus, Silo Guard, Denver, Colorado) demonstrated a various inhibitory activity against some pathogenic micro organisms (table 1). The inhibitory effect, measured by the diameter of the sterile zone around the well containing the probiotic, is dose-dependent and the biggest for the 0.38 ml dose against S. enteritidis, good against Staph. aureus and L.monocytogenes and weaker against E. coli. The suspension experiment revealed that this probiotic inhibited the growth of fore mentioned pathogens (table 2), reducing their counts with 99% in combined cultivation. All this is important for the colonization of avian gut with pathogenic micro organisms and their adherence to intestinal mucosa epithelium.

Table 1. Inhibitory effect of Lactobacillus bulgaricus-LB (Silo Guard) and Streptococcus thermophilus-ST (Temp Lac) on some bacteria by diffusion method
Tabela 1. Inhibitorni efekat Lactobacillus bulgaricus-LB (Silo Guard) i Streptococcus thermophilus-ST (Temp Lac) na neke bakterije metodom difuzije

Volume of LB Zapremina LBa	Sterile zone in millimetres after 24 hrs of cultivation on agar media Sterilna zona u milimetrima nakon 24 h gajenja na agaru			
	E.coli	Staph.aureus	S.enteritidis	L.monocytogenes
0,38ml	18	21	25	21
0,23ml	14	14	22	17
0,21ml	11	14	20	14
0,12ml	12	12	15	14
volume of ST				
0,38ml	20	30	17	21
0,23ml	20	30	14	19
0,21ml	20	30	11	21
0,12ml	20	30	12	12

Our experiments with Streptococcus thermophilus (Temp Lac, Denver, Colorado) by the agar-diffusion method showed (table 1) that the inhibitory effect of this probiotic was poorly dose-dependent and the most expressed against Staphylococcus aureus, followed by L. monocytogenes and E. coli and the least against S. enteritidis. It is interesting that the inhibitory effect was stronger against Gram-positive compared to Gram-negative bacteria. The data from the suspension experiment (table 2) showed that this probiotic inhibited the growth of those pathogenic micro organisms, reducing their counts more than of 99%. It must be emphasized that the achievement of an optimal balance of micro flora in avian gut requires the simultaneous use of probiotics containing lactobacilli, lactostreptococci, bakers and brewery yeasts.

Table 2. Inhibitory effect in percents (%) of Lactobacillus bulgaricus (Silo Guard) and Streptococcus thermophilus (Temp Lac) on some bacteria in broth media after 24 hrs cultivation (suspension method)
Tabela 2. Inhibitorni efekat u procentima (%) Lactobacillus bulgaricus (Silo Guard) i Streptococcus thermophilus (Temp Lac) na neke bakterije na tečnoj podlozi nakon 24 h gajenja (metoda suspenzije)

Kind of bacteria Vrsta bakterije	L.bulgaricus	pH	Str.thermophylus	pH
E.coli	99%	4,5	99,99%	4,0
Staph.aureus	99,99%	4,0	99,99%	4,0
S.enteritidis	99,7%	4,5	99,88%	4,5
L.monocytogenes	99,88%	4,0	99,99%	4,5

The results of our studies evidenced that 4% citric acid and 4% tartaric acid inhibited the growth of several Gram-negative bacteria such as E. coli, Proteus spp., Pseudomonas spp., S.enteritidis and some Gram-positive organisms like L. monocytogenes and Staph. aureus (table 3). Those organic acids reduce considerably the contamination of litter with such organisms and simultaneously, neutralize the ammonia production. On the other side, the balance between Gram-positive and Gram-negative micro organisms is optimized, the risk of reinfection, superinfection and disbacteriosis in birds is diminished.

Table 3. Inhibitory effect in percents (%) of some organic acids on some micro organisms in broth media at exposure time of 48 hrs
Tabela 3. Inhibitorni efekat u procentima (%) nekih organskih kiselina na mikroorganizme na tečnoj podlozi nakon 48 sati izlaganja

Species of	Inhibitory effect in percents (%) of organic acids			Steril zones on agar in mm	
bacteria	4% citric acid	4% tartaric acid	1,5% salycilic acid	4%citr.acid	0,5%c.acd
E.coli	99%	99%	100%	13mm	6mm
L.monocytogenes	99%	99%	100%		
Proteus spp.	99%	99%	100%		
Pseudomonas spp.	99%	99%	100%		
Salm.enteritidis	99%	99%	100%		
Staph.aureus	99%	99%	100%		

The data on the table 4 show a huge inhibitory effect (over the 90%) of all of used probiotics on E. coli in the fodder. The highest effect is recorded (more than of 99%) in the probe with “probiotic” mixture. This result brings out a synergism between lactoso-positives bacteria, yeast and citric acid as well. There is a correlation between this effect and a low pH.

Table 4. Inhibitory effect of some probiotics (L.bulgaricus, Str.thermophylus, baker yeasts and citric acid) on E.coli 078+ 026 serotypes in fodder for chicken broilers in 6 hrs incubation at 37oC
Tabela 4. Inhibitorni efekat nekih probiotika (L.bulgaricus, Str.thermophylus, pekarski kvasac i limunska kiselina) na E.coli 078+ 026 serotipove u hrani za brojlere nakon inkubacije na 37^0C u trajanju od 6 sati

Kind of sample Vrsta uzorka	Inhibitory effect in percents(%) Inhibitorni efekat u procentima (%)	pH of sample pH vrednost uzorka
1.Control feed/kontrolna hrana 50g+	0%	7,57
2ml($3x10^8$cfu*ml) E.coli+		
70ml water		
2. Fodder/Kabasta hrana +E.coli+3% lactoso-	92,66%	7,16
Positive bacteria.		
3.Fodder/kabasta hrana +E.coli+1% b.yeasts	96,52%	6,99
4.Fodder/kabasta hrana +E.coli+0,7%citric		
acid	99,76%	6,63
5.Fodder/kabasta hrana +E.coli+3%lacto-		
positive bacteria+1%yeasts+		
0,7%citric acid	99,98%	6,78

Tables 5 and 6 showed that Gram-positive bacteria formed only 10% of microbial micro flora while Gram-negative organisms – 90% of the micro flora in control broiler chickens. Simultaneously, in experimental groups, treated with probiotic and organic acid, Gram-positive bacteria predominated (77-80%). These data indicated that control chickens have more than of 3,8 to 5 times E.coli in one gramme of colon contains at the age of 20 and 42 days, 6,6 times more coliformes on 20 days of age and less than of 12 to 250 times yeasts comparing with treated birds.

Table 5. Data for the composition and the percentage of gastrointestinal micro flora of broiler chickens at the age of 10, 20 and 42 days (Gram-stained samples)

Tabela 5. Podaci o sastavu i procentu gastro-intestinalne mikroflore brojlera u uzrastu od 10, 20 i 42 dana (Gram obojeni uzorci)

Microorganisms/ mikroorganizmi	Experimental chickens, age/ Eksperimentalni pilići, uzrast			Control chickens, age/ Kontrolni pilići, uzrast		
	10 days/dana	20 days/dana	42 days/dana	10 days/dana	20 days/dana	42 days/dana
Gram-positive	80%	77%	50%	28%	28%	10%
Gram-negative	20%	23%	50%	72%	72%	90%

Table 6. Data for microbial counts per gram caecal and colon content in broiler chickens

Tabela 6. Podaci o mikrobijalnom broju po gramu sadržaja slepog i debelog creva kod brojlera

Microorganisms/ mikroorganizmi	Experimental chickens, age/ Eksperimentalni pilići, uzrast			Control chickens, age/ Kontrolni pilići, uzrast		
	10 days/dana	20 days/dana	42 days/dana	10 days/dana	20 days/dana	42 days/dana
E. coli	4×10^7	3×10^6	7×10^6	4.2×10^7	1.5×10^7	2.7×10^7
Coliforms	4.8×10^8	3×10^6	-	4.6×10^8	2×10^7	-
Aerobic cells	3.2×10^8	4×10^7	1.9×10^8	1.3×10^8	2×10^7	5.4×10^8
Yeasts	5×10^5	5.4×10^5	5.4×10^5	2×10^3	3.8×10^4	4.2×10^4

From the data in table 7 is seen that 75% of experimental broiler chickens reached the technological weight for 42 days while the body weight of control birds was by 26.5% lower than the standard one. There was a statistically significant difference in both weight gain and the live weight in both groups at the age of 20 and 42 days.

Table 7. Data for the live weight of broiler chickens

Tabela 7. Podaci o telesnoj masi brojlera

Age/Uzrast	Live weight/telesna masa, g	
	Experimental birds/ ogledni brojleri, n=30	Control bird/ kontrolni brojleri, n=30
10 days/dana	148 ± 21 (120-175)	104 ± 20 (– 30%) (80-130)
20 days/dana	426 ± 58 (375-520) p< 0.01	298 ± 42 (– 30%) (260-360)
42 days/dana	1677 ± 231 (1350-1850) p< 0.05	1233 ± 42 (– 26.5%) (1150-1400)
Percentage of birds that reached the technological weight at the age of 42 days/ Procenat brojlera koji su dostigli masu iz tehnologije u uzrastu od 42 dana	75%	0%

The fodder expenditure per kg weight gain was 2.3 kg in experimental and 3.1 kg in control chickens respectively. The difference of 0.8kg of fodder in favour of treated birds is due to the supplementation with citric acid, baker's yeasts and yoghurt. The benefit from the treatment was reached for a technological period of 42 days. The difference of 444 g with the slaughtering weight required another 10 days and 1432 g fodder for reaching a technological weight, which increased the cost of weight gain and decreased the benefit by broiler chickens for attainment of a slaughtering weight of 1800 g.

Data from table 8 evidenced a clear difference in the daily weight gain in favour of experimental chickens during the whole technological period of breeding until the attainment of 1800 g weight. The mortality in control birds was 17%, while in treated birds there were no cases of lethality.

Table 8. Data for daily weight gain in broiler chickens
Tabela 8. Podaci o dnevnom prirastu mase brojlera

Groups/Grupe	Daily weight gain/Dnevni prirast mase, g			Dead birds/ uginuli brojleri, %
	10 days	20 days	42 days	
Experimental/ Ogledna, n=30	15.0	28.0	57.0	None/ nijedna
Control/ Kontrolna, n=30	10.4	19.4	42. 5	1 7% (5 birds/ brojlera)

Table 9 shows that fodder expenditure per unit weight gain in treated birds was by 34.8% lower compared to controls. The cost of 1 kg body weight in control chickens was by 0.15 $ higher than in experimental ones.

Table 9. Data for fodder cost and cost of 1 kg of weight gain
Tabela 9. Podaci o ceni hraniva i koštanju 1kg prirasta mase

Groups/Grupe	Fodder expenditure per 1 kg weight gain/ Potrošnja hraniva za 1kg prirasta mase, kg	Cost of 1 kg weight gain/ Cena 1 kg prirasta mase, $	Difference/ Razlika
Experimental/ Ogledna, n=30	2.3 (–34.8%)	0.60	–0.15 $
Control/ Kontrolna, n=30	3.1 (+34.8%)	0.75	+0.15 $

Table 10 showed significant differences in the size and weight of body and viscera between experimental and control birds in favour of the former group. The total body weight in controls was by 28% lower. The crop volume was by 32% lower, the duodenum - by 24% shorter, the thin

bowels – by 19% shorter, the caecum volume - by 60% lower and the liver- by 20% lighter than the respective parameters in treated chickens.

Table 10. Morphometric data for organs of the gastrointestinal tract in experimental and control birds
Tabela 10. Morfometrijski podaci za organe gastro-intestinalnog trakta kod oglednih i kontrolnih brojlera

		Experimental birds/ ogledni brojleri, n=30	Control birds/ kontrolni brojleri, n=30
Total weight/ukupna težina, g		2085±227 (p<0,01) (1770-2285)	1502±141(-28%) (1420-1665)
Crop/voljka	Weight/težina, g	12.6±3.78 (p<0,01) (10-17)	8.0±1,73 (7-10)
	Volume/ zapremina, ml	125 ± 18(p<0,01) (105-140)	86+24.6 (-32%) (70-115)
Stomach (glandular + gizzard) / stomak	Weight/težina, g	101 ± 15 (p<0.01) (80-115)	66 ±15 (25-27)
Duodenum/ dvanaestopalačno crevo	Length/ dužina, cm	34 ± 5(p<0,01) (25-36)	26 ±1 (-24%) (25-27)
	Weight/težina, g	16.8 ± 2.17 (p<0,05) (15-20)	14.5 ± 4 (10-17.5)
	Volume/ zapremina, ml	12 ± 0.7 (p<0,01) (11-12)	8.33 ± 1.52 (7-10)
Caecum/ slepo crevo	Length/ dužina, cm	23.25 ±0.95(p<0,01) (22-24)	16.83 ± 2.56 (-27%) (14-19)
	Weight/težina, g	6.45 ± 0.41(p<0,01) (6-7)	4.16 ± 1.03 (3-5)
	Volume/ zapremina, ml	20 ± 3.55(p<0,01) (15-23)	8 ± 3.46 (-60%) (4-10)
Jejunum-ileum/ prednji i završni deo tankog creva	Length/ dužina, cm	173 ± 20.49(p<0,01) (152-196)	140.33 ± 10.51 (-19%) (130-151)
	Weight/težina, g	63.75 ± 19.73(p<0,01) (45-90)	43.33 ± 12.72 (30-55)
	Volume/ zapremina, ml	104.5 ± 21 (80-130)	84.33 ± 41.79 (-19%) (48-130)
Liver/ jetra	Weight/težina, g	55 ± 6.45(p<0,01) (45-65)	43.33 ± 3.08 (-20%) (40-45)

The results of our observations and microbiological studies showed a shift in the balance of intestinal micro flora in favour of Gram-negative bacteria and their negative impact upon avian health in control group of birds. In such cases the selection of resistant and pathogenic *E. coli* strains that could provoke septicaemia and quick death in conditions of decreased general systemic resistance (overcrowding, high humidity and ammonia, low-quality fodder, alkaline gastrointestinal pH; subclini-

cal mycotoxicosis etc.). Those strains dominate in gastrointestinal micro flora with shifted balance towards Gram-negative organisms. On the other side, those bacteria accumulate in litter – a source of huge infecting and re-infecting doses.

Probiotics could activate the lactoperoxidase-thiocyanate system in intestines. In this system, lactoperoxidase is combined with hydrogen peroxide and oxidizes thiocyanate to an intermediate product that could inhibit bacterial growth and has a bactericide effect when pH is low *(Priells J., Ph. Delahaut, A. Kaekenbeck.1989; Priels J., D. Monnom, Ph. Delahaut, E. Jaquemin, A. Kaeckenbeck, 1989; Perraudine J., 1990,1991*). Probiotics such as live baker's and brewer's yeasts and especially *Saccharomyces cerevisiae*, release manano-oligosaccharides that occupy intestinal receptors and thus preserve them from the influx and attachment of pathogenic bacteria, enhance and improve their transit passage through intestines without colonizing them (*Pal,1999;Ohira,2000*). Therefore, probiotics have to follow the oral application of antibiotics for achieving the effect of balance, i.e. preservation and restoration of healthy micro flora and cleaning the intestine from pathogenic bacteria (*Pal 1999; Ohirra 2000*). Lactobacteria release also bactericin, proteins with antimicrobial properties, used for bio conservation of dairy products (*Giraffa et al. 1994)* that also contributes to optimization of microbial balance in avian gastrointestinal tract. The beneficial effect of the application of probiotics is possibly related to the various types of antagonism (passive: depletion of substrate; forced: bactericide substances; active: acid pH), performed against concurrent Gram-negative bacteria (*Apatenko 1990*).

The use of organic acids also influences the balance of gastrointestinal micro flora especially those of E.coli. Organic acids have an indirect impact on bacteria, decreasing pH and a direct antibacterial activity against Gram-negative organisms, destructing their cellular membrane. Proteases and useful bacteria (i.e. *Lactobacillus spp*.) are optimally active within the pH range 3.5-4.0 while pathogens like *E. coli, Salmonella spp.* slow down and retard their vegetation(Ledoux,2002). Citric, fumaric, orthophosphoric and lactic acids could inhibit the growth of *E. coli* when pH is 5.0. Formic and lactic acids decrease intestinal pH, thus inhibiting pathogenic bacteria and favourizing the growth of lactobacilli. The combination of citric, lactic, propionic, formic and orthophosphoric acids at a dose of 2-8 kg per tonne fodder (or 0.3%-1.8%) is reported to be effective (*Blanchard and Wright 1999; Geboes 1999; Broek 2000)*. Those organic acids decrease considerably the contamination of broiler litter and at the same time, neutralize the production of ammonia. On the other side, the balance between Gram-negative and Gram-positive micro organisms is

optimized, the risk of re-infection, superinfection and dysbacteriosis caused by those pathogens is lower (*Ivanov 2001*). Organic acids drop the pH.This acidification creates a less favourable (even lethal) environment for many micro organisms, prevents lime deposits and improves digestion. They slow the passage of feed through the gut, increasing nutrient absorption and there will be less diarrhoea, which results in drier litter (*Ledoux, 2002*).

It must be emphasized that our results affirm and complete other studies showing that the supplementation with *Lactobacillus acidophilus* as probiotic to bovine forage results in a considerable reduction of *E. coli* 0157:H7 by 50-60% (*Woodard 2000*) Furthermore, *L. acidophilus* increases the weight gain from ingested food. The eradication of pathogenic *E. coli* from live animals in the farm reduces their presence in meat and meat products during their processing. The observations and experimental data show also that this probiotic decreases by 50% the incidence of infection with *E. coli* 0157:H7. On the other side, the use of probiotic is cheap and costs 0.02 $ per cattle daily, achieving t the same time a good utilization of forage. It could be stated that *Lact. acidophilus* markedly reduces the amount of pathogens in animal intestines. Thus, the probiotic is a new means for ensuring health-friendly nutrients from animal origin (beef and poultry meat) with respect to *E. coli* 0157:H7 and *Listeria monocytogenes* presence (*Woodard 2000*).

The use of antibiotics in poultry breeding is related to some negative side effects, such as drug residues in meat and eggs that prolongs the carency period and enables appearance of resistant pathogens, secondary infections, etc. The direct comparison of probiotics and organic acids with antibiotics shows that the former are the best possible alternative to antibiotics as growth promoters, but they could not yield the same result. Several studies report that the decrease and prevention of mortality in poultry farms is achieved with various antibiotics, probiotics and organic acids (*Hooge, 2003*). Some calculations evidence that each dollar invested in a similar programme of prophylaxis gives back 3,5-14 $ benefit (*Becker, 1999*).

The addition of citric acid, baker's yeasts and yoghurt to fodder increased the weight gain via inhibition of the reproduction of Gram-negative bacteria (E.coli) and preventing the colonization of the gastrointestinal tract of birds. It protected the gastric and intestinal mucosa from inflammation, improves the enzymatic degradation and utilization of carbohydrates, lipids and proteins, increases the live weight, the volume and the functional activity of viscera that results in a higher weight gain, lower fodder expenditure and a cheaper production. Our result are similar to those of *Willard et al. (1994*) that reported a significant decrease in both aerobic and anaerobic micro flora in canine

intestines following a fructo-oligosaccharide diet. Furthermore, the effect of the combination probiotic-organic acid is similar to that of the Russian preparation "Lactobacterin" (efficacy 90-97%), inhibiting the development of coliform bacteria in intestines and improving the weight gain in calves (*Veterinaria 1989*).

Conclusions

In conclusion, the maintenance of optimal balance between Gram-positive (77-80%) and Gram-negative (20-33%) micro flora in avian gastrointestinal tract, required antibiotics as well as probiotics and organic acids. Their combined supplementation to the fodder and drinking water of poultry allowed the weakness of one to be compensated by the advantages of others and thus, to achieve at the same time good health and high weight gain in birds. Altering intestinal micro flora population in broiler chickens via acid-producing gram-positive bacteria plus baker's yeasts and citric acid for competitive exclusion appears to be viable approach.

LABORATORIJSKO ISPITIVANJE EFEKTA PROBIOTSKE SMEŠE NA BROJLERE

I. Ivanov

R e z i m e

Ogled je izveden na dve grupe brojlera. Brojleri iz ogledne grupe su tretirani kombinacijom 3% mlečnih bakterija, 1% pekarskog kvasca i 0.7% limunske kiseline koji su dodati u obroke. Utvrđeno je da su tretmani rezultirali u pomeranju mikrobakterijske ravnoteže u gastrointestinalnom traktu brojlera u korist Gram-pozitivnih bakterija (77-80%), dok su kod kontrolnih brojlera preovladale Gram-negativni organizmi (90%). I zapremina i težina unutrašnjih organa oglednih brojlera, koji su dobijeni nakon klanja, su bili 20-60% veći u poređenju sa kontrolnim brojlerima. Takođe, 75% tretiranih brojlera je dostiglo težinu pre klanja (1800 g) za 42 dana sa prosečnimdnevnim prirastom od 57 g i potrošnjom 2.3 kg hraniva za 1 kg prirasta telsne mase, dok je telsna masa brojlera iz kontrolne grupe bila za 26.5% niža od standardne, a potrošnja hraniva 3.1 kg za 1 kg prirasta telsne mase, prosečni dnevni prirast je bio 42 g. Mortalitet kod kontrolnih brojlera je bio 13% dok kod tretiranih brojlera nije bilo uginuća. Cena 1 kg telsne težine kod oglednih brojlera je bila za 0.15 $ niža u poređenju sa kontrolnim brojlerima.

Ključne reči: brojleri, probiotici, organske kiseline, mikroflora.

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