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The Comparative Effects of a Probiotic and a Phytobiotic on the Growth Efficiency, Biochemical and Morphological Blood Indices in Broilers

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The effects of a probiotic (500 ppm; PRO treatment) and a phytobiotic (500 ppm, PHY treatment) in diets on the growth and digestive efficiency and blood indices in broilers were comparatively studied on 3 treatments of chicks (35 birds per treatment, 1-35 days of age). Live bodyweight at 7-35 days of age was significantly improved by the additives due to increases in the digestibility of dietary nutrients. The activity of trypsin in serum in PRO was similar to that in control, in PHY insignificantly lower by 28.3%. The activity of alkaline phosphatase in PRO was lower compared to control, in PHY significantly lower by 24.9% (P<0.05). Concentrations of glucose and triglycerides in PRO remained unaffected while in PHY lower by 20.3% and higher by 50.0%, respectively, compared to control (P<0.05). The phytobiotic affected metabolism in broilers more clearly in compare to the probiotic. The concentration of white blood cells in PHY was significantly lower by 9.2% compared to control (P<0.05) while in PRO this trend was insignificant; the percentage of lymphocytes in PRO was significantly higher by 53.1% compared to control (P<0.05) while in PHY this trend was insignificant. The significant decreases in the percentage of eosinophils (P<0.05) were found: in PHY by 32.7% and in PRO by 57.7%. The conclusion was made that the supplementation of diet for broilers with a probiotic or a phytobiotic beneficially affected live bodyweight and feed efficiency due to the improvement of feed efficiency, optimization of metabolism, and enhancement of the immune reactivity.

Keywords: Broilers; probiotic; phytobiotic; growth efficiency; nutrient digestibility; biochemical blood indices; WBC concentration and differential counts.

INTRODUCTION

Broiler production worldwide is based on the different modern crosses with high and genetically preconditioned productive potential: average daily weight gains (ADWG) over 60 g/bird/day, feed conversion ratio (FCR) 1.35-1.40 kg/kg, mortality level less than 2-3%. The intense genetic selection of broiler crosses has resulted in the acceleration of their metabolism, and the ability of the digestive system to physiologically effective metabolization of dietary nutrients now became a main limiting factor of further progress in the production efficiency. The considerable genetic improvements in the growth rate and feed efficiency achieved during the last 50 years (Havenstein et al. 2003) have led to the significant alterations in the digestive efficiency and in the composition of the intestinal microbiota in broilers (Lumpkins et al. 2010); as a result modern broilers require adequate functional support of the digestive tract and regulation of the microbiota. The function of the latter in the digestion and metabolism is important for the adequate or precise nutrition and for the improvement of productivity and health in poultry (Lenkova et al. 2013b; Lenkova et al. 2015a; Ilyina et al. 2015; Fisinin et al. 2016); it plays a role of a barrier for alimentary pathogens (Van Dijk et al. 2007) and other extremely important roles in the host.

It is known that digestive disorders are the second (after viral diseases) major cause of deaths in the growing poultry. These disorders can result in the imbalances in the intestinal microbiota and decrease in the reactivity of the immune system of the host negatively affected by high density of the birds on restricted areas, management-induced stresses, ecologic conditions, bacterial load and vaccinations, considerable chemical pressing caused by medicines and disinfectants (Panin 2002; Panin and Malik 2006; Lysenko et al. 2007) and alimentary factors like mycotoxins, bacterial toxins, heavy metals, products of lipid oxidation, non-starch polysaccharides and other antitutritive substances within the feed ingredients, as well as imbalances in diet composition (Kornilova et al. 2007). Fast-growing broilers reared to 35-36 days of age are especially susceptible to these stresses; it takes 2.5 weeks for the sustainable microbiota to establish in broilers (Panin and Malik 2006).

Since intestinal microbiota prevents the colonization of the intestinal mucosa by pathogens and with its detoxifying, antimutagenic, anticarcinogenic, synthetic, digestive and other important functions taken into account it is a key factor of the intestinal health providing the efficiency of the digestion, immunity, and productivity in broilers (Gabriel et al. 2006; Vilà et al. 2010).

For many years, until 2006 ban in the EU and later in other countries, in-feed antibiotics were the main agents for the prophylaxis of the digestive disorders in poultry. However, constant and often non-controlled use of the antibiotics has resulted in their low efficiency due to the emergence of the resistant pathogenic and opportunistic strains; the problem of microbial resistibility to antibiotics became increasingly important in animals and human. The resistibility of intestinal pathogens can lead to their accumulation in the intestine and displacement of beneficial species even in the antibiotic-treated poultry; furthermore, the repeated use of certain antibiotics can cause allergy (Iliesh and Goryacheva 2012).

The ban on the in-feed antibiotics impelled the search for the alternatives (Tardatyan 2004). Studies in microbiology, physiology, biochemistry, and nutrition resulted in a wide range of more effective preparations without detrimental effects of the antibiotics (Lenkova et al. 2015b); these biosafe preparations can be effectively utilized by poultry preventing the hazards for the consumers of poultry products and for the environment in general. These preparations include probiotics, prebiotics, symbiotics, synbiotics, phytobiotics, enzyme preparations, feed acidifiers; their common trait is the modulation of the intestinal microbiota (Ryabchik 2012; Lenkova et al. 2013b; Lenkova et al. 2013a).

According to the classic definition by R. Fuller (1989), probiotic is “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance” (Fuller 1989). Probiotics can differ in the number and species of the microorganisms; this type of feed additives also includes symbiotics (combinations of different probiotic microbial species) and synbiotics (combinations of probiotics and prebiotics). Phytobiotics are the combinations of plant extracts and essential oils with antimicrobial and antifungal activities.

The aim of the trial presented was to study comparatively the effects of a probiotic and a phytobiotic on the growth and digestive efficiency, biochemical and morphological blood indices in broilers.

MATERIALS AND METHODS

The trial was performed on a new broiler cross recently selected by the Center for Genetics & Selection “Smena” (Moscow Province). The birds (35 as-hatch broilers per treatment, 1-35 days of age) were kept in cage batteries under standard conditions of management and nutrition. All treatments were fed balanced crumbled compound feeds (based on corn, wheat, and soybean meal) with the contents of metabolizable energy and crude protein 12.98 MJ/kg and 23%, respectively, from 1 to 21 days of age; 13.19 MJ/kg and 21% from 22 to 28 days of age; and 13.40 MJ/kg and 20% from 29 to 35 days of age. The contents of crude fiber in the diets fell within the range 3.7-4.2%.

Control treatment (CON) was fed non-supplemented diets. Phytobiotic treatment (PHY) was fed the same diets supplemented with a phytobiotic (500 ppm) containing 0.64-1.60% of a mixture of essential oils (eucalypt, onion, lemon, thyme), 0.4-1.0% of citric acid, and wheat bran as a carrier (to 100%). Probiotic treatment (PRO) was fed the same diets as CON supplemented with a probiotic (500 ppm) containing live cultures of *Bacillus megaterium* (strain B-4801; no less than $1.0x10^7$ CFU/g) and *Enterococcus faecium* (strain 1-35; no less than $1.0x10^7$ CFU/g) on an inert carrier. Both additives did not contain GMO products; the contents of potentially harmful substances were below the respective permissible levels.

The productive performance in broilers was determined (mortality, live bodyweight, ADWG, feed consumption, FCR, dressing percentage). At 30-35 days of age the balance trial was performed on 3 birds per treatment to assess the digestibility of dietary nutrients.

The blood was sampled at 35 days of age from the axillary vein from the starved birds (10 birds per treatment); the solution of sodium citrate was added with subsequent centrifugation (4,000 rpm) for 3 min. The serum obtained was analyzed on the semi-automatic flow-type analyzer Sinnowa BS3000P (SINNOWA Medical Science & Technology, China) using reagent kits DIAKON-VET (Russia) to determine the concentrations of total protein, triglycerides, cholesterol, glucose, and activity of alkaline phosphatase (ALP). The activity of trypsin in serum was determined by the kinetic method (Vertiprakhov and Grozina 2018). Morphological blood indices were determined on automatic veterinary analyzer DF-50 with attached reagent kits (Shenzhen Dymind Biotechnology, China).

The statistical analysis of the results was performed using paired Student's t-test.

RESULTS AND DISCUSSION

The productive performance in broilers is presented in Table 1. Mortality level in all treatments was 0%. Dressing percentage in treatments PHY and PRO was higher in compare to control by 1.4 and 1.2%, respectively.

The phytobiotic and the probiotic significantly increased live bodyweight in all ages. In PHY treatment average live bodyweight at 7, 21 and 35 days of age was higher by 6.5; 5.5 and 6.3%, respectively, in compare to control; in PRO treatment by 6.3; 5.2 and 5.3%. Live bodyweight at 35 days of age in females and males was significantly higher in compare to control by 6.0 and 6.6%, respectively, in PHY and by 4.5 and 6.0% in PRO.

Feed consumption was similar in all treatments (2.978-3.032 kg/bird); it's interesting to note that the phytobiotic did not stimulate feed consumption. FCR was lower in PHY treatment in compare to control by 7.02%, in PRO by 6.78%. These improvements in feed efficiency were related to better digestibility of dietary nutrients (Table 2).

The digestibility of dietary dry matter in PHY and PRO treatments was higher by 2.5 and 2.3% in compare to control; crude protein by 2.5 and 2.3%, crude fat by 1.6 and 3.1%, nitrogen by 2.4 and 2.6%, calcium by 0.4 and 1.2%, respectively. The digestibility of phosphorus was similar in all treatments.

Biochemical blood indices (Table 3) indicated that the additives studied affected the metabolism in broilers.

The activity of trypsin in serum in PRO treatment was similar to that in control while in PHY treatment it was insignificantly lower by 28.3%; the alterations in tryptic activity in blood serum were earlier reported to be related to the digestibility of dietary protein sources in cattle (Lebedev et al. 2019). The ALP activity in PRO treatment was slightly lower in compare to control, in PHY treatment significantly lower by 24.9% ($P<0.05$). The ALP activity in all treatments fell within the reference range for chickens which is quite wide due to the non-specificity of the enzyme (Meluzzi et al. 1992). Concentration of total protein did not differ significantly between the treatments though the trend to lower concentration was found in PHY treatment; together with lower tryptic activity this can evidence a lowering effect of the phytobiotic on protein metabolism while the effect of the probiotic was rather reverse. However, the concentrations of glucose and triglycerides (indicators of energy metabolism) in PHY treatment were significantly affected ($P<0.05$); the decrease in the concentration of glucose (by 20.3%) evidenced better utilization of energy while the increase in the concentration of triglycerides (by 50.0%) is related to certain alterations in lipid metabolism. Generally, the phytobiotic affected metabolism in broilers more clearly in compare to the probiotic; this is in accordance with the data on the productive performance and efficiency of the digestion.

Blood morphology (Table 4) reflects clinical and immune status and nonspecific resistibility in poultry.

The concentration of white blood cells (WBC) in PHY treatment was significantly lower by 9.2% in compare to control ($P<0.05$) while in PRO treatment this trend was insignificant.

Table 1: The productive performance in broilers fed a phytobiotic (PHY) or a probiotic (PRO) (M±m, n=35)

	Treatments		
	CON	**PHY**	**PRO**
Average live bodyweight (g): at 1 day of age	41.9±0.21	42.0±0.27	42.4±0.22
7 days of age	186.0± 2.31	198.0± 2.40*	197.8± 2.8*
21 days of age	762.5±10.2	804.5±11.21**	802.3±11.49**
35 days of age, in average	1845.5	1961.8	1942.9
in males	1939.0±29.7	2067.3±30.2***	2055.3±27.9***
in females	1752.0 ±27.3	1856.3±25.1***	1830.5±21.4***
Average daily weight gains, g	51.53	54.85	54.30
Feed consumption, kg/bird	3.032	3.001	2.978
Feed conversion ratio, kg/kg	1.681	1.563	1.567
Dressing percentage, %	72.0	73.4	73.2

Differences with control treatment were significant at: *p<0.05; **p<0.01; ***p<0.001.

Table 2: Digestibility of dietary nutrients (%) at 30-35 days of age by broilers fed a phytobiotic (PHY) or a probiotic (PRO) (n=3)

	Treatments		
	CON	**PHY**	**PRO**
Dry matter	72.2	74.7	74.5
Crude protein	90.3	92.8	92.6
Crude fat	87.4	89.0	90.5
Crude fiber	11.8	12.0	12.0
Nitrogen	52.4	54.80	55.0
Calcium	43.0	43.4	44.2
Phosphorus	35.7	35.8	35.7

Table 3: Biochemical blood indices at 35 days of age in broilers fed a phytobiotic (PHY) or a probiotic (PRO) (M±m, n=10)

Показатель	Treatments		
	CON	**PHY**	**PRO**
Trypsin, U/L	53±13.9	38±2.8	53±2.5
Alkaline phosphatase, U/L	3970±137.5	2980±432.1*	3286±710.5
Total protein, g/L	31.5±0.25	30.1±1.43	34.5±1.2
Glucose, mM/L	7.9±0.45	6.3±0.17*	7.7±0.6
Triglycerides, mM/L	0.2±0.01	0.3±0.04*	0.2±0.01
Cholesterol, mM/L	1.9±0.17	1.8±0.10	2.2±0.1

Differences with control treatment were significant at: *p<0.05.

Table 4: Morphological blood indices at 35 days of age in broilers fed a phytobiotic (PHY) or a probiotic (PRO) (M±m, n=10)

	Treatments		
	CON	**PHY**	**PRO**
WBC, 10^9/L	39.3±0.92	35.7±1.27*	38.3±2.14
Neutrophils, %	42.2±2.32	32.7±6.00	23.3±3.27
Lymphocytes, %	48.2±3.92	63.4±7.13	73.8±4.54*
Monocytes, %	0.4±0.04	0.4±0.08	0.4±0.03
Eosinophils, %	5.2±0.7	3.5±0.35*	2.2±0.09*
Basophils, %	0.2±0.04	0.2±0.04	0.2±0.02
RBC, 10^12/L	3,1±0.04	3,0±0.09	3,1±0.07
Hemoglobin, g/L	102±1.08	95.7±4.36	103.0±4.55
Hematocrit, %	24.8±0.31	23.3±1.23	25.0±1.08
MCV, fL	123±1.5	123±0.4	127±0.6*
MCH, pg	51.2±0.66	50.3±0.25	52.2±0.45
MCHC, g/L	416±1.65	410±2.25	411±2.72
RDW-CV, %	10.5±0.08	10.8±0.06	10.5±0.05
RDV-SD, %	52.4±0.27	54.2±0.46	54.2±0.45

WBC = white blood cells; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW-CV = RBC distribution width - variation coefficient; RDV-SD = RBC distribution width - standard deviation. Differences with control treatment were significant at: *p<0.05.

The analysis of WBC differential counts revealed that the percentage of lymphocytes in PRO treatment was significantly higher by 53.1% in compare to control (P<0.05) while in PHY treatment this trend was insignificant. Lymphocytes play the protective role specifically binding the respective antigens while eosinophils are rather related to allergic reactions (Kuznik 2002). The significant decreases in the percentage of eosinophils were found with both additives studied (PHY – by 32.7%; PRO – by 57.7%, P<0.05); these percentages remained within the reference range for chicken and hence these decreases could be regarded as beneficial. The general index of immune reactivity (Ivanov 2014) was higher with both additives: in PHY treatment 167.2 units, in PRO treatment 190.0 vs. 133.5 units in control; the higher effect of PHY was due to the well-known immunostimulating effect of the phytobiotics (Adaszyńska-Skwirzyńska and Szczerbińska 2017).

Concentration and characteristics of the red blood cells (RBC) were not significantly affected by the additives with the exception of mean corpuscular volume of the RBC (higher by 3.2%, P<0.05, in PRO treatment in compare to control). Therefore, supplementation of diets with PHY and PRO primarily affected WBC concentration and differential counts toward the enhancement of general immune reactivity.

CONCLUSION

The data on the growth efficiency, digestibility of dietary nutrients, and blood analysis evidenced that supplementation of diet for broilers with a probiotic or a phytobiotic (500 ppm) beneficially affected live bodyweight and feed efficiency due to the improvement of the digestibility of nutrients, optimization of metabolism, and enhancement of the immune reactivity.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

IAE designed the experiments and wrote the manuscript. TNL and VAM performed experiments on poultry. TAE performed statistical data analysis and reviewed the manuscript. VGV and AAG took the blood samples and performed the biochemical analyses. All authors read and approved the final version.

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