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ANALYSIS OF THE INFLUENCE OF A NUTRACEUTICAL SUPPLEMENT WITH PROBIOTIC EFFECTS ON HEALTH INDEX AND PRODUCTIVE PERFORMANCE IN BROILER CHICKEN

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Abstract

This study was motivated by the fact that the available data on the effects of probiotics and nutraceuticals concerning the health and the productive performance indices in avian species are still controversial. The research consisted in evaluating the influence of BioLactorom nutraceutical product on the main haematological, clinical and biometric indices in Broiler chickens (Ross 308), divided in a control group (n=20) and an experimental group (n=20). According to the manufacturer's instructions, 1 ml of BioLactorom/l water was administered to the chickens in the experimental group in the first 3 days of life, respectively in days 17-19, to reduce the stress generated by manipulation and batching. The chicks were clinically monitored throughout the evaluation, and haematological and microbiological examinations (cultural, on simple agar and McConkey agar, respectively bacterioscopic exam) were performed. All the data obtained emphasized the beneficial effects of BioLactorom, in improving the growth factor, the immunity and the balance of the intestinal flora, and also in reducing the costs of vaccines and other drug products.

Key words*: broiler chickens, nutraceuticals, probiotics, haematological analyses.*

INTRODUCTION

The main purpose of this study, undertaken at the request of ROMVAC Company, was to carry out documentation and investigations to evaluate the safety, tolerance and bioactive potential of the nutritional product BioLactorom (*Lactobacillus plantarum* germs, NCIMB 11974 strain, minimum 1 x 108 CFU/ml, in deproteinized glycerinated whey). With all the remarkable progress made by poultry farming in the last decades, with the expansion of broiler hybrids, the susceptibility of chickens to the major pathogens and precarious environmental conditions has also increased (Huyghebaert et al., 2011), the action of different stress factors becoming a major problem in the intensive poultry farms (Lara and Rostagno, 2013). According to the consulted bibliographies, there is little data available regarding the influence of the nutraceuticals on the blood aspect in broilers raised in intensive system, which fully justifies the investigations carried out in this study. Moreover, there is brief data available on the effect of probiotics and nutraceuticals (a food or part of a food that allegedly provides medicinal or health benefits, including the prevention and treatment of disease) on growth enhancement and production in avian species, as well as on immune system stimulation.

MATERIALS AND METHODS

First, we have to mention that this study was carried out with the Bioethics Commission of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca approval.

Organization of the experiment. The research consisted in evaluating the influence of BioLactorom on the main haematological, clinical and biometric indices in broiler chickens. The biological material (Figure 1) was represented by Broiler (Ross 308) chickens (n=40), one day old, divided in a control group (n=20) and an experimental group (n=20). The chickens were raised on the ground under the

following conditions: kennels with an area of 2 m^2/batch, natural sawdust and daily sanitation; food and water *ad libitum*; 24/7 lighting regime with neon lamps and infrared lamp heating. Initially, crumbled food was administered, containing corn, soy, wheat, vitamin-mineral supplements (calcium carbonate, monocalcium phosphate, oils, L-methionine, sodium chloride, L-lysine, Sacox premix 120, L-threonine, phytase, NSp enzyme) and coccidiostatics (Salinomycin-NA), and after the 18th day with a flour mixture, without the addition of coccidiostatics.

Conduct of investigations. The implementation of the BioLactorom product went through the following sequences: day 0, organization of the study; days 0-3, the first administration of the product (1 ml/l drinking water, for 3 days) and the first weight assessment, following new weighings every week; days 17-19, collection of blood samples for haematological and serological analyses and faecal samples for microbiological examinations, second administration of the product to reduce the stress generated by handling and collecting samples; day 31, the second collection of individual blood samples, respectively individual and mean faecal samples; day 45, completion of the study, including the last collection of blood and faecal samples, as well as repeating clinical and paraclinical examinations.

Figure 1. The appearance of Broiler Ross308 chickens, one day old, when they were introduced to the test

Location and testing period. The investigations were carried out during May-August 2019 and started with the acquisition of the biological material, from a breeding farm of Crăiești-Mureș Avicola Company, following that all the investigations are carried out in the biobase and the laboratories of Physiology, respectively of Microbiology of the Faculty of Veterinary Medicine Cluj-Napoca.

Materials and equipment used. The necessary for this study was composed of the BioLactorom product already presented, the appropriate equipment and consumables. The equipment used included: spectophotometer, centrifuge, drying stove, vortex, colony counting apparatus, microscopes. We also used a set of materials made up of: EDTA and heparin vacutainers, sterile vials for coprology sample collection, gloves, sterile pharyngeal exudate swabs, sanitary alcohol, 5 ml syringes and 22-24G needles, hemocytometers, Natt-Herrick solution, ependorf tubes, well plate, semiautomatic pipettes, ammonia solution, NaCl supersaturated solution, Willis glasses, simple agar plates, McConkey agar plates, gas bulb, loop, degreased blades, DiaQuick Panoptic and May Grunwald-Giemsa dyes; scales, water and feeding pots, bedding, infrared light bulbs.

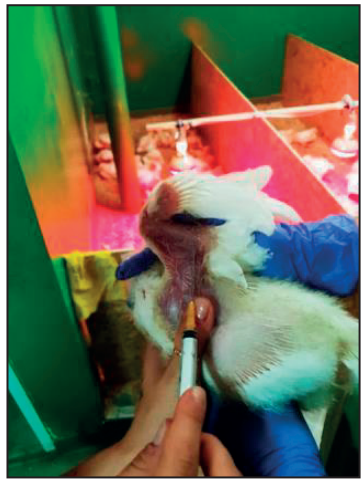

Figure 2. Detail regarding the labor of collecting the blood sample from the basilar vein

Determination of the total number of red blood cells (RBC) and leukocytes (WBC). The count of RBC in the bird shows some differences from the count of RBC in mammals. Prochaska-modified Natt-Herrik dilution fluid is used, which protects all the figured blood elements (erythrocytes, leukocytes, platelets), being considered the standard method (Ognean and Cernea, 2011; Pierson, 2000; Campbell et al., 2007). The working procedure consisted of taking 2 µl of blood from each sample and homogenizing it in an ependorf tube with

400 µl Natt-Herrick solution to obtain a dilution of 1 to 200. After 3 minutes of samples are loaded the hemocytometer and left to settle for 5 minutes. It followed the counting of the WBC in the 4 squares of order I in the corners of the hemocytometer and the count of the red blood cells in 5 squares of order II in the central network (Ognean and Cernea, 2011; Samour, 2006).

Hemoglobin dosage. This determination is difficult due to the presence of the RBC nucleus in the case of blood samples from birds (Hawkey and Samour, 1988; Samour, 2006; Campbell et al., 2007). In order to determine the hemoglobin concentration in birds, the colorimetric method and semi-automatic or automatic methods can be used. We used the semi-automatic spectrophotometric method (Ognean and Cernea, 2011). Thus, we diluted 200 µl of blood with 400 µl of ammonia solution and allowed to stand for 3 minutes, then transferring 20 µl into ELISA-type wells. This determination requires a negative control (ammonia solution) and a positive control (a blood sample whose exact hemoglobin value is known). The reading is done with the help of the spectrophotometer. The result obtained is multiplied by the correction factor 33 for the expression in g/dl as a unit of measurement (Samour, 2006).

Determination of hematocrit. This basic analysis in avian hematology allows a relevant evaluation of the erythrocyte mass. For this purpose we resorted to the evaluation of the macro-hematocrit, in the case of blood samples centrifuged at 2500 rotations/minute and its percentage expression (Ghergariu et al., 2000; Ognean and Cernea, 2006; 2011).

The determination of the erythrocyte constants was based on the use of calculation formulas known in the field (Ghergariu et al., 2000; Samour, 2006; Ognean and Cernea, 2011), these indices being important for birds, especially for the detection of stress of nutritional origin. MCV (mean erythrocyte volume) is an index of cell size and represents the volume occupied by a single red blood cell and has as its unit of measurement femtoliter (fl) (Ghergariu et al., 2000; Ognean and Cernea, 2006; 2011). His knowledge allows the early detection of anemia (Duguy, 1970). MCH (mean eritrocyte hemoglobin) is a color index, which refers to the average amount of RBC hemoglobin. Together with MCHC and other erythrocyte indices, it helps to differentiate between different types of anemia, being expressed in peak grams (pg). MCHC (mean hemoglobin concentration) represents the average hemoglobin concentration in a given volume of erythrocytes, or the ratio of the amount of hemoglobin to the volume of red blood cells and is expressed in g/dl (Ghergariu et al., 2000; Ognean and Cernea, 2006; 2011).

Leukogram determination. Coloring of bird blood smears is based on the use of most Romanovsky-type stains used in mammalian smears (Wright, Gimsa, Wright-Gimsa, Leishman, Wright-Leishman, May-Grunnwald, May-Gundwald-Gimsa, DiaPanoptic etc.). For fastness reasons, in this study we used DiaQuick Panoptic staining, based on the use of 2 dyes (acidophilic and basophilic) and a fixative containing absolute methyl alcohol (Campbell, 1994).

Microbiological analysis of faecal samples. Studies in the field have already demonstrated the positive effects of probiotics on productive performances in animals, mainly stimulating growth and development. It is worth mentioning, however, the beneficial effects of probiotics on increasing resistance to various pathogens, by supporting the proper functioning of the immune system. Starting from the aforementioned, in this study we summarize the quantification of microorganisms content in faecal samples in and establish the dominant taxon. For this purpose, faecal samples were collected, by cloacal sampling, using sterile swabs for pharyngeal exudate, from 5 individuals of each lot. Faecal samples from the litter were also collected for the coproscopic examination of each lot. From the samples taken seedings were carried out on simple agar and McConkey agar, and the resulting cultures, after 12 hours incubation at 30ºC, were used to count the colonies with an automatic microbial colony counting apparatus (Colony Counter CC1).

The populations of intestinal microorganisms were quantified by dilution method, using the microbiological method of determining the number of germs (Zarnea, 1984). For this purpose, 2 mg of faecal sample was added to 1 ml of distilled water, with a dilution of 1:

100,000. The samples thus prepared were vortexed and with the aid of a seeding handle, under the protection of the gas bulb, other series of Petri dishes were seeded on simple agar and McConkey agar, the latter being thermostated at 30ºC for 12 hours, following the final count with the help of the Colony Counter CC1. From the colonies obtained after the dilutions, bacterioscopic examinations were then carried out, on Gram method colored smears, in order to identify and differentiate the microorganisms types, grouping them in Gram-positive and negative, as well as according to the main morphological criteria (shape and grouping of vegetative cells). In parallel, smears were also stained by panoptic methods (May Grunwald-Giemsa).

Monitoring of health status and evaluation of growth enhancement. Chicken lots maintained under optimum conditions according to the "Ross308 strain instruction manual", (http://en.aviagen.com/brands/ross/products/ross-308), were monitored at least 3 times/day, evaluating the level of zoo hygiene and health indices, and as the case may be, the use of general semiotic methods. By inspection, the general health and maintenance status of the chicks was continuously monitored. The evolution of the ventilation and lighting degree, the temperature of the floor and water, the consumption of food and water, the quality of the litter, the appearance of the faeces were also monitored. Thus, any changes were noted and monitored in order to eliminate the various risk factors.

Moreover, according to the protocol, we also monitored the weight gain of the chicks through weekly weighings, both individually and in batches, graphically representing the evolution of the weight of the chicks. According to the protocol, starting on day 18 the coccidiostatic administration was discontinued and coproscopic verification examinations were carried out weekly. We mention that, on days 19-22, we reported the occurrence of isolated cases of feces with blood streaks, which led to an intensification of parasitological examinations. In order to perform the co-parasitological examinations, we used the Willis method, which ensures a good concentration of sporocysts, oocysts, spore cysts, oncospheres, nematode eggs (Cozma et al., 2013).

RESULTS AND DISCUSSIONS

Control group results. The research carried out on this group led to the obtaining of a particularly relevant data set, which statistically processed constituted an important benchmark for interpreting the results from the experimental batches. Following the processing and statistical analysis, we have outlined an overview of the individual variations of the main haematological parameters in broiler chickens. By comparing the values obtained in our research with those of the specialized literature, we were able to outline a relevant image on the dynamics of haematological and leukocyte parameters in broiler chickens.

Evolution of haematological parameters. In Tables 1 and 2, the distribution of data obtained for the statistical analysis of the haematological indices in the control group are presented, which were the basis of the subsequent correlation with the reference values in the specialized literature. The determination of the total number of RBC revealed maximum values of 4.39 T/L at the first harvest and minimum values of 1.75 T/L at the third harvest. The mean values obtained for this parameter ranged from 2.35 ± 0.22 to 2.83 ± 1.04 T/L, being within normal ranges (Wallach and Boever, 1983; Douglas et al., 2010). In the same context, the total number of leukocytes evolved, registering averages (0.29 ± 31.18 and 2.65 ± 28.5 G/L) within physiological ranges (Ghergariu et al., 2000; Douglas et al., 2010). The haematocrit showed an average between 21.89 ± 3.15 and 37.5 ± 4.33%, reconfirming that the blood-cell ratio is slightly lower in meat birds compared to laying hens, interpreted in this context the values obtained can be considered normal. Haemoglobin reached the maximum value of 28.21 g/dl in the case of a chicken at the third harvest, a value that we consider high. In contrast, the minimum values were between 6.5 and 15.12 g/dl, with the average at the first harvest of 8.62 ± 1.37 g/dl, the values being within the reference intervals (Ahmed Al- Nedawi, 2018) and 21.76 ± 6.2 /dl at the third harvest, considered a slightly

increased value according to the studied bibliographies.

Erythrocyte constants were also calculated for each batch separately. Thus, MCV had an average value between 84.77 ± 45.13 and 147.56 ± 39.43 fl, MCH recorded maximum values of 90.79 pg, with a minimum of 27.65 pg values between 37.04 ± 7.54 and 82.3 ± 14.65 pg, evolving within the physiological limits, according to Ahmed M. Al-Nedawi (2018). For the third erythrocyte constant MCHC, the mean values between 39.63 ± 6.38 g/dl and 71.87 ± 52.65 g/dl were within the reference ranges. The distribution of leukocyte subpopulations was characterized by oscillations recorded in their physiological intervals, as evidenced by the data presented in Table 2.

Table 1. Distribution of RBC and leukocyte parameters values in the control group

Parameters	RBC	WBC	Haematocrit	Haemoglobin (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)
	(T/L)	(G/L)	(%)				
			Day 17th				
Average	2.83	2.65	22	12.62	84.77	48.94	71.87
St. Dev.	1.04	2.85	8.36	2.2	45.13	17.92	52.65
			Day 31st				
Average	2.35	2.04	21.89	8.62	94.77	37.04	39.63
St. Dev.	0.22	2.30	3.15	1.37	23.38	7.54	6.38
			Day 45th				
Average	2.64	1.29	37.5	21.76	147.56	82.3	57.44
St. Dev.	0.52	3.11	4.33	6.2	39.43	14.65	12.09

Table 2. Distribution of leucogram values in the control group

WBC counts					
Cells	**Heterophile, %**	**Eosinophils, %**	**Basophils, %**	**Lymphocytes, %**	**Monocytes, %**
			Day 17th		
Average	48.6	2.6	-	39.2	9.6
St. Dev.	8.35	1.94	-	7.91	2.07
			Day 31st		
Average	46.4	4.4	-	37.2	11.8
St. Dev.	10.73	1.14	-	7.01	5.01
			Day 45th		
Average	42.8	3.4	-	40.2	13.6
St. Dev.	12.63	1.67	-	8.4	8.76

Thus, the analysis of the evolution of heterophiles revealed reaching the maximum values of 57-58% and averages of 42.8 ± 12.63 - 48.6 ± 8.35%, within physiological limits. The evolution of eosinophils was also framed by mean values between 2.6 ± 1.94 and 4.4 ± 1.14% and within the physiological intervals (Ghergariu et al., 2000). The basophils were sporadically reported and we did not attach any particular significance to the bird's immune status. The lymphocytes showed maximum values between 47-50% and minimum values between 27-31%, being slightly below the physiological limit. In contrast, the mean values, between 37.2 ± 7.01% and 40.2 ± 8.4%, were within the physiological limits after Hoffmann (1961). Regarding the proportion of monocytes, a maximum value was registered in the case of an individual at the third harvest (29%), this value being considered increased by most bibliographic sources. In contrast, the minimum value was 7%, the averages being in the range 9.6 ± 2.07% and 13.6 ± 8.76%, included in the physiological spectrum (Pârvu et al., 1984; Glystorff, 1983).

Experimental group results. The evolution of the haematological parameters in the chicks from the experimental group, fed with the addition of BioLactorom during the study, are presented in Table 3. In this case, the distribution of the haematological parameters was characterized by large oscillations, within the physiological limits or with insignificant deviations. Thus, the average values of haematocrit and haemoglobin were within the physiological limits (22.00-48.00%, respectively 5.58-15.10 g/dl). In case of the total number of RBC, the mean values (2.27 ± 0.38 - 4.07 ± 5.15) were also recorded within the physiological limits (3.00-5.5 T/L). At the

first harvest, in the case of an individual, a maximum value of 13.3 T/L was noted. For the total number of leukocytes the dominant trend was decreasing with a maximum value at the first harvest of 24.0 G/L and minimum values of 8.0 G/L at the last harvest, but without significantly exceeding the normal limits (4-13 G/T). Mean levels of erythrocyte constants showed significant but circumscribed fluctuations between baseline limits for MCV 112.52 ± 17.73 (72.25-173.85 fl), and MCH 46.8 ± 9.59 (19, 6-58.69 μg), compared to references (21.48-34.84 g/dl) the MCHC values being slightly increased in some of the chicks (66.6 g/dl). Data on the distribution of leukocyte subpopulations showed average values of heterophiles proportion of 42.4 ± 4.27 - 50.8 ± 5.21%, ranging between physiological limits (13.00-49.00%).

Table 3. Distribution of RBC and leukocyte parameter values in the experimental group

Parameter	RBC	WBC	Haematocrit	Haemoglobin (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)
	(T/L)	(G/L)	(%)				
			Day 17th				
Average	4.07	18.72	26	13.84	117.63	61.79	54.62
St. Dev.	5.15	13.38	5.47	1.98	58.34	27.93	9.95
			Day 31st				
Average	2.27	19.36	25	10.69	112.52	46.8	42.76
St. Dev	0.38	43.22	0	3.44	17.73	9.59	13.76
			Day 45th				
Average	3.822	10.4	38	19.86	109.48	56.09	52.52
St. Dev	1.35	1.60	4.47	2.73	39.45	14.69	6.31

Table 4. Distribution of leucogram values in the control group

	WBC counts				
Cells	**Heterophile %**	**Eosinophils %**	**Basophils %**	**Lymphocytes %**	**Monocytes %**
		Day 17th			
Average	42.4	4.2	-	43	10.2
St. Dev.	4.27	3.03	-	8.03	2.38
		Day 31st			
Average	50.8	3.6	-	35.4	10.2
St. Dev	5.21	1.51	-	4.72	4.08
		Day 45th			
Average	46	2.6	-	45.4	6
St. Dev	9.51	1.51	-	8.64	2.91

The population of eosinophils evolved likewise and did not exceed the upper limit of 14%, the highest level of 9% being recorded at the first harvest. The dynamics of the lymphocyte population was also characterized by minor variations, from 29% to 55%, within the physiological limits (31.00-72.00%). In contrast, the proportion of monocytes was characterized by wider oscillations (2-17%), still within the reference limits (2.00-16.5%). It is important to mention that the data obtained in the researches on broiler chickens, especially the haematological ones, are characterized by wide variations depending on age, sex, or even circadian. Thus, the mean values of the total number of RBC in the control group (2.35 ± 0.22 - 2.83 ± 1.04 T/L) were slightly lower than in the experimental group, (2.27 ± 0.38 - 4.07 ± 5.15 T/L), being within the normal limits for both lots. Comparatively, the total number of leukocytes in the control group recorded slightly higher levels (1.29 ± 31.18 - 2.65 ± 28.5 G/l) than the experimental group (1.0416-1.93 ± 43.22 G/L), caused by the higher values of some individuals in the control group. Regarding the evolution of heterophiles, proximate average values were also recorded for the control and experimental group (42.8 ± 12.63 - 48.6 ± 8.35%, respectively 42.4 ± 4.27% - 50.8 ± 5.21%), within physiological limits, according to Hoffman (1961), while other authors consider these values to be

slightly above the upper limit. Eosinophils showed normal values for both groups (2.6 ± 1.94% - 4.4 ± 1.14%, respectively 2.6 ± 1.51 - 4.2 ± 3.03%). The basophils, were sporadically encountered in the case of both batches, being below the limits recorded in the consulted bibliographies, without having a special significance regarding the health of the birds. The average values of lymphocytes were also within the physiological intervals for both groups (37.2 ± 7.01-40.2 ± 8.4% respectively 35.4 ± 4.72-45.4 ± 8.64 %), according to Hoffmann (1961). Also, the average values of the monocytes were within the physiological limits, both in the case of the control group (9.6 ± 2.07 – 13.6 ± 8.76%), as well as of the experimental group (6 ± 2.91 to 10.2 ± 2.38%). There were also slightly above the physiological limits recorded values in the case of several individuals from the control group, towards the end of the experiment. However, we considered that, as a whole, they were within the physiological limits (Gylstorff, 1983). The haematocrit of the control group had average values between 21.89 ± 3.15% and 37.5 ± 4.33, and for the experimental group between 25% and 26%, without being outside the physiological limits, and only a few individuals in the control group had closer to the lower limit values. Hemoglobin recorded mean values between 8.62 ± 1.37 and 21.76 ± 6.2 g/dl, for the control group, and for the experimental group between 10.69 ± 3.44 g/dl and 19.86 ± 2.73 g/dl. According to Bounous and Stedman, 2000, we consider that these values also fall within the normal intervals, except for a few individuals from the experimental group at which they slightly exceeded the upper limit. Regarding the values of the erythrocyte constants, for the control group, MCV had an average value between 84.77 ± 45.13 and 147.56 ± 39.43 fl, the value of the experimental group was 109.48 ± 39.45 and 117.63 ± 58.34 fl. The MCH recorded mean values between 37.04 ± 7.54 and 82.3 ± 14.65 pg for the control group, and for the experimental group the values were 46.8 ± 9.59 pg and 61.79 ± 27.93 pg. MCHC had an average value of between 39.63 ± 6.38 g/dl and 71.87 ± 52.65 g/dl for the control group and 42.76 ± 13.76 g/dl and 54.62 ± 9.95 g/dl, for the experimental group. We observe that the values of the control group are higher than those of the experimental group, but all the values exceed the upper physiological limit. According to some studies, there is a negative relationship between MCH, MCHC and the total number of RBC, so it is normal for a high amount of hemoglobin to increase the value for MCHC while the total number of RBC is maintained normal.

Microbiological investigations results. All microbiological examinations in the case of the control group, revealed the predominance of the bacteria in the genus *Bacillus* of Gram-negative germs (80%) compared to the Gram-positive ones (40%), as well as the presence of lactose-negative and coliform bacteria. Morphologically, the colonies formed were extremely diverse: large, medium and small, smooth or mucous (Figures 3 and 4). At the second harvest, the colonies were counted using the counting apparatus, obtaining a maximum number of 860 million colonies for chicken M1 and a minimum number of 430 million colonies for M2, the average of the whole lot was 645.08 ± 214.9 (Table 5).

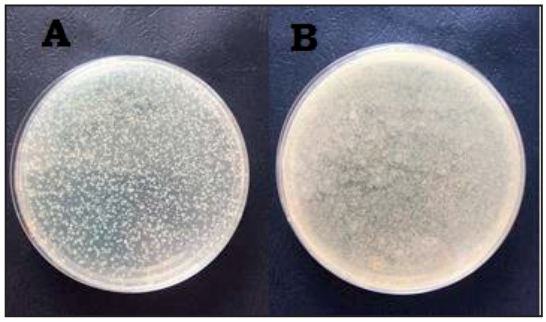

Figure 3. The morphological aspect and density of the developed colonies on simple agar in the experimental group (A) and the control group (B)

Regarding the experimental group supplemented with BioLactorom, only medium and small colonies were identified, and in addition to the control group, the presence of thin filaments belonging to the genus *Lactobacillus* was reported. Also, all the microbiological examinations revealed, for the experimental group, the predominance of Gram-positive bacilli (100%), while the Gram-negative bacilli and cocobacilli were in a much smaller proportion (60%). In the case of the test group, there were no large, mucous colonies, which often merge together, the pathogenic germs being most often responsible for their

formation. Regarding the evolution of the number of colonies, the maximum value of 688 x 10^6 was reached in a chicken in the experimental group, and the minimum value of 113.4 x 10^6 in the case of a chicken in the same group (Figure 3A), with an average of 315.28 x 10^6 ± 228.55. Comparative analysis of the average values recorded in the control group (645 x 10^6 ± 214.9) and in the experimental group (280.08 x 10^6 ± 228.5 (Table 6), with significant differences between the two groups, due to the supplementation of the diet with the investigated nutritional product.

For the control group, we can conclude that, the results of the microbiological examination suggest possible imbalances of the endosymbiotic microflora, the balance being inclined towards the predominance of the Gram-negative germs (80%), while the Gram-positive bacteria had a minor weight (40%).

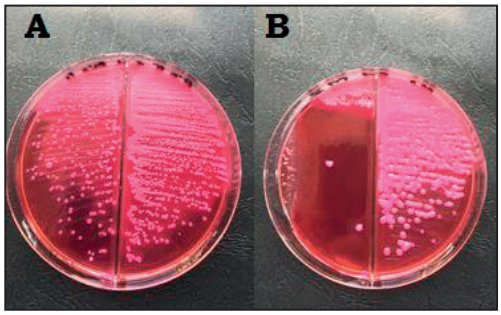

Figure 4. The morphological aspect and density of the developed colonies on McConkey agar in experimental group (A) and control group (B)

The increased number of colonies in the control group suggests an overpopulation of the intestinal tract, an aspect that adversely affects broiler chickens, because the lack of balance of intestinal endosymbionts can lead to the destruction of the epithelial barrier, with negative effects on growth and health in general. We consider that, for the experimental group, the microbiological examination results revealed some significant beneficial effects compared to those obtained in the control group. In this context, we mention that the endosymbionic microflora from the intestinal level is particularly important for the functioning of the defense mechanisms in birds, both the innate and the adaptive ones.

As it is well known, the gut is populated with both organism-beneficial bacteria, such as Gram-positive bacteria, lactobacilli or bifidobacteria, as well as potentially pathogenic bacteria, such as *Clostridium* spp., *Escherichia coli, Salmonella* etc.

Table 5. Distribution of the total number of colonies in the control group

	Parameters	Colony count
M1	**Max.**	860
M2	**Min.**	430
M3	**Average**	645.08
M4	**St. Dev.**	214.9
M5	**Median**	645

It is vital to maintain a constant balance between these two categories. Thus, it is considered that 85% of the intestinal bacterial population should be Gram-positive bacteria, so that digestion works well.

Table 6. Distribution of the total number of colonies in the experimental group

	Parameters	Colony count
B1	**Max.**	688
B2	**Min.**	113.4
B3	**Average**	315.28
B4	**St. Dev.**	228.55
B5	**Median**	280.8

Based on the obtained results we consider this was fulfilled by the addition of the nutraceutical product BioLactorom, formulated by the Company ROMVAC, in the tested diet. Based on the good growth level achieved we consider the BioLactorom product exerts beneficial effects on weight dynamics (Figure 5) in broiler chickens. In this regard, the results of the microbiological tests obtained for the experimental group were also shown, indicating the importance of a balanced intestinal flora in the processes of digestion, absorption and defense in birds, as well as for reducing the effects generated by stress.

The synthesis of the results obtained in the clinical and coproscopic examinations revealed a good general condition and maintenance of the chicks throughout the experiment, expressed by the increased level of appetite, the temperament, the pleasant aspect of the plumage. We also recall that at the coproscopic examinations, no major pathogens were reported, except for days 20-24 of the experiment,

when due to the change of the feeding some chicks presented soft feces with blood streaks.

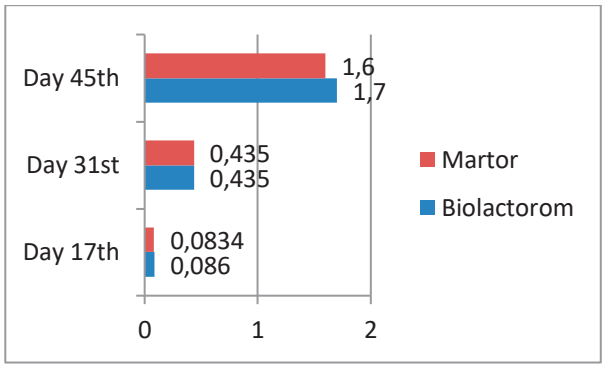

Figure 5. Graphical representation of weight dynamics in control group and fed with BioLactorom chickens

Given that the sub-therapeutic use of antibiotics in feed has been either banned or reduced in most countries (Sugiharto, 2014) due to the fact that they caused the emergence and/or extension of the known phenomenon of "anti-resistance", thus representing a health hazard. For consumers, the use of nutraceuticals in the poultry farming industry is not only increasingly appreciated, but has been used extensively. The exclusion of antibiotics from feed has caused many problems in the industry, as the growth performance decreased and during the breeding period the birds developed subclinical diseases with an increasing mortality rate (Huyghebaert et al., 2011). Moreover, intestinal health has recently been the subject of intense studies aimed at poultry production (Rinttila and Apajalahti, 2013). In addition to improving environmental conditions, nutritional strategies have been developed to partially alleviate the negative impact of stress on birds (Lara and Rostagno, 2013), including diet with high energy density, addition of salts, antioxidant and mineral vitamins in stressed bird diets (Sahin et al., 2009; Das et al., 2011). Recently, dietary supplementation with probiotics, prebiotics and symbiotics has been implemented in poultry to counteract the negative effects of heat stress (Lara and Rostagno, 2013).

We believe that Romvac BioLactorom, a probiotic for the stabilization of intestinal flora, has beneficial effects on growth and stimulating effects on defense mechanisms in birds, even under stressful conditions. However, the evolution of daily growth growth did not reveal the existence of very large differences between the two batches, at the end of the 45 days the average weight of the batch supplemented with the BioLactorom product was slightly higher (1.7 kg) than the control batch (1.6 kg). We can therefore consider that this effect of biometric stimulation is due to the supplementation of the diet with the probiotic investigated, whose action of balancing the intestinal flora has been proved by the microbiological tests performed.

CONCLUSIONS

An important contribution of the nutraceutical BioLactorom has significantly contributed to balancing the intestinal flora and achieving a good level of growth in Broiler chickens and also has been shown to stimulate and support immunity in Broiler chickens (reducing the costs of administering vaccines and medicinal products, including coccdiostatics), expressed by reducing mortality and severe episodes of disease;

Following the administration of the product in the drinking water at a significant number of broiler chickens, the necessity of reformulating the BioLactorom product was outlined, for the administration in feed, which is more suitable for the industrial growth of the birds.

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