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Effects of Antibiotic Substitution with Probiotics and Prebiotics on Broiler's Health

COULIBALY Souhalio[1*], OUSSOU N'Guessan Jean-Baptiste[2], COULIBALY Aboubacar[2], SORO Dofara[1]

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[1]Laboratory of Biology and Animal Cytology, Université NANGUI ABROGOUA, 02 BP 801 Abidjan 02, Côte d'Ivoire
[2]Laboratory of Physiology, Pharmacology and Pharmacopoeia, Université NANGUI ABROGOUA, 02 BP 801 Abidjan 02, Côte d'Ivoire

ABSTRACT

In order to reduce the use of antibiotics in broiler farming, the use of probiotic microorganisms and beneficial food substances has been opted for. So on the first day, 1013 chicks were raised in groups until the 7th day. Then, 999 chicks were randomly divided into 3 comparable groups with three repeats each, on day 8. A control group (Group T) was subjected to an antibiotic prophylaxis program, an experimental group (Group 1) where chicks received only the Polybiote (a probiotic) and the last experimental group (Group 2) where chicks received the Polybiote associated with Renfort⁺ (a prebiotic). Blood samples were weekly collected from 15 chickens from each batch and kept in suitable tubes. Chickens haematological and biochemical parameters were measured. The results of this research work showed that the leukocyte and erythrocyte parameters of chickens fed with the polybiote associated with Renfort⁺ (Group 2) were significantly increased ($p < 0.05$) compared to those of the control group (Group T). As for the biochemical parameters, the total serum protein concentrations of chickens in groups 1 and 2 were significantly increased ($p < 0.05$). However, the activities of the enzymes of chickens in group 2 were significantly lowered ($p < 0.001$) while its serum calcium concentrations were increased compared to group T. It emerged from our experiment that chickens subjected to the Polybiote alone or associated with the Renfort⁺ did not show any abnormalities and had better biological characteristics. It could, therefore, be used as natural substitutes for synthetic antibiotics.

Keywords: Antibiotic, Chicks, Probiotics, Prebiotics and blood

*Corresponding author. Email: Souhaliocoulibaly@yahoo.fr; Phone number: +225 07 58 246 300

INTRODUCTION

The livestock sector has undergone unprecedented changes for a few decades. The booming demand for animal food products in countries experiencing strong economic growth has resulted in a marked increase in livestock production driven by leading technological innovations and structural changes (FAO, 2018). In this context of world population growth, the poultry sector has strongly developed and has begun to industrialize in many regions and countries of the world (Magdelaine, 2014). One of the most significant food manufacturing sectors in the world is poultry farming (Sharopatova and Pyzhikova, 2020).

Thus, poultry meat has recorded a very strong evolution in absolute consumption but also in world consumption per capita. In order to meet the growing demand for this commodity, poultry industries have incorporated antibiotics into poultry feed as growth promoters, mortality reduction and disease incidence in order to improve meat production (Chattopadhyay, 2014).

The poultry's digestive health is a very important factor in animal husbandry which can strongly affect production and animal welfare. It is based on three pillars which are the state of the digestive tract's health of the animal, the balance of the microflora and the state of the immune system.

Following the definitive withdrawal of the use of Growth Factor Antibiotics (GFA) and the gradual withdrawal of those used as a preventive measure, the new regulations

on feed additives and the new expectations of consumers looking for more natural poultry products, many alternatives have been developed. Among them, the most used to improve gastrointestinal health are probiotics, prebiotics, enzymes, and organic acids. The aim of these alternatives is to strengthen the sanitary barrier, maintain a low mortality rate and optimize digestion and avian performance (Idoui et al., 2009). Among these alternatives, the most used to improve gastrointestinal health are probiotics, prebiotics, enzymes, organic acids, immunostimulants, bacteriocins, bacteriophages, phytogenic food additives, nanoparticles and essential oils (Rouissi, 2020).

Several recent studies have demonstrated the importance of the use of probiotics and prebiotics in the poultry industry. According to Rashid et al. (2023), salmonellosis, which is a major pathologie in poultry farming, is poorly managed by antibiotics because of bacterial resistance. Thus, probiotics derived from Bacillus can be used to treat resistant genes. Increasing therapeutic limitations of antibiotics have led to huge investments in probiotics for safe poultry meat production (Gul et al., 2022). Atta et al (2021) showed that probiotics can also reduce tissue antibiotic residues in chickens to the maximum recommended limits (MRLs). The addition of prebiotics to the diet can mitigate the negative effects of high stocking density on production performance, physiological and oxidative stress parameters and production efficiency factors (Karar et al., 2023).

Polybiote is a liquid product with probiotic activity composed of several lactic acid bacteria and yeasts. These lactic acid bacteria were *Bacillus subtilis*, *Lactobacillus acidophilus, Lactobacillus plantarum* and *Lactobacillus casei*. As for the yeasts they were *Saccharomyces boulardii* and *Saccharomyces cerevisiae*. The dosage of Polybiote was 1 ml/L of water and no withdrawal period is prescribed. One milliliter of Polybiote contains $4{,}8.10^9$ CFU/mL of lactic acid bacteria and $2{,}6.10^9$ CFU/mL of yeast. Renfort$^+$ is a mixture of plant extracts rich in non-digestible oligosaccharides. It came in a liquid form consisting of *Moringa oleifera* cloves, garlic cloves, rosemary, sage, turmeric, salt, calcium, vitamin A, vitamin E and Vitamin D_3. The dosage of Reinfort$^+$ was 1 ml/L of water with no waiting period.

It is in this context that this work was undertaken to propose an alternative to the use of antibiotics in broiler farming in order to contribute to food security. Specifically, this study aims to compare the conventional antibiotic-based treatment to alternative treatments on broilers' haematological and biochemical parameters.

MATERIAL AND METHODS

Experimental Site

This study was conducted in Abidjan (Côte d'Ivoire). The breeding took place on the poultry farm of the NANGUI ABROGOUA University (UNA) whose geographical coordinates are: 5°23'33'67896" N and 4°1'5.9034" W.

Animals and Products

The study was carried out on 999 broilers chicks of COBB 500 strain of *Gallus gallus* species. They were one day old and had an average weight of 40 ± 2 g. They were bought from the company ivoire poussin® and raised in the experimental farm of the Université Nangui Abroguoa (UNA). Blood samples were collected every week. Then, the samples were sent to two different laboratories for analysis. The measurement of the haematological parameters took place at the *Institut Pasteur de Côte d'Ivoire (IPCI)* and that of the biochemical parameters was done in a private laboratory.

The antibiotics used were TTS® and ALISERYL®. TTS® is an antibiotic made of Tylosin, Trimethoprim and sulfadiazine sodium. Its dosage was 1 g/L of water. As for ALISERYL®, it was made of erythromicyne thiocyanate, hydrochorid oxytetracycline, Streptomycin sulphate, colistin sulphate and vitamins A, D, E, K and C with a dosage of 100 g/L of water.

Animals grouping

Chicks of cobb 500 strain which were 1013 days old were raised in a single strip in an open 105 m^2 building (7 m x 15 m) under the standard conditions of broiler farming. During the first breeding week, no treatment was administered to the chicks. After this period, the 999 subjects were divided into 3 batches of 333 animals each. The first chicks' batch (Groups T) was subdivided into three subgroups of 111 chicks and fed with antibiotics. The second batch (Group 1) was also subdivided into three subgroups of 111 subjects each and fed with Polybiote (probiotics). Finally, the last batch (Group 2) was also subdivided into three subgroups of 111 subjects and fed with a mixture of Polybiote and Renfort$^+$ (prebiotics).

Chicks' feeding and lighting

All animals were fed *ad-libitum* with the same types of food made by Ivograin®. Thus, three (3) types of food were produced during the breeding cycle. These were pre-starter food (GENESA), starter food (C1) and growth food (C5). The animals went through a transition period of four (4) days from one range to another. The characteristics of the three foods were presented in Table 1.

During the first week of the animal (adaptation period), the chicks received only tap water *ad libitum*. After that, each chick's batch received water in accordance with the experimental procedure. Thus, chicks in batch T received water containing antibiotic intake according to an established medical prophylaxis program. The medical prophylaxis program was presented in Table 2.

Table 1: Characteristics of the food distributed.

Items	Content		
	GENESA	Food (C1)	Food (C5)
Metabolizable energy (kcal/kg)	-	2194	2768
Crude Protein (%)	22.20	30	17
Gross fat (%)	5	7	5
Crude ash (Mr. Miner) (%)	6	10.10	13
Gross cellulose (%)	3.50	5 .40	4.70
Phosphorus (%)	0.46	13.80	5.80
Calcium (%)	0.74	15.90	34.90
Sodium (%)	0.27	0.11	0.17
Lysine (g/kg)	13.4	-	-
Methionine (g/kg)	6.40	-	-
Vitamin A (IU/kg)	1200	10000	8000
Vitamin D3 (IU/kg)	4500	2000	1000
Vitamin E (IU/kg)	42	204	60.18
Vitamin B1 (mg/kg)	3	-	-
Vitamin C (mg/kg)	150	-	-
Iron sulfate monohydrate (mg/kg)	60	-	-
Copper sulfate penta hydrate (mg/kg)	18	-	-
Manganese oxide (mg/kg)	138	-	-
Zinc oxide (mg/kg)	96	-	-
Potassium iodide (mg/kg)	2.40	-	-
Sodium selenite (mg/kg)	0.36	-	-

Table 2: Medical Prophylaxis Program.

Days	Trade names	Nature
1 to 7	Water	-
8 to 10	TTS®	Antibiotic
11 to 13	VMD-AMIN SPECIAL®	Vitamin
14 to 16	ANTICOX SUPER®	Anticoccidial
17	Water	-
18 to 20	ANTICOX SUPER®	Anticoccidial
24 to 26	ALISERYL®	Antibiotic
27 to 36	Water	-

The chicks in batch 1 received water containing probiotics every three days throughout the test period. As for batch 2, the probiotic was served in the morning and the prebiotic in the afternoon. All products were administered according to the indicated dosage.
The hen house was lit 24 hours a day during the breeding using natural artificial lights. This lighting allowed the animals to apprehend the food and the water throughout the trial period.

Blood samples collection

At the end of each week, blood samples were randomly collected from 15 fasted subjects in each chicks batch. Blood samples were drawn in the alar vein precisely at the joint between the humerus and the radius using a syringe.
Two milliliters of blood samples were collected in EDTA tubes for haematological analyses and another two milliliters in sterile tubes for biochemical analyses. The samples were then immediately sent to the laboratory for analysis.

Haematological and biochemical parameters determination

Complete blood counts (CBC) were performed directly using an automated hematology machine (Sysmex X-1000, Japan), on days 14, 21, 28 and 35 to avoid cell autolysis and to obtain reliable results. Erythrocyte counts, hemoglobin and haematocrit levels, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocyte count, neutrophils, eosinophils, basophil polynuclear cells, lymphocytes and monocytes were assessed according to the method described by Langford et al. (2003).
The biochemical assays were based on 12 parameters analyzed by a spectrometer (PIOWAY 3000, China). These parameters included protein metabolism parameters (total protein, albumin, urea, creatinine), lipid metabolism parameters (HDL cholesterol, LDL

Table 3: Leukocyte parameters in broilers during 4 weeks of breeding.

	Items[1]					
Treatments	**WBC (10^3 /µL)**	**H (%)**	**E (%)**	**B (%)**	**Lym (%)**	Mono (%)
Week 1						
Group T	2.47 ± 0.23	44.90 ± 4.26	1.003 ± 0.003	0.05 ± 0.005	55.03 ± 2.62	1.070 ± 0.02
Group 1	2.16 ± 0.07	41.05 ± 0.65	1.003 ± 0.003	0.01 ± 0.013	55.20 ± 0.71	1.060 ± 0.02
Group 2	2.55 ± 0.27	45.66 ± 2.17	1.000 ± 0.000	0.06 ± 0.033	58.53 ± 0.52	1.833 ± 0.14
Week 2						
Group T	2.83 ± 0.24	40.31 ± 0.29[a]	1.020 ± 0.023	1.08 ± 0.051	52.78 ± 1.42	3.347 ± 0.24
Group 1	2.32 ± 0.07	43.73 ± 0.05[b]	1.030 ± 0.033	1.23 ± 0.180	54.65 ± 0.64	3.277 ± 0.19
Group 2	3.84 ± 0.11	43.44 ± 0.97[b]	1.023 ± 0.014	1.11 ± 0.064	51.77± 0.38	3.660 ± 0.03
Week 3						
Group T	3.39 ± 0.05	47.71 ± 0.17[a]	1.007 ± 0.006	0.70 ± 0.057	58.23 ± 0.61[a]	2.020 ± 0.01
Group 1	3.52 ± 0.11	41.22 ± 0.21[b]	1.000 ± 0.000	1.06 ± 0.040	55.96 ± 0.95[a]	2.370 ± 0.29
Group 2	3.40 ± 0.17	43.51 ± 2.06[b]	1.063 ± 0.053	0.14 ± 0.100	54.85 ± 1.41[b]	3.220 ± 0.09
Week 4						
Group T	3.84 ± 0.06	44.72 ± 1.43[a]	1.010 ± 0.005	1.08 ± 0.057	52.57 ± 0.21[a]	3.070 ± 0.04
Group 1	3.74 ± 0.16	32.83 ± 0.42[b]	1.003 ± 0.003	1.03 ± 0.010	62.47 ± 0.86[b]	2.140 ± 0.07
Group 2	4.70 ± 0.09	42.49 ± 0.27[a]	1.003 ± 0.003	1.03 ± 0.015	58.27 ± 0.61[b]	3.250 ± 0.10

WBC=White Blood Cells; H=Heterophils; E=Eosinophils; B=Basophils; Lym=Lymphocyte; Mono=Monocyte. Group T=chickens group treated with antibiotics; Group 1=chickens group treated only with Polybiote; Group 2=chickens group treated with Polybiote associated with Renfort$^+$.

[1]These values are the means followed by the standard error were calculated using 3 replicates (111 chickens /replicate) per treatment.

[a-b] Mean values with different letters in the same column are significantly different ($p < 0.05$) according to the Bonferroni test.

cholesterol, total cholesterol and triglycerides), liver parameters (ALT, ASAT, PAL) and mineral metabolism parameter (calcium).

Statistical Analysis

Data analysis was performed using a post hoc difference test (Bonferonni test) to determine the level of significance between the control and experimental groups with Graph Pad statistical software.

A one-way ANOVA was also performed to test the effect of each product on the parameters assessed in the study. The differences were considered statistically significant when P values were lower than 0.05.

RESULTS

Effects of probiotics and prebiotics on haematological parameters in chickens

Leukocyte parameters

Table 3 shows the results of the evolution of leukocyte parameters in chickens treated either with antibiotics (control batch), with the Polybiote (Group 1), or with the association of Renfort$^+$ and Polybiote (Group 2). The results showed non-significant variations in leukocyte parameters during the first two weeks, except, for the heterophils level on the second week. In fact, the heterophils levels which were 43.73 ± 0.05% in batch 1 and 43.44 ± 0.97% in batch 2 were significantly ($p < 0.05$) higher than that of the control batch (40.31 ± 0.29%).

Heterophils and lymphocyte levels on weeks 1 and 2 were lower than those in the control group. However, the differences in these parameters of batches 1 (41.22 ± 0.21%) and batch 2 (41.22 ± 0.21%) were significant ($p < 0.001$) compared to the control batch (47.71 ± 0.17%). A significant difference ($p < 0.05$) was also recorded in lymphocyte count between batch 2 (54.85 ± 1.4%) and the control group (58.23 ± 0.61%). At the end (week 4), the white blood cells of the chickens in groups 1 and 2 showed no significant variation when compared to the control group. In addition, the heterophils content of batch 1

Table 4: Erythrocyte and thrombocyte parameters in broilers after 4 weeks.

Treatments	RBC (10^6 /µL)	HGB (g/dL)	Hte (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (10^3 /µL)
Week 1							
Group T	2.14 ± 0.12	6.86 ± 0.53	27.93 ± 2.17	123.7 ± 2.33[a]	32.07 ± 1.45[a]	24.57 ± 0.14[a]	26.67 ± 1.20
Group 1	2.08 ± 0.14	6.70 ± 0.35	27.37 ± 1.51	137.3 ± 1.10[b]	32.80 ± 0.80[b]	24.47 ± 0.16[b]	24.67 ± 0.88
Group 2	1.99 ± 0.19	6.00 ± 0.30	26.33 ± 2.45	132.2 ± 0.69[b]	30.37 ± 1.58[b]	22.97 ± 1.07[a]	25.67 ± 0.33
Week 2							
Group T	2.68 ± 0.14	7.36 ± 0.06	33.93 ± 0,40	130.0 ± 0.83[a]	35.43 ± 0.60	27.53 ± 0.27	26.67 ± 0.33
Group 1	2.24 ± 0.08	7.66 ± 0.14	34.37 ± 2.00	127.1 ± 2.21[a]	30.03 ± 0.40	32.87 ± 0.49	25.33 ± 1.66
Group 2	2.21 ± 0.07	6.96 ± 0.43	28.00 ± 1.45	126.5 ± 2.39[b]	31.47 ± 0.93	24.83 ± 0.29	25.33 ± 2.02
Week 3							
Group T	2.22 ± 0.21	7.06 ± 0.47	35.40 ± 1.29[a]	126.5 ± 0.75[a]	32.00 ± 1.55	34.63 ± 0.54	25.00 ± 1.15[a]
Group 1	2.83 ± 0.05	9.53 ± 0.12	39.07 ± 0.46[b]	123.9 ± 0.61[b]	31.10 ± 0.66	35.07 ± 0.44	23.67 ± 0.33[a]
Group 2	3.30 ± 0.17	8.46 ± 0.12	35.70 ± 0.17[a]	122.4 ± 1.51[b]	30.10 ± 0.85	33.80 ± 1.62	27.67 ± 0.66[b]
Week 4							
Group T	2.14 ± 0.12	6.83 ± 0.14[a]	36.17 ± 1.56[a]	122.1 ± 0.23[a]	32.03 ± 1.29[a]	26.23 ± 1.06[a]	24.33 ± 0.88
Group 1	2.53 ± 0.07	7.50 ± 0.20[a]	38.30 ± 1.00[a]	123.0 ± 1.94[a]	32.30 ± 1.45[a]	36.07 ± 0.89[b]	23.00 ± 0.57
Group 2	3.22 ± 0.01	10.3 ± 0.15[b]	45.80 ± 0.20[b]	116.2 ± 0.72[b]	38.37 ± 0.46[b]	34.40 ± 0.43[b]	23.33 ± 0.88

RBC=Red blood cell; HGB=Hemoglobin; Hte=Hematocrit; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; MCHC=Mean corpuscular hemoglobin concentration; PLT=Level of Thrombocyte; Group T=chickens' group treated with antibiotics; Group 1=chickens' group treated only with Polybiote; Group 2=chickens' group treated with Polybiote associated with Renfort$^+$.
[1]These values are the means followed by the standard error were calculated using 3 replicates (111 chickens/replicate) per treatment.
[a-b] Mean values with different letters in the same column are significantly different ($p < 0.05$) according to the Bonferroni test

(32.83 ± 0.4%) and batch 2 (42.49 ± 0.27%) were lower than that of the control batch (44.72 ± 1.43%) with a significant difference ($p < 0.001$) between the control group and group 1. Finally, the lymphocyte levels in groups 1 (62.47 ± 0.86%) and 2 (58.27 ± 0.61%) were significantly ($p < 0.001$) higher than that in the control batch.

Erythrocyte and thrombocyte parameters

The results showed that the mean corpuscular volume (MCV) of Batches 1 (137.3 ± 1.10 fL) and 2 (132.2 ± 0.69 fL) were significantly higher ($p < 0.001$) than that of the control batch (123.7 ± 2.33 fL), on the first week. As for the second week, the MCV of group 2, the Mean corpuscular hemoglobin (MCH) of groups 1 and 2 and the mean corpuscular hemoglobin concentration (MCHC) of group 2 varied significantly ($p < 0.001$) compared to those of the controls. The results on the third week showed a significant difference ($p < 0.05$) between the hematocrit levels (Hte) of group 1, the MCV of groups 1 and 2 and their respective controls.
At the end of the test, the red blood cell values of batches 1 and 2 showed no significant change ($p > 0.05$) compared to the control. In addition, only haemoglobin concentration (HGB),
haematocrit level, MCV and MCH of batch 2 were very high and significantly increased ($p < 0.001$) compared to the control. However, the MCHC of groups 1 and 2 were significantly increased ($p < 0.001$) compared to the control. Throughout the experiment, except the level of thrombocyte in batch 2 (27.67 ± 0.66.103 µL) on the third week, which was significantly higher ($p < 0.001$) than that of the control batch (25 ± 1.15.103 µL), no significant variation ($p > 0.05$) was recorded between the batches (Table 4).

Effects of probiotics and prebiotics on serum's biochemical parameters in broilers

Comparative effects of probiotics and prebiotics on serum ALAT, ASAT, PAL activities and calcium parameters

The serum activities of ASAT (Aspartate aminotransferase), ALT (Alamine amino

Table 5: Serum enzyme activities in experimental chickens.

Treatments	Items[1]			
	AST (IU/L)	ALT (IU/L)	PAL (IU/L)	Calcium (mg/mL)
Week 1				
Group T	137.2 ±1.23[a]	44.43 ± 0.34	106.80 ± 0.97[a]	136.6 ± 0.50[a]
Group 1	128.6 ± 0.99[b]	40.80 ± 2.37	101.00 ± 0.50[b]	135.0 ± 2.22[a]
Group 2	139.9 ± 0.54[a]	46.03 ± 2.39	103.60 ± 1.56[a]	142.8 ± 4.38[b]
Week 2				
Group T	140.5 ± 0.75[a]	40.84 ± 3.68	96.74 ± 1.37	147.1 ± 0.91[a]
Group 1	138.8 ± 2.13[a]	39.14 ± 1.62	91.79 ± 4.10	134.0 ± 0.13[b]
Group 2	130.4 ± 1.54[b]	41.02 ± 3.07	94.95 ± 122	147.4 ± 5.09[a]
Week 3				
Group T	163.2 ± 0.42[a]	43.53 ± 2.31[a]	99.28 ± 3.19[a]	155.4 ± 1.12
Group 1	146.3 ± 1.53[b]	32.58 ± 1.02[b]	93.96 ± 0.89[b]	175.9 ± 4.91
Group 2	156.1 ± 1.67[b]	36.68 ± 0.71[b]	70.01 ± 0.76[b]	180.5 ± 3.98
Week 4				
Group T	202.7 ± 1.06[a]	59.36 ± 2.83[a]	118.9 ± 2.18[a]	141.1 ± 8.04[a]
Group 1	195.4 ± 0.60[b]	44.07 ± 2.12[b]	61.79 ± 1.23[b]	154.9 ± 7.34[b]
Group 2	131.5 ± 4.64[b]	37.83 ± 0.19[b]	92.60 ± 1.48[b]	167.2 ± 1.34[b]

ASAT=Aspartate amino transferase; ALAT=Alanine amino transferase; PAL=Alkaline phosphate; Group T=chickens' group treated with antibiotics; Group 1=chickens' group treated with Polybiote; Group 2=chickens' group treated with Polybiote associated with Renfort[+].

[1]These values are the means followed by the standard error were calculated using 3 replicates (111 chickens /replicate) per treatment.

[a-b] Mean values with different letters in the same column are significantly different ($p < 0.05$) according to the Bonferroni test.

transferase), PAL (alkaline phosphatase) and the serum calcium levels were presented in Table 5. Throughout the experiment, the serum enzyme activities in groups 1 and 2 were less than those in the control groups. However, significant decreases in AST and PAL activities were shown in chickens' batch 1 in the first week. Only the AST activity of group 2 was significantly low ($p < 0.001$) compared to the control during the second week. The ASAT, ALAT and PAL activities of groups 1 and 2 in the last two weeks were all significantly reduced ($p < 0.001$) compared to the activity of their respective controls.

The calcium concentration in the control batch was higher than that in batch 1 but lower than that in batch 2 on the first week. However, a significant difference ($p < 0.05$) was recorded between the control batch and batch 2. On week 4, the calcium concentrations of batches 1 (154.9 ± 7.34 mg/mL) and 2 (167.2 ± 1.34 mg/ml) was significantly high ($p < 0.001$) when compared to that of the control (141.1 ± 8.04 mg/mL).

Comparative effects of probiotics and prebiotics on serum metabolites concentrations in broilers

The concentrations of creatinine, urea, albumin, and total protein in chickens were given in Table 6. Regardless of the type of treatment, the serum creatinine, urea and albumin concentrations showed no significant change. However, on the first week, the total serum protein concentration of group 1 (35.02 ± 1.76 g/L) was approximately 1.2 times higher than that of the control group (28.88 ± 0.16 g/L). The serum total protein concentration during the third week of the control batch (23.62 ± 2.13 g/L) was significantly low ($p < 0.001$) compared to batch 1 (26.97 ± 0.62 g/L) and group 2 (43.69 ± 0.12 g/L). In the last week, the total serum protein concentration of group 1 (17.27 ± 2.16 g/L) was approximately 2 times lower than the control group (34.26 ±1.68 g/L) and this difference was significant ($p < 0.05$).

Comparative effects of probiotics and prebiotics on the blood lipid profile of broilers

The results showed no significant difference ($p > 0.05$) in total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides between the control batch and batches 1 and 2 during the entire experiment. However, the concentration of all these lipid parameters in batches 1 and 2 was lower than those of the control group (Table 7).

DISCUSSION

The study of antibiotic substitution with probiotics and prebiotics on broilers' health showed no clinical signs of toxicity. Indeed, hematological parameters represent the important markers of the physiological and pathological state of humans and animals because of their sensitivity to substances ingested during experiments. Several studies have shown that certain food substances consumed can have deleterious effects on blood parameters (Olayode et al., 2020; Machado et al., 2011). These effects included neutropenia, thrombocytopenia,

Table 6: Serum concentrations of creatinine, urea, albumin and total protein of the chickens.

Treatments	Creatin[1] (mg/L)	Urea[1] (g/L)	Albumin[1] (g/L)	Total Protein[1] (g/L)
Week 1				
Group T	4.46 ± 0.23	0.12 ± 0.022	19.18 ± 0.56	28.88 ± 0.16[a]
Group 1	4.89 ± 0.33	0.07± 0.008	17.53 ± 0.95	35.02 ±1.76[b]
Group 2	4.74 ± 0.06	0.17 ± 0.002	20.9 ± 2.65	29.57 ± 0.67[a]
Week 2				
Group T	6.84 ± 0.61	0.17 ± 0.015	15.73 ± 2.18	35.15 ± 0.46
Group 1	7.38 ± 0.10	0.10 ± 0.010	19.31 ± 0.45	39.58 ± 1.10
Group 2	6.41 ± 0.57	0.14 ± 0.011	15.31 ± 1.92	36.75 ± 1.61
Week 3				
Group T	7.57 ± 0.42	0.15 ± 0.012	16.93 ± 0.37	23.62 ± 2.13[a]
Group 1	8.53 ± 0.09	0.11 ± 0.022	16.86 ± 0.69	26.97 ± 0.62[b]
Group 2	9.19 ± 0.55	0.09 ± 0.018	16.24 ± 0.55	43.69 ± 0.12[b]
Week 4				
Group T	7.74 ± 1.01	0.11 ± 0.016	15.73 ± 2.62	34.26 ± 1.68[a]
Group 1	8.80 ± 0.23	0.14 ± 0.005	18.36 ± 0.28	17.27 ± 2.16[b]
Group 2	8.38 ± 0.09	0.15 ± 0.006	18.7 ± 0.85	38.27 ± 1.18[a]

Group T=chickens group treated with antibiotics; Group 1=chickens group treated only with Polybiote; Group 2=chickens group treated with Polybiote associated with Renfort^{+}.
[1]These values are the means followed by the standard error were calculated using 3 replicates (111 chickens /replicate) per treatment.
[a-b] Mean values with different letters in the same column are significantly different ($p < 0.05$) according to the Bonferroni test

Table 7: Average concentration of lipid parameters.

Treatments	Total- Chol[1] (g/L)	HDL-Chol[1] (g/L)[1]	LDL-Chol[1] (g/L)	Triglycerides[1] (g/L)
Week 1				
Group T	1.60 ± 0.25	0.23 ± 0.02	0.65 ± 0.06	2.23 ± 0.04
Group 1	1.00 ± 0.03	0.25 ± 0.02	0.60 ± 0.05	2.20 ± 0.03
Group 2	1.53 ± 0.21	0.29 ± 0.03	0.84 ± 0.10	2.25 ± 0.03
Week 2				
Group T	1.35 ± 0.13	0.25 ± 0.05	0.72 ± 0.16	3.21 ± 0.04
Group 1	1.45 ± 0.17	0.21 ± 0.03	0.47 ± 0.17	2.31 ± 0.03
Group 2	0.94 ± 0.03	0.23 ± 0.01	0.65 ± 0.02	3.26 ± 0.03
Week 3				
Group T	1.49 ± 0.18	0.20 ± 0.04	0.55 ± 0.14	4.35 ± 0.09
Group 1	0.86 ± 0.04	0.12 ± 0.01	0.53 ± 0.04	4.37 ± 0.08
Group 2	0.79 ± 0.05	0.23 ± 0.04	0.62 ± 0.13	3.35 ± 0.02
Week 4				
Group T	2.50 ± 0.18	0.37 ± 0.20	0.65 ± 0.20	5.23 ± 0.05
Group 1	1.69 ± 0.14	0.17 ± 0.03	0.46 ± 0.09	2.26 ± 0.07
Group 2	1.05 ± 0.31	0.18 ± 0.01	0.48 ± 0.01	3.31 ± 0.04

Chol= Cholesterol; HDL=High-Density Lipoprotein; LDL=Low-Density Lipoprotein; Group T=Chickens group treated with antibiotics; Group 1=chickens group treated only with Polybiote; Group 2=chickens group treated with Polybiote associated with Renfort+.
[1]These values are the means followed by the standard error were calculated using 3 replicates (111 chickens /replicate) per treatment.

hemolytic anemia, aplastic anemia, macrocytic anemia. On the other hand, probiotics and prebiotics have shown stimulating effects (polycythemia, leukocytosis) on blood parameters (Dawood et al., 2019).

In the present study, there is no significant difference between white blood cell levels in control chickens and those treated with polybiote (probiotic), on the one hand, and those treated with the combined action of polybiote and renfort^{+} (prebiotics), on the other hand. These results indicated that the administration of these products did not disrupt the chickens' immune systems. In addition, heterophilic polynuclear and lymphocyte levels of chickens fed with polybiote combined with renfort^{+} were affected compared to the control batch, but the values were within the range described by Kokore et al. (2021) in normal-growing broilers. The norm is 26-63% and 27-67% respectively for Heterophils and lymphocytes, this further confirms that the administration of the polybiote and renfort^{+} better strengthens the immune system of chickens. These measured leukocyte parameters help to

know if the ingested substances have triggered inflammation. Inflammation is the first response to chemical, toxic, microbial, traumatic, and environmental aggression. This process is beneficial for the body, it allows the establishment of a rapid immune response to eliminate the causative agent and repair the damaged tissues (Boukhari, 2022). These results for white blood cell counts are in tandem with those of Nikpiran et al. (2013) who highlighted the probiotic and stimulatory effect on their immune system after administration of lactic bacterium accompanied by prebiotics on broilers (Gallus gallus). Robins et al. (2015) reported that several studies showed that the administration of lactic bacteria increases the ability of lymphocytes to secrete various cytokines that are immune chemical mediators. In the same vein, the beneficial effect of the combination of polybiote and renfort⁺ on the immune system of chickens was confirmed by the study of Ghasemi et al. (2014) who reported that diets supplemented with probiotics and symbiotics improve immune response of animals. These components are characterized by their powerful immunomodulatory effect and their crucial role in the stimulation and reactivity of the immune system. Slawinska et al. (2014) reported that, apart from maintaining beneficial bacteria in the gut, prebiotics induce better immune system development. Other studies have also shown that the injection of prebiotics (inulin) and symbiotics (inulin with *Lactococcus lactis*) led to an improvement in immune responses related to the stimulation of Peyer's plaques, colonization of the caecal tonsils by T cells as well as the development of immune organs such as the spleen and thymus (Madej et al., 2015; Slawinska et al., 2014).

Red blood cells and erythrocyte parameters (hemoglobin, hematocrit, MCV and MCH) have the primary role of ensuring the transport function of oxygen and nutrients in the body (Benchikh, 2022). The red blood cell count of chickens subjected only to the polybiote and chickens subjected to the combination of the polybiote and renfort⁺ showed no significant difference in red blood cell levels compared to the control chicken. On the other hand, the level of hemoglobin, hematocrit, MCV and MCH chickens in the batch subjected to the combination of polybiote and renfort⁺ were significantly increased compared to the control group. However, these values fall within the range described by Kokore et al. (2021). These various observations suggest that the administration of polybiote alone or in combination with renfort⁺ does not induce hemolytic anemia or polycythemia. Therefore, it allows better transportation of nutrients and oxygen in chickens' bodies, resulting in better animal health. Our results are in tandem with those of Majdi et al. (2022) who assessed the level of protein assimilation in broiler feed and reported that the experimental batch of chicken that received the symbiotics had their red blood cell constantly higher than the control batch.

The main role of platelets or thrombocytes is to ensure hemostatic function. The platelet count of all chickens in this study was not affected, indicating that the polybiote used alone or in combination with the renfort⁺ tested did not induce thrombocytopenia. These results are contrary to those of Fathi et al. (2017) which show the addition of 400 g of probiotic in the feed raised the platelet number in rabbits. This difference could be explained by the nature of the bacteria strains used in both studies and the type of animals.

The evaluation of serum biochemical parameters is important in humans and animals ingested with a particular food or drug. Indeed, these parameters concern substances synthesized by the body whose excess or deficit is indicative of its dysfunction (Perri et al., 2017). In this study, the creatinine, urea and blood albumin values of animals subjected only to the polybiote and animals subjected to the combination of polybiote and renfort⁺ did not undergo significant variations compared to the animals in the control batch. Regarding the total protein concentrations, the experimental batches were significantly higher than the control group on the third week but only the batch that received only the polybiote had its rate significantly decreased at the end of the experiment. Note that all values for total protein concentrations recorded were within the range (25 - 45 g/L) recommended by (Thrall et al., 2012) except for the batch subjected only to the polybiote at the end of the test. However, it's not possible to conclude that the animals in batch 2 subjected only to the polybiote suffer from liver damage or insufficiency because proteinemia alone may lack specificity, so it is necessary to couple its interpretation with the results of the albuminemia assay (Eckersall, 2008) which in our case is stable according to standards. Urea and creatinine are considered good markers of renal dysfunction (Mukinda and Eagle, 2010). However, creatinine is the most commonly used clinical serum biomarker to diagnose kidney dysfunction. An elevation in its serum concentration is only observed if the functional nephrons are severely damaged (Haye, 2008; Gbakon et al., 2018). Thus, the results recorded in this study suggest that neither the administration of the polybiote nor the administration of the combination of the polybiote and the renfort⁺ altered the renal function of the chickens because their value was decreased. Moreover, this hypothesis is confirmed by Brosnan et al. (2010) who after administration of a prebiotic (Bioplus) and a probiotic (Primalac) recorded a decrease in creatinine concentration resulting in good functioning of the kidneys of chickens.

For the lipid parameters such as total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride concentrations, no significant changes were observed between chickens in the treated batches and those in the control batch. In contrast, lipid concentrations in the treated batches were lower than in the control batch all the time. This suggests a potential hypocholesterolemic effect and a possible cardioprotective action of renfort⁺ and polybiote. This hypothesis is confirmed by Sharif et al. (2011) who found that giving variable amounts of symbiotics (combination of probiotic and prebiotics) to

quails reduces blood triglycerides. Also, Khajeali et al. (2012) found that probiotics in the diet of broilers significantly reduce blood triglyceride concentration. In addition, this decrease in blood triglycerides could be attributed in some cases to a decrease in fat oxidation, leading to a decrease in acidic fats including cholesterol and triglycerides. According to Dina Bushuty et al. (2012), blood cholesterol levels are significantly reduced in groups treated with Lactobacillus probiotics and the assimilation of cholesterol compared to control groups with a dietary baseline. *Lactobacillus acidophilus and lactobacillus casei* in food or water produce a reduction in biliary vesicle acids in the digestive process, resulting in reduced fat digestion capacity and consequently lower blood lipid levels (Getachew, 2016). Similarly, Navid Hosseini (2010) revealed the cholesterol-lowering effect of feed supplementation with *Lactobacillus acidophilus* and *Lactobacillus casei,* alone or in combination with water during all the rearing phases of chickens. Also, Ignatova et al. (2009) reported that the addition of probiotic strains, *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus reuterii, Bifidobacterium bifidudum, Bifidobacterium animalis, Bifidobacterium infantis* significantly reduces serum cholesterol levels. Finally, a study conducted by Mayahi et al. (2010) showed that supplementation with the probiotics *Enterococcus feacium* and *Bifidobacterium genera* in chickens causes the same effect on blood cholesterol as the other probiotics studied.

Serum enzymes such as AST, ALT and PAL are well-known as good indicators of hepatic cytolysis and as predictive biomarkers of possible toxicity (Merghem et al., 2013). AST and ALT transaminases increase sharply in the bloodstream when hepatocytes or their cell membranes are damaged. ALT is a more specific biomarker of hepatocellular lesions because it is produced exclusively in the liver. AST, on the other hand, is produced to some extent also in the heart, skeletal muscles, kidneys, brain, pancreas and blood cells (Belguet, 2010). In addition, serum ALP activity increases in cases of liver cell damage and bile duct obstruction (Akhtar et al., 2012). Serum AST, ALT and PAL concentrations in the polybiote-treated and polybiote-renfort^{+} combination groups decreased significantly compared to the control group. This could demonstrate that there was no cell damage in the kidneys, liver, muscles and heart. The enzyme activities in broilers' serum were also not influenced by probiotic, prebiotic or symbiotic supplementation in studies done by Fathy et al. (2009). In the same vein, the study of Khalil et al. (2014) showed that the administration of the food supplemented with lactic acid bacteria brought the serum liver enzymes of previously intoxicated rats to normal.

As for the evaluation of calcium levels, the results showed that the administration of the polybiote alone or in combination with the renfort^{+} resulted in a significant increase compared to the control batch. These levels were always within the range of usual values for calcium in broilers (132 - 237 mg/L) as described by Hochleithner (2013). Calcium is very necessary for ossification, regulation of skeletal and cardiac muscle activity, activation of several enzymes, transmission of nerve impulses, hormonal mediation, membrane permeability, blood coagulation and maintenance of osmotic pressure (De Matos, 2008). However, they are not fully available in the body hence the need for an intake in the food ration. Therefore, our results could reflect beneficial effects for the body of broilers and would be in agreement with the work of Fathy et al. (2009), who also recorded a significant increase in calcium in broilers after the administration of prebiotics and symbiotics. Our results are also in agreement with that of Aluwong et al. (2013) which showed that broilers who received a daily and periodic dose of probiotic (NormosilR) showed an increase in hemoglobin, glucose, total protein, albumin, sodium, potassium and calcium from 4.9% to 10.9%, 12.3% to 17.4%, 0.5% to 5.7%, 3.7% to 14.4%, 2.1% to 5.0%, 17.0% to 33.3% and 7.7% to 21.2% ($p<0.05$) respectively after 42 days.

CONCLUSION

The present study revealed that the administration of the polybiote alone or associated with renfort^{+} improves the hematological parameters in chickens. Moreover, their combination better strengthens the immune system of chickens. As for serum biochemical parameters, the administration of polybiote alone or in combination with renfort^{+} regulates the renal, cardiac, hepatic and skeletal muscle activities of broilers. Therefore, polybiote and renfort^{+} can substitute antibiotics in broiler farming in order to contribute to food security worldwide.

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