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Alternative use of probiotic (*Lactobacillus acidophilus, Lactobacillus lactis and Saccharomyces cerevisiae*) in boosting immunity in four breeds of poultry birds

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Abstract

This study researched the effect of *Lactobacillus acidophilus, Lactobacillus lactis and Saccharomyces cerevisiae* as probiotic that can be used to boost immunity in poultry birds.The markers used for the analysis were weight analysis and hematological parameters of 4-weeks old, 5-weeks old, 6-weeks old and 7-weeks old poultry birds respectively. The results showed that average body weights of the birds treated with different probiotics considerably increased from an average of 40 to 107g, 40 to 104g and 41 to 100g for probiotic, antibiotic and control groups respectively in all classes of birds used. The blood parameter (PCV, Hb, MCV, and MCH) of birds fed with *Lactobacillus acidophilus, Lactobacillus lactis and Saccharomyces cerevisiae* were greatly improved in post-test results done at 7weeks in all groups of the birds under studycompared to the pretest done at 3weeks. Blood differential counts in the birds were decreased as seen in Percentage Eosinophil and Neutrophil counts while percentage monocyte and neutrophil counts were increased in control birds. Although the difference in differential blood count was more significant in birds treated with probiotic than those treated with antibiotic (Enrofloxacin). CD4 count was increased in groups of bird treated with probiotics (15.8 to 25.7 in broiler chickens) than those treated with antibiotic and control group.

Keywords: Probiotic, *Lactobacillus acidophilus, Lactobacillus lactis*, Eosinophil, leucocytes, CD4, *Saccharomyces cerevisiae*, Differential count.

1. Introduction

Food security is a major challenge, particularly for developing countries since the world's population is expected to reach more than 9billion by 2050. The livestock sector has been found to be one of the fastest growing agricultural sectors contributing about 40 percent of the global value of agricultural products to support the livelihoods and food security of almost 1.3 billion people (Briunsma,2003).It is expected to produce more with limited resources since economic growth has increased the demand for livestock products. Livestock and its products provide a major source of disposable income for disadvantaged and marginal populations in developing countries and also a major entry point to fight against poverty (Randolph *et al.*, 2007).

The largest processor of chicken in the United States, Tyson foods announced the use of probiotic fed chickens in order to produce healthier birds (US Department of Agric,2017).Other Researchers at Okhlaoma State University's Robert M. Kerr food and Agricultural products centre (FAPC) had also studied the implementation of probiotics in chicken feed. Intensive production systems are playing an increasingly important role in the livestock sector worldwide with a lot of benefits but with a serious public health issue. The sub-therapeutic use of antibiotics as growth promoters in animal feed, prophylaxis, metaphylaxis and therapy has evoked widespread concern, with their use banned in many countries including the European Union (EU), due to the potential to develop antibiotic resistance in microbial populations associated with human and animal diseases (Aarts, 2015).

The joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Working Group defined Probiotics as "live micro-organisms which when administered in adequate amounts confer a health benefit on the host" (FAO/ WHO, 2001). This definition is widely accepted and adopted by the International Scientific Association for Probiotics and Prebiotics (Hill *et al.*, 2014). These microorganisms are most often bacteria, but also include other kinds of organisms such as yeast. Probiotics (or Direct Fed Microbial) are becoming increasingly popular as one of

the alternatives to Antibiotic Growth Promoters (AGP). The use probiotics in animal feed are encouraged to maintain and improve the productivity and growths of the animals. It also prevents and controls enteric pathogens. Due to the growing concern with the sub-therapeutic use of AGP in animal feed and greater appreciation of the role of the microbial ecology of the gastro-intestinal tract (GIT) in determining animal productivity, increasing numbers of Probiotic products are being developed and used in animal nutrition (Abdel-Raheem *et al.*, 2012).

Thus, the objective of this work is to isolate, identify and characterize microorganisms that can act as Probiotic in poultry birds and the overall effect of the probiotics on growth performance, immune response, and blood parameters.

2. Methodology

2.1: Grouping: A total of 60 poultry birds comprising of one-week-old mixture of broiler, layer and cockerel chicks of mixed sex were used in this experiment. They were obtained from Ibadan Agricultural farm (local private hatchery). The chicks were randomly allotted into five groups comprising of broilers, layer, cockerel and local chicks per group. The five groups comprised of three probiotic groups, antibiotic and control groups. The birds were divided into three (3) main groups of twelve birds, this comprise of three numbers of cockerels, broilers and layers and local birds. The groups were tagged as probiotics (1,2 and 3), antibiotics and control.
The probiotic group divided into three subgroups comprised of twelve birds (Three numbers of each breed of birds) in a group, treated with *Lactobacillus acidophilus, Lactobacillus lactis and Saccharomyces cerevisiae).* Also the antibiotic group (Enrofloxacin) and the control group (untreated) were composed of twelve birds each. The control group was fed with only feed and water throughout the course of the research. Probiotic were administered through feed and drinking water. This was achieved by mixing the isolated probiotic culture with the feed of the birds and delivering the same quantity of probiotics through the drinking water supply.

2.2 Accommodation and Management: The chicks were housed in clean well-ventilated room previously disinfected with50% formalin. The room was provided with a gas heater in addition to electric lamps of 60 watts over each partition to obtain the suitable temperature needed for the chicks. Each compartment was bedded with fresh clean wheat straw forming a few centimeter-deep litter beds. Each compartment was provided with a suitable feeder and watering can. The certificate of vaccination against Newcastle disease at days old was given after purchase. Vitamins ADE (in 1 ml/L of drinking water) was given daily during the period of acclimatization to improve vitality of chicks. The acclimatization of the birds was done for 20 days.

2.3 Weighing: The birds were weighed with weighing scale once in a week to check the improvement in weight, feed conversion ability and the agility of the bird. The birds were fed for 20 days with top feed starter and clean water.

2.4 Tests parameters: Packed cell volume (PCV), White blood cell (WBC),Hemoglobin (Hb), Mean corpuscular haemoglobin (MCH)and the differential counts of neutrophils, lymphocytes, eosinophil, monocyte and basophil were carried out at 4 and 7 weeks for all groups of birds used.

2.5 Isolation of Lactic Acid Bacteria (LAB): LAB isolates were isolated from goat milk, the samples were cultivated on deMann Rogossa and sharpe medium (MRS agar) and incubating at 37^{o}C for 24-48 hours. The isolates were later identified and characterized.

2.6 Isolation of yeast: Yeast (*Sacharomyces cerevisiae)* isolates were isolated from fermented maize. The samples were cultivated on Saboraud Dextrose Agar (SDA) at 27^{o}c for 48-72 hours.

2.7 Identification/Characterization of Bacterial Isolates: Bacterial isolates were identified morphologically viz: size, shape, colour, consistency, edges, elevation, and opacity. They were further identified by staining (Gram, Spore and Capsule staining) and biochemically characterized viz: Indole, Motility, Catalase, Urease, Citrate, Methyl-red,Voges Proskauer, Hydrogen sulphide and Sugar fermentation test. The results were interpreted according to Berges Manual of determinative bacteriology.
2.8 Identification/Characterization of yeast Isolates: Yeast isolates were identified morphologically based on their size, shape, consistency, colour, edges, elevation, and opacity. They were further stained with Lactophenol in cotton blue, iodine and methyl-blue stains and their reaction to the stains were recorded.

2.9 Pre and Post-test: Blood samples were collected after acclimatization (4weeks) and after 3 weeks of treatment with probiotics and Antibiotic. Parameters such as PCV, WBC, HB, MCV, MCH and differential counts were measured and recorded.
2.10 Immune level determination: CD4 counts of the birds were done at 4 and 7 weeks to monitor the level of CD4 cells in the blood.

3. **Results and discussion**

Table 1: Average weights of birds fed with *L. acidophilus, L. lactis and Saccharomyces cerevisiae* in grams

Breeds	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Cockerel 1	44	68	70	75	86	90	96
Cockerel 2	44	55	65	70	79	87	90
Cockerel 3	47	57	72	73	85	90	94
Cockerel 4	44	55	70	74	80	82	85
Cockerel 5	45	54	67	70	74	79	82
Broiler 1	45	59	76	77	83	95	106
Broiler 2	40	56	80	94	100	102	105
Broiler 3	49	55	89	96	102	105	107
Broiler 4	40	53	87	93	99	100	104
Broiler 5	42	54	86	79	93	98	100
Layers 1	40	46	59	71	83	87	91
Layers 2	42	45	56	73	85	87	89
Layers 3	41	42	54	70	82	84	88
Layers 4	40	42	57	71	84	87	89
Layers 5	41	44	56	70	81	82	86
Local 1	40	47	52	65	70	73	79
Local 2	44	45	50	68	70	73	77
Local 3	45	48	52	65	69	72	76
Local 4	42	47	49	64	68	73	75
Local 5	45	51	58	62	65	70	74

Note: 1 is *L. acidophilus*, 2 is *L. lactis*,3 is *Saccharomyces cerevisiae*, 4 is Enrofloxacin and 5 is control group

TABLE 2: Blood parameters of different breeds of birds at 4 weeks (Pre-test)

Erythrocytes indices	*L. acidophilus*	*L. lactis*	*S. cerevisiae*	Enrofloxacin	Control
PCVc	29	28	28	26	28
PCVa	32	26	32	31	30
PCVl	34	33	34	32	29
PCVb	28	27	28	25	28
MCVc	76.64	66.63	76.55	73.24	69.96
MCVa	79.62	79.52	70.1	78.06	66.5
MCVl	66	67.22	76	76	66
MCVb	79.21	79.35	78.47	77.54	61.4
Hbc	9.4	9.6	9.6	9.5	9.1
Hba	10.1	9.8	9.9	9.1	9.2
Hbl	9.9	9.8	9.8	9.1	9.5
Hbb	11	10.8	10.2	10.1	9.5
MCHc	71.31	72.16	73.42	70.62	71.45
MCHa	72.23	72.32	72.45	71.81	71.53
MCHl	72.68	74.54	72.2	72.9	72.37
MCHb	72.75	72.01	72.53	71.64	75.38

Note: PCV means % Packed cell volume, MCV is Mean corpuscular volume, Hb is heamoglobin (g/dl), MCV is Mean corpuscular volume (fl), MCH is Mean corpuscular heamoglobin (pg) concentration while the small letters c,a,l and b allotted to each end of the blood parameters are the codes for Cockerel, Layers, Local and Broiler birds respectively.

TABLE 3: Blood parameters of different breeds of birds at 7 weeks (Post-test)

Blood parameters	***L. acidophilus***	***L. lactis***	***S. cerevisiae***	**Enrofloxacin**	**Control**
PCVc	36	32	34	30	28
PCVa	34	30	32	28	24
PCVl	38	39	34	34	24
PCVb	38	34	34	30	28
MCVc	86.96	86.95	86.95	83.24	79.96
MCVa	89.62	89.52	90.1	98.06	76.5
MCVl	86	87.36	86	86	76.13
MCVb	89.41	89.51	88.69	87.69	71.52
Hbc	12.1	12.5	12.2	11.8	11.7
Hba	15.1	15.5	15.2	14.8	13.67
Hbl	15.4	15.6	15.6	14.5	13.1
Hbb	14.1	14.8	14.9	14.1	13.9
MCHc	91.62	81.48	91.62	91.62	91.68
MCHa	91.03	91.52	91.71	91.71	91.76
MCHl	81.78	81.74	81.4	81.4	92.09
MCHb	81.85	81.01	81.85	81.85	91.08

Table 4: Differential counts of different groups of birds at 4 weeks (Pre–test)

Groups	*L. acidophilus*	*L. lactis*	*S. cerevisiae*	Enrofloxacin	Control
Nc	62	63	61	61	64
Na	64	65	63	62	62
Nl	64	65	64	64	63
Nb	64	64	64	63	64
Ec	1.42	1.58	1.59	1.42	1.49
Ea	1.49	1.59	1.48	1.57	1.52
El	1.39	1.49	1.48	1.52	1.52
Eb	1.53	1.52	1.52	1.52	1.46
Bc	0	0	0	1	0
Ba	0	1	0	0	1
Bl	0	0	0	0	0
Bb	1	0	0	1	1
Lc	35	34.9	33.5	34.01	33.46
La	34.89	34.59	35	34.86	34.04
Ll	35	33.76	34	34.64	33.4
Lb	34	33.89	33.1	34	34.64
Mc	1.2	1.2	1.23	1.2	1.20
Ma	1.3	1.24	1.23	1.22	1.21
Ml	1.27	1.24	1.24	1.24	1.22
Mb	1.25	1.25	1.24	1.24	1.20

NB: N means % Neutrophil count, E is % Eosinophil count, B is % Basophil, L is % leucocyte count and M is % Monocyte count

Table 5: Differential counts of different groups of birds at 7 weeks (Post-test)

Groups	*L. acidopilus*	*L. lactis*	*S. cerevisiae*	Enrofloxacin	Control
Nc	60	61	61	61	64
Na	62	62	62	62	64
Nl	62	62	62	62	65
Nb	62	62	62	60	64
Ec	1.26	1.25	1.23	1.22	1.43
Ea	1.23	1.23	1.25	1.26	1.42
El	1.25	1.24	1.25	1.26	1.33
Eb	1.24	1.26	1.25	1.25	1.39
Bc	0	0	0	0	0
Ba	0	0	0	0	0
Bl	0	0	0	0	0
Bb	0	0	0	0	0
Lc	35	34.7	33.5	34	33

La	34	34	35	34.16	33.04
Ll	35	33.01	34	34.42	31.4
Lb	33.5	33.02	33	34	30.61
Mc	1.2	1.2	1.23	1.2	1.24
Ma	1.25	1.24	1.23	1.22	1.24
Ml	1.22	1.24	1.24	1.23	1.24
Mb	1.22	1.23	1.14	1.2	1.22

NB: N means % Neutrophil count, E is % Eosinophil count, B is % Basophil, L is % leucocyte count and M is % Monocyte count

Table 6: Morphological and growth characterization

	L. acidophilus	***Lactobacillus lactis***
Colour	Cream to off white	Cream to off white
Shape	Circular	Circular
Size	0.49um	0.49um
Motility	Non motile	Non motile
Gram reaction	Positive	Positive
Growth at different temperature (Degrees)		
15-20	Negative	Positive
30	Positive	Positive
35	Positive	Positive
37	Positive	Positive
45	Positive	Negative
Oxygen requirement	Positive	Positive
Growth in medium		
4% Nacl	Negative	Positive
6.5% Nacl	Negative	Negative

TABLE 7: Biochemical characterization of lactobacillus isolates

Biochemical characterization	***L. acidophilus***	***Lactobacillus lactis***
CO_2 production from glucose	Negative	Negative
Catalase	Negative	Negative
Indole	Negative	Negative
Methyl red	Negative	Negative
Vogesproskaeur	Negative	Negative
Citrate	Negative	Negative

Urease	Negative	Negative
TSI	Negative	Negative
Nitrate reduction	Negative	Negative
Catalase	Negative	Negative
Oxidase	Negative	Negative
Sugar fermentation		
Dextrose	Positive	Positive
Fructose	Positive	Positive
Galactose	Positive	Positive
Glucose	Positive	Positive
Lactose	Positive	Positive
Maltose	Positive	Positive
Mannitol	Negative	Negative
Sucrose	Positive	Positive
Xylose	Negative	Negative

TABLE 8: Morphological characterization and staining reactions of yeast isolates

Morphological characteristics	of yeast isolates
Colour	creamy
Shape	Cocci shaped while others appeared as budding cells
Consistency	Smooth colonies
Opacity	Transparent
Size	1-2mm
Elevation	slightly raised
Staining	
Lacto-phenol in cotton blue	blue colored cocci shaped isolates
Methyl blue	colorless cells with some dark blue cells
Iodine	bluish black colonies and some purple colonies

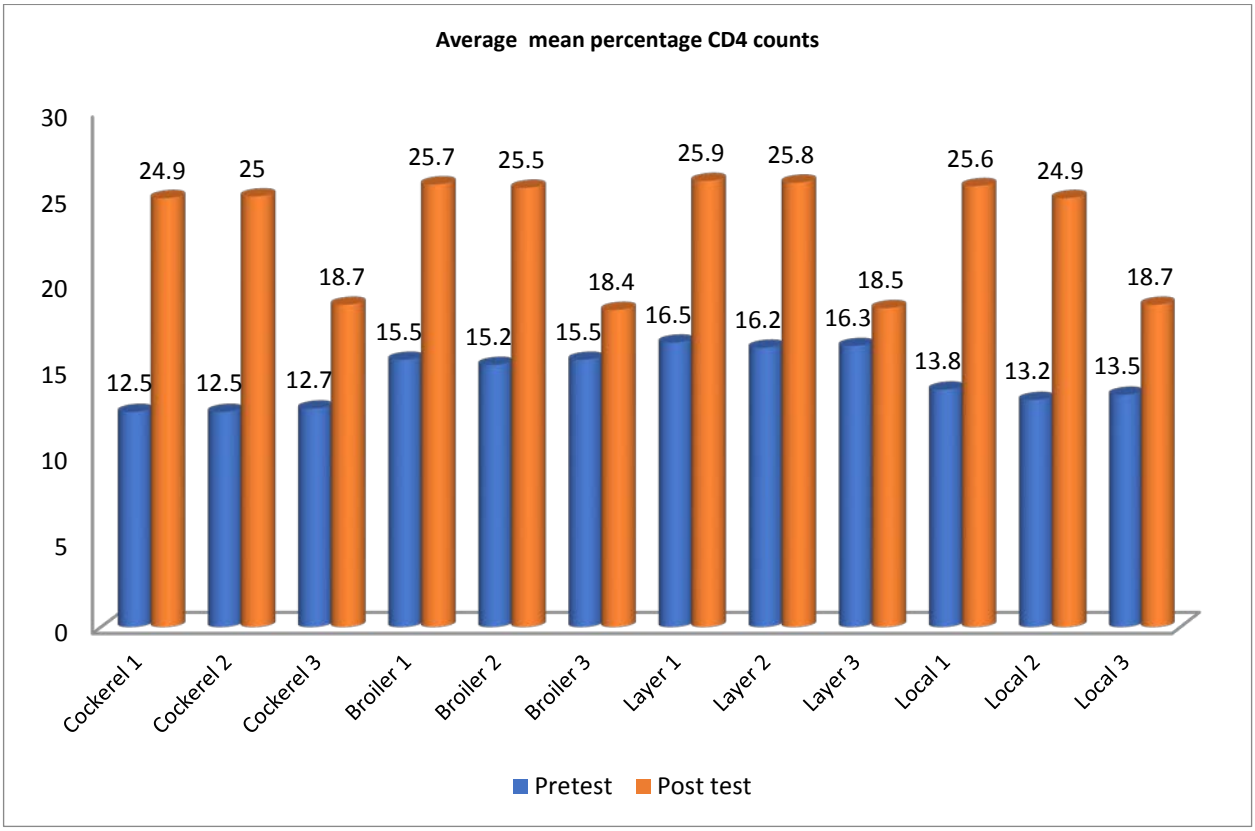

Fig 1: CD4 (Average Mean percentage) count of the birds

Note: 1 is allocated to group of chicks treated with Antibiotic (Enrofloxacin), 2 is allocated to group of chicks treated with probiotics (*Lactobacillus acidophilus, Lactobacillus lactis and Saccharomyces cerevisiae* and 3 is for group of birds that are not treated (control group).

3.1 Discussion

The overall effect of probiotics used as a replacement for antibiotic (enrofloxacin) was measured within four to seven weeks of study and the highest weight gain recorded was in the probiotic group of broiler chickens which increased to an average of 107g, followed by 104 and 100g recorded in enrofloxacin and control groups respectively. The average weight of the poultry birds differs greatly as the chickenstreated with probiotics tend to add more weight when compared to others group as the research week progresses. This is evident as seen in Table 1.The blood parameter values obtained as shown in Table 2 (at 4 weeks) and Table 3 (at 7 weeks) in all groups revealed a similar trend between probiotic and antibiotic used. The Packed Cell Volume (PCV) increased from an average of 34 to 39% and 32 to 34% in probiotic and enrofloxacin groups respectively while the control group reduced from 30 to 28%.The Microcorpuscular volume (MCV) increased from 79.64 to 89.62%, 78.06 to 87.69% and 69.96% to 79.96% for probiotic, antibiotic and control groups respectively. The difference between the Haemoglobin content (Hb) of the Red Blood Cell (RBC) recorded the numbers were very close for the probiotic (which increased from 10.1% to 15.6%) and the antibiotic group (which increased from 14.1 to 14.8%) this was similar to what was obtained by Olaiya *et al.*, (2014) for healthy chickens. The White Blood cell (WBC) count shows that the immune system was not impaired by the treatment with probiotics but rather increases the ability of the birds to fight infections as this was established in the results obtained in WBC and CD4 counts. The difference in the blood parameters experienced during the course of this might be due to the treatment given to the birds as quantity of PCV, MCV, Hb and MCH counts increased significantly during post-test as against what was recorded during pre-test as seen in Table 3. The decrease in Neutrophil (65 to 62%) and monocyte counts in probiotic chicks and increment seen in control birds might be evidence of healthiness and probable reduction of infectious pathogens in the bird's system. The lymphocyte count remained unchanged in probiotic and antibiotic groups but was reduced in control birds. The results were in agreement with Onyishi *et al.*, 2017. The CD4 count of the birds treated with probiotic (25.8) were able to compete with the other birds that were treated with antibiotic (25.9) group which greatly increased from the pre-test to post-test results.

4. Conclusions: The use of probiotics as a safer alternative to regular antibiotics that can be used in poulty business. The need to solve the problems of antibiotic residue in poultry product and antibiotic resistance cannot be over emphasized. The treatment of the different breeds of poultry birds with three probiotics isolated from plant and animal source gave positive results due to the fact that the birds were able to wade off infections due to increased blood parameters, CD4 count and improved differential counts.

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