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Research Article
The Effect of Prebiotics-Phytobiotics Supplement on Broiler Biological and Immunological Performance

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Abstract

Background and Objective: Over the years poultry producers have relied on the use of antibiotics as growth promoters to improve the performance and the health status of their birds. This study was conducted to test the effect of 'chicken protector' a combination of prebiotics and phytobiotics on the immunological, haematological and bacteriological status of broilers for 21 days. **Materials and Methods:** In the experiment, 50 Cobb 500 one-day old broiler chicks obtained from a local hatchery in Ghana were used. They were randomly assigned to two treatments, chicken protector (T_1) and control (T_2) in a Completely Randomized Design (CDR) for 21 days. The parameters that were measured included, haematological parameters, body weight, blood cholesterol levels, immunology, histological performance and gut microbial level. **Results:** The inclusion of the chicken protector in the water of broiler chicks significantly reduced blood cholesterol levels on day 7 and day 14 ($p<0.05$). White blood cell count, red blood cell, haematocrits, neutrophils and platelet large cell ratio were all significantly improved at day 7 with the inclusion of the chicken protector. The chicken protector was effective in reducing cholesterol levels in the blood on days 7 and 14 but no significant difference was seen on day 21. **Conclusion:** Despite the improvement in the haematological parameters of broilers, the inclusion of the chicken protector did not improve the body weight gain of the broiler chickens compared to the control group. Per the results obtained from the study, prebiotics can serve as an alternative for antibiotic usage in broiler production but benefits can best be determined when broilers are grown to the end of production.

Key words: Broiler, antibiotics, prebiotics and phytobiotics, histological performance, gut microbial

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry production involves the rearing of domesticated birds purposely for either their meat or eggs for human consumption and manure for agricultural production. The industry has been known to be the fastest-growing sub-sector of agriculture over the past 50 years[1]. The fast growth in the industry is because demand for animal products has increased as a result of urbanization and population growth[2]. Since poultry has short production cycles and the ability to convert a wide range of agri-food by-products and wastes into meat and eggs, the industry has high prospects to support food security thereby, making a substantial contribution to food security and nutrition, energy, protein and essential micro-nutrients to humans[3].

The top four producers of poultry meats in the world today are, the United States, China, the EU and Brazil with production levels of around 20, 18, 13 and 12 M ton, respectively[1]. On the African continent, the largest producer of poultry meat is South Africa. The poultry industry is the biggest sub-sector of agriculture in South Africa contributing 16% of the gross domestic product of the economy[4]. In Ghana, the poultry industry is one of the important sectors of agriculture. Eggs and meat produced by the industry provide the greatest portion of the daily protein requirement of the people. It also supplements the income generated by farmers and serves as an avenue for employment to the youth[5].

The contribution of the poultry industry to the development of the economy of Ghana cannot be neglected as it accounts for some portion of the country's GDP. Disease outbreak is one of the main causes of the high mortality rate, especially in starter chicks. This is mostly due to the quality of chicks obtained from the various hatcheries[6]. Over the years poultry, producers have relied on the use of antibiotics as growth promoters to improve the performance and the health status of their birds. The antibiotics when administered to the chicks kill harmful bacteria in the gut of the bird, therefore, reducing the activity of the bacteria. The continual use of these antibiotics has led to resistant strains of bacteria in animals and humans[7].

Prebiotics has been identified as the best alternative for antibiotics in poultry production. Prebiotics are feed additives that are non-digestible in the small intestine of the digestive tract of animals and can improve the functions and stimulate the growth of beneficial gastrointestinal (GI) microbes[8]. Prebiotics are made up of short-chain polysaccharides and oligosaccharides and are mostly generated from fermentation products. Even though prebiotics is resistant to gastric acid of the stomach they are digested by microbes in commensal association with the host to produce short-chain fatty acids like propionate, acetate and butyrate[9]. It has been shown that prebiotics can alter the gastro-intestinal microflora, improve immunity, prevent colon cancer, reduce pathogenic invasions such as salmonella enteritidis and *E. coli* as well as reduce cholesterol accumulation[10]. Prebiotics has an antagonistic effect against pathogens by preventing them from binding to the villi. It promotes enzymatic reaction which enhances nutrient utilization, improves performance and reduces production cost[11]. The objective of this study was to determine the effect of prebiotics (chicken protector) on locally hatched broilers in Ghana concerning their immunological and haematological performance.

MATERIALS AND METHODS

Location of experiment: This study was carried out at the Poultry Research Unit of the Department of Animal Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. All experiments were conducted according to the Procedure for Animal Research Ethics Committee (AREC) of the Kwame Nkrumah University of Science and Technology, Kumasi-Ghana, Quality Assurance and Planning Unit (KNUST POLICY 0016 AREC 2018) from February to May, 2021.

Experimental design: A total of fifty Cobb 500 one-day-old broiler chicks were obtained from Topman local hatchery in Ghana. The day-old chicks were randomly assigned to two treatment groups with each group receiving a total of twenty-five birds. The treatments were: T_1: Chicken protector and T_2: No chicken protector. The experiment was arranged in a Completely Randomized Design (CRD).

Housing and management: The day-old chicks were housed in a slatted floor pen for twenty-one days. The pen was raised about 1m from the ground using metallic stands to allow for easy circulation of air. The pen was about 2.5 m high above the metallic stand and was made of wire mesh. The light was provided using two 60 W tungsten bulbs in each division. The light served as the source of heat for the birds. Black plastic sheets were used to cover the side of the structure (pen) to maintain the temperature of the pen. Two plastic drinkers and feeders were provided in each section to enhance the accessibility of birds to water and feed, respectively. Clean water was provided for each group of birds on an ad libitum basis throughout the experimental period. The vaccination schedule recommended by the veterinary services Department in Kumasi was followed throughout the period.

This included the first Gumboro vaccination on the 7th day and *Haemophilus influenzae* B (HB1) vaccine I on day 14th and an intermediate Gumboro vaccination on day 21.

Haematological and immunological parameters: At the beginning of the experiment, the chicks were physically examined and five birds were randomly selected from each of the two treatments. They were euthanized and with the help of a 2 mL syringe and a needle, the blood sample was collected from the jugular vein into separate test tubes that already contained Ethylenediamine tetraacetic acid (EDTA) and another set of test tubes that did not have EDTA. This was repeated on days seven, fourteen and twenty-one. The tubes were kept in a freezer until they were needed for the analysis. The blood samples were analyzed for white and red blood cells count, haemoglobin, packed cell volume and cholesterol.

Yolk sac: After the birds were euthanized and blood samples were taken on the 1st and 7th day, they were dissected from the abdomen and the residual yolk sac of each bird was removed. The yolk sacs were wrapped in an aluminium foil and weighed using a chemical balance. It was then placed in an oven at a temperature of 65°C for 96 hrs after which it was reweighed to determine the dry weight.

Bacteriological parameters: At the end of the 21 days one bird from each treatment were dissected and a portion of the intestine (caecum) was taken for bacterial isolation and identification. Media such as nutrient agar, MacConkey agar and Mannitol salt agar were prepared for the bacteria isolation[12,13].

Statistical analysis: All the data obtained were subjected to Analysis of variance using the Proc GLM procedure of SAS 9.4[14]. The means were separated using Tukey's test at $p \leq 0.05$.

RESULTS

Physical indices of chick quality: Table 1 and 2 show the weekly body weight of chicks, final chick length, shank length and residual yolk sac. The body weights of chicks between the two treatments on day 1 were not different ($p>0.05$). However, chicks subjected to chicken protector (CP) treatment were less heavy compared to control at days 7 (200.56g vs. 219.36 g, $p = 0.0023$) and 21 (895.00 g vrs.975.95 g, $p = 0.0548$). At 14 days the weight of chicks was not different. The chick length and shank length were all not significantly different as measured at day 1 in Table 1.The residual yolk sac weights were also not significantly different between the chicken protector and control treatments at day 1 (4.24 g vs. 4.04, $p = 0.7608$), 7 (2.42 g vs. 3.18 g, $p = 0.1915$), 14 (0.10 g vs. 0.08 g, $p = 0.9055$) and 21 (0.08 g vs. 0.06 g, $p = 0.9120$) in Table 2.

Biochemical indices of chicks: Table 3-6 show the measurements of cholesterol, urea, creatinine, Aspartate transaminase (AST) and Alanine transaminase (ALT) in the blood of broiler chicks over 21 days. On day 1 the blood cholesterol level in the chicken protector group (17.53 mmol L^{-1}) were numerically higher compared to the control (14.47 mmol L^{-1}) but not different ($P = 0.2297$). While not significantly different, the urea was numerically lower

Table 1: Effect of oral administration of chicken protector on body weight and chick quality indicators during brooding over 21 days

	Body weight (g)					
Sources	Day 1	Day 7	Day 14	Day 21	Chick length (mm)	Shank length (mm)
Control	46.15	219.36[a]	503.59	975.95[a]	20.0	3.2
Chicken protector	44.91	200.56[b]	483.08	895.00[b]	19.9	3.2
SEM	0.581	4.063	13.198	28.158	0.163	0.025
p-value	0.1371	0.0023	0.2807	0.0548	0.5445	0.3533

[a-b]Means with different superscripts within the row are significantly different at $p \leq 0.05$

Table 2: Effect of oral administration of chicken protector on the weight of residual yolk sac over 21 days

	Residual yolk sac weight (g)			
Sources	Day 1	Day 7	Day 14	Day 21
Control	4.04	3.18	0.08	0.06
Chicken protector	4.24	2.42	0.10	0.08
SEM	0.430	0.377	0.092	0.069
p-value	0.7508	0.1915	0.9055	0.8120

Table 3: Effect of oral administration of chicken protector on blood serum indicators during brooding at day 1

Sources	Total cholesterol (mmol L^{-1})	Urea (mmol L^{-1})	Creatinine (µmol L^{-1})	AST (µmol L^{-1})	ALT (µmol L^{-1})
Control	14.47	6.00	39.33	218.00	11.33
Chicken protector	17.53	3.70	137.33	253.33	6.67
SEM	1.531	0.638	77.732	11.055	5.270
p-value	0.2297	0.0633	0.4231	0.0867	0.5652

AST: Aspartate transaminase and ALT: Alanine transaminase

Table 4: Effect of oral administration of chicken protector on blood serum indicators during brooding at day 7

Sources	Total cholesterol (mmol L^{-1})	Urea (mmol L^{-1})	Creatinine (µmol L^{-1})	AST (µmol L^{-1})	ALT (µmol L^{-1})
Control	6.20^{a}	0.30^{a}	23.67	211.33	2.67
Chicken protector	4.43^{b}	1.57^{b}	32.33	247.33	4.00
SEM	0.269	0.320	8.715	33.042	0.943
p-value	0.0097	0.0487	0.5207	0.4840	0.3739

AST: Aspartate transaminase, ALT: Alanine transaminase and $^{a\text{-}b}$Means with different superscripts within the roll are significantly different at $p \leq 0.05$

Table 5: Effect of oral administration of chicken protector on blood serum indicators during brooding at day 14

Sources	Total cholesterol (mmol L^{-1})	Urea (mmol L^{-1})	Creatinine (µmol L^{-1})	AST (µmol L^{-1})	ALT (µmol L^{-1})
Control	3.97^{a}	0.90	35.00	324.00	4.00
Chicken protector	2.10^{b}	0.70	38.00	464.67	12.00
SEM	0.243	0.334	3.979	65.967	2.582
p-value	0.0055	0.6939	0.6222	0.2061	0.0936

AST: Aspartate transaminase, ALT: Alanine transaminase and $^{a\text{-}b}$Means with different superscripts within the roll are significantly different at $p \leq 0.05$

Table 6: Effect of oral administration of chicken protector on blood serum indicators during brooding at day 21

Sources	Total cholesterol (mmol L^{-1})	Urea (mmol L^{-1})	Creatinine (µmol L^{-1})	AST (µmol L^{-1})	ALT (µmol L^{-1})
Control	2.80	0.75	33.50	335.00	4.00
Chicken protector	2.80	0.35	29.50	253.00	3.00
SEM	0.204	0.185	3.926	38.28	0.913
p-value	1.00	0.2007	0.5112	0.2044	0.4818

AST: Aspartate transaminase and ALT: Alanine transaminase

on days 1 (3.70 mmol L^{-1} vrs. 6.00 mmol L^{-1}). The creatinine (137.33 µmol L^{-1} vrs. 39.33 µmol L^{-1}), aspartate transaminase (AST) (253.33 µmol L^{-1} vrs. 218 µmol L^{-1}) and alanine transaminase (ALT) (6.67 µmol L^{-1} vrs. 11.33 µmol L^{-1}) levels were not significantly different on day 1 between chicken protector and control groups (Table 3).

There was a significant reduction in blood cholesterol level at day 7 (4.43 vs. 6.20 mmol L^{-1}, p = 0.0097). However, there was a significant increase in urea for the chicken protector on day 7 compared to control (1.57 vs. 0.30 mmol L^{-1}). The creatinine (32.33 vs. 23.67 µmol L^{-1}), AST (247.33 vs. 211.33 µmol L^{-1}) and ALT (4 vs. 2.67 µmol L^{-1}) were not significantly different between the chicken protector and control treatments in Table 4.

On day 14 the cholesterol was again lower in chicken protector chicks compared to control (2.10 vs. 3.97 mmol L^{-1}) but the urea (0.7 vs. 0.9 mmol L^{-1}), creatinine (38 vs. 35 µmol L^{-1}), AST (464.67 vs. 324 µmol L) and ALT (12 vs. 4 µmol L^{-1}) were all not different between chicken protector and control treatments in Table 5. On day 21 the cholesterol level in the blood (2.80 vs. 2.80 mmol L^{-1}), the urea (0.35 mmol L^{-1} vs. 0.75 mmol L^{-1}), (creatinine (29.5 vs. 33.5 µmol L^{-1}), AST (253 vs. 335 µmol L^{-1}) and ALT (3 vs. 4 µmol L^{-1}) were not different between the chicken protector and control groups in Table 6.

Haematological indices: On day 1 and day 21 the red blood cell count was numerically higher in the control group than the chicken protector in Table 7. The red blood cell count was significantly higher in the chicken protector group (200.90×10^3 µL^{-1}) compared to the control (198.00×10^3 µL^{-1}) at day 7. The white blood cell count was also numerically higher in the control group on day 1 and day 21, however, a significant increase was observed in the chicken protector (1.62×10^3 µL^{-1}) compared to the control (1.41×10^3 µL^{-1}) on day 7 ($p<0.05$). The haemoglobin count was not significantly different ($p>0.05$) on days 1, 7, 14 and 21 even though a numerical increase was observed for chicken protector on days 7 and 14. The week by the week number of neutrophils were all not significant between the treatment except for day 7 where a significant increase was seen in the chicken protector (200.9) compare to control (198) (p = 0.0196). There was significant increase in mean platelet volume and platelet large cell ratio at day 14 (p = 0.0238 and p = 0.0365) and day 21 (p = 0.0523 and p = 0.0120) for the chicken protector compared to control, respectfully. A significant increase in hematocrit and a significant reduction in mean cell volume at day 7 for chicken protector were observed.

Table 7: Effect of oral inclusion of chicken protector on haematological indicators during brooding over 21 days

Days	Treatments	WBC× 10^3 µL^{-1}	RBC× 10^6 µL^{-1}	HGB (g dL^{-1})	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})	PLT× 10^3 µL^{-1}	NEU (%)	NEUT#	RDW-SD (fL)	RDW-CV (%)	MPV (fL)	P-LCR (%)
1															
	Control	191.70	2.06	8.10	32.35	157.60	39.45	27.85	18.50	3.65	6.70	53.35	11.35	8.30	20.20
	CP	184.45	1.82	7.50	29.60	163.50	41.80	28.20	6.00	2.75	5.25	62.90	10.50	8.40	18.25
	SEM	8.593	0.164	0.200	2.226	5.378	3.241	0.215	10.277	0.82	1.802	5.242	1.455	1.523	7.984
	p-value	0.6113	0.4090	0.1679	0.4745	0.5191	0.6592	0.3688	0.4804	0.5189	0.6267	0.3266	0.7196	0.9672	0.8849
7															
	Control	198.00^a	1.41^a	6.90	25.15^a	179.00^a	49.10	27.40	3.00	100	198.00^b	93.00^a	12.05a	8.30	19.90
	CP	200.90^b	1.62^b	7.70	27.75^b	171.30^b	47.55	27.75	4.50	100	200.90^a	67.40^b	9.05^b	8.70	21.20
	SEM	0.292	0.013	0.158	0.304	0.552	0.683	0.302	0.791	0	0.292	2.277	0.403	0.781	3.189
	p-value	0.0196	0.0070	0.0700	0.0263	0.0101	0.2497	0.4987	0.3118	0	0.0196	0.0155	0.0343	0.7519	0.8003
14															
	Control	174.05	1.92	7.90	29.10	151.9	41.05	27.05	2.50	3.85	6.80	47.50	12.50	7.90^a	17.00^a
	CP	189.45	2.21	8.89	32.10	146.00	40.00	27.40	0.50	2.60	4.90	41.40	13.65	9.70^b	26.25^b
	SEM	8.123	0.166	0.854	1.981	3.553	2.029	1.175	0.500	0.887	1.851	3.299	0.957	0.200	1.285
	p-value	0.3120	0.3426	0.5340	0.3964	0.3612	0.7495	0.8527	0.1056	0.4423	0.5433	0.3211	0.4851	0.0238	0.0365
21															
	Control	187.05	2.37	9.55	34.30	145.00	40.35	25.00	17.00	100.00	187.05	35.10	12.60	8.00^a	16.55^a
	CP	184.50	2.32	9.45	33.55	144.85	40.80	25.50	5.50	51.65	101.40	37.70	12.50	9.05^b	22.45^b
	SEM	8.940	0.075	0.320	1.381	1.355	0.079	1.421	11.319	34.187	67.674	1.8028	2.140	0.177	0.461
	p-value	0.8588	0.6838	0.8457	0.7379	0.9447	0.0565	0.8268	0.5471	0.4226	0.4653	0.4151	0.9766	0.0523	0.0120

$^{a-b}$Means with different superscripts within the roll are significantly different at $p \leq 0.05$, SEM: Standard error of mean, p-value: Probability value, CP: Chicken protector, RBC: Red blood cells, WBC: White blood cell, HGB: Haemoglobin, HCT: Haematocrits, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, PLT: Platelet test, RDW-SD: Red cell distribution width standard deviation, RDW-CV: Red cell distribution width coefficient of variation, PDW: Platelets distribution width, MPV: Mean platelet volume and P-LCR: Platelet large cell ratio

Bacteriology: At the end of the 21 days *Staphylococcus aureus*, *Salmonella spp* and *Escherichia coli* were isolated in both the chicken protector group as well as the control. The bacterial load for the chicken protector was numerically lower (1.6×10^7 CFU g^{-1}) compared to the control (6.65×10^7 CFU g^{-1}). Similarly, the Enterobacteria count was lower in the chicken protector group (3.3×10^7 CFU g^{-1}) compared to the control (6.75×10^7 CFU g^{-1}).

DISCUSSION

The use of chicken protector (prebiotics and phytobiotics supplement) as a feed additive to supplement broiler feed may be an efficient alternative to the use of antibiotics in broiler production. The results show that the chicken protector was effective in reducing cholesterol levels in the blood. This is a special property of plant-derived prebiotics acting as photobiotic. Plants contain volatile oils as also contained in CP that can inhibit the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase[13]. This is a liver enzyme that regulates the synthesis of cholesterol in the blood. As prebiotics, it may have been efficient in inhibiting the pathway, which resulted in lower cholesterol levels on day 7 and day 14. Ali *et al.*[14] also reported the same result where the inclusion of prebiotics (Saf-mannan) and local Iraqi herbs reduced cholesterol levels in the blood of the broiler. Higher cholesterol level is mostly linked with heart failure as the fat deposit in the blood end up blocking blood vessels. This makes it difficult for blood to flow easily and can lead to the sudden death of a bird.

The supplementation of the chicken protector also had a significant influence by increasing the white blood cells (WBC), red blood cells (RBC) and neutrophils (NEUT) levels in the group given the chicken protector on day 7. This is likely to be a result of the antimicrobial property of the chicken protector and stimulation of the immune system and may result in enhanced immunity of the bird. High levels of red blood cells coupled with high levels of haemoglobin are an indication of higher oxygen circulation in the birds. Haematocrit levels were increased by the inclusion of the chicken protector in this study. However, chicken protector did not significantly increase the levels of AST, ALT and Creatinine, which indicates that the kidney and liver are in good health conditions and are functioning as expected. This result is different from Abdel-Raheem *et al.*[15] who observed a significant increase in AST and ALT enzymatic activities in broilers when supplemented with prebiotics but was in agreement with Das *et al.*[16] who reported no significant difference in AST and ALT for prebiotics inclusion in broiler feed. There was a clear impact on the immunity of the birds by the inclusion of CP in water, which indicates the potential to increase healthy birds without antibiotics in using CP. Janardhana *et al.*[17] also had similar results where the addition of MOS prebiotics in broiler feed improved immunity by enhancing mucin mRNA expression in chicken. This result is also in line with the result obtained by Huang *et al.*[18] when the inclusion of prebiotics in broiler feed improved intestinal immune functions as well as a regulated immune response in the gut-associated lymphoid tissues of chickens.

The study shows variation in the initial body weight but the residual yolk sac was not affected by treatment. While the bodyweight difference experienced within the first 3 weeks is different it may not be a true reflection of the final performance of the birds including final body weight, mortalities and cost of production. It is expected that the CP will result in positive growth performance similar to Ortiz *et al.*[19] who reported that the inclusion of insulin in the feed of broilers did not have a significant effect on body weight.

CONCLUSION

The result from this study shows that chicken protectors can be an alternate for antibiotics in broiler production for increased food safety. Providing chicken protectors in the water of broilers can improve the immunity of the birds against pathogenic invasion by increasing the number of white blood cells in the blood. Again, the inclusion of the chicken protector improved meat quality by reducing the level of cholesterol. Further studies, preferably an on-farm study should be conducted to establish the interaction of the chicken protector with the micro-flora composition and large-scale broiler production.

SIGNIFICANCE STATEMENT

This study discovered that the use of chicken protector (prebiotic and phytobiotics) as a feed additive to supplement broiler feed may be an efficient alternative to the use of antibiotics, especially in the first three weeks of raising broilers. The results show that the chicken protector was effective in reducing blood cholesterol levels, boosting immunity and could be beneficial in healthy meat production in the future. This study will help researchers and industry to uncover the critical areas of broiler meat production by taking advantage of gut health, immune system and enhancing the competitive development of beneficial gut microbial population that is becoming the focus of nutrition in recent research among many researchers.

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