Influence of Garlic, Synbiotic, Organic Acidifier and Synthetic Antibiotics on Blood Parameters of Funaab Alpha Broiler Chickens

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Received: 21 May 2025
Accepted: 3 December 2025

Abstracts

This study evaluated the effects of garlic, synbiotic, organic acidifier, and synthetic antibiotics on hematological and serum biochemical indices of FUNAAB Alpha broiler chickens. Two hundred day-old chicks were randomly assigned to five dietary treatments: control, garlic, synbiotic, organic acidifier, or antibiotics; each with 40 birds in four replicates. At the starter phase, synbiotic and organic acidifier diets significantly increased hemoglobin concentration compared to the control ($P < 0.05$), while garlic and organic acidifier diets raised serum albumin levels. Organic acidifier-fed birds had higher glucose and uric acid concentrations, and garlic reduced total cholesterol while increasing HDL levels compared with the control. In the finisher phase, garlic and antibiotics increased red blood cell counts, garlic elevated albumin, and both garlic and synbiotic reduced LDL concentrations relative to organic acidifier. Across both phases, changes in liver enzyme activities (ALT, AST) remained within normal physiological ranges, indicating no evidence of hepatic stress. These results suggest that garlic, synbiotics, and organic acidifiers can beneficially modulate selected blood and serum biochemical parameters in broilers, offering potential alternatives to synthetic antibiotics for improved health and productivity.

Keywords: antibiotics; garlic; hematology; organic acidifier; synbiotics

Introduction

Broiler chicken production plays a vital role in addressing food insecurity, particularly protein deficiency, in developing countries. Over the past decades, improvements in broiler production and other livestock enterprises have largely relied on synthetic antibiotics as growth promoters. While this approach has helped meet the increasing global demand for poultry products, it has also raised serious public health concerns. The routine use of antibiotic growth promoters contributes to the development of antimicrobial resistance and leads to antibiotic residues in poultry products, posing a significant threat to human and animal health (Rahmatnejad *et al.,* 2009; Van Boeckel *et al.,* 2019). Consequently, there is an urgent need to identify safe and effective alternatives that promote growth and health without the associated risks. Several alternatives to antibiotic growth promoters have been studied, including phytobiotics, prebiotics, probiotics, and synbiotics, with varying degrees of success (Patra *et al.,* 2020; Al-Khalaifah, 2018). Among these, garlic (*Allium sativum*), synbiotics, and organic acidifiers have attracted attention due to their potential to improve gut health, nutrient utilization, and immune function in poultry.

Garlic is widely used as a culinary spice and in traditional medicine. It's antimicrobial, anti-inflammatory, antiparasitic, and immunomodulatory effects are largely attributed to its sulfur-containing compounds, such as allicin and diallyl sulfides (Adibmoradi *et al.,* 2006; Hanieh *et al.,* 2010). In poultry nutrition, garlic supplementation has been associated with improved lipid metabolism, better feed efficiency, and enhanced growth performance (Canogullari *et al.,* 2010; Mansoub, 2011)

Synbiotics (combinations of probiotics and prebiotics) work synergistically to improve gut microbiota balance, suppress pathogenic bacteria, and enhance nutrient absorption (Markowiak and Śliżewska, 2018; Gadde *et al.,* 2017). Probiotics are beneficial live microorganisms that, when administered in adequate amounts, support gut health and immune responses, whereas prebiotics are indigestible carbohydrates that selectively stimulate beneficial gut microbes.

Organic acidifiers, such as citric, lactic, and formic acids, lower gastrointestinal pH, inhibit harmful bacteria, and promote nutrient digestibility (Lückstädt, 2015; Dittoe *et al.,* 2018). Their inclusion in poultry diets has been shown to improve feed conversion and overall performance. In Nigeria, the Federal University of Agriculture, Abeokuta (FUNAAB) developed the Alpha strain of broiler chicken, a locally adapted breed suited to tropical climates. Optimizing its productivity requires effective and sustainable feeding strategies. However, limited information exists on the impact of garlic, synbiotics, and organic acidifiers on the blood and serum biochemical profiles of this strain. Therefore, this study was designed to evaluate the effects of dietary garlic, synbiotics, organic acidifiers, and synthetic antibiotics on the hematological and serum biochemical parameters of FUNAAB Alpha broiler chickens.

Materials and Methods

Experimental Site

The experiment was carried out at the Poultry Unit of the Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria (Latitude 7°13'27"N, Longitude 3°25'29"E, altitude 76 m above sea level). The area has a humid climate, a mean annual rainfall of about 1037 mm, and mean temperature and relative humidity of 34.7 °C and 83%, respectively. The procedures used in this experiment complied with the guidelines of the Research Ethics Committee of FUNAAB (FUNAAB, 2016) under protocol number APH 07-09/23.

Sourcing and Preparation of Test Ingredients

Fresh garlic (*Allium sativum*) was purchased from a local market in Abeokuta, Ogun State. The cloves were separated from each bulb, sorted to remove impurities, minced, and oven-dried at 51 °C until a constant weight was obtained. The dried garlic was then ground into powder and incorporated into the basal diet.

A commercial synbiotic (Innovad Lummance®, Belgium) and organic acidifier (Formycine Gold Px®, Huvepharma, Bulgaria) were sourced through a registered feed miller. The antibiotic (Keproceryl®, KEPRO B.V., Netherlands) was purchased from a licensed veterinary pharmacy. Inclusion levels were based on the manufacturer's recommendations, which are consistent with dosages reported in previous poultry studies for each additive.

Experimental Birds and Management Practices

Two hundred unsexed day-old FUNAAB Alpha broiler chicks were obtained from the university hatchery. Before chick arrival, pens and equipment were cleaned, disinfected, fumigated, and rested for two weeks. Wood shavings were used as litter, and coal pots plus electric bulbs provided heat during brooding. Ambient temperature and humidity were monitored using a thermo-hygrometer.

Upon arrival, chicks were weighed, balanced for body weight across groups, and randomly allocated to five dietary treatments in a Completely Randomized Design (CRD) with four replicates of 10 birds each (40 birds/treatment). The experimental period lasted eight weeks, with standard vaccination schedules followed. Feed and water were offered *ad libitum*.

Dietary Treatments

At day old, the birds were assigned to five treatments with four replicates. There were 40 birds under each treatment; which were further sub-grouped into replicates consisting of 10 birds each, in a Completely Randomized Design. The five treatments consist of: T1 (diet contains no test ingredient/material); T2 (diet mixed with garlic at 90g per 30kg of feed); T3 (diet mixed with synbiotic at 60g per 30kg of feed); T4 (diet mixed with organic acidifier at 45g per 30kg of feed) and T5 (diet mixed with antibiotics at 15g per 30kg of feed).

Table 1: Composition of Experimental Starter and finisher Diet

Ingredients	Starter diet	Finisher diet
Maize	58.00	53.50
Fish Meal	1.6	0.4
Soybean Meal	29.40	20.20
Groundnut cake	6.00	10.35
Bone Meal	2.50	10.80
Oyster Shell	1.5	3.0
Lysine	0.25	0.25
Methionine	0.25	0.25
*Premix	0.25	0.25
Salt	0.25	1.00
Total	**100.00**	**100.00**
Calculated composition		
ME (Kcal/kg)	2,899.95	2,598.34
Crude protein (%)	22.05	20.59
Crude fibre (%)	3.73	3.16
Ether extract (%)	4.02	3.83
Ash (%)	3.12	2.55

*Premix contained: Vitamin A (12,000,000 IU), vitamin D3 (2,500,000 IU), vitamin E (30, 000 IU), vitamin K (2,000mg) vitamin B1 (2,250 mg), vitamin B2 (6,000 mg), vitamin B6 (4,500 mg) vitamin B12 (15 mcg), Niacin (40,000 mg), Pantothenate (15,000 mg), Biotin (50 mcg), Choline chloride (30, 000 mg), Manganese (80,000 mg), Zinc (50,000 mg), Iron (20,000 mg), Copper (5,000 mg), Iodine (1,000 mg), Selenium (200 mg) Cobalt (500 mg), Antioxidant (125,000 mg). Composition/ 2.5kg of broiler vitamin-mineral premix

Data Collection

At 28 and 56 days of age (starter and finisher phases), three average-weight birds per replicate were selected for blood sampling. Approximately 3 mL of blood was collected from the wing vein using sterile syringes.

Hematology: Blood in EDTA tubes was analyzed for packed cell volume (PCV), hemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), and differential leukocytes (lymphocytes, heterophils, eosinophils, basophils, monocytes) using the procedure of Coles (1986) with reference to updated hematological norms in poultry (Bounous and Stedman, 2000).

Serum biochemistry: Blood in plain tubes was centrifuged at 3000 rpm for 10 min to separate serum. Total protein, albumin, glucose, uric acid, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined using commercial kits (Randox Laboratories Ltd., UK; Lot numbers provided upon request) following the manufacturer's protocols. Globulin concentration was obtained by subtracting albumin from total protein. Cholesterol and lipoproteins were determined following Friedewald (1972) and Seigler and Wu (1981), with cross-reference to current laboratory practices.

Statistical Analysis

Data were analyzed using one-way Analysis of Variance (ANOVA) in a CRD with SAS software (Version 9.4, SAS Institute Inc., Cary, NC, USA). When significant treatment effects ($P < 0.05$) were detected, means were separated using Duncan's Multiple Range Test (DMRT). DMRT was chosen because it balances Type I error control with statistical power in multiple pairwise comparisons for balanced designs.

Result and Discussion

Effects of garlic, synbiotics, organic acidifier and antibiotics on heamatological indices of FUNAAB Alpha broiler at starter and finisher phase

At the starter phase, hemoglobin (Hb) concentration was significantly higher in birds fed synbiotics (10.60 g/dL) and organic acidifier (10.48 g/dL) compared to the control (9.15 g/dL), suggesting potential improvements in oxygen-carrying capacity through better nutrient absorption and gut health. WBC counts were significantly lower in the synbiotic group (0.92×10^{12}/L) compared with the antibiotics group (1.78×10^{12}/L). While a lower WBC count may indicate reduced immune activation under non-pathogenic conditions, this remains speculative without immunological markers (e.g., cytokines, acute-phase proteins). Therefore, no definitive claim is made regarding immune stress. Other parameters (PCV, RBC, leukocyte differentials) did not differ significantly ($P > 0.05$) and were within physiological ranges for broilers (PCV: 24–45%; RBC: $2.5–4.0 \times 10^{12}$/L; Bounous and Stedman, 2000).

At the finisher phase, RBC counts were significantly higher in garlic-fed birds (3.67×10^{12}/L) and antibiotic-fed birds (3.67×10^{12}/L) compared to the organic acidifier group (2.44×10^{12}/L).

Table 2: Effects of garlic, synbiotics, organic acidifier and antibiotics on heamatological indices of FUNAAB Alpha broiler at starter phase

Parameters	Control	Garlic	Synbiotic	Organic acidifier	Antibiotics	SEM
Packed cell volume (%)	28.83	27.67	31.17	30.83	28.67	0.54

Haemoglobin (g/dl)	9.15^{b}	9.33^{ab}	10.60^{a}	10.48^{a}	9.83^{ab}	0.21
Red blood cells (x10^{12}/^{L})	3.65	1.99	2.24	2.18	2.06	5.93
White blood cells (x10$^{12/L}$)	1.24^{ab}	1.14^{ab}	0.92^{b}	1.20^{ab}	1.78^{a}	0.11
Heterophil (%)	14.50	16.67	15.33	14.83	20.17	0.93
Lymphocytes (%)	78.83	75.83	75.17	77.67	73.50	1.17
Eosinophil (%)	2.33	3.16	3.83	2.33	2.83	0.34
Basophils (%)	0.83	1.00	0.67	0.67	0.67	0.20
Monocytes (%)	3.50	3.33	5.00	4.50	2.83	0.46

abc:Means with different superscript are significantly different at p<0.05. SEM – Standard Error of Mean

Table 3: Effects of garlic, synbiotics, organic acidifier and antibiotics on heamatological indices of FUNAAB Alpha broiler at finisher phase

Parameters	**Control**	**Garlic**	**Synbiotic**	**Organic acidifier**	**Antibiotics**	**SEM**
Packed cell volume (%)	32.67	33.67	29.33	26.00	31.33	1.12
Haemoglobin (g/dl)	10.90	11.13	9.87	8.87	10.73	0.39
Red blood cells (x10^{12}/^{L})	3.50^{ab}	3.67^{a}	3.32^{ab}	2.44^{b}	3.67^{a}	0.18
White blood cells (x10$^{12/L}$)	1.71	1.45	1.27	1.19	1.47	0.07
Heterophil (%)	10.33	14.33	20.33	16.67	19.67	2.33
Lymphocytes (%)	81.33	79.33	73.00	78.00	71.33	2.41
Eosinophil (%)	3.00	4.00	2.67	3.00	1.67	0.58
Basophils (%)	3.00^{a}	0.00^{b}	0.67^{ab}	0.67^{ab}	1.33^{ab}	0.39
Monocytes (%)	2.00^{ab}	2.33^{ab}	3.33^{a}	1.67^{b}	1.00^{b}	0.62

abc:Means with different superscript are significantly different at p<0.05. SEM – Standard Error of Mean

The enhanced erythropoiesis in garlic-fed birds at this stage may be due to cumulative effects of its bioactive sulfur compounds (e.g., allicin) stimulating red cell production (Khan *et al.,* 2012). This effect could be amplified by higher dietary protein utilization during the finisher phase and potential adaptation to environmental stressors (e.g., heat stress), as antioxidants in garlic may mitigate oxidative damage to erythrocytes. Basophil counts were significantly lower in the garlic group (0.00%) than in the control (3.00%), aligning with garlic's anti-inflammatory properties. Monocytes were highest in the synbiotic group (3.33%), suggesting possible innate immune readiness, though this requires further confirmation with immune biomarkers.

Effects of garlic, synbiotics, organic acidifier and antibiotics on serum biochemical indices of FUNAAB Alpha broiler at starter and finisher phase

At the starter phase albumin was significantly higher in garlic (27.43 g/L) and organic acidifier (26.97 g/L) groups than in the control (25.17 g/L), indicating improved protein synthesis and hepatic function. Serum glucose was highest in the organic acidifier group (5.79 mmol/L), potentially due to enhanced carbohydrate digestibility. Uric acid was highest in the organic acidifier group (0.53 mmol/L) and lowest in the control (0.25 mmol/L). Given that uric acid is the primary nitrogenous waste in birds, elevated levels here may indicate higher protein turnover, possibly linked to better amino acid availability.

Table 4: Effects of garlic, synbiotics, organic acidifier and antibiotics on serum biochemical

Parameters	**Control**	**Garlic**	**Synbiotic**	**Organic acidifier**	**Antibiotics**	**SEM**
Total protein (g/L)	39.53	39.25	39.7	41.65	40.7	0.43
Albumin (g/L)	25.17^{b}	27.43^{a}	26.50^{ab}	26.97^{a}	26.50^{ab}	0.02
Globulin (g/L)	14.36	11.88	13.2	14.68	14.2	0.05
Glucose (mmol/L)	5.51^{bc}	5.47^{c}	5.60^{abc}	5.79^{a}	5.75^{ab}	0.04
AST (U/L)	109.22	83.8	77.92	91.9	104.25	6.83
ALT (U/L)	25.91	31.48	22.85	25.18	29.68	2.01
Uric acid (mmol/L)	0.25^{c}	0.34^{bc}	0.48^{ab}	0.53^{a}	0.32^{c}	0.03
Cholesterol (mmol/L)	4.95^{a}	4.30^{b}	4.63^{ab}	4.61^{ab}	4.65^{ab}	0.06
ALP (U/L)	125.78^{b}	140.07^{ab}	153.72^{a}	153.75^{a}	159.12^{a}	3.66
HDL (mmol/L)	1.00^{b}	1.45^{a}	1.46^{a}	0.88^{b}	1.02^{b}	0.05
LDL (mmol/L)	0.096	0.182	0.194	0.09	0.043	0.04

^{abc}:Means with different superscript are significantly different at p<0.05. HDL- High density lipoprotein; LDL- low density lipoprotein, SEM – Standard Error of Mean

Garlic supplementation significantly reduced cholesterol (4.30 mmol/L) compared to the control (4.95 mmol/L), while HDL was significantly higher in garlic (1.45 mmol/L) and synbiotic (1.46 mmol/L) groups compared to the control (1.00 mmol/L). ALP was elevated in synbiotic, organic acidifier, and antibiotics groups, possibly reflecting increased bone growth and mineral metabolism during the early growth phase.

Table 5: Effects of garlic, synbiotics, organic acidifier and antibiotics on serum biochemical indices of FUNAAB Alpha broiler at finisher phase

Parameters	**Control**	**Garlic**	**Synbiotic**	**Organic acidifier**	**Antibiotics**	**SEM**
Total protein (g/L)	53.37	58.6	56.3	50.77	51.73	1.55
Albumin (g/L)	32.97^{ab}	35.70^{a}	33.23^{ab}	31.70^{b}	33.06^{ab}	0.06
Globulin (g/L)	20.4	22.9	23.07	19.07	18.67	1.29

Glucose (mmol/L)	9.38	6.93	7.13	6.84	8.09	0.43
AST (U/L)	152.3	156.9	157.63	151.6	163.4	3.2
ALT (U/L)	35.67[a]	28.60[ab]	28.57[ab]	25.00[b]	36.47[a]	1.66
Uric acid (mmol/L)	0.36[b]	0.39[b]	0.62[a]	0.47[ab]	0.41[b]	0.03
Cholesterol (mmol/L)	4.7	3.67	3.31	3.25	3.9	0.26
ALP (U/L)	241.43	213.77	214.13	207.87	235.47	5.48
HDL (mmol/L)	1.22	1.0	0.97	0.94	1.16	0.07
LDL (mmol/L)	0.38[ab]	0.28[b]	0.33[b]	0.48[a]	0.38[ab]	0.03

[abc]:Means with different superscript are significantly different at $p<0.05$. HDL- High density lipoprotein; LDL-low density lipoprotein, SEM – Standard Error of Mean

At the finisher phase albumin was significantly higher in garlic-fed birds (35.70 g/L) compared to organic acidifier-fed birds (31.70 g/L), suggesting improved hepatic protein synthesis. ALT activity was significantly lower in the organic acidifier group (25.00 U/L) than in the control (35.67 U/L) and antibiotics group (36.47 U/L). Considering that normal ALT activity in broilers typically ranges from 22–40 U/L (Coles, 1986; Mirfard et al., 2018), all values in this study fall within healthy limits, indicating no hepatic damage. Uric acid was highest in the synbiotic group (0.62 mmol/L) and lowest in control (0.36 mmol/L) and garlic (0.39 mmol/L) groups, consistent with increased protein metabolism in synbiotic-fed birds. LDL cholesterol was significantly lower in garlic (0.28 mmol/L) and synbiotic (0.33 mmol/L) groups compared to organic acidifier (0.48 mmol/L), supporting the cholesterol-lowering effects of these additives.

Conclusions

Garlic, synbiotics, and organic acidifiers improved key hematological and serum biochemical indices in FUNAAB Alpha broilers without causing hepatic stress. Synbiotics and organic acidifiers enhanced hemoglobin at the starter phase, while garlic improved red blood cell counts and lipid profile at the finisher phase. Hence, garlic powder (90 g/30 kg feed) in the finisher phase and synbiotics (60 g/30 kg) or organic acidifiers (45 g/30 kg) in the starter phase as effective alternatives to in-feed antibiotics for promoting broiler health and productivity.

Conflict of interest

The authors declare that there are no conflicts of interest.

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