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STRAIN EFFECT ON HEMATOLOGICAL INDICES OF BROILER CHICKS FED GRADED LEVELS OF *PHYLLANTHUS AMARUS* LEAF EXTRACT

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ABSTRACT

This study examined how varying quantities of *Phyllanthus amarus* leaf extract (PALE) affect the hematological indicators of three broiler chicken strains: Arbor acres, Marshall, and Ross 308. The trial included 200 chicks which were randomly distributed into four treatment groups and five replicates. The experiment aimed to determine the effects of four levels (0, 15, 30, and 45mL/2L of water) of PALE on the hematological indices of three broiler strains using an appropriate design. The hematological indices studied include red blood cell (RBC) count, hemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), platelet count (PCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH). Blood samples were collected from the birds via the right-wing vein using a sterilized syringe. Determinations of the hematological indices were done using standard methods. The results showed that the inclusion of PALE significantly influenced all the hematological indices in the study. There were considerable increases in the RBC, PCV, and Hb levels of the birds maintained with 30mL and 45mL PALE. Therefore, PALE is a viable natural alternative to synthetic antibiotics in broiler production and may be administered as much as 45mL without deleterious effects on the hematological indices of Arbor acres, Marshall, and Ross 308 strains.

Keywords: Antibiotic alternative, Broiler chicken, Hematological indices, Immune system enhancement, *Phyllanthus amarus*, and strains

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1. INTRODUCTION

The livestock industry is the primary source of animal protein and plays an immense and undeniable part in the generation of revenue most nations (Herrero et al. 2013). Livestock production is a major source of socioeconomic transformation in Nigeria, helping to raise incomes and improve the quality of rural life. Poultry, a significant component of livestock production, outnumbers all other forms of livestock produced in Nigeria and it is widely distributed across the country. In recent years, poultry production has evolved from a small-scale backyard activity into a commercially driven industry (Chaiban et al. 2020). Poultry farming in Nigeria is highly profitable and has the potential for rapid returns. This is due to the rapid maturation of chicks. Thus, this sector is expanding rapidly as more consumers are turning away from imported frozen poultry products, which are frequently considered unhealthy (Chibanda et al. 2022). This in turn has led to the increased demand for locally produced poultry products such as chicken, turkey and eggs. However, disease is a critical factor affecting chicken production because it disturbs health and impedes growth and output (Magnoli et al. 2024; Nouri et al. 2024). Salmonellosis is one of these diseases, which is known to cause early death in young birds and also reduce egg production in laying hens.

According to Hafez and Attia (2020) the high cost of feed ingredients, treatments and vaccination, combined with disease outbreaks and a lack of knowledge about their use, are severe constraints for poultry production, especially in the tropics. However, synthetic antibiotics can cause resistance and are increasingly expensive for poultry breeders. The unfavorable characteristics are of great concern to farmers and others considering poultry farming because they can result in significant economic losses due to antibiotic abuse and the development of bacterial resistance (Adebisi et al. 2021; Ade et al. 2024). This demand has led to the search for antibiotic alternatives in broiler management.

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Synthetic antibiotics, while widely used in cattle husbandry, are getting increasingly expensive and can lead to antibiotic resistance. This has spurred researchers to look into plant-based alternatives, which appear to be a potential answer for preventing and treating diseases in livestock. Medicinal plants endowed with biologically active components, which include vitamins, saponins, flavonoids, and phenolic acids inclusive, have been proven to improve poultry health and reduce microbial resistance. As research in this field progresses, medicinal plants like *Moringa oleifera, Syzygium aromaticum, Curcuma longa,* and *P.amarus* are emerging as a potential alternative to antibiotics in animal production, encouraging organic farming and lowering costs while improving poultry health (Khan et al. 2012; Ologhobo and Adejumo 2015; Mahfuz and Piao 2019; Adebisi et al. 2021; Suliman et al. 2021; Abubakar et al. 2023; Akinlade et al. 2024; Osho et al. 2024). According to these researchers, the complementary effects of these unconventional antibiotics were due to their pronounced effect on gut biomass, thereby enhancing feed digestibility cum feed utilization and stimulating the body's immune system.

Owing to the rising cost of synthetic drugs and vaccines and the risks and unavailability of these drugs to small-scale farmers, particularly those in rural areas, Kiambi et al. (2021) advocated for cheaper unconventional feed additives for a sustainable local poultry disease control program. This then necessitates looking inward for alternative antibiotics in leaves and other plant parts that could be included as additives. Hence, this study aims to determine the efficacy of PALE as an unconventional antibiotic in poultry by evaluating its effect on the hematological indices of three strains of broiler birds.

2. MATERIALS AND METHODS

2.1. Study Area

The study was performed at the Poultry Unit of the Teaching and Research Farm Department of Animal Science and Technology, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra State. The location lies between latitude 6.24˚N and 6.28˚N and longitude 7.00˚E and 7.08˚E in the Southeastern part of Nigeria. The climatic features are that of the tropical dry and wet type with a clear season. The average daily maximum temperature is usually 27˚C for a year and can be 34°C in March, having the least during the harmattan month of December- January (Ezenwenyi et al. 2020).

2.2. Preparation Protocol of the Experimental Units

The experimental pen was exceedingly washed and sanitized. All the drinkers and feeders were properly washed and dried before use. The poultry house was allowed to stand unoccupied for one week after cleaning. This was to break the life cycle of any disease-causing organisms that were not killed by the disinfectant. The heating apparatus was engaged for two hours before the arrival of the day-old chicks. Fresh wood shavings were spread on the floor as bedding for the chicks to keep them warm.

2.3. Management of Experimental Animal

The experimental animals were obtained from AGRITED hatchery Lagos-Ibadan expressway, Oyo State, through their major agent, and they were transported at extraordinary hours of the day to minimize transportation stress. Some days before the arrival of the birds, the poultry house was thoroughly scrubbed with water, and a solution of water and disinfectant was used to spray the poultry house, which the house was left for seven days to dry off. Also, feeding troughs and drinkers were thoroughly washed with detergent in water and were allowed to dry under the sun. The chicks, on arrival, were unboxed and placed in the brooding unit; the chicks were given clean water and anti-stress (glucose in conjunction with vitamins) to enable them to recover from stress they might have incurred during transportation from the hatchery to the site of the experiment. During the first two weeks, the birds were brooded in a section of the house, then released to full-house finishing; meal-time feeding was initiated and continued throughout the rest of the growth of the chicks. Drinkers were washed daily, and freshwater was given with treatment material. Fecal droppings mixed with wood shavings were removed from feeding troughs before fresh feeds were apportioned. Litters were changed as when due.

2.4. Experimental Design

Two hundred (200) broiler chicks of different strains (Abor acer, Marshall, and Ross 308) were used for the experiment and randomly distributed into four treatment groups of 50 birds per group (Strain). Each group had five replicates, with 10 chicks per replicate. A randomized complete block design was used where chicken strain served as a block, and the graded levels of PALE dissolved in water became the treatment. In treatment one (P1), birds were not subjected to P. amarus aqueous extract treatment (0mL), a control experiment. Birds in treatments 2, 3, and 4 (P2, P3, and P4, respectively) were subjected to aqueous *P. amarus* extract treatment at concentrations of 15, 30, and 45mL/2L of water. The broiler chicks were housed in deep-litter pens.

2.5. Collection and Preparation of Experimental Material

Fresh *P. amarus* were collected every morning or evening from the Awka metropolis, where they are grown for human consumption. Fresh whole *P. amarus* plant was harvested, sorted to remove unwanted parts, and washed several times to remove dirt. 200g of the thoroughly washed *P. amarus* were weighed using electronic balance MT 5000D and milled with clean 600mL distilled water in an electric blending machine Power Deluxe electric blender Model PDB-823I-T. The blended *P. amarus* leaves were filtered with a fine filtration sieve. During the filtration process, an additional 750mL of water was added with the aid of a measuring cylinder to ensure that all the extracts were removed, making a total of 1350mL of aqueous filtrate, and the different treatment percentages were poured accordingly into P2, P3, and P4. The drinkers containing aqueous *P. amarus* liquid filtrate were placed in the pens of the replicates apportioned to the treatment pen and kept in the pens for the birds to access as the only source of drinking water.

The proximate composition of *P. amarus*, as reported in the literature, includes 10.5-11.50% moisture, 6.30-7.00% ash, 5.80-6.21% fat, 16.60-16.80% CP, 24.00-25.00% fiber, and 45.00-46.00% carbohydrates. This experiment used commercial feed (Chikum feed). The experimental birds had access to feed and water without restriction throughout the brooding period, and light was also provided for at least 18 hours.

2.6. Broiler Starter Diet

The starter diet contains 22% crude protein and an energy content of 2,900 Kcal/kg. The chicks were fed the stipulated diets from day-old to four weeks of age.

2.7. Broiler Finisher Diets

The diet contains 18% Crude Protein and an Energy content of 3000kcal/kg. This ration was fed to the birds from 28 days of age until the end of the experiment. An adequate number of drinkers of the correct sizes (3L drinkers) was provided. The drinkers were washed daily and served fresh, cool water with the different inclusion levels of the treatment daily throughout the experiment. The drinkers were placed on raised platforms to minimize water wastage and contamination.

2.8. Medication and Sanitation

On arrival, Vitaconc (vitamin and glucose) was administered as an anti-stress in the drinking water to control transportation stress. Vaccination was given to the chicks on all treatments as they were due. For the birds in treatment P2, P3, and P4, an extract of P. amarus leaf was added to their water as follows: P2 (15mL of filtrate + 2L water), P3 (15mL of filtrate + 2L water) and P4 (45mL of filtrate + 2L water). The chicks were vaccinated during this research against Newcastle and Gumboro disease. The immunization protocol was strictly maintained throughout the experimental period to prevent the birds from common viral diseases, and the vaccines were administered as per the manufacturer's instructions.

Commercial bedding material consisting of wood shavings was used. The litter was changed from the pen bi-weekly. However, wet bedding materials were promptly removed to discourage bacteria outbreaks, and the litter was turned once a week to avoid caking and ammonia buildup. The drinkers were washed and feeders cleaned daily to remove their droppings and wood shavings particles. Also, a foot dip containing disinfectant was used and changed when due throughout the experiment.

2.9. Data Collection

At the end of the study period, the birds were bled using a 2mL disposable syringe, and the needle and the blood collected were placed in an EDTA bottle to prevent coagulation for hematological studies, as described by Unigwe et al. (2022). The samples were cooled at 4^0C using ice packs and transferred to the laboratory for analysis within 12hrs of collection. Parameters analyzed from the sample include WBC, Hb concentration, RBC, and PCV. The PCT was also determined. In addition, the MCV, MCH, and the MCHC were calculated.

2.10. Statistical Analysis

Data collection from this study was statistically handled using a two-way analysis of variance (ANOVA). The SPSS 2021 statistical package, as described by Jalolov (2024), was employed, and the differences between means were differentiated using Duncan's multiple range test at the 5% significance level.

3. RESULTS

3.1. Effect of Chicken Strains on Blood Characteristics

Table 1 shows the hematological indices of three chicken strains (Arbor acres, Marshall, and Ross 308) fed varying levels of PALE. The strain of the chickens significantly influenced ($P<0.05$) the hematological indices of the birds. Arbor acres recorded the highest ($P<0.05$) RBC, WBC, Hb concentration, and PCT compared to the other

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strains. The highest PCV, MCV, MCHC, and MCH values were found in Marshall Strain, while Ross 308 strains recorded the poorest values for most indices.

Table 1: Blood characteristics of various Chicken Strains fed PALE

Parameters	Units	Arbor Acres	Marshall	Ross 308
RBC	$10^6/\mu L$	2.87±0.69a	2.49±0.60b	2.27±0.57c
WBC	$10^3/\mu L$	154.83±8.25a	138.55±8.27c	146.05±8.23b
Hb	g/dL	10.70±1.20a	8.20±0.90c	9.98±1.10b
PCV	%	31.73±1.11b	36.36±1.26a	28.50±1.20c
PCT	$10^3/\mu L$	118.25±7.24a	98.25±7.22b	89.25±7.28c
MCV	fL	145.50±8.20b	148.38±8.24a	143.65±8.25c
MCH	Pg	49.78±1.30b	55.30±1.35a	48.85±1.32c
MCHC	g/dL	34.75±1.08c	37.38±1.30a	36.58±1.28b

Values (Mean+SE) bearing different alphabets in a row differ significantly (P<0.05). White blood cells (WBC), Hemoglobin concentration (Hb), Red blood cell count (RBC), Packed cell volume (PCV), Platelet count (PCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC) and Mean corpuscular hemoglobin (MCH).

3.2. PAL Effect on Blood Characteristics of Chickens

Table 2 presents the effect of PALE on the blood profile of the chicken. Generally, MCV, MCH, and MCHC did not significantly differ (P>0.05), irrespective of the concentration of the administered PALE. Also, when 0mL and 15mL of PALE were added to the water, the birds showed no significant variation (P>0.05) in the WBC, PCV, and PCT of the birds. The same is true for 30mL and 45mL PALE inclusion. The PALE treatment significantly affected all the hematological indices except for MCV and MCHC. There was a marginal increase in WBC, PCV, MCH, and PCT across the treatments due to the administration of PALE.

Table 2: Blood characteristics of Chicken administered graded levels *Phyllanthus amarus* leaf extract

Parameters	Unit	P1 (0.0mL)	P2 (15mL)	P3 (30mL)	P4 (45mL)
WBC	$10^3/\mu L$	133.73±6.25c	132.07±6.23d	156.37±6.27b	163.73±6.29a
Hb	g/dL	128.23±5.73a	132.07±5.23a	156.37±6.03b	163.73±6.23b
PCV	%	25.53±1.01d	26.33±1.03c	28.40±1.08b	30.33±1.07a
MCV	fL	146.77±5.53	144.03±5.43	146.40±5.83	146.17±6.00
MCH	Pg	49.07±3.09c	51.83±3.11b	51.27±3.09b	53.07±3.15a
MCHC	g/dL	37.90±2.08	35.53±2.07	35.07±2.13	36.43±2.09
PCT	$10^3/\mu L$	121.67±5.71a	104.33±5.73b	77.33±5.99c	104.33±5.73b

Values (Mean+SE) bearing different alphabets in a row differ significantly (P<0.05).

4. DISCUSSION

4.1. Effect of Chicken Strains on Blood Characteristics

Hematological indices have been trustworthy health indicators in livestock. The fluctuation should follow a consistent pattern. Different strains of chicken have unique hematological indices and biochemical characteristics. This research showed an observable increase in the three strains of chicken after the administration of PALE. This present study indicated that the RBC and PCT of experimental animals increased across the breeds. Thus, the different breeds significantly affected the RBC and PCT. According to Vallverdú-Coll et al. (2019), WBC plays a vital part in producing and recognizing antibodies and foreign bodies, respectively. WBC in this study ranged from 138.55-154.83(x10^3/mm^3), with Arbor Acres having the highest value (154.83). The PCV readings obtained were slightly higher than the values of 24-33 and 22-35 reported by Onyishi et al. (2017) and Oguntade et al. (2021), respectively, but similar to the value reported by Upah et al. (2024). Marshall Strain reported higher MCV, MCH, and MCHC values of 148.38, 55.30, and 37.38, respectively. The MCV values ranged from 143.65-148.38 cell/$mm^{3,}$ slightly higher than the 92.42-136.80fL reported by Onyishi et al. (2017) for Nigerian domestic chicken. This could be attributed to the variation in strains or environmental factors during bird rearing. The MCH values in this research are far greater than the 39-41 as reported by Kyakma et al. (2022) and Sultan (2023). Any increase in the MCH and MCHC could indicate anemia, as Ojediran et al. (2012) suggested. However, the values reported in this study showed that the experimental birds were not anemic.

4.2. PALE Effect on Blood Characteristics of Chickens

The Hematological indices obtained in this work fall within the literature values (Okafor et al. 2015; Gana et al. 2018; Akintomide et al. 2021). According to Herrero et al. (2013), Okonkwo and Uba (2017), Unigwe et al. (2022)

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and Ezejesi et al. (2023), birds fed with herbal ingredients (such as cloves, ginger, *P. amarus*, and ginger) have higher levels of vital hematological constituents such as WBC, Hb, PCV, MCH, and PCT, indicating improved oxygen-carrying capacity of cells and improved nutrient availability for birds welfare. The PALE supplement may boost hematopoietic activity by decreasing competition between pathogenic stomach micro-flora and the host bird. The MCH and MCHC values observed in this experiment were slightly higher than those reported by Wumnokol et al. (2019), which might result from other environmental discrepancies.

5. CONCLUSION

Following the findings of this result, it is advised to utilize PALE as a natural supplement in chicken farming, particularly at a concentration of 30 or 35mL/2L water. These levels dramatically increase hematological indices, which are important for immunological function, nutrition transport, and overall welfare of broiler chickens. This shows that PALE could be a viable alternative to synthetic antibiotics, lessening the dangers of antibiotic resistance and reducing the overall cost of poultry production. Further research should look into the impact of PALE on long-term broiler performance. The study maintained that PALE is an effective ingredient in poultry feed capable of supporting broilers' health and reducing reliance on synthetic antibodies in commercial poultry farming.

Authors' Contributions

Ezejesi HC, Okonkwo JC, and Nwankwo CA designed the project and analyzed the data statistically. Ike OO, Ekugba CU, and Ezenyilimba BN carried out the fieldwork. Onwumelu IJ, Okonkwo AP, and Ejivade OM were involved in supervising and proofreading the work.

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