

Research Article

Effects of Varying Inclusion Levels of Different Parts of *Costus afer* on the Packed Cell Volume of Broilers

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Abstract

Broilers are very important components of the poultry industry that serve as sources of animal protein and income. A study to determine the effects of varying inclusion levels of different parts of *Costus afer* on the packed cell volume (PCV) of broilers was carried out in the animal house of the Department of Zoology, Nnamdi Azikiwe University, Awka, Anambra state, Nigeria. 150-day-old broiler chicks with mean initial body weight of 35g were randomly distributed into ten dietary treatment groups, A – J, in a 3 x 4 factorial design (3 plant parts of *Costus afer* and 4 inclusion levels). The plant parts were leaf meal, stem meal and leaf + stem meal, while the four inclusion levels were 0%, 1%, 2% and 3%. Each treatment group contained 15 birds, divided into three replicates of five weight-matched birds. The experiment lasted for 10 weeks. The data collected on PCV was entered into a Microsoft Excel spreadsheet (version 2016) and subjected to statistical analysis using the Statistical Package for Social Science (SPSS) software for windows (version 26). Descriptive analysis results were represented as mean $\pm$ standard deviation and the statistical significance difference between the different treatment groups was analyzed using two-way ANOVA. Duncan's multiple range test was used to separate the mean significant differences between the plant parts and inclusion levels. The level of significance was set at $P < 0.05$. The result of the PCV of the broilers showed that there was a statistically significant effect ($P < 0.05$) of plant part ($P = 0.036$) on the mean PCV of the broilers at 3 weeks. Also, there was a statistically significant effect ($P < 0.05$) of the interaction between plant part and inclusion level ($P = 0.019$) on the final mean PCV of the broilers. From the findings of the study, the different inclusion levels of the experimental plant produced different favourable outcomes, thus it is recommended that *Costus afer* should be used as an additive when compounding the feed of broilers.

Keywords

Varying, Inclusion Levels, *Costus afer*, Packed Cell Volume, Broilers

1. Introduction

The Food and Agriculture Organization (FAO) reported that in 2019, global chicken production comprised 35% of total meat production, surpassing pork to be the most-produced meat [21]. Conversely, the demand for poultry meat in Nigeria has been on the rise, with projections indicating a potential increase in consumption by 200% from

2010 to 2020, with the trend expected to continue at a rate of 6-10% annually through 2025 [2]. This is in line with a recent report by United States Department of Agriculture (USDA) that global increase in the demand for chicken meat has been predicted for the decades to come [40]. With the anticipated increase in the world population to about 9 billion people by

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Received: 8 September 2025; **Accepted:** 22 September 2025; **Published:** 18 October 2025



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2050, this implies that there would be scarcity of natural resources and food shortages, especially protein [20].

Over the years, growing concerns about the negative impact of antibiotic growth promoters (AGPs) have triggered various research into the use of feed additives in poultry production in order to enhance better performance of birds as well as ensure safety in the food chain. Nutritional strategies that enhance growth performance, feed efficiency, and meat quality in broilers, even under challenging environmental conditions include the use of feed additives like microbial feed additives [13, 24], enzyme additives [41], nanoparticles [6], and phytogetic feed additives [28]. The effects of different feed additives on the physiology of broilers can be determined through blood. [11] reported that feed additives, such as *Costus afer*, significantly improved haematological indices (red blood cell count and packed cell volume) of broilers. Similarly, [32] observed that administration of *Costus afer* leaf extract on broilers increased white blood cell counts, indicating a boosted immune response. *Costus afer* is a tall, monocot, medicinal, unbranched tropical plant that is native to West Africa, and belongs to the Costaceae family [7]. The aim of this study was to determine the effects of varying inclusion levels of different parts of *Costus afer* on the packed cell volume of broilers.

2. Materials and Methods

2.1. Experimental Site

The study was carried out at the animal house of Zoology Department, Nnamdi Azikiwe University, Awka, Anambra state, Nigeria. The study site lies between latitude 6°15'18.06"N and longitude 7°06'41.37"E. Awka is located at latitudes 6°9'19"N 7°07'12"E and stretches 8 kilometers East-West along the Enugu-Onitsha expressway and about 5 kilometers North-South. The area is a humid, Guinea Savannah vegetation, with an average rainfall of 1798.52mm annually between 1977 and 2019 [33].

2.2. Procurement of Experimental Animals

150-day-old broiler chicks were procured from Emmason Agro Farms, along Upper Iwaka, adjacent Eastern Academy Secondary School, Onitsha, Anambra state, Nigeria, and were transported in perforated cartons to the study site.

2.3. Experimental Design

The experimental design utilized for the study was 3 x 4 factorial arrangement, comprising 3 plant parts (leaf, stem, and leaf + stem) of *Costus afer* and 4 inclusion levels (0%, 1%, 2%, and 3%). The day-old broiler chicks were randomly assigned to the ten dietary treatment groups (A – J) in a 3 x 4 factorial arrangement. Each group contained 15 birds, divided into three replicates of five weight-matched birds.

Treatment A was the control containing no additive (0%) in the feed of the birds. Birds in treatments B, C and D were offered graded levels (1%, 2% and 3% respectively) of *Costus afer* leaf meal. Also, birds in treatment E, F and G were given 1%, 2% and 3% of *Costus afer* stem meal respectively. Lastly, birds in treatments H, I and J were given feed containing 1%, 2% and 3% of an equal mixture (1:1) of *Costus afer* leaf and stem meal respectively. Each of these treatment groups were replicated three times.

2.4. Management of Experimental Animals

On arrival to the study site, the chicks were weighed, properly identified with permanent markers, and randomly assigned into sterile brooding boxes. They were then offered 2 litres of distilled water containing two teaspoons of glucose [23] which was provided in the different drinking troughs. Additionally, heat was supplied from medium-sized lanterns that were kept in each of the brooding boxes. At three weeks old, the birds were visually inspected for health, after which they were transferred to a netted cage, with each duplicate cell measuring 60 × 60 × 40 cm [1].

Throughout the period of the study, all the broilers were kept under the same sanitary, management, and environmental conditions. Additionally, their feeding schedule was according to the nutrient recommendation for broiler chickens of the National Research Council (NRC). This consisted of starter mash which was given to the chicks from day old until they were 21 days old, then grower mash which was given to them from 21 days old till they were 42 days old, and lastly, finisher mash which they were given when they were 42 days old until the completion of the study [31].

2.5. Identification of the Experimental Plant

The experimental plant was collected from the bush around the study site and identified using taxonomic keys as *Costus afer* by a plant taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra state, Nigeria. Specimens of the experimental plant were deposited in the herbarium of the same Department and Institution. The voucher number is NAUH-166A.

2.6. Harvesting and Preparation of the Experimental Plant

The experimental plant was harvested from the bush around the study site in the early hours of the morning, after which the leaves were plucked out from the stem, washed separately in a big bowl of distilled water and allowed to drain in different sieves. Later, the leaves and stems of *Costus afer* were chopped into small bits and dried in a room under ambient temperature. Upon drying, the leaves and stems of the experimental plant were ground separately into

powder in a clean dry electric blender (FD-989, 1800W), after which they were stored in separate labelled air-tight containers for future use.

Every week, different diets for the different treatment groups (A – J) were formulated. Firstly, graded levels of the powdered parts (leaf, stem, and leaf + stem) of *Costus afer* were determined using a sensitive weighing scale (ADAM AFP-4,100L), then, the broiler mash was weighed with a commercial weighing scale. The graded levels of the leaf, stem and leaf + stem of *Costus afer* and the appropriate amount of broiler mash were then mixed thoroughly in succession, to obtain treatments B to J, after which they were stored in a cool dry place in separate labelled empty bags of feed. This process was repeated throughout the study.

To compound 5kg (5,000g) of feed for birds in treatment B, E and H, 1% additive (1% leaf meal, 1% stem meal and 0.5% leaf meal + 0.5% stem meal respectively) required 50g of each of the additives, with leaf + stem meal requiring 25g leaf meal and 25g stem meal. Likewise, for 5kg of feed, 2% additive required 100g of additive, while 3% additive required 150g of additive. Every morning, the same quantity of feed was weighed and served to all the birds in the different treatment groups, A - J.

2.7. Determination of Proximate Composition of the Leaf and Stem of *Costus Afer*

The leaf and stem of the experimental plant was analyzed for their proximate composition using the standard methods of Association of Official Analytical Chemists (AOAC) International to determine their moisture content, crude protein, crude ash, crude fat, crude fiber, and total carbohydrate contents. Each analysis was done in duplicates [8].

2.8. Estimation of Packed Cell Volume

The broilers were fasted overnight before collecting blood very early the following morning, to avoid postprandial lipemia, which can interfere with the result of the laboratory analyses [14]. Thereafter, two birds were randomly selected from each of the replicates per treatment group for blood collection. Each of the birds was placed on its side with the ventral surface toward the researcher, while the wing lying uppermost was turned back [17].

The PCV of the broilers was determined using the methods described by [16]. During the venipuncture of each of the birds, three micro-hematocrit capillary tubes were filled to two-thirds length of the hematocrit capillary tubes with blood samples, after which the tube was sealed with a sealant and labelled accordingly. Thereafter, the capillary tubes were placed in a microhematocrit centrifuge and spun at 10,000 revolutions per minute (rpm) for 5 minutes. After centrifugation, the packed cell volume was calculated using a microhematocrit reader, and the results were estimated and recorded in percentages.

2.9. Statistical Analysis

The data collected on PCV was entered into a Microsoft Excel spreadsheet and subjected to statistical analysis using the Statistical Package for Social Science (SPSS) software for windows (version 26). Descriptive analysis results were represented as mean $\pm$ standard deviation and the statistical significance difference between the different treatment groups was analyzed using two-way ANOVA. Duncan's multiple range test was used to separate the mean significant differences between the plant parts and inclusion levels. The level of significance was set at $P < 0.05$.

3. Results

3.1. Proximate Composition of *Costus afer* Leaf and Stem

Table 1 shows that the carbohydrate content of *Costus afer* leaf had the highest value (66.51%), followed by fiber (10.50%), then fat (9%), ash (7.25%), moisture (4.98%), with crude protein having the least constituent. On the other hand, the stem of *Costus afer* had the highest carbohydrate content (45.85%), followed by the fiber content (33.70%), then fat (7.50%), ash (6.25%), crude protein (3.95%), and moisture (2.75%) had the least value.

Table 1. Proximate composition of *Costus afer* leaf and stem.

Parameter	Leaf	Stem
Moisture (%)	4.98	2.75
Crude protein (%)	1.76	3.95
Fat (%)	9.00	7.50
Fiber (%)	10.50	33.70
Ash (%)	7.25	6.25
Carbohydrate (%)	66.51	45.85

3.2. Packed Cell Volume of Broilers Subjected to Varying Inclusion Levels of Different Plant Parts of *Costus afer*

The result of the PCV of broilers subjected to varying inclusion levels of different plant parts of *Costus afer* is presented in Table 2. Broilers administered 2% leaf meal had the highest mean PCV ($36.73 \pm 1.52\%$) at 3 weeks, followed by those administered 0% leaf meal ($36.33 \pm 1.52\%$), then those administered 3% leaf meal ($34.80 \pm 1.52\%$), with the least mean PCV at 3 weeks recorded by broilers administered 1% leaf meal ($33.83 \pm 1.52\%$) (Figure 1). On the other hand, broilers administered 3% stem meal had the highest mean

PCV ($39.60 \pm 1.52\%$) at 3 weeks, followed by those administered 1% stem meal ($39.10 \pm 1.52\%$), then those administered 2% stem meal ($38.50 \pm 1.52\%$), and the least mean PCV at 3 weeks was recorded in broilers administered 0% stem meal ($36.33 \pm 1.52\%$) (Figure 1). Finally, broilers administered 2% *Costus afer* leaf + stem meal had the highest mean PCV ($38.50 \pm 1.52\%$) at 3 weeks, followed by those administered 1% leaf + stem meal ($37.40 \pm 1.52\%$), then those administered 3% leaf + stem meal ($36.43 \pm 1.52\%$), and broilers administered 0% leaf + stem meal had the least mean PCV ($36.33 \pm 1.52\%$) at 3 weeks (Figure 1). Table 1 further shows that there is a statistically significant effect ($P < 0.05$) of plant part ($P = 0.036$) on the mean packed cell volume of the broilers at 3 weeks.

The final PCV of broilers administered various inclusion levels of *Costus afer* leaf, stem and leaf + stem (Table 1) shows that broilers administered 3% leaf meal had the highest final mean PCV ($37.60 \pm 1.34\%$), followed by those administered 0% leaf meal ($35.50 \pm 1.34\%$), then those

administered 2% leaf meal ($34.30 \pm 1.34\%$), with the least final mean PCV recorded by broilers administered 1% leaf meal ($33.37 \pm 1.34\%$) (Figure 2). On the other hand, broilers administered 1% stem meal had the highest final mean PCV ($37.93 \pm 1.34\%$), followed by those administered 0% stem meal ($35.50 \pm 1.34\%$), then those administered 2% stem meal ($34.37 \pm 1.34\%$), and the least final mean PCV of *C. afer* stem meal was recorded in broilers administered 3% stem meal ($31.03 \pm 1.34\%$) (Figure 2). In addition, broilers administered 1% *Costus afer* leaf + stem meal had the highest final mean PCV ($38.17 \pm 1.34\%$), followed by those administered 2% leaf + stem meal ($36.57 \pm 1.34\%$), then those administered 0% leaf + stem meal ($35.50 \pm 1.34\%$), and broilers administered 3% leaf + stem meal had the least final mean PCV ($35.43 \pm 1.34\%$) (Figure 2). On the other hand, there is a statistically significant effect ($P < 0.05$) of the interaction between plant part and inclusion level ($P = 0.019$) on the final mean packed cell volume of the broilers (Table 2).

Table 2. PCV of broilers subjected to varying inclusion levels of different parts of *Costus afer*.

Plant part	Inclusion level	PCV at 3 weeks (%)	Final PCV (%)
Leaf	0%	36.33	35.50
	1%	33.83	33.37
	2%	36.73	34.30
	3%	34.80	37.60
Stem	0%	36.33	35.50
	1%	39.10	37.93
	2%	38.50	34.37
	3%	39.60	31.03
Leaf + Stem	0%	36.33	35.50
	1%	37.40	38.17
	2%	38.50	36.57
	3%	36.43	35.43
SEM		1.52	1.34
Main effect			
Plant part			
Leaf		35.43a	35.19a
Stem		38.38b	34.71a
Leaf + stem		37.17a, b	36.42a
SEM		1.07	0.95
Inclusion level			
0%		36.33a	35.50a
1%		36.78a	36.49a

Plant part	Inclusion level	PCV at 3 weeks (%)	Final PCV (%)
2%		37.91a	35.08a
3%		36.94a	34.69a
SEM		1.24	1.09
Effect (P-value)	df		
Plant part	2	0.036	0.198
Inclusion level	3	0.637	0.408
Plant part x inclusion level	6	0.596	0.019

Means in the same column with different superscripts are different ($P < 0.05$).

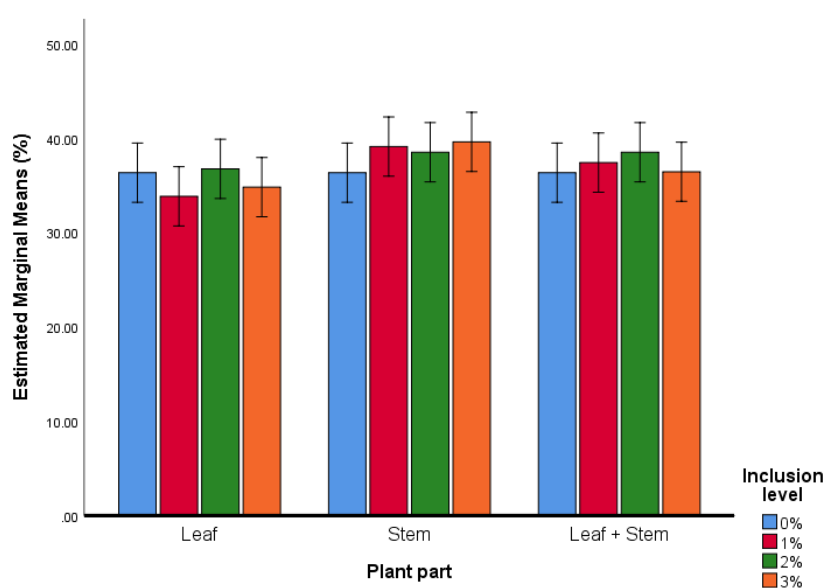


Figure 1. Estimated marginal means of PCV at 3 weeks.

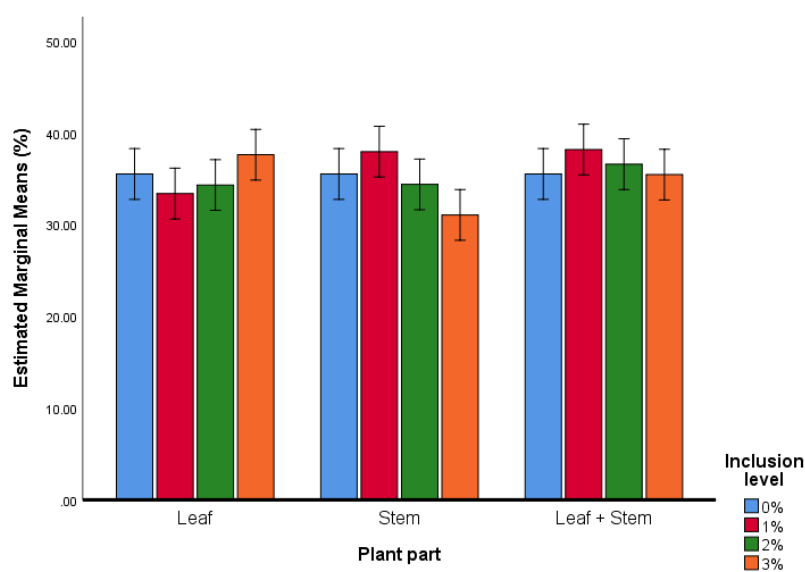


Figure 2. Estimated marginal means of final PCV.

4. Discussion

The observed values of the nutritional profile of the leaf of *Costus afer* differ from those reported by [1, 5]. However, the trend of carbohydrate having the highest value, followed by fiber, is similar. The same trend of carbohydrate and fiber having the highest values in *Costus afer* stem was reported by [2, 4]. The differences in the reported values of proximate composition of the leaf and stem of *Costus afer* could be due to variations in sample collection, extraction and analysis techniques, natural variation, environmental factors (soil, climate and growing conditions), and age/maturity of the plant [3].

The result in Table 2 showed that the type of plant part (leaf, stem, and leaf + stem) significantly ($P = 0.036$) influenced the PCV values of the broilers at 3 weeks. Broilers administered *C. afer* stem meal had the highest PCV (38.38%) at 3 weeks, followed by those administered leaf meal + stem meal (37.17%), and the least PCV was recorded by those administered only leaf meal (35.43%), with a difference that was statistically significant. This trend indicates that *Costus afer* stem may possess bioactive compounds that could stimulate erythropoiesis or enhance oxygen transport capacity at the early stage of administration to broilers [12]. On the other hand, for the final PCV, there was no significant difference ($P = 0.198$) among plant parts. This may imply a potential adaptation or homeostatic regulation over time [34, 36, 43], which may have normalized effects of treatment on the broilers.

Regardless of plant part, increasing inclusion level numerically increased the PCV of the broilers at 3 weeks compared to the control, however, there was no statistically significant effect ($P = 0.637$) of inclusion level on the PCV of the broilers at 3 weeks. On the other hand, broilers administered 1% inclusion of *C. afer* had the highest final PCV values (36.49%), compared to those administered 3% inclusion (34.69%), however, there was no statistically significant effect ($P = 0.408$) of inclusion level on the final PCV of the broilers. The lack of trend in the inclusion levels may indicate that the erythropoietic response to *Costus afer* is not linear and may be influenced more by the type of plant part or by the interaction between plant parts, rather than only inclusion levels. It may also mean that there are some confounding variables [5, 15, 26, 27, 42] that are not accounted for.

Importantly, there was a significant interaction ($P = 0.019$) observed between plant parts and inclusion levels for the final PCV of the broilers. This implies that the effect of *Costus afer* on erythropoiesis is context-dependent. Particularly, broilers administered 3% *Costus afer* stem meal had the lowest final PCV value (31.03%), which could indicate suppressive toxic effects at higher inclusion levels. This aligns with the findings of [28] who reported that the efficacy of phytogenic applications in poultry nutrition depends on several factors, such as feed composition, inclusion level of phytogenic preparations, bird

genetics, and overall diet composition. Conversely, broilers administered 1% *Costus afer* leaf + stem meal recorded the highest final PCV (38.17%). This could suggest a synergistic effect, which may enhance the production of red blood cells when the plant parts are combined at moderate levels. This is in line with the findings of [10] that certain phytochemicals in *Costus afer* can exert haematopoietic effects when they are present at optimal concentrations.

From a physiological perspective, the PCV values observed across all the treatment groups were within the normal range for healthy broilers. Specifically, the range of the initial PCV values of the broilers at 3 weeks old was 33.83% - 39.10% irrespective of the treatments. These values fall within the normal PCV values for 3 weeks old broilers (32-40%) reported by [22]. Similarly, [35] noted that most caged birds have a packed cell volume of 35 to 55 per cent, while [25] reported that the normal range of PCV values for broilers is 27 - 42%. On the other hand, the range of final PCV values of the broilers administered varying inclusion levels of the different parts of the experimental plant at 10 weeks old is 31.03% - 38.17%, which also falls within the ranges of PCV values, 31-41% and 35-55%, documented by [22, 35] respectively. Contradictorily, [9] reported that the normal PCV value for broilers aged 1 to 42 days that are reared under experimental conditions is 27.53%, however, the variation could be as a result of biological (age, sex and genetic) factors [3, 18, 30, 35], environmental factors [3, 4, 19, 23, 35], nutrition [3, 19, 37, 39], or diurnal variations [15]. The differences could also be as a result of the environment in which the experimental plant was grown [29], harvest conditions, and method of processing, as well as storage conditions like temperature, light, oxygen tension, and time [38]. The normal PCV range observed, however, indicates that even at varying inclusion levels and plant parts, *Costus afer* did not induce pathological hematological deviations.

5. Conclusion

This study demonstrated that the values of PCV of broilers across all the treatment groups remained within the normal range for healthy broilers, indicating that *Costus afer*, even at varying inclusion levels and parts, did not induce pathological haematological deviations. However, the observed fluctuations at higher inclusion levels of 3% warrant caution, as it may impair haematological function. Inclusion of *Costus afer* leaf meal, stem meal and leaf + stem meal in the diet of broilers, thus have potential haematopoietic benefits. Further research is recommended.

Abbreviation

PCV

Packed Cell Volume

Author Contributions

Afoemezie Ifeyinwa Philippa: Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft
Ufele-Obiesie Nwogor Angela: Conceptualization, Formal Analysis, Investigation, Methodology, Supervision, Validation, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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