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Effects of Ascleipiadaceae *Secamone afzelii* (Rhoem. and Schult.) aerial parts meal on growth performance and health of broiler chickens

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ABSTRACT: Broiler chicken production in developing countries faces challenges in balancing growth performance, health, and food safety. This study pointed out the effects of *Secamone afzelii* aerial parts meal on broiler growth, and their health. A total of 300 broiler chicks of twenty-one days old were randomly assigned to six dietary treatments containing 0, 0.5, 1, 1.5, 2, or 2.5% *S. afzelii* aerial parts meal for 49 days. Performance parameters, serum lipid profiles, Hematological indices and carcass traits were assessed. Inclusion of 1.5-2.5% *S. afzelii* significantly increased final body weight and average daily gain while improving feed conversion ratio. Birds fed supplemented diets exhibited no morbidity, in contrast to control birds receiving medical treatments. Serum analyses revealed decreased total cholesterol and LDL and elevated HDL levels with increasing *S. afzelii* content. Carcass yield improved and abdominal fat decreased, particularly in diets containing 1.5-2.5% of *S. afzelii* aerial parts meal in supplementation. Moreover, total white blood cell and lymphocyte counts were significantly reduced in birds receiving diets containing 1-2.5% of *S. afzelii* aerial parts meal in supplementation. Overall, *S. afzelii* aerial parts meal is a promising natural feed supplement that promotes broiler growth and health, providing a sustainable alternative to synthetic additives in poultry nutrition.

KEYWORDS: natural additive, poultry, food safety, sustainability, cholesterol.

1 INTRODUCTION

Improving broiler chicken quality and performance remains a major challenge in modern agriculture, particularly in developing countries. Poultry production plays a crucial role in the livestock sector due to its ability to rapidly supply high-quality animal protein to a growing population [1].

Among the most widely raised poultry species, broiler chickens (*Gallus gallus domesticus*) stand out as an essential source of animal protein and food for many societies, especially in sub-Saharan Africa. In Côte d'Ivoire, where poultry farming is a key component of food security and economic development, maximizing the productivity of poultry production systems while maintaining meat quality is essential [2].

However, the pursuit of optimal productivity has led to the increased use of synthetic feed additives, including antibiotics and growth promoters. While effective, this approach raises concerns regarding antimicrobial resistance and chemical residues in poultry products [3].

In this context, the inclusion of natural feed supplements in poultry diets has attracted growing interest. Exploring natural alternatives is therefore necessary to ensure food safety while enhancing the growth performance of poultry. Among these

alternatives, medicinal plants are increasingly recognized for their beneficial effects on the health and growth of livestock [4], [5].

Thus, the use of plant-based dietary supplements represents a promising strategy to improve the zootechnical performance of broiler chickens. These supplements, rich in bioactive compounds, can influence growth, health, and disease resistance in birds. *S. afzelii,* a climbing plant from the Asclepiadaceae family, is widely distributed across Asia and Africa. It is frequently used in traditional medicine to address a range of health issues, such as digestive problems, respiratory infections, gonorrhea, diabetes, kidney disorders, back pain, spinal conditions, catarrh, and reproductive system irregularities [6], [7].

Moreover, in many African indigenous communities, herbs and vegetables, including *S. afzelii* leaves, are incorporated into the diets of pregnant and lactating women to support well-being, prevent anemia, and enhance milk production [8]. The plant exhibits antioxidant [9], [10], antimicrobial [11], insecticidal [12], and anti-inflammatory activities [13]. Studies have shown that *S. afzelii* contains phenols, alkaloids, coumarins, tannins, cardiac glycosides, and saponins [14], [6], [7] and exhibit liver-protective effects [15].

While research has indicated that this plant species holds potential as a source of bioactive compounds for the food industry, especially in creating new functional foods or dietary supplements, its application in broiler chicken nutrition has been explored in only a limited number of studies.

The aim of this study was to evaluate the impact of dietary supplementation with *S. afzelii* aerial parts meal on the growth performance, serum lipid profile and carcass yield of broiler chickens.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL AREA AND ETHICAL STATEMENT

The study was conducted from March to May 2024 at the experimental farm of the National Polytechnic Institute Félix Houphouët-Boigny (INP-HB) in Yamoussoukro, Côte d'Ivoire, in collaboration with the French National Institute for Agriculture, Food, and Environment (INRAE). Animals were raised and slaughtered in accordance with regulations on the care and use of animals in research, following European Directive 86/609/EEC (National authorization for experiments on alive animals No. 3502 issued by the French Ministry of Agriculture). Birds were managed according to Council Directive 1999/74/EC and were weighed only within the scope of this study.

2.2 PRODUCTION OF S. AFZELII AERIAL PARTS MEAL

The aerial parts of *S. afzelii* were harvested within the INP-HB perimeter in January 2024. The harvested forage was dried using a ventilated oven set at 55°C until a constant weight was reached. Once dried, the forage underwent an initial coarse grinding using a plant grinder. The coarse powder was then further milled using a blender to obtain a finer, flour-like texture suitable for incorporation into pelleted broiler chicken diets.

2.3 ANIMALS AND EXPERIMENTAL DESIGN

A total of 300 broiler chicks of twenty-one days old were divided into thirty homogeneous batches of ten birds and randomly allocated into thirty pens of 4 $m^{2,}$ on deep wood shavings bedding. Six groups, each consisting of five batches, were randomly formed. Each group was randomly assigned to receive either a control diet or diets supplemented with 0.5, 1, 1.5, 2, or 2.5% of *S. afzelii* aerial parts meal (five replicates per diet) for a period of forty-nine days. Birds were fed and watered *ad libitum*. All diets were formulated to be iso-nitrogenous and iso-caloric to meet the nutritional requirements of the birds and were prepared in the Animal Science Laboratory of INP-HB, Yamoussoukro, Côte d'Ivoire. The ingredients and composition of the broiler chicken diets are presented in Table 1.

Table 1. Ingredients and composition of S. afzelii aerial parts and diets used in broiler chicken

Item	*S. afzelii*	Diets					
		T_0	$T_{0.5}$	T_1	$T_{1.5}$	T_2	$T_{2.5}$
Ingredients (g/kg)							
Corn	-	660	656.7	653.40	650.1	646.8	643.5
Soybean meal	-	300	298.5	297	295.5	294	292.5
S. Afzelii	1000	0	5	10	15	20	25
Premix	-	40	39.8	39.6	39.4	39.2	39
Total	1000	1000	1000	1000	1000	1000	1000
Proximate composition amalyses[1]							
DM (% CM)	92.11	89.33	89.45	89.5	89.51	90.03	89.51
Ash (% DM)	12.50	2.90	2.95	3.00	3.04	3.09	3.14
Fiber (% DM)	28.91	3.64	3.76	3.89	4.01	4.14	4.27
Protein (% DM)	15.75	18.30	18.30	18.29	18.28	18.27	18.26
Fat (% DM)	6.35	5.56	5.56	5.56	5.57	5.57	5.58
Calculated AMEn (MJ/kg DM)[2]	-	13.55	13.55	13.54	13.54	13.53	13.53
Phenolic compounds and antioxidant activity[1]							
Tot_P (mg GAE/g DM)	102.80	3.16	3.65	4.15	4.65	5.15	5.65
Tot_F (mg QE/g DM)	123.00	1.54	2.15	2.76	3.36	3.97	4.58
Tot_T (mg TAE/g DM)	473.51	0.58	2.94	5.31	7.67	10.03	12.40
Cond_T (mg CE/g DM)	84.78	0.05	0.47	0.90	1.32	1.74	2.17
Anti_A (µmol TE/g DM)	2.28	8.22	8.19	8.16	8.13	8.10	8.07
Fatty acid profile (in % of total fatty acids)[1]							
∑SFA	25.65	24.04	22.48	22.41	24.59	23.77	23.98
∑MUFA	11.70	31.85	32.06	27.87	31.57	31.45	31.28
∑PUFA	62.65	44.11	45.46	45.02	43.84	44.78	44.74
∑n-6	14.99	41.62	43.08	42.32	40.74	41.58	41.31
∑n-3	47.66	2.50	2.38	2.70	3.11	3.20	3.43
PUFA/SFA ratio	2.53	2.01	2.19	2.26	1.96	2.07	2.04

DM: Dry matter; CM: Crude matter; ME: Metabolizable energy; Tot_P: Total polyphenols; Tot_F: Total flavonoids; Tot_T: Total tannins; Cond_T: Condensed tannins; Anti_A: Antioxidant activity; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TAE: Tannic acid equivalent; CE: Catechin equivalent; TE: Trolox equivalent; ∑SFA: saturated fatty acids; ∑MUFA: monounsaturated fatty acids; ∑PUFA: polyunsaturated fatty acids; ∑n-6: Sum of omega 6 fatty acids; ∑n-3: Sum of omega 3 fatty acids; PUFA/ SFA ratio = 18: 2n-6 + 18: 3n-3/14: 0 + 16: 0 + 18: 0 [16]. [1] Values are the means of three analyses per sample. [2] Apparent metabolizable energy, corrected for nitrogen, was calculated based on the chemical composition [17]: AMEn (MJ/kg) = 0.1551 × % crude protein + 0.3131 × % crude fat + 0.1669 × % starch + 0.1301% total sugar.

2.4 ANIMAL PERFORMANCE

Health status and mortality were monitored throughout the trial. Body weight of the broiler chickens was recorded at the start and weekly during the experimental period. Feed intake per pen was measured daily throughout the trial. Daily weight gain, daily feed intake, and feed conversion ratio were calculated.

At the end of the trial, blood samples were collected from fasted broiler chickens into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for hematological analysis, and into plain tubes containing a coagulation activator such as factor XII (Hageman factor) for serum analysis. The EDTA tubes were gently mixed by hand to prevent clot formation. The plain tubes were first left in room temperature for 20 minutes to allow clotting. Subsequently, plasma was separated by centrifugation at 2000×g for 15 minutes at 4°C, then frozen at -20°C for serum analysis.

Fasted birds were stunned, slaughtered, scalded in 60°C water, manually plucked, and eviscerated. Internal organs were removed, and abdominal fat was separated from the viscera. Legs were cut off at the hock joint, and the head was removed at the cranio-atlas junction. Carcasses were weighed and then dissected according to the method described by [16]. Different cuts (breast including bone and both pectoral muscles, whole thighs, and wings) and abdominal fat separated from the viscera were weighed.

2.5 CHEMICAL ANALYSES

Extracts obtained from *S. afzelii* were subjected to biochemical analyses aimed at determining their total polyphenol content, total flavonoid content, and antioxidant activities. Quantification of total polyphenols and total flavonoids in *S. afzelii* and diets was performed following the method described by [18] after alcoholic extraction (70% ethanol) and lyophilization, as reported by [7]. Analyses were conducted using an Agilent Technologies 6890 N gas chromatograph (Bios Analytic, Toulouse, France), with gallic acid and rutin as standards to quantify total polyphenols and total flavonoids, respectively.

For antioxidant activity assessment, the radical cation 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{*+}$) was generated [19]. The $ABTS^{*+}$ solution was diluted with methanol to achieve an absorbance of 0.7 ± 0.02 at 734 nm. For measurements, 3.9 mL of the diluted ABTS*+ solution was mixed with 100 µL of extract. After vortexing, the mixture was incubated for 6 minutes in the dark at 30 ± 2°C. The residual $ABTS^{*+}$ radical absorbance was then measured at 734 nm using a UV-visible spectrophotometer (Shimadzu UV-1601 PC, Kyoto, Japan). Antioxidant activity was calculated using Equations (1) and (2).

$$I\ (\%)\ =\ [(AbsControl - AbsSimple)\ /AbsControl]\ x\ 100 \quad (1)$$

Where: I (%) is the percentage of inhibition; $Abs_{Control}$ is the absorbance of diluted $ABTS^{*+}$; Abs_{Sample} is the absorbance of diluted $ABTS^{*+}$ with the extract.

$$Extract\ Concentration\ =\ (I\ (\%)\ x\ DF)\ /4.9901 \quad (2)$$

The number 4.9901 represents the slope of the Trolox standard curve, and DF is the dilution factor.

Results were expressed as micromoles of Trolox equivalent per liter (µmol TE/L) of extract. These values were then converted to micromoles of Trolox equivalent per gram (µmol TE/g) of sample extract by multiplying by a factor of 2.4 [4 mL / (5/3 mg)]. Here, 4 mL corresponds to the final volume of the medium used for analysis, and 5/3 mg represents the sample weight in this solution.

Total tannins in the samples were determined according to the method described by [18]. Briefly, 7.5 mL of distilled water was added to 100 µL of extract to prepare a solution. Then, 0.5 mL of pure Folin-Ciocalteu reagent and 1 mL of 35% sodium carbonate were added. Subsequently, 0.9 mL of distilled water was added to bring the solution to the final volume after reagent addition. The mixture was thoroughly mixed and incubated for 30 minutes in room temperature (25°C). Absorbance was finally measured at 700 nm using a UV-visible spectrophotometer against a blank consisting of distilled water. A tannic acid standard series (0, 1.95, 3.9, 7.81, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL) was used to construct the calibration curve. Total tannin contents were expressed as micrograms of tannic acid equivalents per liter of extract (µg TAE/L) and subsequently converted to milligrams of tannic acid equivalents per gram of dry matter (mg TAE/g extract). Total tannins were calculated using Equation (3).

$$Ce\ (mg\ TAE.g-1)\ =\ Cal/0.6 \quad (3)$$

Where (Ce) is the total tannin content in the sample, and (Cal) is the concentration read on the spectrophotometer.

Condensed tannins were determined using the vanillin-acid method described by [21]. This method is based on the ability of vanillin to react with the condensed tannin units in the presence of acid to form a colored complex measured at 500 nm. Notably, vanillin reactivity involves only the first unit of the polymer. Briefly, 3 mL of 4% methanolic vanillin was added to 50 µL of hydro-alcoholic extract. The mixture was then vortexed and supplemented with 1.5 mL of concentrated hydrochloric acid. The solution was supposed to react in the dark for 15 minutes before measurement. Each assay was performed in triplicate. Absorbance was read at 500 nm against a blank consisting of 4% vanillin in methanol using a spectrophotometer. Catechin was used as the standard, and results were expressed as micrograms of catechin equivalents per milliliter (µg CE/mL). These values were subsequently converted to milligrams of catechin equivalents per gram of dry matter (mg CE/g). Condensed tannin content was calculated using Equation (4).

$$Ce\ (mg\ CE.g-1)\ =\ CL/0.546 \quad (4)$$

Where (Ce) is the condensed tannin content in the sample, and (C_L) is the concentration read on the spectrophotometer.

Diet and *S. afzelii* aerial parts meal samples were analyzed to determine dry matter (DM), ash, and crude protein (CP, N × 6.25) contents in accordance with [22]. Total fiber content in the diets was measured using a Fibertec System 1021 cold extraction unit (Saint André de Cubzac, France). Lipids were extracted from muscle according to the chloroform/methanol method of [23].

Triglycerides, total cholesterol (CHRL), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were quantified in plasma using commercial kits (Giesse Diagnostics SRL, Guidonia Montecelio, Italy) following a colorimetric method. Hematological analysis was performed using a SYSMEX KX 21N hematology analyzer (Zhejiang Xinke Medical Technology Co., Ltd, Zhejiang City, China) at the National Blood Transfusion Center in Yamoussoukro, Côte d'Ivoire. Various hematological parameters were evaluated, including hemoglobin (HGB), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), lymphocytes (LYM), granulocytes (GRAN), and hematocrit (HCT).

Fatty acid (FA) profiles were analyzed for each growth diet and breast muscle sample. FA composition was determined after sample methylation. Fatty acid methyl esters (FAMEs) were prepared using methanol-bromotrimethyl [16] and analyzed on an Agilent Technologies 6890 N gas chromatograph (Bios Analytic, Toulouse, France), with an internal standard (C21: 0, Sigma-Aldrich, Saint-Quentin-Fallavier, France) for FA quantification (g/100 g total FAs). The nutritional quality of lipids was assessed using the polyunsaturated fatty acids (PUFA) /saturated fatty acids (SFA) ratio [16].

2.6 DATA ANALYSIS

The data were analyzed using the ANOVA option of the Generalized Linear Model (GLM) in R software version 4.3.2 (Copyright © 2023 The R Foundation for Statistical Computing; Platform: x86_64-apple-darwin20, 64-bit), with diet as the main effect. The statistical model used was: Yiik = μ + Di + Rij + γijk, where Yijk represents the response variable for each individual replicate, μ was the overall mean, Di was the effect of diet, Rij was the inter-experimental unit error (replicates), and γijk was the intra-experimental unit error. Least Significant Difference (LSD) tests were performed to compare means for diets when the main effects had a significant F-value. Statistical significance was considered at $p < 0.05$, unless otherwise stated.

3 RESULTS

3.1 PROXIMATE COMPOSITION, BIOACTIVE COMPOUNDS OF S. AFZELII AERIAL PARTS MEAL

The protein and lipid contents of *S. afzelii* aerial parts meal were respectively 15.57% and 8.26% of dry matter. Bioactive compounds in the meal included polyphenols (102.80 mg GAE/g of dry matter), flavonoids (123.00 mg QE/g of dry matter), and tannins (473.51 μg TAE/g of dry matter). The antioxidant activity of *S. afzelii* aerial parts meal was 2.28 μmol TE/g of dry matter. Moreover, the meal was rich in polyunsaturated fatty acids (PUFAs), with omega 3 fatty acids accounting for over 47.66% of total fatty acids, resulting in a high omega 3 PUFA content in the supplemented diet (Table 1).

3.2 BROILER PERFORMANCE ACCORDING TO DIETS

Table 2 presents the performance of broilers fed the different diets. Supplementing the T_0 diet with 0.5% and 1% *S. afzelii* meal had no effect on final body weight or average daily gain (ADG). However, supplementation with 1.5%, 2%, and 2.5% *S. afzelii* meal significantly improved both final body weight and ADG ($p = 4.1 \times 10^{-2}$ and 9.60×10^{-3}, respectively). Birds fed diets containing 2% or 2.5% *S. afzelii* meal showed similar final weights and ADG, with mean values of 2386.82 ± 0.01 g and 39.11 ± 0.15 g/day. The heaviest birds (2429.2 g) and highest ADG (39.90 g/day) were observed with the $T_{1.5}$ diet.

Furthermore, supplementation of T_0 with *S. afzelii* meal had no effects on feed intake ($p = 5.6 \times 10^{-1}$), which averaged 98.1 ± 1.23 g/day. Broilers fed diets supplemented with 1.5%, 2%, and 2.5% *S. afzelii* meal had the same feed conversion ratio (FCR) of 2.50, which was lower than that of birds receiving T_0, $T_{0.5}$, and T_1 diets, all with an FCR of 2.60 ($p = 9.0 \times 10^{-3}$).

Birds fed diets supplemented with increasing levels of *S. afzelii* ($T_{0.5}$ to $T_{2.5}$) did not require any medical treatments. In contrast, birds receiving the control diet (T_0) underwent three anticoccidial treatments and three antibiotic treatments when signs of morbidity were observed.

Table 2. Performance of broilers according to diets

Item	Diets						SEM	p-value
	T_0	$T_{0.5}$	T_1	$T_{1.5}$	T_2	$T_{2.5}$		
PI (g)	451.20	461.21	466.43	476	465.61	475.63	14.68	5.56×10^{-1}
PF (g)	2336.42^{c}	2320.81^{c}	2325.62^{c}	2429.23^{a}	2386.81^{b}	2386.82^{b}	75.10	4.10×10^{-2}
FI (g/j)	97.51	99	99.2	98.5	95.9	98.5	2.31	1.52×10^{-1}
ADG (g)	38.51^{c}	38^{c}	37.90^{c}	39.90^{a}	39.21^{b}	39^{b}	0.40	9.60×10^{-3}
FCR	2.60^{a}	2.60^{a}	2.60^{a}	2.50^{b}	2.50^{b}	2.50^{b}	0.08	9.00×10^{-3}
MF (%)	12.20	0	0	0	0	0	-	-

IW: Initial weight; FW: Final weight; ADG: Average daily gain; FI: Feed intake; FCR: Feed to gain ratio; MF: Morbidity frequency; SEM: Standard error of the mean. $^{a–c}$ Means within rows sharing a common superscript do not differ significantly ($p \geq 0.05$).

3.3 Serum Biochemical Indices In Broiler Chickens According To Diets

Serum parameters of birds fed the six diets are presented in Table 3. Serum triglyceride concentrations did not differ significantly among diets ($p = 1.0 \times 10^{-1}$). Broilers fed the control diet (T_0) had the highest total cholesterol and low-density lipoprotein (LDL) levels compared with the other groups, whereas birds receiving the $T_{2.5}$ diet had the lowest concentrations ($p < 2.0 \times 10^{-16}$ for both) (Figure 1). Total cholesterol and LDL levels decreased progressively with increasing supplementation.

Compared with birds fed the T_0 diet, serum high-density lipoprotein (HDL) increased significantly by 19.57%, 39.30%, 61.20%, 69.20%, and 71.09% in birds receiving 0.5%, 1%, 1.5%, 2%, and 2.5% of *S. afzelii* meal, respectively ($p < 2.0 \times 10^{-16}$). In addition, serum LDL/HDL and CHRL/HDL ratios decreased with supplementation levels of 0.5%, 1%, and 1.5%, and then remained stable at 2% and 2.5% supplementation ($p < 2.0 \times 10^{-16}$) (Figure 1).

Table 3. Serum parameters of broiler chickens according to diets

Item (mg/dl)	Diets						SEM	p-value
	T_0	$T_{0.5}$	T_1	$T_{1.5}$	T_2	$T_{2.5}$		
CHRL	313.58^{a}	228.18^{b}	202.56^{c}	181.07^{d}	179.32^{d}	174.01^{e}	2.51	$< 2 \times 10^{-16}$
TRIG	110.10	113.47	111.88	111.51	113.89	113.78	1.54	1.03×10^{-1}
HDL	73.72^{d}	88.15^{c}	102.69^{b}	118.84^{a}	124.74^{a}	126.13^{a}	2.54	$< 2 \times 10^{-16}$
LDL	217.84^{a}	117.33^{b}	77.49^{c}	39.92^{d}	31.80^{de}	25.12^{e}	4.70	$< 2 \times 10^{-16}$
LDL/HDL	2.97^{a}	1.34^{b}	0.76^{c}	0.35^{d}	0.25^{d}	0.20^{d}	0.08	$< 2 \times 10^{-16}$
TC/HDL	4.27^{a}	2.60^{b}	1.97^{c}	1.53^{d}	1.44^{d}	1.38^{d}	0.08	$< 2 \times 10^{-16}$

CHRL: Total cholesterol; TRIG: Triglycerides; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; SEM: Standard error of the mean. $^{a-b}$ Means within rows sharing a common superscript do not differ significantly ($p \geq 0.05$).

Serum total cholesterol concentration was mainly correlated with serum HDL (r = -0.90) and LDL (r = 0.99) levels, as well as with the level of *S. afzelii* meal supplementation in the diet (r = -0.86). Additionally, abdominal fat proportion was strongly correlated with serum HDL (r = -0.92) and LDL (r = 0.91) concentrations, and with the supplementation level of *S. afzelii* meal (r = -0.94). Except for serum triglyceride concentration, both HDL and LDL levels were strongly correlated with the level of dietary *S. afzelii* meal supplementation (r = 0.94 and r = -0.90, respectively). The changes in the different serum parameters measured are clearly illustrated by Figure 1.

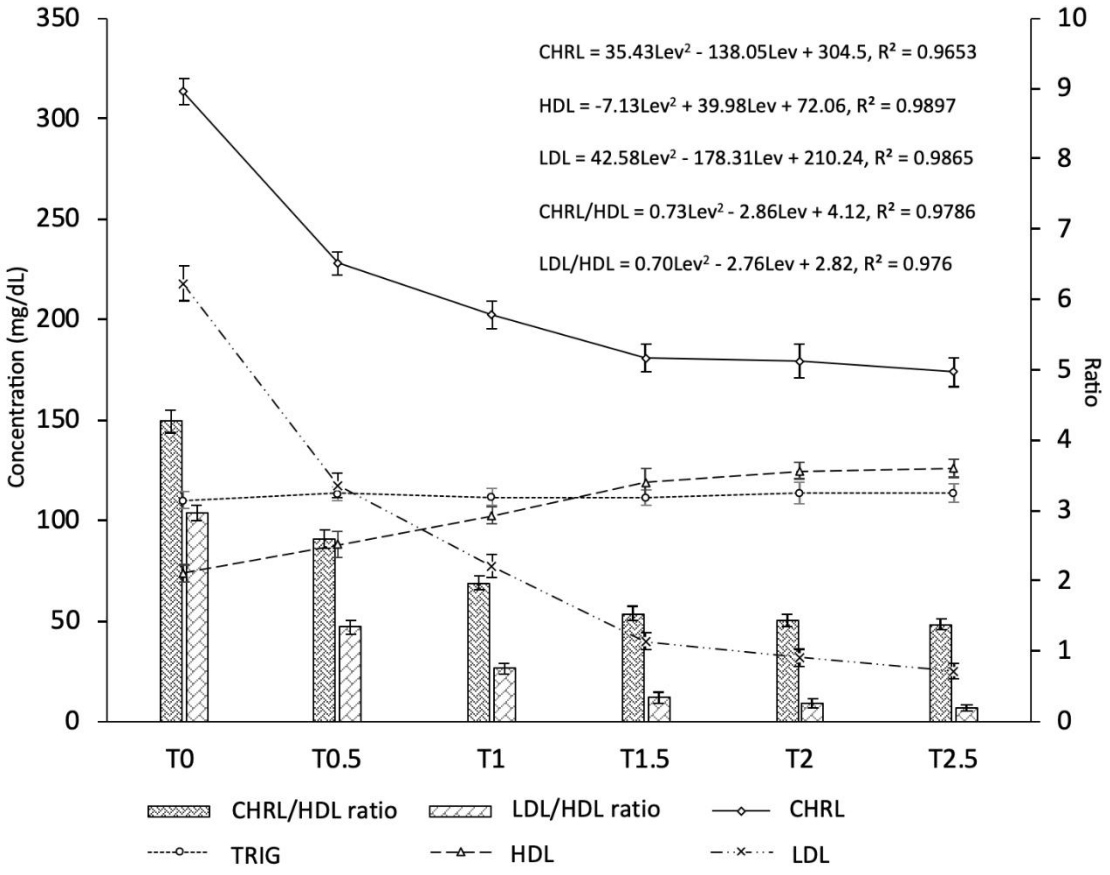

Fig. 1. Changes in serum parameters of broiler chickens according to diets

CHRL: Total cholesterol; TRIG: Triglycerides; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

3.4 HAEMATOLOGICAL INDICES IN BROILER CHICKENS ACCORDING TO DIETS

The hematological profile revealed significant variations among the dietary treatments. Total white blood cell (WBC) and lymphocyte (LYM) counts were significantly reduced in birds receiving diets from T_1 onward compared to T_0 and $T_{0.5}$ ($p = 1.58 \times 10^{-2}$ and $p = 1.36 \times 10^{-2,}$ respectively), indicating a diet-related influence on immune cell dynamics. Similarly, granulocyte (GRAN) values decreased notably at T_1 ($p = 3.16 \times 10^{-2}$), although values remained relatively stable across higher inclusion levels (Table 4).

Red blood cell (RBC) counts showed a progressive decline, with the lowest values recorded in the T_2 and $T_{2.5}$ groups ($p = 4.40 \times 10^{-2}$). Correspondingly, hemoglobin (HGB) concentration and hematocrit (HCT) percentage were significantly reduced with increasing dietary levels, suggesting possible effects on oxygen-carrying capacity ($p = 1.42 \times 10^{-2}$ and $p = 4.55 \times 10^{-2,}$ respectively). In contrast, mean corpuscular volume (MCV) increased significantly from $T_{1.5}$ onwards, reflecting larger red cell size ($p = 3.89 \times 10^{-2}$). However, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were not significantly influenced by the dietary treatments, ($p \geq 0.05$).

Table 4. Hematological indices in broiler chickens according to diets

Item	Diets						SEM	p-value
	T_0	$T_{0.5}$	T_1	$T_{1.5}$	T_2	$T_{2.5}$		
WBC (10^3/µL)	51.41^a	51.04^a	46.19^b	44.94^b	45.12^b	45.06^b	1.17	1.58×10^{-2}
LYM (10^9/L)	45.18^a	45.14^a	41.27^b	39.45^b	40.24^b	40.15^b	1.18	1.36×10^{-2}
GRAN (10^9/L)	5.55^a	5.47^a	4.35^b	4.43^a	4.39^a	4.39^a	0.38	3.16×10^{-2}
RBC (10^6/µL)	2.66^a	2.48^{ab}	2.45^{ab}	2.34^a	2.24^b	2.21^a	0.11	4.40×10^{-2}
HGB (g/dL)	13.20^a	12.60^a	11.70^{ab}	11.37^b	11.23^b	10.70^b	0.53	1.42×10^{-2}
HCT (%)	35.57^a	33.60^a	32.13^{ab}	30.57^b	30.70^b	30.30^b	0.59	4.55×10^{-2}
MCV (fL)	134.10^a	134.03^a	136.23^{ab}	137.73^b	137.43^b	138.97^b	0.59	3.89×10^{-2}
MCH (pg)	49.80	49.70	47.77	49.30	50.20	51.47	1.59	3.89×10^{-1}
MCHC (g/dL)	37.13	37.10	36.37	37.17	36.57	37.03	0.45	3.83×10^{-1}

WBC: White blood cell; GRAN: Granulocyte; LYM: Lymphocyte; RBC: Red blood cell; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscle volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; SEM: Standard error of the mean. $^{a-b}$ Means within rows sharing a common superscript do not differ significantly ($p \geq 0.05$).

3.5 Carcass Traits Of Broiler Chickens According To Diets

Carcass yields, cut-up parts, and abdominal fat values are presented in Table 5. The carcass yield of broilers fed the diet supplemented with 2.5% *S. afzelii* meal was significantly higher than that of the other groups, whose carcass yields did not differ significantly from each other ($p = 2.9 \times 10^{-3}$). Birds receiving the diet containing 2.5% *S. afzelii* meal also showed reduced feather coverage compared with the other groups, which had similar feathering levels ($p = 1.9 \times 10^{-2}$).

Broilers fed the control diet (T_0) exhibited the highest proportions of abdominal fat compared with the other groups, while those receiving *S. afzelii* meal-supplemented diets had lower abdominal fat levels ($p = 3.1 \times 10^{-6}$). Abdominal fat content decreased significantly as the level of *S. afzelii* meal supplementation increased. However, the different diets had no effect on the proportions of the various cut-up parts ($p \geq 0.05$).

Table 5. Carcass yield and cut-up parts of broiler chickens according to diets

Item (%)	Diets						SEM	p-value
	T_0	$T_{0,5}$	T_1	$T_{1.5}$	T_2	$T_{2.5}$		
Carcass yield	73.75^b	73.79^b	74.75^b	74.09^b	73.48^b	76.99^a	0.70	2.91×10^{-3}
Feather	7.31^a	6.82^a	6.96^a	7.58^a	7.95^a	3.69^b	0.98	1.85×10^{-2}
Breast	36.63	36.15	34.77	38.30	38.85	37.15	2.07	4.40×10^{-1}
Legs	31.72	31.38	32.83	29.68	29.81	31.49	1.58	3.80×10^{-1}
Thighs	17.33	17.68	18.19	16.67	16.35	17.58	0.89	3.80×10^{-1}
Wings	11.37	10.59	11.12	11.35	13.38	10.65	1.38	4.10×10^{-1}
Head	3.11	3.11	3.40	3.01	2.96	3.37	0.27	5.00×10^{-1}
Feet	5.01	5.11	5.54	5.26	4.91	4.95	0.65	9.30×10^{-1}
Gizzard	3.19	3.57	3.10	3.14	3.18	3.16	0.32	7.30×10^{-1}
Liver	2.38	2.70	2.42	2.53	2.44	2.32	0.29	8.20×10^{-1}
Heart	0.61	0.55	0.50	0.50	0.50	0.50	0.09	7.30×10^{-1}
Spleen	0.21	0.14	0.10	0.17	0.15	0.13	0.03	6.00×10^{-2}
Abdominal fat	1^a	0.76^{ab}	0.61^{bc}	0.41^{cd}	0.23^d	0.24^d	0.08	3.11×10^{-6}

SEM: Standard error of the mean. $^{a-d}$ Means within rows sharing a common superscript do not differ significantly ($p \geq 0.05$).

4 Discussion

The absence of morbidity in birds fed diets supplemented with *S. afzelii* meal, along with the improved production performance observed at inclusion levels of 1.5, 2, and 2.5%, could be attributed to the presence of *S. afzelii* in the diet. This medicinal plant is known to be rich in total phenolic compounds and flavonoids, with a high accumulation of kaempferol 2-glycosides [7]. Indeed, secondary metabolites found in medicinal plants contribute to controlling parasitic diseases by reducing

stress and alleviating oxidative stress, thereby enhancing nutrient utilization, improving overall health, and boosting productivity [24].

The inclusion of *S. afzelii* dry leaf meal as a dietary supplement in broiler feed significantly reduced the white blood cell count. These findings are consistent with those by [5], who reported a 20.6% decrease in leukocyte numbers when 3% Acacia auriculiformis leaf powder was incorporated into broiler diets. Similarly, the addition of doxycycline (1 g/kg of feed) to broiler rations reduced white blood cell counts from $9.29 \times 10^3/\mu L$ to $6.5 \times 10^3/\mu L$, representing a 30.03% decrease [25]. Such observations suggest that *S. afzelii* may exert antibiotic-like effects and, consequently, act as a phytobiotic, with potential implications for the health and physiological status of the birds. This could explain the absence of morbidity observed in broilers fed diets containing *S. afzelii* dry leaf meal.

The inclusion of herbal medicinal supplements in poultry diets covering broilers, layers, indigenous chickens, quails, ducks, and ornamental birds has been shown to elevate HDL while reducing total cholesterol and LDL levels [26], [24]. A comparable trend was observed in our experiment, where broilers receiving diets enriched with *S. afzelii* meal exhibited increased HDL and decreased total cholesterol and LDL concentrations. This effect may be linked to the elevated levels of n-3 PUFAs present in *S. afzelii* meal and consequently in the supplemented diet. Two mechanisms have been proposed to explain the reduction in plasma LDL and total cholesterol following PUFA intake. The first suggests that enhanced β-oxidation lowers the pool of free fatty acids available for triglyceride formation, thereby limiting triglyceride synthesis. The second points to a suppression of hepatic fatty acid production through reduced activity of lipogenic enzymes. These processes may act concurrently, leading to decreased very-low-density lipoprotein (VLDL) and LDL output, ultimately resulting in lower cholesterol concentrations [16]. However, given the significant reduction in cholesterol, there appears to be synergy between several mechanisms of action of different secondary metabolites [27].

The reduction in abdominal fat observed in this study is consistent with the findings of [28], [29], [30] in poultry fed plant-based supplements. According to these authors, the presence of bioactive compounds in plants may influence lipid metabolism in chickens. Polyphenolic constituents identified in *S. afzelii* including phytol, mequinol, catechol, general phenolics, flavonoids, alkaloids, coumarins, tannins, cardiac glycosides, saponins, and ascorbic acid [7], [31] act in synergy to contribute to its pancreatic lipase inhibitory effect [31]. This inhibitory action is consistent with previous findings showing that phenolic compounds can modulate pancreatic lipase activity and influence lipid digestion and absorption through the formation of complexes [32], [33].

The $T_{2.5}$ diet produced the highest carcass yield (77%), while the other diets resulted in average yields of about 74%, with no significant differences among them. This outcome is consistent with the findings of [34], who reported similar results when incorporating Moringa oleifera into broiler diets in Nigeria. The higher carcass yield observed under $T_{2.5}$ was linked to the reduced feather coverage of the birds, an advantage in tropical regions where ambient temperatures are high. Indeed, previous studies have shown that reduced feathering promotes faster growth and improved meat yield in broilers exposed to elevated temperatures [35], [36], [37]. The use of low-feathered birds has already been reported in Ghana [38], [39] and Nigeria [40], and it is expected to become more widely adopted in hot climates in the future [36]. In contrast, heavily feathered birds tend to drink more water and reduce feed intake under heat stress, since high temperatures increase their water requirements for thermoregulation while suppressing appetite [37].

At the carcass cut-up level, no significant effects were observed on the proportions of the different parts (breast, drumsticks, thighs, wings). This indicates that supplementation with *S. afzelii* up to 2.5% does not alter the carcass composition of broiler chickens in terms of muscle mass distribution. Similar findings were reported by [41], who also found no significant changes in cut-up part proportions in broilers fed diets containing various plant-based additives.

5 CONCLUSION

Incorporating *S. afzelii* aerial parts meal into broiler diets, particularly at 1.5-2.5% inclusion levels, enhanced growth performance, improved feed efficiency, and reduced morbidity. Supplementation positively influenced serum lipid profiles by lowering total cholesterol and LDL while increasing HDL levels. Carcass yields were improved, and abdominal fat deposition decreased without affecting the distribution of muscle cuts. Moreover, *S. afzelii* supplementation enriched the Pectoralis major muscle with n-3 polyunsaturated fatty acids, enhancing meat nutritional quality. These results indicate that *S. afzelii* meal is a safe, natural feed additive that can improve both the productivity and health benefits of broiler meat, supporting sustainable poultry production strategies.

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