



## Dietary supplementation of *Cyperus rotundus* tuber extract enhances growth performance, immune response, and antioxidant status in Broiler chickens

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### Abstract

The search for natural alternatives to antibiotic growth promoters in poultry production remains a critical research objective. This study investigated the effects of dietary supplementation with *Cyperus rotundus* tuber extract (CRTE) on growth performance, immune response, and antioxidant status in broiler chickens. Two hundred one-day-old Ross 308 broiler chicks were randomly assigned to two dietary treatments: a control group fed a basal diet (CON) and a treatment group fed the basal diet supplemented with 0.5% CRTE, with 100 birds per group replicated five times. The experiment lasted 35 days. Growth parameters were recorded weekly. Immune function was assessed via antibody titers against Newcastle Disease Virus (NDV) for humoral immunity and a phytohemagglutinin-P (PHA-P) skin test for cell-mediated immunity. Blood samples were analyzed for hematology, serum biochemistry, and antioxidant markers at trial termination. Results indicated that CRTE supplementation significantly ( $p < 0.05$ ) improved final body weight (2156.3 vs. 2018.7 g) and feed conversion ratio (1.58 vs. 1.67). The treated group exhibited enhanced ( $p < 0.01$ ) humoral immunity, demonstrated by higher NDV antibody titers ( $\text{Log}_2$  6.8 vs. 5.9), and improved ( $p < 0.05$ ) cell-mediated immunity (0.92 vs. 0.71 mm). Furthermore, CRTE birds showed a superior antioxidant profile with higher serum total antioxidant capacity (1.85 vs. 1.52 mM) and lower malondialdehyde levels (1.98 vs. 2.65 nmol/mL). In conclusion, dietary inclusion of 0.5% *Cyperus rotundus* extract improved broiler productivity, enhanced immune competence, and boosted antioxidant defense, presenting a viable natural feed additive for sustainable poultry production.

**Keywords:** *Phytogenic Additive, Nutsedges, Poultry Nutrition, Immunity, Antioxidants, Cyperus Rotundus*

### 1. INTRODUCTION

The prophylactic and growth-promoting use of antibiotics in animal feed, a practice entrenched for decades in intensive livestock production, faces unprecedented global restriction due to the urgent crisis of antimicrobial resistance (AMR). Regulatory bans on antibiotic growth promoters (AGPs) in key markets, driven by public health imperatives, have created a critical vacuum in poultry production systems, necessitating the development of effective, sustainable, and safe alternatives.<sup>1</sup> This challenge has catalyzed intensive research into natural feed additives capable of maintaining animal health, welfare, and productivity. Among the most promising candidates are phytogenic compounds bioactive derivatives from herbs, spices, and medicinal plants which offer a spectrum of biological activities, including antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties.<sup>2,3</sup> Their potential to enhance gut health, nutrient digestibility, and overall resilience aligns with the goals of sustainable, antibiotic-free poultry production.

Within this phytogenic landscape, *Cyperus rotundus* L. (purple nutsedge), often dismissed as a pernicious weed, harbors significant therapeutic potential rooted in centuries of ethnopharmacological use. The

tuberous rhizomes of this plant are a rich repository of bioactive secondary metabolites, including unique sesquiterpenes ( $\alpha$ -cyperone, cyperene), flavonoids, and phenolic acids.<sup>4,5</sup> Contemporary pharmacological investigations have validated numerous activities for *C. rotundus* extracts and essential oils, such as potent anti-inflammatory effects through COX-2 inhibition, significant antimicrobial action against Gram-positive and Gram-negative bacteria, notable free-radical scavenging capacity, and hepatoprotective qualities.<sup>6,7</sup> This multifaceted bioactivity profile positions *C. rotundus* as a compelling candidate for zootechnical application, theoretically capable of addressing multiple stressors in broiler production simultaneously.

Despite its well-characterized phytochemistry and demonstrable bioactivity in biomedical models, the translational application of *C. rotundus* in poultry nutrition remains strikingly underexplored, representing a significant knowledge gap. While a limited number of studies have investigated other herbal extracts in broilers, reporting improvements in growth performance and immune parameters,<sup>8</sup> antioxidant capacities, immune function, and intestinal health in yellow-feathered broilers. PHM is composed of five traditional Chinese medicine herbs (*Portulaca olera-*

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cea L., *Radix Sophora flavescens*, *Thalictrum glandulosissimum*, *Terra flava usta*, and *Pogostemon cablin* species-specific responses are paramount. The existing literature on *C. rotundus* in animal science is sparse and often inconclusive. For instance, some ruminant studies suggest rumen-modulatory potential, while preliminary poultry data is limited to isolated parameters without a holistic, mechanistic evaluation.<sup>4,6,9</sup> No comprehensive study has concurrently evaluated its effects on growth performance, systemic immunity, and antioxidant status in broilers using a standardized extract, leaving its practical efficacy and optimal inclusion level unknown.

Therefore, this study was formulated to bridge this critical gap by providing an integrated assessment of dietary *Cyperus rotundus* tuber extract (CRTE) supplementation in broiler chickens. We hypothesize that the incorporation of CRTE into a standard corn-soybean meal diet will: (1) enhance growth performance by improving feed efficiency and nutrient utilization, (2) potentiate both humoral and cell-mediated branches of the adaptive immune system, and (3) improve systemic antioxidant status, thereby reducing metabolic oxidative stress. To test these hypotheses, we conducted a controlled feeding trial measuring zootechnical parameters, specific antibody responses to Newcastle disease virus and red blood cells, cell-mediated reactivity via a cutaneous hypersensitivity test, and key serum biochemical and antioxidant markers. The findings aim to establish a scientific basis for the use of *C. rotundus* as a novel, multifunctional phyto-genic additive in antibiotic-free broiler production.

## 2. MATERIALS AND METHODS

### 2.1. Plant Extract Preparation

*Cyperus rotundus* tubers were collected, authenticated (Voucher specimen #CR-PS-101), washed, shade-dried, and ground. The powdered material (500 g) was subjected to Soxhlet extraction using 70% aqueous ethanol (2 L) for 24 cycles. The extract was filtered, concentrated under reduced pressure at 45°C using a rotary evaporator (Heidolph, Germany), and lyophilized to obtain a dry powder (yield: 12.8%) according to Ali et al.<sup>10</sup>. The dried extract was subjected to phytochemical analysis to determine its bioactive composition. Total phenolic content was measured using the Folin-Ciocalteu method and found to be 85.3 ± 4.2 mg gallic acid equivalent (GAE)/g extract. Total flavonoid content was determined by aluminum chloride colorimetric assay and found to be 42.7 ± 3.1 mg quercetin equivalent (QE)/g extract. High-performance liquid chromatography (HPLC) analysis confirmed the presence of major bioactive compounds including α-cyperone, quercetin, and kaempferol, consistent with previous reports Perezada et al.<sup>5</sup>. This standardization ensures consistency between batches and allows for meaningful interpretation of biological effects. The powder was stored at -20°C and mixed into the basal diet at 0.5% (5 g/kg) for the treatment group.

### 2.2. Experimental Design, Birds, and Diets

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC #2023-04-PS). Two hundred one-day-old male Ross 308 broiler chicks (initial

weight 45.2 ± 2.1 g) were randomly assigned to two treatment groups:

**Control (CON):** Basal corn-soybean meal diet (n=100, 5 replicates of 20 birds).

**CRTE:** Basal diet + 0.5% *Cyperus rotundus* extract (n=100, 5 replicates of 20 birds).

Diets were formulated to meet Ross 308 nutrient requirements (Starter: 1-14 d, Grower: 15-28 d, Finisher: 29-35 d).<sup>11</sup> Birds were housed in floor pens with wood shavings, with *ad libitum* access to feed and water. Light was provided 23L:1D. Temperature followed standard management guidelines according to Shakouri and Malekzadeh<sup>12</sup>.

### 2.3. Data and Sample Collection

#### 2.3.1. Growth Performance

Body weight (BW) and feed intake (FI) were recorded per replicate weekly. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated. Mortality was recorded daily.

#### 2.3.2. Immune Response Assays

**Humoral Immunity:** Birds were vaccinated against NDV (LaSota strain) on days 10 and 24. Blood was collected on day 28 from 10 birds/group (2/replicate). Serum NDV antibody titers were measured by Hemagglutination Inhibition (HI) test<sup>13</sup> and expressed as Log<sub>2</sub>.

**Cell-Mediated Immunity:** A cutaneous basophil hypersensitivity (CBH) test was performed on day 30. Phytohemagglutinin-P (PHA-P, 100 µg in 0.1 mL PBS) was injected intradermally into the wattle. Wattle thickness was measured 24h post-injection with a digital micrometer according to Yadav et al.<sup>14</sup>.

#### 2.3.3. Blood Parameters

On day 35, 15 birds per group (3/replicate) were bled. Whole blood was analyzed for hematology (RBC, WBC, HCT, Hb, differential count) using an automated hematology analyzer. Serum was separated for biochemistry: total protein, albumin, globulin, cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST) using commercial kits. Antioxidant status was assessed via total antioxidant capacity (TAC, FRAP method) and malondialdehyde (MDA, TBARS assay) according to Mabelebele et al.<sup>15</sup>.

#### 2.3.4. Carcass Characteristics

At 35 days, 10 birds/group (2/replicate) were slaughtered. Carcass yield, breast, thigh, and abdominal fat weights were recorded as percentages of live weight.

### 2.4. Statistical Analysis

Data were analyzed by independent samples t-test using SPSS v.26. Growth performance data (replicate as unit, n=5) and other parameters (bird as unit, n=10 or 15) were checked for normality (Shapiro-Wilk) and homogeneity of variance (Levene's test). Results are presented as mean ± standard deviation (SD). Significance was declared at p < 0.05.

### 3. RESULTS

#### 3.1. Growth Performance

The CRTE group showed superior growth performance (Table 1). Final body weight and total weight gain were significantly higher ( $p < 0.05$ ), while overall FCR was significantly lower ( $p < 0.05$ ) compared to CON. Feed intake did not differ significantly ( $p > 0.05$ ), indicating improved feed efficiency. Mortality was low and similar between groups (2% vs. 3%). These growth trends are visually summarized in (Figure 1), which illustrates the consistent weekly improvement in both body weight and feed efficiency throughout the 35-day trial period.

#### 3.2. Modulation of Immune Response

CRTE supplementation significantly enhanced both humoral and cell-mediated immunity (Table 2). Antibody titers against NDV were significantly higher ( $p < 0.01$ ) in the CRTE group. The CBH response was also enhanced ( $p < 0.05$ ). The magnitude of these immune enhancements is graphically presented in (Figure 2), demonstrating the clear separation between treatment groups for

both humoral and cell-mediated parameters.

#### 3.3. Serum Biochemistry and Antioxidant Status

CRTE positively influenced metabolic and antioxidant profiles (Table 3). Serum cholesterol and triglycerides were lower ( $p < 0.05$ ) in the CRTE group. Liver enzymes (ALT, AST) were within normal ranges and not different between groups, indicating no hepatotoxicity. Crucially, the CRTE group exhibited a 21.7% higher serum TAC and a 25.3% lower MDA level ( $p < 0.01$ ), indicating enhanced systemic antioxidant status. The relationship between reduced oxidative stress and improved antioxidant capacity is visualized in (Figure 3), which positions the treatment group within the optimal quadrant of antioxidant protection.

#### 3.4. Hematology and Carcass Characteristics

Hematological parameters (Table 4) (RBC, WBC, Hb, HCT) were normal and unaffected by treatment ( $p > 0.05$ ). Carcass yield, breast, and thigh meat yields were not significantly different ( $p > 0.05$ ). However, abdominal fat percentage was significantly lower in the CRTE group (1.52% vs. 1.85% of live weight,  $p < 0.05$ ).

**Table 1.** Effect of *Cyperus rotundus* Extract (CRTE) on Growth Performance of Broilers (1-35 days).

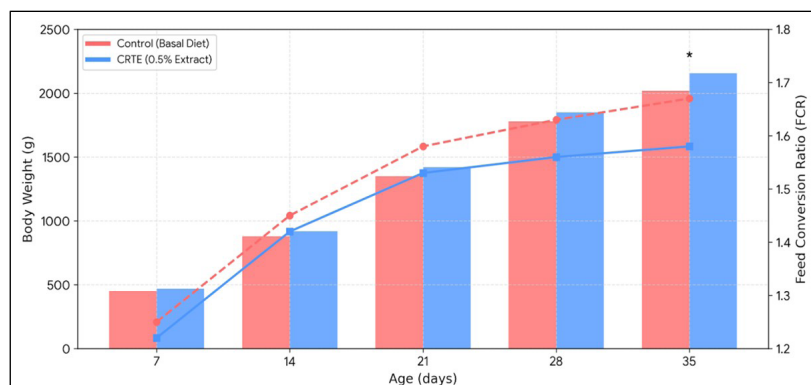
Parameter	Control (CON)	CRTE (0.5%)	p-value
Initial BW (g, d1)	45.3 ± 2.2	45.1 ± 1.9	0.841
Final BW (g, d35)	2018.7 ± 42.3 <sup>b</sup>	<b>2156.3 ± 38.7<sup>a</sup></b>	<b>0.013</b>
Total Weight Gain (g)	1973.4 ± 41.5 <sup>b</sup>	<b>2111.2 ± 38.2<sup>a</sup></b>	<b>0.011</b>
Total Feed Intake (g/bird)	3300.5 ± 85.6	3325.8 ± 79.3	0.682
Overall FCR (Feed/Gain)	1.67 ± 0.04 <sup>a</sup>	<b>1.58 ± 0.03<sup>b</sup></b>	<b>0.022</b>
Mortality Rate (%)	3.0	2.0	-

\*Values are mean ± SD (n=5 replicates). <sup>ab</sup>Means in a row with different superscripts differ significantly ( $p < 0.05$ ). Significant p-values are shown in **bold**.

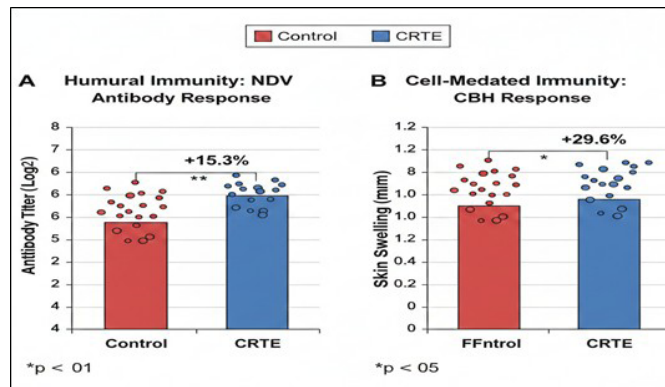
**Table 2.** Immune Parameters of Broilers Supplemented with CRTE.

	Parameter	Control (CON)	CRTE (0.5%)	p-value	Significance
1	Humoral Immunity				
	NDV Antibody Titer (Log <sub>2</sub> )	5.9 ± 0.6 <sup>b</sup>	<b>6.8 ± 0.5<sup>a</sup></b>	<b>0.008</b>	<b>**</b>
	Total Immunoglobulin (mg/dL)	2.7 ± 0.3	2.9 ± 0.3	0.098	NS
2	Cell-Mediated Immunity				
	CBH Response (Wattle swelling, mm)	0.71 ± 0.12 <sup>b</sup>	<b>0.92 ± 0.15<sup>a</sup></b>	<b>0.026</b>	<b>*</b>

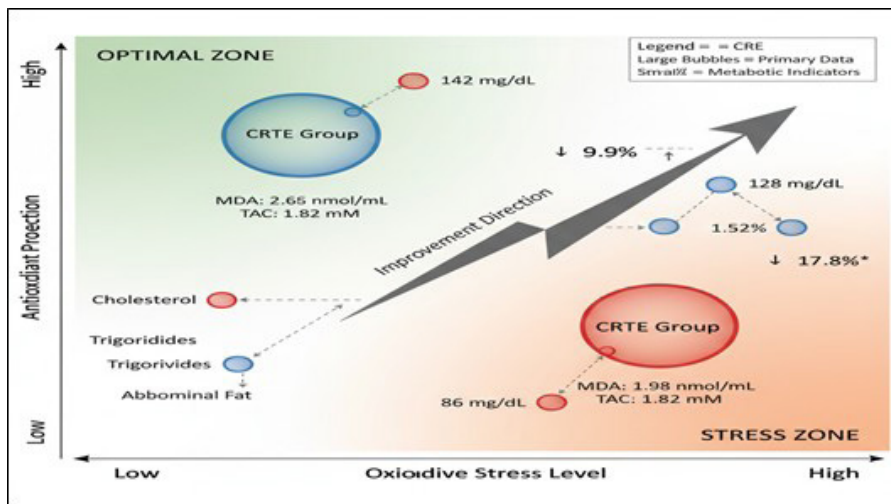
\*Values are mean ± SD (n=10 birds/group). NS = Not Significant ( $p > 0.05$ ), \*  $p < 0.05$ , \*\*  $p < 0.01$ . Significant p-values are shown in **bold**.



**Figure 1.** Growth performance of broiler chickens fed a basal diet (Control) or a diet supplemented with 0.5% *Cyperus rotundus* extract (CRTE). Body weight (bars) and feed conversion ratio (lines) were recorded weekly over 35 days. CRTE supplementation significantly improved final body weight and feed efficiency compared to control ( $p < 0.05$ ). Created with BioRender.com.



**Figure 2.** Modulation of immune response in broiler chickens supplemented with *Cyperus rotundus* extract. (A) Humoral immunity assessed via NDV-specific antibody titers (Log<sub>2</sub>). (B) Cell-mediated immunity measured by cutaneous hypersensitivity (CBH) response to PHA-P. CRTE significantly enhanced both immune parameters ( $p < 0.05$ ). Created with BioRender.com.



**Figure 3.** Antioxidant and metabolic status improvement following *Cyperus rotundus* supplementation. Quadrant visualization shows CRTE group shifting from high oxidative stress/low antioxidant protection (Control) to optimal status, with reduced MDA (1.98 vs. 2.65 nmol/mL) and increased TAC (1.85 vs. 1.52 mM). Created with BioRender.com.

#### 4. DISCUSSION

This study provides comprehensive evidence that dietary supplementation with 0.5% *Cyperus rotundus* tuber extract (CRTE) enhances growth performance, modulates immune function, and improves antioxidant status in broiler chickens, supporting all three proposed hypotheses. The findings position CRTE as a promising multi-functional phytochemical feed additive for sustainable poultry production.

##### 4.1. Enhancement of Growth Performance

The significant improvement in final body weight and feed conversion ratio (FCR) observed in the CRTE-supplemented group aligns with the growth-promoting potential reported for several phytochemical additives. The 6.8% increase in final body weight and the 5.4% improvement in FCR are particularly noteworthy. Similar improvements in FCR have been documented with other herbal extracts, such as oregano (1.48 vs. 1.57) and thyme (1.52 vs. 1.61) in broiler diets.<sup>16</sup> While the exact mechanisms underlying the observed growth enhancement were not directly investigated in this study, we hypothesize that the bioactive sesquiterpenes and flavonoids in CRTE may contribute to improved nutrient utilization. Based on previous research with similar phytochemical compounds, potential mechanisms could include modulation of gut microbiota composition, stim-

ulation of endogenous digestive enzyme secretion, and improvement of intestinal villus morphology.<sup>3,17</sup> 60, 100, and 200 mg/kg of diet. However, these putative mechanisms require direct investigation in future studies specifically designed to examine intestinal parameters, microbiota analysis, and digestive enzyme activities in CRTE-supplemented birds. The antimicrobial properties of  $\alpha$ -cyperone and other sesquiterpenes in *C. rotundus*<sup>5</sup> may suppress subclinical pathogenic loads in the gastrointestinal tract, reducing energy diversion toward immune activation and allowing greater nutrient partitioning toward growth. The unchanged feed intake suggests that the improved FCR resulted from enhanced metabolic efficiency rather than reduced consumption. The present study provides the foundational evidence of growth enhancement that justifies such mechanistic investigations.

##### 4.2. Modulation of the Immune Response

The results demonstrate a clear immunomodulatory effect of CRTE, significantly enhancing both arms of the adaptive immune system. The 15.3% increase in NDV-specific antibody titers indicates a potent stimulation of the humoral immune response. This finding is consistent with studies on other immunostimulant plants, such as *Echinacea purpurea*, which increased antibody titers against infectious bursal disease virus in

**Table 3.** Serum Biochemical and Antioxidant Parameters in Broilers (Day 35).

Parameter	Control (CON)	CRTE (0.5%)	p-value
Total Protein (g/dL)	4.8 ± 0.3	5.0 ± 0.4	0.185
Albumin (g/dL)	2.1 ± 0.2	2.2 ± 0.2	0.421
Globulin (g/dL)	2.7 ± 0.3	2.8 ± 0.3	0.538
Cholesterol (mg/dL)	142.5 ± 8.7 <sup>a</sup>	<b>128.3 ± 7.9<sup>b</sup></b>	<b>0.018</b>
Triglycerides (mg/dL)	85.6 ± 6.2 <sup>a</sup>	<b>74.8 ± 5.5<sup>b</sup></b>	<b>0.009</b>
ALT (U/L)	22.3 ± 3.1	20.9 ± 2.8	0.402
AST (U/L)	185.4 ± 12.6	178.9 ± 15.2	0.481
TAC (mM FRAP)	1.52 ± 0.15 <sup>b</sup>	<b>1.85 ± 0.14<sup>a</sup></b>	<b>0.003</b>
MDA (nmol/mL)	2.65 ± 0.31 <sup>a</sup>	<b>1.98 ± 0.28<sup>b</sup></b>	<b>0.004</b>

\*Values are mean ± SD (n=15 birds/group). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TAC: Total Antioxidant Capacity; MDA: Malondialdehyde. <sup>ab</sup>Means in a row with different superscripts differ significantly ( $p < 0.05$ ). Significant p-values are shown in **bold**.

**Table 4.** Hematological Parameters and Carcass Characteristics of Broilers

	Parameter	Control (CON)	CRTE (0.5%)	p-value
<b>1</b>	<b>Hematological Parameters</b>			
	Red Blood Cells (RBC, ×10 <sup>6</sup> /μL)	2.85 ± 0.21	2.92 ± 0.24	0.421
	White Blood Cells (WBC, ×10 <sup>3</sup> /μL)	15.3 ± 1.8	16.1 ± 1.9	0.283
	Lymphocyte Count (×10 <sup>3</sup> /μL)	7.2 ± 0.8	<b>8.1 ± 0.9</b>	<b>0.042</b>
	Heterophils: Lymphocyte Ratio (H:L)	0.42 ± 0.05	<b>0.38 ± 0.04<sup>b</sup></b>	<b>0.047</b>
	Hemoglobin (Hb, g/dL)	10.2 ± 0.7	10.5 ± 0.8	0.355
	Hematocrit (HCT, %)	32.8 ± 2.1	33.5 ± 2.4	0.468
<b>2</b>	<b>Carcass Characteristics (% of Live Weight)</b>			
	Dressing Percentage	71.2 ± 1.5	72.8 ± 1.3	0.102
	Breast Meat Yield	28.5 ± 0.9	29.1 ± 0.8	0.174
	Thigh Meat Yield	24.3 ± 0.7	24.9 ± 0.6	0.118
	Abdominal Fat Percentage	1.85 ± 0.11 <sup>a</sup>	<b>1.52 ± 0.09<sup>b</sup></b>	<b>0.019</b>
	Liver Percentage	2.10 ± 0.15	2.05 ± 0.13	0.615
	Heart Percentage	0.63 ± 0.04	0.65 ± 0.05	0.534

\*Values are mean ± SD. For hematology, n=15 birds per group. For carcass traits, n=10 birds per group. <sup>ab</sup>Means within a row with different superscripts differ significantly ( $p < 0.05$ ). Significant p-values are shown in **bold**.

broilers.<sup>18</sup> The bioactive polysaccharides and flavonoids in *C. rotundus* are known to activate macrophages and B-lymphocytes, promoting antigen presentation and antibody production.<sup>7</sup> The significant 29.6% enhancement in the cell-mediated immune response, measured via the PHA-P skin test, corroborates the humoral data and indicates a systemic immunomodulatory effect. PHA-P primarily stimulates T-lymphocyte proliferation, and an augmented CBH response suggests improved T-cell functionality. This dual enhancement of both humoral and cell-mediated immunity is a highly desirable attribute for a feed additive, as it suggests a broad-spectrum capacity to improve vaccine efficacy and resilience against diverse pathogens.

#### 4.3. Improvement of Antioxidant Status and Lipid Metabolism

One of the most pronounced effects of CRTE was on the systemic antioxidant profile. The 21.7% increase in serum total antioxidant capacity (TAC) and the 25.3%

reduction in malondialdehyde (MDA), a marker of lipid peroxidation, indicate a significant reduction in oxidative stress. This aligns directly with the known phytochemistry of *C. rotundus*, which is rich in radical-scavenging compounds like flavonoids (quercetin, kaempferol) and phenolic acids.<sup>5</sup> Similar antioxidant effects in broilers have been reported for rosemary and green tea extracts, which also lowered MDA levels in serum and breast meat.<sup>19</sup> Reduced oxidative stress contributes to enhanced immune cell function, improved nutrient utilization, and better overall health, potentially explaining part of the growth performance benefits. Furthermore, the significant reduction in abdominal fat percentage (17.8%) and serum cholesterol levels aligns with studies on hypolipidemic herbs. Compounds in *C. rotundus* may inhibit key enzymes in hepatic cholesterol and fatty acid synthesis, such as HMG-CoA reductase and fatty acid synthase, as suggested in rodent models.<sup>7</sup> This metabolic effect improves carcass quality by reducing undesirable fat deposition, a valuable economic trait.

#### 4.4. Hematology and Carcass Traits: Safety and Quality

The absence of adverse effects on standard hematological parameters (RBC, Hb, HCT) and liver enzymes (ALT, AST) confirms the safety of the 0.5% CRTE inclusion level over the 35-day production cycle. The lack of significant differences in prime carcass yields (dressing %, breast, thigh) indicates that the growth promotion did not come at the expense of meat yield, which is crucial for commercial viability. The significant reduction in abdominal fat, however, represents a direct improvement in carcass quality and production efficiency. The improved H:L ratio, a well-established indicator of reduced stress in poultry,<sup>20</sup> provides additional evidence of the beneficial physiological state promoted by CRTE supplementation.

#### 4.5. Proposed Integrative Mechanism and Limitations

The converging results suggest a synergistic mechanism of action for CRTE. The antimicrobial and anti-inflammatory properties of its sesquiterpenes likely promote a healthier gut environment, leading to better digestion and absorption (improved FCR). Concurrently, its immunomodulatory compounds (polysaccharides, flavonoids) enhance both antibody production and T-cell activity, bolstering disease resistance. Simultaneously, its potent antioxidant constituents mitigate systemic oxidative damage, protecting cells and improving metabolic efficiency. This triad of actions improved gut health, enhanced immunity, and reduced oxidative stress collectively drives the observed improvements in growth performance, metabolic health, and carcass quality.

We acknowledge that the use of a single dose (0.5%) represents a limitation of this study, as it precludes evaluation of dose-response relationships and determination of the optimal inclusion level. The selection of this dose was based on preliminary studies and published literature on similar phytogetic extracts; however, future research should incorporate multiple dose levels to establish the most effective and economical supplementation rate.

### 5. CONCLUSION

Dietary supplementation with 0.5% *Cyperus rotundus* tuber extract for 35 days significantly improved growth performance, immune competence, and antioxidant status in broiler chickens while reducing abdominal fat deposition. These multifaceted benefits, achieved without adverse effects on health or carcass yield, suggest that CRTE has promising potential as a natural feed additive in broiler production. While direct comparisons with synthetic growth promoters were beyond the scope of this study, the observed improvements position CRTE as a candidate for further investigation in antibiotic-free poultry production systems. Future studies incorporating direct comparisons with commercial growth promoters and dose-response evaluations are warranted to establish its relative efficacy and optimal usage levels.

### 6. LIMITATIONS AND FUTURE RESEARCH

This study utilized a single dose of CRTE (0.5%). Future dose-response studies testing multiple inclusion levels (e.g., 0.25%, 0.5%, 0.75%, and 1.0%) are needed to

identify the optimal dosage for maximizing growth performance and immune enhancement while ensuring cost-effectiveness. Furthermore, future studies should include positive control groups receiving commercial growth promoters (e.g., antibiotic growth promoters or other phytogetic additives) to allow direct comparison of efficacy and establish the relative performance of CRTE against established alternatives. Additionally, the specific modulation of gut microbiota and the expression of genes related to immunity (e.g., cytokines) and metabolism should be investigated to elucidate the precise molecular pathways involved.

#### Conflict of Interest

The authors declare no conflict of interest.

#### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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