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RESEARCH ARTICLE
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Anticoccidial and Antioxidant Effects of Organic Charcoal and its Impact on Gut Integrity, Meat Quality, Blood Parameters and Immunity in Broiler Chickens Challenged with Coccidiosis

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ABSTRACT

Coccidiosis is considered as one of the most economically significant diseases in poultry. It requires the need for sustainable alternatives to synthetic anticoccidials. This study aimed to evaluate the efficacy of organic charcoal as a dietary supplement in broiler chickens experimentally infected with *Eimeria* spp. The investigation focused on its anticoccidial and antioxidant properties and its impact on growth, gut health, and immunity. A total of 375 chicks were randomly allocated into five groups (T_{ch1}, T_{ch2}, T_{-ve1}, T_{-ve2}, and T_{+ve}), with three replicates of 25 birds each. Charcoal was supplemented to the feed at 1g/kg (Tch1) and 3g/kg (Tch2), while positive control received sulphadimidine + diaveridine HCl. Birds were reared for 35 days; dietary treatments began on day 12 and infection was induced on day 14. Charcoal supplementation significantly improved feed conversion ratio, lesion score, oocyst index, and fecal oocyst shedding as compared to controls ($P<0.05$). It also enhanced total antioxidant capacity, reduced malondialdehyde concentrations, and improved intestinal morphology (villus height and surface area). Notably, these benefits were achieved without compromising growth or mineral balance. In conclusion, organic charcoal demonstrates dual anticoccidial and antioxidant effects. It acts as a natural feed additive that alleviates the pathological and oxidative effects of coccidiosis. These findings support its ability as a sustainable strategy to reduce reliance on synthetic anticoccidials in intensive poultry production.

Keywords: Broiler, Coccidiosis, Antioxidant, Gut integrity, Antioxidant defense, Charcoal supplementation.

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INTRODUCTION

The poultry industry is considered as the key contributor to global food security. This industry is major supplier of affordable protein in the form of meat and eggs. However, infectious diseases remain a major barrier to production efficiency. Among these, coccidiosis is particularly destructive. It is caused by intracellular protozoa of the genus *Eimeria*. These protozoans have special organelles within the apical complex that are essential for invading the host's intestinal cells. These parasites damage intestinal epithelial cells and result in malabsorption, impaired growth, and elevated mortality. There are seven recognized species of *Eimeria*, each targets specific region of the intestines and exhibits different pathogenicity (Chapman, 2014; Jones & Garcia, 2021; Mesa-Pineda et al., 2021; Qaid et al., 2022). Globally, coccidiosis accounts for losses exceeding USD 14.5 billion, largely through reduced performance and high treatment costs (Blake et al., 2020).

Currently, poultry industry relies on anticoccidial drugs and live or attenuated vaccines (Chand et al., 2016). While effective, these approaches face major limitations. These limitations include widespread drug resistance, residue concerns in poultry products, and increase in consumer's demand for antibiotic-free meat (Chapman et al., 2010). Live vaccines represent another method for controlling coccidiosis. They are useful but can impair growth under poor management and do not fully eliminate infection risk. These challenges highlight the urgent need for sustainable, residue-free alternatives to conventional strategies (Acharya and Acharya, 2017).

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Recent advances in *Eimeria* biology, including host-parasite-microbiome interactions, have informed novel therapeutic approaches (Gao et al., 2024). Moreover, a comprehensive review of coccidiosis management outlines sustainable alternatives such as phytochemicals and probiotics show promise in reducing infection severity. Field trials further demonstrate that combinations of anticoccidial drugs, probiotics, phytochemicals, and vaccines can offer improved protection and broiler performance in *Eimeria*-infected chickens (Ahmad et al., 2023).

Coccidiosis may result in multiple pathological and performance-related issues. These issues include intestinal leakage of plasma proteins, villous atrophy, malabsorption, disruption of nutrient digestion, decreased weight gain (WG), reduced feed and water intake, elevated feed conversion ratio (FCR), higher mortality, accelerated intestinal transit, greater susceptibility to other diseases, and increased medication cost (Martins et al., 2022). In addition, many countries have imposed restrictions on various feed additives, due to concerns about drug residues in humans and associated health risks. The recent shift toward alternative feed additives, such as enzymes and medicinal herbs, reflects the growing recognition that gastrointestinal health is critical for poultry productivity, as even minor disruptions can compromise function and overall performance (M'Sadeq, 2023).

Charcoal is a carbon-rich product of pyrolyzed organic matter. It has long been used in medicine, agriculture, and environmental management due to its strong adsorptive and detoxifying properties. In animal nutrition, charcoal can bind toxins, reduce pathogen load, and modulate intestinal microbiota. In addition, it also supports digestion and nutrient absorption (Mabe et al., 2018; Enyenihi et al., 2022). Its beneficial effects in poultry include improved growth, survival, feed efficiency, and gut morphology. Furthermore, charcoal possesses antioxidant activity that may be helpful to alleviate oxidative stress during intestinal infections (Abd El-Maksoud et al., 2014).

Despite these findings, evidence on the dual anticoccidial and antioxidant functions of dietary charcoal in broilers under *Eimeria* challenge is limited. The establishment of its efficacy, optimal inclusion levels, and systemic effects is critical to validate the charcoal as a sustainable alternative to synthetic anticoccidials. Therefore, this study evaluated the impact of supplementation of broiler diets with hardwood charcoal (1g/kg and 3g/kg) on growth performance, anticoccidial efficacy, antioxidant status, immune responses, gut integrity, and meat quality under experimental coccidiosis challenge. We hypothesized that, dietary charcoal would alleviate the pathological and oxidative impacts of *Eimeria* infection and enhance overall broiler health and productivity.

MATERIALS & METHODS

Study Place

This research was conducted at the poultry production unit at Jordan University Station for Dry Land Research at Al-Muwaqqar, located 45km southeast of Amman, Jordan. This semi-arid region presents challenging environmental conditions representative of those under which a significant portion of global poultry production operates. Conducting the trial in such an environment enhances the practical relevance of the findings to real world, water-scarce settings. The experimental facility was a closed housing system consisting of 10 rooms, each divided into three floor pens and equipped with stoves, drinkers, feed troughs, and a lighting system.

Management of Experimental Birds

A total of Three hundred seventy five day-old broiler chicks (Ross 308) were obtained from a local source and acclimatized for 10 days. On day 12, chicks were randomly allocated into five equal treatment groups (three replicates of 25 birds per group). All chicks had a similar initial body weight (~48±0.05g). Feed and water were provided *ad libitum*, and birds were reared on floor litter (wood shavings). Standard nutrient requirements and husbandry practices were followed according to Ross 308 management guidelines. All chicks were provided a broiler starter ration from placement until week 2, followed by a finisher ration (Table 1). Pen temperature was maintained at 29-32°C during the first week and then reduced by ~0.5°C per week. Continuous lighting (24h) was provided throughout the experiment. On day 12, birds were segregated into their assigned treatment groups and the experimental diets/medications were initiated. Chicks were vaccinated for infectious bronchitis on day 1 and Newcastle disease on day 7 (Hayajneh et al., 2024).

Table1: Composition of broiler basal feed (g/kg)

Ingredients	Starter (0-21d)	Finisher (22-35d)
Maize	540.0	710.0
Soybean meal (480g/kg CP)	360	250.0
Dicalcium phosphate	20	20.0
Soybean oil	13	13.0
Fish meal	60	0
DL-Methionine	1.5	0.5
Vitamin-mineral premix[a,b]	3.5	3.5
Sodium chloride	2.0	3.0
Chemical composition (g/kg diet as fed basis)		
MEn (MJ/kg)	12.6	13.0
Crude protein	235.0	182.0
Calcium	12.3	8.0
Total Phosphorus	6.5	5.1
Lysine	17.0	10.0
Methionine	9.0	8.0

[a]Vitamin premix provided per kilogram of diet: Vitamin A=0.5760; Vitamin K=?IU; Vitamin B5=19.4532mg; Tocopherol=4.6799mg; Vitamin B1=3.5016mg; Vitamin B2=1.6994mg; Vitamin B6=6.4911mg; Vitamin B12=16mg; Biotin=0.1382mg; Folic Acid=1.2692mg; Pantothenic=7.8104mg; Vitamin K3=0.9071mg; [b]Trace mineral premix per kg diet were: Iron= 62.0061mg; Zinc=43.065mg; Copper=6.855mg; Iodine=0.0589mg; Selenium=1.3466mg. Soybean concentrate and mono-calcium phosphate were also provided.

Experimental Design

The experiment was arranged as a randomized complete block design (RCBD) with five treatment groups (as described above) and three replicates per treatment (25 chicks per replicate).

Drugs and Administration

Anticoccidial Medication Protocol: Beginning on day 12 post-hatch, birds in the positive control group (T+ve) received an anticoccidial treatment via drinking water. The treatment consisted of a commercial formulation containing sulphadimidine (320mg) and diaveridine HCl (10mg) (Avico, Amman, Jordan), administered at 100mg of product per 200mL of water. The treatment regimen was cyclical: medicated water was provided for three consecutive days, followed by two days of plain water, and then a final two-day course of medicated water.

Dietary Treatment Protocol: The experimental diets were prepared at the feed mill. Hardwood charcoal was incorporated into the basal diet at two concentrations: 1g/kg of feed for the Tch1 group and 3g/kg of feed for the Tch2 group. Both groups received their respective charcoal-supplemented diets during the seven-day treatment period. Two negative control groups (T–ve1 and T–ve2) received the unsupplemented basal diet. A summary of treatment groups is provided in Table 2.

Histopathological Examination

Tissue samples from affected intestinal segments were collected for histopathology. The samples were preserved in 10% buffered formalin, dehydrated, embedded in paraffin, sectioned at 4–5µm thickness, and stained with hematoxylin and eosin, following standard protocols (Hayajneh et al., 2020). Slides were examined under a light microscope.

Evaluation of Sensitivity or Resistance

Seventeen chicks from each group were randomly selected and weighed on day 12, then reweighed on day 21 to determine the body weight gain. On day 21, five chicks per group were sacrificed for lesion scoring (Raman et al., 2011). Gross lesion scores (GLS) were assigned on a scale of 0 (no lesion) to 4 (most severe lesion). Microscopic examination of intestinal scrapings was used to determine the oocyst index (0–5) as described by Arabkhazaeli et al. (2013). Anticoccidial sensitivity was evaluated using the global index (GI) (Table 9).

Coccidiosis Induction

Infective oocysts were isolated from naturally infected chickens on local farms as per standard protocol of Hayajneh et al. (2020). Speciation was based on infection site and morphological characteristics of sporulated oocysts (size, shape, and color). The inoculum was a mixed *Eimeria* suspension containing *E. acervulina* (10%), *E. brunetti* (13%), *E. maxima* (12%), *E. necatrix* (12%), and *E. tenella* (57%). On day 14, all birds were orally challenged with 3×10^5 sporulated oocysts per bird (Abbas et al., 2011).

Performance Parameters

Feed intake, body weight gain, and feed conversion ratio (FCR) were recorded on days 21, 28, and 35. Oocysts per gram of feces, lesion scores, and oocyst indices were also measured on days 14, 21, and 28. General health and mortality were monitored daily. Anticoccidial efficacy was expressed as a percentage of the GI relative to the normal negative control (NNC). Meat quality was assessed as described by Jalal et al. (2023).

Lesion scoring and Oocyte Index

On days 7, 14 and 21 post-inoculations (corresponding to experimental days 21, 28, and 35), three birds from each replicate were randomly selected and sacrificed humanely for lesion scoring. Lesions were graded from 0 (no lesion) to +4 (severe lesion) (Raman et al., 2011). Oocyst index scores (0–5) were determined from intestinal scrapings of the same birds (Arabkhazaeli et al., 2013).

Statistical Analysis

Data were analyzed using SPSS software. One-way analysis of variance (ANOVA) with repeated measures was applied, and treatment means were compared using Tukey's test. Statistical significance was set at $P \leq 0.05$.

Measurements

Serum biochemical parameters were determined using commercial diagnostic kits. Magnesium, creatinine, and uric acid were measured with Biolab kits (Jordan). Albumin, cholesterol, total protein, bilirubin, and phosphorus were measured with Biomed kits (MDSS GmbH, Germany). Glucose was measured with an Atlas Medical kit (Jordan). Zinc concentration was determined spectrophotometrically using a kit from Ita (Italy). Malondialdehyde (MDA) and total antioxidant capacity (TAC) were analyzed with assay kits from Creative Proteomics (USA).

Serum antibody titers against Newcastle disease virus (NDV) and infectious bursal disease (IBD) were quantified using BioChek ELISA kits (Netherlands), following the manufacturer's instructions, with a cut-off value of <0.35. All laboratory analyses were performed at Feedco Labs (Jordan). Villus surface area was calculated for 15 villi per bird using the formula:

2 × (villus width / 2) × villus height, as described by De Los et al. (2005).

Table 2: Group arrangement in the experiment

Group	Drug Used	Dose	Coccidiosis Introduced
T_{ch1}	Charcoal	1gm/kg feed	Yes
T_{ch2}	Charcoal	3gm/kg feed	Yes
T_{+ve}	Sulphadimidine 320mg and diaverdine HCL 10mg	100mg/200L drinking water for three days, followed by plain water /2days, then the medication was added in water 100mg/200L water/2days	Yes
T_{-ve1}	Infected medicated with bentonite 1.5mg/kg	1.5mg/kg	No
T_{-ve2}	None infected none medicated	None	No

RESULTS

Performance, Feed Conversion Ratio (FCR) and Lesion Score

In the current study, the feed conversion ratio was significantly affected by the addition of charcoal (Table 3, Fig. 3). The charcoal-supplemented groups (Tch1 and Tch2) showed improved FCR, with the lowest FCR values observed in these groups, whereas the untreated infected control group (T−ve2) had the highest (poorest) FCR. Correspondingly, charcoal supplementation reduced disease severity: the lowest fecal oocyst shedding and lesion scores were noted in the high-charcoal group (Tch2), while the highest lesion score was recorded in the infected negative control (T−ve2) (Table 3; Fig. 2 and 4). As shown in Table 3, both FCR and lesion scores were significantly improved with charcoal treatment (ANOVA $F=5.178$, $P=0.002$ for FCR; $F=5.846$, $P=0.001$ for lesion score). These results demonstrate that dietary charcoal effectively enhanced feed efficiency and mitigated gut damage under coccidial challenge.

Oocyte Index and Fecal Oocyte Count

Charcoal supplementation markedly improved the oocyte index in infected birds. The oocyte index was significantly higher in the charcoal-treated groups than in controls ($F=19.958$, $P<0.001$; Table 3, Fig. 2). In parallel, fecal oocyst counts (shedding) were substantially lower in the charcoal-fed groups (especially Tch2) compared to the untreated infected group. This indicates that charcoal reduced the intestinal parasite load and improved gut health. Despite these benefits in feed efficiency and parasite control, there were no significant differences in body weight gain or overall feed intake among the groups. Weight gain ($F=0.803$, $P=0.608$) and feed intake ($F=0.829$, $P=0.588$) remained statistically similar across all treatments (Table 3), suggesting that charcoal primarily enhanced feed utilization efficiency rather than accelerating growth rate per se.

Antioxidant and Oxidative Stress Markers

Charcoal had a pronounced effect on oxidative status in coccidia-challenged broilers. Birds receiving charcoal showed a significantly higher total antioxidant capacity (TAC) and lower lipid peroxidation levels than controls. Specifically, charcoal supplementation increased TAC ($F=101.011$, $P<0.001$) and decreased malondialdehyde (MDA, an indicator of oxidative stress) levels ($F=48.270$, $P<0.001$) compared to no-charcoal groups (Table 7). These results indicate that charcoal helped neutralize oxidative stress induced by infection. In contrast, charcoal had no significant effect on systemic immune responses to vaccinations, as the concentrations of Newcastle disease and infectious bronchitis virus antibody titers did not differ notably between groups (Table 4).

Histopathology and Gut Health

Intestinal histology was improved in charcoal-fed chickens (Fig. 7 & 8). Charcoal supplementation led to increases in villus length and overall healthier gut morphology compared to controls (Table 6). In the charcoal-treated groups, villi were visibly longer and more structurally intact, indicating better maintenance of the intestinal lining (Table 8). These structural enhancements in the gut likely facilitated more efficient nutrient absorption. The improvement in villus architecture was reflected in downstream performance measures, such as a trend toward enhanced growth and better carcass yield in charcoal-treated birds, compared to those not receiving charcoal.

Anticoccidial Effects

Charcoal demonstrated notable anticoccidial activity in this experiment. Both charcoal-supplemented groups (Tch1 and Tch2) exhibited the lowest coccidial oocyst output and the mildest intestinal lesion scores among the infected groups (Fig. 1 and 2, 4). In contrast, the infected chickens that did not receive charcoal (T−ve2) showed much higher oocyst shedding and more severe gut lesions, indicative of intense coccidial infection. By reducing oocyst shedding and lesion severity, charcoal effectively curtailed the replication of *Eimeria* protozoa and protected the gut from coccidiosis-related damage. Charcoal also attenuated the infection-induced oxidative stress in the gut: the elevated oxidative stress observed with coccidiosis was significantly reduced in both Tch1 and Tch2, as evidenced by lower MDA concentrations in these groups (Fig. 5 and 6). Consequently, charcoal-fed birds had less intestinal damage and recovered better from the coccidial challenge, relative to the untreated infected birds.

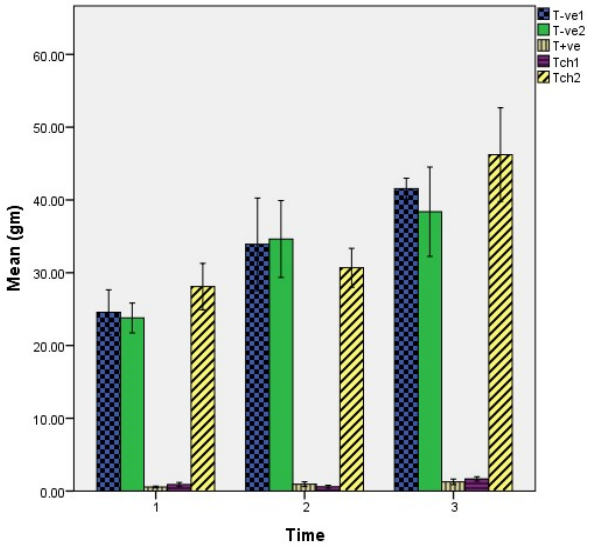

Fig. 1: Mean weight of spleen in the different groups.

Biochemical and Mineral Parameters

Key blood mineral levels remained stable with charcoal supplementation. There were no significant changes in serum phosphorus, magnesium, or zinc concentrations between charcoal-treated and control groups (Table 5). This indicates that the inclusion of charcoal in the diet did not deplete or interfere with the absorption of these essential minerals. In other

Table 3: Performance parameters in the different groups

		Sum of Squares	df	Mean Square	F	P value
FCR	Between Groups	0.441	8	0.055	5.178	0.002
Lesion score	Between Groups	22.519	8	2.815	5.846	0.001
Oocyte index	Between Groups	70.963	8	8.870	19.958	0.000
Fecal oocyte count	Between Groups	4661717419.333	8	582714677.417	1.168	0.370
Weight gain	Between Groups	127826.775	8	15978.347	0.803	0.608
Feed intake	Between Groups	149809.895	8	18726.237	0.829	0.588

Table 4: Meat quality values in the different groups

		Sum of Squares	df	Mean Square	F	P value
MWT	Between Groups	56671.760	8	7083.970	19.223	0.000
FWT	Between Groups	49835.920	8	6229.490	23.645	0.000
L	Between Groups	2234686.667	8	279335.833	12.569	0.000
A	Between Groups	5343475.728	8	667934.466	1.316	0.250
B	Between Groups	818197.580	8	102274.698	4.041	0.001
PH	Between Groups	0.003	8	0.000	0.737	0.658
CL	Between Groups	202.552	8	25.319	12.107	0.000
WHC	Between Groups	399.512	8	49.939	34.140	0.000
SHF	Between Groups	15.970	8	1.996	5.766	0.000

MWT: muscle weight, FWT: fillet weight, (L, A, B) values of meat color, WHC: water holding capacity, SHF: shear force, LI: lesion score index, OI: Oocyte index, FOC: fecal oocyte index.

Table 5: Blood parameters values in the different groups

		Sum of Squares	df	Mean Square	F	P value
Albumin	Between Groups	0.115	8	0.014	1.306	0.255
	Within Groups	0.795	72	0.011		
	Total	0.910	80			
Cholesterol	Between Groups	2318.673	8	289.834	.857	0.557
	Within Groups	24356.817	72	338.289		
	Total	26675.489	80			
Glucose	Between Groups	15030.612	8	1878.827	1.116	0.363
	Within Groups	121186.893	72	1683.151		
	Total	136217.505	80			
Total Protein	Between Groups	0.595	8	0.074	.198	0.990
	Within Groups	27.075	72	0.376		
	Total	27.670	80			
Magnesium	Between Groups	17.644	8	2.205	1.196	0.314
	Within Groups	132.743	72	1.844		
	Total	150.387	80			
Uric Acid	Between Groups	4.718	8	0.590	.344	0.946
	Within Groups	123.504	72	1.715		
	Total	128.222	80			
Phosphorus	Between Groups	150.839	8	18.855	1.099	0.374
	Within Groups	1235.379	72	17.158		
	Total	1386.219	80			
zinc	Between Groups	24484.425	8	3060.553	1.693	0.116
	Within Groups	124768.467	69	1808.239		
	Total	149252.891	77			

Table 6: IB and Newcastle disease antibody titers in the blood of the different groups

		Sum of Squares	df	Mean Square	F	P value
IB Titer	Between Groups	1339151.556	8	167393.944	1.202	0.310
ND concentration	Between Groups	512002.691	8	64000.336	0.565	0.803
	Within Groups	8154342.444	72	113254.756		

Table 7: Total antioxidant and malondialdehyde concentrations in the blood of the different groups

		Sum of Squares	df	Mean Square	F	S P value
TAC	Between Groups	448237.158	8	56029.645	101.011	0.000
MAD	Between Groups	4160.245	8	520.031	48.270	0.000
	Within Groups	775.680	72	10.773		

Table 8: Villus parameters in the different groups

		Sum of Squares	df	Mean Square	F	P value
VL	Between Groups	2822946.009	8	352868.251	4.604	0.000
VW	Between Groups	19127.778	8	2390.972	1.020	0.429
CD	Between Groups	24269.958	8	3033.745	1.285	0.265
SA	Between Groups	228187808768.512	8	28523476096.064	.602	0.773

VL: villus length, VW: villus width, CD: crypt depth, SA: villus surface area.

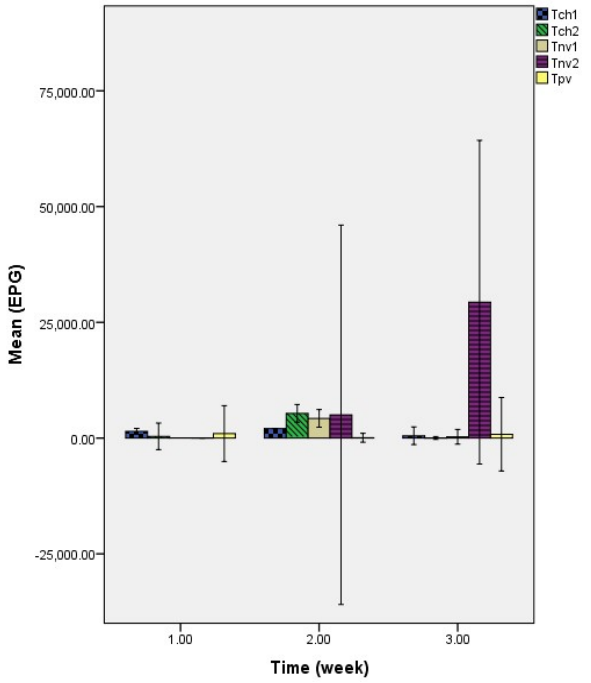

Fig. 2: Fecal egg shedding in the different groups.

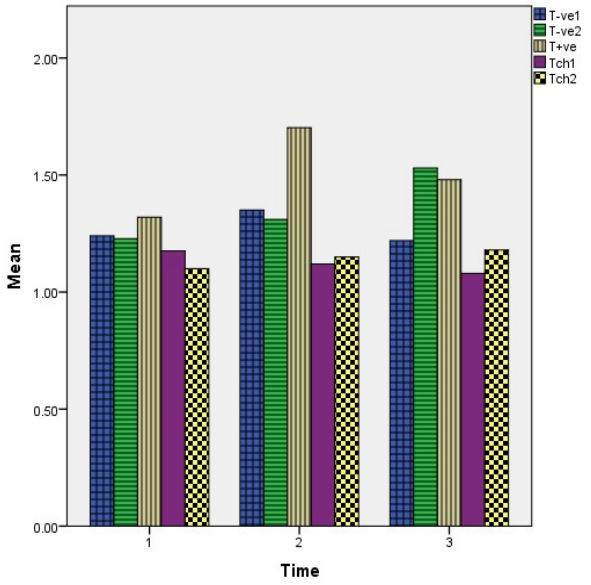

Fig. 3: Feed conversion ratio in the different groups.

biochemical measures, charcoal-supplemented birds showed normal profiles comparable to controls, suggesting that charcoal's adsorptive action was selective for harmful substances and did not indiscriminately remove vital nutrients or alter baseline blood chemistry.

Table 9: Comparative values of FCR, lesion score, oocyst index, mortality percentage, global index, and efficacy status

Group	FCR (g/g)	Lesion score	Oocyte index	mortality	GI %	The global index of NNC%	Efficacy status
Tch1	1.18	2	1	2	≥90%	68.6.2	1
Tch2	1.1	1	2	3	≥90%	65.3	1
T+ve	1.48	3	3		Limited efficacy	77	3
TNNC (T-ve2)	1.35	4	5				
TINC (T-ve1)	1.39	4	5				

Tch1, 2= charcoal -medicated group; NNC= Noninfected, nonmedicated control (T-ve2). INC=Infected nonmedicated controls (T-ve1); Global index (GI) = %WGNNC − [(FM− FNNC) × 10] − (OIM − OIINC) − [(LSM − LSINC) × 2] − (%mortality/2), where WG is weight gain, F is the FCR, OI is the oocyst index, LS is the lesion score, M is the medicated group, NNC is the noninfected, nonmedicated control group, and INC is the infected nonmedicated control group. Efficacy status was calculated as a percentage of the GI for the NNC. The following 5 categories were used for testing resistance to anticoccidials: 1) very good efficacy, ≥90% GINNC; 2) good efficacy, 80 to 89% GINNC; 3) limited efficacy, 70 to 79% GINNC; 4) partially resistant, 50 to 69% GINNC; and 5) resistant.

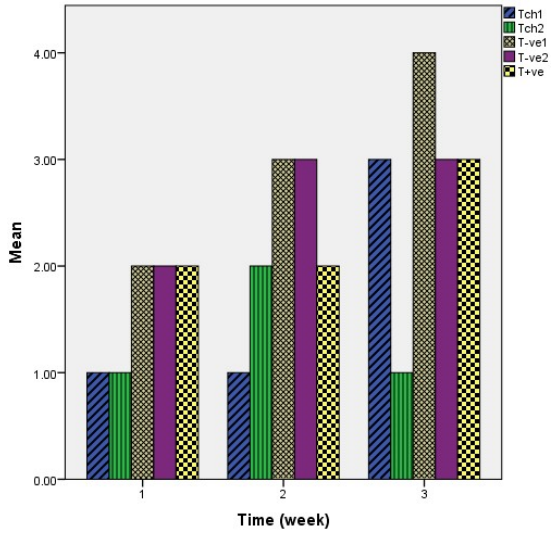

Fig. 4: Lesion score in the different groups.

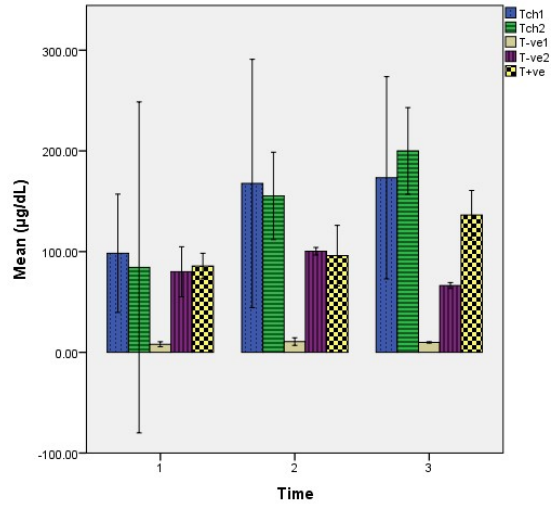

Fig. 5: Total antioxidant capacity in the different groups.

Meat Quality

Charcoal had beneficial effects on certain meat quality and production traits. Birds fed charcoal achieved a higher final body weight and improved meat water-holding capacity compared to those not fed charcoal. Final live weight at slaughter was significantly greater in charcoal groups (FWT, F=23.645, P<0.001), and the breast muscle water-holding capacity (an important meat quality parameter) was also higher (WHC, F = 34.140, P<0.001) in charcoal-supplemented birds (Table 4). These improvements suggest that charcoal enhanced nutrient utilization and muscle development, leading to better growth and meat quality outcomes. No adverse effects on carcass characteristics were observed; on the contrary, charcoal-fed broilers yielded well-developed carcasses with good quality attributes, consistent with their improved growth performance.

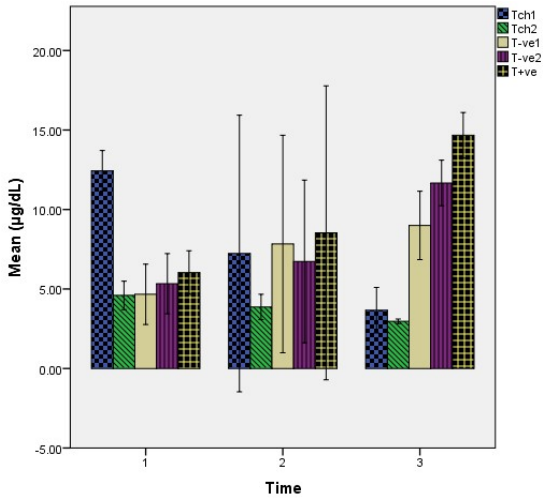

Fig. 6: Malondialdehyde concentrations in the different group.

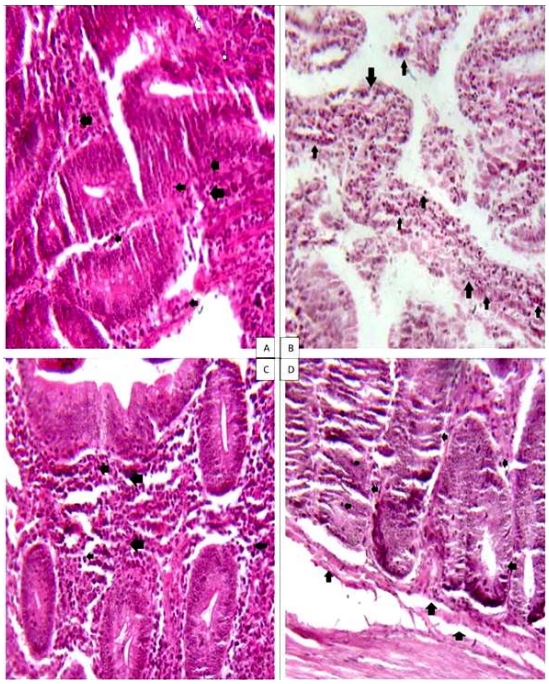

Fig. 7: Histopathology of the intestine in different groups showing different stages of *Eimeria* in the intestine T_{-ve1} and T_{-ve2} (B, D), cell necrosis and sloughing in T_{-ve2} group (B), T_{-ve1} (D), T_{+ve} (A), T_{-ve1} (A), A, B, C, D at 40X.

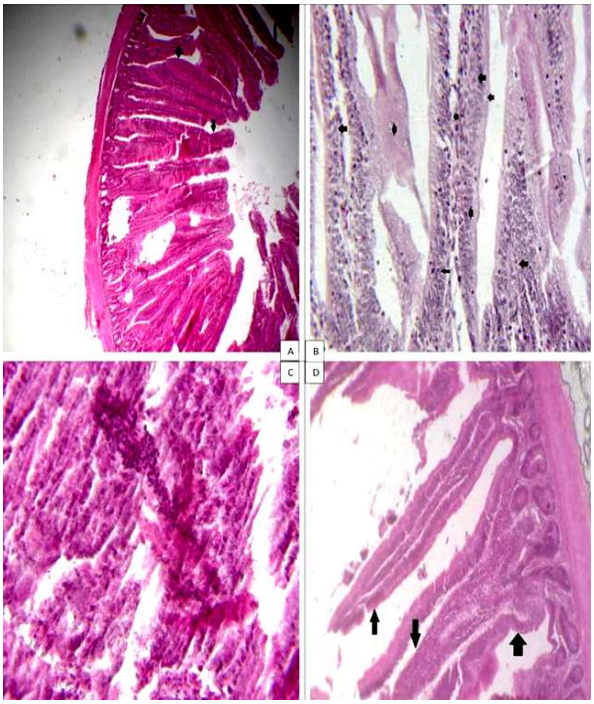

Fig. 8: Histopathology of the intestine in different groups, normal villi in Tch1 (A) and Tch2 (D) showing different stages of Eimeria in the intestine and changes in the columnar mucosal epithelium shape and lymphocyte infiltration in T-ve2, T-ve1 (B, C),A,B,C,D at 40X.

DISCUSSION

The significant improvement in FCR observed with charcoal supplementation in this study indicates enhanced feed efficiency, which aligns with numerous reports in poultry science. Many researchers have found that adding charcoal to broiler diets benefits growth performance and efficiency. For instance, Majewska et al. (2011) reported that 21-day-old broilers fed 0.3% charcoal were about 5% heavier than control birds. Other studies across different contexts echo these positive effects: Majewska et al. (1999) with 0.3% dietary charcoal, Edrington et al. (1997) with 4.4%, Shareef et al. (1998) with 0.5% in turkey diets, and Kutlu et al. (2000) all observed improvements in weight gain or FCR following charcoal inclusion. These consistent findings underscore charcoal's role in enhancing nutrient utilization. However, not every study found long-lasting effects; Abu Bakr (2008) reported that citrus-wood charcoal improved broiler weight only during the first week of supplementation, suggesting that the timing and duration of charcoal's benefits may vary. Overall, a broad body of poultry research supports the efficacy of dietary charcoal in boosting performance metrics.

In our study, charcoal's efficacy was evident not only in better FCR but also in lower lesion scores, indicating improved gut health. This dual benefit of enhanced feed efficiency and gut integrity is in line with recent findings by Hassan et al. (2023), who observed that charcoal or biochar additives improved feed utilization and maintained intestinal health in birds under dietary or disease stress. Nair et al. (2023) highlighted a plausible mechanism for these effects, noting that biochar's adsorptive properties can bind intestinal toxins and irritants, thereby mitigating enteric inflammation. By reducing gut inflammation, charcoal likely allows nutrients to be absorbed more effectively, explaining the better feed conversion seen in charcoal-treated groups. It is noteworthy that in our trial, weight gain and feed intake were not significantly different among groups despite the improved FCR. This suggests charcoal doesn't necessarily promote faster growth in healthy birds but makes the birds more efficient at converting feed into body mass.

Charcoal's impact on gut health was evident through the improved oocyte (oocyst) index and the dramatic reduction in fecal oocyst counts in treated birds. These outcomes indicate a potent anticoccidial effect, which is supported by similar findings in the literature. Zhu et al. (2023) reported that broilers given biochar had improved intestinal barrier function and significantly lower *Eimeria* oocyst shedding, attributing this to charcoal's ability to modulate the gut environment and inhibit parasitic proliferation. In our study, the charcoal-fed groups experienced far fewer oocysts in the feces compared to controls, implying that charcoal either impeded the replication of *Eimeria* or enhanced the birds' ability to cope with the infection.

When assessing anticoccidial efficacy, researchers commonly evaluate multiple parameters: body weight gain, FCR, lesion scores, and oocysts per gram of feces (OPG). In the current study, charcoal improved several of these metrics, most notably reducing lesion scores and oocyst output, which demonstrates its protective effect against coccidiosis. Typically, oocyst shedding in coccidial infections peaks around 5–7 days post-infection (Al-Badri and Barta, 2012) and interventions aim to reduce this peak and hasten recovery. Our findings of lowered oocyst count in charcoal-treated birds around the expected peak period suggest that charcoal successfully suppressed the parasite's life cycle. It should be noted that the relationship between oocyst load and infection severity can be complex due to the "crowding effect," where extremely high parasite doses lead to competition and a reduction in oocyst output per parasite. Nevertheless, monitoring OPG remains valuable for gauging infection levels and the success of interventions. In our case, the substantially lower OPG in charcoal groups clearly reflected a milder infection and effective control measure. This agrees with Chasser et al. (2020), who emphasized that reduced oocyst shedding indicates a lower replication rate of the pathogen and can be used to judge the efficacy of anticoccidial strategies. Thus, the charcoal-induced decline in oocyst shedding and lesions in our study strongly supports its role as a natural anticoccidial additive.

Coccidial infection is known to induce oxidative stress and inflammation in the gut, compounding tissue damage (El-Ghareeb et al., 2023). In the present study, charcoal supplementation significantly bolstered the antioxidant defense system (higher TAC) and reduced markers of oxidative damage (lower MDA) in infected broilers. El-Ghareeb et al. (2023) noted that adding certain antioxidants and adsorptive plant extracts to diets effectively neutralizes ROS in the gastrointestinal tract,

thereby diminishing inflammation. Our results align with this principle, as charcoal-treated birds showed mitigated oxidative stress despite the coccidial challenge. In fact, the charcoal groups had MDA levels comparable to uninfected or less-stressed birds, indicating that charcoal helped counteract the pro-oxidant effects of *Eimeria* infection. Abd El-Maksoud et al. (2014) similarly documented that interventions reducing oxidative stress (in their case, antioxidant supplements) led to better outcomes in coccidiosis, paralleling the reduction in MDA we observed (Fig. 10 and 11).

The histopathological improvements with charcoal, such as longer villi, suggest an enhancement in the gut's capacity to absorb nutrients and recover from injury. Damage to the intestinal lining by coccidia typically shortens villi and impairs absorption, but charcoal-fed birds maintained significantly greater villus length (Table 6) and overall mucosal integrity than controls. This outcome is consistent with findings by Ruttanavut et al. (2009) and Yamauchi et al. (2010), who reported that certain dietary additives (including charcoal in some cases) preserved villus architecture and even improved villus height, leading to better growth performance. The improved villus morphology in our study can be attributed to charcoal's ability to bind and neutralize harmful substances (such as coccidial toxins or inflammatory molecules) in the gut environment, thereby protecting the epithelial lining.

The clear reduction of coccidiosis severity in charcoal-fed groups highlights charcoal as an effective natural anticoccidial agent. We observed markedly lower lesion scores and oocyst shedding in Tch1 and Tch2, mirroring the results of recent studies that have tested biochar against *Eimeria*. Zhu et al. (2023) reported significant reductions in intestinal lesion severity and oocyst output in broilers supplemented with biochar, affirming its efficacy against coccidial infections. These similar outcomes across independent studies strengthen the evidence that charcoal/biochar can inhibit *Eimeria* proliferation or at least mitigate its pathological effects. The exact mechanisms are still being investigated, but possibilities include toxin adsorption, modulation of gut pH, or providing a beneficial matrix for gut microbiota that outcompete or antagonize *Eimeria*.

One concern with using adsorbents like charcoal in feed is the potential binding of essential nutrients, which could lead to deficiencies. However, our results showed no significant changes in serum phosphorus, magnesium, or zinc levels with charcoal supplementation, indicating that the charcoal did not appreciably impede the absorption of these important minerals. This finding underscores the selective adsorption properties of charcoal: it tends to bind toxic compounds or excess moisture and gases, while having a minimal effect on minerals at the inclusion levels used.

The enhancement in final body weight and meat quality parameters (such as water-holding capacity) in charcoal-supplemented broilers suggests that the benefits of charcoal extend all the way to processing and product quality. By improving feed conversion and gut health, charcoal likely allowed birds to channel more nutrients into growth and muscle development, yielding heavier birds with better-quality meat. Tufarelli et al. (2022) and Laudadio et al. (2021) both reported that adding biochar to broiler diets led to superior carcass yields and improved meat characteristics, which is in agreement with our findings. They attributed these improvements to more efficient nutrient uptake and metabolism, as well as reduced subclinical health issues, during the rearing period. In our study, the higher water-holding capacity in meat from charcoal-fed birds is particularly notable, as WHC is linked to juiciness and tenderness in poultry meat. A possible explanation is that the improved antioxidant status in charcoal birds protected muscle cells from oxidative damage, thereby preserving cell integrity and the ability of muscle tissue to retain water post-mortem. Additionally, the absence of any negative effects on meat (such as no off-odors, discoloration, or textural problems observed) indicates that charcoal is a safe feed additive that does not impart any undesirable qualities to the animal or its products. Overall, the enhancements in growth and meat quality reinforce the idea that charcoal can be a multifaceted additive: not only controlling disease and improving gut function, but also contributing to better production and product outcomes.

Implications and Future Directions

This study highlights the multifaceted benefits of dietary charcoal in broiler production, from improved feed efficiency and growth performance to enhanced gut health and coccidiosis control. The evidence suggests that charcoal could be integrated as a natural feed additive to promote poultry health and productivity, potentially reducing the need for certain medications. Looking ahead, there are several avenues for future research. One important direction is to explore the synergistic effects of combining charcoal with other feed additives, such as probiotics, prebiotics, or organic acids. Charcoal might work in concert with beneficial microbes or acidifiers to further stabilize the gut environment and inhibit pathogens. Additionally, long-term studies are needed to assess how continuous charcoal supplementation affects the gut microbiota diversity and whether it might select for a healthier microbial community that confers resistance to diseases. Monitoring for any development of tolerance or changes in efficacy over multiple flock cycles would also be valuable. Another consideration is the environmental impact: using charcoal in feed could potentially influence manure composition and nutrient content, so research should evaluate how excreted charcoal affects litter quality and whether it offers any benefits for waste management (for example, odor reduction or improved fertilizer value of manure). Finally, economic analyses would help determine the cost-effectiveness of large-scale charcoal use in commercial operations. By addressing these questions, future investigations can build upon our findings and help optimize the use of charcoal in sustainable poultry production systems.

Conclusion

The present findings demonstrate that supplementing

broiler diets with charcoal can alleviate the adverse effects of coccidiosis and improve overall performance. The beneficial outcomes were attributed to the presence of bioavailable microelements in charcoal and its detoxifying action, which reduces the surface tension of intestinal digesta and supports liver function in fat digestion. In summary, dietary charcoal improved feed conversion ratio (FCR), enhanced gut health (through reduced lesion scores and better villus structure), and bolstered the immunity and antioxidant status of broiler chickens under coccidial challenge. These results suggest that charcoal is a promising natural additive to improve poultry health and productivity in the face of enteric pathogens.

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