poultry

Communication

Assessing the Effects of Phytogenic Feed Additives on Broilers during a Necrotic Enteritis Challenge

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Abstract: Subclinical necrotic enteritis (NE) is an enteric disease that inflicts significant economic losses in the poultry industry, primarily by reducing performance in commercial flocks but without significant mortality. This study evaluated the effects of a variety of phytogenic blends on broilers' performance and carcass composition during an induced NE challenge. In this study, 1120 day (d)-old male broilers were allocated to four treatments groups (14 replicate floor pens, 20 birds/pen): the control (CONT) group, fed a basal corn-soybean diet, and three phytogenic blend dietary additives (PHYTO1, PHYTO2, and PHYTO3) added to the basal diet at 150, 250, and 500 mg/kg feed, respectively. Subclinical NE was induced by spraying a concentrated coccidiosis vaccine onto the feed and litter 24 h post-placement. On day 8, two birds/pen were necropsied for NE lesions. On days 8, 14, 28, and 42, the average daily gain (ADG), feed intake (ADFI), and feed conversion ratio (FCR) were calculated. On day 42, two birds/pen were euthanized to assess carcass composition using dual-energy X-ray absorptiometry (DXA). Statistical analyses for all data were performed using the ANOVA procedure (JMP, Pro 16) and significance ($p \leq 0.05$) between treatments was determined by the LSD test. There was no effect of treatment on NE lesions. PHYTO1, PHYTO2, and PHYTO3 significantly improved FCR from days 9 to 14, 0 to 14, and 0 to 42 and resulted in greater ADG from days 9 to 14, 29 to 42 and cumulatively on days 0 to 42. Carcass composition data revealed a numerically higher lean-to-fat ratio in the PHYTO groups compared to the CONT group. These results indicate that the dietary supplementation of phytogenic blends could alleviate the adverse effects of NE challenge on broilers' performance and carcass composition.

Keywords: necrotic enteritis; phytogenic; broiler; performance; body composition

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1. Introduction

Necrotic enteritis (NE) is primarily caused by *Clostridium perfringens* types A and G, leading to both clinical and subclinical infections [1]. This condition significantly impacts the poultry industry, with a global estimated annual cost of USD 6 billion [2]. Subclinical NE infections result in reduced body weight gain (BWG), feed intake (FI), and digestibility, along with increased feed conversion ratio (FCR), intestinal lesions, and occurrences of wet litter and diarrhea [3].

Consumer preferences have prompted the industry to transition to "antibiotic-free" or "no antibiotics ever" production methods, contributing to a rise in NE cases among broiler chickens [4]. Consequently, there has been a growing interest in evaluating alternative products, such as phytogenics, to enhance bird performance and mitigate the adverse effects of bacterial and environmental stressors [5,6]. Phytogenics, also known as phytobiotics, encompasses a diverse range of natural plant-derived bioactive compounds incorporated into animal feed or water [7,8]. These compounds include phenols, flavonoids, tannins, saponins, and essential oils [9]. Phytogenics have gained attention due to their safety profiles, productivity-enhancing properties [10], antimicrobial activity [11], pathogen prevalence reduction [12], and digestibility [13].

Despite increased interest, uncertainty remains regarding the effects of phytogenic blends on broiler performance and carcass composition during NE due to variations in their content. Therefore, this study aimed to investigate the impact of specific phytogenic blends on the average daily gain (ADG), FCR, gross lesion scores, and body composition of broiler chickens challenged with NE.

2. Materials and Methods

2.1. Bird Management and Diets

This study was conducted under the Institutional Animal Care and Use Committee guidelines. A total of 1120 day-old Cobb male broiler chicks were sourced from a local hatchery, weighed in groups of 20, and allocated to 28-floor pens. Floor pens were ~1.22 m × 2.44 m and covered with fresh pine shavings as starting litter. There were four treatments, including a control group (CONT), birds fed a corn-soybean meal basal diet; phytogenic group 1 (PHYTO1), birds fed the CONT diet supplemented with an encapsulated combination of three purified phytogenic essential oils (the full composition is proprietary) at 150 mg/kg; phytogenic group 2 (PHYTO2), birds fed the CONT diet supplemented with essential oils of thyme and star anise and *Quillaja saponaria* at 250 mg/kg; and phytogenic group 3 (PHYTO3), birds fed the CONT diet supplemented with prebiotics, probiotics, plant extracts, and butyric acid at 500 mg/kg. All additives were utilized at the manufacturers' recommendation levels. The diets were crumbled for the starter phase (days 0 to 14) and pelleted for the grower (days 15 to 28) and finisher (days 29 to 42) phases (Table 1). Chicks had ad libitum access to feed and water using bucket-type feeders and nipple drinker lines, and environmental temperatures and light conditions followed the recommendations outlined in the *Cobb Broiler Management Guide*.

Table 1. Composition of basal diets (as-fed basis, %) [14].

	Feeding Phase (Days) [a]		
Ingredients (%)	**Starter (1–14)**	**Grower (14–28)**	**Finisher (28–42)**
Corn (7.81% CP)	59.53	64.12	65.70
Soybean meal (48% CP)	33.5	28.80	26.86
Soybean oil (9000 kcal/kg)	2.18	2.60	3.50
Dicalcium phosphate (18.5% P, 22% Ca)	2.05	1.92	1.70
Calcium carbonate (37% Calcium)	1.11	1.00	0.90
Sodium chloride	0.3	0.3	0.3
Sodium bicarbonate	0.07	0.07	0.05
DL-methionine (990 g/kg) [b]	0.38	0.34	0.29
L-lysine hydrochloride (788 g L-Lysine/kg) [c]	0.37	0.35	0.24
L-threonine (985 g/kg) [d]	0.15	0.14	0.10
Vitamin/trace mineral premix [e]	0.36	0.36	0.36
Calculated analysis (% unless specified)			
ME (kCal/kg)	3007	3087	3168
Crude protein	21.81	19.90	18.94
Total phosphorus	0.76	0.71	0.66
Available phosphorus	0.45	0.42	0.38
Calcium	0.90	0.84	0.76
Chlorine	0.33	0.33	0.29
Sodium	0.16	0.16	0.15
Potassium	0.85	0.77	0.73
Methionine	0.67	0.61	0.55
Methionine + cysteine	0.98	0.89	0.82
Lysine	1.32	1.19	1.05

Table 1. *Cont.*

	Feeding Phase (Days) [a]		
Ingredients (%)	**Starter (1–14)**	**Grower (14–28)**	**Finisher (28–42)**
Threonine	0.86	0.78	0.71
Linoleic acid	1.44	1.52	1.55
Dietary cation–anion balance (mEq)	194	174	170

[a] The supplements were added to the basal mixes to provide the six experimental diets in every feeding phase. [b] Rhodimet® NP9, ADISSEO. [c] L-Lysine HCl, AJINOMOTO HEARTLAND. [d] FENCHEM Ingredient Technology. [e] Vitamins supplied per kg diet: retinol 3.33 mg, cholecalciferol 0.1 mg, α-tocopherol acetate 23.4 mg, vitamin K3 1.2 mg, vitamin B1 1.6 mg, vitamin B2 9.5 mg, niacin 40 mg, pantothenic acid 9.5 mg, vitamin B6 2 mg, folic acid 1 mg, vitamin B12 0.016 mg, biotin 0.05 mg, and choline 556 mg. Minerals supplied per kg diet: Mn 144 mg, Fe 72 mg, Zn 144 mg, Cu 16.2 mg, I 2.1 mg, and Se 0.22 mg.

2.2. Necrotic Enteritis Challenge and Lesion Scoring

Feed and litter were sprayed 24 h post-placement with 10 × coccidiosis vaccine (Coccivac-B52; Merck Animal Health, Omaha, NE, USA). This vaccine contains live oocysts of *Eimeria acervulina*, *E. maxima*, *E. maxima MFP*, *E. mivati*, and *E. tenella*, which, when coupled with the presence of *C. perfringens* spores in the barn environment, leads to the development of an NE outbreak around one week after the challenge [15].

On day 8, two birds were randomly selected based on the average body weight of each pen and euthanized by cervical dislocation, and the small intestines were removed to examine NE lesions. The duodenum, jejunum, and ileum were scored separately based on a 0–4 scale system: 0 = no gross lesions, normal intestinal appearance; 1 = thin-walled or friable, gray appearance; 2 = thin-walled, focal necrosis, gray appearance, small amounts of gas production; 3 = thin-walled, sizable patches of necrosis, gas-filled intestine, small flecks of blood; 4 = severe extensive necrosis, marked hemorrhage, large amounts of gas in the intestine [16]. Scoring was conducted by personnel blinded to the treatments.

2.3. Performance

Birds were weighed on a per-pen basis on days 0, 8, 14, 28, and 42. If any dead birds were found, their body weight was recorded. Adjustments were made when calculating the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) for the starter, grower, finisher, and overall experimental phases, as previously described [17].

2.4. Carcass and Body Composition

On day 42, two birds per pen were wing-banded and euthanized via cervical dislocation. Birds were individually tagged, de-feathered, weighed, and stored at −20 °C until further analysis. Carcasses were then thawed and scanned by DEXA/DXA (dual-energy X-ray absorptiometry) using a GE Healthcare Lunar Prodigy Advance System (General Electric, Madison, WI, USA). The Prodigy Small Animal Software was used to calculate de-feathered carcass fat and lean composition (%).

2.5. Statistical Analysis

Performance and body composition data analyses were subjected to one-way ANOVA using the JMP Pro 16.2 program, and a Chi-squared test was used for lesion scores. The LSD test compared separate means when significant differences were noted. Statistical differences were considered significant at $p \leq 0.05$.

3. Results and Discussion

The present study was designed to investigate the effects of various phytogenic blends on the performance, gross lesions, and body composition of 42 day-old broilers during an NE challenge. Broiler performance is chiefly assessed by ADG and FCR. In poultry production, optimizing feed utilization without compromising growth is crucial due to the substantial cost of broiler feed, which constitutes around 75% of the total expenses [18]. As the use of some or all antibiotics in poultry diets is being reduced, there is a growing need to explore alternative protective measures for enteric diseases, such as NE, that can enhance chicken production efficiency while addressing food safety concerns [7]. Incorporating phytogenic blends into poultry diets has shown promising effects in improving health and performance in broilers challenged with NE [19,20]. In this study, supplementation with PHYTO1, PHYTO2, and PHYTO3 resulted in improved feed efficiency during subclinical NE, as evidenced by increased cumulative ADG (days 0 to 42) and a significant reduction in FCR from days 8 to 14, 0 to 14, and 0 to 42 compared to the CONT group (Table 2). Earlier research also reported similar improvements in FCR and weight gain when using either a single phytogenic or a blend of phytogenics [21–23]. However, the efficacy of phytogenic blends can vary based on the inclusion levels of their components, ranging from neutral to beneficial effects [10,24,25]. Typically, during a subclinical NE challenge, there are lower mortality rates compared to a clinical challenge [1], and a similar trend was seen in our study. There were no significant differences ($p = 0.92$) among treatments with the following cumulative mortality: CONT 7.67%, PHYTO1 7.69%, PHYTO2 6.92%, and PHYO3 6.54%.

Table 2. Effects of phytogenic feed additives on average daily gain (g/bird), average daily feed intake (g/bird), and feed conversion ratio (g/g bird).

	Dietary Treatments [1]				*Statistics*	
	CONT	**PHYTO1**	**PHYTO2**	**PHYTO3**	***SEM***	***p*-Value**
Days 0 to 8						
ADG	25.12	25.14	24.43	24.68	0.31	0.2959
ADFI	27.63	27.65	27.71	27.43	0.32	0.9308
FCR	1.10	1.10	1.13	1.11	0.01	0.1535
Days 9 to 14						
ADG	34.40	37.77	35.84	37.97	1.04	0.0617
ADFI	51.58	52.47	51.85	52.40	0.97	0.8958
FCR	1.52 ^{a}	1.39 ^{b}	1.45 ^{ab}	1.38 ^{b}	0.03	0.0123
Days 0 to 14						
ADG	33.51	34.89	33.33	34.82	0.55	0.0879
ADFI	43.06	43.23	42.88	43.33	0.57	0.9464
FCR	1.29 ^{a}	1.24 ^{b}	1.29 ^{a}	1.25 ^{b}	0.01	0.0088
Days 15 to 28						
ADG	79.41	77.55	77.67	81.84	1.33	0.1222
ADFI	120.81	122.59	123.16	126.96	1.74	0.1123
FCR	1.53	1.58	1.59	1.55	0.02	0.1225
Days 0 to 28						
ADG	59.05	59.29	57.48	60.61	0.92	0.1334
ADFI	87.00	88.37	87.13	90.15	1.11	0.1731
FCR	1.48	1.49	1.52	1.49	0.01	0.3093
Days 29 to 42						
ADG	116.71	126.82	127.38	123.78	3.19	0.0824
ADFI	199.96	206.31	204.94	206.41	2.23	0.1488
FCR	1.74	1.63	1.62	1.67	0.04	0.1249
Days 0 to 42						
ADG	60.61	62.97	63.77	63.66	1.28	0.2748
ADFI	97.32	99.73	101.13	100.52	2.09	0.5923
FCR	1.61 ^{a}	1.58 ^{b}	1.59 ^{b}	1.58 ^{b}	0.01	0.0241
% Mortality	7.67	7.69	6.92	6.54	1.42	0.9213

In each row, numbers with different letters (a–b) differ significantly. [1] Treatments included control (CONT): corn–soybean meal basal diet, CONT + 150 mg/kg of phytogenic blend (PHYTO1), CONT + 250 mg/kg of phytogenic blend (PHYTO2), and CONT + 500 mg/kg of phytogenic blend (PHYTO3).

To gain deeper insights into the observed positive effects on performance, DXA is an in-depth analysis that can estimate the fat, lean, and bone mineral composition of bird carcasses [15,26,27]. Figure 1 illustrates that, while there were no statistical differences in lean and fat tissues among the four treatments, all PHYTO supplementation groups resulted in numerically elevated levels of lean muscle and reduced fat. During enteric infections, a decrease in lean tissue or body fat may indicate birds reallocating energy to immune processes [26] or improving nutrient digestion and absorption [6,28]. Additionally, the positive correlation observed between lean tissue, increased ADG, and improved FCR supports the use of body composition as a predictor of the economic and market value of broiler production [29]. Commercially, even subtle improvements in lean-to-fat ratios could translate into significant economic benefits for producers.

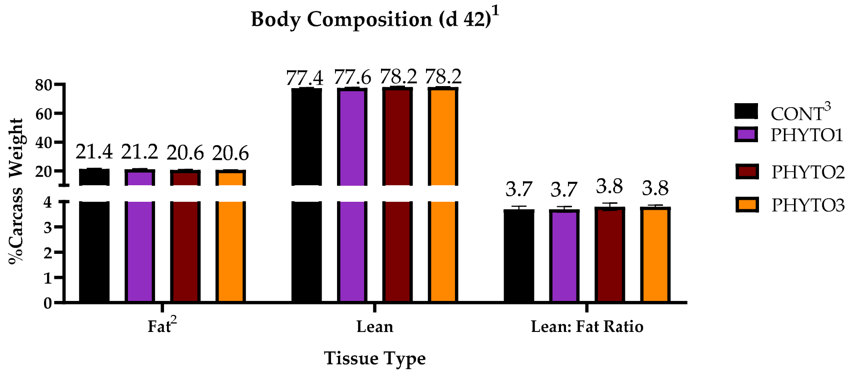

Figure 1. Effects of phytogenic feed additives on the body composition of broilers at day 42 during a subclinical necrotic enteritis challenge. [1] Each bar represents the mean ± SE values of 14 replicate pens and two birds/pen. [2] Fat and lean are represented as the percent composition of the defeathered whole carcass. [3] Treatments included control (CONT): corn–soybean meal basal diet, CONT + 150 mg/kg of phytogenic blend (PHYTO1), CONT + 250 mg/kg of phytogenic blend (PHYTO2), and CONT + 500 mg/kg of phytogenic blend (PHYTO3).

Broiler performance and intestinal lesion scores are important parameters for assessing the severity of enteric diseases including coccidiosis and NE [15]. Although NE lesions were observed, no statistical differences in lesion scores were found among treatments; however, all three PHYTO groups exhibited lower lesions compared to CONT (Figures 2 and 3). Phytogenics are known to increase thickness in the small intestine and promote mucus production, which can reduce the possibility of adhesion to the epithelium and pathogen colonization [30]. Certain plant extracts reduce oocyst shedding of *Eimeria*, the proliferation of *C. perfringens*, and intestinal lesions under both *Eimeria* and NE infections, thus mitigating intestinal damage and lowering the risk of disease-related mortality in birds [31,32].

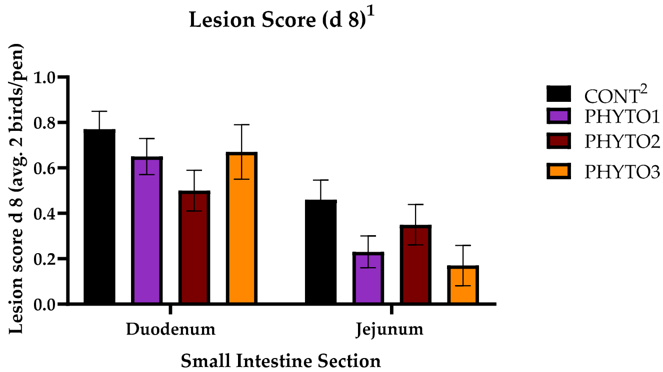

Figure 2. Effects of phytogenic feed additives on the necrotic enteritis lesion scores of broilers at day 8 during a subclinical challenge. [1] Data represent gross lesion scores in the small intestine sections (duodenum, jejunum, and ileum). Each bar represents the mean ± SE values of 14 replicate pens and two birds/pen. [2] Treatments included control (CONT): corn–soybean meal basal diet, CONT + 150 mg/kg of phytogenic blend (PHYTO1), CONT + 250 mg/kg of phytogenic blend (PHYTO2), and CONT + 500 mg/kg of phytogenic blend (PHYTO3).

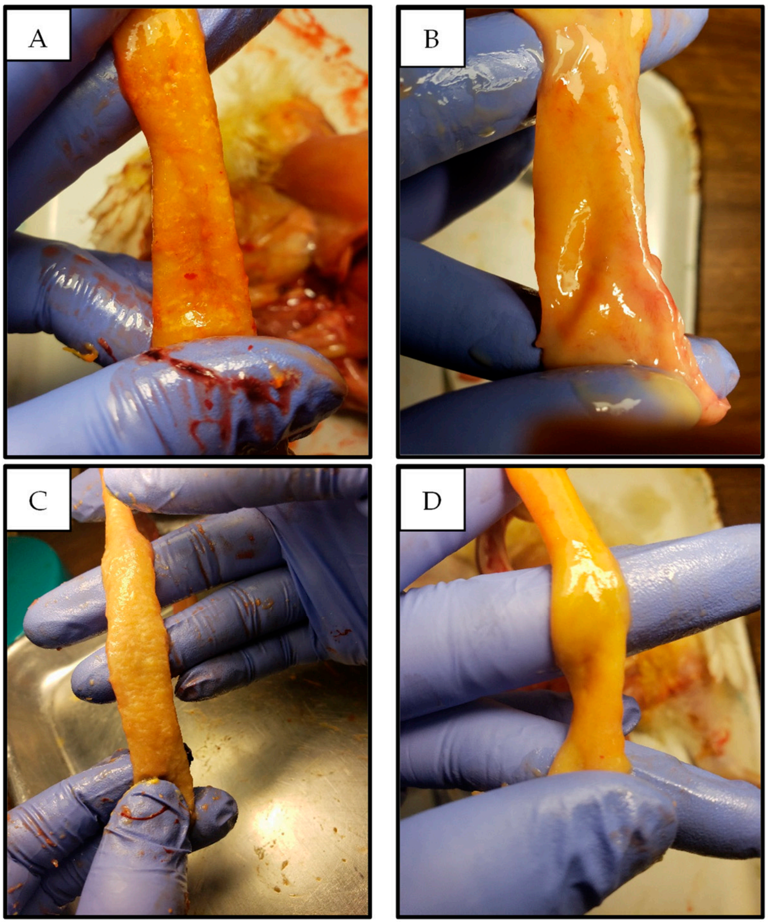

Figure 3. Gross lesions in the jejunum experimentally induced during a subclinical necrotic enteritis challenge. (**A**) CONT; (**B**) PHYTO1; (**C**) PHYTO2; (**D**) PHYTO3. Treatments included control (CONT): corn–soybean meal basal diet, CONT + 150 mg/kg of phytogenic blend (PHYTO1), CONT + 250 mg/kg of phytogenic blend (PHYTO2), and CONT + 500 mg/kg of phytogenic blend (PHYTO3).

4. Conclusions

In this subclinical necrotic enteritis model, the phytogenic blends resulted in greater average daily gains and lower FCR throughout the experimental trial compared to the control birds. Although not statistically significant, all phytogenic-supplemented groups resulted in reduced intestinal lesion scores in the duodenum and jejunum while also increasing the lean-to-fat ratio in market-age birds. Therefore, this study provides valuable insights into the potential mechanisms by which these phytogenic blends could improve performance while alleviating the effects of an enteric challenge. By demonstrating improved feed efficiency and favorable differentials in body composition and pathology, the results suggest that phytogenics may enhance broiler health and productivity under conditions of subclinical necrotic enteritis. Understanding these mechanisms is crucial in optimizing poultry nutrition strategies and addressing challenges posed by enteric diseases in the absence of antibiotics. Further research exploring the specific modes of action of phytogenic compounds and their interactions within the avian gastrointestinal tract could lead to more targeted and effective interventions in poultry production.

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