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Original Article

Efficacy and Optimal Feeding Level of Rumex Nervosus Leaves on Blood Biochemistry, Carcass Characteristics, Productivity Indices, and Anticoccidial Indicators of Broiler Chickens Infected or Not Infected with Eimeria Tenella

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Anticoccidial efficiency, broiler chickens; *Rumex nervosus* leaves; performance indicators; small intestine.

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ABSTRACT

The current study investigated the efficacy and optimal feeding level of *Rumex nervosus* leaves (RNL) in broilers infected and uninfected with *Eimeria tenella*. In a factorial design of 2 (coccidial challenge or not) 5 (feed treatments), 250-day-old broiler chicks were randomly assigned to one of ten groups and reared for 28 days. Intestinal measurements, carcass traits, serum biochemical indices, leukograms, performance and anticoccidial indicators were investigated. All selected parameters were not significantly (p>0.05) altered by the interactions between the experimental diet and challenge at 7 days post-infection, with the exception of serum levels of albumin and globulin, as well as the anticoccidial index. Infected broilers had a lower performance indicator, poorer production efficiency, poor anticoccidial indices, heavier gizzard, longer and heavier small intestine, shorter cecum, and higher proportion of eosinophils compared with uninfected broilers. The RNL had marked anticoccidial efficacy at a dose of 5 g. The current study found that *Rumex nervosus* leaf powder had mild to marked, dose-dependent anticoccidial preventive efficacy in broilers. However, further studies are needed to fully understand the anticoccidial mechanisms.

INTRODUCTION

The poultry industry is one of the most important agricultural subsectors in the world, contributing to meet the increasing demand for meat and eggs (Neethirajan, 2022). In recent decades, the enormous production sometimes led to an increase in diseases such as microbial or parasitic diseases. In broiler chickens, Eimeriosis caused by intestinal protozoa results in high mortality, stunted growth, and poor feed conversion. Coccidiosis leads to economic losses of up to $3 billion worldwide (Khater *et al.*, 2020). *Eimeria tenella*, *Eimeria necatrix*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria praecox*, and *Eimeria mitis* cause avian coccidiosis. The incidence of intestinal lesions is determined by the *Eimeria* species. *Eimeria tenella*, for example, can penetrate and damage cecal cells (López-Osorio *et al.*, 2020). A natural product such as garlic has been used as a natural feed additive for prophylaxis and cure of coccidiosis to reduce economic losses caused by *Eimeria tenella* (Kuraa *et al.*, 2021). The use of feed additives has played an important role in improving production, growth efficiency, disease prevention, and feed utilization.

Measures to control coccidia in broiler chickens are currently primarily single coccidia control measures or vaccines. However, when ionophores are used alone, infestation with *Eimeria tenella* results in increased mortality and reduced growth performance, and when chemical coccidial drugs are used alone, there is a risk of developing drug resistance, limiting their efficacy (Harfoush *et al.*, 2010). When coccidial vaccines are used alone, chickens are at higher risk of enteritis at younger ages and at higher risks of necrotic enteritis with age due to inadequate coccidiosis prevention and control, and they have lower production performance (Williams, 2002). In addition, the effects of a single vaccine and/or combinations with different coccidiostats on broiler chickens are varied (Lei *et al.*, 2022). Due to growing concerns about drug resistance, researchers are forced to search for natural, safe, and non-therapeutic alternatives to synthetic ionophores, such as herbs and essential oils (Hussein *et al.*, 2019; Mustafa *et al.*, 2021; Liu *et al.*, 2022).

Natural strategies have been investigated for use in poultry production recently. Supplementing poultry feed with natural products containing bioactive components has shown promising results (Alagawany *et al.*, 2019; Gado *et al.*, 2019; El-Hack *et al.*, 2020; Liu *et al.*, 2022). The coccidial inhibitory effect of 3,4,5-trihydroxybenzoic acid can be enhanced by the addition of oregano extracts to improve animal performance and eliminate adverse effects in coccidiosis-infected broilers for 34 days (Nawarathne *et al.*, 2022). Several of the approximately 200 species of the genus Rumex are considered good sources of traditional medicines for the treatment of inflammation, cancer, and various bacterial infections due to bioactive compounds, particularly quercetin 3-O-glucoside, nepodin, emodin, trans-resveratrol, and torachryson (Vasas *et al.*, 2015). Rumex nervosus “Othrob”, a medicinal plant of the family Polygonaceae, has traditionally biological and pharmacological properties for the treatment of various diseases (Nigussie, 2020) and acts as an antiparasitic (Ali *et al.*, 2016).

Previous studies using RNL as an extract included pharmacognostic studies such as Al-Sunafi (2016), *in vitro* antimicrobial and antioxidant studies such as Desta *et al.* (2016) and AlMousa *et al.* (2022), and *in vitro* and *in vivo* studies such as Al-Quraishy *et al.* (2020) and Qasem *et al.* (2020). Thus, our research contributed to the knowledge on the efficacy of dietary *Rumex nervosus* leaves powders as an alternative to salinomycin in broilers infected with *Eimeria tenella* and compared it with uninfected broiler chickens. Previously, RNL extract was administered orally as a therapeutic agent after *Eimeria tenella* oocysts infected broilers chickens to affect the final stage of the parasite’s life cycle, whereas here RNL was administered with broiler chick feed as a preventive agent to affect the parasite’s early stages of life. Therefore, this study was conducted to determine the effects of *Rumex nervosus* leaves on intestinal measurements, carcass traits, serum biochemical indices, leukogram, and anticoccidial index in broilers infected and uninfected with *Eimeria tenella*.

MATERIALS AND METHODS

Ethical Approval

Parameters were taken in accordance with Saudi Arabian standards for the use of animals and with the approval of the KSU Animal Care and Welfare Committee (KSU-SE-20-44).

Birds and experimental diets

The study was conducted at the Animal Production Experimental Farm of King Saud University (KSU), in Riyadh, Saudi Arabia. A total of 250 one-day-old, mixed-sex Ross 308 broiler chicks (45.71 ± 0.02 g) were obtained from a local hatchery (Al Khumasia Company) distributed into 50 cages and reared for 28 days. At 21 days of age, *Eimeria tenella* oocysts were orally administered into the five replicate cages of each positive group. The 1 mL suspension of distilled water contained $4*10^4$ sporulated oocysts/chicken of *Eimeria tenella* and were injected directly into the pharynx into each of the five replicate cages of the positive groups at 21 days of age using a long injector pipette (Lee *et al.*, 2012; Al-Shaibani *et al.*, 2020). Thus, five replicate cages per treatment with five birds (2♀ and 3♂) per cage were randomly assigned to ten groups (Figure 1) in 2 (coccidial challenge or not)×5 (feeding treatments):

1. 1 g *Rumex nervosus* leaves (RNL)/kg, without coccidia challenge (negative 1 g RNL).
2. 1 g RNL/kg, with coccidia challenge (positive 1 g RNL).
3. 3 g RNL/kg, without coccidia challenge (negative 3 g RNL).
4. 3 g RNL/kg, with coccidia challenge (positive 3 g RNL).
5. 5 g RNL/kg, without coccidia challenge (negative 5 g RNL).
6. 5 g RNL/kg, with coccidia challenge (positive 5 g RNL).
7. 66 mg/kg Salinomycin, without coccidia challenge. Salinomycin as standard anticoccidial ionophore drug (negative Salinomycin).
8. 66 mg/kg Salinomycin, with coccidiosis challenge (positive Salinomycin).
9. Basal diet without coccidia challenge (negative control).
10. Basal diet with coccidia challenge (positive control).

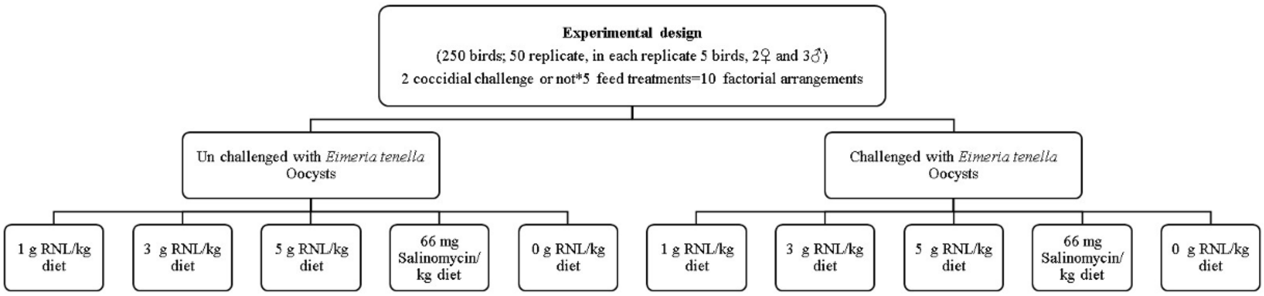

Figure 1 – Experimental design illustration.

The ingredients, chemical composition, and energy value of broilers diets are described in our previous paper (Qaid *et al.*, 2021b). Feed and water were provided *ad libitum* throughout the study. Housing conditions were set at 33 °C on the first day of chick life. Thereafter, the temperature was gradually decreased by about 0.5°C per day until it reached 22 °C at 21 days of age, and then remained at this temperature for the rest of the growth cycle in the poultry house (Maiorka *et al.*, 2006; Zahraa, 2008). The relative humidity was 65-85%. An illumination program (23L "23 h on":1D"1 h off") was used as the photoperiod.

Measurement of blood parameters

From 10 birds per group (5 from the challenged and 5 from the non-challenged groups), blood samples were collected at 28 days of age from the wing vein in tubes without EDTA. For biochemical analysis, serum was separated by centrifugation at 3000 x g for 15 minutes. The serum was aspirated with a pipette and stored in Eppendorf tubes at 20°C until analysis. Total protein (TP), albumin (ALB), glucose (GLU), total cholesterol (CHOL), alanine aminotransferase (ALT), aspirate aminotransferase (AST), and creatinine were measured spectrophotometrically (Randox, U.K.) using reagent kits (Randox, London, U.K.). Serum globulin levels were calculated by subtracting albumin levels from total protein levels (Alqhtani *et al.*, 2022). For the determination of leukocyte differential count, a drop of fresh blood was prepared, smeared, and stained with Giemsa stain. Leukograms (heterophils, lymphocytes, eosinophils, basophils, and monocytes) were counted in 100 cells per field using a microscope (Nikon Corp., Japan), and the ratio of heterophils to lymphocytes was calculated (Qaid *et al.*, 2016; Abdelhady *et al.*, 2021).

Slaughter variables and small intestine measurements

At 28 days of age (7 dpi), one bird from each replicate pen was randomly selected and weighed individually (*n* = 10 birds/treatment). Birds were slaughtered by severing the jugular vein with a sharp knife. Slaughter weight (SW) and carcass weight (CW) were recorded to calculate carcass yield (CY; (CW/SW)*100). Bursa, spleen, thymus, proventriculus, gizzard, heart, liver, and pancreas, were all weighed. In addition the pectoral muscle without skin with whole legs (thighs with drumsticks) and keel bone were also weighed. All organ weights were expressed in g/100 g carcass weight. For example, gizzard yield (GY = (GW/CW)*100) was calculated as GW relative to CW. Total weight and length of small intestine and its parts were separated and measured.

Performance and anticoccidial indicators

Growth performance and production efficiency factor (PEF) was measured on day 28 (7 dpi) as described in (Qaid *et al.*, 2021a).

The sporulated oocysts were collected from the cecum of naturally infected Baladi chickens and passaged three times in healthy broilers at two weeks of age to assess oocyst infectivity, oocyst value, and confirm the site of lesion. After oocyte collection, the species of Eimeria isolates were validated by amplification of the internal transcribed spacer 1 (ITS-1) partial area and sequence analysis. To evaluate the efficacy or anticoccidial index (ACI) of RNL powder in each group, relative weight gain, survival rate, Oocysts value, and lesion scores were calculated as anti-coccidial indicators according to the formula described by Nouri (2022). Briefly, an ACI value below 120 was evaluated as inactive anticoccidial effect, 120 to 140 as mild or slight, 140 to 160 as moderate, 160 to 180 as marked and above 180 as excellent.

Statistical analysis

Data were analyzed with ANOVA for a randomized complete block design (RCBD) using the general linear models (GLM) of SAS software (SAS, 2012). A 5*2 factorial design was used to analyze the data, which included five levels of treatment and two levels of challenge, and their interactions. The model equation is described below:

$$\gamma_{ij} = \mu + T_i + C_j + T_{ij} + \varepsilon_{ij}$$

Where Y_{ij} is the individual observation, μ is the general experimental mean, T_i is the main effect of the i^{th} treatment, C_j is the main effect of the th challenge; TC_{ij} is the effect of the interaction of dietary treatment and coccidial challenge, e_{ijk} is a random error. When the fixed effects means and their interactions were significant, Duncan's multiple range test with a 5% probability was used to separate them.

RESULTS

The bioactive components of RNL (Gallic acid was detected with the highest concentration in RNL extracts and other 11 volatile compounds in different ratios) were detected in our previous study using GCMS and HPLC analysis (Qaid *et al.*, 2021a). Also, the total phenolics in the leaves of *Rumex nervosus* were found to be 171.15 mg GAE/g for RNL (Al-Garadi *et al.*, 2022). In addition, proximity analysis revealed that the nutritional value of this shrub is present in different ratios (Qaid *et al.*, 2021a).

The interaction effects of total protein, globulin, and anticoccidial index differed significantly (p<0.05) as shown in Figures 2 and 3, whereas other parameters did not differ (p>0.05), and thus the illustration of interactions in both infected and non-infected conditions was not required.

Leukogram and serum biochemical indices

The biochemical indices analyzed in serum blood collected on the 7th dpi are shown in Table 1. The experimental treatments, challenge, and interactions

Table 1 – Serum biochemistry parameters at 28 d (7 d post inoculation) of broiler chickens fed diets containing different levels of *Rumex nervuses* leaves and challenged with *Eimeria tenella* oocysts.

Treatment[1]	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G ratio	Glucose (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)	ALT (U/L)	AST (U/L)
Main effects of experimental diet									
1 g RNL	2.16[b3]	1.04	1.12[b]	0.98	247	114	0.456	12.8	126
3 g RNL	2.61[b]	1.18	1.43[b]	1.09	289	124	0.501	15.2	120
5 g RNL	2.86[b]	1.09	1.77[ab]	0.95	267	116	0.522	18.7	152
Salinomycin	2.63[b]	1.04	1.60[b]	0.97	260	120	0.425	11.9	125
Control	3.70[a]	1.27	2.43[a]	0.65	237	107	0.418	12.3	139
SEM±[2]	0.288	0.0673	0.276	0.167	17.11	7.94	0.0556	2.02	14.9
Main effects of coccidial challenge									
No	2.79	1.11	1.68	0.93	261	121	0.491	13.6	126
Yes	2.79	1.14	1.65	0.92	258	112	0.438	14.8	139
SEM±	0.182	0.0425	0.174	0.106	10.82	5.02	0.0352	1.27	9.41
p-value									
Diet	0.01	0.087	0.025	0.422	0.275	0.631	0.61	0.112	0.563
Challenge	0.998	0.624	0.905	0.932	0.86	0.193	0.285	0.509	0.337
Interaction	0.013	0.816	0.008	0.325	0.466	0.061	0.885	0.388	0.188

[1]The treatments consisted of a basal diet alone (control), or a basal diet plus Rumex nervosus leaves (RNL) at doses of 1, 3, and 5 g/kg, or a basal diet plus 66 mg Salinomycin/kg, with each dietary group birds subjected to infection and non-infection coccidial challenge at 21 d. [2]SEM, standard error of the mean; [3]Means of ten birds per treatment group; [a and b]Means in the column with different superscripts indicate significant differences (p<0.05). A:G ratio; Albumin: Globulin ratio.

had no significant effect on TP, albumin, globulin, albumin to globulin ratio, glucose, cholesterol, creatinine, ALT, and AST. However, the interaction between experimental diet and coccidial challenge resulted in significantly higher levels of TP (p=0.0098) and globulin (p=0.0128) in the infected non-supplemented RNL group (positive control) compared to other groups (Figure 2). However, the significant differences in TP and globulin were within normal range.

As shown in Table 2, a leukogram of chicken blood collected 7 dpi revealed no significant differences between treatments, challenged bird, or their interaction. However, the treatments had a significant effect on heterophil, eosinophil, and H/L ratio. Lower

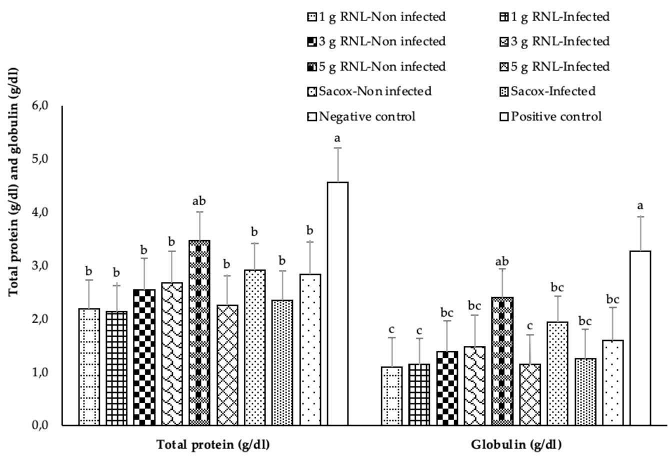

Figure 2 – Effect of interaction between experimental diets treatments and *Eimeria tenella* challenge on total protein (g/dl; SEM±1.22) and globulin (g/dl; SEM±0.39) at the seventh day of infection at the seventh day of infection.

Table 2 – Leukogram of blood samples taken on the seventh day of infection (28 days of age) in broiler chickens fed *Rumex nervosus* leaves and challenged with *Eimeria tenella* oocysts.

Treatment[1]	Heterophil	Lymphocytes	Eosinophil	Basophil	Monocytes	Heterophil: Lymphocyte ratio
Main effects of experimental diet						
1 g RNL	31.70[c3]	58.7	3.60[b]	1.6	4.3	0.55[b]
3 g RNL	33.40[bc]	57.5	3.80[b]	1.2	4.1	0.59[ab]
5 g RNL	34.40[abc]	54.6	5.40[a]	1.4	4.4	0.63[ab]
Salinomycin	36.20[ab]	55.6	2.20[c]	1.5	4.5	0.66[a]
Control	36.40[a]	55.2	4.00[b]	1.3	3.3	0.67[a]
SEM±[2]	0.937	1.253	0.418	0.322	0.348	0.03
Main effects of coccidial challenge						
No	34.56	57.04	2.96	1.44	4.12	0.61
Yes	34.28	55.6	4.64	1.36	4.12	0.62
SEM±	0.593	0.792	0.265	0.204	0.22	0.019
p-value						
Diet	0.005	0.133	<0.001	0.914	0.126	0.035
Challenge	0.74	0.206	<0.001	0.783	1.00	0.832
Interaction	0.632	0.874	0.098	0.294	0.344	0.736

[1]The treatments consisted of a basal diet alone (control), or a basal diet plus *Rumex nervosus* leaves (RNL) at doses of 1, 3, and 5 g/kg, or a basal diet plus 66 mg Salinomycin/kg, with each dietary group birds subjected to infection and non-infection coccidial challenge at 21d. [2]SEM, standard error of the mean; [3]Means of ten birds per treatment; [abc]Means in the column with different superscripts indicate significant differences ($p<0.05$).

RNL doses decreased heterophil and H/L ratios, possibly indicating that RNL relieves stress when compared to control, especially at lower doses. In addition, the blood of infected chickens had more eosinophils than that of uninfected chickens, suggesting the presence of parasite effects.

Slaughter variables

At the 7th dpi, there was no significant association between treatments, challenge, and their interactions on avian slaughter (Table 3). On the other hand, the treatments had a significant ($p<0.05$) effect on breast, and coccidial challenge had a significant ($p<0.05$) effect on gizzard. Compared to non-infected broilers, the infected birds had the highest gizzard weight relative to dressed weight. The control group had the highest percentage of breast weight, while the birds treated with 3 g RNL without coccidial challenge had the lowest percentage.

Table 3 – Effect of experimental diets treatments and *Eimeria tenella* challenge on broiler chicken carcass traits based on dressed carcass weight (% DCW) at 28 days of age.

		Carcass components and lymphoid organ weights relative to dressed carcass weight (% DCW)										
Treatment[1]	DCW	Heart	Liver	Proventriculus	Gizzard	Bursa	Spleen	Thymus	Breast	Leg	FAT	Pancreas
Main effects of experimental diet												
1 g RNL	670[3]	0.78	3.18	0.67	3.66	0.35	0.16	0.62	40.4[ab]	28.97	0.86	0.43
3 g RNL	659	0.74	3.28	0.71	3.53	0.31	0.18	0.54	37.4[b]	29.28	1.22	0.40
5 g RNL	619	0.73	3.21	0.69	3.76	0.29	0.15	0.69	38.2[ab]	30.02	1.06	0.76
Salinomycin	694	0.75	2.80	0.59	3.54	0.29	0.14	0.63	40.6[ab]	30.34	1.13	0.36
Control	711	0.70	3.10	0.61	3.60	0.27	0.12	0.55	41.6[a]	29.54	0.73	0.41
SEM±[2]	15.775	0.013	0.084	0.023	0.042	0.014	0.010	0.028	0.788	0.247	0.090	0.073
Main effects of coccidial challenge												
No	708[a]	0.77	3.03	0.65	3.38[b]	0.31	0.15	0.63	40.0	30.22	1.13	0.55
Yes	633[b]	0.71	3.19	0.65	3.85[a]	0.29	0.15	0.58	39.3	29.04	0.87	0.39
SEM±	14.37	0.016	0.060	0.015	0.083	0.014	0.008	0.025	0.455	0.309	0.078	0.078
p-value												
Diet	0.246	0.648	0.095	0.050	0.873	0.422	0.134	0.358	0.014	0.636	0.255	0.493
Challenge	0.008	0.064	0.167	0.925	0.004	0.537	0.951	0.380	0.366	0.065	0.088	0.312
Interaction	0.493	0.804	0.474	0.060	0.105	0.287	0.710	0.822	0.576	0.683	0.640	0.544

[1]The treatments consisted of a basal diet alone (control), or a basal diet plus *Rumex nervosus* leaves (RNL) at doses of 1, 3, and 5 g/kg, or a basal diet plus 66 mg Salinomycin/kg, with each dietary group birds subjected to infection and non-infection coccidial challenge at 21 d. [2]SEM, standard error of the mean; [3]Means of ten birds per treatment; [a and b]Means in the column with different superscripts indicate significant differences ($p<0.05$). Measurements of each organ percentage weight were calculated based on dressed carcass weight; Organ relative weight to dressed carcass weight = organ weight/ dressed carcass weight. FAT: abdominal fat.

Small intestine measurements

The morphometric parameters of the intestinal data after 28 days (7th dpi) are shown in Table 4. There were no significant differences in total or partial intestinal length or intestinal weight or intestinal relative weight (IRW) due to treatment or the treatment-challenge interaction (p>0.05). However, there were significant differences in total intestinal length, percent cecal length, and jejunum and ileum weight percentage due to experimental diet. Salinomycin-treated birds had a lower percentage of cecal length than the other RNL treatment groups. On the other hand, cocccidial challenge had an effect on total weight and length of the small intestine as well as the percentage of duodenal weight (p<0.05). Challenged fowl had longer and heavier total small intestine and lower percent of jejunum length. Challenged birds had a higher IRW than non-challenged birds.

Table 4 – Dietary *Rumex nervosus* leaves supplementation and anticoccidial effects on small intestine measurements at 28d of broiler chicken challenged with *Eimeria tenella* oocysts.

Treatment[1]	Total Length (cm)	Total Weight (g)	Length (%) Duodenum	Jejunum	Ileum	Ceca	Weight (%) Duodenum	Jejunum	Ileum	Ceca	IRW % DW#	% BW¥
Main effects of experimental diet												
1 g RNL	152.80^{a}	33.75[3]	15.75	41.99	42.25	20.11^{a}	20.66	45.01^{a}	34.32^{b}	19.86	5.14	3.24
3 g RNL	148.10^{a}	35.45	15.60	41.57	42.83	20.93^{a}	18.96	43.05^{ab}	37.99^{ab}	25.33	5.51	3.52
5 g RNL	152.34^{a}	34.30	14.95	41.55	43.50	19.63^{ab}	19.05	40.83^{b}	40.12^{a}	24.80	5.67	3.56
Salinomycin	134.75^{b}	28.44	16.41	42.62	40.97	16.92^{b}	19.30	45.62^{a}	35.08^{b}	25.34	4.25	2.75
Control	153.72^{a}	33.64	15.80	42.46	41.74	18.23^{ab}	18.87	44.74^{a}	36.40^{b}	23.42	4.78	3.13
SEM±[2]	3.583	1.262	0.283	0.271	0.486	0.762	0.381	0.917	1.092	1.084	0.306	0.197
Main effects of coccidial challenge												
No	140.40^{b}	30.30^{b}	16.41^{a}	41.99	41.60	20.13^{a}	19.48	44.50	36.02	25.63	4.41^{b}	2.84^{b}
Yes	156.28^{a}	35.93^{a}	15.00^{b}	42.09	42.92	18.20^{b}	19.25	43.20	37.55	21.87	5.74^{a}	3.64^{a}
SEM±	2.414	1.047	0.336	0.318	0.339	0.517	0.365	0.561	0.594	1.069	0.203	0.121
p-value												
Diet	0.011	0.184	0.761	0.730	0.154	0.044	0.540	0.035	0.012	0.432	0.061	0.082
Challenge	<0.001	0.005	0.038	0.879	0.051	0.050	0.793	0.212	0.166	0.108	0.001	0.001
Interaction	0.303	0.356	0.104	0.058	0.860	0.067	0.631	0.280	0.671	0.823	0.070	0.097

[1]The treatments consisted of a basal diet alone (control), or a basal diet plus *Rumex nervosus* leaves (RNL) at doses of 1, 3, and 5 g/kg, or a basal diet plus 66 mg Salinomycin/kg, with each dietary group birds subjected to infection and non-infection coccidial challenge at 21d. [2]SEM, standard error of the mean; [3]Means of ten birds per treatment; [a and b]Means in the column with different superscripts indicate significant differences (p<0.05). Measurements of each organ percentage weight were calculated based on dressed carcass weight; Organ relative weight to dressed carcass weight = organ weight/ dressed carcass weight.

Performance indicators and production efficiency factor

The impact of diet, challenge, and their interactions on the performance indicators and production efficiency factor (PEF) of the birds' exposure to Eimeria tenella oocysts at 7 dpi are shown in Table 5. Infestation had a significant effect (p<0.05) on body weight, body weight gain (BWG), feed intake, feed conversion ratio, and PEF. Non-infected chickens gained more weight, consumed more feed, had better feed conversion, and had higher PEF than infected chickens. Diet and the interaction between diet and challenge had no effect (p>0.05) on performance indicators or the production efficiency factor.

Anticoccidial indicators

The amount of oocysts shed on feces in infected bird groups decreased with increasing RNL dose as compared to infected untreated bird groups (Figure 3).

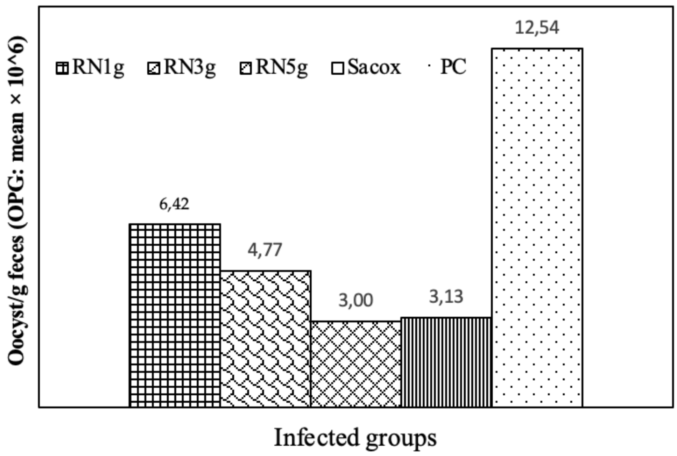

Figure 3 – Oocysts number of infected birds groups shed in gram of feces.

The effects of diet, challenge, and their interactions on anticoccidial indicators of broiler chickens' exposure to *Eimeria tenella* oocysts at 7 dpi are shown in Table 6. Infection had a significant effect (p<0.05) on Log_{10}/g excreta, relative ratio of BWG, survival rate, lesion score,

Table 5 – Effect of experimental diets treatments and *Eimeria tenella* challenge on growth performance and production efficiency factor (PEF) of broiler chicken at the seventh day of infection.

Treatment[1]	Performance productivity indicators[2]				
	BW (kg)	BWG (g)	FI (g)	FCR (g:g)	PEF
Main effects of experimental diet					
1 g RNL	1.228	70.1	108.3	1.57	294.4
3 g RNL	1.206	65.7	108.0	1.66	272.1
5 g RNL	1.214	66.6	109.4	1.67	275.2
Salinomycin	1.271	70.0	114.5	1.68	288.2
Control	1.197	70.0	108.3	1.61	283.4
SEM±[3]	0.031	2.78	3.02	0.061	14.88
Main effects of coccidial challenge					
No	1.282[a]	80.14[a]	120.21[a]	1.516[a]	318.95[a]
Yes	1.164[b]	56.82[b]	99.17[b]	1.7596[b]	246.36[b]
SEM	0.019	1.756	1.909	0.0385	9.41
p-value					
Diet	0.486	0.661	0.519	0.671	0.822
Challenge	0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction	0.31	0.882	0.972	0.664	0.562

1The treatments consisted of a basal diet alone (control), or a basal diet plus *Rumex nervosus* leaves (RNL) at doses of 1, 3, and 5 g/kg, or a basal diet plus 66 mg Salinomycin/kg, with each dietary group birds subjected to infection and non-infection coccidial challenge at 21d. [2]BW: Body weight, BWG: Body weight gain, FI: Feed intake, FCR: Feed conversion ratio. [3]SEM, standard error of the mean; n=10 per treatment; [a and b]Means in the column with different superscripts indicate significant differences ($p<0.05$).

Table 6 – Effect of experimental diets treatments and *Eimeria tenella* challenge on anticoccidial indicators at the seventh day of infection.

Treatment[1]	Anticoccidial indicators						
	Log_{10}/g excreta	Relative ratio of BW gain	Survival rate	Lesion score	Oocyst value	Anticoccidial index (ACI)	
Main effects of experimental diet							
1 g RNL	3.4	93	100	0.9	25.6	166[b]	Moderate
3 g RNL	3.3	91	100	0.8	19.0	171[ab]	Marked
5 g RNL	3.2	94	100	0.7	12.0	181[a]	Excellent
Salinomycin	3.2	96	100	0.4	12.5	184[a]	Excellent
Control	3.5	88	98.3	1.3	50.0	135[c]	Slight
SEM±[2]	0.07	1.48	0.75	0.13	5.86	8.76	
Main effects of coccidial challenge							
No	0.0[b]	101[a]	100[a]	0.0[b]	0.0[b]	200.7[a]	Excellent
Yes	6.6[a]	84[b]	99.3[b]	1.6[a]	47.6[a]	133.7[b]	In active
SEM	0.05	8.54	0.472	0.085	3.704	6.7	
p-value							
Diet	0.486	0.661	0.519	0.671	0.822	0.015	
Challenge	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.02	
Interaction	0.31	0.882	0.972	0.664	0.562	0.035	

[1]The treatments consisted of a basal diet alone (control), or a basal diet plus *Rumex nervosus* leaves (RNL) at doses of 1, 3, and 5 g/kg, or a basal diet plus 66 mg Salinomycin/kg, with each dietary group birds subjected to infection and non-infection coccidial challenge at 21d. [2]SEM, standard error of the mean; n=10 per treatment; [abc]Means in the column with different superscripts indicate significant differences ($p<0.05$).

Oocyst value, and anticoccidial index (ACI). Infected chickens had a higher discharge of fecal *Eimeria tenella* oocysts per gram, a lower relative ratio of BWG, higher mortality, a higher lesion score, and a higher active anticoccidial index (133.7) than non-infected chickens. Diet and the interaction between diet and challenge had no effect ($p>0.05$) on Anticoccidial indicators. However, anticoccidial index was affected by diet and challenge interaction (Figure 4). The anticoccidial index of all uninfected groups indicating that no lesions or *Eiemeria tenella* oocysts were found in their feces. The interaction effect of the experimental diet and coccidial challenge resulted in marked ACI efficacy (marked anticoccidial index) of salinomycin at a dose of 66 mg and RNL at a dose of 5 g. The results of the current study showed that *Rumex nervosus* leaves powder as a natural product exhibited dose-dependent anticoccidial preventive efficacy in challenged birds (mild, marked and excellent) in broilers at doses of 1 g, 3 g and 5 g, respectively. Results showed broilers fed RNL as a natural feed additive reduced numerically fecal oocyte count and cecal lesion scores to a degree comparable or equivalent to salinomycin.

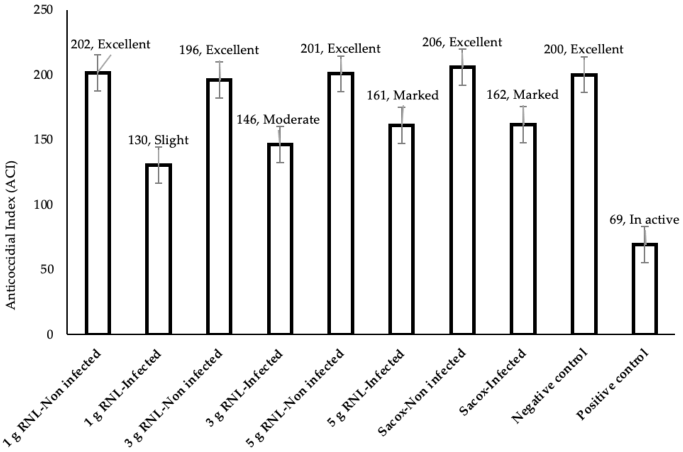

Figure 4 – Effect of interaction between experimental diets treatments and *Eimeria tenella* challenge on anticoccidial index (ACI) at the seventh day of infection.

DISCUSSION

Phenolic compounds are secondary plant metabolites containing an aromatic ring and one or more hydroxyl substituents. Our previous *in vitro* study Al-Garadi *et al.* (2022), found that total phenolic content was 171.15 mg GAE/g in the ethanolic extract for RNL. The chemical composition of the leaves of RN was investigated in the current study using HPLC and GC-MS analysis, which led to the identification of gallic acid and 13 compounds, respectively. Gallic acid is a phenolic compound found in plants and foods and has a variety of activities such as antioxidant, anti-inflammatory, and antimicrobial properties. As a result, natural or synthetic antioxidants are commonly used in the poultry industry as feed supplements to reduce oxidative stress caused by the high production of free radicals during the host cell immune response to *Eimeria* sporozoite invasion and inflammation (Zhu *et al.*, 2019; Al-Quraishy *et al.*, 2020; Adjei-Mensah and Atuahene, 2022).

Nutritional analysis revealed that RNL contained 13.63% crude protein and 52.91% carbohydrate, which resulted in a higher nutritional value (327.66 Kcal/100 g) in this study. According to the pharmacognostic study of Al-Sunafi (2016) on RNL grown in Yemen, there were no adverse effects of the currently used anticoccidial drugs on broiler chickens or even in humans. He found that the ethanolic extract 70% of RNL is not toxic up to a dose of 7.1 g/kg body weight. We believe that RNL can be considered non-toxic at the dose ranges used. Consistent with previous findings (Ashraf *et al.*, 2020; Lahlou *et al.*, 2021; Nouri, 2022), the natural product and an anticoccidial such as salinomycin significantly had mild to marked anticoccidial efficacy, including suppression of oocysts in feces, reduction of lesion levels in the cecum, and increase in anticoccidial efficacy. Amprolium, a thiamine antagonist with a high affinity for maturing first-generation schizonts, was tested (McDougald *et al.*, 1997). While salinomycin interferes with the early life cycle of the parasite during initial nuclear replication by destroying or significantly delaying schizonts maturation, it reduces activity against oocyst shedding at the asexual stage by 80-90% compared to controls (Chappel, 1979). In this study, RNL was administered as a preventive agent with broiler feed to affect the early stage of the parasite's life cycle; however, further studies are needed to determine if RNL has the same mode of action as salinomycin in destroying early-stage oocysts of *Eimeria tenella*. Although RNL, especially at a dosage of 5 g, is a similar effective agent to salinomycin that increased production efficiency while reducing the number of *Eimeria tenella* oocysts shed in feces and coccidiosis symptoms in infected birds, further studies are needed to understand the mechanism of RNL action on both the host and the parasite.

Many studies have found that coccidial challenge has negative effects on broiler health and performance (Al-Shaibani *et al.*, 2020; Balta *et al.*, 2021; Beski, 2023). It is critical to develop effective remedies against coccidiosis in animals. The poultry manufacturing is under increasing pressure to reduce the use of anticoccidiosis drugs and to search for natural sources of agents that improve immune function in animal.

The efficacy of anticoccidial products derived from natural herbs in the treatment of coccidiosis has already been demonstrated (Lan *et al.*, 2016; Al-Shaibani *et al.*, 2020; Balta *et al.*, 2021; Beski, 2023). In this study, the main effect of *Eimeria* infection resulted in significant weight loss and low feed intake, as well as a negative effect on FCR and PEF in broiler chickens. This could be due to the coccidia infection leading to poor nutrient absorption, reduced immune response, and then intestinal tissue damage (Chapman, 2014; Mehlhorn, 2014; Beski, 2023).

The relative weight of the lymphatic system organs can be used to assess immune function; the greater the weight of these organs, the stronger the immune functions (Ravis *et al.*, 1988). In this study, neither RNL treatments nor *Eimeria* infection had an effect on lymphoid organs compared with broilers not infected with *Eimeria*d. In the current study, the relative weight of cecum atrophy and intestine increased significantly in infected animals, demonstrating the deleterious effects of infection. Abudabos *et al.* (2017) confirmed our results and documented that the relative weight of the intestine increased significantly in infected birds.

Serum biochemical parameters can provide information about the physiological state of the body and nutrient metabolism. Serum albumin, total protein and glucose levels were within the ranges of 0.8-2 g/dL and 2.5-4.5 g/dL and (200-250 mg/dL), respectively, as reported by Campbell (2004) and Thaxton *et al.* (2006). Serum albumin, glucose, cholesterol, creatinine, and the enzymes ALT and AST were not affected by *Eimeria* infection in this study. In addition, according to Toghyani *et al.* (2011) the dietary treatments had no effect on serum protein, albumin, or triglyceride levels. Feeding phytogenic feed additives increased serum albumin, total protein, and cholesterol levels (Al-Kassie, 2009; Amad *et al.*, 2013; Sadek *et al.*, 2014; Khafaji, 2018). Serum cholesterol levels were highest in the positive salinomycin treated group and lowest in the negative salinomycin treated group, indicating a treatment-challenge interaction. Since the additives and challenge had no negative effects on the safety profile of the chickens, serum levels of ALT and AST were present at low levels in the bloodstream and were not affected in this study, indicating normal hepatic metabolic function without liver or muscle damage (Brancaccio *et al.*, 2010; Wang *et al.*, 2013). Infected chickens had more eosinophils than uninfected chickens, which is consistent with the results of Ahmad *et al.* (2020), but not with the results of Nahed *et al.* (2020) and El-Maddawy *et al.* (2022), where there was no change in the percentage of eosinophils. Lower RNL doses decreased heterophil and H/L ratios, possibly indicating that RNL relieves stress compared with control, especially at lower doses. Toghyani *et al.* (2011) found that administration of antibiotics or thyme powder had no effect on the H/L ratio. In addition, Ali (2010) found that the addition of *Thymus vulgaris* leaves powder to the diet of broiler chicken resulted in a decrease in the H/L ratio.

At 7 dpi, the anticoccidial index of all uninfected groups was excellent, indicating that no lesions or *Eiemeria tenella* oocysts were found in their feces and that their relative weight gain was higher. *Rumex nervosus* leaves powder, as a natural product, demonstrated dose-dependent anticoccidial preventive efficacy in challenged birds (mild, marked, and excellent) in broilers at doses of 1 g, 3 g, and 5 g, respectively. The results showed that broilers fed RNL as a natural feed additive reduced fecal oocyte count and cecal lesion scores to the same extent as salinomycin.

In parallel with the effects of coccidiosis on relative weight gain and productive efficiency, anticoccidial index were affected by coccidial challenge, which is in agreement with Beski (2023) and Dubey (2019). As a result, the anticoccidial efficacy, growth performance, and productive efficiency of RNL should be considered in future prescriptions for both growth and health. Further research is needed, either alone or in combination with other natural products, to determine if the consequences of coccidial challenge can be reduced, thus contributing to improved performance. The presence of agents that change microflora modulation, reduce fecal oocyst excretion, reduce intestinal inflammation, enhance immune system, and enhance antioxidant status may be associated with the effects of RNL as an anticoccidiosis herb on PEF compared to controls (Ali *et al.*, 2020; Khater *et al.*, 2020; Yang *et al.*, 2020; Habibi *et al.*, 2022).

Supplementary Table S1 – Ingredients and calculated nutrients of broilers starter and finisher diets.

Ingredient	Period	
	Starter	Finisher
Yellow corn	53.218	58.09
Soybean meal	37.85	32.15
Wheat bran	2.00	2.2
Corn gluten meal	1.4	0
Choline chloride CL 60	0.05	0.05
Corn oil	1.5	4.2
Dicalcuim phosphate DCP	1.98	1.615
Ground limestone	0.9	0.79
Salt	0.400	0.30
DL-methionine	0.292	0.25
Lysine-HCL	0.21	0.105
Vitamin–mineral premix[1]	0.200	0.200
Total	100	100
Metabolic energy, kcal/kg	3000	3200
Crude protein, %	23.0	20.0
Non phytate P, %	0.48	0.405
Calcium, %	0.96	0.81
D-lysine, %	1.28	1.06
Sulfur amino acids, %	0.95	0.83
Threonine, %	0.86	0.71

[1] The vitamin-mineral premix components per kg: vitamin A, 12000000 IU; vitamin D3, 5000000 IU; vitamin E, 80000 IU; vitamin K3, 3200 mg; vitamin B1, 3200 mg; vitamin B_2, 8600 mg; vitamin B_3, 65000 mg; pantothenic acid, 20000 mg; vitamin B_6, 4300 mg; biotin 220 mg; antioxidant(BHA+BHT), 50000 mg; B_9, 2200 mg; B_{12}, 17 mg; copper, 16000 mg; iodine, 1250 mg; iron, 20000 mg; manganese, 120000 mg; selenium, 300 mg, and zinc, 110000 mg.

CONCLUSIONS

In conclusion, the current study indicated that RNL powder, particularly at high dose, had a marked anticoccidial efficacy in the prevention and control of coccidiosis in coccidia-infected broiler chickens by reducing oocyst shedding and decreasing cecal lesion levels, comparable to that of the anticoccidial drug salinomycin. Regardless of coccidial challenge, the current investigation found that RNL treated groups at level 1 g had a numerical improvement in production efficiency when compared to groups treated with RNL at high levels (3 g or 5 g). Considering all these factors, RNL feed has the potential to be used as a health-promoting and production-enhancing agent that can alleviate the effects of coccidia and improve the production efficiency of broilers.

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