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Anti-coccidial effects of dietary chamomile against experimentally induced coccidiosis in broiler chicken

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Abstract: A 4 × 2 factorial experiment was conducted to investigate the effect of dietary supplementation levels (0, 5 and 10 g/kg) of chamomile flower powder and a level (60 mg/kg) of salinomycin on performance and gut health of broiler chickens under coccidiosis challenge. A total of 320-day-old Ross 308 broilers were assigned to eight treatments of four replicates with 10 birds. Oral inoculation of the challenged groups with *Eimeria tenella* occurred on day 8. On day 10, disease challenge and additive level significantly ($P = 0.003$) interacted, decreasing the feed intake. Feed intake significantly ($P \leq 0.001$) decreased in birds supplemented with 10 g/kg of chamomile. At 35 days, birds fed chamomile and anticoccidial significantly ($P = 0.001$) increased body weight (BW) and weight gain (WG). Disease challenge adversely influenced ($P = 0.001$) BW and WG. Additive level and disease challenge significantly ($P = 0.001$) interacted on feed conversion ratio (FCR). Anticoccidial and chamomile improved ($P = 0.001$) FCR of birds regardless of the rearing conditions. Significantly ($P = 0.001$) better FCR was observed in the unchallenged birds. The interaction of experimental factors was significant ($P \leq 0.04$) on the relative weight of bursa. Disease challenge significantly ($P = 0.005$) increased the relative weight of liver whereas that of bursa decreased. Cholesterol ($P = 0.002$), albumin/globulin ($P = 0.009$), aspartate aminotransferase (AST) ($P = 0.002$) and alanine transaminase (ALT) ($P = 0.001$) significantly increased in challenged birds. The interaction of experimental factors was significant ($P = 0.002$) on villus length, crypt depth and villus height/crypt depth ratio ($P = 0.001$). Longer ($P = 0.001$) villi were found in anticoccidial and chamomile supplemented birds. Coccidiosis adversely ($P = 0.001$) influenced the jejunum morphology. Crypt depth decreased and villus height/crypt depth increased in chamomile offered birds ($P = 0.001$) regardless of the challenge conditions. Bursal morphology was significantly influenced by experimental factors. Dietary supplementation of chamomile had positive effects on broiler performance, immunity and intestinal morphology during exposure to the *E. tenella* parasite. Chamomile could be used as a potential natural anticoccidial in broiler nutrition.

Keywords: phytogenic; medical plants; *Eimeria tenella*; Ross 308; jejunum morphology

The rearing environment has a strong impact on animal growth and feed efficiency. Broilers are well-known for their sensitivity to health challenges because of the intensive production practices. Caused by various species of *Eimeria* that are different in prevalence and pathogenicity, coccidiosis is the most widely spreading parasitic disease of poultry. Impaired growth, malnutrition, intestinal tissue injury, inefficient nutrient utilization, high morbidity and mortality are the major

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consequences of coccidiosis (Tsiouris et al. 2021). Fourteen billions USD are the annual costs of the massive negative impacts of coccidiosis in poultry industry (Blake et al. 2020). The use of in-feed anticoccidials to control avian coccidiosis has been predominant for decades. However their usage has been publicly under concern after the emergence of resistant strains of *Eimeria*, with concomitant reductions in the efficacy of many currently used coccidiostats and changes in the tolerance to the use of medication in livestock production by consumers (Stringfellow et al. 2011). This has resulted in an increased avoidance of anticoccidial chemotherapeutics. Following the restriction of the use of in-feed medications and growing public concerns about the chemical residues in poultry products (Blake and Tomely 2014) animal nutrition strategies to control diseases and promote growth performance of animals are being modified in some countries. New natural products have been introduced to animal nutrition to support the growth performance and health of animals in the conventional unsanitary conditions. The exploration of natural alternative strategies to in-feed medications such as plant-derived compounds seems to have some potential in controlling avian coccidiosis with no negative impacts on the performance (Galli et al. 2021). These herbs produce bioactive phytochemicals that are effective in controlling poultry coccidiosis while being safe, nutritious, and therapeutic for poultry meat consumers (El-Shall et al. 2022). Immunomodulatory, antioxidant and anti-inflammatory properties of herbal phytogenic compounds preserve the gut ecology and health and thus enhance the host's defences against coccidial parasites (Irawan et al. 2021). The anticoccidial effect of phytochemicals emerges by directly interrupting the life cycle of the parasites, altering the formation of oocyst walls and inhibiting sporulation (Fatemi et al. 2015) and destroying the sporozoites (Kim et al. 2013). In accordance the use of phytogenic feed additives, which comprise a wide variety of herbs, has recently gained increasing interest. Among them is chamomile (*Matricaria chamomilla*), which is one of the important medicinal herbs that were used in the ancient history (Singh et al. 2011). Chamomile flowers (*Matricaria chamomilla* L.) have anti-inflammatory, antiseptic, carminative, diaphoretic, sedative properties due to their content of several health benefiting phytonutrients including essential oils with azulene, bisabolol, flavonoids, glycosides and fatty acids (Mahmmod 2013). Dietary chamomile positively affected the growth performance of broiler chickens (Abaza et al. 2003). Higher weight gain and lower FCR and an improvement in the intestinal morphology were found when a mixture of peppermint, chamomile and prebiotic was given to broilers under normal (Beski et al. 2021) and experimentally induced coccidiosis (Hussien et al. 2021). Upadhaya et al. (2019) found that cumulative body weight gain was increased and feed conversion ratio was improved when natural herbs were provided to the birds under coccidiosis challenge. Qaid et al. (2022) recommended the use of dietary *Rumex nervosus* leaves and *Cinnamomum verum* bark as potential coccidiostats to treat poultry coccidiosis in the field. The objective of this study is to evaluate the effect of dietary supplementation of chamomile on subsequent broiler performance, and digestive development and function under experimentally induced coccidiosis. The hypothesis that was tested is that chamomile would support better growth and immune responses and eliminate the negative impact of coccidiosis on broilers.

MATERIAL AND METHODS

The experiment was approved by the Animal Ethics Committee of Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok (Approval No.: UoD AEC120120202).

Experimental design and bird management

This experiment was conducted to assess the effect of dietary supplementation of chamomile flowers on performance and gut health of coccidiosis challenged broilers up to 35 days of age. Three supplementary levels (0, 5 and 10 g/kg diet) of chamomile flower powder and a level (60 mg/kg) of the anticoccidial (salinomycin) were given to the birds in either normal or disease (*E. tenella*) challenged conditions. Three feeding phases were applied: a starter diet which was fed from hatch to 10 days of age, then the birds were switched to a grower diet which was fed from 11 to 24 days, and thereafter to a finisher diet which was fed to 35 days. The formulation of all three diets was identi-

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cal in the nutrient profile to meet the specifications of Ross 308 broiler chickens (Table 1). The active compounds of chamomile are presented in Table 2.

Table 1. Ingredient and nutrient composition (%) of starter, grower and finisher diets

Ingredients	Starter	Grower	Finisher
Corn	53.06	56.9	61.8
Soybean meal	31.48	32.89	28.23
Fish meal	4.0	–	–
Vegetable oil	3.0	4.48	4.6
Limestone	2.0	1.39	1.4
Dicalcium phosphate	2.72	0.95	0.82
Salt	0.11	0.19	0.1
D,L-methionine	0.38	0.32	0.24
L-lysine HCl	0.5	0.26	0.21
L-threonine	0.25	0.13	0.1
Broiler premix[1]	2.5	2.5	2.5
Nutrient composition			
ME (kcal/kg)	3 000	3 150	3 200
Crude protein	23	21	19.16
Crude fiber	2.25	2.38	2.33
Digestible arginine	1.29	1.14	0.99
Digestible lysine	1.29	1.14	0.99
Digestible methionine + cysteine	0.87	0.84	0.73
Digestible tryptophan	0.226	0.24	0.21
Digestible isoleucine	0.87	0.81	0.73
Digestible threonine	0.82	0.73	0.63
Digestible valine	0.99	0.92	0.83
Calcium	1.60	0.9	0.85
Available phosphorus	0.844	0.450	0.42
Sodium	0.16	0.160	0.16
Chloride	0.35	0.312	0.23

[1]The broiler premix contained per kg: vitamin A, 400 000 IU; vitamin D_3, 160 000 IU; vitamin E, 1 200 mg; vitamin B_1, 120 mg; vitamin B_2, 280 mg; vitamin B_6, 160 mg; vitamin B_{12}, 1 400 mcg; biotin, 4 mg; niacin, 1 600 mg; folic acid, 40 mg; vitamin K_3, 100 mg; calcium D-pantothenate, 600 mg; choline chloride, 12 000 mg; choline, 10 411.2 mg; Cu, 0.4 g; Mn, 3.2 g; Zn, 2.4 g; Fe, 2.0 g; I, 40 mg; Se, 10 mg; lysine, 113.2 g; methionine, 113.5 g; methionine + cysteine, 113.9 g; tryptophan, 0.4 g; threonine, 58.9 g; valine, 1.4 g; arginine, 2.2 g; calcium, 62.0 g; available phosphorus, 121.3 g; sodium, 50.0 g; chloride, 64.0 g; endo-1,3(4)-beta-glucanase, 2 800 IU activity; endo-1,4-beta-xylanase, 10 800 IU activity; BHT (E321) 1.34 g; propyl gallate (E310) 0.112 g; citric acid (E330) 0.2 g; mycotoxin binder 40 g

In a 4 × 2 factorial arrangement, a total of 320 Ross 308-days-old chicks were randomly allocated to eight treatments that were replicated four times, with 10 birds per replicate. Chickens were reared in floor pens (100 × 100 cm) bedded with wood shavings in two different rooms. Feed and water were offered *ad libitum*. On day one the room temperature was set up at 33 °C, then it was gradually decreased to 24 °C on 35 days. The applied lighting program was 23L : 1D on the first seven days, then it was switched to 18L : 6D till the end of the experimental period. To calculate the feed intake and body weight, the leftover feed and birds were weighed on days 10, 24 and 35 of bird age. Mortality was recorded whenever it occurred and the feed conversion ratio (feed intake/weight gain) was corrected accordingly. On day 24, two birds were randomly selected from each replicate and euthanized by cervical dislocation, then they were dissected. The internal organs were excised and their relative weight was calculated as mass per unit of body weight (g/kg of body weight). Approximately 5 ml of blood were collected from the jugular vein and centrifuged at 3 000 rpm for 15 min after clotting. Then the serum was collected and stored at –20 °C until used for measurement of serum biochemistry.

Preparation of coccidial inoculant

Fresh faecal samples of coccidiosis infected broilers were collected to isolate the *E. tenella* sporulated oocysts in a laboratory of the College of Veterinary Medicine, University of Duhok. The samples were mixed with saline solution in 2-ml microfuge tubes. The supernatant was discarded after centrifugation of samples at 6 000 *g* for 5 min. The oocysts were allowed to sporulate in 2.5% potassium dichromate for three days at 27 °C.

Coccidiosis challenge model

An infectious dose of 5 000 *E. tenella* sporulated oocysts/ml was used. On day 8 of age each bird of the challenged groups received 1 ml of the parasite suspension separately using a crop needle and a 10-ml syringe with a flexible tube connected. However, unchallenged birds were given 1 ml of sterile normal saline instead of the parasite suspension. To prevent the incidence of cross-contamination, unchallenged

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Table 2. Antioxidant inhibition activities, phenolic and flavonoid contents and neuroprotective enzyme activities in water and methanol extracts of chamomile (own analysis)

Concentration (µg/ml)	DPPH analysis (inhibition%)		CUPRAC analysis (inhibition%)	
	water extract	methanol extract	water extract	methanol extract
1 000	84.09	88.17	1.11	1.11
500	70.53	73.11	0.74	0.76
250	65.22	67.20	0.42	0.42
125	40.96	48.03	0.27	0.27
62.5	31.63	32.65	0.19	0.19
Content (inhibition% ± SD)				
Total phenolic	42.12 ± 0.62	26.38 ± 1.05		
Total flavonoid	4.19 ± 0.02	6.19 ± 1.08		
AChE	49.35 ± 1.21	32.16 ± 1.05		
BChE	60.86 ± 0.14	56.50 ± 0.92		

AChE = acetylcholinesterase; BChE = butyrylcholinesterase; CUPRAC = cupric ion reducing antioxidant capacity; DPPH = diphenyl-2-picrylhydrazil

birds were always treated first. The inoculation process was accomplished inside the pens.

Identification of coccidial oocysts in excreta

To confirm the challenge model, excreta samples of both challenged birds and unchallenged controls were collected on days 7, 8, 9, 10 and 11 after coccidial inoculation, samples were microscopically examined for the presence of oocysts.

Serum biochemistry

Five ml of blood were taken from the jugular vein and poured into non-heparinized tubes. Thereafter, blood samples were centrifuged and the serum was collected and stored for analyses. Serum biochemical parameters including total protein, albumin, cholesterol, alanine transaminase (ALT) and aspartate aminotransferase (AST) were determined using an automatic Cobas Integra 400 plus analyser (Cedex Bio HT Analyser; Roche CustomBiotech, Penzberg, Germany).

Jejunal and bursal histomorphology

Tissue samples of the proximal jejunum (approximately 1 cm) and the whole bursa were collected, flushed with buffered saline and fixed in 10% neutral buffered formalin for histomorphological analysis. The staining of samples with haematoxylin and eosin occurred after sample embedding in paraffin wax and sectioning. Sample sections were captured at 10× magnification using a digital camera under microscope (Dino-Eye Microscope Eyepiece Camera, DinoCapture 2.0; ANMO Electronics Corporation, Taipei, Taiwan) and morphometric indices were determined by Dino-Eye program. Images were digitized and the villus height (from the tip of the villus to the villus/crypt junction) and crypt depth (from the villus/crypt junction to the muscular junction) were measured in 7–10 well-orientated villi for each jejunal section. The apparent absorptive surface area of the villus was determined using the formula: [(villus tip width + villus base width)/2] × villus height (Iji et al. 2001). The bursal follicular area was measured with DinoCapture software by tracing round the image of the selected follicles.

Statistical analysis of data

Data analysis of the present study was performed using the General Linear Model (GLM) procedure of Minitab v17 (Minitab Inc., Pennsylvania, PA, USA) for the main effect of each of the experimental factors and their interactions. Duncan's multiple range test was used to determine the differences between mean values.

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RESULTS

To confirm the infection, excreta samples of both negative and positive control (challenged, unsupplemented) were taken. Excreta samples of challenged control broilers were collected from seven to 11 days after inoculation, and the presence of oocysts was confirmed. In addition to the existence of oocysts, the clinical signs of coccidiosis including ruffled feathers and bloody droppings appeared on challenged birds, indicating that the inoculation of challenged groups of birds with the used parasite suspension was sufficient to cause an infection. The collected excreta samples of unchallenged control broilers were free from oocysts on days 7–11 after receiving the saline solution.

Growth performance

Up to day 10 the significant interaction (P = 0.003) of the experimental factors indicated the lower feed intake in the unchallenged birds that received the highest level of chamomile followed by those on the positive control diets (Table 3). Feed intake significantly (P = 0.001) decreased in birds that were offered diets containing the higher level of chamomile compared to the control and other experimental groups. The average body weight gain and feed conversion ratio of 10 days old chicks were not affected by the experimental factors or by their interaction. When assessed over the 35 days experimental period, feed intake was not affected by the treatments. However, birds on chamomile- and anticoccidial-containing diets were significantly (P = 0.001) heavier and gained more weight compared to those on the control diet regardless of the rearing conditions. Unchallenged birds had higher body weight (P = 0.001) and weight gain than the disease challenged birds. A significant interaction (P = 0.001) was observed between the experimental factors revealing the higher FCR in the challenged control followed by challenged birds that were on diet containing the higher level

Table 3. Feed intake (FI, g), body weight (BW, g), weight gain (WG, g) and feed conversion ratio (FCR) of broiler chickens receiving dietary chamomile under coccidiosis challenge

Challenge	Additive level (g/kg)	Response							
		FI		BW		WG		FCR	
		1–10	1–35	10	35	1–10	1–35	1–10	1–35
No	Control	295.5^{ab}	2 794.3	287.8	1 934.9	247.8	1 894.9	1.199	1.47^{bc}
	Anticocc	302.6^{ab}	2 829.4	293.6	1 996.9	253.6	1 956.9	1.194	1.45^{c}
	Cham5	300.1^{ab}	2 920.7	298.3	2 021.9	258.3	1 981.9	1.162	1.47^{bc}
	Cham10	275.1^{c}	2 933.6	269.5	2 030.6	229.5	1 990.6	1.215	1.47^{bc}
Yes	Control	293.1^{b}	2 858.7	274.5	1 737.8	234.5	1 697.8	1.253	1.67^{a}
	Anticocc	296.8^{ab}	2 783.8	289.3	1 942.8	249.3	1 902.8	1.193	1.46^{bc}
	Cham5	303.8^{a}	2 810.0	290.3	1 914.4	250.3	1 874.4	1.213	1.50^{bc}
	Cham10	295.3^{ab}	2 824.1	284.8	1 914.1	244.8	1 874.1	1.206	1.51^{b}
SEM		1.161	14.716	2.848	9.872	2.848	9.872	0.014	6.475
Main effects									
Additives	Control	294.3^{a}	2 826.5	281.1	1 836.4^{b}	241.1	1 796.4^{b}	1.226	1.580^{a}
	Anticocc	299.7^{a}	2 806.6	291.4	1 969.8^{a}	251.4	1 929.8^{a}	1.193	1.454^{b}
	Cham5	301.9^{a}	2 865.3	294.3	1 968.2^{a}	254.3	1 928.2^{a}	1.188	1.486^{b}
	Cham10	285.2^{b}	2 878.8	277.2	1 972.3^{a}	237.2	1 932.3^{a}	1.211	1.490^{b}
Challenge	no	293.6	2 869.5	287.3	1 996.1^{a}	247.3	1 956.1^{a}	1.193	1.466^{b}
	yes	297.3	2 819.1	284.7	1 877.3^{b}	244.7	1 837.3^{b}	1.216	1.538^{a}
Main effects and interaction (P-value)									
Additives		0.001	0.298	0.132	0.001	0.132	0.001	0.770	0.001
Challenge		0.102	0.100	0.657	0.001	0.657	0.001	0.413	0.001
Additives × challenge		0.003	0.147	0.334	0.110	0.334	0.110	0.786	0.001

Anticocc = anticoccidial; Cham5 = chamomile 5 g/kg; Cham10 = chamomile 10 g/kg

$^{a–c}$Mean values in the same column not sharing a superscript letter are significantly different at the P-level shown for the main effect

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of chamomile. FCR was significantly (P = 0.001) improved in birds that were offered diets containing anticoccidial and chamomile than in the control group regardless of the rearing conditions. Significantly (P = 0.001) better FCR was observed in the unchallenged birds than in the challenged ones.

Visceral organ weight

The interaction of the experimental factors was not significant in the relative weight of internal organs except for the bursa which was significantly (P = 0.04) higher in the challenged control and in those that received the anticoccidial compared to the other experimental groups in both rearing conditions (Table 4). At the same time, the relative weight of small intestine tended (P = 0.055) to significantly increase in birds that received the lower level (5 g/kg) of chamomile. The relative weight of bursa showed a tendency (P = 0.062) to decrease with the rising level of chamomile in broiler diets. In general, the relative weight of spleen decreased in birds that received chamomile or anticoccidial compared to the control. As a main effect, the challenge model significantly (P = 0.005) increased the relative weight of liver whereas that of bursa significantly decreased in the challenged birds compared to those of unchallenged groups.

Serum biochemistry

The interaction of the experimental factors significantly (P = 0.041) increased the globulin level in the serum of unchallenged birds that were offered diets containing 10 g/kg chamomile (Table 5). The lower serum globulin was recorded in unchallenged birds given an anticoccidial. As a separate factor, the challenge model had effects on some chemicals in the serum. Cholesterol (P = 0.002) and albumin/globulin (P = 0.009), AST (P = 0.002)

Table 4. The relative weight of internal organs of *Eimeria* challenged broilers given different dietary levels of chamomile

Challenge	Additive level (g/kg)	Sm.int	Giz+pro	Liver	Spleen	Bursa	Heart
No	Control	7.08	3.73	3.11	0.104	0.15^{b}	0.78
	Anticocc	6.64	3.82	3.19	0.089	0.15^{b}	0.71
	Cham5	7.69	3.63	3.17	0.073	0.16^{b}	0.55
	Cham10	6.86	3.66	3.00	0.096	0.15^{b}	0.66
Yes	Control	6.54	3.96	2.82	0.104	0.21^{a}	0.61
	Anticocc	6.06	3.73	2.77	0.099	0.21^{a}	0.57
	Cham5	7.63	4.12	2.76	0.096	0.16^{b}	0.55
	Cham10	6.34	3.91	2.77	0.091	0.13^{b}	0.68
SEM		0.166	0.125	0.055	3.357	5.625	0.017
Main effects							
Additives	Control	6.81	3.84	2.96	0.104	0.18	0.69^{a}
	Anticocc	6.35	3.78	2.98	0.094	0.18	0.64^{ab}
	Cham5	7.66	3.88	2.97	0.085	0.16	0.55^{b}
	Cham10	6.60	3.79	2.89	0.093	0.14	0.67^{a}
Challenge	no	7.07	3.71	3.12^{a}	0.091	0.15^{b}	0.67
	yes	6.64	3.93	2.78^{b}	0.098	0.18^{a}	0.60
Main effects and interaction (P-value)							
Additives		0.055	0.991	0.928	0.260	0.062	0.040
Challenge		0.215	0.388	0.005	0.313	0.049	0.055
Additives × challenge		0.942	0.878	0.914	0.483	0.042	0.168

Anticocc = anticoccidial; Cham5 = chamomile 5 g/kg; Cham10 = chamomile 10 g/kg; Giz+pro = gizzard + proventriculi; Sm.int = small intestine

[a,b]Mean values in the same column not sharing a superscript letter are significantly different at the P-level shown for the main effect

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Table 5. Serum biochemistry of *Eimeria* challenged broilers given different dietary levels of chamomile

Challenge	Additive level (g/kg)	Chol	TP	Albumin	Globulin	a/b	AST	ALT
No	Control	91.3	2.53	1.06	1.48^{ab}	0.72	158.3	2.43
	Anticocci	86.0	1.80	0.71	1.09^{b}	0.65	135.8	2.30
	Cham5	94.8	2.49	0.98	1.52^{ab}	0.66	183.8	2.20
	Cham10	98.5	2.78	0.96	1.82^{a}	0.50	169.0	1.23
Yes	Control	141.3	2.35	1.02	1.34^{ab}	0.76	244.0	4.70
	Anticocc	119.8	2.57	1.07	1.51^{ab}	0.74	212.8	3.80
	Cham5	113.3	2.67	1.17	1.50^{ab}	0.79	187.5	3.20
	Cham10	98.5	2.11	0.94	1.17^{b}	0.80	181.0	3.40
SEM		3.957	0.098	0.044	0.061	0.024	6.271	0.179
Main effects								
Additives	Control	116.3	2.44	1.04	1.41	0.74	201.3	3.56
	Anticocc	102.9	2.18	0.89	1.30	0.69	174.3	3.05
	Cham5	104.0	2.58	1.07	1.51	0.72	185.6	2.70
	Cham10	93.6	2.44	0.95	1.49	0.64	175.0	2.31
Challenge	no	90.19^{b}	2.40	0.93	1.47	0.63^{b}	161.7^{b}	2.04^{b}
	yes	118.19^{a}	2.43	1.05	1.38	0.77^{a}	206.4^{a}	3.78^{a}
Main effects and interaction (*P*-value)								
Additives		0.274	0.553	0.461	0.594	0.557	0.406	0.115
Challenge		0.002	0.890	0.182	0.495	0.009	0.002	0.001
Additives × challenge		0.313	0.096	0.359	0.041	0.287	0.055	0.561

a/b = albumin/globulin; ALT = alanine transaminase; Anticocc = anticoccidial; AST = aspartate aminotransferase; Cham5 = chamomile 5 g/kg; Cham10 = chamomile 10 g/kg; Chol = cholesterol; TP = total protein

^{a,b}Mean values in the same column not sharing a superscript letter are significantly different at the *P*-level shown for the main effect

and ALT (P = 0.001) significantly increased in the challenged birds.

Jejunal histomorphology

The interaction of the experimental factors was significant (P = 0.002) in the villi indicating the shorter villi in both challenged and unchallenged controls compared to other experimental groups (Table 6, Figure 1). Regardless of the challenge conditions, the villus height significantly increased (P = 0.001) in birds that were fed diets containing anticoccidial and chamomile compared to those on the control diets. Longer (P = 0.001) jejunal villi were observed in unchallenged birds than in the challenged ones. A significant (P = 0.001) interaction was indicated among the experimental factors revealing the deeper crypts in birds on the positive control whereas the lower crypt depth was found in challenged birds that received the highest chamomile level. The crypt depth significantly (P = 0.001) decreased in chamomile offered birds compared to the control and anticoccidial supplemented birds regardless of the challenge condition. The crypt depth significantly (P = 0.001) decreased in the unchallenged birds compared to those that were challenged with coccidia. The lower villus height/crypt depth was observed in the challenged control due to the significant (P = 0.001) interaction of the experimental factors. The villus height/crypt depth significantly (P = 0.001) increased with the rising levels of chamomile compared to the other groups regardless of the challenge conditions. The villus height/crypt depth was higher in the unchallenged birds than in those that were exposed to coccidia. The intestinal muscle thickness was decreased in the chamomile supplemented birds in both challenged and unchallenged birds due to the significant (P = 0.001) interaction of the experimental factors. Regardless of the challenge conditions, the muscle thickness was decreased (P = 0.001) with the rising

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Table 6. Jejunal histomorphology of 24-day-old *Eimeria* challenged broilers given different dietary levels of chamomile

Challenge	Additive level (g/kg)	Jejunum				
		villi	crypt	v/c	muscle	VSA
No	Control	944.8^{c}	183.3^{cd}	5.55^{abc}	204.5^{c}	1.67
	Anticocc	1 108.6^{a}	210.8^{c}	5.43^{bc}	265.3^{b}	1.73
	Cham5	1 025.4^{b}	188.3^{cd}	6.02^{ab}	169.1^{c}	1.71
	Cham10	1 034.7^{b}	181.5^{cd}	5.84^{ab}	186.7^{c}	1.74
Yes	Control	761.6^{d}	402.3^{a}	1.91^{e}	330.2^{a}	1.46
	Anticocc	1 007.7^{b}	310.9^{b}	3.82^{d}	262.1^{b}	1.82
	Cham5	849.7^{d}	200.8^{cd}	4.46^{cd}	188.7^{c}	1.25
	Cham10	1 000.8^{bc}	164.7^{d}	6.33^{a}	173.2^{c}	1.73
SEM		6.889	5.924	0.124	4.412	0.015
Main effects						
Additives	Control	888.4^{c}	250.8^{a}	4.43^{b}	243.2^{a}	1.58
	Anticocc	1 064.2^{a}	254.8^{a}	4.72^{b}	263.9^{a}	1.77
	Cham5	944.9^{c}	194.0^{b}	5.30^{ab}	178.1^{b}	1.47
	Cham10	1 020.4^{b}	174.4^{b}	6.05^{a}	181.0^{b}	1.74
Challenge	no	1 050.5^{a}	194.4^{b}	5.68^{a}	216.3^{b}	1.72
	yes	954.2^{b}	250.8^{a}	4.57^{b}	225.2^{a}	1.64
Main effects and interaction (*P*-value)						
Additives		0.001	0.001	0.001	0.001	0.064
Challenge		0.001	0.001	0.001	0.003	0.156
Additives × challenge		0.002	0.001	0.001	0.001	0.122

Anticocc = anticoccidial; Cham5 = chamomile 5 g/kg; Cham10 = chamomile 10 g/kg; v/c = villus height/crypt depth; VSA = villus surface area

$^{a–d}$Mean values in the same column not sharing a superscript letter are significantly different at the *P*-level shown for the main effect

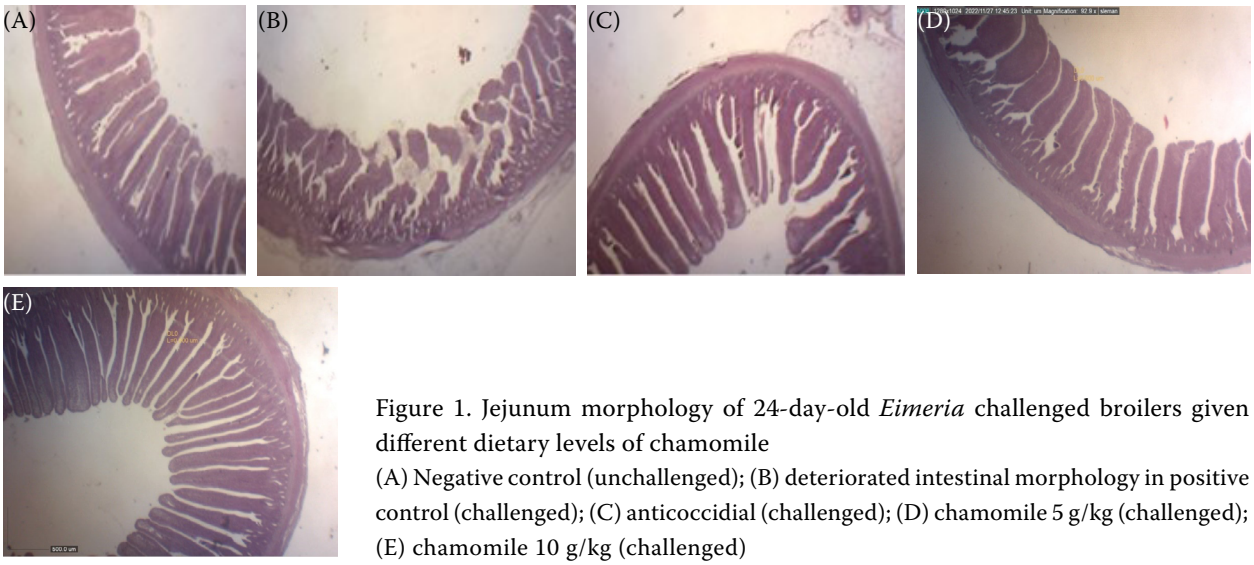

Figure 1. Jejunum morphology of 24-day-old *Eimeria* challenged broilers given different dietary levels of chamomile

(A) Negative control (unchallenged); (B) deteriorated intestinal morphology in positive control (challenged); (C) anticoccidial (challenged); (D) chamomile 5 g/kg (challenged); (E) chamomile 10 g/kg (challenged)

levels of chamomile in the broiler diets. The intestinal muscle thickness significantly (P = 0.003) increased in the challenged birds compared to the unchallenged groups. Furthermore, the villus surface area (VSA) tended (P = 0.062) to significantly decrease when the lower level (5 g/kg) of chamomile was supplemented to the broiler diets irrespective of the challenge conditions.

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Morphology of bursa of Fabricius

The experimental factors significantly (P = 0.007) interacted indicating the longer bursal follicles in the challenged control groups followed by challenged groups that received an anticoccidial in their diets (Table 7, Figure 2). The challenged birds that received the highest level of chamomile had shorter bursal follicles than other experimental groups. A significant (P = 0.001) interaction was detected among the experimental factors indicating the higher bursal follicle area in the challenged control than in the other experimental groups. In general the follicle area was decreased (P = 0.03) with

Table 7. Bursal histomorphology of 24-day-old *Eimeria* challenged broilers given different dietary levels of chamomile

Challenge	Additive level (g/kg)	Bursal follicles		
		length	width	area
No	Control	5.02^{bc}	2.99	9.72^{cd}
	Anticocc	4.56^{bc}	3.02	11.56^{cd}
	Cham5	5.03^{bc}	2.84	11.61^{cd}
	Cham10	5.28^{b}	2.91	12.46^{bcd}
Yes	Control	6.58^{a}	3.99	21.53^{a}
	Anticocc	5.61^{ab}	3.60	16.54^{b}
	Cham5	5.10^{bc}	3.11	13.80^{bc}
	Cham10	4.19^{c}	2.55	8.67^{d}
SEM		0.130	0.104	0.553
Main effects				
Additives	Control	5.80^{a}	3.49	16.47^{a}
	Anticocc	5.09^{ab}	3.31	14.05^{ab}
	Cham5	5.07^{ab}	2.98	12.70^{ab}
	Cham10	4.73^{b}	2.73	10.57^{b}
Challenge	no	4.97	2.94	11.45^{b}
	yes	5.37	3.32	15.13^{a}
Main effects and interaction (P-value)				
Additives		0.052	0.072	0.031
Challenge		0.141	0.084	0.003
Additives × challenge		0.007	0.153	0.001

Anticocc = anticoccidial; Cham5 = chamomile 5 g/kg; Cham10 = chamomile 10 g/kg

$^{a–d}$Mean values in the same column not sharing a superscript letter are significantly different at the P-level shown for the main effect

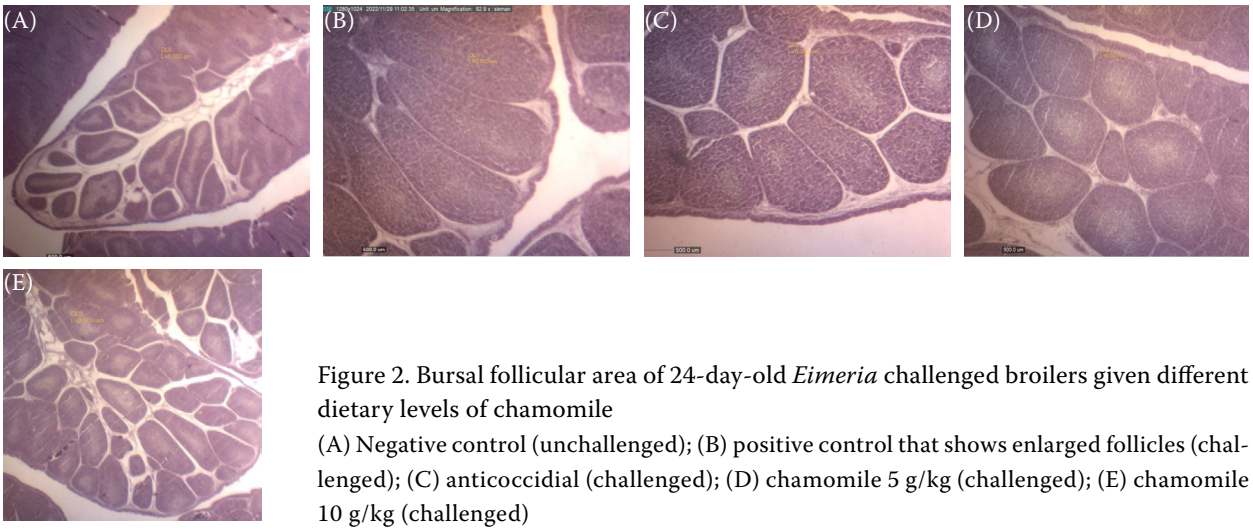

Figure 2. Bursal follicular area of 24-day-old *Eimeria* challenged broilers given different dietary levels of chamomile

(A) Negative control (unchallenged); (B) positive control that shows enlarged follicles (challenged); (C) anticoccidial (challenged); (D) chamomile 5 g/kg (challenged); (E) chamomile 10 g/kg (challenged)

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the rising levels of chamomile in the broiler diets regardless of the challenge conditions. The area of the bursal follicle significantly (P = 0.003) increased in the challenged birds compared to those reared under normal conditions.

DISCUSSION

Clinical symptoms of challenged birds

Infected birds showed the clinical signs of coccidiosis after 24 h from inoculation. Clinical symptoms were most obvious in the challenged control group. Chicks huddling together showed somnolence, ruffled feathers and they were generally depressed. Feed and water consumption decreased and bloody diarrhoea appeared in most challenged birds. Most of the clinical symptoms were temporary and lasted for a few days, then gradually disappeared, recovery being complete within 14 days. The inoculation of isolated oocytes of *E. tenella* was successful to induce coccidiosis. This was evidenced by the oocyst existence in the faeces of the challenged control which the laboratory examined.

Growth performance

The results of the present study confirm the destructive impact of coccidiosis on performance and intestinal morphology of broiler chickens. Challenged birds had lower body weight, weight gain and poorer FCR than unchallenged birds. This could be a consequence of distraction of the intestinal integrity that was also obtained in this study, thereby shorter villi and less villous surface area. This may affect the feed utilization efficiency and the absorption of nutrients, which are other most common effects of coccidiosis (Walk et al. 2011).

This study shows the possibility of offering chamomile to eliminate the negative impact of *E. tenella* on broiler chickens. Dietary supplementation of chamomile alleviated the deleterious impact of the experimental infection and was as efficient in improving BW, BWG and FCR as the coccidiostats used. Throughout the experimental period, there was an improvement in the body weight and FCR of broilers due to the consumption of chamomile while they were reared in sanitary conditions or subjected to a pathogenic challenge. The improved growth and feed conversion of broiler chickens with in-feed chamomile in the present study could be due to the improvements in the health of gastrointestinal tract which have positive effects on feed digestibility, nutrient absorption (Persia et al. 2006), and disease resistance (Abdullah and Al-Barwary 2020). Dietary herbs and their extracts have been confirmed to have positive impacts on the broiler performance via enhancing the feed consumption, improving the release and activity of digestive enzymes and increasing the intestinal digestion and better utilization of nutrients (McCrea et al. 2005). The results of the present study were in accordance with those of Hussein et al. (2021), who showed that a mixture of powdered chamomile and peppermint had positive effects on WG and FCR when added to the diet of broilers affected by coccidiosis. The beneficial effect of dietary chamomile on the performance has also been confirmed in unchallenged broilers (Mahmmod 2013) and those that were challenged with *E. coli* (Khishtan and Beski 2020). The same results have also been obtained by Beski et al. (2021), when chamomile was fed to broilers in combination with peppermint.

Relative organ weight and bursal morphology

In this study, higher relative weight of bursa and increased bursal follicle length, width and higher follicular area were observed in the challenged birds. The entire life cycle of *E. tenella* in the bursa was noticed by Helal et al. (2019). Therefore, the hypertrophy of the bursa of Fabricius and the significantly longer bursal follicles with the higher follicular area in the challenged birds of the present study could be due to the exposure of birds to *Eimeria*, which may expand the epithelial lining of the bursa (Helal et al. 2019). In addition, the complexity in the life cycle of *Eimeria* which includes intracellular and extracellular stages that trigger a powerful inflammatory response that causes tissue injury (El-Shall et al. 2022) leading to inflammation, accumulation of fluids and subsequently causing enlargement in the size of the infected tissue. This may indicate the early cellular immune response against *E. tenella* (Ilic et al. 2004). However, in the present study, dietary supplementation of chamomile eliminated the negative impacts of coccidiosis on the bursa and prevented the hypertrophy

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and inflammation of bursa in coccidiosis infected birds. Phytochemicals have a direct cytotoxic effect on parasites thereby disrupting their life cycle, altering the wall formation of oocysts and impairing sporulation (Fatemi et al. 2015). The findings were in line with those of Hussien et al. (2021), who found that the relative weight of bursa and bursal follicular length of coccidiosis challenged broilers were decreased by dietary supplementation of chamomile in combination with peppermint and yeast. In this study the relative weight of liver decreased in the challenged birds. This may indicate that coccidiosis could negatively influence the development and hepatic functions of broiler chickens.

Serum chemistry

In the present study, globulin slightly decreased and the ratio of albumin to globulin increased in challenged birds. This may confirm that the parasitic infection would not directly stimulate the specific immunity, most particularly humeral immunity and immunoglobulin production. Antibodies do not play a major role in response to coccidiosis (Kim et al. 2019). However, the exact reason for decreased globulin in challenged birds with the higher level (10 g/kg) of chamomile is unknown. In this experiment higher serum cholesterol was observed in the serum of challenged birds than in the unchallenged ones. This could be attributed to the disruption of fat metabolism (Freitas et al. 2008). The higher levels of ALT and AST in the challenged broilers compared with those of unchallenged groups could be due to the improper liver function in coccidia-infected birds as a result of injuries in the epithelial lining of gut and liver that are caused by coccidiosis as reported by Hesabi Nameghi et al. (2019). The elevated levels of AST and ALT indicate the hepatic mitochondrial damage caused by *E. tenella* infection (Elmahallawy et al. 2022). Free radical generation that accompanied the different stages of coccidiosis could be the most possible reason for the liver cell injury (Srivastava et al. 2012). The findings were in accordance with those of Hussien et al. (2021), when dietary supplementation of chamomile in combination with peppermint and yeast were fed to broilers. Similar findings have been obtained by Beski et al. (2021), when a mixture of chamomile, peppermint and yeast was offered to the broiler chickens.

Jejunum histomorphology

In the present experiment, dietary supplementation of chamomile was effective in improving the intestinal morphology of birds in either normal or disease challenge conditions. Coccidiosis may cause detrimental effects on the intestines, bring about inflammations and increase the mucus production. In addition, imbalanced intestinal homeostasis due to the distraction of the gastrointestinal environment caused by *Eimeria* spp. parasite infection could increase the incidence of infection with other pathogens such as *Clostridium* species (Cvikova and Papp 2006). *Eimeria* spp. are spore-forming parasites that replicate in the enterocytes (Felici et al. 2021) causing severe damage to the host mucosal cells. This results in pathological changes including increased cell permeability, leaking of nutrients and plasma protein, diminished digestion and protein absorption (Nabian et al. 2018; Madlala et al. 2021). Additionally, it alters the causes of morphology of the intestinal mucosa reducing the absorptive surface area (Nabian et al. 2018), and thus compromising the well-being and productivity of chickens (Madlala et al. 2021). Chamomile consumption reduced the negative impacts of coccidiosis on the intestinal morphology. This was evidenced by the longer villi, better villi/crypt ratio and thinner intestinal muscle wall in birds that were fed chamomile. The effect of chamomile on the gut morphology was most likely the same as that of the anti-coccidial. The increased intestinal absorptive capacity, efficient nutrient uptake and improved performance are the usual responses of the good gut morphology. The positive effects of chamomile on the gut health could be attributed to the possibility of altering the microbial community in the gastrointestinal tract such as beneficial *Lactobacillus* and pathogenic *Salmonella* and *E. coli* populations (Shanmugasundaram and Selvaraj 2012). Kamasolen and a-bisabolol oxide, bisabolol oxide essential oils and chamazulene are the most frequently found biological active compounds in chamomile (Dada et al. 2015). These herbal active compounds could reduce the virulence of pathogens via interaction with its cell membrane causing modifications to its permeability for cations like H and K which lead to leakage (Windisch et al. 2008). In addition, flavonoid and phenolic compound that are present in most herbs could inhibit the wall formation and sporulation of pathogen oocysts (Fatemi et al. 2015).

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They also cause damage to sporozoites (Kim et al. 2013), reduce the number of oocysts and slow down the parasite growth and reproduction (Abbas et al. 2012). The findings of the present study were in accordance with the findings of Hussien et al. (2021), who found that the intestinal morphology of coccidiosis challenged broilers was improved by dietary supplementation of chamomile in combination with peppermint and yeast. Similar findings were obtained by Beski et al. (2021) when a mixture of chamomile, peppermint and yeast was offered to the broiler chickens. Improved jejunal morphology was noticed by Kishtan and Beski (2020) in *E. coli* challenged broilers that were provided diets containing chamomile powder. Improved jejunal histomorphology was reported by Tsiouris et al. (2021), when a dietary polyherbal formula was offered to coccidiosis challenged broilers. This could be attributed to the antimicrobial activity of chamomile when in this study unchallenged birds presented longer jejunal villi than those of challenged groups.

CONCLUSION

The present study was conducted to create a model of coccidiosis infection. It illustrated that chamomile was as effective as salinomycin in preventing the negative impacts of coccidiosis on the performance of broiler chickens. Dietary supplementation of chamomile also improved the intestinal health and integrity of either challenged or unchallenged broilers. This was evidenced via increasing villous length, villous height to crypt depth ratio and higher absorptive surface area of villi compared with untreated coccidiosis challenged broilers. The findings of the present study indicate chamomile as a potential natural alternative to artificial coccidiostats in broiler nutrition. In addition, chamomile had growth promoting effects; this was evidenced by significantly increased body weight, weight gain and improved FCR throughout the experimental period particularly in challenged groups.

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Conflict of interest

The author declares no conflict of interest

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