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PAPER

Different combinations of peppermint, chamomile and a yeast prebiotic have different impacts on production and severity of intestinal and bursal abnormalities of broilers challenged with coccidiosis

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ABSTRACT

Various mixtures of two phytogenics and a prebiotic were assessed for their impacts on performance of Ross 308 broilers experimentally challenged at 8 d-of-age with *Eimeria tenella* sporulated oocysts. The seven treatment groups (4 pens/treatment, 10 chicks/pen) were: Negative Control, unchallenged, un-supplemented; Positive Control, challenged (C), un-supplemented; C P + CH, challenged, supplemented (per kg diet) with 5 g peppermint (P) and 5 g chamomile (CH); C P + Y, challenged, supplemented with 5 g of P and 5 g prebiotic yeast cell wall (YCW) product; C CH + Y, challenged, supplemented with 5 g CH and 5 g YCW; C P + CH + Y, challenged, supplemented with 5 g each of P, CH and YCW; C Sal, challenged, supplemented with 60 mg salinomycin (Sal)/kg diet. At 24 and 35 d-of-age, all supplemented groups had higher weight gains ($p < .0001$) and lower feed conversion ratios ($p < .05$) to Positive Controls. At 24 d-of-age, 2 chicks/pen were killed. Groups C P + Y and C P + CH + Y had higher intestinal relative weights than other treatments ($p < .0001$). Positive Controls had significantly shorter villi, deeper crypts, lower villus height:crypt depth ratios and thicker jejunal muscle than other treatments. Lymphoid follicles of bursa of Fabricius were longer in Positive Controls than all groups except C Sal and C P + CH + Y and, except for Group C Sal, lymphoid follicle areas were larger ($p < .05$). Thus, the various dietary combinations of peppermint, chamomile and YCW had similar outcomes to that of the anticoccidial salinomycin in preventing a coccidiosis-induced decrease in performance and gut health.

HIGHLIGHTS

- Phytogenic feed additives and yeast cell wall prebiotic prevented weight loss and gut pathology in coccidiosis challenged broilers.
- Mixtures of peppermint, chamomile and yeast cell prebiotic had similar effects to the coccidiostat salinomycin.
- The oral treatment mixtures are potential alternatives to pharmaceutical coccidiostats.

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Introduction

Caused by *Eimeria* protozoa, coccidiosis is one of the most serious disease problems for the World's poultry industry and causes significant negative economic impacts (Abdul Rasheed and Matsler 2020; Adhikari et al. 2020). Based on 2016 data, coccidiosis caused a global loss of more than USD 13.75 billion/annum, equivalent to about USD 0.21/chicken, to the poultry industry (Blake et al. 2020). Coccidiosis has a high rate of spread or dispersal in intensive poultry farms (Shirley et al. 2007) and causes reduced productivity, comprised welfare, impaired growth, suppressed immune function and a high mortality rate. The widespread use over many years of in-feed anticoccidial drugs to control the disease has led to the development of resistance to the drugs in *Eimeria* strains (Pop et al. 2019). Development of drug resistance and an increased concern for the effects that the overuse of pharmaceutical chemicals in food animals can have on disease control and prevention in people has led to bans and restrictions on their use (Abdul Rasheed and Matsler 2020). As a result, new and relatively cheaper

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control methods using in-feed herbs, either singly or in mixtures, are being investigated for the prevention and treatment of coccidiosis in poultry (Pop et al. 2019) and, in some countries, combinations of herbs are produced commercially as coccidiostats (Abbas et al. 2012). Such coccidiostats are considered less likely to cause resistance than chemical drugs (Srinivasu et al. 2020).

Active phytogenic compounds in herbs can enhance the host's defences against coccidial parasites through their immunomodulatory, antioxidant and anti-inflammatory effects in the gut (see reviews by Abbas et al. 2012; Wunderlich et al. 2014; Irawan et al. 2021) or they can directly affect the viability of the parasites by altering the formation of oocyst walls, thus inhibiting sporulation (del Cacho et al. 2010; Fatemi et al. 2015) and damaging the sporozoites (Kim et al. 2013). Improvement in post coccidiosis recovery can thus be enhanced (Arczewska-Włosek and Świątkiewicz 2013). Soft stemmed plants such as peppermint, oregano, sage and basil and those with woody stems such as rosemary contain complex mixtures of many different chemical compounds such as flavonoids, polyphenols and polypeptides (Masood et al. 2013) that are reported to be responsible for the majority of their anticoccidial potential (Masood et al. 2013). Herbs or their extracts (e.g. from turmeric and *Aloe vera)* are also able to enhance performance of poultry through appetite stimulation, stimulation of saliva secretion, improved gastro-intestinal tract function, stimulation of digestive enzyme secretion, better feed utilisation, improved weight gain (WG) and lower feed conversion ratio (g feed eaten:g weight gain, FCR) (Vinus et al. 2018). Dietary prebiotics are also being used against coccidia: they have been defined as 'selectively fermented ingredients' that can improve health by altering the composition and activities of the microbiota of the gut (Gibson et al. 2010) and subsequently enhance productivity of poultry (Ahiwe et al. 2020; Solís-Cruz et al. 2020). Yeast cell walls (YCW) derived from *Saccharomyces cerevisiae* are rich sources of prebiotics such as mannan-oligosaccharides, β-glucans, d-mannoses and α methyl-d-mannosides (Shanmugasundaram and Selvaraj 2012). However, commercial products of YCW may be derived from a variety of sources and the concentrations of their prebiotic compounds can vary depending on the strain of yeast, the substrates they are grown on and methods of preparation. As a result, their positive effects on performance and health of broilers (e.g. Morales-López et al. 2010; Shanmugasundaram et al. 2013; M'Sadeq, Wu, Choct et al. 2015) may differ (Shurson 2018).

The current experiment forms part of a series of on-going experiments on the efficacy of peppermint (*Mentha piperita L.*), chamomile (*Matericaria chamomille* L.) and yeast in the control and treatment of coccidiosis in broilers. Hussein (2021) has shown that powdered peppermint (P, *M. piperita L.*) added at 0.5 or 1.0 g/100 g to broiler diets had beneficial effects on growth rate, feed conversion ratio (FCR) and gut morphology of broilers orally challenged with *Eimeria tenella* oocysts (Hussein 2021). No published experiments have been found on the effects of chamomile in poultry and experiments with chamomile and YCW as single supplements are in preparation by Beski and M'Sadeq (personal communications email to Hussein July 2021).

As the mechanisms by which different in-feed herbal supplements reduce the effects of coccidiosis and improve health of poultry depend on the origin of an herb and composition of its organic compounds and these can vary among plant species and are different from YCW, the current experiment was designed to compare the effects of various mixtures of two phytogenic feed additives, peppermint (P) and chamomile (CH) and a YCW product on coccidiosis-challenged broilers. Growth performance, FCR, jejunal and bursal histomorphology and serum biochemistry of coccidiosis-challenged broilers were measured or examined to determine whether the impacts the different combinations of herbs and YCW had were similar to each other and to those of the coccidiostat, salinomycin (Sal).

To the best of our knowledge, there are no published data on the effects of similar combinations of the phytogenic feed additives, peppermint and chamomile, and YCW on the performance and gastrointestinal health of coccidiosis challenged broilers.

Material and methods

The experimental procedures were approved by Animal Ethics Committee at Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok under the approved number UoD AEC120120202.

Birds, husbandry and experimental design

A total of 280 d-old mixed sex Ross 308 strain chicks were obtained from a local hatchery (Qendil Hatchery, Erbil province, Kurdistan region, Iraq) that sources their breeding stock and eggs from Turkey. The chicks were randomly assigned to seven treatments groups

Table 1. Composition of the basal starter, grower and finisher diets as g/100 g (as fed basis).

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
Dicalciun phosphate	2.72	0.95	0.82
Salt	0.11	0.19	0.10
DL-methionine	0.38	0.32	0.24
L-lysine HCl	0.5	0.26	0.21
L-threonine	0.25	0.13	0.09
Broiler premix[a]	2.5	2.5	2.5
Nutrient composition			
Metabolisable energy (kcal/kg)	3000	3150	3200
Crude protein (%)	23	21	19.16
Crude fibre (%)	2.25	2.38	2.33
Digestible arginine (%)	1.29	1.14	0.99
Digestible lysine (%)	1.29	1.14	0.99
Digestible meth + cyst (%)	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	0.45	0.42
Sodium (%)	0.16	0.16	0.16
Chloride (%)	0.35	0.312	0.23
Linoleic acid (%)	2.18	2.64	2.73

[a]The broiler premix contained per kg: Vitamin A, 400,000 U; Vitamin D_3, 160,000 U; Vitamin E, 1200 mg; Vitamin B_1, 120 mg; Vitamin B_2, 280 mg; Vitamin B_6, 160 mg; Vitamin B_{12}, 1400 mcg; Biotin, 4 mg; Niacin, 1600 mg; Folic acid, 40 mg; Vitamin K_3, 100 mg; Calcium-D-pantothenate, 600 mg; Choline chloride, 12,000 mg; Choline, 10411.2 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; Meth + Cyst, 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, 2800 U activity; Endo-1,4-Beta-xylanase, 10,800 U activity; BHT (E321) 1.34 g; Propyl gallate (E310) 0.112 g; Citric acid (E330) 0.2 g; Mycotoxin binder 40 g.

of 4 pen replicates, with 10 chicks/pen, in a completely randomised design.

The chicks were reared on wood shavings in wire-mesh sided, floor pens (100 × 100 cm) in two partitioned spaces in the same environmentally controlled facility under high sanitary conditions to protect from infection the Negative Control chicks that were not challenged with coccidia and were not treated. Temperature and lighting were set according to the management guidelines of the Ross 308 broiler strain (Aviagen 2018). All chicks were reared from d-old to 35 d-of-age on diets formulated with the Concept5 feed formulation program (Creative Formulation Concepts, Pierz, Minnesota 5636, USA, https://cfctech.com/contact.aspx, accessed August 2021) in accordance with the nutrient specifications for Ross 308 broilers (Aviagen 2019) and management guide. The starter, grower and finisher diets (Table 1) were fed from d 0 to 10, d 11 to 24 and d 25 to 35, respectively. Feed was provided *ad libitum* in plastic, hanging feeders and water from cup drinkers was available at all times.

From d-old the six groups of coccidiosis-challenged (C) broilers were fed the following treatments: Positive Control group, fed basal diets without herbs or YCW supplement; Group C P + CH, fed basal diets supplemented on top with 5 g each of peppermint powder and chamomile powder/kg diet; Group C P + Y, fed basal diets supplemented on top with 5 g each of peppermint powder and YCW product (Alimaya Catch Myco, Alimaya Company, W2N, La Forê, 03130 Neuilly-en-Donjon, France)/kg diet; Group C CH + Y fed basal diets supplemented on top with 5 g each of chamomile powder and YCW product/kg diet; Group C P + CH + Y, fed basal diets supplemented on top with 5 g each of peppermint powder, chamomile powder and YCW/kg diet; Group C Sal, fed basal diets supplemented on top with 60 mg of the anticoccidial salinomycin/kg diet. The Negative Control group was unchallenged and was fed the basal diets without supplement. Methods of preparation and analysis of peppermint and chamomile extracted from dried herb powders, results of evaluation of antioxidant activity of phytogenics and their content of phenolic compounds and flavonoids are described in the Appendix.

At 8 d-old, chicks in the six coccidia challenged groups were inoculated orally with 5000 sporulated oocysts of *Eimeria* (see below for preparation). Unchallenged birds were given 1 mL of 1 g/100 g sterile saline solution. At 10, 24 and 35 d-of-age, chicks and leftover feed were weighed on a pen basis to measure WG, feed intake and FCR. At 24 d-of-age, 2 chicks/pen were randomly selected, weighed, blood samples taken and euthanized by cervical dislocation. Five millilitres of blood were taken from the jugular vein, allowed to clot, centrifuged at 3000 rpm for 5 min and the serum was stored at −20 °C until used for measurement of blood biochemistry. Visceral organs from each chick were collected and weighed. The relative organ weights were calculated as g/100 g live BW. Approximately, 1 cm of the jejunum from each chick was gently flushed and cleaned with phosphate buffered saline (pH 7.4). The jejunal samples and the whole bursa of Fabricius were then fixed in 10% buffered formalin (M'Sadeq, Wu, Choct et al. 2015) for subsequent morphometric analysis.

Preparation of coccidial inoculant

Sporulated oocysts of coccidia were isolated in the laboratory of College of Veterinary Medicine, University of Duhok from fresh, blood-stained

Table 2. Effect on weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) at 10, 24 and 35-d-of-age in *Eimeria* challenged broilers given different dietary supplements (mean, pooled SEM, $n = 4$ pens).

Period	0–10 d			0–24 d			0–35 d		
Treatments*	WG (g)	FI (g)	FCR	WG (g)	FI (g)	FCR	WG (g)	FI (g)	FCR
Negative control	248	296^{a}	1.20	982^{b}	1369	1.39^{b}	1895^{a}	2794	1.48^{b}
Positive control	234	293^{a}	1.25	882^{c}	1467	1.67^{a}	1698^{b}	2859	1.69^{a}
C P + CH	246	297^{a}	1.21	1050^{a}	1430	1.36^{bc}	1853^{a}	2767	1.49^{b}
C P + Y	249	303^{a}	1.22	1079^{a}	1394	1.29^{c}	1859^{a}	2802	1.51^{b}
C CH + Y	244	296^{a}	1.22	1069^{a}	1456	1.36^{bc}	1865^{a}	2814	1.51^{b}
C P + CH + Y	243	270^{b}	1.11	1068^{a}	1405	1.32^{bc}	1872^{a}	2799	1.50^{b}
C Sal	249	297^{a}	1.19	1054^{a}	1418	1.35^{bc}	1903^{a}	2784	1.46^{b}
SEM	2.45	2.71	0.02	14.36	11.16	0.02	15.37	14.88	0.02
p Value	.72	.02	.35	<.0001	.23	<.0001	.001	.82	<.0001

a, b, c – Means within the same column with different superscripts differ significantly ($p < .05$).

*Negative control = unchallenged, un-supplemented diet; Positive control = *Eimeria* challenged, un-supplemented treatment diet; C P + CH = *Eimeria* challenged, peppermint and chamomile supplemented diet; C P + Y = *Eimeria* challenged, peppermint and yeast supplemented diet; C CH + Y = *Eimeria* challenged, chamomile and yeast supplemented diet; C P + CH + Y = *Eimeria* challenged, peppermint, chamomile and yeast supplemented diet; C Sal = *Eimeria* challenged, salinomycin supplemented diet.

droppings of chickens maintained in the Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok. The samples were transferred into 2 mL microfuge tubes and mixed with saline solution, then centrifuged at 6000*g* for 5 min and the supernatant discarded. The oocysts were allowed to sporulate in an aqueous solution of 2.5 g/100 mL potassium dichromate solution for 3 d at 27 °C: they were then counted and made up to a concentration of 5000 sporulated oocysts in 1 mL of saline.

Identification of coccidial oocysts in excreta

On days 7, 8, 9, 10 and 11 post inoculation with coccidia, 24 h samples of excreta were collected from Positive Control (challenged, un-supplemented) and Negative Control (unchallenged, un-supplemented) and examined under a low power light microscope for the presence of oocysts.

Tissue histological processing and measurements

The fixed samples of jejunum and bursa of Fabricius were dried, cleared and embedded in paraffin wax for histomorphological analysis (M'Sadeq, Wu, Choct et al. 2015). Sequential 7 µm longitudinal sections were placed individually onto Superfrost® slides (Thermo Scientific, Rockville, MD, USA) and stained with haematoxylin and eosin. Villus height (VH), villus apical width at the tip of the villus, villus basal width at the crypt-villus junction and crypt depth (CD) were measured on 10 villi per chick. Cross-sectional jejunal muscle widths were measured in each chick and in the bursa of Fabricius, length of lymphoid follicle, width of lymphoid follicle and follicular area in 10 lymphoid follicles were measured per chick. The Dino-eye program was used to measure and analyse images captured with a colour video camera (DinoCapture 2.0, ANMO Electronics Corporation). The villus height:crypt depth ratio (VH:CD) was calculated by dividing the average of the 10 measured villi heights by the average of the 10 measured crypt depths of the same chick. The apparent villus surface area was determined using the formula: {[(villus width at tip + villus width at base)/2] × villus height} (Iji et al. 2001). The area of bursal lymphoid follicles was measured with DinoCapture software by tracing round the image of the selected follicles.

Serum biochemistry

Serum samples were thawed and used to determine the total protein, albumin, cholesterol, alanine transaminase (ALT) and aspartate aminotransferase (AST) in an automatic COBAS *INTEGRA400* plus analyser (Cedex Bio HT Analyser).

Statistical analysis

The SAS statistical package (PROC GLM) was used to test the homogeneity of variances and normality of data as well as to determine the significant differences between treatments using a one-way analysis of variance (ANOVA) (SAS 2013). When there was a significant difference among treatments, means were compared with Duncan's multiple range test to detect differences among individual treatment means.

Results

Oocysts were detected in the excreta of Positive Control (challenged, un-supplemented) broilers collected from 7 to 11 days post inoculation. This,

Table 3. Effect on relative internal organs weights (g/100g body weight) in 24 d-old *Eimeria* challenged broilers given different dietary supplements (mean, pooled SEM, $n = 4$ pens).

Treatments*	Liver	Heart	Spleen	Gizzard	Small intestine	Bursa of fabricius
Negative control	3.11	0.78^{a}	0.10	3.73	7.08^{bc}	0.15
Positive control	2.82	0.61^{b}	0.10	3.96	6.54^{bcd}	0.21
C P + CH	2.65	0.71^{ab}	0.11	3.93	6.44^{cd}	0.15
C P + Y	2.87	0.60^{b}	0.09	3.78	8.28^{a}	0.16
C CH + Y	3.07	0.60^{b}	0.09	3.65	7.32^{b}	0.17
C P + CH + Y	2.99	0.61^{b}	0.10	4.24	8.25^{a}	0.16
C Sal	2.77	0.57^{b}	0.10	3.73	6.06^{d}	0.21
SEM	0.076	0.020	0.003	0.083	0.180	0.007
p Value	.70	.03	.85	.58	<.0001	.08

a, b, c, d – Means within the same column with different superscripts differ significantly ($p < .05$).
*See Table 2 for details of treatments.

together with the ruffled feather and bloody droppings of challenged birds, indicated that the coccidial inoculation of challenged groups of birds, was sufficient to cause an infection. No oocysts were detected in the excreta of Negative Control broilers on days 7–11 after giving the saline solution.

From d-old to 10 d-of-age (which included a period of 2 d after challenge), *Eimeria* challenged broilers given the diet supplemented with P + CH + Y consumed less feed compared to other treatments ($p < .05$): there were no other significant differences in broiler performance between treatments over that time (Table 2). During the period from 0 to 24 d-of-age, the Negative Control broilers (non-challenged and un-supplemented) and all the challenged broilers given a diet containing a treatment supplement, had greater WG ($p < .0001$) and improved FCR ($p < .0001$) than the Positive Control (challenged and un-supplemented) broilers. Negative Control broilers also had lower WG compared to all the challenged, supplemented broilers ($p < .0001$). Moreover, challenged broilers in Group C P + Y had a better FCR compared to Negative Control broilers ($p < .0001$).

From 0 to 35 d-of-age, the Positive Control broilers had lower WG ($p < .05$) and poorer FCR ($p < .0001$) than the challenged, supplemented broilers (including Group C Sal) and those of the Negative Control (Table 2).

Relative weight of internal organs

Significant differences in RW of organs were only observed in heart and intestines (Table 3). For the heart, RW was highest in the Negative Controls and, compared with all groups except that of Group C P + CH, the differences were significant ($p < .05$). The RW of the small intestines of Groups C P + Y and C P + CH + Y were heavier ($p < .0001$) than those of all other groups: intestinal RW of the C CH + Y group was heavier ($p < .0001$) than C P + CH and C Sal groups: intestinal RW of the Negative Control was heavier ($p < .0001$) than Group C Sal. No differences were observed between Positive Control, C Sal and C P + CH groups although RW of the C Sal group was lowest of all treatment groups. No differences in RW of bursa of Fabricius were observed among the groups.

Gut morphology

Villus heights (Table 4) of all groups were greater than those of Positive Control ($p < .0001$). The VH of the C Sal group was longer ($p < .0001$) than those of C P + CH, C P + Y and C P + CH + Y groups: although VH of the C Sal group was longest of all groups, it was not significantly different from the Negative Control and C CH + Y groups. Crypt depths of the Positive Control and C Sal broilers were deeper than those of all the other treatments ($p < .0001$). No significant differences were observed in CD among the other groups. The ratio of VH:CD was lowest in the Positive Control and it was significantly lower than all other groups (Table 4). The VH:CD of the C Sal group was significantly lower than the C CH + Y group and not significantly different from the Negative Control or the C P + CH, C P + Y and C P + CH + Y groups.

Although there were no significant differences (Table 4) among treatments in terms of villus tip and base widths, and apparent villus surface areas ($p > .05$), the apparent villus areas of the Negative Control and C Sal groups of broilers approached being significantly greater ($p = .0535$) than those of the other treatments.

Jejunal muscle of the Positive Control was thicker ($p < .0001$) than those of all the other groups and broilers of the C Sal and C P + Y groups had thicker jejunal muscle compared to those of C P + CH, C CH + Y, and C P + CH + Y groups ($p < .0001$). There were no significant differences between Negative Control, C Sal and C P + Y or between Negative Control, C P + CH, C CH + Y and C P + CH + Y groups.

Histomorphology of the bursa of Fabricius

Positive Control broilers had longer lymphoid follicles in the bursa of Fabricius ($p < .05$) compared with those of all the other treatments except C Sal and C P + CH + Y groups (Table 5). No significant differences in widths of lymphoid follicles were observed among

Table 4. Effect on villus height (VH), crypt depth (CD), VH:CD, apparent villus widths and surface area and jejunum muscle thickness in 24 d-old *Eimeria* challenged broilers given different dietary supplements (mean, pooled SEM, $n = 4$ pens).

Treatments*	VH (μm)	CD (μm)	VH:CD ratio	Width villus tip (μm)	Width villus base (μm)	Villus surface area (mm^2)	Jejunal muscle thickness (μm)
Negative control	945^{ab}	183^{b}	5.55^{ab}	241	247	0.230	204^{bc}
Positive control	762^{c}	402^{a}	1.91^{c}	178	207	0.146	330^{a}
C P + CH	912^{b}	203^{b}	4.57^{ab}	143	149	0.136	177^{c}
C P + Y	907^{b}	188^{b}	4.83^{ab}	145	162	0.139	258^{b}
C CH + Y	946^{ab}	160^{b}	5.90^{a}	136	141	0.130	156^{c}
CP + CH + Y	877^{b}	171^{b}	5.29^{ab}	169	174	0.150	172^{c}
C Sal	1008^{a}	311^{a}	3.82^{b}	182	242	0.211	262^{b}
SEM	12.70	14.83	0.22	11.57	14.49	0.011	9.79
p Value	<.0001	<.0001	.0002	.30	.20	.05	<.0001

a, b, c – Means within the same column with different superscripts differ significantly ($p < .05$).
*See Table 2 for details of treatments.

Table 5. Effect on histomorphology of bursa of Fabricius follicles in 24 d-old *Eimeria* challenged broilers given different dietary supplements (mean, pooled SEM, $n = 4$ pens).

Treatments*	Follicle length (μm)	Follicle width (μm)	Follicle area (μm)
Negative control	5018^{bc}	2985	$12{,}909^{b}$
Positive control	6576^{a}	3995	$21{,}532^{a}$
C P + CH	4485^{c}	3023	$10{,}803^{b}$
C P + Y	4622^{bc}	2724	$10{,}314^{b}$
C CH + Y	4924^{bc}	2925	$11{,}498^{b}$
C P + CH + Y	5572^{ab}	2904	$12{,}712^{b}$
C Sal	5610^{ab}	3604	$16{,}538^{ab}$
SEM	169.9	132.4	967.0
p Value	.004	.1	.01

a, b, c – Means within the same column with different superscripts differ significantly ($p < .05$).
*See Table 2 for details of treatments.

treatments ($p > .05$). The areas of the lymphoid follicles in the Positive Control group were larger ($p < .05$) than those of all the other treatments except those of Group C Sal. No other differences in areas were observed.

Serum biochemistry

The only differences in serum biochemistry values (Table 6) were seen in cholesterol and ALT: in the Negative Control, serum cholesterol concentration was lower ($p < .05$) than in the Positive Control and the C P + Y group had lower cholesterol ($p < .05$) than the Positive Control. Serum ALT concentrations of the Negative Control were lower ($p < .05$) than the Positive Control, C P + CH, C CH + Y and C Sal, groups. The Positive Control had significantly higher concentrations of AST than the C P + Y group. No differences among the groups were observed in serum AST, total protein, albumen and globulin concentrations.

Discussion

The isolated oocysts of *E. tenella* used to challenge the 8 d-old chicks successfully induced coccidiosis within 24 d as shown by presence of ruffled feathers and oocytes, and bloody droppings of Positive Control birds (challenged, un-supplemented). The significant reduction in WG and significantly greater FCR in the Positive Control group compared with the Negative Control group (unchallenged, un-supplemented) were also indicative of the presence of coccidiosis (Williams and Catchpole 2000). In addition, the significantly adverse effects on VH, CD, VH:CD, jejunal muscle thickness, bursal follicle length and area following administration of the coccidial inoculant in the Positive Control also indicate that coccidiosis was successfully induced by the oocyst preparation. The various combinations of phytogenic feed additives used here, with or without the prebiotic, YCW, had beneficial, though sometimes different, effects on the broilers challenged with *E. tenella* compared with un-supplemented Positive Control broilers. Beneficial effects on challenged broilers were also observed with salinomycin treatment.

Hussein (2021) has shown that the powdered peppermint additive alone had beneficial effects on WG and FCR of broilers affected with coccidiosis. Improvements in WG and FCR in uninfected broilers fed dried peppermint leaves compared with un-supplemented broilers were reported by Ocak et al. (2008) and, in heat-stressed (Arab Ameri et al. 2016), *E. coli* challenged broilers (Hasan and M'Sadeq 2020) and in unchallenged quail (Abdel-Wahab et al. 2018) beneficial effects on WG and FCR have been reported with peppermint. In combination with phytogenics other than chamomile, peppermint has been shown to be beneficial in broilers: growth and FCR in broilers challenged with different *Eimeria* species, was improved in those treated with peppermint and eucalyptus oils (Barbour et al. 2015); a combined dietary supplement of peppermint, thyme and eucalyptus oils (Hesabi Nameghi et al. 2019) resulted in improved performance, immune response, ileal morphology and microflora in broilers; WG was improved in broilers sprayed

Table 6. Effect on serum biochemistry in 24 d-old *Eimeria* challenged broilers given different dietary supplements (mean, pooled SEM, $n = 4$ pens).

Treatments*	Cholesterol (mg/dL)	ALT (μL/dL)	AST (μL/dL)	Total Protein (g/dL)	Albumen (g/dL)	Globulin (g/dL)
Negative control	91^{bc}	2.43^{c}	158	2.53	1.06	1.48
Positive control	141^{a}	4.70^{a}	244	2.35	1.02	1.34
C P + CH	128^{ab}	4.43^{ab}	188	2.34	1.08	1.26
C P + Y	83^{c}	3.25^{bc}	169	2.08	0.86	1.22
C CH + Y	114^{abc}	3.88^{ab}	180	2.45	1.05	1.40
C P + CH + Y	106^{abc}	3.48^{abc}	182	2.53	1.05	1.48
C Sal	120^{abc}	3.80^{ab}	213	2.57	1.07	1.51
SEM	5.412	0.187	8.270	0.080	0.035	0.054
p Value	.03	.01	.07	.73	.73	.74

a, b, c – Means within the same column with different superscripts differ significantly ($p < .05$).
ALT: alanine aminotransferase; AST: aspartate aminotransferase.
*See Table 2 for details of treatments.

with a mist of peppermint in combination with thyme (Witkowska et al. 2019). In the current experiment on broilers, peppermint combined in diets with chamomile or YCW or both chamomile and YCW were equally effective (with the single exception of FCR at 24 d in Group C P + YCW), at overcoming the deleterious effects on WG and FCR of a challenge with coccidiosis.

Chamomile on its own has been used in diets at 2.5–15 g/kg with beneficial effects on WG and FCR in unchallenged broilers (Al-Kaisse and Khalel 2011; Mahmmod 2013) and in *E. coli* challenged broilers (Khishtan and Beski 2020). However, Dada et al. (2015) found no benefit on the growth of broilers of 2 or 4 g dried chamomile powder or extract/kg diet. M'Sadeq, Wu, Choct et al. (2015) have shown that broilers challenged with *Eimeria* and infected with *Clostridium perfringens* to induce necrotic enteritis, had improved WG and FCR when given a YCW preparation and, in *Eimeria* challenged broilers fed a different YCW from that of M'Sadeq, Wu, Choct et al. (2015), there were improvements in production and immune function (Shanmugasundaram et al. 2013). In unchallenged broilers, Ahiwe et al. (2020) showed improved WG in those supplemented with YCW and Liu et al. (2018) have shown improvements in broilers challenged with *C. perfringens*. The improvements in growth and feed conversion with the various combinations of peppermint, chamomile and prebiotic used as in-feed additives in the current experiment could have been the results of improvements in gut health and thus improvement in digestibility of feed and absorption of nutrients (Adams et al. 1996; Persia et al. 2006), as well as improvements in disease resistance (Abdullah and Al-Barwary 2020). In the current study, treatments containing peppermint and YCW (Groups C P + Y and C P + CH + Y) appeared to have a greater effect on small intestinal RW than did treatments without both these supplements (C Sal, C P + CH and C CH + Y). Chamomile with or without YCW, appeared to have a greater effect on jejunal muscle thickness (Groups C P + CH, C CH + Y and C P + CH + Y) than the treatment without chamomile (Group C P + Y) or the group treated with the antibiotic salinomycin (C Sal).

Eimeria challenge in untreated chicks (Positive Control) resulted in shorter VH, greater CD and lower VH:CD ratio compared to unchallenged Negative Control and is consistent with published reports of the morphological damage resulting from coccidiosis (e.g. M'Sadeq, Wu, Choct et al. 2015). A shorter VH in relation to CD results in reduced absorption and more secretory cells (Schneeman 1982) whereas with a greater VH and VH:CD ratio and an increase in passage rate of digesta through the gut, the absorption capacity of the intestines is increased (Haldar et al. 2014). Thus, the changes to the gut morphology of the Positive Control would have contributed to reduce nutrient absorption and to decrease performance. The differences observed in the effects of the various combinations of peppermint, chamomile and YCW and of salinomycin although not always significant suggest that the treatments acted differently on different mucosal surface cells or intestinal structures: treatments containing both CH and YCW resulted in a more beneficial effect on the VH:CD ratio than other treatments, including salinomycin, and may reflect the chemical and physical compositions of the treatments. Combining CH with YCW may have resulted, unlike that observed with only YCW (M'Sadeq, Wu, Choct et al. 2015), in a positive effect on *Eimeria* challenged broilers.

In the current study, the increased relative weight of the bursa of Fabricius, though not significant, and the significantly increased length and area of the lymphoid follicles in the Positive Control could have been due to expansion of the bursal lining epithelium resulting from the presence of *Eimeria* in the bursa of Fabricius (Anderson et al. 1976; Helal et al. 2019). Anderson et al. (1976) and Helal et al. (2019) have observed the complete life cycle of *E. tenella* in the

bursa of infected broilers and that enlargement of the bursal fold and lymphoid follicles is likely to be an indication of an early cellular immune response against the pathogen (Ilić et al. 2004).

In the present study, the high serum levels of cholesterol and ALT in the Positive Control broilers compared with that of Negative Control and all treated groups (but especially the Group C P + Y) could be due to fat disturbed metabolism (Freitas et al. 2008): reduced liver function caused by injuries to liver and gut epithelium has been reported in coccidial infected broilers (Basith et al. 2000; Hesabi Nameghi et al. 2019).

Precise mechanisms of action of peppermint, chamomile and YCW on inducing beneficial effects in broilers are not known however some of the beneficial effects may be a result of changes to the concentrations of microflora in the gut such as beneficial *Lactobacillus* and pathogenic *Salmonella* and *E. coli* populations (Hashemi and Davoodi 2011; Shanmugasundaram et al. 2013). Active antimicrobial ingredients of peppermint have been shown to be menthol, menthone, menthyl acetate, 1,8-cineole, limonene and β-pinene (e.g. Jirovetz et al. 2009; Adaszyńska-Skwirzyńska and Szczerbińska 2017) and in chamomile, active compounds are kamasolen and α-bisabole oxide, bisaboldaxid essential oils and chamazulene (Al-Kaisse and Khalel 2011; Dada et al. 2015; Adaszyńska-Skwirzyńska and Szczerbińska 2017). The antimicrobial mode of action of essential oils derived from herbs is through their interactions with the cell membrane of microorganisms causing changes to membrane permeability for cations such as H^+ and K^+ resulting in leakage and subsequently a reduction of microbial virulence (Ultee et al. 1999; Windisch et al. 2008). In addition, phytogenics and herbal extracts have antiparasitic and coccidiostatic properties through the action of their flavonoid and phenolic compounds on oocyst wall formation and inhibition of sporulation (del Cacho et al. 2010; Fatemi et al. 2015). They also cause damage to sporozoites (Kim et al. 2013), slow the growth and reproduction of the parasite and reduce the oocyst population (Allen et al. 1997; Youn and Noh 2001; Abbas et al. 2012). On the other hand, YCW act to enhance immune function of the host and alter types or proportions of gut microflora (M'Sadeq, Wu, Swick et al. 2015, M'Sadeq, Wu, Choct et al. 2015).

Conclusions

The present study was successful in producing a model of coccidiosis infection. It illustrated that combinations of peppermint, chamomile and prebiotic YCW were as effective as salinomycin in preventing the decline in the WG and FCR performance of coccidiosis-challenged broilers. The treatments also enhanced the health and integrity of challenged broilers through increasing VH, VH:CD ratio and reducing CD compared with untreated *Eimeria* infected broilers. Outcomes of the current research indicate that combinations of peppermint, chamomile and YCW show promise as alternative treatments to pharmaceutical anticoccidiostats for preventing or reducing the effects of coccidiosis in broilers. In addition, all treatments had growth promoting effects as they significantly increased WG at 24 d compared with the un-supplemented Negative Control however, except for the C P + Y Group, FCR was not improved among the treated chicks. By d 35, the treatments retained positive effects on challenged broilers, overcoming the effects of coccidiosis challenge but there were no added production effects as treated groups were not significantly different from the un-supplemented Negative Control.

We believe the results of this study show the benefits that different combinations of peppermint, chamomile and YCW as in-feed additives can have as effective anticoccidial agents. Although differences in activities of phenol, flavonoid, AChE and BChE inhibition and antioxidant activities were detected between peppermint and chamomile (see Appendix and Tables A1 and A2) and there are differences in the active components of peppermint (Jirovetz et al. 2009; Adaszyńska-Skwirzyńska and Szczerbińska 2017) and chamomile (Al-Kaisse and Khalel 2011; Dada et al. 2015; Adaszyńska-Skwirzyńska and Szczerbińska 2017) it is not possible to identify from the results of the current experiment the relative advantage of one phytogenic over the other. It is possible that polyherbal mixtures with or without probiotics may provide greater benefits than single in-feed phytogenics however, this would require further work.

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Ethical approval

The experimental protocol and all procedures were approved by the Animal Ethics Committee of the Department of Animal Production, College of Agricultural

Engineering Sciences, University of Duhok, Kurdistan Region, Iraq under the approval number UoD AEC120120202.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Data availability statement

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

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Appendix

Samples of dried peppermint (*M. piperita*) and chamomile (*Matricaria chamomilla*) were analysed by Kilis 7 Aralik University, Turkey. Briefly, samples of the dried leaves of peppermint and chamomile were dissolved and extracted with dimethyl sulfoxide and efficiency of extraction determined as 29.5 g/100 g for peppermint and 15.8 g/100 g for chamomile.

The extracts were dissolved in water and in methanol for analysis of

1. total phenolic content using methods of Singleton and Rossi (1965) and Gezici and Sekeroglu (2019) and total flavonoid content using methods of Woisky and Salatino (1998) and Gezici and Sekeroglu (2019),
2. neuroprotective enzyme activity by measuring acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition (Ellman et al. 1961),
3. antioxidant activity using the method of Gezici and Sekeroglu (2019) for both diphenyl-2-picryl-hydrazil (DPPH) activity and cupric ion reducing capacity (CUPRAC).

Results for total phenolic, total flavonoid contents and inhibition activity for AChE and BChE enzymes (Table A1) show that peppermint contains 2–3 times higher concentrations of phenol and 4–5 times higher concentrations of flavonoids than chamomile. Griss et al. (2019) have shown that the adenosinergic and cholinergic systems are involved in the pathogenesis of *Eimeria* infections and in the immune and inflammatory responses poultry. The enzymes, AChE and BChE are important in maintaining the anti-inflammatory properties of acetylcholine.

Antioxidant inhibition activities (Table A2) measured by DPPH show similar activities in peppermint and chamomile whereas measurements with the CUPAC assay show that peppermint has about 3 times the activity of chamomile. Oxidative stress and production of free radicals occurs during the initiation and progression of infection by *Eimeria* infections (Griss et al. 2019) and some beneficial effects of phytogenics may be through an antioxidant function.

Table A1. Phenolic and flavonoid contents and neuroprotective enzyme activities in water and methanol extracts of peppermint (P) and chamomile (CH) (mean ± SD).

		Water extract	Methanol extract
Total phenolic	P	102.67 ± 0.98	90.88 ± 1.02
(mg gallic acid equivalents/g dried plant material)	CH	42.12 ± 0.62	26.38 ± 1.05
Total flavonoid	P	21.46 ± 0.36	26.02 ± 0.61
(mg quercetin equivalents/g of dried plant material)	CH	4.19 ± 0.02	6.19 ± 1.08
AChE	P	90.28 ± 0.36	94.16 ± 0.25
(Inhibition % ± SD)	CH	49.35 ± 1.21	32.16 ± 1.05
BChE	P	86.06 ± 1.12	88.55 ± 0.19
(Inhibition % ± SD)	CH	60.86 ± 0.14	56.50 ± 0.92

AChE: Acetylcholinesterase inhibition; BChE: Butyrylcholinesterase inhibition.

Table A2. Antioxidant inhibition activities in water and methanol extracts of peppermint (P) and chamomile (CH).

	DPPH analysis (inhibition %)				CUPRAC analysis (inhibition %)			
	Water		Methanol		Water		Methanol	
Concentration (μg/mL)	P	CH	P	CH	P	CH	P	CH
1000	81.03	84.09	85.19	88.17	3.74	1.11	3.63	1.11
500	64.78	70.33	74.78	73.11	2.32	0.74	2.31	0.76
250	47.75	65.22	56.75	67.20	1.26	0.42	1.25	0.42
125	34.57	40.96	42.57	48.03	0.81	0.27	0.80	0.27
62.5	27.85	31.63	31.85	32.65	0.54	0.19	0.54	0.19

DPPH: diphenyl-2-picryl-hydrazil inhibition (antioxidant capacity); CUPRAC: CUPric Reducing Antioxidant Capacity.