Protective Efficacy of *Lepidium sativum*, *Capsicum frutescens* and their Mixtures against Experimentally Induced *Eimeria tenella* Infection in Broiler Chickens

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Abstract: This study was aimed at evaluating the anticoccidial efficacy of *Lepidium sativum* (Garden cress (GC)), *Capsicum frutescens* (Hot red pepper (Hrp)) and their mixtures powder in broiler chickens. A total of 144 Cobb-500 broiler chickens were randomly allocated into six treatment groups with three replications. The experiment lasted for 42 days. Rations were fortified with 0.75% GC, 0.75% Hrp, 0.38% GC+0.38% Hrp and 0.0125% amprolium in groups 3, 4, 5, and 6, respectively and fed to the chicks starting on day two of age. Ration fed to chicks in group 1 and 2 were without any additives. Chicks in groups 2, 3, 4, 5 and 6 were infected with ~10^5 sporulated *Eimeria tenella* oocyst per chick at the age of 15 days. Animal performance, oocysts output, cecal lesion score, carcass and serum parameters were recorded during the experiment. Uninfected-unfortified and amprolium ration groups in the starter period and group that received ration fortified with a mixture of GC+Hrp and uninfected-unfortified ration in the finisher phase resulted in a higher body weight gain (BWG). Across the entire experimental period, BWG was higher in the uninfected-unfortified ration group. The average feed intake in the entire period was higher in uninfected-unfortified ration, GC and amprolium groups. Broiler chicks fed a diet fortified with GC 0.75% or amprolium (0.0125%) as additive were equally effective to reduce *E. tenella* oocyst shed at day 6, 7, 8 and average total count post inoculation. Infected chickens fed diet fortified with GC, Hrp and their mixtures showed cecal lesion similar to those fed with infected-unfortified diet group at day 7 post inoculation. Highest intestinal length at 27 days post inoculation was observed in the uninfected-unfortified ration group and the shortest length was noticed in infected-unfortified ration group. In conclusion, broilers fed diet fortified with GC 0.75%, Hrp 0.75%, GC 0.38%+Hrp 0.38% mixture and amprolium 0.0125% showed better BWG at the end of the production phase than infected-unfortified ration group. Garden cress and amprolium lowered oocyst shed indicating better protection against *E. tenella* infection.

Keywords: *Cecal lesion; Eimeria tenella; Feed additive; Oocyst shed; Phytogenic*

Introduction

Coccidiosis is a protozoan disease caused by *Eimeria* species that affect the intestinal tract of poultry and cause considerable economic loss to the poultry industry (Dalloul and Lillehoj, 2005). This disease causes moderate to severe damage to the intestinal epithelium, resulting in reduced growth rate, impaired feed conversion and often overt morbidity and mortality in chicken (Chandrakesan *et al*., 2009). Among the nine different *Eimeria* species known to infect chickens, *E. tenella* infection causes a severe disease characterized by hemorrhagic lesion development in the cecum leading to high morbidity and mortality, and reduced weight gain (McDougald and Fitz-Coy, 2008). In Ethiopia, the incidence of poultry coccidiosis has been reported to exist in many parts of the country. Lobago *et al.* (2005) reported 38% mortality in Kombolcha Poultry Multiplication Center and Dinka and Yacob (2012) noted 72% mortality in Debre zeit Agricultural Research Center poultry farm due to coccidiosis outbreak.

Currently, coccidiosis control programs in the poultry industry across the world are largely relying on chemotherapy and immunoprophylaxis measures. While these methods are effective for the control of avian coccidiosis, the continuous uses of anticoccidial drugs have led to the emergence of drug-resistant strains (Abbas *et al*., 2008). Furthermore, drug residue in poultry products has been a potential deterrent to the use of poultry products by consumers. Producers also incur high costs for medication, which results to increased cost of poultry products. Hence, searching for natural, cheap and safe alternative means of treatment against the control of coccidiosis infection has been an area of research in the recent past (Williams, 2006). Accordingly, anticoccidial properties of various natural products were reported (Abbas, 2012; Meskerem and Bookaewan, 2013). Herbs and spices rich in alkaloids, antioxidants (Naidoo *et al.,* 2008) and diets high in n-3 fatty acids (Allen *et al.,* 1996, 1997) are reported to have value for treating coccidiosis in chickens.

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Diwakar *et al.* (2010) noted that the essential oil derived from *Lepidium sativum* (garden cress (GC)) seed contains tocopherol, carotenoid, oleic acid and α-linolenic acid. Garden cress seed possesses varied medicinal properties and is known as "versatile medicine". It has been used to treat various kinds of human and animal ailments such as diarrhea, dysentery, unidentified gastrointestinal disorder, stomach-ache, indigestion, febrile disease and skin disorders (Teklehaymanot *et al.*, 2007). It also has antihypertensive, diuretic and hypoglycaemic effects (Maghrani *et al.*, 2005), known to improve asthmatic attacks (Paranjape and Mehta, 2006), and possesses aperients, alterative, tonic and carminative activities (Sumangala *et al.,* 2004; Patel *et al.,* 2009). Similarly, *Capsicum frutescens* (hot red pepper (Hrp) is said to have a pungent taste because of capsaicin and it is used as spice, feed additive and drug (Nwaopara *et al.*, 2007). Other biologically active phytochemical constituents in this plant include alkaloids, mucilages, reducing compounds, sterols and polyterpenes (Dougnon *et al.*, 2014). These components gives Hrp several pharmacological properties especially against obesity, hyperglycemia, hypercholesterolaemia, hyperlipidaemia (Takashi *et al.,* 2004), pain, gastric ulcer (Suk-Hyun *et al.*, 2006), pneumonia (Newall *et al.,* 1996), diarrhea and inflammation. Moreover, Hrp has been reported to have antioxidative (Sun *et al.,* 2007), immunomodulatory (Takano *et al.,* 2007) and anti-carcinogenic (Suk-Hyun *et al.*, 2006) effects. Furthermore, there is preclinical and clinical evidence that Hrp may have beneficial actions in protecting against lesion formation in the gastric and intestinal mucosa in part by alleviating oxidative stress (Holzer, 2004). Despite the aforementioned manifold health benefits of GC and Hrp, their natural anticoccidial effects against broiler coccidiosis was not sufficiently investigated. Therefore, the present study aimed to investigate the effects of Garden cress, Hot red pepper and their mixtures powder against *E. tenella* in experimentally infected Cobb-500 broiler chickens.

Materials and Methods

Experimental Site, Feed Ingredients and Ration Formulation

The experiment was conducted at Haramaya University poultry farm, Ethiopia, east of the capital Addis Ababa. The feed ingredients used to formulate the experimental rations are presented in Table 1. After contaminant materials were removed, the GC and Hrp were hammer milled to powder. Corn grain, noug seed meal and limestone were milled through a sieve size of 5 mm. Six standard isocaloric and isonitrogenous starter and finisher treatment rations were formulated (Table 1). The rations were formulated to meet the nutrient requirement of starter and finisher broilers as described by Leeson and Summers (2005).

Table 1. Ingredients used to formulate experimental rations and their chemical composition

	Treatment groups											
	G1		G2		G3		G4		G5		G6	
Ingredients%	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher
Corn grain	50	57	50	57	50	57	50	57	50	57	50	57
Noug Seed Meal	17	17.3	17	17.3	16	17.3	16	16.6	16.5	17.3	17	17.3
Soybean Meal	30	23	30	23	30	23	30	23	30	23	30	23
Garden Cress	-	-	-	-	0.75	0.75	-	-	0.38	0.38	-	-
Hot red pepper	-	-	-	-	-	-	0.75	0.75	0.38	0.38	-	-
Amprolium	-	-	-	-	-	-	-	-	-	-	0.0125	0.0125
Premix*	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4
Limestone	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DL-meth.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
L-lysine HCL	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Dry matter (%) and nutrient contents (%DM) of the ration												
Dry Matter	93	93	93	93	92.7	93	92.7	93	92.7	93	93	93
Crude Protein	22.6	20.3	22.6	20.3	22.5	20.3	22.55	20.3	22.55	20.3	22.6	20.3
Ether extract	4.75	4.62	4.75	4.62	4.66	4.62	4.66	4.62	4.66	4.62	4.75	4.62
Crude fiber	7.17	7.0	7.17	7.0	7.2	7.0	7.4	7.0	7.3	7.0	7.17	7.0
ME (kcal/kg DM)	3171	3218	3171	3220	3178	3221	3172	3218	3174	3221	3189	3218
Calcium	0.93	0.91	0.93	0.91	0.92	0.91	0.92	0.91	0.92	0.91	0.93	0.91
Phosphorus	0.58	0.3	0.58	0.3	0.58	0.3	0.58	0.3	0.58	0.3	0.58	0.3

**Vitamin and mineral premix =25 kg contains: Vitamins: Vit. A (E672), 75,000,000 IU; Vit. D3 (E671), 25,000,000 IU; Vit. E (all-rac-alpha tocopherylacetate) (3a700), 20,000 mg; Vit. K3, 2,000 mg; Vit. B1, 1,500 mg; Vit. B2 (riboflavin), 5,000 mg; Vit. B3 (calcium-D-pantothenate), 9,001 mg; Vit. B6 (3a831), 5,000 mg; Vit. B12 (cyanocobalamin), 25,000 mcg; Vit. pp (nicotinic acid), 30,003 mg; Folic Acid, 1,000 mg; Biotin, 100,000 mcg, Choline, 648,750 mg; Minerals: Iron, 45,000; Copper (Cu, E4), 15,000 mg; Manganese (Mn, E5), 75,001 mg; Zinc oxide-Zinc (Zn, E6), 70,001 mg; Iodine (I, E2), 2,000 mg; Selenium (Se, E8), 400,050 mcg; Calcium, 1,231,662 mg; Magnesium, 12,687 mg; Sodium, 952 mg; Chloride, 185,313 mg; BHT, 500 mg. DM: Dry matter; ME: Metabolizable energy; G1: Uninfected-unfortified ration; G2: Infected-unfortified ration; G3: Infected-0.75% Garden cress; G4: Infected-0.75% Hot red pepper; G5: Infected-0.38% Garden cress+0.38% Hot red pepper; G6: Infected-0.0125% Amprolium.*

Managements of Experimental Broilers

The experimental house and pens, watering and feeding troughs were thoroughly cleaned, disinfected and sprayed against external parasites before placing the birds. The chicks were vaccinated with HB1 at day 7 as eye drop as a preventive treatment against Newcastle disease. The chicks were brooded using 250 watt infrared electric bulbs as sources of heat and light. *Teff* straw was used as a litter material at a depth of approximately 7 cm. Feed and clean tap water were offered *ad libitum* throughout the experiment.

Experimental Design and Treatments

The experiment was carried out using a completely randomized design (CRD). One hundred forty four unsexed day old Cobb-500 broiler chickens with initial body weight of 38.5 ± 0.82 g (mean ± SD) were randomly allotted into six dietary groups each consisting three replicates of 8 chicks (Table 2). At the age of 15 days, all chickens except those in group 1 were orally inoculated with ~10^5 sporulated *E. tenella* oocysts per 1 mL of inoculums (Long *et al.,* 1976) using a calibrated syringe. Chicken in group 3, 4, 5 and 6 were fed with ration fortified with 0.75% GC, 0.75% Hrp, 0.38% GC+0.38% Hrp mixture and 0.0125% amprolium, respectively from 2 to 42 days of age. The birds were monitored daily for the presence of clinical signs.

Table 2. Layout of the experiment

Groups		Chicks/ treatment	Oocysts challenge
G1	Uninfected-unfortified ration	24	–
G2	Infected-unfortified ration)	24	+
G3	0.75% *Lepidium sativum* seed powder	24	+
G4	0.75% *Capsicum frutescens* powder	24	+
G5	0.38% *L. sativum* + 0.38% *C. frutescens* mixture	24	+
G6	0.0125% amprolium	24	+

Oocysts challenge = Challenge with ~100,000 sporulated E. tenella oocysts; (-) = No infection; (+) = Infection with sporulated E. tenella oocysts.

Isolation and Propagation of Eimeria tenella Oocysts

Eimeria tenella oocysts were identified by a combination of oocysts size, location in the gut and appearance of the lesions (McDougald and Fitz-Coy, 2008). Following evisceration at post-mortem of coccidia suspected birds, the cecal contents were washed into a beaker using tap water and the oocysts were isolated using a flotation procedure (Permin and Hansen, 1998). Oocysts were sporulated by incubating concentrated suspension of oocysts in distilled water with 2.5% potassium dichromate solution ($K_2Cr_2O_7$) and with forced aeration at room temperature for 72 hours (Bowman, 2009). The sporulated *E. tenella* oocysts were suspended in 2.5% $K_2Cr_2O_7$ solution and refrigerated at 4 ºC until oral administration. The $K_2Cr_2O_7$ solution was removed through 5 times centrifugation by distilled water and the sporulated *E. tenella* oocysts were suspended in distilled water at the time of oral administration. The sporulated *E. tenella* oocysts were orally inoculated in three chickens for oocyst multiplication. Chickens were monitored daily for the development of clinical coccidiosis and the presence of *Eimeria* oocysts in their feces. Then the reproduced oocysts were sporulated, stored and washed as described earlier for the actual experiment.

Data Collection

Intake and body weight gain: The amount of feed offered and refused was recorded daily and the amount consumed was determined as the difference between the feed offered and refused. Birds were weighed weekly in a group per pen and pen average was calculated by dividing the total pen weight by the broilers alive on that day. Body weight (BW) change was calculated as the difference between the final and initial BW. Average daily gain (ADG) was calculated as BW change divided by the number of experimental days. Feed conversion ratio was computed as the ratio of daily DM consumption per ADG. The general health of the birds daily and mortality as occurred were also recorded.

Estimation of oocyst: Fresh fecal droppings from the ground of each pen were collected in sterile universal plastic bottles from all experimental groups on day 14 and oocysts counted were recorded as pre-inoculation measure. Oocyst count per gram of feces (OPG) was determined by McMaster egg counting technique and calculated using the technique described by Permin and Hansen (1998). During the post-inoculation period, fecal samples in each pen were collected from randomly selected sites on days 4, 5, 6, 7, 8, 9, 14, 20 and 27, mixed well, and the oocysts were counted and recorded.

Pathological study: On day 7 and 27 post inoculation, three randomly selected chickens from each replicate were euthanized by cervical dislocation for cecal lesion scoring according to the method of Johnson and Reid (1970). The scoring scales ranged from 0 to +4, where: 0 = no lesion (the wall is thin and presents characteristic longitudinal groves and mucosal folds; the contents are homogenous and creamy and do not contain any particles; the color is chestnut), +1= mild lesion (with few scattered petechia, the cecal contents are normal; the petechiae are more visible on the serous membrane than on the mucosal side), +2= moderate lesion (with numerous petechia, bleeding and the cecal wall is slightly thickening; visible bloody contents at the proximal end), +3= severe lesion (with severe bleeding and clotting, the caecal walls are greatly thickened; the feacal contents have practically disappeared) and +4= extremely severe lesion (with severe bleeding, a much thickened or

rupture of caecal wall, feacal debris is no longer visible; it is enclosed in the caseous plugs; gangrene or death, assigned to dead fowl). Cecal and intestinal length (gizzard to cloaca) was also measured.

Carcass measures: At the end of the experiment, three randomly selected broilers' used for cecal lesion scoring at 27 days post-mortum were selected randomly from each replicate, starved for 12 hours, weighed and slaughtered. Birds were eviscerated and carcass cuts and non-edible offal components were determined according to the procedure described by Kekeocha (1985). Dressed weight was measured after the removal of blood and feather and the dressing percentage was calculated as the proportion of dressed carcass weight to slaughter weight. Eviscerated carcass weight was determined after removing blood, feather, shank, head, kidney, lungs, pancreas, crop, proventriculus, small intestine, large intestine, caeca and urogenital tracts. The eviscerated percentage was determined as the proportion of slaughter weight. Drumstick, thigh, breast meat, heart, gizzard and liver were separated, weighed and calculated as a percentage of slaughter weight. Fat around the proventriculus, gizzard, against the abdominal wall and the cloacae were separated, weighed and expressed as a percentage of slaughter weight.

Serum biochemical parameters: Blood for serum biochemical analysis were collected from the same birds slaughtered for caecal lesion analysis. Each blood sample was collected without anticoagulant for serum biochemical analysis. Serum was separated after centrifugation at 3,000 rpm x g for 15 min and stored at -20 ^{0}C until analyzed. Total serum protein was determined by refractometer (George, 2001). Total serum immunoglobulin concentration was determined by serum zinc sulfate turbidity test by reading the optical density of the test and the control separately at 545 nm by using spectrophotometer (Mondesire, 2004).

Data Processing and Analysis

Data were analyzed using GLM model in a completely randomized design (CRD). Data analysis was done using SAS (SAS Institute, 2008). Duncan's multiple range test was used to detect the differences ($p<0.05$) among group means. Prior to statistical testing, mortality values were logarithmically transformed [$\log10^{(X+1)}$] to create a normal distribution. The difference between treatment groups were considered significant at $p < 0.05$ level.

Results

Feed Intake, Body Weight Gain, Feed Conversion Ratio, and Mortality

Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) during the first 15 days (pre-inoculation period) were not different ($p>0.05$) among treatment groups (Table 3). However, BWG and FCR were better during the third week (first week after inoculation) in uninfected-unfortified group followed by GC and amprolium received groups. At the end of week 4 higher BWG was recorded for uninfected-unfortified group. Overall BWG at the end of starter phase (1–21 days) was higher ($P<0.05$) in uninfected-unfortified and amprolium ration groups (Table 3). In the finisher phase (22–42 days) BWG in groups uninfected-unfortified and group consumed ration fortified with GC+Hrp mixture were not different and higher ($p<0.05$) than GC, Hrp and amprolium ration groups. When considered for the entire period of broiler production (1-42 days) BWG was highest for the uninfected-unfortified ration group, lowest for the infected-unfortified ration group, and intermediate for the other groups ($p<0.05$). However, FCR for the entire period was unaffected by treatment groups ($p>0.05$). Feed intake was lower for infected-unfortified treatment as compared to the uninfected-unfortified, 0.75% GC and amprolium groups.

Mortality was higher in the starter (1–21 days) than the finisher phase of the experiment with no deaths recorded in the group consumed ration fortified with 0.75% GC and 0.38% GC+0.38% Hrp mixture. In the finisher phase (22–42 days), mortality was recorded only in uninfected-unfortified and amprolium ration groups. However, mortality rate was not significant among the groups over the entire production period.

Table 3. Performance and mortality rate of broilers fed ration fortified with garden cress, hot red pepper, their mixture or amprolium and inoculated with *Eimeria tenella* oocysts

		Treatment groups						
Age	Parameters	G1	G2	G3	G4	G5	G6	SEM
Week 1	Initial weight (g/bird)	39.3	38.7	38.6	39.4	37.6	37.6	0.8
	BWG (g/bird)	104	107	109	102	91.0	108	6.12
	FI (g/bird/day)	23.6	23.1	22.5	23.5	21.7	23.7	0.96
	FCR (feed:gain)	1.54	1.52	1.48	1.62	1.70	1.55	0.08
Week 2	BWG (g/bird)	149	143	146	144	141	154	6.23
	FI (g/bird/day)	50.4	48.6	48.5	46.3	46.4	50.1	1.39
	FCR (feed: gain)	2.37	2.39	2.35	2.27	2.32	2.28	0.10
Week 3	BWG (g/bird)	286^{a}	204^{c}	237^{b}	219^{bc}	207^{c}	241^{b}	9.93
	FI (g/bird/day)	80.5	76.4	79.0	76.4	71.2	76.4	3.48
	FCR (feed:gain)	1.97^{d}	2.62^{a}	2.34^{bc}	2.44^{b}	2.44^{b}	2.24^{c}	0.04
Week 4	BWG (g/bird)	301^{a}	231^{b}	233^{b}	237^{b}	246^{b}	248^{b}	13.7
	FI (g/bird/day)	107	94.1	90.9	91.5	94.2	100	4.98
	FCR (feed:gain)	2.50^{bc}	2.85^{a}	2.51^{bc}	2.72^{ab}	2.44^{c}	2.83^{a}	0.07
Week 5	BWG (g/bird)	377^{a}	289^{c}	329^{bc}	345^{ab}	387^{a}	354^{ab}	13.9
	FI (g/bird/day)	138	114	132	125	127	134	6.43
	FCR (feed:gain)	2.56^{ab}	2.76^{a}	2.81^{a}	2.55^{ab}	2.30^{b}	2.64^{ab}	0.11
Week 6	BWG (g/bird)	320	349	340	358	380	325	17.0
	FI (g/bird/day)	137	120	130	135	135	134	6.08
	FCR (feed:gain)	2.97a	2.41^{c}	2.68^{b}	2.63^{b}	2.49^{c}	2.88^{a}	0.04
Week 1-3	BWG (g/bird)	543^{a}	453^{bc}	485^{bc}	465^{bc}	437^{c}	502^{ab}	16.3
	FI (g/bird/day)	51.5	49.4	49.7	48.8	46.4	50.3	1.41
	FCR (feed:gain)	1.96^{b}	2.18^{a}	2.05^{ab}	2.11^{ab}	2.15^{a}	2.02^{ab}	0.05
	Mortality (%)	0.00^{b}	0.46^{ab}	0.00^{b}	0.83^{a}	0.00^{b}	0.23^{ab}	0.14
Week 4-6	BWG (g/bird)	998^{a}	869^{c}	922^{b}	939^{b}	1019^{a}	927^{b}	17.2
	FI (g/bird/day)	127^{a}	110^{c}	120^{ab}	117^{bc}	115^{bc}	123^{ab}	2.94
	FCR (feed:gain)	2.68^{a}	2.69^{a}	2.65^{a}	2.63^{a}	2.41^{b}	2.78^{a}	0.11
	Mortality (%)	0.23	0.00	0.00	0.00	0.00	0.23	0.06
Week 1-6	BWG (g/bird)	1541^{a}	1322^{c}	1407^{b}	1404^{b}	1456^{b}	1429^{b}	21.4
	FI (g/bird/day)	89.2^{a}	79.4^{c}	84.7^{ab}	82.9^{bc}	81.6^{bc}	86.5^{ab}	1.77
	FCR (feed:gain)	2.32	2.43	2.36	2.37	2.28	2.40	0.04
	Mortality (%)	0.23	0.46	0.00	0.83	0.00	0.37	0.21

[a-c]Means within a row and under treatment groups with different superscripts differ ($p<0.05$); SEM: Standard error of the mean; BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio

Oocysts Shed, Lesion Score and Intestinal Length

Peak oocysts shed was observed on day 6 and 7 post inoculation and then after reduced until day 9 post inoculation. However, amprolium supplemented groups showed fast reduction of oocysts shed on day 8 post inoculation. Diet consisting 0.75% GC or 0.0125% amprolium significantly reduced oocyst counts compared to the group infected and not fortified with the feed additives, feed fortified with Hrp or a mixture of GC+Hrp groups (Table 4). The total oocyst shed for the entire count was not significantly different between amprolium and GC fortified groups. The highest average oocysts shed across the post inoculation period were detected in infected-unfortified, Hrp and GC+Hrp fortified groups.

Table 4. *Eimeria tenella* oocyst excretions of broilers fed diet fortified with garden cress, hot red pepper, their mixtures or amprolium inoculated with *Eimeria tenella* oocysts

	Treatments (Oocyst per gram of feces * 1000)						
Days	G1	G2	G3	G4	G5	G6	SEM
Pre-inoculation	0.00	0.00	0.12	0.02	0.02	0.03	0.05
Day 4 PI	0.03	0.25	0.22	0.10	0.12	0.07	0.06
Day 5 PI	0.52^{b}	8.80^{a}	6.37^{ab}	12.3^{a}	5.85^{ab}	11.2^{a}	2.42
Day 6 PI	0.10^{c}	99.6^{a}	23.7^{bc}	101^{a}	71.0^{ab}	54.4^{abc}	20.9
Day 7 PI	0.00^{b}	176^{a}	77.7^{b}	86.7^{ab}	174^{a}	60.0^{b}	28.5
Day 8 PI	0.50^{d}	47.0^{bc}	42.9^{bc}	75.4^{b}	136^{a}	15.2^{cd}	12.5
Day 9 PI	0.07	9.67	18.4	9.73	11.1	7.03	4.8
Day 14 PI	1.70	19.7	15.6	32.9	19.9	15.0	14.2
Day 20 PI	26.4	11.2	9.87	17.4	3.00	0.73	9.37
Day 27 PI	5.85	0.90	0.60	0.58	7.90	0.38	3.54
Total count	35.2^{c}	373^{a}	196^{b}	337^{a}	429^{a}	164^{b}	31.8

$^{a-d}$ Means within a row with different superscripts differ (p<0.05); SEM: Standard error of the mean; PI: Post-inoculation; G1: Uninfected-unfortified ration; G2: Infected- unfortified ration; G3: Infected-0.75% Garden cress; G4: Infected-0.75% Hot red pepper; G5: Infected-0.38% Garden cress+0.38% Hot red pepper; G6: Infected-0.0125% Amprolium.

Cecal lesion was significantly different (p<0.05) among treatment groups at 7 days post inoculation (Table 5; Fig. 1). Infected broilers fed a diet for tified with 0.75% GC, 0.75% Hrp and their mixtures showed higher cecal lesion similar to those in infected-unfortified ration group. As expected, no lesions were observed in the cecum of uninfected-unfortified and amprolium fortified groups at 7 days post-inoculation. Significantly higher cecal lesion score was observed in the uninfected-unfortified ration group followed by 0.75% GC fortified ration groups at 27 days post-inoculation. Shortest intestinal length (from gizzard to cloaca) at 7 days post-inoculation was recorded in uninfected-unfortified ration group followed by infected 0.38% GC+0.38% Hrp fortified ration groups. Longest length at 27 days post-inoculation was observed in the uninfected-unfortified ration group followed by infected 0.75% GC ration group and the shortest length was noticed in infected-unfortified ration group.

Table 5. Intestinal length and cecal lesion score of broilers fed with diet fortified with garden cress, hot red pepper, their mixtures, or amprolium and inoculated with *Eimeria tenella* oocysts

	Treatment groups						
Parameters	G1	G2	G3	G4	G5	G6	SEM
22 days of age (7 days post inoculation)							
Slaughter weight (g)	529	510	506	519	458	535	34.0
Cecal length (cm)	11.5	12.7	11.3	12.2	10.6	12.1	0.71
Intestinal length (cm)	121^{c}	137^{ab}	140^{ab}	147^{a}	130^{bc}	147^{a}	4.94
Cecal lesion score	0.14^{b}	1.83^{a}	2.33^{a}	2.19^{a}	2.17^{a}	0.00^{b}	0.47
42 days of age (27 days post inoculation)							
Slaughter weight (g)	1574	1398	1568	1524	1541	1523	72.8
Intestinal length (cm)	195^{a}	158^{c}	187^{ab}	179^{abc}	180^{abc}	170^{bc}	7.61
Cecal length (cm)	17.2	17.2	18.5	18.6	17.8	17.5	0.83
Cecal lesion score	0.67^{a}	0.00^{b}	0.25^{ab}	0.00^{b}	0.00^{b}	0.00^{b}	0.15

$^{a-c}$ Means within a row and under treatment groups with different superscripts differ (p<0.05); SEM: Standard error of the mean; G1: Uninfected-unfortified ration; G2: Infected-unfortified ration; G3: Infected-0.75% Garden cress; G4: Infected-0.75% Hot red pepper; G5: Infected-0.38% Garden cress+0.38% Hot red pepper; G6: Infected-0.0125% Amprolium.

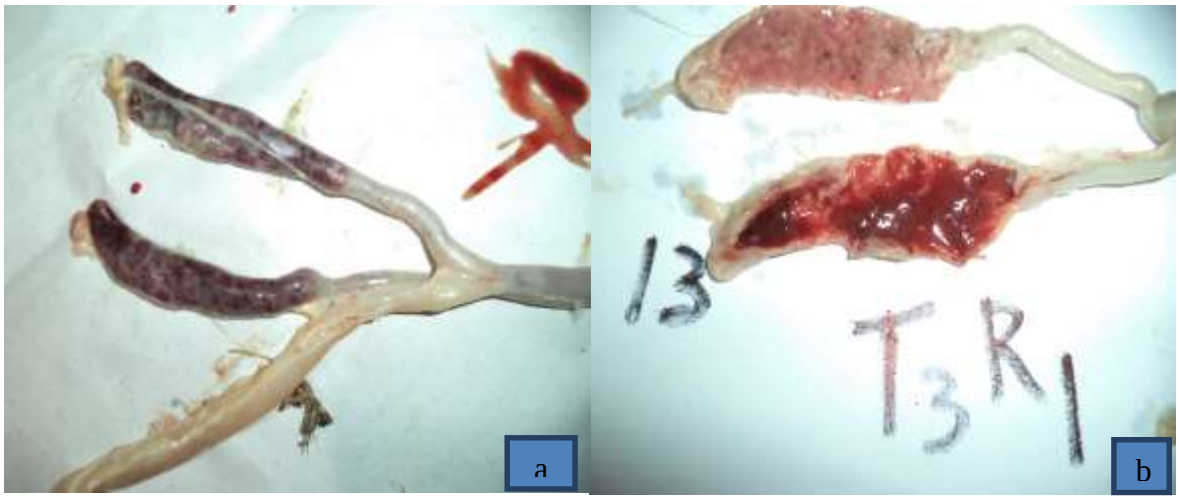

Figure 1. Gross lesions of *Eimeria tenella* oocysts infected broiler chicken caeca. a: Unopened ceca severely affected and distended with blood; b: Opened filled with blood.

Carcass Measures

Slaughter weight, dressed weight, eviscerated percentage, carcass, breast, drumstick, thigh, heart, liver and abdominal fat percentage were not significantly different ($p>0.05$) among the treatments. However, lower percentage of gizzard was observed in amprolium followed by GC+Hrp mixture and infected-unfortified ration groups (Table 6).

Table 6. Carcass characteristics of *Eimeria tenella* oocysts inoculated broilers fed with diet fortified with garden cress, hot red pepper, their mixtures or amprolium

	Treatment groups						
Parameters	G1	G2	G3	G4	G5	G6	SEM
Slaughter weight (g)	1574	1398	1568	1524	1541	1523	72.8
Dressed weight (g)	1438	1284	1437	1391	1412	1393	70.6
Dressing percentage	91.3	91.8	91.6	91.2	91.6	91.4	0.47
Eviscerated weight (g)	1143	1017	1117	1107	1097	1098	60.2
Eviscerated percentage	72.6	72.6	71.3	72.6	71.1	71.8	1.24
Carcass weight (g)	1065	952	1042	1032	1027	1030	58.2
Carcass percentage	67.7	67.9	66.5	67.7	66.6	67.4	1.32
Breast percentage	24.1	22.7	24.3	23.0	23.5	23.6	1.04
Drumstick percentage	8.68	8.51	8.88	8.70	8.92	8.71	0.27
Thigh percentage	10.2	10.4	10.2	10.5	10.4	10.0	0.33
Thigh + drumstick (%)	18.9	18.9	19.0	19.2	19.3	18.7	0.52
Heart percentage	0.58	0.67	0.64	0.68	0.61	0.64	0.06
Liver percentage	2.14	2.08	2.25	2.24	2.21	2.26	0.10
Gizzard percentage	1.93^{a}	1.86^{ab}	1.91^{a}	2.11^{a}	1.82^{ab}	1.55^{b}	0.11
Abdominal fat (%)	1.68	1.93	1.42	2.06	2.07	2.13	0.37

[a,b] Means within a row and under treatment with different superscripts differ ($p<0.05$); SEM: Standard error of the mean; G1: Uninfected-unfortified ration; G2: Infected- unfortified ration; G3: Infected-0.75% Garden cress; G4: Infected-0.75% Hot red pepper; G5: Infected-0.38% Garden cress+0.38% Hot red pepper; G6: Infected-0.0125% Amprolium.

Serum Biochemical Parameters

The effect of experimental diets on some serum biochemical parameters of broiler chickens at 22 days of age (7 days post-inoculation) is given in Table 7.

There was no significant differences ($p<0.05$) among groups in serum total protein and total serum immunoglobulin concentration.

Table 7. Serum parameters of broilers fed diet fortified with garden cress, hot red pepper, their mixtures or amprolium and inoculated with *E. tenella* oocysts at 7 days post inoculation

	Treatment groups						
Parameters	G1	G2	G3	G4	G5	G6	SEM
Total protein (g/dl)	3.10	2.80	2.70	3.03	2.33	3.20	0.23
TIG (mg/dl)	2.03	1.61	1.91	1.84	1.24	1.73	0.24

SEM: Standard error of the mean; TIG: Total immunoglobulin; G1: Uninfected-unfortified ration; G2: Infected-unfortified ration; G3: Infected-0.75% Garden cress; G4: Infected-0.75% Hot red pepper; G5: Infected-0.38% Garden cress+0.38% Hot red pepper; G6: Infected-0.0125% Amprolium.

Discussion

Improved growth performance and feed intake of *E. tenella* infected broilers fed ration fortified with GC, Hrp, their mixture or amprolium than the infected-unfortified ration group in the finisher and entire production period indicate that such feed additives play a role in the prevention of *E. tenella* in broilers. This could be an attribute of the immuno-stimulant or the appetite and digestive enzymes stimulating properties of GC and Hrp (Jang *et al.,* 2007) due to the different bioactive ingredients in these additives (Frankic *et al.,* 2009). Feed supplements with herbs and spices increase stability of feed and beneficially influence the gastrointestinal ecosystem mostly through growth inhibition of pathogenic microorganisms, which consequently reduce exposure of the digestive system to the toxins of microbiological origin (Windisch *et al*., 2008). Subsequently, herbs and spices help to increase the resistance of the animals exposed to different stress situations and increase the absorption of essential nutrients, and improve the growth of animals (Windisch *et al*., 2008). In his review, Rosen (1995) noted that supplementation with ionophores or phenolic compounds improve performance of fast-growing broilers. Meskerem and Bookaewan (2014) also reported significantly higher BWG for chickens challenged with *E. tenella* and fed diet containing *L. sativum* than those fed the control diet. Significantly lower BWG in infected-unfortified diet group following the infection was a consequence of reduced feed intake and the probably disrupted intestinal integrity, which might have affected the absorption of nutrients and the efficiency of feed utilization, which is ascribed to be the common effects of coccidiosis (Walk *et al*., 2011).

No mortality was observed in all phases of production period in groups fed diet fortified with GC or GC+Hrp even though no significant difference was recorded among the treatments. The result of the work by Meskerem and Bookaewan (2014) also demonstrated that supplementation with *L. sativum* following *E. tenella* infection significantly reduced mortality rate than those fed the control diet. Although broilers in the infected-unfortified, Hrp and GC+Hrp mixture ration groups had larger number of excreted oocysts, mortality remain lower across the production period. This was in agreement with the study conducted by Almeida *et al.* (2014) who infected broilers with *E. acervulina* and *E. maxima* and found larger oocyst count in the positive group, but similar mortality compared with the group not infected. Lower mortality might be due to relatively lower cecal lesion scores across all the treatment groups. Sever infection that may lead to death should display cecal lesion score of +4 (Johnson and Reid, 1970), but in this experiment the average higher cecal score recorded was 2.33, which is not sever enough to cause high mortality.

The parasite was not completely suppressed in any of the infected treatment groups. However, the coccidial oocyst loads of GC and amprolium groups were lower than the infected-unfortified and GC+Hrp groups. Lower oocyst in the GC group might be linked with the effect of the phenolic compounds, antioxidants and high n-3 fatty acids. *L. sativum* seed oil is rich in tocopherol, carotenoid and fatty acids such as oleic and α-linolenic acids (Diwakar *et al.,* 2010). The hydrophobic essential oils possess the ability to intrude the bacterial cell membrane and disintegrate membrane structure and cause ion leakage (Windisch *et al*., 2009). It has been also reported that antioxidant-rich plant extracts and high n-3 fatty acids have a potential benefit in treating cecal coccidiosis infections in chickens (Allen *et al.,* 1996; 1997; Naidoo *et al.,* 2008).The result highlights the importance of commercial preparations of plant-based products to reduce the effect of coccidiosis infection in organic production systems (Abbas *et al*., 2012). Such products will improve intestinal health of chickens and thus reduce the effects caused by coccidiosis infection (Waldenstedt, 2003). Regarding anticoccidial property of the chemical amprolium, it acts as a thiamine analog that competitively inhibits the active transport of thiamine, negatively affecting *Eimeria* species without harming the broilers due to the comparatively greater sensitivity of the parasite than the host (Ruff and Chute, 1991).

Broilers in the amprolium ration group showed no lesion in the cecum caused by *E. tenella*. Uninfected-unfortified group showed oocyst excretion between days 14 to 27 post-inoculation, even though there was no significant differences among treatments at these ages. As a result, cecal lesions at 27 days post inoculation were observed. This suggests contamination of pen litter of *Eimeria* parasites, even with the strict hygiene measures applied during the study. Flies, ants, other insects, and also the feet and hands of technicians may have served as vectors for the dissemination of the *Eimeria* parasites (Henken *et al*., 1994). But, BWG in the finisher and entire period was higher in uninfected-unfortified group than other treatment groups, suggesting that the low infection loads that occurred as a result of contamination

did not influence performance attributes (Rosen, 1995). All infected chicken groups showed typical signs of coccidiosis including bloody diarrhea and weight loss compared to uninfected-unfortified group at the early age of infection.

The higher cecal lesion occurred in GC during 27 days post-inoculation might be related to certain anti-nutritional factors present in GC. According to Agarwal and Sharma (2013), whole GC seed flour contains tannins, phytic acids, oxalic acid and cyanogens which might have an effect on lesion development in the cecum. Meskerem and Bookaewan (2014) noted that non-infected chickens fed a diet containing GC seed powder showed cecal lesions and mortality. The longer intestinal tract (gizzard to cloaca) recorded for the uninfected-unfortified group at 42 days of age might have provided larger absorptive capacity; hence the birds grew rapidly than infected-unfortified ration group. The shorter intestinal length observed in the infected-unfortified ration group might be due to the infection, thus, less surface area is available for absorption and consequently less tissue mass gain (Miles *et al*., 2006), which resulted to reduced performance of the birds.

Carcass characteristics of broilers relative to live body weight did not significantly differ among treatment groups, which were in agreement with Ocak *et al.* (2008) who found no significant effect on carcass traits in broiler chickens supplemented with thyme (*Thymus vulgaris*). Inclusion of GC and Hrp into the diet of broilers also did not affect the development of liver and heart differently. Al-kassie *et al.* (2012) also reported no difference between treatments consumed different levels of Hrp and black pepper mixture on liver and heart percentage.

Conclusion

Broilers fed ration fortified with GC 0.75%, Hrp 0.75%, GC 0.38%+Hrp 0.38% and amprolium 0.0125% in the entire production phase showed better BWG than infected-unfortified group. Garden cress (0.75%) and amprolium showed comparable reduction of *E. tenella* oocyst shed which is also an additional advantage over 0.75% Hrp and 0.38% GC+0.38% Hrp. However, 0.75% GC or 0.75% Hrp or 0.38% GC+0.38% Hrp can be used as an alternative feed additive to improve the body weight of broilers under areas where *E. tenella* infestation is common in all levels of production because of their availability, affordability and easy usage at farm level. However, the reason for the presence of slight cecal lesion in the GC group should be further investigated.

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Conflict of Interests

The authors declare that they have no competing interests.

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