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Full Length Research Paper

Supplementation of broiler feed with leaves of *Vernonia amygdalina* and *Azadirachta indica* protected birds naturally infected with *Eimeria* sp.

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Anticoccidial efficacy of the herbal mixture of *Vernonia amygdalina* (VA 10%) and *Azadirachta indica* (AI 10%) was tested against naturally infected broiler coccidiosis. A total of 100 four week old broilers naturally infected with coccidiosis were divided into five treatment groups based on the number of coccidian oocyst they contained. Group I which contained 20,700 oocyst counts served as a negative control. Group II contained 20,000 oocyst counts served as a positive control and was treated with a synthetic drug, amprolium, at recommended dose of 0.6 g/L for seven days. Groups III and IV with 20,200 and 20,500 oocyst counts were treated with 10% of *V. amygdalina* and 10% *A. indica*, respectively. Group V contained 20,400 oocyst count and was treated with the mixture of 10% each of *V. amygdalina* and *A. indica.* Groups II, III, IV and V were treated continuously for seven days. Oocysts were identified using the sodium chloride floatation method. The number of oocysts egg per gram (EPG) of faeces was determined by modified McMaster counting technique. Oocysts EPG decreased steadily in all the treated groups with a marked reduction in groups II and V. The packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) count were determined using standard haematological methods. PVC, haemoglobin (Hb) and RBC count of the treated birds were significantly ($p< 0.05$) higher than those of the infected untreated groups. Hematological parameters and histological features showed a significant increase in WBC, RBC and lymphocytic infiltration; indicative of cell mediated immune response. Lower heterophil/lymphocytic ratio of the groups III, IV and V after treatment suggested immunomudulatory effect of the herbs (*V. amygdalina* and *A. indica*). Hence, the plants are capable of boosting the immunity of birds with either clinical or sub-clinical coccidia infection following subsequent exposure.

Key words: *Vernonia amygdalina*, *Azadirachta indica,* haematology, immunity, coccidiosis.

INTRODUCTION

Coccidiosis of chicken is an enteric parasitic disease caused by multiple species of the protozoan parasite genus Eimeria (Apicomplexa: Eucoccidia: Eimeriidae) and is one of the commonest and economically most important diseases of poultry worldwide (Shirley et al.,2005). Yunus et al., 2008) and Lee (2009) also reported coccidiosis as one of the most important diseases of poultry worldwide. It is characterized by marked anorexia, bloody diarrhoea, morbidity, mortalities and reduction in productivity and feed conversion efficiency of affected chicken (Jang et al., 2007). The control of coccidiosis has relied mainly on the preventive use of anticoccidial drugs (coccidiostats), together with the induction of species-specific natural immunity in chicken flocks (Shirley et al., 2004, 2005). In Nigeria, the use of sulphonamides and pyrimidine

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Abbreviations: EPG, Egg per gram; **PCV,** packed cell volume; **RBC,** red blood cell; **WBC,** white blood cell; **Hb,** haemoglobin.

derivatives such as amprolium as coccidiostats is common practise (Oladoja and Olusanya, 2007). Lillehoj et al. (2007) reported the use of anticoccidial drugs in feed or water and/or vaccines using live strains of *Eimeria* in intensively reared poultry settings.

Due to the emergence of drug-resistant strains of *Eimeria* in populations of commercial flocks and increasing public concern about drug residues in meat (Williams, 2006; Bafundo et al., 2008), alternative treatments are urgently needed (Dongjean et al., 2011). Medicinal plants and herbs have been used for many years in the treatment of various diseases in animal and man. Plants native to Nigeria have also been shown to possess anticoccidial activity (Nweze and Obiwulu, 2009). Nigeria herbal plants *Vernonia amygdalina* (VA) and *Azadirachta indica* (AI) have been considered potentially effective chemo-preventive and chemo-therapeutic agents against breast cancer cells (Luo et al., 2010; Kumar et al., 2009). *V. amygdalina* was reported to have an anthelmintic and hepatoprotective properties (Adedapo et al., 2007; Adesanoye and Farombi, 2010). Recently, Ademola and Eloff (2011) reported anthel-minthic activity of *V. amygdalina* against *Haemonchus contortus* eggs and larvae.

Therefore, several studies have been undertaken to identify various dietary supplements and probiotics to control *Eimeria* infections (Jang et al., 2007; Molan et al, 2009). Here, we examined dietary supplementation with amprolium (reference drug), *V. amygdalina* and *A. indica* to control coccidiosis in naturally infected broiler chicken.

MATERIALS AND METHODS

A total of 100 day old broilers were obtained from Obasanjo Hatchery, Oluyole Estate, Ibadan. The broiler chicks were reared under standard management practices in the experimental animal house of the Faculty of Veterinary Medicine, University of Ibadan, for eight weeks. The birds were administered orally with antibiotic (*Doxygen* 20/20; Kepro B.V Holland) containing gentamycin sulphate and doxycline hydrate at the dose rate of 1 g per 3 L of drinking water for the first five days. The birds were vaccinated against *Infectious bursa* disease and Newcastle disease on days 8 and 28 respectively, using potent locally produced vaccine from National Veterinary Research Institute, Jos (NVRI). The birds were fed with a well standard broiler feed (anticoccidial free feed) and water *ad libitum* for the first few weeks of life on deep litter before transferred into battery cage system. *V. amygdalina* leaves and *A. indica* leaves were air-dried at room temperature, grounded and incorporated into broiler starter at the rate of 10%.

Grouping and dosage of experimental birds

Broiler chicks were then divided into five treatment groups (I, II, III, IV and V) of 10 birds of two replicates per group. Group I containing average oocyst count of 20,700, was left untreated (negative control). Group II containing average oocyst count of 20,000 was treated with amprolium hydrochloride (1 g per 2.5 kg body weight) for five days. Group III containing 20,200 average oocysts count was supplemented with 10% *V. amygdalina* leaves in feed. Group IV containing 20,500 average oocyst count, was treated with 10% *A. indica* leaves in feed, while group V containing 20,400 average oocyst count was supplemented with the mixture of 10% *V. amygdalina* and 10% *A. indica*.

Clinical signs typical of coccidiosis (Mc Dougald and Reid, 1997), oocyst egg per gram (EPG) of faeces, red blood cell (RBC) counts, packed cell volume (PCV) and other erythrocyte indices were used to evaluate the anticoccidial activity of amprolium hydrochloride (standard drug), *V. amygdalina* and *A. indica*. The modified McMaster technique as described by Vassilev (2002) was used to estimate EPG.

Blood collection

Blood samples (5 ml) were collected from the jugular vein into lithium heparinized tubes. The PCV was determined by microhaematocrit method (Goldenfarb et al., 1971). The values found were expressed as a percentage of the total blood volume. RBC and white blood cells (WBC) were counted using the haemocytometer (Jain, 1986). Haemoglobin concentration was determined by standard cyanometahemoglobin method as described by Jain (1986) and its values were expressed in g/% ml of blood. The mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were calculated according to Jain (1986). Blood samples were collected twice (before the commencement of the treatment and after the treatment). All the chickens that died during the study were subjected to necropsy.

Statistical analysis

All values are expressed as mean ± standard deviation (SD). Statistical analysis were performed using the analysis of variance (ANOVA) followed by Turkey post test. Differences were considered to be significant at $p<0.05$.

RESULTS

Clinical observation

The experimental birds exhibited a diarrhoeic brownish blood tinged feaces characteristic of coccidial infection. Mortality was only recorded in group I (infected-untreated) and group III (treated with *V. amygdalina).*

Growth performance

Changes in body weight of broiler chickens infected with coccidiosis after treatment were as shown in Table 1. Infected broiler chickens (group I) exhibited 15% reduction in body weight while group II treated with amprolium resulted in 22% increase in body weight. However, group III (*V. amygdalina*), group IV (AI) and group V (*V. amygdalina and A. indica*) resulted in 5, 11 and respectively. Though the group treated with *V. amygdalina* has the least weight gain compared to all other treated groups (Table 1), there is a strong indication that treatment with the mixture of *V. amygdalina and A. indica* significantly alleviated the weight loss in broiler coccidiosis in a similar manner to amprolium ($p<0.05$).

Table 1. Daily weight gain during treatment.

Experimental groups	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group I	618.30±20.07	579.30±10.14*	560.00±29.66*	540.30±21.51*	532.00±29.77*	528.30±25.78*	526.00±37.48*
Group II	610.30±36.67	640.70±22.57*	670.30±46.58	700.50±49.37	710.30±34.39*	720.00±29.60*	744.00±24.15*
Group III	640.00±27.33	566.00±15.92*	572.70±29.06	600.30±21.20	620.00±30.89	645.00±36.77*	670.30±32.08*
Group IV	635.00±36.22	650.70±30.92	662.30±46.79	680.90±28.68	690.00±29.77	700.30±30.73*	705.50±22.13*
Group V	620.70±37.45	635.70±17.06	650.40±37.12	670.00±35.78	690.00±14.72*	720.50±6.29*	732.00±15.12*

Values are expressed as mean±SEM (N=10). Asterisks (*) indicate significant difference (P<0.05) when days 2, 3, 4, 5, 6 and 7 are compared with day 1. Group I: control, group II: amprolium, group III: *Vernonia amygdalina* (VA), group IV: *Azadirachta indica* (AI), group V: VA+AI.

Haematological parameters

Tables 2a, 2b and 2c show haematological parameters of broiler chickens infected with *Eimeria* sp. and treated with amprolium, *V. amygdalina* and *A. indica*. There was significant (p<0.05) increase in parked cell volume of birds that were treated with *V. amygdalina* (27.20± 2.68), and *A. indica* (25.80±2.17), compared with infected-untreated (22.20±3.96). Similarly, there was significant (p<0.05) increase in heamoglobin concentration in birds treated with *V. amygdalina* and *A. indica* compared with the control (infected-untreated). In the same vein, red blood cell count also increased significantly (p<0.05) in birds treated with *V. amygdalina* (7.32±0.88) and *A. indica* (7.20±0.67), compared with control (5.85±1.47). The WBC of broiler chickens treated with AI (5.68±0.76) and VA+AI (5.50±0.53) were significantly higher (p<0.05) compared with control (4.32±1.29). However, the erythrocyte sedimentation rate of bird treated with *V. amygdalina* and *A. indica* (3.80±0.84) increased significantly (p<0.05) compared with control (1.6±0.89).

Effect on oocyst count

Table 3 shows the effect of amprolium (AP), VA and AI on oocyst counts of broiler chickens infected with *Eimeria* sp. Initial oocyst egg per gram of infected broiler chicken before treatment were: control (20,700), AP (20,000), VA (20,200), AI (20,200) and VA+AI (20,400), respectively. There was gradual decrease in oocyst EPG of feaces for all the treatment groups except infected-untreated (control). The EPG for the treatment groups AP (50), VA (150), AI (100), VA+AI (50) and control (22,750) differ significantly (p<0.05) compared with initial values (Table 2). Also, there were significant reductions in EPG in birds treated with AP (0), VA (100), AI (50), VA+AI (50) two weeks post treatment compared with the control (25,000).

Immuno-modulatory effect

Figure 1 shows immuno-modulatory property of *V. amygdalina* and *A. indica* on broiler chickens naturally infected with *Eimeria* sp. using mean heterophil/lymphocyte ratio. The level of mean heterophil/lymphocyte ratio of all the birds in the groups were ascertained before treatment (Figure 1). The mean results obtained after treatment showed reduction in mean heterophil/lymphocyte ratio in infected-untreated birds compared with birds treated with AP, VA, AI and VA+AI (Figure 2). This reduction was also closely followed with AI and VA+AI treated groups. This result therefore shows induction of natural immunity in birds naturally infected with *Eimeria* sp. but left untreated. Similarly, the result shows evidence of immuno-modulatory effect of leaves of *A. indica* and *V. amygdalina*. However, the groups treated with the leaves *A. indica* and *V. amygdalina* have their immunity maintained and even boosted in AI group.

DISCUSSION

In this study, a gradual but significant weight in both infected-untreated and infected-treated groups was recorded, although, the weight gain observed in the infected-untreated group was not consistent. Improvement in weight gain and decreased weight loss has also been reported in birds infected with *Eimeria tenella* strain treated with medicinal plant in reference to amprolium (El-Abasy et al., 2003; Ogbe et al., 2009). The birds in groups III, IV and V did not have appreciable weight gain, possibly due to the bitterness of the leaves.

According to our findings, there were significant reductions in haematological parameters such PCV, RBC, haemoglobin (Hb), and WBC in birds that were naturally infected with *E. tenella*. This is in line with findings of Ellakany et al. (2011) who

Table 2a. Haematological parameters of naturally infected broiler chickens before and after treatment with amprolium, *Vernonia amygdalina* and *Azadirachta indica*.

Parameter	Group I	Group II	Group III	Group IV	Group V
PCV1 (%)	22.20±3.96	23.40±2.79	27.20±2.68	25.80±2.17	24.40±0.83
PCV2 (%)	14.0±7.0*	14.67±7.23*	26.67±7.37	27.75±4.5*	26.25±2.22*
Hb1 (g/dl)	6.50±2.42	7.68±0.92	8.98±0.89	8.48±0.74	8.00±0.31
Hb2 (g/dl)	4.6±2.35*	4.73±2.40*	7.10±2.48	7.98±1.52	8.65±0.09
RBC1(×10^6/µL)	5.85±1.47	6.60±0.57	7.32±0.88	7.20±0.67	6.64±0.53
RBC2 (×10^6/µL)	3.74±2.11*	3.72±1.70*	6.57±4.33	7.20±2.87	7.09±1.41

Values are expressed as mean±SEM (N=10). Asterisks indicate significant difference (P<0.05) when groups II, III, IV and V are compared with control. Group I, control; group II, amprolium (AP); group III, *Vernonia amygdalina* (VA); group IV, *Azadirachta indica* (AI); group V, VA+AI. PCV, Packed cell volume; RBC, red blood cell; Hb, haemoglobin.

Table 2b. Haematological parameters of naturally infected broiler chickens before and after treatment with amprolium, *Vernonia amygdalina* and *Azadirachta indica*.

WBC1(×10^3/µL)	WBC2 (×10^3/µL))	MCHC1 (fl)	MCHC2 (fl)	MCV1 (pg)	MCV2 (pg)
4.32±1.29	3.33±1.01	11.54±4.21	12.47±2.80	39.06±6.12	38.10±7.44
4.56±0.48	4.33±1.03	11.62±0.56	12.55±0.57	35.25±6.37	37.04±3.95*
5.54±1.09	7.13±5.00*	36.74±0.85	38.14±12.75*	12.35±0.45	12.45±0.57
5.68±0.76	7.40±0.88*	35.88±0.7	34.78±6.15	11.78±0.26	11.68±2.09
5.5±0.53	9.10±2.58*	36.74±0.88*	12.49±2.20*	12.05±0.31	37.92±6.58*

Values are expressed as mean±SEM (N=10). Asterisks (*) indicate significant difference (P<0.05) when groups II, III, IV and V are compared with control. Group I, control; group II, amprolium (AP); group III, *Vernonia amygdalina* (VA); group IV, *Azadirachta indica* (AI); group V, VA+AI. MCHC, Mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin.

Table 2c. Haematological parameters of naturally infected broiler chickens before and after treatment with amprolium, *Vernonia amygdalina* and *Azadirachta indica*.

Parameter	Group I	Group II	Group III	Group IV	Group V
MCHC1 (g/dl)	32.84±0.43	32.82±0.09	33.10±0.92	32.86±0.15	32.79±0.11
MCHC2 (g/dl)	32.63±1.09	32.14±0.41	28.45±7.40*	32.84±0.43	32.94±0.25
ESR1 (×10^3/µL)	1.6±0.89	1.80±0.84	2.60±1.34	1.60±0.89	3.80±0.84
ESR2 (×10^3/µL)	7.0±1.0*	3.67±0.58*	2.33±0.58	4.25±0.05*	2.25±1.26*

Values are expressed as mean±SEM (N=10). Asterisks indicate significant difference (P<0.05) when groups II, III, IV and V are compared with control. Group I, control; group II, amprolium (AP); group III, *Vernonia amygdalina* (VA); group IV, *Azadirachta indica* (AI); group V, VA+AI. MCHC, Mean corpuscular haemoglobin concentration; ESR, erythrocyte sedimentation rate.

Table 3. Oocyst egg per gram (EPG) of broiler chickens naturally infected with *Eimeria* species and treated w*ith Amprolium, Vernonia amygdalina* and *Azadirachta indica*.

Day	Group I	Group II	Group III	Group IV	Group V
0	20,700	20,000	20,200	20, 200	20,400
1	20,800	12,000	9,000	7,500	6,000
2	21,000	3,000	3,500	2,500	1,500
3	21,200	1,400	2,000	1,650	1,000
4	21,400	800	1,100	900	600
5	22,000	100	400	500	150
6	22,750	50	150	100	50
7	23,000	-	100	50	50

Group I, control; group II, amprolium (AP); group III, *Vernonia amygdalina* (VA); group IV, *Azadirachta indica* (AI); group V, VA+AI.

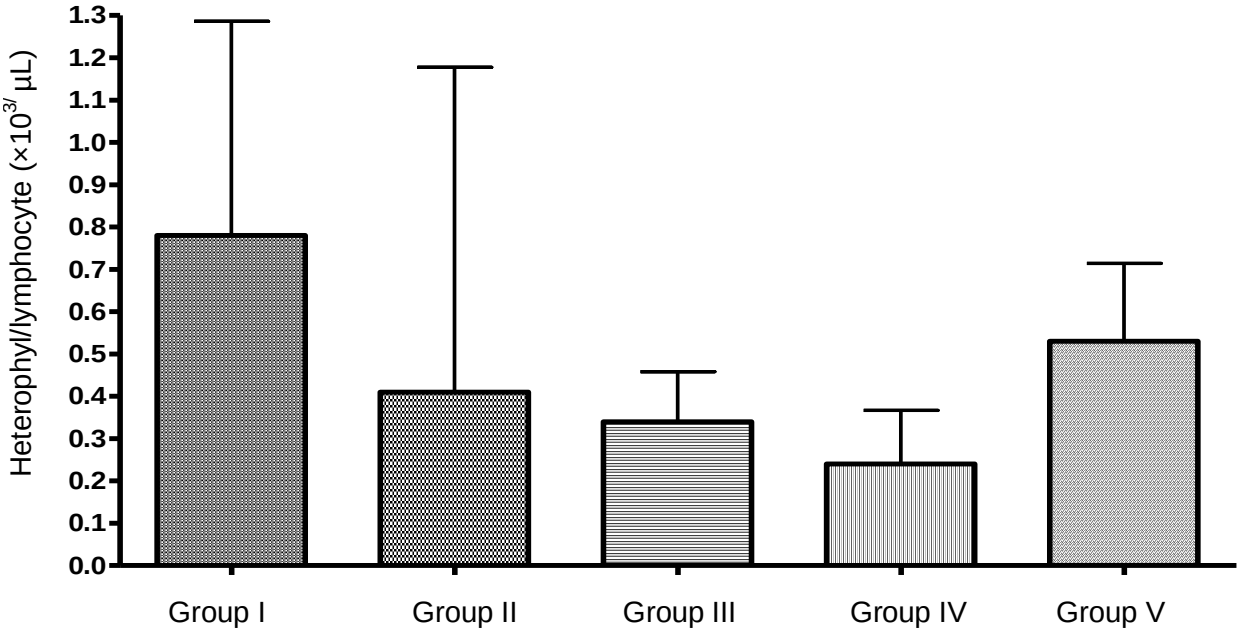

Figure 1. Mean heterophil/lymphocytes ratio (×10^3/µL) of broiler chickens before treatment. Group I, control; group II, amprolium (AP); group III, *Vernonia amygdalina* (VA); group IV, *Azadirachta indica* (AI); group V, VA+AI.

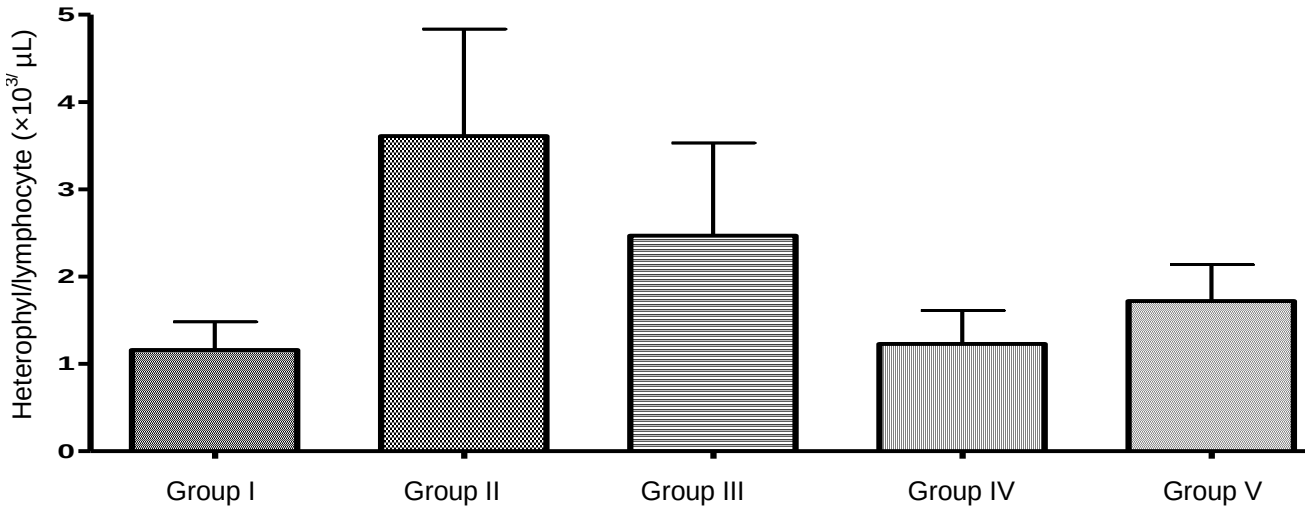

Figure 2. Mean heterophil/lymphocytes ratio (×10^3/µL) of broiler chickens after treatment. Group I, control; group II, amprolium (AP); group III, *Vernonia amygdalina* (VA); group IV, *Azadirachta indica* (AI); group V, VA+AI.

reported significant reduction in packed cell volume, haemoglobin content and lymphocyte percentage of *E. tenella* infected broilers. Broiler chickens supplemented with either VA or AI had significant increase in the values of their haematological parameters seven days post treatment. This therefore confirms the haematinic property of the supplemented diet. Similarly, dietary supplementation with either VA or AI also improved haematological parameters of the broiler chickens compared with infected-untreated birds 14 days post treatment. The improvement in the haematological parameters of the infected-treated birds was consistent throughout the course of the experiment.

As part of the underlying protective mechanisms responsible for coccidiosis, humoral immune responses have also been extensively studied in coccidiosis (Lee et al., 2009; Dalloul et al., 2003). In this study, anticoccidial antibody responses were significantly influenced by the diet supplemented with VA and AI; although significantly lower mean heterophil/lymphocyte ratio detected in the group supplemented with AI was closely followed with VA as compared to the infected-untreated group (control)

seven days post dietary supplementation. Lower heterophil/lymphocyte ratio which is a strong pointer to increased potentiation of the immune system has been reported (Oyagbemi et al., 2008). This work therefore collaborates the findings of other workers who reported significant association between lower heterophil/ lymphocyte ratio and potentiation of immune system (Aengwanich and Suttajit, 2010; Butler et al., 2010). Also, according to the findings, infected-untreated birds showed significant lower heterophil/lymphocyte ratio after one week compared to other birds that were supplemented with either VA or AI. This confirms natural immunity against coccidiosis, although with characteristics morbidity and mortality. As part of the underlying protective mechanisms responsible for coccidiosis, humoral immune responses have also been extensively studied in coccidiosis (Waihenya et al., 2002a, b; Dalloul et al., 2003). Recently, the use of alternative treatment including *Aloe vera* and medicinal plant (extract) has been reported for controlling avian coccidiosis (Anosa and Okoro, 2011; Yim et al., 2011).

In this study, dietary VA and or AI resulted in significantly reduced faecal oocyst shedding as compared to the infected group fed the amprolium (reference drug) seven days post treatment. In the same vein, dietary supplementation with either VA or AI reduced faecal oocyst shedding near normal (amprolium) 14 days post treatment. There was observable and continuous increase in faecal oocyst shedding in infected-untreated group.

Conclusion

The reduced faecal oocyst shedding obtained in this study conferred a protective role against Eimeria infection. However, supplementation with *V. amygdalina* and *A. indica*-based diet for chickens could be associated with more cellular-mediated responses than humoral responses. In conclusion, the results of this study suggest that dietary *V. amygdalina* and *A. indica* can inhibit invasion and/ or replication of *E. tenella* in the gut tissues of chickens. However, the mode of action remains to be determined. The findings of this study suggest that *V. amygdalina* and *A. indica* could be a cheaper, safe, and beneficial dietary supplement to control coccidiosis in sub-Saharan Africa. Hence, the promising results obtained from the mixture of 10% each of A.I and V.A justify its ethno-veterinary importance in broiler coccidiosis.

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