THE POTENTIAL EFFICACY OF ALOE VERA GEL AND YUCCA SCHIDIGERA EXTRACT ON GROWTH PERFORMANCE, INTESTINAL LESIONS AND INFLAMMATORY RESPONSE IN BROILER CHICKENS CHALLENGED WITH COCCIDIA

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ABSTRACT

This study was conducted to investigate the effect of Aloe vera gel and Yucca schidigera extract supplementation on growth performance, oocyst shedding, intestinal lesions and inflammatory response in broilers challenged with coccidia. Two hundred and ten, one-day old Cobb chicks were randomly allocated to seven equal groups (30 birds/group). (G1): non-infected non-treated group, (G2): infected non-treated group, (G3)&(G4): infected and treated with Aloe vera gel 5gm/L and Yucca schidigera extract 200 mg/ L respectively from 7th day of age till the end of the experiment, (G5)&(G6): infected and treated with Aloe vera gel 5gm/L and Yucca schidigera extract 200 mg/L respectively from 15th day of age till the end of the experiment and (G7): infected and treated with Amprolium 1g/ 2 L from 15th day for five successive days. Chickens in infected groups were challenged with 50,000 sporulated oocysts of field strain of Eimeria spp. on the 14th days of age. The average body weight gains, feed intake and feed conversion ratio were recorded all over the experimental period. The anticoccidial evaluation post infection depended on severity of bloody diarrhea, lesion scores as well as the oocyst shedding. In addition, histopathological changes in intestine and serum level of inflammatory cytokines were evaluated. Aloe vera gel and Yucca schidigera extract supplementation were able to mitigate the devastating effects of coccidia challenge in broilers. Growth performances represented by bodyweight gain, and feed conversion ratio were significantly improved. The Lesion score and oocysts shedding were significantly reduced as well as the serum level of pro-inflammatory (IL-6 and IL-1β) were significantly down regulated in challenged treated groups. The histopathological changes of intestine were ameliorated in treated supplemented groups. In conclusion, Aloe vera gel and Yucca schidigera extract can be a promising candidate to be used as a natural, low cost alternative to control coccidiosis in chickens.

INTRODUCTION

Avian coccidiosis is one of the most economically important protozoan diseases in the broiler, caused by genus Eimeria. (Noack et al., 2019). E.acervulina, E.maxima and E.tenella are most frequently found in intensively poultry system and the latter is highly pathogenic (Thenmozhi et al., 2014; Clark et al., 2016). Coccidial infection damage the epithelial cells of the intestinal mucosal leading to nutrient malabsorption, inefficient feed utilization, poor growth rate, high mortality and secondary bacterial infections (Lee et al., 2012; Huang et al., 2018). Also, it cause economic losses due to increase costs of treatment and prophylaxis. Indeed in a subclinical form, it may cause immunosuppression that makes the bird more vulnerable to secondary disease conditions (Akhtar et al., 2012).

Chemoprophylaxis and anticoccidial feed additives have long been used as the main strategy to control avian coccidiosis. Unfortunately, the development of Eimeria strains resistance to multiple drugs and residues in poultry products may be potentially hazardous to consumers have made increasing interest for alternative products to control coccidiosis (Cervantes 2015). One of the ways, using botanicals in the control of avian coccidiosis, as they are novel natural products contains new therapeutic molecules to which immunity has not yet developed.

Aloe vera (AV) (Aloe barbadensis Miller) is one of plants, having a great many medicinal and antibiotic properties (Christaki and Florou-Paneri 2010; Kar and Bera 2018). Aloe vera (AV) contains over 75 bio-logically active compounds include anthraquinones, polysaccharides, vitamins, enzymes and low molecular weight compounds (Choi and Chung 2003) which gives Aloe vera antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant and immunomodulatory properties (Ahlawat and Khatkar 2011; Radha and Laxmipriya 2015; Akram et al., 2019). Aloe vera gel contains polysaccharides, including acemannan which can improve the humoral immune response and cellular immunity (Du et al., 2011). Previous studies explained that Aloe vera has positive influence of on growth performance, gut microflora, and immune response (Kar and Bera 2018). Also, Aloe vera improved growth performance, reduced fecal oocyst shedding and lesion score in broilers challenged with coccidia (Yim et al., 2011; Akram et al., 2019; Ahmad et al., 2020).

Yucca schidigera (YS) is a natural, nontoxic product used commercially as a source of steroidal saponins. Moreover, it is a source of many polyphenolic compounds, such as resveratrol and a number of other phytochemicals like yuccaols (Alagawany et al., 2015). These chemical constituents have an antibacterial, anti-inflammatory, antioxidant and immunomodulatory effect (Cheeke et al., 2006). Dietary supplementation of Yucca schidigera extract improve nutrient digestibility, growth performance in broilers (Sahoo et al., 2015; Sun et al., 2017). Saponins improve the absorption of nutrients by the intestinal mucosal surface (Barnes et al., 2003). Several studies have shown Anticcoccidial effect of Yucca schidigera extract in broilers (Djezzar et al., 2014; Bafundo et al., 2020).

Keeping in view of the facts stated above, the present study was planned to investigate the beneficial effects of Aloe vera gel and Yucca schidigera extract on growth performance, lesion score, oocyst shedding, histopathological changes and inflammatory response in broilers experimentally infected with coccidia.

MATERIALS AND METHODS

Herb extracts and anticoccidial drug:

Aloe vera gel: The plant part (leaves) was identified by a taxonomist in Faculty of Agriculture Mansoura University. The mucilaginous leaf gel was separated from A. vera leaves within 3-4 h post collection to avoid aero deterioration of gel contents according to the method described by Lin et al. (2005) and Isah et al. (2019). Gently, the prewashed A. vera leaves were incised longitudinally with the
help of a sharp sterilized knife followed by gentle scraping of gel using a spatula. The gel was homogenized, filtered through cheesecloth and stored in screw-capped jars at 4°C till further use.

**Yucca schidigera extract:** Yucca extract as a commercial product (Santufo Logestics Corporation, Canada). Each liter contain Yucca schidigera extract 200gm (saponin 10gm), Mann oligosaccharides 10gm, citric acid (98%) 10 gm, sodium benzoate 5 gm and purified water up to 1 liter.

**Eimeria Challenge:** Field intestinal strains of Eimeria spp were subjected to isolation, propagation, purification and sporulation as described by (Tanweer et al., 2014). Briefly, the intestine of the infected birds were collected. The contents of the gut were collected and soaked overnight in 2.5 % potassium dichromate solution. The suspension was filtered and centrifuged at 1500 rpm for 3 min. The supernatant was discarded, and the sediment was resuspended in a saturated solution of sodium chloride and centrifuged at 1500 rpm for 3 min. The sediment containing oocysts was separated and kept in incubator at 30 °C for 24–72 h. The sporulated oocysts were stored at 4 °C in potassium dichromate solution. McMaster technique was used for counting of oocyst (Gibbons et al., 2016). Then were inoculated intra crop in broiler chickens in a dose of o 50,000 sporulated oocysts at 14th day of age (Munir et al., 2018) except negative control.

**Experimental Animals and study groups:** A total of 210 one-day old Cobb chicks were divided into equal seven groups, each of 30 birds (3 replicates / group). The birds were fed with coccidiostat free experimental feed. The birds were vaccinated with New Castle Disease (ND) and Infectious Bronchitis (IB) at day 1, Infectious Bursal Disease (IBD) at day 8 and then ND at 14 day.

Group 1(G1): non challenged and nontreated.
Group 2(G2): challenged and non treated.

Group 3(G3): challenged and treated with Aloe vera gel 5gm/L from 7th day of age till the end of the experiment.

Group 4(G4): challenged and treated with Yucca schidigera extract 200mg/L from 7th day of age till the end of the experiment.

Group 5(G5): challenged and treated Aloe vera gel 5gm/L from 15th day of age till the end of the experiment.

Group (G6): challenged and treated with Yucca schidigera extract 200mg/L from 15th day of age till the end of the experiment.

Group 7 (G7): challenged and treated with Amprolium1g/ 2 L of water from 15th day of age for five successive days.

The broiler chickens were fed ad libitum and they received a lighting regimen of 23 h light: 1 h darkness. The initial temperature was 32 °C which gradually reduced according to breeding standards. Control parameters, such as temperature, humidity, light and ventilation, were the same for all treatments.

**Evaluation of supplementation effectiveness:**
**Performance parameters:** Feed intake and body weight gain were weekly recorded throughout the study. Feed Conversion ratio were calculated (total feed intake / body weight gain) (Sainsbury 1984).

**Anticoccidial evaluation:** At days 7 and 14 post infection, three chickens from each group were selected randomly and killed. Intestines along with caeca were removed from the infected birds for the lesion scores (0 to + 4 counting system) as described by (Tanweer et al., 2014). The lesions were comprised of petechial hemorrhages, thickening of wall, bloody fecal contents, and mucoid discharge. Based upon the severity of the lesions, a score of 0 (no lesions), 1 (mild lesions), 2 (moderate lesions), 3 (severe lesions), or 4 (extremely severe lesions). The bloody diarrhea score for fecal materials as excreted droppings from each group was
determined on a scale from 0 to 4 using previously described methods (Youn and Noh 2001) as well as the number of oocyst per gram feces excreted (OPG) on 3rd, 5th, 7th, 9th and 11th days post infection (dpi) by the modified McMaster technique as described by (Alnassan et al., 2013). The reduction in oocyst production (ROP) was calculated with the formula ROP = (oocyst output of positive control group - oocyst output of negative control or medicated groups) × 100/oocyst output of the positive control group.

Inflammatory responses in serum: At 5 and 10 day post challenge three blood samples were collected from each group. Serum sample were centrifuged at 3000 rpm for 5 min. The collected sera were subjected to determination of interleukin-1β and IL6 concentrations by using ELISA kits (Becton, Dickinson and Company, Franklin Lakes, NJ) following the manufacturer’s instructions.

Pathological assay: At first week post infection (two weeks post treatment for G3&G4 and one week post treatment for G5, G6 &G7) and after 2 weeks post infection (three weeks post treatment for G3&G4 and two weeks post treatment for G5, G6&G7), three bird /group were euthanized by neck severing and necropsied. Samples of about two centimeters from the intestine (duodenum, jejunum and ileum) and cecum were cut, pinned and stowed in 10% buffered formalin solution. The samples were processed by routine histology methods, embedded longitudinally in paraffin, sectioned 5 µm thick parallel to the cut edge and stained with hematoxylin and eosin (H&E) (Suvarna et al., 2018).

Statistical analysis: Differences between groups were analyzed by using One-Way ANOVA and Duncan's multiple comparison Post Hoc tests

**RESULTS**

**Growth performance parameters:**
Our results showed that there was significant increase (p<0.05) in body weight gain and significant decrease (p<0.05) in feed conversion ratio in all challenged treated groups as compared with control positive group (G2). Chickens in groups (G3&G4) administered with Aloe vera gel and Yucca schidigera extract before infection showed significantly higher (p < 0.05) body weight gains as compared with (G7) treated with amprolium and (G5&G6) administered with Aloe vera gel and Yucca schidigera extract after infection. In addition, there was significant difference (p<0.05) in body weight and body weight gain between G3&G4. while, there was no significant difference (p>0.05) in body weight and body weight gain between G5&G6. Birds from the positive control treatment showed highest FCR (P < 0.05) compared to all other challenged treatments. There was no significant difference (p>0.05) in FCR between all challenged treated groups (Table 1).

**Clinical signs:**
In this study, no clinical abnormalities were observed in chickens of the control group (G1), while, chickens in positive control group (G2) exhibited the typical symptoms of coccidiosis including depression, dullness, ruffled feathers, reduction of feed intake, and blood stained whitish to brownish diarrhea, accompanied with progressive weakness leading to death within 3–5 days after infection. The severity of clinical signs in G7, G3 and G4 was less sever than G5&G6.

**Bloody diarrhea:**
After infection, bloody diarrhea was observed in control positive groups (G2) from 3 DPI and in infected treated groups, started one day later. The severity of bloody diarrhea in all infected treated groups was milder compared with the infected non treated groups. The severity of bloody diarrhea was milder in (G7, G3 &G4) than (G5&G6). Only one chick died from G3,G4&G6, 2 out of G5, while 4 from G2, whereas no deaths were recorded in both G1 and G7 as shown in Table(2).

**Lesion score:**
Regarding to the effect of different treatments on lesion score, our results showed that there
was a significant decrease (p< 0.05) in intestinal lesion score in all infected treated groups as compared with control positive group (G2). The most significant decrease was observed in G7 and G4 as compared with G3, G5 and G6 (P< 0.05) (table3).

**Oocysts output:**
The effect of the dietary treatments on fecal oocyst excretion in broiler chickens post infection is presented in table 4. Fecal oocyst excretion peaked at 5, 6 and 7 dpi and then declined again gradually. The faecal oocyst counts in G3, G4, G5, G6 & G7 were significantly (P < 0.05) lower as compared with positive control group (G2). The G7, G4 & G3 had the lowest number of oocyst count with an ROP value of 76.52%, 45.31% & 39.89% respectively.

**Pro inflammatory cytokine serum level:**
Our results showed that coccidia infection significantly (p<0.05) up regulate pro inflammatory cytokine IL-1β and IL-6 in all challenged groups as compared to control negative group (G2). Aloe vera gel and Yucca schidigera extract significantly down regulate (P<0.05) expression of pro inflammatory cytokines as compared to control positive group (G2). There was significant decrease (P<0.05) in pro inflammatory cytokines levels in G3, G4 & G7 as compared to G5 & G6. (fig1 A&B).

**Histopathological finding:**
At first week post infection(two weeks post treatment for G3&G4 and one week post treatment for G5,G6&G7), intestine in infected untreated group (G2) showed involvement of the intestinal crypts and glands by the different developmental stages of coccidia, desquamated mucosal epithelia, inflammatory cells (eosinophils & plasma cell) and extravasated erythrocytes in the intestinal lumina (Fig 2A). Epithelial cells were markedly degenerated and necrotic. Ball-Like collections of coccidial developmental stages were seen replacing most of the intestinal glands. Superficial mucosal necrosis and desquamation could be detected (Fig 2B). Large intestine in (G3) showed different developmental stages of coccidia including gametes and oocytes and moderate round cells infiltration, mainly lymphocytes and plasma cells in submucosa (Fig3). The superficial cecal mucosa revealed surface epithelial atrophy and degeneration. While, in G5 large intestine represented severe submucosal vascular hyperemia, hemorrhages, large number of coccidial developmental stages with partial or complete replacement and destruction of the glandular epithelium (fig4). Cecum in (G4) showed moderate involvement of intestinal crypts by degenerated developmental stages of coccidian with moderate round cells infiltration (plasma cells) in submucosa (fig5). While, in (G6) large intestine showed developmental stages of coccidia in the intestinal crypts and glands with complete or partial replacements of their epithelial lining (Fig6) and moderate submucosal infiltration by round cells. Large intestine in G7 demarcated failure of the traditional anticoccidial drug from complete relief and healing of the pre-existing coccidial lesions at this time schedule of the experiment with still presence developmental stages, although some were degenerated particularly in the intestinal glands (fig7).

After 2 weeks post infection(three weeks post treatment for G3&G4 and two weeks post treatment for G5, G6 & G7) large intestine of (G3) revealed characteristic therapy effect of the used supplementation, where large number of the still existing coccidial developmental stages were deformed (Fig8). The submucosa showed mild to moderate infiltration of round cells. The mucosal and submucosal epithelial cells were regenerated and appeared healthy with presence of variable number of goblet cells. While, (G5) showed large number of coccidial oocytes in cecal lumen, some of them appeared deformed or degenerated with absence of inflammatory or blood exudates. The intestinal crypts and glands showed moderate involvement of their epithelia by developmental stages of coccidia, many of them were degenerated (Fig 9). Examined sections from intestines of (G4) pointed out the most promising and innovated plant extract compounds as nearly most of the intestinal crypts and glands were free from any of the coccidial developmental stages,
with marked regenerative changes in the epithelial cells (Fig 10). While in (G6) large intestine showed a previously existing remnants from coccidial developmental stages, most of them were degenerated. The mucosal and glandular epithelium appeared regenerated with large hyperchromatic nuclei (Fig 11). Large intestine of G7, most of the intestinal crypts and glands were free from any of the parasitic developmental stages and the previously infested cells exhibited regenerative changes. Mild submucosal infiltration of round cells. (Fig 12).

Table 1: Effect of Aloe vera gel and Yucca schidigera extract on growth performance parameters in broilers chickens experimentally challenged with coccidia.

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<td>165.3±0.75a</td>
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SEM=Standard Error of Mean. a-e Values followed by different superscript letters were significantly different at (P <0.05).

(G1): non challenged and non treated; (G2): challenged and non treated. (G3): challenged and treated with Aloe vera gel 5gm/L from 7th day of age till the end of the experiment; (G4): challenged and treated with Yucca schidigera extract 200mg/L from 7th day of age till the end of the experiment. (G5): challenged and treated Aloe vera gel 5gm/L 15th day of age till the end of the experiment. (G6): challenged and treated with Yucca schidigera extract 200mg/L from 15th day of age till the end of the experiment. (G7): challenged and treated with Amprolium1g/ 2 L of water from 15th day of age for five successive days.
Table 2: Effect of *Aloe vera* gel and *Yucca schidigera* extract on severity of bloody diarrhea and number of deaths in broilers chickens experimentally challenged with coccidia

<table>
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<th>GROUP</th>
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Table 3: Effect of *Aloe vera* gel and *Yucca schidigera* extract on lesion scores in broilers chickens experimentally challenged with coccidia

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Table 4: Effect of *Aloe vera* gel and *Yucca schidigera* extract on oocyst output in broilers chickens experimentally challenged with coccidia

<table>
<thead>
<tr>
<th>groups</th>
<th>Oocysts excretion (10^3/g excreta)</th>
<th>3dpi</th>
<th>5dpi</th>
<th>7dpi</th>
<th>9dpi</th>
<th>11dpi</th>
<th>ROP(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>G2</td>
<td>28.276±463a</td>
<td>62.166±811a</td>
<td>182.9±1493a</td>
<td>39.303±585a</td>
<td>11.390±593a</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>12.700±564c</td>
<td>20.933±548c</td>
<td>35.345±642c</td>
<td>12.500±472c</td>
<td>4.450±444c</td>
<td>39.89</td>
<td></td>
</tr>
<tr>
<td>G4</td>
<td>11.433±611c</td>
<td>18.633±845d</td>
<td>33.66±683d</td>
<td>10.300±472d</td>
<td>4.450±444c</td>
<td>45.31</td>
<td></td>
</tr>
<tr>
<td>G5</td>
<td>14.733±405b</td>
<td>24.400±472b</td>
<td>37.600±435b</td>
<td>14.33±423b</td>
<td>5.543±166b</td>
<td>32.43</td>
<td></td>
</tr>
<tr>
<td>G6</td>
<td>14.433±448b</td>
<td>23.200±838b</td>
<td>37.400±682b</td>
<td>13.233±491bc</td>
<td>5.266±202b</td>
<td>34.30</td>
<td></td>
</tr>
<tr>
<td>G7</td>
<td>8100±550c</td>
<td>12.200±519e</td>
<td>16.600±240e</td>
<td>6750±240e</td>
<td>2.100±57d</td>
<td>76.52</td>
<td></td>
</tr>
</tbody>
</table>
**Fig. (1):** Effect of *Aloe vera* gel and *Yucca schidigera* extract on serum level of proinflammatory cytokines in broilers chickens experimentally challenged with coccidia.

**Fig. (2):** Large intestines from (G 2) showing developmental stages of coccidia (green arrows). Infiltration of submucosa by inflammatory cells, eosinophils, plasma cells and extravasated erythrocytes (black arrows) (A). Epithelial cells were degenerated and necrotic (blue arrow) coocidial developmental stages replacing the intestinal glands (red arrow) (B). Scale bars 100 um, 200 um. H&E stain
Fig. (3): Cecum from (G3) showing different developmental stages of coccidia including gametes and oocytes (black arrow) and Submucosal infiltration by lymphocytes and plasma cells (red arrow). Scale bar 100um. H&E stain.

Fig. (4): Large intestine from (G5) showing submucosal vascular hyperemia, hemorrhages (blue arrow). Destruction of glandular epithelium (black arrow). Infiltration of the submucosa by round cells (orange arrow). Scale bars 100 um H&E stain.

Fig. (5): Large intestine from (G4) showing degenerated coccidial stages (blue arrows) and infiltration of submucosa by round cells mainly plasma cells (green arrow). Scale bar 100 um H&E stain.

Fig. (6): Large intestine from (G6) showing different developmental stages of coccidia in the intestinal crypts and glands (green arrow) and Submucosal infiltration by round cells (black arrow). Scale bar 100 um H&E stain.

Fig. (7): Large intestines from (G7), showing degenerated coccidial developmental stages in the intestinal glands (blue arrow). The submucosa is moderately infiltrated by round cells (red arrow). Scale bar100 um. H&E stain.
Fig. (8): large intestine from G3 showing regenerated epithelium with large hyperchromatic nuclei in glandular epithelium (red arrow) with degenerated bcoccidial developemental stages (black arrow). Scale bar 100 um. H&E stain.

Fig. (9): large intestine from G5 showing developmental stages of coccidia in intestinal crypts and glandular epithelium (yellow arrows). Regenerative changes in the glandular epithelium (green arrow). Scale bar 100 um H&E stain.

Fig. (10): large intestines from (G 4) showing free intestinal crypts and glands from coccidial developmental stage. Regenerative changes in the crypt and glandular epithelial cells (green arrow). Scale bar 200 um. H&E stain.

Fig. (11): large intestines from (G 6) showing degenerated coccidial developmental stages (black arrows) (A). Infiltration of submucosa by round cells (orange arrow). Scale bar 100 um. H&E stain.

Fig. (12): large intestines from (G 7) showing free intestinal crypts and glands from the parasitic elements (green arrow) Mild submucosal infiltration of round cells (black arrow). Regenerative changes in previously infested cells (orange arrow). Scale bar 100 um. H&E stain.
DISSCUSSION

For long periods anticoccidial drugs are used successfully to ameliorate the adverse effect coccidia infection in poultry. However, alternatives to combat coccidiosis has become of great importance due to increase in resistance against anticoccidial drugs (Oelschlager et al., 2019). Phytochemicals are emerging as new alternative methods to control coccidiosis (Kaingu et al., 2017). Keeping in view the wide range of Aloe vera and Yucca schidigera activities, the present study was conducted to investigate their ability to alleviates the negative impact of Eimeria infection in broiler chickens.

In the current study, Eimeria challenge significantly reduced growth performance of infected birds including body weight gain, feed intake, and increase FCR as compared with non challenged birds. These results were in close agreement with previous observation showing that growth performance of broiler chickens impaired by coccidia challenge (Kaingu et al., 2017; Oelschlager et al., 2019). Pathological damage of the intestinal wall during Eimeria infection lead to poor nutrient absorption which, in turn decrease weight gain (Kipper et al., 2013). Regarding to effects of Aloe vera gel and Yucca schidigera extract on body weight gain, there was significant increase in body weight gain (p<0.05) in (G3&G4) supplemented with these treatment as prophylactic than G7 treated with amprolium and (G5&G6) supplemented with these treatment as therapeutic. On the other hand, there was a significant difference (P<0.05) in body weight gain between G3&G4 while, there was no significant difference (P>0.05) in body weight gain between G5&G6. There was no significant difference (p>0.05) in FCR between all challenged treated groups.

Similar to the finding of the current study, Alfaro et al. (2007) and Bafundo et al. (2020) reported that dietary supplementation of Yucca schidigera extract (YSE) induced significant increase in bodyweight gain and better FCR in broilers challenged with Eimeria as compared to infected non treated groups. On the contrast, Oelschlager et al. (2019) reported that Yucca schidigera extract had no significant influence on growth performance include body weight gain and FCR of broilers challenged with Eimeria. The growth-promoting effects of YSE is attributed to synergistic effect of steroidal saponins and phenolic components that improve nutrient absorption by increasing intestinal permeability (Alagawany et al., 2016). Also, dietary supplementation with saponins result in the emulsification of oil fats, promoting their digestion. (Begum et al., 2015).

Isah et al. (2019) reported that supplementation of drinking water with AV gel significantly increase body weight gain in broilers challenged with coccidia. Also, Ahmad et al. (2020) reported that supplementation of drinking water with AV gel (5gm/L) significantly increase body weight gain and decrease FCR in broilers challenged with coccida as compared to control positive groups. On the contrary, Mmereole (2011) reported that dietary inclusion of Aloe vera leaves powder in broilers at 1% has no significant difference in body weight gains as compared to the control group. Growth hormones (auxins and gibberellins) and polysaccharide (glucomannan) components of Aloe vera able to reduce the inflammation and haemorrhages in the intestines caused by Eimeria infection that led to better digestion and enhanced weight gain (Surjushe et al., 2008). In addition, AV gel contains several beneficial ingredients including vitamins, minerals, enzymes, organic acids, and carbohydrates which could improve productive traits in broilers (Yim et al., 2011).

Regarding to anticoccidial activity, our results showed that yucca schidigera extract and Aloe vera gel significantly reduced the intestinal lesion as compared to positive control group. Among treated groups (G7) and (G4) had less severe lesion score as compared with (G3), (G5) and (G6). These observations agree with those described by Ahmad et al. (2020) who reported that Aloe vera gel reduced intestinal lesions in broilers chickens challenged with
coccidia as compared to positive control group. Likewise, Bafundo et al. (2020) reported that dietary *yucca schidigera* extract reduced intestinal lesion in broilers chickens challenged with coccidia. Concerning the fecal oocyst output, *yucca schidigera* and *aloe vera* gel supplementation significantly lower oocyst per gram (OPG) as compared with positive control group, yet, it failed to do as the infected medicated group with amprolium (G7). The highest reduction in (OPG) was (76.25%, 45.31%&39%) in G7, G4&G3 respectively. While, G5&G6 had the lowest reduction in OPG (34.3% and 32.43%). The reduction in OPG is an indicative of a lower degree of infection (Kucukyilmaz et al., 2012). The reduction in oocyst count is an evidence of improvement bird immunity to resist coccidian infection. (Iee et al., 2013). These findings corresponded with Alfaro et al. (2007) and Bafundo et al. (2020) who reported that dietary *Yucca schidigera* reduced oocyst output in broilers challenged with coccidia. The anticoccidial activity of *Yucca schidigera* might be ascribed to saponins content of *Yucca schidigera* that inhibit the development of protozoa by interacting with the cholesterol present in the parasite cell membrane, resulting in parasite death (Francis et al., 2002). Also, It was assumed that saponin did not destroy the oocyst’s wall but entered the wall through the micropyle cap or gap and directly disturbed the sporocyst (Wina 2018).

In addition, (Yim et al., 2011; Kaingu et al., 2017; Ahmad et al., 2020) reported that dietary supplementation of *Aloe vera* significantly reduced mean of OPG in coccidia challenged broilers. *Aloe vera* may interfere with critical stages of *Eimeria* development and reduces damages to the intestinal wall of the chicken by reducing oocysts count, lesion score and haemorrhage (Yim et al., 2011; Kaingu et al., 2017; Ahmad et al., 2020). The polysaccharide derivatives, in *Aloe vera* gel, has antisporulation effect by interfering in the physiological process necessary for sporulation process like preventing access of oxygen and inhibition of various enzymes responsible for sporulation (Khan 2012). The chemical constituents in *Aloe* extract as 1, 8 dihydroxyanthraquinone and its derivatives include Aloeemodin, aloetic acid and isobarbaloin act as laxative agents that increase bowel motility. This leads to the quick discharge of coccidial oocysts that are lodged in faecal matter thereby reducing the oocyst count (Nghonjuyi et al., 2015).

Cocidiosis is well known to result in strong intestinal inflammation that is partly mediated by an overexpression of cytokines (Lillehoj et al. 2004). In the current study, *Eimeria* challenge induced significant increase (p<0.05) in IL6& IL-1β level in serum of positive control group as compared to other treated groups. Our findings corroborate previous reports increase level of IL-6, IL-1β due to *Eimeria* infection in chickens, (Hong et al., 2006; Grenier et al., 2016; Moraes et al., 2019). While, supplementation of *Yucca schidigera* and *Aloe vera* downregulate levels of IL6& IL-1β. These finding indicate that these supplements exerted an anti-inflammatory effect. Oelschlager et al. (2019) reported that *Yucca schidigera* extract ameliorated the upregulation of IL-1β level in broiler experimentally challenged with coccidia. Saponin supplementation may possess some measurable immunomodulatory effects during *Eimeria* infection. YSE is a rich source of polyphenols such as resveratrol (Piacente et al., 2005). Resveratrol could inhibit the NF-jB pathway that result in the decrease transcription of pro inflammatory cytokines like IL-1β and TNF-α. (Zhang et al., 2014). Acemannan, a major polysaccharide present in AV gel, appeared to be able to binds to macrophage receptors and stimulates the synthesis of cytokines (interleukin 1 (IL-1), interleukin 6 (IL-6)) and tumor necrosis factor-alpha (TNF-α) (Djeraba and Quere 2000.; Darabighane et al., 2012).

The severity of lesions and the number of parasites observed at histological pathological examination are in accordance with the recordings for the macroscopic lesion score and the oocysts counts. At first week after infection,
all challenged treated groups showed less inflammatory and degenerative changes and reduced coccidial developmental stages as compared to control positive groups. Among treated groups, the most improvement in inflammatory changes and reduction in coccidial developmental stages was observe in (G7) treated with amprolium followed by (G 4) treated with yucca and (G3) treated with aloe vera as prophylactic. Similarly, after two weeks post infection (G7) treated with amprolium showed the most improvement where the intestinal crypts and glands were free from any of the parasitic elements and the previously infested cells exhibited regenerative changes. (G4) treated with yucca as prophylactic showed improvement similar to (G7) treated with amprolium where the large intestine appeared free from coccidial stages with marked regenerative changes in crypts and glandular epithelium and absence of inflammatory or hemorrhagic complicating changes of avian coccidiosis. These finding indicates that these supplementation is able to kill or inhibit the growth and development of oocysts, regenerate the damaged cecal tissues and the decrease in inflammatory cells.

Oelschläger et al. (2019) reported that saponin supplementation modified morphological parameters of the jejunum on 14th day following Eimeria challenge by reducing mucosal thickness. Saponin supplementation may promote regeneration and reconstitution of the intestinal mucosal layer back to normal levels as evidenced by saponin treated birds not being significantly different from the unchallenged birds.

Previous studies reported increased Lactobacillus count and reduced Escherichia coli count in the gut of broilers by supplementing diets with AV (Sujatha et al., 2017). Short-chain fatty acids, as the final product of fermentation by Lactobacillus bacteria, improve intestinal morphology and might have stimulated the proliferation of epithelial cells of the bowel (Olnood et al., 2015). In addition, lower crypt depth with Aloe vera supplementation indicated for slow tissue turnover preventing the pathogens from tissue destruction in the gut (Ghazanfari et al., 2015).

CONCLUSION

In conclusion, the results of the present study demonstrated that Yucca schidigera extract and Aloe vera gel supplementation has great potentials for improving growth performance, intestinal lesions and inflammatory response in broilers under coccidia challenge. Therefore, Yucca schidigera extract and Aloe vera gel may be a potential and valuable candidate to be used as a low cost alternative drugs to control coccidiosis in chickens. Further researches should be done to investigate the therapeutic effect and mechanism of action of Yucca schidigera extract and Aloe vera gel against coccidiosis in broiler chickens.

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