

## THERAPEUTIC POTENTIAL OF SOME ORGANIC ACIDS AND ESSENTIAL OILS AGAINST *EIMERIA SPP.* ISOLATES COLLECTED FROM BROILER CHICKEN FARMS IN ASSIUT

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### ABSTRACT

Different *Eimeria* isolates were collected and identified in 50 intestinal specimens. The therapeutic potential of the organic acids and essential oils blends against *E. tenella* was compared with the anticoccidial drugs toltrazuril and amprolium in broiler chickens. Results revealed that *E. tenella* was the most frequent identified *Eimeria* species (48 %) followed by *E. acervulina* (30 %) and *E. mitis* (28 %), while the least detected were *E. mivati* (2%) and *E. brunetti* (2 %). Organic acids medication reduced the clinical signs and mortality to 33.3 % compared to amprolium (40 %), Essential oils (40 %), and toltrazuril (53.3 %) medicated groups and the positive control (60 %). The best relative body weight gain percent during the experimental course was achieved by birds medicated with organic acids (40 %) and toltrazuril (40 %) followed by amprolium (30 %) and essential oils (30 %) medicated group and the least in the positive control (10 %). The severity of the cecal lesions was reduced similarly by 34 % in all the medicated groups; all scored 2.3 on average in comparison to 3.5 in the positive control. Both organic acids and essential oils were unable to reduce the output of oocysts by over 17% and 3%, respectively, as opposed to 37% in the toltrazuril and 20.6% in the Amprolium treated groups. Microscopic observations revealed their healing effect of on the cecal tissue (epithelium and inflammatory response). The results support the notion that integrating the organic acids and or essential oils may have the ability to induce positive therapeutic effects similar to some chemical drugs such as toltrazuril and amprolium.

**Keywords:** *E. tenella*, Organic acids, Essential oils, Toltrazuril, Amprolium

### INTRODUCTION

Chicken coccidiosis triggers nonstop economic losses and outbreaks despite the

continuous use of anticoccidial and improved management conditions in broiler rearing (Blake *et al.*, 2020). In broiler chickens, the intestine can be resided by one or more of 7 *Eimeria* species; known as *E. acervulina*, *E. praecox*, *E. mivati*, *E. maxima*, *E. mitis*, and *E. tenella*. Variations among these *Eimeria* species comprised the pathogenicity, high degree of host and

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location specificity, type of lesion produced, and oocysts morphometrics (López-Osorio, 2020). The *Eimeria* life cycle, in general, is complicated and triggers a strong inflammatory reaction, oxidative stress, and lipid peroxidation, leading to tissue damage, diarrhea, impaired growth, increased susceptibility to other disease agents, and in severe cases, mortality (López-Osorio, 2020). *E. tenella* produces hemorrhagic typhlitis accompanied by white dots (schizonts and oocysts) that can be visible from the serosal surface (Schneiders *et al.*, 2020). Although advanced immunological and especially molecular techniques are available, microscopic detection for parasitic elements, for its simplicity and specificity, remains the main diagnostic method for coccidiosis (Holdsworth *et al.*, 2004). Various anticoccidial drugs are widely used to control coccidiosis comprised toltrazuril, amprolium, ionophores, and sulfonamides (Mustafa *et al.*, 2021). Chapman, (1989) recorded the high efficiency of toltrazuril against *E. tenella* over the sulfaquinoxaline /pyrimethamine and Amprolium /ethopabate. Chemotherapy is the first choice for controlling coccidiosis outbreaks however many drawbacks were reported including drug residues and drug resistance. The international regulations prohibiting the use of chemical therapy on livestock intended for human consumption encouraged researchers to look for natural and secure alternatives (Muthamilselvan *et al.*, 2016). Organic acids and essential oils are attractive natural alternatives to anticoccidial drugs. The anticoccidial alternatives depended on diminishing the oocyst shedding through retarding parasite invasion, replication, and development in the gut tissues. Some alternatives as essential oils (carvacrol, terpenoids, cinnamon bark, and anise oils) and phenolics react with cytoplasmic membranes stimulating coccidial cell death; reducing lipid peroxidation to an acceptable level; allowing epithelial repair; and reducing the intestinal permeability brought on by *Eimeria* species (El-Shall *et al.*, 2021). Furthermore, essential oils are efficient antimicrobial

agents against Gram-negative and Gram-positive bacteria (El-Tarabily *et al.*, 2021). Organic acids are efficient for feed and food preservation as well as having positive impacts on intestinal health and bird performance. Organic acids were reported as a favorable supplement for swine and poultry due to boosting animal physiology and immunity, gut protection, modifying microbiota, and reducing the gut pH (Mustafa *et al.*, 2021). The organic acids such as short-chain fatty acids (majorly propionic acid, acetic acid, and butyric acid) can replace the antimicrobial growth promoters from broiler diets (Scicutella *et al.*, 2021). In their undissociated forms, organic acids can enter the microbial cell and dissociate into H<sup>+</sup> ions and anions (RCOO<sup>-</sup>) which run out the microbial energy and disrupt protein synthesis (Mani-López *et al.*, 2012). Commonly, these alternatives were used in poultry diets as growth-promoters and natural immuno-stimulators (El-Shall *et al.*, 2021). The effect of organic acids and essential oils as anticoccidial therapies has been not often investigated. So, this study highlights the therapeutic efficiency and the clinical outcome of 2 anticoccidial alternatives (organic acids and essential oils blends) against cecal coccidiosis in view of their effect on the performance and intestinal morphology of healthy and challenged broiler chickens

## MATERIALS AND METHODS

### I. Frequency of *E. species* in broiler chickens

#### 1) Sampling

Multiple *Eimeria* isolates were collected from 50 representative intestinal specimens of different clinically-ill broiler farms (birds aged 10-40 days) and submitted (September 2021 - March 2022) to the research laboratory of Avian and Rabbit Medicine Department, Faculty of veterinary medicine, Assiut University, with a history of bad feed conversion, depression, and or bloody diarrhea.

The fresh intestines were partitioned into duodenum, jejunum, ileum, and two ceca. The contents of each part were directly examined for *Eimeria* oocysts and other Eimerian developmental stages through “unstained wet mount” using light microscopy (10X and 40X objectives, Olympus CH30, Olympus Optical Co., Ltd., Tokyo, Japan) (Garcia, 2001; Conway and McKenzie, 2007).

## 2). *Eimeria* Oocysts identification

The oocysts were sporulated, purified, and specified according to the standard techniques (Ojimelukwe *et al.*, 2018). The *Eimeria* spp. were identified, conventionally, based on the affected site and morphometries (length, width, shape index, and presence or absence of micropyle and micropylar cap) of the sporulated oocysts using light microscopy (Levine, 1985; Castañon *et al.*, 2007; Haug *et al.*, 2008).

## II. The therapeutic possibility of the organic acids and essential oils against cecal coccidiosis

### a. *Eimeria* isolate

An *Eimeria tenella* isolate was obtained from the ceca of a 28-day-old broiler chicken flock with typical signs of cecal coccidiosis; bloody diarrhea, abnormal growth rate, hemorrhagic typhlitis, and mortality. For further *E. tenella* isolate identification, cecal tissue samples from these birds were fixed in 10% neutral buffered formalin and submitted to histopathological processing according to Talukder, (2007). The isolated oocysts were propagated, sporulated, and identified following the standard measures described by Ryley *et al.* (1967) and Levine, (1985), then stored at 4 °C until use.

### b. Experimental birds and design

Ninety 1-day-old Arbor Acres broiler chicks (Assiut for investment and development Co., Egypt) were divided into 6 groups (15 birds/each); negative control (non-infected non-medicated), positive control (infected non-medicated), infected-organic acid blend, Acidene Idro New, medicated (dose:

1 mL/L drinking water), infected-essential oils blend, Orgacol, medicated (dose: 1 cm/L drinking water), infected toltrazuril, Nalcoxi<sup>®</sup> suspension 5 %, medicated (dose: 7.5 mg/kg. BW), and infected-amprolium HCl medicated (dose: 73 mg/kg B. W. /day).

Birds were raised following the ethical guidelines of Assiut University, Assiut, Egypt. The experimental groups accessed freely to feed and water and continuous constant lightening for 24 hrs throughout the experiment. Standard starter and grower pelleted rations were supplied without any anticoccidial medication.

At 23 days old, all birds, except the negative control group, were challenged with  $4.1 \times 10^4$  sporulated oocysts/bird. Birds received the experimental drugs 48 hrs after infection for two consecutive days.

The anticoccidial potential of the experimental drugs was assessed on the 7<sup>th</sup> day post-infection (30 days old) through clinical signs including the mortality ratio, the mean relative body weight gain percent (RBWG %), the mean cecal lesion score (MLS), the reduction % of the MLS (RLS), and the oocyst reduction % according to Haug *et al.* (2008), Conway and McKenzie, (2007), and Li *et al.* (2019).

### Mortality %

The cumulative mortality during the challenge period was recorded and the mortality % was calculated;

$$\text{Mortality \%} = \frac{\text{Number of dead birds}}{\text{Number of initial chickens in the group}} \times 100$$

### The mean relative body weight gain percentage (RBWG%)

All birds of each group were weighed prior to infection (initial BW) and at day 7 post-infection (final BW). The RBWG % between the negative control, positive control, and challenged-medicated groups was calculated as assigned by Li *et al.* (2019) via the following equation:

$$\text{Mean RBWG \%} = \frac{\text{Final BW} - \text{Initial BW}}{\text{Initial BW}} \times 100$$

### The cecal lesion score

After sacrifice, the cecal lesions caused by *E. tenella* were scored in 7 birds/group grossly following Johnson and Reid, (1970) scale; from 0 to 4 depending on their severity, 0 being no lesions and 4 the most severe. The average lesion score (MLS) for each group was calculated and used for estimating the reduction % in lesion severity (RLS %) from the equation:

$$RLS \% = 100 - \left( \frac{MLS \text{ of medicated}}{MLS \text{ of challenged}} \times 100 \right)$$

### Cecal oocysts burden

Oocysts / gram cecal contents (OPG) were counted for each bird sacrificed for lesion score at 7<sup>th</sup> day post-challenge. The average OPG of each group was for calculation of the oocyst's reduction percent as follow:

$$\text{Oocysts Reduction \%} = \frac{\text{Shedding of control challenged} - \text{Shedding of medicated}}{\text{Shedding of control challenged}} \times 100$$

### Microscopic alterations of the cecal tissue

Cecal section from 5 birds per group were histopathologically processed according to Talukder, (2007) for judging the histological condition and findings for each treatment. Cecal tissue pieces (1 cm length) were excised during necropsy, rinsed briefly with normal saline, clamped on a piece of clean cardboard and fixed with freshly prepared 10 % neutral buffered formalin. The tissues were dehydrated, infiltrated, embedded in wax following standard histological practices, thin sectioned, mounted on a glass slide, and stained with hematoxylin and eosin.

## RESULTS

### I. The verified *Eimeria* species in broiler farms

The recovered *Eimeria* species and their frequency is summarized in Table 1, Fig. 1, 2, and 3. At least one or more *Eimeria* species was identified in each intestinal specimen. *Eimeria tenella* was the most dominant coccidian species among the examined specimens (Fig. 1, 2) with a frequency rate of 48 % and mean oocysts

size 22 x 19 μm (20-24 x 17 – 21 μm), followed by *E. acervulina* (30 %) with oocysts size of 18.54 x 14.7 μm (17.5-19.58 x 13.49 – 15.9 μm), *E. mitis* (28 %) with oocysts size 15x14 μm (11 - 18 x 11 – 17.5), *E. maxima* (24 %) with mean oocysts size 31 x 21 μm (22-40 x 17-25), and *E. praecox* (10 %) with oocyst size of 22.25x18 (21-23.3 x 16-20). The least detected species were *E. mivati* (15.5 x 12.9) and *E. brunetti* (25.5 x 20 μm, 21.5-30.5 x 18 -22) with the same percent (2 %).

### II. The therapeutic potential of the organic acids and essential oils against cecal coccidiosis

#### The clinical observations

The therapeutic effect of organic acids and essential oils compared with the toltrazuril on the clinical signs and mortality % of the broiler chickens challenged with *E. tenella* (Fig. 3) are summarized in Fig 4, 5. All infected birds expressed the typical signs of coccidiosis at the 5<sup>th</sup> day post challenge that represented in bloody diarrhea, decreased appetite, weakness, feather-ruffling, depression, and mortalities. Signs were relatively less severe in the organic acids medicated groups. The mortalities were treatment dependent. The mortality was comparatively the lowest in the organic acids medicated group (33.3 %, 5/15) followed by the amprolium and essential oils medicated (40 %, 6/15). Toltrazuril medicated group revealed the highest mortality rate (53.3 %, 8/15) among the medicated groups. Mortality rate was 60 % (9/15) in the positive control group. The negative control group didn't exhibit any abnormal signs till end of the experiment.

#### The relative body weight gain percent (RBWG %)

The effect of medication using organic acids and essential oils on the RBWG % are showed in Table 2. At the 7<sup>th</sup> dpi (30 days old), a significant improvement in the RBWG % was observed in the medicated groups compared to the PC group (*P-value* < 0.05). Nevertheless, the performance of the challenged-medicated wasn't as good as the

negative control ( $P$ -value  $< 0.05$ ). The organic acids medication expressed improving effect on the RBWG % (40 %,  $0.4 \pm 0.1$ ) which was similar to the toltrazuril (40 %,  $0.4 \pm 0.0$ ), comparatively, medication with the essential oils and amprolium HCl induced somewhat a lower RBWG % (30 %,  $0.3 \pm 0.0$  each). There were statistically non-significant variations between the medicated groups during the challenge period ( $P$ -value  $\geq 0.05$ )

### The Cecal gross observations and lesion score

The gross observations, mean cecal lesions scores (MLS), and reduction % in the MLS (RLS) related to the tested therapies are shown in Table 3 and Figs. 6. On the 7<sup>th</sup> day post challenge, birds belonging to the negative control group had no lesions. Whereas for the positive control group, birds had average scoring, 3.5. All birds medicated with the organic acids, essential oils, toltrazuril, and amprolium HCl scored  $2.3 \pm 2$ ,  $2.3 \pm 2$ , and  $2.3 \pm 1$  on average, respectively, so they were capable of reducing the severity of the lesions by 34 % which was significantly lower than the MLS of the positive control ( $P$ -value  $> 0.05$ ). Cecal sections of the medicated groups showed numerous serosal and mucosal petechiae and slight thickening of the wall (+ 2). Cecal sections of the positive control group were very distended, engorged with blood, and contained hardened cecal cores (score +4).

### The oocyst shedding

Oocysts per gram of the cecal contents, post challenge with *Eimeria* species, was decreased non-significantly in the medicated groups ( $P$ -value  $\geq 0.05$ ), Table 4, Fig.7. However, using the organic acids blend and essential oils blend didn't behave as the toltrazuril and amprolium HCl against oocysts shedding. The oocysts shedding was the lowest in the toltrazuril medicated group (37 %,  $8.7 \pm 2$ ), followed by the amprolium HCl medicated (20.6 %,  $11 \pm 2$ ) and organic acids medicated group (17 %,  $11.4 \pm 3$ ). The essential oils didn't reduce the oocysts

shedding over 3 % ( $13.4 \pm 4$ ) which was near the PC ( $13.8 \pm 1$ )

### Microscopic findings

The histological lesions in the ceca observed on 7<sup>th</sup> day post challenge are represented in Table 5 and Fig. 8. Normal histological appearance of the cecal sections from the control negative birds was observed. Cecal sections of the positive control birds displayed severe mucosal destruction which was represented in loss of plica peculiars, epithelial necrosis to complete villus and cryptal loss, necrosis of goblet cells, and diffuse mucosal hemorrhages. Diffuse eosinophilic and mononuclear cells infiltration in lamina propria and submucosa, and submucosal congestion. Numerous various developmental stages of *E. tenella* inhabited the mucosal layers (epithelium and lamina propria). The toltrazuril medicated group showed short to atrophied plica, fewer localized inflammatory cells infiltration, hyperplasia and distension of goblet cells. Necrosis of villar, cryptal, and glandular epithelial. Mucosal and submucosal layers were markedly thickened and edematous. Fewer congestion was noticed. The amprolium medicated cecal sections revealed epithelial regeneration, marked submucosal edema, relatively few inflammatory cells infiltration, and most of the parasitic stages were destructed. To the organic acids medicated group, cecal sections displayed histological changes similar to the toltrazuril medicated except little epithelial damage. Goblet cells were minimally hyperplastic. More diffuse inflammatory cells infiltration in the lamina propria and submucosa.

Although extensive parasitological stages in the essential oils medicated group, the epithelial lining of the cecal sections was in a good intact condition. Epithelial hyperplasia and interepithelial eosinophil and mononuclear inflammatory cells infiltration occurred. Goblet cells were within normal range. The parasite mainly localized subepithelial in focal areas. Less

thickened submucosal layer which was diffusely infiltrated with mononuclear inflammatory cells and no congestion. Although there was no interaction between organic acids and essential oils and coccidial challenge, the therapy improved the cecal tissue. All the medicated groups revealed increase in the surface area of the ceval villi in comparison to the negative and positive control groups as explained in table 5.

**Table 1:** Frequency of different *Eimeria sp.* in the examined farms.

| <i>Eimeria species</i> | n/t   | %  |
|------------------------|-------|----|
| <i>E. acervulina</i>   | 15/50 | 30 |
| <i>E. mivati</i>       | 1/50  | 2  |
| <i>E. praecox</i>      | 5/50  | 10 |
| <i>E. maxima</i>       | 12/50 | 24 |
| <i>E. mitis</i>        | 14/50 | 28 |
| <i>E. brunetti</i>     | 1/50  | 2  |
| <i>E. tenella</i>      | 24/50 | 48 |

**Table 2:** Effect of the organic acids blend, essential oils blend, toltrazuril, and amprolium HCl on the RBWG % of broiler chickens infected with  $4.1 \times 10^4$  *E. tenella* oocysts. The chickens were weighed prior to *Eimeria* infection and at day 7 post-infection:

|                  | Mean RGWG            |                 |
|------------------|----------------------|-----------------|
| Negative control | 0.7±0.0 <sup>a</sup> | 70 <sup>a</sup> |
| Positive control | 0.1±0.0 <sup>c</sup> | 10 <sup>c</sup> |
| Toltrazuril      | 0.4±0.0 <sup>b</sup> | 40 <sup>b</sup> |
| Amprolium HCl    | 0.3±0.0 <sup>b</sup> | 30 <sup>b</sup> |
| Organic acids    | 0.4±0.1 <sup>b</sup> | 40 <sup>b</sup> |
| Essential oils   | 0.3±0.0 <sup>b</sup> | 30 <sup>b</sup> |

The RBWG is expressed as the mean ± standard errors (%). The differences between the groups are statistically significant (ANOVA, P-value < 0.001). Means within columns sharing similar superscripts within a column are not significantly different (P-value ≥ 0.05).

**Table 3:** Mean lesion score (MLS) and lesion severity reduction % (RLS) per chicken in response to Organic acids blend, Essential oils blend, Toltrazuril, and Amprolium HCl as therapies against *E. tenella* infection in broiler chickens in comparison to the challenged non-medicated and the non-challenged non-medicated (7 dpi).

| Experimental groups | MLS*                 | RLS% |
|---------------------|----------------------|------|
| Control negative    | 0±0.0 <sup>c</sup>   | -    |
| Control positive    | 3.5±0.1 <sup>a</sup> | -    |
| Toltrazuril         | 2.3±.1 <sup>b</sup>  | 34   |
| Amprolium HCl       | 2.3±.1 <sup>b</sup>  | 34   |
| Organic acids       | 2.3±2 <sup>b</sup>   | 34   |
| Seessential oils    | 2.3±2 <sup>b</sup>   | 34   |

\*: Values= Mean ± standard error. <sup>a-c</sup>: Means with dissimilar letters are significantly different (P-value < 0.05). - : non-calculated.

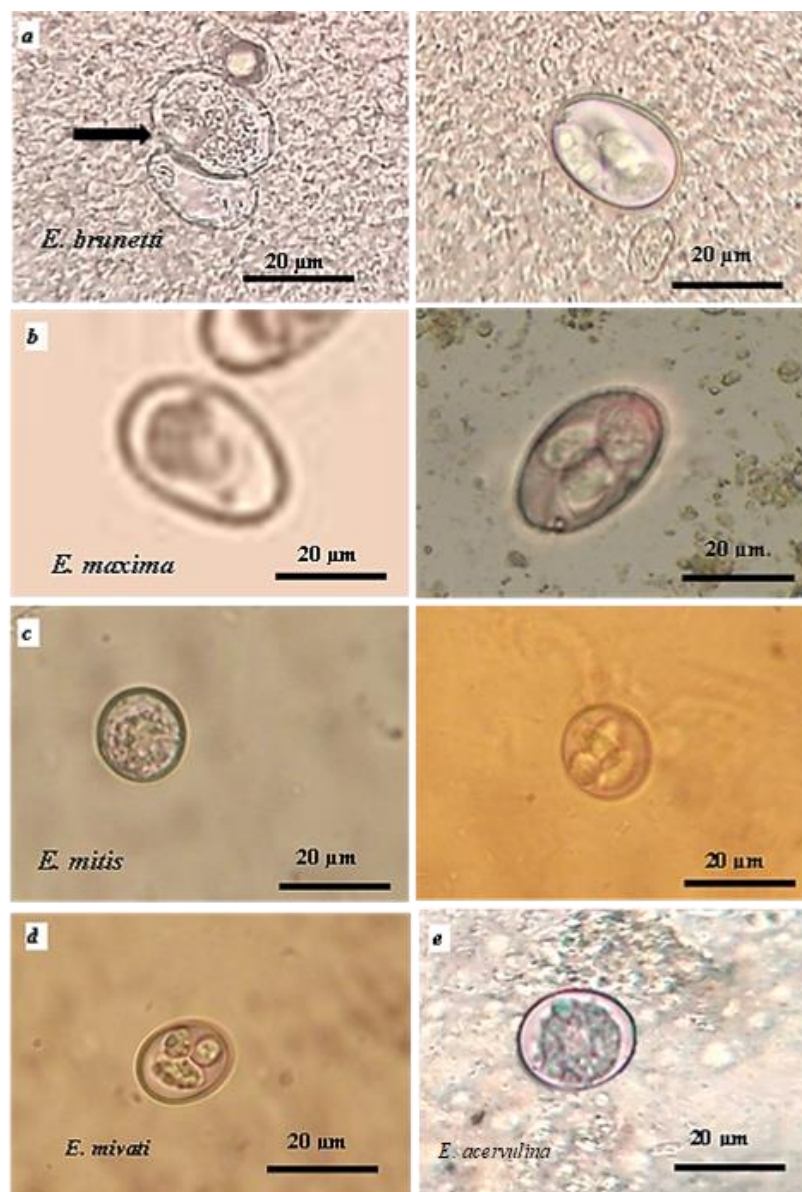
**Table 4:** Means of oocysts number per chicken in response to toltrazuril (Tz), Organic acids blend (OrgA), and Essential oils blend (EssO) as therapies against *E. tenella* infection in Arbor Acres broiler chickens in comparison to the challenged non-medicated (PC) and the non-challenged non-medicated (NC) (7 dpi).

| Experimental groups | Shedding reduction % |
|---------------------|----------------------|
| Control negative    | -                    |
| Control positive    | -                    |
| Toltrazuril         | 37                   |
| Amprolium HCl       | 20.6                 |
| Organic acids       | 17                   |
| Essential oils      | 3                    |

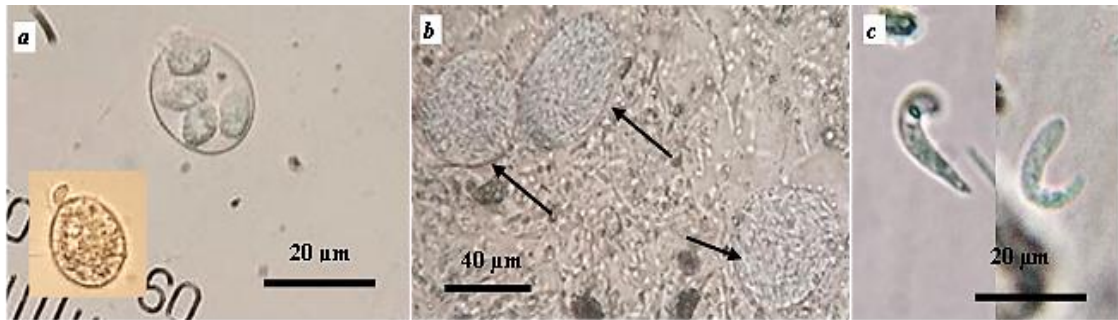
<sup>a-c</sup>: The mean difference is significant at lower than 0.05 level (P-value < 0.05)  
-: non-calculated

**Table 5:** The comparative effect between organic acids blend, essential oils blend, and the toltrazuril medicated groups in comparison to the positive and negative control on the cecal villar dimensions of *E. tenella* challenged broiler chickens (7 days post challenge)

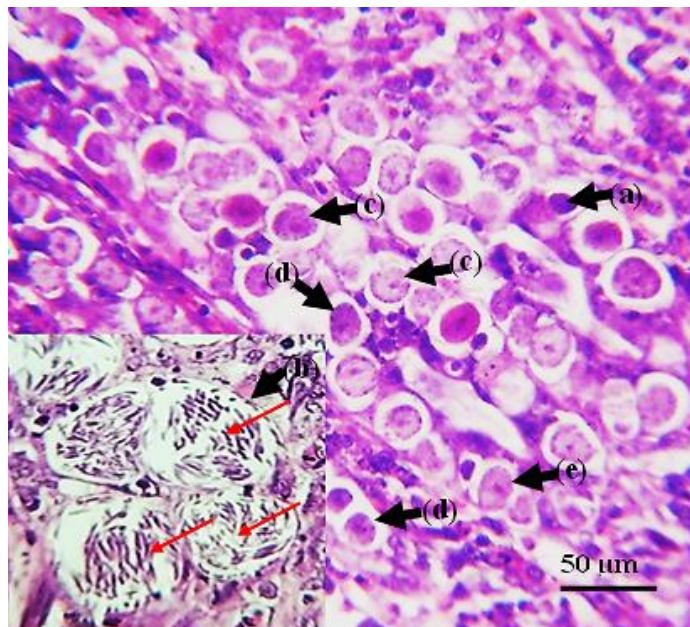
| Group            | Mean villus Dimensions  |                          |                        |
|------------------|-------------------------|--------------------------|------------------------|
|                  | Width ( $\mu\text{m}$ ) | Height ( $\mu\text{m}$ ) | Area ( $\text{mm}^2$ ) |
| Negative control | 88.03571                | 212.8393                 | 0.059                  |
| Positive control | 106.3333                | 91.83333                 | 0.03                   |
| Toltrazuril      | 107.9078                | 245.2551                 | 0.083                  |
| Organic acids    | 140.8571                | 198.1667                 | 0.088                  |
| Essential oils   | 121.8                   | 217.1778                 | 0.083                  |



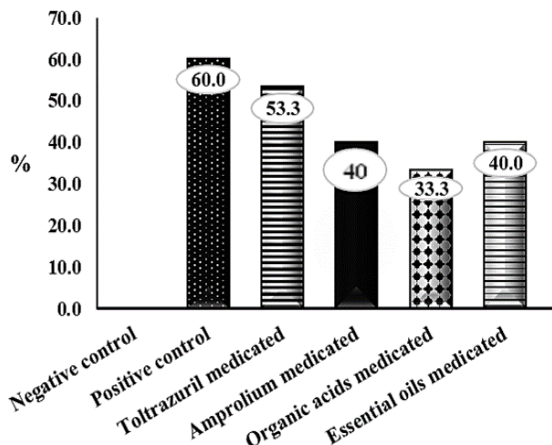
**Fig. (1):** *Eimeria* spp. oocysts. (a) *E. brunetti*, non-sporulated (left) and sporulated (right) oocysts (b) *E. maxima*, non-sporulated (left) and sporulated (right) oocysts (c) *E. mitis*, no-sporulated (left) and sporulated (right) oocysts. (d) *E. mivati*, sporulated oocyst. (e) *E. acervulina*, non-sporulated oocysts. Bar 20  $\mu\text{m}$ .



**Fig. (2):** Micrograph showing the different developmental stage characteristic to *E. tenella* (a) Non-sporulated and sporulated oocysts- sporulation time 19 hr, oocysts are ovoid, averaging 22 x19 µm (20-24 x 17 – 21 µm) (shape index 1.16). Bar 20 µm, (b) A cluster of mature 60 µm schizonts containing several banana shape merozoites, Bar 40 µm, (c): Banana-like shape sporozoite, oocysts were mechanically and chemically disrupted. Mean length 22 µm. Bar 20 µm. Morphology was accessed via light microscopy 40X.



**Fig. (3):** Photomicrograph to a cecal section from *E. tenella* naturally infected broiler chicken displaying the parasite characteristic developmental stages within the epithelium; (a) Single developing schizont, (b) Mature schizont contains numerous elongated crescent-shaped merozoites (arrow), (c) Macrogametes with bright peripheral eosinophilic plastic bodies, coronal granula, and a big central nucleus, (d) Microgametes containing multiple gametocytes, (e) Oocyst (400X) Bar, 50 µm

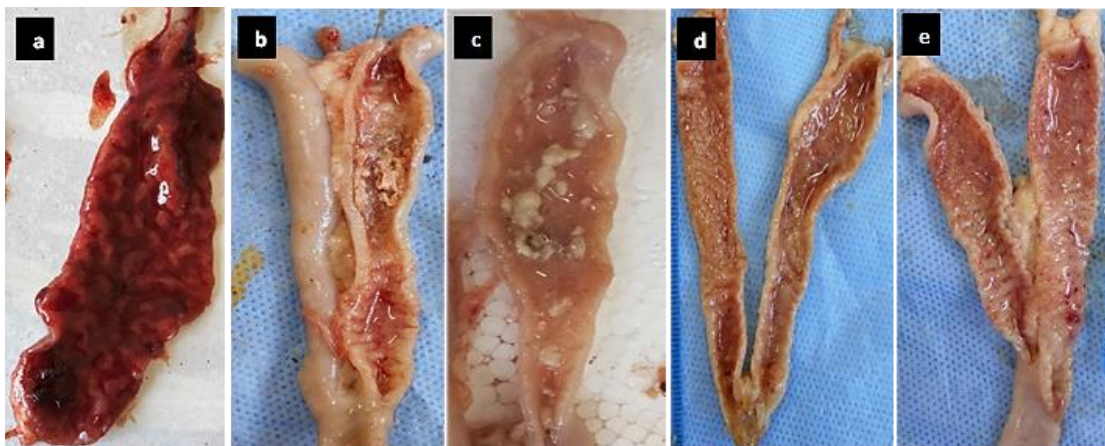


**Fig. (4):** Effect of the organic acids blend, essential oils blend, toltrazuril, and amprolium HCl on the mortality % of Arbor Acres broiler chickens challenged with  $4.1 \times 10^4$  sporulated *E. tenella* oocysts. Data were recorded at the 7<sup>th</sup> day post-challenge and represent the cumulative mortality during the challenge period

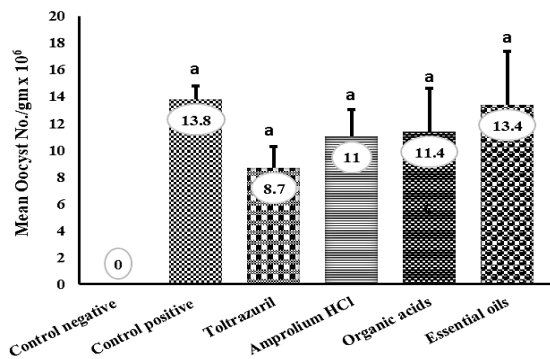




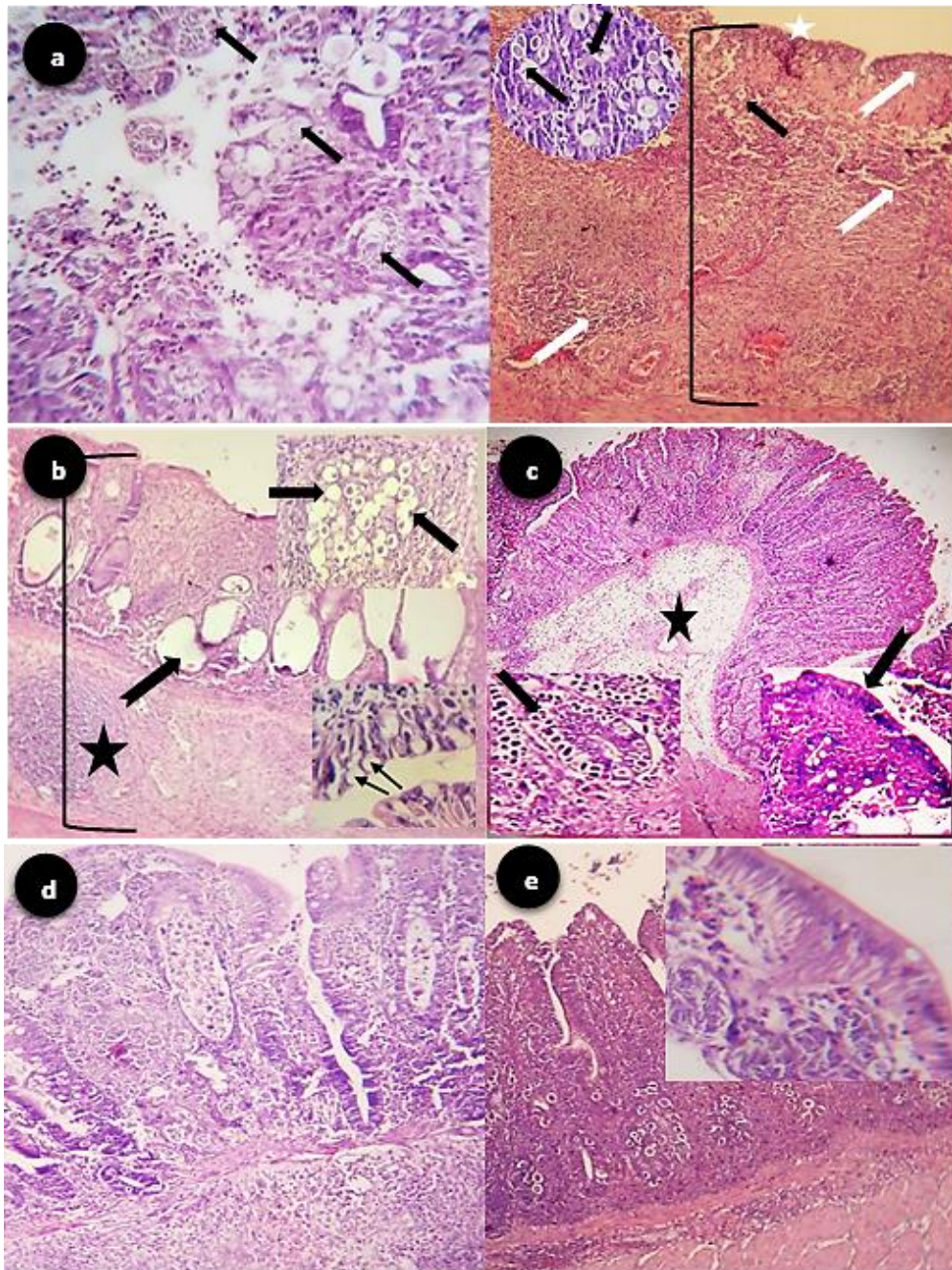
**Fig. (5):** Clinical symptoms due to cecal coccidiosis in broiler chickens after 5 days from challenge with  $1.3 \times 10^4$  oocysts of *E. tenella*, **a:** Bloody diarrhea, **b:** Weakness, feather ruffling, and mortality, **c:** Dullness, depression, and huddling.



**Fig. (6):** Gross pathological findings in the ceca of *E. tenella* ( $4.1 \times 10^4$  oocysts/bird) infected broiler chickens (7 dpi) allocated to different medications. **(a):** Lesion score +4 in the control +ve group revealing extensive necrosis, coagulated blood sheet covering the mucosa, complete loss of the longitudinal cecal corrugations and ulceration of the surface mucosa. **(b-e):** Lesion score +2 in the organic acids blend, essential oils blend, toltrazuril, and amprolium HCl (respectively) medicated groups showing petechiae, which were apparent on the serosal surface, slight thickening of the cecal wall, noticeable loss in the mucosal corrugations, and somewhat normal contents.



**Fig. (7):** Changes in mean number of *E. tenella* oocyst per gram ( $\times 10^6$ ) in the different experimental groups at 7 dpi. Error bars represent standard errors of the mean. The columns with different letters are significantly different (P-value < 0.05)



**Fig (8):** Photomicrographs revealing the cecal tissue changes in response to the effect of organic acids and essential oils as therapeutic anticoccidials in broiler chickens in comparison to toltrazuril, amprolium HCL, and the positive control. **(a) Positive control:** Extensive epithelial necrosis and complete villar and crypts loss (star), diffuse inflammatory cells infiltration in the mucosa and submucosa (notched arrow), numerous developmental stages of *E. tenella* inhabiting the mucosal and submucosal layers (black arrow). **(b) Toltrazuril:** medicated birds, Mucosal and submucosal thickening and edema (Left bracket), Marked cryptal and cecal glands atrophy (notched arrows). Disintegration of most parasitic developmental stages leaving empty Parasitophorous vacuoles or crescent shape structures inside the vacuoles (arrows). Hyperplasia and hypertrophy of goblet cells (line arrow), submucosal focal inflammatory cells infiltration (star). **(c) Amprolium HCl** medicated group had marked death of developmental stages of the parasite (arrow), submucosal edema (star), epithelial regeneration (notched arrow). **(d) Organic acids** medicated group revealing epithelial, mucosal, and submucosal thickening, epithelial hyperplasia and moderate goblet cells number and form **(e) Essential oils** medicated group showed mucosal thickening, epithelial regeneration and infiltration with inflammatory cells, no thickening in the submucosa which was diffusely infiltrated with mononuclear inflammatory cell. *E. tenella* developmental stages were numerous.

## DESCUSSION

Chickens' coccidiosis led to significant economic losses, globally, due to the capacity of *Eimeria* spp. to exploit the host digestive micro-environment for growth and survival (Balta *et al.*, 2021). In addition to the widely spread drug resistance of *Eimeria* species, phasing out the use of anticoccidials is a global target that started by the US Food and Drug Administration which introduced its first ban on an agricultural drug in 2005 followed by European countries, in 2006. As a result, numerous studies looked for less harmful alternatives for the general public's health (Muthamilselvan *et al.*, 2016; Canal *et al.*, 2022). So, the current study verified the *Eimeria* species in the clinically-ill broiler chickens in Assiut, Egypt, during September 2021 -March 2022 and concentrated on the therapeutic potential of the organic acids and essential oils blends against the most frequent *Eimeria* species during the study period.

According to the description of Castañon *et al.*, (2007), Haug *et al.*, (2008), and Cervantes *et al.*, (2020), seven *Eimeria* spp. (*E. tenella*, *acervulina*, *mivati*, *mitis*, *brunetti*, *maxima*, and *praecox*) were discriminated in the current study. This finding is in agreement with earlier investigative observations was made in Sohage, Egypt, (Mohamed *et al.*, 2021), indicating that there are 7 *Eimeria* spp., prevalent in the broiler chickens. Conversely, data doesn't support the statement of Ahmad, (2018) which identified only four *Eimeria* species (*E. tenella*, *E. necatrix*, *E. maxima* and *E. mitis*) in the farm raised chicken in Assiut. This discrepancy could be due to the differences in the type of sample as Ahmed, (2018) depended on apparently health broiler chickens which collected from poultry markets.

*E. tenella* evidenced the highest frequency (48 %) among broiler chickens in comparison to other *Eimeria* spp; *E.*

*acervulina*, *E. mitis*, and *E. maxima* (30 %, 28 %, and 24 % rates, respectively) during the study period. *E. praecox* was less noticed (10 %) and the least to be detected was *E. mivati* and *E. brunetti* with the same percent (2 %).

The relative higher frequency of *E. tenella*, regardless of the short study period, may be attributed to that all the examined birds were introduced to the diagnostic labs due to an acute clinical disease outbreak. Also, many poultry breeders associate coccidiosis with the bloody diarrhea and mortality. This high frequency of *E. tenella*, *E. acervulina*, and *E. maxima* may be a warning sign of probable endemicity and need to be ascertained in large-scale studies. The high detection % of *E. mitis* call for the concern of subclinical coccidiosis that leads to impaired feed conversion, as feed costs 70% of production cost (Haug *et al.*, 2008).

Even though different *Eimeria* spp. screening scale and clinical condition of the examined birds, partially parallel results had been reported in earlier studies done. In Assiut, Ahmed, (2018) found that *E. tenella* was the most common while *E. brunetti* was the least recorded. Diverse results were recorded by Mohamed *et al.* (2021), they stated that *E. acervulina* was the most prevalent followed by *E. tenella*, while *E. mitis* was the least verified species.

The further histopathological identification of an *E. tenella* within cecal sections of a chicken sample with heavy oocyst intensity, revealed the unique large sized secondary stage meronts (schizonts) characteristic to *E. tenella* arranged in clusters within the cecal mucosa and submucosa. The noticed large multiple (40- 55 × 30-40 µm) in the current study were previously described by Tyzzer, (1929) and Fernando *et al.* (1983) in *E. tenella* infection in poultry where large second-generation meronts develop in the lamina propria within displaced epithelial cells.

Susceptibility of *Eimeria* spp. to organic acids and essential oils has been demonstrated by several records through studying infection level in chickens after their feed or water supplementation and *in vitro* parasite susceptibility (El-Shall *et al.*, 2021s). Both were considered a potential alternative to anti-biotic growth promoters, AGPs, (Mustafa *et al.*, 2021; Canal *et al.*, 2022). Organic acids are natural organic compounds with acidic properties, the most public example are the short-chain fatty acids, SCFAs, (commonly propionic acid, acetic acid, and butyric acid). SCFAs are formed after carbohydrates fermentation by the beneficial intestinal microbiota (Rawi *et al.*, 2021). Organic acids have the ability to retard the flourishing of pathogenic and opportunistic bacteria, e.g., *Salmonella* and *E. coli*, in the bird's feed and gut, impacting positively on performance (Nguyen *et al.*, 2020). Organic acids acidify the microbial cell cytoplasm due to anions ( $\text{RCOO}^-$ ) and  $\text{H}^+$  ions accumulation. Essential oils are volatile phytochemicals which obtained via distillation or mechanical cold pressing. Essential oils have antibacterial, antioxidant, antiparasitic, anti-inflammatory, antidiarrheal, and antimycotic properties. Using Organic acids blend or essential oils blend is more advantageous than single component in broiler chickens. The combination optimizes a synergistic effect between them and their most bioactive elements, hence become more effective than individual acid or oil (Polycarpo *et al.*, 2017; Canal *et al.*, 2022). The characteristics of each element will define the final effects of each oil and acid, at the same time, different combinations will result in different properties. Lately, branded marketable blends, as a water and feed additive of Organic acids and Essential oils, have been established and developed in broiler chickens reared without antibiotics (Canal *et al.*, 2022).

Due to being an important pathogen in the poultry industry and the most encountered species in the current study, an *E. tenella* isolate was used for studying the therapeutic

potential of Organic acids and Essential oils as anticoccidials. The clinical signs, mortality, RBWG %, MLS, RLS, oocysts shedding reduction %, and scoring the histopathological findings in the cecal tissue were assessed after concurrent therapy. Blends of organic acids (included orthophosphoric, propionic, acetic, lactic, citric, and formic acids) and essential oils (included cinnamaldehyde, carvacrol, anethol, terpinene, carvaone, menthol, and eucalyptol oils) were studied as anticoccidial therapies against *E. tenella* for 2 successive days and compared with the toltrazuril 5 % suspension.

Organic acids blend successfully diminished the clinical signs of coccidiosis and decreased the mortality rate to 33.3 % compared with 40 % amprolium HCl and 40 % in the Essential oils, and 53.3 % in the toltrazuril medicated groups. All gave better results than the PC (60 % mortality). This agreed with Balta *et al.* (2021).

The best RBWG % at the end of the trial was achieved in birds treated with the Organic acids (40 %) which equaled RBWG % in the toltrazuril medicated group, signifying a candidate of better weights during cecal coccidiosis outbreaks, with no significant differences for birds treated with the amprolium HCl (30 %) and essential oils (30 %). This came in line with Balta *et al.* (2021) and Canal *et al.* (2022).

Both the organic acids and essential oils blend behaved equally as the toltrazuril and the amprolium HCl in reducing the severity of cecal lesions induced by *E. tenella* in the challenged broiler chickens (34 %). This reduction % in lesions severity was higher than that obtained by Canal *et al.* (2022) (19.3 %) after using essential oils blend against *E. tenella* in broiler chickens. Balta *et al.* (2021) recorded the success of Organic acids blend in reducing the *E. tenella* induced cecal lesions.

Compared to birds from the positive control ( $13.8 \times 10^6$  OPG) 7 dpi, chickens medicated

with organic acids blend, toltrazuril, and amprolium HCl had non-significantly lower ( $P$ -value  $\geq 0.05$ ) oocysts burden in the cecal content ( $8.7 \times 10^6$  and  $11.4 \times 10^6$  OPG, respectively), being able to reduce it by 37 %, 20.6 %, and 17 %, respectively. When contrasted with those obtained from the Essential oils treatment ( $13.4 \times 10^6$  OPG) and positive control, it can be concluded that the difference in the results was not significant. The failure of Essential oils blend to diminish oocyst count per gram cecal content refer to the indirect antiparasitic effect of these compounds against *E. tenella*. That wasn't in concurrence with the report of Canal *et al.* (2022) which stated that Essential oils blend able to reduce oocysts shedding by 94.92%. This objection could be conveyed to the difference in medication regimen as in the current study either Organic acids or Essential oils were applied for brief time (2 days) during the prepatent period of the parasite challenge (2 days post-infection). Most previous studies used organic acids and essential oils as feed or water additives during the bird life time.

The present results demonstrated that supplementing an organic acid and or essential oils blend during coccidiosis outbreak can share in enhancing recovery from infection.

## REFERENCES

- Ahmed, R. (2018): Prevalence of Protozoan Parasites in Farm and House Raised Chicken in Assiut. M. V. Sc. Thesis, Fac. Med., Assiut University.
- Balta, I.; Marcu, A.; Linton, M.; Kelly, C.; Stef, L.; Pet, L.; Ward, P.; Pircalabioru, G. G.; Chifiriuc, C.; Gundogdu, O., Callaway, T. and Corcionivoschi, N. (2021): The in vitro and in vivo anti-virulent effect of organic acid mixtures against *Eimeria tenella* and *Eimeria bovis*. Scientific Reports, 11:16202
- Blake, D.P.; Knox, J.; Dehaeck, B.; Huntington, B.; Rathinam; T.; Ravipati, V.; Ayoade, S.; Gilbert, W.; Adebambo, A.O.; Jatau, I.D.; Raman, M.; Parker, D.; Rushton, J. and Tomley, F.M. (2020): Re-calculating the cost of coccidiosis in chickens. Vet. Res. 51:115
- Canal, B.; Mesas, L. and Puyalto, M. (2022): Investigating the effect of an essential oils blend on coccidiosis. International Poultry Production, 26 (7)
- Castañon, C.A.; Fraga, J.S.; Fernandez, S.; Gruber, A. and Costa, L.D.F. (2007): Biological shape characterization for automatic image recognition and diagnosis of protozoan parasites of the genus *Eimeria*. Pattern Recognit., 40:1899–1910
- Cervantes, H.M.; McDougald, L.R. and Jenkins, M.C. (2020): Coccidiosis. In Swayne, D. E.; Boulianne, M.; Logue, C. M.; McDougald, L. R.; Nair, V.; Suarez, D. L. (Eds.), Diseases of poultry., 14<sup>th</sup> ed. Wiley Blackwell Publishing, Ames, IA, pp. 1193–1212.
- Chapman, H.D. (1989): Chemotherapy of caecal coccidiosis: efficacy of toltrazuril, sulphaquinoxaline/pyrimethamine and amprolium/ethopabate, given in drinking water, against field isolates of *Eimeria tenella*. Res Vet Sci., 46(3):419-20.
- Conway, D.P. and McKenzie, M.E. (2007): Poultry coccidiosis: diagnostic and testing procedures. John Wiley and Sons.
- El-Shall, N.A.; Abd El-Hack, M.E.; Albaqami, N.M.; Khafaga, A.F.; Taha, A.E.; Swelum, A.A.; El-Saadony, M.T.; Salem, H.M.; El-Tahan, A.M.; AbuQamar, S.F.; El Tarabily, K.A. and Elbestawy, A.R. (2021): Phytochemical control of poultry coccidiosis: a review. Poultry Science 101:101542.
- El-Tarabily, K.A.; El-Saadony, M.T.; Alagawany, M.; Arif, M.; Batiha, G.E.; Khafaga, A.F.; Elwan, H.A.; Elnesr, S.S. and Abd El-Hack, M.E. (2021): Using essential oils to

- overcome bacterial biofilm formation and their antimicrobial resistance. *Saudi J. Biol. Sci.* 28:5145–5156.
- Fernando, M.A.; Lawn, A.M.; Rose, M.E. and Al-attar, M.A. (1983):* Invasion of chicken caecal and intestinal lamina propria by crypt epithelial cells infected with coccidia. *Parasitology*, 86: 391–398
- Garcia, L.S. (2001):* Diagnostic Medical Parasitology. ASM Press, Washington, DC.
- Haug, A.; Gjevne, A.G.; Thebo, P.; Mattsson, J.G. and Kaldhusdal, M. (2008):* Coccidial infections in commercial broilers: epidemiological aspects and comparison of *Eimeria* species identification by morphometric and polymerase chain reaction techniques. *Avian Pathol.* 37:161–170
- Holdsworth, P.A.; Conway, D.P.; McKenzie, M.E.; Dayton, A.D.; Chapman, H.D.; Mathis, G.F.; Skinner, J.T.; Mundt, H.C. and Williams, R.B. (2004):* World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anticoccidial drugs in chickens and turkeys. *Vet. Parasitol.*, 121: 189-212.
- Johnson, J. and Reid, W.M. (1970):* Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. *Exp. Parasitol.*, 28: 30–36.
- Levine, N.D. (1985):* Veterinary Protozoology. Iowa State University Press, Ames, Iowa, USA.
- Li, C.; Yan, X.; Lillehoj, H.S.; Oh, S.; Liu, L.; Sun, Z.; Gu, C.; Lee, Y.; Xianyu, Z. and Zhao, H. (2019):* *Eimeria maxima*-induced transcriptional changes in the cecal mucosa of broiler chickens. *Parasites Vectors*, 12:285.
- López-Osorio, S.; Chaparro-Gutiérrez, J.J. and Gómez-Osorio, L.M. (2020):* Overview of Poultry *Eimeria* Life Cycle and Host-Parasite Interactions. *Front. Vet. Sci.* 7:384. doi: 10.3389/fvets.2020.00384
- Mani-López, E.; García, H.S. and López-Malo, A. (2012):* Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. *Food Res Int* 45:713–721. <https://doi.org/10.1016/j.foodres.2011.04.043>
- Mustafa, A.; Bai, S.; Zeng, Q.; Ding, X.; Wang, J.; Xuan, Y.; Su, Z. and Zhang, K. (2021):* Effect of organic acids on growth performance, intestinal morphology, and immunity of broiler chickens with and without coccidial challenge. *AMB Expr.*, 11(140): 2-18.
- Muthamilselvan, T.; Kuo, T.; Wu, Y. and Yang, W. (2016):* Herbal Remedies for Coccidiosis Control: A Review of Plants, Compounds, and Anticoccidial Actions. Evidence-Based Complementary and Alternative Medicine.
- Nguyen, D.H.; Seok, W. J. and Kim, I.H. (2020):* Organic Acids mixture as a dietary additive for pigs—a review. *Animals* 10:952. <https://doi.org/10.3390/ani10060952>
- Ojimelukwe, A.E.; Emedhem, D.E.; Agu, G.O.; Nduka, F.O. and Abah, A.E. (2018):* Populations of *Eimeria tenella* express resistance to commonly used anticoccidial drugs in southern Nigeria. *International Journal of Veterinary Science and Medicine*, 6: 192-200
- Polycarpo, G.V.; Andretta, I.; Kipper, M.; Cruz-Polycarpo, V.C.; Dadalt, J.C.; Rodrigues, P.H.M. and Albuquerque, R. (2017):* Meta-analytic study of organic acids as an alternative performance-enhancing feed additive to antibiotics for broiler chickens. *Poult Sci* 96:3645–3653. <https://doi.org/10.3382/ps/pex178>
- Rawi, M.H.; Abdullah, A.; Ismail, A. and Sarbini, S.R. (2021):* Manipulation of gut microbiota using acacia gum

- polysaccharide. ACS Omega 6:17782–17797. <https://doi.org/10.1021/acsomega.1c00302>
- Ryley, J.F. (1967): Methylbenzoate, a new wide-spectrum coccidiostat for chickens. British Vet. J., 123: 513–20.
- Schneiders, G.H.; Foutz, J.C.; Fuller, A.L.; Nelson, Rekaya, J.R. and Aggrey, S.E. (2020): The effect of increased temperatures on viability, morphology, infectivity, and development of *E. tenella*. Journal of Parasitology, 106(3): 428–437.
- Scicutella, F.; Mannelli, F.; Daghighio, M.; Viti, C. and Buccioni, A. (2021): Polyphenols and organic acids as alternatives to antimicrobials in poultry rearing: a review. Antibiotics 10:1010. <https://doi.org/10.3390/antibiotics10081010>
- Talukder, S. (2007): Histopathology techniques: tissue processing and staining. [www.talukderb.com](http://www.talukderb.com).
- Tyzzer, E.E. (1929): Coccidiosis in gallinaceous birds. Am. J. Hyg., 10: 269–383.

## القدرة العلاجية لبعض الأحماض العضوية والزيوت العطرية ضد عزلات الأيميريا التي تم جمعها من مزارع الدجاج اللاحم بأسسيوط

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تم الكشف عن انواع الأيميريا المختلفة في ٥٠ عينة معوية لمزارع دجاج التسمين بأسسيوط خلال سبتمبر ٢٠٢١-مارس ٢٠٢٢. كما تم مقارنة الإمكانيات العلاجية لمزيج من الأحماض العضوية والزيوت الأساسية ضد الأيميريا تينيليا مع التولترازوريل وهيدروكلوريد الأمبروليم في دجاج التسمين. ووجد أن الأيميريا تينيليا كانت أكثر الأنواع شيوعا (بمعدل ٤٨%) يليها الأيميريا أسيرفيولينا (٣٠%) و الأيميريا مايتس (٢٨%) ، بينما أقل الأنواع كانت الأيميريا ميفاتي (٢%) والأيميريا بروني تي (٢%). ووجد أن استخدام الأحماض العضوية كعلاج قلل من العلامات السريرية للكوكسيديا وخفض معدل الوفيات (٣٣,٣%) مقارنة بالأمبروليوم (٤٠%) والزيوت الأساسية (٤٠%) والتولترازوريل (٥٣,٣%). كما أنها حققت أفضل زيادة نسبية في وزن الطيور خلال التجربة (٤٠%) والتي تشبه التولترازوريل (٤٠%) بينما الأمبروليوم والزيوت الأساسية حققوا زيادة أقل نسبيا ومتساوية (٣٠%) وبدورهم كانوا أفضل من الطيور المصابة الغير معالجة (١٠%). أما بالنسبة للصفة التشريحية فكل العلاجات التي تم تقييمها قللت شدة الآفات الأعورية بالمثل بنسبة ٣٤% حيث سجلت جميع المجموعات العلاجية درجة أذى أعورية بمتوسط ٢,٣ مقارنة ب ٣,٥ في الطيور التي لم يتم علاجها. لم تتمكن كلا من الأحماض العضوية والزيوت الأساسية من تقليل إنتاج البويضات بأكثر من ١٧% و ٣% على التوالي ، مقابل ٣٧% في تولترازوريل و ٢٠,٦% في المجموعات المعالجة بالأمبروليوم. على العكس من ذلك، على الرغم من التفاعل الطفيف نسبيا بين الأحماض العضوية والزيوت الأساسية ضد الطفيل إلا أن الملاحظات المجهرية كشفت عن تأثيرها المحسن على الأنسجة الأعورية. ومن ثم وجدت هذه الدراسة أن الأحماض العضوية والزيوت الأساسية قد يكون له القدرة على إحداث آثار علاجية إيجابية مماثلة لبعض الأدوية الكيميائية مثل تولترازوريل وأمبروليوم.