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Effects of white wormwood (*Artemisia herba-alba* Asso), during an experimental coccidiosis in broilers

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ABSTRACT

The aim of this study is to evaluate effects of white wormwood, Artemisia herba-alba Asso, against an experimental Eimeria tenella infection. 120 broiler chicks, divided into four groups: (UIUT) uninfected untreated group; (IUT) infected untreated group; (ITT) infected treated group with 0.025g/l of toltrazuril and (ITA) infected treated group with the dried leaves of (Artemisia herba-alba Asso) incorporated at a level of (5%) in animals feed. Infected animals received each 10^5 sporulated oocysts of Eimeria tenella. Anticoccidial effects were evaluated through: feed intake, live body weight gain, oocyst shedding and variations of blood total proteins and total lipids. Despite symptoms of coccidiosis, no mortality was recorded in (ITA) group, while the two other infected groups recorded mortality rates of 13.79% and 20.69% respectively for (IUT) and (ITT) groups. On day 6 post-infection, oocyst shedding was significantly reduced ($p<0.05$) in white wormwood treated group (ITA) $5.34\pm6.59\ 10^6$ OPG, compared to the infected untreated group (IUT) $15.54\pm6.34\ 10^6$ OPG. On day 7 post-infection, all three infected groups showed hypoproteinemia, however, unlike the untreated group, consumption of white wormwood seems to have prevented the occurrence of hypolipidimia. Despite an interesting anticoccidial activity, one adverse effects of the plant incorporation was its negative effect on body weight gain. The long period of use (4 consecutive weeks) and also the possible antinutritional effect of the plant's tannins could be incriminated.

Key words : *Artemisia herba-alba* Asso, broilers, coccidiosis, *Eimeria tenella*.

INTRODUCTION

Avian coccidiosis is a serious infectious protozoan disease. Seven *Eimeria* species (Apicomplexa family), have been identified to be responsible of this important economical infection in broilers [1]. *Eimeria tenella*, cecal coccidiosis agent, is the most pathogenic species. It causes a hemorrhagic diarrhea often leading to death. Since coccidian contamination is almost inevitable in broiler farms [2], the current development of poultry industry is partly due to the use of coccidiostats in feed [3]. Nevertheless, the emergence of resistant strains of coccidia and restrictions on the use of anticoccidials imposed by recent regulation, have prompted the search for alternative control methods against this protozoan. Among the natural alternatives to anticoccidials, studies on artemisinin, a substance extracted for the first time from *Artemisia annua* showed an interesting anticoccidial activity [4]. Other works done with other species of the genus *Artemisia* showed similar results [5-6]. The genus *Artemisia* (Asteraceae) includes about 400 species distributed in the Mediterranean region, Northern Africa, Western Asia and Southwestern Europe, and in Arabian Peninsula [7]. *Artemisia herba-alba* Asso (White wormwood) is a common species in Algeria, this perennial shrub is known for many therapeutic properties [8-10].

In this study, an experimental infection by *Eimeria tenella* was produced to evaluate the effects of *Artemisia herba-alba* against this highly pathogenic cecal coccidiosis. The following parameters were used to evaluate the plant's

anticoccidial effects: feed intake, live body weight gain, oocyst shedding and blood biochemical variations of total proteins and total lipids.

MATERIALS AND METHODS

Experiment procedures used in this study were approved by the scientific council of the Institute of veterinary sciences (University of Constantine1. Algeria) and were conform to international guidelines of animal care and use in research and teaching (NIH publications no 85-93 revised 1985).

Animals

One hundred twenty, one day-old broiler chicks of either sex (ISA^{15} strain) were used in this study. Animals were housed in floor pens and fed *ad libitum* throughout the experiment, first with starter diets offered from 1 to 14 days of age. Then a growth and finisher diets offered from 15 to 45 and 46 to 53 days of age respectively. All diets were free of coccidiostats and were formulated to cover the nutrient requirements of chicken [11]. Standard management practices of commercial broiler production were applied. Chicks were vaccinated against Newcastl's disease and Infectious bronchitis disease.

Characteristics of experimental groups

On day 17, animals were assigned into 4 groups. First group served as a control (UIUT), while the other groups were inoculated with *Eimeria tenella* to produce coccidiosis infection (Table 1).

Among the infected groups, one group was left untreated (IUT) while the two other infected groups received treatments: group (ITT) was treated with 0.025g/l of toltrazuril and group (ITA) received dried leaves of white wormwood (*Artemisia herba-alba* Asso) incorporated in animals feed.

Table 1: Distribution and characteristics of experimental groups

Group	Characteristics	Designation
01	Negative control: uninfected untreated.	**UIUT**
02	Positive control: infected untreated.	**IUT**
03	Infected treated with toltrazuril (a commercial anticoccidial).	**ITT**
04	Infected received dried leaves of white wormwood (*Artemisia herba-alba* Asso) incorporated in animals feed (5%) during four consecutive weeks.	**ITA**

Challenge Infection of Chicken

On the 18th day of age each animal of the groups 2, 3 and 4 received, by gavage, 1 ml of a suspension containing 10^5 sporulated oocysts of *Eimeria tenella* conserved in a 2.5% potassium dichromate ($Cr_2K_2O_7$) solution. The used oocysts were first isolated from a broiler farm in Constantine region (north east Algeria), and then maintained in our research laboratory PADESCA (Institute of veterinary sciences, University of Constantine1, Algeria).

Treatments

Toltrazuril

From the onset of coccidiosis symptoms (bloody diarrhea characteristic of *Eimeria tenella* coccidiosis) animals of group 3 received in drinking water 0.025g/l of Toltrazuril (Baycox® 2.5% oral solution. Bayer plc). This anticoccidial is a coccidicide drug active on various intracellular stages of coccidia [12]. The treatment was installed for two consecutive days.

Artemisia herba alba Asso

The aerial parts of *Artemisia herba-alba* Asso were harvest in April 2011 in the region of T'kout in Batna province (north east Algeria). A voucher specimen is kept in PADESCA research laboratory, University of Costantine1 Algeria. After drying in shade, the aerial parts were finely cut and incorporated in feed distributed to group 4 animals (ITA) as a 5% supplementation during four consecutive weeks. On age 30 days, supplementation was stopped, and animals of this group received the same feed distributed to other groups.

Studied parameters

Feed intake

Quantification of feed intake was done daily during the period of infection (days 1 to 6 post-infection, corresponding to the 19th day to the 24th day of age).

Live body weight gain

Ten chicks from each group were randomly selected and weighted to obtain live body weight. Weightings were made at age 17 days, 19, 21, 23, 25, 27, 33, 39, 45 and at age 53 day.

Oocyst excretion
From day 5 to day 8 post-infection, feces were collected daily from 6 birds in each infected group. Quantification of oocyst output was carried out using a McMaster cell according to the method described by Bussieras and Chermette, 1992 [12]. Oocysts excretion was expressed as 10^6 OPG (Oocysts Per Gram of feces).

Blood sampling
One day before infection and on day 7 and day 10 post-infection, blood samples were collected after bleeding six animals from each group. Blood was collected in heparinized tubes and centrifuged at 3000rpm for 15mn.

Statistical analysis
Data obtained were expressed as mean±SEM. Statistical analysis was performed using Kruskal-Wallis Test followed by Mann-Whitney Test, using XLSTAT 2010 statistical analysis software (Addinsoft SARL). *p.value* < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Clinical observations
On day 5 post-infection, corresponding to age 23 days, animals of infected groups showed coccidiosis symptoms : immobility, depression, nervousness, haemorrhagic diarrhea. No mortality was recorded in (ITA) group, despite a deep depression and manifestation of bloody diarrhea which is a pathognomonic sign of the disease. The two other infected groups presented mortality rates of 13.79% and 20.69% respectively for (IUT) and (ITT) groups. The pathogenicity of coccidia in a farm depends, not only on its species and the quantities of ingested oocysts, but also on the responsiveness of the chicken and environmental factors [3]. In our study, from the 5th day post-inoculation, corresponding to the prepatent period of *Eimeria tenella* coccidiosis [13], all infected animals showed signs of coccidiosis. (ITA) group showed no mortality. Animals of this group seem to be more resistant subsequent to white wormwood consumption. While the high mortality rate recorded in toltrazuril treated group (ITT) could be explained by a possible resistance of the inoculation strain.

Feed intake
During the infection period (day 1 to day 6 post-infection), the infected groups showed less cumulative quantities of consumed feed compared with the control one (Table 2).

Table 2: Cumulative consumption of feed during the period of infection.

Groups	UIUT	IUT	ITT	ITA
Consumed feed (g/chick)	355.11	285.5	273.26	277.53

The decrease in feed intake during infection period coincides with the phase of intracellular multiplication of oocysts, which causes destruction of intestinal epithelial cells [1]. Loss of appetite and reduced feed intake are among the most important signs of coccidial infections, whatever is the affected enteric segment [14].

Live body weight gain
Starting from day 4 post-infection, all animals of infected groups showed a slowdown of their body weight gain. This latter remained slow compared to the control uninfected untreated group (UIUT) which animals showed a steady increase of their body weight throughout the experiment, and had at age 53 days the best mean weight (2191.5g/chick). Among the infected groups, growth of (ITA) animals was the weakest (1502.1 g/chick) (Figure 1).

Animals of infected groups suffered a weight loss and a slowdown of their body weight gain. These growth disturbances are common in coccidiosis infected chicken and have been reported by many studies [14][15]. This growth slowdown seems mainly due to a decrease in feed intake [16].

Among the infected groups, growth of (ITA) group animals was the weakest, that could be explained by a possible effect of antinutrients, mainly tannins [17] present in the aerial parts of white wormwood incorporated in animals' diet during four consecutive weeks. This long period of use could be also incriminated.

Oocysts excretion
On day 6 and 8 post-infection, oocysts excretion was significantly reduced in both toltrazuril (ITT) and white wormwood (ITA) groups in comparison with the infected untreated group (IUT). While, comparison between treated groups (ITT) and (ITA) showed no significant differences (Table 3).

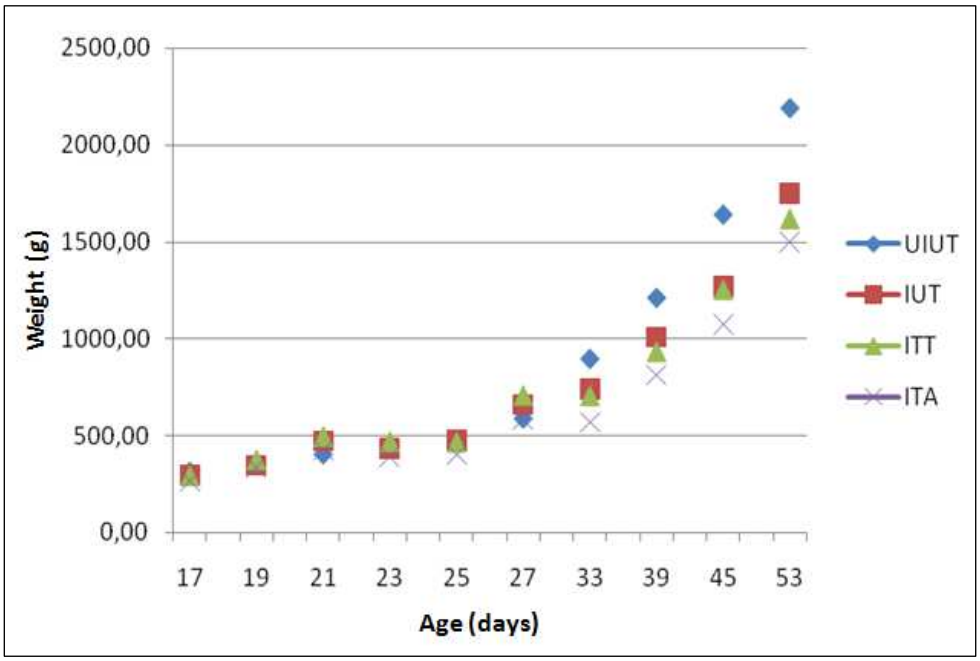

Figure 1: Evolution of animal's body weight from day 17 to day 53.

Table 3: Oocyst output in infected animals (10^6OPG)

Days post-infection	Infected groups			$p < 0.05$		
	IUT	ITT	ITA	a	b	c
5	**2.45±0.94**	2.25±2.29	3.03±1.89	*ns*	*ns*	*ns*
6	**15.54±6.34**	6.72±3.44	5.34±6.59	*s*	*s*	*ns*
7	**77.77**±72.**44**	37.21±19.10	36.20±37.67	*ns*	*ns*	*ns*
8	**27.57±17.82**	5.32±2.2	5.78±6.13	*s*	*s*	*ns*

Values are expressed as mean ±SEM (n=6). SEM: Stan dards Means of Errors.
a: comparison between IUT and ITT. b: comparison between IUT and ITA. c: comparison between ITT and ITA. s: significant difference (p<0.05). ns: non significant difference.

According to Yvoré *et al*., 1982 [2], the importance of parasite development in the host can be measured by the number of oocysts shed in the feces at the end of the prepatent period. In our study, since day 6 post-infection, dried *Artemisia herba-alba* leaves incorporated during four weeks in animals'diet, significantly reduced oocyst shedding in (ITA) group compared with the infected untreated group (IUT). Similar results to ours were obtained with other *Artemisia* species: *Artemisia annua*, tested by Allen *et al*., 1997 [4] and *Artemisia sieberi* tested by Arab, 2006[5]. This reduction in the production of oocysts could be attributed to the effect of artemisinin, which generates oxidative stress in coccidia [18] [6].

Plasma levels of total protein

On day 7 post-infection, animals of all three infected groups (IUT), (ITT) and (ITA) showed a decrease in total protein levels, the lowest rate was recorded in the (IUT) group. Upon recovery corresponding to day 10 post-infections, the highest rates were recorded respectively in (ITA) and (ITT) groups (Table 4). However, diferences were not statistically significant based on the pre-chosen alpha level ($p < 0.05$)

Table 4: Plasma levels of total proteins (g/l)

Days post-infection	UIUT	Infected groups			$p < 0.05$		
		IUT	ITT	ITA	a	b	c
- 1	**22.43±3.97**	22.70±2.79	23.12±1.17	23.46±1.58	*ns*	*ns*	*ns*
7	**26.54±5.18**	18.29±5.68	23.39±3.51	22.45±2.67	*ns*	*ns*	*ns*
10	-	23.13±3.11	24.14±1.51	24.48±2.29	*ns*	*ns*	*ns*

Values are expressed as mean ±SEM (n=6). SEM: Stan dards Means of Errors.
a: comparison between IUT and ITT. b: comparison between IUT and ITA. c: comparison between ITT and ITA.
s: significant difference (p<0.05). ns: non significant difference.

In *E. tenella* infections, falling of plasma total proteins levels has been demonstrated since 1963 by Schlueter [19]. This important decrease in the level of total plasma protein is evident during infection [20]. It appears to be one of the most significant metabolic disturbances recorded during various coccidial infections in chicken. The role of

malabsorption and protein leakage through the injured intestine seems very important in the apparition of hypoproteinemia associated with coccidial infections. According to Kumar-Mondal *et al*., 2011[20] and Turk, 1974 [21], acute hemorrhage on day 7 post-infection causes large loss of plasma protein followed by rapid movement of interstitial fluid without protein into the plasma compartment to induce acute hypo-proteinemia. Other factors explaining the hypoproteinemia in the coccidia infected birds might be the acute stress that leads to cortisol secretion and catabolism of protein [22].

Plasma levels of total lipids

Hypolipidimia was observed on day 7 post-infection in (IUT) group animals; their total lipids plasma levels remained low until day 10 post-infection. While both treated groups (ITT) and (ITA) were not affected by such disturbances (Table 5).

Table 5: Plasma levels of total lipids (g/l)

Days post-infection	UIUT	Infected groups			$p < 0.05$		
		IUT	ITT	ITA	a	b	c
- 1	**2.74±0.50**	2.55±0.25	2.84±0.32	3.61±0.93	*ns*	*ns*	*ns*
7	**2.95±1.23**	1.82±0.49	2.91±0.42	3.32±0.45	*s*	*s*	*ns*
10	/	1.99±0.27	3.11±0.39	2.82±0.55	*ns*	*ns*	*ns*

Values are expressed as mean ±SEM (n=6). SEM: Standards Means of Errors.
a: comparison between IUT and ITT. b: comparison between IUT and ITA. c: comparison between ITT and ITA.
s: significant difference (p<0.05). ns: non significant difference.

The fall of plasma lipid levels seems, like hypoproteinemia, a sensitive and early setting parameter in coccidial infection. Allen (1988) [23] and Pascalon-Pekelniczky *et al*., 1994) [16] reported fall in plasma triglyceride in chick and duck infected with *Eimeria tenella* and *Eimeria mulardi* respectively. Ruff *et al*., 1990 [24] attribute observed hypolipemia to anorexia and malabsorption.

In our study consumption of white wormwood seem to have prevented the occurrence of hypolipidimia.

CONCLUSION

Artemisia herba-alba Asso could have an interesting anticoccidial activity, especially through its effect in decreasing excretion of *E. tenella* oocysts and helping to reduce animals' mortality. However, we have noticed an adverse effect of the plant use on live body weight gain. The long period of time (four weeks) during which the aerial parts of the plant have been incorporated into animals feed could be the cause of this adverse effect. Other studies with shorter time of use are necessary to a better knowledge of the anticoccidial effect of white wormwood.

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