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Effect of *Artemisia annua* L. as Substitute to Sulfonamides (Sodium Sulfadimerzine) on Coccidiosis and Growth Performance in Rabbits

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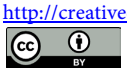

Abstract

Coccidiosis is a disease caused by protozoa of the genus *Eimeria* which seriously affects young rabbits. Treatment based on the use of anticoccidial drugs is increasingly ineffective due to the rapid emergence of resistant strains of coccidia and the high cost of drugs. Consumer demand for rabbit products without chemical residues led to a growing interest in the use of medicinal plants as an alternative treatment for coccidiosis. The present study was carried out during the period of August to December 2020 to assess the anticoccidial effect of hydro-ethanolic extract of leaves of *Artemisia annua* L., in young rabbits. The antiparasitic efficacy of Artemisia extract was tested on 15 young rabbits (whose age varied between 7 and 9 weeks) divided into 5 lots of 3 animals. The average weight of these animals was 790 g. The results of this study show that the feces samples and the weight of young rabbits before administration of the treatment and the coprological examination (every 7 days for 4 weeks) show a fecal excretion reduction rate (FECRT) of 55.13% in the lot treated by sulfonamide. On the other hand, in animals received treatments extract of the leaves of *Artemisia annua* L., the average FECRT is evaluated at 69.64%, 79.22%, and 96.36% for respective doses of 400, 800 and 1200 mg/kg bodyweight and proves their anticoccidial effect. Furthermore, the variation in mean Eggs Per Gram (EPG) of coccidia and the average weekly weight gain (AWWG) of each lot were significant in the lots treated with hydro-ethanolic extract ($P < 0.05$). The greatest reductions in oocystal excretion and weight gain obtained were those of lot 5, treated at 1200 mg/kg of hydro-ethanolic leaves extract of *Artemisia annua* L.

Keywords

Coccidiosis, Rabbit, *Artemisia annua* L., Sulfonamide, Hydroethanolic Extract, Anticoccidial Activity

1. Introduction

Livestock farming is increasingly directed towards an intensification of production systems in order to meet the socio-economic requirements linked to the population explosion and a food deficit. This intensive production has comparative advantages in terms of animal productivity [1]. To face this problem, the promotion of livestock farming, especially short-cycle species, remains a necessity to produce meat [2]. Rabbit (*Oryctolagus cuniculus*), due to its ease of rearing combined with high productivity, is a species that can effectively contribute to the increase in animal protein needed by African populations [3] [4]. According to Lebas and Colin [5], rabbit meat production in Cameroon is 0.6 tons/year for an average consumption of 0.41 g/inhabitant/year for an estimated population of 25 million inhabitants. It remains insufficient, hence the need to move towards a large-scale production. However, it intensifies zoohygiene problems [6]. According to many studies [2] [7] [8] coccidiosis is one of the main constraints that damage the development of livestock and causes enormous economic losses. In rabbit breeding, it remains one of the major health problems [9]. In addition, it causes important economic losses worldwide [10] [11].

Coccidiosis (infection with coccidia) is a disease of rabbits caused by a class of single-celled organism known as protozoa which is developed in the digestive tract [12]. This disease can be contracted from the environment and usually is present in multi rabbit situation as well as in shelters/breeding establishments where stocking rate are high and rabbits are kept in communal runs. Generally, the infections remain subclinical with consequences such as reduced growth or weight loss. But eimeriosis can also be deadly, especially in young rabbits [12]. Furthermore, by reducing the immunity of the host, infections by Eimeria species could favour other diseases [13]. The control of this infection in livestock farms is essential for the improvement of productivity, especially in cuniculture. This is why molecules with anticoccidial activity were developed. However, a lot of anti-coccidial medications to prevent Eimeria infection have revealed the decreased efficacy because some *Eimeria* species have developed resistance activity to anti-coccidials [14]. Recently, phytochemicals from different types of botanical elements have been explored as sustainable alternatives to combat coccidiosis and much more, influence zootechnical performance [15]. Due to the growing demand of "organic meat" and the development of antimicrobial resistance on the other hand, current research efforts are moving towards the use of plant extracts. Due to their therapeutic properties, several species belonging to the genus Artemisia, have attracted more attention from researchers in recent years. Many

of them have antiparasitarian properties [16]. The anticoccidial effects of certain species as well as their effects on the parameters of growth have also been showed with the active ingredient lactone sesquiterpenes, of which artemisinin is the most important [17] [18] [19]. This study aimed in evaluating the anticoccidial activity of the hydro-ethanolic extract of the leaves of *Artemisia annua* L. and the weight gain of animals treated with both phytotherapy and conventional therapy.

2. Material and Methods

2.1. Description of the Study Area

This study was conducted within the National Veterinary Laboratory (LANAVET) of Bocklé located in the North region of Cameroon in the Benoué department and Garoua 3 sub-division (9˚15'35.19618" North latitude; 13˚27'18.20196" East longitude). The study was conducted from August to December 2020. The climate in this area is tropical type characterized by a long dry season from October to April and a short rainy season from May to September. The average annual rainfall is 1000 mm. Temperatures remain high with an average of 28˚C with maxima reaching 40˚C - 45˚C in March and April.

2.2. Plant Material Collection

A. annua L. was collected in August 2020, in the Adamawa region of Cameroon, whose altitude is about 1200 m and identified at the National Herbarium of Cameroon in comparison with the material of Ngansop 682 of the specimen of the herbarium collection N˚67448/HNC. An herbarium was made for this purpose. The harvest of the leaves of *A. annua* L. consisted of pinching one end of the twigs between thumb and forefinger to tear leaves along the stem. This method has the advantage of giving very clean material and solving the problem of subsequent separation of leaves and stems [20]. The leaves were dried in the oven at 30˚C for constant drying without temperature variation for 5 days. After drying, they were crushed with an electric grinder (KENWOOD brand) to obtain a powder which was sieved (1 mm of mesh) and then kept in a hermetically closed box and placed in a place away from light and heat.

2.3. Animal Material

Fifteen (15) post-weaned young rabbits of common breed whose age varied between 7 and 9 weeks were the subject of the experiments. The average weight of these animals was 790 g. Water and feed were distributed *ad libitum* to the animals. The feed (**Table 1**) was formulated within the LANAVET, without anticoccidials and distributed till the end of the experiment, as well as banana leaf tops which serve as fiber source.

2.4. Preparation of the Hydroethanolic Extract

The dried leaves of *Artemisia annua* L. were ground as describe above for *A.*

Table 1. Nutritive value of the diet.

Components	Quantity (%)
Maize	32
Cotton cake	13
Corn bran	53
Salt	0.97
Bone powder	0.7
Olivitasol	0.06

annua L. The powder (128.5 g) was macerated with a hydroalcoholic mixture (1285 ml) in the proportions 70 volumes of ethanol/30 volume of distilled water at room temperature and away from light for 24 hours. Subsequently, the mixture obtained was first filtered on gauze and a second time on filter paper (**Figure 2**). This operation was repeated a 2nd and a 3rd time with renewal of the solvent. The three hydro-ethanolic extracts have been brought together [21] [22] and put in the oven for drying at 40˚C. The dry extract was stored in the refrigerator at 4˚C till use.

2.5. Plant Yield

After drying operation of the leaves of the annual mugwort we obtain 300g of powder from 1200 g fresh leaves, for a yield of 25%. The extraction by maceration of this powder in ethanol/distilled water in the proportions 70/30 allow us to obtain 31.25 g of dry crude extract of dark green color with a yield of 24.3% relative to the total weight of the leaves powder. Hydroethanolic solution was used for extraction because many studies have already showed the anti-coccidial activity of ethanol extract of plants [23] [24]. In addition, ethanol has the capacity to extract many actives compounds like tannins [25] which showed their efficiency against *Eimeria* species [26].

2.6. Animal Care

The young rabbits were placed in individual iron cages. Each was 70 cm long, 50 cm wide and 50 cm high. These cages were individually isolated to facilitate the collection of fecal matter. Each cage was equipped with a feeder and a drinker.

2.7. Sampling

The rabbits were divided into 5 lots of 3 animals according to the treatments to be administered.

Lot 1: Infected, Not treated;

Lot 2: Infected, Treated with sodium sulfadimerzine 50 mg/kg live weight for 10 days;

Lot 3: Infected, treated with hydro-ethanolic extract of leaves of *A. annua* L. at 400 mg/kg live weight, single dose;

Lot 4: Infected, treated with hydro-ethanolic extract of *A. annua* L. leaves at 800 mg/kg live weight, single dose;

Lot 5: Infected, treated with hydro-ethanolic extract of *A. annua* L. leaves at 1200 mg/kg live weight, single dose.

The reference drug used in this study was an anticoccidial named Antiococsuper (active ingredients: sodium sulfaimerzine, lot number: PBO-002361, laboratory: QALIAN)

The anticoccidial was administered to the animals in drinking water as indicated by the manufacturer and administered by gavage using an electronic pipette to ensure that the extract was actually taken by the young rabbits.

2.8. Parasitological Data

Individual fecal samples were taken and analyzed on days 7; 14; 21; 28. Fresh droppings were collected using gloves in plastic jars early in the morning. Each jar was labelled with the information of the animal and the lot.

2.8.1. Coprologic Analysis

The flotation method for a qualitative approach and that of Mc Master for egg research and enumeration were used for coproscopic analyses [27].

The flotation method consisted of mixing 3 g of feces in 45 ml of NaCl solution and then sieving with, forming a meniscus on the surface of the tube and finally affixing a lamella on said meniscus. After about ten minutes, the lamella was deposited on the slide so as to adhere to it for observation under a microscope [28].

For the Mc Master method, four grams (4 g) of feces were taken from each sample and crushed in a porcelain mortar by adding gradually 60 ml of the saturated solution of NaCl (Willis solution). Subsequently, the mixture was filtered through a tea strainer, lined with a gauze previously placed in the tea strainer. The filtrate was homogenized 20 minutes later using a spatula. During stirring, a sample of the suspension was taken using a pipette for filling the Mc Master blade chambers, with preventing the formation of air bubbles. The filled slide was placed on the microscope and left five minutes before observation [29]. Egg counting was done by chambers or cells. Thus, the number of parasite eggs per gram of feces (EPG) was obtained by multiplying the total number of eggs observed in a cell by 50.

2.8.2. Evaluation of the Efficacy of Medicinal Products Administered by the Fecal Egg Excretion Reduction Test

The efficacy of the drugs was determined by calculating the fecal excretion reduction rate (FECRT) of coccidia eggs in each lot after treatment according to the [30] formula following:

$$\text{FECRT} = \frac{(T_1 - T_2)}{T_1} \times 100$$

with:

T_1: EPG before treatment in the treated lot (at D_0).

T_2: EPG after treatment in the treated lot (at D_7, D_{14}, D_{21}, D_{28}).

2.9. Determination of Weight Gain

The weight of the animals was recorded during the administration of treatment and every week during the experiments. This allowed us to calculate the average weekly weight gain (AWWG) of each lot.

2.10. Data Analysis

Data from *in vivo* excretion of coccidia oocysts in rabbits, as well as weight gain were subjected to one-factor analysis of variance (ANOVA) using the general linear model.

The statistical model used was:

$$X_{ij} = \mu + \alpha_i + e_{ij}$$

X_{ij} = Observation on the animal j that received the treatment i

μ = Overall average

α_i = Effect of the dose of anticoccidial in treatment i

e_{ij} = Residual error observed in the animal j that received the treatment i.

When differences existed between treatments, the means were separated by the Duncan test at the 5% threshold.

Data on excretion *in vivo* of coccidia oocysts were compared between lots by Student's t test ($p < 5\%$).

3. Results

3.1. Anticoccidial Effect of *Artemisia annua* L.

3.1.1. Fecal Egg Excretion Reduction Test

Rates of reduction of fecal excretion of coccidia eggs per treatment lot (Table 2) shows that the rabbits of the various lots formed excreted eggs throughout the test in a variable way. Differences in the degree of animal infestation (expressed as EPG) and the rates of reduction in egg excretion levels were observed between the control lot and the different lots treated with sulphamide and plant extract at different doses. Treatment with 1200 mg of plant extract induced a decrease of 98.54% in average EPG on day 14 and 100% on day 28. The same observation was made for Lot 3 and Lot 4 which recorded respectively 94.14 and 97.58% of reduction in egg excretion on day 28. In the lot treated with sodium sulfadimerzine, there was also a maximum reduction at the rate of 80.20% on the 28th day.

3.1.2. Average Evolution of Numbers of Oocysts per Lot

The evolution of the number of oocysts per gram of feces (EPG) in the different groups (Figure 1) shows that the rabbits being naturally infested, the enumeration of eggs per gram of feces (parasitic load on day 0) was done before administration of any treatment, then every week after treatment within seven days. A decrease in oocystal load per gram of feces was observed from D_0 to D_7 in lots 2,

Table 2. Reduction rate of coccidia egg excretion for different treatments.

Post-processing period	FECRT (%)				
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
Day_7	−850.88	17.18	31.18	52.63	88.35
Day_{14}	−547.37	52.98	64.52	76.31	98.54*
Day_{21}	−314.03	70.17	88.71	90.35	98.54*
Day_{28}	76.84	80.20	94.14	97.58*	100*
FECRT Average (%)	−408.86	55.13	69.64	79.22	96.36*

FECRT: "*Fecal Eggs Reduction Count*"; *: A reduction > 95% indicates the absence of resistance to the product used for treatment.

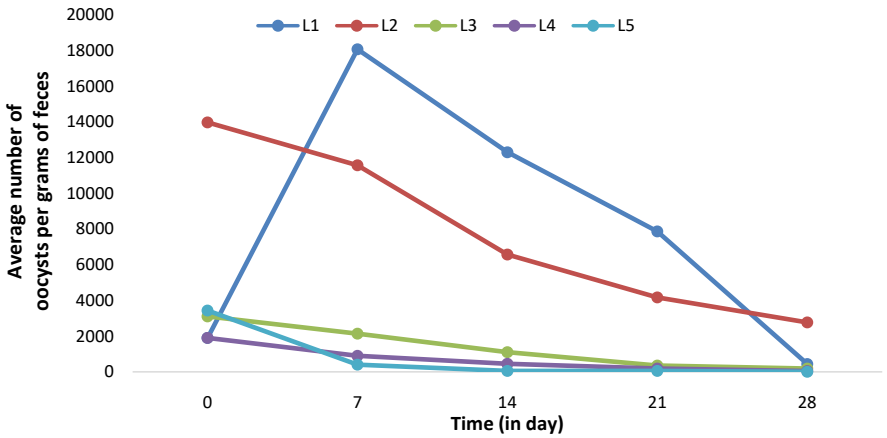

Figure 1. Evolution of number of oocysts per gram (EPG) of feces.

3, 4 and 5 respectively treated with sodium sulfadimerzine at 50 mg/kg live weight, and 400, 800 and 1200 mg/kg live weight of the hydroethanolic extract of leaves of *Artemisia annua* L. However, there is an increase in excretion for the same period in lot 1 that received no treatment. This average load per lot has decreased considerably for all lots from the 7th day, to tend towards zero for lots 3, 4 and 5. It was 440 EPG on day 28 for lot 1, 2766 EPG on the same day for lot 2 and 0 EPG for lot 5.

3.1.3. Average Parasitic Load per Lot of Treatment

The average parasitic loads obtained during the 28 days of follow-up (**Figure 2**) were significantly higher for lot 2 (sulfonamide-treated) and 1 (untreated) and low for lots 3, 4 and 5, all treated with a hydro-ethanolic extract of leaves of *A. annual* L. Thus, the parasitic loads of lots 1 and 2 were significantly higher ($P < 0.05$ with $F = 65.69$ and $N = 3$) than those of lots 3; 4 and 5.

3.1.4. Mean Parasite Load Compared to Sodium Sulfadimerzine

The average parasitic load of the batch treated with sodium sulfadimerzine (L2) and of the untreated lot and those treated with the hydroethanolic extract of leaves of *Artemisia annua* L. at doses of 400, 800 and 1200 mg/kg live weight respectively (**Figure 3**) shows that the parasitic load of lot 2 is significantly higher

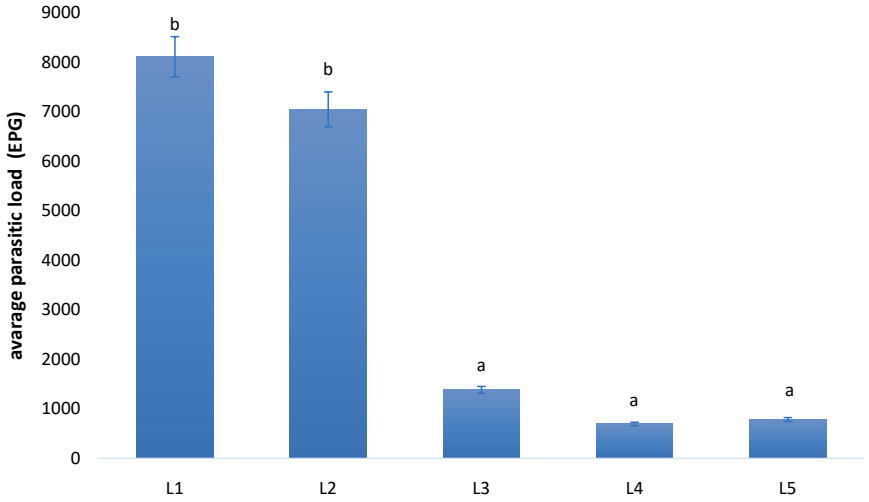

Figure 2. Average parasitic load per lot. (a, b) Average EPGs with the same letters are statistically comparable (P > 0.05).

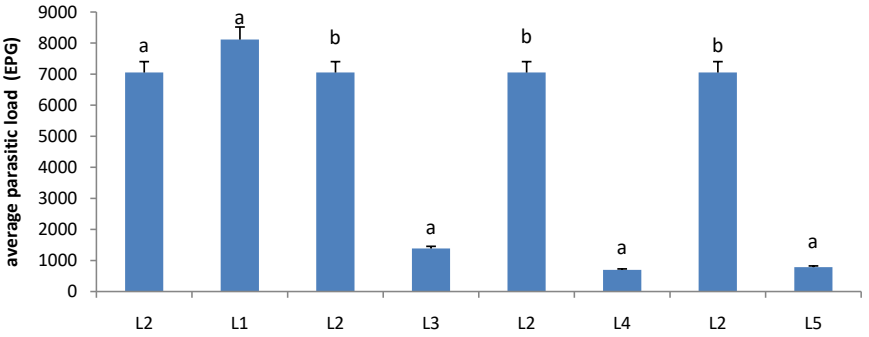

Figure 3. Average parasitic load of animals treated with sulfonamide and plant extract. (a, b) Average EPGs with the same letters are statically comparable (P > 0.05).

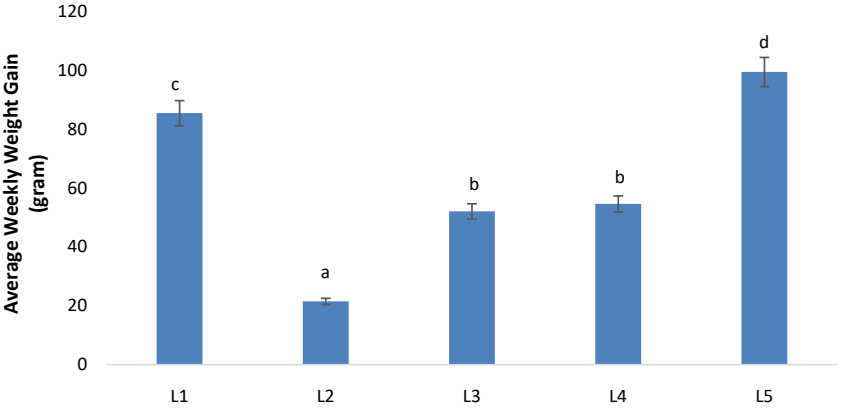

Figure 4. Average weekly weight gain of each lot (a, b, c, d) AWWGs with the same letters are statistically comparable (P > 0.05).

(P < 0.05) than those of lots 3 (with F = 6.149 and N = 3) and 4 (with F = 6.459 and N = 3). The same observation was made between lots 2 and 5 (with F = 6.335 and N = 3). However, the parasitic loads of lots 1 and 2 are comparable and not significant (P > 0.05 with F = 2.528 and N = 3).

3.2. Weekly Weight Gain of Young Rabbits

Growth expressed as weight gain (**Figure 4**) shows that the average weekly weight gain (AWWG) of each batch after treatment increased significantly ($P < 0.05$) with the increasing doses of hydro-ethanolic extract of leaves of *A. annua* L. Although, lots 3 and 4 remained comparable ($P > 0.05$), the highest AWWG was recorded with in lot 5 that received 1200 mg of hydro-ethanolic extract of mugwort leaves per kilogram of live weight. However, the lowest was recorded in lot 2 treated with sulfonamide.

4. Discussion

Throughout the control phase of oocystal excretions, no subject treated with the extract presented an adverse clinical effect, although no biochemical test was performed to assess the toxicity of the extract. This would explain the widespread use of mugwort. In addition, the absence of mortality in lots treated with *Artemisia annua* testifies to a protection conferred to the animals. [31] obtained similar results with *Artemisia annua* L. following the incorporation of the *Artemesia* into the broiler diet during an experimental infection with *Eimeria tenella*.

The reduction in oocyst excretion observed in the control group, which received no treatment (lot 1), could be explained by the occurrence of intense destruction of cells in the intestine of these rabbits, which causes the interruption of *Eimeria* in the *in vivo* development cycle [32].

The growth of the animals in the different treated lots was significantly higher than that of the lot treated with sodium sulfadimerzine. In addition, the best growth was obtained with lot 5. The use of the hydro-ethanolic extract of *Artemisia annua* L. at 1200 mg per kg of live weight therefore significantly improved the growth of the animals during treatment compared to other lots. These results are consistent with those of [33] for whom, the addition of leaf powder and Artemisia extract in poultry feed have the potential to improve daily weight gain and feed conversion rate. These findings are also similar to those of [15], who found that the use of *Artemisia annua* (5%) mitigates the negative effect of Eimeria *tenella* infection on broiler performance. Indeed, the chemical analysis of *Artemisia annua* showed a good balance of nutrients with high levels of antioxidants that potentiates its successful integration into poultry feed. [34] also showed that *Artemisia annua* extracts improve weight gain and feed efficiency during an infestation by *Eimeria tenella* in poultry.

During this study, the toxicological effects of the use of the hydroethanolic extract of *Artemisia annua* L. have not been established. However, the observation of the animals which received this extract by gavage did not suggest any abnormality in their behavior.

5. Conclusion

At the end of this study on the effect of *Artemisia annua* L. as substitute to sulfonamides (sodium sulfadimerzine) on coccidiosis and growth performance in

rabbits it appears that the evaluation of the effectiveness of the ethanolic extract of *Artemisia annua* L. leaves at concentrations of 400 mg/kg, 800 mg/kg and 1200 mg/kg in the treatment of rabbit coccidiosis caused by *Eimeria* sp. naturally infested rabbits, have shown that this plant has significantly superior anticoccidial properties to that of sodium sulfadimerzine at a dose of 400 mg/kg. Also, the result at the zootechnical level showed an improvement in the growth performance of the rabbits and the best improvement was at the dose of 1200 mg/kg live weight. Although the results of this study are satisfactory with 400 mg of *A. annua* L. extract, we suggest emphasizing this work by isolating the active ingredient from the extract to allow easier use.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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