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Comparative Anticoccidial Potential of Maxiban 160 and Herb-All COCC-X against Eimeria *Species* Infestation on Intestinal Lesion and Oocyte Shedding in Broilers

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ABSTRACT

Coccidiosis is the most serious protozoan disease of poultry industry worldwide. Infected birds shed the infective oocysts in high numbers in the droppings which enables the persistence of the disease in the flock. Traditionally, the infestation is controlled by chemical anticoccidial preparations. However, the extensive use of chemical preparations represented a serious problem to public health and lead to the emergence of resistant coccidian strains. To overcome this problem, commercial herbal products are available since the last few decades. The present work aims to evaluate and compare the efficiency of the anti-coccidial herbal product (Herb-All COCC-X) and the chemical preparation (Maxiban 160) in the control of coccidiosis in broilers and to determine their effect on shed oocysts. For this purpose, 320 one-day-old broiler chicks were randomly and equally divided in four groups. All groups (with the exception of the negative control group) were subjected to an infestation with *Eimeria acervulina* and *Eimeria tenella.* One group was treated with Herb-All COCC-X, the second with Maxiban 160, and the third remained untreated as a positive control group. The birds were slaughtered after 41 days for PM examination. The oocysts count in droppings was significantly lower in both treated groups in comparison to the positive control one. The efficiency of Herb-All COCC-X was significantly higher than that of Maxiban 160 in the reduction of the number and sporulation potential of shed oocyst. Other parameters including the FCR, body weight gain, and mortality rate did not vary significantly in both treated groups.

Keywords: Coccidia, *Eimeria acervulina*, *Eimeria tenella*, Herb-All COCC-X, Maxiban 160, phytobiotics.

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INTRODUCTION

Coccidiosis is the most economically significant contagious diseases of poultry which represents a great challenge for the broiler industry worldwide (**Tewari and Maharana 2011**) with a global losses up to $14 billion annually **(Fabia *et al.* 2021; Teng *et al.*, 2021)**. The economic losses in USA alone due to coccidiosis are estimated to reach 127 million during the last 7 year (**Lahlou *et al.*, 2021**). Moreover, the infestation with coccidia enhances the increase in population of other enteric pathogens such as *Clostridium perfringens*, *Salmonella enterica*, *Enterococcus*, *Shigella*, *Klebsiella*, and *Streptococcus* species accompanied by a clear reduction in the abundance of beneficial bacteria such as Lactobacilli and bifidobacterial **(Chen *et al.*, 2020 and Sidiropoulou *et al.*, 2020)**. ***The*** disease is caused by diverse members of the obligate intracellular parasite Eimeria species (family Eimeriidae, phylum Apicomplexa). Eimeria strains are tissue specific (i.e. every species can invade and colonize specific sites in the intestine from the duodenum to the cecum). The field isolates have different level of pathogenicity (**Fabia *et al.*, 2021 and Teng *et al.*, 2021**).

At the time, more than 1800 species of *Eimeria* could be identified which can infect different bird species, mammals, and even humans. Out of these, nine Eimeria species can infect the domestic poultry (*Gallus gallus domesticus*), namely: the highly pathogenic (*E. brunetti*, *E. maxima*, *E. necatrix* and *E. tenella), the less pathogenic species (E. acervulina*, *E. mivati*, and *E. hagani*), in addition to two species which are mostly non-pathogenic (*E. praecox*, and *E. mitis*) **(Quiroz-Castañeda and Dantán-González 2015 and Fabia *et al.*, 2021)**.

Coccidiosis is an enteric disease. The infestation results in a wide range of clinical signs ranging from asymptomatic infestation to fatal conditions **(Lahlou *et al.*, 2021 and Teng *et al.* 2021).** Clinical cases suffer from dysentery, bloody diarrhoea, poor growth, emaciation, and even death in severe cases (**Moryani *et al.*, 2021**). The death occur due to the poor absorption, in addition to the dehydration, loss of nutrients and electrolytes due to the bloody diarrhea, and the enhancement of secondary infestations by opportunistic enteric bacteria (**Chen *et al.*, 2020; Chauhan *et al.*, 2021 and Kalkal *et al.*, 2021**). Coccidiosis is traditionally treated with commercial chemical products (such as Amprolium, Clopidol, and Halofuginone), Ionophores (such as Salinomycin, Maduramycin, monensin, lasalocid), and sulfadimethoxine (**Quiroz-Castañeda and Dantán-González 2015; Kalkal *et al.*, 2021 and Sharma *et al.*, 2021**). Such preparations usually require a withdrawal time of 3–7 days before slaughter to ensure the absence of drug residues from the carcass.

However, due to the high susceptibility of broilers to coccidia infestation, the risk of coccidiosis outbreaks seriously increases during the withdrawal period. Ignoring the withdrawal period will result in public health hazards and the contamination of human food with drug residues. In addition, the massive use of anticoccidial drugs results in the development of resistance against the used preparations, occurrence of toxic side effects on the birds, and contamination of the environment with unabsorbed drug residues excreted in the manure which can be later used as fertilizers **(Sidiropoulou *et al.*, 2020 and Kalkal *et al.*, 2021**). Moreover, the use of several coccidiostats is now contraindicated for farm animals **(Noack *et al.*, 2019**). To overcome all these disadvantages, herbal products "phytobiotics" were established as efficient and safe alternatives to chemotherapeutics, they have a broad-spectrum therapeutic effect even against other protozoan parasites, don't require withdrawal period, and usually provide additional bioactivities (for instance being growth enhancers, immunostimulants, antioxidants or anti-inflammants).

At present, several promising commercial coccidicidal products are available in the market such as Natustat® (Alltech, USA) and Herb-All COCC-X (Life Circle Nutrition AG, Switzerland). They deliver clear therapeutic benefits against coccidiosis (**Abbas *et al.*, 2012; Quiroz-Castañeda and Dantán-González 2015; Sidiropoulou *et al.*, 2020; Chauhan *et al.*, 2021 and Kalkal *et al.*, 2021)**. The aim of the present study is to evaluate the efficacy of Herb-All COCC-X (herbal blend contains many plants such as *Holarrhena antidysenterica* & *Alium sativum*) in an experimental model compared to other well established anticoccidial product of chemical origin (Maxiban 160: active ingredients Narasin and Nicarbazin). The study focusses on the ability of Herb-All COCC-X to affect the excretion of oocysts in the feces to prevent the persistence of these parasites in the farm. The efficacy of the tested products in experimental coccidiosis in broilers was measured by calculating the number of post-infestation shed oocysts / gram faeces (OPG), and the Post-mortem intestinal lesion score (LS).

MATERIALS AND METHODS

1.Preparation of the experiment:

The trial was carried out using 320 one-day-old broiler chicks (Ross males). The chicks were obtained from a commercial hatchery belonging to a specialized research unit in Ploufragan, France. The pure herbal blend (Herb-All COCC-X) was kindly provided by the manufacturer (Life Circle Nutrition AG). The birds received only 50% of the recommended dose from the manufacturer in the starter feed (0.5 Kg / Ton instead of 1 Kg/ton) to investigated the efficiency of the preparation at this dose in young chicks. Meanwhile, older chicks got the recommended dose of 0.5 Kg / Ton in the grower/finisher feed, respectively.

2. Experimental design:

During the experiment, the birds were kept on litter in 32 pens (1 m^2 pens and 10 birds per pen) until the age of 41 days. The birds were randomly and equally distributed to one of 4 trial groups according to their live weight, so that the average bird weight remained similar in each treatment group. The four groups are (A) Negative control: non-infected animals, with no medication, (B) Positive control: infected animals with no medication, (C) Maxiban 160 treated group: infected animals with supplementation of Maxiban 160, and finally (D) Herb-All COCC-X treated group: infected animals with supplementation of the herbal mixture Herb-All COCC-X (Table 1). During this period, the birds received ad libitum starter broiler crumb feed during their first 20 days of life, followed by growing pellet feed until the end of the experiment (from the 21th to the 41th day). The feeds of the group (C) and (D) were supplemented with Maxiban 160 (500 g / ton feed) or Herb-All COCC-X (500 g / ton feed), respectively, as shown in table (2),

Table 1: Shows the different treatments of the birds involved in the present investigation.

Group	Treatment	Birds (n)
A	negative control (non-infected,)	80
B	positive control (infected)	80
C	infected birds	80
D	infected birds	80

Table 2: The feeding protocol of the broilers during the experiment:

Group	Feed (*ad libitum*)		Duration
A	commercial broiler feed without antibiotics and anticoccidials (no supplementation).	starter broiler crumb feed	first 20 days
		growing pellet feed	21- 41 d
B	commercial broiler feed without antibiotics and anticoccidials (no supplementation).	starter broiler crumb feed	first 20 d
		growing pellet feed	21- 41 d
C	commercial broiler feed without antibiotics and anticoccidials (supplemented with Maxiban 160).	starter broiler crumb feed	first 20 d
		growing pellet feed	21- 41 d
D	commercial broiler feed without antibiotics and anticoccidials (supplemented with Herb-All COCC-X).	starter broiler crumb feed	first 20 d
		growing pellet feed	21- 41 d

3. Artificial infestation with Eimeria:

Two strains of Eimeria were used for the experimental infestation (*Eimeria (E.) acervulina* strain PA3 and *Eimeria tenella* strain PT5). The strains were supplied by the parasitological laboratory of ANSES at Ploufragan, France. These two strains were selected to be inoculated as they are the most common pathogenic strains in the field. The inoculation process was carried out according to the used protocol if the parasitological laboratory of ANSES at Ploufragan, France as will be mentioned later. In order to simulate the dynamic of infestation under filed conditions, a pre-inoculation was done at day 6 and day 11 of the trial which represents about 1/10 of the infective dose required to induce infestation in the birds. The received full dose was (80,000 *(E.) acervulina* oocyt/inoculation/bird) and (8000 oocyst /bird *E. tenella*). Both Eimeria containing solutions were mixed and were given together. All birds, except those belonging to the negative control group, were pre-inoculated twice with a low dose containing 10’000 sporulated oocysts of *E. acervulina* and 1’000 sporulated oocysts of *E. tenella* (1ml by oesophageal route directly into the crop with a pipette). At 16 days of age the same birds were infectiously challenged by individual inoculation with an inoculum containing the full dose as described above.

- **4. Oocyst collection and determination of their sporulation potential:**

The excreted oocysts in the droppings were counted using the standard McMaster method according to **Haug *et al.*, (2006)**. Briefly, in the 5th and 6th days post infestation, 2 g of feces were collected and dispersed in saturated salt solution (60 mL). The solution was vigorously mixed before it was filtrated. The solution was loaded onto a McMaster Egg Slide for oocyst counting. The number of oocysts / g feces can be calculated as (the number of oocysts in 2 Chambers x 100) (**Haug *et al.*, 2006**). For measuring the sporulation potential of the collected oocysts, hemocytometer chamber was used as described by Habibi and his team (**Habibi *et al.*, 2016**).

- **5. Post-mortem examination of the gut:**

Examination of the intestinal lesions were scored according to the **Johnson and Reid (1970)** following a scale from 0 to 4 (0 = no lesions to 4 = massive haemorrhage with brownish mucus contents and complete ballooning) at day 22 of the trial (i.e. 6 days after the main challenge) on 2 birds per pen for *E. acervulina* and *E. tenella*.

6. Statistics analysis:

The statistics analysis was performed on body weight and ADWG using ANOVA followed by multiple comparison tests (Newman and Keuls test) after checking of data normality. In case of abnormal distributions, non-parametric tests were applied. Statistical tests on lesion scores were performed with Kruskall-Wallis test followed by paired comparison between control groups and test groups adjusted with Bonferroni p-value. Mortality was tested with Pearson chi-square test or, in case of low number of the sample size by Fisher’s exact test **(Toothaker 1993 and De Muth 2006).**

RESULTS

Effect of herbal product on oocyst excretion and other physiological parameters

The average oocysts excretion per gram of faeces (OPG) varied significantly among the four groups after 5 days following the main challenge at the 5th day. The OPG count ranged from 69,000 to 865,000 OPG in the two control groups, and was 98,000 and 68,000 OPG in Maxiban 160 and Herb-All COCC-X treated groups, respectively. At the 6th day following the challenge, the OPG count was 12,000, 440,000, 30,000, and 11,500 in A, B, C, and D groups, respectively (Table 3).

Table 3: Average oocytes excretion per gram of faeces (OPG) after 5 and 6 days following the main challenge at the 16th day.

Group	OPG / g faeces at day 5 after challenge	OPG / g faeces at day 6 after challenge	Sporulation potential
A	69,000	12,000	+++
B	865,000	440,000	+++
C	98,000	30,000	+++
D	68,000	11,500	++

The differences in the other parameters did not differ significantly among the groups. For instance, no significant differences in body weight gain and daily weight gain could be detected among birds of group B, C and D. During the first 10 days of the experiment (before the birds get challenged with the parasite) there was no need to calculate the feed consumption or the feed conversion rate (FCR) as the four groups were kept under the same treatments and circumstances. Starting from the 11th day, FCR was calculated. Statistically, there were none significant differences among the four groups concerning the feed consumption and FCR. Statistically significant differences could only be detected in body weight gain between the negative control group and the remaining three groups (positive control, and the two treated groups) on the other side. Similarly, there was no significant differences in bird mortality rate among the four groups (average mortality was 5.8%). The main reason for mortality was due to unabsorbed yolk in early life. Based on the post-mortem investigations, none of the losses was attributed to the coccidial infestation.

Effect of herbal product on the prevention of intestinal lesions:

The damage of intestinal mucosa in response to the infestation could be determined after slaughtering the birds. The severity of the lesions could be scored from 0 to 4 (0 = no lesions to 4 = massive haemorrhage with brownish mucus contents and complete ballooning) according to the **Johnson and Reid (1970)** (table 4 and figure 1).

Effect of herbal product on performance:

The mortality rate among the different groups was 4.26% (negative control group), 8.51% (positive control group), 6.12% (Maxiban 160 treated group), and finally 4.17% (Herb-All COCC-X supplemented group) with an average mortality of 5.8% in the period from day 6 to end of the trial at day 41. The average body weight at slaughter at 41 days of age was 3’725 g in the negative control group, 3’631 g in the positive control, 3’596 g in the Maxiban 160 and 3’559 g in the Herb-All COCC-X group, respectively. There are no significant differences in body weight, except the negative control group with no challenge and no supplement (p= 0.007). The corresponding results for the average daily gains over the whole fattening period were 89.9 g, 87.6 g, 86.7 g and 85.8 g. Feed consumption was not recorded for the first 10 days of the trial. Thus, feed conversion rate (FCR) could not be calculated for this period. Nevertheless, a comparison of the mean FCR during the period from day 11 to day 41 showed no significant differences between all groups (p = 0.469).

Table 4: The score of PM intestinal lesions in slaughtered birds (n=16/ group).

E. acervulina					
Score	0	1	2	3	4
A	11	3	0	2	0
B	1	4	10	1	0
C	15	1	0	0	0
D	15	1	0	0	0
E. tenella					
Score	0	1	2	3	4
A	15	1	0	0	0
B	6	4	3	1	2
C	16	0	0	0	0
D	11	3	2	0	0

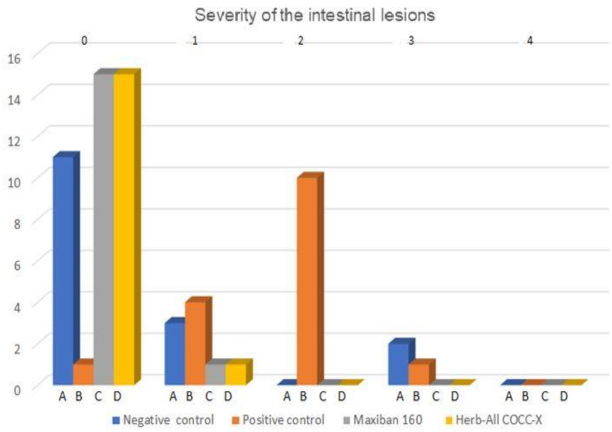

Fig. 1: The graphic illustrates the distribution of the birds in four groups according to the severity of PM intestinal lesions. The intestinal lesions due to infestation with *E. acervulina* were scored according to the Johnson and Reid following a scale from 0 to 4 (0 = no lesions to 4 = massive haemorrhage) at day 22 of the trial. From every group 16 birds were slaughtered for PM evaluation. Every column in the graphic shows the number of birds (out of 16 birds).

DISCUSSION

Due to the disadvantages and side effects of using chemical coccidiostats on birds, workers, consumers and environment **(Additives and Feed (FEEDAP 2010)**) an international trend of replacing chemical products with natural ones started to grow worldwide. In the last few years, commercial herbal products were developed. Several studies were carried out to compare the efficiency of herbal products with that of chemical ones (**Sidiropoulou *et al.,* 2020**) and to investigate whether they provide safe, effective and economic alternative for the control of avian coccidiosis **(Abbas *et al.,* 2012 and Moryani *et al.,* 2021).**

The present work compared the potential of a classical coccidiostat to control coccidiosis with that of a pure natural herbal alternative. The obtained data revealed that the use of both medications (Maxiban 160 and Herb-All COCC-X) was effective against coccidian infestation. However, although no significant differences could be detected among the four groups involved in the trial in terms of ADWG and FCR, the number of shed oocysts in the faeces was significantly lower in the Herb-All COCC-X group than in the second group which is in agreement with the PM findings of the intestinal lesions. The used herbal product was found to reduce but not completely eliminate the shedding of oocysts in the feces. The presence of oocysts which partially lost their ability to sporulate and re-infect new birds is important to advocate the immune system in a way similar to (life) vaccination in a similar way like described in previous works and according to the manufacturers **(Rafqi *et al.,* 2017 and Soutter *et al.,* 2020).**

Although the intestinal lesions resulting from infestation with *E. acervulina* in both treated groups (C and D) were comparable, the treated group with Maxiban 160 showed less intestinal lesions upon being challenged with by *E. tenella*. This might explain the recommendation of the manufacturer to use full dose (1 kg / ton) for the complete control of coccidiosis and not half dose as was carried out in the present study as *E. tenella* usually target the distal pert of the intestine (the cecum and cecal tonsils present at the ileocecal junction) **(Yun *et al*., 2000).**

In opposite to our results, the efficiency of Maxiban 160 to control coccidiosis was higher than that of herbal extracts of (1) *Artemisia annua* and *Curcuma longa* according to Almeida and his team (**Almeida *et al.,* 2014)** and (2) *oregano; Quillaja;* combination of *Curcuma, saponins,* and *inulin* (**Scheurer *et al*., 2013)**.

The lack of efficiency of the tested plant extracts in comparison to the delivered results from this study can be attributed to many reasons, (1) the difference in the composition of the included plants between Herb-All COCC-X and the other blends, (2) the involvement of the whole plants in Herb-All COCC-X and not only the plant extract like the case of other tested products, (3) differences in the used knowhow for the preparation of the products. However, testing the efficiency of herbal products against other chemical preparations such as monensin was also carried out and revealed comparable anti-coccidial efficiency. For instance, the comparing with the herbal preparations (1) *Artemisia sieberi* extract (**Quiroz-Castañeda and Dantán-González 2015**), (2) Oregano essential oil **(Sidiropoulou *et al.,* 2020**), (3) *Cnidium monnieri* and *Taraxacum mongolicum* (**Song *et al.,* 2020**), or (4) the commercial herbal preparation (adiCox®AP) (**Lukasiewicz *et al.,* 2014**). Reported the potential of natural products to provide potential coccidiostatic effects comparable to the traditional commercial products. Similarly, the comparison of the anti-coccidial potential of Amprolium with Neem (**Barbour *et al.,* 2015**), *Artemisia sieberi*, or *Curcuma longa* (**Mousavinasab *et al.,* 2020**) had similar results.

CONCLUSION

Newly designed pure herbal products showed promising results concerning their ability to control coccidiosis in poultry flocks when used in the recommended dose. The present study, supported by findings from previous researches and recommends the replacement of the traditionally used anti-coccidial products with herbal commercial products. This overcomes the public health hazards concerning the chemical residues, and the development of resistances. It provides also several advantages to the broiler industry regarding efficiency, safety and costs.

Declaration of Conflicting Interests

The authors revealed that there is no potential conflicts of interest.

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