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Use of coccidiostat in mineral salt and study on ovine eimeriosis

Uso de coccidiostático no sal mineral e estudo da eimeriose ovina

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Abstract

Coccidiosis is a serious obstacle to sheep production, which is becoming a limiting factor, especially with regard to lamb production. However, there are few studies on this parasite in the State of Rio Grande do Norte. The aim of this study was to evaluate the action of decoquinate, added to mineral salt, for controlling *Eimeria* infection in lambs, and to identify which species are infecting sheep in the eastern region of the state. This study was carried out from August 2009 to January 2010, and used 76 animals. These were divided into two treatment groups: one with common mineral salt, and the other with mineral salt enriched with 6% micronized decoquinate. Fecal samples and body weight measurements were taken every 14 days for parasitological diagnosis, weight gain follow-up and quantitative analysis. The study showed that there was a significant difference in OPG only at the 7th collection, but no significant difference in weight gain. The *Eimeria* species found were *E. absata*, *E. crandallis*, *E. granulosa*, *E. intricata*, *E. ovina*, *E. faurei*, *E. ovinoideal*, *E. pallida* and *E. parva*. It was concluded that addition of decoquinate to mineral salt gave rise to lower oocyst elimination, thus favoring eimeriosis control in sheep.

Keywords: Eimeriosis, lambs, coccidiostatic, mineral salt, Macaíba – RN.

Resumo

A coccidiose constitui-se num sério obstáculo à ovinocultura, a qual vem se tornando um fator limitante para a exploração, especialmente para a produção de cordeiros precoces. Porém, poucos são os estudos com esse parasito no Estado do Rio Grande do Norte. O objetivo deste trabalho foi avaliar a ação do decoquinato, adicionado ao sal mineral, no controle da infecção causada por parasitas do gênero *Eimeria* em cordeiros, e identificar quais as espécies infectam ovinos na região leste Potiguar. O trabalho foi desenvolvido entre agosto de 2009 e janeiro de 2010, e foram usados 76 animais, distribuídos em dois tratamentos, um com sal mineral comum e o outro com sal mineral enriquecido com decoquinato a 6% micronizado. Amostras fecais e pesagens dos animais foram feitas a cada 14 dias para o diagnóstico parasitológico, acompanhamento do ganho de peso ponderal e análise quantitativa. O estudo evidenciou que houve diferença significativa na redução do OoPG apenas na 7ª semana de experimento, mas não houve diferenças significativas para ganho de peso dos animais. As espécies encontradas foram *E. absata*, *E. crandallis*, *E. granulosa*, *E. intricata*, *E. ovina*, *E. faurei*, *E. ovinoideal*, *E. pallida* e *E. parva*. Conclui-se que a adição de decoquinato ao sal mineral propiciou uma menor eliminação de oocistos favorecendo o controle da eimeriose ovina.

Palavras-chave: Eimeriose, cordeiro, coccidiostático, sal mineral, Macaíba – RN.

Introduction

Sheep farming is a traditional activity in northeastern Brazil, but sanitary problems still exist, and these are largely to blame for the low zootechnical and economic performance of the herds and are factors that limit increased productivity. Economic losses

from mortality and unsatisfactory performance are caused mainly by parasites like those responsible for coccidiosis, which is one of the main diseases harming the breeding of small ruminants. Mortality in young animals affected by eimeriosis is high under certain circumstances, sometimes reaching rates of more than 20% (VIEIRA, 2002).

These animals are parasitized by a large number of *Eimeria* species with marked host specificity, and young animals have greatest susceptibility (VIEIRA, 2002; LIMA, 2004; LAGARES,

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2008). *Eimeria* species found in the State of Rio Grande do Norte were first studied by Ahid et al. (2009), in the municipality of Mossoró, in the western part of the state, but there are no reports about which species infect sheep in the other regions.

A large number of drugs have been recommended for treating coccidiosis in ruminants. They can be added to feed, water or mineral salt and, depending on the drug, its use can be continuous or strategically periodic. Among the most widely use are sulfa, amprolium (SANTIAGO et al., 1977), decoquinate, antibiotics, ionophores (monensin, salinomycin and lasalocid) (HEINRICH; BUSH, 1991; WAGGONER et al., 1994; NUSSIO et al., 2002; VIEIRA et al., 2004; VIEIRA et al., 2005; LIMA et al., 2009) and toltrazuril (SANTIAGO et al., 1977; SILVA et al., 2007). Decoquinate interrupts the development of coccidians from the outset of their life cycle, by acting on the first stages of their lives to delay their development before they can cause any intestinal damage over the entire period of their life cycle in the small intestine. This period represents 71% of the protozoan cycle in the host.

In light of the need to study ways of preventing coccidiosis or eimeriosis in the State of Rio Grande do Norte (RN), the aims of the present study were to evaluate the efficiency of decoquinate in mineral salt fed to lambs, for preventing and controlling ovine eimeriosis and for promoting growth and weight development among these animals, and to identify which *Eimeria* species infect these animals, in the municipality of Macaíba in the eastern portion of Rio Grande do Norte.

Material and Methods

The experiment took place in the municipality of Macaíba, RN, in the eastern mesoregion of the state, from August 2009 to January 2010. The examinations were done at the Animal Parasitology Laboratory, Animal Nutrition Laboratory and Aquatic Microbiology Laboratory of the Federal University of Rio Grande do Norte (UFRN).

A total of 76 crossbred Santa Inês-Dorper lambs (32 males and 44 females), aged seven days, were randomly selected and divided into two groups: group T1, without the coccidiostat (control group), and group T2, with the coccidiostat (treated group). They were given water and mineral salt, with or without the coccidiostat, ad libitum, depending on the group to which they belonged.

All the animals were weighed every two weeks. Using a surgical glove, feces were collected directly from the rectal ampoule, and were placed inside duly identified plastic collectors, packed in Styrofoam® containers and sent for oocyst examination and induced sporulation.

The feces were evaluated by means of the floatation technique in saturated sugar solution. Quantitative analysis was done using the oocyst per gram of feces (OPG) counting method, in accordance with the technique developed by Gordon and Whitlock (1939), as modified by Ueno and Gonçalves (1998).

Positive fecal material from animals belonging to the same group (T1 or T2, separately) was subjected to qualitative analysis, using induced sporulation of oocytes in an aqueous solution of feces added to a solution of 2.5% potassium dichromate ($K_2Cr_2O_7$),

at proportions of one part of feces to two parts of potassium dichromate. Next, the mixture was passed through sieves to remove any fragments and poured in fine layers into Petri dishes. It was then sporulated at ambient temperature for 10 days for later identification of *Eimeria* species according to morphological traits (SILVA et al., 2007; SKIRNISSON, 2007; TOULAH, 2007; YAKHCHALI; GOLAMI, 2008; AHID et al., 2009).

After sporulation, the material was centrifuged and the oocysts were suspended in a saturated sugar solution, placed between a slide and coverslip and identified based on morphology and sporulated oocyst measurements. Two hundred random oocysts (100 from group T1 and 100 from group T2) were morphometrically studied (measured using an ocular micrometer with a 100x lens) to identify each species. The criteria adopted for differentiating the species were based on color, presence or absence of an operculum and oocyte size (REGINSSON; RICHTER, 1997; FREITAS et al., 2005; SILVA et al., 2007; AHID et al., 2009; DENIZ, 2009). All the measurements were presented as mean \pm SD.

The mean number of oocysts in T2 feces was calculated and compared with the mean found in T1, and this was also done for the animals' weights. Before analysis of variance (ANOVA) was performed, it was found that the OPG variable did not obey normal distribution. The following logarithmic transformation was used to stabilize occurrences of OPG: $OPGT = \log(OPG + 20)$.

The least-squares method was used in analysis of variance, in which the statistical model used for OPG and for weight was: $y_{ijk} = \mu + t_i + a_j(t_i) + c_k + (tc)_{ik} + e_{ijk}$, where y is the k^{th} observation of OPG or of weight, in the i^{th} treatment on the j^{th} animal; μ is the parametric mean; t is the fixed effect of the i^{th} treatment ($i = 1, 2$); a is the effect of the j^{th} animal on the i^{th} treatment; c is the effect of the k^{th} observation of OPG or of weight; tc is the effect of the interaction between the i^{th} treatment and the k^{th} observation of OPG or of weight; and e_{ijk} is the random error. All the analyses were conducted using the SAS software (2001).

Results and Discussion

During the experiment, a significant treatment difference ($p < 0.05$) was observed only in relation to the reduced OPG count (Table 1) in the 7th collection week. However, in some weeks, the OPG count in group T2 was lower than that of group T1 (Figure 1), thus resulting in a lower overall mean for T2 than for T1. It should be pointed out that this slight difference between treatments may have been due to the food given to the animals, given that they received nutritional supplementation, which promotes a certain degree of immunological development, thereby contributing towards eimeriosis control.

According to Lima (2004), the treatment for this disease, when specific drugs are used, is efficient if it is applied early. The treatment is effective and yields good results when used at the onset of the disease, since most drugs act on the early stages of coccidian multiplication.

Despite the high OPG values observed, clinical symptoms of eimeriosis were found in the groups only in the first weeks, when the lambs were still being nursed. From the 8th week of the experiment (5th collection), no clinical symptoms were detected

Table 1. Mean number of OPG in sheep in 11 assessments. Data transformed to log (OPG + 20). (T1 = Control group; T2 = Treated group).

| Collections | T1 | T2 |
|-------------|-------------------------|---------------------------|
| 1 | 1.66 ^{a A} | 1.79 ^{abcdefg A} |
| 2 | 2.56 ^{abcd A} | 2.22 ^{abcde A} |
| 3 | 1.95 ^{cde A} | 1.30 ^{g A} |
| 4 | 2.63 ^{abc A} | 1.89 ^{abcdefg A} |
| 5 | 1.84 ^{de A} | 1.60 ^{adefg A} |
| 6 | 2.10 ^{bcd A} | 2.14 ^{abcdef A} |
| 7 | 2.68 ^{ab A} | 1.74 ^{acdefg B} |
| 8 | 2.99 ^{a A} | 2.45 ^{abc A} |
| 9 | 2.19 ^{bcd A} | 2.50 ^{ab A} |
| 10 | 2.38 ^{abcde A} | 2.58 ^{a A} |
| 11 | 1.78 ^{e A} | 2.27 ^{abcd A} |
| Mean | 2.25 ^A | 2.04 ^A |

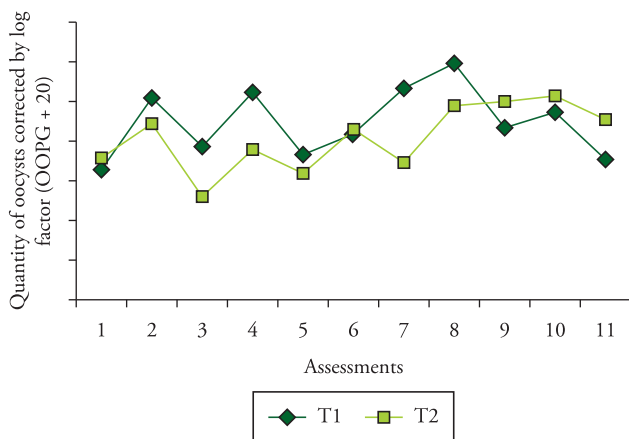


Figure 1. Results from oocyst examinations on the lambs, according to treatments, corrected using the log factor (OPG + 20) ($p < 0.05$), in 11 assessments.

in either of the groups. This may have been due to parasitism by non-pathogenic *Eimeria* species or because of the small numbers of pathogenic species. Several authors have reported that high population density may be responsible for massive contaminations, and have confirmed that young animals are more susceptible to coccidiosis than are older individuals, in any animal species (MENEZES; LOPES, 1997; REGINSSON; RICHTER, 1997; HASSUM et al., 2002; BARBOSA et al., 2003; FREITAS et al., 2005; HASSUM; MENEZES, 2005; SARTOR et al., 2007; TOULAH, 2007; YAKHCHALI; GOLAMI, 2008).

The first sign that the disease has spread throughout the herd is the fact that the lambs are not developing as expected. In addition to presenting with pleated skin and loss of wool, some of the animals show feces on the hindquarters, due to diarrhea (DENIZ, 2009).

The diagnosis of coccidiosis is based on anamnesis, in which information on handling and the rearing system, clinical signs, macroscopic lesions at necropsy, presence of endogenous forms of the parasite in the affected tissues and feces examination is

correlated (LIMA, 2004). The prophylaxis for the disease very often consists of eliminating overcrowded conditions and fecal contamination of drinking water or food. Improved hygiene and sanitation conditions reduce the infection level and incidence of clinical outbreaks (DENIZ, 2009).

The pathogenic effects of eimeriosis on sheep production are stronger among animals raised in intensive systems, in which animal concentrations are much higher than in extensive systems. The infection may be asymptomatic, according to the species of *Eimeria*, seriousness and rate of acquiring the disease, age of the animal and the presence or absence of predisposing factors. Thus, the coccidiosis may be clinical or subclinical, and may or may not exhibit clinical symptoms. Both the clinical and the subclinical form of the disease compromise animal health, possibly leading to their death (LAGARES, 2008). The most serious form of coccidiosis is characterized by severe diarrhea, dehydration, anorexia, lethargy and a high mortality rate (LIMA, 2004). Subclinical coccidiosis is the most common form of the disease and, even though the losses are imperceptible to the producer, they are significant and may have greater economic impact than losses due to death or clinical manifestations.

The quantitative OPG analysis was followed by qualitative assessment using sporulation to identify *Eimeria* species. The species found were *E. ovina* (9.0%); *E. absata* (4.0%); *E. crandallis* (19.0%); *E. faurei* (4.0%); *E. intricata* (2.0%); *E. ovinoidalis* (15.0%); *E. pallida* (17.0%); *E. parva* (21.0%); and *E. granulosa* (9.0%) (Figure 2). These results are in partial agreement with those obtained by Ahid et al. (2009), in the municipality of Mossoró, which is located in the western part of the state. They did not find the species *E. pallida* or *E. intricata*, but they did identify *E. caprovina*, a species that was not observed in the present study. Therefore, the present investigation can be considered to be a pioneering study on ovine eimeriosis in western Rio Grande do Norte.

The morphometric assessments on oocysts from *Eimeria* species found in the lambs of the present study (Table 2) are similar to those observed by Ahid et al. (2009), with a slight difference for *E. faurei*, *E. crandallis* and *E. granulosa*, such that the previous authors recorded a larger polar diameter. They also reported slight individual differences. However, the oocyst dimensions should be considered to be specific characteristics.

Ovine coccidiosis is present in practically all the countries where sheep are raised. In addition to this, many papers have shown that some *Eimeria* species are found more often than others: *E. parva*, *E. absata*, *E. faurei*, *E. ovinoidalis*, *E. crandallis*, *E. intricata*, *E. bakuensis*, *E. granulosa*, *E. pallida*, *E. ovina*, *E. weybridgeensis*, *E. punctata*, *E. caprovina*, *E. arloingi*, *E. marsica*, and *E. ninaekohlyakimovi* (TORRES, 1945; SILVA et al., 1987; AMARANTE et al., 1993; HIDALGO-ARGÜELLO et al., 1997; REGINSSON; RICHTER, 1997; KAYA, 2004; HASSUM; MENEZES, 2005; RIVERA et al., 2005; SILVA et al., 2007; SKIRNISSON, 2007; TOULAH, 2007; SILVA et al., 2008; YAKHCHALI; GOLAMI, 2008; AHID et al., 2009).

The species *E. ovinoidalis* is considered to be the most pathogenic of the species, capable of causing death in male lambs, followed by *E. crandallis*. Although the latter is only moderately pathogenic,

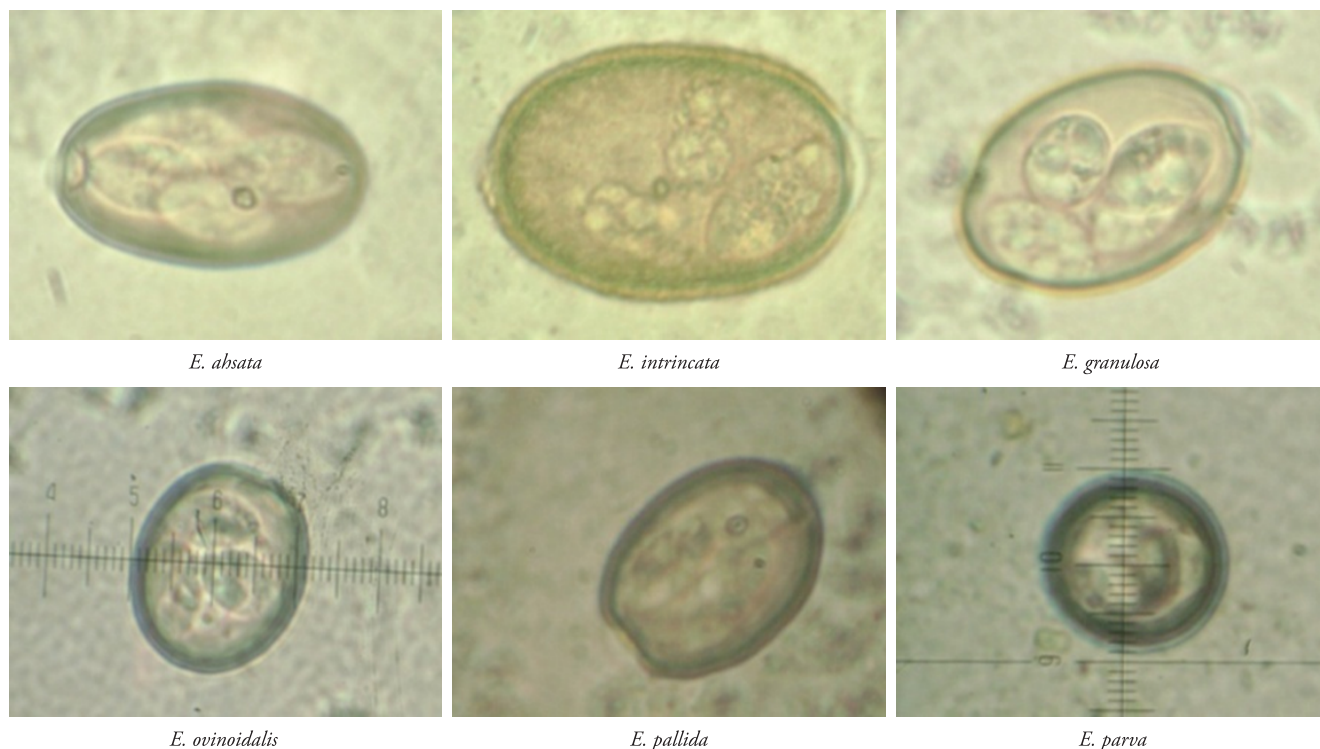


Figure 2. Some species of *Eimeria* were identified in the feces of lambs raised in an intensive system in the eastern part of Rio Grande do Norte.

Table 2. Morphometric indices of sporulated oocysts from *Eimeria* species in lambs raised in an intensive system in the municipality of Macaíba, Rio Grande do Norte, Brazil.

| <i>Eimeria</i> species | Operculum | Oocysts (μm) | | MI |
|------------------------|-----------|--------------|--------------|-------------|
| | | Diameters | | |
| | | Polar | Equatorial | |
| <i>E. absata</i> | Yes | 31.88 ± 3.56 | 22.00 ± 3.12 | 1.46 ± 0.14 |
| <i>E. crandallis</i> | Yes | 22.41 ± 2.60 | 18.46 ± 2.08 | 1.22 ± 0.12 |
| <i>E. granulosa</i> | Yes | 24.88 ± 1.76 | 19.41 ± 2.06 | 1.29 ± 0.12 |
| <i>E. intricata</i> | Yes | 50.20 ± 5.72 | 38.20 ± 2.95 | 1.31 ± 0.11 |
| <i>E. ovina</i> | Yes | 30.61 ± 0.98 | 21.78 ± 2.05 | 1.42 ± 0.12 |
| <i>E. faurei</i> | No | 26.89 ± 2.37 | 20.56 ± 0.73 | 1.31 ± 0.10 |
| <i>E. ovinoidalis</i> | No | 23.21 ± 3.06 | 18.41 ± 2.41 | 1.27 ± 0.14 |
| <i>E. pallida</i> | No | 15.70 ± 1.70 | 12.30 ± 1.40 | 1.29 ± 0.17 |
| <i>E. parva</i> | No | 18.98 ± 3.87 | 17.17 ± 3.46 | 1.11 ± 0.12 |

it exacerbates the pathogenic effects of the former. *E. bakuensis* is equally pathogenic, especially in the asexual phase. *E. parva* is a mildly pathogenic species. Under experimental conditions, *E. absata* is highly pathogenic. The pathogenicity of *E. faurei*, *E. intricata* and *E. weybridgensis* remains unknown (LAGARES, 2008; SILVA et al., 2008).

With regard to weight gain, decoquinate was not found to have any statistical influence ($p > 0.05$) over the course of the experiment (Figure 3). However, group T2 animals gained more weight than group T1 animals (difference of 2.829 kg).

Further studies should be performed using decoquinate, with the aim of determining what percentage supplementation of this coccidiostat in the diet of lambs provides efficient weight gain.

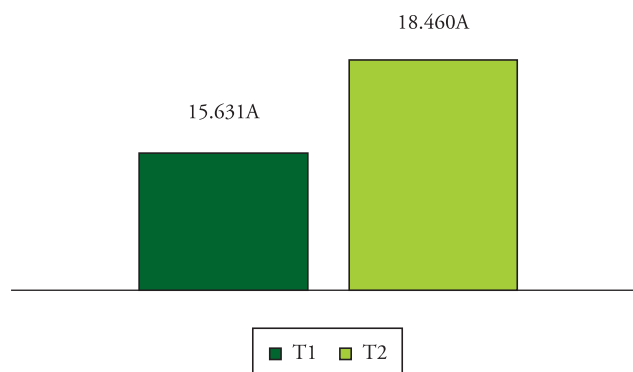


Figure 3. Mean weight gain of lambs according to treatment.

Conclusion

Preventive treatment with decoquinate, added to mineral salt, was efficient in preventing clinical and subclinical coccidiosis, but did not result in maximum weight gains in the treated lambs. On the other hand, it always produced better outcomes than among untreated animals.

The following *Eimeria* spp. species were identified: *E. ovina*; *E. absata*; *E. crandallis*; *E. faurei*; *E. intricata*; *E. ovinoidealis*; *E. pallida*; *E. parva*; and *E. granulosa*. This was a pioneering study with regard to identifying coccidian species in the eastern region of the State of Rio Grande do Norte, Brazil.

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