Influence of fasting on the efficacy of anthelminthics in naturally infected sheep

Influência do jejum na eficácia de antihelmínticos em ovinos naturalmente infectados

Influencia del ayuno en la eficacia de los antihelmínticos en ovejas infectadas naturalmente

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ABSTRACT
The aim of this study was to verify the effect of fasting on the anti-helminthic efficiency of ivermectin-albendazole-levamisole (IAL) combination (first stage) and the closantel and albendazole (second stage) in sheep naturally infected with gastrointestinal nematodes. To this end, fecal samples from young (4 to 6 months), male and female sheep were evaluated seven and 14 days after the treatments, using the Egg count Per Gram of feces (EPG) and the Fecal Egg Count Reduction Test (FECRT). In the first stage of the experiment, the mean EPG of the animals treated and fasted beforehand was lower than the mean EPG of the animals treated, but not fasted 14 days after treatment. Moreover, the group of fasted animals exhibited a better reduction in fecal egg count rate. In the second stage, there was no significant decrease in the mean EPG of the animals in any group (closantel and albendazole) and the efficiency calculated by FECRT was low for the closantel group and zero for the albendazole group. However, the decrease in EPG in animals treated with closantel after fasting was 326.8% greater than the decrease in EPG in animals treated without prior fasting. It was evident that fasting had a positive influence on the reduction of the genera *Haemonchus, Cooperia, Trichostrongylus* and *Oesophagostomum* in the animals treated with closantel. The anthelminthic effect of closantel, albendazole and ivermectin-albendazole-levamisole (IAL) combination in sheep was significantly enhanced by previous fasting. Therefore, it is strongly recommended to withhold solid food for a minimum of 12 hours before administering these treatments.

Keywords: Albendazole. Closantel. Ivermectin. Nematodes. Small Ruminants.
ao jejum 14 dias depois do tratamento. Melhor taxa de RCOF também foi observada no grupo dos animais submetidos ao jejum. Já na segunda etapa não houve diminuição significativa nas médias dos OPGs nos animais de nenhum grupo (closantel e albendazole) e a taxa de RCOF foi baixa para o grupo closantel e nula para o grupo albendazol. No entanto, a diminuição nos OPGs dos animais tratados com closantel depois do jejum foi 326,8% maior que a diminuição do OPG dos animais tratados sem o jejum prévio. Evidenciou-se que o jejum influenciou positivamente na diminuição dos gêneros *Haemonchus, Cooperia, Trichostrongylus* e *Oesophagostomum* nos animais tratados com closantel. Concluiu-se neste estudo que o efeito antihelmíntico do closantel, do albendazole e da associação albendazole-levamisole-ivermectina na espécie ovina foi potencializado pelo jejum prévio, sendo, portanto, recomendada a retirada de alimentos por no mínimo 12 horas antes da administração destas drogas.


**RESUMEN**
El objetivo de este estudio era comprobar el efecto del ayuno sobre la eficacia antihelmíntica de la combinación ivermectina-albendazol-levamisol (IAL) (primera etapa) y closantel y albendazol (segunda etapa) en ovejas infectadas naturalmente por nematodos gastrointestinales. Para ello, se evaluaron muestras fecales de ovejas machos y hembras jóvenes (de 4 a 6 meses) siete y 14 días después de los tratamientos, mediante el recuento de huevos de nematodos (HPH) y la prueba de Reducción del Recuento de Huevos en Heces (RRHH). En la primera fase del experimento, el promedio de HPH de los animales tratados y ayunados antes de la cria fue inferior al promedio de los animales tratados pero no ayunados 14 días después del tratamiento. También se observó una mejor tasa de RCOF en el grupo de animales ayunados. En la segunda etapa, no se observó una disminución significativa de la media de HPH de los animales de ninguno de los dos grupos (closantel y albendazol) y la tasa de RRHH fue baja en el grupo tratado con clo-santel y nula en el grupo tratado con albendazol. Sin embargo, la disminución de HPH de los animales tratados con closantel tras el ayuno fue un 326,8% mayor que la disminución de HPH de los animales tratados sin ayuno previo. Era evidente que el ayuno influía positivamente en la reducción de los gérmenes *Haemonchus, Cooperia, Trichostrongylus* y *Oesophagostomum* en los animales tratados con closantel. En este estudio se llegó a la conclusión de que el efecto antihelmíntico del closantel, el albendazol y la asociación albendazol-levamisole-ivermectina en ovinos se veía potenciado por el ayuno previo, por lo que se recomienda retirar la comida durante al menos 12 horas antes de administrar estos fármacos.

1 INTRODUCTION

Gastrointestinal helminthiases are one of the main causes of losses in domestic ruminants and horses. Although chemical control remains the most widely used approach worldwide, nowadays various alternatives for controlling helminthiasis are available. Nevertheless, the intensive use of anthelminthic drugs in production animals has elevated high levels of resistance through parasites and the negative impacts linked to animal production (Charlier et al., 2020; Kaplan, 2020).

When deciding which drug to use for this control, the veterinarian should consider the age, weight, gender, general condition of animals and other habits. The fasting period preceding treatment has been approved for decades, depending on the chosen drug, via administration and animal species (Sánchez et al., 1999; Sánchez et al., 2000).

Hall (1926) suggested that dogs and cats should be subjected to fasting overnight, while the pigs not be fed for a longer period for a more extended period. For effectiveness, sheep and other ruminants should be in a prolonged fast for the anthelmintics, while horses should be fasted for 36 hours to eliminate the worm of the large intestine, as to Ascarids and Habronema spp. 12 to 24 hours of fast would be enough. However, on occasion, the use of copper sulfate, carbon tetrachloride, bephenium hydroxynaphthoate and sodium arsenite as vermicide for different animals, including humans.

It is known that low gastrointestinal absorption hampers the clinical efficacy of anthelmintics composed of benzimidazoles due to their poor water solubility. Some alternatives have already been tested to improve the absorption and systemic bioavailability of benzimidazoles in cattle, mice and pigs, such as the use of amphoteric surfactants, cyclodextrin binding and lipid nanocapsules (Alí & Hennessy, 1995; Castro et al., 2013; Ceballos et al., 2011; Ceballos et al., 2015; Hennessy et al., 1995; Sánchez et al., 2000; Virkel et al., 2003).

Temporary food restriction in ruminants, before administering specific benzimidazoles, enhances their absorption and bioavailability. This improvement in absorption kinetics and metabolite disposition increases plasma and tissue
availability (Sánchez et al., 2000). Hunger resulting from fasting slows down the passage of ingesta, thereby prolonging the drug's presence in the gastrointestinal tract and enhancing its absorption (Alí & Hennessy, 1995).

On the other hand, prolonged food restriction leading to low body condition affects the drug's pharmacokinetics, resulting in lower bioavailability of the anthelminthic due to reduced hepatic biotransformation (Sánchez et al., 1996). Conversely, plasma concentration is higher and persists longer in well-fed animals (Craven et al., 2002).

The oral bioavailability of macrocyclic lactones in sheep has also been shown to be altered, depending on the diet. Ewes treated with ivermectin and fed alfalfa pellets exhibited lower plasma drug concentration when compared to ewes treated and fed tannin-containing plant-based pellets (Gaudin et al., 2016).

The identification of factors that affect the pharmacokinetics and metabolism of anthelmintics as well as their effectiveness is relevant for correcting and optimizing helminth control in animals. Therefore, the objective of this study was to evaluate the influence of fasting on the anthelminthic efficacy of closantel, albendazole and a multiple compound of ivermectin, levamisole and albendazole in sheep naturally infected with gastrointestinal nematodes.

2 MATERIAL AND METHODS

This study was conducted in two stages on a rural property in the municipality of Ribeirão Claro, in the northern region of the state of Paraná, Brazil. In the first stage, conducted in April 2017, the efficacy of an oral anthelminthic, a combination of ivermectin, albendazole and levamisole, was tested. The effect of fasting was evaluated seven and 14 days after treatment by Egg count Per Gram of feces (EPG) and the Fecal Egg Count Reduction Test (FECRT) in of young sheep (4 to 6 months old), both males and females, who were naturally infected.

In the second stage, conducted in April 2018, the oral anthelminthics closantel and albendazole were evaluated in naturally infected adult ewes seven days after treatment. In addition to counting nematode eggs per gram of feces (EPG) and the FECRT, a larval culture (fecal egg count) was performed to assess
the effect of fasting on each identified genus of nematode.

In the first stage, following randomization, the animals were divided into four experimental groups as follows: Group 1 (G1) - 15 lambs (4 to 7 months old), comprising 9 females and 7 males, with an average of 2,337 EPG, treated with an oral anthelminthic containing ivermectin (200µg/kg), levamisole (7.5mg/kg) and albendazole (5mg/kg) after a 12-hour solid food fast; Group 2 (G2) - 15 lambs (4 to 7 months old), with an average of 2,693 EPG, including 10 females and 5 males, in the control group that fasted and received 5mL of oral saline solution after a 12-hour solid food fast; Group 3 (G3) - 15 lambs (4 to 7 months old), consisting of 9 females and 7 males, with an average of 2,353 EPG, treated with an oral anthelminthic containing ivermectin (200µg/kg), levamisole (7.5mg/kg) and albendazole (5mg/kg) without fasting, meaning they were kept on pasture; and Group 4 (G4) - 15 lambs (4 to 7 months old), comprising 9 females and 7 males, with an average of 2,633 EPG, in the control group, that did not fast and received 5mL of oral saline solution.

In the subsequent phase, after randomization, the animals were divided into six experimental groups as follows: Group 1 (G1) - seven adult ewes, with an average of 1,050 EPG, treated with oral 10% closantel (10mg/kg) after a 12-hour solid food fast; Group 2 (G2) - seven adult ewes, with an average of 1,057 EPG, treated with oral 10% closantel (10mg/kg) without prior fasting; Group 3 (G3) - six adult ewes, with an average of 1,192 EPG, treated with oral 5% albendazole (5mg/kg) after a 12-hour solid food fast; Group 4 (G4) - seven adult ewes, with an average of 1,036 EPG, treated with oral 5% albendazole (5mg/kg) without prior fasting; Group 5 (G5) - six adult ewes, from the control group, untreated, received 5mL of oral saline solution and remained fasting for 12 hours; and Group 6 (G6) - seven adult ewes, from the control group, untreated, received 5mL of oral saline solution but without prior fasting.

The fasted animals were confined in a pen with free access to water, whereas the non-fasted animals were allowed to graze freely in the pasture with unrestricted access to water and natural shelter.

Fecal samples were collected before the treatments and up to 14 days after for egg counting, performed using the Gordon & Whitlock (1939) technique.
and the assessment of the fecal egg count reduction test (FECRT%). In stage 2, samples from the fecal pools of each group were subjected to coproculture, following the Roberts and O’ Sullivan (1950) methodology. The L3 larvae were recovered and identified using Keith’s criteria (1953) before the treatments (day zero) and seven days later (Day 7).

For each group and experimental days, the arithmetic means of the EPG counts were calculated. From these values, the drug’s efficacy percentage was calculated through the fecal egg count reduction test (FECRT) using the following formula (Wood et al., 1995): \[
\text{FECRT} = 1 - \left( \frac{\text{mean EPG of the treated group on day } x}{\text{mean EPG of the control group on day } x} \right) \times 100.
\] In the second stage, the efficacy of treatments on the nematode genera identified in coprocultures was calculated using the RESO program (1990).

For the statistical analyses, the mean EPGs were compared using the "Wilcoxon" and "Mann-Whitney" tests and considered significant when \( p < 0.05 \). Statistical analyses were performed using the GraphPad Prism 5.0 statistical software (GraphPad Software Inc., San Diego, CA).

2 RESULTS AND DISCUSSION

2.1 FIRST STAGE (Albendazole-Levamisole-Ivermectin combination):

Animals treated with the ILA combination (ivermectin, levamisole and albendazole) experienced a decrease in their mean EPGs, which began seven days after treatment and persisted until the 14th day. However, the mean EPG of the animals treated and subjected to prior fasting (G1) was 97.63% lower (17 EPG) than the mean of the treated animals but not subjected to fasting (G3, 717 EPG) 14 days after treatment (Figure 1, Table 1).
Figure 1. Evolution of fecal egg counts (EPG) in groups treated with the anthelminthic combination ivermectin+levamisole+albendazole with [G1(T/F)] and without [G3(T/NF)] prior fasting and in control groups (untreated) with [G2(Cont/F)] and without [G4(Cont/NF)] prior fasting.

Different letters on the same line represent significant difference (p < 0.05) between days in the same group by the "Wilcoxon" test, calculated by the GraphPad Prism program, version 5.0

Source: prepared by the authors, 2024

As expected, in untreated animals subjected (G2) or not subjected (G4) to fasting, no decrease in mean EPGs was observed (p > 0.05). However, untreated animals not receiving anthelminthic treatment but subjected to fasting (G2) showed a significant increase (p = 0.0067) in mean EPG 14 days after the start of the experiment (Figure 1). This increase could be attributed to the stress caused by keeping the animals in the pen (fasted animals), even though they were kept confined every night on the property for protection against attacks by wild felids and canids. Stress might have caused the emergence of hypobiotic trichostrongyloid larvae or increased exposure to infectious larvae in the pen, although the latter is less likely given the long prepatent period of most sheep nematodes.
Table 1. Means ± standard error of egg counts in feces (EPG) of animals in groups G1 (treated with anthelminthic and fasted), G2 (untreated control and fasted), G3 (treated with anthelminthic and not fasted) and G4 (untreated and not fasted) before treatment (day zero), seven and 14 days later

<table>
<thead>
<tr>
<th></th>
<th>Day zero</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>2,337 ± 551.5</td>
<td>7(a) ± 6.7</td>
<td>17(a) ± 9.3</td>
</tr>
<tr>
<td>G2</td>
<td>2,693 ± 623.3</td>
<td>4,270(b) ± 1,632</td>
<td>5,157(b) ± 1,460</td>
</tr>
<tr>
<td>G3</td>
<td>2,353 ± 566.5</td>
<td>197(a) ± 196.7</td>
<td>717(a) ± 633.4</td>
</tr>
<tr>
<td>G4</td>
<td>2,633 ± 634.8</td>
<td>1,733(b) ± 540.2</td>
<td>3,750(b) ± 1,278</td>
</tr>
</tbody>
</table>

Different letters represent significant difference (p <0.05) between groups in the same day (column), by the “Mann-Whitney” test, calculated by the GraphPad Prism program, version 5.0.

Source: prepared by the authors, 2024

Despite this, the group of animals treated and subjected to prior fasting (G1) exhibited a greater reduction in EPG count on days seven and 14 (RCOF = 99.84 and 99.68%, respectively) than the animals in the treated group not subjected to fasting (G3) on these same days (RCOF = 88.65 and 80.89%, respectively). It is worth noting that, despite the fact that the anthelminthic used is no longer commercially available, its recommendations (labeling) did not contain any mention of fasting as an alternative to enhance its efficiency.

A higher absorption rate of albendazole and ivermectin through oral administration after fasting in sheep has been described (Sánchez et al., 2000; Craven et al., 2002). On the other hand, levamisole, which is commonly associated with cases of intoxication in various animal species, including humans (Xu et al., 2009), is probably not recommended after fasting due to the higher risk of intoxication, as its effectiveness is more closely related to the peak plasma concentration rather than the duration of presence in the animal’s body (Fernández et al., 1998).

In cattle and sheep, the oral administration of levamisole reduces bioavailability by 42% due to degradation in the gastrointestinal tract or association with digesta. Pre-fasting can prevent this degradation, although it increases the risk of intoxication if the therapeutic dose (7.5-8mg/kg) is exceeded. Reports of levamisole intoxication are, in their entirety (Atessahin et al., 2004; Babish et al., 1990; Rather et al., 2020; Dar et al., 2020), the result of drug overdose.

In dogs, Watson et al. (1988) demonstrated that administering oral levamisole along with food reduced the drug’s bioavailability, with its absorption...
delayed and possibly reduced. However, the researchers stated that, although fasting improves effectiveness, it may increase the frequency and severity of side effects related to high levamisole concentrations in the blood.

The safety margin for levamisole is four to eight times the recommended therapeutic dose of 7.5mg/kg. For instance, studies conducted by Duarte et al. (2012) and Bastos et al. (2017) evaluated the efficacy of oral levamisole in sheep and kept the animals fasting prior to drug administration and they did not report signs of intoxication in the animals.

Following the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP), helminths are classified as resistant when the FECRT (reduction in the count of eggs per gram of feces) seven days after treatment is less than 95% and the lower limit of the 95% confidence interval is less than 90% (Coles et al., 1992). Therefore, when considering the results of FECRT as indicative of anthelmintic resistance, the helminths in this study will be deemed resistant if the test is calculated in animals without prior fasting. Conversely, they will be considered sensitive if the same test is calculated in animals subjected to fasting, as the FECRT for G3 (animals treated without prior fasting) was 94.61% and the 95% confidence interval was 83.06%.

According to Kaplan and Vidyashankar (2012), the FECRT (Fecal Egg Count Reduction Test) is an indirect phenotypic test for detecting resistance, widely accepted by regulatory agencies and the pharmaceutical industry. Variations in experimental protocols in conducting this resistance test can lead to variable results. Therefore, the development of new methods and the standardization and validation of existing ones are essential for enabling data comparison and their incorporation into routine laboratory practices (Chagas et al., 2011; Molento et al., 2013).

When the FECRT is less than 95%, it does not necessarily indicate that a particular anthelmintic is failing due to parasite resistance to the drug. The anthelmintic's efficacy depends on exposure to an adequate concentration for a sufficient duration. If this is not achieved, the efficacy will be low even if there is no resistance of the parasite to the drug (Morgan et al., 2022). Furthermore, an FECRT above 95% can lead to a false sense of security and misguided
management decisions. Therefore, it is important to understand the pharmacodynamics and pharmacokinetics of the drugs to determine the correct action of the medication (Lifschitz et al., 2017; Lanusse et al., 2018).

2.2 SECOND STAGE (Closantel and Albendazole):

There was no significant decrease in mean EPGs in any of the groups evaluated (Figure 2) and the efficacy calculated by FECRT was low for the group treated with closantel (11.9 - 77.1%) and null for the group treated with albendazole.

Figure 2 – Evolution of fecal egg counts (EPG) in animals treated with closantel (Clo) with (F) and without (NF) prior fasting (G1 and G2, respectively) or albendazole (Al) with and without prior fasting (G3 and G4, respectively) and untreated control (Cont) groups with and without prior fasting (G5 and G6, respectively)

However, even though it was not statistically significant (p > 0.05), the decrease in EPGs in animals treated with closantel after fasting was 326.8% greater than the decrease in EPGs in animals treated without prior fasting (Table 2). Since the objective of this study is not to assess the efficacy of the drugs themselves but rather the effect of fasting on this efficacy, we can assert that fasting also positively influenced the reduction of parasitic infection in the treated animals.
Table 2. Means ± standard error of egg counts in the feces (EPG) of animals in the groups treated with Closantel (G1 and G2 – “Clo”) and Albendazole (G3 and G4 – “Alb”) with (F) or without (NF) prior fasting before treatment (day zero) and seven days later and the decrease in the means of EPGs for each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day zero</th>
<th>Day 7</th>
<th>Decrease in EPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (Clo/F)</td>
<td>1050 ± 400.3</td>
<td>193 ± 73.54</td>
<td>81.61%</td>
</tr>
<tr>
<td>G2 (Clo/NF)</td>
<td>1057 ± 398.7</td>
<td>793 ± 311.2</td>
<td>24.97%</td>
</tr>
<tr>
<td>G3 (Alb/F)</td>
<td>1192 ± 395.7</td>
<td>1042 ± 480.5</td>
<td>12.58%</td>
</tr>
<tr>
<td>G4 (Alb/NF)</td>
<td>1036 ± 377.3</td>
<td>1357 ± 613.1</td>
<td>-23.65%</td>
</tr>
</tbody>
</table>

Source: prepared by the authors, 2024

The anthelmintic efficacy of closantel and albendazole, calculated using the RESO (1990) method for each nematode genus, was assessable under the genera *Haemonchus* and *Trichostrongylus* in all experimental groups (Table 3). Closantel demonstrated better anthelmintic efficacy when administered after a 12-hour fasting period under these genera. However, for *Cooperia* and *Oesophagostomum*, it was 100% effective when administered after fasting. Unfortunately, a comparison with the efficacy of the same drug without prior fasting was not possible in these cases because these nematode genera did not appear in the fecal cultures on days zero and seven (G2).

Table 3. Larvae per gram of feces (LPG) of *Cooperia, Haemonchus, Trichostrongylus* and *Oesophagostomum* on days zero (D0) and seven (D7) and the efficacy (Ef%) of anthelmintics closantel with and without prior fasting (G1 and G2) and albendazole with and without prior fasting (G3 and G4)

<table>
<thead>
<tr>
<th>Cooperia</th>
<th>Haemonchus</th>
<th>Trichostrongylus</th>
<th>Oesophagostomum</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>D7</td>
<td>Ef%</td>
<td>D0</td>
</tr>
<tr>
<td>G1</td>
<td>11</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>G2</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>G3</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>G4</td>
<td>41</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

* Negative values represent an increase in larval count after treatment (D7)  
** Ef%: Efficacy calculated by the RESO Program (1990).

Source: prepared by the authors, 2024

The null efficacy of albendazole (FECRT = zero), coupled with a 100% reduction in *Trichostrongylus* and *Cooperia* larvae (G3 and G4) with only *Haemonchus* remaining, likely indicates resistance of *Haemonchus* to this anthelmintic. Furthermore, despite the low efficacy of albendazole when administered after fasting (G3), as evidenced by only a 6.0% reduction in LPG
(larvae per gram of feces) under *Haemonchus*, there was a 43.9% increase in LPG of this nematode in animals treated without prior fasting (G4) (Table 3).

The anthelminthic activity of benzimidazoles relies on the prolonged presence of drug concentrations at the site where the parasite resides (Lacey, 1990). The extended period of exposure and increased availability of the medication are correlated with enhanced clinical efficacy (Prichard *et al*., 1978).

It is well known that the rumen can influence the absorption pattern and pharmacokinetic behavior of benzimidazole anthelminthics when administered via the enteral route. The large volume of the rumen and the extended retention time of ingesta help drug absorption by slowing the drug’s passage through the gastrointestinal tract. The ruminal pH and the degree of ionization of a compound determine the absorption rate through the epithelium, which is permeable only to the lipophilic, non-ionic form of a drug (Hogben *et al*., 1959; Iseki *et al*., 1992; Avdeef *et al*., 1998).

Therefore, a delay in gastrointestinal transit time induced by fasting increases the availability of albendazole and its metabolites in the plasma and abomasal fluid in cattle. Sánchez *et al*. (2000) concluded that the increase in concentration profiles of the active drug, measured in the tissues where parasites reside, provides strong scientific evidence to recommend fasting as a method for optimizing parasite control in cattle. In sheep, Alí and Hennessy (1995), Hennessy *et al*. (1995) and Lifschitz *et al*. (1997) demonstrated that reduced food consumption resulted in increased plasma availability of oxfendazole and albendazole.

Fasting also contributed to the efficacy of closantel under the genus *Trichostrongylus*. While there was a 100% reduction in LPG count in G1, there was an 8.8% increase in these larvae in G2 (Table 3).

Reduced food consumption for 24 hours decreases digesta flow and prolongs the residence time of anthelmintics like albendazole (Hennessy & Alí, 1995), oxfendazole (Alí & Hennessy, 1995) and ivermectin (Alí & Hennessy, 1996). Closantel is poorly absorbed, with nearly 50% of the dose excreted in feces within 36 hours of oral administration (Michiels *et al*., 1987). Therefore, decreased food intake also increases the residence time of closantel in the
gastrointestinal tract, enhancing the quantitative absorption of the drug. The duration of closantel at the absorption site is one of the main determinants of its systemic availability (Morgan et al., 2022).

In sheep receiving 800g of food daily, about 50% of the administered closantel was recovered in the feces 50 hours after administration. In contrast, sheep fed half of this ration took approximately 90 hours for 50% of the dose to appear in the feces. This indicates that sheep with reduced digesta flow in the gastrointestinal tract, i.e., those consuming less food, experienced increased closantel absorption, resulting in higher plasma drug concentrations (Hennessy & Ali, 1997).

In our study, we observed that fasting had a positive influence on the reduction of *Haemonchus, Cooperia, Trichostrongylus* and *Oesophagostomum* in animals treated with closantel. Hennessy and Ali (1997) also demonstrated that reducing food intake before the administration of closantel extended the period of protection against *Haemonchus* larvae due to increased drug absorption and plasma concentration.

### 3 CONCLUSIONS

This study aimed to assess whether or not fasting would enhance the effect of the anthelmintics closantel, albendazole and a combination of albendazole-levamisole-ivermectin. In this way, we concluded that fasting the animals before administering the anthelmintics increases the effectiveness of the drugs, even though they have not achieved satisfactory anthelmintic potential for the sheep species as conventionally determined by research bodies and scholars on the subject.

Despite the parasites’ resistance to the drugs tested (in the flock evaluated) as determined by the Fecal Egg Count Reduction Test (FECRT), it is advisable to suspend feeding for at least 12 hours before administering anthelmintics with the active ingredients closantel, albendazole, ivermectin and levamisole (orally) when they have shown efficacy in the flock.
Although several studies have already reported parasitic resistance to these anthelminthics, veterinarians and producers must be aware of the importance of fasting in potentiating these drugs.

In addition, using FECRT test results to indicate parasite resistance without taking into account the correct use of anthelminthics (the possibility of prior fasting, for example), may represent a mistake or underestimation of the potential of these drugs.

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