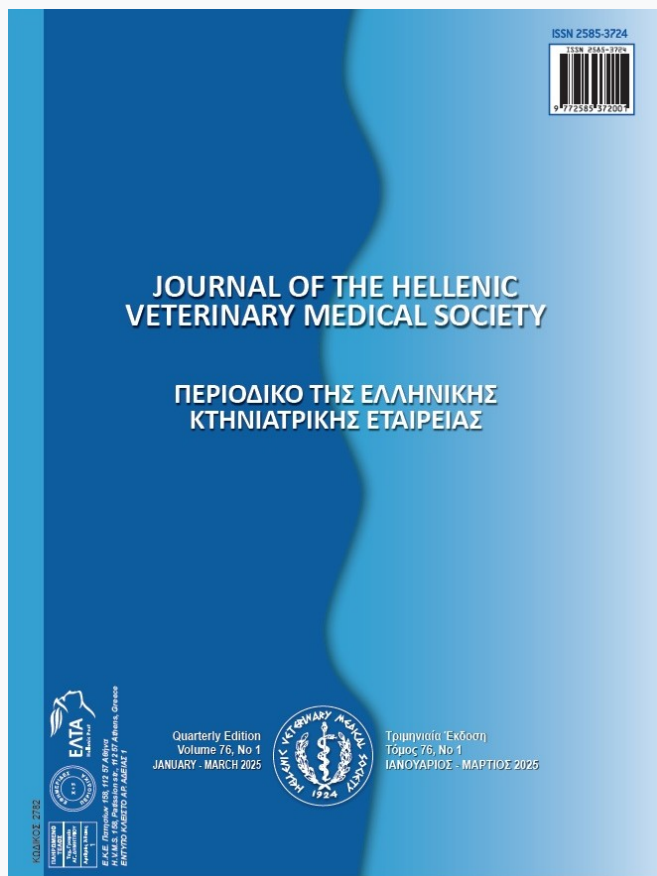


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**Fasciolicidal efficacy comparison between Amaro (*Chuquiraga weberbaueri*) and triclabendazole in calves experimentally infected with *Fasciola hepatica* metacercaria**

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## Fasciolicidal efficacy comparison between Amaro (*Chuquiraga weberbaueri*) and triclabendazole in calves experimentally infected with *Fasciola hepatica* metacercaria

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**ABSTRACT:** The Cajamarca valley is an endemic area for cattle affected by *Fasciola hepatica*. Despite demonstrated resistance to triclabendazole, the use of this anthelmintic remains prevalent. Therefore, constant efficacy evaluations of this active ingredient and exploring alternatives for parasite control are necessary. This study aimed to determine the effectiveness of Amaro (*Chuquiraga weberbaueri*) and triclabendazole in controlling *F. hepatica* in experimentally infected calves. Three groups (T0, T1, and T2) of four weaned Holstein calves each were formed and infected with 200 metacercariae. The control group (T0) received no antiparasitic treatment, group T1 was dosed with lyophilized aqueous extract of *C. weberbaueri* (200 mg.kg<sup>-1</sup>, orally, for four consecutive days), and group T2 received a single dose of triclabendazole (12 mg.kg<sup>-1</sup>, orally). Clinical efficacy was assessed using the Egg Count Reduction Test, and absolute efficacy was determined by adult parasite counts at necropsy. The mean clinical efficacy of *C. weberbaueri* was 46.70% (95% CI: 33.27 - 60.13) and of triclabendazole 25.00 (95% CI: 12.62 - 37.38) ( $p=0.029$ ). The efficacies increased in the count of adult parasites at necropsy but were not statistically different ( $p=0.686$ ), *C. weberbaueri* showed an efficacy of 58.63% (95% CI: 45.24 - 72.02), and triclabendazole 66.22% (95% CI: 53.36 - 79.08). Both treatments proved insufficiently active against *F. hepatica* in calves, falling short of the category of effective fasciolicides. Nevertheless, using *C. weberbaueri* could be considered an environmentally friendly option to reduce the parasite burden in cattle.

**Keywords:** efficacy; ethnopharmacology; liver fluke; parasite control; parasite resistance

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## INTRODUCTION

The helminth *Fasciola hepatica*, a parasite widely distributed worldwide among various animal species, has been associated with considerable diseases and production losses (Beesley et al., 2018). Infection by this trematode leads to significant economic and production losses, primarily due to the condemnation of infected livers and weight loss in dairy cattle (Mp-isana et al., 2022). This issue arises because *F. hepatica* has developed advanced mechanisms to deceive, evade, and alter the host's immune response. The infection by *F. hepatica* itself represents a source of oxidative stress affecting slaughtered animals, resulting in biochemical and metabolic alterations in the early postmortem period (Nasreldin and Zaki, 2020).

Parasite control in domestic animals has relied primarily on chemical drugs for many years. However, this practice poses challenges, as residues of these products can accumulate in tissues, milk, meat, and other animal-derived by-products intended for human consumption, posing an imminent risk to public health (Rana et al., 2019). Additionally, the disposal of chemical residues into the environment through feces and urine raises additional concerns. Since the soil serves as the ultimate destination for residues of chemical anthelmintics, these compounds can pose an environmental threat, being toxic to beneficial soil organisms, thereby disrupting local microflora (Goode-nough et al., 2019; Villar and Schaeffer, 2022).

On the other hand, the extensive use of anthelmintic medications has led to a concerning and dramatic level of resistance to these drugs in various parts of the world, affecting nearly all animal species and demonstrating resistance to different anthelmintic groups across several continents. Factors such as frequent administration, insufficient doses, parasite genetics, and the selection and timing of mass treatment have contributed to the emergence of anthelmintic resistance (Fissiha and Kinde, 2021).

Given the harmful effects of chemical antiparasitic, there has been a shift towards more environmentally friendly alternatives, such as natural products, including bioactive plants. Ethnoveterinary medicine, practiced since historical times worldwide, has recently resurged as an alternative for controlling parasites or countering anthelmintic resistance (Githiori et al., 2005; Nyahangare et al., 2015; Benlarbi et al., 2023). In Peru, it has been identified that the Amaro plant (*Chusquea weberbaueri*) is used for the treatment of animal fasciolosis, with ovicidal activity attributed

to phenolic components such as chlorogenic acid and ethyl caffeate, as well as the presence of flavonoids, saponins, and lactones (Ortiz et al., 2023).

Given the global significance of the challenge posed by anthelmintic resistance, it is crucial to implement proper use of existing anthelmintics and reduce dependence on these drugs to mitigate the risk of resistance (Fissiha and Kinde, 2021). Cajamarca is an endemic region for *F. hepatica*, a parasite that has been reported in various domestic animals, including guinea pigs, cattle, and horses, among others (Torrel et al., 2022; Torrel et al., 2023; Rázuri et al., 2023). Furthermore, resistance of *F. hepatica* to triclabendazole has been confirmed in this area (Ortiz et al., 2013).

The present study determined the efficacy of a native Andean plant, Amaro (*C. weberbaueri*), and triclabendazole in controlling *F. hepatica* in experimentally infected calves. Despite the well-documented resistance of *F. hepatica* to triclabendazole, this anthelmintic was selected due to its widespread use in local livestock practices. The study aims to raise awareness, discourage its continued use, and explore alternative methods for controlling fasciolosis in cattle from the Cajamarca region by highlighting the need for further research on this anthelmintic.

## MATERIALS AND METHODS

### Place and experimental design

The research was conducted in the Cajamarca valley at the facilities of the Facultad de Ciencias Veterinarias (FCV), National University of Cajamarca (UNC), Peru. In the cattle pens of the FCV livestock farm, 12 Holstein calves were selected and individually identified with ear tags after being weaned at three months of age.

Despite being exclusively fed with milk for the first three months, fecal samples were collected in the mornings by anal sphincter stimulation (approximately 100 g) and processed using the Natural Sedimentation Technique (Dennis et al., 1954) at the Laboratorio de Parasitología Veterinaria y Enfermedades Parasitarias of the UNC. This was done to ensure the absence of *F. hepatica* eggs in the fecal samples.

After confirming the absence of infection, the calves were infected with 200 metacercariae per animal on day 100 of life. The metacercariae, produced at the Laboratorio de Inmunología of the UNC, were administered orally in gelatin capsules using anatom-

ical forceps. A period of 77 days was allowed after infection to permit the development of larval forms to the adult stage in the bile ducts and the production and excretion of eggs.

After the completion of 77 days post-infection, new fecal samples were collected in the morning and processed similarly to the initial sampling. The egg count per gram of feces facilitated the formation of three homogeneous groups based on parasitic load and the number of animals: the control group (T0), the Amaro group (T1), and the triclabendazole group (T2).

On day 78 post-infection, group T1 received a lyophilized aqueous extract of *Chuquiraga weberbaueri* in capsules at 200 mg.kg<sup>-1</sup> of body weight, for four consecutive days. Since 200 mg of plant capsules were formulated, one capsule was administered for each kg of body weight. These were placed with the hand behind the tongue roll and the mandible was slightly raised to ensure swallowing. Group T2 received a single dose of triclabendazole at a rate of 12 mg.kg<sup>-1</sup> of body weight, orally. The body weight of each calf was calculated using a bovine measuring tape, and the dosage per animal was determined by multiplying the therapeutic dose of the anthelmintic by the weight in kg, divided by the product concentration.

The metacercaria were produced through the breeding and infection of *Lymnaea* snails. Initially, eggs of *F. hepatica* were obtained from the bile ducts of cattle processed in the slaughterhouse. These eggs were incubated for two weeks at 28°C, after which hatching was stimulated using light at 2000 lumens. Each snail, aged 2 to 3 months, was infected with 2 to 3 miracidia in wells of recycled ELISA plates and maintained under controlled breeding conditions for 1.5 to 2 months. Subsequently, the snails were placed in polyethylene bags with water and exposed to sunlight and thermal shocks (refrigeration and incandescent light) 2 to 4 times per day. Following this process, the metacercaria encysted on the walls of the bags were counted under a stereoscope and finally placed into empty gelatin capsules.

### Amaro tablets production

Fresh Amaro plants were dried for 24 hours at 45°C in an oven, then de-leaved and crushed. One hundred grams of the resulting powder were weighed, placed in a 4 L flask with 2 L of distilled water, and brought to a boil for 10 minutes. Subsequently, it was

allowed to cool and filtered through gauze and filter paper in a Büchner funnel under vacuum to remove solids. The filtrate was vacuum-concentrated in a rotary evaporator until it reached one-fourth of its initial volume. The concentrated extract was transferred to a lyophilization flask, where it underwent a cooling bath at 42°C for 15-20 minutes to impregnate and solidify the extract. Finally, it was placed in an ultra-freezer at -80°C for 72 hours and then lyophilized at 66 mTorr at -80°C for 48 hours.

Amaro (200 mg) capsules were produced in a controlled environment at 24°C and 50% humidity. Gelatin capsules (00) were used, and their average empty weight was determined from 20 capsules. Encapsulation was performed using a semi-automatic double-zero capsule filler, incorporating 50 empty capsules with the larger part facing downward. The weighed sample was added and uniformly distributed into the capsules with a spatula. The upper part of the capsule filler was attached, joining both parts of the capsules. Twenty filled capsules were weighed to obtain the average weight, which was compared with the average weight of the empty capsules. The capsules were stored in a plastic bag to prevent degradation and kept in a tightly sealed high-density polypropylene container.

### Dosage controls

Fecal samples were collected directly from the rectum, approximately 100 g each, by stimulating the anal sphincter on four occasions with eight-day intervals until day 32. These collections aimed to conduct egg count per gram of feces.

The decision was made to end the egg per gram of feces (EPG) count on day 32 because during this period *F. hepatica* is in the early immature stage (1 to 4 weeks). It does not mature or produce eggs within the first 30 days (Wood et al., 1995).

### Clinical efficacy estimate

Clinical efficacy (% C.E.) was determined by calculating the percentage reduction in *F. hepatica* EPG counts using the following formula (Young et al., 1999).

$$\% C.E. = \frac{\text{Average EPG Day 0} - \text{Average EPG Day 8-32}}{\text{Average EPG Day 0}} \times 100$$

Following the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP), the efficacy percentage in each group was

classified according to the following ranges: highly effective (>98%), effective (90 - 98%), moderately effective (80 - 89%), and insufficiently active (<80%) (Wood et al., 1995).

### Absolute efficacy estimate

To calculate absolute efficacy through observation of adult *F. hepatica* in the liver, the 12 calves were transported to the municipal abattoir in Cajamarca for slaughter on day 34 post-treatment. During postmortem examination, livers were selected, and longitudinal cuts were made through the bile ducts for identification, recovery, and counting of adult *F. hepatica*. The percentage of absolute efficacy (E) was calculated using the geometric means (GM) of the count of adult flukes in the bile ducts, comparing the control group and the treatment group, following the guidelines of WAAVP (Wood et al., 1995).

$$\% E = \frac{GM F. hepatica control group - GM F. hepatica treated group}{GM F. hepatica control group} \times 100$$

### Statistical analysis

The collected data were organized using Microsoft Excel. Averages of EPG and corresponding standard deviations were calculated. Additionally, efficacies

along with their 95% confidence intervals were determined.

The EPG values and the number of adult *F. hepatica* (NAP) were subjected to the Shapiro-Wilk normality test. Since not all values conformed to the assumptions of normality or variance were high, these were analyzed using the Kruskal-Wallis test to identify potential statistical differences in mean EPG or NAP among the three groups and across different days. The comparison of clinical and absolute efficacies between treatment groups was performed using the Mann-Whitney U test, a non-parametric method suitable for data that do not follow a normal distribution. All analyses were conducted using IBM SPSS Statistics version 27.0.1 and a significance level of  $p < 0.05$  was considered.

## RESULTS

The EPG decreased in both groups, T1 (*C. weberbaueri*) and T2 (triclabendazole), compared to T0 (Control Group). However, no statistical differences were observed between groups or days (Table 1). Similarly, efficacies between both treatment groups did not show statistical differences in days, except for

**Table 1.** Fecal count egg per gram of feces (EPG) in groups of calves experimentally infected with *Fasciola hepatica*

Day	Count	T0: Control	T1: 200 mg.kg <sup>-1</sup> × 4 d <i>C. weberbaueri</i>	T2: 12 mg.kg <sup>-1</sup> Triclabendazole	<i>p</i> -value <sup>a</sup>
Day 0	EPG $\bar{x} \pm SD$	9.00 ± 2.94	13.25 ± 0.96	11.75 ± 2.87	0.094
Day 8		9.25 ± 2.75	7.75 ± 2.87	8.75 ± 4.79	0.734
Day 16		8.25 ± 0.96	6.75 ± 2.06	9.25 ± 2.06	0.258
Day 24	EPG $\bar{x} \pm SD$	6.25 ± 0.96	7.00 ± 1.15	8.25 ± 0.96	0.082
Day 32		8.5 ± 0.58	6.75 ± 2.06	9.00 ± 1.41	0.300
<i>p</i> -value <sup>a</sup>		0.379	0.070	0.057	

SD: Standard deviation

<sup>a</sup>No statistical difference between rows and or columns ( $p > 0.05$ , Kruskal-Wallis test)

**Table 2.** Clinical efficacy compared between *Chuquiraga weberbaueri* extract and triclabendazole in experimentally infected calves with *Fasciola hepatica*

Days	% Efficacy (95% CI)		<i>p</i> -value	Status
	T1: 200 mg.kg <sup>-1</sup> × 4 d <i>C. weberbaueri</i>	T2: 12 mg.kg <sup>-1</sup> Triclabendazole		
Day 8	41.51 (28.24 - 54.78)	25.53 (13.06 - 38.00)	0.886	IA
Day 16	49.06 (35.60 - 62.52)	21.28 (9.58 - 32.98)	0.200	IA
Day 24	47.17 (33.73 - 60.61)	29.79 (16.71 - 42.87)	0.200	IA
Day 32	49.06 (35.60 - 62.52)	23.40 (11.30 - 35.50)	0.200	IA
Average	46.70 (33.27 - 60.13)	25.00 (12.62 - 37.38)	0.029*	IA

CI: Confidence interval

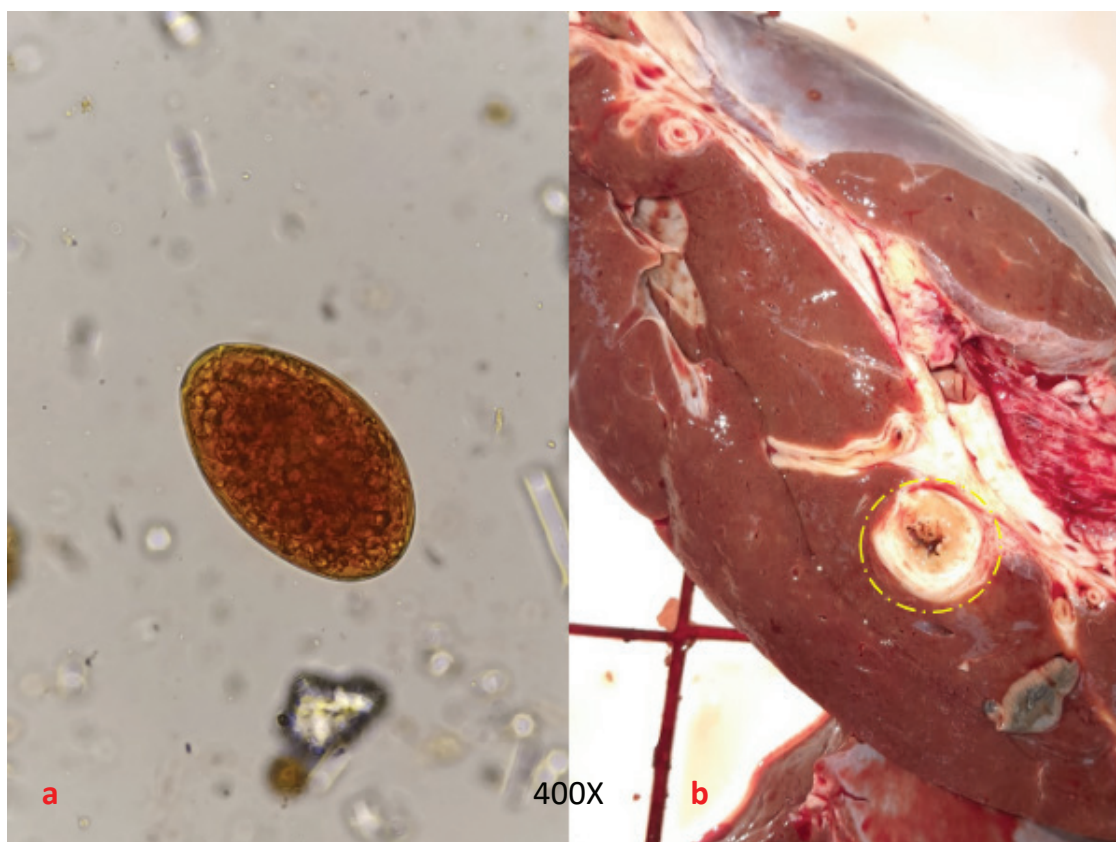
\*Statistical difference between both treatment groups ( $p < 0.05$ , Mann-Whitney U test)

IA: Insufficiently active

the general efficacy ( $p < 0.05$ ) (Table 2).

During the necropsy, the fecal egg count and the number of adult *F. hepatica* parasites in the bile ducts of the calves were recorded (Figure 1). The highest number of adult parasites (NAP) was observed in the

Control Group, followed by T1, and finally T2, although these differences were not statistically significant ( $p > 0.05$ ) (Table 3). Furthermore, the percentage reduction of NAP (efficacy) between T1 and T2 also did not show a significant difference ( $p > 0.05$ ) (Table 4).



**Figure 1.** Egg observed in Natural Sedimentation (a) and adult *Fasciola hepatica* parasite in the bile ducts of the calves (b). Calcification of the bile ducts is observed (yellow circle)

**Table 3.** Count of the number of adult parasites (NAP) in groups of calves experimentally infected with *Fasciola hepatica* at postmortem

Group	Calve	NAP Count	Mean <sup>(G)</sup> NAP ± SD	<i>p-value</i> *
T0: Control	I	4	8.85 ± 13.22	0.204
	II	12		
	III	4		
	IV	32		
T1: 200 mg.kg <sup>-1</sup> ×4 d <i>C. weberbaueri</i>	I	3	3.66 ± 0.96	
	II	4		
	III	5		
	IV	3		
T2: 12 mg.kg <sup>-1</sup> Triclabendazole	I	2	2.99 ± 1.50	
	II	2		
	III	5		
	IV	4		

(G) Geometric mean

SD: Standard deviation

\*No statistical difference between groups ( $p > 0.05$ , Kruskal-Wallis test)

**Table 4.** Comparative absolute efficacy of *Chuquiraga weberbaueri* extract and triclabendazole in calves experimentally infected with *Fasciola hepatica*

Group	% Efficacy (95% CI)	<i>p</i> -value*	Status
T1: 200 mg.kg <sup>-1</sup> ×4 d <i>C. weberbaueri</i>	58.63 (45.24 - 72.02)	0.686	IA
T2: 12 mg.kg <sup>-1</sup> Triclabendazole	66.22 (53.36 - 79.08)		IA

CI: Confidential interval

\*No statistical difference between groups ( $p > 0.05$ , Mann-Whitney U test)

IA: Insufficiently active

## DISCUSSION

The egg count in the control and treated group with *C. weberbaueri* and triclabendazole did not reveal any statistical differences ( $p > 0.05$ ). Similarly, the count of adult *F. hepatica* during necropsy was comparable across the three groups ( $p = 0.204$ ).

The plant *C. weberbaueri* has been evaluated in sheep infected with *F. hepatica* at a dose of 100 mg.kg<sup>-1</sup> over five consecutive days using an aqueous extract, achieving an absolute efficacy of 51.79% (Ortiz et al., 2023). In this study, the absolute efficacy at a double dose (200 mg.kg<sup>-1</sup>) was slightly higher (58.63%), indicating that the plant is more effective at higher doses, as the NAP was lower (min. 3 and max. 5) compared to the findings of Ortiz et al. (2023), who reported NAPs ranging from 20 to 80. The same authors suggest that the fasciolicidal activity of the plant is due to its phenolic compounds, such as chlorogenic acid, ethyl caffeate, flavonoids, saponins, and lactones.

It is important to note that no clinical signs or symptoms of toxicity were observed at the administered dose. However, further toxicity studies at different concentrations of the plant are necessary to confirm these findings and determine a safe dosage range for cattle. At a dose of 100 mg.kg<sup>-1</sup>, liver enzymes and hepatic markers in sheep did not indicate any significant toxicity of the aqueous extract after the third week of treatment (Ortiz et al., 2023).

On the other hand, in local cattle farming in Cajamarca, triclabendazole has been reported as ineffective for several years. A study with a clinical trial and an *in vivo* efficacy test in sheep showed an efficacy of 25.2% against *F. hepatica* isolated from dairy cattle in Cajamarca, Peru (Ortiz et al., 2013). In a recent study, efficacy ranging from  $13.36 \pm 4.30$  to  $62.24 \pm 9.60\%$  was found in fasciolosis control in naturally infected

dairy cattle (Rojas-Moncada et al., 2024).

Triclabendazole has been reported as insufficiently active in other regions of Peru, particularly in dairy cattle from the Mantaro valley for example (Chávez et al., 2012; Zárate-Rendón et al., 2023). In various countries around the world, *F. hepatica* has been reported as resistant to triclabendazole due to its prolonged use in combating fasciolosis in both animals and humans (Cabada et al., 2016; McMahon et al., 2016; Ramadan et al., 2019).

In light of the results of the present study, measures recommended by various authors need to be implemented. The proper use of anthelmintic drugs and the exploration of alternative approaches are essential strategies to curb the development of anthelmintic resistance. As anthelmintic resistance poses a serious challenge worldwide, the proper use of existing anthelmintics and reducing dependence on these drugs should be implemented to address this challenge (Fisaha and Kinde, 2021).

Although *C. weberbaueri* was insufficiently active (efficacy <80), its use could be beneficial in reducing the parasitic loads of *F. hepatica* in dairy cows or animals with parasites resistant to multiple drugs. On one hand, it represents a more environmentally friendly alternative because chemical anthelmintics alter soil microorganisms (Goodenough et al., 2019; Villar and Schaeffer, 2022). On the other hand, it would provide greater safety for humans as chemical drugs can accumulate in the animal's body and derive products for consumption (Rana et al., 2019).

## CONCLUSION

The clinical efficacy of the *C. weberbaueri* extract was superior to that of triclabendazole; however, in terms of absolute efficacy, triclabendazole exhibited a slightly better performance, although without reach-

ing statistical differences. Both anthelmintics proved insufficiently active as they did not significantly reduce the presence of eggs or adult parasites of *F. hepatica* in the calves, suggesting they cannot be considered effective fasciolicides. Despite these results, *C. weberbaueri* could be useful as an ecological alter-

native to reduce the parasitic load in dairy cattle and obtain products free from synthetic drugs.

## CONFLICT OF INTEREST

None declared.

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