

# Efficacy of Contoya plant (Lobelia decurrens Cav.) to control coccidiosis in calves

Eficacia de la planta Contoya (Lobelia decurrens Cav.) para controlar la coccidiosis en terneros

Eficácia do Lobelia decurrens Cav. no controle de coccidiose em bezerras

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#### **Abstract**

Background: Cattle are susceptible to infections by parasitic protozoa, which often require chemical treatments. However, using these products can contaminate the soil and negatively affect ecosystems (flora, fauna and microbiota), affecting the food chain and safety by leaving residues in milk and meat and posing risks to consumers. Therefore, it is crucial to seek sustainable alternatives, such as using bioactive plants and their extracts. **Objective:** To evaluate the efficacy of contova plant (Lobelia decurrens Cav.) at single doses of 1 g.kg-1 and 2 g.kg<sup>-1</sup> of body weight for controlling coccidiosis in naturally infected calves under extensive rearing conditions. **Methods:** Groups of calves with similar parasitic loads (ANOVA p>0.05) were formed and orally administered pulverized leaves and flowers of contova plant. Efficacy was calculated as the percentage reduction of oocysts per gram of feces using a McMaster chamber. Results: The 2 g.kg<sup>-1</sup> dose significantly reduced the parasitic load compared to the 1 g.kg<sup>-1</sup> dose (p<0.05). With a dose of 1 g.kg<sup>-1</sup>, a minimum efficacy of 38.04% was observed on the third day and a maximum efficacy of 86.93% on the fifteenth day. With the 2 g.kg<sup>-1</sup> dose, a minimum efficacy of 73.91% was achieved on the third day and a maximum efficacy of 95.45% on the fifteenth day. Conclusion: Our results indicate that contoya at a dose of 2 g.kg<sup>-1</sup> body weight can control coccidiosis in calves. This could be advantageous for cattle producers, providing a superior alternative to conventional drugs in terms of costs, environmental impact, as well as food safety and quality.

**Keywords:** alternative control; bioactive plant; biocontrol; calves; cattle; <u>Eimeria</u> spp.; parasitic control; parasite; phytochemical; therapeutic dose.

#### Resumen

Antecedentes: El ganado bovino es susceptible a infecciones por protozoarios parásitos, que a menudo requieren tratamientos

químicos. Sin embargo, el uso de estos productos puede contaminar el suelo y afectar negativamente la flora y fauna, además de dejar residuos en la leche y la carne, representando un riesgo para los consumidores. Por lo tanto, es crucial buscar alternativas menos dañinas para el ambiente, como el uso de plantas bioactivas. **Objetivo:** Evaluar la eficacia del pulverizado de la planta contoya (*Lobelia decurrens* Cav.) en dosis única de 1 g.kg<sup>-1</sup> y 2 g.kg<sup>-1</sup> de peso vivo para el control de la coccidiosis en terneras infectadas naturalmente en condiciones de cría extensiva. **Métodos:** Se formaron grupos de terneras con cargas parasitarias similares (ANOVA, p>0.05) a las que se les administró vía oral el pulverizado de hojas y flores de contoya. La eficacia se calculó como el porcentaje de reducción de ooquistes por gramo de heces utilizando una cámara McMaster. **Resultados:** La dosis de 2 g.kg<sup>-1</sup> mostró un efecto significativamente mayor en la reducción de la carga parasitaria en comparación con la dosis de 1 g.kg<sup>-1</sup> (p<0.05). Con una dosis de 1 g.kg<sup>-1</sup> se observó una eficacia mínima del 38.04% al tercer día y una eficacia máxima del 86.93% al día quince. Con la dosis de 2 g.kg<sup>-1</sup>, se alcanzó una eficacia mínima del 73.91% al tercer día y una eficacia máxima del 95.45% al día quince. **Conclusión:** Estos resultados indican que la contoya, a dosis de 2 g.kg<sup>-1</sup> p.v., puede controlar la coccidiosis en terneras. Esto podría ser ventajoso para los productores de ganado, proporcionando una alternativa superior a los fármacos convencionales en términos de costo, impacto ambiental, sanidad y calidad de los alimentos.

**Palabras clave:** biocontrol; control alternativo; control parasitario; dosis terapéutica; <u>Eimeria</u> spp.; fitoquímico; ganado; parásito; planta bioactiva; terneras.

## Resumo

Antecedentes: O gado bovino é suscetível a infecções por protozoários parasitários, cujo tratamento geralmente envolve o uso de produtos químicos. No entanto, a utilização desses produtos pode contaminar o solo e afetar negativamente a flora e a fauna, além de deixar resíduos no leite e na carne, representando um risco para os consumidores. Portanto, torna-se fundamental buscar alternativas menos nocivas ao meio ambiente, como o uso de plantas bioativas. **Objetivo:** Avaliar a eficácia da planta contova (Lobelia decurrens Cav.) pulverizada, em doses únicas de 1 g.kg<sup>-1</sup> e 2 g.kg<sup>-1</sup> de peso corporal, no controle da coccidiose em novilhas naturalmente infectadas, criadas em sistema extensivo. Métodos: Foram formados grupos de novilhas com cargas parasitárias semelhantes (ANOVA p>0,05) e foram administradas oralmente com as folhas e flores pulverizadas da planta contoya. A eficácia foi calculada como a porcentagem de redução de oocistos por grama de fezes, utilizando a câmara de McMaster. **Resultados:** A dose de 2 g.kg<sup>-1</sup> reduziu significativamente a carga parasitária em comparação com a dose de 1 g.kg<sup>-1</sup> (p<0,05). Com a dose de 1 g.kg<sup>-1</sup>, observou-se uma eficácia mínima de 38,04% no terceiro dia e uma eficácia máxima de 86,93% no décimo quinto dia. Com a dose de 2 g.kg<sup>-1</sup>, atingiu-se uma eficácia mínima de 73,91% no terceiro dia e uma eficácia máxima de 95,45% no décimo quinto dia. Conclusões: Esses resultados indicam que a contoya na dose de 2 g.kg<sup>-1</sup> p.c. pode ser uma opção para o controle da coccidiose em novilhas. Isso poderia ser vantajoso para produtores de gado, pois oferece uma alternativa superior aos medicamentos convencionais em termos de custo e impacto ambiental.

Palavras-chave: bezerros; biocontrole; controle alternativo; controle parasitário; dose terapêutica; <u>Eimeria</u> spp.; fitoquímico; gado; parasita; planta bioativa.

## Introduction

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Coccidiosis, a common disease in ruminants worldwide, generates significant economic losses to the livestock industry. It is caused by host-specific species of *Eimeria* (Keeton and Navarre, 2018; Bangoura and Bardsley, 2020). Although not all *Eimeria* species are equally pathogenic, young animals are the most affected, as the infection disrupts the digestive process and homeostasis.

This manifests through diarrhea, subclinical production losses, and clinical disease, leading to reduced growth rates and occasional deaths (Daugschies and Najdrowski, 2005; Keeton and Navarre, 2018).

For many years, parasite control in animals has primarily relied on chemical drugs. However, this practice poses challenges, as drug residues can accumulate in tissues and products intended

for human consumption, posing a risk to public health (Rana et al., 2019). Furthermore, since soil is the ultimate repository for antiparasitic residues, these components may represent an environmental threat due to their toxicity to beneficial soil organisms (Villar and Schaeffer, 2022).

Effective control of coccidiosis largely depends on management measures and use of anticoccidial chemical drugs, such as toltrazuril (Keeton and Navarre, 2018; Bangoura and Bardsley, 2020). Although several studies have demonstrated high efficacy rates of toltrazuril in calves (Jonsson et al., 2011), other researchers have shown variable efficacy over time (Philippe et al., 2014; Zechner et al., 2015; Beltrán et al., 2022). Resistance of *Eimeria* spp. to toltrazuril has been confirmed in lambs (Odden et al., 2018). Drug resistance should be considered a potential threat in the treatment of coccidiosis (Bangoura and Bardsley, 2020).

In environments with high parasitic presence and resistance, finding alternative control methods is urgently needed to prevent the spread of parasitic resistance. These alternatives should minimize the need for pharmaceutical interventions and optimize animal production (Burke and Miller, 2020). In this context, several plants have emerged as a safe alternative for consumers and the environment. Bioactive components in plants have shown promising results for controlling various animal parasites (Worku et al., 2009; Silva et al., 2014). Several phytochemicals have been reported to optimize production by promoting growth and improving animal health (Lillehoj et al., 2018). Among these plants, Lobelia decurrens Cav. contains a series of bioactive components such as flavonoids, steroids, alkaloids, tannins, quinones, and saponins, which have shown activity against Eimeria spp. in rabbits (Reyna-Cotrina et al., 2024).

Given that *Eimeria* infection affects productive performance in ruminants and may even result in fatal outcomes for younger cattle (Daugschies and Najdrowski, 2005; Keeton and Navarre,

2018), and the potential of *Lobelia decurrens* Cav. to control coccidiosis in rabbits (Reyna-Cotrina et al., 2024), research in cattle is warranted. Therefore, this study aimed to evaluate the efficacy of powdered *Lobelia decurrens* Cav. (contoya) leaves for controlling coccidiosis in naturally infected and extensively raised calves.

#### Materials and methods

## Ethical considerations

The study complied with the Animal Protection and welfare Law of Peru (Law No. 30407). The animals used in the study were granted the five freedoms of animal welfare.

## Location

The study was conducted on two farms within the Cajamarca region. Farm I was in the hamlet of Nitisuyo, district and province of San Miguel. Farm II was in the Huacariz San Antonio sector, situated in the district and province of Cajamarca, at a linear distance of 71 kilometers from Farm I.

Both farms are situated at elevations between 2700 and 2900 masl, sharing a similar climate and extensive cattle-rearing conditions. The cattle grazed on pastures predominantly composed of ryegrass (*Lolium multiflorum*) and clover (*Trifolium repens*).

## Selection of animals and formation of groups

Initially, coprological analysis was performed on all Creole calves from Farm I (N = 40), aged between 3 and 6 months (5  $\pm$  1.05), with a live weight range of 51 to 150 kg (108  $\pm$  27.21). From this group, 21 calves with a parasitic load equal to or greater than 500 oocysts per gram of feces were selected. These 21 calves were distributed into three groups of 7 individuals each, maintaining homogeneity in parasitic load (ANOVA, p>0.05).

On Farm II, following the same criteria and conditions, 40 Holstein calves were analyzed, of which 18 that met the established criteria were selected. These calves had an age range of 3 to 6 months  $(5.06 \pm 1.00)$  and live weights ranging

from 51 to 140 kg ( $110.11 \pm 22.08$ ). These 18 calves were divided into three groups of 6 individuals each and were assigned identifiers using ear tags.

The control and treatment groups were randomly assigned by two laboratory researchers who did not participate in any field activities. Fieldwork was conducted by two additional researchers who were not involved in laboratory sample processing. Another researcher served as an observer throughout all stages of the study.

The World Association for the Advancement of Veterinary Parasitology indicates that groups of at least 6 individuals should be formed to evaluate the clinical efficacy of antiparasitic agents in cattle with natural parasite infection under field conditions (Wood et al., 1995). On Farm I, groups of 7 calves were formed to avoid wasting the number of animals that met the selection criterion for parasitic load.

## Contoya powder preparation

The plants were found in their natural habitat in a populated area called La Mascota, located at a longitude of 79° 10′ 15.42″ West and a latitude of 7° 5′ 56.55″ South, at an altitude of 1238 masl, in El Prado district of San Miguel province, Cajamarca region (Figure 1).

The leaves and flowers of the flowering plant were collected and then washed with running water to remove any soil or inert matter residues. Subsequently, the cleaned leaves were spread out on blankets and left to air dry in a well-ventilated, sheltered area, being regularly turned over for 21 to 30 days. Once dry, contoya leaves were ground using a manual mill, and the resulting powder was packaged in paper envelopes and stored in expanded polystyrene boxes until enough amount was obtained for administration to the calves.

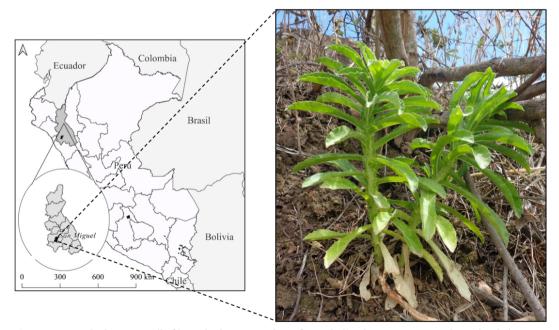


Figure 1. Study location (left) and photography of a Lobelia decurrens Cav. Plant (right).

## Administration of contoya to the animals

First, a bovine measuring tape was used to calculate the weight of the calves. Then, a scale was used to weigh the amount of contoya required by each calve. The pre-measured doses for each animal were then placed in glass bottles (630 mL capacity) and between 250 and 500 mL of cold, clean water was added to the powder before administration.

In both stages of the study, three groups were formed: a control group, a first treatment group (T1) where contoya was administered orally at a single dose of 1 g/kg body weight, and a second treatment group (T2) under the same conditions but at a dose of 2 g/kg body weight. The contoya solution was administered using the same glass bottles in which it had been previously mixed with water, ensuring proper homogenization before administration. The doses used were based on a previous study with the same plant (Reyna-Cotrina et al., 2024).

## Parasitological analysis and controls

To quantify the number of oocysts per gram of feces, coproparasitological analysis

was performed using the McMaster technique modified by Gordon and Whitlock (Ueno and Gonçalves, 1998). The calves were properly restrained, and approximately 100 g of feces were collected directly from the rectum of the animals. These samples were placed in  $15 \times 20$  cm polyethylene bags, which were properly labeled. The fecal samples were collected by stimulating the anal sphincter through manual massage.

In Farm I, controls were conducted on days 3 and 15 after dosing. In Farm II, controls were carried out on days 3, 17, and 21. All analyses were performed at the Veterinary Parasitology and Parasitic Diseases Laboratory of the Faculty of Veterinary Sciences at the National University of Cajamarca.

## Calculation of the efficacy percentage

Efficacy of contoya was determined by calculating the percentage reduction of oocysts using the oocyst per gram of feces (OPG) reduction test compared to the control group (Kassai, 1998):

# %Efficacy = (Mean OPG Control Group-Mean OPG Treated Group)(Mean OPG Control Group) × 100

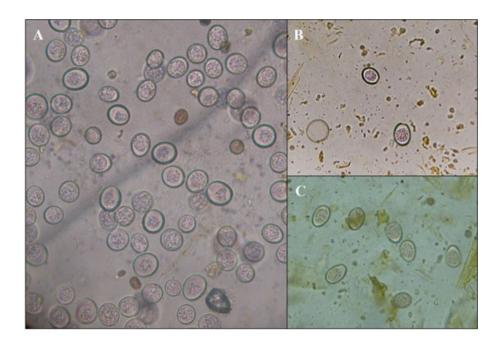
## Statistical analysis

Collected data were organized using MS Excel, and efficacies with 95% confidence intervals were calculated. The IBM SPSS Statistics 27.0.1 software was used to analyze potential differences among groups and days in terms of parasitic load, and data normality was assessed with the Shapiro-Wilk test. As the data did not exhibit a normal distribution (p<0.05), analysis was conducted using the non-parametric Kruskal-Wallis test, followed by Dunn's post hoc test to compare the independent groups (Control, T1, and T2). The comparison

of oocyst counts within the same group across different days was analyzed using the Wilcoxon test, as the data were paired. Significance level of p<0.05 was established for all statistical tests.

## **Results**

*Eimeria* spp. oocysts were still observed after administration of contoya. However, a notable decrease in OPG concentration (Figure 2) was noted. Additionally, no diarrhea or clinical signs indicating toxicity were observed post-treatment in any of the calves.



**Figure 2.** Microscopic view of *Eimeria* spp. oocysts in calf feces before (A), and after (B-C) pulverized *Lobelia decurrens* Cav. administration.

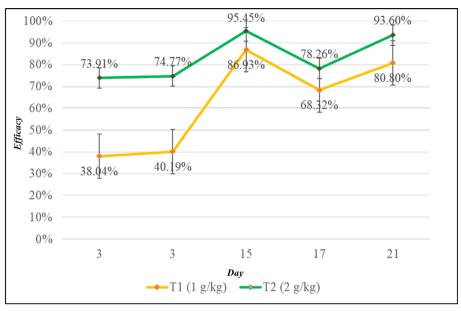
Reduction in parasitic load was observed in both treatment groups compared to the controls in both farms. No variation in parasite load over time was observed in the control group of each farm (p>0.05). Additionally, in both treatment groups (T1 and T2), a difference in parasite load

was observed only in Farm I between the first and second or third analysis (p<0.05). In contrast, the OPG count remained statistically unchanged across all three controls (p>0.05) at Farm II (Table 1). Efficacy of contoya was higher in the T2 group (2 g/kg b.w.) in both evaluations (Figure 3).

**Table 1.** Average parasitic load and percentage reduction of eggs (%Efficacy) after administration of pulverized leaves and flowers of contoya plant, *Lobelia decurrens* Cav. for coccidiosis control in calves.

Farm	Day	Control	T <sub>1</sub> : 1 g/kg		T <sub>2</sub> : 2 g/kg	
		Mean ± SD OPG	Mean ± SD OPG	%Efficacy (95% IC)	Mean ± SD OPG	%Efficacy (95% IC)
I	Day 3	1528.57 ± 292.77 <sup>ax</sup>	$914.29 \pm 536.75^{\text{bx}}$	40.19 (39.26 - 41.12)	385.71 ± 234.01 <sup>cx</sup>	74.77 (73.95 - 75.59)
	Day 15	2514.29 ± 2992.45 <sup>ax</sup>	328.57 ± 335.23 <sup>by</sup>	86.93 (86.43 - 87.43)	$114.29 \pm 106.90^{\text{by}}$	95.45 (95.14 - 95.76)
II	Day 3	1533.33 ± 320.42 <sup>ax</sup>	950.00 ± 578.79 <sup>ax</sup>	38.04 (37.05 - 39.03)	400.00 ± 252.98 <sup>bx</sup>	73.91 (73.01 - 74.81)
	Day 17	2683.33 ± 3256.63 <sup>ax</sup>	$850.00 \pm 771.36^{ax}$	68.32 (67.60 - 69.04)	583.33 ± 783.37 <sup>ax</sup>	78.26 (77.62 - 78.90)
	Day 21	2083.33 ± 3980.16 <sup>ax</sup>	400 ± 442.72 <sup>bx</sup>	80.80 (80.11 - 81.49)	$133.33 \pm 163.30^{\mathrm{bx}}$	93.60 (93.17 - 94.03)

OPG: oocysts per gram of feces; SD: Standard deviation; CI: Confidence Interval. Different letters between groups (abc) [Kruskal-Wallis, Dunn's post hoc test] and days (xyz) [Wilcoxon's test] indicate statistical differences (p<0.05).



**Figure 3.** Efficacy of *Lobelia decurrens* Cav. at two concentrations for controlling *Eimeria* spp. in calves.

## Discussion

The parasitic load decreased after administering contova to the calves in both groups. A significant reduction was observed in the group that received contoya at a dose of 2 g/kg compared to the control group (p<0.05), reachingd 95.45% efficacy on day 15. This effect is attributed to the chemical composition of the plant. Members of the genus Lobelia contain a series of bioactive components, such as flavonoids, steroids, alkaloids, tannins, quinones, saponins, and others (Chen et al., 2014; Stolom et al., 2016; Reyna-Cotrina et al., 2024). These components have shown activity against various parasites. In vitro and in vivo studies indicate that plant extracts and isolated secondary metabolites can inhibit protozoan and other intestinal parasites (Wink, 2012; El-Seedi et al., 2022; Ranasinghe et al., 2023).

The highest efficacy achieved were 95.45% and 93.60%. According to the World Association for the Advancement of Veterinary Parasitology guidelines, an antiparasitic is considered effective when the efficacy percentage ranges between 90 and 98%, thus our results are highly

promising. Even in the T1 group (1 g/kg b.w.) efficacies greater than 80% were achieved, a value within the 80 to 89% range, which is considered moderately effective (Wood et al., 1995).

Although none of the concentrations reached 100% efficacy, this might not be entirely disadvantageous; on the contrary, it may be beneficial, as a lower parasitic load would remain, stimulating the animal's immune system and thus enhancing its immune response against Eimeria spp. It is known that parasitic load enhances the immune response by the host to fight infection (Hayward et al., 2019; Hofmeester et al., 2019; Tombak et al., 2020). Therefore, maintaining a proportion of protozoan parasites in the animal would be beneficial. This approach could be useful in integrated parasite control programs or in organic farming to avoid chemical residues in milk, derivatives, and other animal products.

Since the pulverized plant was used, it could be argued that the amount administered is excessive and impractical compared to pure chemical products where therapeutic doses are offered in small quantities. When the active metabolite(s) in contoya are isolated, the therapeutic dose would substantially decrease. Nevertheless, using the plant is a more convenient option due to its low cost, making it accessible for small or medium-scale farmers. Moreover, it has been suggested that there is a greater tendency to develop resistance to purified products than to unpurified extracts (Amoah et al., 2015). Therefore, using the plant in its natural form could be recommended to avoid or reduce parasitic resistance.

A single species and a specific age group of calves were used in this study. Therefore, our findings may only apply to this species and age group. Nonetheless, given its potential, this plant could be a promising alternative for cattle farmers, as it is a low-cost, readily accessible natural product. Alternative administration methods and assessment of long-term effects on animal health and environmental impact should be investigated.

Contoya, a relatively unknown and undervalued plant, is regarded as a weed due to its non-palatability for farm animals, yet this should be reconsidered due to its antiparasitic properties. Contoya cultivation should be encouraged for potential commercial production. Additionally, bioactive compounds with antibacterial (Choi and Lee, 2016) and anticancer properties (Chen et al., 2014; Luo et al., 2024) have been identified in other species of the same *Lobelia* genus, which opens new avenues for future research on *L. decurrens* in parasitology and other medical sciences.

#### Conclusion

Pulverized leaves of contoya plant, *Lobelia decurrens* Cav., administered orally in a single dose of 2 g/kg b.w., highly reduced *Eimeria* spp. oocysts in naturally infected calves. Efficacy was 95.45% (95% CI 95.15 – 95.76), thus contoya can be considered an effective antiparasitic.

According to our results, using contoya is advised for farms in areas where the plant grows,

given its ease of acquisition and low cost of processing. Additionally, its use provides an ecofriendly alternative for animal-derived products intended for human consumption, free from chemical antiparasitic residues.

## **Recommendations**

Administering contoya at an oral dose of 2 g/kg b.w. is recommended for controlling coccidiosis in calves, with no visible adverse side effects. Although it was not evaluated in this study, it is possible that contoya does not leave residues in meat or milk, which would enhance food and environmental safety. Additional research is needed to confirm these findings, including different concentrations, dosing regimens and administration routes. Moreover, contoya is an economically viable option for controlling coccidiosis in small farms. Contoya cultivation in cattle farms should be promoted as an economical and eco-friendly solution to coccidiosis.

## **Declarations**

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## Conflicts of interest

The authors declare that they have no known financial interests or personal relationships that could have influenced the work presented in this article.

## **Author contributions**

TT and JR-C conceptualized, designed the methodology, supervised, and managed the research. JV and FP executed and carried out field and laboratory work. LV-R contributed to the validation, data curation, visualization, and writing-preparation of the original draft.

All authors collaborated in the visualization, writing-revising, and editing of the manuscript. All authors approved the final manuscript and accept responsibility for its content.

# Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

## Data availability

The data sets used in the current study are available from the corresponding author on request.

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