1	This unedited manuscript has been accepted for future publication. The
2	manuscript will undergo copyediting, typesetting, and galley review before fire
3	publication. Please note that this advanced version may differ from the fina
4	version.
5	SHORT COMMUNICATION
6	Efficacy of powdered leaf and flower of Lobelia decurrens Cav. to
7	control coccidiosis in calves
8	Eficacia del pulverizado de la hoja y flor de <u>Lobelia decurrens C</u> av. para el control de la
9	coccidiosis en terneros
10	Eficácia do pó de folhas e flores de <u>Lobelia decurrens</u> Cav. no controle de coccidiose em bezerras
11	Teófilo Torrel ¹ ^(b) ; Julissa Valle ¹ ^(b) ; Fredesbinda Pérez ¹ ; Juan Rojas-Moncada ¹ ^(b) ; Luis Vargas-Rocha ¹
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19	Abstract
20	Background: Cattle are susceptible to infections by parasitic protozoa, which often require chemical
21	treatments. However, using these products can contaminate the soil and negatively affect ecosystems
22	(flora, fauna, and microbiota) while also affecting the food chain and safety, leaving residues in milk
23	and meat, posing risks to consumers. Therefore, it is crucial to seek sustainable alternatives, such as

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using bioactive plants and their extracts. This study aimed to evaluate the efficacy of the pulverized 24 plant contoya (Lobelia decurrens Cav.) at single doses of 1 g.kg⁻¹ and 2 g.kg⁻¹ of body weight in 25 controlling coccidiosis in naturally infected calves under extensive rearing conditions. Methods: 26 Groups of calves with similar parasitic loads (ANOVA p>0.05) were formed and orally administered 27 28 to the pulverized leaves and flowers of the contoya plant. Efficacy was calculated as the percentage reduction of oocysts per gram of feces using a McMaster chamber. Results: The 2 g.kg⁻¹ dose 29 significantly reduced the parasitic load compared to the 1 g.kg⁻¹ dose (p<0.05). With a dose of 1 g.kg⁻¹ 30 ¹, a minimum efficacy of 38.04% was observed on the third day and a maximum efficacy of 86.93% 31 on the fifteenth day. With the 2 g.kg⁻¹ dose, a minimum efficacy of 73.91% was achieved on the third 32 day and a maximum efficacy of 95.45% on the fifteenth day. Conclusions: These results indicate that 33 contoya at a dose of 2 g.kg⁻¹ of b.w. can be an option for controlling coccidiosis in calves. This could 34 be advantageous for cattle producers, providing a superior alternative to conventional drugs in terms 35 of costs, environmental impact as well as food safety and quality. 36

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

39 **Resumen**

Antecedentes: El ganado bovino es susceptible a infecciones por protozoarios parásitos, que a 40 menudo requieren tratamientos químicos. Sin embargo, el uso de estos productos puede contaminar 41 el suelo y afectar negativamente la flora y fauna, además de dejar residuos en la leche y la carne, 42 representando un riesgo para los consumidores. Por lo tanto, es crucial buscar alternativas menos 43 dañinas para el ambiente, como el uso de plantas bioactivas. El objetivo de este estudio fue evaluar 44 la eficacia del pulverizado de la planta contoya (Lobelia decurrens Cav.) en dosis única de 1 g.kg⁻¹ y 45 2 g.kg⁻¹ de peso vivo en el control de la coccidiosis en terneras infectados naturalmente en condiciones 46 de cría extensiva. Métodos: Se formaron grupos de terneras con cargas parasitarias similares (p>0.05) 47 a las que se les administró vía oral pulverizado de la hoja y la flor de la planta contoya. La eficacia 48 se calculó mediante el porcentaje de reducción de ooquistes por gramo de heces utilizando cámara 49 McMaster. **Resultados**: La dosis de 2 g.kg⁻¹ de mostró un efecto significativamente mayor en la 50 reducción de la carga parasitaria en comparación con la dosis de 1 g.kg⁻¹ (p < 0.05). Con una dosis de 51 1 g.kg⁻¹, se observó una eficacia mínima del 38.04% al tercer día y una eficacia máxima del 86.93% 52 al día quince. Con la dosis de 2 g.kg⁻¹, se alcanzó una eficacia mínima del 73.91% al tercer día y una 53 eficacia máxima del 95.45% al día quince. Conclusiones: Estos resultados indican que contoya a 54 dosis de 2 g.kg⁻¹ p.v. puede ser una opción para el control de la coccidiosis en terneras. Esto podría 55 ser ventajoso para los productores de ganado, proporcionando una alternativa superior a los fármacos 56

convencionales en términos de costos y el impacto ambiental así como sanidad y calidad de los
alimentos.

59 Palabras clave: biocontrol; carga parasitaria; control alternativo; control parasitario; dosis
60 terapéutica; eficacia; Eimeria spp; fitoquímico; planta bioactiva; terneras.

61 **Resumo**

Antecedentes: O gado bovino é suscetível a infecções por protozoários parasitários, cujo tratamento 62 geralmente envolve o uso de produtos químicos. No entanto, a utilização desses produtos pode 63 contaminar o solo e afetar negativamente a flora e a fauna, além de deixar resíduos no leite e na carne, 64 representando um risco para os consumidores. Portanto, torna-se fundamental buscar alternativas 65 menos nocivas ao meio ambiente, como o uso de plantas bioativas. Este estudo teve como objetivo 66 avaliar a eficácia da planta contova (Lobelia decurrens Cav.) pulverizada, em doses únicas de 1 g.kg⁻ 67 ¹ e 2 g.kg⁻¹ de peso corporal, no controle da coccidiose em novilhas naturalmente infectadas, criadas 68 em sistema extensivo. Métodos: Foram formados grupos de novilhas com cargas parasitárias 69 semelhantes (ANOVA p>0.05) e administradas oralmente com as folhas e flores pulverizadas da 70 planta contoya. A eficácia foi calculada como a porcentagem de redução de oocistos por grama de 71 fezes, utilizando a câmara de McMaster. **Resultados**: A dose de 2 g.kg⁻¹ reduziu significativamente 72 a carga parasitária em comparação com a dose de 1 g.kg⁻¹ (p < 0.05). Com a dose de 1 g.kg⁻¹, observou-73 se uma eficácia mínima de 38,04% no terceiro dia e uma eficácia máxima de 86,93% no décimo 74 quinto dia. Com a dose de 2 g.kg⁻¹, atingiu-se uma eficácia mínima de 73,91% no terceiro dia e uma 75 eficácia máxima de 95,45% no décimo quinto dia. Conclusões: Esses resultados indicam que da 76 contoya na dose de 2 g.kg⁻¹ p.c. pode ser uma opção para o controle da coccidiose em novilhas. Isso 77 poderia ser vantajoso para produtores de gado, pois oferece uma alternativa aos medicamentos 78 convencionais em termos de custo e impacto ambiental. 79

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

82 Introduction

Coccidiosis is a common disease in ruminants worldwide, causing significant economic losses in 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, disrupting 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 residues from these products can accumulate in tissues and products intended for human consumption, posing an imminent risk to public health (Rana *et al.*, 2019). Furthermore, since soil acts as the ultimate repository for antiparasitic residues, these components could 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 chemical control through anticoccidial drugs (Keeton and Navarre, 2018; Bangoura and Bardsley, 2020). The primary product used has been toltrazuril, and although some studies have demonstrated high efficacy rates in calves (Jonsson *et al.*, 2011), over time, other studies have shown variable efficacy (Philippe *et al.*, 2014; Zechner *et al.*, 2015; Beltrán *et al.*, 2022). Resistance of *Eimeria* spp. to toltrazuril has even been confirmed in lambs (Odden *et al.*, 2018). Drug resistance must be considered a significant potential threat in the treatment of coccidiosis (Bangoura and Bardsley, 2020).

102 In environments with high parasitic presence and resistance, searching for alternative control methods is urgently necessary to prevent the spread of parasitic resistance. This involves utilizing as 103 many tools as possible to minimize the need for pharmaceutical interventions and optimize animal 104 production (Burke and Miller, 2020). In this context, using natural plants has emerged as a safe 105 alternative for consumers and the environment. Bioactive plants have shown promising results in 106 controlling various animal parasites (Worku et al., 2009; Silva et al., 2014). Some phytochemicals 107 have even been indicated to optimize production, promoting growth and improving animal health 108 (Lillehoj et al., 2018). Among these plants, Lobelia decurrens Cav has been identified as containing 109 a series of bioactive components such as flavonoids, steroids, alkaloids, tannins, quinones, and 110 saponins, which have shown activity against *Eimeria* spp. in rabbits (Reyna-Cotrina et al., 2024). 111

Given the diverse negative clinical manifestations that diminish the productive parameters in ruminants and may even result in fatal outcomes for younger cattle due to *Eimeria* infection (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, as this species holds significant economic importance in Latin American countries and globally. Therefore, this study aimed to evaluate the efficacy of powdered *Lobelia decurrens* Cav. (contoya) leaves in controlling coccidiosis in naturally infected and extensively raised calves. 119 Materials and methods

120 *Ethical considerations*

121 The authors complied with the Protección y Bienestar Animal Law of the Peruvian State (Law 122 No. 30407). The animals used in the study enjoyed the five freedoms of animal welfare.

123 Location

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

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

131 Selection of animals and formation of groups

In an initial evaluation, a coprological analysis was performed on all Creole calves from the first farm (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).

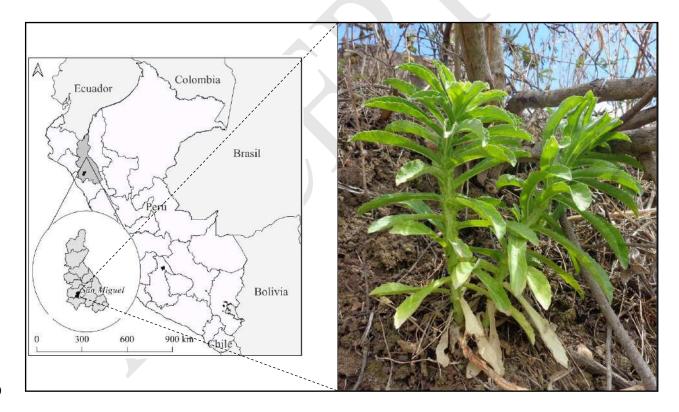
On the second farm, following the same criteria and conditions, 40 Holstein calves were analyzed, from which 18 met the established criteria were selected. These calves had an age range of 3 to 6 months (5.06 ± 1.00) and live weights ranging from 51 to 140 kg (110.11 ± 22.08) . These 18 calves were divided 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. A final researcher served as an observer throughout all stages of the study.

145 The World Association for the Advancement of Veterinary Parasitology indicates that to evaluate 146 the clinical efficacy of antiparasitic agents in cattle under field conditions with natural parasite infection, groups of at least 6 individuals should be formed (Wood *et al.*, 1995). On the first farm,
groups of 7 calves were formed to avoid wasting the number of animals that met the selection criterion
for parasitic load.

150 *Obtaining contoya powder*

The plants were found in their natural habitat in a populated center called La Mascota, located at 151 a longitude of 79° 10' 15.42'' West and a latitude of 7° 5' 56.55'' South, at an altitude of 1238 meters 152 above sea level, in the El Prado district of the San Miguel province, in the Cajamarca region (Figure 153 154 1). For analysis, 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 155 spread out on blankets and left to air dry in a well-ventilated, sheltered area, being constantly turned 156 over for 21 to 30 days. Once the contoya leaves were dry, they were ground using a manual mill, and 157 the resulting powder was packaged in paper envelopes, and stored in expanded polystyrene boxes 158 until the necessary amount for administration to the calves in all groups was obtained. 159



160

Figure 1. Location of the plant *Lobelia decurrens* Cav. in the El Prado district of the San Miguel
 province, Cajamarca region (Peru)

163 Preparation and administration of contoya

The preparation and administration of contoya were carried out as follows: First, a bovine measuring tape designed for cattle was used to calculate the weight of the calves. Based on the weight obtained for each individual, a balance was used to weigh the exact amount of contoya required. The pre-measured doses for each animal were then placed in glass bottles (630 mL capacity), and cold, clean water was added in an amount varying between 250 and 500 mL to mix the powder before administration.

In both stages of the study, three groups were formed: a control group, a first treatment group (T_1) where contoya was administered orally at a single dose of 1 g/kg of body weight, and a second treatment group (T_2) under the same conditions but at a dose of 2 g/kg of b.w. The contoya solution was administered using the same glass bottles in which it had been previously mixed with water, ensuring proper homogenization before application. The doses used were selected based on a previous study with the same plant (Reyna-Cotrina *et al.*, 2024).

176 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 15×20 cm polyethylene bags, which were duly labeled. The fecal samples were collected by stimulating the anal sphincter through manual massages.

In the first farm, controls were conducted on days 3 and 15 after dosing. In the second farm, controls were carried out on days 3, 17, and 21. All analyses were performed in the Laboratorio de Parasitología Veterinaria y Enfermedades Parasitarias, part of the Facultad de Ciencias Veterinarias at the Universidad Nacional de Cajamarca.

186 *Calculation of efficacy percentage*

The 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):

$$\% Efficacy = \frac{Mean OPG Control Group - Mean OPG Treated Group}{Mean OPG Control Group} \times 100$$

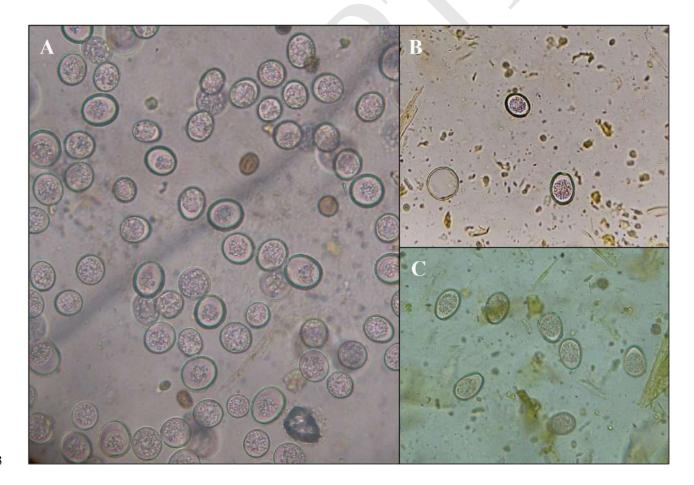
190 Statistical analysis

The collected data were organized using MS Excel, and efficacies with 95% confidence intervals were calculated. To analyze potential differences among groups and days in terms of parasitic load, IBM SPSS Statistics 27.0.1 software was employed, and data normality was assessed using 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 post hoc tests with Mann-Whitney U. A significance level of p<0.05 was established for all statistical tests.

197 **Results**

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

202



203

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

In both the first and second farms, the reduction in parasitic load was lower in both treatment groups compared to the control group. There was no variation in parasite load over time in the control group at each farm (p>0.05). Additionally, in both treatment groups (T₁ and T₂) a difference in parasite load was observed only at the first farm between the first analysis and the second or third analysis (p<0.05). In contrast, at the second farm, the OPG count remained statistically unchanged across all three controls (p>0.05) (Table 1). The efficacy of the contoya plant was higher in the T₂ group (2 g/kg b.w.) in both evaluations (Figure 3).

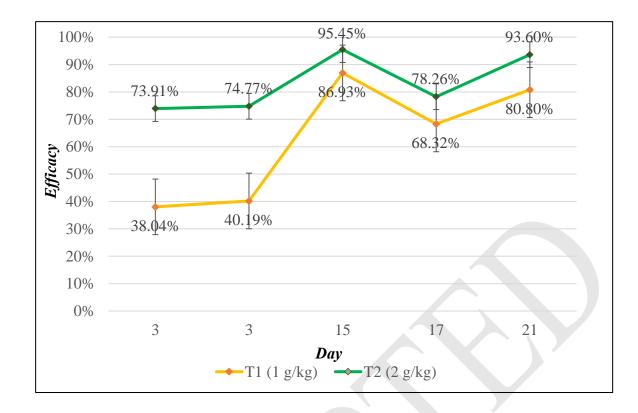
Table 1. Average parasitic load and percentage reduction of eggs (%Efficacy) of the pulverized leaves and flowers of the contoya plant, *Lobelia decurrens* Cav., in the control of coccidiosis in calves.

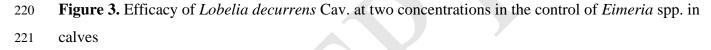
Farm	Day	Control	T1: 1 g/kg		T2: 2 g/kg	
		$Mean \pm SD \ OPG$	$Mean \pm SD \ OPG$	%Efficacy (95% IC)	Mean ± SD OPG	%Efficacy (95% IC)
т	Day 3	1528.57 ± 292.77^{ax}	914.29 ± 536.75^{bx}	40.19 (39.26 - 41.12)	385.71 ± 234.01^{cx}	74.77 (73.95 - 75.59)
1	Day 15	2514.29 ± 2992.45^{ax}	328.57 ± 335.23^{by}	86.93 (86.43 - 87.43)	114.29 ± 106.90^{by}	95.45 (95.14 - 95.76)
	Day 3	1533.33 ± 320.42^{ax}	950.00 ± 578.79^{ax}	38.04 (37.05 - 39.03)	400.00 ± 252.98^{bx}	73.91 (73.01 - 74.81)
II	Day 17	2683.33 ± 3256.63^{ax}	850.00 ± 771.36^{ax}	68.32 (67.60 - 69.04)	583.33 ± 783.37^{ax}	78.26 (77.62 - 78.90)
	Day 21	2083.33 ± 3980.16^{ax}	400 ± 442.72^{bx}	80.80 (80.11 - 81.49)	133.33 ± 163.30^{bx}	93.60 (93.17 - 94.03)

216 OPG: oocysts per gram of feces; SD: Standard deviation; CI: Confidence Interval. Different letters between groups (abc) and days

217 (xyz) indicate statistical differences between each farm (Kruskal-Wallis, Mann-Whitney U post hoc, p<0.05).

218





222 Discussion

219

After administering the pulverized contoya to the calves, the parasitic load decreased in both 223 groups. A significant reduction was observed in the group that received contoya at a dose of 2 g/kg 224 compared to the control group (p < 0.05). Therefore, this group showed greater efficacy, reaching 225 95.45% on day 15. This effect is attributed to the chemical composition of the plant. It has been 226 evidenced that members of the genus Lobelia contain a series of bioactive components, such as 227 flavonoids, steroids, alkaloids, tannins, guinones, saponins, and others (Chen et al., 2014; Stolom et 228 al., 2016; Reyna-Cotrina et al., 2024). In other studies, these components have been indicated to 229 possess activity against various parasites. It has been stated that plant extracts and isolated secondary 230 metabolites can inhibit protozoan parasites and other intestinal parasites both in vitro and in vivo 231 (Wink, 2012; El-Seedi et al., 2022; Ranasinghe et al., 2023). 232

The highest efficacies 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 is between 90 and 98%, so the results are promising and highly useful. Even in the T_1 group (1 g/kg b.w.), efficacies greater than 80% were achieved, a value within the 80 to 89%, considered moderately effective (Wood *et al.*, 1995).

Although none of the concentrations reached 100% efficacy, this scenario might not be entirely 238 disadvantageous; on the contrary, it would be quite appropriate, as a lower parasitic load would 239 remain, stimulating the animal's immune system and thus enhancing its immune response against 240 Eimeria spp. It has been identified that the parasitic load influences the intensity of the immune 241 242 response generated by the host to combat the infection (Hayward et al., 2019; Hofmeester et al., 2019; Tombak et al., 2020). Therefore, a proportion of protozoan parasites in the animal would be 243 beneficial. Additionally, this approach could be useful in integrated parasite control programs or in 244 organic farming to avoid chemical residues in milk, derivatives, and other animal products. 245

Since the pulverized form was administered in grams, various researchers might argue that the 246 amount is excessive and impractical compared to pure chemical products where therapeutic doses are 247 measured in small quantities in milligrams. However, it should be considered that the whole plant 248 leaf was used; if the active metabolite or metabolites were isolated, the concentration of the 249 therapeutic dose would substantially decrease. In its natural state, the plant represents a highly 250 effective option due to its low cost, making it accessible for small or medium-scale livestock farmers. 251 252 Moreover, it has been suggested that there is a greater tendency to develop resistance to a purified product than to an unpurified extract, as in the case of plant extracts (Amoah et al., 2015). Therefore, 253 using the plant in its natural state (without purifying the active principles) would be a highly 254 recommended option to avoid or reduce parasitic resistance in livestock 255

Considering that this study employed a single species and a specific age group of calves, this may represent a limitation due to the particular responses associated with species and age. Therefore, the findings may only apply to this species and age group, which could limit the generalizability of the results to other species. Nonetheless, given its potential, this plant could represent a promising alternative for cattle farmers, as it is a low-cost, readily accessible natural product. Exploring alternative administration methods and assessing long-term effects on animal health and environmental impact would be beneficial.

This plant is relatively unknown and undervalued, even considered a weed due to its nonpalatability for farm animals, its antiparasitic properties could support its revaluation and encourage cultivation 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 another species of the same genus, *Lobelia*, which opens new avenues for future research on *L. decurrens* in parasitology and other medical sciences.

269

270 Conclusion

The pulverized leaves of the contoya plant, *Lobelia decurrens* Cav., administered orally in a single dose of 2 g/kg b.w., achieved the highest percentage reduction of *Eimeria* spp. oocysts in naturally infected calves. It reached an efficacy of 95.45% (95% CI 95.15 – 95.76), thus being considered an effective antiparasitic.

These suggest a cost-effective and practical option for farmers in areas where this plant grows, given its ease of acquisition and low-cost processing. Additionally, its use provides an eco-friendly alternative that could facilitate the production of animal-derived products for human consumption, free from chemical antiparasitic residues.

279 **Recommendations**

The use of contoya at a dose of 2 g/kg b.w. in calves is recommended for the control of coccidiosis, as no visible adverse side effects have been observed, and there is a possibility that it does not leave residues in meat or milk, enhancing food and environmental safety. However, additional studies are needed to confirm these findings, including different concentrations, dosing regimens, and administration routes. Moreover, this alternative presents itself as an economically viable option for small-scale farmers in controlling bovine coccidiosis, promoting the cultivation of contoya on their farms as an economical and eco-friendly solution.

287 Declarations

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294 Conflict 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.

297 *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 drafts. 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.

- 303 Use of artificial intelligence (AI)
- 304 No AI or AI-assisted technologies were used during the preparation of this work.

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