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Influence of Anthelmintic Treatment on Immune Response and Oxidative Stress in Cattle Vaccinated against Lumpy Skin Disease

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ABSTRACT

Background: Vaccination and deworming are routine programs in cattle health management. However, the anthelmintic's effectiveness on the immune response in vaccinated cattle is limited. Therefore, this study investigated whether anthelmintic administration affects the oxidative stress and immunity induced by vaccination against lumpy skin disease (LSD) in cattle.

Methods: Thirty-seven Thai beef cattle were divided into two groups. Group 1) 19 cattle were vaccinated against LSD with a Neethling LSD virus (LSDV) vaccine as the control group and Group 2) 18 cattle were vaccinated against LSD with a Neethling LSDV vaccine and received albendazole treatment. Then, the intensity of gastrointestinal parasite infestation, level of malondialdehyde, inflammatory cytokines and antibody titer to LSDV were investigated in both groups on day 30 of the experimental period.

Result: The results showed that the types and intensity of gastrointestinal parasites were decreased in the anthelmintic-treated group. There were no significant differences in the levels of malondialdehyde, IFN-γ, TNF-α and LSD-specific antibody titers between the control and the anthelmintic treatment group ($P>0.05$), while the expression level of IL-4 in the anthelmintic treatment group was significantly lower than in the control group ($P<0.05$). Our study indicated that albendazole treatment did not affect oxidative stress and innate and adaptive immunity against LSDV. Moreover, single-dose albendazole therapy led to a reduction in the expression level of IL-4, which is involved in defense against parasites.

Key words: Albendazole, Beef cattle, Gastrointestinal parasite, Lumpy skin disease virus vaccine.

INTRODUCTION

Lumpy skin disease (LSD) is an emerging bovine disease caused by the lumpy skin disease virus (LSDV). LSDV infection can cause several skin nodules, fever, anorexia and enlarged lymph nodes in cattle (Tuppurainen *et al*., 2020) and substantially cause substantial economic losses in livestock industries due to increasing costs and loss from production, control and treatment (Roche *et al*., 2020). LSD outbreaks have been reported in Africa, the Middle East and Asia. Recently, it has spread to China, Thailand and other countries (Arjkumpa *et al*., 2021; Roche *et al*., 2020).

Gastrointestinal parasites are known to be the major worldwide cause of health and productive potential of livestock, especially ruminants (Foster and Elsheikha, 2012). The helminth infestation in animals may vary from subclinical to severe, which can lead to death depending on the type and number of parasites and animal factors, including age, breed and health status (Schutz *et al*., 2012). The clinical signs of parasitic infestations are associated with a reduction in productive performance due to anorexia, emaciation, poor growth and anemia (Das *et al*., 2018; Mohanta *et al*., 2016). Recent studies showed that infestation with gastrointestinal nematodes could impair the host's immune mechanisms (Hendawy, 2018). The nematode parasites secrete specific components, for example, ES-62, that could alter the host immune response by inhibiting eosinophil infiltration, inducing mucosal mast cell hyperplasia and suppressing the proliferation of B and T cells (Shin *et al*., 2009). The cystatins (cysteine protease inhibitors) secreted by nematodes could mount upregulation of IL-10, resulting in impairment of the Th2 immune response (Cooper and Eleftherianos, 2016.). It was found that such helminth-induced immunosuppression interferes with the immune mechanism of response to vaccination (Motran *et al*., 2018). Recent studies reported that helminth infestation alters the efficacy of different

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vaccines such as Newcastle disease (Pleidrup *et al*., 2014), *Mycoplasma hyopneumoniae* (Steenhard *et al*., 2009) and malarial vaccine (Esen *et al*., 2012). Accordingly, parasite infestations are a global health problem and are likely to worsen immunization outcomes in animals.

Anthelmintics are used for parasite control in cattle. Benzimidazole anthelmintics such as fenbendazole and albendazole are widely used in ruminants (Lanusse *et al*., 2018). The efficacy of anthelmintic drugs depends on parasite type, animal species, mode of application, dosage and duration of usage (Baiak *et al*., 2018). In Thailand, the use of albendazole against gastrointestinal helminths of cattle had high efficiency, particularly on a farm with no prior albendazole usage (Rukkwamsuk *et al*., 2005). A previous study has shown the interaction between anthelmintic treatment and inflammatory response to vaccination in ponies (Nielsen *et al*., 2015). In addition, Schutz *et al*. (2012) reported that deworming of calves two weeks before or at the time of vaccination against the infectious bovine rhinotracheitis virus had a beneficial effect.

Emergency vaccination, especially if applied before the LSDV enters a high-risk area, is an effective way to control and prevent the outbreak of LSD (Roche *et al.,* 2020). The applicability of live attenuated vaccines, especially the LSDV Neethling strain, was demonstrated to protect cattle from LSDV during the first outbreaks in Thailand in 2021-2022. However, some studies have shown vaccine failure (Tuppurainen *et al*., 2020; Haegeman *et al*., 2021). The reasons for the failure of immunizations include the difference of strain between the virus vaccine and field outbreak strain, failure to develop detectable antibodies from the vaccine, vaccination of calves with the interfering titre of the maternal-derived antibody, vaccination of animals already incubating LSD and errors during storage and administration of vaccine (Hunter and Wallace, 2001; Ayelet *et al*., 2013). Therefore, the reasons for vaccination failures are undoubtedly complex, yet several reports suggest parasite infestation can significantly alter animals' immunity to vaccinations. However, there have not been studies on the impact of parasitic infestation on LSDV-vaccinated cattle.

In this study, we hypothesized that gastrointestinal parasites and malondialdehyde levels (oxidative stress parameter) would decrease in beef cattle following deworming and vaccination with a live attenuated LSDV Neethling strain vaccine while LSD immunity would rise. In addition, IL-4, IFN-γ and TNF-α levels may be higher in the cattle without anthelmintic treatment caused by the gastrointestinal parasite. Therefore, this experiment aimed to study the effects of anthelmintic administration on the intensity and type of parasites, oxidative stress, specific antibody titre to the LSDV vaccine and the expression level of IL-4, IFN-γ and TNF-α in LSDV-vaccinated beef cattle.

Benefits from this study will help establish a beneficial vaccination program against lumpy skin disease and help properly administer anthelmintic drugs to effectively stimulate the immune system, especially in countries experiencing the first outbreak of lumpy skin disease.

MATERIALS AND METHODS

The experimental procedures were approved by the Institutional Animal Care and Use Committee, Mahasarakham University (IACUC-MSU-5/2023).

Experimental design

The study was conducted from January to February 2022. Thirty-seven beef cattle were divided into two groups: the first group (the control group with no deworming; n = 19) and the second group: the experimental group with deworming; n = 18) to compare the effect of anthelmintic use on the types and intensity of parasites, oxidative stress, antibody levels to LSDV and IL-4, IFN-γ and TNF-α in LSD-vaccinated beef cattle.

The 37 beef cattle used in this study were raised in small farms inside Non-Mueang Village, Phon District, Khon Kaen Province, northeastern Thailand. Beef cattle were between 6-18 months old, regardless of sex, weighing 150-250 kilograms. Farmers raise these cattle by grazing in the grassland and water sources around the village or nearby areas, with some concentrated feed. The cattle had never received any anthelmintic drug at least six months before the start of the trial. All cattle received a vaccine against foot-and-mouth disease every six months, but they had never been vaccinated against lumpy skin disease. At the start of the experiment, beef cattle (experimental group) received albendazole suspension (112.5 mg/mL, Albentel, Atlantic Laboratories Corporation, Thailand) at 10 mg/kg of body weight. Both groups (control and experimental group) were vaccinated subcutaneously with 1 mL of a live attenuated LSDV Neethling strain vaccine (KEMIN, Mevac, Egypt). After 30 days, cattle manure and blood were collected to check for worm eggs and immunological levels.

Fecal collection

About 20 g of fresh fecal samples from cattle were collected from the rectum and individually placed in clean bags, then placed in buckets with ice and transferred to the laboratory at the Faculty of Veterinary Sciences, Mahasarakham University.

Blood collection

10 ml of blood was collected aseptically via jugular venipuncture using tubes containing sodium EDTA for the peripheral blood mononuclear cells (PBMCs) isolation and into anticoagulant-free tubes for preparation of the serum. The blood samples without anticoagulant were allowed to clot for 30 min at room temperature, followed by centrifugation at 1500 × g for 10 min. The serum was aliquoted into centrifuge tubes and stored at -20°C.

Simple flotation method

A simple floatation method (isolation of nematode eggs and protozoan oocysts) was performed according to the previously described (Pawar *et al*., 2019). Briefly, 1-3 grams of feces were mixed with 20 mL of saturated salt

solution in a cup and filtered through gauze into a centrifuge tube, then filled to the top with a saturated salt solution. Place a coverslip on the top and examine the coverslips by light microscopy.

Formalin ethyl-acetate concentration technique (FECT)

FECT was carried out as described previously (Anamnart *et al.,* 2013). Briefly, 10 g of feces was mixed well with 10 mL of 9% NaCl and strained the mixture through gauze into a centrifuge tube. After centrifugation at 500 × g for 5 min, the sediment was suspended with formalin and ethyl acetate solution. The mixture was vigorously shaken and centrifuged. All supernatants were decanted and 1 mL of formalin was adjusted. Place two drops of recovered sediment on the slide and examine the sample under a light microscope. Identification of parasites using morphological criteria guidelines described by William (1997).

Antibody titre investigation

LSD-specific antibodies detection *via* enzyme-linked immunosorbent assay (ELISA) was assessed using a commercially available ELISA kit ID Screen® Capripox Double Antigen Multi-species ID vet® (Montpellier, France) according to the manufacturer's instructions. The results calculated the percentage of sample-to-positive control ratio (S/P%). Samples with the S/P ratio ≥30% were positive, while samples with the S/P ratio <30% were negative.

Malondialdehyde

The malondialdehyde (MDA) levels in serum were determined by a thiobarbituric acid reactive substance assay. Briefly, an aliquot of serum (100 µL) was transferred into a test tube, followed by additions of 200 µL of 0.12 M thiobarbituric acid, 450 µL of 0.9% NaCl and 1000 µL of 10% trichloroacetic acid and then placed in a 60°C water bath for 30 min. After cooling in running tap water, the mixture was centrifuged at 1100 × g for 10 min. The reaction mixture was analyzed by a microplate reader (Tecan Trading AG, Männedorf, Switzerland) at 532 nm and compared with 1,1,3,3-tetraethoxypropane.

PBMCs isolation

PBMCs were isolated using Ficoll-paque plus (CytivaTM) in accordance with the manufacturer's instructions. Briefly, the whole blood was diluted with phosphate buffered saline (PBS). Cell suspensions were layered above Ficoll-paque plus (CytivaTM) for density separation centrifugation at 800 × g for 25 min at room temperature. Isolated PBMCs were collected and washed with PBS at 1,400 × g for 10 min. RBC lysis was performed using RBC lysis buffer (Sigma-Aldrich, St. Louis, MO). The cells were washed with PBS and then the separated cells were used for RNA extraction.

RNA isolation and Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted using a commercial kit (Nucleospin RNA kit, Macherey-Nagel, Germany), following the manufacturer's instructions. Total RNA was reverse transcribed into cDNA using the ReverTra Ace qPCR RT Kit (TOYOBO, Japan), following the manufacturer's instructions. RNA quality and quantity were subsequently performed by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). The qRT-PCR was performed using Maxima Sybr Green qPCR Mastermix (Thermo Inc, USA) on a QuantStudio™ 3 Real-Time PCR System (Applied Biosystems) instrument. Gene expression was analyzed with the $2^{-\Delta\Delta Ct}$ method employing GAPDH as the reference genes. The primer sets are provided in Table 1.

Statistical analysis

The normal distribution of data was tested. A comparison of the parameters between groups was performed using an unpaired *t*-test. All data were expressed as mean ± SD. A *P*-value P<0.05 was accepted as significant.

RESULTS AND DISCUSSION

From 19 fecal samples of beef cattle (the control group), 94% of cattle were infested with gastrointestinal parasites. There were three nematodes, one trematode and one cestode. Of these, 46% were single-infested (Strongyle type) and 48% were co-infested with more than one type (Strongyle type, *Strongyloides* spp., *Moniezia* spp., *Trichuris* spp. and Rumen fluke). In the anthelmintic treatment group, after examining 18 samples for parasite infestation, 50% of the samples in this group were infested with parasites. Parasites detected in this group were three nematodes and one trematode. In the anthelmintic treatment group, the highest single-infested was Strongyle type (39%),

Table 1: The real-time quantitative PCR primers.

Cytokine gene	Sequences (5′ - 3′)	Reference	Accession number
GAPDH	F: GGC GTG AAC CAC GAG AAG TAT AA		
	R: CCC TCC ACG ATG CCA AAG T	Norian *et al.*, 2017	U22385
IL-4	F: CAA AGA ACA CAA CTG AGA AG		
	R: AGG TCT TTC AGC GTA CTT GT	Norian *et al.*, 2017	M77120
IFNG	F: TTGAATGGCAGCTCTGAGAAAC		
	R: TCTCTTCCGCTTTCTGAGGTTAGA	Bai *et al.*, 2019	FJ263670
TNFA	F: TGACGGGCTTTACCTCATCT		
	R: TGATGGCAGACAGGATGTTG	Bai *et al.*, 2019	NM_173966

GAPDH- Glyceraldehyde 3-phosphate dehydrogenase, IL-4- Interleukin 4, IFNG- Interferon gamma, TNFA- Tumor necrosis factor alpha.

followed by *Trichuris* spp. (6%). Among the co-infested (5%), three distinct parasite species were found, including Strongyle type, *Strongyloides* spp. and Rumen fluke. Overall, the different parasite infestations in the anthelmintic treatment and control group are shown in Fig 1.

From the egg count in the control group, it was found that there were five parasites in this group, *i.e.*, Strongyle type, *Strongyloides* spp., *Moniezia* spp., *Trichuris* spp. and Rumen fluke. Egg counts of each species were 13.33±20.40, 7.87±26.32, 5.75±20.53, 0.51±1.42 and 0.32±1.25 eggs per gram, respectively. In the anthelmintic treatment group, three types of parasites were found, *i.e.*, Strongyle type, *Trichuris* spp. and Rumen fluke. Egg counts of each type were 1.41±3.34, 0.18± 0.62 and 0.14±0.47 eggs per gram, respectively (Table 2).

Investigating fecal samples revealed that gastrointestinal parasite infestation was 94%. Additionally, 48% of the fecal samples were co-infested, including Strongyle type, *Strongyloides* spp., *Moniezia* spp., *Trichuris* spp. and Rumen fluke. This result was congruent with the report of Sakwiwatkul *et al*. (2017) and Thanasuwan *et al*. (2021), who found that the prevalence of gastrointestinal parasitic infections was 93% and 84.24%, respectively, while Income *et al*. (2021) reported a relatively lower prevalence of the infections (35.7%). It is possible that agroecology and climate alteration could influence the infection rate and type of gastrointestinal parasitic infestation (Junsiri *et al*., 2021; Income *et al*., 2021; Thanasuwan *et al*., 2021). Considering the infestation rate, the percentage of infested animals decreased from 94% to 50% on day 30 after the treatment period. This result is consistent with the previous report on the efficacy of albendazole against gastrointestinal parasites in ruminants (Rukkwamsuk *et al*., 2005).

As shown in Fig 2, the malondialdehyde of the control group was not significantly different from the anthelmintic treatment group (P>0.05).

The LSD-specific antibody titers at day 30 post vaccinations of the control group were positive in 8 out of

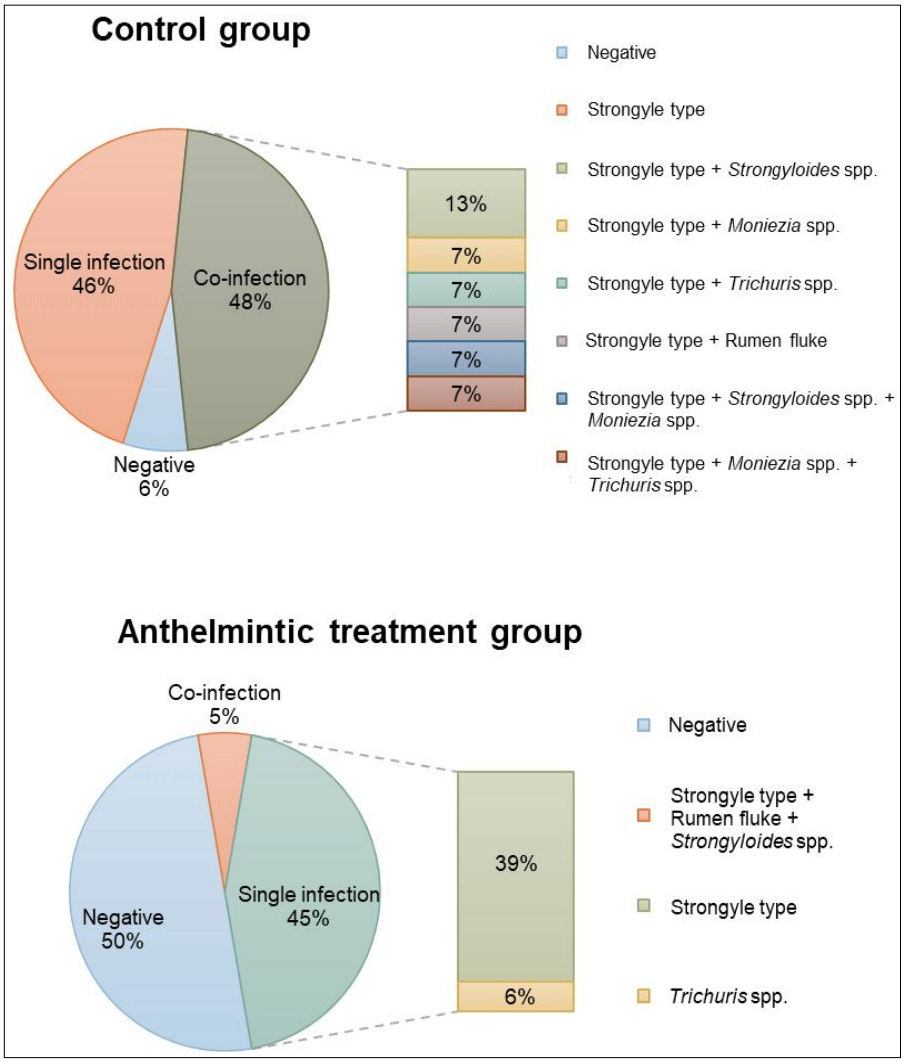

Fig 1: Percentages of the different gastrointestinal parasites in fecal samples of control and the anthelmintic treatment group.

19 serum samples (42.11%). While in the anthelmintic treatment group, there were 12 positives out of 18 serum samples (66.67%). The S/P ratios of the antibodies against LSDV showed positive ELISA results (cut-off S/P ratio ≥30%) at days 30 after the LSD vaccination in both groups. However, the LSD-specific antibody titers between the control and anthelmintic treatment group were not significantly different (P>0.05) (Table 3).

Vaccination elicits humoral immunity through antibody production. In this study, the immunogenicity of the LSDV vaccine was investigated using an ELISA test to determine immune responses following a single-dose vaccination in cattle. The number of LSD-specific antibody samples in the anthelminthic treatment group was higher than in the control group, but no statistical difference between the two groups after vaccination at day 30. This phenomenon indicated that albendazole did not increase the level of antibodies in beef cattle that received the LSDV vaccine. The result was consistent with the report by Nielsen *et al*. (2015), who found no difference in vaccine-specific antibody titers between ivermectin and pyrantel pamoate treatment and the control group on days 1, 14, 29 and 42 in ponies. In addition, Brückner *et al*. (2015, 2016) also reported that albendazole did not alter the outcomes of influenza, meningococcal and cholera vaccines after treatment in children. However, Cooper and Eleftherianos (2016) found an elevation of oral cholera vaccine-induced immune responses following the albendazole anthelminthic treatment. These variations may be related to the differences in parasite type in the host, parasite burden, host species, diagnostic technique and experimental design (Amoani *et al*., 2021).

IL-4 gene expression of the control group was significantly higher than the anthelmintic treatment group (P<0.05). However, IFN-γ and TNF-α gene expression of the control group and the anthelmintic treatment group were not significantly different (P>0.05) (Fig 3).

Table 2: The average number of parasite eggs per gram of fecal samples.

Groups	Type of gastrointestinal parasites	Average per gram (Infected cattle)
Control group	Strongyle type	13.33±20.40
	Strongyloides spp.	7.87±26.32
	Moniezia spp.	5.75±20.53
	Trichuris spp.	0.51±1.42
	Rumen fluke	0.32±1.25
Anthelmintic treatment group	Strongyle type	1.41±3.34
	Trichuris spp.	0.18±0.62
	Rumen fluke	0.14±0.47

Table 3: LSD-specific antibody titer of the control group and the anthelmintic treatment groups at day 30 after vaccination.

Group	Number of samples	Number of positive samples	Number of negative samples	Positive samples (%)	*P*-value
Control group	19	8	11	42.11	P>0.05
Anthelmintic treatment group	18	12	6	66.67	

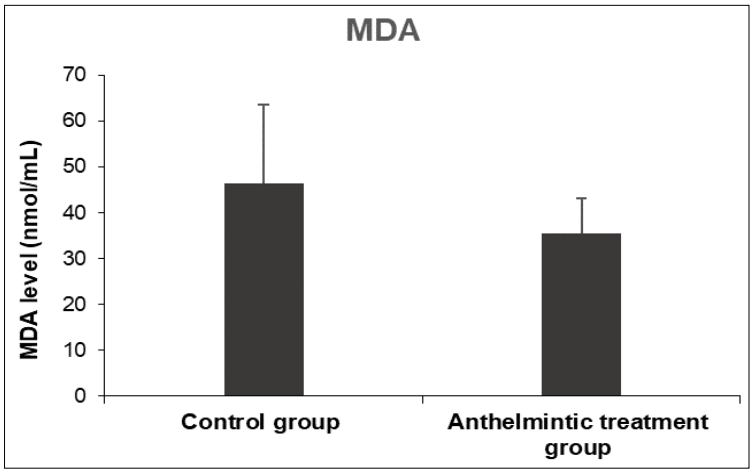

Fig 2: Serum MDA of the control and the anthelmintic treatment groups at day 30 after vaccination.

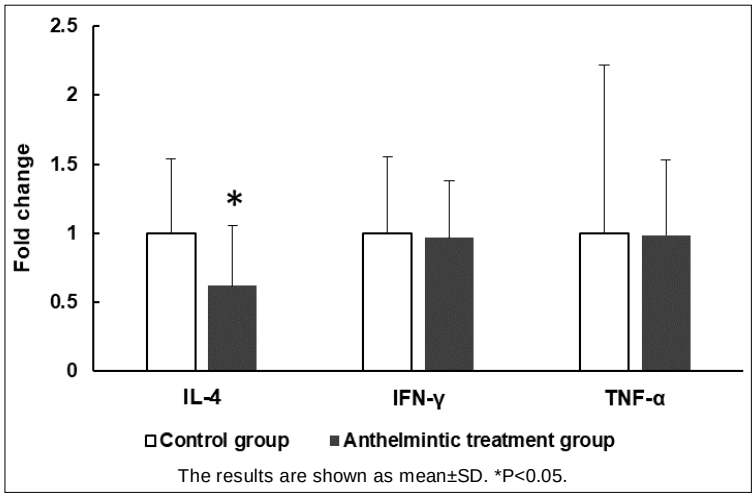

Fig 3: The expression of IL-4, IFN-γ and TNF-α in PBMCs of the control and the anthelmintic treatment group after GAPDH normalization at day 30 after vaccination.

Gastrointestinal parasite infestation induces Th2, manifested as increased production of IL-4, IL-5 and IL-13 (Foster and Elsheikha, 2012; Hendawy, 2018). These cytokines are involved in enhanced parasite expulsion. In addition, IL-4 also drives class switching of B cells to produce IgE antibodies, which helps against parasites. In this study, the cytokine mRNA analyses of PBMC showed that Th2 cytokines IL-4 of the anthelminthic treatment group were lower than in the control group, which is consistent with previous reports of Nielsen *et al*. (2015) and Anuradha *et al*. (2017) in which the decrease of IL-4 was related to a lower number of parasites in the anthelmintic treatment group. These data suggest that concurrent administration of the LSDV vaccine and anthelmintic drug ameliorated the Th2 cytokines IL-4 response caused by the gastrointestinal parasite. This study corroborates previous data that single doses of albendazole and Schisandra B reduced IL-4 levels during *Angiostrongylus cantonensis* infection (Lam *et al*., 2020). However, this study investigated the gene expression of IL-4 *in vitro* (PBMCs), which may not represent host-parasite interaction in the gastrointestinal tract (Nielsen *et al*., 2015).

CONCLUSION

In conclusion, albendazole could reduce the type and intensity of gastrointestinal parasite infestations in beef cattle. The LSD vaccination concurrently treated with a single dose of albendazole in beef cattle did not sufficiently improve immune responses to LSDV. Finally, single-dose albendazole significantly reduced the level of IL-4, which is the response to intestinal parasite infestation.

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Conflict of interest

The authors declare no conflict of interest.

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