FULL ARTICLE

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Pharmacopuncture with a low dosage of B complex vitamins in the Da Zhui acupoint (Governing Vessel 14) for controlling gastrointestinal parasitism in periparturient ewes

Farmacopuntura com dose baixa de vitaminas do complexo B no acuponto Da Zhui (Vaso Governador 14) para o controle da parasitose gastrintestinal em ovelhas no periparto

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ABSTRACT

Gastrointestinal parasites are a significant challenge in sheep farming, and periparturient ewes are one of the most susceptible categories in sheep flocks. This study aimed to verify whether the injection of B vitamins at the acupuncture point governing vessel 14 (GV14) can influence the parasitic load or host-parasite interaction in naturally infected ewes during the peripartum period. Four weeks before parturition, 25 ewes were divided into five treatment groups: (1) Dose Control: 0.2 mL of B complex via IM; (2) Drug Control: 2 mL Complex B via IM; (3) Acupoint Control: 0.2 mL of Water at GV14 point; (4) Control; (5) Test group: 0.2 mL of B complex at the GV14 point. Treatments were performed at weeks -3, -1, 2, 4, and 6 about the parturition time. Fecal samples to estimate the parasitic load by fecal egg counts (FEC) and total blood samples for hemogram examination were performed seven days after the treatment, coinciding with weeks -2, 3, 5, and 7 about the parturition time. The number of peripheral eosinophils was higher in group 3 than in group 1 ($p < 0.05$). Although groups 3 and 5 had lower mean values of FEC and higher hematological values at the time of weaning, these differences were not statistically significant ($p > 0.05$) according to the F test. However, all animals in groups 3 and 5 maintained low parasitism levels until the end of the experiment. Further studies should be performed with larger sample sizes and minor changes to the experimental protocol to elucidate the role of GV14 acupoint stimulation in the host-parasite relationship.

Keywords: Acupoint injection. Aquapuncture. Integrated management of parasites. *Haemonchus.* Periparturient egg rise.

RESUMO

A parasitose gastrointestinal é o principal desafio sanitário para a criação de ovinos a pasto, e as ovelhas no periparto são uma das categorias mais susceptíveis a esta infeção no rebanho. O objetivo deste trabalho foi verificar se a injeção de vitaminas do complexo B no acuponto Vaso Governador 14 (VG14) poderia influenciar a carga parasitária ou modular a interação hospedeiro-parasito em ovelhas naturalmente infectadas no período de periparto. Quatro semanas antes da data prevista para o parto, 25 ovelhas foram divididas em cinco grupos de tratamento (1) Controle da dose: 0,2 mL de vitaminas do complexo B via IM; (2) Controle do fármaco: 2mL complexo B via IM; (3) Controle do Ponto: 0,2 mL de água destilada no ponto VG14; (4) Controle sem tratamento; (5) Grupo teste: 0,2 mL de complexo B no pontoVG14. Estes tratamentos foram realizados nas semanas -3; -1; 2; 4 e 6 em relação à data do parto. Amostras de fezes para contagem de ovos por grama de fezes (OPG), assim como amostras de sangue para realizar o hemograma, foram obtidas dos animais sete dias após os tratamentos, coincidindo com as semanas -2, 3, 5 e 7 do parto. O número de eosinófilos periféricos foi superior no grupo 3 se comparado ao grupo 1 ($p < 0,05$). Os animais dos grupos 3 e 5 tiveram menores valores de OPG e maior hematócrito, mas estas diferenças não foram consideradas significativas pelo teste F ($p > 0,05$). No entanto, todas as ovelhas dos grupos 3 e 5 se mantiveram com níveis baixos de parasitismo durante todo o experimento. Sugere-se que novos estudos sejam realizados com algumas modificações deste protocolo a fim de aprofundar o conhecimento do potencial imunomodulador do acuponto VG14.

Palavras-chave: Acuinjeção. Aquapuntura. Controle integrado de parasitas. *Haemonchus.* Aumento peripuerperal.

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Introduction

Gastrointestinal worms are the primary health problem in sheep farming and pose an economic threat. Breeding ewes and growing lambs are the groups with greater susceptibility to the effects of parasites. During pregnancy and lactation, the acquired immune response to parasites is not manifested due to physiological immunosuppression combined with the high nutritional requirement for the development of the fetus and udder (Jimenez-Sanz et al., 2016; Salgado et al., 2019a). Therefore, ewes in the peripartum period are an essential target of parasite control actions.

Among the gastrointestinal parasites, *Haemonchus contortus* is considered the most frequent species in tropical and subtropical production systems (Salgado et al., 2019a). This hematophagous helminth is pathogenic and has been reported to be resistant to anthelmintic drugs. In South and Southeast Brazil, the resistance to these gastrointestinal parasites has become so advanced that there are no active ingredients capable of controlling parasites in most sheep farms (Salgado & Santos, 2016; Salgado et al., 2019b; Veríssimo et al., 2012). Thus, alternative parasite control tools have been researched all over the world.

Acupuncture is a word that is derived from the Latin *accus* and *pungere,* which means "to puncture with needles." It is a treatment that is part of Chinese Traditional Medicine, whose origin is related to the pre-history of Asian territory (4000 BC). The basis of this technique is the insertion of a dry needle to stimulate specific points of the human or animal body, the acupoints, to produce a therapeutic or homeostatic effect. The effect can be obtained locally, systemically, or at sites distant from the stimulus due to the transmission of the nociceptive stimulation (Scognamillo-Szabó & Bechara, 2010).

Variations of this technique are called aquapuncture, aqua injection, or pharmacopuncture, which is the injection of clinical or subclinical doses of drugs in acupoints. It aims to produce a clinical outcome equal to the effect obtained with the usual amount of injectable medications. Studies show that pharmacopuncture in large animals reduces undesirable drug side-effects, residues in animal consumption products, and treatment costs and provides comparable results to the pharmacological dosage recommended by the industry (Araujo et al., 2019; Scallan et al., 2021). The administration of 10-20% of drugs dosage into acupuncture points has been employed in people and animals, leading to the synergistic effect of acupuncture and drugs (Araujo et al., 2019; Bottecchia, 2005; Cruvinel & Vianna, 2017; Luna et al., 2015; Pons et al., 2017; Santos et al., 2015; Scallan et al., 2021).

Cruvinel & Vianna (2017) reviewed the most frequent forms of aqua injection use in veterinary medicine. Among the most frequent objectives of this technique is immunostimulation. As host resistance to parasitic infection results from an acquired immune response (Amarante, 2015), it is possible that the immunomodulatory effect of an acupoint such as governing vessel 14 (GV14) can be added to the hematopoietic effect of vitamin B12 to treat animals with gastrointestinal parasites.

This paper aims to verify if an injection of B vitamins in the GV14 acupoint can influence the parasite load, red blood cell production, and immune response of periparturient ewes naturally infected with gastrointestinal parasites.

Materials and Methods

The experiment was conducted at the Capim Branco Experimental Farm, the Federal University of Uberlandia, located in Uberlandia, Minas Gerais, Brazil (18º53'19" S, 48°20'57" W, and 863 m above sea level), from August to November 2017. Twenty-five crossbred Santa Inês and Dorper ewes between two and five years of age were used in this study. Sheep grazed in an area composed of Urochloa brizantha cv. Gastrointestinal nematodes naturally infect Marandu. In addition to pasture, the ewes were supplemented with a protein salt formulated for 0.1% of their body weight. Five weeks before the expected date of parturition, animals received a dose of monepantel (2.5 mg/kg) orally, whose estimated efficacy in the herd was 75% of the reduction in fecal nematode egg counts (FEC).

Seven days after this treatment, the individual FEC was determined using the Gordon & Whitlock (1939) technique. On the same day of collection, the sheep were

removed from the pasture and remained confined in pens with wood shavings as bedding, where they received a balanced diet according to the reproductive phase (National Research Council, 2007). The roughage: concentrate ratio was 60%:40%, respectively, and the amount per serving was calculated to be 10%.

Newborn lambs remained with their mothers until 30 days after parturition, from when the females had access to a paddock composed of Marandu palisade grass during the day but returned to the same pen as their offspring at night. This management of controlled suckling lasted until the lambs were weaned in the eighth week after parturition. Throughout the experiment, the animals had access to water and mineral salt *ad libitum*.

The sheep were divided into five experimental groups: (1) Dose Control: 0.2 mL of B complex via IM; (2) Drug Control: 2 mL Complex B via IM; (3) Acupoint Control: 0.2 mL of Water at GV14 point; (4) Control; (5) Test group: 0.2 mL of B complex at the GV14 point. Treatments were performed at weeks -3, -1, 2, 4, and 6 about the parturition time. Intramuscular (IM) injections were administered into the lateral neck region. Acupoint governing vessel 14 (GV14) was in the dorsal midline, in the depression cranial to the spinous process of the T1 vertebra, as described by Xie & Priest (2011).

Sample collections were always performed seven days after the administration of the experimental treatments, which coincided with the second week before and the first, third, fifth, and seventh week after the parturition. The collections were always conducted between 9 and 11 am. The sheep were manually restrained while standing, so that blood and feces could be collected. Blood samples were obtained by jugular puncture in vacuum tubes containing ethylenediaminetetraacetic acid. Fecal samples were obtained in plastic bags directly from the rectal ampulla. All samples were kept refrigerated until they were sent to the laboratory, and clinical analysis was performed. At most, the time between blood collection and sample analysis was 5 h.

Hematological examinations were performed at the Clinical Pathology Laboratory of the Veterinary Hospital, Federal University of Uberlandia. The procedures always started around 1 pm, on the same collection date. Hemogram analysis was performed on an automatic Poch-100iv Diff® analyzer to obtain the hematocrit (Ht), hemoglobin (Hb), red blood cell (RBC), and white blood cell (WBC) values of each sample. The differential leukocyte count was performed on blood smears stained by May-Grüwald-Giemsa. To establish the relative and absolute leukocyte values, 100 cells were identified and counted. All procedures were performed according to routine laboratory methods described by Ferreira Neto et al. (1982).

Parasitological examinations were performed at the Laboratory of Parasitic Diseases, Federal University of Uberlandia. The fecal samples remained refrigerated for a maximum of 48 h until the coproparasitological tests were performed. The modified method of Gordon & Whitlock (1939) was used to determine the FEC value of everyone. A dilution factor of 1:50 was used, and the process was sensitive to 50 eggs per gram of feces (epg). Samples from five animals of each group were pooled and cultured for seven days at 25 °C to obtain third-stage larvae (L3). These were obtained from cultures using the Roberts & O'Sullivan (1950) technique and identified by morphometry with the aid of optical microscopy, according to the key of van Wyk et al. (2004).

The FEC results were transformed for statistical analysis to homogenize the variances (log FEC+1). Data from all variables obtained were processed by the GLM procedure of the SAS program, considering a completely randomized design with five treatments and five replications. When there was a significant difference in the F test, the treatment means were compared using the student-Newman-Keuls (SNK) test, considering a p-value of <0.05.

Results and Discussion

During the entire parasitological follow-up of the sheep, strongylid eggs were the most found in the coprological examinations and the only ones tabulated for statistical analysis. *Strongyloides papillosus*, *Moniezia expanza* eggs, and coccidian oocysts were found in low proportions. Among the strongylids, *Haemonchus* sp. was the most identified parasite in the coprocultures (80% on average). Larvae of *Trichostrongylus* sp., *Cooperia* sp., and *Oesophagostomum* sp. were also found. This proportion of parasites described in the parasitological findings is consistent with previous studies conducted in the Southeast region of Brazil (Wilmsen et al., 2014).

After the treatment with monepantel that was performed at the beginning of the experiment, the FEC means were low and equivalent between the groups (Figure 1). There was a progressive increase in the elimination of eggs in the ewes' feces from parturition to weaning of the lambs. This phenomenon may reflect the peripuerperal phenomenon, by which the increase in the elimination of parasite eggs in the feces of sheep during this phase of the reproductive cycle is described (Amarante, 2015). Despite the lower averages observed in the groups with water or vitamin B

application at the GV14 point, there was no significant difference in the FEC values (p=0.0901).

Ueno & Gonçalves (1998) defined that FEC values lower than 2,500 epg would indicate a mild infection by *Haemonchus* sp., that values greater than 8,000 epg would mean a massive condition, and that values in between these cutoffs would suggest a moderate degree of infection. During the experiment, only one ewe in the dose control group manifested 7,900 epg and required anthelmintic treatment on the last day of data collection.

Although lactating ewes are susceptible to gastrointestinal parasitosis (Amarante, 2015; Salgado et al., 2019b), there was no need to perform any emergency treatment to avoid clinical signs or death of the animals in this study. It is possible that the concentrated protein supplementation provided the animals with a favorable host-parasite interaction, as proposed by Louvandini et al. (2015) and Mendes et al. (2018). In addition, the sheep used in this experiment were crossbred Santa Inês, considered in several studies to be more resistant to parasites (Albuquerque et al., 2017; Amarante, 2015).

Despite the analysis of variance not showing a significant difference between the experimental groups, all the animals in the groups that received some stimulation in the GV14 acupoint maintained FEC values compatible with a mild infection of *Haemonchus* sp. (Table 1), even for three months of the experiment without any anthelmintic treatment.

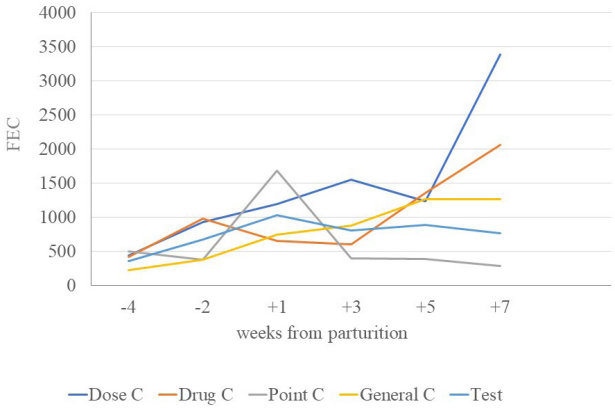

Figure 1 – Mean fecal egg counts (FEC) from the ewes by week before or after parturition (-4; -2; +1; +3; +5; +7) and group of treatment: (1) Dose C: 0.2 mL of B complex via IM; (2) Drug C: 2 mL of B complex via IM; (3) Point C: 0.2 mL of water at the GV14 point; (4) General C: no intervention; (5) Test: 0.2 mL of B complex at the GV14 point, August to November 2017.

The sheep hemogram results revealed that the average hematocrit, hemoglobin concentration, and the number of red blood cells per microliter varied little throughout this study, slightly decreasing at the end of lactation (Figure 2). However, no significant differences were recorded between the treatment groups (p>0.05).

As *Haemonchus* sp., a highly hematophagous helminth (Salgado et al., 2019a), was the most frequent parasite in animals, a depletion in hematological indices was expected as the parasite load increased. Rosalinski-Moraes et al. (2012) considered the occurrence of a negative correlation between hematocrit and FEC (-0.43 to -0.69) in sheep herds with proportions of *Haemonchus* sp. like those found in the present study, which may explain the reduction in the value of the hematological variables of the ewes at the end of the lactation period.

The leukogram showed a high variability of responses between the ewes and throughout the experiment. No significant difference was detected in the number of total leukocytes between the treatment groups (Figure 3), nor in the absolute and relative values of neutrophils, basophils, lymphocytes, and monocytes. Among the leukocytes, only eosinophils showed a difference in the mean values obtained in the experimental groups (Table 2). These data corroborate those obtained by Santos et al. (2014), who got elevated levels of eosinophils in peripheric blood and the gastric mucosa of lambs experimentally infected with *Haemonchus* sp. Still, the concentration of the other leukocytes did not differ from the control animals that were not infected.

It is known that the protective immune response against gastrointestinal parasites in sheep and goats derives from an immune response modulated by Th2 cells, characterized by the action of mast cells and eosinophils, in addition to the production of IgG, IgA, and IgE antibodies (Amarante, 2015; Estrada-Reyes et al., 2017). Rosalinski-Moraes et al. (2011) obtained a correlation between FEC and peripheral eosinophils

Table 1 – Periparturient ewe distribution by the level of parasite infection (Ueno & Gonçalves, 1998) by fecal egg counts (FEC) in the last fecal collection before weaning, August to November 2017

	Mild infection FEC lower than 2,000	Moderate infection FEC 2,000 to 8,000	Massive infection FEC up to 8,000
Dose Control[1] (n=5)	2	3	0
Drug Control[2] (n=5)	2	3	0
Acupoint Control[3] (n=5)	5	0	0
General Control[4] (n=5)	3	2	0
Test Group[5] (n=5)	5	0	0

[1]0.2 mL of B complex via IM; [2]2 mL of B complex via IM; [3]0.2 mL of water at the GV14 point; [4]No treatment; [5]0.2 mL of B complex at the GV14 point.

Table 2 – Mean peripheral eosinophil counts (cells/μL) of crossbred Santa Inês and Dorper periparturient ewes by week from parturition and group of treatment, August to November 2017

Group	Week from parturition					
	-4	-2	+1	+3	+5	+7
Dose Control[1] (n=5)	1118 A	449.8 A	849.8 A	625.2 A	254.6 B	250.4 B
Drug Control[2] (n=5)	660.6 A	590.4 A	287.4 A	414.75 A	1002.4 AB	515.2 AB
Acupoint Control[3] (n=5)	460.6 A	599.6 A	396.2 A	683 A	1429.8 A	1166.6 A
General Control[4] (n=5)	777.2 A	450.6 A	420.6 A	814.75 A	869.8 AB	482.4 AB
Test Group[5] (n=5)	739.2 A	275 A	705.8 A	326 A	996.6 AB	852.8 AB

[1]0.2 mL of B complex via IM; [2]2 mL of B complex via IM; [3]0.2 mL of water at the GV14 point; [4]No treatment; [5]0.2 mL of B complex at the GV14 end. Different letters in the column indicate significant differences for the SNK test ($p<0.05$).

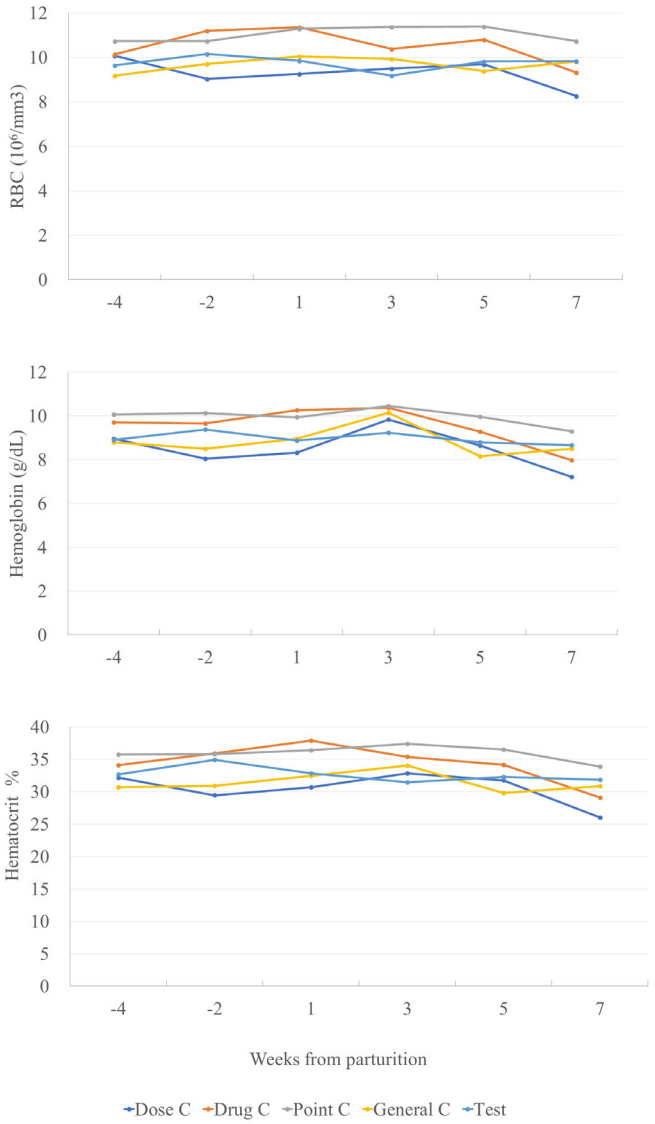

Figure 2 – Mean red blood cell counts (RBC), hemoglobin and hematocrit from the ewes by week before or after parturition (-4; -2; 1; 3; 5; 7) and group of treatment: (1) Dose C: 0.2 mL of B complex via IM; (2) Drug C: 2 mL of B complex via IM; (3) Point C: 0.2 mL of water at the GV14 point; (4) General C: no intervention; (5) Test: 0.2 mL of B complex at the GV14 point, August to November 2017.

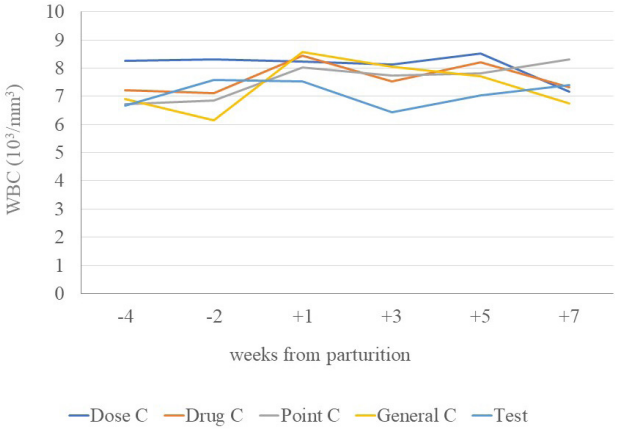

Figure 3 – Mean white blood cell counts (WBC) from the ewes by week before or after parturition (-4; -2; +1; +3; +5; +7) and group of treatment: (1) Dose C: 0.2 mL of B complex via IM; (2) Drug C: 2 mL of B complex via IM; (3) Point C: 0.2 mL of water at the GV14 point; (4) General C: no intervention; (5) Test: 0.2 mL of B complex at the GV14 point, August to November 2017.

of -0.64. They found that sheep resistant to parasites had higher concentrations of eosinophils in the peripheral blood, and lower values of FEC than sheep considered susceptible. Thus, it is concluded that the number of peripheral eosinophils can be a good indicator for evaluating the immune response of sheep to gastrointestinal parasites.

The Th2 response does not always benefit individuals because it is linked to all immediate hypersensitivity responses, such as respiratory allergies. Some studies explored the effect of acupuncture on Th2 response in different models of asthma. Wu et al. (2012) found decreased eosinophil counts and increased eosinophil apoptosis in rats with respiratory asthma under ST36 point stimulation. Yang et al. (2013) found lower IgE, IgA, and peripheral eosinophil titers and higher concentrations of CD3+, CD4+, and CD8+ lymphocytes in the peripheral blood of human patients with allergic asthma subjected to stimulation at

the GV14, BL12, and BL13 acupoints three times a week. The authors concluded that acupuncture could regulate mucosal immunity and benefit patients.

Scognamillo-Szabó (1998) verified that guinea pigs subjected to infestations with *Rhipicephalus sanguineus* together with stimulation in the acupoints ST36, SI01, LI11, *Yin Tang,* and *Er Jian* showed less immediate hypersensitivity response and greater delayed hypersensitivity when stimulated with the mite antigen than those who did not receive acupoint stimulation. In this model of host-parasite interaction, the response pattern potentiated with acupuncture was linked to better resistance to tick infestation. Bottecchia (2005) observed lower *Rhipicephalus microplus* in cattle vaccinated with Bm86 antigen at the posterior *Bai Hui* point. However, this response was accompanied by a lower antibody titer, which suggests a difference in the modulation of Th1/Th2 to favor the host-parasite interaction in the animals that received the stimulus in the acupoint. Kim & Bae (2010) reviewed a series of experiments that followed Th1/Th2 modulation in different models and with stimulation at different acupoints and concluded that, in general, acupuncture tends to establish homeostasis that benefits the host.

In the present study, the group which received stimulation at the GV14 point with water showed a more significant number of peripheral eosinophils than the group that received 10% of the dose of B vitamins intramuscularly. Although the small sample size meant no statistical difference was/ apparent, the two groups of ewes that received some stimulus at the GV14 point had higher means of peripheral eosinophils and lower standards of FEC in the last data collection before weaning of the lambs. This finding reflects how each animal was able to respond to the parasitic challenge throughout the entire lactation period. In addition, all sheep in these groups were classified as having a mild parasitic infection (Table 1).

Araujo et al. (2019) evaluated the efficiency of the administration of subdoses of eCG in estrus synchronization protocols in goats. They found that 20% of the Bai Hui point dose efficiently synchronized the estrus. The same result could not be achieved when the dose was 10%. Therefore, increasing the dose of B vitamins on the VG14 acupoint could result in a better immune and hematologic response on the ewes of the present study. Most papers that report the synergistic effect of acupuncture and drugs use 10-20% of the standard dosage (Cruvinel & Vianna, 2017; Luna et al., 2015; Pons et al., 2017; Santos et al., 2015; Scallan et al., 2021).

The results obtained in the present work show that the stimulation of the GV14 acupoint has the potential to modulate the balance between host and parasite, and this mechanism should be further investigated. As this is the first study reported in the literature that aims to find an acupuncture protocol to improve host-parasite interaction in haemonchosis, some modifications in the methodology could be implemented for better effects in animals. Weekly stimulation of the point, an increase of the B complex dosage for acupoint stimulus, systematic collection of blood samples, evaluation of other variables that allow a better assessment of the animal's immune response, and expansion of sampling are some proposed suggestions. According to Cruvinel & Vianna (2017), other acupoints have immunomodulatory potentials, such as LI11, SP6, SP10, GV13, posterior *Bai Hui*, ST36, BL11, BL20, BL23, BL28, and CV12. Therefore, the stimulation of these other points could also be evaluated.

Conclusion

Under the conditions in which this experiment was conducted, the average parasite load of lactating ewes, estimated by FEC, did not show a significant difference in animals with stimulation at the GV14 point about the control groups. There was also no difference in the values of hemoglobin, hematocrit, and RBC of sheep belonging to the distinct groups.

However, all ewes that received stimulation at the GV14 point had a light parasite load throughout the experimental period, and animals that received stimulation with water at the acupoint had a more significant number of peripheral eosinophils than the group that received 10% of the vitamin dose B complex intramuscularly. Further studies should be conducted with a larger sample size and minor modifications to the experimental protocol to elucidate the role of GV14 acupoint stimulation in the host-parasite relationship.

Conflict of Interest

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Ethics Statement

All procedures involving animals were approved and followed the ethical standards for animal experimentation and were approved by the Ethics Committee on the Use of Animals of the Federal University of Uberlandia (CEUA/UFU process 074/17).

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