

## Vitamin and mineral supplementation improves erythrocytic recovery and productive performance of infected by gastrointestinal parasites

*A suplementação de vitaminas e minerais melhora a recuperação eritrocítica e o desempenho produtivo de cordeiros infectados por parasitas gastrointestinais*

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### ABSTRACT

The objective of the present study was to evaluate the effect of a vitamin and mineral complex, associated with the application of an efficient anthelmintic, in parasitized lambs, with characteristic signs of gastrointestinal nematode infection, on hematological clinical parameters of clinical improvement and weight gain. 60 lambs Australian Merino breed, with 8 and 9 months of age, were segregated in four groups: control (no supplementation); formula 1 (Iron Dextran, Organic Phosphorus, Cyanocobalamin and Vitamin k); formula 2 (Iron Dextran, Organic Phosphorus and Cyanocobalamin); and formula 3 (Vitamin k). Hematocrit, prothrombin time, total plasma proteins, color of the conjunctiva and weight gain were analyzed. The results were submitted to analysis of variance (ANOVA) through the repeated measures test. There was no statistical difference between treatments for the variables of hematocrit ( $P = 0.564$ ), prothrombin time ( $P = 0.911$ ) and plasma proteins ( $P = 0.6$ ), for the conjunctiva color variable there was a difference ( $P = 0.052$ ), with greater results for the groups supplemented with Vitamin K, Butafosfan, Cyanocobalamin and Iron (groups F1 and F3), as well as those same groups reached higher body weight at D35 ( $P = 0.023$ ). It can be concluded that the administration of vitamin and mineral complex,

associated with efficient anthelmintic, promoted a better performance in parasitized lambs.

**Keywords:** anemia, erythrocytes, lambs, nematodes

## RESUMO

O objetivo do presente estudo foi avaliar o efeito da aplicação de um complexo vitamínico e mineral, aplicado junto com a administração de um anti-helmíntico eficiente, em cordeiros parasitados, com sinais característicos de infecção por nematóide gastrointestinal, sobre os parâmetros clínicos hematológicos de melhora clínica e ganho de peso. Foram utilizados 60 cordeiros da raça Merino Australiano, com 8 e 9 meses de idade, segregados em quatro grupos: controle (não suplementado); fórmula 1 (Ferro Dextrano, Fósforo Orgânico, Cianocobalamina e Vitamina k); fórmula 2 (Ferro Dextrano, Fósforo Orgânico e Cianocobalamina); e fórmula 3 (Vitamina k). Foram analisados hematócrito, tempo de protrombina, proteínas plasmáticas totais (PPT), coloração da conjuntiva e ganho de peso. Os resultados foram submetidos à análise de variância (ANOVA) através do teste de medidas repetidas. Não houve diferença estatísticas entre os tratamentos para as variáveis de hematócrito ( $P=0,564$ ), tempo de protrombina ( $P=0,911$ ) e PPT ( $P=0,6$ ), para a variável de coloração da conjuntiva houve diferença ( $P=0,052$ ), com resultados superiores para os grupos suplementados com Vitamina K, Butafosfan, Cianocobalamina e Ferro (grupos F1 e F3), assim como esses mesmos grupos atingiram peso vivo no D35 superior ( $P=0,023$ ). Podendo concluir que a administração de complexo vitamínico e mineral, administrado em conjunto com anti-helmíntico eficiente, promoveu um melhor desempenho em cordeiros parasitados.

**Palavras-chave:** anemia, cordeiros, eritrócitos, nematoides

## INTRODUCTION

Gastric infection by nematodes is one of the main challenges of sheep farming. Nematodes are thought to infect 87.5% of the sheep in the state of Rio Grande do Sul, the most prevalent being parasites in the *Strongylida* order (Ferraz et al. 2019).

The treatment consists of periodic anti-helminthics; however, research suggest that dietary supplementation during this process improves the response reducing the animal's recovery time. These consist of vitamin and mineral supplements which improve coagulation and stimulate erythropoiesis. This has a positive impact on blood cell counts, and on the sheep's energetic recovery (MOTTIN et al., 2019; CASANOVA et al., 2018).

Vitamin K is one of the utilized supplements. This vitamin is responsible for the biosynthesis of coagulation factors, acting directly in the production of the gamma-carboxyglutamic amino acid (Gla). Other substances such as iron and vitamin B12 are also used because they act in the hematopoietic system accelerating the recovery of red blood cell counts to revert anemia (DEUBAX et al., 2019; ROCHA et al., 2018; AHMAD et al., 2019).

Phosphorus is also a valuable mineral during recovery since it acts in energy metabolism (ATP and ADP). Butafosfan is a commercially available phosphorus supplement. It is an injectable organic form of phosphorus which mitigates the impacts of heat stress by reducing cortisol and increasing insulin levels, which

increases the glucose availability improving cell metabolism and energy storage in the form glycogen, triglycerides, and proteins (MORAIS et al., 2018; TABELÃO et al., 2017).

Currently there are few studies evaluating vitamins and minerals as aids to the recovery of animals undergoing anti-helminthic treatment. Therefore, the goal of this study was to evaluate a vitamin and mineral supplement combined with an anti-helminthic and their effect on clinical parameters in helminth (*Strongylida* order) infected lambs. The parameters chosen were blood cell count, clinical improvement and weight gain of the lambs.

## MATERIALS AND METHODS

This study was developed at a grazing sheep farm in the state of Rio Grande do Sul, Brazil (coordinates 31 ° 30 '37,2 "south 53 ° 26' 11,3" west) between the months of March and April of 2017. All the procedures involving animals were approved by the Animal Experimentation Ethical Committee of the Federal University of Pelotas

(UFPEL) (project number 23110004100/2017-10).

Sixty Australian Merino lambs were utilized, 26 males and 34 females all ranging between 8 and 9 months of age. To calculate sample size, we considered 95% confidence interval, the standard deviation of continuous variables, and an error of 5%. In the beginning of the study the lambs weighed  $19,8 \pm 2,95$  kg on average and were randomly divided into four groups: control, formula 1, formula 2 and formula 3. To maintain homogeneity, the body weight and parasitic infection (eggs/g of feces) were considered for the allocation of the lambs into groups. The composition of the formulas was the following: 1 - iron dextran (625 mg), organic phosphate (250 mg) and cyanocobalamin (0,0125 mg), and vitamin K (7,5 mg); 2 - iron dextran (625 mg), organic phosphate (250 mg) and cyanocobalamin (0,0125 mg); 3 - vitamin K (7,5 mg). Lambs in the control group received 5 mL of saline solution.

**Table 1.** Number of animals, average initial weight, and eggs/g of feces in Australian Merino lambs

Characteristics	Control (c) <sup>1</sup>	Treatment <sup>2</sup>		
		F1	F2	F3
Number of animals	14	15	14	17
Weight	19,64±3,32	19,48±2,75	20,16±3,76	20,14±2,10
Eggs/g of feces	4407±1975	5007±4638	4392±2026	4829±2170

<sup>1</sup> Control group (c) received only the anti-helminthic;

<sup>2</sup> (F1) Formula 1: iron dextran-625 mg, organic phosphate-250 mg, cyanocobalamin-0,0125 mg e vitamin K-7,5 mg; (F2) Formula 2: iron dextran-625 mg, organic phosphate-250 mg e cyanocobalamin-0,0125 mg e (F3) Formula 3: vitamin K-7,5 mg.

All the lambs were treated anti-helminthic (10 mg / kg de Closantel Sodium - Diantel®, Hipra), and each group received their respective

supplement. Dosage was calculated based on the individual weight of the animals. Both the anti-helminthic and the supplements were delivered by a single

intra-muscular injection of D0. Additionally, on D0 we collected feces for parasitic analysis using the Gordon e Whitlock (1939) method.

Blood samples were also obtained in tubes containing sodium citrate to verify prothrombin time (Teste PT Hemostasis®). This test was repeated six hours after treatment at which point blood samples were collected in Vacutainer® tubes containing EDTA for further analysis in the biochemistry lab of the Center for Livestock Farming Research, Teaching and Extension (NUPEEC) at UFPEl. In the lab we performed red blood cell counts and evaluated total plasmatic protein (TPP). Red blood cell counts were carried out using the microhematocrit method, capillary tubes, and 5 minutes centrifugation at 10.000 rpm. TPP was determined using a commercial kit (Labtest Diagnóstica S.A., Brasil) and a visible light spectrophotometer (FEMTO 435®, Brasil). The total number of erythrocytes was determined using the automatic cell counter CC-530® (CELM, SP, Brasil), after diluting the samples in the liquid handler DA-500® (CELM, SP, Brasil).

On day 7 the fecal matter was analyzed to verify the efficacy of the anti-helminthic. Additionally, blood samples were drawn to evaluate the hematocrit, total plasmatic protein (TPP) and total erythrocytes. This was repeated on day 35.

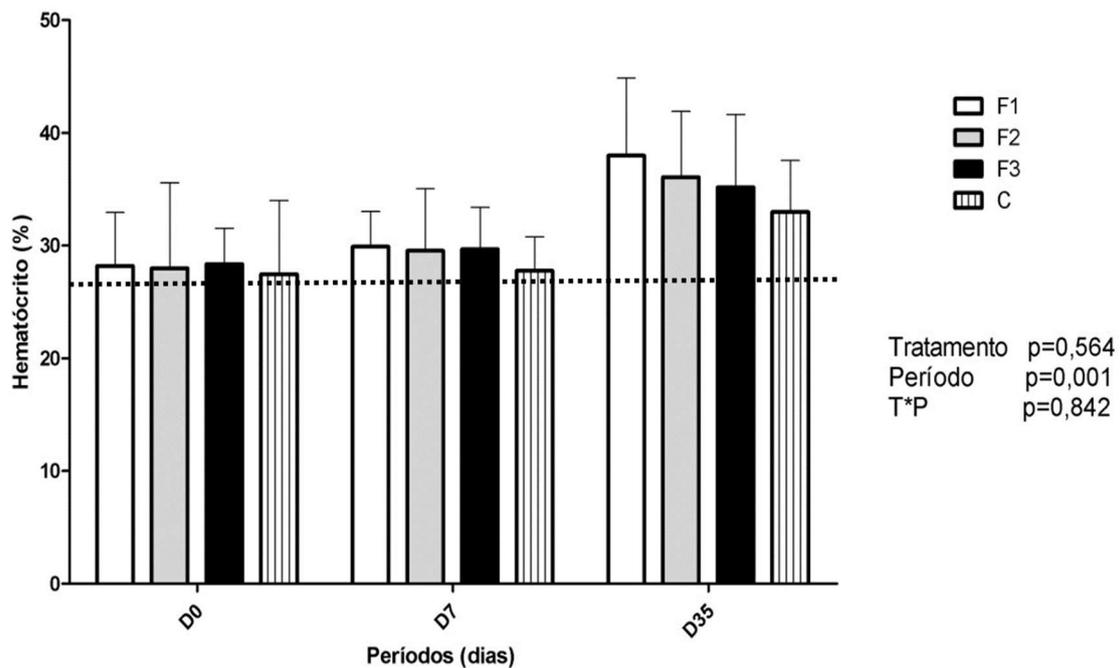
Animals were weighed on days 0, 7 and 35 using an electronic scale. Levels of anemia were also estimated on these

days based on the coloration of mucosal tissues according to the Famacha® score card (CINTRA et al., 2018).

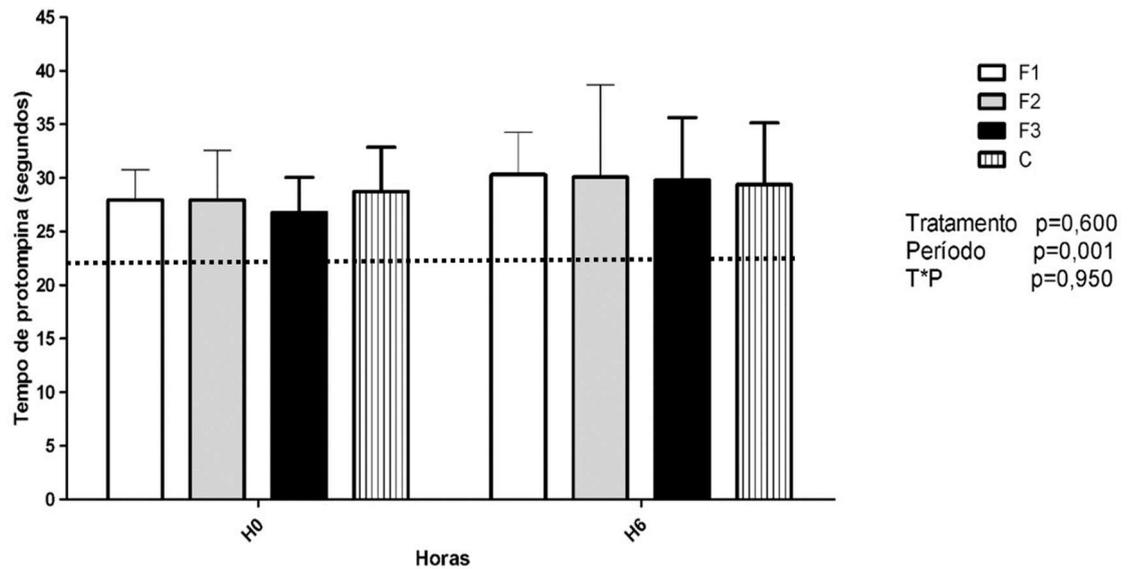
Normality tests were performed on all the variables using the Shapiro-Wilk test. Analysis of variance (ANOVA) was carried out on all the data using Mixed Procedure (PROC MIXED) and SAS (2001). Means were determined using LSMEANS; and comparisons between them were done by PDIFF and the Tukey test at 10% significance. Post-hoc tests were performed when the treatment by period interaction was significant ( $P < 0,10$ ). We considered  $P < 0,10$  significant since this was an exploratory study with few other like it.

## RESULTS

Lambs had high nematode (*Strongylida*) burdens on D0 (the global mean calculates based on 4 groups was  $4664 \pm 2826$  egg/g of feces). On D7 the average eggs/g was  $2,6 \pm 2,1$  demonstrating the efficacy of the anti-helminthic used in the treatment of parasitic gastrointestinal infections (Closantel Sodium - Diantel®, Hipra). The different formulations used as supplements did not affect the red blood cell counts ( $P = 0,564$ ), and there was no treatment by period interaction ( $P = 0,843$ ). Nevertheless, we observed an increase in the red blood cells throughout the experiment (Figure 1) ( $P < 0,10$ ). Treatments did not affect Prothrombin time, only the periods were different (Figure 2)



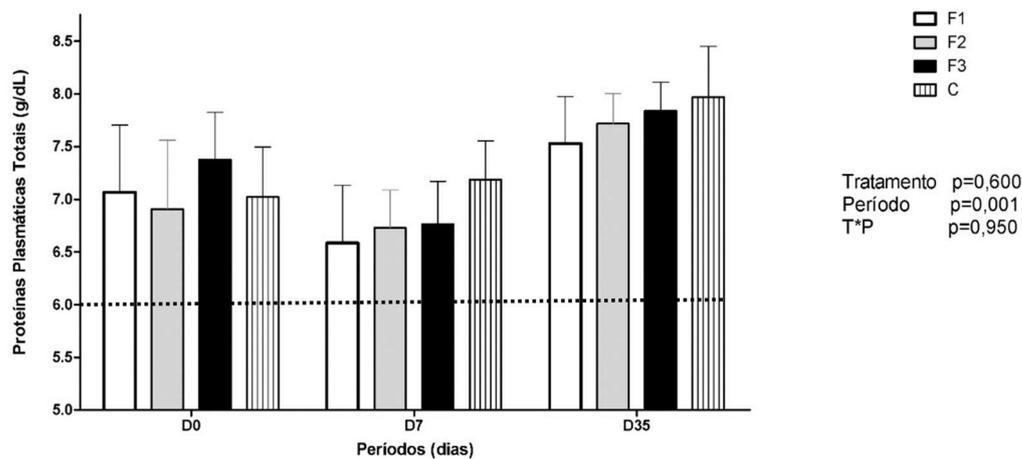
**Figure 1.** Mean hematocrit of Australian Marino lambs used in the experiment to evaluate recovery and outcome of anti-helminthic treatment under different supplementation regimens. [F1= formula 1 comprised of iron dextran (625 mg), organic phosphate (250 mg), cyanocobalamin (0,0125 mg) and vitamin K (7,5 mg); F2= Formula 2 comprised of iron dextran (625 mg), organic phosphate (250 mg) and cyanocobalamin (0,0125 mg); F3=Formula 3 containing only vitamin K (7,5 mg); and C= control, no supplementation]; P\*T = period\* treatment; Tukey test at 10% de significance; dotted line= inferior reference limit for the species



**Figure 2.** Prothrombin time (expressed in seconds) of Australian Marino lambs used in the experiment to evaluate recovery and outcome of anti-helminthic treatment under different supplementation regimens. [F1= formula 1 comprised of iron dextran (625 mg), organic phosphate (250 mg), cyanocobalamin (0,0125 mg) and vitamin K (7,5 mg); F2= Formula 2 comprised of iron dextran (625 mg), organic phosphate (250 mg) and cyanocobalamin (0,0125 mg); F3=Formula 3 containing only vitamin K (7,5 mg); and C= control, no supplementation]; P\*T = period\* treatment; Tukey test at 10% de significance; dotted line= inferior reference limit for the species

The results observed in figure 3 for total plasmatic protein show no difference between treatments or a treatment by

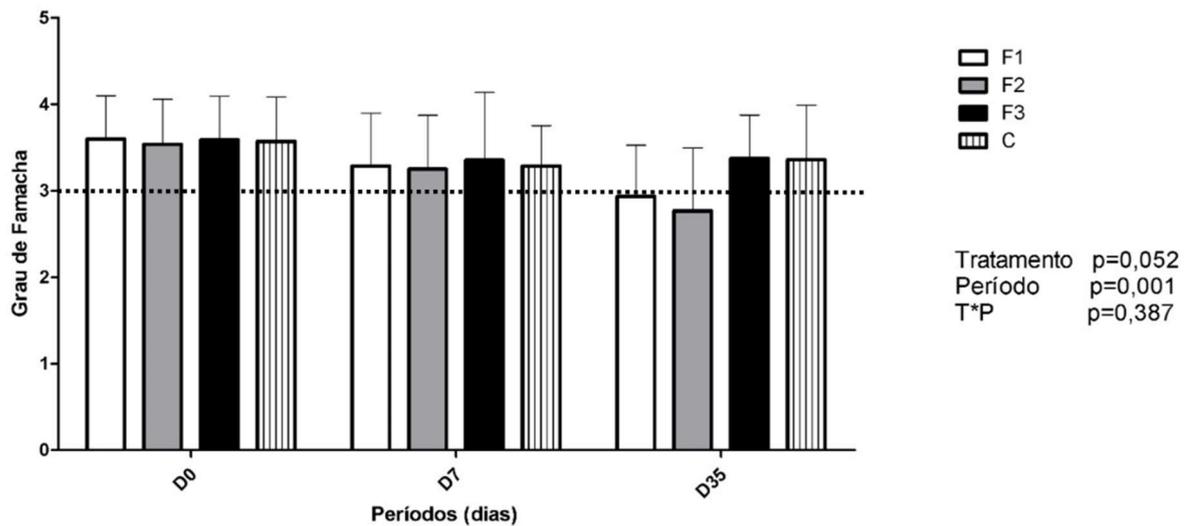
period interaction (Figure 3), with a statistical difference occurring only between (P>0,001).



**Figure 3.** Mean total plasmatic protein of Australian Marino lambs used in the experiment to evaluate recovery and outcome of anti-helminthic treatment under different supplementation regimens. [F1= formula 1 comprised of iron dextran (625 mg), organic phosphate (250 mg), cyanocobalamin (0,0125 mg) and vitamin K (7,5 mg); F2= Formula 2 comprised of iron dextran (625 mg), organic phosphate (250 mg) and cyanocobalamin (0,0125 mg); F3=Formula 3 containing only vitamin K (7,5 mg); and C= control, no supplementation]; P\*T = período\* treatment; Tukey test at 10% de significance; dotted line= inferior reference limit for the species.

We observed no period by treatment interaction in the mucosal tissue coloration ( $P=0,3870$ ), although there was a difference between treatments ( $P=0,052$ ). The group supplemented with iron, phosphate, cyanocobalamin, and vitamin K (F1) and the group supplemented with vitamin K (F3) had a

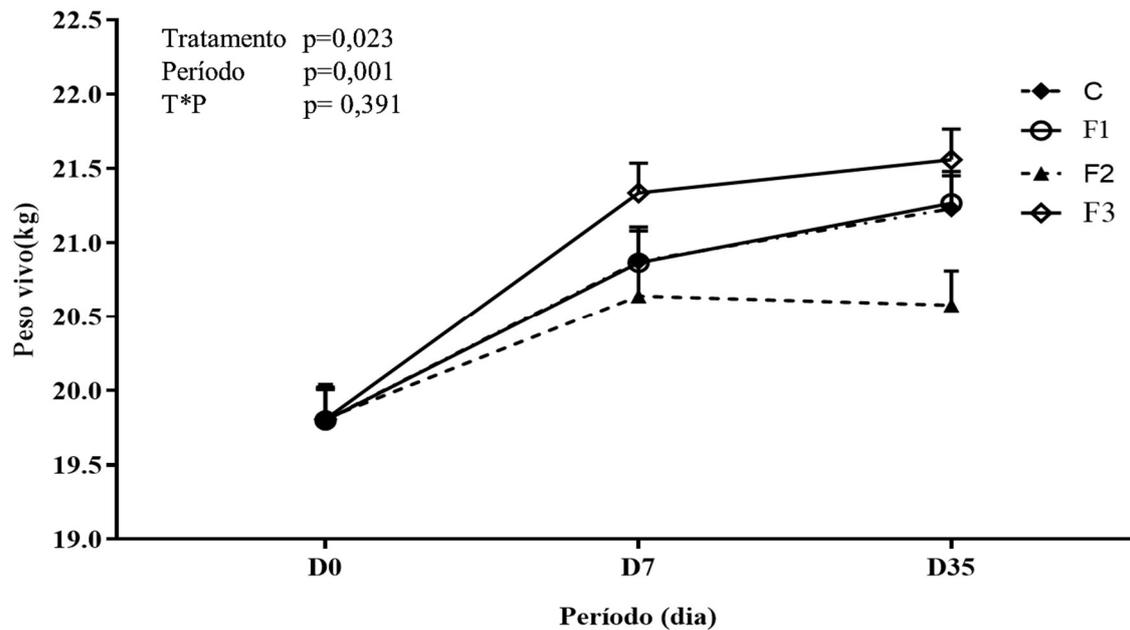
lower Famacha® score, indicating that the animals mucosal tissue had more pigmentation.



**Figure 4.** Mean Famacha® score in three evaluations done on Australian Marino lambs used in the experiment to evaluate recovery and outcome of anti-helminthic treatment under different supplementation regimens. [F1= formula 1 comprised of iron dextran (625 mg), organic phosphate (250 mg), cyanocobalamin (0,0125 mg) and vitamin K (7,5 mg); F2= Formula 2 comprised of iron dextran (625 mg), organic phosphate (250 mg) and cyanocobalamin (0,0125 mg); F3=Formula 3 containing only vitamin K (7,5 mg); and C= control, no supplementation]; P\*T = period\* treatment; Tukey test at 10% de significance; dotted line= inferior reference limit for the species.

A statistically significant difference was observed between the periods for weight ( $P < 0,001$ ), with lambs displayed higher weight starting on D7. At the end of the experiment (D35) the animals had gained 1.35 Kg (Figure 5). The control

group gained the least amount of weight in the experimental period in comparison with the other groups. The group supplemented with iron dextran, phosphate and cyanocobalamin had the highest weight gain ( $P < 0,10$ ) (figure 5).



**Figure 5.** Mean weight (live animal weight in Kg) of Australian Marino lambs used in the experiment to evaluate recovery and outcome of anti-helminthic treatment under different supplementation regimens. [F1= formula 1 comprised of iron dextran (625 mg), organic phosphate (250 mg), cyanocobalamin (0,0125 mg) and vitamin K (7,5 mg); F2= Formula 2 comprised of iron dextran (625 mg), organic phosphate (250 mg) and cyanocobalamin (0,0125 mg); F3=Formula 3 containing only vitamin K (7,5 mg); and C= control, no supplementation]; P\*T = period\* treatment; Tukey test at 10% de significance; dotted line= inferior reference limit for the species.

## DISCUSSION

There was a linear increase in the red blood cell counts of the animals between periods. This suggests erythrocytic recovery post infection, since these parasites are hematophagous agents which significantly reduce the red blood cell levels in their host (HERNANDEZ et al., 2016; ROUAUTBI et al., 2016). However, lambs in the control group had a reduction in the first week of treatment, which is the opposite of what we observed in the other groups. This probably occurred because these animals did not receive vitamin and mineral supplementation.

An increase in red blood cells had been previously reported in iron supplemented lambs (Casanova et al., 2018). This probably occurs since iron is an important component of non-enzymatic metalloproteins such as hemoglobin, ferredoxin and ferritin. These results were also consistent with data by Rocha et al. (2018). Lambs supplemented with phosphate and cyanocobalamin (vitamin B12) also displayed better recovery since B12 is essential for the formation of red blood cells as well as formation and maturation of erythrocytes (MORAIS et al., 2018; DEUBAX et al., 2019; ROCHA et al., 2018; AHMAD et al., 2019).

Similarly, vitamin K also leads to positive outcomes since it acts on

homeostasis, facilitating blood clotting after parasitic death since nematode parasites cause temporary hemorrhaging. Nevertheless, we observed no difference in prothrombin time, between groups ( $P = 0,96$ ) (CHENG et al., 2015).

In this study the total plasmatic protein (TPP) of lambs in different groups were similar. This result is consistent with Hupp et al. (2018), the only difference were the periods in which the increase in TPP occurred. This suggests hematological recovery after the anti-helminthic. Moraes et al. (2012) also detected a negative correlation between TPP levels and nematode infection levels.

When correlating the TPP and red blood cell results we noticed that both parameters were lower in the control group. This suggests that the control animals had slower recoveries in comparison to the others.

A previous study demonstrated that animals infected with a gastrointestinal nematode (*H. contortus*) had lower TPP and red blood cell counts since these parameters are directly affected by the level of anemia.

The animals supplemented with iron, phosphate, cyanocobalamin, and vitamin K (F1); and those supplemented with vitamin K (F3) had a more noticeable reduction in coloration of mucosal tissue (SOTOMAIOR et al. 2012).

The positive response of these groups was probably due to supplementation with vitamin K. This vitamin acts as a co-factor for carboxylating specific residues of glutamic acid to form gamma carboxy-glutamic acid (Gla). This amino acid is present in coagulation factors II, VII, IX e X. Considering this, it is possible that supplementation with

vitamin K influenced the mucosal coloration leading it to recover the physiologically healthy color faster.

Gastrointestinal parasites cause not only sanitary problems but are also the main limiting factor in zotechnical aspects, including delaying growth and productive parameters. Considering this, we evaluated weight gain since 43% of the sheep in our state is for meet and 49% for the wool and meet. Therefore, weight gain and final weight are used indicators of productivity for 92% of the state's sheep herd (MOTTIN et al., 2019; SILVA et al. 2013).

In this study, the weights of the lambs on D35 were different between groups and periods. Groups supplemented with iron, phosphate, cyanocobalamin, and vitamin K (F1); or just vitamin K (F3) had the highest weights, respectively.

The mechanism of action of vitamin K is directly linked to blood clotting, attenuating the momentary hemorrhage caused by the parasitic death allowing a faster recovery for the animals. This likely allowed the lambs in this study to allocate more energy towards weight gain since these other physiological needs had been met (DEUBAX et al. 2019; CHENG et al. 2015; KLACK & CARVALHO, 2006).

We expected the group supplemented with phosphate and cyanocobalamin to have the highest final weight since these components act positively on energy metabolism either through the Krebs Cycle increasing gluconeogenesis or by acting directly in ATO synthesis (ROLIN et al. 2010). Dairy cattle supplemented with phosphate and cyanocobalamin increase their dry matter intake, which would contribute to the higher weight gain (Pereira et al., 2013). However, in this study lambs did not increase their dry matter intake.

One of the limiting factors of this study was the allocation of animals into groups. Since hematological and clinical parameters were not considered this may have affected the results. Furthermore, it would be interesting to evaluate the clinical recovery of the sheep in shorter time intervals to obtain more detailed data.

In conclusion, supplementation of lambs, treated with an effective anti-helminthic, with a mineral and vitamin complex promoted better recovery of animals infected with nematodes of the *Strongylida* order, increasing weight gain and final weight on D35.

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