Effects of protein supplementation on dairy goats naturally infected with gastrointestinal nematodes in Valle de Lerma, province of Salta, Argentina

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ABSTRACT

This work aimed to evaluate the effects of protein and copper supplementation in dairy goats naturally infected with gastrointestinal nematodes (GIN) in Valle de Lerma, province of Salta, Argentina. Three groups of 10 animals each were tested: GSP (group supplemented with 19% protein and copper administered subcutaneously at a rate of 0.5 ml per 20 kg of weight at the beginning of the trial), GSPD (group supplemented with protein and copper as GSP and dewormed monthly with Derquantel + Abamectin), and CG (control group, without supplementation or deworming). The animals were monitored every 15 days from the beginning of March 2021 until kidding, at the end of July 2021. The animals were clinically examined and Famacha and body condition were determined. In addition, hematocrit and serum protein were detected and coprological analyses were performed to confirm EPG and nematode prevalence. After kidding, daily milk production of each animal was measured up to 90 days of lactation. The prevalent GIN genera were *Haemonchus* and *Trichostrongylus*. At parturition, CG differed significantly in the average GIN egg count from GSP and GSPD, with 4035 (±4873.8) for CG, 1225 (±1173.5) for GSP and 983 (±1854.8) for the GSPD. Regarding physiological parameters, the hematocrit was 27.7% (±4.4) in CG, 31.7% (±5.1) in GSP, and 32% (±3.5) in GSPD; total serum proteins were 4 g/dl (±0.62) in CG, 5.8 g/dl (±0.69) in GSP and 6.2 g/dl (±0.61) in GSPD. Milk production in the treated groups (GSPD: 167.7l ± 18.2 l; GSP: 153.3 ± 18.2 l) was higher than in CG (85.7 ± 19.2 l). Protein supplementation had a positive effect on parasitological, physiological, and productive parameters. Under the conditions of this trial, it would be advisable to supplement the animals 45 days before kidding to enhance GIN control and milk production.

Keywords: gastrointestinal nematodes, caprine, protein, supplementation, productivity.

RESUMEN

El objetivo de este trabajo fue evaluar los efectos de la suplementación con proteína y cobre en cabras lecheras naturalmente infectadas con nematodos gastrointestinales (NGI) en el Valle de Lerma, provincia de Salta, Argentina. Se analizaron tres grupos de 10 animales cada uno: GSP (grupo suplementado con 19% de proteína y cobre administrado por vía subcutánea a razón de 0,5 ml por cada 20 kg de peso al inicio del ensayo), GSPD (grupo suplementado con proteína y cobre como GSP y desparasitados mensualmente con Derquantel + Abamectina), y GC (grupo control,

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sin suplementación ni desparasitación). Se realizaron monitoreos cada 15 días desde principios de marzo de 2021 hasta el parto, a fines de julio de 2021. Además, los animales fueron examinados clínicamente y se determinó su condición de Famacha y corporal. Se examinó hematocrito y proteínas séricas, y se realizaron análisis coprológicos para confirmar y determinar la prevalencia de nematodos y EPG. Luego del parto, se midió la producción diaria de leche de cada animal hasta los 90 días de lactancia. Los géneros NGI prevalentes fueron Haemonchus y Trichostrongylus. En el momento del parto, CG difirió significativamente en el recuento de huevos NGI promedio de GSP y GSPD, con 4035 (±4873.8) para CG, 1225 (±1173.5) para GSP y 983 (±1854.8) para GSPD. En cuanto a los parámetros fisiológicos, el hematocrito fue de 27,7% (±4,4) en GC; 31,7% (±5,1) en GSP y 32% (±3,5) en GSPD; las proteínas séricas totales fueron 4 g/dl (±0,62) en GC, 5,8 g/dl (±0,69) en GSP y 6,2 g/dl (±0,61) en GSPD. La producción de leche en los grupos tratados (GSPD: 167,7 l ± 18,2 l; GSP: 153,3 ± 18,2 l) fue mayor que en el GC (85,7 ± 19,2 l). La suplementación proteica tuvo un efecto positivo sobre los parámetros parasitológicos, fisiológicos y productivos. Bajo las condiciones de este estudio, sería recomendable suplementar a los animales 45 días antes del parto para mejorar el control de NGI y la producción de leche.

***Palabras clave**: nematodos gastrointestinales, caprino, proteína, suplementación, productividad.*

INTRODUCTION

The impact of gastrointestinal nematode (GIN) infestation on production is one of the most important health problems in pastoral goat production systems worldwide (Stear *et al*., 2011; Suarez *et al.*, 2013, 2021). Currently, the most widespread and almost exclusive tool to control GINs is anthelmintic treatment (Cerutti *et al*., 2018). However, the high frequency of deworming (Suarez and Cristel, 2014) without considering the regional epidemiology of GINs and the dynamics of the larvae in the refuge (Van Wyk, 2001) were the main causes that triggered the development of anthelmintic resistance (AR), making GIN control difficult. Another factor that contributes to AR development is underdosing, which in the case of goats is due to the low availability on the market of anthelmintic drugs approved for use in this species (Anziani and Fiel, 2015). Therefore, in this AR context, there is a need to search for alternatives to the chemical control of GIN.

One of the alternative GIN control methods is the use of different chemical compounds, such as copper oxide and/or concentrated tannins (Waller *et al*., 2014; Sandoval *et al*., 2018; Suarez *et al.*, 2019). Supplementation with protein concentrates has also been shown to decrease GIN loads in sheep (Abbott *et al*., 1986, 1988; Van Houtert *et al*., 1994; Bricarello *et al*., 2005) and goats (Nnadi *et al*., 2009). In those works, the GIN loads in sheep subjected to different protein supplementation schemes were measured. The results showed that the animals supplemented with proteins had a lower GIN load than a control group. Therefore, protein supplementation could be an alternative in farms where the AR phenomenon occurs. In the northwestern region of Argentina (NOA region), where AR has been detected in goats (Aguirre *et al*., 2000 a,b; 2002; Suarez *et al*., 2012), the study of this type of alternative is of great regional interest. Therefore, the objective of this work was to evaluate the effects of protein and copper supplementation on dairy goats infected with GINs in Valle de Lerma, province of Salta.

MATERIALS AND METHODS

Study area: The work was carried out in the Dairy Goat Unit of the Salta Agricultural Experimental Station of INTA, Cerrillos, province of Salta (24°53’32.65°28’26.4”W), Valle de Lerma, Argentina. The area is characterized by a sub-Andean mountain climate, an average altitude of about 1050 m a.s.l., and a summer rainfall regime, followed by a winter-spring dry period (Bianchi and Bravo, 2008).

Experimental design: The study involved pregnant goats of the Saanen breed and was conducted from March 5 2021 until the end of October 2021. The animals were divided into three groups (10 animals each) according to the number of kiddings, previous milk production and estimated time of calving: GSP, goats supplemented with 19% protein (200 g of commercial balanced Starter Calf Concentrate 40% Rearing 30%, Santa Sylvina) and copper (administered subcutaneously at a rate of 0.5 ml per 20 kg of weight at the beginning of the trial); GSPD, goats supplemented with the same amount and type of protein and the same amount of copper as GSP, and dewormed monthly with Derquantel + Abamectin at a rate of 1.2 ml/5 kg LW (STARTEC Derquantel 1% + Abamectin 0.1%); and CG, control group with no protein supplementation (PS) or deworming. In the three groups, goats with an egg count per gram of feces (EPG) greater than 3000 or with clinical signs compatible with GIN infection (diarrhea, Famacha scores 4-5, poor condition) were treated immediately with Monepantel at a rate of 1ml/10 kg LW (Zolvix Monepantel 2.5%).

The three groups grazed together alfalfa (13% protein) for 8 h daily. A rotational grazing system was used on daily strips and at a rate of 15 goats per ha. After grazing, in the afternoon, goats were provided the protein supplement in feeders, ensuring 30 cm of feeding frontage per animal. In mid-May, 45 days before the expected kidding date, goats were penned until parturition and received a daily ration of 4 kg DM of premium quality alfalfa hay and 0.5 kg of corn. Finally, after parturition, the goats were taken to an oat pasture paddock.

Sampling and parameters evaluated: From March 5 until the moment of kidding in July, fecal and blood samples were taken every 15 days. Fecal samples were processed using the McMaster technique modified by Roberts and O’Sullivan (1949) to determine eggs per gram of feces (EPG). To determine the parasitic genera, pool samples from each of the experimental groups were cultured according to the technique described by Suarez *et al*. (1997). Parasitic genera were identified following the keys described by Niec (1968). Serum protein was determined by refractometry, copper dose in blood by atomic absorption spectrophotometry and hematocrit (HCT) from blood samples. In addition, at the time of sampling, the body condition (BC) was scored based on palpation of the lumbar vertebrae, as described by Russel *et al*. (1969), and weight and Famacha score were recorded to clinically evaluate the level of anemia in all the animals (Van Wyk and Bath, 2002). Finally, individual daily milk production was measured during the first 90 days of lactation. At kidding, the individual weight of the kids born per goat was recorded.

Statistical analysis: The variables EPG, HCT, serum protein, Famacha score, BC, total weight of the kids born per goat, and total milk production at 45 and 90 days with normal distribution were subjected to analysis of variance; those values that were not normally distributed were subjected to the Kruskal Wallis test. The EPG was naturally log transformed before analysis (x= ln (x+1)) (Di Rienzo *et al*., 2008). Analyses were adjusted to goats with less or more than one kidding and previous milk production of less or more than 2 liters per day.

RESULTS

Figure 1 shows the dynamics of EPG from the start of the trial to parturition in the three experimental groups. Before confinement, EPG of GSPD was significantly ($p<0.03$) lower than that in the other two groups. At kidding, significant differences ($p<0.0011$) were observed between the control group (CG) and the groups supplemented with protein and copper (GSP and GSPD). The average EPG at parturition was 4035 (±4873.8) for CG, 1225 (±1173.5) for GSP, and 983 (±1854.8) for GSPD.

The distribution of the parasitic genera showed a predominance of *Haemonchus* spp. and *Trichostrongylus* spp. The former showed its highest prevalence until May. Then, *Trichostrongylus* spp. prevailed up to the time of kidding. The genus *Teladorsagia* sp. was observed in a low percentage throughout the study. Nematode genera had the same prevalence in all the study groups. Figure 2 shows the dynamics of the average genus prevalence relative to the average EPG count of the three experimental groups.

Differences in HCT (table 1) were statistically significant ($p<0.05$) between CG and the treated groups (GSP and GSPD) only at the time of kidding, with mean values of 27.7% (±4.4) in CG, 31.7% (±5.1) in GSP and 32% (±3.5) in GSPD.

Serum protein showed statistically significant differences ($p<0.014$) between CG and GSPD at the time of kidding, with no differences being observed between CG and the GSP ($p>0.05$) (table 1).

Copper did not show statistically significant differences among groups, with average values of 89 µg/dl for CG, 93 µg/dl for GSP, and 94 µg/dl for GSPD.

Clinical examination of the animals during the trial (table 2) showed no statistical differences in BC. However, in the case of the Famacha scale, statistically significant differences ($p<0.023$) were observed between the CG and the treated

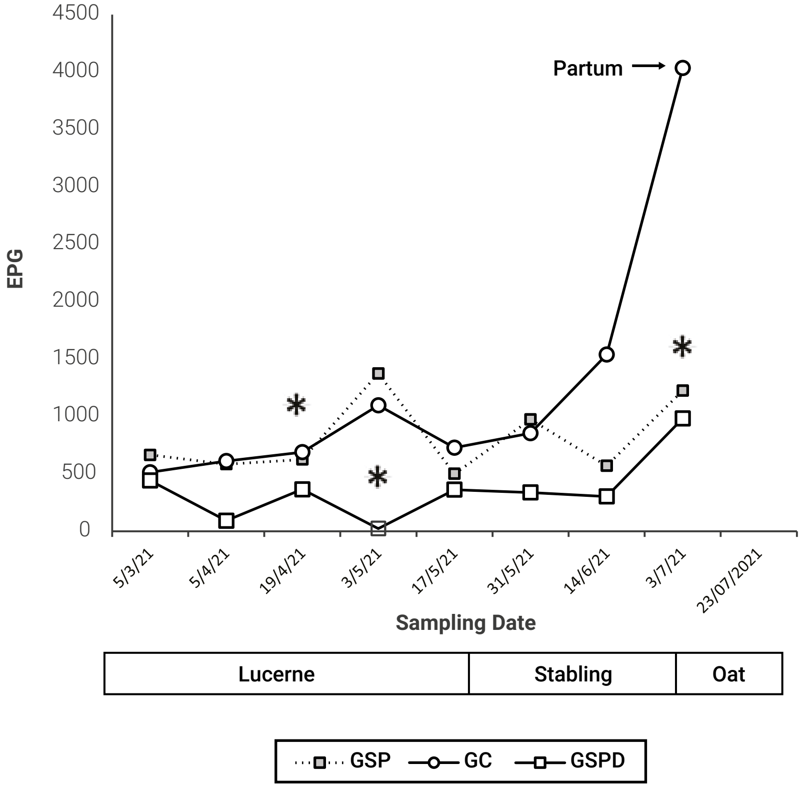

Figure 1. Dynamics of EPG in the experimental groups throughout the trial. GSP: Group with protein supplementation; GSPD: group with supplementation and dewormed with Abamectin-Derquantel; CG: Control group*.
*Means with significant differences ($p<0.05$).

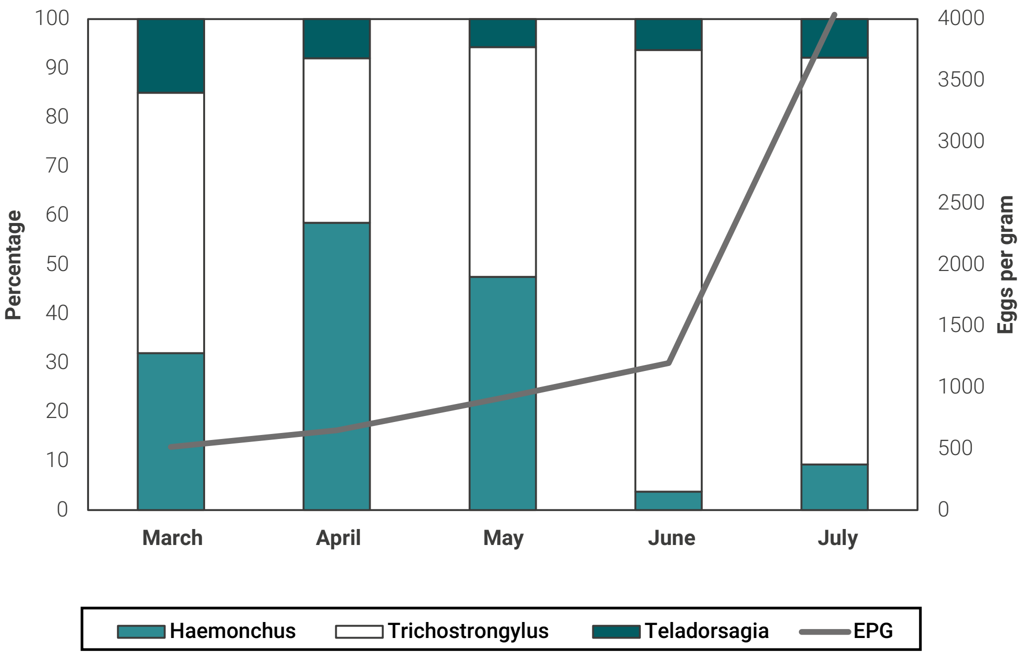

Figure 2. Percentage of GIN genera relative to the average EPG of the three experimental groups throughout the sampling period (from March to July 2021).

Parameter	Group	5/3/21	5/4/21	19/4/21	3/5/21	17/5/21	31/5/21	14/6/21	28/6/21
Hematocrit (%)	GSP	30.4	29.3 a	29.3	28.2	28.9	29.2	29.6	31.7 b
	CG	32.9	32.8 b	31.2	30.5	31.4	30.8	29.4	27.8 a
	GSPD	31.4	31.0 b	30.6	30.3	30.8	31.5	32.6	32.0 b
Serum Protein (g/dl)	GSP	6.2	6.2 a	6.1	5.9	5.9	6.0	5.6	5.8 a
	CG	6.7	6.6 b	6.5	6.3	6.1	6.0	5.7	5.4 ab
	GSPD	6.6	6.6 b	6.5	6.4	6.2	6.2	6.2	6.2 b

Table 1. Mean hematocrit and total protein in the three experimental groups (GSP: Group supplemented with protein and copper; GSPD: Group supplemented with protein and copper, and dewormed with Abamectin-Derquantel; CG: Control group) throughout the sampling period*.
*Different letters indicate significantly different values (p<0.05).

Parameter	Group	5/3/21	5/4/21	19/4/21	3/5/21	17/5/21	31/5/21	14/6/21	28/6/21
Body Condition	GSP	2.8	2.9	3.1	3.2	3.2	3.1	2.8	2.7
	CG	3.1	3.2	3.1	3.2	3.1	3.0	2.9	2.7
	GSPD	2.8	3.0	3.1	3.1	3.1	3.1	2.9	2.9
Famacha (%)	GSP	7.7	7.7	0.0	0.0	0.0	0.0	0.0	8,3 b
	CG	0.0	0.0	0.0	7.7	7.7	7.7	7.7	30.8 a
	GSPD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 b

Table 2. Average body condition and percentages of animals with Famacha 4 and 5 scores in the experimental groups (GSP: Group supplemented with protein and copper; GSPD: Group supplemented with protein and copper, and dewormed with Abamectin-Derquantel; CG: Control group)*.
*Different letters indicate significantly different values (p<0.05).

groups (GSP and GSPD) only at kidding, with the percentage of goats with Famacha scores 4 and 5 in CG (30.8%) being higher than that of GSP (8.3%). Finally, none of the animals presented any health problem that prevented them from continuing to participate in the study.

Total milk production at 45 days postpartum was significantly (p<0.0091) higher in GSP (83.7 ± 9.5 l) and GSPD (90.6 ± 9.5 l) than in CG (46.9 ± 10.1 l). At 90 days postpartum, GSP (153.3 ± 18.2 l) and GSPD (167.7 ± 18.2 l) also showed a significantly (p<0.03) higher yield than that of the CG (85.7 ± 19.2 l). Figure 3 shows the lactation curves of the three experimental groups from kidding to 90 days.

Production of kids -evaluated as the total weight produced by each goat showed no differences (p<0.41) among groups. Live weight at birth was on average 5.41 ±0.74 (CG), 5.96 ±0.77 (GSP), and 6.83 ±0.74 (GSPD) kg.

DISCUSSION

Results of parasitological parameters showed that the positive impact of supplementation was evident mainly at the time of kidding, when there was a significant reduction in the elimination of GIN eggs. Similarly, differences in parasitic *H. contortus* loads were reported in groups of lambs with protein supplementation (PS) and a control group (Abbott *et al*., 1985, 1988; Bricarello *et al*., 2005). Van Houtert *et al*. (1994) also observed that in the group with PS, the load of *T. columbriformis* based on the count of adult specimens was lower than in a control group (without PS). By contrast, Abbott *et al*. (1986) did not observe significant differences in EPG and the load of adult specimens of *H. contortus* in the digestive tract of lambs between groups treated with high (169 g/kg DM) and low (88g/kg DM) PS proportion. Our results also disagree with the findings of Van Houtert *et al*. (1995) in sheep, who reported no significant differences in the effect of PS on EPG and the load of adult GIN of *H. contortus* and *T. columbriformis*, between a control group and a group treated with PS. However, both Abbott *et al*. (1986) and Van Houtert *et al*. (1995) conclude that PS would reduce the impact of parasites on animals. Although neither work reported statistically significant differences between groups, in both works the group treated with PS had a greater live weight gain than the control group. In turn, animals with high protein concentration were found to be less markedly affected by *H. contortus* infestation than a group with low protein concentration (Abbott *et al*., 1986).

Regarding works involving goats, Nnadi *et al*. (2009) studied West African Dwarf goats in Nigeria from the first day of gestation until 6 weeks postpartum and evaluated the effect of PS on productivity relative to *H. contortus* burden. The authors found that PS increased the animal capacity to resist the establishment of *H. contortus*. In the present study, an abrupt drop in the prevalence of this species was observed from the middle of the trial until the moment of kidding, both in the treated groups and the CG. In this work, the effect of PS on *H. contortus* burden was not evaluated.

The pathogenic effect of *Haemonchus* spp. and *Trichostrongylus* spp., measured through biochemical and clinical parameters, differed between animals supplemented or not with protein and copper, as observed in FAMACHA and Hematocrit results at the time of kidding. Interestingly, in the case of FAMACHA, the results showed that the highest values of the scale (4-5) were observed when the prevalence of Haemonchus spp. was lower. This could be related to the fecal matter pool, which had a higher representation of animals with lower FAMACHA values. This effect of PS is relevant from a productive and epidemiological point of view, because pro-

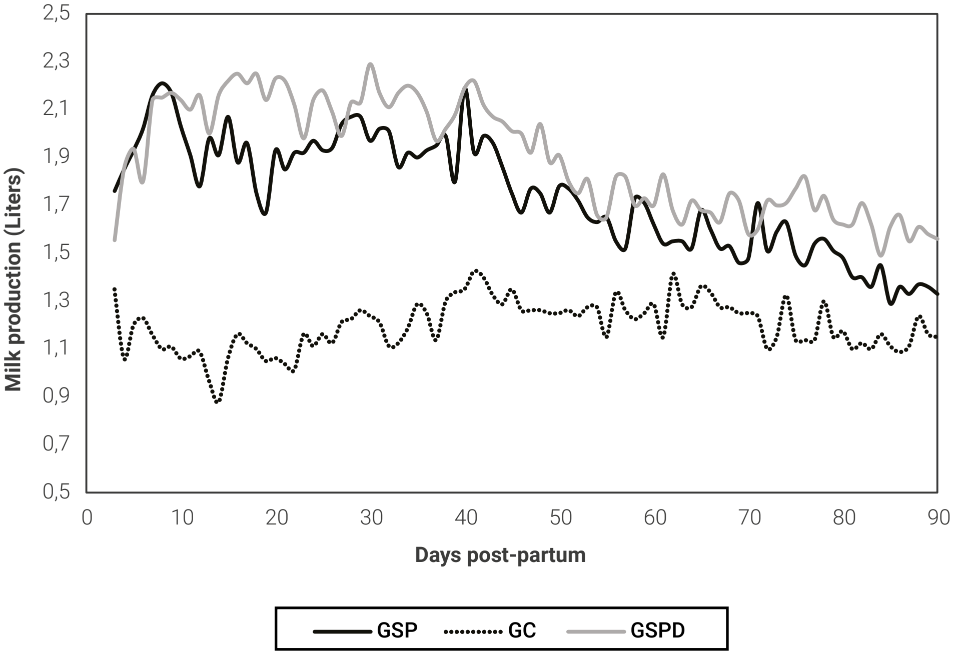

Figure 3. Average daily milk production of the experimental groups. (GSP: Group supplemented with protein and copper; GSPD: Group supplemented with protein and copper, and dewormed with Abamectin-Derquantel; CG: Control group).

tein-supplemented animals are in better conditions to face the negative energy balance of the first third of lactation (Nsalahi *et al*., 2004). These results agree with several works describing the mitigating effects of PS on infestations by *H. contortus* (Abbott *et al*., 1985, 1986,1988; Van Houtert *et al*., 1994; Bricarello *et al*., 2005), by *T. columbriformis* in lambs (Van Houtert *et al*., 1994) and by *H. contortus* in goats (Blackburn *et al*., 1991,1992; Nnadi *et al*., 2007,2009). On the other hand, in terms of serum protein values, no statistically significant differences were observed between groups until the time of kidding, when a higher percentage of animals was below the range of reference values (6.0-7. 8 mg/dl) (Matthews, 1999) in the CG (53.8%) than in the GSP (41.7%) and GSPD (23.1%). By contrast, previous reports did not find an effect of different levels of PS on serum protein, which reached values between 4.4 g/dl and 2.6 g/dl (Abbot *et al*., 1985,1986,1988). It is important to note that the pathogenic effect of GINs on animals was controlled through the treatment with Monepantel, with no differences in the number of treatments between CG and GSP. However, GSPD did not require treatment with Monepantel, which may be related to the previous deworming of the group with the combination of Abamectin + Derquantel.

The greatest differences among groups were observed in the impact of PS on milk production. Indeed, GSP and GSPD produced a significantly higher quantity of milk than CG. On the other hand, there were no differences in the live weight of the kids at birth. This result may be explained by the lack of periods of deficiencies in the nutritional requirements during pregnancy throughout the trial and that the influence of PS on GIN was not evaluated at the time the goats were served.

The response of the evaluated parameters to PS was evidenced at parturition and in the subsequent milk production. The results show a positive effect of PS, since animals with high production are considered more susceptible to GIN infestations (Hoste *et al*., 2005). In this sense, the negative effect of GIN on milk production has been frequently reported in goats (Hoste and Chartier, 1993; Chartier and Hoste, 1994; Hoste *et al*., 1999; Suarez *et al*., 2017). Similarly, Chartier *et al*. (2002) suggest that PS in goats before parturition could reduce the magnitude of the peripartum EPG peak described in small ruminants. In this line, Etter *et al*. (1999) observed that in animals supplemented with different protein concentrations, the peripartum peak of EPG was lower than in a control group, demonstrating that such peak, which is caused by relaxation of immunity of the animals in that period, may be related to their nutritional levels. Overall, our observations agree with previous works, since the groups with PS had higher milk yields and lower EPG than the control group.

CONCLUSIONS

The results of this work allow us to determine that PS has an effect on most of the studied parameters, except for BC. The greatest difference was observed in the response of the parameters at kidding, with the supplemented animals being the supplemented animals less affected by the GIN infestation and presenting better values of productive parameters than the untreated animals. In contexts of anthelmintic resistance, where GINs affect the health of animals and, consequently, their production, the use of protein supplementation can support anthelmintic treatment. Thus, supplementation of the animals 45 days before parturition could be recommended to enhance the anthelmintic effect, mitigating the effect of GIN and increasing postpartum milk production.

AUTHOR CONTRIBUTIONS

LH Olmos, GM Martínez and VH Suárez contributed to the study conception and design. Material preparation, data collection and analysis were performed by LH Olmos, JP Díaz, LA Colque-Caro, E Alfaro and J Alfaro. The first draft of the manuscript was written by LH Olmos and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

DECLARATIONS

Ethics approval: This study has been approved by a research ethics committee of the National Institute of Agricultural Technology of Argentina (Regional Centre Salta—Jujuy). Acta No 14/20 of 18th August 2020.

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Consent to participate: Authors have permission to participate.

Consent for publication: Authors have permission for publication.

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