https://doi.org/10.52973/rcfcv-e34438 Revista Científica, FCV-LUZ / Vol. XXXIV, rcfcv-e34438

Effect of supplementation on estimated parasite load in periparturient ewes and their offspring

Efecto de la suplementación sobre la carga parasitaria estimada en ovejas en periparto y su descendencia

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ABSTRACT

The objective of the essay was to determine the influence of protein-energy supplementation on the gastrointestinal nematode population during spring rise in ewes and their offspring as a non-chemical alternative in Integrated Parasite Control. One hundred twenty six Corriedale ewes were divided into two groups, one supplemented with protein-energy blocks from one month before lambing until weaning and the other without supplementation. Every 17 days, faecal matter was randomly collected from 20 ewes and 20 lambs from both groups. Modified McMaster and coproculture were performed, estimating the pathogenicity index for each gender. Lambs´ weight at birth, marking and weaning were recorded and the daily weight gains from birth to marking and from marking to weaning were calculated. Eggs count per gram of faeces were higher (P<0.05) in the non-supplemented group and their lambs. The predominant genus in dams and lambs were *Haemonchus contortus* and *Trichostongylus* spp. The pathogenicity index in the non-supplemented dams was higher than 1 from the faecal egg count increase. The lamb body weights and average daily gains were higher in those lambs born to the supplemented dam group (P<0.05). In conclusion, the supplementation contributed to the non-chemical control of the most prevalent gastrointestinal nematode in periparturient ewes, *H. contortus*, and environmental contamination for lambs at the dam foot.

Key words: Supplementation; sheep; lamb; gastrointestinal nematode

RESUMEN

El objetivo del ensayo fue determinar la influencia de la suplementación proteico-energética sobre la población de nematodos gastrointestinales durante el alza de lactación en ovejas y sus corderos como alternativa no química en el Control Integrado de Parásitos. Se dividieron 126 ovejas Corriedale en dos grupos, uno fue suplementado con bloques proteico-energéticos desde un mes antes del parto hasta el momento del destete y el otro grupo permaneció durante el mismo período sin suplementación. Cada 17 días se recogió materia fecal de 20 ovejas y 20 corderos de ambos grupos elegidos al azar. A estas muestras se les realizó las técnicas de McMaster modificado y coprocultivo, estimándose además el índice de patogenicidad para cada género. Se registró el peso de los corderos al nacimiento, en el momento de la realización de la marca de propiedad en la oreja y al destete. Se calculó la ganancia diaria de peso entre el nacimiento y el momento de la marcación en la oreja y entre este último y el destete. El contaje de huevos por gramo de materia fecal fue mayor (P<0,05) en el grupo no suplementado y en sus corderos. Los géneros predominantes en las madres y en los corderos fueron *Haemonchus contortus* y *Trichostongylus* spp. El índice de patogenicidad en las madres no suplementadas fue superior a 1 debido al aumento del recuento de huevos en materia fecal. El peso vivo y la ganancia media diaria de los corderos fueron mayores en los corderos nacidos del grupo de madres suplementadas (P<0,05). En conclusión, los bloques proteico-energéticos contribuyeron al control no químico de *H. contortus* (alza de lactación), retrasando dos semanas su presentación, además, con la disminución significativa del pico de huevos en materia fecal, la contaminación ambiental de los corderos al pie de la madre disminuyó, lo que resultó en un recuento de huevos en materia fecal significativamente más bajo y en corderos con mayor peso vivo al nacer y ganancia media diaria. *Trichostrongylus* spp. Fue el segundo con mayor índice de patogenicidad.

Palabras clave: Suplementación; ovinos; corderos; nematodos gastrointestinales

Received: 05/04/2024 Accepted: 10/06/2024 Published: 31/08/2024

INTRODUCTION

Sheep (*Ovis aries*) production faces different daily challenges, and parasitosis is one of the most important [1]. Among them, gastrointestinal nematodes (GIN) are the main ones, and, in Uruguay, the genus diagnosed are *Haemonchus contortus* (43%), *Trichostrongylus axei* (12%), *Nematodirus* spp. (11%) and *Trichostrongylus* spp. (26%) [2]. The incidence of the different GIN is determined by their pathogenic potential (PP), biotic potential (BP), and the number of parasites present, and all of them make the Pathogenicity Index (PI) vary [3].

GIN reduce voluntary intake by 10%, generating a body weight (BW) reduction of 33% in adult animals [4] and of 23.6% in the rearing [1]. Moreover, if infection occurs before the lambs are one month old, there is no effect on their body condition score (CS) [4, 5].

Adult sheep have immunity to GIN that allows them to perform productively [6], while lambs (3–6 months) do not eliminate their first infection, generating parasitic disease where *Trichostrongylus* spp. produces acquired and specific immunity faster than *H. contortus* [1]. The response of sheep to a parasitic challenge depends on age and nutritional status level [7, 8, 9, 10, 11, 12, 13, 14], and frequency of challenges and genetic factors [15]. Despite this power of response, in breeding ewes, immune weakening occurs in the period immediately after lambing, known as "spring rise" [16, 17, 18, 19, 20]. This epidemiological phenomenon is measurable through the significant increase in egg elimination per gram of faeces (EPG) of GIN. In addition, it allows massive contamination of the paddock and acts as a source for susceptible lambs before weaning [2, 18]. In Corriedale sheep, the spring rise has been determined between the sixth and eighth week postpartum in Uruguay [21] and United Kingdom [22]. In Australian Merino ewes, this phenomenon coincided with peak milk production (2–4 weeks postpartum) [23].

Increases in prolactin levels associated with parturition and lactation have been shown to have a suppressive effect on the immune system, reducing IgA levels, which favours increased fertility of *H. contortus* and, therefore, of EPG [24]. Immunorelaxation also occurs due to increased energy and protein requirements in postpartum and lactation [9]. In addition, at 2 to 3 weeks postpartum, the emergence of hypobiotic larvae (L4) of *H. contortus* from the mucosa is another critical factor in spring rise [1]. Pastures have higher parasite loads due to this increase in faecal egg counts (FEC), which is more marked when lambing takes place in spring [19, 25, 26].

The lamb is subjected to two sources of parasitic infectation, one being the dam herself and the other due to residual ingestion of infesting larvae (L3) from previous grazing [27, 28].

The emergence and development of anthelmintic resistance (AR) in sheep, mainly *Trichostrongylus* spp. and *H. contortus* was generated by the exclusive use of anthelmintics as the sole control measure [29, 30].

However, changes in nutritional management in sheep can influence GIN behaviour [7, 8, 9, 10, 11 ,12, 30]. Donaldson *et al.* [25] indicated that the increase in EPG that occurs in peripartum in Coopworth ewes and the environmental contamination produced from this EPG can be reduced by protein supplementation to ewes from the month before lambing. Nutritional protein supplementation benefits the immune response in sheep [1, 13, 14, 31], although Provenza *et al*. [32] indicated that protein supplementation without an energy source could generate adverse effects due to excess ammonia.

Therefore, this research aimed to determine the influence of protein–energy supplementation on the GIN population at spring rise in ewes and their offspring as a non–chemical alternative in Integrated Parasite Control (IPC).

MATERIALS AND METHODS

The trial was conducted at the Experimental Station Nº 1 of the Facultad de Veterinaria (Canelones, Uruguay, 34°37'28" Latitude; 55°60'27" Longitude) from May to December 2015.

Animals

A total of 126 full–mouths Corriedale ewes, individually identified, were used. The ewes were selected from a flock of 300 animals, blocked by age, CS, body weight (BW), tooth and hoof condition, thus homogenising the sample. The ewes were synchronised in March with intravaginal sponges containing 160 mg of progesterone (Cronipres® CO, Biogénesis–Bagó) previous artificial insemination [33]. Transrectal ultrasonography was carried out in May when 126 ewes with single gestation were selected. Lambing lasted from August 22nd to August 31st.

During the whole trial, the animals were grazing on natural pasture, mainly composed by *Cynodon dactylon*. In February, the ewes were dosed with Naftalophos 80% (Baymetin, Laboratorio BayerR, Uruguay).

The ewes were supplemented with protein–energy blocks (Metabolisable Energy = 8.8 MJ·kg^{-1}, protein = 15%; Compañía Cibeles SA.) at a rate of 300 g·day^{-1} from August 12th to December 9th (weaning of the lambs). An adaptation period of 10 days (d) to feeding with the blocks was carried out in a small paddock. During this period, one person was responsible for identifying those ewes that ate the supplement, making four daily observations of 1 hour. At the end of this period, the animals were divided into two groups: the supplemented group (GS, n= 54) and the non–supplemented group (GC, n= 72), and were located in a new paddock, which was divided into equal parts by an electric fence.

Determinations in sheep

From August 12th to November 22nd, faecal samples were collected every 17 d from 20 animals (randomly) from both experimental groups. These were directly obtained from the rectum [34], conditioned and identified in nylon bags (without air) and transported refrigerated to the laboratory [34, 35].

Determinations in lambs

From October 7th to December 9th, faecal samples were taken every 17 d from 20 lambs (randomly) born to dams of both experimental groups and were obtained and conditioned in the same way as in the dams.

BW was determined in all lambs at birth, at marking (average age: 22 d) and at weaning (average age: 98 d) using a digital scale (Baxtran, UCS30, Spain). Average daily gain (ADG) for the birth–marking and marking–weaning periods were calculated.

Laboratory analysis

Faecal samples from ewes and lambs were processed at the Facultad de Veterinaria Parasitology Laboratory. Modified McMaster technique was carried out using a McMaster camera (INTA, Argentina) and a microscopy with 40× sensitivity (Olympus, model CX21, Tokyo, Japan) [36]. The technique was based on FEC, and the result was expressed in eggs per gram of faeces (EPG). Coproculture (CL) was carried out using Roberts and O'Sullivan technique (modified) [36, 37] and subsequent morphological identification of the L3 obtained using the Niec´s key [38], counting a minimum of 100 L3, and was expressed in percentage of each genus.

Pathogenicity index

Based on the EPG and CL and according to the BP of each genus (*H. contortus*, 5000–10000 eggs·day^{-1}; *Trichostrongylus* spp., 200 eggs·day^{-1}, and *Oesophagostomum* spp., 3000 eggs·day^{-1}), the probable number of GIN females was estimated according to the following formula:

$$N^{o}\ females = \frac{\left(EPG\ of\ the\ genus \times g\ faecal\ matter\ per\ d\ \{5\%\ BW\}\right)}{BP\ of\ the\ genus}$$

The number of males was calculated as 70% of females. The pathogenicity index (PI) of each of the nematodes was determined using the following formula:

$$PI = \frac{(N^{o}\ females + N^{o}\ males)}{Theorethical\ pathogenicity\ factor}$$

The theoretical pathogenicity factor used was 500 for *H. contortus*, 4000 for *Trichostrogylus* spp. and 100 for *Oesophagostomum* spp. The parasite/host ratio is considered to be in favour of the former whenever the PI is greater than 1 and in favour of the latter whenever the PI is less than 1. Furthermore, a PI value of 2 initiates parasitic symptomatology in sheep [3, 39].

Meteorological records

Daily average records of temperature (ºC) and rainfall (mm) were taken at the Migues Experimental Station using a meteorological station (Vantage Vue, Davis Instruments, USA). FIG. 1 shows the average meteorological records during the trial.

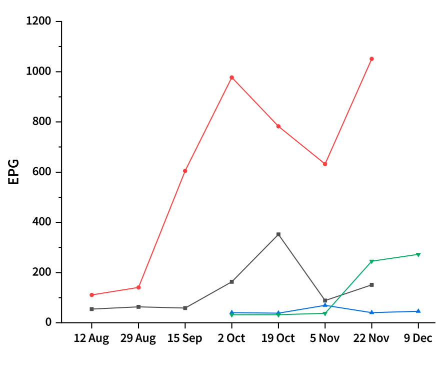

FIGURE 1. Average meteorological records of temperature and rainfall during the assay

Statistical analysis

EPG values obtained were normalised and expressed in logarithm in base 10. Descriptive statistics (averages ± SEM) of the variables analysed (EPG, lambs´ body weight, and mean daily gains) were performed. The effect of treatment (GS, GC) on EPG was determined by analysis of variance (ANOVA). Live weights of lambs at birth, marking and weaning, and daily gains in birth-marking and marking-weaning periods were analysed by ANOVA, determining the effect of treatment (GS, GC) and sex and their interaction. The significance level was $P<0.05$, and P values between 0.05 and 0.10 were considered as trend. The statistical package STATA was used for the analysis.

RESULTS AND DISCUSSION

EPG in ewes

As shown in FIG. 2, in the first sampling, no significant difference in the egg count/gram of faecal matter was found (54.3 and 110.4, GS and GC, respectively). From the second sampling (August 29th) until the last sampling (November 22nd), the GC presented significantly higher values ($P<0.05$) than the GS, except in the fifth sampling (October 19th), where no significant difference between treatment groups was found.

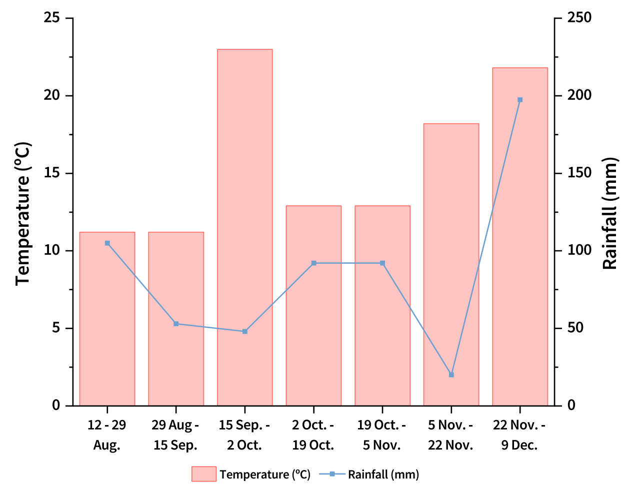

FIGURE 2. Mean EPG of the lambs and their dams from the GS and GC (GS: supplemented group and GC: control group)

The maximum peak of eggs per gram occurred first in the GC in the seventh week postpartum (October 2nd; 977.5 EPG). This EPG behaviour is similar to that described by Crofton [40], who defines this short-duration phenomenon related to the time of parturition as spring rise. Furthermore, these results agreed with those reported by Cardozo and Berdie [21] and Nari and Cardozo [2], who described that the spring rise occurs between the sixth and eighth week postpartum. Donaldson *et al.* [25], Beasley *et al.* [19] and Mederos *et al.* [26] further suggested that this increase is more marked when lambing takes place in spring, which would coincide with the results of the present trial.

In the GS, the peak of EPG occurred at nine weeks post-lambing (October 19th; 352 EPG), with EPG values lower than in the control group. At this time, no significant difference was found between treatment groups. These results agreed with those reported by Donaldson *et al.* [25] who showed that ewes supplemented with protein in peripartum showed a lower increase in postpartum FEC. These authors found no evidence of inhibition larval development or GIN egg-laying, which suggests that the main effect of protein supply to dams would be to improve resistance at the larval establishment stage of GIN.

Likewise, de Melo *et al*. [31], Torres–Acosta *et al*. [14], and Molento *et al*. [13] indicated that protein supplementation improved nutritional intake and resistance to helminth infection in sheep, thus increasing the capacity of the animals to resist the adversities of parasitism. Therefore, it could be used as a non–chemical control method for parasitosis, as indicated by Castells *et al*. [1], stating that a high nutritional level would benefit the health of the sheep, improving the immune response.

EPG in lambs

In the first two samplings, no significant differences were found between treatment groups in egg counts/gram of faecal matter. In the third sampling (November 22nd), lambs born to GC dams had significantly higher EPG values than those born to GS dams (245.3 and 40 EPG, respectively; $P<0.05$). In the last sampling, the EPG values of lambs born to GC dams continued to be significantly higher (272.2 and 45.7, respectively; $P<0.05$) (FIG. 2). The EPG peaks presented by both experimental groups would generate an increase of L3 in the pastures, which can be ingested by susceptible animals (lambs) [2]. Castells *et al*. [1] indicated that environmental contamination generated by *H. contortus* and its high BP would be the primary source of infection for the lamb at the foot of the dam. This result would coincide with those of the present trial, as the lambs born to the GC had significantly higher EPG values than those born to the GS from the third sampling onwards, which continued until the end of the trial. According to Castells *et al*. [1], lambs would be susceptible at first contact with this L3 as they have a less developed immune system.

Larval culture in ewes and lambs

Concerning larval culture in dams, the predominant GIN genus was *H. contortus*, followed by *Trichostongylus* spp. (FIG. 3), while *Teladorsagia* spp., *Cooperia* spp. and *Oesophagostomum* spp. genus were present in percentages lower than 10% (TABLE I). This coincided with those reported by Nari and Cardozo [2] and Castells *et al*. [6], which indicate that the predominant genus were *H. contortus* and *Trichostrongylus* spp.

In the lambs the predominant genus were *H. contortus* and *Trichostongylus* spp. In addition, 10% of *Oesophagostomum* spp. was present in the November 22nd sampling. In this category, the genus of GIN identified was in agreement with the results obtained in Uruguay by Nari and Cardozo [2], Castells *et al*. [6], Valledor [41] and de Melo *et al*. [31], who also diagnosed *Oesophagostomum* spp. as the third prevalent genus.

In both categories, *H. contortus* was the GIN genus that started to be identified in late winter, when temperatures began to rise. These results agreed with Castells *et al*. [1], van Dijk *et al*. [42] and McMahon *et al*. [43], who described this nematode as the most prevalent in warm climates (autumn and spring, with mild temperatures and the presence of rainfall), when its BP was expressed at its highest level (5000–10000 eggs·d^{-1}) [1].

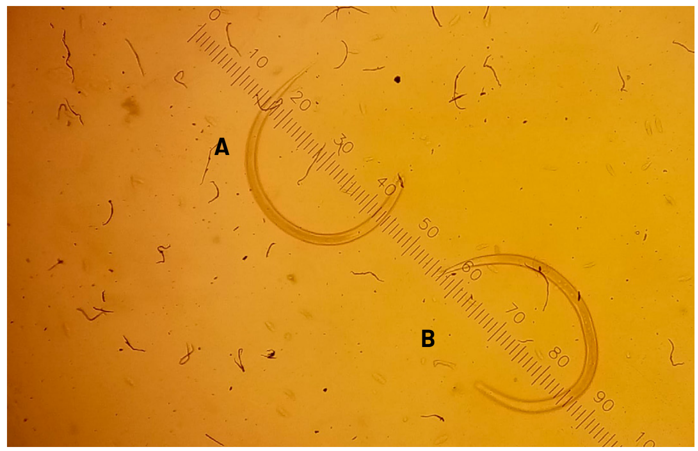

FIGURE 3. Image of larval culture. (A): L3 *Haemonchus contortus* and (B): L3 *Trichostrongylus* spp.

TABLE I
Larval culture and Pathogenicity Index of GS and GC dams

	August 12nd		August 29th		September 15th		October 2nd		October 19th		November 5th		November 22nd	
	GS	GC	GS	GC	GS	GC	GS	GC	GS	GC	GS	GC	GS	GC
Larval culture (%)														
Haemonchus contortus	40	43	38	45	63	68	57	65	60	66	60	66	88	92
Trichostongylus spp.	38	40	45	38	20	23	13	19	21	19	21	19	8	7
Teladorsagia spp.	7	2	8	6	7	5	15	4	6	4	6	4	0	0
Cooperia spp.	0	0	4	8	5	2	6	7	5	4	5	4	0	0
Oesophagostomum spp.	15	15	5	3	5	2	9	5	8	7	8	7	4	1
IP														
Haemonchus contortus	0.1	0.1	0.0	0.1	0.0	0.4	0.2	1.0	0.3	0.8	0.1	0.6	0.2	1.5
Trichostongylus spp.	0.0	0.0	0.1	0.2	0.1	1.1	0.2	0.1	0.2	0.7	0.1	0.6	0.0	0.4
Teladorsagia spp.	0.0	0.1	0.0	0.1	0.0	0.2	0.0	0.3	0.3	0.2	0.0	0.2	0.0	0.0
Cooperia spp.	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.1	0.3	0.0	0.1	0.0	0.0
Oesophagostomum spp.	0.0	0.0	0.1	0.3	0.0	0.2	0.1	0.3	0.4	0.5	0.1	0.6	0.0	0.1
Totales	0.1	0.3	0.3	0.6	0.2	2.2	0.5	1.9	1.4	2.5	0.3	2.1	0.2	2.0

GS= supplemented group; GC= control group; IP= Pathogenicity Index

Pathogenicity Index in ewe and lambs

The PI determined in the dams in both experimental groups is presented in TABLE I. In the GC, the PI higher than 1 coincided with the beginning of the increase in EPG (September 15th) and remained elevated until the end of the trial (November 22nd). On the other hand, the GS presented a PI above 1 only at the EPG peak (October 19th) at the expense of the sum of the GIN.

The PI in those lambs born to GS dams at no time exceeded the value of 1, while in the GC, it was higher than 1 in the last sampling (December 9th) (TABLE II).

According to Ueno and Goncalves [44], PI values above 1 favours the parasite over the host. In the present work, this situation was present in the GC dams from the EPG peak and maintained until the end of the trial. However, in the GS, it was only observed at the peak of EPG due to the addition of the GINs. In lambs, only in the GC last sampling the PI was higher than 1, and *Oesophagostomum* spp. was the most prevalent genus.

TABLE II
Larval culture and Pathogenicity Index of GS and GC lambs

	Oct. 19th		Nov. 5th		Nov. 22nd		Dec. 9th	
	GS	GC	GS	GC	GS	GC	GS	GC
Larval culture (%)								
Haemonchus contortus	55	65	58	64	60	69	75	78
Trichostongylus spp.	15	20	20	19	15	10	12	12
Teladorsagia spp.	18	5	7	4	14	8	0	0
Cooperia spp.	6	7	6	5	4	3	5	3
Oesophagostomum spp.	6	4	9	8	7	10	8	7
IP								
Haemonchus contortus	0.01	0.01	0.00	0.01	0.00	0.03	0.00	0.20
Trichostongylus spp.	0.02	0.02	0.00	0.04	0.03	0.03	0.03	0.20
Teladorsagia spp.	0.05	0.05	0.10	0.11	0.08	0.09	0.08	0.50
Cooperia spp.	0.03	0.04	0.10	0.08	0.00	0.24	0.00	0.00
Oesophagostomum spp.	0.05	0.87	0.10	0.11	0.16	0.18	0.16	1.00
Totales	0.15	0.98	0.30	0.35	0.40	0.76	0.21	1.90

Oct= October; Nov.= November; Dec.= December; GS= supplemented group; GC= control group; IP= Pathogenicity Index

Body weight in lambs

BW at birth, marking and weaning were significantly higher in lambs born to the supplemented dam group ($P<0.05$). Average daily gains at birth–marking and marking–weaning periods were also significantly higher in lambs born to the GS ($P<0.05$) (TABLE III). None of the variables were affected by sex or the interaction between treatment group and sex. The higher BW at birth, marking and weaning, and higher ADG obtained in both periods evaluated in lambs born to the supplemented dams demonstrated that supplementation could be helpful for parasitic control in susceptible categories. De Melo *et al*. [31] showed that lambs born to Ile de France and Dorper/Santa Inês ewes supplemented with a protein concentrate and deworming treatments from lambing to weaning obtained lower EPG values and higher daily weight gains compared to lambs born to unsupplemented control group dams.

TABLE III
Body weight of the lambs at birth, marking and weaning, average daily gain between birth and marking and between marking and weaning (Mean ± SEM)

	GS	GC
Birth (kg)	5.26 ± 0.10^a	4.96 ± 0.08^b
Marking (kg)	10.02 ± 0.37^a	8.82 ± 0.27^b
Weaning (kg)	18.90± 0.48^a	17.11 ± 0.37^b
AGD Birth–marking (g·day^{-1})	240.00 ± 20.00^a	170.00 ± 10.00^b
AGD Marking–weaning (g·day^{-1})	150.00 ± 0.00^a	140.00 ± 0,00^b

BW= body weight; GS= supplemented group; GC= control group; AGD= average daily gain; $^{a-b}$= $P<0.05$

CONCLUSION

According to the results, the protein–energy blocks contribute to the non–chemical control of the spring rise phenomenon and environmental contamination for lambs at the foot of the dam. They significantly decreased the peak of EPG and delayed its presentation by two weeks. *Haemonchus contortus* was the most prevalent GIN genus, and *Trichostrongylus* spp. was the second one with had the highest PI. In lambs, maternal supplementation also resulted in a significantly lower EPG and lambs with higher BW at birth and AGD. Further studies on the non–chemical control of GIN are required because of its importance in integrated control and environmental preservation.

Conflict of interest

The authors declare that they have no competing interests.

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