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Original article

Effect of gastrointestinal nematodes on ovulation rate of merino Booroola heterozygote ewes (Fec^B Fec^+)

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Abstract – Twenty-five multiparous Merino ewes, each carrying one copy of the Booroola gene (Fec^B Fec^+), were divided into two experimental groups (Control, n = 13 and Challenged, n = 12). The treatments were applied in the autumn between 14 March and 21 May. The sheep in the Control group were dosed orally twice-weekly with 10 mL water, and dosed with Levamisole every 14 days. Each sheep in the Challenged group was dosed orally twice-weekly with 6000 nematode larvae in 10 mL water. Challenged sheep shed more eggs per gram of faeces, lost live weight (40 vs. 38.2) and lost body condition score (3.5 vs. 3.0 condition score) during the study compared with the Control sheep. While live weight, body condition and the number of developing follicles all decreased in association with a nematode larval challenge, the proportion of developing follicles which formed a corpora lutea were unaffected. Thus a larval challenge during joining with nematode larvae at doses comparable to those encountered under field conditions can be expected to affect subsequent lambing performance.

ewe / nematode / ovulation rate / Booroola

Résumé – Effet des nématodes gastro-intestinaux sur le taux d'ovulation chez la brebis Mérinos Booroola hétérozygote (Fec^B Fec^+). Vingt-cinq brebis Mérinos multipares ayant une copie du gène Booroola (Fec^B Fec^+) ont été réparties en deux groupes (Témoin, n = 13 et Défi Larvaire, n = 12). L'expérience s'est déroulée du 14 mars au 21 mai. Le groupe Témoin a été traité oralement avec 10 mL d'eau, deux fois par semaine pendant 10 semaines et tous les 14 jours avec une dose de Lévamisole. Les brebis du groupe Défi Larvaire ont été traitées oralement avec 6000 larves de nématodes deux fois par semaine. Au début et à la fin de la période expérimentale les brebis ont été pesées et l'évaluation de l'état corporel a été déterminée, ainsi que l'activité ovarienne observée par laparoscopie. Des prélèvements de fèces ont été collectés tous les 14 jours pour déterminer le nombre d'œufs de parasites par gramme de fèces (OPG). A la fin de la période expérimentale chez les brebis du groupe Défi Larvaire, le poids vif (40 vs. 38,2 kg) et l'état corporel (3,5 vs. 3,0) étaient significativement inférieurs. Dans le groupe Témoin, les caractéristiques corporelles n'ont pas changé

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pendant toute la période expérimentale. Les brebis du Défi Larvaire présentent un nombre d'OPG significativement supérieur à celui du groupe Témoin. A la fin de l'expérience, le taux d'ovulation et l'activité ovarienne ont été significativement inférieurs chez le groupe Défi Larvaire. Le moindre taux d'ovulation est la conséquence d'un nombre inférieur de follicules développés. Ces résultats indiquent que l'ingestion de larves de nématodes, à des doses comparables à celles rencontrées par des brebis sur un pâturage hautement contaminé, pouvait affecter la fécondité.

brebis / nématodes / taux d'ovulation / Booroola

1. INTRODUCTION

Nematode infections in grazing sheep depress feed intake [13], lamb growth [3, 12, 18], wool growth [2] sheep and lamb survival and ewe fertility [6, 15]. However, the reproductive experiments referred to lambing rate, lamb birth weight, lamb survival and peri-parturient rise in faecal egg counts in ewes [8, 10, 17, 19]. Few studies have investigated whether the various steps associated with either follicle development or the shedding of viable ova is affected by exposure of the ewe to a nematode challenge [7, 9].

The purpose of this study was to measure the effect of a nematode challenge during the autumn on follicle development and ovulation rate. A prolific genotype with a naturally high ovulation [14] rate was used to increase the chance of detecting a measurable response.

2. MATERIALS AND METHODS

The experiment was carried out under natural lighting conditions during the autumn in the Experimental Station of the Faculty of Agronomy in Salto, in northern Uruguay (31.23 °S), at an altitude of 30 metres above sea level with an average annual rainfall of 1100 mm and mean autumn temperature of 15.5 °C.

2.1. Animals

Twenty-five multiparous Merino ewes, each carrying one copy of the Booroola gene (Fec^B Fec^+), were divided into two experimental groups (Control, n = 13 and Challenged, n = 12) on the basis of live weight and body condition. The ewes were shorn during the previous summer (January). Beginning on March 14, the sheep in the Control group were dosed orally twice-weekly with 10 mL water and every 14 days the water treatment was combined with a dose of Levamisole (0.25 mL (7.5 mg of Levamisole chlorhydrate) per kg body weight; Ripercol®, Fort Dodge Laboratory). In contrast, sheep in the Challenged group were dosed orally twice-weekly with 6000 nematode larvae in 10 mL water. The larvae were cultivated [4] from a field pool (collected rectally from a grazing flock), that comprised on average: 87% *Haemonchus contortus*, 10% *Trichostrongylus* spp. and 3% *Oesophagostomum* spp. The last treatment was given on 21 May completing a 10 week treatment period. During the treatment period the ewes were maintained on natural pasture which had not been grazed by sheep during the previous 12 months.

2.2. Sampling procedures

Live weight and body condition score [11] of each ewe were recorded at the beginning and at end of the treatment period. Faecal samples were collected directly from the rectum every 14 days and the number of nematode eggs per gram of faeces (epg) counted by a modification of the Mc Master method [1].

A month after starting the treatment oestrus was synchronised using intra-vaginal sponges containing 60 mg of medroxiprogesterone acetate (MPA)

Table I. Mean live weight and body condition score ± standard error of mean for the Control (n = 13) and Challenged (n = 12) groups.

Parameter	Treatment group	Start of treatment	End of treatment
		14 March	21 May
Live weight (kg)	Control	40.0 ± 0.8	40.0 ± 0.6
	Challenged	40.1 ± 0.9	38.2 ± 1.2
	Treatment effect	NS	*
Body condition	Control	3.54 ± 0.07	3.40 ± 0.04
score	Challenged	3.52 ± 0.09	3.02 ± 0.07
	Treatment effect	NS	**

* $P < 0.05$, ** $P < 0.01$, NS: not significant.

(Santa Elena Laboratory, Uruguay) which were inserted for 14 days. The ovaries were examined by laparoscopy [16] using a 6.5 mm telescope with a 0° angle (Richard Wolf, GMBH Knittlingen, Germany). Ovarian activity was assessed by recording the number of unruptured follicles (UF) and the number of corpora lutea (CL) visible across the surface of both the ovaries in each sheep. The number of corpora lutea present in the ovaries equals the number of follicles that ovulated in the previous oestrous cycle. Ovulatory efficiency was calculated as the ratio CL/(CL+UF).

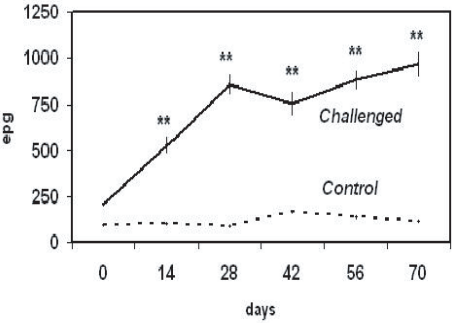

Figure 1. Mean epg during the experimental period (** $P < 0.01$). (Error bars indicate the SEM.)

2.3. Statistical analysis

Live weight, body condition, ovulation rate, ovarian activity and ovulatory efficiency values were analysed using the SAS statistical package (Statistical Analysis System Institute, Windows Version 6.08, 1995). The ovarian and epg data were log transformed for analysis. A difference was considered significant at a probability level of 5%.

3. RESULTS

3.1. Body characteristics

Mean live weight and body condition score + standard error of mean (SEM) of the two groups of sheep at the start and end of the trial are given in Table I. While the live weight and body condition of the Control group did not change during the trial, the Challenged group lost 1.9 kg live weight and 0.5 units of body condition. The loss in live weight is equivalent to approximately 5% of the pre-treatment live weight.

3.2. Faecal nematode egg counts (epg)

Mean ± SEM for the mean count of nematode eggs in the faeces of each treatment group at each sampling are shown in Figure 1. The Challenged group of ewes shed significantly more nematode eggs in their

Table II. Mean ovulatory activity ± standard error of mean for the Control (n = 13) and Challenged (n = 12) groups.

Parameter	Treatment group	Start of treatment 14 March	End of treatment 21 May
No of unruptured follicles (UF) per sheep	Control	1.4 ± 0.2	1.7 ± 0.2
	Challenged	1.7 ± 0.3	0.9 ± 0.1
	Treatment effect	NS	**
No of corpora lutea (CL) per sheep	Control	3.3 ± 0.6	3.5 ± 0.5
	Challenged	3.4 ± 0.5	2.2 ± 0.2
	Treatment effect	NS	**
Ovulatory efficiency (CL/(CL+UF)	Control	0.7 ± 0.1	0.7 ± 0.2
	Challenged	0.7 ± 0.1	0.7 ± 0.1
	Treatment effect	NS	NS

* $P < 0.05$, ** $P < 0.01$, NS: not significant.

faeces at each sampling after the first with the difference increasing in a step-wise fashion throughout the trial. On the contrary, the Control group had significantly lower counts throughout the experimental period (range: 96–163 epg).

3.3. Ovarian activity

Mean ± SEM for the mean ovulatory data are shown in Table II. At the end of the experiment, both ovulation rate and ovarian activity were significantly lower in the larval Challenged group ($P < 0.01$). No differences were found in efficiency ($P > 0.05$); the lower ovulation rate was a consequence of a lower number of recruited follicles developing to the unruptured stage.

4. DISCUSSION

In essence, this study showed that challenging ewes with nematode larvae (87% *Haemonchus contortus*, 10% *Trichostrongylus* spp. and 3% *Oesophagostomun* spp.) resulted in substantial reduction in ovulation rate and ovarian activity. Previous studies have shown that both parameters tend to be lower in larval Challenged groups [9] or in non-drenched groups in grazing conditions [7], than the drenched groups. In our study, the use of Booroola prolific sheep increased the differences between treatments. However, the percentage difference is the same in prolific and non-prolific sheep (15–20 percent). In addition, the nematode infection used could explain the significant differences obtained in our work. Jeffcoate et al. [9] used *Teladorsagia* (*Ostertagia*) which is less pathogenic than *Haemonchus contortus and Trichostrongylus* spp.

The ovulation rate is a consequence of terminal follicle development. There are three events in the development process: recruitment, selection and follicular dominance. These events determine the number of corpus lutea [5]. This study showed that nematode infection reduces follicular development. In addition, in similar

conditions using non-prolific sheep, Fernandez Abella et al. [7] observed that 20–28% of the corpus lutea did not develop normally (small CL), affected pregnancy rates and increased early foetal losses. These results suggest that both ovulation rate and ovulation quality are affected by nematode infection.

The ovulatory efficiency obtained in this work was higher (in our conditions it is normally ≤ 0.4 [7]) indicating difficulties in identifying unruptured follicles on the surface of individual ovaries in Booroola Merino ewes, due to their smaller corpora lutea [14].

The ewes in the Challenged group lost weight (5 per cent loss of bodyweight and 0.5 unit loss in body condition). Jeffcoate et al. [9] reported similar losses in animals of larval challenged groups, each given 12000 *Teladorsagia* (*Ostertagia*) *circumcincta*, three times a week for 10 weeks.

The lower follicular development may have been caused by a secondary effect associated with live weight loss induced by nematode challenge affecting aspects of protein metabolism [18]. The larval challenge in the present experiment was comparable to larval intake expected from grazing contaminated pasture [7]. Economically, the reduction in ovarian activity is very important since it could potentially cause a 20% reduction in lambing rate.

In conclusion, in our conditions, nematodes affect reproduction performance decreasing follicular development and ovulation rate.

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