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Short communication

Efficacy and productive performance of moxidectin in feedlot calves infected with nematodes resistant to ivermectin

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ABSTRACT

Anthelmintic resistance (AR) of gastrointestinal nematodes to macrocyclic lactones is an increasingly common worldwide phenomenon limiting cattle production. This has motivated the search for alternatives, such as new active compounds, added drug synergisms, different doses, and alternate administration routes. The aim of this study was the assessment of moxidectin (MXD) performance in feedlot calves with a history of AR to ivermectin (IVM). Crossbred female calves aged 6–7 months and weighing 163 kg (SD = 34 kg) were divided into 3 groups of 35 animals each. They were assigned to the following antiparasitic treatment groups: IVM group (0.2 mg/kg IVM); MXD group (0.2 mg/kg MXD), and ricobendazole + levamisole (RBZ + LEV) group (7.5 mg/kg RBZ + 8 mg/kg LEV). On days 0, 26, and 47, fecal samples were taken and the weight of each animal was registered. Anthelmintic efficacy (by fecal egg count reduction), total weight gain (TWG) and average daily weight gain (AWG) were compared between the groups. A mixed SAS procedure was used for statistical analysis. Fecal egg count reduction 26 days post-treatment (PT) was calculated at 28% for the IVM group, 85% for the MXD group, and 99% for the RBZ + LEV group. AWGs (Standard Error) of 1.095 g (56), 1.264 g (49), and 1.340 g (52) were registered for the IVM, MXD, and RBZ + LEV groups, respectively ($p < 0.05$). Coprocultures revealed that MXD more effectively reduced *Haemonchus* spp. and *Cooperia* spp. egg counts than IVM. This resulted in higher AWGs and TWGs for this group; similar results were seen for the RBZ + LEV group as well. In this study, animals treated with MXD gained about 160 more g/day than animals treated with IVM. This represents a gain of 16 USD per animal over the 47 day trial.

1. Introduction

Gastrointestinal nematode (GIN) infections in calves economically affect cattle production (Kaplan, 2004). This is especially pertinent in Argentina, where 3,640,000 animals a year are fattened in feedlots (Robert et al., 2009). Fattening cycles for calves weighing 150–200 kg on arrival are short (3–4 months) (Pordomingo, 2013), meaning that even if reinfection is not possible, weight loss due to GINs can still be important (Fazzio et al., 2014).

Subcutaneous administration of macrocyclic lactones (ML), mainly ivermectin (IVM), has been widely adopted in feedlots due to the broad-spectrum activity of the drug against nematodes and arthropods. Unfortunately, the frequent use of IVM, particularly in areas of *Rhipicephalus microplus* infestation, has increased the prevalence of GIN resistance (Fiel et al., 2005). Administration of IVM to animals infected with ML-resistant nematodes may lead to an 8.3% weight loss, requiring an extension of the fattening cycle (Fazzio et al., 2012, 2014). This has limited the use of this group of drugs, in spite of the advantages. This is particularly a problem in Argentina, where the earliest cases of ML resistance against *Cooperia* were recorded 15 years ago (Anziani et al., 2001).

Moxidectin (MXD) is a milbemicyn ML. Although its mechanism of action and antiparasitic spectrum are similar to that of IVM, it does exhibit some pharmacodynamic and pharmacokinetic differences, including a better efficacy profile (Prichard et al., 2012; Lloberas et al., 2013). For example, MXD resulted in an egg count reduction of over 90% when given to Holstein calves infected with IVM-resistant *Cooperia oncophora* and *Ostertagia ostertagi* (De Graef et al., 2012); this level of activity is consistent with expectations for effective use (Wood et al., 1995). In a separate sheep trial, MXD

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was even more effective against IVM-resistant *Hemonchus contortus* (Lloberas et al., 2013). However, MXD resistance has also been documented (Anziani et al., 2001; Kaplan, 2004; Sutherland and Leathwick, 2011)

Thus, the aim of this study was to test MXD performance in feedlot calves that previously demonstrated anthelmintic resistance (AR) to IVM. To accomplish this, fecal egg count reduction and weight gain were assessed over the first 47 days of fattening in a commercial feedlot.

2. Materials and methods

This study was carried out from July to August 2014 in a commercial feedlot located in Marcos Paz, Buenos Aires, Argentina (−34.7968, −58.9002), a region with a mild climate. The crossbred *Bos indicus* × *Bos Taurus* female calves that were used were shipped 900 km from Esquina, Corrientes, Argentina (−30.0173, −59.5496), a subtropical climate where AR has previously been demonstrated (Fazzio et al., 2014). Cattle in this region graze together on the same fields with sheep. None of the calves used in this study had any previous record of any kind of deworming treatment, let alone MXD use.

During the first 72 h, the calves remained in pens with free access to hay and water. After 72 h, one dose of Policlostrigen®, a polyvalent clostridial vaccine (Biogénesis Bagó, Garín, Argentina), and one dose of Maxitil, a macrolide antibiotic (Biogénesis Bagó), were administered. The calves were put on a diet of corn grain, sunflower meal, and wheat bran, and were given a regular vitamin and mineral supplement. Protein and fiber content accounted for 15% and 25% of the calves' diet in the first 26 days, respectively, after which point they were transitioned to a finishing diet containing protein and fiber content of 12% and 8%, respectively.

After 72 h from the point of arrival, 105 healthy calves were randomly selected and ear-tagged. Calves were between 6–7 months old and had an average weight of 163 kg (SD = 34 kg). These calves were then immediately divided into 3 groups of 35 animals each and were treated as follows: IVM group (IVM 1%, 0.2 mg/kg subcutaneous; Ivomec®, Merial, Lyon, France), MXD group (MXD 1%, 0.2 mg/kg subcutaneous; Cydectin Alfa®, Fort Dodge Animal Health, Overland Park, USA), Ricobendazole (RBZ) + Levamisole (LEV) group (RBZ, 7.5 mg/kg subcutaneous; Axilur® PI, Intervet, Vicente Lopez, Argentina; LEV, 8 mg/kg subcutaneous; Fosfamisol® MV, Biogénesis Bagó).

Out of the 35 calves in each group, 18 were selected randomly and were individually sampled for feces on days 0, 26 and 47. The Fecal Egg Count Reduction (FECR) test was performed on day 26 due to reports of more reliable results (Das Neves et al., 2014). The eggs per gram (EPG) parameter was calculated using a modified version of the McMaster method, where each egg represents 20 eggs/g of feces (Roberts and O'sullivan, 1950). FECR was then calculated using the following formula: $100 \times [1-(T2/T1)]$ (Cristel and Suárez, 2006), where T2 is the EPG arithmetic mean of each group on day 26 post-treatment (PT) and T1 is the arithmetic mean of the same group on day 0. Egg reduction confidence intervals of 95% were calculated according to Coles et al. (1992). Pooled fecal cultures were carried out twice per group and per date (days 0 and 26) for third stage larvae (L3) differentiation (Van Wyk et al., 2004).

Each calf was individually weighed on days 0, 26, and 47. The EPG value, average daily weight gain (AWG), and total weight gain (TWG) over the whole study were statistically analyzed by a mixed procedure using SAS 9.1 software (Cary, USA). Random effects for each animal (experimental unit), along with the fixed effects of time (0 vs. 26 vs. 47), treatment (IVM vs. MXD vs. RBZ + LEV), and the interaction of the two, (time x treatment) were included in the analysis. The Slice option was used to detect statistical differences at each time. A p-value < 0.05 was considered statistically significant, while a value of $0.05 \leq p < 0.10$ was seen as a tendency.

Table 1
Fecal egg count reduction in feedlot calves, at 26 days post-treatment with ivermectin, moxidectin or ricobendazole + levamisole.

	All genera	Cooperia Efficacy (95% CI)	Haemonchus
IVM	28% (0–69)	0% (0–51)	66% (21k86)
MXD	85% (72–92)	79% (60–89)	91% (82–95)
RBZ + LEV	99% (97–100)	100% (100–100)	100% (100–100)

IVM: ivermectin group (0.2 mg/kg ivermectin 1%).
MXD: moxidectin group (0.2 mg/kg moxidectin 1%).
RBZ + LEV: ricobendazole + levamisole (7.5 mg/kg ricobendazole and 8 mg/kg levamisole).
CI: confidence interval.
n = 18 animals per group.

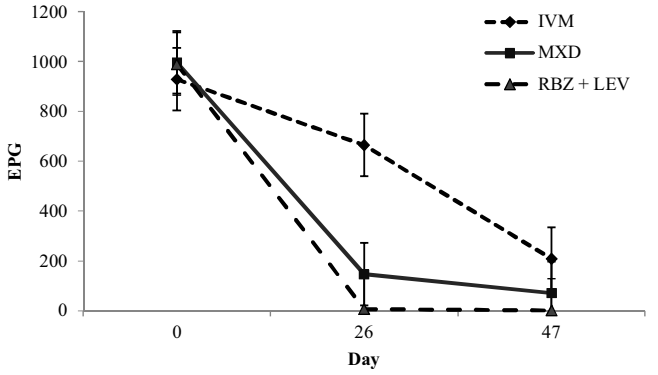

Fig. 1. Effect of anthelmintic treatment on fecal eggs per gram (mean per group) in feedlot calves.
IVM: ivermectin group (0.2 mg/kg ivermectin 1%).
MXD: moxidectin group (0.2 mg/kg moxidectin 1%).
RBZ: ricobendazole + levamisole (7.5 mg/kg ricobendazole and 8 mg/kg levamisole).
**p-value <0.001.
n = 18 animals per group.

3. Results and discussion

FECR revealed that egg count reduction was high (99%) for the RBZ + LEV group, moderate (85%) for the MXD group, and insufficient (28%) for the IVM group (Wood et al., 1995) (Table 1). No significant difference was seen in PT EPG comparing the MXD and RBZ + LEV groups (Fig. 1).

Coprocultures revealed a relatively equal distribution of *Cooperia* and *Haemonchus* at the beginning of the experiment, but *Cooperia* predominated on day 26. The lower bound of the confidence interval calculated for day 26 PT for MXD use against *Haemonchus* is considered to be consistent with moderate activity (Wood et al., 1995) (Table 1; Fig. 2). No L3 were harvested in the RBZ + LEV group after treatment.

Differences in FECR were correlated with significant differences in TWG and AWG (Table 2, Fig. 3). This shows that the MXD and RBZ + LEV groups behaved similarly, whereas the IVM group performed more poorly. These findings are similar to those of Stromberg et al. (2012), who observed a drop in feed intake and AWG reduction in feedlot animals infected with ML-resistant *C. punctata*.

The negative effects of GIN on feedlots have been studied even before the recent rise in AR levels. Results vary depending on the antiparasitic drug used, age of the animal, and parasite burden at the beginning of the fattening period. Yet, as noted by Stockdale and Harries (1979), AWG, feed intake, and other indicators are still consistently adversely affected.

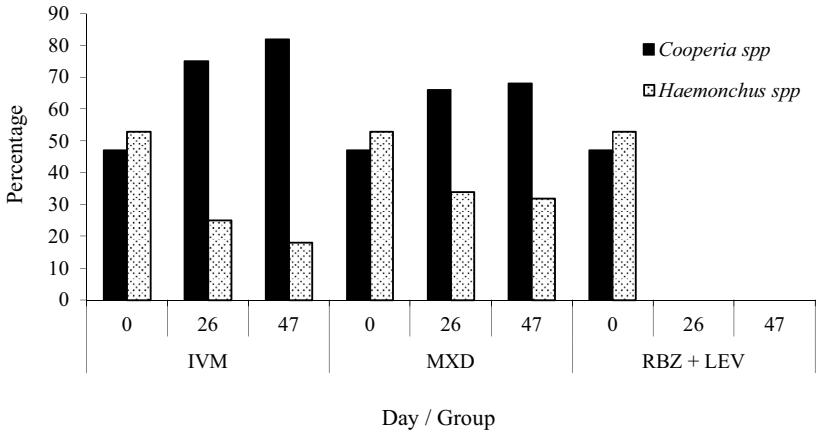

Fig. 2. Effect of anthelmintic treatment on the fecal cultures' composition in feedlot calves.
IVM: ivermectin group (0.2 mg/kg ivermectin 1%).
MXD: moxidectin group (0.2 mg/kg moxidectin 1%).
RBZ + LEV: ricobendazole + levamisole (7.5 mg/kg ricobendazole and 8 mg/kg levamisole).
n = 18 animals per group.

Table 2
Average daily weight gain in feedlot calves with different antiparasitic treatments.

	IVM	MXD LSM g (SE)	RBZ + LEV
AWG[1]	892(61)[a]	1098(64)[b]	1142(62)[b]
AWG[2]	1347(87)[y]	1471(81)	1586(87)[z]
AWG[t]	1095(56)[a]	1264(49)[b]	1340(52)[b]

IVM: ivermectin group (0.2 mg/kg ivermectin 1%).
MXD: moxidectin group (0.2 mg/kg moxidectin 1%).
RBZ + LEV: ricobendazole + levamisole (7.5 mg/kg ricobendazole and 8 mg/kg levamisole).
LSM: least squared means (g).
SE: Standard Error.
AWG[1]: average daily weight gain (g) from days 0 to 26.
AWG[2]: average daily weight gain (g) from days 27 to 47.
AWG[t]: average daily weight gain (g) throughout the study (days 0–47).
[a–b]: different letters within rows mean $p < 0.05$.
[y–z]: different letters within rows mean $p > 0.05$ to <0.10.
n = 35 animals per group.

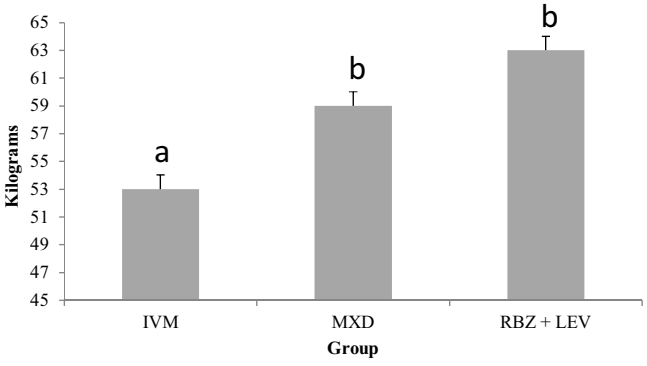

Fig. 3. Total weight gain during the first 47 days of fattening in feedlot calves assigned to different antiparasitic treatments.
IVM: ivermectin group (0.2 mg/kg ivermectin 1%).
MXD: moxidectin group (0.2 mg/kg moxidectin 1%).
RBZ + LEV: ricobendazole + levamisole (7.5 mg/kg ricobendazole and 8 mg/kg levamisole).
Different letters mean p < 0.05.
n = 35 animals per group.

The calves used in this study were typical of weaning calves in Argentina, with an average weight of 150–170 kg and an average age of 6–7 months. However, greater losses would be expected in younger animals, such as in early weaning calves aged 3–4 months and weighing 80–120 kg.

Drug combinations have shown promise is minimizing the continued spread of AR (Leathwick et al., 2012; Gasbarre, 2014). Different mechanisms of action increase the spectrum of antiparasitic activity and delay increased resistance (Geary et al., 2012). In this study, as well as in previous trials (Fazzio et al., 2012, 2014), the simultaneous use of RBZ and LEV showed better production performance than IVM. However, RBZ + LEV does not address ectoparasites like mites and lice, which are also a concern in feedlots.

So far, there have been no reports of resistance to LEV in Argentina as confirmed by FECR, while resistance to RBZ is still low, but increasing (Caracostantogolo et al., 2005; Fiel, personal communication). Thus, combining RBZ + LEV with ML should be effective (Geary et al., 2012). Moreover, there is no risk of spreading resistance due to reinfection because the animals are slaughtered (Gasbarre, 2014).

In this study, MXD showed superior EPG reduction when compared to IVM. Although MXD is already considered a viable alternative to IVM (Prichard et al., 2012; Lloberas et al., 2013), the added benefit, in this case, is moderate (Wood et al., 1995). Moreover, resistance to MXD has already been reported, and is growing (Kaplan, 2004; Sutherland and Leathwick, 2011).

Avermectins persist from 14 to 28 days, with MXD lasting even longer (Prichard et al., 2012). Additionally, since reinfection in the feedlot is exceedingly unlikely (Gasbarre, 2014), it can be assumed that the EPG found on PT day 26 belonged only to parasites which survived treatment. De Graef et al. (2012) discussed how MXD can temporarily reduce the oviposition of *Cooperia*, which can survive at least 7 days after treatment. This makes it fairly easy to overestimate drug efficacy when testing EPG reduction on PT day 14 (Coles et al., 2006). Research by Das Neves et al. (2014) supports this, in that they detected MXD resistance by sampling feces on day 28 PT, when the females that survived treatments had recovered their fertility.

The present study does not fully account for the lack of differences between the groups on day 47. However, according to Armour (1989), calves acquire a relatively good immune response to *Cooperia,* which can partially reduce worm burdens when initial stress factors from arrival to the feedlot decrease.

Using MXD as the only drug upon arrival to the feedlot should be monitored; even if it is more effective than IVM, remaining burdens

can result in weight loss (Fazzio et al., 2012, 2014). Combining MXD with RBZ or LEV could avoid flawed deworming.

In this study, animals given MXD gained about 160 g/day more than those that were given IVM. According to current livestock prices in Argentina, this represents an added value of 16 USD per animal during the first 47 days of fattening. (http://www.mercadodeliniers.com.ar/) This contrasts with the RBZ + LEV group, which showed an added value of 22 USD per animal in the same period.

Conflict of interest

The authors have no conflicts of interest.

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