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FEEDING HORSES WITH INDUSTRIALLY MANUFACTURED PELLETS WITH FUNGAL SPORES TO PROMOTE NEMATODE INTEGRATED CONTROL

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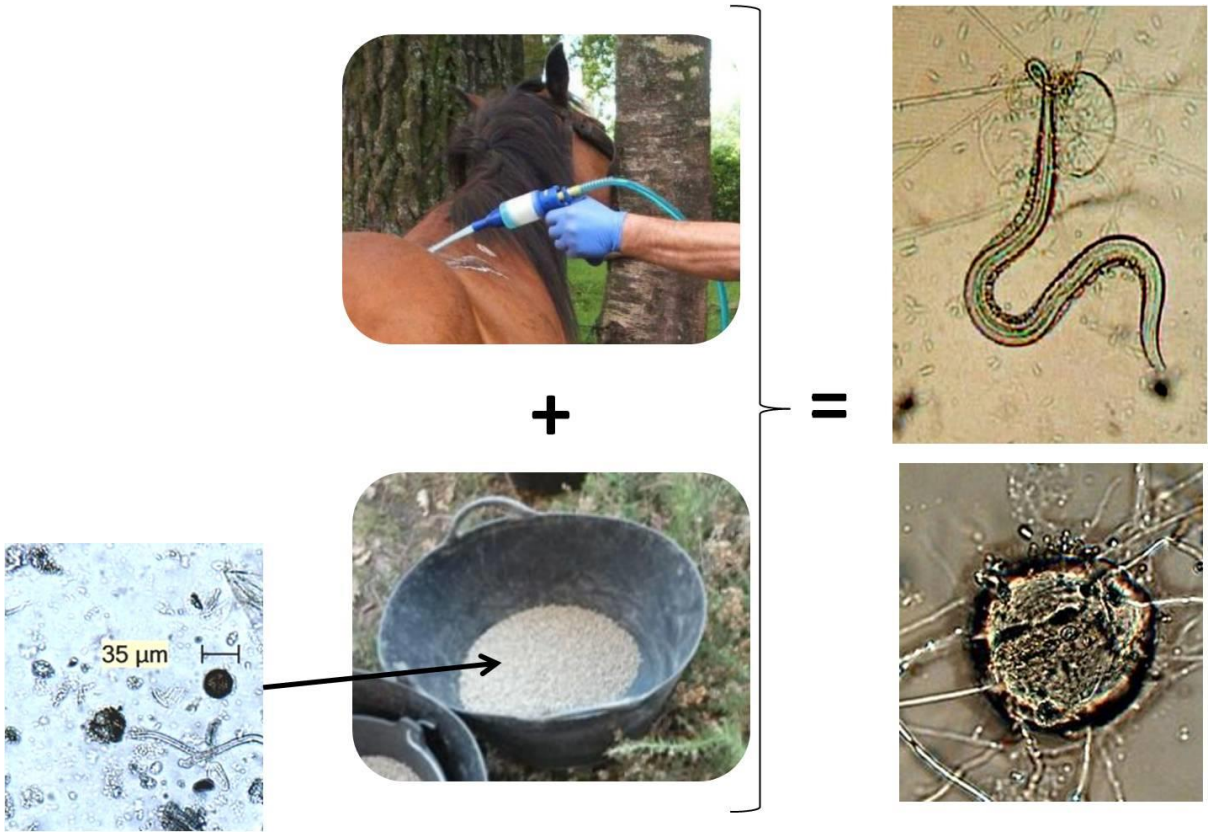
Graphical abstract



Highlights

- An integrated control strategy of horse nematodes has been assayed
- We manufactured pellets with spores of *Mucor circinelloides* and *Duddingtonia flagrans*
- Horses provided with pellets containing added spores did not need deworming for 15 months
- Undesirable effects on horses feeding on pellets containing fungal spores were not observed

Abstract

The usefulness of pellets industrially manufactured with spores of parasiticide fungi as a contribution to integrated nematode control was assessed in grazing horses throughout sixteen months. Two groups of 7 Pura Raza Galega autochthonous horses (G-T and G-P) were dewormed pour-on (1 mg Ivermectin / Kg bw) at the beginning of the trial, and other group (G-C) remained untreated. The G-P was provided daily with commercial pellets to which was added a mixture of fungal spores during the industrial manufacturing ($2x10^6$ spores of *Mucor circinelloides* and same dose of *Duddingtonia flagrans* / Kg), and G-T and G-C received pellets without spores. The efficacy of the parasiticidal strategy was assessed by estimating the reduction in the faecal egg counts (FECR) and in the number of horses shedding eggs in the faeces (PHR), and also the egg reappearance periods (ERP). Blood analyses were performed to identify the changes in the red and white cell patterns. To ascertain if horses developed an IgG humoral response against the fungi, antigenic products collected from *M. circinelloides* and *D. flagrans* were exposed to the horse sera by using an ELISA.

The faecal elimination of eggs of *Parascaris equorum* and strongyles ceased 2 weeks after treatment in G-T and G-P, thus the values of FECR and PHR were 100%. No *P. equorum*-eggs were detected later, and the strongyle egg reappearance period was 28 weeks in G-P, and 8 weeks in G-T. Strongyle egg-output values remained lower than 300 eggs per gram of faeces in the G-P, whereas numbers between 330 and 772 in G-C and G-T were recorded. Normal values for the erythrocytes, haemoglobin and haematocrit in horses consuming pellets with spores were recorded, and lower than normal in the other groups. Sensitization of horses to the fungal species was disproven. It is concluded that feeding horses with pellets industrially manufactured with fungal spores represents a very useful tool to implement an

integrated control of helminths affecting horses. This strategy allows a decrease in their risk of infection, aids in reducing the frequency of anthelmintic treatment.

Key words: Pellet, horses, nematodes, integrated control, *Duddingtonia flagrans*, *Mucor circinelloides*

Introduction

As described for other animal species, grazing horses are at high risk of infection by helminth parasites, mainly cestodes and nematodes (ascarids, strongyles and oxyurids) (Lyons et al., 2007; Relf et al., 2013; Rehbein et al., 2013). The possibility also exists that horses on pastures could be exposed to the liver fluke *Fasciola hepatica* and become infected (Arias et al., 2012; Soykan & Oge, 2012; Sanchís et al., 2015). Ingestion of *P. equorum* eggs containing second-stage larvae leads to infection especially in horses younger than 15 months, although infection has been detected in adult horses also (Francisco et al., 2009; Larsen et al., 2011; Burk, 2013). Infection by strongyles occurs when horses ingest third stage larvae with the herbage, where they can live for at least three months under appropriate conditions (high humidity and warm temperature) (Corning, 2009).

By considering that free-living stages of different parasites (ascarid and trematode eggs, cysticercoid-containing mites, strongyle larvae) can be present in pasture, administration of anthelmintics to the horses provides only a temporary solution. In the last years the intense use of anthelmintic drugs has led to the development of resistance in most of nematode parasitic populations (Reinemeyer, 2009; von Samson-Himmelstjerna, 2012; Canever et al., 2013). According to the selective treatment when horses exceed a predetermined threshold is strongly recommended (Uhlinger, 2007; Francisco et al., 2012; Nielsen et al., 2014).

Some approaches rely on biological control procedures through the distribution of parasiticidal fungi in theenvironment. Favourable results against the larval stages of strongyles by means of the nematode-trapping fungi *Duddingtonia flagrans* or even *Monacrosporium thaumassium* have been reported (Fernández et al., 1999; Araújo et al., 2004). There has been little research conducted on the usefulness of predator (ovicidal) fungi against the eggs of trematodes, cestodes or ascarids, most involving *Pochonia chlamydosporia* (Silva et al., 2010; de Carvalho et al., 2014). Recent investigation showed the ability of *Mucor circinelloides* to destroy the eggs of *Fasciola hepatica* and *Parascaris equorum* in the faeces of infected animals (Arroyo et al., 2016).

Different fungal formulations consisting of oral suspensions and feeding supplements have been assayed (Terrill et al., 2004; Waller et al., 2006; Ojeda-Robertos et al., 2008; Sagüés et al., 2011). Under laboratory conditions, mass mycelia have been embedded in alginate pellets for facilitating the administration of *D. flagrans* or *M. thaumassium* spores to livestock in tropical and temperate regions (Braga et al., 2009; Tavela et al., 2013). Data regarding their inclusion during the industrial manufacturing of commercial pellets are not available.

Pelleted feeds are frequently provided for horse nutrition because of the presence of all the ingredients in every pellet ensures a complete and balanced ration. In previous works, the ability of the spores of *M. circinelloides* (ovicidal) or *D. flagrans* (larvicidal) to survive the industrial manufacturing of pelleted feed without losing their activity has been described (Arias et al., 2015; Arroyo et al., 2016). Considering the presence of parasitic eggs and larvae in thepasture, it was decided to investigate their combined effect against both.

In the current investigation, the preventive effect on nematode infection by feeding horses with pellets manufactured with a mixture of spores of *M. circinelloides* and *D. flagrans* was evaluated.

Material and methods

Area of study

This study was carried out in Castro Riberas de Lea (Lugo, northwest Spain, 43º15'83''N - 7º05'0''W).

Fungal specimens

Two fungal species with proven parasiticidal activity were utilized in the current study, *Mucor circinelloides* (ovicidal) and *Duddingtonia flagrans* (larvicidal) (Cortiñas et al., 2015; Arias et al., 2015). With the aim to obtain their spores, both fungi were simultaneously cultured in a submerged medium (COPFr; patent Nr PCT/ES2014/070110) for 1.5–2 months at room temperature until reaching a concentration higher than $1 \cdot 10^8$ spores / L medium (Arias et al., 2013).

Experimental design

Twenty-one autochthonous Pura Raza Galega mares (2-8 yr) were randomly divided into three groups of 7 each. These are indigenous horses feeding natural pastures in forests and wooded areas (a regime called silvopasturing). Due to difficulties in their handling, deworming consists of the pour-on administration of macrocyclic lactones (Francisco et al., 2009).

The horses of group G-P were dewormed in August 2014 (1 mg Ivermectin pour on / Kg bw, Noromectin 0.5%, Norbrook Laboratories, UK) and provided daily pelletswith fungal spores; G-T was also dewormed in August 2014 (1 mg Ivermectin / Kg bw pour on) and received daily pelleted feed without fungal spores; G-C remained without treatment as control and was given daily concentrate without fungal spores.

Horses were maintained in three different 3 Ha fenced meadows provided with drinkers, feeders and wooden shelters. Water was available ad libitum, and a quantity of 2.5 Kg of concentrate given daily to each horse. The equines were supplemented with wheat straw and barley when the grass was scarce (December to February and July-August).

Pelleted horse feed

Horses belonging to G-C and G-T were given a commercially available pelleted feed (*ProHorse Club*®, Nanta, Padrón, Spain), which contains cereal grains and by-products, oil seeds and derivatives, sugar cane processing by-products, minerals, forages and amino acids. The analytical composition comprises crude protein (14%), crude fat (2.9%), crude fibre (12.5%), Calcium (1.5%), Phosphorus (0.65%), Sodium (0.53%), Magnesium (0.54%), vitamin A (10000 UI / Kg), vitamin D3 (1500 UI / Kg) and vitamin E (42 UI / Kg).

Every 3 months, one batch of concentrate was inoculated with spores of *M. circinelloides* and *D. flagrans* and provided to the G-P horses. After milling the feed ingredients, a dosage of $2x10^6$ spores of each fungus was added per kilogram of meal in the feed mixer, and the complete blend conditioned by injecting steam (75ºC for 90 seconds) before entering the pelletizer. The final product was cooled, dried and finally packed into 25 Kg bags.

Coprological probes

Faeces were collected monthly directly from the rectum, and 5 grams analysed by using the saline flotation test (ρ= 1.20 g/L), with a sensitivity of 30 eggs per gram (EPG) (Francisco et al., 2009). Then, a quantity of 15-20 g faeces from every horse in each group were mixed and pooled, and finally incubated for 20 days at 22-25°C. Four replicates were performed for each group. Third-stage larvae (L3) were collected by means of the Baermann technique,

observed under a light microscope and identified according to morphological keys (MAFF, 1986).

Evaluation of the efficacy

The efficacy of the parasiticide procedures was measured by estimating the reduction of the faecal egg counts (FECR) as well as of the number of horses shedding eggs in the faeces (PHR) (Francisco et al., 2012):

$$FECR\ (\%) = [1 - (FEC_{post\text{-}treatment} / FEC_{pre\text{-}treatment})] \times 100$$

$$PHR\ (\%) = [1 - (\text{number of positive horses}_{post\text{-}treatment} / \text{positive horses}_{pre\text{-}treatment})] \times 100$$

According to the American Association of Equine Practitioners (AAEP, 2013), the egg reappearance period (ERP) was considered as the week after treatment when the percent reduction of faecal egg counts decreased below a cut-off value of 90% (with a 95% Confidence Interval).

Blood analysis

Horses were bled monthly and two samples individually collected, one preserved with EDTA, and the other without it. Blood samples with anticoagulant were processed by means of an automated Coulter-Counter, the Abacus Junior Vet haematology analyzer (Spain) for measuring the values of Red Blood Cells, Haemoglobin and Haematocrit, as well as the counts of White Blood Cells and percentages of Lymphocytes, Granulocytes and Monocytes. The blood samples without anticoagulant were allowed to clot at room temperature for 45 min and the sera kept at -35ºC until used.

Sensitization against the fungal species

The exposure of the horses to the fungal spores was determined by analysing for the presence of serum antibodies against products from both fungi. Firstly, mycelium of each specimen was washed in PBS (phosphate buffered saline, pH 7.2), homogenized under a tissue grinder and dialyzed extensively against water. Then, the final products were lyophilized and the protein concentration estimated by means of the BCA test.

ELISAs were performed by adding 3 concentrations of the fungal products (2.5, 5 and 20 µg / mL) to the wells of U-bottom microtiter plates (Costar®, Barcelona, Spain). After an incubation period of 8 hours at 4ºC, sera (tested in duplicate) diluted at 1:100 in 10% PTL (PBS–0.3% Tween 20 and 10% skimmed milk) were added to the wells and maintained at 37ºC for 1 h. Finally, horseradish peroxidase (HRP) conjugated with rabbit anti-Horse IgG (Nordic Immunology Laboratories, The Netherlands) was used at a 1:1000 dilution and incubated for 1 h. Substrate consisting of 10 mg of ortho-phenylenediamine in 12 mL of citrate buffer and 10 µL of 30% H_2O_2 were then placed into each well, and the absorbance was read using a spectrophotometer (Titertek Multiskan) at 492 nm.

Adverse effects

With the purpose to discard that the addition of the fungal spores to the pellets could transfer them awful odour, consistency and/or taste, it was also observed if any of the mares in G-P refused to feed the pellets with the spores.

The appearance of side effects in the horses belonging to the three groups was evaluated throughout the assay. The analysis of the digestive activity was based on the mares showed normal appetite, and the presentation of disorders as drooling, constipation, diarrhoea and dehydration was recorded. The respiratory function was assessed by checking the possible

manifestation of signs as cough, nasal discharge, abnormal temperature and breathing, increased respiratory rate or effort at rest, and slow recovery and frequent swallowing after exercise.

The reproductive functionality was based on that all mares cycle properly. Finally, the examination of the skin was also conducted.

Statistical analysis

The FECR and PHR values were expressed as percentages and 95% confidence intervals. The kinetics of the egg-output was represented in the graph (Fig. 1) as the mean and the standard deviation, so that these results could be compared with those of previous investigations.

Because of the Levene's probe showed that faecal egg count values did not adjust to a normal distribution (Statistic= 8.127, P= 0.001), these numbers were analysed by means of the non-parametric Mann-Whitney U two-sided test (α= 0.05) (Francisco et al., 2012). On the contrary, data of FECR, PHR and the IgG antibody values were normally distributed and therefore an ANOVA with repeated measures was performed.

Significant differences were considered when $P < 0.05$. All tests were done using SPSS for Windows (20.0; SPSS Inc., Chicago, IL, USA).

Results

Coprological analyses

Eggs of *P. equorum* and strongyles were observed in the faeces at the first week of study. Larvae were identified as *Cyathostomum sensu latum* type A (63%), type B (1%), type C (12%), type D (19%), and type H (1%), and *Gyalocephalus capitatus* (4%) (Madeira de Carvalho *et al*., 2008).

As shown in Figure 1, at the beginning of the study the percentages of horses shedding eggs of *P equorum* ranged from 14% (1 out of 7 in G-T) to 29% (2 out of 7 in G-C and G-P); all the horses passed eggs of strongyles, and the egg-output values were higher than 500 EPG (eggs per gram of faeces) in the three groups (Fig. 1). Two weeks after the pour-on administration of Ivermectin (IVM) to the horses of G-P and G-T, eggs of *P. equorum* and strongyles were not observed in the faeces, thus 100% values for the FECR and PHR were recorded. In the two dewormed groups (G-P and G-T), eggs of *P. equorum* did not appear again after treatment, whereas a discontinuous egg-excretion in the G-C was observed throughout the study, with values ranging from 0 to 150 EPG (Table 1).

In the horses receiving the feed pelleted with the fungal spores (G-P), the strongyle faecal counts ranged from 44 (8^{th} weeks after treatment, wat) to 247 EPG (60^{th} wat) (Fig. 1). The egg-output values in the horses of G-T (treated and given pellets without spores) increased significantly from the 8^{th} wat and reached a similar pattern to that observed in the G-C (non-dewormed horses), oscillating between 310 (20^{th} wat) and 773 EPG (44^{th} wat) (Fig. 1). Significant differences among the G-P and the G-T and G-C were recorded (U= -11.166, *P*= 0.001, and U= -11.532, *P*= 0.001, respectively).

FECR in the horses fed pellets with spores (G-P) was higher than 90% until the 24^{th} wat (87%; 95% CI 85-91) (Table 2), and the ERP until 28 weeks. Percentages ranging from 57% to 90% were achieved till the end of the trial. The PHR values remained higher than 50% until the 24^{th} wat.

In the horses of G-T, the FECR numbers were lower than 50% from the 8^{th} wat to the end of the study, and the ERP was 8 weeks (Table 2). From the 12^{th} wat, all the horses in this group were positive to the flotation test.

Haematological parameters

Erythrocyte count and haemoglobin concentration are shown in Figures 2-4. Values of erythrocytes and haematocrit lower than the minimum were observed in the horses of G-C and G-T, whereas significantly higher counts were recorded in G-P (F= 60.740, P= 0.001 and F= 43.600, P= 0.001, respectively). Normal concentrations of haemoglobin were recorded in all groups, but the highest values were obtained in the horses of G-P (F= 48.265, P= 0.001). Regarding the white blood cells, significant differences were achieved for the leukocytes only, with the highest values among the horses of G-C and G-T (F= 11.938, P= 0.001).

After the administration of Ivermectin, the values of red blood cell parameters significantly increased in the two treated groups until the 8th week after treatment. In the horses of G-T (dewormed and receiving pellets without spores) a new reduction in these parameters was then observed, decreasing to values lower than the normal range. In contrast, the values of red blood cell parameters maintained elevated in the G-P until the end of the study.

When considering data collected in the 3 groups, significant negative correlations were recorded between the strongyles EPG and the values of erythrocytes (Pearson correlation, PC= -0.469, P= 0.001), haemoglobin (PC= = -0.460, P= 0.001) and haematocrit (PC= -0.404, P= 0.001). The correlation with the leukocyte counts was positive (PC= 0.245, P= 0.001).

Sensitization to fungal antigens

By testing the horse sera against the antigens collected from the two fungal species, no IgG humoral response was detected in any case, and the values reached in the G-P were closed to those of G-T and G-C.

Adverse effects

None of the mares in the current study exhibited side effects concerning respiratory, digestive, reproductive or cutaneous problems. The mares in G-P never refused to take the pellets with the fungal spores.

Discussion

A strategy for controlling gastrointestinal nematodes affecting grazing horses, based on integrating deworming and prevention has been assayed in autochthonous Pura Raza Galega (PRG) mares shedding eggs of *P. equorum* and strongyles. At the beginning of the current research, all the horses shed more than 500 strongyle eggs per gram of faeces (EPG), and 14-29% of them passed eggs of *P. equorum*. Two groups of 7 each were dewormed pour-on and other remained untreated as control. The egg-output of *P. equorum* and strongyles ceased 14 days after treatment, providing a 100% value for the reduction of faecal egg counts (FECR) and of the horses positive to the flotation test (PHR). These results are partly in agreement with previous investigations comprising the oral administration of the macrocyclic lactone (Lind et al., 2007; Schougard & Nielsen, 2007; Lyons et al., 2008; Larsen et al., 2011). The PRG horses are seldom dewormed due to the handling difficulties. Efficacy of ivermectin against strongyles and ascarids affecting PRG horses has been previously reported, without observation of adverse effects (Francisco et al., 2009, 2011).

Despite the efficacy of different anthelmintics, a short-term effect is achieved in horses with access to grass, due to the frequent presence of free-living stages (eggs, larvae, cysts) in the pasture. This reinforces the requirement to apply preventive measures to reduce their presence in the environment. Prior investigations showed the ability of certain fungal spores to survive and maintain their biological properties after the industrial extrusion of pellets (Cortiñas et al., 2015; Arroyo et al., 2016), thus a novel possibility to decrease the risk of nematode infection was assayed. With this aim, commercial pellets manufactured with a

mixture of spores of *Mucor circinelloides* (egg-parasitic fungus) and *Duddingtonia flagrans* (larva-trapping fungus) were given daily to horses previously dewormed. Egg of *P. equorum* were not detected throughout the study (15 months), and the eggs of strongyles remained lower than 300 EPG until the end of the assay, thus it was concluded that they did not need additional treatment throughout this period. In contrast, in the dewormed horses receiving pellets without fungal spores, the strongyle EPG counts reached values higher than 300 from the 8th week after treatment, close to those recorded in the untreated group. Former studies conducted on horses receiving Ivermectin and then weekly provided handmade pellets containing mycelium of trapping fungi reported a significant reduction in the EPG numbers during 6 months (30.5%-73.2% with *D. flagrans*, 35.2%-87.5% with *Monacrosporium thaumasium*) (Braga et al., 2009; Tavela et al., 2011). Other formulations involving the oral administration of chlamydospores of *D. flagrans* as a feed supplement also provided successful results (Larsen et al., 1996; Mendoza de Gives et al., 2006; Arias et al., 2013). There is a lack of information regarding the efficacy of ovicidal fungi on horse parasites, and *Pochonia chlamydosporia* is the only described to date (Silva et al., 2010; Braga et al., 2012). A significant reduction of viable eggs of *Ascaris suum* (60%) and *Fasciola hepatica* (67%) in the faeces of piglets and calves given mash feed-added *Mucor circinelloides* spores has been recently reported (Cortiñas et al., 2015).

The mere presence of worm eggs in faeces does not always justify an anthelmintic treatment, and a reduction in the frequency of deworming has been widely advised with the objective to limit the selection for anthelmintic resistance. According to the Parasite Control Guidelines stated by the American Association of Equine Practitioners (AAEP, 2013), foals should be dewormed when they are over 8-12 weeks old and then repetitions done every 3 months until they are yearlings. Other methods focused on reducing the use of anthelmintics and the development of anthelmintic resistance are the targeted treatments, consisting of the

deworming of a whole herd according to a predetermined threshold faecal egg count (FEC), while targeted selective treatments (TST) promote the application of treatment only to the animals that are in need, mainly when health or productivity can be significantly lessened (Kenyon et al., 2009; Kenyon & Jackson, 2012). In the present investigation, there was a demonstrated negative correlation between the strongyle egg-output counts and the values of erythrocytes, haemoglobin and haematocrit, in agreement with Francisco et al. (2009). The untreated horses attained values of red blood cell parameters significantly lower than the normal levels throughout the study. After the administration of Ivermectin, these parameters significantly increased until the 8^{th} week after treatment in the two treated groups. Whereas in the horses feeding pellets without spores the red blood cell parameters decreased beneath the normal levels again, those equids receiving pellets with spores maintained normal levels till the end of the study. Braga et al. (2009) reported a significant weight gain in mares feeding handmade pellets with *D. flagrans* in respect to the control group.

Some points concerning the utilization of fungal spores to prevent nematode infection in horses require more attention. Firstly, a possible interference of the anthelmintic (Ivermectin) on the parasiticide fungi could be suspected. There is no available information concerning this point, but a preliminary assay conducted in our Lab demonstrated in Petri plates with Ivermectin showed that the fungi *M. circinelloides* and *D. flagrans* could develop, and differences in relation to the absence of the macrocyclic lactone were not obtained (unpublished data). The second point refers to the dosage of spores required. Previous investigations pointed out the need of giving high numbers of fungal spores to horses to reach successful results. Several studies recommended a dose of $5x10^6$ *D. flagrans* chlamydospores / Kg bodyweight for horses and small ruminants (Larsen et al., 1995; Epe et al., 2008). The distribution of $2x10^6$ chlamydospores of *D. flagrans* / Kg bodyweight as a premixed feed reduced the strongyle egg counts by more than two thirds among equids from a zoological

park (Arias et al., 2013). Araújo et al. (2004) provided $2x10^6$ conidia of *M. thaumassium* orally twice a week during 4 months, and a 53.81% of reduction in the faecal eggs of gastrointestinal nematodes in relation to non-treated calves was observed. Furthermore, the weekly administration of an oral dose of handmade sodium alginate pellets containing 1 g mycelium / 10 Kg bodyweight decreased the cyathostomin egg-output numbers (35%-73%) in grazing horses (Braga et al., 2009).

It has been demonstrated that *Duddingtonia flagrans* can adapt to the cyathostomin egg-output, and as a results, elevated percentages of reduction are obtained without increasing the numbers of chlamydospores (Paz-Silva et al., 2011), This might be very useful for achieving satisfactory results by using lower quantities of spores than initially proposed. In the current investigation, horses were fed 2.5 Kg pellets per day, which resulted in $5x10^6$ spores / horse / day. By considering an average weight of 400 Kg, a dosage of $1.25x10^4$ spores/Kg bodyweight was administered to the mares daily, and the strongyles egg-output values were down by two thirds in comparison to the groups given pellets without the fungi. Moreover, a threefold increase in the egg reappearance period (28 weeks) was recorded in the mares receiving pellets containing spores compared with that observed in the horses given pellets without spores (8 weeks). It is remarkable that more than half the horses in this group did not shed eggs of strongyles in the faeces until the 28^{th} wat.

Finally, the analysis of horse sera by means of an ELISA and antigenic products collected from the utilized fungi showed the absence of a serological response to either *M. circinelloides* or *D. flagrans*, confirming previous investigations indicating that horses ingesting the spores are not exposed to them systematically and participate in the distribution of the fungi via the faeces to the soil (Braga et al., 2009; Tavela et al., 2013). It is needed to emphasize that the shelf-life of packed commercially pelleted feed is warranted for 3 months, and the spores are able to maintain their anti-parasitic effect in the pelleted feed through 6

months, without showing signs of alteration or fungal growth (Arias et al., 2015; Arroyo et al., 2016). This mode of administering fungal spores would not imply an additional work for the horse-keepers.

Conclusions

Control of horse nematodes relies on deworming for suppressing the infection in the animals, and prevention of their reinfection. The pour-on administration of Ivermectin eliminates the presence of adult stages of *P. equorum* and strongyles. Our results demonstrate the possibility to reduce the risk of infection by gastrointestinal nematodes by feeding horses with pellets industrially manufactured with a blend of spores of an ovicidal fungus (*M. circinelloides*) and a larval-trapping species (*D. flagrans*). No adverse effects have been observed after feeding the horses with pellets containing spores for 15 months, and these equids reached normal values for the cell blood parameters. This procedure allows for reduced reliance for the control of nematodes affecting horses.

Conflict of interest

All authors declare the absence of any financial or personal interests that could inappropriately influence the current work. The final article has been approved by all authors.

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Caption of figures

Figure 1.- Kinetics of strongyles egg-output in Pura Raza Galega (PRG) grazing horses under an integrated nematode control strategy. Points represent the average and bars 2*SD. 1: significant differences between G-C and G-T; 2: between G-C and G-P; 3: between G-T and G-P.

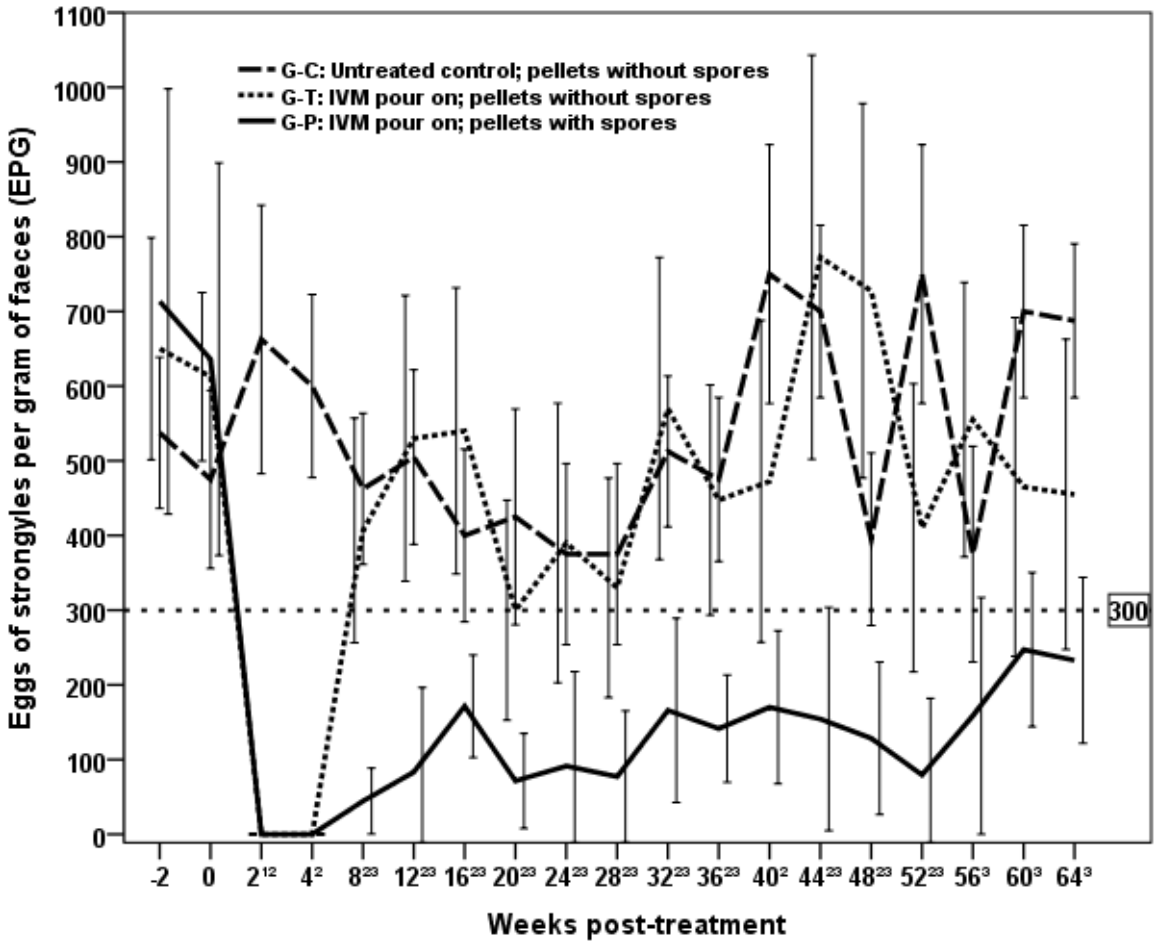

Figure 2.- Dynamics of blood cell parameters in Pura Raza Galega (PRG) grazing horses under an integrated nematode control strategy. Points represent the average and bars 2*SD. Dashed lines mean the upper and lower limit physiological (healthy) values, respectively. 1: significant differences between G-C and G-T; 2: between G-C and G-P; 3: between G-T and G-P.

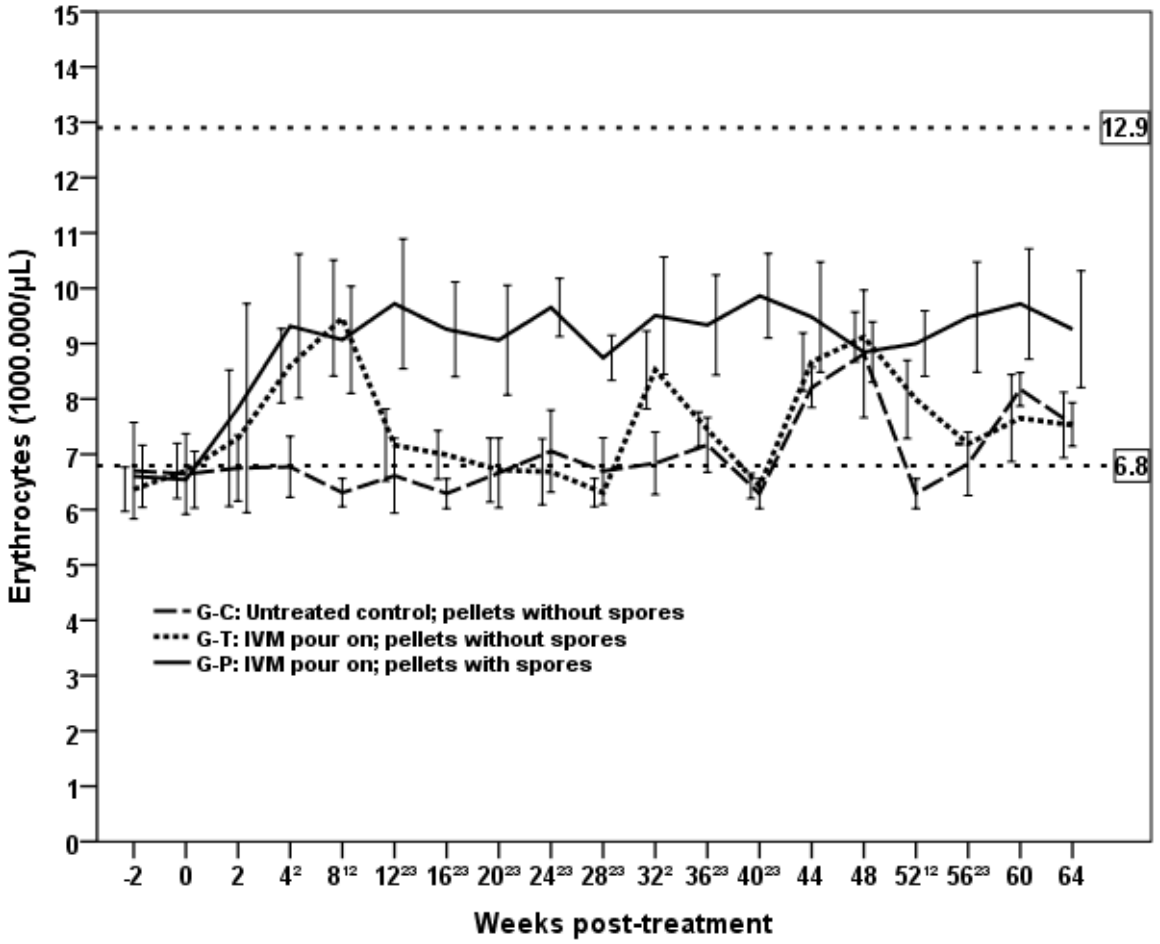

Table 1.- Values of the *Parascaris equorum* egg-output, Faecal Egg Count Reduction (FECR) and coprological Positive Horses Reduction (PHR) in Pura Raza Galega (PRG) grazing horses under an integrated nematode control strategy. G-P: horses dewormed (1 mg Ivermectin / Kg bw pour on, Noromectin 0.5%, Norbrook Laboratories, UK) and provided daily pellets with fungal spores; G-T: horses dewormed (1 mg Ivermectin / Kg bw pour on) and given pelleted feed without fungal spores. G-C: horses without deworming and feeding on pellets without fungal spores. (CI: Confidence Interval; WAT: weeks after treatment).

	G-C (*n*= 7)	G-T (*n*= 7)					G-P (*n*= 7)				
WAT	EPG (x ± SD)	EPG (x ± SD)	FECR	95% CI	PHR	95% CI	EPG (x ± SD)	FECR	95% CI	PHR	95% CI
-2	37 ± 48	50					39 ± 24				
0	50 ± 71	44 ± 62					46 ± 48				
2	12 ± 25	0	100		100		0	100		100	
4	12 ± 25	0	100		100		0	100		100	
8	0	0	100		100		0	100		100	
12	38 ± 75	0	100		100		0	100		100	
16	0	0	100		100		0	100		100	
20	12 ± 25	0	100		100		0	100		100	

24	0	0	100	100	0	100	100
28	0	0	100	100	0	100	100
32	150 ± 63	0	100	100	0	100	100
36	0	0	100	100	0	100	100
40	12 ± 25	0	100	100	0	100	100
44	0	0	100	100	0	100	100
48	0	0	100	100	0	100	100
52	38 ± 48	0	100	100	0	100	100
56	0	0	100	100	0	100	100
60	0	0	100	100	0	100	100
64	12 ± 25	0	100	100	0	100	100

Table 2.- Values of the strongyles Faecal Egg Count Reduction (FECR) and coprological Positive Horses Reduction (PHR) in Pura Raza Galega (PRG) grazing horses under an integrated nematode control strategy. G-P: horses dewormed (1 mg Ivermectin / Kg bw pour on, Noromectin 0.5%, Norbrook Laboratories, UK) and provided pellets with fungal spores; G-T: horses dewormed (1 mg Ivermectin / Kg bw pour on) and given pelleted feed without fungal spores. (CI: Confidence Interval; WAT: weeks after treatment).

	G-P (*n*= 7)				G-T (*n*= 7)			
WAT	FECR	95% CI	PHR	95% CI	FECR	95% CI	PHR	95% CI
2	100		100		100		100	
4	100		100		100		100	
8	93	91 – 95	71	38 – 100	34	30 – 37	29	0 – 62
12	89	86 – 91	57	20 – 94	13	11 - 16	0	
16	88	86 – 91	29	0 – 62	12	9 – 14	0	
20	89	87 – 92	29	0 – 62	51	47 - 55	0	
24	87	85 – 91	57	20 – 94	36	32 – 40	0	
28	76	73 – 80	14	0 – 40	46	42 – 50	0	
32	74	71 – 77	14	0 – 40	7	5 – 9	0	
36	78	75 – 81	14	0 – 40	27	23 – 30	0	
40	73	70 – 77	14	0 – 40	23	19 – 26	0	

44	76	72 – 79	14	0 – 40	0		0
48	80	77 – 83	14	0 – 40	0		0
52	88	85 – 90	43	6 – 80	33	29 – 37	0
56	75	72 – 78	29	0 – 62	9	7 – 12	0
60	61	57 – 65	0		24	21 – 27	0
64	63	60 – 67	0		26	22 – 29	0