

Differences in the fly-load of *Haematobia irritans* (Diptera: Muscidae) on cattle is modified by endophyte infection of pastures

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Abstract

Background: The horn fly, *Haematobia irritans*, is an obligate bloodsucking ectoparasite of pastured cattle and is a major pest of livestock production in North and South America and Europe. In this study, we investigated the potential to use cattle pastures, infected with non-toxic, “friendly” fungal-endophyte-infected (E+) tall fescue, *Festuca arundinacea* Schreb., as a strategy for reducing horn fly loads in cattle, and to evaluate the possible bioinsecticide effect on horn fly larvae.

Results: When cattle grazed in E+ tall fescue, a decrease in fly-load was observed, compared with other pastures (endophyte-free (E-) pastures). The infestation of horn fly load decreased according to an increase in the percentage of endophyte present in the different pastures (0 to 100%). Moreover, two groups of animals with significant differences in the fly-load (high and low fly-load) in the same herd were observed ($P < 0.05$). Additionally, it was possible to determine a bioinsecticide effect of cattle dung, upon horn fly larvae (80%), from animals fed E+ tall fescue.

Conclusions: These results constitute the first report on the potential for exploiting pasture management for controlling 1) horn fly-loads on cattle and 2) the normal development of horn fly larvae. In conclusion, this information provides preliminary understanding of the role of cattle pasture diet management for controlling horn flies as part of an integrated pest management strategy for this major pest of farmed livestock.

Keywords: endophyte-infected tall fescue, fly-load, horn fly, pasture.

INTRODUCTION

The horn fly, *Haematobia irritans* (Diptera: Muscidae), is an obligate bloodsucking ectoparasite of pastured cattle and currently represents a serious pest problem for livestock production in North and South America and Europe (Byford et al. 1992; Byford et al. 1999). Cattle are continuously annoyed by the feeding activity of horn flies (Oyarzún et al. 2008). Foil and Hogsette (1994) described that both male and female *H. irritans* spend their entire life on the host, feeding 24-38 times/day, using their piercing sucking proboscis. An insect can consume between 11 to 21 mg blood/day, and an animal infected with 500 flies can potentially lose up to 7 ml blood/day (Cupp et al. 1998; Tommasi, 1999). The economic impact of horn fly biting on weight gain in beef cattle and milk production has been reviewed by Oyarzún et al. (2008). An infestation between 200 and 400 flies per animal can cause a 4-20% decrease in weight gain and 4-12% decrease in milk production (Guglielmone et al. 1998; DeRouen et

al. 2003). In the US, horn flies cause annual losses of over US\$ 730 million (Byford et al. 1992). In Chile, Velasco et al. (2001) and Lanuza et al. (2005) estimated that annual losses due to horn flies were US\$ 25 million approximately. Currently, control of *H. irritans* is based on the use of broad-spectrum insecticides (organophosphates and pyrethroids). However, this practice has caused the emergence of insecticide-resistant fly populations, and, therefore, new control strategies are needed (Steelman et al. 2003). The most promising strategies comprise 1) selection of horn fly-resistant cattle (Kunz and Kemp, 1994), and 2) use of semiochemicals (naturally-occurring behaviour and development-modifying chemicals) that are involved in conferring such host resistance (Pickett et al. 2010). Previous studies have reported a variation in the number of the flies carried by individual animals (Jensen et al. 2004). This uneven distribution of flies, and the variation within and between breeds, has been described by several authors (Steelman et al. 1993; Jensen and Jespersen, 1994; Guglielmo et al. 2000). Brown et al. (1992) and Donald (1996) suggested that the variation in the number of flies appears to be heritable, suggesting that the selection of cattle with low fly-load (or resistant animals) may offer an alternative to chemical control. Furthermore, several pioneering studies have elucidated the role of volatile semiochemicals involved in the process of identifying or discriminating between host and non-host cattle (Birkett et al. 2004; Logan and Birkett, 2007; Oyarzún et al. 2009).

A further possible approach for horn fly control could be *via* manipulating cattle diet. According to Dougherty and Knapp (1994), changes in animal diet modify the response of *Musca autumnalis* De Geer (Diptera: Muscidae). Tall fescue, *Festuca arundinacea* Schreb., and perennial ryegrass, *Lolium perenne* L., (Poaceae), are commonly used as pasture for animal feed. These species are often infected with fungal endophytes, such as *Neotyphodium* spp., and this symbiotic relationship results in the synthesis of fungal alkaloids that confer resistance to the plant against insect herbivores (Hoveland, 1993; Schardl et al. 2004; Potter et al. 2008). For example, the resistance of infected plants to the Argentine stem weevil, *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae), a major insect pest in Europe and America, is due to peramine alkaloids produced by the endophyte *N. lolii* (Rowan and Gaynor, 1986; Rowan et al. 1990). Dougherty et al. (1999) demonstrated that alkaloids extracted from endophyte-infected *N. coenophialum*, present in seeds of tall fescue, and incorporated in cattle dung, present a larvicidal effect against horn flies and other insect. The horn fly life cycle starts when females deposit their eggs in fresh cattle dung (Hogsette and Farkas, 2000), with first instar larvae appearing 1-2 days after hatching, and adult emergence occurring about 6 and 8 days after oviposition in summer (Foil and Hogsette, 1994). The insecticidal activity of alkaloids present in forage infected with endophytic fungi could take place either via the blood (Hill et al. 2000) or the cattle dung (Dougherty et al. 1998; Dougherty et al. 1999). However, nothing is known about the possible insecticidal role of diet based on the use of endophyte-infected pastures. Additionally, one of the major difficulties of this type of study is the rearing of horn flies for obtaining larval instars, under laboratory conditions. In this work, we explore, for the first time, the effect of pastures with endophytic fungi on both the horn fly-load on cattle and bioinsecticide activity on larval stages. Furthermore, we describe a facile and inexpensive method to obtain immature stages of the horn fly. Based on these antecedents we hypothesize that the horn fly-load can be modified by pastures associated with endophytic fungi. Thereby, the aims of this study were: 1) to validate variations in the fly-load of *H. irritans* in animals fed on endophyte-infected and endophyte-free pastures and, 2) to determine the bioinsecticide activity of cattle dung on horn fly larvae when animals graze on endophyte-infected and endophyte-free pastures.

MATERIALS AND METHODS

Study site

Field experiments were conducted at Centro Regional de Investigación INIA Carillanca, Vilcún, Chile (38°41'SL, 72°25'WL, 200 masl), during the summer period (January to February 2011). The experiments were performed in pastures of different botanical composition: 1) endophyte-infected (E+) tall fescue cultivar K-31 (100% endophyte), which is non-toxic to cattle (Jennings et al. 2002), 2) mixed pasture (ryegrass/white clover) (0% endophyte) and 3) brome Poker-INIA cultivar (0% endophyte) (E-). Steer beef cattle herd (n = 25) was monitored daily during the study. Each day, between 10:00 a.m. and 15:00 pm, the following toxicosis symptoms were monitored: staggers, skin change and hoof problems (Stuedemann and Thompson, 1993; Ball et al. 2002).

Cattle

The animals were selected from a crossbreed herd consisting of Herford x A. Angus steers with similar weight. All the animals were marked individually with numbered ear tags ($n = 25$), and to avoid grazing interference with other herd, hence, the animals of the study were separated by a distance of approximately 500 m from other grazing animals. Grazing was only source of feed through the season using a rotational management. The herd was used for all bioassays: fly-load, effect of feed on fly-load and bioinsecticide effect of cattle dung. Cattle did not receive any treatment against ectoparasites before or during the study period.

Identification of steers of low and high fly-load in different types of pasture

For this experiment, the methodology described by Jensen et al. (2004) and modified by Oyarzún et al. (2009) was used. The herd made up of 25 animals was kept for two weeks in each pasture (E+ tall fescue, mixed pasture and brome grass) between January and February 2011. Two observers recorded all flies present on Overo Colorado steers when the animals grazed between 10:00 am and 15:00 pm. According to the methodology proposed by Castro et al. (2008), each observer counted flies visible on each animal's upper body, from the head to tailhead, left and right flank of each animal, as well as foreleg and hind legs. Paired counts from the two observers were used to rank, numerically, cattle into two groups: low fly-load (I-V) and high fly-load (VI-X), each consisting of 5 animals, as suggested by Pruett et al. (2003).

Effect of feed on the fly-load

This experiment consisted of moving the herd from brome pasture to mixed (ryegrass/white clover) pasture, and then to E+ tall fescue pasture. In order to eliminate the residual effects from the previous pastures, the herd was left for 4 days in each pasture for acclimatization before fly-load assessment began. The counts were performed daily (10:00 am) from day 5 to 9. Total counts were standardized and averaged for determining differences in the populations of horn flies between the different pastures.

Presence of endophyte in tall fescue

Verification of the presence of endophytic fungi in tall fescue pasture (cultivar K-31) was checked by the method described by Saha et al. (1988). Briefly, 20 tillers obtained from different plants were cut and the inner epidermis of a leaf sheath was peeled off and placed on a glass slide. Two drops of rose Bengal stain were applied to each sample. After 60 sec the samples were covered with a cover slip. Finally, microscopic examination (200X) was performed, identifying as positive those samples presenting typical fungal mycelia. The percentage infection was determined from the number of samples with endophyte examined over the total samples examined.

Horn fly rearing

Horn flies required for laboratory experiments were obtained using a protocol modified from Oyarzún et al. (2009). Steers previously selected were placed in a chute. *H. irritans* feeding on these animals were trapped in 1-liter glass flask (50-60 insect per flask). Insects were then transferred to other glass flasks with filter paper attached to the walls and covered with dung from animals fed with E- pasture (mixed and brome pasture). The flask were sealed with gauze mesh and transported to the laboratory in a cooler at 8-10°C. Once in the laboratory, the flies were maintained at 26°C and a photoperiod of 14:8 (L:D).

Bioinsecticide effect of cattle dung on horn fly larvae

This bioassay was based on the methodology proposed by Dougherty et al. (1999). Parallel to the insect collection, dung samples were obtained from E- pasture and E+ tall fescue pastures. These samples were transferred to the laboratory, where 15 g quantities of cattle dung were weighed and placed in Petri dishes (8.5 by 1.5 cm). Second Instar larvae, 5-6.5 mm long and 3-5 days old (Krafsur and Ernst, 1986), were placed into the dishes which were covered with aluminum paper which had

holes to facilitate gas exchange. The dishes were stored at 26°C and in the dark. Twenty replicates were performed per treatment, which were reviewed at 48 hrs. Bioinsecticide effect was defined as the percentage of larvae that did not development into pupae under the conditions of the experiment.

Statistical analysis

Significant differences between animals with both low and high fly-load were determined by a Kruskal-Wallis test ($P < 0.05$). Data for determining the effect of feed on fly-loads were analysed by a Friedman test ($P < 0.05$), whilst data for determining the bioinsecticide effect of cattle dung on horn fly larvae were analyzed by parametric a Tukey test (Conover, 1999).

RESULTS

Identification of animals of high and low fly-load

Fly counting data showed that *H. irritans* populations were not distributed homogeneously over the animals in any of the pastures evaluated. Regardless of the pasture, there were, in the same herd, two groups of animals with significant differences in the fly-load ($P < 0.05$) (Figure 1). Steers VI to X were defined as high fly-load individuals and I to V as low fly-load individuals.

Effect of animal feed on fly-load variation

Animals grazing on E+ tall fescue pasture had a significantly lower amount of horn flies than animals grazing on mixed and brome pastures (Figure 2, $P < 0.05$). The infestation of *H. irritans* decreased according to an increase in the percentage of endophyte present in the different pastures (0 to 100%) (Figure 2). The average fly-load in the herd decreased from 127.3 (± 20) in the brome pasture, to 113.1 (± 13.1) in the mixed pasture, and finally to 75.1 (± 6.9) in E+ tall fescue pasture. The effect of decreasing fly-load was only significant on high-fly load cattle (Figure 3a). The fly population decreased from 214 \pm 23.9 (brome pasture) and 170.7 \pm 14.4 (mixed pasture) to 105.9 \pm 6.8 (E+ tall fescue pasture).

Presence of endophyte

The phytopathological analysis of tall fescue plant material indicated that the species of endophyte fungus present in cultivar K-31 was *Neotyphodium coenophialum*. Table 1 shows that K-31 was completely infected by the endophyte. In contrast, *N. coenophialum* was not found in the brome and mixed pasture.

Table 1. Percentage (%) of infection of *Neotyphodium coenophialum* in tall fescue pastures in the Centro Regional de Investigación INIA Carillanca, Vilcún, Chile. Season 2011.

Pasture	Plant +	Plants -	Endophyte (%)
E+ tall fescue	10	0	100
Mixed (ryegrass/ white clover)	0	10	0
Brome	0	10	0

Bioinsecticide effect of cattle dung

Significant differences ($P = 0.0481$) in larval mortality of *H. irritans* were observed between treatments at 48 hrs (Figure 4). 80 \pm 11.8% of the larvae died when they fed on dung of cattle kept on pasture with endophytic fungi. The natural mortality of larvae in the E- cattle dung was estimated at 50 \pm 9.2% (Figure 4).

DISCUSSION

An uneven distribution of *H. irritans* between individuals within cattle herds, with animals being identified with high and low fly load, was observed. This result was consistent with those reported from previous studies (Jensen et al. 2004; Oyarzún et al. 2009). Following the methodology of Oyarzún et al. (2009), phenotypically similar animals were used to avoid variation in the study. However, the horn flies were differentially attracted to individual steers. Pruett et al. (2003) summarized several studies indicating that factors such as breed differences, skin colour, skin temperature, hair density and sebaceous secretions are responsible for the unequal distribution of *H. irritans* on individual animals. Another factor that could affect the fly-load and which has gained prominence in the last decade comprises host-derived volatile semiochemicals. Birkett et al. (2004) and Oyarzún et al. (2009) showed the importance of volatile semiochemicals in conferring different levels of attraction of horn flies and other dipterans to animals within a herd. 1-Octen-3-ol and 6-methyl-5-hepten-2-one were identified as attractants under laboratory conditions, but in the field, they reduced the population of horn flies in cattle, suggesting that these compounds may mask cattle odour, leading to a reduction of fly-load of *H. irritans* (Birkett et al. 2004). A similar effect was reported by Oyarzún et al. (2009), who showed that the *m*-cresol and *p*-cresol, produced via microbial degradation of urine, were attractive to *H. irritans* in olfactometry bioassays, but possessed a repellent effect under field conditions.

Our fly-load data (Figure 2) showed that animals grazing on a E+ tall fescue pasture had lower fly loads, in contrast to animals that grazed on the E- pastures, in which the fly-load per animal was significantly higher. This effect was only observed on the high fly-load group (Figure 3). These results suggest that infestation of *H. irritans* in bovines could be manipulated via use of pastures with endophyte present, through the presence of endophyte-produced natural products that exert a negative effect on fly loads on animals. The symbiotic relationship between plant and endophytic fungi is mutualistic, whereby the fungus receives shelter and food from the plant. The fungus spreads within the plant through its hyphae, and through the seeds for new plants. In return, the fungus protects the plant from attack of herbivores by producing alkaloids, which either confer a repellent or insecticidal effect (Prestidge et al. 1991; Artigas, 1994). It should be noted that the first record of fly-load was conducted at the fourth day of grazing in each pasture, so that the digestive process of animals coming from the previous pasture did not interfere with the effect produced by feeding. When the E+ tall fescue is ingested by the animal, the alkaloids are stored in the rumen fluid and then absorbed through the rumen wall remaining in the blood stream (Stuedemann et al. 1998). Because the horn fly is totally dependent upon bovine blood as a food source, alkaloid flowing via the blood stream can exert a deterrent action (Kuramochi, 2000), and it can be hypothesized that flies will be deterred from feeding on animals containing alkaloids in their blood, and seek alternative suitable hosts. This coincides with Pruett et al. (2003), who indicated that if the flies were unsuccessful in obtaining blood from their initial host, they would seek other more suitable hosts. This behaviour could result in animals with low horn fly-loads, as observed in this study and elsewhere (Figure 1).

The phytopathological results indicated that the species of endophyte fungus present in cultivar K-31 was *Neotyphodium coenophialum* (Clavicipitaceae) (Table 1). This species is an asexual fungus that shares a mutualistic relationship with tall fescue (Schardl et al. 2004). Endophytes in pasture grasses confer important agronomic advantages such as persistence on poor soils and improving its drought tolerance (Jennings et al. 2002; Schardl et al. 2006). However the E+ tall fescue may cause toxicosis problems in the cattle, known as fescue toxicosis. According to Stuedemann and Hoveland (1988) and Seman et al. (1999), fescue toxicosis affects adversely cattle performance and alters ruminant grazing behaviour. Many studies have documented adverse effects of endophytic pastures on insect herbivores. However, the specific role of particular alkaloids in such interactions is less clear (Potter et al. 2008). In some cases, alkaloids can affect animal health (Prestidge et al. 1991), and therefore, their presence is considered, under certain conditions, as a major risk to livestock production. Endophytes produce alkaloids that can be classified into three categories based on structure: lolitrem B (pyrrolizidine), ergovaline (ergot alkaloids) and peramine (pyrrolopyrazine) (Schardl et al. 2004). Previous studies have reported health problems such as staggers and heat stress (fescue toxicosis) caused by lolitrem B and ergovaline (Porter, 1995). On the other hand, peramine has been associated with insect resistance because of its' deterrent effect but, importantly has not been linked to a toxic effect in animals (Fletcher, 1999; Pennell et al. 2005). Efforts to resolve this dilemma of fescue toxicosis have focussed on creating novel associations of high-yielding grass cultivars with endophytes that are relatively non-toxic to bovines and others animals (Bouton and Easton, 2004; Hunt and Newman, 2005; Panaccione, 2005), including production of non-toxic "friendly" E+ tall fescue (Jennings et al. 2002) that was used in this study. According to this, in our study the steers did not show the

toxicosis symptoms described by Stuedemann and Thompson (1993) and Ball et al. (2002). This is consistent with the report of Jennings et al. (2002), indicating that friendly E+ tall fescue is an effective alternative to E- tall fescue due to plant persistence, good cattle performance and no toxicity problems in animals.

The results referring to the larval mortality of *H. irritans* (Figure 4) are consistent with the results reported by Dougherty et al. (1998), who obtained 100% mortality of horn fly larvae using cattle dung supplemented with alkaloids (acyl lolines). Rotter and Phillips (1991) suggested that the ecology of cattle dung may be influenced depending on the alkaloid concentration in the E+ forage consumed, which would be the cause of this bioinsecticide activity. With respect to the interaction between alkaloids and haematophagous insects, it has been shown that leaf extracts of *Ervatamia coronaria* (Apocynaceae) contain alkaloids that are responsible for the larvicidal activity against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* mosquitoes (Diptera: Culicidae), (Mathivanan et al. 2010). Dougherty and Knapp (1994) reported that cattle dung from E+ tall fescue pastures caused a decrease in oviposition and an increase in the larval mortality of *Musca autumnalis*. According to the authors, the reason for this effect was probably due to the presence of alkaloids in E+ tall fescue. Our results indicate that the toxicity of endophytes due to alkaloids could be conferred to dung.

There have been several investigations into the potential use of alkaloids in the protection of crops and livestock (Krzyszowska et al. 1998). The resistance provided by alkaloids against insect herbivore has been well established and has generated interest in using these pastures or grasses containing alkaloids to manage insect pests (Salminen et al. 2005). It has been reported that alkaloid extracts influence food, larval development and mortality of many insect. For example, decreases in larval weight and survival of fall armyworm, *Spodoptera frugiperda* JE Smith (Lepidoptera: Noctuidae) have been observed in E+ tall fescue and perennial ryegrass (Hardy et al. 1985; Bultman and Conard, 1998). The inoculation of endophyte in forage crops was developed for addressing the persistence caused by the Argentine stem weevil, *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae). The larvae and adult stages cause damage, affecting the establishment of plants by foliar consumption of newly emerged seedlings. This detrimental effect produces a decrease in plant population, which means replanting and/or production losses, decreasing pasture quality and reducing of half-life (Norambuena and Gerding, 1985; Prado, 1991; Artigas, 1994). Rowan et al. (1990) and Rowan (1993) demonstrated that peramine, present in both endophytic perennial ryegrass and tall fescue was responsible for feeding deterrence by *L. bonariensis*.

In summary, this study provides the first evidence that friendly E+ tall fescue could contribute to a significant reduction in horn fly loads in cattle and a reduction of larvae in cattle dung. The fact that it was possible to reduce the fly-load on steers with a high fly-load (Figure 3a) would indicate that the effect of the pasture is more effective on fly-susceptible cattle. However, future studies should be performed to determine the mechanism by which horn flies are deterred from feeding on the animals, *i.e.* whether the effect is caused by a change in host volatile semiochemistry that modifies fly behaviour at a distance, or whether it is due to a close range antifeedant effect, and, if they are responsible, to identify the alkaloids present in E+ tall fescue that are responsible for deterrence. Moreover, similar studies should be focused on identifying the compounds present in the cattle dung responsible for the larval mortality of *H. irritans*.

CONCLUDING REMARKS

Our results constitute the first evidence on the potential for exploiting pasture management for controlling 1) horn fly-loads on cattle and 2) the normal development of horn fly larvae. This information will provide an understanding of the role of endophytes in mediating horn fly host selection, and provide the scientific basis for utilizing diet-specific pastures in an integrated horn fly management strategy.

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Figures

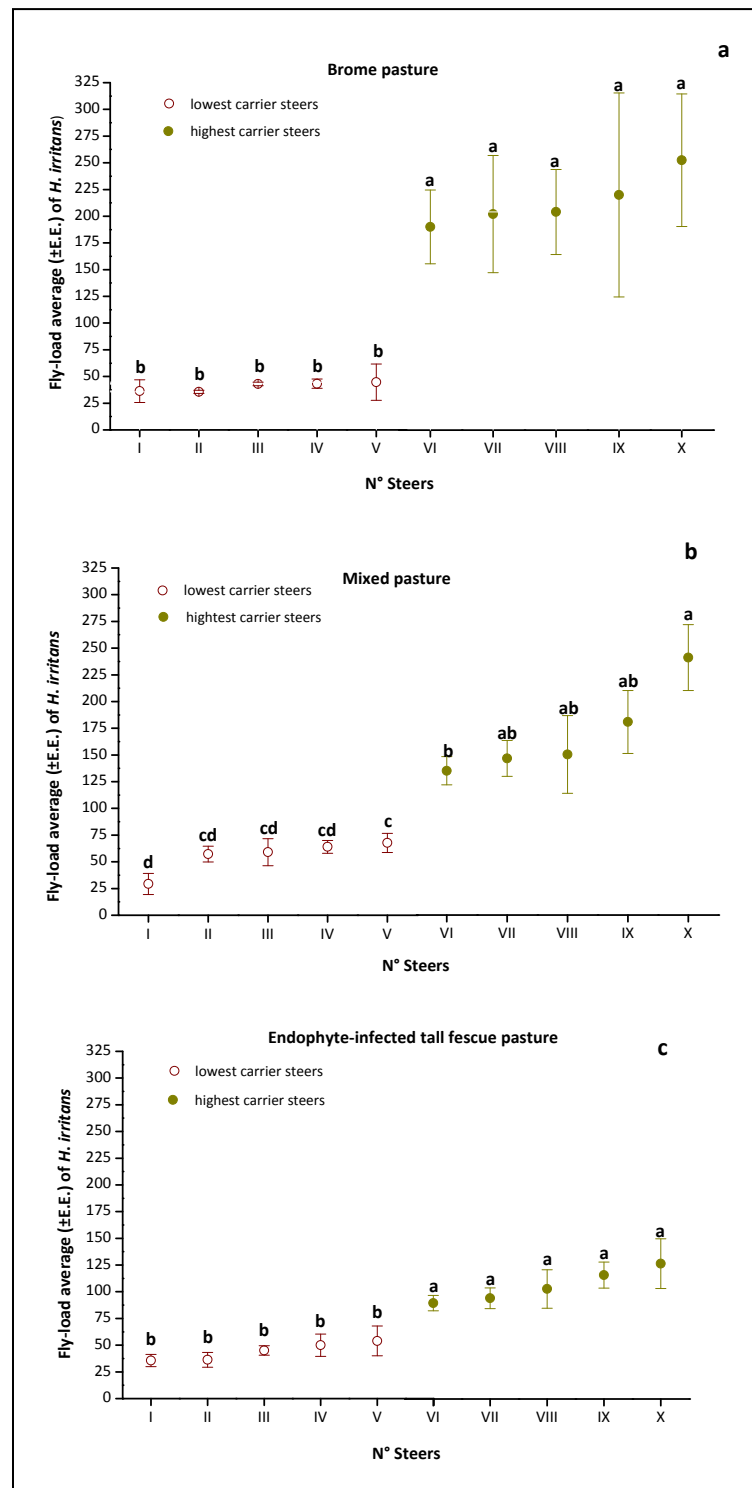


Fig. 1 Average fly-load of *H. irritans* in Overo Colorado steers grazing on: brome pasture (A), ryegrass/white clover pasture (B) and E+ tall fescue (C) at Centro Regional de Investigación INIA Carillanca, Vilcún, Chile, during 2011 season. I-V= low fly-load; VI-X= high fly-load. Different letters indicate significant differences by the nonparametric Kruskal-Wallis test followed by Conover-Inman test ($P < 0.05$).

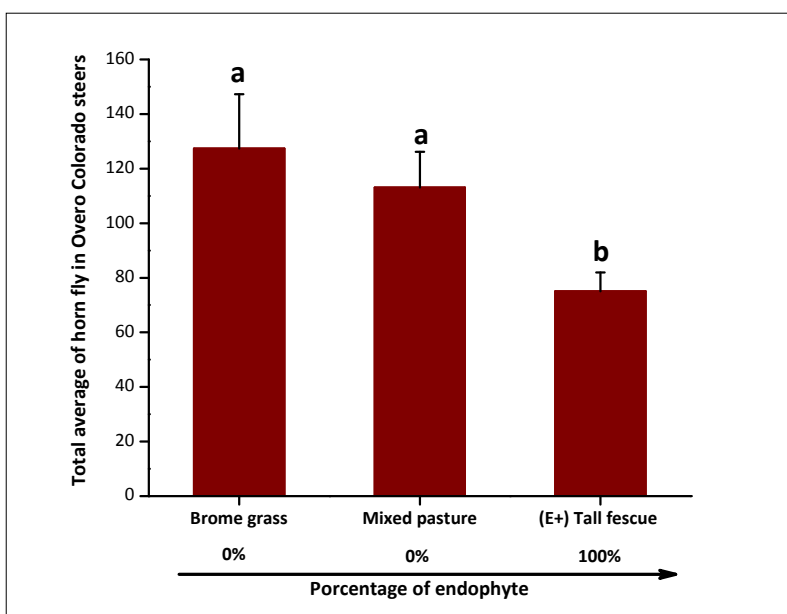


Fig. 2 Average load of *H. irritans* on Overo Colorado steers grazing on: brome pasture, mixed pasture (ryegrass/white clover) and E+ tall fescue during 2011 season (n = 25). Arrow indicates the percentage of endophyte in each pasture. Different letters indicate significant differences according to nonparametric Friedman test ($P < 0.05$).

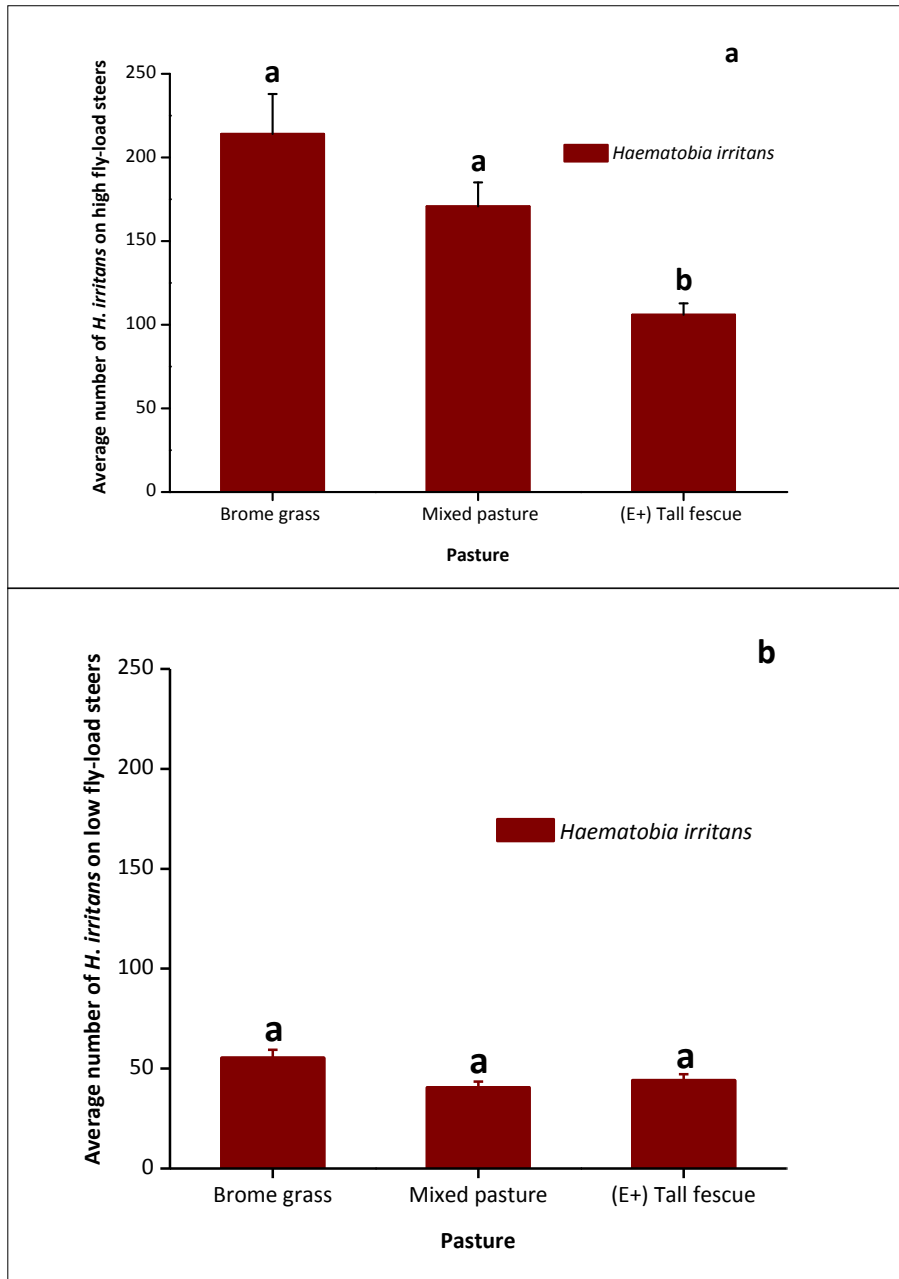


Fig. 3 Effect of animal feed on fly-load variation in Overo Colorado steers grazing in three pastures: brome, rieggrass/white clover and E+ tall fescue. 3A = effect on high fly-load animals, 3B = effect on low fly-load animals. The effect of change of pasture on the population of *H. irritans* was measured every two weeks. Averages were compared by a nonparametric Friedman test and different letters indicate significant differences ($P < 0.05$).

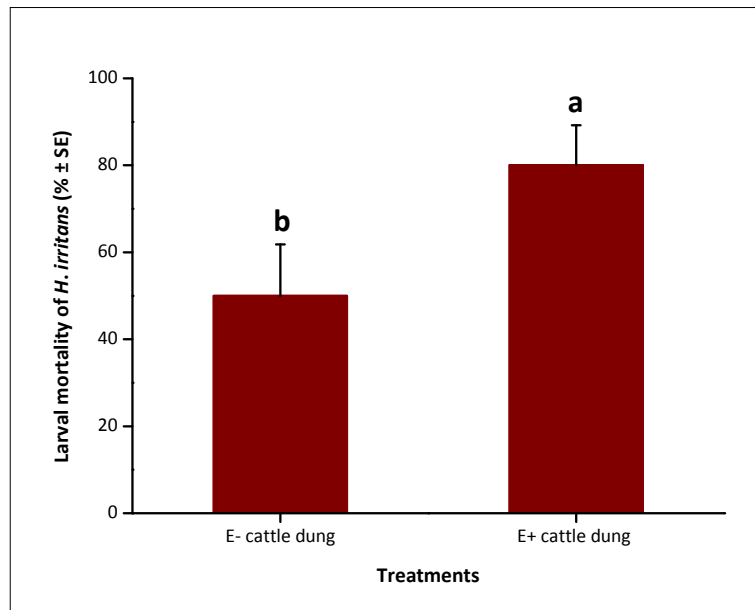


Fig. 4 Bioinsecticide effect of cattle dung (E+ and E-) on horn fly larvae at 48 hrs of evaluation. Different letters indicate significant differences according to Tukey test ($P < 0.05$). $n = 40$.