AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Infestation assessment with *Haematobia irritans* in grazing cattle and stress behaviors in tropical regions

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ABSTRACT

Objective: To evaluate the infestation with flies in grazing cattle, and its relationship with some behaviors (tail butting, head butting, kicking and rubbing) that alter animal welfare, through direct observation and use of photographs.

Design/Methodology/Approach: At two times (7:00 and 14:00 h) the variables were measured on thirty naturally infested cows and randomly distributed in two treatments: TS: control without deworming and TD: chemically dewormed.

Results: The fly infestation were higher ($p<0.001$) in TS cows (483.7 flies/animal), they also, expressed with greater intensity ($p<0.001$) and frequency of upset behaviors: tail-tapping (10.84 movements min^{-1}), head-butting (1.66), kicking (0.51) and rubbing (0.33) in order to drive away the annoying contact and aggression of the ectoparasite.

Limitations: More in deep research is needed in order to assess the physiological disorders that this parasite could cause by altering well-being of grazing cattle in the tropics.

Findings/Conclusions: It is concluded that the greater the fly infestation, the movements that alter the welfare of the animals' increase; however, more research is required to know the physiological welfare consequences that the infestation of this parasite implies.

Keywords: Hun flies, dual purpose cattle, ectoparasites, well-being, stress behaviors

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INTRODUCTION

Ectoparasites associated with livestock are a major concern worldwide due to their economic, health and welfare impacts, which can be direct through tissue damage and blood loss or indirect due to their role as vectors of viral, bacterial, protozoan and helminths pathogens (Trout-Frixell *et al.*, 2021). A second category of indirect effects are

those that result from the alteration of the behavior of cattle induced by the attack of ectoparasites (Eiras *et al.*, 2021). Effective control is challenging and relies primarily on the use of chemical insecticides and miticides (Sarwar and Arfa, 2018; Madhav *et al.*, 2020). The horn fly (*Haematobia irritans*) is a natural hematophagous ectoparasite of cattle, especially grazing. Adult flies spend most of their lives attached to cattle, they tend to congregate on the back and shoulders or on their belly and legs during the hottest hours of the day (Almazán-García *et al.*, 2001; Pérez de León *et al.*, 2020). Flies usually feed 20 to 30 times a day, they only separate from their host to oviposit. Cattle infested with 200 flies have been reported to cause a loss of 520 mL of milk per day and 28 g of live weight per animal per day (Fuentes-Castillo *et al.*, 2016). In tropical livestock, the fly is present throughout the year, with greater abundance in the warmer and humid months from August to October (Cruz *et al.*, 2000; Galindo-Velazco *et al.*, 2008) and provoke stress behaviors in cattle, such as movements of ears, head blows, kicks, skin movement, muscle contractions, tail movements, licks, in order to ward off flies attached to the body and reduce discomfort, generating a greater expenditure of energy and changes in your eating habits normal (Cruz *et al.*, 2000; Almazán-García *et al.*, 2001; Vitela-Mendoza *et al.*, 2016; Barragán-Hernández *et al.*, 2019). The way described to measure the degree of infestation is to count the number of flies by direct observation (Cruz *et al.*, 2000; Alonso-Díaz *et al.*, 2007; Galindo-Velazco *et al.*, 2008; Fuentes-Castillo *et al.*, 2016; Vitela-Mendoza *et al.*, 2016). However, under grazing conditions it is a determine challenge the flies number by visual counting, mainly when the density is high, since the flies have the ability to fly easily, adhere to another part of the same animal or land on other animals in only seconds, also the movement of cattle to keep flies away, are factors that interrupt the count (Smythe *et al.*, 2017; 2020). It is essential in animal welfare evaluations to have an easy and fast method to measure the number of flies adhering to the body of animals. The objective was to evaluate, through direct observation and the use of photographs, the degree of flies' infestation on grazing cattle, and its relationship with some behaviors (number of tail butting, head butting, kicking, rubbing and flies count) that alter animal welfare.

MATERIALS AND METHODS

Study area

The study was carried out in Guerrero, Mexico (18° 25' NL and 100° 43' WL), during the rainy season (July). The climate is considered hot dry (Aw0) with rains in summer, with a temperature between 36 to 39 °C and an average relative humidity of 85%. Annual rainfall of 750 mm (June to September) and an altitude of 250 m.

Production units

The study was developed in six production units with a semi-extensive system and continuous grazing (day and night) in mixed native grasslands with Bermuda grass (*Cynodon dactylon*), purple nutsedge (*Cyperus rotundus*) and muhly grass (*Muhlenbergia macroura*). The herd structure was between 20 and 40 animals of heterogeneous ages and sex (young, adult, male and female). The racial compositions were hybrid animals *Bos indicus* × *Bos taurus* (Brahaman, Gyr, Sardo negro × Brown Swiss, Simmental, Beefmaster, Charolais). In each

production unit, 5 cows with dull fur were randomly selected, accustomed to aflight zone between 1 and 2 m away.

Experimental design

The animals of each ranch were randomly distributed in two treatments, one control without deworming (TS) and another experimental subject to an external deworming scheme (TD: alternate baths every three days by spraying with 15% cypermethrin at a dose of 1 mL L^{-1} and chlorpyrifos at 24% at doses of 1 mL L^{-1} during the 36 days of the evaluations), in this way each treatment had 15 cows as experimental units, on which the measurement of the variables was developed.

Variables measured

The study lasted 36 days, in all animals the variables were measured and recorded by the same evaluator under the same criteria, through direct observation and the use of photography (Table 1). The variables were evaluated in repeated measures for six days at two times 7:00 and 14:00 h for ten minutes in each cow. Five cows of a ranch per day at a time, in the grazing areas.

The degree of fly infestation was evaluated by counting the number of flies adhering to the body of the animal: scapula-back, legs, belly (lateral and lower part) and neck of one side of the animal and the result was multiplied by two to obtain the total number of flies per animal, at the same time photographs were captured to relate the images with the counts of the ectoparasite. During the observation time, the number of times that the animals performed some behavior related to the attempt to drive away or repel the flies from their body was also recorded as described in Table 1

Analysis of data

The data of the variables of each treatment were analyzed using the Man Whitney non-parametric test and a minimum significance of 0.05 was used. In addition, a Pearson correlation analysis was developed considering the degree of infestation by flies as an independent variable and as dependent variables the number of behaviors (tail butting, head butting, kicking and body rubbing) developed by the cows.

Table 1. Cattle movements related to the attempt to ward off or repel aggression or infestation by horn flies (*Haematobia irritans*).

Behaviors	Description
Pigtails	The tail movements were considered when it hit the side of the animal or exceeded the back of the animals in order to keep flies away.
Heading	The times that the animals performed head movements towards the back or belly to ward off the flies were counted.
Kicks	The movements that the animals performed with their hind legs to keep flies away from the lower part of their belly were considered.
Rub	The number of times the animals rubbed some part of their body with bushes, stems or tree branches was counted.

RESULTS AND DISCUSSION

The results firstly show that any scientifically proven control method can help to reduce the infestation of flies in cattle. Figure 1 shows that the degree of infestation in cows with deworming (TD) was low, while in cows without deworming (TS), oscillating from moderate to high and with a higher incidence the extreme.

The number of flies adhering to the body surface of the cows was higher ($p \leq 0.05$) in the non-dewormed animals (TS) compared to the dewormed cows (TD) (Figure 2). Also linearly, the cows of the TS that had a higher degree of infestation were those that developed more behaviors to repel flies ($p \leq 0.05$) and of these the tail movement was the most predominant (108.04 movements cow^{-1} 10 min^{-1}), followed by head butting (16.65 movements cow^{-1} 10 min^{-1}), kicking (5.17 movements cow^{-1} 10 min^{-1}) and rubbing their body (0.33 movements cow^{-1} 10 min^{-1}), compared to those expressed by TD cows, respectively (Figure 3). These observations show that the attack of flies on grazing cattle is uncomfortable and annoying for the animals, which causes stress and additional energy expenditure, in addition, the presence of these vectors increases the risk of diseases, and all this deteriorates the conditions of animal welfare. Mullens *et al.* (2017) reported that the main defensive behaviors exhibited by fly-infested cattle were head throws, leg strikes, panic reflex and tail flick. The tail is the part of the body that animals use the most to temporarily drive away flies and it is likely that they do so because it is a part that they can move more easily to easily reach a large part of the body where the flies. The head movement was the second most performed by the animals, this movement requires more effort and the animals only performed it when the intensity of discomfort was greater, including at the same time the movement of the ears and lick. The movement of legs (kicks)

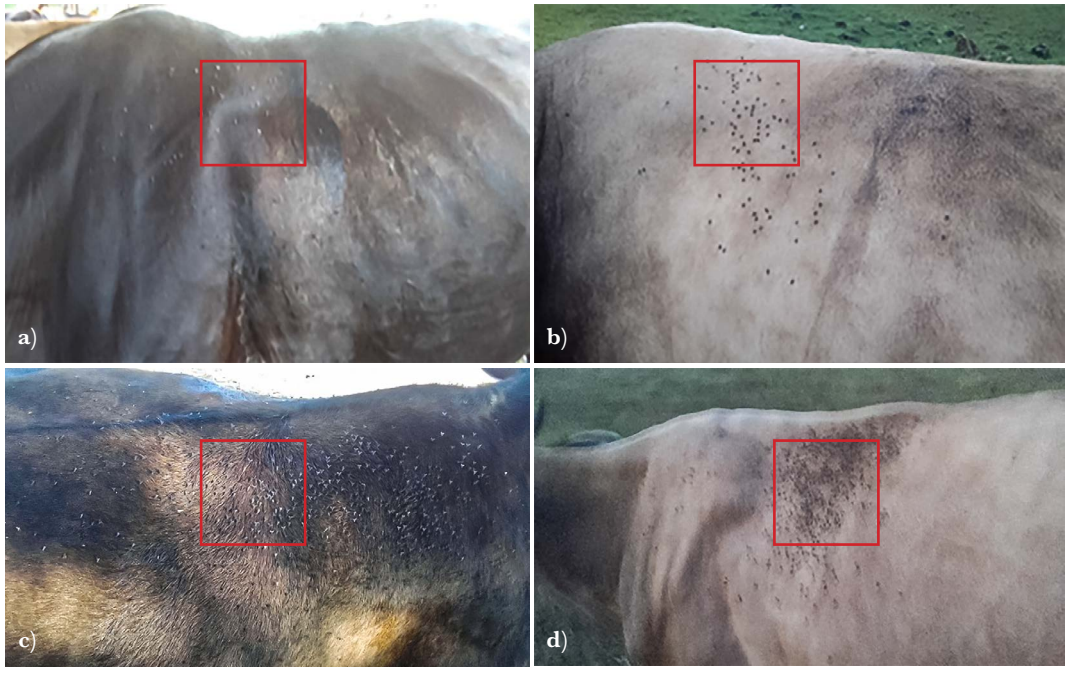

Figure 1. Horn fly (*Haematobia irritans*) infestation degrees observed in the cows with deworming (TD) (low: a) and cows without deworming (TS): moderate (b), high (c) and extreme (d).

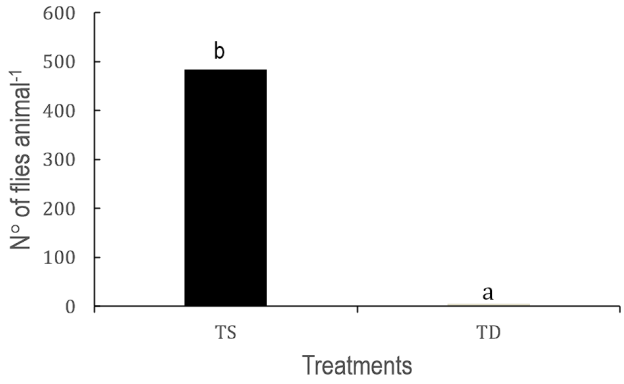

Figure 2. Number of flies per animal in non-dewormed animals (TS) and dewormed cows (TD) grazing under tropical conditions. Means with different literal indicates statistical difference (p≤0.05, Mann-Whitney test).

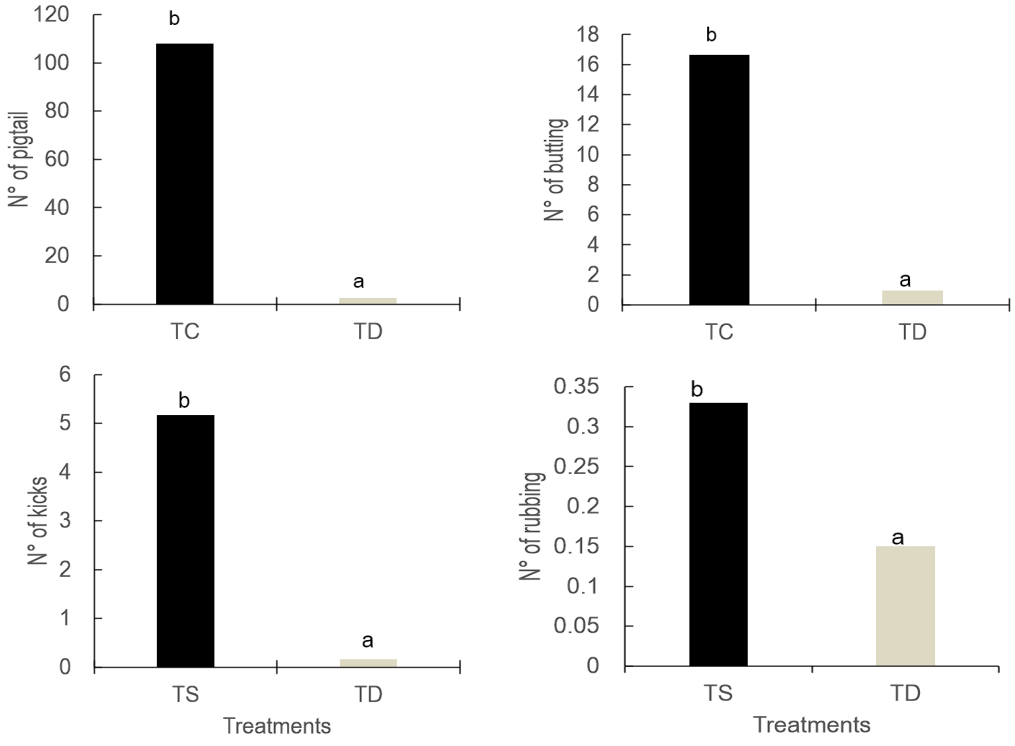

Figure 3. Movements number of non-dewormed (TS) and dewormed cows (TD) grazing under tropical conditions. Means with different literal indicates statistical difference (p≤0.05, Mann-Whitney test).

is carried out when the flies are attached to the lower part of the belly and the extremities. In addition to the body movements that animals use to keep flies away, resort to other alternatives such as rubbing your body on the branches of shrubs or the stem of trees. The number of behaviors performed by the animals during the observation period were compared to those obtained by Kojima *et al.* (2019) who reported a total of 54 movements in cow and an infestation of 120 flies, and also positively related the number of flies with the stress behaviors developed by the animals.

The number of flies was different ($p \leq 0.05$) with 483.77 and 5.51 flies cow^{-1} for TS and TD, respectively (Figure 2). The infestation of TS cows is classified as high within the standard of Smythe *et al.* (2017) which considers low level 0, medium 250, high 500 and extreme 1000 flies. It was also confirmed that flies are responsible for affecting the behavior and welfare of livestock. The numbers of flies were higher during the morning count (07:00 h) on both sides of the back of the animals. The count at 14:00 h registered a lower amount of the parasite perched on the body of the animals, in the ventral body regions (belly, legs and chest) seeking shelter from the sun's rays (Smythe *et al.*, 2020), which made counting difficult at these hours. The quantities of flies/cow obtained are much higher than those reported by other authors such as Kojima *et al.* (2019) with 120 flies cow^{-1}, and Vitela-Mendoza *et al.* (2016) with 55 flies cow^{-1}, while Galindo-Velazco *et al.* (2008) reported three peaks during the year with values of 156, 236 and 120 flies $animal^{-1}$. Fuentes-Castillo *et al.* (2016) found infestations of 50 to 56 flies $animal^{-1}$ over the course of the year. Almazán-García *et al.* (2001) reported maximum values during the year of 200 flies $animal^{-1}$.

Correlation between the infestation degree and stress behaviors

The correlation between the number of behaviors developed by the animals and the number of flies adhering to their bodies was significant. Table 2 shows that the number of tail butting, head butting, kicking and body rubbing against bushes and other animals increased significantly and gradually with the degree of fly infestation with a correlation value of 0.98 ($p \leq 0.05$), 0.96 ($p \leq 0.05$), 0.95 ($p \leq 0.05$) and 0.89 ($p \leq 0.05$), respectively.

The correlation analysis showed that the flies altered the normal behavior and well-being of the animals by causing body movements to repel and drive away the ectoparasite. Also, Trout-Frixell *et al.* (2021) observed that the behaviors developed by cattle to ward off flies are related to the intensity of the infestation. The images show that when the number of flies is lower, they adhere separately on the back of the animal, mainly during the cooler hours of the day (07:000-09:00 h) and when the density is high, they are placed at distances shorter between them (Figure 1a-d).

Smythe *et al.* (2017) they suggest that digital photographs taken of infested cattle provide estimates that are just as accurate as traditional visual counts. However, they argue that more research is needed to standardize this technique. Mochi *et al.* (2009) and Mullens *et al.* (2016) recommended the use of high-resolution digital cameras to capture images

Table 2. Correlation of the infestation degree (flies' number) by horn flies (*Haematobia irritans*) with the behaviors that reflected stress in grazing cattle in a tropical environment.

Behaviors	Flies number				Correlation
	0-100	101-200	201-400	>401	
Pigtail	5.9	70	147	214	0.98*
Butting	1.7	10	20	32	0.95*
Kicks	0.3	5.5	7.5	7.7	0.96*
Rubbing	0.3	0.4	0.5	0.8	0.88*

*$p \leq 0.05$

of infested cattle and improve the practicality of counting. Unfortunately, this method is not without its challenges since avoidance behaviors of cattle and human interaction make it difficult to obtain images clear and reliable. Smythe *et al.* (2020) indicated that deep thinking, computer vision, and object detection frames can be adapted for future fly counting. Trout-Frixell *et al.* (2021) mentioned that the estimation of face flies in horses is carried out by producers through direct observation, counts and by lesions observed in the animals' eyes.

Recommendations for the reliability of the technique

It is suggested that the evaluation of the fly infestation on the animals is carried out by observation during the hours of less solar radiation (morning or afternoon). It is important to carry out the evaluation in 10% of mainly adult animals, with dark fur and that allow human approach at short distances to favor observation. Observe the lateral dorsal part of either side of the animal to classify the observation in one of the categories low, moderate, high or extreme. Considering the low category as a normal density that animals can support without compromising their welfare. The moderate category can be considered as the limit threshold that the animals can withstand, from this density the animals express a lack of well-being and decrease in production. In the high and extreme categories, the welfare of the animals is null and obviously the production is affected considerably.

CONCLUSION

Visual observation can be used as a viable alternative to estimate the infestation by the horn flies (*Haematobia irritans*) adhering to cattle grazing under tropical conditions to classify the degree of infestation of the animals. The intensity of the behavioral movements that grazing cattle perform to keep flies away from their bodies are related to the number of flies attached. It is important to consider more research in this regard that allows us to obtain results that assess the physiological disorders that this parasite could cause by altering well-being.

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