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Effect of Fly Annoyance on Buffalo Behavior and Milk Production

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Abstract: This study was carried out to investigate the fly count during different periods of the day during fly season and to show the effect of fly annoyance on buffalo behaviour and milk production and to investigate the efficacy of Butox ®, a commercially available fly repellent in reducing fly numbers. Five Egyptian adult female buffalo cows,6 years old, were used in the study. Both fly count and fly avoidance behaviors were recorded for two weeks before application of Butox ® and for the same period after application. Each animal was observed for 10 min, 3 times a day to estimate the fly count per fore and hind legs and the frequency of specified responses occurrence to fly annoyance. Milk production per individual buffalo was recorded for 2 weeks before and after the fly repellent application. The obtained results indicated that there was highly significant differences in the fly count between non treated and treated buffalo cows with Butox ®. The mean fly count was 13.48 and 3.22 fly/leg/min for the untreated and the treated animal , respectively. The highest fly count was recorded at 12 at noon period of the day (11.77 fly/leg/ min), while the lowest fly count was recorded at 8 am period of the day (4.91 fly/leg/min). There was highly significant difference in the fly count per minute between the fore and hind legs of buffalo as it was 11.33 and 5.37 fly/min for the fore and hind legs, respectively. There were highly significant differences ($p \leq 0.001$) in the performance of fly avoidance behaviour between treated and non-treated buffaloes with Butox ®. The highest observed fly avoidance behaviour was the skin twitching (30.44times/min) followed by tail switch (18.75/min) and ear flicking (16.54times/min) for the non treated buffaloes, while the lowest observed fly avoidance were hind leg stamp (0.55times /min) followed by head shaking (0.74times/min) and fore leg stamp (0.79/min) for the treated buffaloes. The highest avoidance behaviour was that which was recorded at 12 at noon period of the day followed by at 4 PM period while the lowest avoidance behaviour was that recorded at 8 AM period of the day.There was no significant difference in the daily milk production between treated and non-treated buffaloes with the fly repellent. The daily milk yield was 4.8 and 4.9 Liter/day for non-treated and treated buffaloes, respectively. It was concluded that deltametherin application has a significant effect in reducing fly population landing on dairy buffaloes and treated buffaloes with the fly repellent (deltametherin) displayed significantly fewer avoidance behaviour to fly attack than non-treated buffaloes. Although, there was no significant effect of fly repellent application on daily milk production, the reduced fly population on dairy buffaloes and the reduced performance of avoidance behaviour in treated buffaloes has a good impact on welfare and health condition of dairy buffaloes.

Key words: Fly Annoyance-Buffalo Behavior-Deltametherin-Milk Production

INTRODUCTION

Ruminants such as cattle, sheep, goats, etc. are world wide important sources of human food and different economically important goods such as leather and/or wool, etc. Therefore, they are reared often in masses and in monoculture, which are highly attractive for many ectoparasite [1].

Biting insects impose a number of costs on ungulate hosts, including blood loss, decreased feeding or resting time caused by disturbance and disease transmission [2]. Harassment by biting insects decreases feeding and resting time in goats and cattle and increases time spent standing and moving [3]. Loss of feeding time and increased energy expenditure results in a loss of weight gain for growing cattle [4]. Flies are the greatest

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ectoparasite threat to livestock behavior as they cause reduced efficiency in feed conversion, reduced weight gain and decreased milk production. Biting flies, like stable fly and horn fly, have mouth parts that are very well adapted for biting to get their blood meals. These flies feed about once every hour during the day [5]. An economic threshold has been cited at two stable flies per fore leg, which is set because of disruption and alteration of eating patterns and increased energy expenditure in avoidance behavior [6, 7].

It is widely accepted that sever insect harassment could result in a negative energy balance and eventually in poor physical condition of animals [8]. Face flies are non-biting ectoparasities, they feed on tears, sweat, saliva and other body secretions and thus tend to congregate around the eyes and muzzle when they are not actively feeding [9].

Nuisance fly also can be involved in the spread of disease including pink eye and mastitis [10]. Insect-repelling behaviors, such as ear-flicking, head-shaking, stamping, tail-switching and muscle-twitching, are exhibited by many ungulates to repel or dislodge biting insects and thus reduce the pain and blood loss from bites [11]. In addition, harassed ungulates might seek to reduce attacks from biting insects by lying down (reducing exposed body surface and attractants, such as carbon dioxide), seeking suitable microhabitat (e.g., moving to windy ridges or bare areas) and grouping or bunching to dilute insect attacks [12]. Some insect-repelling behavior is evoked by the cutaneous pain of insect bites and the associated visual and auditory stimuli of biting insects, which termed 'peripheral stimulation' [13]. Ectoparasites present a serious challenge to the welfare of livestock. It is clear that there is a stress response to fly infestation, which will lead to distress if it is sufficiently large and long-lasting to cause clinical signs of damage [14]. Humans are also targets for nuisance flies, so deterring fly presence and controlling their numbers through treatment of animals can reduce the irritation and painful bites experienced by the people managing and milking the herd.

Unfortunately, research on entomological topics has been decreased during the last decades so that basic data of many important ectoparasites are rather old or even lacking. Thus, there is an extreme need to update such data in order to get the most efficient control and to avoid development or increase of potential resistances [15]. Several insecticidal products are registered in European countries for use on the skin of ruminants. Among the different pyrethroids (such as cyfluthrin, cypermethrin, permethrin, cyhalothrin, deltamethrin, fenvalerate, etc.), deltamethrin (the active compound in Butox ® 7.5 pour-on) is one of the most effective compounds [15]. Different to other pyrethroids, deltamethrin is a single cis-isomer. Cis-isomers are considered as more effective than isomer combinations. Deltamethrin repels ectoparasites by the "hot foot effect", which is typical for pyrethroids. An insect then redraws its feet suddenly from treated hair after it had a "touch down" on such an animal. Even after a very short contact for only a few seconds to treated hair, a "knock down" effect occurs since insects (and ticks) die soon after the open nerve ends at their feet got into contact with the insecticide [16]. The objectives of the present study were to determine the fly count during different periods of the day during fly season, show the effect of fly nuisance on dairy buffalo avoidance behavior and milk production and to investigate the efficacy of Butox ® a commercially available fly repellent in reducing fly numbers and irritation.

MATERIALS AND METHODS

The present study was carried out at the teaching farm, Faculty of veterinary medicine, Benha University during May, 2011.

Animals and Management: Five Egyptian buffalo cows, 6 years old, were selected from a total number of 15 females with an average live body weight of 434±24.16 kg. The selected buffalo cows were nearly within the same reproductive (the 3rd calving for each female) and productive (one month after weaning their calves) conditions. Animals were housed under a semi closed barn, each buffalo was tied to a metal ring about 30cm below the rim of the manger and with about one meter space between each two successive rings and standing on soil type floor. Animals received 3.5kg of concentrated ration twice/head/day (El-Salam feed Factory- El-Marg. Ministry of Agriculture. Egypt) beside an armful of hay twice per day.

The air temperature and relative humidity percentages during the present study were recorded by using digital thermohygrometer. The average air temperature recorded during May, 2011 was 33.8°C while the average relative humidity percentage was 67.5%.

Fly Count and Behavioral Measures: Both fly count and fly avoidance behavior performed by buffaloes were recorded for two weeks before the application of the fly repellent Butox ® and for the same period after application.

We used focal individual sampling (FIS) according to Altmann [17] as, follows: an individual buffalo was chosen at random and sampled for up to 10 min, three times daily (8 a.m., 12 at noon and 4 p.m.), to estimate the fly count per fore and hind limbs and the frequency of occurrence of specified behavioural responses to fly harassment according to Toupin, Huot and Mansean [18].

Fly Count: Fly count was made by approaching an animal slowly from the side. Once it was visible, an estimate of flies was made by counting the number of flies landing on both fore and hind limbs.

Blood sucking stable fly, Stomoxys calcitrans is a chronic problem on most farms, usually feeding on the lower limbs of animal and because of this preferred feeding area, fly counts in the present study was made on the limbs according to McNeal and Campbell [19]. Also the number of stable flies on the fore limbs comprises about 45% of the total fly number on an animal according to Lysyk [20].

Fly landing per 1 min observation on each limb were counted. Each limb was observed from below the level of the flank to the claws (for the hind limbs) and from the level of the shoulder to the claws (for the fore limbs). The number of flies were counted on the outside of one fore limbs and the inside of the other and so on for the hind limbs according to Mullens *et al.* [21].

Behavioral Measures: Following completion of fly count, the frequencies of occurrence of specified avoidance behaviors as a result of fly harassment were recorded. The behavioral responses recorded were: tail switching, skin twitching, ear flicking, head shaking and foot stamping [18]. Head and ear movements, apparently induced by flies attack were counted per 1 min [22]. An ear movement was defined as "the initiation of ear movements which may be single twitch or continuous rotation of one or both ears, either clockwise or counter clockwise". A head throw or movement was recorded if the head was tossed backward to the observer side sufficiently that the nose of the animal crossed an imaginary plane across the front of the animal's chest.

A leg stamp was recorded if either fore or hind limbs was raised to the point that the foot cleared the ground. A tail switch was defined as "the movement of the tail from its resting position to one side" and if the tail recrossed its resting position, another tail movement was recorded.

Skin twitching was counted for one minute on one side of each animal. Skin twitches are caused by the contraction of the cutaneous trunic muscle (panniculus reflex) [23]. One twitch in a localized area or a continuous shiver over the whole flank for several seconds was recorded as one event according to Dougherty *et al*. [22].

Milk Production: Milk production was recorded per individual buffalo twice per day at 6 a.m. and 3 p.m. for 14 days before the application of the fly repellent and for the same period after application.

Application of Fly Repellent: In the present study, we used a commercially available fly repellent Butox ® with active principle deltamethrin 5%, a highly active pyrethroid which is odour free and used safely.

Butox ® pour-on is a useful remedy for protection from ectoparasites [15]. It contains deltamethrin that has a rather safe LD_{50} (6000 mg/kg) in rats specially for young and lactating animals.

A dose of 15ml of Butox ® with a concentration of 5% deltamethrin was used per animal in a 5-10cm wide strip along the midline of the animal from the poll to the base of the tail by using a T-shape rubber spreader as recommended by Alexander and Whealan [24]. When Butox ® applied down the midline of animal, it works by moving through the natural oil on the skin surface to cover the entire body.

Statistical Analysis: Analysis of variance was calculated by using SAS procedure guide, (1996), while means were compared by the "Duncan" multiple comparison [25].

RESULTS AND DISCUSSION

The results in Table (1) showed the means and standard errors of fly count per minute on the fore and hind limbs during different periods of the day before and after fly repellent application. Results showed high significant difference in the fly count/limb/min between non treated and treated buffaloes. The mean fly count were 13.48 and 3.22fly/limb/min for the non treated and treated buffaloes respectively. The obtained results indicated that deltametherin application has a significant effect in reducing fly population landing on dairy buffaloes. The obtained results are in agreement with those recorded by Alexander and Whealan [24] who recorded that pour on treatment with deltametherin has been shown to reduce the herd-associated fly population under New Zealand conditions. They added, the number of observed fly landing on the cattle significantly decreased on treated animals compared to untreated ones.

Table 1: Means and standard error of fly count on fore and hind limbs during different periods of the day before and after fly repellent application

Item	Fly count/min
Treatment (T)	
T_1 (Non treated Buffaloes)	13.48 ± 0.12^a
T_2 (Treated Buffaloes)	3.22 ± 0.12^b
Period (P)	
P_1 (8 a.m.)	4.91 ± 0.15^c
P_2 (12 at noon)	11.77 ± 0.15^a
P_3 (4 p.m.)	8.37 ± 0.15^b
Limb (L)	
L_1 (fore limb)	11.33 ± 0.12a
L_2 (hind limb)	5.37 ± 0.12^b
T x P x L interaction	
T_1 x P_1 x L_1	10.67 ± 0.30^c
T_1 x P_1 x L_2	5.22 ± 0.30^g
T_1 x P_2 x L_1	26.40 ± 0.30^a
T_1 x P_2 x L_2	11.42 ± 0.30^e
T_1 x P_3 x L_1	16.85 ± 0.30^b
T_1 x P_3 x L_2	10.32 ± 0.30^f
T_2 x P_1 x L_1	2.60 ± 0.30^e
T_2 x P_1 x L_2	1.15 ± 0.30^h
T_2 x P_2 x L_1	7.02 ± 0.30^d
T_2 x P_2 x L_2	2.25 ± 0.30^g
T_2 x P_3 x L_1	4.45 ± 0.30^e
T_2 x P_3 x L_2	1.85 ± 0.30^h
ANOVA	
Treatment	***
Period	***
Leg	***
Treat x Period x Leg	***

- Means followed by different superscripts in the column for each trait are significantly different (P<0.001).
- *** (highly significant) at (P<0.001).

Regarding the fly count during different periods of the day, the obtained results revealed that there was highly significant differences in the fly count during different periods of the day where the highest fly count was recorded at noon (12pm) (11.77 fly/limb/min) followed by that recorded at 4 p.m. (8.37 fly/limb/min) while the lowest fly count was recorded in the early morning (8 a.m.) 4.91 fly/limb/min.

These finding could be attributed to the normal fly behavior where they prefer to adhere to walls and other surfaces until the temperature rises, so peak activity is around noon for feeding (biting the animals), then the flies return to rest on the wall or surfaces, with fewer feeding bouts [22,26,27].

Regarding the mean fly count per fore and hind limbs, the obtained results in Table (1) showed that there was highly significant differences in the fly count per minute between the fore and hind limbs. The mean fly count were 11.33 and 5.37 fly/min for the fore and hind limb respectively. These results indicated that fly landing per minute on the fore limbs were nearly 2-fold more than those for the hind limbs. The obtained results agreed with the results reported by [22, 28-30] who recorded that the ratio of alighted flies on the fore limbs to alighted flies on the hind limbs was nearly 2 [28] while the ratio was 3:1 [29] and it was 5-7 times as many alighted flies on the fore limbs than on the hind limbs [30]. These results may be due to that feeding of stable flies (Stomoxys calcitrans) congregate on the fore limbs and to a lesser extent on the hind limbs of their hosts, possibly because the hair is thinner [31] or because the blood vessels are close to the surface of the skin in the fore limbs, or because fly-deterrent responses, such as tail switching, skin twitching displace flies from other feeding areas [22]. Also the lower surfaces of the fore limbs have weakly developed skin twitching ability and are not swept by the tail [22].

The obtained results in the present study in Table (1) revealed that there were highly significant interactive effect on the fly count due to deltametherin treatment X period of the day X the limbs of animal. The highest fly count was recorded for (T_1 x P_2 x L_1) which means the non treated buffaloes during the second period of the day (at 12 at noon) and on the fore limbs and the count was 26.40 fly/min. While the lowest fly count was recorded for (T_2 x P_1 x L_2) which means the treated buffaloes during the first period of the day at (8 a.m.) and on hind limbs as the count was 1.15 fly/min.

The obtained results for the interaction between treatment with deltametherin X period of the day X limbs of animal support the above mentioned results and are in agreement with the previous studies [22, 24, 26, 30].

Results in Table (2) show the means and standard errors of fly avoidance behavior performed by buffaloes during different periods of the day before and after fly repellent application. Regarding the effect of treatment with deltametherin on the fly avoidance behavior, it is clear that there were highly significant differences in the fly avoidance behavior between treated and non treated buffaloes. The means of fly avoidance behavior count/min were (18.75 and 11.97), (30.44 and 14.17), (16.54 and 10.34), (2.14 and 0.74), (1.18 and 0.79) and (1.05 and 0.55) for tail switch, skin twitch, ear flicking, head shaking, fore limbs and hind limb stamp for non treated and treated buffaloes respectively. The obtained results are in agreement with the results recorded by Alexander and Whealan [24] and Ralley, Galloway and Crow [26] who found that

Table 2: Means and standard error of fly avoidance behaviors performed by buffaloes during different periods of the day before and after fly repellent application

	Avoidance behavior count/min					
Item	Tail switch	Skin twitching	Ear flicking	Head shaking	Fore leg stamp	Hind leg stamp
Treatment (T)						
T_1 (Non treated buffaloes)	18.75 ± 0.16[a]	30.44± 0.20[a]	16.54± 0.15[a]	2.14± 0.04[a]	1.18± 0.03[a]	1.05± 0.03[a]
T_2 (Treated buffaloes)	11.97 ± 0.16[b]	14.17± 0.20[b]	10.34± 0.15[b]	0.74± 0.04[b]	0.79± 0.03[b]	0.55± 0.03[b]
Period (P)						
P_1 (8 a.m.)	9.78 ± 0.20[c]	11.75± 0.24[b]	9.23 ± 0.18[b]	0.53± 0.05[c]	0.01± 0.04[c]	0.00 ± 0.04[c]
P_2 (12 at noon)	18.85 ± 0.20[a]	27.76 ± 0.24[a]	15.53± 0.18[a]	2.08± 0.05[a]	1.71±0. 04[a]	1.36± 0.04[a]
P_3 (4 p.m.)	17.46 ± 0.20[b]	27.41± 0.24[a]	15.55± 0.18[a]	1.70± 0.05[b]	1.23± 0.04[b]	1.03± 0.04[b]
T x P interaction						
T_1 x P_1	10.67 ± 0.28[d]	14.10± 0.34[c]	10.80± 0.26[c]	1.07± 0.06[c]	0.02± 0.05[c]	0.00± 0.05[d]
T_1 x P_2	23.80 ± 0.28[a]	38.75 ± 0.34[a]	19.47± 0.26[a]	3.07± 0.06[a]	2.27± 0.05[a]	1.97 ± 0.05[a]
T_1 x P_3	21.80 ± 0.28[b]	38.47 ± 0.34[a]	19.35± 0.26[a]	2.27± 0.06[b]	1.25± 0.05[b]	1.17± 0.05[b]
T_2 x P_1	8.90 ± 0.28[e]	9.40± 0.34[d]	7.67± 0.26[d]	0.00± 0.06[d]	0.00± 0.05[c]	0.00± 0.05[d]
T_2 x P_2	13.90 ± 0.28[c]	16.77± 0.34[b]	11.60± 0.26[b]	1.10± 0.06[c]	1.15± 0.05[b]	0.75± 0.05[c]
T_2 x P_3	13.12 ± 0.28[c]	16.35± 0.34[b]	11.75± 0.26[b]	1.12± 0.06[c]	1.22± 0.05[b]	0.90± 0.05[c]
ANOVA						
Treatment	***	***	***	***	***	***
Period	***	***	***	***	***	***
Treat x Period	***	***	***	***	***	***

- Means followed by different superscripts in each column for each Trait are significantly different (P<0.001)
- *** highly significant (P<0.001)

animals treated with fly repellent deltametherin [24] or cypermetherin [26] displayed significantly fewer avoidance behavior to biting fly attack than non treated animals.

The highest observed fly avoidance behavior in the present study was skin twitch, it was (30.44/min), for the non treated buffaloes followed by tail switch (18.75/min) and ear flicking (16.54/min) while the lowest observed fly avoidance behavior performed by non treated buffaloes was 1.05, 1.18 and 2.14 per minute for the hind limb, the fore limb stamp and head shaking respectively.

The obtained results were in accordance with the results recorded by Mullens *et al*. [21] who divided the fly avoidance behavior in dairy cattle into less frequent and energy-intensive acts (head throw and leg stamp) and more frequent and less energy-intensive acts (skin twitch and tail switch) as they recorded one head throw/min, 1.6 fore limb stamp/min, 8 skin twitches/min and 9 tail switches/min as behavioral responses of dairy cattle to stable flies in an open filed environment. They added that skin twitches and tail switches occurred more frequently and tended to occur at a low level, even when there were no flies counted at 2-minutes observation interval.

Also Dougherty *et al*. [30] and Eicher *et al*. [32] found that tail swings were the most frequent fly-avoidance behaviour in dairy cow. They added that stable flies did not alight on the heads of dairy cows and so they recorded a head movements of less than 1/min and they also mentioned that stable flies induced few movements of fore and hind limbs of grazing cows as it reached less than 1/min when 100 stable flies were released.

Results in Table (2) revealed that there were highly significant differences in the fly avoidance behavior during different periods of the day. The highest recorded avoidance behavior were those which recorded at 12 at noon. The avoidance behavior count were 18.85, 27.76, 15.53, 2.08, 1.71 and 1.36 for tail switch, skin twitch, ear flicking, head shaking, fore and hind limb stamp, respectively.

On the other hand, the lowest recorded avoidance behavior were those which recorded at 8 a.m. The avoidance behavior count were 9.78, 11.75, 9.23, 0.53, 0.01 and 0.00 per minute for tail switch, skin twitch, ear flicking, head shaking, fore and hind limb stamp, respectively.

These results reflect the increased repelling behavior during period 2 (12 at noon) and period 3 (4 p.m.) which parallel with the increased fly count and activity during these periods (around noon) and these results are in agreement with those reported by Phipps, Mathews and Vrkerk [33] who found that, there were increased fly avoidance behavior in dairy cattle at 12.00 and 15.00 p.m., but not at 07.00 a.m.

Also Eicher *et al*. [32] observed that, the fly avoidance behavior reflected the increased fly counts on dairy cattle. Dougherty *et al*. [29] and Dougherty *et al*. [30] observed that, fly avoidance behaviors of beef cattle, including head and ear movements, panniculus reflex and tail swings increased linearly with increased numbers of released flies.

The obtained results in Table (2) revealed highly significant differences in the fly avoidance behavior due to interaction between treatment with deltametherin x periods of the day. From the obtained results it is clear that the highest observed fly avoidance behaviors were those which recorded for (T_1 x P_3) (The non treated buffaloes during period 2 (12 at noon). The means were 23.80, 38.75, 19.47, 3.07, 2.27 and 1.97 per minute for the tail switch, skin twitch, ear flicking, head shaking fore and hind limb stamp respectively. On the other hand, the lowest recorded fly avoidance behaviors were those which recorded for (T_2 x P_1) (The treated buffaloes during period 1 (at 8 a.m.). The means were 8.90, 9.40, 7.67, 0.00, 0.00 and 0.00 per minute for tail switch, skin twitch, ear flicking, head shaking, fore and hind limbs stamp respectively.

The obtained results for the interaction between treatment with deltametherin x period of the day were paralleled with the above mentioned results and were in agreement with the previous recorded results [22, 24, 26, 30, 32, 33].

Results in table (3) show the means and standard errors of daily milk production produced by buffaloes before and after fly repellent application. From the obtained results it is clear that, there was no significant differences in the daily milk production between treated and non treated buffaloes with deltametherin. The means of daily milk production were 4.8 and 4.9 Liter/day for the non treated and treated buffaloes, respectively.

The obtained results in the present study for milk production disagreed with several studies who pointed to decreased milk yield associated with fly bites [6, 7] and Minar, Kostenko and Riha [34].

Campbell *et al*. [7] recorded an economic threshold has been cited at two stable flies per fore leg, which is set because of disruption and alteration of eating patterns and increased energy expenditure in avoidance behavior.

While Jonsson and Mayer [35] predicted a threshold number of flies (n = 30) below which no adverse effects on milk yield or weight gain could be detected. In the present study, the mean fly count per fore limbs was (13.48/leg/min) which is under the threshold number reported by Jonsson and Mayer [35] which may explain the non significant effect of fly annoyance on milk production.

Table 3: Means and standard error of daily milk production produced from buffaloes 14 days before and after fly repellent application

Item	Amount of milk (Liter/day)
Non treated buffaloes	4.80 ± 0.08^a
Treated buffaloes	4.90 ± 0.08^a

Means followed by the same superscripts are not significantly different at $p \leq 0.05$

Dougherty *et al*. [21] reported that there were no visible relationships between stomoxys flies and milk yield. They explained that, lactation is influenced substantially by environmental, husbandry and selective breeding and they added, the only factor that was significantly related to milk yield was the day of lactation. They explained the non significant effect of fly bit on milk yield may be due to that the fly loads were not high enough to detect economic effects as the fly loads were (3-3.5 flies per leg) and at the end they added that more work in an economic context is needed on behavioral responses of animals to fly pressure.

In conclusion, deltametherin application has a significant effect in reducing fly population landing on buffaloes and the treated buffaloes with the fly repellent (deltametherin) displayed significantly fewer avoidance behavior to fly attack than non-treated buffaloes. Although, there was no significant effect of fly repellent application on daily milk production, the reduced fly population on buffaloes and the reduced performance of avoidance behavior in treated animals has a good impact on welfare and health condition of animals.

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