Bulgarian Journal of Veterinary Medicine, 2017, **20**, Suppl. 1, 123–129
ISSN 1311-1477 (print); ISSN 1313-3543 (online)

REDUCED STRESS AND FATIGUE INDICATORS (CORTISOL AND CREATININE KINASE) IN DAIRY CATTLE DUE TO FLY REPELLENCY USING DELTAMETHRIN (BUTOX®, MSD)

K. ARSENOPOULOS[1], E. TRIANTAFILLOU[2] & E. PAPADOPOULOS[1]

[1]Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Independent Veterinarian, Owner of Vet-Analysis Microbiology Laboratory, Larissa, Greece

Summary

Arsenopoulos, K., E. Triantafillou & E. Papadopoulos, 2017. Reduced stress and fatigue indicators (cortisol and creatinine kinase) in dairy cattle due to fly repellency using deltamethrin (Butox®, MSD). *Bulg. J. Vet. Med.*, **20**, Suppl. 1, 123–129.

Fly infestation is a perennial problem of cattle herds, affecting animal welfare and leading to economical losses of the farmers, due to the reduction of subsequent productivity. Both licking and blood sucking flies provoke discomfort, annoyance and painful bites, affecting normal behaviours of the cows, favouring increased stress and fatigue status of the animals. The use of deltamethrin is a necessary step towards the decrease of the fly population and their consequences on the animals. Twenty multiparous dairy cows were divided in 2 groups. Ten cows were individually dressed on their back with deltamethrin, while no treatment was applied to the other 10 cows. In the deltamethrin-treated group, serum cortisol and creatinine kinase (CK) concentration decreased on day 20, in contrast to control group, in which they remained unaffected. The results of our study showed that the fly population of the farm was positively related to the cortisol and CK concentration, which are indicative of stress and fatigue status of the animals. The administration of the fly repellent, Butox® reduced fly abundance, normalising the cortisol and creatinine kinase level.

Key words: cortisol, creatinine kinase, fatigue level, fly, stress level

INTRODUCTION

Fly control is a perennial problem of dairy cattle herds worldwide (Hillerton *et al.*, 1985). Fly numbers increase during spring and early summer, causing severe welfare and productivity problems in cattle herds (Scott, 2008; Taylor *et al.*, 2012). Flies, usually, disrupt normal feeding behaviour, stimulating defensive behaviours (head throwing, skin and tail twitching) (Dougherty *et al.*, 1993; 1994; Mullens *et al.*, 2006) and decrease relaxation time (fatigue) due to provoked annoyance (Vitela-Mendoza, 2006; 2007). In addition, flies act like competent vectors of infectious pathogens, causing infectious keratoconjuctivitis, mastitis through teat injury, dermatophilosis, papillomatosis and others (Scott, 2008).

Horn flies (*Haematobia* spp.), blackflies (*Simulium* spp.), stable flies (*Sto-*

moxys calcitrans) and common flies (*Musca domestica*) can cause annoyance, painful bites and skin lesions to the cattle. Other fly species, such as *Phormia regina,* lay eggs on the active wounds leading to severe myiasis or "strike". The clinical appearance of fly infestation of dairy cattle include restlessness, irritability, decreased feed intake, painful skin lesion and a negative impact on subsequent productivity (Scott, 2008).

At a herd level, management practices such as draining swamps, stagnant water, removal of the manure, dead animals or placentas, that focus on the reduction of fly breeding areas consist a major concern towards the prevention and treatment of fly infestation (Scott, 2008; Taylor & Berkebile, 2011). These practices when combined with specially designed insecticides for premises enhance the level of control. In animal level, fly burdens can be controlled by the application of fly repellents or insecticides on the cattle, themselves (Mehlhorn *et al.*, 2010).

A variety of insecticidal products is registered in Europe for application on ruminants' skin. Among them, the pyrethroids, such as cyfluthrin, cypermethrin, permethrin and deltamethrin, are widely used. The later one, i.e. deltamethrin (Butox®, MSD) is one of the most effective pyrethroid compounds, due to its cis-isomer form (Mehlhorn *et al.*, 2010). Deltamethrin repels flies by the "hot foot effect," which is typical for pyrethroids. More precisely, the flies die due to "a knock down" effect, after a short contact (a few seconds are adequate) to the treated cattle. This is the result of the immediate contact of the feet nerves of the fly with the insecticide on the treated skin of the cattle (Eckert *et al.*, 2009; Dettner & Peters 2010). This efficacy constantly decreases the rate of annoyance, biting or other insect attacks, leading to reduced stress of the animals.

Measurement of stress level is an essential aspect of animal welfare and management (Moberg, 2000). Serum cortisol and creatinine kinase (CK) concentration can be used as potential stress indicators. Adrenal cortex response is activated when animals are submitted to aversive or noxious stimuli (e.g. fly annoyance or bites). Therefore, increased cortisol concentration may be indicative of stress provoked on cows by fly infestation. In addition, muscle cells contain high levels of CK, an enzyme which is associated with muscle cell damage. In case of restlessness (reduced relaxation time) due to fly infestation, CK leaks from muscle cells into serum (Brancaccio *et al.*, 2010), leading to increased serum CK concentration. Consequently, both of them may be used as estimators of the stress and fatigue status of the cattle due to fly abundance.

The aim of this study was to assess the possible effect of fly repellency, using deltamethrin (Butox®, MSD), on cortisol and CK concentration of dairy cattle.

MATERIAL AND METHODS

Herd history

The herd was consisted of 120 dairy cows of Holstein breed in lactation, reared under intensive management, in Thessaly (Greece). The animals consumed total mixed ratio (TMR) according to the nutrient requirements of their productive stage. The average milk production of the herd was approximately 27 liters. The structure of the farm buildings permitted the animals to have adequate rest area as well as to express normal feeding and drinking behavior. The routine vaccination program included vaccination against *Clostridium* spp. (Covexin®, MSD) and rotavi-

rus, coronavirus and *Escherichia coli* K99 (Rotavec Corona®, MSD). In addition, a vaccination against mastitis was implicated in all animals (Startvac®, Hipra) of the farm.

Experimental design

Twenty multiparous cows, aged between 3 to 5 years old, were included in the study. All animals were clinically healthy and weighed on average 650 kg. These animals were divided in 2 groups; cows of Group A (n=10) were individually dressed on their back with Butox® 7.5 pour-on post morning milking. No treatment was applied to the cows of Group B (n=10, control group). The study was conducted between June and July 2016.

Sampling

Blood samples were taken by jugular venipuncture (BD Vacutainer®) without anticoagulant from the total of the animals, on day of treatment (day: 0) and 20 days after the application of Butox® pour-on in Group A. Cows were walked calmly to the milking parlor, where they were individually trapped, while the blood samples were taken. This procedure was not supposed to affect stress and fatigue levels significantly, because they were habituated to walk through these facilities before every milking session. At the same time, fly traps were set in order to estimate the fly burden of the farm and to identify the fly species, as well.

Cortisol and creatine kinase assay

Blood samples were transported immediately to the Laboratory of Parasitology and Parasitic Diseases of the Veterinary Faculty of the Aristotle University of Thessaloniki in order to separate the serum through centrifugation at 1000 rpm for 15 min at environmental temperature. The samples were frozen (−20 °C) until cortisol and CK concentration to be measured. Roche Cobas E601 (Diamond Diagnostics, U.S.A.) immunology analyzer was used to estimate cortisol concentration with electrochemiluminescence immunoassay method (ECLIA). The estimation of CK concentration was performed with the use of Roche Cobas C501 (Diamond Diagnostics, U.S.A.) chemistry analyzer though spectrophotometric method.

Statistical analysis

The non parametric Wilcoxon signed rank test, for 2 dependent samples was used to compare cortisol and CK concentration, on day 0 and 20, between treated with Butox® pour-on (Group A) versus untreated (Group B) cows. A P value ≤ 0.05 was considered statistically significant. The statistical analysis was conducted with the help of statistical programme SPSS (v. 22.0).

RESULTS

Fly species

The most prevalent fly species found on the fly traps were common house flies (*Musca domestica),* horn flies (*Haematobia* spp.), stable flies (*Stomoxys calcitrans*) and blackflies (*Simulium* spp.).

Cortisol concentration

The average serum cortisol concentration for Group A on day 0 was 7.20 ng/mL (1.40, 12.30). In contrast, the same group had smaller ($P \leq 0.05$) average cortisol concentration [1.35 ng/mL (1.00, 2.40)] on day 20. According to statistical analysis, there was no significant ($P \geq 0.05$) difference regarding cortisol concentration in Group B between day 0 and 20 (Table 1).

Table 1. Median (Min, Max) of cortisol concentration (ng/mL) per day of sampling per group

Group	Day	Median (Min, Max) of cortisol concentration (ng/mL)
Deltamethrin-treated	0	7.20 [a] (1.40, 12.30)
	20	1.35 [b] (1.00, 2.40)
Control	0	7.15 [a] (1.70, 10.10)
	20	4.90 [a] (2.70, 13.40)

[a,b] different superscripts in the same column indicate statistical significant differences between cortisol concentrations (P≤0.05).

Table 2. Median (Min, Max) of creatine kinase concentration (U/L) per day of sampling per group

Group	Day	Median (Min, Max) of creatine kinase concentration (ng/mL)
Deltamethrin-treated	0	459.70 [a] (219.60, 1565.00)
	20	185.30 [b] (144.40, 230.70)
Control	0	526.30 [a] (231.40, 2101.00)
	20	483.95 [a] (212.60, 1763.00)

[a,b] different superscripts in the same column indicate statistical significant differences between cortisol concentrations (P≤0.05).

CK concentration

The average CK concentration followed the same frame. More precisely, there was a statistically significant effect of deltamethrin treatment on CK concentration in Group A cows between day 0 and day 20, while the other group (control group) remained unaffected (Table 2).

DISCUSSION

The aim of this study was to assess the possible effect of the fly repellent deltamethrin (Butox®, MSD), on stress (cortisol concentration) and fatigue (CK concentration) indicators in a dairy cattle herd.

In temperate climates, flies reproduce fast under certain circumstances (e.g. humidity, sufficient food), showing a temperature dependent appearance during the year (Mehlhorn *et al.*, 2010). Among them, *Musca domestica* presents licking activity, while other flies species such as *Stomoxys calcitrans, Haematobia irritans* and *Simulium* spp. present enhanced blood sucking activity (Mehlhorn *et al.*, 2010). In our study, the most prevalent fly species found on fly traps were the common flies (*Musca domestica),* horn flies (*Haematobia* spp.), stable flies (*Stomoxys calcitrans*) and blackflies (*Simulium* spp.).

Both licking and blood sucking activities provoke discomfort, annoyance and painful bites, affecting normal behaviors of the cows (Mullens *et al.*, 2006). As a result, cows increase both their physiological activity and behavioral responses in order to protect themselves, conditions that favor increased stress and fatigue level of the animals (Vitela-Mendoza *et al.*, 2016). Several studies showed a lineal relationship between flies' burden (e.g. *Stomoxys calcitrans*) and defensive be-

haviors. More precisely, the higher numbers of flies were estimated, the more defensive behaviors from the cows were observed (Eicher *et al.*, 2001; Taylor *et al.*, 2012). A reduction in defensive behaviors leads to more comfort and less stress for the animals improving the welfare status and the subsequent productivity as well (Vitela-Mendoza *et al.*, 2016).

Nowadays, there is a constantly increasing interest regarding the quantification of stress level, a potential indicator of welfare and health status (Mostl & Palme, 2002; Saco *et al.*, 2008). Serum cortisol is the most predominant indicator of stress studied in cattle (Earley *et al.*, 2011). In our study, serum cortisol concentration (1.35 ng/mL) was lower in day 20 of deltamethrin treated cows ($P \leq 0.05$), while in control group the average cortisol concentration was higher at both Day 0 (7.15 ng/ml) and 20 (4.90 ng/mL) ($P \geq 0.05$). In accordance with our study, Schwinghammer *et al.* (1986) reported high serum cortisol concentration (10 ng/ml) due to heavy infestation of beef cattle by stable flies. Vitela-Mendoza *et al.* (2016) concluded that plasma cortisol concentration is linearly related to the number of flies in dairy cows herds. On the contrary, Estienne *et al.* (1991) found that serum cortisol concentrations were similar among groups of steers exposed to 0, 10, 20 or 30 stable flies, three times (15 min each time) per day, for a 2 week period. However, the above numbers of flies used by Estienne *et al.* (1991) were not very high, according to our experience, to provoke significant reactions. In our case, the numbers of flies were much higher (approximately >100 individuals per cow). Therefore, serum cortisol concentration can be used as a potential stress indicator, taking into consideration some limitations, such as the circadian rhythms (Earley *et al.*, 2011).

In order to further elucidate the beneficial effect of the administration of deltamethrin, CK was measured as a fatigue indicator. The activity of CK increases after muscle cell damage or as a result of increased muscular exertion (Mitchell *et al.*, 1982; 1988) due to restlessness and defensive behaviors in cases of heavy fly infestation of the dairy cattle herds. In the literature, there is little information regarding the relationship between CK and restlessness due to fly abundance. In our study, serum CK concentration (185.30 U/L) was lower in day 20 of Group A cows ($P \leq 0.05$), while in Group B the average CK concentration remained unaffected at both Day 0 (526.30 U/L) and 20 (483.95 U/L) ($P \geq 0.05$). CK activity was significantly higher in serum of grazing ruminants due to muscular exertion compared to control, suggesting damage to muscle cells (Forcados *et al.*, 2016). Another example, which supports the relationship between CK concentration and restlessness (fatigue level) is given by Earley *et al.* (2011), who found that CK activity was higher on day 6 in bulls during transportation.

Other stressful and restless factors, in herd management level, can lead to misinterpretation of the evaluation of the cortisol and CK measurements. Feed and water deprivation (inadequate feeding and drinking area) due to overcrowding consists a major management problem for the Greek dairy cattle herds. As a result, animals suffer from nutritional stress leading to high concentration of serum cortisol. Due to overcrowding, the resting area is inadequate leading the animals to physical exhaustion and restlessness and thus, to high concentration of CK. In our study, the structure of the farm buildings permit-

ted the animals to have adequate rest area as well as to express normal feeding and drinking behavior. All animals, participating in the trial, have been clinically examined in order to avoid current clinical or sub-clinical diseases (e.g. mastitis, pneumonia) which cause serum hypercortisolemia. In this way, our measurements were fully attributed to the fly repellency.

CONCLUSIONS

Avoidance of animal stress and fatigue represents an essential key point of welfare and management in dairy cattle practice. The results of our study showed that the fly population of the farm was positively related to the serum cortisol and CK concentration, which are indicative of stress and fatigue status of the animals. The administration of the fly repellent deltamethrin (Butox®, MSD) reduced fly abundance, favouring a more stressless and restful environment. Consequently, fly control using an effective repellent constitutes a valuable strategy to maintain animal welfare and ethology.

REFERENCES

Bradley, 1993. Multiple releases of stable flies (*Stomoxys calcitrans L.*) and behavior of grazing beef cattle. *Applied Animal Behaviour Science*, **38**, 191–212.

Brancaccio, P., G. Lippi & N. Maffulli, 2010. Biochemical markers of muscular damage. *Clinical Chemistry and Laboratory Medicine*, **48**, 757–767.

Dettner, K. & W. Peters, 2010. Lehrbuch der Entomologie. Spektrum, Heidelberg.

Dougherty, C. T., F. W. Knapp, P. B. Burrus, D. C. Willis, P. L. Cornelius & N. W. Dougherty, C. T., F. W. Knapp, P. B. Burrus, D. C. Willis & P. L. Cornelius, 1994. Moderation of grazing behavior of beef cattle by stable flies (*Stomoxys calcitrans L.*). *Applied Animal Behaviour Science*, **40**, 113–127.

Earley, B., B. McDonnell, M. Murray, D. J. Prendiville & M.A. Crowe, 2011. The effect of sea transport from Ireland to the Lebanon on inflammatory, adrenocortical, metabolic and behavioural responses of bulls. *Research in Veterinary Science*, **91**, 454–464.

Eckert, J., K.T. Friedhoff, H. Zahner & P. Deplazes, 2009. Lehrbuch der Parasitologie für die Tiermedizin. Enke, Stuttgart.

Eicher, S. D., J. L. Morrow-Tesch, J. L. Albright & R. E. Williams, 2001. Tail-docking alters fly numbers, fly-avoidance behaviours, and cleanliness, but no physiological measures. *Journal of Dairy Science*, **84**, 1822–1828.

Estienne, M. J., F. W. Knapp, J. A. Boling & J. G. Burg, 1991. Physiological and nutritional responses of beef steers exposed to stable flies (Diptera: Muscidae). *Journal of Economic Entomology*, **84**, 1262–1265.

Forcados, G. E., A. S. Lohlum, Y. Usman, B. K. Tondo & A. A. Atiku, 2016. Changes in serum biochemical parameters and oxidative stress biomarkers in grazing cattle. *Comparative Clinical Pathology*, **25**, 1013–1016.

Hillerton, J. E., A. J. Bramley & N. H. Yarrow, 1985. Control of flies (Diptera: Muscidae) on dairy heifers by flectron ear-tags. *British Veterinary Journal*, **141**, 160–167.

Mehlhorn, H., K. A. S. Al-Rasheid, F. Abdel-Ghaffar, S. Klimpel & H. Pohle, 2010. Life cycle and attacks of ectoparasites on ruminants during the year in Central Europe: Recommendations for treatment with insecticides (e.g. Butox®). *Parasitology Research*, **107**, 425–431.

Mitchell, G., J. Hattingh & M. Ganhao, 1988. Stress in cattle assessed after handling, after transport and after slaughter. *The Veterinary Record*, **123**, 201–205.

Mitchell, G., J. J. Heffron & J. H. Dreyer, 1982. Muscle fiber type, fiber diameter and pH values of *M. Longissimus* dorsi of normal, malignant hyperthermia and PSE

susceptible pigs. *British Veterinary Journal*, **138**, 45–50.

Moberg, G. P., 2000. Biological response to stress: Implications for animal welfare. In: *The Biology of Animal Stress,* eds G. P. Moberg & J. A. Mench, CABI Publishing, pp. 123–146.

Mostl, E. & R. Palme, 2002. Hormones as indicators of stress. *Domestic Animal Endocrinology*, **23**, 67–74.

Mullens, B. A., K. S. Lii, Y. Mao, J. A. Meyer, N. G. Peterson & C. E. Szijj, 2006. Behavioral responses of dairy cattle to the stable fly, *Stomoxys calcitrans*, in an open field environment. *Medical and Veterinary Entomology*, **20**, 122–137.

Phillips, C., 2002. Cattle Behavior and Welfare. Blackwell Science Ltd, England.

Saco, Y., M. Fina, M. Gimenez, R. Pato, J. Piedrafita & A. Bassols, 2008. Evaluation of serum cortisol, metabolic parameters, acute phase proteins and faecal corticosterone as indicators of stress in cows. *The Veterinary Journal*, **177**, 439–441.

Schwinghammer, K. A., F. W. Knapp, J. A. Boling & K. K. Schillo, 1986. Physiological and nutritional response of beef steers to infestations of the stable fly. *Journal of Economic Entomology*, **79**, 1294–1298.

Scott, D.W., 2008. Skin diseases. In: *Rebhun's Diseases of Dairy Cattle*, 2nd edn, eds T. Divers & S. Peck, St. Louis, Missouri, pp. 295–326.

Taylor, D. B. & D. R. Berkebile, 2011. Phenology of stable fly (Diptera: Muscidae) larvae in round bale hay feeding sites in eastern Nebraska. *Environmental Entomology*, **40**, 184–193.

Taylor, D. B., R. D. Moon & D. R. Mark, 2012. Economic impact of stable flies (Diptera: Muscidae) on dairy and beef cattle production, *Journal of Medical Entomology*, **49**, 198–209.

Vitela-Mendoza, I., C. Cruz-Vazquez & A. Orihuela, 2006. A note on the effect of controlling stable flies (*Stomoxys calcitrans*) in the resting activity and pen distribution of dairy cows. *Journal of Applied Animal Welfare Science*, **9**, 241–248.

Vitela Mendoza, I., C. Cruz-Vazquez, J. J. Solano & A. Orihuela, 2007. A note on the associations between the prevalence of stable flies (*Stomoxys calcitrans*) and the behaviour of dairy cows under semi-arid conditions. *Journal of Animal Veterinary Advances*, **6**, 1284–1290.

Vitela-Mendoza, I., C. Cruz-Vázquez, J. Solano-Vergara & A. Orihuela-Trujillo, 2016. Short communication: Relationship between serum cortisol concentration and defensive behavioral responses of dairy cows exposed to natural infestation by stable fly, *Stomoxys calcitrans. Journal of Dairy Science*, **99**, 9912–9916.