

Forensic veterinary use of the fly *Lucilia sericata* (Diptera: Calliphoridae) in the aspect of determining the time of death using tissues treated with calcium diclofenac

KATARZYNA CZEPIEL-MIL¹, PIOTR LISTOS², ROBERT STRYJECKI¹,
DANUTA KOWALCZYK-PECKA¹, MAREK NIEOCZYM¹

¹Department of Zoology and Animal Ecology, Faculty of Environmental Biology,
University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

²Department of Pathomorphology and Forensic Medicine, Faculty of Veterinary Medicine,
University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland

Received 10.03.2021

Accepted 04.01.2023

Czepiel-Mil K., Listos P., Stryjecki R., Kowalczyk-Pecka D., Nieoczym M.

Forensic veterinary use of the fly *Lucilia sericata* (Diptera: Calliphoridae) in the aspect of determining the time of death using tissues treated with calcium diclofenac

Summary

Non-steroid anti-inflammatory drugs are commonly used both in medicine and veterinary medicine. The aim of the paper was to determine the effect of diclofenac calcium, the active ingredient of a medicine called diclofenac, on the development rate and survivorship of the fly *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae). Diclofenac was used at three concentrations: it was mixed with pork meat in proportions 25 mg of the drug 23 g of meat (dose 1), 50 mg/23 g (dose 2) and 75 mg/23 g (dose 3). To compare the results, a control sample was used (23 g of drug-free meat). All diclofenac calcium doses delayed the development of *L. sericata*. Moreover, all the drug doses (25 mg, 50 mg, 75 mg) resulted in body weight loss of *L. sericata* larvae. Finally, all the drug doses increased the mortality of *L. sericata*. The findings indicate that forensic entomology and forensic veterinary analyses involving *L. sericata* need to take into account a potential factor modifying the fly natural life cycle – the presence of diclofenac calcium in the body. A reduction of the body mass and a longer, than normal developmental cycle of *L. sericata* may be of significant in correctly determining the time of death of humans or animals in cases of diclofenac application.

Keywords: forensic entomology, forensic veterinary medicine, entomotoxicology, drugs, xenobiotics, post mortem interval

The multifaceted nature of veterinary protection of animals in Poland is reflected in the implementation of scientific achievements of one of the disciplines of veterinary science, which is forensic veterinary medicine. The analyzed area of animal veterinary protection is a different and at the same time specific form. Forensic veterinary medicine, as a scientific discipline, does not cover animal protection issues (26). Only its practical application makes it possible mainly for procedural authorities and private persons to implement animal protection, a significant part of which is veterinary protection.

A large and diverse group of drugs belonging to the non-steroid al anti-inflammatory drugs (NSAIDs) is frequently used in both medicine and veterinary

medicine (27). This group includes carboxylic acids, among the diclofenac. The analgesic, antipyretic, anti-inflammatory and anticoagulant effects of these drugs cause that they are often recommended by veterinarians for the treatment of small animals.

Therapeutic effect of non-steroidal anti-inflammatory drugs is strongly related to toxic effects (28). Some of these drugs have a destructive effect on the gastric mucosa, and the intensity of the changes increases with time of use (27). A few cases of anaphylactic reaction to diclofenac were also reported (3, 8, 20). The tests on laboratory animals showed that diclofenac can cause hepatic and kidney degenerative changes, especially when it is dosed together with other anti-inflammatory drugs (5, 31, 34). In addition to their therapeutic

effects, non-steroidal anti-inflammatory drugs can also cause poisoning. These poisonings can be accidental or can be caused by the owner's failure to self-medicate the animal, or sometimes by an overdose of the drug. Unfortunately, sometimes the poisoning can be the cause of the animal's death (28).

During decomposition of organic matter of animal origin, including animal and human bodies, a specific microhabitat develops for different animal taxa as dead bodies can provide a suitable protection for the fauna, favorable features of environment (such as temperature, humidity) are present in such habitats as well as high nutritious resources are constantly provided for invertebrates that colonize corpses (2, 15, 16, 32). Insects, and dipterans in particular, are one of the most important groups of invertebrates that colonize animal and human corpses (4). Insects gathered from a dead body may provide vital information, particularly when the body is in an advanced state of decomposition or is skeletonized (10). A correct analysis of entomological data provided by insects that colonize corpses is of enormous significance in establishing the time that has elapsed from death (PMI, post mortem interval) (17, 23).

Exogenous substances, such as non-steroid anti-inflammatory drugs, can affect the development rate of insects living on corpses as well as their number which also need to be taken into account while estimating the post mortem interval (24). There were some reports concerning the influence of diclofenac on development rates of some forensic blowfly species (35). But no data about this subject is available for *Lucilia sericata* (Meigen, 1826) – the widespread fly species that is commonly utilized in forensic entomology to estimate the post mortem interval (19, 25). This dipteran is a carrion-breeder and facultative parasite as well as important sheep myiasis fly (13). *L. sericata* eggs may occur on corpses as early as several hours after death. Larvae hatch after several hours. The time of the emergence of the consecutive instars depends primarily on ambient temperature and humidity. Apart from being used in forensic medicine as well as being a veterinary problem, these fly larvae are also used in larvae therapy owing to the fact that they secrete an antibacterial substance termed lucifensin (9, 11, 22, 37, 38). For this the reason this fly species is bred on a mass scale (14).

The presented study seeks to determine the effect of diclofenac calcium, an active ingredient of a drug termed diclofenac, on the development rate and survivorship of the fly *L. sericata* feeding on the corpses of humans and animals. The findings may provide valuable information in the case of an investigation when a potential cause of death of a human or animal was overdosing this medicine. Diclofenac calcium is a foreign substance (xenobiotic) for *L. sericata* therefore a hypothesis was made that this chemical compound

would have a negative effect on the fly's development, affecting its larvae mass, the length of the development cycle, as well as the mortality of individual instars.

Material and methods

Laboratory trial. The experiment was conducted using a population of *Lucilia sericata* – a common fly species from the Calliphoridae family. The substrate for the larvae culture was pork liver. At first, a portion of the meat without the drug was exposed in an outdoor environment to attract the flies and for them to lay eggs. After the egg packets appeared on the meat medium, the subsequent procedure was carried out under laboratory conditions. The egg packets were carefully transferred to weighed portions of meat with the appropriate dose of the drug. The drug used in the experiment was Voltaren Acti Forte (manufacturer: Novartis Consumer Health GmbH) in the form of film-coated tablets. One tablet contained 25 mg of Diclofenacum kalicum. The tablets were crushed and thoroughly mixed with portions of pork liver. The following variants of the experiment were prepared: 25 mg of the drug for 23 g of pork liver (dose 1), 50 mg/23 g (dose 2) and 75 mg/23 g (dose 3). To compare the results, a control sample was used (23 g of drug-free meat). Forty *L. sericata*, the second in star larvae (L2, 4th day of development), were used respectively for the control and for each of the three variants of the trial which gives all together 160 larvae used in the experiment. In the course of the experiment 5 weightings were made. The weightings took place every three days while the condition of the larvae (their vitality, ability of movement) was checked on every day. Larvae were weighed to the nearest 0.00001 g. The experiment lasted until the moment of pupation of the last L3 larva. The larvae used in the experiment eventually yielded adults of *Lucilia sericata*, confirming the proper determination of larval material. The experiment was performed at the temperature ~ 23.5°C and relative humidity RH ~ 54%. An analytical balance (WPA 40/160/C/1 RADWAG) was used for larval weightings.

Statistical analyses. Descriptive statistics (sums, means, range, standard deviation) were calculated using PAST ver. 3.16/2017 software (21). The normality of the data distribution was checked by the Shapiro-Wilk test. The data were tested for homogeneity of variance using Bartlett's test. As the data had normal distribution and the variances were equal, the one-way ANOVA test was used to compare multiple independent samples and then post hoc LSD Fisher's tests were used to compare two independent samples. All statistical tests were applied using the Statistica 13.1 software. The statistical significance level was set at $p = 0.05$.

Results and discussion

Larvae body mass. Significant statistical differences in the larvae body mass between the variants of the experiment were found (one-way ANOVA $F_{3,276} = 12.40, p = 0.00000$). Fisher's post hoc LSD tests detected statistically significant differences between the control sample and the samples with the three doses of the drug, whereas no statistically significant differences were found in the body mass of the larvae

reared on the feedstuff with the three doses of the drug. The mean larval body mass was higher in the control sample ($0.04520 \text{ g} \pm 0.0059$) than at doses 1, 2 and 3 of the drug (respectively: $0.04001 \text{ g} \pm 0.0044$, $0.03983 \text{ g} \pm 0.0048$ and $0.04096 \text{ g} \pm 0.0057$) – Figure 1. The maximum weight of the larvae (0.05938 g) during the experiment was found in the control sample, and the minimum weight (0.02568 g) in dose 3.

Subsequent control weightings found a significant reduction of larvae mass in the samples with the diclofenac calcium doses (Fig. 2). We also detected differences in the minimal larvae mass before pupation depending on the variant of the experiment. The smallest body mass before pupation in the control sample was 0.03436 g , in dose 1: 0.02816 g , in dose 2: 0.02996 g , in dose 3: 0.02568 g .

Larvae development rate. During the weightings, larvae were present in all the variants of the experiment only on the I, II and III date of the weighing (4th, 7th and 10th day of development, respectively – Table 1). On later dates, larvae were present only in some of the variants of the experiment (Tab. 1, Fig. 2). On day 10 of the developmental cycle in the control sample only 9 larvae were found as the rest had transformed into pupae. In drug doses 1-3 larvae still predominated. On day 13 of the development cycle no larvae were present in the control sample and in the sample with drug dose 1. On day 16 of development the larvae were present only in drug dose 3 (Tab. 1).

Larvae mortality. During the experiment, different larvae mortality was found depending on the variant of the experiment. One larva died in the sample with drug dose 1, 3 larvae died in the control sample, 5 in dose 2 and 8 in dose 3.

The specific nature of the implementation of veterinary protection of animals by forensic veterinary medicine is influenced by the fact that in recent years its importance as an applied science has clearly increased (26). This is due to the development of medical and veterinary legislation and legislation related to the protection of animals. The increased interest in forensic veterinary medicine is also fueled by the increased awareness of the society and the ensuing demands for animal protection.

Forensic entomology is considered to be an important instrument in criminal investigations (36). The analysis of entomological material can also be used in forensic veterinary medicine, being one of the practical tools that enable procedural authorities and private persons to implement animal protection, a significant part of which is veterinary protection (26).

Xenobiotics may affect the length of the growth cycle of necrophagous insects in a diversified way. Some delay insect development and disrupt corpse colonization, while others reduce developmental processes compared to controls (7, 18). Entomotoxicological studies conducted in Turkey on the domestic pig

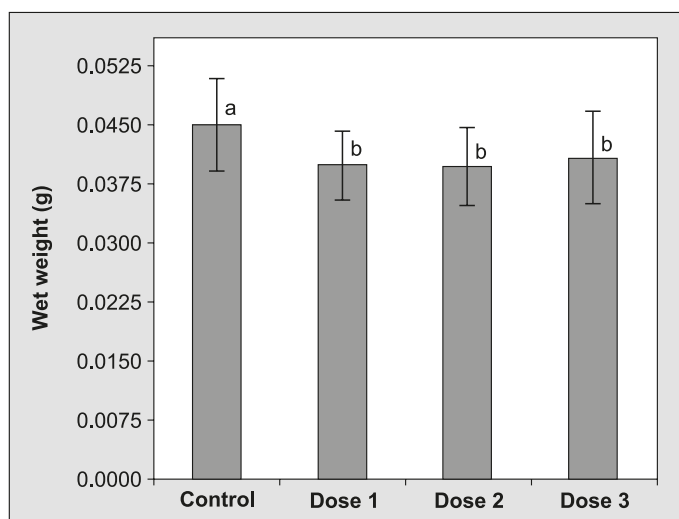


Fig. 1. Differences in *Lucilia sericata* larval body mass (mean and $\pm$ SD) in the different variants of the experiment

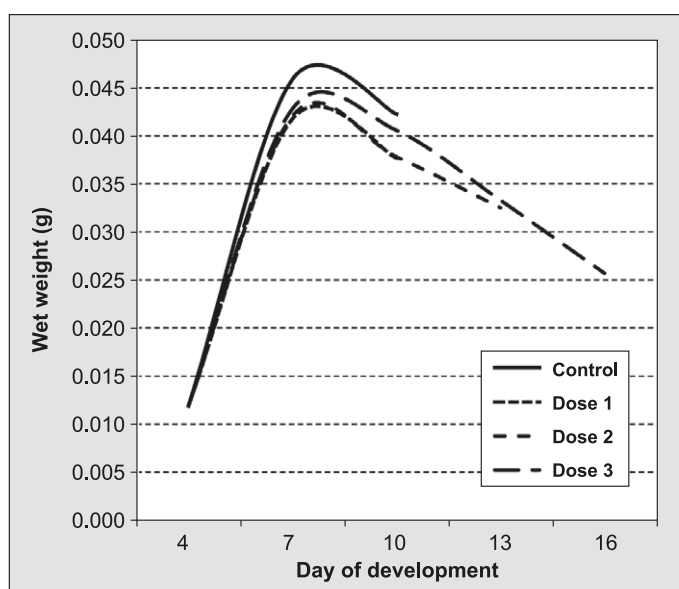


Fig. 2. Mean larval masses in individual control weightings/ days of development. For dose 2 on day 13 of development and dose 3 on day 16 of development – data from individual records

Tab. 1. The number of larvae on successive days of the experiment

Variant	Day of development				
	4	7	10	13	16
Control	40	39	9	0	0
Dose 1	40	40	37	0	0
Dose 2	40	37	37	1	0
Dose 3	40	37	37	5	1

(*Sus scrofa domestica*) clearly indicate that toxic substances have altered the life cycle of *Chrysomya albiceps* (1). This confirms the validity of performing the above analyses to keep PMI estimation errors as small as possible.

Similarly, analgesic drugs may have a diversified effect on the growth of necrophagous insects. Some

accelerate it, others delay it. Necrophagous insects react to drugs in a diversified manner. Note that discrepancies are observed even between species belonging to the same fly family. As Wyman (39) reports, a factor affecting insects growth cycle, apart from the duration of drug action, is also the stage of decomposition of the corpse. It has been noted that paracetamol accelerates the growth rate of *Calliphoravicina* larvae from day 2 to day 4 of their development (29). This may result in a 12-hour discrepancy in PMI estimate. Ketamine, an anesthetic and analgesic substance, reduces the larval instar in *L. sericata*, whereas morphine delays the growth cycle of *L. sericata* (6, 40). Tramadol hydrochloride delays the growth cycle of *Chrysomya albiceps* (Diptera: Calliphoridae) by 2 days compared to the control group (12). Such different findings may result from alternations in drug concentration and its metabolites (7). Shamsuddin et. al (35) found that diclofenac delayed *Chrysomya megacephala* development for up to 24 hours.

The experiment presented in this paper demonstrated that diclofenac calcium resulted in delaying larval development of *L. sericata* by 3-6 days compared to the control group. In the control group the last larva was noted on day 10 from the ovi position, whereas in drug dose 2 larvae were still present on day 13 of the cycle, and in drug dose 3 even on day 16 of the development cycle (see Tab. 1). Thus, the study showed that the compound extended the growth cycle at the third larval instar (L3) compared to the control group. This finding may be of great significance to estimate the PMI in forensic medicine.

In the presented experiment with the doses of diclofenac calcium it was also discovered that all the doses of the drug resulted in *L. sericata* larvae body mass reduction. It was partially connected with the slowing of the development rate of the larvae reared on substrates containing the drug. Shamsuddin et. al (35) found that the larvae of *Chrysomya megacephala* grown on diclofenac-containing substrates were shorter in comparison to the larvae from control samples. Similar results were obtained by Oliveira (30) with reference to the analgesic and spasmolytic drug Buscopan and *Chrysomya megacephala* fly. She found that under the influence of Buscopan the growth cycle of *C. megacephala* slowed and the body mass was reduced.

Some substances may cause huge mortality among insect larvae, like thiopental in *Calliphoravicina* (33). The present study has found that diclofenac calcium in larger doses also contributed to significant mortality of larvae.

Conclusions:

1. All the doses of diclofenac calcium slowed the development of *L. sericata* larvae. The relationship was found: the higher the diclofenac calcium dose, the slower the development of *L. sericata*.

2. All the doses of diclofenac calcium resulted in *L. sericata* larvae body mass reduction.

3. All the doses of diclofenac calcium resulted in increased mortality of *L. sericata* larvae. The relationship was found: the higher the diclofenac calcium dose, the higher the mortality of larvae.

4. A reduction of the body mass and a longer than normal developmental cycle of *L. sericata* may be of significant in correctly determining the time of death of humans or animals in cases of diclofenac overdose.

References

1. Açıkgöz H. N.: Multiple drug analysis of *Chrysomya albiceps* larvae provides important for ensicins sights to unravel drug-associated mortalities. Entomological News 2018, 128, 1, 99-107.
2. Aguirre S., Barragán A.: Datos preliminares de la entomofauna cadavérica en la provincia de Pichincha. Ecuador Rev. Ecu. Med. Ciens Biol. 2015, 36 (1), 67-72.
3. Alkhawajah A. M., Eifawal M., Mahmoud S. F.: Fatal anaphylactic reaction to diclofenac. Forensic Sci. Int. 1993, 60, 107-110.
4. Amendt J., Richards C. S., Campobasso C. P., Zehner R., Hall M. J. R.: Forensic entomology: applications and limitations. Forensic Sci. Med. Pathol. 2011, 7, 379-392.
5. Aydın G., Gokcimen A., Oncü M., Çiçek E., Karahan N., Gökalp O.: Histopathologic changes in liver and renal tissues induced by different doses of diclofenac sodium in rats. Turkish J. Vet. Anim. Sci. 2003, 27, 1131-1140.
6. Bourel B., Hédouin V., Martin-Bouyer L., Bécart A., Tournel G., Deveaux M., Gosset D.: Effects of morphine in decomposing bodies on the development of *Lucilia sericata* (Diptera: Calliphoridae). J. Forensic Sci. 1999, 44 (2), 354-358.
7. Carvalho L. M. L. de, Linhares A. X., Palhares F. A. B.: The effect of cocaine on the development rate of immatures and adults of *Chrysomya albiceps* and *Chrysomya putoria* (Diptera: Calliphoridae) and its importance to post mortem interval estimate. Forensic Sci. Int. 2012, 220, (1-3), 27-32.
8. Colak S., Gunes H., Afacan M. A., Kandis H., Erdogan M. O., Ayrançi M., Saritas A.: Anaphylaxis after intramuscular injection of diclofenac sodium. Am. J. Emerg. Med. 2014, 32 (7), 815.e1-2, doi: 10.1016/j.ajem.2013.12.049.
9. Čerovský V., Žďárek J., Fučík V., Monincová L., Voburka Z., Bém R.: Lucifensin, the long-sought antimicrobial factor of medicinal maggots of the blow fly *Lucilia sericata*. Cell Mol. Life Sci. 2010, 67 (3), 455-466.
10. Chopi R., Sharma S., Sharma S., Rajinder S.: Forensic entomotoxicology: Current concepts, trends and challenges. J. Forensic Leg. Med. 2019, 67, 28-36.
11. Cruz-Saavedra L., Díaz-Roa A., Gaona M. A., Cruz M. L., Ayala M., Cortés-Vecino J. A., Patarroyo M. A., Bello F. J.: The effect of *Lucilia sericata* and *Sarconesiopsis magellanica* derived larval therapy *Leishmania panamensis*. Acta Trop. 2016, 164, 280-289.
12. Ekrakene T., Odo P. E.: Comparative developmental effects of tramadol hydrochloride and cypermethrin on *Chrysomya albiceps* (Weid.) (Diptera: Calliphoridae) reared on rabbit carrions. Sci. World J. 2017, 12 (1), 28-32.
13. Erzincinlioglu Y. Z.: The early larval in stars of *Lucilia sericata* and *Lucilia cuprina* (Diptera, Calliphoridae): myiasis blowflies of Africa and Australia. Journal of Natural History. 1989, 23, 5, 1133-1136, doi: 10.1080/00222938900771021.
14. Firoozfar F., Moosa-Kazemi H., Baniardalani M., Abolhassani M., Khoobdel M., Rafinejd J.: Mass rearing of *Lucilia sericata* Meigen (Diptera: Calliphoridae). Asian Pac. J. Trop. Biomed. 2011, 1 (1), 54-56.
15. García A. B., Donoso D. A.: Casos sin resolver y la entomología forense en Ecuador. Rev. Ecu. Med. Ciens Biol. 2015, 36 (1), 61-66.
16. Gemmellaro D.: 2015. A user's guide for forensic entomology. Rev. Ecu. Med. Ciens Biol. 2015, 36 (1), 11-18.
17. Gennard D. E.: Forensic entomology: an introduction. John Wiley and Sons, Chichester (UK) 2007, p. 272.
18. Goff M. L., Omori A. I., Goodbrod J. R.: Effect of cocaine in tissues on the development rate of *Boettcherisca peregrina* (Diptera: Sarcophagidae). J. Med. Entomol. 1989, 26 (2), 91-93.
19. Grassberger M., Reiter C.: Effect of temperature on *Lucilia sericata* (Diptera: Calliphoridae) development with special reference to the isomegalen- and isomorphen-diagram. Forensic Sci. Int. 2001, 120 (1-2), 32-36.
20. Hadar A., Holcberg G., Mazor M.: Anaphylactic shock after diclofenac sodium (Voltaren). Medicine Harefuah. 2000, 1, 138 (3), 211-212, 270.

21. Hammer Ø., Harper D. A. T., Ryan P. D.: Past: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* 2001, 4, 1, 4, 9pp., 178kb. Available: http://palaeo-electronica.org/2001_1/past/issue1_01.htm.15.
22. Huberman L., Gollop N., Mumcuoglu K. Y., Breuer E., Bhusare S. R., Shai Y., Galun R.: Antibacterial substances of low molecular weight isolated from the blowfly, *Lucilia sericata*. *Med. Vet. Entomol.* 2007, 21, 127-131.
23. Introna F., Campobasso C. P., Goff M. L.: Entomotoxicology. *Forensic Sci. Int.* 2001, 120, 42-47.
24. Kaczorowska E., Draber-Mońko A.: Introduction to forensic entomology [in Polish]. Gdańsk University Press., Gdańsk 2010, p. 292.
25. Karabey T., Sert O.: The analysis of pupal development period in *Lucilia sericata* (Diptera: Calliphoridae) forensically important insect. *J. Legal. Med.* 2014, 40, 47-56.
26. Listos P.: Weterynaryjna medycyna sądowa, [in:] Teresiński G. (red.): *Medycyna sądowa. T. 2*, Wydawnictwo Lekarskie PZWL, Warszawa 2020, 869-873, 978-83-200-6108-6.
27. Nicpoń J., Kubiak K., Dzimira S., Sapikowski G.: Wpływ wybranych niesterydowych leków przeciwzapalnych na błonę śluzową żołądka u psów. *Med. Weter.* 2000, 56 (9), 582-585.
28. Niemczycka E. A., Pakula A., Łach M.: Wybrane ostre zatrucia u małych zwierząt. *Życie Wet.* 2021, 96 (10), 720-724.
29. O'Brien C., Turner B.: Impact of paracetamol on *Calliphoravicina* larval development. *Int. J. Legal Med.* 2004, 118 (4), 188-189.
30. Oliveira H. G., Gomes G., Morlin J. J. Jr., Von Zuben C. J., Linhares A. X.: The effect of Buscopan on the development of the blow fly *Chrysomya megacephala* (F.) (Diptera: Calliphoridae). *J. Forensic Sci.* 2009, 54 (1), 202-206.
31. Ong C. K., Lirk P., Tan C. H., Seymour R. A.: Anevidence-based update on nonsteroidal anti-inflammatory drugs. *Clin. Med. Res.* 2007, 1, 5 (1), 19-34.
32. Pruna W., Guarderas P., Donoso D. A., Barragán Á.: Life cycle of *Lucilia sericata* (Meigen 1826) collected from Andean mountains. *Neotrop. Biodivers.* 2019, 5 (1), 3-9.
33. Sadler D. W., Robertson L., Brown G., Fuke C., Pounder D. J.: Barbiturates and analgesics in *Calliphoravicina* larvae. *J. Forensic Sci.* 1997, 42 (6), 1214-1215.
34. Salih R. A.: Clinical and histopathological study of diclofenac sodium-acetylsalicylic acid toxic effect on liver of mice. *Indian J. Forensic Med. Toxicol.* 2021, 15, 2314-2321, doi:10.37506/IJFMT.V15I1.13747.
35. Shamsuddin S., Zulkifli N., Zuraina M. Y., Mohamed K., Rashid R.: Influence of diclofenac on development rates of forensic blowfly *chrysomya megacephala*, [in:] *Bioresources Technology in Sustainable Agriculture*. 2018, 153-165, doi: 10.1201/9781315365961-11.
36. Sharma R., Garg R. K., Gaur J. R.: Various methods for the estimation of the post mortem interval from Calliphoridae. A review. *Egypt J. Forensic Sci.* 2015, 5 (1), 1-12.
37. Valachova I., Prochazka E., Bohova J., Novak P., Takac P., Majtan J.: Antibacterial properties of lucifensin in *Lucilia sericata* maggots after septic injury. *Asian Pac. J. Trop. Biomed.* 2014, 4 (5), 358-361.
38. Valachova I., Majtan T., Takac P., Majtan J.: Identification and characterisation of different proteases in *Lucilia sericata* medicinal maggots involved in maggot debridement therapy. *J. Appl. Biomed.* 2014, 12 (3), 171-177.
39. Wyman J. F., Dean D. E., Yinger R., Simmons A., Brobst D., Bissell M., Silveira F., Kelly N., Short R., Ohr J., Howard R., Lewis B.: The temporal fate of drugs in decomposing porcine tissue. *J. Forensic Sci.* 2011, 56, 694-699.
40. Zou Y., Huang M., Huang R., Wu X., You Z., Lin J., Huang X., Qiu X., Zhang S.: Effect of ketamine on the development of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) and preliminary pathological observation of larvae. *Forensic Sci. Int.* 2013, 226 (1-3), 273-281.

**Corresponding authors: Piotr Listos DVM, PhD, Master of law, PhD, Głęboka 30, 20-612 Lublin, Poland; e-mail: piotr.listos@up.lublin.pl
Robert Stryjecki PhD, Akademicka 13, 20-950 Lublin, Poland; e-mail: robert.stryjecki@up.lublin.pl**