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Ivermectin causes adverse effects on the metabolic rate and thermoregulatory capacity of Dung beetles

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Ivermectin (IVM), a commonly used endectocide in livestock, has been shown to produce adverse effects in dung beetle ecology, physiology, reproduction, and even their ecosystem services. However, the ever-growing ecological importance of thermoregulation and its associated metabolic demand in dung beetles has not received as much focus regarding the effects of this drug. Here, we evaluated experimentally the effects caused by IVM in the metabolic rate and thermoregulation of *Ateuchetus cicatricosus* (Lucas, 1846), using a standardized ecotoxicity test based on thermolimit respirometry combined with infrared thermography (TLR-IR). The total capacity of excess heat regulation (iTR) and the caloric metabolic rate (MR) gave the most sensitive responses to IVM ingestion. The inhibition concentration at 50% (IC_{50}), a relevant toxicity threshold used to calculate the concentration of IVM that provokes a response half way among the inhibition responses obtained showed that iTR was impacted at 0.39 µg g^{-1}, while the MR was compromised at 0.24 µg g^{-1}. Applying a TLR-IR procedure has not only revealed how the MR of active thermoregulators is crucial for their adaptation in warm and competitive environments, but also shown the potential of this method to be applied in real-world scenarios like cattle-farming regions, where the need to assess the direct impact of endectocides like IVM is vital to update and enhance regulation guidelines of these pharmaceuticals.

Keywords Antiparasitic, Ecotoxicology, Ecophysiology, Endothermy, Respirometry, Scarabaeidae

Ivermectin (IVM) is the most widely used antiparasitic drug in cattle in some regions of the world because it is both relatively inexpensive and highly effective against endo- and ectoparasites[1]. Due to their actions on glutamate-gated chloride ion channels (GluCl), the glycine receptor (GlyR) and the γ-aminobutyric acid receptor (GABA), IVM interferes with the transmission among nervous and muscular cells, which are present in nematodes and arthropods[2,3]. Invertebrates (target and non-target organisms) display ataxia and death as a result of inhibition of inter-neural and neuromuscular transmission. Among the non-target organisms affected by these substances, dung beetles are particularly sensitive[4,5]. The evidence has overwhelmingly pointed to the silent, but devastating effects IVM has on dung beetles[4,6]. As early as 1993 there were already descriptions on how the ingestion of cattle dung with traces of the drug was causing a gradual loss of mobility and weight loss, culminating in the death of coprophagous insects[7]. Since then, a host of other studies have discovered the effects of IVM on dung beetles across their life cycle including female reproduction[8], fecundity rates[9,10], adult emergence rates[11–14], and even at the physiological level in their sensory and locomotor capacity[5,15] as well as a biomagnification of the drug in various tissues of these insects[16].

The thermoregulatory capacity, and its associated metabolic requirement, in dung beetles is another physically demanding trait that has not received as much focus in the literature with regard to the possible effects of IVM ingestion[17,18]. Recognized now as an important trait that can determine a species' thermal niche[19], and thus its competitive advantage for resources[20–24], the thermoregulatory capacity in dung beetles is thought to be modulated by, among other traits, their endothermy[25–27], body mass[28], exoskeletal structure[29–31], endoskeletal and tracheal system anatomy[23], time of activity[25,32] elevational distribution[33,34], and even seasonality[35]. Of particular importance in winged dung beetles, thermoregulation has been specifically analyzed through the aerobic respiration of the individuals (O_2 uptake and CO_2 production) during flight[19,24,36] where the rate of heat transfer from the thorax to the abdomen during this activity greatly determines its thermoregulatory capacity[23,37].

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Based on what is known so far on the mode of action of IVM on the neuromuscular system in dung beetles[5], it is very likely that ingesting the drug will also affect their ability to reduce excess body heat through the respiratory spiracles along with the help, sometimes, of abdominal contractions that accelerate the decrease in body temperature[23]. Related to respiration, the metabolic rate of dung beetles could also be altered in situations of thermal stress due to excess heat.

In order to demonstrate this potential impact of IVM, an ecotoxicity test is first proposed, where individual dung beetles are fed with a controlled amount of dung spiked with different concentrations of IVM, followed by a thermolimit-respirometry (TLR-IR) method to measure both the metabolic rate and the excess heat regulation capacity, taking the total amount of drug ingested as the main determining factor for the observed results. To analyze these possible effects of IVM, an ecotoxicological test was performed using a thermolimit respirometry technique with infrared thermography (TLR-IR). The objective of the present study was to determine, through dose vs. response curves, the concentrations of IVM that significantly affected the thermoregulation capacity and metabolic rate of dung beetles, using *Ateuchetus cicatricosus* (Lucas, 1846) as a model species.

Results

Of the 68 individuals that underwent the ecotoxicity test, 52 completed the tests and were used in the TLR-IR phase of the experiment. Of the 20 individuals assigned to the T1000 treatment, the highest concentration of IVM, seven did not survive long enough to pass on to the thermolimit-respirometry trials. One individual from the T1 treatment was discarded given its rapid deterioration in health observed at an early stage of the ecotoxicity test. No bias was observed due to possible starvation caused by the ingestion of feces with high IVM concentrations, so the average amount of IVM ingested by the individuals increased linearly as the IVM concentration of the selected treatments increased ($F = 73.7$, Adj. $R^2 = 0.84$, $p < 0.001$, d.f. = 50; Table 1; Fig. 1).

Symptoms of intoxication appeared at different times depending on the treatment used, with earlier symptoms being observed at higher concentrations of IVM used in the treatments (Table 1). The T1000 group, which had the highest concentration of IVM in its manure treatment, was the first group to show slow and uncoordinated movement when moving, and an inability to extend the antennal lamellae. These symptoms of intoxication for this group were observed in all individuals, varying in time between 3 and 12 days (8.50 ± 3.43 days, on average; $n = 12$), of treatment with IVM (Table 1). The T100 group started to present the same symptoms between 9 and 21 days (16.13 ± 4.22, on average; $n = 11$) and as in the previous case, all treated individuals suffered symptoms of intoxication. The T10 group presented these symptoms at 24 days, although they were only observed in two individuals of the total (24.00 ± 0.00, on average; $n = 10$). Finally, in the T1 and Control groups, no individual ($n = 10$ and $n = 9$, respectively) showed symptoms of intoxication during the development of the bioassays.

Thermoregulation capability (iTR) and metabolic rate (MR) of *A. cicatricosus* decreased as a function of the concentration of IVM contained in the feces and ingested by the individuals (iTR: $F = 23.69$, Adj. $R^2 = 0.32$, $p < 0.001$, d.f. = 50; MR: $F = 15.07$, Adj. $R^2 = 0.23$, $p < 0.001$, d.f. = 50; Table 1). For iTR, an IC_{50} value of 0.39 µg g^{-1} (95% CI: 0.11 to 1.90 µg g^{-1}) was found, while for the MR we obtained an IC_{50} value of 0.24 µg g^{-1} (95% CI: 0.02 to 1.54 µg g^{-1}), signaling the minimum amount of drug that causes a 50% inhibition response. An increase in IVM ingestion accompanied a strong reduction in the ability of *A. cicatricosus* to release the excess heat generated in the thorax as well as diminish its metabolic rate (Fig. 2).

Discussion

Thermolimit-respirometry coupled with infrared thermography has allowed the visualization of differences in the state of health of individuals with varying degrees of intoxication with IVM. This procedure incorporates the observation of the behavior of the test subject, the body temperature measurement with infrared thermography, and the exhalation of CO_2 per breath ($\dot{V}CO_2$) of the individual, all taking place and being recorded simultaneously with the least possible amount of interference, unnecessary manipulation, or damage to the individuals[24,25,38]. The data logging of the $\dot{V}CO_2$ across the entire heat stress experiment has been fundamental in further understanding the physiological mechanism of thermoregulation of *A. cicatricosus*[23]. Real-time infrared thermography has allowed us to accurately identify the Heat Regulation Temperature (HRT) and the Maximum Critical Temperature (CT_{max})[25], which were necessary to calculate the two ecophysiological variables selected for the study (iTR and MR).

Although continuous flow respirometry has been previously used to study the effect of cadmium exposure on the metabolic rate of Hymenoptera[39], or the metabolic rate of *Thaumatotibia leucotreta* (Meyrick, 1913) under

Treatments	Dung ingested (g)	IVM intake (µg g^{-1})	Symptoms[a] (days)	iTR[b] (°C min^{-1})	MR[c] (W g^{-1})	HRT[d] (°C)	CT_{max}[e] (°C)
Control	78.47 ± 3.47	0	N.S.	2.96 ± 1.38	1.32 ± 0.58	43.02 ± 2.01	49.34 ± 2.01
T1	69.32 ± 3.90	0.02 ± 0.01	N.S.	3.00 ± 0.78	1.08 ± 0.45	45.08 ± 1.99	49.76 ± 1.49
T10	60.80 ± 5.93	0.20 ± 0.05	24.00 ± 0.00	2.56 ± 0.49	1.20 ± 0.39	44.82 ± 1.40	49.81 ± 1.17
T100	17.65 ± 1.77	0.41 ± 0.24	16.13 ± 4.22	1.46 ± 0.78	0.42 ± 0.16	43.06 ± 1.25	49.69 ± 1.65
T1000	8.44 ± 0.55	1.21 ± 0.70	8.50 ± 3.43	0.63 ± 0.78	0.39 ± 0.22	44.04 ± 3.07	49.80 ± 2.65

Table 1. Values of Ivermectin (IVM) ingested by *Ateuchetus cicatricosus*, appearance of intoxication symptoms and ecophysiological variables measured from TLR-IR assays (mean ± SD) for each treatment. [a]Days until first symptoms began manifesting. [b]Capacity of excess heat regulation. [c]Metabolic rate. [d]Heat Regulation Temperature. [e]Maximum Critical Temperature.

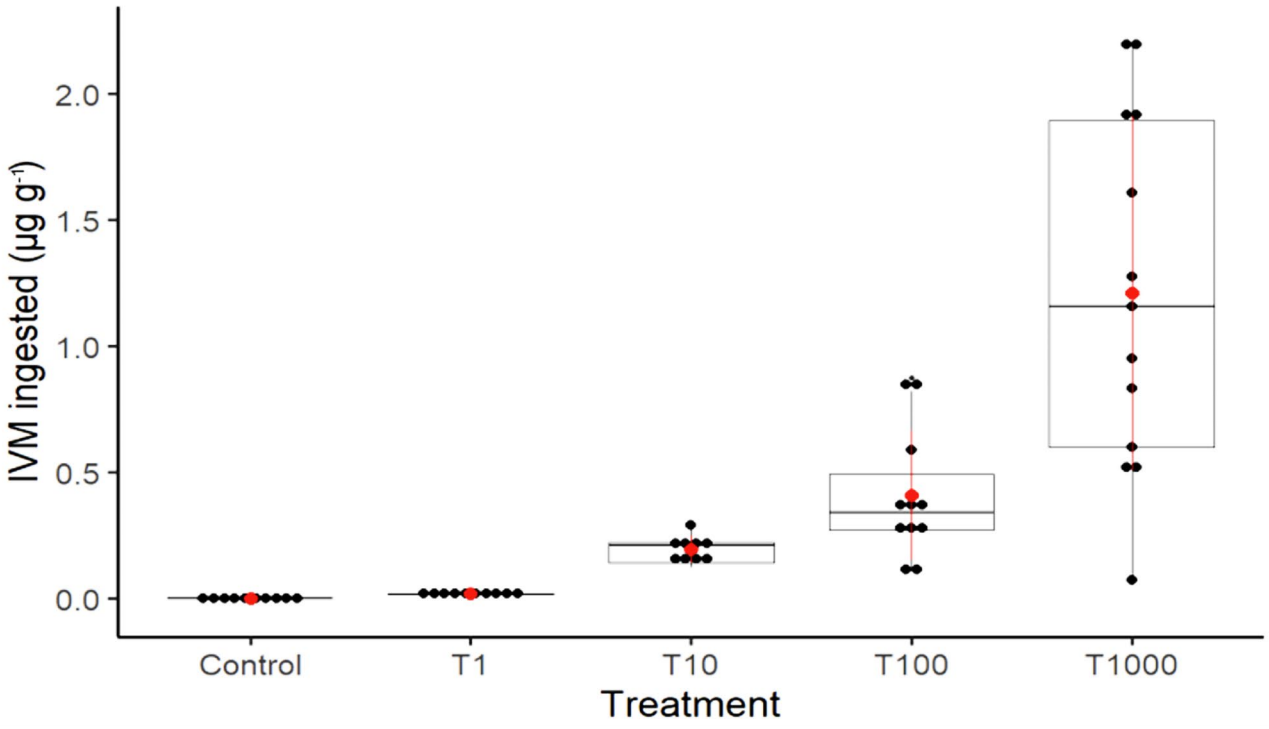

Fig. 1. Boxplots for the ingestion of ivermectin (IVM) across the treatment groups. Four concentrations of IVM (1, 10, 100, and 1000 µg kg^{-1} dung fresh weight) and a control were used for the ecotoxicity test. The red dots denote the average amount of ingested endectocide per treatment, normalized by body weight (Control = 0, without IVM, T1 = 0.019 µg g^{-1}, T10 = 0.196 µg g^{-1}, T100 = 0.409 µg g^{-1}, T1000 = 1.212 µg g^{-1}) while the red bars show the standard deviations. Controls not included because of log transformation.

thermal and water stress situations[40], few studies have applied the combined TLR-IR procedure[38]. A study on the effects of three types of anesthesia (cold, CO_2 anoxia, or N_2 anoxia) on the thermal tolerance and metabolic rate of *Drosophila suzukii* (Matsumura, 1931) observed sex differences in MR and CT_{max} values after exposure to low temperatures[41].

The current approach combining TLR with IR provides several options to investigate in greater detail the ecophysiological mechanisms of insects when subjected to different poisoning scenarios by endectocides, pesticides, and other ecotoxic substances, which could help in the development of updated environmental regulations for veterinary medical products and similar drugs.

The results showed that an increasing ingestion of IVM reduces the ability of *A. cicatricosus* to remove excess body heat under conditions of thermal stress. This phenomenon could be related to the progressive paralysis of the muscular system observed in arthropods[4,5,7,42]. The inability of the studied individuals to effectively contract their abdominal muscles may lead to a malfunction of the active thermoregulation mechanism through abdominal pumping[23], and therefore, the ability to decrease their body temperature under thermal stress (iTR). Likewise, the metabolic rate (MR), during the thermal stress phase, decreased as the intake of IVM increased, which is directly related to the analyzed rate of CO_2 production ($\dot{V}CO_2$). Respiration in this group of insects is regulated by the system of opening and closing of the respiratory spiracles and, in the case of *A. cicatricosus*, also by the abdominal pumping mechanism that acts as a facilitator of the expulsion of CO_2 through the tracheal system. Since the spiracles depend, in turn, on the capacity of the abdominal muscles to function correctly, the inhibition that occurs as a result of the action of IVM could explain the reduction in the MR of the affected individuals. Contrary to the results, Villada-Bedoya et al.[18] observed an increase in the MR in individuals of *Euoniticellus intermedius* (Reiche, 1848) that were fed with IVM-containing dung. This difference in results is probably due to the methodological limitations of this study. First, a single and very low concentration of IVM was used in the study (10 µg kg^{-1}), which limits the potential real impact of the drug on the MR of dung beetles over a logarithmic series of concentrations. In addition, the CO_2 analyzer used does not allow the detection and recording of individual measurements of such small organisms, so the measurements were made using several individuals at a time, which could lead to a lack of precision in the measurements obtained and the interpretation of the results. In the present work, the TLR-IR assay allowed the recording of the entire MR sequence of each individual separately under various degrees of intoxication, allowing the fitting of the best dose-response models and an accurate calculation of IC_{50} values for each physiological mechanism.

The IC_{50} values obtained from the iTR and MR responses to IVM ingestion were extremely low. Given that the concentrations used are sublethal in the short term, with the exception of 1000 µg kg^{-1} that can produce ataxia and death in a few days, it is necessary to extrapolate these results in a real field scenario. Thus, for

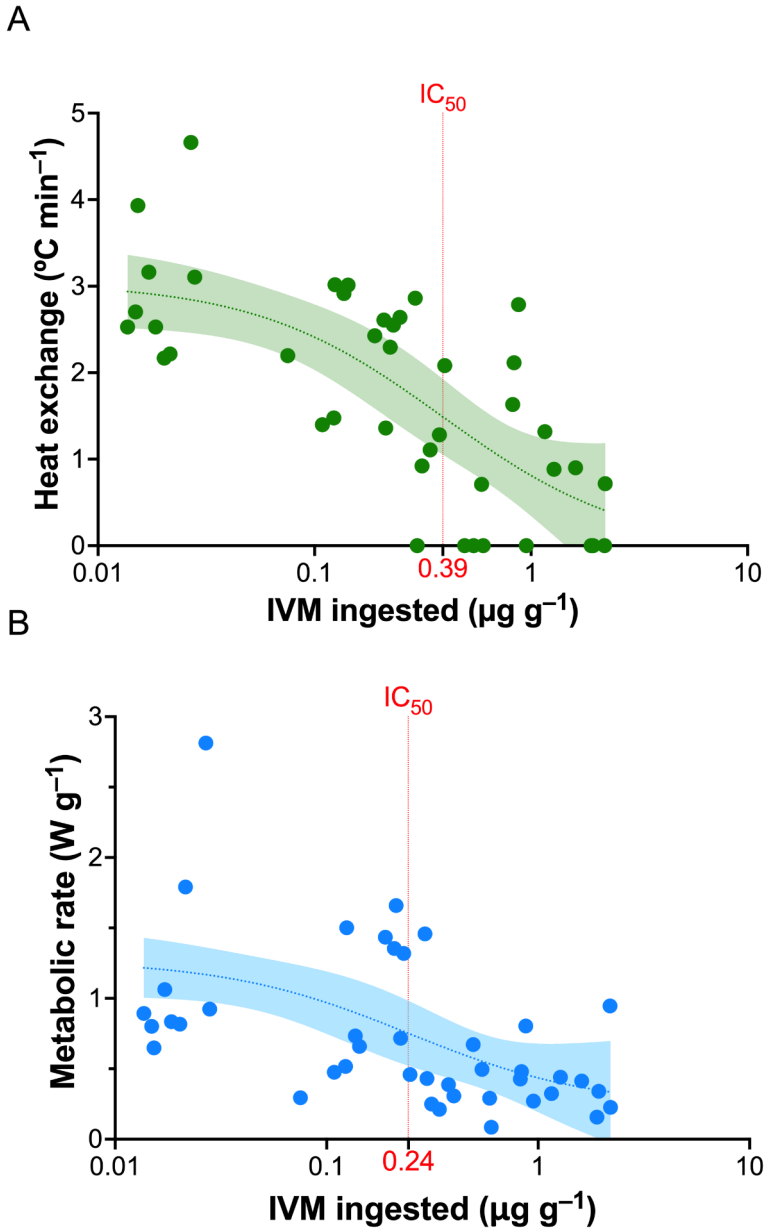

Fig. 2. Ivermectin ingestion response curves for (A) the heat exchange capability (iTR) and (B) the metabolic rate (MR) of *Ateuchetus cicatricosus*. The shaded areas represent the 95% confidence intervals. The IC_{50} values denote the amount of IVM ingested per gram of individual that provokes a response half way among the inhibition responses obtained.

greater understanding, taking any of the two IC_{50} values, a theoretical calculation of the number of days required feeding in cattle dung with different residual concentrations of the drug to reach or even surpass these IC_{50} values could be established. First, we would have to estimate the average daily amount of excrement consumed by a healthy adult of *A. cicatricosus*. Based on the results of the "Control" group, an individual of *A. cicatricosus* presented an average daily intake of 0.76 g of fresh cow excrement. Considering that the concentration of IVM in cow excrement after the first day of treatment to cattle is 500 µg kg^{-1} (Iglesias et al.[43]), it would only take one day of ingestion for an individual to reach the highest IC_{50} value of 0.39 µg g^{-1}, while from the third day of treatment, with an IVM concentration of 530 µg kg^{-1}, it would take less than a day of feeding to exceed the ecotoxicity parameters. Even after a week of elimination of IVM residues[43], the concentration found in cattle excrement by the authors was 270 µg kg^{-1}, an amount that would only require between one and two days of ingestion for the thermoregulation capacity and metabolic rate of *A. cicatricosus* to be affected. What is more serious is that the excrement of cattle treated with IVM presents significant IVM residues up to 60 days after treatment[43], which implies a devastating effect on the health of dung beetle species. Although this estimate is based exclusively on one species, the IC_{50} values were normalized by the weight of the individual, which could represent a value that can be extrapolated to other species. However, the volume of excrement ingestion at the individual level for other

species is unknown; therefore, we cannot generalize to all known species, since we must consider other variables that could vary the IC_{50} obtained. For example, the type of cattle excrement could influence the amount of IVM ingested per gram of dung[6,44–46], the type of digestive tract in different species of dung beetles[47] (Miller 1961) could determine the amount of excrement ingested per day and the digestion time, the types of mouthparts determines the size of the particles they filter or crush (hard diet *versus* soft diet)[48–50], among other traits. Given the diversity of dung beetles, it is impossible to generalize the possible scenarios of exposure, however, the results obtained showed that IVM residues in dung are harmful throughout the entire elimination period, even when concentrations could already be considered relatively low, which accentuates their danger for the survival of coprophagous fauna in extensive livestock systems.

Materials and methods

Collection, selection and Preparation of beetles

Ateuchetus cicatricosus (Lucas, 1846) was selected as the model species for the experiment. This species, in addition to having been used in previous ecotoxicology studies[5,15,51], has had its thermoregulation behavior studied[23]. Adult dung beetles of *A. cicatricosus* were collected from the Doñana Biological Reserve (DBR-ICTS), an ivermectin-free site within Doñana National Park (Huelva), in southern Spain during October of 2022. The individuals were kept in plastic containers (60 × 40 × 40 cm) at 20 °C until they were placed in a climate chamber at the laboratory at 29 ± 1: 21 ± 1 °C (L: D), 80 ± 5% relative humidity with a photoperiod of 14:10 (L: D) to simulate the conditions from the site of collection. For the bioassays, only individuals in good health were used, without amputated body parts or bruises produced in the field or during collection and transport. For the selected individuals, the ecotoxicity test began no later than two weeks after the date of collection from the field to minimize the bias generated by stress as a result of being in an unfamiliar environment. This work conforms to the Spanish legal requirements, including those relating to conservation and welfare. Additionally, beetle collection was conducted with the relevant permissions related to collection and field study within Doñana National Park (PI 2022/03).

Ecotoxicity test

Collection and Preparation of Dung

Cow dung from untreated cattle was collected at the DBR-ICTS in Doñana National Park. The feces (~ 20 kg) were homogenized using an electric paint mixer. If not used immediately, dung was cooled (1 °C) until its usage. Ivermectin concentrations were selected according to the literature[5,15]. Four concentrations in fresh dung, 1.0, 10, 100, and 1000 µg kg^{-1} plus an untreated control were used. These treatments were made by dissolving ivermectin (1% IVM; Ivomec® Merial) in absolute ethanol (Merck KGaA, Darmstadt, Germany). For each treatment (T1, T10, T100, and T1000), a 2 ml aliquot of IVM was added to 200 g portions of fresh dung and subsequently mixed for 30 min with a kitchen machine mixer. For the untreated control, absolute ethanol was added at the same quantity of dung. Residual ethanol was removed by evaporation for one hour before transferring the dung treatments to the individual experimental units, with the rest being stored at 1 °C until their use.

Laboratory bioassay design

Each individual experimental unit consisted of a plastic container (15 × 10 × 7) cm with shredded moist paper towels as substrate containing one dung beetle. No food was given to the selected individuals for three consecutive days, immediately before the start of the trial, to induce their appetite and homogenize their feeding state. Twelve individuals were randomly assigned to the different treatments (Control, T1, T10, and T100) and 20 individuals were assigned to the T1000 group due to the increased possibility of mortality during the first days of the diet. Beetles were sexed, numbered, and weighed (fresh body mass) prior to the first day of the trial. Based on previous protocols[5,15,51], dung was supplied in 3 ml portions on a 6 cm Petri dish, avoiding contact with the substrate to better estimate the amount of IVM ingested per individual. Every three days, the unconsumed dung was removed and measured (in ml) using an assigned calibrated plastic syringe. Following this, the plastic containers were cleaned, new moist, shredded paper towels were placed, and a new 3 ml portion of dung treatment was given. Following the symptom pattern described and used in a previous study[5], (1) the ability of the individual to walk in a coordinated manner, and (2) reflex avoidance movements of the scape-pedicel joint of the antennae were recorded. For every individual upon positive confirmation of these symptoms, which are sequential in time, the date and description of these were logged down. The experimental units were placed in the laboratory at a relative humidity of 80 ± 5% and temperatures of 25 ± 1: 21 ± 1 °C in a light/dark (LD) regime respectively. The selected LD was 14:10 throughout the course of the ecotoxicity test. Upon the first symptoms recorded in the treatments the TLR-IR phase would begin by selecting the affected individuals along with a random selection of a control individual, reducing a possible bias in the commencement time of the tests for the treatments. For each individual, on the day of the TLR-IR procedure its final weight and symptoms was registered.

Thermolimit respirometry and infrared thermography (TLR-IR) test

To determine the effects of IVM ingestion on the thermoregulation and metabolic rate of *A. cicatricosus*, carbon dioxide emissions ($\dot{V}CO_2$) were recorded using the TLR-IR technique following the protocol previously described by Verdú et al.[24,25]. Each individual of *A. cicatricosus* was placed into a flow-through respirometer measurement chamber made of methacrylate (15 × 5 × 5 cm). The selected individual was adjusted to a strip of polyurethane at the roof of the chamber by a metal pin that was adhered to the pronotum of the subject with melted silicon, leaving the specimen unable to make any other point of contact within the chamber. Alongside the individual, 3 cm apart, a dead specimen previously killed by freezing and dehydrated at 80 °C for 48 h, was placed in the same manner to act as a reference to ambient temperature. A clear polypropylene film, 80 µm thick, was used as the cover of the frontal face of the chamber. This film was transparent to infrared radiation but retained the CO_2

within the chamber. The chamber was placed above a precision digital hotplate (J.P. Selecta, Barcelona, Spain), and a temperature increase rate of 0.32 °C min^{-1}, from 25 to 60 °C, was applied inside the chamber. Next, dry CO_2-free air at 150 ml min^{-1} was pumped through the chamber by an inlet hose connected at the side closest to the individual, and evacuated through an outlet hose connected at the opposite extreme. A gas pump Q-P103 (Qubit Systems Inc., Kingston, Ontario, Canada) was used for the described air flow, and the rate was controlled by a gas pressure blow-off valve (Qubit Systems Inc., Kingston, Ontario, Canada) connected to a G-265 gas controller and monitor (Qubit Systems Inc., Kingston, Ontario, Canada). The relative humidity, dew point, and water vapor in the chamber were monitored by an RH-300 system (Sable Systems Int., North Las Vegas, U.S.A.). The carbon dioxide concentration of air produced by the individual was detected and measured with a Li7000 IR gas analyzer (LiCor, Lincoln, NE, U.S.A.). Carbon dioxide data was recorded using a UI2 interface. A BL-2 unit (Sable Systems International, North Las Vegas, USA) was used for the automated baselining of gas measurements. Data conversion into carbon dioxide production ($\dot{V}CO_2$, in ml h^{-1}), and measurements corresponding to metabolic rate calculation were performed using Expedata software (Sable Systems Int., North Las Vegas, U.S.A.).

Synchronized to the TLR assays, body temperature and beetle activity were recorded with a FLIR ThermaCAM P620 thermal infrared camera placed 20 cm from the chamber to record the body temperatures and movements of the test subject through IR sequences at 10 frames s^{-1}. Body temperatures for each individual were measured using temperature profiles through the ThermaCAM TM Researcher software (v. 2.9). Cuticle emissivity of *A. cicatricosus* was adjusted to 0.81 according to a previous study[23]. Correction of temperature by partial polypropylene absorption was made using a beetle partially covered with the polypropylene film and by measuring the IR emission at different temperatures (20–60 °C).

Of the various ecophysiological traits that have been described previously in the literature employing the TLR-IR procedure[24,25], the total capacity of excess heat regulation (iTR) and the total emission of CO_2 ($\dot{V}CO_2$), as a measure of the metabolic rate (MR), were selected as the main response variables under analysis in relation to the amount of ingested IVM. Both variables were measured within the range of body temperatures from the Heat Regulation Temperature (HRT) to the maximum Critical Temperature (CT_{max}) (Fig. 3). $\dot{V}CO_2$, measured in ml h^{-1}, was subsequently transformed into metabolic units (W)[24,52,53].

Data analyses

Since the logarithmic increase in IVM concentration of the treatments can sometimes cause a significant reduction in the capacity to ingest feces[5], the relationship between the increase in IVM concentration and intake by each individual was analyzed. In addition, the relationship between the ecophysiological variables selected and the IVM concentration ingested by the individuals of each treatment was analyzed. These analyses were performed using linear regression and slope tests[5,15] with the 'stats' package (v. 4.4.0) and the 'ggplot2' package (v. 3.5.1).

The IVM concentration that inhibited 50% of both the iTR and MR variables (IC_{50}) was calculated from the dose-response curves using dose-response and inhibition models. GraphPad Prism software (v10, San Diego, USA) was used to perform these statistical analyses.

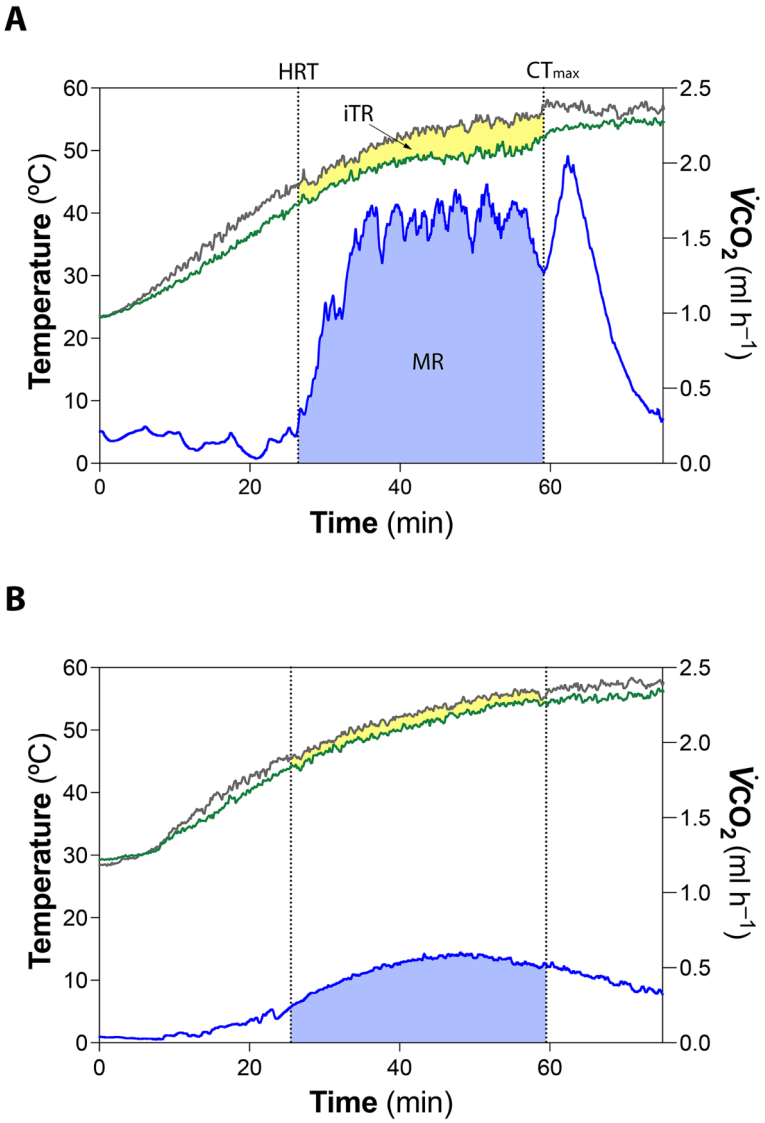

Fig. 3. Representative TLR-IR bioassays showing the ecophysiological characteristics of: (**A**) an individual of *Ateuchetus cicatricosus* fed with dung without IVM during the heat stress response, and (**B**) an individual of *A. cicatricosus* fed with dung with IVM (100 µg kg^{-1}). CO_2 rates are shown in blue, body temperature of the live individual in green and the control (dead individual) in grey. Shaded areas represent the total capacity of excess heat regulation (iTR) (in yellow) and the metabolic rate (MR) (in blue) measured from the Heat Regulation Temperature (HRT) to the maximum Critical Temperature (CT_{max}).

Data availability

The dataset analysed during the current study are available from http://hdl.handle.net/10045/147878.

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References

1. Campbell, W. C. *Ivermectin and Abamectin* (Springer, 1989). https://doi.org/10.1007/978-1-4612-3626-9
2. Lanusse, C. et al. Comparative plasma disposition kinetics of Ivermectin, moxidectin and doramectin in cattle. *J. Vet. Pharmacol. Ther.* **20**, 91–99. https://doi.org/10.1046/j.1365-2885.1997.00825.x (1997).
3. Prichard, R., Ménez, C. & Lespine, A. Moxidectin and the avermectins: consanguinity but not identity. *Int. J. Parasitol. Drugs Drugs Resist.* **2**, 134–153. https://doi.org/10.1016/j.ijpddr.2012.04.001 (2012).
4. Lumaret, J. P., Errouissi, F., Floate, K. D., Römbke, J. & Wardhaugh, K. G. A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. *Curr. Pharm. Biotechnol.* **13**:1004–1060. https://doi.org/10.2174/138920112800399257(2012).
5. Verdú, J. R. et al. Low doses of Ivermectin cause sensory and locomotor disorders in Dung beetles. *Sci. Rep.* **5**, 1–10. https://doi.org/10.1038/srep13912 (2015).

6. Ambrožová, L. et al. Lasting decrease in functionality and richness: effects of Ivermectin use on Dung beetle communities. *Agric. Ecosyst. Environ.* **321**, 107634. https://doi.org/10.1016/j.agee.2021.107634 (2021).
7. Strong, L. & Overview The impact of avermectins on pastureland ecology. *Vet. Parasitol.* **48**, 3–17. https://doi.org/10.1016/0304-4017(93)90140-I (1993).
8. Weaving, H., Sands, B. & Wall, R. Reproductive sublethal effects of macrocyclic lactones and synthetic pyrethroids on the Dung beetle *Onthophagus similis*. *Bull. Entomol. Res.* **110**, 195–200. https://doi.org/10.1017/S0007485319000567 (2019).
9. Martínez-Morales, I., Lumaret, J. P., Ortiz, R. Z. & Kadiri, N. The effects of sublethal and lethal doses of Ivermectin on the reproductive physiology and larval development of the Dung beetle *Euoniticellus intermedius* (Coleoptera: Scarabaeidae). *Can. Entomol.* **149**, 1–12. https://doi.org/10.4039/tce.2017.11 (2017).
10. Pérez-Cogollo, L. C., Rodríguez-Vivas, R. I., Reyes-Novelo, E. & Delfín-González, H. Muñoz-Rodríguez, D. Survival and reproduction of *Onthophagus landolti* (Coleoptera: Scarabaeidae) exposed to Ivermectin residues in cattle Dung. *Bull. Entomol. Res.* **107**, 118–125. https://doi.org/10.1017/S0007485316000705 (2017).
11. Wardhaugh, K. G. & Rodriguez-Menendez, H. The effects of the antiparasitic drug, Ivermectin, on the development and survival of the Dung-breeding fly, *Orthelia cornicina* (F.) and the scarabaeine Dung beetles, *Copris hispanus* L., *Bubas bubalus* (Oliver) and *Onitis belial* F. *J. Appl. Entomol.* **106**, 381–389. https://doi.org/10.1111/j.1439-0418.1988.tb00607.x (1988).
12. Krüger, K. & Scholtz, C. H. Lethal and sublethal effects of Ivermectin on the dung-breeding beetles *Euoniticellus intermedius* (Reiche) and *Onitis alexis* Klug (Coleoptera, Scarabaeidae). *Agric. Ecosyst. Environ.* **61**, 123–131. https://doi.org/10.1016/S0167-8809(96)01108-5 (1997).
13. González-Tokman, D. et al. Ivermectin alters reproductive success, body condition and sexual trait expression in Dung beetles. *Chemosphere* **178**, 129–135. https://doi.org/10.1016/j.chemosphere.2017.03.013 (2017).
14. Rodríguez-Vivas, R. I. et al. Evaluation of the attraction, lethal and sublethal effects of the faeces of ivermectin-treated cattle on the Dung beetle *Digitonthophagus gazella* (Coleoptera: Scarabaeidae). *Aust. Entomol.* **59**, 368–374. https://doi.org/10.1111/aen.12450 (2020).
15. Verdú, J. R. et al. First assessment of the comparative toxicity of Ivermectin and moxidectin in adult Dung beetles: Sub-lethal symptoms and pre-lethal consequences. *Sci. Rep.* **8**, 1–9. https://doi.org/10.1038/s41598-018-33241-0 (2018).
16. Verdú, J. R. et al. Biomagnification and body distribution of Ivermectin in Dung beetles. *Sci. Rep.* **10**, 1–8. https://doi.org/10.1038/s41598-020-66063-0 (2020).
17. González-Tokman, D. et al. Effect of chemical pollution and parasitism on heat tolerance in Dung beetles (Coleoptera: Scarabaeinae). *J. Econ. Entomol.* **114**, 462–467. https://doi.org/10.1093/jee/toaa216 (2020).
18. Villada-Bedoya, S. et al. Heat shock proteins and antioxidants as mechanisms of response to Ivermectin in the Dung beetle *Euoniticellus intermedius*. *Chemosphere* **269**, 128707. https://doi.org/10.1016/j.chemosphere.2020.128707 (2021).
19. Verdú, J. R. & Lobo, J. M. Ecophysiology of thermoregulation in endothermic dung beetles: Ecological and geographical implications. *Research Signpost* 37/661 (2). In: *Insect Ecology and Conservation*, : ISBN: 978-81-308-0297-8 (2008).
20. Heinrich, B. & Bartholomew, G. A. Roles of endothermy and size in inter- and intraspecific competition for elephant Dung in an African Dung beetle, *Scarabaeus laevistriatus*. *Physiol. Zool.* **52**, 484–496. https://doi.org/10.1086/physzool.52.4.30155939 (1979).
21. Scholtz, C. H., Davis, A. L. V. & Kryger, U. *Evolutionary Biology and Conservation of Dung Beetles* 1st edn (Pensoft, 2009).
22. Simmons, L. W. et al. *Ecology and Evolution of Dung Beetles* 1st edn (Blackwell Publishing Ltd., 2011).
23. Verdú, J. R., Alba-Tercedor, J. & Jiménez-Manrique, M. Evidence of different thermoregulatory mechanisms between two sympatric *Scarabaeus* species using infrared thermography and micro-computer tomography. *PLoS ONE*. **7**, e33914. https://doi.org/10.1371/journal.pone.0033914 (2012).
24. Verdú, J. R., Cortez, V., Oliva, D. & Giménez-Gómez, V. C. Thermoregulatory syndromes of two sympatric Dung beetles with low energy costs. *J. Insect Physiol.* **118**, 103945. https://doi.org/10.1016/j.jinsphys.2019.103945 (2019).
25. Verdú, J. R., Oliva, D., Giménez-Gómez, V. C. & Cortez, V. Differential ecophysiological syndromes explain the partition of the thermal niche resource in coexisting eucraniini Dung beetles. *Ecol. Entomol.* **47**, 689–702. https://doi.org/10.1111/een.13153 (2022).
26. Gallego, B., Verdú, J. R., Carrascal, L. M. & Lobo, J. M. Thermal tolerance and recovery behaviour of *Thorectes lusitanicus* (Coleoptera, Geotrupidae). *Ecol. Entomol.* **42**, 758–767. https://doi.org/10.1111/een.12447 (2017).
27. Gallego, B., Verdú, J. R. & Lobo, J. M. Comparative thermoregulation between different species of Dung beetles (Coleoptera: Geotrupinae). *J. Therm. Biol.* **74**, 84–91. https://doi.org/10.1016/j.jtherbio.2018.03.009 (2018).
28. Verdú, J. R., Arellano, L. & Numa, C. Thermoregulation in endothermic Dung beetles (Coleoptera: Scarabaeidae): effect of body size and ecophysiological constraints in flight. *J. Ins Physiol.* **52**, 854–860. https://doi.org/10.1016/j.jinsphys.2006.05.005 (2006).
29. Verdú, J. R. Chill tolerance variability within and among populations in the Dung beetle *Canthon humectus hidalgoensis* along an altitudinal gradient in the Mexican semiarid high plateau. *J. Arid Environ.* **75**, 119–124. https://doi.org/10.1016/j.jaridenv.2010.09.010 (2011).
30. Amore, V., Hernández, M. I. M., Carrascal, L. M. & Lobo, J. M. Exoskeleton May influence the internal body temperatures of Neotropical Dung beetles (Col. Scarabaeinae). *PeerJ* **5**, e3349. https://doi.org/10.7717/peerj.3349 (2017).
31. Carrascal, L. M., Ruiz, Y. J. & Lobo, J. M. Beetle exoskeleton May facilitate body heat acting differentially across the electromagnetic spectrum. *Physiol. Biochem. Zool.* **90**, 338–347. https://doi.org/10.1086/690200 (2017).
32. Gotcha, N., Machekano, H., Cuthbert, R. N. & Nyamukondiwa, C. Heat tolerance May determine activity time in coprophagic beetle species (Coleoptera: Scarabaeidae). *Insect Sci.* **28**, 1076–1086. https://doi.org/10.1111/1744-7917.12844 (2021).
33. Verdú, J. R., Arellano, L., Numa, C. & Micó, E. Roles of endothermy in niche differentiation for ball-rolling Dung beetles (Coleoptera: Scarabaeidae) along an altitudinal gradient. *Ecol. Entomol.* **32**, 544–551. https://doi.org/10.1111/j.1365-2311.2007.00907.x (2007).
34. Herzog, S. K. et al. Elevational distribution and conservation biogeography of phanaeine Dung beetles (Coleoptera: Scarabaeinae) in Bolivia. *PLoS ONE*. **8** https://doi.org/10.1371/journal.pone.0064963 (2013).
35. Agoglitta, R., Moreno, C. E., Zunino, M. E., Bonsignori, G. & Dellacasa, M. Cumulative annual Dung beetle diversity in mediterranean seasonal environments. *Ecol. Res.* **27**, 387–395. https://doi.org/10.1007/s11284-011-0910-8 (2012).
36. Chown, S. L. & Nicolson, S. W. *Insect Physiological Ecology: Mechanisms and Patterns* 1st edn (Oxford University Press, 2004).
37. Giménez-Gómez, V. C., Verdú, J. R. & Zurita, G. A. Thermal niche helps to explain the ability of Dung beetles to exploit disturbed habitats. *Sci. Rep.* **10**, 1–14. https://doi.org/10.1038/s41598-020-70284-8 (2020).
38. Stabentheiner, A., Kovac, H., Hetz, S. K., Käfer, H. & Stabentheiner, G. Assessing honeybee and Wasp thermoregulation and energetics - New insights by combination of flow-through respirometry with infrared thermography. *Thermochim Acta*. **534**, 77–86. https://doi.org/10.1016/j.tca.2012.02.006 (2012).
39. Gao, S., Zheng, F., Yue, L. & Chen, B. Chronic cadmium exposure impairs flight behavior by dampening flight muscle carbon metabolism in bumblebees. *J. Hazard. Mater.* **466**, 133628. https://doi.org/10.1016/j.jhazmat.2024.133628 (2024).
40. Boardman, L., Sørensen, J. G. & Terblanche, J. S. Physiological responses to fluctuating thermal and hydration regimes in the chill susceptible insect, *Thaumatotibia leucotreta*. *J. Insect Physiol.* **59**, 781–794. https://doi.org/10.1016/j.jinsphys.2013.05.005 (2013).
41. Putero, F. A., Mensch, J. & Schilman, P. E. Effect of brief exposures of anesthesia on thermotolerance and metabolic rate of the spotted-wing fly, *Drosophila suzukii*: differences between sexes? *J. Insect Physiol.* **149**, 104549. https://doi.org/10.1016/j.jinsphys.2023.104549 (2023).
42. Bai, S. H. & Ogbourne, S. M. Eco-toxicological effects of the avermectin family with a focus on abamectin and Ivermectin. *Chemosphere* **154**, 204–214. https://doi.org/10.1016/j.chemosphere.2016.03.113 (2016).

43. Iglesias, L. E. et al. Environmental impact of Ivermectin excreted by cattle treated in autumn on Dung fauna and degradation of faeces on pasture. *Parasitol. Res.* **100**, 93–102. https://doi.org/10.1007/s00436-006-0240-x (2006).
44. Marriner, S. E., McKinnon, I. & Bogan, J. A. The pharmacokinetics of Ivermectin after oral and subcutaneous administration to sheep and horses. *J. Vet. Pharmacol. Ther.* **10**, 175–179. https://doi.org/10.1111/j.1365-2885.1987.tb00097.x (1987).
45. Forbes, A. B. A review of regional and Temporal use of avermectins in cattle and horses worldwide. *Vet. Parasitol.* **48**, 19–28. https://doi.org/10.1016/0304-4017(93)90141-9 (1993).
46. Holter, P. & Scholtz, C. H. What do Dung beetles eat? *Ecol. Entomol.* **32**, 690–697. https://doi.org/10.1111/j.1365-2311.2007.00915.x (2007).
47. Miller, A. The mouth parts and digestive tract of adult Dung beetles (Coleoptera: Scarabaeidae), with reference to the ingestion of helminth eggs. *J. Parasitol.* **47**, 735–744 (1961).
48. Cambefort, Y. From saprophagy to coprophagy. In: (eds Hanski, I. & Cambefort, Y.) Dung Beetle Ecology. Princeton University Press, 22–35. (1991).
49. Holter, P. Particle feeding in *Aphodius* Dung beetles (Scarabaeidae): old hypotheses and new experimental evidence. *Funct. Ecol.* **14**, 631–637. https://doi.org/10.1046/j.1365-2435.2000.00464.x (2000).
50. Verdú, J. R. & Galante, E. Behavioural and morphological adaptations for a low-quality resource in semi-arid environments: Dung beetles (Coleoptera, Scarabaeoidea) associated with the European rabbit (*Oryctolagus cuniculus* L). *J. Nat. Hist.* **38**, 705–715. https://doi.org/10.1080/0022293021000041707 (2004).
51. Verdú, J. R. et al. Nontoxic effects of thymol, carvacrol, cinnamaldehyde, and Garlic oil on Dung beetles: A potential alternative to ecotoxic anthelmintics. *PLoS ONE.* **18**, 0295753. https://doi.org/10.1371/journal.pone.0295753 (2023).
52. Lighton, J. R. B. *Measuring metabolic rates: A manual for scientists.* (New York ; online edn, Oxford Academic, 2008).
53. Duncan, F. D. & Byrne, M. J. Discontinuous gas exchange in Dung beetles: patterns and ecological implications. *Oecologia* **122**, 452–458. https://doi.org/10.1007/s004420050966 (2000).

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Author contributions

J.R.V., V.C., and M.A.U. conceived and designed the research; J.R.V. and V.C. collected the biological samples. J.R.V., V.C., and M.A.U. designed and performed the physiological and behavioural tests. J.R.V. and M.A.U. applied the statistical tests. M.A.U., J.R.V. and V.C wrote and revised the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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