

WAGENINGEN UNIVERSITY LABORATORY OF ENTOMOLOGY

# Ammonia, nitrous oxide, methane, and carbon dioxide emissions from edible insect species

No: 09.23 Name: Joost Van Itterbeeck Period: August 2009 Thesis: ENT - 80424 1e Examinator: Prof. Dr. Ir. Arnold van Huis. 2e Examinator: Dr. Ir. Joop van Loon

## SUMMARY

Experiments are conducted with climatised respiration chambers to measure the emissions of ammonia, nitrous oxide, methane, and carbon dioxide from different larval instars of *Locusta migratoria* and *Acheta domesticus*, and last larval instars of *Tenebrio molitor* and *Pachnoda marginata*, and mixed stages of *Blaptica dubia*. Four trials for each species (n = 4, *L. migratoria* n = 6) of approx. three consecutive days were performed at the experimental farm 'de Haar' (Wageningen University). Methane and carbon dioxide emissions were measured continuously in nine minute intervals, ammonia was detected with Kitagawa tubes (20 ppm), and air samples taken with a syringe (approx. 60 ml) were analysed for nitrous oxide at the Environmental Laboratory of Wageningen University.

All five insect species produce carbon dioxide, ranging from 0.019  $\pm$  0.002 kg CO<sub>2</sub> kg<sup>-1</sup> of insect live weight day<sup>-1</sup> (*B. dubia*) to 0.088  $\pm$  0.007 kg CO<sub>2</sub> kg<sup>-1</sup> day<sup>-1</sup> (*L. migratoria*). Methane is only produced by *B. dubia* (0.081  $\pm$  0.017 g CH<sub>4</sub> kg<sup>-1</sup> day<sup>-1</sup>) and *P. marginata* larvae (0.190  $\pm$  0.060 g CH<sub>4</sub> kg<sup>-1</sup> day<sup>-1</sup>). Nitrous oxide seems to be produced only by *T. molitor* larvae (0.002  $\pm$  0.0001 g N<sub>2</sub>O kg<sup>-1</sup> day<sup>-1</sup>) and *L. migratoria* instars (0.002  $\pm$  0.001 g N<sub>2</sub>O kg<sup>-1</sup> day<sup>-1</sup>). Ammonia is emitted in small amounts by *L. migratoria* and *A. domesticus* larval instars (0.005  $\pm$  0.001 g NH<sub>3</sub> kg<sup>-1</sup> day<sup>-1</sup>) and *B. dubia* (0.003  $\pm$  0.001 g NH<sub>3</sub> kg<sup>-1</sup> day<sup>-1</sup>). Conversion of the emissions of greenhouse gases to CO<sub>2</sub> equivalents shows that the emissions of methane and nitrous oxide make a minor contribution to total greenhouse gas emissions.

Values from conventional livestock (cattle, pigs, and poultry) have been compiled from the literature, but comparison of these values with those from the insects is debated. Assuming that comparison is valid, only *B. dubia* produces less greenhouse gases than conventional livestock. *Acheta domesticus* and *L. migratoria* are more than double as polluting as pigs. *Tenebrio molitor* produces almost twice as much greenhouse gas as pigs. All insect species produce at least seven times less ammonia than conventional livestock.

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## INTRODUCTION

In *'Livestock's Long Shadow'*, the FAO reported the ecological problems associated with conventional livestock (Steinfeld et al., 2006). The report elaborated on the role of livestock production in land degradation, water depletion and pollution, climate change and air pollution, and on its impact on biodiversity. Overgrazing, deforestation, compaction and erosion are proclaimed primary causes of land degradation, and water is polluted through animal waste, antibiotics, hormones, and fertilizers and pesticides applied to feedcrops. With regard to climate change, the livestock sector emits globally 9 percent of anthropogenic CO<sub>2</sub>, 37 percent of anthropogenic methane (25 times the global warming potential (GWP) of CO<sub>2</sub>; Forster et al., 2007), and 65 percent of anthropogenic nitrous oxide (298 times the GWP of CO<sub>2</sub>; Forster et al., 2007). The main sources of these greenhouse gases are: deforestation for pastures and feedcrops (CO<sub>2</sub>), enteric fermentation (methane), and manure (nitrous oxide). Conventional livestock also emits 64 percent of anthropogenic ammonia, playing an important role in acidification of the natural environment.

While the FAO suggests that "improving the resource use efficiency of livestock production can reduce environmental impacts" (Steinfeld et al., 2006) other scholars look for alternatives to conventional meat. Alternatives are deemed necessary as already 70 percent of all agricultural land is used for livestock production (Steinfeld et al., 2006). Furthermore, as the world's population is estimated to top 9 billion in the year 2050 (United Nations, 2009) with an expected rise in demand for animal products (Myers and Kent, 2002), it will be impossible to keep up with these demands. One such alternative is eating insects (mini-livestock). Throughout Latin America, Africa, and South-east Asia, edible insects are considered a normal class of food, contributing to, especially, rural people's quality of diet (Dufour, 1987; Balinga et al., 2004; Yhoung-Aree and Viwatpanich, 2005). They are nutritious (Bukkens, 2005; Yhoung-Aree and Viwatpanich, 2005), but also take up little space to rear, and require less food intake per produced kilogram of edible biomass compared to conventional livestock (Nakagaki and DeFoliart, 1991). Ramos-Elorduy (1997) discussed the potential of edible insects as a sustainable food source for both developing and developed countries.

This study is part of a research project<sup>1</sup> focussing on ecological and nutritional aspects of mass-rearing edible insects for consumption in developed countries. As conventional livestock production systems make a major contribution to the widely recognized threats of global warming and climatic change, here, we study the emissions of carbon dioxide ( $CO_2$ ), methane ( $CH_4$ ), nitrous oxide ( $N_2O$ ), and ammonia ( $NH_3$ ) for a number of edible insect species, calculate the amount of gas emitted per animal unit (AU = 500 kilogram of animal live weight), and compare these findings with those of conventional livestock reported in literature.

<sup>1</sup> PhD research conducted by Dennis Oonincx

## **MATERIALS & METHODS**

#### Insects

Final larval stage mealworms (*Tenebrio molitor*), rose chafer larvae or rose beetle larvae (*Pachnoda marginata*; L3), and 5<sup>th</sup> - 6<sup>th</sup> (final) house cricket instars (*Acheta domesticus*) were obtained from commercial companies<sup>2</sup>. *Locusta migratoria* instars of stage L3 – L4 were provided by a laboratory culture<sup>3</sup>. Cockroaches (*Blaptica dubia*), a mix of all stages, were acquired from a private person. *P. marginata* and *B. dubia* were fed dry chicken flour, *A. domesticus* and *T. molitor* were fed dry bran. Carrots were offered as a source of water. *Locusta migratoria* were fed a diet of dry wheat bran and daily fresh grass. Feed was supplied by the insect providers, as was the substrate for *P. marginata* (on two occasions this substrate was not suitable and replaced by potting compost).

#### Experimental set-up

The experiments were conducted at the experimental farm 'De Haar' (Wageningen University). Two climate respiration chambers<sup>4</sup> were at our disposal in which the insects were housed, and adjusted for humidity and temperature for each of the species (Table 1). Photoregimes of 12L:12D were applied to all species, except for *T. molitor* which was kept in the dark at all times by covering the respiration chambers with large black plastic.

Four trials (n=4; *L. migratoria* n=6) of approx. three consecutive days (Table 1) were run for each insect species. The respiration chambers were left closed during each trial (unless feed ran out), except for *L. migratoria* due to daily requirement o fresh grass.

Mealworm larvae and rose beetle larvae were divided over two plastic trays, mixed with feed by hand for an even distribution, and carrot was added. The trays were placed on top of each other, leaving some space between them. In the case of *P. marginata*, moist substrate was put in the plastic trays first. Because of possible damage to wiring and sensors inside the respiration chambers by activity of *A. domesticus*, *L. migratoria*, and *B. dubia*, aluminium cages of the Laboratory of Entomology ( $\pm$  45 cm L,  $\pm$  41 cm H,  $\pm$  37.5 cm W) with aluminium netting at four sides and a plastic bottom were used. In the case of *A. domesticus* and *B. dubia*, each cage was filled for approximately  $\frac{3}{2}$  in height with plastic tubes ( $\pm$  20 cm L,  $\pm$  3 cm D; sanded down for grip) to create hiding places and room. Carrots and an aluminium tray with feed were placed on top of the tubes. The tube openings were directed to incoming air flow. At the end of each trial, the cages were hosed down with water and the tubes were brushed off, rinsed with water, and left overnight in a water filled bucket with chlorine. Aluminium netting was placed inside each cage to create room for *L. migratoria*. Grass and an aluminium tray with wheat bran were put on the bottom of the

<sup>2</sup> Kreca (The Netherlands) - P. marginata & A. domesticus / Van der Ven (The Netherlands) - T. molitor

<sup>3</sup> Laboratory of Entomology (Wageningen University, The Netherlands)

<sup>3</sup> For technical details of similar respiration chambers see Verstegen et al., 1987

<sup>4</sup> Used densities of carbon dioxide and methane are respectively 1.96 g/L and 0.71 g/L

cages. Each cage was closed off with a glass plate.

	P. marginata	T. molitor	B. dubia	A. domesticus	L. migratoria
Temperature, ⁰C	28.0 ± 0	25.0 ± 0	28.0 ± 0	28.03 ± 0.04	32.01 ± 0.01
Humidity, %	84.3 ± 3.3	79.8 ± 0.2	$70 \pm 0.0$	69.9 ± 0.1	69.7 ± 0.2
Ventilation, L/min	6.46 ± 2.06	6.82 ± 1.31	5.16 ± 0.05	11.18 ± 1.80	4.98 ± 0.39
Duration, days	2.98 ± 0.04	$3 \pm 0.00$	$2.99 \pm 0.04$	2.96 ± 0.05	2.99 ± 0.02

Table 1: Temperature (°C), relative humidity (%), ventilation (L/min), and duration (days) of the experiments; mean  $\pm$  standard deviation.

#### Gas measurements

Methane and carbon dioxide emissions were measured continuously in nine minute intervals with sensors placed inside the climate respiration chambers and averaged per hour. These hourly means were nevertheless in unit: L (liter) day<sup>-1</sup> (provided by Marcel Heetkamp). Ammonia levels were monitored with ammonia detector tubes (Kitagawa, 20 ppm detection threshold) twice a day (12:00h and 0:00h; but three times on day 1 of trial 1 of *P. marginata* and *B. dubia*) via a small plastic tube running through one wall of the respiration chambers that can be closed with a tap from outside the chambers. Air samples were similarly taken once a day (12:00h) with a syringe (approx. 60 ml), including of incoming air, and analysed by gas chromatography for nitrous oxide at the Environmental Laboratory (Wageningen UR).

#### Calculations and analysis

Many of the hourly means of methane (L day<sup>-1</sup>) showed negative values presumably due to inaccuracy of the sensor (Marcel Heetkamp, personal communication). Lower and upper limits were then determined at respectively minus and plus 0.035 L day<sup>-1</sup>, values within this range were replaced by 0. The hourly means of methane and carbon dioxide emissions (L day<sup>-1</sup>) were then averaged per trial (L day<sup>-1</sup>).

Methane and carbon dioxide emissions (possibly) require correction (= subtraction) for emissions from flour/bran (*T. molitor*, *B. dubia*, *A. domesticus*, *L. migratoria*), grass (*L. migratoria*), and the moist mixes of substrate and flour (*P. marginata*) also present inside the climate respiration chambers. These emissions were measured but not extensively. In the case of *P. marginata*, no correction for carbon dioxide emission could be made and therefore the carbon dioxide emission from the insects could not be determined (explained in the discussion). Corrections for emissions from the different kinds of dry flour/bran, even though expected to be very low and possibly 0, could neither be made due to insufficient testing (explained in the results) and therefore assumed to be 0.

In order to correct for carbon dioxide emission from grass (*L. migratoria*), the following equation was applied per trial:

$$E_{i} = E - \boxed{\begin{array}{c} (F_{b} + F_{e}) \\ 2 \end{array}} x E_{F}$$

where  $E_i$  is the emission from the insects in one trial (L day<sup>-1</sup>), E is the emission as measured during the trial (L day<sup>-1</sup>),  $F_b$  is the amount of grass (kg, dry matter) at the beginning of the trial,  $F_e$  is the amount of grass (kg, dry matter) at the end of the trial, and  $E_F$  is the CO<sub>2</sub> emission of grass (L kg<sup>-1</sup> day<sup>-1</sup>, dry matter).  $E_F$  (L kg<sup>-1</sup> day<sup>-1</sup>) is calculated from a separate experiment in which only grass was placed inside a climate respiration chamber, by dividing the measured emission of grass (L day<sup>-1</sup>) by the dry weight of the grass (kg).

The emissions of methane and carbon dioxide from the insects are divided by LW (live weight = (initial weight + final weight) / 2) of the respective trials resulting in the emissions of carbon dioxide and methane in litre  $CO_2$  and  $CH_4$  per kg of insect per day. These emissions are recalculated to kg  $CO_2$  kg<sup>-1</sup> day<sup>-1</sup> and g  $CH_4$  kg<sup>-1</sup> day<sup>-1</sup> based on the known density of the gases<sup>5</sup> and averaged over all trials of an insect species. The means of each trial (in kg  $CO_2$  kg<sup>-1</sup> day<sup>-1</sup> and g  $CH_4$  kg<sup>-1</sup> day<sup>-1</sup>) are also multiplied by 500 kg since a common used unit in animal science is AU<sup>-1</sup> (AU = animal unit = 500 kg live weight), and averaged over all trials (kg  $CO_2$  AU<sup>-1</sup> day<sup>-1</sup> and g  $CH_4$  AU<sup>-1</sup> day<sup>-1</sup>). This multiplication is made in order to be able to compare the emissions of insects presented in this report with those reported for conventional livestock in the literature. The majority of the consulted literature on conventional livestock report emissions per 500 kg of live weight, not per kg of live weight. The multiplication of the emissions from the insects should then be interpreted as a multiplication of the conducted experiments, not as further growth of the insects into higher developmental stages.

Daily values of nitrous oxide (ppm) were calculated (measurement of the entire respiration chamber minus measurement of incoming air) and measurements of ammonia (ppm) were averaged per day of each trial. Negative numbers that were returned when calculating daily values of nitrous oxide were replaced by 0 (considered to be negligible). Daily averages of ammonia emission lower than 0.1 ppm but higher than 0.0 ppm were replaced by the former value. The daily averages of ammonia and nitrous oxide (in ppm) were recalculated with the following equations to daily emission rates (ER; g day<sup>-1</sup>):

NH<sub>3</sub>: ER = [NH<sub>3</sub>] x VR x (17 / 0.0224) x 60 x 24

 $N_2O$  : ER = [ $N_2O$ ] x VR x (44 / 0.0224) x 60 x 24

where  $[NH_3]$  and  $[N_2O]$  are respectively average daily concentrations of ammonia and nitrous oxide (ppm x 10<sup>-6</sup>), VR is average ventilation rate (m<sup>3</sup> min<sup>-1</sup>), (17 / 0.0224) is density of ammonia (g m<sup>-3</sup>), and (44 / 0.0224) is density of nitrous oxide (g m<sup>-3</sup>). These daily emission rates were averaged to

<sup>5</sup> Density of carbon dioxide is 1.96 g/L, density of methane is 0.71 g/L

ER per trial (g day<sup>-1</sup>) and divided by live weight (LW = (initial weight + final weight) / 2) of the respective trial to result in mean emissions per kg of insect (g kg<sup>-1</sup> day<sup>-1</sup>), which are then averaged over all trials. The ER of each trial (in g kg<sup>-1</sup> day<sup>-1</sup>) is also multiplied by 500 kg (AU) and averaged over all trials (g NH<sub>3</sub> AU<sup>-1</sup> day<sup>-1</sup> and g N<sub>2</sub>O AU<sup>-1</sup> day<sup>-1</sup>).

Analysis of variance (ANOVA) and Tukey's tests were performed to test for differences between animal species.

## RESULTS

#### Animal performance

Table 2 summarizes measured and calculated live weights, daily gain and daily intake of feed (dry matter). Acheta domesticus showed the highest growth rate  $(182.19 \pm 57.88 \text{ g FW day}^{-1})$  calculated over the first three trials. The fourth trial was excluded from calculations due to unexplained and unexpected much lower growth rate. Growth rate of *L. migratoria* was lowest of all insect species ( $32.35 \pm 2.92 \text{ g day}^{-1}$ ). *Pachnoda marginata* showed remarkable differences in growth rates between trials:  $25.34 - 139.33 \text{ g day}^{-1}$  on substrate provided by the commercial company (trials 1 and 2) and  $61.15 - 87.81 \text{ g day}^{-1}$  on potting compost (trials 3 and 4). Due to human error at weighing *B. dubia* final live weight, trial one had to be excluded from further calculations. The number of animals that died during the experiments was minimal, ranging from 0 to less than 5 per trial (data not shown). Chemical analysis of the offered feed has not been conducted yet.

The emissions from *T. molitor*, *A. domesticus*, and *L. migratoria* generally show consistency in trials 1 and 2, and trials 3 and 4 (and trials 5 and 6 of *L. migratoria*). Each set of two trials was conducted at the same time: one batch of animals was divided over the two climate respiration chambers. Animal performance and differences in the developmental stages represented in each batch most likely explain differences in emissions between sets of trials. All trials of *P. marginata* and *B. dubia* were conducted at different times.

#### Emissions from grass, substrate, and flour/bran

The grass offered as food produced carbon dioxide (23.411 L kg<sup>-1</sup> day<sup>-1</sup>), but not methane. Both substrates without the presence of *P. marginata* larvae, made moist and mixed with flour in similar quantities and ratios as in the four trials with larvae, were tested and produced carbon dioxide, but they did not produce methane. Similarly, moist substrates without flour and larvae did produce carbon dioxide, but not methane. Only the flour offered to *P. marginata* and *B. dubia* was tested for carbon dioxide and methane emissions, but moistened. This was done so because the substrate of *P. marginata* was made a little moist. While *B. dubia* were offered dry flour, the measured emissions from moist flour could not account for emissions from the flour offered to the

#### Table 2: Animal performance

		Pachnoda marginata	Tenebrio molitor	Blaptica dubia	Acheta domesticus	Locusta migratoria
Trial 1	Live weight (g) Initial live weight (g) Final live weight (g) Daily gain (g) Daily intake bran (g; DM) Daily intake carrot (g; DM) Daily intake grass (g; DM)	2064.50 2027.00 25.34 58.68 4.74 n.a.	2161.24 2019.67 2302.80 94.38 86.33 35.25 n.a.	n.a. 2087.00 n.a. n.a. n.a. n.a. n.a.	2375.57 2071.00 2680.14 208.61 269.29 35.60 n.a.	261.65 206.69 316.61 37.14 22.58 n.a. 61.68
Trial 2	Live weight (g) Initial live weight (g) Final live weight (g) Daily gain (g) Daily intake bran (g; DM) Daily intake carrot (g; DM) Daily intake grass (g; DM)	2214.14 2007.93 2420.34 139.33 n.a. n.a. n.a. n.a.	2136.06 1984.92 2287.19 100.76 87.37 40.46 n.a.	2230.96 2072.00 2389.91 108.87 n.a. n.a. n.a. n.a.	2336.68 2012.33 2661.02 222.15 271.46 36.49 n.a.	258.02 207.79 308.25 33.94 23.73 n.a. 56.41
Trial 3	BLive weight (g)2187Initial live weight (g)2094Final live weight (g)2280Daily gain (g)61.1Daily intake bran (g; DM)n.aDaily intake carrot (g; DM)n.aDaily intake grass (g; DM)n.a		1929.22 1684.72 2173.71 163.00 98.36 40.34 n.a.	2586.44 2352.61 2820.26 153.83 33.21 41.47 n.a.	1984.38 1810.66 2158.10 115.81 142.71 36.58 n.a.	192.00 145.37 238.62 31.08 16.21 n.a. 68.48
Trial 4	Live weight (g) Initial live weight (g) Final live weight (g) Daily gain (g) Daily intake bran (g; DM) Daily intake carrot (g; DM) Daily intake grass (g; DM)	1892.17 1762.20 2022.13 87.81 n.a. n.a. n.a. n.a.	1815.93 1582.24 2049.61 155.79 91.23 42.75 n.a.	2479.90 2257.62 2702.17 148.18 47.91 37.54 n.a.	1835.72 1781.50 1889.94 36.15 105.40 39.63 n.a.	195.11 145.93 244.28 32.78 19.14 n.a. 64.28
Trial 5 Live weight (g) Initial live weight (g) Final live weight (g) Daily gain (g) Daily intake bran (g; DM) Daily intake carrot (g; DM) Daily intake grass (g; DM)						193.66 149.54 237.78 29.41 16.06 n.a. 59.61
Trial 6	Live weight (g) Initial live weight (g) Final live weight (g) Daily gain (g) Daily intake bran (g; DM) Daily intake carrot (g; DM) Daily intake grass (g; DM)					194.32 149.67 238.97 29.77 19.22 n.a. 57.73
Average	Live weight (g) Initial live weight (g) Final live weight (g) Daily gain (g) Daily intake bran (g; DM) Daily intake carrot (g; DM) Daily intake grass (g; DM)	2089.48 ± 146.77 1972.82 ± 145.20 2206.13 ± 178.93 78.41 ± 48.01 n.a. n.a. n.a. n.a.	2010.61 ± 166.28 1817.89 ± 217.47 2203.33 ± 117.52 128.48 ± 35.91 90.82 ± 5.45 39.70 ± 3.17 n.a.	2432.43 ± 182.43 2227.41 ± 142.72 2637.45 ± 222.36 136.96 ± 24.49 n.a. n.a. n.a. n.a.	2232.21 ± 215.50 1964.66 ± 136.56 2499.75 ± 296.03 182.19 ± 57.88 197.21 ± 85.85 37.07 ± 1.76 n.a.	$215.79 \pm 34.15$ $167.50 \pm 30.84$ $264.09 \pm 37.61$ $32.35 \pm 2.92$ $19.49 \pm 3.17$ $n.a.$ $61.37 \pm 4.47$

Note: not all dry matter determinations were available yet

Note: numbers in grey are excluded from calculations

n.a.: not available

cockroaches. The measured emission of carbon dioxide (no methane is produced) from moist flour is much higher than expected from dry flour. Carbon dioxide emissions from the two kinds of dry bran offered to *T. molitor*, *A. domesticus*, and *L. migratoria* and the flour offered to *B. dubia* were then assumed to be 0, as were emissions of methane. Emissions of ammonia and nitrous oxide were not measured for grass, substrate, nor flour/bran, and assumed to be 0.

#### Carbon dioxide emissions

Carbon dioxide emissions are shown in Table 3. The emissions range from  $0.019 \pm 0.002$  kg CO<sub>2</sub> kg<sup>-1</sup> day<sup>-1</sup> (*B. dubia*) to  $0.088 \pm 0.007$  kg CO<sub>2</sub> kg<sup>-1</sup> day<sup>-1</sup> (*L. migratoria*). Trials 1 and 2, trials 3 and 4, and trials 5 and 6 of *L. migratoria* show similar ranges in carbon dioxide emissions, with respective means  $\pm$  SD of  $0.079 \pm 0.001$ ,  $0.095 \pm 0.001$ , and  $0.088 \pm 0.000$  kg CO<sub>2</sub> kg<sup>-1</sup> day<sup>-1</sup> (data not shown). *Tenebrio molitor* and *A. domesticus* emitted respectively  $0.061 \pm 0.001$  and  $0.071 \pm 0.007$  kg CO<sub>2</sub> kg<sup>-1</sup> day<sup>-1</sup>, with the emissions from *A. domesticus* being consistent similarly to those from *L. migratoria*. Carbon dioxide emissions from *T. molitor* larvae seem to show consistency over all trials. Carbon dioxide emission from *P. marginata* could not be calculated (see discussion).

#### Methane emissions

Neither *T. molitor*, *A. domesticus*, nor *L. migratoria* produce methane. The calculated mean methane emission from *P. marginata* is  $0.190 \pm 0.060$  g CH<sub>4</sub> kg<sup>-1</sup> day<sup>-1</sup> and from *B. dubia* is  $0.081 \pm 0.017$  g CH<sub>4</sub> kg<sup>-1</sup> day<sup>-1</sup>. Our findings are consistent with Hackstein and Stumm (1994) who could only report methane production from a few classes of insects: cockroaches (Blattaria), termites (Isoptera), and scarab beetles (Scarabaeidae). *Pachnoda marginata* larvae in trials 3 and 4 (potting compost) produce higher amounts of methane than in trials 1 and 2. This can not be explained by emission by the substrate as it does not emit methane. The substrate can yet influence animal performance (e.g. movement, encountering feed) which in turn affects methane production.

#### Nitrous oxide emissions

Nitrous oxide is only produced in small amounts. *Tenebrio molitor* and *L. migratoria* produced respectively  $0.002 \pm 0.000$  and  $0.002 \pm 0.001$  g N<sub>2</sub>O kg<sup>-1</sup> day<sup>-1</sup>. *Blaptica dubia* emitted little nitrous oxide in trials 3 and 4 (0.001 g N<sub>2</sub>O kg<sup>-1</sup> day<sup>-1</sup>), but no emission was detected in trial 1. None of the other examined species produced nitrous oxide in any considerable amounts. In fact, *P. marginata* emitted 0.0001 g N<sub>2</sub>O kg<sup>-1</sup> day<sup>-1</sup> in trials 3 and 4, the same explanation as for their methane emission is possibly applicable. Recalculating the emissions to larger amounts of insects (g N<sub>2</sub>O AU<sup>-1</sup> day<sup>-1</sup>) does reveal production by all researched species, yet in small amounts ranging from 0.025 ± 0.018 (*P. marginata*) to 1.167 ± 0.574 (*L. migratoria*) g N<sub>2</sub>O AU<sup>-1</sup> day<sup>-1</sup>.

#### Ammonia emissions

Ammonia is produced in small amounts by *B. dubia*  $(0.003 \pm 0.001 \text{ g NH}_3 \text{ kg}^{-1} \text{ day}^{-1})$ , and *A. domesticus* and *L. migratoria* (both 0.005 ± 0.001 g NH<sub>3</sub> kg<sup>-1</sup> day<sup>-1</sup>). Similar to nitrous oxide, recalculating the emissions to larger amounts of insects (g NH<sub>3</sub> AU<sup>-1</sup> day<sup>-1</sup>) shows production by all researched species ranging from 0.038 ± 0.047 (*T. molitor*) to 2.519 ± 0.345 (*L. migratoria*) g NH<sub>3</sub> AU<sup>-1</sup> day<sup>-1</sup>. Ammonia is only produced by *T. molitor* larvae of the first batch (trials 1 and 2).

Table 3: Gaseous emissions	from fiv	e insect	species
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		Pachnoda marginata	Tenebrio molitor	Blaptica dubia	Acheta domesticus	Locusta migratoria
Trial 1	NH <sub>3</sub> , g kg ' d '	0.000	0.000	n.a.	0.004	0.006
	$NH_3$ , g AU <sup>-1</sup> d <sup>-1</sup>	0.000	0.055	n.a.	2.155	2.765
	N <sub>2</sub> O, g kg ' d '	0.000	0.002	n.a.	0.000	0.002
	N <sub>2</sub> O, g AU <sup>+</sup> d <sup>+</sup>	0.023	0.807	n.a.	0.025	1.125
	CH <sub>4</sub> , g kg ' d '	0.069	0.000	n.a.	0.000	0.000
	CH <sub>4</sub> , g AU <sup>-1</sup> d <sup>-1</sup>	34.256	0.000	n.a.	0.000	0.000
	CO <sub>2</sub> , kg kg <sup>-'</sup> d <sup>-'</sup>	n.a.	0.061	n.a.	0.074	0.078
	CO <sub>2</sub> , kg AU <sup>+</sup> d <sup>+</sup>	n.a.	30.461	n.a.	37.028	39.189
Trial 2	$NH_{\alpha} \alpha k \alpha^{-1} d^{-1}$	0.000	0.000	0.002	0.004	0.006
That 2	$NH_{2}$ a $AU^{-1}$ d <sup>-1</sup>	0.000	0.097	1 139	1 761	2 989
	$N_2Q_1 = kg^{-1} d^{-1}$	0.000	0.001	0.000	0.000	0.004
	$N_2O_1 g A U^{-1} d^{-1}$	0.000	0.659	0.004	0.089	1 912
	$CH_4$ , a ka <sup>-1</sup> d <sup>-1</sup>	0.000	0.000	0.062	0.000	0.000
	$CH_4$ , g $AU^{-1} d^{-1}$	74 594	0.000	31 155	0.000	0.000
	$CO_2$ , kg kg <sup>-1</sup> d <sup>-1</sup>	n a	0.062	0.016	0.075	0.080
	$CO_2$ , kg $AU^{-1} d^{-1}$	n.a.	30,884	8,200	37,736	40.219
	002, i.g / io u	ind.	00.001	0.200	01.100	10.210
Trial 3	NH <sub>3</sub> , g kg <sup>-1</sup> d <sup>-1</sup>	0.000	0.000	0.003	0.006	0.005
	NH <sub>3</sub> , g AU <sup>-1</sup> d <sup>-1</sup>	0.134	0.000	1.548	2.935	2.529
	N <sub>2</sub> O, g kg <sup>-1</sup> d <sup>-1</sup>	0.000	0.002	0.001	0.000	0.001
	N <sub>2</sub> O, g AU <sup>-1</sup> d <sup>-1</sup>	0.038	0.820	0.250	0.138	0.373
	CH <sub>4</sub> , g kg <sup>-1</sup> d <sup>-1</sup>	0.162	0.000	0.096	0.000	0.000
	CH <sub>4</sub> , g AU <sup>-1</sup> d <sup>-1</sup>	80.987	0.000	47.977	0.000	0.000
	CO <sub>2</sub> , kg kg <sup>-1</sup> d <sup>-1</sup>	n.a.	0.060	0.020	0.063	0.094
	CO <sub>2</sub> , kg AU <sup>-1</sup> d <sup>-1</sup>	n.a.	29.900	9.789	31.326	47.112
	-1 -1					
Trial 4	NH <sub>3</sub> , g kg ' d '	0.000	0.000	0.002	0.006	0.005
	$NH_3$ , g AU <sup>+</sup> d <sup>+</sup>	0.213	0.000	1.012	2.907	2.570
	$N_2O, g kg^2 d^2$	0.000	0.002	0.001	0.000	0.002
	$N_2O, g AU^{-1}d^{-1}$	0.039	0.757	0.264	0.15	0.820
	$CH_4$ , g kg d	0.259	0.000	0.083	0.000	0.000
	$CH_4$ , g AU d	129.573	0.000	41.739	0.000	0.000
	$CO_2$ , kg kg d	n.a.	0.062	0.020	0.061	0.096
		n.a.	30.844	10.204	30.562	47.815
Trial 5	NH₃, a ka⁻¹ d⁻¹					0.004
i nar o	NH₃, g AU <sup>-1</sup> d <sup>-1</sup>					2.204
	$N_2O_1 a ka^{-1} d^{-1}$					0.002
	N <sub>2</sub> O, a AU <sup>-1</sup> d <sup>-1</sup>					1.037
	CH₄, g kg <sup>-1</sup> d <sup>-1</sup>					0.000
	CH <sub>4</sub> , g AU <sup>-1</sup> d <sup>-1</sup>					0.000
	$CO_2$ , kg kg <sup>-1</sup> d <sup>-1</sup>					0.088
	$CO_2$ , kg $AU^{-1} d^{-1}$					44.201
Trial 6	NH <sub>3</sub> , g kg <sup>-1</sup> d <sup>-1</sup>					0.004
	NH <sub>3</sub> , g AU <sup>-1</sup> d <sup>-1</sup>					2.057
	N <sub>2</sub> O, g kg <sup>-1</sup> d <sup>-1</sup>					0.004
	N <sub>2</sub> O, g AU <sup>-1</sup> d <sup>-1</sup>					1.734
	CH <sub>4</sub> , g kg <sup>-1</sup> d <sup>-1</sup>					0.000
	$CH_4$ , g $AU^{-1}$ d <sup>-1</sup>					0.000
	CO <sub>2</sub> , kg kg <sup>-1</sup> d <sup>-1</sup>					0.088
	CO <sub>2</sub> , kg AU <sup>-1</sup> d <sup>-1</sup>					44.069
	l					

#### Table 3 (cont.)

Average	NH₃, g kg⁻¹ d⁻¹	0 <sup>a</sup>	0 <sup>a</sup>	0.003 ± 0.001 <sup>b</sup>	0.005 ± 0.001 <sup>c</sup>	0.005 ± 0.001 <sup>c</sup>
-	NH <sub>3</sub> , g AU <sup>-1</sup> d <sup>-1</sup>	$0.087 \pm 0.105^{a}$	$0.038 \pm 0.047^{a}$	1.233 ± 0.280 <sup>b</sup>	$2.284 \pm 0.598^{\circ}$	$2.519 \pm 0.345^{\circ}$
	N <sub>2</sub> O, g kg <sup>-1</sup> d <sup>-1</sup>	0 <sup>×</sup>	$0.002 \pm 0.000^{\text{y}}$	0 <sup>x,y</sup>	0 <sup>×</sup>	$0.002 \pm 0.001^{y}$
	N <sub>2</sub> O, g AU <sup>-1</sup> d <sup>-1</sup>	$0.025 \pm 0.018^{\times}$	$0.761 \pm 0.073^{y,z}$	0.173 ± 0.146 <sup>x,y</sup>	$0.084 \pm 0.057^{\times}$	1.167 ± 0.574 <sup>z</sup>
	CH <sub>4</sub> , g kg <sup>-1</sup> d <sup>-1</sup>	$0.190 \pm 0.060^{\circ}$	O <sup>y</sup>	$0.081 \pm 0.017^{z}$	O <sup>y</sup>	O <sup>y</sup>
	CH <sub>4</sub> , g AU <sup>-1</sup> d <sup>-1</sup>	$79.853 \pm 33.073^{\times}$	Оу	$40.290 \pm 8.504^{z}$	Oy	Оу
	CO <sub>2</sub> , kg kg <sup>-1</sup> d <sup>-1</sup>	n.a.	$0.061 \pm 0.001^{a}$	$0.019 \pm 0.002^{b}$	0.071 ± 0.007 <sup>a</sup>	$0.088 \pm 0.007^{\circ}$
	CO <sub>2</sub> , kg AU <sup>-1</sup> d <sup>-1</sup>	n.a.	$30.522 \pm 0.457^{a}$	9.397 ± 1.058 <sup>b</sup>	35.364 ± 3.514 <sup>a</sup>	43.768 ± 3.504 <sup>°</sup>

Note: numbers in grey are excluded from calculations

n.a.: not available

 $^{\rm a,b,c}\!\!:$  values in one row with different notations significantly differ at the 0.05 level

<sup>x,y,z</sup>: values in one row with different notations significantly differ at the 0.10 level

#### Greenhouse gas emissions in CO<sub>2</sub> equivalents

The emissions of methane and nitrous oxide from each insects species are converted to  $CO_2$  equivalents and added to the  $CO_2$  emissions, resulting in total greenhouse gas emissions (Table 4). The emissions of methane and nitrous oxide are respectively multiplied by 25 and 298, which stand for their global warming potential (time period assumed: 100 years) (Forster et al., 2007).

	Pachnoda marginata	Tenebrio molitor	Blaptica dubia	Acheta domesticus	Locusta migratoria
1 1					
N <sub>2</sub> O, g kg <sup>-1</sup> d <sup>-1</sup>	0.000	0.596	0.000	0.000	0.596
N <sub>2</sub> O, g AU <sup>-1</sup> d <sup>-1</sup>	7.450	226.778	51.554	25.032	347.766
CH₄, g kg⁻¹ d⁻¹	4.750	0.000	2.025	0.000	0.000
CH <sub>4</sub> , g AU <sup>-1</sup> d <sup>-1</sup>	1996.325	0.000	1007.250	0.000	0.000
CO₂, kg kg⁻¹ d⁻¹	n.a.	0.061	0.019	0.071	0.088
CO <sub>2</sub> , kg AU <sup>-1</sup> d <sup>-1</sup>	n.a.	30.522	9.397	35.364	43.768
Total, kg kg <sup>-1</sup> d <sup>-1</sup>	n.a.	0.062	0.021	0.071	0.089
Total, kg AU <sup>-1</sup> d <sup>-1</sup>	n.a.	30.749	10.456	35.389	44.116

Table 4: Greenhouse gas emissions from five insect species in CO<sub>2</sub> equivalents

Greenhouse gas emissions range from 0.021 (*B. dubia*) to 0.089 (*L. migratoria*) kg CO<sub>2</sub> equivalents kg<sup>-1</sup> d<sup>-1</sup>. Total greenhouse gas emission from *P. marginata* could not be calculated. The emissions range from 10.456 to 44.116 kg CO<sub>2</sub> equivalents AU<sup>-1</sup> d<sup>-1</sup> when multiplied by 500 kg to account for larger amounts of insects. *Blaptica dubia* is then least polluting, followed by *T. molitor* and *A. domesticus*. In CO<sub>2</sub> equivalents, the emissions from *L. migratoria*, *T. molitor*, and *B. dubia* hardly differ from their respective carbon dioxide emissions. The contribution of both nitrous oxide and methane emissions to total greenhouse gas emissions is then minimal.

		Cattle	Pig		Poultry	
		Source		Source		Source
NH₃, g AU⁻¹ day⁻¹	15.25 12.5	Casey et al., 2006	310 128	Casey et al., 2006	216 307.2	Casey et al., 2006
	19.4	Casev et al. 2006	72	Casey et al. 2006	45	Casev et al. 2006
	43	Casev et al. 2006	58.9	Casev et al. 2006	24.6	Casev et al. 2006
	12.9	Demmers et al., 2001	18.1	Casey et al., 2006	7.1	Casey et al., 2006
N₂O, g AU⁻¹ day⁻¹	0.9 1.6	Chadwick et al., 1999 Jungbluth et al., 2001	0.4 11.11 42.9 2.64 1.6	Chadwick et al., 1999 Nicks et al., 2003 Nicks et al., 2003 Blanes-Vidal et al., 2008 Osada et al., 1998	26 0 0 10	Chadwick et al., 1999 Guiziou and Béline, 2005 Fabbri et al., 2007 Chadwick et al., 1999
CH₄, g AU <sup>-1</sup> day <sup>-1</sup>	234.25 524.64 71.25 150.15 223	Lassey, 2007 Snell et al., 2003 Beauchemin and McGinn, 2005 Beauchemin and McGinn, 2005 Jungbluth et al., 2001	66.72 48.77 23.77 54	Blanes-Vidal et al., 2008 Nicks et al., 2003 Nicks et al., 2003 Osada et al., 1998	24.96 51.6 0	Fabbri et al., 2007 Fabbri et al., 2007 Guiziou and Béline, 2005
CO <sub>2</sub> , kg AU <sup>-1</sup> day <sup>-1</sup>	n.a. n.a. n.a. n.a. n.a.		14.2 14.81 10.809 14.573 10.222	Nicks et al., 2003 Nicks et al., 2003 Cabaraux et al., 2009 Cabaraux et al., 2009 Cabaraux et al., 2009	n.a. n.a. n.a. n.a. n.a.	

Table 5: Gaseous emissions from conventional livestock reported in literature

n.a.: no literature available

Data Nicks et al. (2003) was per pig (16.2 kg) and recalculated assuming a linear relationship

Data Blanes-Vidal et al. (2008), Snell et al. (2003) and Fabbri et al. (2007) was hourly and recalculated assuming a linear relationship (multiply by 24) Data Lassey (2007) is in fact per head of 712 kg

Data Demmers et al. (2001) and Osada et al. (1998) was yearly and recalculated (divided by 365, and corrected for the unit)

Data Beauchemin et al. (2005) was in fact per animal with average weight of 433 kg

#### Gaseous emissions from conventional livestock

Table 5 shows some values of emissions of ammonia, nitrous oxide, methane, and carbon dioxide reported in literature for cattle, pigs, and poultry. No data on carbon dioxide emissions was available for cattle nor poultry. The carbon dioxide emission is often not taken into account when studying cattle as they are considered to be CO<sub>2</sub>-neutral (S. van Zijderveld, personal communication). This may apply to at least *L. migratoria* as well since their primary food is fresh grass. Admitting that it is dubious, values reported in literature were averaged per gas and per livestock category (cattle, pigs, and poultry) and compared to average emissions calculated for the insect species (from Table 3). Values for conventional livestock show unexplained strong variations (large standard deviations). This variation could not be understood from the applied research methodologies. Yet, these values (and the means acquired from these values) are used in this report.

It then seems that *Blaptica dubia* is the only insect species to emit little less carbon dioxide than pigs. The emissions from *T. molitor*, *A. domesticus*, and *L. migratoria* are at least double that of pigs. The amounts of methane produced by *P. marginata* and *B. dubia* are less than those from cattle. The emission from *B. dubia* seems to be within the range of the emission from pigs, but is higher than from poultry. Especially *P. marginata* larvae and *A. domesticus* instars produce little nitrous oxide in comparison to conventional livestock. *Locusta migratoria*, as the insect species with highest production of nitrous oxide in these experiments, emits a comparable amount of nitrous oxide as cattle, but much less than pigs and poultry. Similarly, *L. migratoria*, emitting the highest amount of ammonia of the five examined insect species, produces much less ammonia than conventional livestock. Ammonia production is at least seven times lower by insects compared to conventional livestock.

Assuming that the values from the consulted literature are fit for comparison, nitrous oxide and methane emissions from conventional livestock were also converted to  $CO_2$  equivalents (Table 6) and compared to those from the insect species (Table 4).

	Cattle	Pigs	Poultry
N <sub>2</sub> O, g AU <sup>-1</sup> d <sup>-1</sup>	372.500	3495.540	2682.000
CH <sub>4</sub> , g AU <sup>-1</sup> d <sup>-1</sup>	6016.450	1207.875	638.000
CO <sub>2</sub> , kg AU <sup>-1</sup> d <sup>-1</sup>	n.a.	12.923	n.a.
Total, kg AU <sup>-1</sup> d <sup>-1</sup>	6.389	17.626	3.320

Table 6: Greenhouse gas emissions from conventional livestock in CO<sub>2</sub> equivalents

Note: values in grey do not take into account carbon dioxide emission

With no values available for carbon dioxide emissions from cattle nor poultry, total greenhouse gas emissions are incomplete and incorrect. Unless, if it is correct to assume that cattle in fact is  $CO_2$ -neutral, their total greenhouse gas emission is 6.389 kg  $CO_2$  equivalents  $AU^{-1}$  d<sup>-1</sup>. The total greenhouse gas emission from pigs is 17.626 kg  $CO_2$  equivalents  $AU^{-1}$  d<sup>-1</sup> including carbon dioxide emission. Poultry is excluded as from here as it is uncertain whether or not poultry can be considered  $CO_2$ -neutral as well. More effort has to be made to find either reported carbon dioxide emissions from poultry (broilers) or strong arguments to regard this conventional livestock category as  $CO_2$ -neutral.

A comparison of the emissions from insects (Table 4) and the emissions from conventional livestock (Table 6) shows that the insects, except for *B. dubia* (and *P. marginata*), produce higher amounts of greenhouse gas and are therefore more polluting than conventional livestock. *Acheta domesticus* and *L. migratoria* are more than double as polluting as pigs. *Tenebrio molitor* produces almost twice as much greenhouse gas than pigs.

This outcome can be debated with regard to carbon dioxide emissions ( $CO_2$ -neutrality) and by the validity of using the values for conventional livestock in Table 5 in the comparison made in this report. A more thorough examination of the literature on emissions from conventional livestock is a necessity.

### DISCUSSION

The calculations presented in this report are not complete. The values given are insufficiently corrected for the emissions from other components (e.g. feed, substrate) inside a climate respiration chamber besides the insects. Furthermore, no correction has been applied for emission from faeces. Insects do not digest their feed completely and active plant cells can be found in their faeces (J.J.A. van Loon, personal communication).

Carbon dioxide emission from P. marginata could not be determined due to insufficient measuring of carbon dioxide emission from the moist mixes of substrate and flour. Figure 1 shows the carbon dioxide production of the four trials. In trials 2 to 4, carbon dioxide production rises rapidly but declines towards the end of the third (and final) day of a trial. Without animals, carbon dioxide production of the moist mixes of substrate and flour rises until approx. halfway the second day (Figure 2). All the mixes are in similar quantities and ratios of substrate, flour, and water. Two of the four trials without larvae were only conducted for two days, the other two unfortunately only for one day. For the latter two, it is then unsure how the carbon dioxide production would continue on the second day. At the end of these trials though, fungi had multiplied because of: the presence of sufficient feed that otherwise would be consumed by P. marginata, and the absence of movement of the larvae through the substrate that could hamper fungi to attach to grains of feed. Fungi most likely multiplied until all feed had been consumed after which carbon dioxide production declines. This may well be the case from the middle of the second day onwards (two-day trials; figure 2). This idea could supported by the fact that in trial 1 of figure 1, P. marginata was offered less feed (200 g) than during the other three trials (694.30  $\pm$  20 g). The steep rise in carbon dioxide production at the beginning of day one in trials 2 to 4 may then (partly) be explained by growth of fungi. With less feed available in trial 1, fungal growth is hampered. The four trials without P. marginata larvae are not representative for carbon dioxide production of the mixes of substrate and flour and can not be used as such to calculate the emission of *P. marginata* larvae. It needs to be understood how the P. marginata larvae affect fungal activity and how this affects carbon dioxide emission.



Figure 1: Carbon dioxide production of P. marginata larvae in moist mixes of substrate and flour



Figure 2: Carbon dioxide production of moist mixes of substrate and flour

*L. migratoria* was fed fresh grass on a daily basis. The climate respiration chambers were opened but this did not strongly affect the continuous measurement of carbon dioxide nor methane since the chambers were opened only briefly. Leftovers of grass at the end of a day were left inside the climate respiration chamber and fresh grass was added. In order to correct the measured emission of carbon dioxide from the entire climate respiration chamber for the carbon dioxide emission of grass (grass does not produce methane), an attempt was made to apply the correction factor for food respiration developed by Axelsson and Agren (1979). But to use this correction factor, which is more accurate than the calculations made here, daily leftovers should have been taken out of the climate respiration chambers and their weight determined.

The flour/bran offered to *T. molitor*, *B. dubia*, *A. domesticus*, and *L. migratoria*, although not expected to emit significant amounts of carbon dioxide, need to be studied for emissions of carbon dioxide and methane.

The values given per 500 kg of live weight (AU<sup>-1</sup>) should be interpreted as a multiplication of the conducted experiments with the same developmental stages, not as further growth of the animals into higher stages. Hardly any literature could be consulted that report emissions from conventional livestock per kg of live weight or per kg of live weight gain.

The comparison made between emissions from insects (this report) and conventional livestock (literature) is very debatable: (1) the values used in this report, compiled from different studies, were averaged to acquire a single value for each category of conventional livestock and per gas, and (2) carbon dioxide emissions from cattle and poultry are lacking.

## CONCLUSION

We determined the emissions of carbon dioxide, methane, ammonia, and nitrous oxide from some insect species. *Locusta migratoria* instars emitted most carbon dioxide per kg animal live weight (0.088 ± 0.007 kg CO<sub>2</sub> kg<sup>-1</sup> day<sup>-1</sup>), followed by *Acheta domesticus* instars (0.071 ± 0.007 kg CO<sub>2</sub> kg<sup>-1</sup> day<sup>-1</sup>), *Tenebrio molitor* larvae (0.061 ± 0.001 kg CO<sub>2</sub> kg<sup>-1</sup> day<sup>-1</sup>), and *Blaptica dubia* (0.019 ± 0.002 kg CO<sub>2</sub> kg<sup>-1</sup> day<sup>-1</sup>). Carbon dioxide emission from *Pachnoda marginata* larvae could not be determined. Methane is produced by *B. dubia* (0.081 ± 0.017 g CH<sub>4</sub> kg<sup>-1</sup> day<sup>-1</sup>) and *P. marginata* larvae (0.190 ± 0.060 g CH<sub>4</sub> kg<sup>-1</sup> day<sup>-1</sup>). Neither *T. molitor* larvae, *A. domesticus* instars, not *L. migratoria* instars produce this greenhouse gas. Nitrous oxide only seems to be produced by *T. molitor* larvae (0.002 ± 0.000 g N<sub>2</sub>O kg<sup>-1</sup> day<sup>-1</sup>) and *L. migratoria* instars (0.002 ± 0.001 g N<sub>2</sub>O kg<sup>-1</sup> day<sup>-1</sup>). When calculated to rearing in larger quantities (500 kg live weight), it shows that *P. marginata* larvae, *B. dubia*, and *A. domesticus* instars do produce nitrous oxide. Ammonia is emitted in small amounts by *L. migratoria* larvae and *A. domesticus* instars (0.005 ± 0.001 g NH<sub>3</sub> kg<sup>-1</sup> day<sup>-1</sup>) and *B. dubia* (0.003 ± 0.001 g NH<sub>3</sub> kg<sup>-1</sup> day<sup>-1</sup>). But multiplication of the experiments to 500 kg live weight also shows that both *P. marginata* larvae and *T. molitor* larvae produce ammonia, but only in small quantities.

Ammonia is produced by insects at least seven times less than by conventional livestock (cattle, pigs, poultry). Only *B. dubia* emits less greenhouse gases (carbon dioxide, methane, and nitrous oxide) than conventional livestock. *Acheta domesticus* and *L. migratoria* are more than double as polluting as pigs. *Tenebrio molitor* produces almost twice as much greenhouse gas than pigs. It is debated that the literature on emissions from conventional livestock needs more thorough examination.

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