Fasting increases shelter use in house crickets (*Acheta domesticus*)

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Abstract

Fasting, i.e. depriving the animals of food prior to harvesting, has been practised in the production of house crickets (*Acheta domesticus*). However, the effectiveness of this method in reducing microbial loads is still unclear. In addition, there may be costs of fasting to the organism, which could compromise product quality and animal welfare. Here, we analysed spontaneous behaviour displayed after 0, 24 or 48 hours of fasting. We show that after 48 hours of fasting, shelter use was increased and the duration spent on the exposed area of the floor was decreased. The same trend was seen after 24 hours fasting. Moreover, in both fasting treatments, a strong reduction in grooming was seen. We conclude that, in the absence of a microbial lowering effect of fasting and considering the current finding that fasting induces behavioural responses in crickets, there is currently no scientific support for using fasting in cricket production.

Keywords: insect production, starvation, behaviour

1. Introduction

In house crickets (*Acheta domesticus*), the gut contains high microbial loads as well as a high diversity of bacterial communities, including spore-forming species (Fernandez-Cassi *et al.*, 2020; Klunder *et al.*, 2012; Vandeweyer *et al.*, 2017). Some insect production facilities apply a 24 to 48 hour fasting period prior to harvesting to reduce the gut content. However, we recently discovered that total aerobic counts (TAC) were increased rather than decreased in crickets fasted for 48 hours (Inácio *et al.*, in press). Also in mealworm larvae (*Tenebrio molitor*), a 48 hour starvation did not bring about a shift in the composition of the microbial community (Wynants *et al.*, 2017). Hence, fasting does not seem to have the intended effect on insect gut microbiota. In addition, there may be a cost of fasting to the organism, which could compromise product quality and animal welfare.

Insects show similar metabolic responses to fasting as vertebrates, i.e. a three-stage paradigm of fuel shifting (Belkhou *et al.*, 1991). After an initial reliance on carbohydrates in the haemolymph (stage I), a longer steady-

state is reached where lipids are the predominant source of energy (stage II). During the third stage, lipid oxidation decreases and protein oxidation progressively increases. Indeed lipids provide the most important fuel for fasting animals and starvation resistance is often positively correlated with lipid content both between individuals (Hoffmann and Harshman, 1999) and between species (Djawan *et al.*, 1998).

Using diets enriched with stable isotopes (¹³C-palmitic acid or ¹³C-leucine as a marker for lipid resp. protein oxidation), McCue *et al.* (2015) reported that in adult house crickets, lipid oxidation is increased as early as 24 hours post feeding (earlier could not be quantified in this design) and from 60 hours, protein oxidation increases. This suggests that both at 24 and 48 hours post feeding, house crickets have not reached the tissue damaging stage of protein catabolism. Nevertheless other starvation responses may have set in. For example, in *Drosophila melanogaster*, genes involved in immune response and reproduction become downregulated already 24 hours post feeding while 50% of the flies survive for at least 60 hours (Fujikawa *et al.*, 2009).

Even if fasting would not affect the physical health of the animal, it may still negatively influence animal welfare if the animal ‘wants something it does not have’ (Dawkins, 2004). Animals may be highly motivated to obtain food and can become restless, try to find food or attempt to escape if they are deprived of food. Freedom from thirst, hunger or malnutrition is, after all, the first of the five freedoms devised by the Farm Animal Welfare Council (FAWC, 1993). Behavioural measures provide a non-invasive, non-intrusive way of assessing animal welfare and shed light on the choices and preferences of animals (Dawkins, 2004).

Here, we assess whether fasting for 24 or 48 hours alters the behaviour of house crickets. We observed freely occurring behaviours in adult male-female pairs with access to a shelter and drinking water. This is a first attempt to evaluate the consequences of fasting for insect welfare.

2. Materials and methods

Animals and housing

The adult house crickets used in this experiment were from our outbred laboratory population established from and regularly backcrossed with wild-caught individuals collected in Sweden (Vaga *et al.*, 2020). The animals were reared in plastic boxes in groups of 5–50 individuals on a standard wheat- and oat-based pellet feed (Vaga *et al.*, 2020) in a climate controlled room at 30 ± 1 °C, 45–50% relative humidity and a 12 hour light/dark cycle with light on at 07:00 CET. During rearing, animals had *ad libitum* access to feed, tap water for drinking (in 10 ml cotton-plugged tubes) and a shelter known to be preferred by house crickets (Vaga *et al.*, 2018; black plastic tubes, $6 \times \varnothing 2.5$ with plastic straws $\varnothing 0.5$ or 0.8 cm).

Experimental treatment

The experiment was carried out in December 2019 and February 2020 at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden. At the start of the experiment, animals were randomly assigned to one of three treatments: ‘control’ (24 hours in experimental boxes with *ad libitum* access to standard feed and water), ‘24h’ or ‘48h’ (24 resp. 48 hours in experimental boxes without feed but with *ad libitum* access to water). Sixteen male and sixteen female crickets were used per treatment (total of 96 crickets). Crickets were placed in male/female pairs (one pair is one replicate) in transparent experimental plastic boxes ($12 \times 12 \times 6$ cm or $20 \times 17 \times 14$ cm) with air holes. All the boxes contained a shelter (see previous section) and a tube with tap water.

Behavioural scoring

Animals were video recorded in their experimental boxes using an iPhone 8 (Apple, Cupertino, U.S.A.) at experimental time 24:00 – 25:00 hours (control and 24h treatment) or 48:00 – 49:00 hours (48h treatment), 1–3 hours into the light phase, for a duration of one minute, during six subsequent occasions (every tenth minute). Using Boris behavioural observation software (Friard and Gamba, 2016), the duration (s) of time that the animal spent hidden behind the shelter, on the shelter, inside the shelter, on the floor or on the wall was manually scored. The duration (s) spent moving included both movement of the bodies’ centerpoint as well as movement of the limbs while the centerpoint kept the same location. Duration (s) grooming (cleaning the head or legs with the mouth), eating, drinking, egg laying, copulating and vocalising (male calling) were also scored. Since the individual’s sex could not be distinguished in each video, the individual’s behaviour in one minute could not be linked to the next. The experimenter that performed behavioural scoring (L.E.V.) did not have formal knowledge of the assigned treatment at the time of scoring, although the control group could be distinguished from the other treatments by the presence of food.

Statistical analyses

Statistical analyses were performed in R version 4.0.2 (R Core Team, 2020). Eating, drinking, egg laying and copulating behaviour occurred too infrequently to be analysed statistically. The remaining seven behaviours were divided by the duration that the animal was visible (i.e. not behind the shelter) to obtain proportions, which were *logit* transformed and analysed with seven linear mixed-effects models (LMERs) containing a fixed effect of fasting treatment and nested random effects of month, box and minute.

3. Results

Fasting influenced where in the arena the animals were located. Animals that were fasted for 48 hours spent more time inside the shelter than controls (28 vs 7%; least square means, $t_{2,46} = -2.237$, $P = 0.030$, Figure 1A) and less time on the floor (6 vs 22%; least square means, $t_{2,45} = 2.330$, $P = 0.024$, Figure 1B). A tendency for spending less time on the floor was also detected for the 24h group (11 vs 22%; least square means, $t_{2,45} = 1.649$, $P = 0.106$, Figure 1B). No effect of fasting was detected for the duration of time spent on top of the shelter (LMER, $F_{2,45} = 0.314$, $P = 0.733$) or on the wall (LMER, $F_{2,45} = 0.607$, $P = 0.549$). Duration spent moving was not affected by fasting treatment (LMER, $F_{2,45} = 1.082$, $P = 0.348$), although the 48h group showed a trend for moving less (Figure 1C).

Figure 1. Effect of fasting treatment on behaviour. Duration (%) of time spent in shelter (A), on the floor (B), moving (C) or grooming (D) in experimental groups with access to food (control), fasting for 24 hours (24h) or fasting for 48 hours (48h). Dots with error bars represent mean $\pm$ S.E.M.

Fasting strongly reduced grooming behaviour (LMER, $F_{2,45}=10.818$, $P<0.001$, Figure 1D) in both the 24h group (2 vs 12%; least square means, $t_{2,46}=4.610$, $P<0.001$) and the 48h group (6 vs 12%; least square means, $t_{2,44}=2.801$, $P=0.008$). We did not detect an effect of fasting on male call duration (LMER, $F_{2,45}=2.400$, $P=0.102$).

4. Discussion

Food availability inherently fluctuates in nature, therefore many animals possess evolved behavioural, physiological and biochemical responses to survive periods of food scarcity. In terms of behaviour, the animal must at any one point in time choose between attempting to find novel food, or conserve energy by reducing its locomotory activity. In the current experiment, we found no evidence for increased food-searching behaviour or increased activity. Rather, at 48 hours post-feeding captive house crickets increased their use of the shelter and avoided the exposed area on the floor, which could be a strategy to conserve energy (minimising heat loss via radiation and convection) and to minimise the risk of predation while doing so. Also, both fasted groups displayed less grooming which may be explained by the general reduction in activity and/or by the reduced need to clean the exoskeleton of food particles.

Shelter seeking is also seen in response to a (mock) predator (Adamo *et al.*, 2013) and after social defeat (Rillich *et al.*, 2011), therefore this behaviour could be indicative of an aversive situation (Dawkins, 2004). Alternatively, the shelter might be the preferred location for rest or sleep-like behaviour (Hendricks *et al.*, 2000), in which case fasting

could be a 'neutral' situation that the animals simply wait out. This distinction is relevant since insects are capable of nociception and can even learn to avoid noxious stimuli (Sneddon *et al.*, 2014). Additional experiments are needed to evaluate how motivated fasting crickets are to obtain food, for example by letting the animal perform work in order to reach a food compartment (Olsson *et al.*, 2002), and contrasting access to food to other resources, such as potential mates or environmental enrichment (Mason *et al.*, 2001). Physiological measurements, such as the (neuro) hormone octopamine (Adamo *et al.*, 1995) and adipokinetic hormone (Lee and Park, 2004) can also contribute to the assessment of whether fasting influences welfare (Dawkins, 2004). In rats, the final stage of fasting (protein oxidation) is associated with a rise in corticosterone level (Belkhou *et al.*, 1991).

It should be noted that the effect of fasting on locomotory activity is likely dependent on the circadian rhythm. In *D. melanogaster*, fasting leads to an overall increase in locomotory activity (Knoppien *et al.*, 2000), due to an elongation and intensification of the daily activity peaks at dawn and dusk (Lee and Park, 2004). However, when activity was low (during the day and middle of the night), starved flies showed both higher and lower activity compared to fed conspecifics (Lee and Park, 2004). We conducted behavioural observations during the inactive part of the day, hence it would be interesting to quantify behaviour of crickets during the entire light/dark cycle.

To conclude, in the absence of clear beneficial effects of fasting in reducing microbial loads and considering the

current findings that fasting induces behavioural responses in crickets, some of which could be aversive, we see no need to apply this method.

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