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Impact of starvation on fat content and microbial load in edible crickets (*Acheta domesticus*)

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SHORT COMMUNICATION

Abstract

Interest in insects as food is increasing worldwide, particularly in industrialised countries. Insect-based ingredients are considered novel foods in Europe and there are unresolved concerns regarding food safety. Microbial counts in insects can be high, posing potential health risks to consumers and possibly causing rapid deterioration by spoilage microorganisms. Gut emptying by starvation prior to killing could reduce the microbial load in the insect gut but could also lead to fat loss and lower energy content, reducing the profitability of production. This study evaluated the microbial load (total aerobic counts (TAC), *Enterobacteriaceae*) in house crickets (*Acheta domesticus*) starved for 0 h (control), 24 h, and 48 h, and the corresponding fat losses. The 24 h starvation group showed significantly lower (P=0.004) *Enterobacteriaceae* counts of one log cfu/g, but not TAC, (compared to the control group). TAC was significantly increased (P=0.002), by almost one log cfu/g in the 48 h starvation group compared with the control. Sex of the insects had no significant effect on microbial numbers (P=0.72 and P=0.46 for TAC and *Enterobacteriaceae*, respectively). Starvation for 24 h decreased fat content in crickets (P=0.02), indicating potential production losses. This shows that starvation is not an effective method for reducing microbial loads in edible crickets.

Keywords: insects as food, food safety, starvation, plate count, lipid content

1. Introduction

Edible insects are being promoted as an alternative source of protein, due to environmental benefits, compared with some conventional animal production systems, deriving from potentially higher feed conversion ratios and higher proportion of edible weight (Oonincx *et al.*, 2015; Van Huis, 2013). Within the European Union, insects are considered as novel foods (Regulation (EU) No. 2015/2283) (EU, 2015). Although no microbiological criteria specific for edible insects have yet been developed in the EU, it has been suggested to use those intended for meat and shellfish in Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (IPIFF, 2019). More precisely, to use the food safety acceptance criteria laid down in Annex 1-1.2. (*Listeria monocytogenes* in ready-to-eat foods), 1.16. (*Salmonella* in cooked crustaceans and molluscan shellfish,), 1.6. (*Salmonella* in minced meat and meat preparation made from other species than poultry). Furthermore, it was suggested to use the process hygiene criteria as laid down in 2.1.6. (*Escherichia coli* and aerobic colony count in minced meat, where out of 5 samples no sample should have more than 500 cfu/g and at most 2 samples with numbers between 50 and 500 cfu/g for *E. coli,* and no sample more than 5 million cfu/g and at most 2 samples with numbers between 0.5 and 5 million 5 cfu/g). Moreover, some European countries such as the Netherlands, Belgium and Finland have already established national criteria on food safety of edible insects (Evira, 2018; FASFC, 2018; NFCPSA, 2014). The European Food Safety Authority (EFSA) has therefore adopted a conservative approach to production and consumption of insects (EFSA, 2015). There are still knowledge gaps regarding food safety of insects, e.g. their microbiological composition (Garofalo *et al.*, 2019). It has been demonstrated that the microbial

loads in raw edible or processed crickets can be high (Fernandez-Cassi *et al.*, 2018; Grabowski and Klein, 2017a; Vandeweyer *et al.*, 2017). This is important as processed cricket products are usually made from whole insects, including the gastrointestinal tract, which increases the microbial load and may affect food quality, safety, and shelf-life. Hence, adequate processing and storing methods must be applied (Klunder *et al.*, 2012). It has been reported that starvation before killing could be one way of emptying the gut, and thus reducing the microbial load in edible crickets. Frequently reported starvation periods include 24 and 48 h (Finke, 2013; Mancini *et al.*, 2019; Wynants *et al.*, 2017). However, starvation may induce significant weight loss and affect the nutritional composition of the insects, as well as increasing cannibalism, resulting in reduced profitability of production (Simpson *et al.*, 2006; Wynants *et al.*, 2017).

This study evaluated the microbial loads in house crickets (*Acheta domesticus*) after different periods of starvation, and their corresponding fat content.

2. Material and methods

Study design and materials

House crickets were reared in a designated facility at the Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, under climate-controlled conditions. Until the start of the experiment, the crickets were kept in plastic boxes (16.5×14×14 cm or 28×20×28 cm) with a thin steel net fitted in one of the sides to enable ventilation. The boxes were enriched with hiding units made of black piping (6 × Ø 2.5 cm) (Vaga *et al.*, 2018) and plastic straws (5 × Ø 0.53 cm and 5 × Ø 0.8 cm). The temperature was kept at 30±1 °C and relative humidity at 45-55%, with a 12 h lighting regime. Feed was provided *ad libitum* and consisted of a pelleted feed mixture of oat bran, wheat bran, wheat meal, rapeseed meal, limestone, and a premix of vitamins and minerals (Vaga *et al.*, 2020). Water was given in plastic tubes (10 × Ø 1.2 cm) that were refilled every 5-10 days. The nymphalid stage lasted about eight weeks and the crickets were collected for analysis within 2-7 days after the last moult to adult stage.

A pilot study was performed to assess whether there was a significant effect of sex of the insects on total aerobic counts (TAC) and *Enterobacteriaceae* counts. Three replicates (each consisting of 10 crickets) of each sex were starved for 24 h. The crickets were kept and managed as described above.

Next, three groups of crickets were compared: a control group fed *ad libitum*, which was euthanised by freezing at -20 °C at the start of the experiment, and a second and third group initially fed the same diet, but starved for 24 and 48 h, respectively. Crickets from the two study groups were euthanised as described for the control immediately on completion of the experiment. The crickets were stored in the freezer immediately after having been euthanised. The storage time varied between one week and two months.

Eight replicates per group were used for the microbiological analyses, with each replicate containing 7-9 crickets (both females and males). For fat quantification, three replicates of each sex were used, with each replicate consisting of six crickets. In order to monitor mortality and reduce cannibalism during starvation, the crickets were kept in pairs in smaller plastic boxes (11×7.5×4 cm and 12.5×12.5×5 cm). These pairs consisted of one male and one female, to prevent male fighting and to obtain equal numbers of individuals of each sex.

Microbiological analyses

The samples (7-9 crickets) for microbiological analysis were thawed at room temperature for 30 min., transferred to a Stomacher bag, and crushed in a mortar. After weighing (weight varied between 1.96 and 3.58 g), the crushed material was suspended in sterile buffered peptone water (1:9) and homogenised in a Stomacher for 2 min. For analysis of TAC, series of 10-fold dilution were prepared and 1-ml aliquots of dilutions 10^{-5} to 10^{-9} were pour-plated onto standard plate count agar (Oxoid, Basingstoke, UK) and homogenised. The methods used were according to the Nordic Committee on Food Analysis (NMKL) method no. 86, 5th edition 2013.

Detection and enumeration of *Enterobacteriaceae* was performed according to NMKL method No. 144, 3rd edition 2005. From the previous serial dilutions, 1-ml aliquots of dilutions 10^{-4} to 10^{-7} were cultured by pour-plating onto violet red bile glucose medium (Becton, Dickson and Company, Sparks Glencoe, MD, USA) and homogenised.

Fat quantification

Individuals from the fat quantification groups were cut transversely into three to four pieces and weighed. The samples (each containing six crickets) were then freeze-dried and frozen at -80 °C. The frozen samples were crushed in a mortar. Total fat content in the cricket material was determined by Soxhlet extraction, using Soxtec/Hydrotec™ 8000 Total Fat Solution (FOSS, Hillerød, Denmark) and expressed on a dry matter (DM) basis.

Statistical analyses

For the microbiological analyses, log-transformed data were analysed using Microsoft Office Excel for Windows (Microsoft Corporation by Impressa Systems, Santa Rosa, CA, USA), with a Wilcoxon Signed Rank test and significance level $P<0.05$. Statistical analysis on the fat

content data was performed in R, using the 'aov' function (Package in R, version 1.4, Wax Begonia: 'stats'; RStudio, Boston, MA, USA factorial analysis of variance (ANOVA) and Tukey's post-hoc analysis, with significance level $P<0.05$.

3. Results and discussion

Microbiological analyses

The pilot study showed that the mean *Enterobacteriaceae* count was 6.5 log cfu/g in female crickets and 6.8 log cfu/g in males. The mean TAC level was 7.8 and 7.6 log cfu/g in females and males, respectively. There were no significant differences between the sexes for either TAC (P=0.72) or *Enterobacteriaceae* (P=0.46). Therefore, the samples used for microbiological analyses in the experiment comprised females and males.

The TAC level in the crickets subjected to starvation for 24 h did not differ from that in the control group (P=0.08), but the level in crickets subjected to 48 h of starvation was significantly higher (P=0.002) than in the control (Figure 1). There was a significant decrease in the level of *Enterobacteriaceae* between 0 h and 24 h of starvation (P=0.004), but not between 0 h and 48 h (P=0.5). Thus the TAC levels in house crickets were not significantly reduced by starvation for 24 h and 48 h, which is similar to findings by Wynants *et al.* (2017) for *Tenebrio molitor*. This might be explained by changes in the gut microbial community caused by reduced feed intake and also by stress, which might favour growth of certain bacteria (Martín-Peláez *et al.*, 2009). The values found for TAC in the control group (no starvation) are comparable to those reported in the literature for fresh *A. domesticus* (Fernandez-Cassi *et al.*, 2020; Grabowski and Klein, 2017b; Klunder *et al.*, 2012; Vandeweyer *et al.*, 2017). The only significant microbial reduction observed in the present study was in *Enterobacteriaceae* counts, which decreased with 24 h of starvation. The differences between this and previous studies may be related to differences in rearing conditions, feed used, and the period of starvation. Typically, in feed and food microbiology, when determining the microbial load of a sample by plate counting as in this study, samples are not frozen but instead analysed in a fresh way. The reason for this is that any freezing treatment will kill part of the micro-organisms present in the sample, which, upon thawing of the sample, are not included in the count. Some micro-organisms may have also become sub-lethally injured after freezing. They will not fully recover during thawing and hence remain uncounted. In our study the freezing step may have affected the living microbiota of the samples and the effect may have been different in samples, explaining the variable *Enterobacteriaceae* counts. However, freezing could not be avoided in our study, since it was applied as the killing method for the crickets.

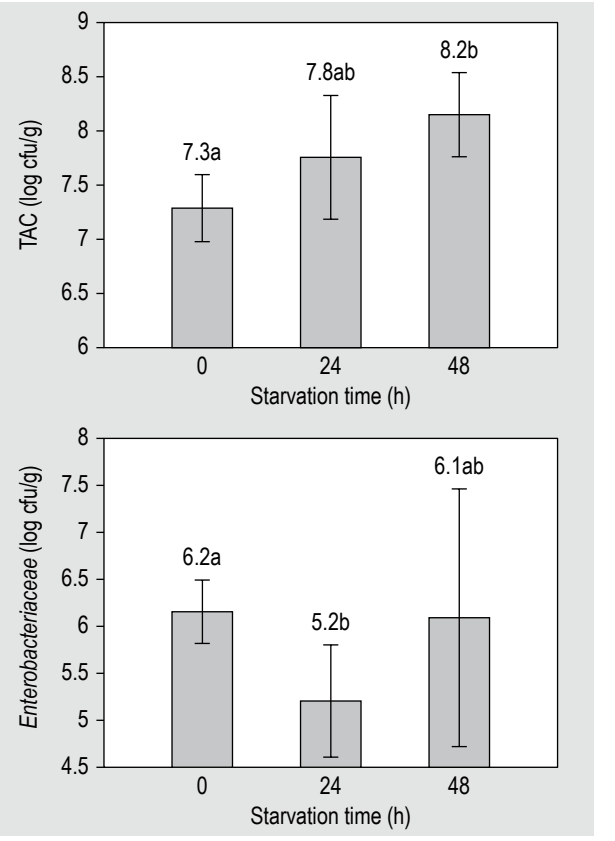

Figure 1. (A) Mean total aerobic counts (TAC; log cfu/g); and (B) mean *Enterobacteriaceae* counts (log cfu/g) in crickets starved for 0 h (control), 24 h, and 48 h. Bars indicate standard deviation, means with different letters differ significantly ($P<0.05$).

Fat quantification

The mean fat content in all crickets after 24 h of starvation (301 g/kg DM) was lower (P=0.02) than in control crickets (357 g/kg DM). However, the mean fat content in crickets after 48 h of starvation (433 g/kg DM) showed a tendency to be higher (P=0.07) than in crickets starved for 24 h and was similar (P=0.76) to that in control crickets (Table 1). These results for the group starved for 48 h agree with those of Woodring (1984), who found no reduction in plasma lipid concentration of *A. domesticus* nymphs after 48 h of starvation. McCue *et al.* (2015) found that lipid oxidation in crickets increases shortly after onset of fasting and that peak lipid oxidation in adult *A. domesticus* occurs at around 36 h within the fasting period.

On combining the data from 0 h, 24 h, and 48 h of starvation, it emerged that males had a higher fat content than females (P=0.015). This contradicts findings by Kulma *et al.* (2019) that females have a higher total fat content. Sex-dependent variation in fat content could be explained by the variation in body composition that occurs with age and developmental stage (Lipsitz and McFarlane, 1971). The exact age (in days) of the crickets in the study by Kulma

Table 1. Total fat content (g/kg DM) in male and female crickets starved for 0 h (control), 24 h, and 48 h. Each sample consisted of six crickets. *P*-values for starving indicate differences from the control crickets.

Starvation period	Fat content (g/kg DM) ± SD			SEM[1]	*P*-value		
	Males	Females	Mean		Starving	Sex	Starving×Sex
0 h	384±36	329±21	357±40				
24 h	322±24	280±11	301±28	9.37	0.019	0.015	0.647
48 h	355±16	333±27	345±25		0.762		

[1] Standard error of mean (n=18).

et al. (2019) is not reported, but the crickets used in the present study had only recently undergone their last moult to adults. Fully mature female ovaries and eggs have high lipid concentrations (Grapes *et al.*, 1989), but the females used in the present analysis were very young, which might have affected their total fat content.

4. Conclusion

This study examined the relationships between starvation, microbial load, and fat content of edible crickets. The results were somewhat contradictory as regards the effectiveness of using starvation to reduce levels of TAC and *Enterobacteriaceae*. There was an initial reduction in *Enterobacteriaceae*, but longer periods of starvation did not reduce levels of bacteria in the gut of crickets and had no clear effect on fat content in the crickets. Therefore, this small study does not support the use of starvation to reduce microbial counts in edible insects. Larger studies are needed to confirm these findings.

Conflict of interest

The authors declare no conflict of interest.

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