

Rearing of honeybee larvae under aseptic conditions

Aufzucht von Honigbienenlarven unter aseptischen Bedingungen



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- Introduction -

The *in vitro* rearing of honeybee larvae under controlled conditions is an important tool to study the effects caused by pesticides or pathogens on honeybees. Keeping larvae in a warm environment with a constant high humidity and the permanent exposure to a protein-sugar solution sometimes leads to bacterial infections and death of the larvae. To prevent infections, the application of antibiotics has been suggested. We had a particular interest in the degradation of penicillin G in honeybee larvae and in the food remaining in cells. In this study, we fed penicillin G to larvae at two different concentrations and investigated the residual penicillin G after 24 and 48-hour incubation periods.

- Material and Methods -

We used a slightly modified method following Aupinel et al. (2005) [1] for rearing of larvae (Fig. 1). Penicillin G was added to the first diet at a concentration of 124 ppm and 375 ppm, respectively. Newly emerged larvae of honeybees were placed into starter cells at the age of 5-10 h. After 24 and 48 h, respectively, the remaining food in the starter cell and the bee larvae were removed. The larvae were washed thoroughly in sterile honeybee Ringer solution and either added to culture medium (thioglycolate, brain-heart-infusion, MRS; each n = 6) or kept frozen at -20°C until penicillin G analysis. For the evaluation of penicillin G concentrations in larvae, an HPLC-MS quantitative method was developed using electrospray ionization in positive mode [2]. In brief, bee larvae were homogenized using ultrasound and penicillin G was extracted with a water/acetonitrile mixture. The homogenised liquid was then subjected to a cleanup using solid phase extraction (SPE, RP-18). Chromatography was done using an RP-18 analytical column and penicillin G was detected on basis of its MS/MS fragmentation pattern from the parent ion [M+H]⁺ at m/z 335 leading to ion fragments of 176 and 160, respectively (Fig. 2). The limit of the detection for the method used was found to be 2 ng per injection on HPLC equalling a concentration in the larvae of > 0.1 ppm. A representative run in LC-MS is given in Fig. 3.



Fig. 1. Rearing of honeybee larvae with food containing penicillin G (lower three rows) and control (upper three rows).

- Results -

Bacterial growth was not detected in the culture media used for any of the artificially reared larvae fed with the penicillin G containing diet. This was assessed by observation of clear medium even after 14 d of incubation with the macerated larvae. Development and size of larvae fed with penicillin G did not differ from the control group (Fig. 1).

A strong decrease in concentration of penicillin G was observed in larvae of the 375 ppm group (Fig. 4). From the age of 4-4.5d on, penicillin G could not be detected anymore. The results of larvae fed at a concentration of 124 ppm were not as consistent, a fact that needs to be addressed in further studies.

Penicillin G could still be detected in both concentrations in the remaining diet after 24h and to a lesser extent after 48h. This may be explained by the dilution with food and the instability of the antibiotic.

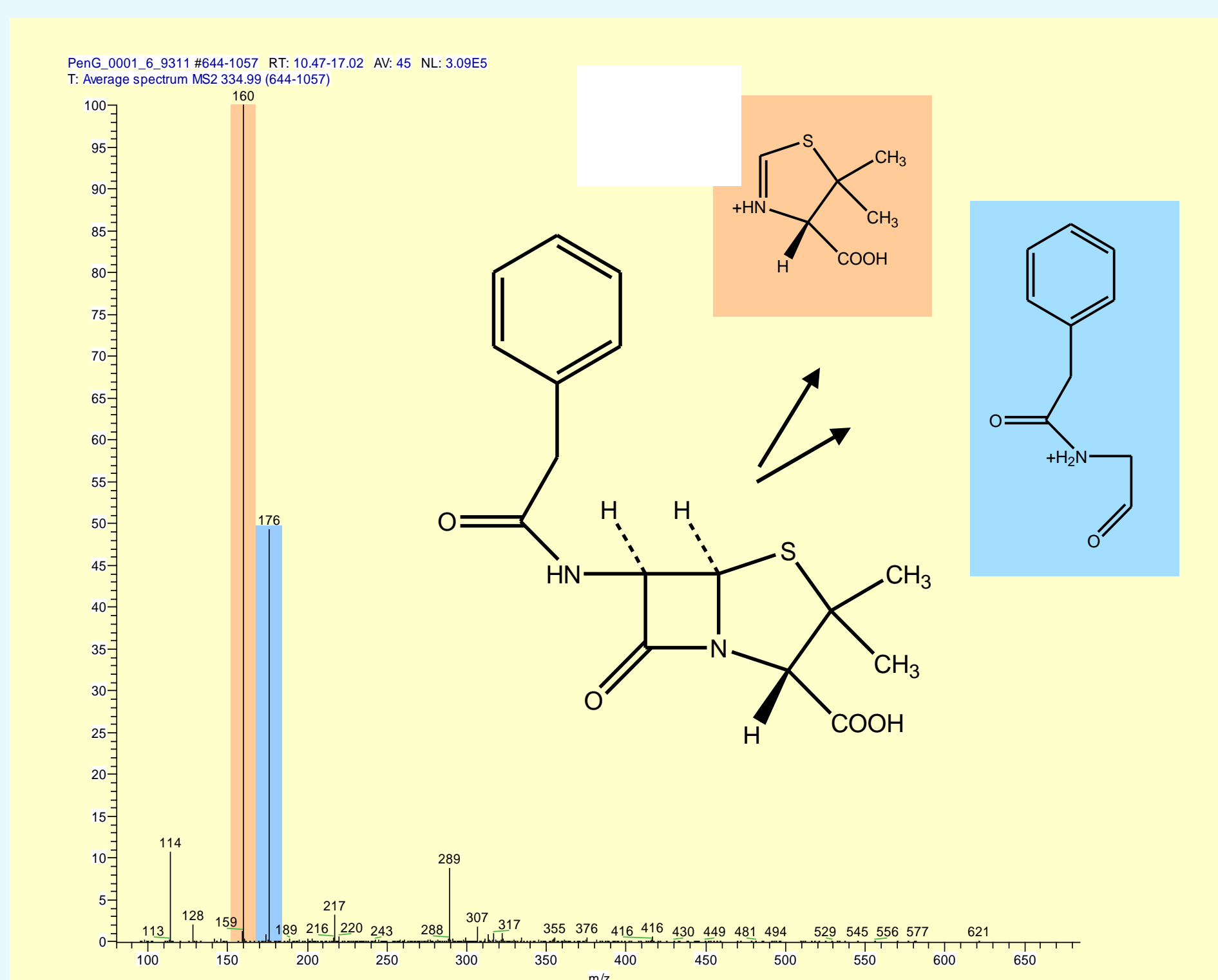


Fig. 2. Penicillin G and its major hypothetical fragment ions at m/z 176 and 160, respectively.

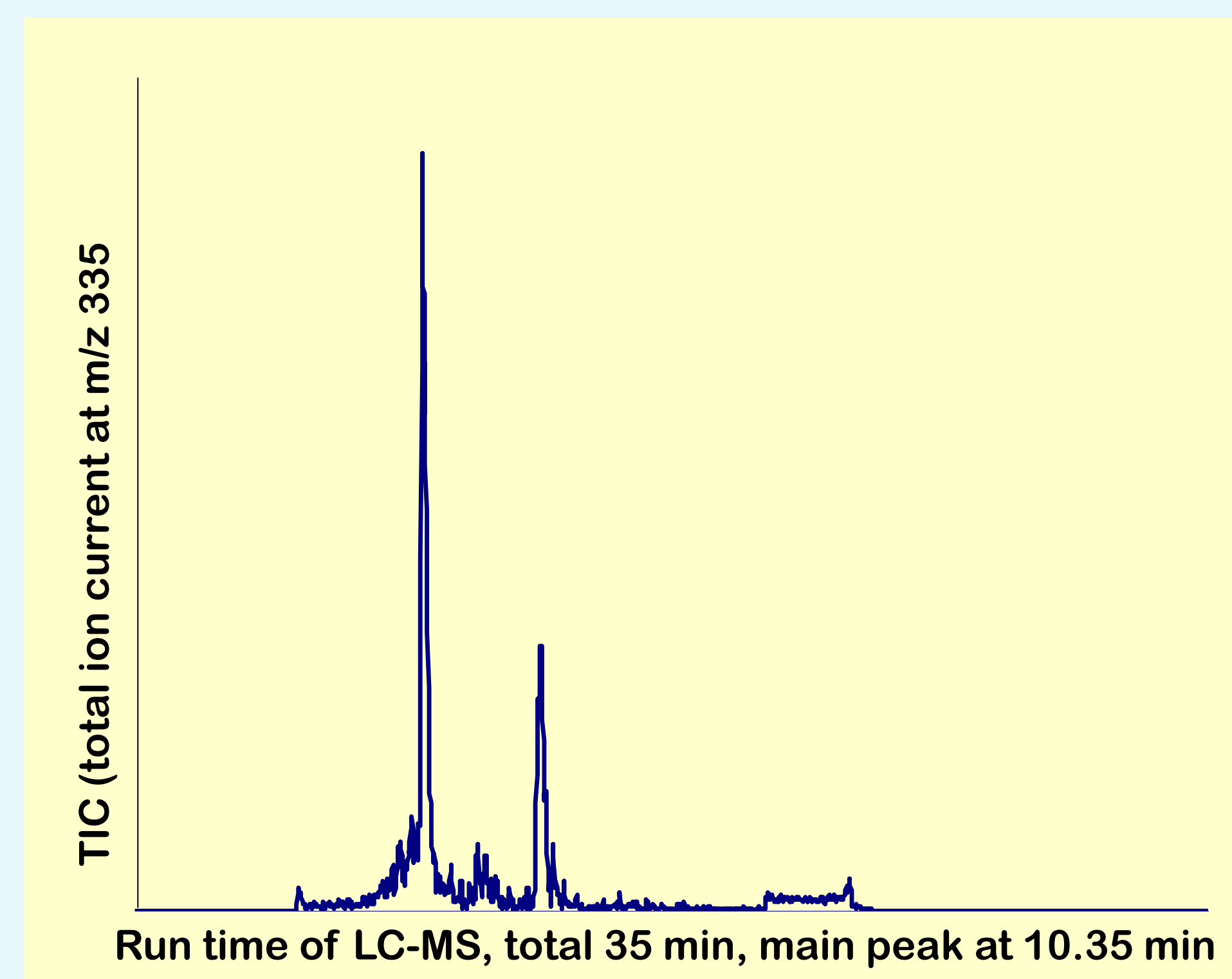


Fig. 3. Penicillin G detected at m/z 335 in a 1-1.5d old larva.

- Discussion -

The findings indicate that the application of a low dose of penicillin G at an early stage may not affect studies that are starting on day 4, but could lead to a higher survival rate of larvae. This could be an important consideration when conducting acute intoxication tests (e.g. with pesticides), which start at this time. The concentration of penicillin G at that age is definitively below 0.1 ppm.

Further studies with a tighter monitoring of the penicillin G concentration in larvae will be carried out. Penicillin G has been reported to degrade in tissue, which makes an accurate determination of tissue levels difficult [2].

The results point to an effective prevention of the establishment of an intestinal bacteria flora in bee larvae and also indicate that the ingested penicillin G is rapidly metabolized. The development of this method has allowed us to create an aseptic test organism, which could be used to conduct future studies to assess the possible protective role of the bacterial flora in honeybee larvae midguts to infection.

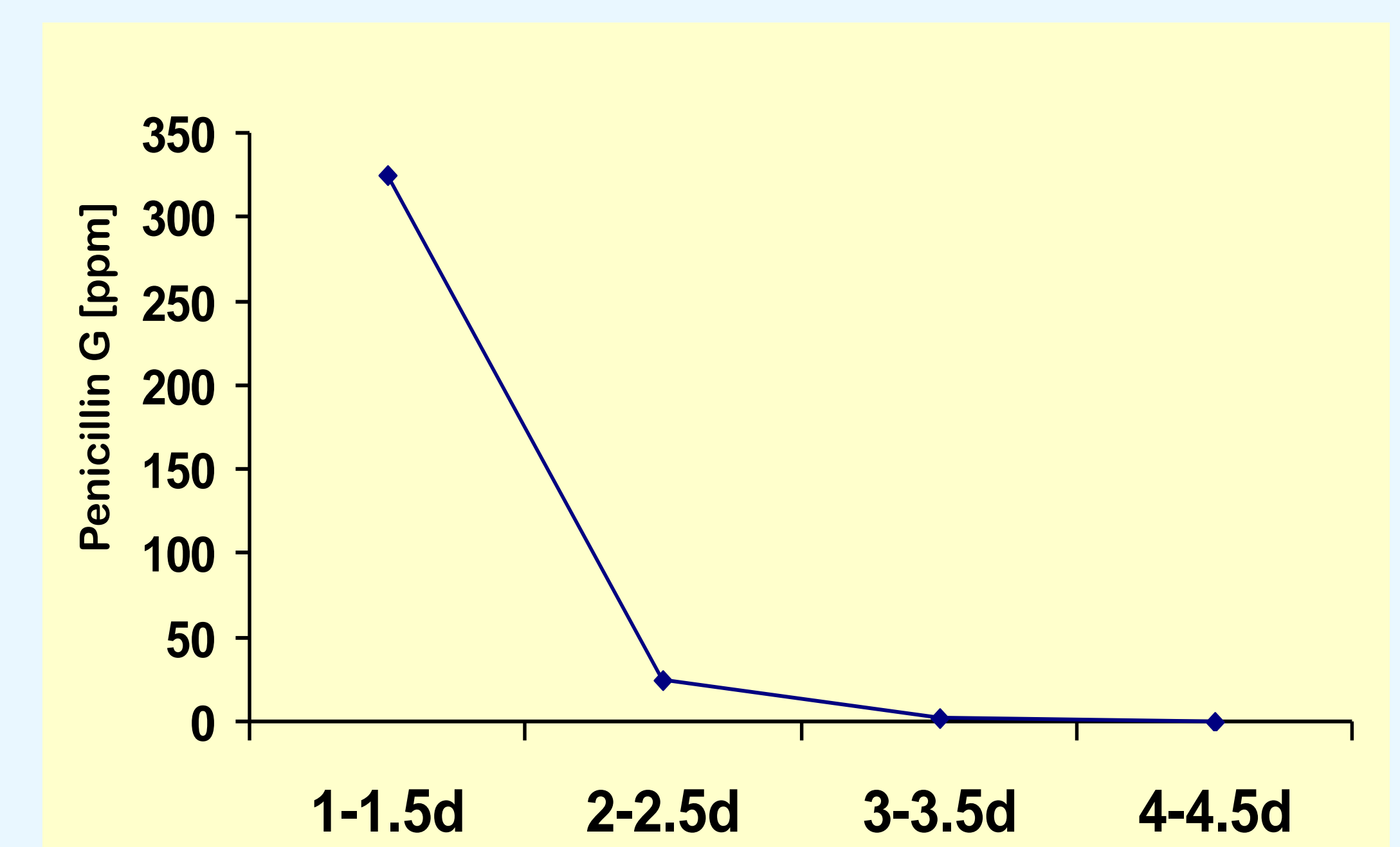


Fig. 4. Concentration (ppm) of penicillin G in larvae of the 375 ppm group at different ages (n = 2 - 3).

- Acknowledgement -

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- Literature -

[1] Aupinel P., Fortini D., Dufour H., Tasei J.-N., Michaud B, Odoux J.-F., Pham-Delègue M.-H. Improvement of artificial feeding in a standard in vitro method for rearing *Apis mellifera* larvae. *Bulletin of Insectology* 58 (2): 107-111, (2005)

[2] Heller, D.N., Smith, M.L., and Chiesa, O.A. LC/MS/MS measurement of penicillin G in bovine plasma, urine, and biopsy samples taken from kidneys of standing animals. *J. Chrom. B.* 830: 91-99 (2006)