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EFFECT OF *Ligilactobacillus salivarius* subsp. *salivarius* A3iob ON THE ABDOMINAL MORPHOLOGY AND THE HISTOLOGICAL STRUCTURE OF THE MIDGUT OF *Apis mellifera*

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In recent years, global concern has grown regarding the weakening of honeybee colonies (*Apis mellifera*), which are increasingly vulnerable to infections and environmental xenobiotics. Their immune system is closely linked to the gut microbiome, which plays key roles in digestion, detoxification, and defense against pathogens. The use of probiotic bacteria has been proposed as a therapeutic tool to improve their health. In this context, *Ligilactobacillus salivarius* subsp. *salivarius* A3iob, a lactic acid bacterium isolated from the gut of a worker bee, has shown beneficial effects when applied to productive colonies. To evaluate the effects, three groups were established and supplied with: 1) viable bacteria in 0.1% w/v peptone water at a final concentration of 10^9 CFU/mL, and 2) bacteria killed by heat treatment (98°C for 10 minutes) in 0.1% w/v peptone water. The experimental groups were compared with a control group that received only sterile 0.1% w/v peptone water. A total of 13 colonies were used: 4 in the control group, 5 in the group treated with viable bacteria, and 4 in the heat-treated bacteria group. For statistical analysis, the non-parametric Kruskal-Wallis method was employed, with differences considered significant at $p < 0.05$. Approximately 400 bees were collected from each hive; 10 were selected for abdominal morphometric studies, and 5 for histological analysis of the midgut. These sample were processed using paraffin embedding techniques and stained with hematoxylin and eosin for observation under light microscopy. The analyses focused on the average epithelial height (AEH) and peritrophic membrane secretion (PMS). The results showed a significant difference ($p = 0.0001$) in abdomen weight in the group that received the viable bacteria ($58.8 \text{ mg} \pm 16.2$) compared to the control group ($43.8 \text{ mg} \pm 13.2$) and the heat-treated group ($45.9 \text{ mg} \pm 12.3$). Regarding AEH, bees treated with live *L. salivarius* A3iob showed a significant increase ($p = 0.0001$) of $259 \text{ } \mu\text{m} \pm 46$ compared to the control group ($194 \text{ } \mu\text{m} \pm 14$) and the heat-treated group ($199 \text{ } \mu\text{m} \pm 18$). In terms of PMS, the group treated with live bacteria showed a significant increase ($p = 0.01$) compared to the control, while the heat treatment did not produce significant differences. The findings suggest that the positive effects of *L. salivarius* A3iob

on bee health may be linked to modifications in the midgut epithelium, involved in the production and secretion of digestive enzymes, as well as nutrient absorption, and to the increase in the degree of peritrophic membrane secretion, which serves as protection against pathogens and various harmful environmental compounds. Although the non-viable bacteria did not induce the same benefits as its live form, further research is needed to evaluate this treatment, aiming to determine whether it could also act as a potential postbiotic that contributes to bee health and productivity.

Palabras clave: honeybee – lactic acid bacteria - probiotics – histological analysis