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PP-410 [Bee Biology]

# THE ROLE OF NITRIC OXIDE IN HONEYBEE EPITHELIAL IMMUNITY

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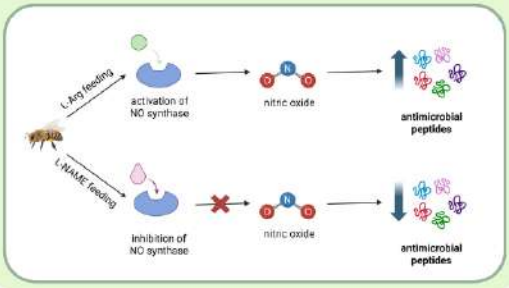
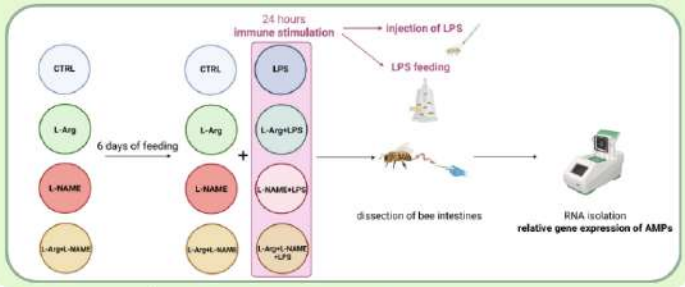
## INTRODUCTION

The digestive tract of invertebrates is essential for nutrient absorption and immune defense, acting as a **primary barrier against orally acquired pathogens.** Epithelial immunity, which involves the symbiotic microbiome and epithelial cells, plays a key role by producing **immune molecules such as antimicrobial peptides (AMPs) and nitric oxide (NO).** NO is synthesized from L-arginine by NO synthase[1] and functions as both a signaling and cytotoxic molecule, which makes it an essential component of invertebrate immune responses. For example, **NO has been shown to regulate AMP expression** in *Drosophila melanogaster*[2,3]. The hypothesis is that **L-arginine, as a substrate for NO synthase, stimulates NO production and thereby increases AMP expression**, whereas **L-NAME inhibits NO synthase, reducing NO levels and AMP expression**.

## HYPOTHESIS

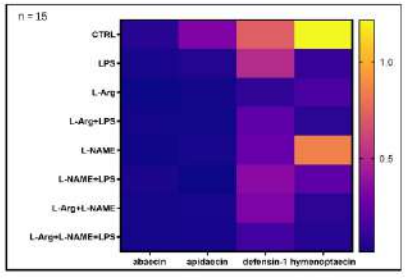



## MATERIAL AND METHODS



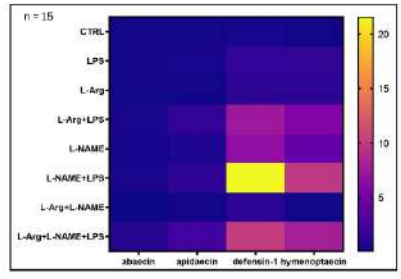

## RESULTS

### Immune stimulation by LPS via oral administration




- oral LPS stimulation did not induce a clear epithelial immune response, complicating interpretation of the results
- qPCR analysis of *aba*, *api*, *def-1*, and *hym* showed only limited and inconsistent effects of NO synthase inhibition
- overall relative gene expression of AMPs was low across all treatments

### Immune stimulation by LPS via injection




- injection of LPS elicited stronger AMP responses, particularly for def-1 and hym
- relative gene expression of *def-1* and *hym* was higher with the NO synthase inhibitor (L-NAME) than with its substrate (L-Arg)
- the combined treatment (L-Arg+L-NAME+LPS) resulted in expression levels of *def-1* and *hym* falling between those observed with L-Arg+LPS and L-NAME+LPS

## DISCUSSION

Relative gene expression of AMPs was generally low in groups receiving LPS via feeding, indicating **weak immune activation via oral administration**.

In contrast, injection of LPS induced stronger responses, particularly for *def-1* and *hym*, demonstrating that **the route of administration strongly affects immune activation.**

Relative gene expression of *aba* and *api* remained unchanged across all treatment groups, whereas relative expression of *def-1* and *hym* was unexpectedly higher in L-NAME+LPS compared to L-Arg+LPS within the injected groups, suggesting that **NO inhibition may enhance AMP expression through compensatory mechanisms.** Treatments combining L-Arg+L-NAME+LPS often resulted in intermediate expression levels, highlighting the complex and context-dependent effects of NO.

Overall, these results suggest that **NO can modulate AMP expression in honeybees.**

## CONCLUSION

**The initial hypothesis** that NO plays a key role in regulating AMP expression in the honey bee gut **was not conclusively supported**. **Relative AMP expression differed only modestly and inconsistently** between treatment groups. **Oral administration of bacterial LPS was insufficient** to elicit robust immune responses, whereas **injection of LPS provided a more reliable immune challenge**. These findings indicate that honey bee immune responses are influenced by **multiple overlapping factors** and cannot be attributed solely to NO availability. Further studies, particularly at the protein level, are necessary to clarify the precise role of NO in epithelial immunity.

## REFERENCES

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## PP-410 [Bee Biology]
## The Role of Nitric Oxide in Honeybee Epithelial Immunity

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Research on honeybee immunity has a long tradition due to the critical role of bees as pollinators in sustaining ecosystems and agriculture. The alarming trend of global colony losses underscores the urgent need to understand factors affecting honeybee health. One key area of investigation in this field is epithelial immunity, which safeguards the honeybee's digestive tract from pathogens and supports overall resilience. This research aims to examine the role of nitric oxide (NO) in epithelial immunity. In fruit flies, NO has been shown to enhance antimicrobial peptide (AMPs) synthesis, contributing to pathogen defence. AMPs combat pathogens by disrupting their membranes. However, the role of NO in honeybee epithelial immunity remains unknown.
To investigate the impact of NO on epithelial immunity, we fed honeybees L-arginine, a precursor of NO, to enhance its production. In addition, they were given bacterial lipopolysaccharides to stimulate AMPs production. To assess the role of NO, an NO synthase inhibitor was administered to block its synthesis. AMPs expression levels were then quantified using qPCR, while AMPs localization and abundance in the honeybee's digestive tract were analyzed through immunohistochemistry.

Our results show a strong link between NO production and immune response. Reduced NO levels due to NO synthase inhibition decreased AMPs production. The main advantage is that we can stimulate AMPs production by increasing NO levels. These findings emphasize the crucial role of NO in honeybee immunity and provide insights into supporting their health. These findings have important practical implications for beekeepers striving to maintain healthy colonies. By understanding the role of NO in honeybee immunity, beekeepers may explore dietary supplements or natural sources of L-arginine to enhance bees' immune defences. Strengthening AMPs production through targeted nutrition could help bees combat infections more effectively, potentially reducing losses caused by bacterial or fungal diseases.
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