



# Comparative analysis of the intestinal microbiota of Gallus gallus domesticus fed with Hermetia illucens larvae

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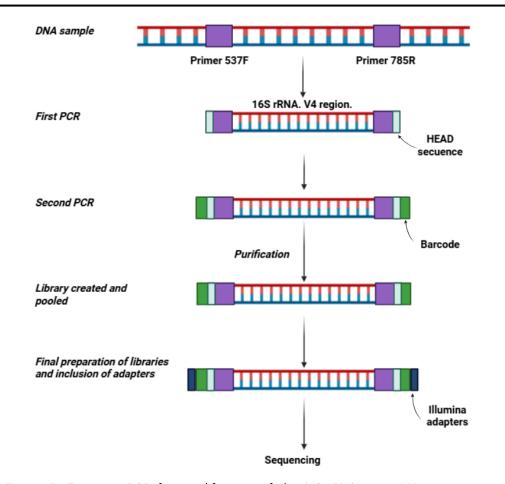
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## **INTRODUCTION**

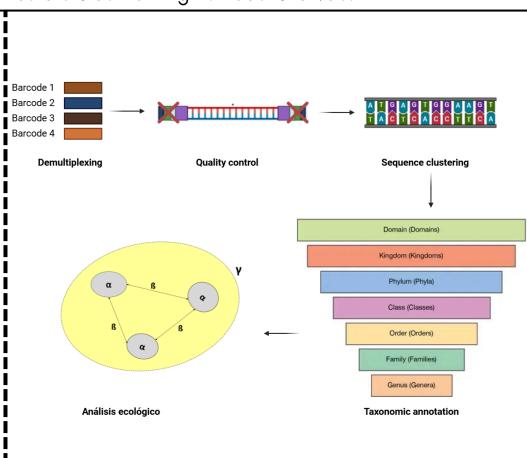
Broiler chickens (*Gallus gallus domesticus*) represent a key component of poultry production in Ecuador; however, the reliance on fishmeal as a protein source for their feed raises concerns about sustainability and resource availability [1]. The black soldier fly (*Hermetia illucens*) has emerged as a promising alternative protein source due to its high nutritional value and ability to recycle organic waste [2,3]. Previous studies have reported beneficial effects of *H. illucens* larvae on the gut microbiota of poultry [4,5]; however, their impact on Ecuadorian production conditions remains to be explored. Therefore, this study seeks to compare the diversity and potential functionality of the gut microbiota in broiler chickens fed conventional fishmeal-based diets with those fed alternative diets containing *H. illucens* larvae.

# Feeding treatments T1: 100% FM N = ~ 4 T4: 100% BSF N = ~ 5 Diet for 4 weeks Intestinal samples DNA

**Figure A:** DNA extraction from 19 intestinal samples from chickens subjected to different feeding treatments (T1: 100% F.M., T2: 75% F.M., 25% B.S.F., T3: 50% B.S.F., 50% B.S.F., T4: 100% B.S.F.) F.M. refers to fish meal and B.S.F. refers to black soldier fly.

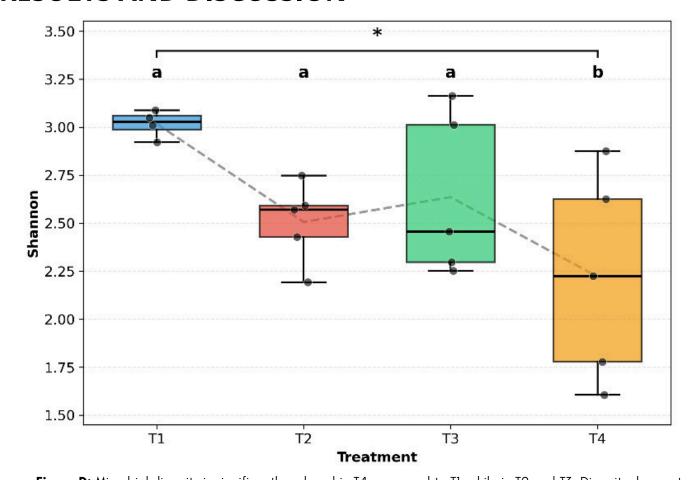


**Figure B:** Two-step PCR for amplification of the 16S rRNA gene, V4 region, using primers 537F and 785R combined with the universal HEAD sequence for barcoding. Illumina sequencing at 2x250 bp.



**Figure C:** Data processing, obtaining zOTUS tables, and taxonomic classification. Alpha and beta diversity analysis, as well as prediction analysis of the potential functionality of microbial communities.

# **RESULTS AND DISCUSSION**



**Figure D:** Microbial diversity is significantly reduced in T4 compared to T1, while in T2 and T3. Diversity does not show a significant impact in relation to T1.

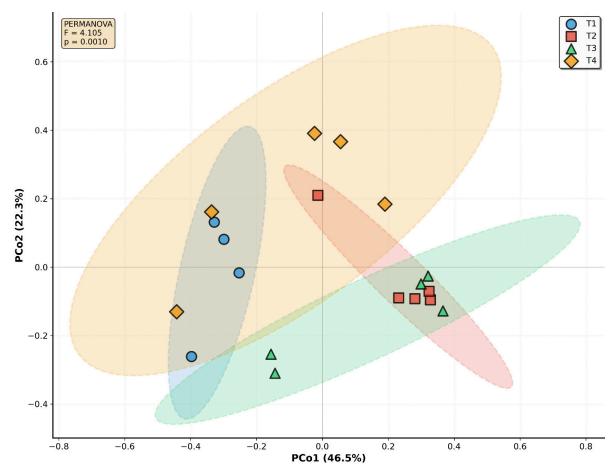


Figure E: PCoA (Bray-Curtis) of gut microbiota. Microbial composition differed significantly among treatments

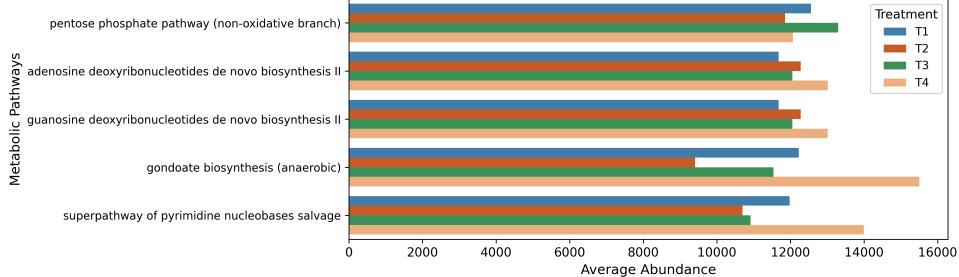


Figure F: Main predicted metabolic pathways showing difference at T1. the increase in the gondoate biosynthesis (anaerobic) pathway is shown.

Alpha diversity decreased with higher inclusion of *H. illucens*, particularly at 100%, indicating reduced microbial evenness. Beta diversity analysis (PERMANOVA, p=0.001) revealed community shifts, but the overlap among groups suggests that factors beyond diet also influence microbiota structure. Functional predictions showed some increase pathways for T4, highlighting potential changes in host-microbe interactions.

# **CONCLUSION**

Replacing fishmeal with *H. illucens* meal significantly influenced the gut microbiota of broiler chickens. Full substitution (100%) reduced microbial diversity and altered functional pathways, while partial replacement maintained a more stable microbial profile. These results suggest that insect meal has potential as a sustainable protein source, but its inclusion level is critical to preserve gut microbial balance.

