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IN-FEED PROBIOTICS AND WATER BIOREMEDIATION IMPROVED GROWTH, BIOMASS, SURVIVAL, WATER CHEMISTRY AND MICROBIOTA IN WHITELEG SHRIMP *Litopenaeus vannamei* IN VIETNAM

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LALLEMAND ANIMAL NUTRITION

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INTRODUCTION & OBJECTIVE

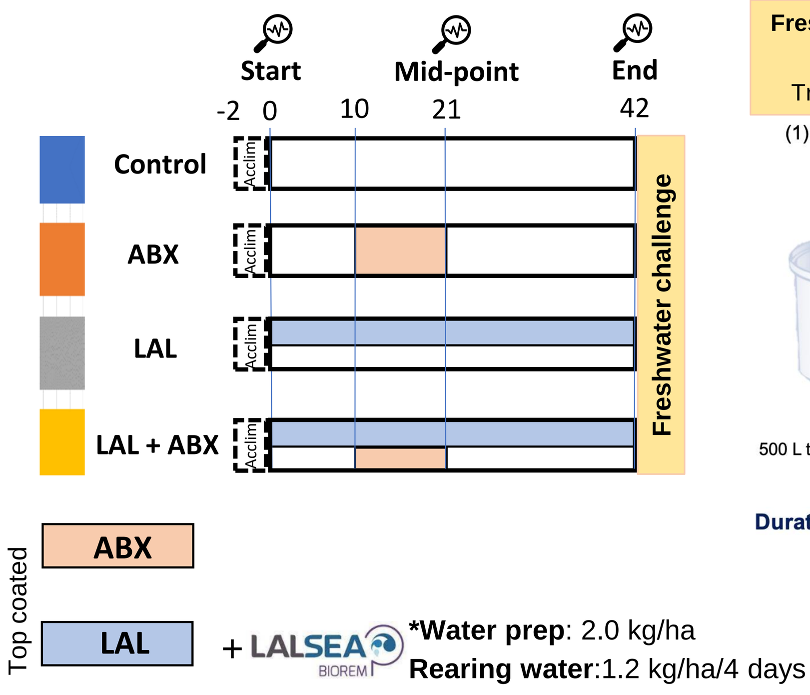
- Intensive shrimp farming suffers from a lack of predictable production performance inherent to fluctuations in shrimp and water quality.
- The use of antibiotic (ABX) as prophylactic or growth promoter remains frequent, despite increasing awareness on the need for demedication to prevent the development of antimicrobial resistance in particular
- There is a need to better understand the impact of positive bacterial and antibacterial intrants on the functionality of the water microbiota and production performance.

OBJECTIVE
Investigate the benefit of in-feed and water probiotics in comparison to prophylactic antibiotic use during *L. vannamei* grow-out under commercial-like conditions

MATERIAL & METHODS

Experimental design

- Whiteleg shrimp; SPF, BW: 0.49 ± 0.07g; n = 150/m³
- 4 Treatment in quadruplicate; 16 outdoor tanks (50m³, plastic-lined)
- Pre-treated pumped ashore water 10 ppt; 29°C; pH 7.85; DO > 80% sat. Water exchanged at 20%/4 day from DOC7 to 15 then daily;
- Commercial feed (CP 40% , CL 5-7%; Fiber 4%, 11% moisture)
- Hand-feeding to visual satiation; 4 times daily using feeding trays



Freshwater (abiotic) challenge
Abrupt salinity shift:
Transfer from 14 ppt to 0 ppt

(1) Filled up with water, 0 ppt salinity
Well-aerated tanks

(2) Allocate experimental shrimp from 60m3 tanks into the challenge tanks

[Shrimp will be randomly selected from each respective 60m3 tank]

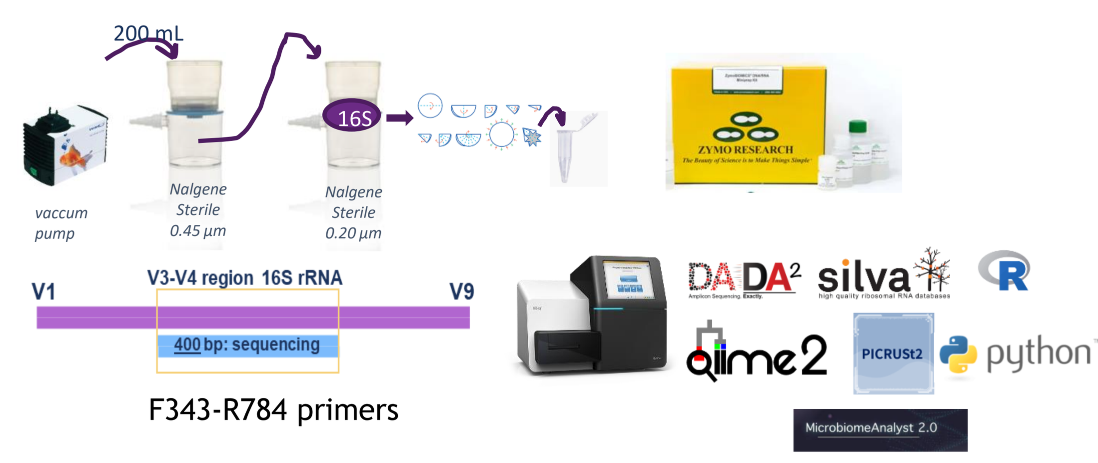
500 L tanks for stress challenge (400L$_{op}$) (1 group x 5 replicates)

Duration: 2 challenge days

Stocking density	**50 shrimp/tank**
Total number of tanks	20 tanks (4 groups x 5 reps)
Sampling	Mortality count and removal
Mortality checking time-point	Every 30 minutes

DNA sequencing & Bioinformatics:

At end of trial (day 42), 4 water samples per tank collected and pooled n=12



F343-R784 primers

RESULTS

- **Growth** (**Fig. 1, Table 1**):
  - Higher growth in LAL or ABX compared to Control (Biomass gain +12%; ADG +15%).
  - Similar growth improvement between LAL and ABX
  - Numerically lower survival with ABX (**Table 1**).
  - Significant negative effect of ABX on condition factor (K; **Fig. 1**).
- **Abiotic challenge**: survival to an abrupt exposure to FW was higher in LAL and LAL+ABX group with a minor benefit of ABX-only (**Fig. 2**).

Fig 1. Biomass and K factor (Mean ± SE; P<0.05)

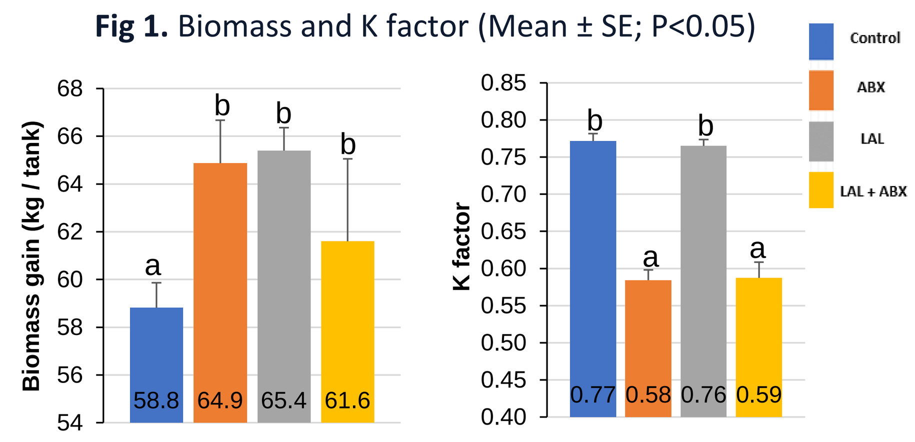
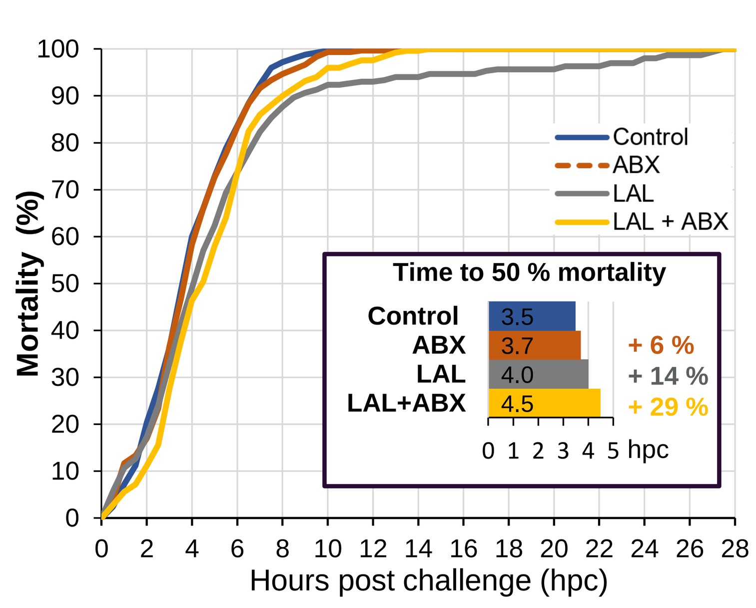


Table 1. Growth and feed performance (Mean ± SD; P < 0.05)

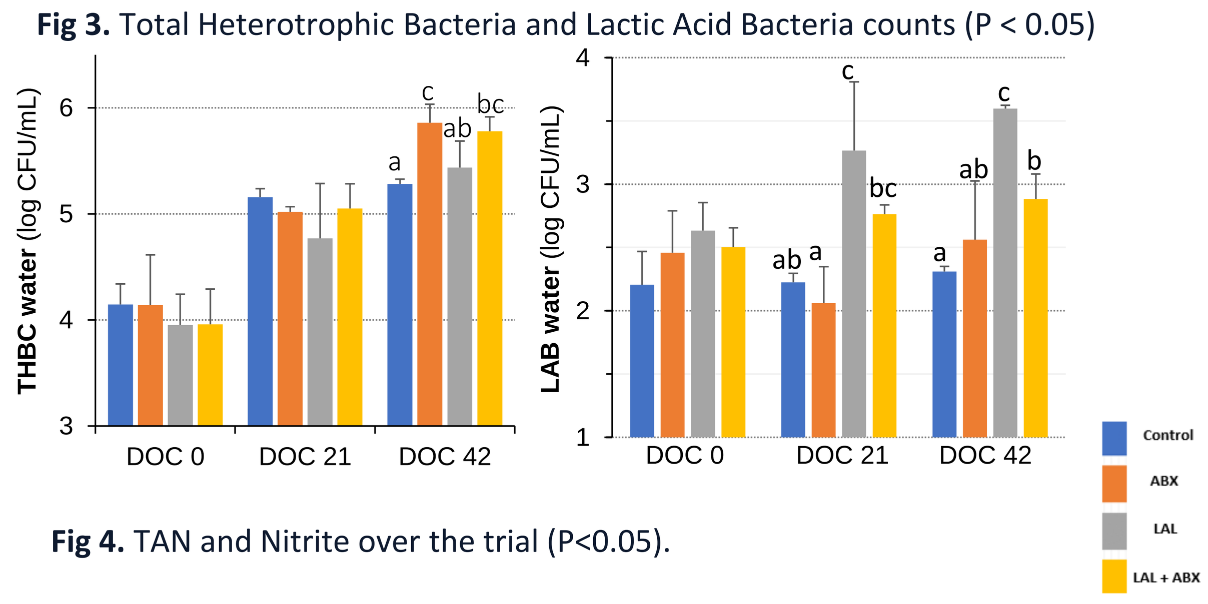
Treatment	Control	ABX	LAL	LAL + ABX
Final body-weight (g)	8.3 ± 0.3**a**	9.9 ± 0.2**b**	9.5 ± 0.5**b**	9.2 ± 0.7**b**
Final biomass (Kg)	58.8 ± 1.0**a**	64.9 ± 1.8**bc**	65.4 ± 1.0**c**	61.6 ± 3.5**ab**
ADG (g/day)	0.19 ± 0.01**a**	0.22 ± 0.01**b**	0.21 ± 0.01**b**	0.21 ± 0.02**b**
FCR	1.14 ± 0.01**a**	1.21 ± 0.05**a**	1.21 ± 0.02**a**	1.30 ± 0.07**b**
Survival (%)	93.0 ± 3.6	87.3 ± 4.3	92.0 ± 4.8	89.9 ± 10.0

Fig 2. Mortality during the abiotic challenge.



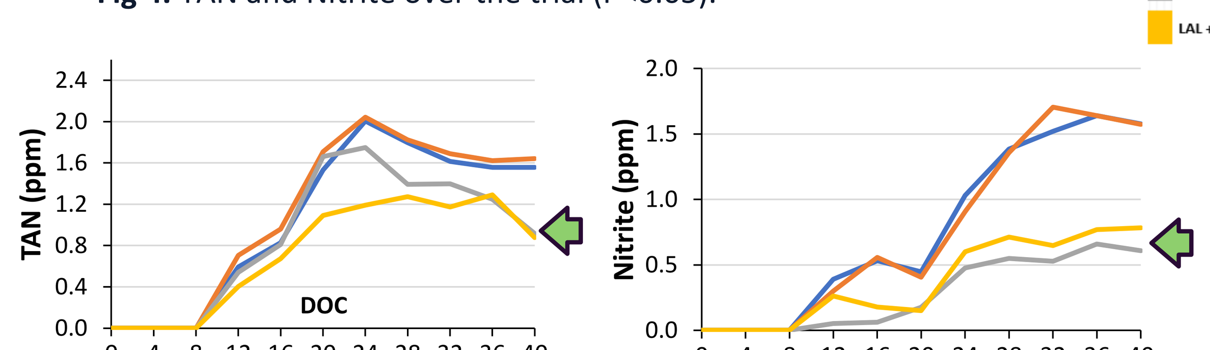
- **THB and LAB counts**: ABX promoted higher THBC post-intervention and LAL supported higher LAB in water from DOC 21 towards the end (**Fig. 3**).

Fig 3. Total Heterotrophic Bacteria and Lactic Acid Bacteria counts (P < 0.05)



- **TAN and Nitrite levels:** Lower accumulation of TAN and Nitrite in both LAL groups (**Fig. 4**).

Fig 4. TAN and Nitrite over the trial (P<0.05).



- **Water microbiota:** ABX had no impact on microbial alpha diversity (**Table 2**). Discriminant analysis at ASV level identified differences in the water microbial composition between treatments (**Fig. 5**; P < 0.05):
  - Control group had a higher abundance of Microbacteriaceae, Cryomorphaceae and *Erythrobacter*.
  - LAL group had a higher abundance of *Candidatus Aquiluna* and *Litoricola* and a lower abundance of NS3a marine group and *Izemoplasmatales*
  - LAL+ABX had a higher abundance of *Idiomarina*.

Table 2. Alpha diversity (Mean ± SD)

	Control	ABX	LAL	LAL+ABX
Shannon-index	4.84 ± 0.23	4.89 ± 0.54	4.71 ± 0.55	4.89 ± 0.83
Observed ASVs	107.2 ± 20.6	113.6 ± 23.8	101.6 ± 18.5	107.2 ± 28.8

Fig 5. sPLSDA showing a clear separation of the groups (P < 0.05)

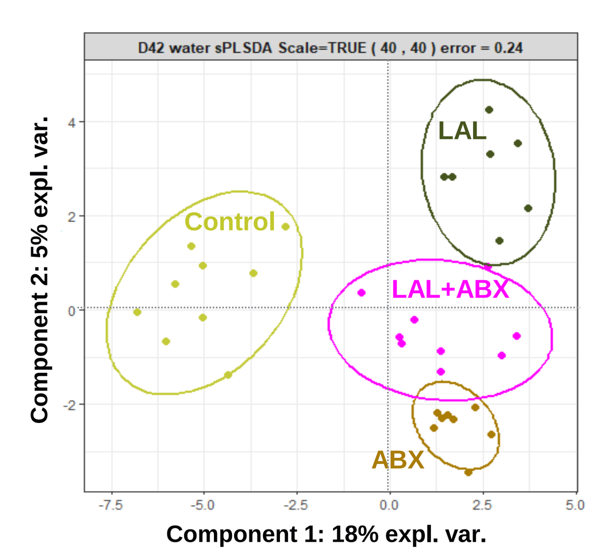
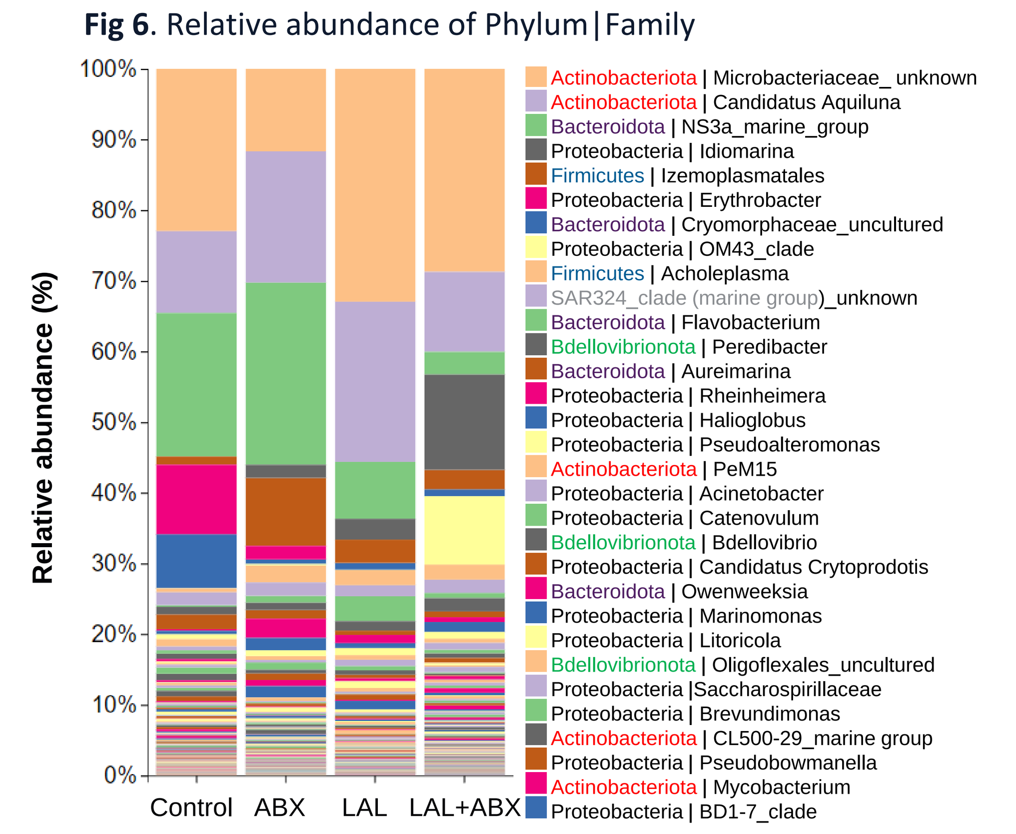


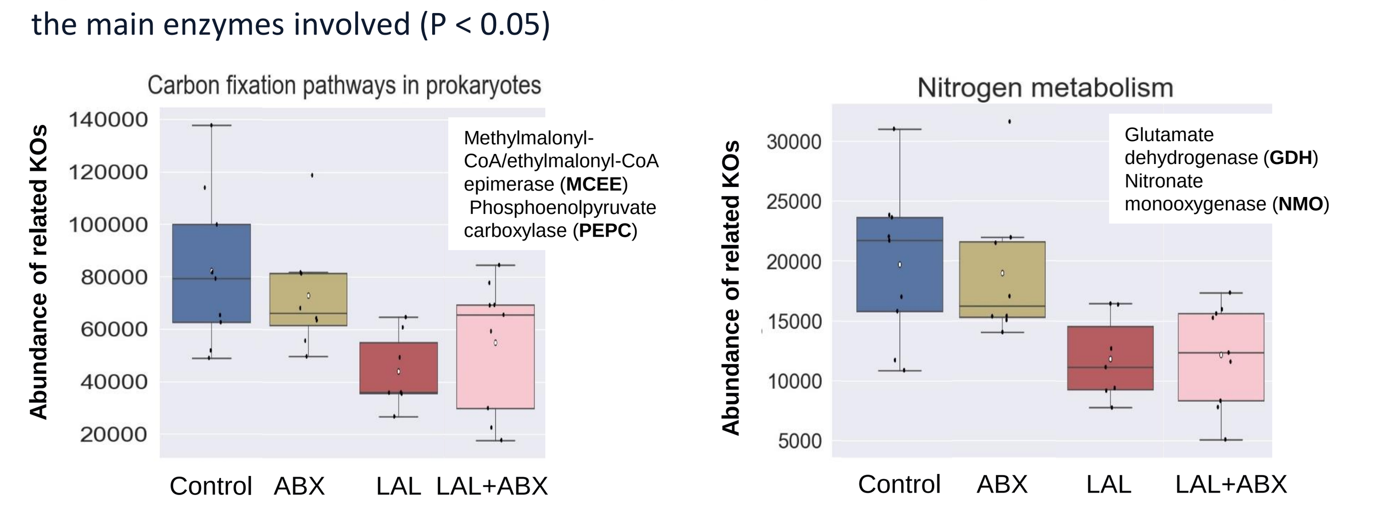
Fig 6. Relative abundance of Phylum|Family



- **Functional inference (PICRUSt)**

At the end of the trial, the predicted abundance of MCEE, PEPC, GDH and NMO pathways in the water microbiota was reduced in the LAL and LAL+ABX groups (**Fig. 7**), in accordance with the lower TAN and Nitrite concentration in the water (**Fig. 4**).

Fig 7. PICRUSt of Carbon fixation pathways in prokaryotes and Nitrogen metabolism at day 42 with the main enzymes involved (P < 0.05)



CONCLUSION

Superior benefits of selected in-feed probiotics and water bioremediation strains compared to prophylactic antibiotic use:

POSITIVE EFFECT ON WATER MICROBIOTA & QUALITY

- Enhanced LAB and lower THB counts in the water
- Enhanced Nitrogen and Carbon cycling
- Modulation of the bacterial composition towards a more balanced environment:
  - ↑ *Candidatus Aquiluna* ↑ *Litoricola* (enhanced carbon turnover and nutrient acquisition)
  - ↓ *NS3a_marine group* ↓ *Izemoplasmatales* (related to dinoflagellates blooms)

POSITIVE EFFECT ON PERFORMANCE

- Improved shrimp growth, biomass gain and resilience to an abiotic stressor to similar or higher levels than achieved by the prophylactic use of antibiotic
- No negative impact on nutrition condition (K) unlike when using antibiotics

PRACTICAL ON-FARM APPLICATION

- **LAL-solutions (In-feed + water) are a promising nutritional and environmental strategy to secure shrimp health, water quality and stability.**