

# IN VITRO VIABILITY OF A PROBIOTIC COMPOSED OF LACTOBACILLUS SPP. STRAINS ASSOCIATED WITH SALMONELLA SUBUNIT VACCINE.

ALCEU KAZUO HIRATA¹; LUIZ EDUARDO TAKANO¹; CARLOS ADELINO DALLE MOLE¹; FABRIZIO MATTɹ; SHERRY LAYTON², LARISSA JUSTINO³, JOÃO VITOR DA SILVA COSTA³, EVELIN LURIE SANO³, ANA ANGELITA SAMPAIO BAPTISTA³

<sup>1</sup>VETANCO DO BRASIL IMPORT AND EXPORT, CHAPECÓ, BRAZIL

<sup>2</sup>VETANCO USA, SAINT PAUL, MN, USA

<sup>3</sup>LABORATORY OF AVIAN MEDICINE, STATE UNIVERSITY OF LONDRINA, LONDRINA, BRAZIL

## INTRODUCTION

Concerns about antimicrobial resistance have led to the search for alternatives that maintain animal health and production efficiency. Probiotics are pointed out as a promising alternative (BERGER and LOEWY, 2024). The early administration of probiotics to animals allows the colonization of the gastrointestinal system and prevents pathogenic bacteria from compromising the host's health (BHOGOJU and NAGASHON, 2022).

Probiotics can be administered in poultry through different routes, however, the application via drinking water deserves attention due to its application logistics. This study was developed with the objective of verifying the cell viability of probiotic strains (Floramax-B11) in the face of the Biotech Vac Salmonella vaccine diluent.

#### MATERIALS AND METHODS

Bacterial: The commercial pool of Lactobacillus spp. (PL)

<u>Trataments</u>: PL was resuspended in various diluents (G1- Vaccine Diluent- BTVS; G2 – Sterile water; G3- PBS) -(0.6g/100mL - 0.6%) and incubated at 23°C for specified time intervals (0, 6, 9 and 12 hours) to evaluate the viability of the constituent bacterial strains.

At the end of each time interval, an aliquot from each treatment was taken, serially diluted, and plated using the spread-plate technique on MRS agar for the determination of colony-forming units (CFU/mL).

Immediately after the preparation of the Lactobacillus strain suspensions, one aliquot (1 mL) from each group was taken for dilution and plating at time zero (0), and the remaining product was incubated at 21-23°C. At each time point, 1 mL from each group was taken for serial dilution and plating to determine CFU/mL. Dilutions and plating were performed in triplicate at each time point.

Data were subjected to analysis of variance (ANOVA), followed by a mean comparison test, adopting a 5% significance level.

#### RESULTS AND DISCUSSION

The results obtained from the assay are presented in Table 1. It was observed that at time zero, there was no significant difference between the treatments, indicating that the dilution PL in the BTVS vaccine does not result in a loss of cell viability of the probiotic strains. However, after 6, 9, and 12 hours of contact dilution between the probiotic strains and the BTVS vaccine, a significant reduction in quantification (Log CFU/mL) of the probiotic strains was observed, suggesting that the vaccine diluent compromises cell viability over time

Table 1. Mean Log CFU/mL of probiotic strains present in the Floramax B11 product after dilution in BTVS, water, and PBS and incubated for different time periods.

Treatment	Time (hours)			
		6	9	12
PL¹+ BTV\$2	6.927 Aa	6.520 Bb	6.223 Bb	6.450 Bb
PL + Sterile water	7.143 Aa	7.053 <b>A</b> a	7.130 Aa	6.953 Aa
FB11 + PB\$3	7.290 Aa	7.140 Aa	7.083 Aa	7.033 Ab

<sup>&</sup>lt;sup>1</sup> Pool Lactocillus; <sup>2</sup>Biotech Vac Salmonella; <sup>3</sup>Phosphate buffered saline;

Means followed by uppercase letters in the same column are significantly different from each other according to the Tukey test (P < 0.05). Means followed by lowercase letters in the same row are significantly different from each other according to the Tukey test (P < 0.05)."

# CONCLUSION

It was concluded that the interaction of probiotic and subunit vaccine did not show significant losses in the recovery of *Lactobacillus* strains during the 12-hour period. Thus, the supply of the associated products to the birds is effective without causing negative interaction between them.

### REFERENCES





