

T128 Effect of a three strain blend of *Bacillus subtilis* on ileal and caecal microbiota dynamics of broilers challenged with *Salmonella* Heidelberg isolated in the south of Brazil

Ricardo Gonzalez-Esquerro*1, Ricardo Mitsuo Hayashi2, Raquel Bighetti Araujo1, Marcelo Falzarella Carazolle3, Eduardo Leonardecz4, Anderson Ferreira da Cunha4, Paulo Monzani4, Juliana Costa de Azevedo2, César Gonçalves de Lima5, Elizabeth Santin2 1 Novus International Inc; 2 Universidade Federal do Paraná; 3 UNICAMP; 4 Universidade Federal de São Carlos; 5 Universidade de São Paulo

The potential of feeding a blend of three strains of *Bacillus subtilis* (SPORULIN® - SPR) on gut microbiota changes was evaluated in broilers challenged with a strain of *Salmonella* Heidelberg (SH) isolated in Brazil. Intestinal samples were collected at 21d of age from birds randomly submitted to 4 treatments and 4 replicates of 12 chicks each: T1) Negative control (NC), T2) SH- challenged birds (SHC), T3) SHC fed 250g of SPR/ton, T4) SHC fed 500g of SPR/ton. At 3d of age, half birds from groups T2, T3 and T4, were orally challenged with 107 CFU/chick of SH. Ileum and cecum contents from 12 birds ([U1] for T2, T3 and T4) were frozen in liquid nitrogen and stored at -80°C. Genomic DNA was purified and pooled to form 4 replicates per treatment. In order to characterize the most abundant bacterial communities, V3 and V4 region of 16S rRNA gene were amplified and sequenced using Illumina MiSeq platform. Bacteria with abundance higher than 1%, *Salmonella* sp., *Bacillus* sp. and ecological indexes were analyzed by ANOVA and Tukey test at $P \leq 0.05$. As expected, microbiota analysis presented a high coefficient of variation illustrating the difficulties in establishing statistical differences when studying changes in bacterial populations, and the need for greater replication. From the 16S rDNA library, an unidentified member (U.m.) from RF39 order in cecum, U.m. of *Enterococcus* genus and U.m. of *Streptophyta* order in ileum showed significant changes ($P < 0.05$) among treatments. *Salmonella* was absent from the ileum but present at the cecum. Feeding 250 or 500g/ton of SPR significantly reduced the frequency of *Salmonella* in cecum, while feeding SPR T 500G/TON INCREASED *Bacillus* spp in ileum. Results on ecological indexes showed that SPR 500g/ton group presented a more diverse microbiota (Shannon-Wiener index) ($p < 0.05$) compared to NC and SHC, while SPR 250g/ton improved richness (Jackknife index) compared to NC. Evenness test (Hill) revealed that SPR 500g/ton tended ($P = 0.08$) to promote higher microbiota heterogeneity than SHC group. SPR at both dosages showed equivalent cecum *Salmonella* sp. abundance in relation to NC and a significant reduction ($P < 0.01$) regarding SHC group. Ileum *Bacillus* sp. abundance increased ($P < 0.01$) compared to SHC when SPR was added at 250 or 500g/ton. In summary, SPR was effective in reducing *Salmonella* in cecum at 250 or 500g/ton, and promotes positive alterations in gut microbiota resulting in a more stable ecosystem which is by itself correlated with better gut health.

Key Words: *Bacillus subtilis*, *Salmonella*, Broilers, Gut health, Gut microbiota