

## **SAL MONELLA TYPHIMURIUM VACCINE TO CONTROL A BRAZILIAN SALMONELLA HEIDELBERG STRAIN IN BROILER CHICKENS**

### **VACINA DE SALMONELLA TYPHIMURIUM PARA CONTROLAR CEPA BRASILEIRA DE SALMONELLA HEIDELBERG EM FRANGOS DE CORTE**

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#### **SUMMARY**

Foodborne *Salmonella* infections in humans, which results from the consumption of contaminated poultry meat and eggs, are a major public health concern. Vaccination of animals against *Salmonella* is one strategy to prevent these infections and reduce the risks to public health. Live attenuated *Salmonella enterica* vaccines can confer protection against salmonellosis by inducing both cell-mediated and mucosal immune responses. This study assessed a live, attenuated *Salmonella enterica* Typhimurium (ST) vaccine in broiler chickens against a heterologous challenge with *Salmonella* Heidelberg (SH) by evaluating bacterial quantification, immune cells infiltration, and cytokine gene expression in the cecum. The treatments were: T1, non-vaccinated, non-challenged; T2, non-vaccinated, SH-challenged; T3, ST-vaccinated and SH-challenged. At 28 days of age, the ST-vaccinated group had significantly recovered reduction of SH in the crop ( $P < 0,01$ ) and cecum ( $P = 0,021$ ) compared to the non-vaccinated SH-challenged group, with no significant changes ( $P > 0,05$ ) in macrophages, T CD4+, or T CD8+ cells dynamics during the same period. Aerosol vaccination on the first day promoted greater interleukin-12 expression in the liver ( $P < 0,05$ ) and interleukin-10 expression and T CD8+ cells in the ileum 16 hours after housing. After prime-boosted oral immunization on the 13th day, the vaccinated group had greater expression of macrophages and T CD4+ cells in the liver ( $P < 0,05$ ) than the control group. Two doses of a live ST-attenuated vaccine promoted a partial cross-protective effect against SH strain UFPR1 challenge in broilers.

**KEY-WORDS:** Interleukins. Lymphocytes. Macrophages. Salmonellosis. UFPR1 strain. Vaccination.

#### **RESUMO**

Infecções por *Salmonella* transmitidas por alimentos como consumo de carne de frango e ovos contaminados em seres humanos constituem um importante problema de saúde pública. A vacinação de animais contra *Salmonella* é uma estratégia para prevenir essas infecções e reduzir o risco para a saúde pública. As vacinas vivas atenuadas de *Salmonella enterica* podem conferir proteção contra a salmonelose, induzindo respostas imunológicas mediadas por células e em mucosas. Este estudo avaliou uma vacina viva e atenuada de *Salmonella enterica* Typhimurium (ST) em frangos de corte contra um desafio heterólogo com *Salmonella* Heidelberg (SH), avaliando a quantificação de *Salmonella*, infiltração de células imunes e a expressão de genes de citocinas no ceco. Os tratamentos foram: T1, não vacinado, não desafiado; T2, não vacinado, desafiado com SH; T3, ST-vacinado, desafiado com SH. Aos 28 dias de idade, o grupo vacinado com ST apresentou significativa redução de SH no papo ( $P < 0,01$ ) e no ceco ( $P = 0,021$ ) comparado ao grupo T2-não vacinado SH-desafiado, sem alterações significativas na dinâmica celular de macrófagos, T CD4+ ou T CD8+ ( $P > 0,05$ ) durante o mesmo período. A vacinação por aerossol no primeiro dia promoveu maior expressão de IL-12 no fígado ( $P < 0,05$ ), maior expressão de IL-10 e células T CD8+ no íleo, 16 horas após o alojamento. Após o reforço de imunização oral ao 13º dia, o grupo vacinado apresentou maior expressão de macrófagos e células T CD4+ no fígado ( $P < 0,05$ ) do que o grupo controle. Duas doses de uma vacina viva atenuada de ST promoveram um efeito de proteção cruzada parcial contra o desafio da cepa de *Salmonella* Heidelberg cepa UFPR1 em frangos de corte.

**PALAVRAS-CHAVE:** Cepa UFPR1. Interleucinas. Linfócitos. Macrófagos. Salmoneloses. Vacinação.

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## INTRODUCTION

Non-typhoidal *Salmonella* is a pathogen, but is also a transient member of the intestinal microbiome of animals, without causing disease (BARROW et al., 1987; MUNIZ et al., 2015). Most types of *Salmonella enterica* does not often affect poultry performance, and as a result, asymptomatic infection may increase the likelihood of zoonotic transmission through the food chain (CARTER et al., 2009). **S. Heidelberg (SH)** ranks among the top three serovars isolated from patients with salmonellosis in North America (CDC, 2014), and causes more invasive infections (e.g. myocarditis and bacteremia) than other non-typhoidal *Salmonella* serovars (HOFFMANN et al., 2014). The Brazilian SH strain (UFPR1) used in this trial was isolated from commercial broiler carcasses in the south of Brazil. A recent study reported its complete genome associated with high and intermediate resistance against short-chain organic acids and some antibiotics, respectively (SANTIN et al., 2017).

Vaccination is one of the best prophylaxes against infectious diseases, and acts by inducing innate and/or adaptive immune responses (GIRARD et al., 2006). There have been several attempts to prevent salmonellosis via vaccination (MATSUDA et al., 2011; NANDRE et al., 2012; WIGLEY et al., 2005), and some studies showed that cross-protection effects enhanced pathogen clearance (SIEVE et al., 2009). More recently, cross-protection immunity among different *Salmonella* serovars has been reported in animals and humans (BEARSON et al., 2016; LI et al., 2016; WAHID et al., 2016).

In recent years, the use of genetically modified *S. Typhimurium* (ST) strains as immunization agents has gained remarkable popularity, since these strains have no

clinical side effects (CHAUDHARI; LEE, 2013) and may provide some protection against other serovars (LEE, 2015). According with HUANG et al., (2016), an attenuated ST vaccine strain has shown to offer protection against *S. Enteritidis* virulent challenge in mice. Another report from LIU et al., (2016) demonstrated that outer membrane vesicles derived from ST mutants with modified LPS induce cross-immunity against other *S. enterica* serovars.

The objective of this trial was to evaluate the ability of a live genetically modified ST *Salmonella* Typhimurium vaccine to reduce *Salmonella* Heidelberg counts in gut and liver, its effects on immune cell dynamics (macrophages, T CD4+ and T CD8+ cells), and changes in interleukin-10 and interleukin-12 in broiler chickens.

## MATERIAL AND METHODS

### Animals and Experimental Design

The experiment was conducted at Center of Immune Response in Poultry (CERIA), at Federal University of Paraná, in Curitiba, Brazil, and was approved by the Agricultural Sector Ethics Committee of the university (process number: 053/2014).

A total of 90 male Cobb 500 broilers ranging from 1 to 28 days of age were distributed in a completely randomized design with three treatments (T) with 30 replicates each, with one bird representing one replicate, as shown in Table 1. All birds used in this trial came from the same broiler breeder flock (which was not immunized with any *Salmonella* vaccine) incubated in the same commercial hatchery.

**Table 1** - Treatment description.

Treatment	ST Vaccine*	Challenge
T1	---	---
T2	---	SH
T3	Two doses* (1 <sup>st</sup> day: spray and 13 <sup>rd</sup> day: drinking water)	SH

\* *Salmonella* Typhimurium AWC591  $\geq 3 \times 10^7$  CFU/dose (Poulvac ST, Zoetis).

The birds were housed in three identical isolated rooms with negative pressure which were located side-by-side and disinfected prior to the study. The birds were individually weighed at the time of housing to ensure uniformity of treatments. The birds were kept at a comfortable temperature according to their age, with water and feed *ad libitum*, for the entirety of the trial. Their diet was based on corn and soymeal, following Brazilian nutritional recommendations for poultry (ROSTAGNO, 2011). The basal diet (containing all the ingredients except amino acids and vitamin and mineral premix) was sterilized in an autoclave at 120°C for 15 minutes. Next, the amino acids and vitamin and mineral premix were added and mixed properly. No ingredient to inhibit *Salmonella* (e.g. organic acids, prebiotics or probiotics) was provided in the drinking water or feed.

### Vaccination and Dosage

Poulvac ST (Zoetis, São Paulo, Brazil) is a commercial vaccine that contains a live non-virulent strain of AWC 591 *Salmonella* Typhimurium (STM-1) in lyophilized form with titer  $\geq 3 \times 10^7$  CFU/dose. This strain was selected for vaccine production because of its colonizing ability, immunogenicity and growth during *in vitro* fermentation. This variant of *S. Typhimurium* was altered by deleting two genes, *aroA* and *serC*. The deletion of these genes results in an organism that retains the structure of the cell wall and flagella, maintaining immunizing antigens intact (ALDERTON et al., 2007). Under an electron microscope, the strain resembles any typical *Salmonella* without any distortions; the vaccine immune response mimics a natural infection. The

mutation of the *aroA* gene impedes the vaccine strain's ability to produce H<sub>2</sub>S in XLD or XLT4 medium, permitting differentiation from the wild type in microbiological analysis.

The animals of group 3 were immediately vaccinated after housing, with Poulvac ST, using a total volume of 20 mL of water sprayed on the 30 chicks (vaccine was diluted in chlorine-free water, with 1 dose/bird). At 13 days of age, the birds in group 3 were given the second dose of vaccine via drinking water.

### *Salmonella enterica serovar Heidelberg*

The UFPR1 strain of *Salmonella* Heidelberg (sequences submitted to the NCBI database/biosample identified as SAMN06560104, GenBank: CP020101) was isolated from commercial broiler carcasses obtained from a broiler farm located in south Brazil. At day one, ten ceca and ten livers were collected from each treatment group to assess the absence of *Salmonella* sp. (qualitative analysis). At 14 days of age, chicks in T2 and T3 groups were orally challenged with 10<sup>8</sup> CFU/chick of SH. At 28 days of age, 10 birds from all treatments were subjected to cervical dislocation and necropsied, and crop, liver and cecum samples were collected to count *Salmonella* sp. The presence or absence of *Salmonella* sp. was confirmed in the crop, and quantified in liver and cecum. In order to quantify typical colonies of *Salmonella* sp. (quantitative analysis), samples were processed using the modified methodology (PICKLER et al., 2012).

### *Macrophage, CD4+ and CD8+ Cell Quantification*

At 16 hours after housing (AH), 14 and 28 days of age, 10 birds from each treatment were euthanized to collect liver (accessory lobe) and ileum (5 cm after Meckel's diverticulum) samples. Immunohistochemistry was performed to obtain macrophage, CD4+, and CD8+ lymphocyte counts (LOURENÇO et al., 2015). The labeled cells were counted using an optical microscope (Nikon Eclipse E200, São Paulo, Brazil) at 100X magnification. Five fields were measured per bird, totalizing 25 microscopic fields per treatment of liver (cells per field) and ileum (villi per field).

### *Cytokine mRNA Expression*

**Table 2** - Sequences of forward and reverse primers of chickens cytokines for RT-qPCR.

Primer	Primer Sequence
IL-10	Forward 5'-CGGGAGCTGAGGGTGAA-3'
	Reverse 5'-GTGAAGAAGCGGTGACAGC-3'
IL-12	Forward 5'-AGACTCCAATGGGCCAAATGA-3'
	Reverse 5'-CTCTTCGGCAAATGGACAGT-3'
GAPDH	Forward 5'-GGTGGTGCTAAGCGTGTTAT-3'
	Reverse 5'-ACCTCTGTCTCTCTCCACA-3'

### *Statistical Analysis*

The data were evaluated using Statistix 9 statistical software (Analytic Software, Tallahassee, FL, USA) and analyzed using the Shapiro-Wilk normality

Six birds per treatment were euthanized 16 hours after housing and samples of their livers and ilea were stored in RNAlater solution (Thermo Fisher Scientific, Waltham, MA, USA) at -20°C until further analysis. Total RNA from the tissues was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. A Turbo-DNase kit (Applied Biosystems, Foster City, CA, USA) was used to treat the samples. RNA concentrations were quantified with a NanoDrop Spectrophotometer (Thermo Scientific, Bonn, Germany) and integrity was determined with a 700-7000 Experion Automated Electrophoresis System (Bio-Rad, Hercules, CA, USA). RNA samples were reverse transcribed and RT-qPCR analysis performed with a MyiQ System (Bio-Rad). One microgram of RNA was converted to cDNA in a 20 µL reaction volume using the iScript Reverse Transcription Supermix kit (Bio-Rad) at 25°C for one hour, 42°C for 30 minutes, and then 85°C for 5 minutes.

The genes analyzed by RT-qPCR were IL-10, IL-12 and GAPDH (primers described in Table 2). The final 20 µL PCR reaction contained 2 µL reverse transcription product, 2 µL of the forward and reverse gene, and 10 µL of iTaq Universal SYBR Green Supermix (Bio-Rad). PCR cycle conditions for all primer pairs used an initial 60s denaturation step at 95°C, followed by 40 cycles of denaturation (15s at 95°C), annealing, and extension (30s at 60°C). The melting profile of each sample was analyzed after every qPCR run to confirm PCR product specificity and was determined by heating samples at 65°C for 30s and then increasing the temperature to 95°C at a linear rate of 20°C/s while continuously monitoring fluorescence. Sample's PCR amplification efficiencies were determined in the log-linear phase with the LinRegPCR program (RAMAKERS et al., 2003). Additionally, the delta-delta equation subtracts sample and reference Ct values from an endogenous control; however, the endogenous control (GAPDH) Ct was affected by treatments in this study (P<0,05), and was therefore removed from the equation. All data were normalized to the mRNA level of the control group (non-challenged group and without SCOA) and reported as the fold-change from the reference, which was calculated as  $E_S^{(40-Ct_{Sample})}/E_R^{(40-Ct_{Reference})}$ , where E<sub>S</sub> and E<sub>R</sub> are the sample and reference PCR amplification efficiencies, respectively (HUMPHREY, 2004).

test. Parametric data were subjected to analysis of variance (ANOVA) and the Tukey test to establish differences between treatment means. Nonparametric data were submitted to the Kruskal-Wallis test at a 5% probability value. When the presence or absence of

*Salmonella* was assayed, the chi-square test was used to establish statistical differences.

## RESULTS

### Microbiology

As expected, the T1 group (negative control) was negative for *Salmonella*, so the data analyzed used the SH-challenged treatments only to obtain a completely randomized design.

In the microbiological analysis (Table 3), it was observed an interesting effect of the ST vaccine against SH challenge. The ST-vaccinated group (T3) had all

negative crop samples for *Salmonella* sp., while the challenged group (T2) reached 20% positive crop samples ( $P < 0,01$ ) at 28 days of age. In liver, no statistical differences were observed between all 3 groups ( $P > 0,05$ ). In ceca, even though there was a significant numerical difference between the T2 and T3 groups (47%), the vaccine did not generate statistical reduction in *Salmonella* counts when all groups were analyzed. However, comparisons between only the challenged groups (T2 and T3) showed a significant ( $P = 0,021$ ) reduction in *Salmonella* recover in the vaccinated group compared with the non-immunized group.

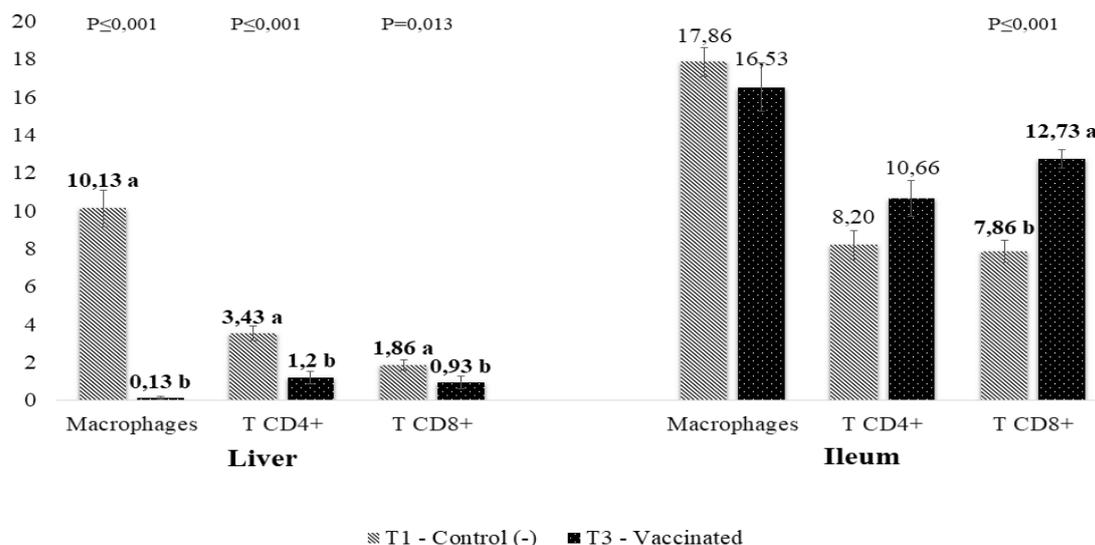
**Table 3** - Percentage of *Salmonella* sp. in crop and counts (Log CFU/g) in liver and cecum at 28 days of age in different treatments. <sup>a,b</sup> Different letters in the same column indicate significant differences at  $P < 0,05$  according to the chi-squared\* and Kruskal-Wallis\*\* tests.

Treatment	Crop*	Liver**	Cecum**	Cecum** (no T1)
	% (+/total)	Log CFU/g	Log CFU/g	Log CFU/g
T1 - Control (-)	0% (0/9) b	0,00 ± 0,00	0,00 ± 0,00 b	-
T2 - Control (+)	20% (2/10) a	1,30 ± 0,48	4,34 ± 1,77 a	4,34 ± 1,77 a
T3 - SH + vaccine	0% (0/10) b	1,10 ± 0,31	2,08 ± 2,30 ab	2,08 ± 2,30 b
P value	≤0,001	0,142	≤0,001	0,021

### Immunogenic profile of the vaccine

After the first immunization with the ST vaccine (aerosol), the T3 group showed a significant decrease in macrophages, T CD4+, and T CD8+ cell counts in the liver ( $P < 0,05$ ) and increased T CD8+ cell mobilization in the ileum at 16 AH ( $P < 0,001$ ) (Figure 1). We also

measured mRNA expression for IL-10 and IL-12 (Tables 4 and 5); T3 group had higher IL-12 mRNA expression in the liver than the T2 group ( $P < 0,05$ ), as well as increased IL-10 mRNA expression in the ileum ( $P < 0,05$ ) at 16 AH.



**Figure 1** - Quantification of macrophages, CD4+ and CD8+ cells in the liver (cells per field) and ileum (cells per villi) at 16 hours after housing (AH) in the T1 and T3 groups. <sup>a,b</sup> Different letters in the same column are significantly different at  $P \leq 0,05$  according to the Kruskal-Wallis test.

**Table 4** - Mean and standard error for quantification of IL-10 and IL-12 in the liver at 16 hours after housing (AH) in different treatments.

Treatment	IL-10	IL-12
Control (-)	3,45 ± 1,62	0,11 ± 0,01 b
Vaccinated	1,23 ± 0,63	3,93 ± 0,10 a
<b>P value</b>	<b>0,157</b>	<b>0,049</b>

<sup>a,b</sup> Different letters in the same column are significantly different at P<0,01 as determined by the Kruskal-Wallis test.

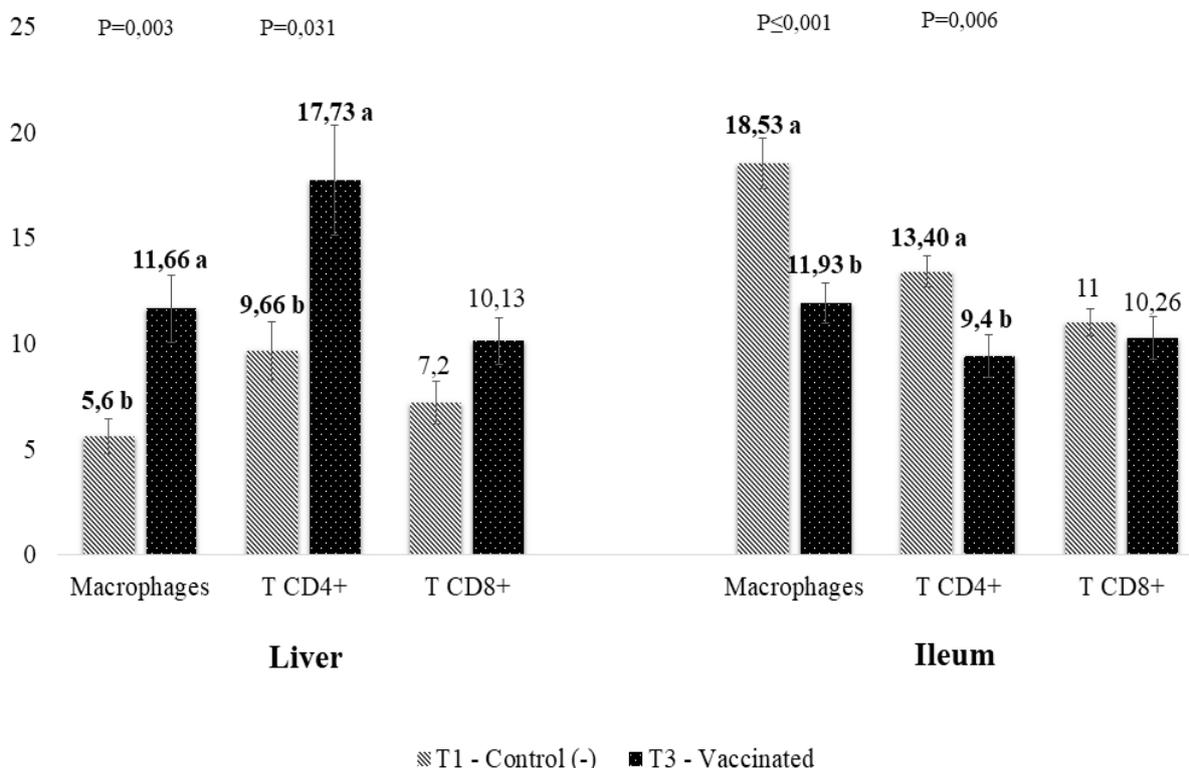
**Table 5** - Mean and standard error for quantification of IL-10 and IL-12 in the ileum at 16 hours after housing (AH) in different treatments.

Treatment	IL-10	IL-12
Control (-)	4,50 ± 0,23 b	231,79 ± 12,76
Vaccinated	31,14 ± 6,16 a	218,29 ± 79,91
<b>P value</b>	<b>0,040</b>	<b>0,222</b>

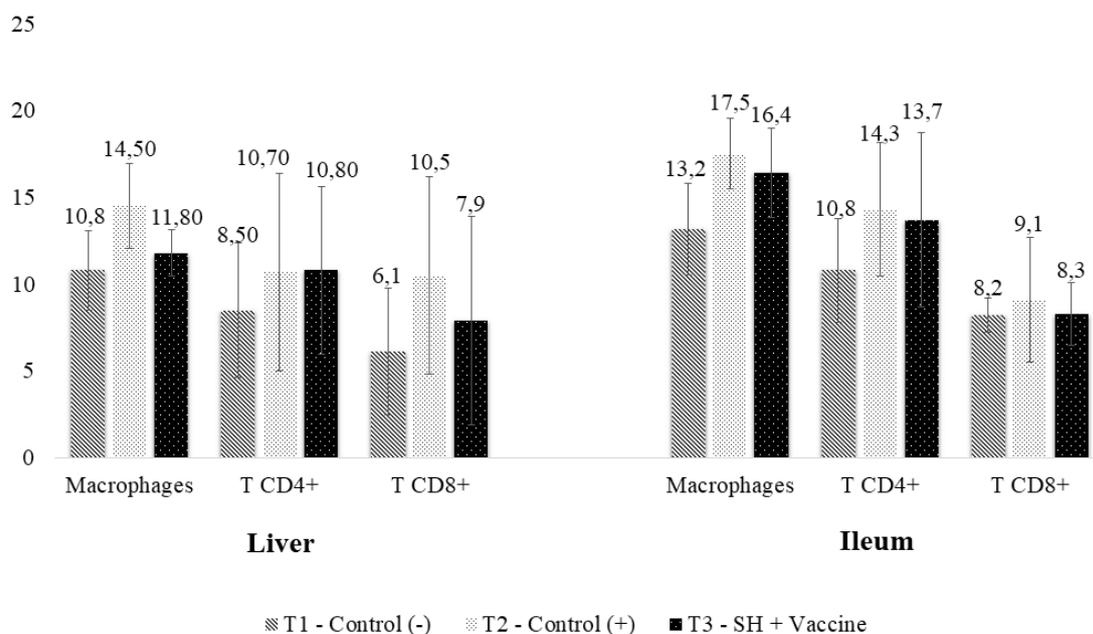
<sup>a,b</sup> Different letters in the same column are significantly different at P<0,01 as determined by the Kruskal-Wallis test.

At 14 days of age, 24h after the second immunization in the drinking water, expression of macrophages and T CD4+ cells in the liver increased in the vaccinated group (P<0,05), while there were fewer macrophages and CD4+ cells in the ileum mucosa (P<0,05) (Figure 2) compared to challenge non-vaccinated group.

At 28 days of age, 14 days after T2 and T3 were challenged with SH, no differences (P>0,05) were observed in the immune cells dynamics in the liver and ileum between all 3 groups (Figure 3).



**Figure 2** - Quantification of macrophages, CD4+ and CD8+ cells in the liver (cells per field) and ileum (cells per villi) at 14 days of age in the T1 and T3 groups. <sup>a,b</sup> Different letters in the same column are significantly different at P≤0,05 as determined by the Kruskal-Wallis test.



**Figure 3** - Quantification of macrophages, CD4+ and CD8+ cells in the liver (cells per field) and ileum (cells per villi) at 28 days of age in the T1, T2 and T3 groups. <sup>a,b</sup> Different letters in the same column are significantly different at  $P \leq 0,05$  as determined by the Kruskal-Wallis test.

## DISCUSSION

Previously, live *Salmonella* vaccines were reported to induce cross-immunity against related serovars (MATULOVA et al., 2013; MOHLER et al., 2008). Although ST infection is common in chickens, it does not cause severe disease in poultry (CHAPPELL et al., 2009); consequently, attenuated ST strains have been used successfully to express foreign antigens and have been suggested as safe strains (LEE, 2015). In this study, administering a ST vaccine did not induce mortality or any adverse effects in the vaccinated birds.

Our results demonstrated that immunizing broiler chickens with ST vaccine conferred a certain degree of protection against challenge with SH strain, since the SH count in the ceca of the vaccinated birds was statistically identical to the count in the non-challenged group. Vaccination significantly reduced *Salmonella* counts in the crop (all negative samples) and the cecum (47%) at 28 days of age, demonstrating the cross-protective effect of the ST vaccine against SH infection. Lee (2015) demonstrated a similar effect using a live attenuated ST strain to decrease *Salmonella* Enteritidis and *Salmonella* Gallinarum in laying hens. Another interesting study has shown that a vaccine consisting of attenuated *S. Enteritidis*, *S. Typhimurium* and *S. Infantis* protected chickens against challenge with the wild type strains of the same serovars and also partially protected chickens against challenge with isolates from serovars Dublin or Hadar (VARMUZOVA et al., 2016). Homologous immunity between strains of the same serovar is known to be considerably stronger than between strains of different (heterologous) serovars (SPRINGER et al., 2000). It seems likely that lipopolysaccharide (O-antigen) is a major component of the key immunogenic component and that protection

between strains within a serovar is likely to be much greater. For this trial, both ST (antigenic formula 4,5,12:i:1,2) and SH (antigenic formula 1,4,[5],12:r:1,2) share the same antigen (O:4), which belongs to serogroup B. Because the Poulvac ST vaccine is developed from a strain of *Salmonella enterica* subsp. *enterica* serovar Typhimurium (STM-1), this serovar contains immunological antigens which are the same as those seen in other group B *Salmonellae* (such as SH). This similarity in the somatic and flagellar antigens explains the good heterologous immune response (MUNIZ et al., 2017).

Previous studies with aerosol vaccination (ATTERBURY et al., 2010; DE CORT et al., 2015) showed that multiple sites including the digestive tract, respiratory tract, and conjunctiva are stimulated during aerosol vaccination, resulting in a systemic immune response, while oral administration of the vaccine stimulates a localized immune response in the intestinal tract. De Cort et al. (2015) also suggested that oral immunization with live attenuated vaccine induces a mechanism that inhibits colonization and protects the bird from subsequent challenge, since young chickens are still immature and vaccination is not effective protection against *Salmonella* infection during the first days of life.

In this present study, aerosol vaccination on the first day promoted increased IL-12 gene expression in the liver. IL-12 is an interleukin which is naturally produced by dendritic cells, heterophiles, and macrophages, leading to a pro-inflammatory response (XING et al. 2000). Even though IL-12 is known to stimulate T cells, we did not observe enhancements in the macrophages, T CD4+, and T CD8+ cells in this organ at 16 AH, probably because more time is needed to see this effect on the tissue.

At the same time, we found higher mRNA expression for IL-10 and more T CD8+ cells in the ileum

in the vaccinated group than in the control group. IL-10 is generally characterized as a regulatory cytokine because of its role in mediating tissue protection, limiting pro-inflammatory responses and preventing autoimmune diseases (OUYANG et al., 2011). In some situations, IL-10 could act as a chemoattractant to CD8<sup>+</sup> T cells (JINQUAN et al., 1993; XI et al., 2017), activate cytotoxic T cell activity (SANTIN et al., 2000), and increase the maturation of CD8<sup>+</sup> T memory cells (LAIDLAW et al., 2015). Shanmugasundaram et al., (2015) and Kogut et al., (2016) have observed that T-regulatory cells increase expression of IL-10 cells after *Salmonella* infection, which suggests a “tolerogenic” response allowing the bacteria to persist in the lumen. Like the vaccine, this could be a mechanism to inhibit colonization, since maintaining the live vaccine in the lumen could have inhibited colonization after the *Salmonella* challenge.

At 14 days of age, the vaccinated exhibited a higher number of macrophages and T CD4<sup>+</sup> cells in the liver group after prime-boosted oral immunization on the 13th day, compared to the control group. Vendrell et al. (2016) reported that immunization with *Salmonella* Typhi could elicit an early immune response in the liver, associated with increased T CD4<sup>+</sup> and dendritic cell populations in mice. Based on these findings, we hypothesized that 24 hours after the booster vaccine was administered via drinking water, the immune cells continuously migrate to the liver and maintain a systemic and specific immune response, even though *Salmonella* recovery was not statistically reduced in this organ at 28 days. Muniz et al. (2017), in a study of the same live ST vaccine administered via spray on the first day, observed reduced SH recovery in the ceca of 21-day-old broilers, results similar to those obtained herein.

No statistical differences were observed in the liver and ileum for macrophages, T CD4<sup>+</sup>, and T CD8<sup>+</sup> counts at 28 days of age. At this time, *Salmonella* control was statistically lower in the crop and ceca compared to the SH group. Protection from live *Salmonella* vaccine is attributed to induction of cellular and humoral immune responses (BEAL et al., 2006). Even though the dynamics of innate and adaptive immune response measured in this trial did not change, we suspect and highlight the role of humoral immune response and/or inhibition of colonization. Nandre et al. (2015) observed that *Salmonella* Enteritidis vaccination induced significantly higher levels of systemic Ig and mucosal sIgA antibodies, and that these levels were markedly increased after booster vaccination. This situation is the result of immunological memory, defined as a faster and stronger immune response after exposure to the same or a related antigen (DUTTON et al. 1998). Systemic antibodies are essential to kill *Salmonella*, since IgA is more likely to be effective in providing a protective immune response after secretion into the gut lumen (BERTHELOT-HÉRAULT et al., 2003), while gut mucosal sIgA prevents the bacterium from entering the intestinal epithelium (STRINDELIUS et al. 2004). The colonization inhibition phenomenon has been described by Barrow et al. (1987) as a primarily microbial physiological process, and not the result of adaptive immunity or bacteriophage/bacteriocin activity. Methner et al. (2011) has stated that this

mechanism is poorly understood, and that inhibition between different strains within the same O-group did not exceed a value of 2 log<sub>10</sub> units compared with the control, which is approximately the same level we observed in our study comparing ST-vaccinated and non-vaccinated SH-challenged birds.

## CONCLUSION

In summary, the two administrations of a live attenuated ST vaccine was able to reduce *Salmonella* recovery in the crop and cecum of broilers challenged with the SH strain UFPR1, but did not reduce these levels in the liver. This partially cross-protective effect may be related to the physiological microbial process as well as an early increase of IL-12 in the liver and IL-10 in the intestine, but further studies should be conducted to better understand this associated mechanism.

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