

Cellular immune response after vaccination with SG 9R in birds fed Aflatoxin and a mycotoxin absorbent in the diet

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INTRODUCTION

Most developed countries around the world have eradicated *Salmonella Gallinarum* (S.G.) and *S. Pullorum* (S.P.) from commercial flocks, and now concentrate mainly in the prevention of infections caused by the paratyphoid group. However, a high percentage of the commercial layer's world population is still affected by S.G., therefore vaccination with a live attenuated vaccines of *S. Gallinarum* (SG 9R) is a strategy frequently used in some areas. After vaccination, developing immunity is critical to get the right protection against field challenge. One serious factor affecting the development of immunity is the presence of mycotoxins, including Aflatoxin (AFL). The objectives of this experiment were threefold: measuring the immune response in birds vaccinated with SG 9R, quantifying the impact of AFL on the immune response, and determining if the mycotoxin absorbent used had an effect on the immune response.

MATERIALS AND METHODS

This experiment was conducted at Imunova Laboratory in the Federal University of Paraná (UFPR) in Brazil. A total of 240-day-old commercial baby chicks (Hendrix®, ISA- Brown®) free of *Salmonella*, were raised in isolation units since day one, to avoid cross contamination among treatments. All birds had *ad libitum* access to feed and water. Pullets were divided in a complete randomized design with 8 treatments of 26 birds each. T1 was the negative control (no vaccine, no AFL, no absorbent), T2 was vaccinated, intoxicated (2.5 ppm), and treated with the absorbent (2.5 kg/ton), T3 was only vaccinated, T4 was vaccinated and intoxicated (2.5 ppm), T5 was only intoxicated with AFL (2.5 ppm), T6 intoxicated (2.5 ppm) and treated with the absorbent (2.5 kg/ton), T7 was vaccinated and intoxicated with AFL (1 ppm), and T8 was vaccinated, intoxicated (1 ppm), and treated with the absorbent (1 kg/ton).

The feed was analyzed to detect any possible contamination with *Salmonella* or AFL. SG 9R®, was obtained from Merck Animal Health, USA. AFL was obtained from LAMIC, Federal University of Santa Maria in Brazil and mixed with the feed using two inclusion rates. Mycoad®, the mycotoxin absorbent (clay) was added using two inclusion rates, and bought from Special Nutrients in Florida, USA. The immune response was measured with flow cytometry (FACSCalibur®/ Becton Dickinson). The data obtained was analyzed with software from one of the following companies: FlowJo® (TreeStar) or WinMDI 2.9 (Joseph, Trotter). Four types of T lymphocytes were measured with flow cytometry. Body weight and mortality were recorded during the duration of the trial and all birds were euthanized at the end of the trial. After *post mortem* sessions were performed, macroscopic changes were recorded and histopathological studies were performed at UFPR.

IMMUNE RESPONSE/BLOOD RESULTS

The statistical analyses were performed separating the results for each blood collection. All treatments were compared against each other. An analysis of variance (Anova) with the method of Fisher PLSD (P 0,05) comparing the media of all groups among themselves was done. StarView® (SAS Institute, Inc.) was the statistical program used for this evaluation.

Table 1. Summary of activities during the whole experiment.

Day	Activity
1	• Placement in isolation units • Aflatoxin mixed with the ration (T2, T4, T5, T6, T7, T8) • Mycoad mixed with the ration (T2, T6, T8) • Weigh birds
28	• Vaccination via subcutaneous injection with SG 9 R (T2, T3, T4, T7, T8)
31	• Weigh birds • Blood samples collection (26 birds/treatment)
41	• Blood samples collection (26 birds/treatment)
42	• Weigh birds
49	• Blood samples collection (26 birds/treatment) • Birds euthanized (all)

0.2 ml of SG 9R vaccine with a titer of 10⁴ colony forming units (CFU) was injected subcutaneously

RESULTS/DISCUSSION

Performance. The control treatment (T1), vaccinated treatment (T3), and the vaccinated/intoxicated treatment treated with Mycoad-1 kg/mt (T8) showed the same statistically significant body weight during the whole experiment (table2). At 31 and 42 days, the treatment only intoxicated with AFL (T5), showed a significantly lower body weight than the control (T1), vaccinated treatment (T3), and the vaccinated/intoxicated treatment treated with Mycoad-1 kg/mt (T8). No mortality was reported in any of the groups. All intoxicated treatments not treated with the absorbent showed a significant reduction in body weight when compared to the non-intoxicated birds. This reduction in body weight was also reported in young commercial layers in a previous publication (4).

Macroscopic observations. Vaccinated birds (T3) showed enlarged livers, slightly greenish with rounded lobules (picture 1). Birds intoxicated with AFL (T5) and birds intoxicated and vaccinated (T4, T7) showed a fragile liver with a yellowish appearance and the presence of multiple red dots. Other groups didn't show any lesions.

Microscopic observations. Histopathological evaluation of the liver in T5 showed the typical lesions produced by an intoxication with AFL, that is, lipidic vacuolization of the hepatocytes, areas of necrosis and hypertrophy of the biliary ducts.

Immune response. This experiment shows a significant reduction in the concentration of helper T lymphocytes in the treatments intoxicated with AFLA. Peripheral helper T lymphocytes (table 3) and mucosal auxiliary T lymphocytes (table 4) showed a significant reduction when comparing the vaccinated treatment (T3) with the vaccinated and intoxicated treatment (T4). A significant reduction in the concentration of cytotoxic T lymphocytes (tables 5 and 6) is observed at 42 days. This reduction is also observed when comparing the vaccinated treatment (T3) with the vaccinated and intoxicated treatment (T4). Other researchers (3) have reported that the inclusion of corn naturally contaminated with AFL and Fumonisin significantly decreased CD4 + and CD8 + T lymphocytes in the intestinal mucosa of broilers. Feeding naturally contaminated corn with *Fusarium* toxins, another report (2) found a reduction in white blood cells, B lymphocytes, CD4 + and CD8 + T lymphocytes.

Picture 1. Control (T1) vs. vaccinated birds (T3) at 49 days of age.

Picture 2. Treatments intoxicated with AFL (T4, T5, T7), without the inclusion of Mycoad vs. treatments exposed to AFL and fed the absorbent (49 days of age).

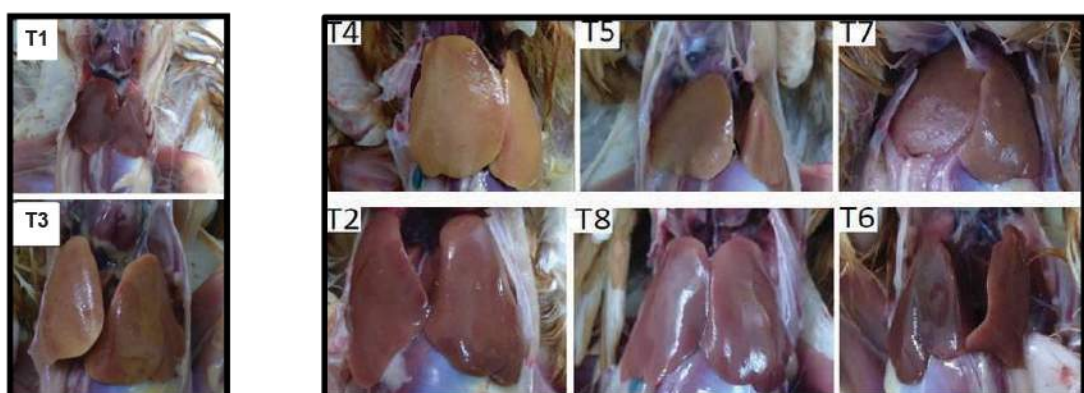


Table 2. Average body weight of commercial brown pullets at different ages.

	Treatments			Age		
	Vaccine	AFLA	Absorbent	1 Day	31 Days	42 Days
T1	-	-	-	42,2 ^a	415,7 ^a	505,1 ^a
T2	SG 9R	2,5 PPM	2,5 kg	42,84 ^a	316,6 ^b	444,7 ^a
T3	SG 9R	-	-	41,3 ^a	371,7 ^a	483,2 ^a
T4	SG 9R	2,5 PPM	-	41,87 ^a	221,8 ^c	322,2 ^b
T5	-	2,5 PPM	-	41,07 ^a	270,7 ^c	360,7 ^b
T6	-	2,5 PPM	2,5 kg	43,39 ^a	338,3 ^b	422,5 ^b
T7	SG 9R	1 PPM	-	44,01 ^a	369,7 ^b	452,3 ^a
T8	SG 9R	1 PPM	1 kg	42,95 ^a	412,8 ^a	481,5 ^a

^{a, b, c} Values within one column with different letters are significantly different (P ≤ 0,05).

Table 3. Percentage of peripheral auxiliary T lymphocytes (CD4+TCRVβ1-) in blood.

	Treatments			Blood collections		
	Vaccine	AFLA	Absorbent	31 Days	42 Days	49 Days
T1	-	-	-	11,36 ± 8,99 ^a	7,38 ± 4,11 ^a	2,01 ± 1,17 ^a
T2	SG 9R	2,5 PPM	2,5 kg	1,45 ± 1,17 ^b	5,96 ± 1,72 ^a	1,64 ± 0,47 ^a
T3	SG 9R	-	-	3,11 ± 0,98 ^a	4,99 ± 1,41 ^a	2,00 ± 0,83 ^a
T4	SG 9R	2,5 PPM	-	2,78 ± 2,23 ^b	3,11 ± 0,45 ^b	1,71 ± 0,50 ^a
T5	-	2,5 PPM	-	11,91 ± 7,82 ^a	4,40 ± 1,34 ^a	1,37 ± 0,40 ^a
T6	-	2,5 PPM	2,5 kg	5,44 ± 5,76 ^a	5,47 ± 2,07 ^a	1,22 ± 0,37 ^a
T7	SG 9R	1 PPM	-	3,10 ± 2,15 ^a	5,16 ± 2,84 ^a	1,26 ± 0,30 ^a
T8	SG 9R	1 PPM	1 kg	2,80 ± 1,89 ^a	4,33 ± 1,27 ^a	1,30 ± 0,51 ^a

^{a, b} Values within one column with different letters are significantly different (P ≤ 0,05).

Table 4. Percentage of mucosal auxiliary T lymphocytes (CD4+TCRVβ1+) in blood.

	Treatments			Collections		
	Vaccine	AFLA	Absorbent	31 Days	42 Days	49 Days
T1	-	-	-	12,77 ± 5,86 ^a	0,92 ± 0,32 ^a	5,17 ± 3,20 ^a
T2	SG 9R	2,5 PPM	2,5 kg	7,69 ± 5,58 ^a	0,77 ± 0,32 ^a	3,46 ± 0,81 ^a
T3	SG 9R	-	-	9,74 ± 6,34 ^a	0,66 ± 0,34 ^a	4,46 ± 2,22 ^a
T4	SG 9R	2,5 PPM	-	3,35 ± 0,91 ^b	0,46 ± 0,15 ^b	3,90 ± 1,21 ^a
T5	-	2,5 PPM	-	4,22 ± 2,71 ^b	0,74 ± 0,28 ^a	4,85 ± 1,56 ^a
T6	-	2,5 PPM	2,5 kg	3,87 ± 3,15 ^b	1,02 ± 0,42 ^a	3,81 ± 1,05 ^a
T7	SG 9R	1 PPM	-	4,59 ± 4,08 ^b	0,87 ± 0,55 ^a	3,86 ± 0,88 ^a
T8	SG 9R	1 PPM	1 kg	3,64 ± 3,26 ^b	0,75 ± 0,19 ^a	3,81 ± 1,46 ^a

^{a, b} Values within one column with different letters are significantly different (P ≤ 0,05). Mean ± Standard Deviation.

Table 5. Percentage of mucosal cytotoxic T lymphocytes (CD4-TCRVβ1+) in blood.

	Treatments			Collections		
	Vaccine	AFLA	Absorbent	31 Days	42 Days	49 Days
T1	-	-	-	2,61 ± 2,13 ^a	1,62 ± 0,58 ^a	3,11 ± 1,96 ^a
T2	SG 9R	2,5 PPM	2,5 kg	0,83 ± 0,61 ^a	1,23 ± 0,71 ^a	1,85 ± 0,56 ^a
T3	SG 9R	-	-	2,15 ± 1,81 ^a	1,33 ± 0,62 ^a	2,33 ± 1,22 ^a
T4	SG 9R	2,5 PPM	-	2,38 ± 1,99 ^a	0,64 ± 0,17 ^b	1,71 ± 0,76 ^a
T5	-	2,5 PPM	-	1,43 ± 0,99 ^a	1,25 ± 0,47 ^a	2,00 ± 0,69 ^a
T6	-	2,5 PPM	2,5 kg	1,73 ± 1,03 ^a	1,15 ± 0,27 ^a	1,60 ± 0,76 ^a
T7	SG 9R	1 PPM	-	2,09 ± 1,52 ^a	1,28 ± 0,68 ^a	1,51 ± 0,26 ^b
T8	SG 9R	1 PPM	1 kg	1,71 ± 1,61 ^a	1,32 ± 0,42 ^a	2,48 ± 1,12 ^a

^{a, b} Values within one column with different letters are significantly different (P ≤ 0,05). Mean ± Standard Deviation.

Table 6. Percentage of activated cytotoxic T lymphocytes (CD8+CD28-) in blood.

	Treatments			Collections		
	Vaccine	AFLA	Absorbent	31 Days	42 Days	49 Days
T1	-	-	-	9,53 ± 7,38 ^a	1,26 ± 0,97 ^a	2,53 ± 1,27 ^a
T2	SG 9R	2,5 PPM	2,5 kg	0,78 ± 0,68 ^b	1,11 ± 0,49 ^a	0,87 ± 0,27 ^b
T3	SG 9R	-	-	2,24 ± 0,90 ^b	1,36 ± 0,97 ^a	1,00 ± 0,46 ^b
T4	SG 9R	2,5 PPM	-	1,51 ± 1,13 ^b	0,59 ± 0,15 ^b	1,38 ± 0,63 ^a
T5	-	2,5 PPM	-	9,86 ± 5,80 ^a	2,00 ± 1,17 ^a	2,78 ± 1,21 ^a
T6	-	2,5 PPM	2,5 kg	1,58 ± 1,72 ^b	1,14 ± 0,43 ^a	1,92 ± 0,62 ^a
T7	SG 9R	1 PPM	-	1,50 ± 1,52 ^b	1,31 ± 1,04 ^a	1,74 ± 0,56 ^a
T8	SG 9R	1 PPM	1 kg	1,08 ± 0,99 ^b	1,29 ± 0,49 ^a	1,86 ± 0,57 ^a

^{a, b} Values within one column with different letters are significantly different (P ≤ 0,05). Mean ± Standard Deviation.

CONCLUSIONS

SG 9R vaccination and the inclusion of a mycotoxin binder in birds vaccinated and intoxicated did not have a significant negative effect on body weight. SG 9R vaccination promoted a statistically significant reduction in the concentration of activated cytotoxic lymphocytes T (CD8-CD28-). This marker can be measured as a parameter before and after vaccination in commercial layers. AFL showed an important suppressive effect on mucosal auxiliary T lymphocytes (CD4+TCRVβ1+), peripheral auxiliary T lymphocytes (CD4+TCRVβ1-), and mucosal cytotoxic lymphocytes (CD4-TCRVβ1+) in vaccinated birds. A significant difference (p<0,05) was detected in blood samples taken 3 and 14 days after vaccination. The inclusion of 2.5 kg/ton of the mycotoxin absorbent in feed prevented the negative effects on the immune parameters already mentioned. A significant increase (p< 0,05) in the concentration of mucosal auxiliary T lymphocytes (CD4+TCRVβ1+), auxiliary peripheral T lymphocytes (CD4+TCRVβ1-) and mucosal cytotoxic T lymphocytes (CD4-TCRVβ1+) was detected at 42 days of age.

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