Infection of Commercial Laying Hens with *Salmonella* Gallinarum: Clinical, Anatomopathological and Haematological Studies

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ABSTRACT

This study aimed at evaluating the susceptibility of commercial laying hens to *Salmonella* Gallinarum (SG). Two experiments were carried using a mutant strain of *Salmonella* Gallinarum resistant to nalidix acid (SGNAL^r). In the first trial, the resistance of birds was evaluated based on clinical signs, faecal shedding, and mortality. It was carried out with six lines of commercial layers being three light white layers, considered to be resistant to SG (W1, W2, W3), and three semi-heavy brown varieties (B1, B2, B3), considered susceptible to SG. Each group contained 15 one-day-old birds. Hens were inoculated in the crop at 5 days of age with 0.2 mL of SGNAL^r neat culture. In addition, to each brown variety, a new group of 15 birds was challenged with 0.2mL of the same SGNAL^r culture diluted at 10^{-3}. At the end of the first experiment, the surviving birds were sacrificed, and microbiological culture of liver and spleen was performed. In the second experiment, white and brown birds were inoculated with neat culture at five days of age. Samples were collected for evaluation of blood parameters and histopathology assessment at 1, 3, 5, 7, 9, 12, and 14 days post-infection. The results of the first experiment showed higher resistance of white birds (p<0.05), although there was no uniformity in the responses against fowl typhoid among the birds within these groups. In the second experiment, there were differences between white and brown birds both in blood parameters and in organ lesion intensity.

INTRODUCTION

Fowl typhoid is caused by *Salmonella* Gallinarum, and it is a serious systemic disease that affects birds. According to Shivaprasad (2000), fowl typhoid causes clinical signs in young and adult birds, including anorexia, diarrhea, dehydration, weakness, as well as gross and microscopic lesions in organs, such as spleen, liver, ovary, heart etc.

Fowl typhoid has a very complex epidemiology. Birds are mainly contaminated by horizontal route. The contact between healthy and sick birds, cannibalism, and the presence of dead birds, wild birds, and workers contribute for dissemination of *Salmonella* Gallinarum in flocks (Berchieri, 2000a).

Therefore, *Salmonella* Gallinarum affects production parameters, resulting in high mortality and lower laying rates, causing economic losses (Pomeroy, 1987; Berchieri, 2000a; Shivaprasad, 2000). Although vaccination programs against this disease have been adopted, fowl typhoid is still reported in Mexico, Central America, South America, Africa, India, and South Korea (Berchieri, 2000a; Shivaprasad, 2000; Ji-Dong *et al.*, 2006).

The knowledge on the resistance mechanisms of birds to this infection is limited. The resistance of some birds to salmonellosis may be related

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to their ability to host the bacteria, and to control bacterial growth inside the cells of the reticuloendothelial system, and also fecal shedding, without eliciting clinical signs. Some studies indicate that resistance to salmonellosis occurs due to a better ability of the phagocytic mononuclear system in restraining infection onset (Qureshi, 1998; Wigley *et al.*, 2002). Nevertheless, doubts still exist whether such efficacy is due to the presence of a high number of phagocytic cells at infection onset, or if the number of cells involved, more than the type of cells, is what really determines the success in clearing up the disease (Qureshi, 1998).

Some studies describe the differences in bird resistance to the infection caused by *S.* Gallinarum (Oliveira *et al.*, 2005; Prince, 1966; Wigley *et al.*, 2002). However, the information provided by such sources are not useful to currently produced birds . The modern poultry industry demands better animal performance, which in turn compromises the immune system of birds, adversely affecting their resistance to diseases. The objectives of the present study were to evaluate layer genetic lines considered to be resistant and susceptible to fowl typhoid, taking into account mortality, fecal shedding, clinical signs, macroscopic and microscopic lesions, as well as to monitor changes in hematological parameters of birds experimentally challenged with *S.* Gallinarum.

MATERIAL AND METHODS

Inoculum

In both experiments, birds were challenged using a *Salmonella enterica* serovar Gallinarum strain resistant to nalidixic acid (SGNal^r). The strain was prepared, and kept at the Department of Animal Pathology, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (FCAVJ-UNESP), according to the methodology described by Smith (1955) and Berchieri *et al.* (2001).

Bacteria were grown in nutrient broth, and incubated with shaking at 37°C for 24 h. The culture contained approximately 3.3×10^8 colony forming units per milliliter (CFU/mL).

Experiment 1

One hundred and thirty-five females of six commercial layer genetic lines were obtained at 1 day of age from commercial hatcheries. Three light white (considered to be resistant) and three semi-heavy brown lines (considered to be susceptible to fowl

typhoid) were used. Chicks were housed in battery cages with electrical heating, located at the isolating units of the Laboratory of Animal Pathology of the Department of Animal Pathology of FCAVJ-UNESP. Food and water were provided *ad libitum*. A fast seroagglutination test was performed, and cloacal swabs were taken immediately after the birds arrived in order to confirm that they were free from *Salmonella*.

Birds were randomly distributed into nine groups. Fifteen birds from light lines (W$_1$, W$_2$, and W$_3$) were inoculated in the crop at five days of age with 0.2 mL of SGNal^r neat culture (3.3×10^8 CFU /mL). Each semi-heavy variety (B$_1$, B$_2$, and B$_3$) was divided into two groups of 15 birds each. The birds of first one group were inoculated with the neat culture of SGNAL^r, whereas the birds of the second one group were inoculated with 0.2 mL of the culture diluted at 10^{-3} (approximately 3.3×10^5 CFU SGNal^r/mL). Bacteriology tests were performed twice a week using cloacal swabs. The surviving birds were sacrificed at 23 days post-infection, and swab samples were taken from the liver and the spleen. Bacteriological examination of these samples was carried out according to a previously described methodology (Berchieri *et al.*, 2001). Briefly, the swabs were placed in tubes with 2.0 mL of Selenite-Novobiocin broth (SN), and then streaked onto brilliant green agar with nalidixic acid (25μg/mL) and Novobiocin (1μg/mL) (BG Nal Nov). Both the plate and the tube were incubated at 37°C for 24 hours. In the absence of growth, new plating was performed from the incubated swab. During the experimental period, clinical signs and mortality were daily recorded.

Experiment 2

In this experiment, 160 one-day-old male birds were used, belonging to W$_1$ (white) and B$_3$ (brown) commercial genetic lines. Birds were housed in battery cages with electrical heating, located at the isolation units of the Laboratory of Pathology of FCAVJ-UNESP, and received water and food *ad libitum*. The absence of *Salmonella* was confirmed as described above.

Fifty semi-heavy birds and 50 light birds were housed in separate rooms, and challenged with SGNal^r at five days of age. They were orally inoculated with 0.2 mL of neat culture SGNal^r in the crop. Thirty birds of each strain were housed separately, and used as non-inoculated controls. In order to perform blood analyses and evaluation of gross and microscopic lesions, five birds from each group were randomly selected to collect blood samples and samples of the following organs: brain, cerebellum, heart, lungs, kidney, liver,

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spleen, thymus, bursa of Fabricius, duodenum, and cecal tonsils. The first sampling was performed one day after infection, and then every 48 hours, with a total of seven samplings for light birds and five samplings for semi-heavy birds. Such difference was due to higher mortality presented by the semi-heavy birds. Blood for hematological analyses was also collected 1 day before experimental inoculation.

Blood samples were collected using anticoagulant (0.1 mL heparin/ mL blood). Globular volume (GV) was determined using microhematocrit method (Jain, 1986), hemoglobin (Hb), according to the cyanomethemoglobin technique (Jain, 1986), and red cell and white cell counts using a Neubauer hemocytometer (Natt & Herrick, 1952). Differential leukocyte count was performed using blood smears stained according to the Rosenfeld method (Lucas & Jamroz, 1961). Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (CHCM), and mean corpuscular volume (MCV) were then calculated (Jain, 1986; Lucas & Jamroz 1961). Albumin concentration (Alb) and the activity of the enzyme aspartate aminotransferase (AST) were determined using commercial kits and a semi-automatic spectrophotometer*, according to bromocresol green and Reitman-Frankel methods, respectively.

Organs were fixed for at least 24 hours in 4% buffered formaldehyde, pH 7.2, at a ratio of 20:1 (buffer:organ fragment). Fragments were further processed at the Histopathology Laboratory of FCAVJ-UNESP according to routine methods (Behmer *et al.*, 1976); 4-µm sections were taken and stained with hematoxylin-eosin. The stained sections were analyzed under a light microscope equipped with a digital camera (NICKON ECLIPSE E 200 x COOLPIX 5400)

Statistical analysis

Chi-square was used to determine differences in mortality among the groups in the first experiment. In the second experiment, the Tukey Studentized Range test (HSD) was used to determine mean, standard deviation, analysis of variance, and the significant differences among groups. In both experiments, the Statistical Analysis System (SAS) software was used to perform the analyses, and differences were considered significant at a 5% level.

RESULTS

In the first experiment, serum agglutination test evidenced that the birds were free from *Salmonella* when they arrived. Higher mortality ($p<0.05$) was observed in the semi-heavy bird group as compared to the light birds when the neat culture was used as inoculum (3.3 x10^8 CFU SGNal^r/ml). On the other hand, there were no significant differences between groups ($p>0.05$) when the diluted inoculum was used. Mortality was different ($p<0.05$) between W_1 and W_3 (light lines); mortality rate was 40% in W_3 and 7% in W_1. There was no statistical difference in mortality among semi-heavy lines inoculated with neat culture ($p>0.05$), neither there were differences when diluted culture was used as inoculum. Mortality rate varied between 7% and 40% in light birds, and between 40 and 100% in semi-heavy birds (Table 1).

Bacteriological assessment of feces showed the presence of *Salmonella* in one semi-heavy bird (B_3) 7 days post-inoculation; sampling was performed just before the bird died. At 23 days post-inoculation, SGNal^r was isolated from the liver of 5/12, 4/9, 2/14 of the resistant W_2, W_3 and W_1 lines, respectively, whereas only one semi-heavy bird (B_2) was positive for SGNal^r when neat culture was used (3.3 x 10^8 CFU/mL).

Considering the clinical evaluation, light W_2 and W_3 birds showed signs of disease, such as depression, weakness, loss of appetite, ruffled feathers, dropped

**Table 1** - Mortality of experimental birds inoculated with *Salmonella* Gallinarum Nalr:

	Variety	Number of Birds	Days after SG inoculation																			Mortality (%)
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
			Number of dead birds in each day																				(Tot)
Susceptible	B1	15	0	0	0	1	3	4	4	1	-	-	-	-	-	-	-	-	-	-	-	-	13	87
	B2	15	0	0	0	1	3	2	4	2	0	0	1	-	-	-	-	-	-	-	-	-	13	87
	B3	15	0	0	0	0	1	8	1	5	-	-	-	-	-	-	-	-	-	-	-	-	15	100
	B1*	15	0	0	0	0	2	0	2	2	1	-	-	-	-	-	-	-	-	-	-	-	7	47
	B2*	15	0	0	0	0	3	1	2	1	1	-	-	-	-	-	-	-	-	-	-	-	8	53
	B3*	15	0	0	0	0	3	2	3	1	1	-	-	-	-	-	-	-	-	-	-	-	10	67
Resistant	W1	15	0	0	0	0	0	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	1	7
	W2	15	0	0	0	0	0	1	0	1	1	-	-	-	-	-	-	-	-	-	-	-	3	20
	W3	15	0	0	0	0	0	0	2	1	1	0	1	0	0	0	0	0	1	-	-	6	40

B1, B2, and B3= semi-heavy brown birds; W1, W2 and W3 = light white birds: Groups B1, B2, B3, W1, W2 and W3 received 0.2 mL of culture with 3.3 x 10^8 CFU SG Nal^r /mL, while B1*, B2* and B3* received 0.2mL of the same culture diluted at 10^{-3}.

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wings, and closed eyes. In addition, these birds presented mortality five days post-inoculation. On the other hand, semi-heavy birds died suddenly at five to seven days post-inoculation, and showed no clinical signs.

In the second experiment, there was no evidence of bacterial infection by serology. As to mortality, no birds died in the control (non-inoculated) and light groups. The number of dead birds in the semi-heavy groups was higher than in the other groups, being nine, nine, and six at days 5, 6, and 7 post-inoculation, respectively.

Gross changes were observed in the organs of semi-heavy birds, mainly in the liver, which was larger, with green-yellowish color, and friable. There was also spleen congestion and splenomegaly. There were no evident anatomopathological changes in light birds (Figure 1).
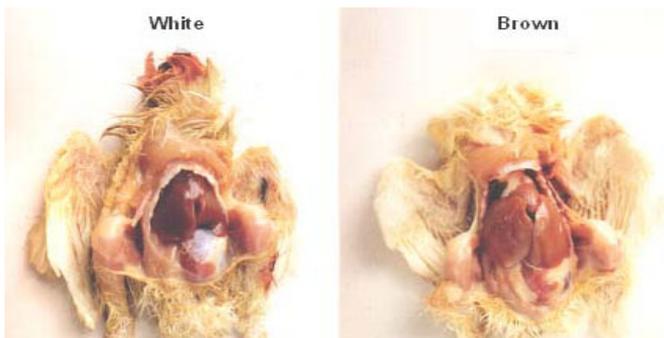

Figure 1 - Gross changes in white (W) and brown birds (B) experimentally infected with SG NaL[r].

The most important microscopic changes were observed in the liver, heart, and kidney in both genetic lines (Figures 2, 3, and 4). In semi-heavy birds, the liver presented multifocal necrosis, whereas light birds showed vacuolar degeneration. The heart of semi-heavy birds had inflammatory infiltrates with predominantly heterophils; light birds had inflammatory infiltrate with no predominance of polymorph populations. There was an inflammatory reaction with polymorph infiltration in the kidney of semi-heavy birds, but rare polymorphs were seen in the kidney of light birds. The lungs of birds of both lines presented mild inflammatory infiltrate.

As for hematological changes (Table 2), the group of semi-heavy birds presented significant reduction in total red cell count at five and nine days post-inoculation as compared to the same group of birds before being inoculated (p<0.05). In light birds, there
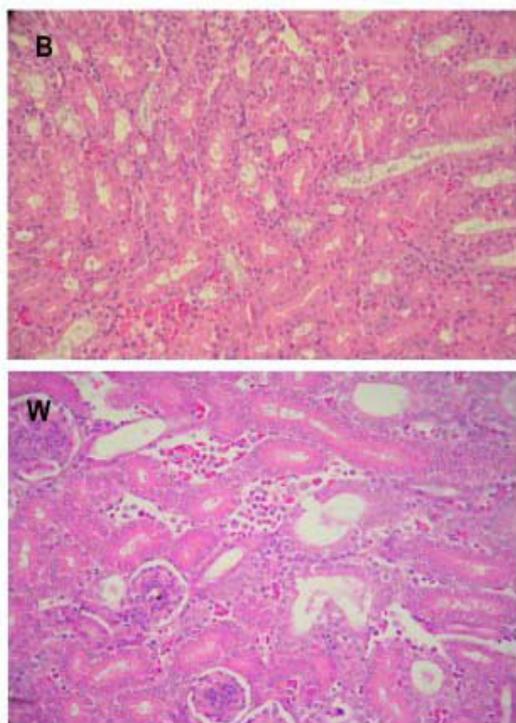
were no differences in mean values during the experimental period (p>0.05). For both strains, there were no significant differences in mean hemoglobin concentration values. There were also no significant differences in globular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, or in mean corpuscular in either bird strain.
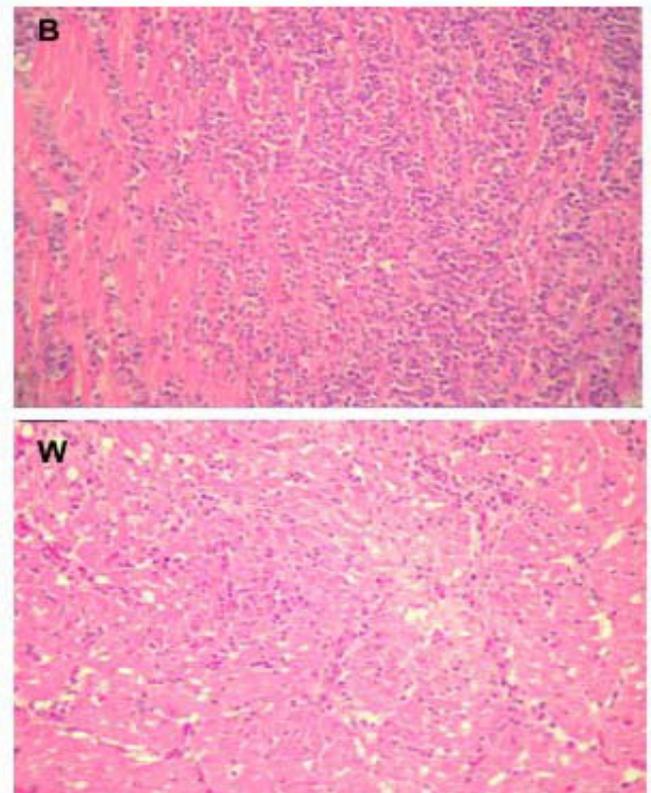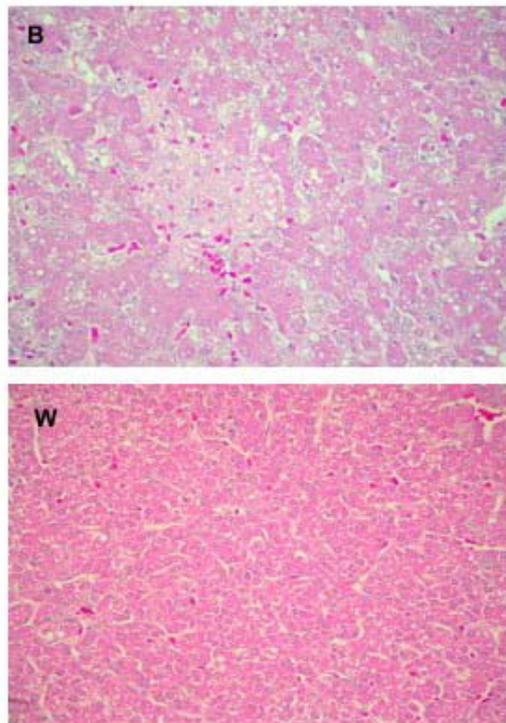

Figure 2 - Photomicrography (40x) of the heart of brown (B) and white birds (W) experimentally challenged with SGNaL[r] (second experiment). Observed that in the upper picture, the inflammatory reaction is more intensive than in the lower picture.

Serum albumin levels of semi-heavy birds presented no statistical differences during the experiment, but there was a marked decrease five days post-inoculation. In light birds, there was a significant increase in albumin values 12 days post-inoculation as compared to the values reported before inoculation (Table 3).

Aspartate aminotransferase enzymatic activity of semi-heavy birds was significantly higher (p<0.05) five days post-inoculation as compared to the mean value previous to inoculation (Table 3), whereas there was a significant increase in light birds at 5, 7, 12, and 14 days post-inoculation as compared to the values before inoculation (p<0.05).

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Figure 3 - Photomicrography (40 x) of the kidney of brown (B) and white birds (W) experimentally challenged with SG Nal[r]. Observe in the upper picture, the intensive inflammatory reaction. In the lower picture, there is less inflammation.

Figure 4 - Photomicrography (40 x) of the liver of brown (B) and white birds (W) experimentally challenged with SG NaL[r]. See in the upper picture multifocal necrosis of hepatocytes (irreversible lesion). In the lower picture, there is a vacuolar degeneration of hepatocytes (reversible lesion).

Table 2 - Means of red blood cell counts and some hematological parameters of birds of brown (B) and white (W) lines experimentally challenged with *S.* Gallinarum Nal[r].

Days post -inoculation (Dpi)	Rc x 10^5/µL		GV (%)		Hb (g/dL)		MCV		MCH (pg)		MCHC (g/dL)	
	B	**W**	**B**	**W**	**B**	**W**	**B**	**W**	**B**	**W**	**B**	**W**
- 1 Dpi	2074[B]	2000[A]	28.00[A]	28.4[A]	13.07[A]	14.47[A]	135.88[A]	144.93[A]	64.20[A]	72.78[A]	47.00[A]	51.42[A]
1º Dpi	1700[AB]	1955[A]	26.25[A]	29.5[A]	13.98[A]	14.77[A]	149.15[A]	157.25[A]	82.05[A]	79.55[A]	53.45[A]	50.15[A]
3º Dpi	1938[AB]	1824[A]	28.40[A]	32.8[A]	15.40[A]	15.28[A]	147.98[A]	194.07[A]	79.40[A]	91.70[A]	55.29[A]	46.74[A]
5º Dpi	1300[A]	1856[A]	23.00[A]	30.0[A]	*	*	186.07[A]	161.71[A]	*	*	*	*
7º Dpi	1722[AB]	2436[A]	28.80[A]	30.4[A]	12.50[A]	*	169.62[A]	125.88[A]	71.57[A]	*	41.94[A]	*
9º Dpi	1233[A]	1858[A]	22.33[A]	30.8[A]	12.81[A]	12.55[A]	182.72[A]	167.85[A]	112.61[A]	68.68[A]	61.35[A]	68.68[A]
12º Dpi	*	2976[B]	*	28.6[A]	*	15.71[A]	*	96.11[A]	*	52.91[A]	*	54.96[A]
14º Dpi	*	2024[A]	*	30.8[A]	*	9.18[A]	*	139.74[A]	*	41.88[A]	*	30.11[A]

Rc = Red blood cell counts; GV = globular volume; Hb = hemoglobin; *MCH = Mean corpuscular hemoglobin; MCHC = mean corpuscular hamoglobin Concentration; MCV = mean corpuscular volume; * = lost sample. Means followed by different letters in the same column indicate significant differences by Tukey's test (p < 0.05).

Table 3 - Albumin concentration (Alb) and of aspartate aminotransferase enzyme activity (AST) of birds of brown (B) and white (W) lines experimentally challenged with *S.* Gallinarum Nal[r].

Days post-inoculation (Dpi)	Albumin (g/dL)		Aspartate aminotransferase (U/L)	
	Brown	**White**	**Brown**	**White**
- 1 Dpi	1.59[A]	1.60[A]	214.4[A]	180.72[A]
1º Dpi	1.46[A]	1.42[A]	228.73[A]	183.35[A]
3º Dpi	*	*	*	*
5º Dpi	1.10[A]	1.27[A]	394.14[B]	309.32[B]
7º Dpi	1.25[A]	1.41[A]	208.56[A]	276.40[B]
9º Dpi	1.16[A]	0.99[A]	211.23[A]	223.52[A]
12º Dpi	*	2.32[B]	*	299.70[B]
14º Dpi	*	1.73[A]	*	267.40[B]

* = lost sample. Means followed by different letters in the same column indicate significant differences by Tukey's test (p < 0.05).

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Mean total leukocyte count of semi-heavy birds presented a significant decrease (p<0.05) five days post-infection as compared to the counts before infection, followed by a significant increase (p<0.05) at 7 and 9 days after infection (Table 4). Light birds showed a marked reduction in this parameter on the fifth day post-inoculation, but which was not statistically significant (p>0.05), followed by a significant increase (p<0.05) at 12 and 15 days post-infection. Therefore, the behavior of total leukocyte count curves was similar for both strains (Figure 5).

Lymphocytes of semi-heavy birds significantly decreased (p<0.05) seven days post-inoculation as compared to the values seen in the birds of the same group before infection. In light white birds, there was no significant difference (p>0.05) in this parameter throughout the experimental period (Table 4). The other white cell counts (heterophils, eosinophils, monocytes, and basophils) presented no significant differences (p>0.05) in both varieties.

DISCUSSION

Experiment 1

The comparatively higher percentage of mortality of semi-heavy birds indicates higher resistance of light birds to fowl typhoid, which is consistent with previous studies (Pomeroy and Nagaraja, 1991; Berchieri *et al.*, 2001). Nevertheless, there was a variation in resistance among white birds, which are generally considered as resistant to this disease. W3 line was more susceptible than the other lines, and presented the highest mortality. The behavior shown in the present study may be due to organic variations resulting from the genetic selection pressure to which the birds are submitted in order to achieve better performance results, which in the end adversely affects immune responses and resistance to diseases (Swaggerty *et al.*, 2003).
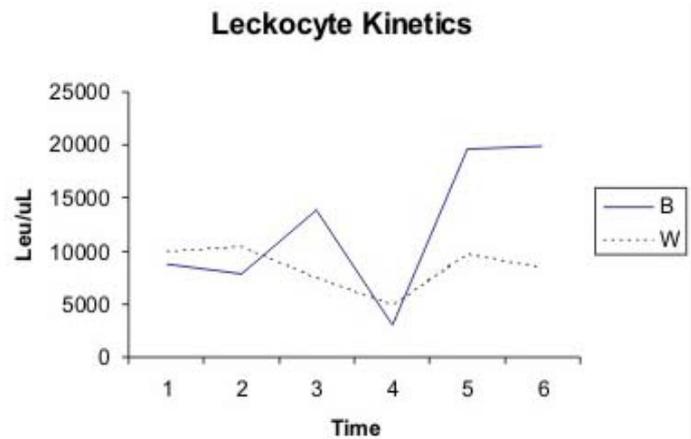

Figure 5 - Graph of leukocyte kinetics of brown (B) and white birds (W) experimentally challenged with SG Nalr.

As to the mortality rate of semi-heavy birds, there was a significant dose-dependent response. The results corroborate an earlier study that showed that disease depends on infecting dose and bird genetic line (Oliveira *et al.*, 2005). On the other hand, Solomon (1968) considers that bird resistance to fowl typhoid depends on the age of the bird at the time of infection.

Fecal shedding was observed seven days post-infection in one semi-heavy bird that was sick. According to Oliveira *et al.* (2005), although birds do not constantly shed SG, birds that survive the disease may do it.

The results of liver swabs taken 23 days post-infection indicated that SG persisted more markedly in light birds, although there were no clinical signs of the disease. Nevertheless, the presence of bacteria in semi-heavy birds was insignificant. These findings are consistent with a previous study, which suggested that bird lines genetically resistant to fowl typhoid may carry the agent for weeks without showing clinical signs (Berchieri *et al.*, 2000a). This indicates that, although

Table 4 - Means of total leucocytes counts and differential leukocyte counts of birds of brown (B) and white (W) lines experimentally challenged with *S.* Gallinarum Nal[r].

Days post-Inoculation (Dpi)	White cell counts											
	Leucocytes /µL		Heterophils (%)		Lymphocytes (%)		Monocytes (%)		Eosinphils (%)		Basophils (%)	
	B	W	B	W	B	W	B	W	B	W	B	W
- 1 Dpi	8800[B]	10000[A]	35.8[A]	44.4[A]	59.2[B]	51.2[A]	2.0[A]	2.6[A]	1.8[A]	0.4[A]	1.2[A]	1.4[A]
1º Dpi	7833[AB]	10375[A]	33.0[A]	53.0[A]	61.0[B]	44.0[A]	3.3[A]	1.5[A]	0.7[A]	0.25[A]	2.0[A]	1.25[A]
3º Dpi	13900[BC]	7400[A]	55.4[A]	49.2[A]	39.2[B]	47.2[A]	5.0[A]	2.2[A]	0.2[A]	0.8[A]	0.2[A]	0.6[A]
5º Dpi	3000[A]	4833[A]	56.0[A]	43.7[A]	40.05[B]	51.3[A]	2.25[A]	3.0[A]	0.5[A]	1.0[A]	0.75	1.0[A]
7º Dpi	19600[C]	9700[A]	58.0[A]	44.0[A]	34.4[A]	50[A]	4.0[A]	3.4[A]	1.8[A]	0.8[A]	1.8[A]	1.8[A]
9º Dpi	19833[C]	8400[A]	40.3[A]	47.8[A]	56.0[B]	47.2[A]	3.3[A]	3.6[A]	0.3[A]	0.6[A]	0.0[A]	0.8[A]
12º Dpi	*	13700[B]	*	47.0[A]	*	48.8[A]	*	2.2[A]	*	1.4[A]	*	0.6[A]
14º Dpi	*	16100[B]	*	40.8[A]	*	55.0[A]	*	3.0[A]	*	0.6[A]	*	0.6[A]

* = lost sample. Means followed by different letters in the same column indicate significant differences by Tukey's test (p < 0.05).

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the birds do not constantly shed SG, in the presence of cannibalism they may become sources of infection of other birds.

In terms of clinical evaluation, light birds showed characteristic signs of fowl typhoid before dying, such as depression, prostration, anorexia, dropped wings, ruffled feathers, and dirty vents. On the other hand, semi-heavy birds died suddenly with no clinical signs. Smith (1955) and Assoku *et al.* (1970) described similar signs in young broilers three to four days after oral inoculation with *S.* Gallinarum; some inoculated birds developed the acute form of the disease, while others developed the sub-acute form of the disease.

Experiment 2

In this experiment, no light birds died, whereas semi-heavy birds showed considerable mortality. There were no clinical signs of the disease, and the birds died suddenly. The results are similar to those reported by Assoku *et al.* (1970), Allan and Duffus (1971), Hall (1991), and Christensen *et al.* (1996), all of which reported high mortality rates during the development of the disease, which was up to 50% among birds affected between five and seven days post-infection.

Interestingly, the mortality rate among light birds varied between the first and the second experiment, although the same inoculum concentration was used in both experiments. Whereas mortality was high among these birds in the first experiment, in the second there were no dead birds. This may be explained by the fact that females were used in the first experiment, whereas males were used in the second one. Physiologically, males of domestic birds are usually heavier at hatching as compared to females. Therefore, it may be inferred that the development of the disease would be dose-dependent, under the conditions of this experiment, as previously described (Oliveira *et al.*, 2005).

Gross lesions were restricted to the liver and the spleen of semi-heavy birds. They were larger, and some presented white spots five days after inoculation. The liver was green-yellowish and friable, similar to the lesions described when birds were inoculated with *Salmonella* Gallinarum (Smith, 1955; Assoku *et al.*, 1970; Pomeroy, 1987; Berchieri, 2000b). There was no evidence of anatomical changes in light birds.

Microscopically, the liver and the heart were the organs that sufffered the greater damage in their cellular structure, considering both bird lines. On the other hand, the kidneys showed a mild degree of lesion, as well as the lungs . Liver lesions were more intense in semi-heavy birds, which presented an irreversible pathological change (hepatocyte necrosis). These lesions were more evident five days after inoculation. Histological liver lesions in light birds were less intense (vacuolar degeneration of hepatocytes). Hall (1991) described histopathological lesions in the liver after experimental infection of birds with SG, such as hepatocyte degeneration around the centrilobular veins that would eventually result in substitution of the parenchyma for conjunctive tissue and an interstitial fibrosis. Later, a process of inflammatory infiltration develops, which is macroscopically evidenced as white spots on the surface of the organ. In the analysis of the histological sections, the heart of both semi-heavy and light birds presented inflammatory infiltration; in the former, it was characterized by the presence of heterophils. Similarly, Smith (1955) described a proliferative cell reaction in this organ, with nodular white masses disseminated in the myocardium.

The results of the present study showed no differences between lines in terms of globular volume (GV), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), or mean corpuscular volume (MCM) values caused by experimental infection with SG. There was only a decrease in erythrocytes value in semi-heavy birds at five and nine days post-infection. These findings are different from a previous study, which reported changes in erythrocyte counts during the acute phase of the infection due to an increase in the destruction of these cells (Assoku *et al.*, 1970). As fowl typhoid may develop as an acute, sub-acute, or chronic disease, hematological changes will be different in each situation, and this may explain the differences found in the present study. In sub-acute fowl typhoid, there is no evidence of marked anemia or erythrocyte fragility. There is only a slight reduction in globular volume, which also returns to normal values after the animal has recovered (Pomeroy, 1987).

Total leukocyte counts in semi-heavy birds showed a decrease at five days post-infection (leukopenia) relative to the values determined before infection. At seven days post infection, there was an increase that indicated leukocytosis. Nevertheless, light birds that showed a biologically marked and non-significant decrease in leukocyte counts also presented leukocytosis after the 12th day post-infection. Assoku *et al.*(1970), Allan and Duffus (1971), and Cardoso *et al.* (2003) reported similar increases using SG inoculation under experimental conditions. The decrease in total leukocyte counts may be explained

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by the cytolytic effect of *Salmonella* on leukocytes during the initial infection (Lam and Munn, 2002). It may also be due to the high susceptibility of these cells to the cytopathic effect of SG lipopolysaccharides (Buxton and Allan, 1963). It is interesting to note that the behavior of leukocyte kinetics shown during the initial infection with *Salmonella* Gallinarum in the present study, both in semi-heavy and light birds (Figure 5), has not been described in the retrieved studies with birds, despite being described in mammals (Santos *et al.*, 2002).

Lymphocyte percentage values in semi-heavy birds decreased (lymphopenia) after the 7th day post-infection as compared to the same group of birds before being inoculated. Values increased after the 9th day post-infection up to normal values, similar to those before inoculation. In the light birds, leukocyte numbers varied within normal ranges during the experiment. In a similar study, Allan and Duffus (1971) found no changes in lymphocyte counts during the course of fowl typhoid. On the other hand, Assoku *et al.* (1970) worked with SG in birds, and found lymphocyte counts lower than normal values. Lymphopenia is common in acute inflammatory responses, because inflammatory mediators stimulate the migration of heterophils and lymphocytes from the blood and lymphoid tissues to the inflammation site (Jain, 1993).

The marked increase in heterophil counts observed after the 5th day post-infection was not statistically significant. However, leukocytosis was previously reported caused by an increase in heterophils up to five times higher than before inoculation (Assoku *et al.*, 1970). Leukocytosis is usually due to heterophilia, and common causes are general infections due to septicemias caused by infectious agents, such as salmonella (Morgulis, 2002).

Monocyte, eosinophil, and basophil percentage values in birds both susceptible and resistant to fowl typhoid, in the present study, showed no relevant changes, and were consistent with previous reports (Allan and Duffus, 1971; Cardoso *et al.*, 2003).

Although serum albumin levels were normal in both groups, they were lower in susceptible birds five days post-inoculation as compared to healthy birds of the same strain.

There was an increase in the activity of aspartate aminotransferase (AST) in semi-heavy birds five days post-inoculation as compared to the mean value in birds of the same group, before being infected, and the values decreased after the 7th day post-inoculation. Light birds showed an increase in mean enzyme value at 5, 7, 12, and 14 days post-inoculation.

In this study, when the values of albumin and AST in semi-heavy birds are compared with the degree of liver lesion at five-days post-inoculation, a coincidence is observed among highest lesion score (multifocal necrosis), higher AST levels, and lower albumin levels in these birds. This may be interpreted as an incapacity of the liver to synthesize protein due to the lesion intensity, microscopically evidenced by hepatomegaly and loss of protein in the affected kidney. Therefore, the damage in the glomerular filtration barrier may result in the presence of plasma proteins in the urine; in addition, inflammation of the renal parenchyma or epithelial damage of the tubules may cause loss of protein to the urine (Reldford and Lees, 1996).

In conclusion, the absence of a similar behavior in the response of the defense system of the birds in the presence of fowl typhoid makes it difficult to understand the relationship between *Salmonella* Gallinarum and affected birds. Similarly, the observations of the present study raise questions regarding the pathogenesis of fowl typhoid. Further studies are necessary to elucidate the relationship between birds and this bacterium.

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