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Aspects clinical and pathological associated with the eletrophoretic profile of turkeys (*Meleagris gallopavo*) inoculated with *Salmonella enteritidis*

Aspectos clínicos e anatomopatológicos associados ao perfil eletroforético de perus (*Meleagris gallopavo*) inoculados com *Salmonella enteritidis*

Aspectos clínicos y anatomopatológicos asociados al perfil electroforético de pavos (*Meleagris gallopavo*) inoculados con *Salmonella enteritidis*

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ABSTRACT
The study evaluated clinical, anatomopathological, immunological aspects, and the electrophoretic profile of serum proteins in turkeys experimentally inoculated

with *Salmonella enteritidis* at different concentrations. A total of 160 one-day-old turkeys were used, distributed into four groups: control (saline solution) and three treatment groups with inocula of 6.0×10^2, 7.0×10^5, and 8.0×10^9 CFU/mL. Blood was collected at specific post-inoculation time points (1 to 24 hours, and on days 3, 4, 38, and 49) for protein analysis. After euthanasia, liver, spleen, and bursa of Fabricius fragments were collected for bacteriological and histopathological examinations. The results showed significant differences in total serum proteins from 6 hours post-inoculation, with higher values observed in the group that received the highest concentration of *Salmonella enteritidis*. Bacterial isolation from the liver was detected from 6 hours up to four days post-inoculation. Hepatic alterations, such as vacuolar degeneration and inflammatory infiltrates, were observed up to 49 days. In the spleen and bursa, lymphocytic depletion and heterophilic infiltrates were noted. Clinically, the turkeys exhibited drowsiness, apathy, and dirty cloaca, with some birds dying without symptoms while others recovered between the third and fourth weeks. Mortality was high in the first week, decreasing in the following weeks. It is concluded that *Salmonella enteritidis* induces significant protein and histopathological alterations, with more pronounced effects at high doses and in the early days following infection.

Keywords: Electrophoresis. Avian Salmonellosis. Histopathology. Lymphocytes.

RESUMO

O estudo avaliou aspectos clínicos, anatomopatológicos, imunológicos e o perfil eletroforético de proteínas séricas em perus inoculados experimentalmente com *Salmonella enteritidis* em diferentes concentrações. Foram utilizados 160 perus de um dia de idade, distribuídos em quatro grupos: controle (solução salina) e três tratamentos com inóculos de $6,0x10^2$, $7,0x10^5$ e $8,0x10^9$ UFC/mL. Sangue foi coletado em momentos específicos pós-inoculação (1 a 24 horas, e nos dias 3, 4, 38 e 49) para análise proteica. Após a eutanásia, fragmentos de fígado, baço e bursa de Fabricius foram coletados para exames bacteriológicos e histopatológicos. Os resultados mostraram diferenças significativas nas proteínas séricas totais a partir de 6 horas, com valores mais elevados no grupo que recebeu a maior concentração de *Salmonella enteritidis*. O isolamento da bactéria no fígado ocorreu desde 6 horas até quatro dias pós-inoculação. Alterações hepáticas, como degeneração vacuolar e infiltrados inflamatórios, foram observadas até os 49 dias. No baço e na bursa, verificaram-se depleções linfocitárias e infiltrados heterofílicos. Clinicamente, os perus apresentaram sonolência, apatia e cloaca suja, sendo que algumas aves morreram sem sintomas e outras se recuperaram entre a terceira e a quarta semanas. A mortalidade foi alta na primeira semana, diminuindo nas subsequentes. Conclui-se que *Salmonella enteritidis* induz alterações proteicas e histopatológicas significativas, sendo os efeitos mais pronunciados em doses elevadas e nos primeiros dias após a infecção.

Palavras-chave: Eletroforese. Histopatologia. Linfócitos. Salmonelose Aviária.

RESUMEN

El estudio evaluó los aspectos clínicos, anatomopatológicos, inmunológicos y el perfil electroforético de proteínas séricas en pavos inoculados

experimentalmente con *Salmonella enteritidis* en diferentes concentraciones. Se utilizaron 160 pavos de un día de edad, divididos en cuatro grupos: control (solución salina) y tres tratamientos con inóculos de $6{,}0x10^2$, $7{,}0x10^5$ y $8{,}0x10^9$ UFC/mL. Se recogieron muestras de sangre en momentos específicos post-inoculación (1 a 24 horas, y en los días 3, 4, 38 y 49) para análisis de proteínas. Después de la eutanasia, se recolectaron fragmentos de hígado, bazo y bolsa de Fabricio para exámenes bacteriológicos e histopatológicos. Los resultados mostraron diferencias significativas en las proteínas séricas totales desde las 6 horas post-inoculación, con valores más altos en el grupo que recibió la mayor concentración de *Salmonella enteritidis*. El aislamiento bacteriano en el hígado ocurrió desde las 6 horas hasta los cuatro días post-inoculación. Las alteraciones hepáticas, como la degeneración vacuolar y los infiltrados inflamatorios, se observaron hasta el día 49. En el bazo y la bolsa de Fabricio, se detectaron depleciones linfocitarias e infiltrados heterofílicos. Clínicamente, los pavos presentaron somnolencia, apatía y cloaca sucia, algunas aves murieron sin síntomas y otras se recuperaron entre la tercera y la cuarta semanas. La mortalidad fue alta durante la primera semana, disminuyendo en las semanas siguientes. Se concluye que *Salmonella enteritidis* induce cambios significativos en las proteínas y alteraciones histopatológicas, con efectos más pronunciados a dosis elevadas y durante los primeros días post-infección.

Palabras clave: Electroforesis. Histopatología. Linfocitos. Salmonelosis Aviar.

1 INTRODUCTION

The *Salmonella* genus comprises intracellular microorganisms that cause enteric and/or systemic infections and can lead to carrier status. Studying the development and diagnosis of infection by this pathogen in turkeys is important, as the presence of *Salmonella* species creates a sanitary barrier, restricting the trade of their meat and products (Chen *et al.*, 2023; Liu *et al.*, 2022). However, establishing a rapid and low-cost diagnosis in avian pathology is challenging. Different methods, such as electrophoretic profiling of serum proteins, are increasingly being used. Although these methods do not provide specific information, they are helpful in diagnosis when their values are analyzed and associated with clinical findings and conventional tests, thus constituting an important tool for prognostic assessment and the course of infectious diseases (Kim *et al*., 2023).

An effective diagnosis for *Salmonella* spp. is based on pathogen isolation, identification, and serological typing, complemented by historical data, clinical signs, lesions, and epidemiological analyses (Park *et al*., 2022; Jones *et al*., 2023). Using more than one laboratory procedure is essential for identifying microorganisms, as various factors can influence results in the isolation and identification of infectious agents (Zhang *et al*., 2021), as well as their development in the affected organism. In addition to methods for diagnosing clinical disease, procedures are used to detect asymptomatic carrier birds.

Determining the electrophoretic profile or the acute-phase proteins (albumin, alpha-globulins, beta-1-globulins, beta-2-globulins, and gamma-globulins) aids both in diagnosis and prognosis of diseases. Tissue alterations and lymphocyte depletion analyses suggest impairment of the lymphoid organs and immune system of the affected animals (González *et al*., 2022; Lee *et al*., 2023). Alongside the electrophoretic study, understanding the kinetics of *Salmonella* invasion in the liver and its tissue alterations is crucial, as proteins are primarily synthesized in this organ (Rahman *et al*., 2023). These values, when analyzed together, may clarify elements involved in determining the infection in susceptible hosts (Wang *et al*., 2023).

The analysis of lymphocyte numbers in organs such as spleens and Bursa of Fabricius helps evaluate lymphoid organ impairment. These cells reflect the involvement of the immune system in acute inflammatory processes, responding to inflammatory mediators that move from the circulatory system and lymphoid tissues to the site of inflammation. The intensity of lymphopenia often reflects the severity of the inflammatory response (Yu *et al*., 2023).

Given the above, this study was conducted to evaluate clinical, pathological, and immunological aspects and determine the electrophoretic profile in turkeys experimentally inoculated with different concentrations of *Salmonella enteritidis*.

2 MATERIALS AND METHODS

The experiment was conducted at the Experimental Center for Avian Diseases and the Bacteriology Laboratory of the Veterinary Medicine Department at the School of Veterinary Medicine and Animal Science (EVZ) at the Federal University of Goiás (UFG), Goiânia, Goiás, Brazil, and at the Departments of Pathological Anatomy and Animal Breeding, Health, and Production at the University of Murcia (UM), Murcia, Murcia Province, Spain.

The experimental protocol used in this study was approved by the UFG Research Ethics Committee, under number 103/09, and complies with the Ethical Principles in Animal Experimentation, adopted by the Brazilian Society of Laboratory Animal Science (SBCAL).

2.1 EXPERIMENTAL DESIGN

A total of 160 one-day-old turkeys from the British United Turkeys of America (BUTA) strain, obtained from commercial hatcheries, were divided into four treatments:

- Treatment 1: Consisted of 40 one-day-old turkeys, inoculated orally with 0.1 mL of buffered and sterilized saline solution at 0.85% (control);
- Treatment 2: Consisted of 40 one-day-old turkeys, inoculated orally with 0.1 mL of 0.85% saline solution containing approximately $6.0x10^2$ CFU/mL of *Salmonella enteritidis* (low concentration);
- Treatment 3: Consisted of 40 one-day-old turkeys, inoculated orally with 0.1 mL of 0.85% saline solution containing approximately $7.0x10^5$ CFU/mL of *Salmonella enteritidis* (medium concentration);
- Treatment 4: Consisted of 40 one-day-old turkeys, inoculated orally with 0.1 mL of 0.85% saline solution containing approximately $8.0x10^9$ CFU/mL of *Salmonella enteritidis* (high concentration).

The challenged and non-challenged birds were housed in separate rooms, with the same environment, in four-story galvanized steel cages equipped with linear feeders and drinkers, as well as trays for collecting excreta. The cages

were heated with 60W incandescent light bulbs per floor until the birds reached 21 days of age.

2.2 INOCULUM PREPARATION

The inoculum was prepared with *S. enteritidis* isolated from samples obtained from broiler chickens, provided by ANDRADE *et al*. (2009). The strain was replicated on XLT4 agar and incubated at 37°C for 18-24 hours. Afterward, the cells were suspended in 0.85% buffered saline solution and stored at 4°C. The concentrations of $6.0x10^2$ CFU/mL, $7.0x10^5$ CFU/mL, and $8.0x10^9$ CFU/mL for *Salmonella enteritidis* were adjusted using the MacFarland scale (Férnandez *et al*., 2001).

The concentration was confirmed by plating serial decimal dilutions onto XLT4 agar, followed by incubation at 37°C and counting CFUs of *Salmonella*.

2.3 STUDIED VARIABLES ELECTROPHORESIS

Serum samples from the turkeys were collected at 1, 3, 6, 12, 18, and 24 hours of life, and at 3, 4, 38, and 49 days of age. The separation of serum protein fractions was performed using the agarose gel electrophoresis technique, according to a commercial kit (Celmgel, Companhia Equipadora de Laboratórios Modernos, Barueri, São Paulo, Brazil), using 80 mL of Tris buffer at pH 9.5, at temperatures between 2ºC and 8ºC. Samples (0.4 µL) were applied to the wells of the agarose gel, placed on a support film after confirming the alignment of the negative poles of the film and the tank. The tank was then covered, and the electrophoretic run was programmed for 20 minutes at 100 volts.

At the end of the run, the support film was removed, placed on filter paper to remove excess buffer from the edges of the film, then immersed in a protein fixation and staining solution containing 0.2% starch black in 5% acetic acid for five minutes. After staining, the gel was decolorized in 5% acetic acid solution and completely dried in an oven at 60°C before being subjected to densitometry at 520nm using a digital densitometer model DS35. The absolute and relative

values of each fraction (albumin, alpha, beta, and gamma globulins) were obtained and presented as means and standard deviations.

Total serum protein and its fractions (albumin and globulins) were determined using the colorimetric biuret reaction method with a commercial kit, with readings at 540nm in a spectrophotometer (SB-210).

2.4 *SALMONELLA* ENTERITIDIS DETECTION

For detection of *Salmonella enteritidis*, liver samples were collected at 1, 3, 6, 12, 18, and 24 hours of life, and at 3, 4, 38, and 49 days of age, homogenized, and transferred to tubes containing enrichment broth, processed according to Georgia Poultry Laboratory (1997) and Brasil (2003). The samples were placed in test tubes containing 9 mL of Selenite Cystine broth and incubated at 37°C for 18-24 hours. After this period, aliquots were streaked onto Brilliant Green and Hektoen agars and incubated at 37°C for 24 hours. Three to five colonies with *Salmonella* morphological characteristics were selected and subcultured onto triple sugar iron agar (TSI) and incubated at 37°C for 24 hours.

Isolates that presented reactions characteristic of the *Salmonella* genus were subjected to biochemical tests, including indole production, H2S production, motility, urease, lysine decarboxylation, methyl red, malonate utilization, and Simmons citrate. Biochemically confirmed samples were subjected to serological testing using polyvalent anti-“O” serum. The samples confirmed both biochemically and serologically were sent to the Oswaldo Cruz Institute (FIOCRUZ-RJ) for serological typing.

2.5 HISTOPATHOLOGICAL EXAMS AND LYMPHOCYTE DEPLETION

Fragments of spleen, Bursa of Fabricius, and liver were collected from turkeys at 1, 3, 6, 12, 18, 24 hours of life, and at 3, 4, 38, and 49 days of age, according to the conventional methodology by Luna (1968). Once the samples were fixed for 24 hours in 10% neutral buffered formalin, fragments were cut, placed in cassettes, and labeled. They were then washed in running water to

remove excess formalin pigments, dehydrated in a series of increasing ethanol concentrations from 70% to absolute ethanol.

The tissue was cleared with xylene and impregnated with histological paraffin with a melting point of 56ºC. 5 µm sections were obtained using a rotary microtome (American Optical, Spencer-820 model) and stained with Hematoxylin-Eosin (HE). Liver, spleen, and Bursa of Fabricius lesions were classified qualitatively, with severity classified as pronounced, moderate, or slight, and distribution as focal, multifocal, or diffuse.

2.6 STATISTICAL ANALYSIS

For histopathological changes and *Salmonella enteritidis* recovery, simple frequency was performed. Electrophoresis data were analyzed using variance analysis with the SAS statistical software (version 9.2). Additional analyses were performed using the R software, considering a significance level of $p<0.05$. When significant differences were found, means were compared using the Dunn post-test applied to the Kruskal-Wallis test, a non-parametric approach widely used in recent microbiological studies to assess categorical or ordinally distributed data.

3 RESULTS

3.1 ELECTROPHORESIS

The results related to the analysis of the acute-phase proteins in the sera of the turkeys are shown in Table 1.

Table 1. Mean values and standard deviation of total serum proteins, albumin, and alpha-globulins of turkeys at different ages experimentally inoculated with three concentrations of *Salmonella enteritidis*.

Age	Treatment	1h	3h	6h	12h	18h	24h	3d	4d	38d	49d
PST	1	2.73±0.11	2.87±0.11	2.76±0.20b	3.10±0.01c	3.31±0.01c	3.32±0.11b	3.34±0.05c	3.13±0.28b	3.17±0.32	3.04±0.05
	2	2.73±0.11	2.83±0.25	3.23±0.23ab	3.46±0.05b	3.59±0.00c	4.06±0.23a	3.36±0.05c	3.40±0.00b	3.16±0.32	3.23±0.11
	3	2.63±0.35	2.63±0.41	3.23±0.23ab	3.60±0.17b	3.93±0.23b	4.33±0.05a	3.86±0.05b	3.47±0.06b	3.27±0.14	2.96±0.41
	4	2.70±0.10	2.83±0.23	3.30±0.00a	4.26±0.11a	4.35±0.05a	4.40±0.00a	4.20±0.00a	4.23±0.05a	3.36±0.05	3.03±0.30
P		0.9159	0.7239	0.0328	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	0.7024	0.6562
Albumin	1	1.64±0.28	1.61±0.09	1.64±0.21	1.96±0.04	2.45±0.26a	2.75±0.30a	2.62±0.31a	2.05±0.30	1.93±0.37	1.95±0.04
	2	1.50±0.09	1.64±0.29	1.99±0.04	1.94±0.27	2.11±0.06b	2.02±0.30b	2.19±0.02ab	1.87±0.42	1.85±0.46	1.91±0.05
	3	1.41±0.31	1.30±0.28	1.71±0.16	2.06±0.27	2.20±0.30ab	2.65±0.23ab	2.44±0.05ab	2.00±0.15	2.02±0.32	1.67±0.43
	4	1.58±0.20	1.74±0.10	2.06±0.29	2.49±0.28	1.82±0.01b	1.85±0.12c	1.86±0.27b	2.61±0.30	2.20±0.28	1.88±0.08
P		0.6801	0.1476	0.0898	0.0719	0.0028	0.0051	0.0112	0.0800	0.6870	0.4726
Alpha-globulin	1	0.23±0.05	0.23±0.03	0.28±0.10	0.33±0.04	0.27±0.09	0.26±0.13	0.32±0.33	0.25±0.06	0.29±0.04	0.20±0.15
	2	0.20±0.01	0.19±0.02	0.51±0.60	0.71±0.45	0.39±0.11	0.46±0.11	0.82±0.49	0.50±0.16	0.41±0.15	0.29±0.11
	3	0.24±0.04	0.65±0.48	0.89±0.52	0.30±0.02	0.40±0.11	0.51±0.16	0.24±0.08	0.56±0.47	0.32±0.00	0.34±0.17
	4	0.26±0.11	0.66±0.46	0.27±0.03	0.40±0.08	0.65±0.51	0.68±0.09	0.62±0.02	0.36±0.02	0.33±0.02	0.30±0.11
P		0.7459	0.2314	0.2710	0.2054	0.7016	0.5258	0.1220	0.9410	0.4068	0.6429

* Different letters in the same column differ significantly by the Kruskal-Wallis test ($p<0.05$). PST = Total Serum Proteins. Treatment 1: Placebo / Treatment 2: $6x10^2$ CFU/mL / Treatment 3: $7x10^5$ CFU/mL / Treatment 4: $8.0x10^9$ CFU/mL.
Source: Authors.

Table 2. Mean values and standard deviation of beta 1-globulins, beta 2-globulins, gamma-globulins, and the albumin/globulin ratio (A/G) of turkeys at different ages experimentally inoculated with three concentrations of Salmonella enteritidis.

Age	Treatment	1h	3h	6h	12h	18h	24h	3d	4d	38d	49d
Beta 1-globulin	1	0.21±0.05	0.17±0.03	0.27±0.09	0.39±0.02	0.28±0.19	0.11±0.09b	0.36±0.15	0.38±0.17	0.38±0.17	0.28±0.10
	2	0.17±0.04	0.21±0.06	0.19±0.14	0.54±0.52	0.35±0.27	0.64±0.11a	0.86±0.37	0.45±0.06	0.45±0.06	0.14±0.11
	3	0.23±0.02	0.27±0.15	0.46±0.57	1.01±0.20	0.32±0.20	0.73±0.05a	0.77±0.60	0.46±0.05	0.46±0.05	0.26±0.18
	4	0.25±0.11	0.29±0.14	0.49±0.04	1.05±0.34	0.57±0.19	0.72±0.08a	1.03±0.35	0.32±0.20	0.32±0.20	0.24±0.15
P		0.5973	0.5317	0.5820	0.0918	0.0614	0.0013	0.2913	0.5854	0.5854	0.6636
Beta 2-globulin	1	0.43±0.33	0.58±0.30	0.22±0.21	0.27±0.11	0.22±0.19	0.12±0.06	0.14±0.27	0.12±0.08	0.38±0.24	0.55±0.32
	2	0.65±0.18	0.54±0.19	0.33±0.15	0.16±0.16	0.27±0.10	0.34±0.20	0.04±0.03	0.09±0.15	0.41±0.22	0.46±0.04
	3	0.42±0.18	0.30±0.29	0.03±0.04	0.08±0.01	0.52±0.20	0.31±0.02	0.43±0.28	0.06±0.03	0.41±0.22	0.40±0.09
	4	0.40±0.37	0.01±0.00	0.35±0.29	0.29±0.30	0.65±0.11	0.50±0.20	0.36±0.01	0.19±0.12	0.34±0.12	0.30±0.25
P		0.6817	0.0573	0.2721	0.4847	0.0614	0.7864	0.1338	0.5434	0.9718	0.5892

Gamma-globulin	1	0.21±0.12	0.25±0.25	0.34±0.21	0.17±0.03	0.09±0.07	0.08±0.11b	0.11±0.21	0.32±0.09a	0.16±0.22	0.08±0.05
	2	0.19±0.13	0.24±0.12	0.19±0.11	0.11±0.06	0.15±0.06	0.13±0.08b	0.10±0.14	0.06±0.04b	0.03±0.02	0.41±0.13
	3	0.32±0.23	0.09±0.04	0.12±0.02	0.39±0.33	0.17±0.17	0.12±0.05b	0.43±0.20	0.07±0.04b	0.08±0.09	0.27±0.21
	4	0.19±0.13	0.12±0.01	0.11±0.13	0.09±0.07	0.64±0.33	0.65±0.33a	0.29±0.01	0.04±0.03b	0.17±0.27	0.29±0.18
P		0.7158	0.4498	0.2513	0.3330	0.3330	0.0460	0.0656	0.0013	0.7808	0.1612
A/G	1	1.58±0.64	1.29±0.05	1.50±0.43	1.71±0.10	1.09±0.02	1.43±0.30	1.31±0.41	1.92±0.31	1.65±0.61	1.81±0.02
	2	1.22±0.05	1.38±0.30	1.61±0.19	1.30±0.34	1.52±0.11	1.08±0.50	1.89±0.17	1.38±0.79	1.43±0.69	1.45±0.23
	3	1.14±0.22	0.96±0.10	1.12±0.06	1.35±0.27	1.37±0.50	1.60±0.34	1.72±0.04	1.40±0.33	1.70±0.60	1.31±0.42
	4	1.47±0.45	1.69±0.52	1.77±0.58	1.46±0.47	1.67±0.41	1.73±0.45	1.72±0.49	1.66±0.44	1.97±0.56	1.68±0.33
P		0.5583	0.1074	0.2719	0.4617	0.2477	0.3070	0.2577	0.5555	0.8178	0.2418

*Different letters in the same column differ significantly by the Kruskal-Wallis test ($p<0.05$). Treatment 1: Placebo / Treatment 2: $6x10^2$ CFU/mL / Treatment 3: $7x10^5$ CFU/mL.
Source: Authors.

The results indicate that, according to Table 1, there was no significant variation ($p>0.05$) in acute-phase proteins between the evaluated groups 1 and 3 hours after inoculation. However, significant increases in total serum proteins were observed at 6 and 12 hours post-inoculation, particularly in the group inoculated with the highest concentration of *Salmonella enteritidis* ($8.0x10^9$ CFU/mL).

At 18 hours, significant increases were also noted in albumin levels. By 24 hours, significant differences ($p<0.05$) were observed in total serum proteins, albumin, beta 1-globulins, and gamma-globulins. These differences continued at three days post-inoculation for total serum proteins, albumin, and beta 1-globulins. By the fourth day post-inoculation, differences were only observed in total serum proteins and gamma-globulins. However, no differences were observed at 38 and 49 days post-inoculation.

The highest levels of total serum proteins and protein fractions were found in the group inoculated with the highest concentration of *Salmonella enteritidis*, and the control group had the lowest values. The control group showed almost no variation in these values throughout the study period, as depicted in Figure 1.

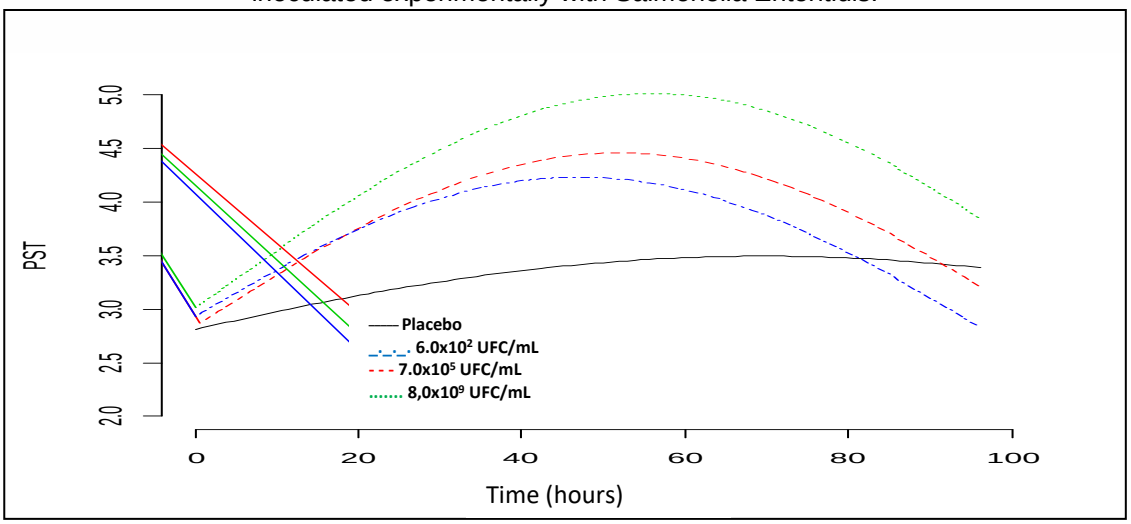
Table 3. Pearson Correlation Results for Total Serum Proteins and Their Fractions

	PST	Albumin	Alpha-globulin	Beta1-globulin	Beta2-globulin	Gamma-globulin	A/G ratio
PST	1.00	0.56	0.50	0.46	-0.22	-0.21	-0.11
Albumin	0.56	1.00	0.43	0.19	-0.55	-0.49	0.75
Alpha-globulin	0.50	0.43	1.00	-0.25	-0.47	-0.11	0.10
Beta1-globulin	0.46	0.19	-0.25	1.00	-0.26	-0.27	-0.12
Beta2-globulin	-0.22	-0.55	-0.47	-0.26	1.00	-0.05	-0.48
Gamma-globulin	-0.21	-0.49	-0.11	-0.27	-0.05	1.00	-0.41
A/G ratio	-0.11	0.75	0.10	-0.12	-0.48	-0.41	1.00

* Correlation values above 0.40 and below -0.40 are statistically different from zero ($p<0.05$), based on the t-test with a 5% significance level.
Source: Authors.

The total serum proteins showed variations with positive correlations ($p<0,05$) for albumin, alpha-globulin, and beta 1-globulin. Additionally, significant negative correlations ($p<0,05$) were observed between albumin and beta 2-globulin, as well as between albumin and gamma-globulin, and between alpha-globulin and beta 2-globulin (Table 2).

Figure 1. Non-linear regression model for mean total serum protein values over time in turkeys inoculated experimentally with Salmonella Enteritidis.



Source: Authors.

The highest total serum protein (PST) value for the control group was 3.49 g/dL at 69.58 hours after housing; in the group inoculated with 6.0x10² CFU/mL of *Salmonella enteritidis*, it was 4.23 g/dL at 55.82 hours post-inoculation and housing; in the group inoculated with 7.0x10⁵ CFU/mL, it was 4.45 g/dL at 51.83 hours; and in the group inoculated with 8.0x10⁹ CFU/mL, it was 5.01 g/dL at 46.65 hours.

It is observed that the higher the inoculum concentration used, the higher the PST value and the shorter the period of protein production.

At 38 and 49 days post-inoculation, although not represented in Figure 1, there was a decline in PST values in all the groups studied.

3.2 *SALMONELLA* ENTERITIDIS INVESTIGATION

In parallel with the study of acute-phase proteins, bacteriological analyses of livers were conducted (Table 3), emphasizing that *Salmonella enteritidis* was not isolated from any of the animals in the control group (treatment 1).

Table 4. Recovery of *Salmonella enteritidis* from the liver of turkeys experimentally inoculated

Age	6.0x10² CFU/mL	7.0x10⁵ CFU/mL	8.0x10⁹ CFU/mL
1 hour	0% (0/2)	0% (0/2)	0% (0/2)
3 hours	0% (0/2)	0% (0/2)	0% (0/2)
6 hours	50% (1/2)	50% (1/2)	100% (2/2)
12 hours	100% (2/2)	100% (2/2)	100% (2/2)
18 hours	100% (2/2)	100% (2/2)	100% (2/2)
24 hours	100% (2/2)	100% (2/2)	100% (2/2)
3 days	50% (1/2)	100% (2/2)	100% (2/2)
4 days	50% (1/2)	100% (2/2)	100% (2/2)
38 days	0% (0/2)	0% (0/2)	0% (0/2)
49 days	0% (0/2)	0% (0/2)	0% (0/2)
Total	39% (7/18)	50% (9/18)	55% (10/18)

Source: Authors.

From the data obtained (Table 4), it is observed that bacterial isolation from the liver occurred only 6 hours post-inoculation. Isolation was found in 50% (1/2) of the livers in the groups inoculated with concentrations of 6.0x10² and 7.0x10⁵ CFU/mL, while 100% (2/2) of the livers from the group inoculated with 8.0x10⁹ CFU/mL of *Salmonella enteritidis* tested positive.

At 12 and 24 hours post-inoculation, 100% (2/2) of the livers from all groups showed isolation of the inoculated pathogen. This continued until four days post-inoculation, with 50% (1/2) of the livers from the group inoculated with the lowest concentration of *Salmonella enteritidis* (6.0x10² CFU/mL) testing positive, and 100% (2/2) from the groups inoculated with concentrations of 7.0x10⁵ CFU/mL and 8.0x10⁹ CFU/mL.

3.3 HISTOPATHOLOGICAL EXAMS

By analyzing the histopathological results summarized in Table 5, it was found that the different concentrations of *Salmonella enteritidis* resulted in varying levels of lesions in the organs of the inoculated turkeys, whereas no histopathological lesions were observed in the control group.

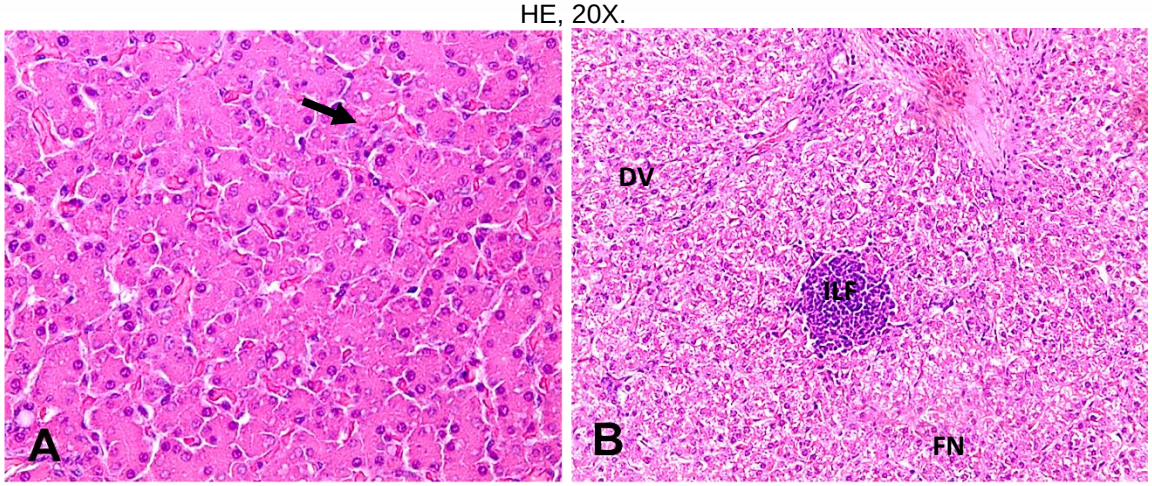
Table 5. Main histopathological alterations observed in organs of turkeys inoculated with *Salmonella enteritidis*

Organs / Lifespan	**Organs / Lifespan Inoculum with concentration of 6.0x10² CFU/mL**									
	1h	3h	6h	12h	18h	24h	3 d	4 d	38 d	49 d
Spleen	-	-	DL*,IIH**	DL*,IIH***	DL**, IIH***	DL**, IIH*	DL*,IIH*	DL*, IIH*	DL*, IIH*	DL*, IH*
Bursa of Fabricius	-	-	DL*	DL**	DL**	DL*	DL**, DC*	DL*	DL*	DL*
Liver	-	-	DV*	DV**	DV**	DV*	DV**	DV*	DV**	-
Organs / Lifespan	**Inoculum with concentration of 7.0x10⁵ CFU/mL**									
	4 d	38 d	6h	12h	18h	24h	3 d	4 d	38 d	49 d
Spleen	-	-	DL**	DL***	DL**	DL*, IIH*	DL**	DL*,IIH***	DL*, IIH*	DL*
Bursa of Fabricius	-	-	-	DL**, IIH*	DL***	DL**	DL**	DL*	DL*	-
Liver	-	-	DV*	DV*	DV**, IIH*	DV**, IIH**	DV**, IIH*	DV**, IIH*	DV*, DC**	DV**
Organs / Lifespan	**Inoculum with concentration of 8.0x10⁹ CFU/mL**									
	4 d	38 d	6h	12h	18h	24h	3 d	4 d	38 d	49 d
Spleen	-	-	DL*, IIH**	DL**, IIH**	DL**, IIH**,	DL**, IIH**	DL**, IIH*, PH**	DL**, IIH**	DL*, IIH*	DL*, IIH*
Bursa of Fabricius	-	-	DL*	DL**, IIH	DL***	DL**	DL*, IIH*	DL**, IIH*	DL**	DL*
Liver	-	-	DV*	DV**	DV**, HI*	DV**, HI**	DV**, HI**	DV**, HI*	DV**	DV*

IIM = mononuclear inflammatory infiltrate; IIH = heterophilic inflammatory infiltrate; - = no histological change; RF = follicular rarefaction; HG = glandular hyperplasia; E = enteritis; DL = lymphoid depletion; DV = vacuolar degeneration; DC = slight congestion; HI = interstitial hepatitis; PH = hemorrhagic points. *Slight, **Moderate, ***Marked.
Source: Authors.

In animals inoculated orally with a concentration of 6.0x10² CFU/mL, the spleen showed lymphoid depletion and intense heterophil infiltration in 80% (16/20) of the cases. Meanwhile, in the Bursa of Fabricius, mild to moderate lymphoid depletion was observed in 80% (16/20) of the animals analyzed. In the liver, the only alteration detected was the presence of mild to moderate vacuolar degeneration in 70% (14/20) of the histologically evaluated fragments (Figure 2-A).

Figure 2. Photomicrographs of turkey liver challenged with 6x10² and 7x10⁵ CFU/mL of *S. enteritidis*, (A) and (B), respectively. A) Liver with mild vacuolar degeneration. HE, 40X. B) Liver with severe vacuolar degeneration (VD), necrotic foci (NF), and focal lymphocytic infiltration (FLI). HE, 20X.



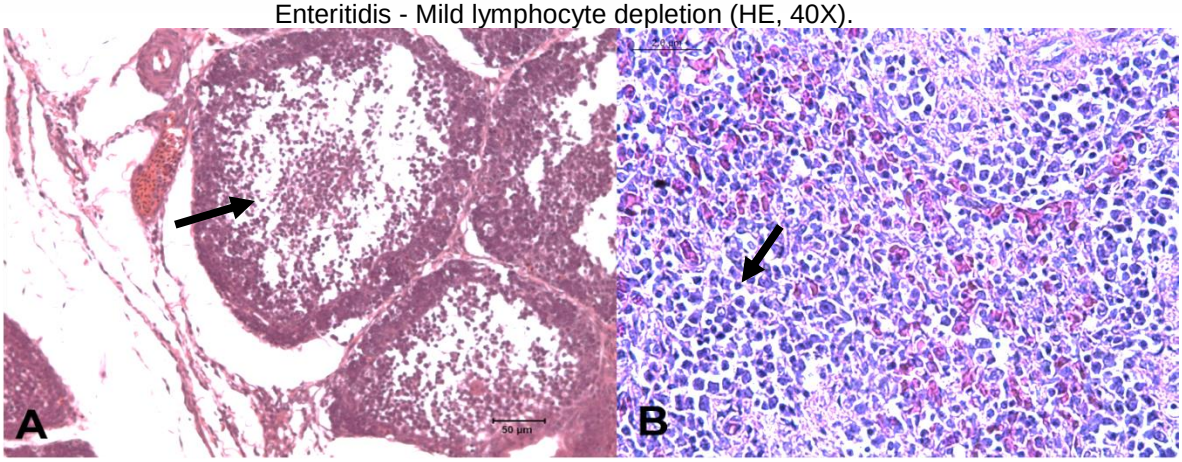
Source: Authors.

Histological examinations of animals inoculated orally with an inoculum concentration of 7x10⁵ CFU/mL revealed mild to moderate lymphoid depletion in the spleen (Figure 2-B) in 80% (16/20) of cases, along with heterophilic inflammatory infiltrates in 30% (6/20). In the Bursa of Fabricius, lymphoid depletion (Figure 2-A) was observed in 60% (12/20) of samples, with heterophilic and mononuclear cell infiltrates in the lamina propria present in only 10% (2/20) of the organs analyzed.

In the liver, mild to severe vacuolar degeneration was identified in 80% (16/20) of the evaluated samples, along with focal interstitial hepatitis with lymphocyte infiltration (Figure 1-B) in 40% (8/20) of the fragments analyzed.

Turkeys inoculated with an inoculum concentration above 8.0x10⁹ CFU/mL of *Salmonella enteritidis* exhibited, in histological analysis of the spleen, lymphoid depletion in 80% (16/20), moderate to diffuse heterophilic inflammatory infiltrates in 80% (16/20), and small hemorrhagic points in 20% (2/20). In the Bursa of Fabricius, lymphoid depletion was identified in 80% (16/20) of the analyses, with mild to moderate follicular rarefactions in 30% (6/20) and heterophilic inflammatory infiltrates in 30% (6/20). In the liver, mild to moderate vacuolar degeneration and focal interstitial hepatitis with lymphocyte infiltration were observed in 40% (8/20) of the analyzed fragments.

Figure 3. Photomicrographs of the Bursa of Fabricius and spleen from challenged turkeys. A) Bursa of Fabricius from turkeys inoculated with 6.0x10² CFU/mL of *S. enteritidis* - Severe lymphocyte depletion (HE, 20X). B) Spleen from turkeys inoculated with 7.0x10⁵ CFU/mL of S. Enteritidis - Mild lymphocyte depletion (HE, 40X).



Source: Authors.

3.4 CLINICAL AND PATHOLOGICAL FINDINGS

During the 49-day experiment, the main clinical signs observed included lethargy, dirty cloaca, uneven body development, drooping wings, and apathy. Notably, some birds died without exhibiting clinical signs, particularly those challenged with higher inoculum concentrations.

The primary macroscopic alterations detected included yolk sac abnormalities such as omphalitis and yolk sac retention. Pale and enlarged livers were also observed in some birds. In one bird, changes in the air sac were noted, with the presence of a foamy substance suggestive of airsacculitis.

Between the third and fourth weeks of the experiment, some animals showing clinical signs recovered clinically but still exhibited mild histological alterations in the liver.

3.5 MORTALITY

The highest mortality rates occurred during the first week of life (Table 6). The main signs observed in this age group included locomotion difficulties, with legs extended and unable to support the body, leading to immobility and death by

starvation. Clinical examination revealed that these turkeys weighed less than their birth weight and appeared emaciated.

Table 6. Frequency of mortality in turkeys during the experimental period.
Different letters in the same row indicate statistically significant differences according to the chi-square test.

Age (weeks)	Control	$6x10^2$ CFU/mL	$7x10^5$ CFU/mL	$8.0x10^9$ CFU/mL
1st	10.0% (4/40)^d	17.5% (7/40)^c	22.5% (9/40)^b	32.5% (13/40)^a
2nd and 3rd	2.5% (1/40)^d	7.5% (3/40)^c	12.5% (5/40)^b	17.5% (7/40)^a
4th and 5th	0.0% (0/40)^c	0.0% (0/40)^c	2.5% (1/40)^b	7.5% (3/40)^a
6th and 7th	0.0% (0/40)	0.0% (0/40)	0.0% (0/40)	0.0% (0/40)
Cumulative	12.5% (5/40)^d	25.0% (10/40)^c	37.5% (15/40)^b	57.5% (23/40)^a

Source: Authors.

Although the previous observations applied to all treatments, distinct mortality rates were recorded among groups inoculated with different concentrations of *Salmonella enteritidis*. The group inoculated with the highest concentration ($8.0x10^9$ CFU/mL) showed the highest mortality rate ($p<0.05$) compared to the group inoculated with the medium bacterial inoculum concentration ($7x10^5$ CFU/mL), which, in turn, exhibited a higher mortality rate than the group inoculated with the lowest concentration ($6x10^2$ CFU/mL).

Throughout the experimental period, the total mortality rates were 57.5% (23/40), 37.5% (15/40), 25.0% (10/40), and 12.5% (5/40) for the groups inoculated with high, medium, and low concentrations of *Salmonella enteritidis* and the control group, respectively. No turkey mortality was recorded during the sixth and seventh weeks of age.

Based on the results of mortality, the group inoculated with *Salmonella enteritidis* at a concentration of $6x10^2$ CFU/mL showed a mortality rate 2.33 times higher than the control group, with confidence intervals ranging from 0.71 to 5.58. This difference compared to the control group is not considered significant.

When turkeys were inoculated with *Salmonella enteritidis* at a concentration of $7.0x10^5$ CFU/mL, the recorded mortality rate was significant, being 4.20 times higher than the control group, with confidence intervals ranging from 1.35 to 13.06.

The group inoculated with *Salmonella enteritidis* at a concentration of $8.0x10^9$ CFU/mL showed a highly significant mortality rate, 9.47 times higher than the control group, with confidence intervals ranging from 3.06 to 29.24.

4 DISCUSSION

The use of laboratory tests is essential for understanding the pathogenesis of infectious diseases. Among these, the serum protein electrophoresis test stands out as a method that separates blood serum proteins into fractions based on electrical potential. In birds, these fractions are referred to as albumin, alpha-globulin, beta 1-globulin, beta 2-globulin, and gamma-globulin.

In the present study, total serum protein concentrations in the control and inoculated groups ranged between 2.63 ± 0.35 and 4.40 ± 0.00, consistent with reference ranges for birds, which vary between 2.5 and 6.0 g/dL, according to recent studies (Jain *et al*., 2023; Thrall *et al*., 2020). These values, lower than those observed in mammals, reflect metabolic and physiological differences between species.

No significant differences in the kinetics of acute-phase proteins were observed among the different groups at one and three hours post-inoculation ($p > 0.05$). These findings align with the data of Singh *et al*. (2022), which highlighted that changes in protein fractions become more evident during later stages of infection.

From 18 hours post-inoculation, a significant reduction in albumin levels proportional to the inoculum concentration was observed. Albumin, which represents approximately 60% of plasma proteins, is synthesized exclusively by the liver and is associated with functions such as substance transport and maintenance of oncotic pressure (Kumar *et al*., 2022). Similar results were reported by Tan *et al.* (2021) in studies with birds exposed to bacterial infections, indicating that albumin is a sensitive marker for liver dysfunction.

Histopathological changes in the liver, such as vacuolar degeneration and inflammatory infiltrates, were evident from six hours post-inoculation, as described by Wang *et al*. (2020). These alterations, along with the reduction in total serum proteins, suggest liver impairment caused by *Salmonella spp.*, as previously reported in other studies (Zhang *et al*., 2023).

Between 24 hours and three days post-inoculation, an increase in beta 1-globulin and gamma-globulin levels was observed, indicating an active immune

response. According to Lin *et al*. (2022), the elevation of these globulin fractions is typical of chronic inflammatory or infectious processes. This increase reflects the activation of humoral immunity, with greater immunoglobulin production by plasma cells.

Finally, the alterations observed in the spleen and Bursa of Fabricius, including heterophilic inflammatory infiltrates and lymphocytic depletion, demonstrate an acute inflammatory response. Recent studies confirm that these organs are primary targets of *Salmonella spp.*, where bacterial replication and immune activation occur (Li *et al*., 2023).

The clinical and laboratory findings of this study emphasize that electrophoretic parameters, combined with histopathological analyses, are valuable tools for understanding the pathogenesis and host response to bacterial infection.

5 CONCLUSION

Salmonella enteritidis alters acute-phase protein levels and causes histopathological lesions in the liver, as well as lymphocytic depletion in the spleen and Bursa of Fabricius, particularly at higher infection doses. These findings are relevant to both academia and society, as they contribute to the understanding of the pathogenic mechanisms of this bacterium, enabling the improvement of control and prevention strategies, especially in the agricultural sector and food safety.

However, this research has some limitations, such as the restricted sample size and the inability to assess the long-term immune response. Future studies should explore the dynamics of the inflammatory response over extended periods and investigate potential therapeutic strategies to mitigate the damage caused by *Salmonella enteritidis* in vulnerable hosts.

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