Macroscopic, histological, and microbiological characterization of broiler tibiotarsal joints

Caracterização macroscópica, histológica e microbiológica das articulações tibiotársicas de frangos de corte

Caracterización macroscópica, histológica y microbiológica de las articulaciones tibiotarsianas de pollos de engorde

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ABSTRACT
To begin to develop a safe and efficient system for broiler condemnation the tibiotarsal joints of 180 carcasses from a commercial slaughterhouse were graded visually from 0 to 5 based on their size and color. Microbiological counts and histological scored were compared with this visual assessment. Avian reovirus (12.7%), *Escherichia coli* (28.3%), *Staphylococcus aureus* (18.3%), and *Streptococcus* spp. (12.7%) but not *Mycoplasma* spp. was cultured from the joints evaluated. Except for group 5, the bacterial and viral numbers were within the acceptable thresholds for food safety. The control group bacterial count was not statistically different from the other grades. The observed histological lesions were not correlated with the presence of bacteria suggesting a sterile inflammatory process which does not present a risk to public health.

Keywords: arthritis, avian reovirus, locomotor problems, synovitis, tenosynovitis

RESUMO
Para começar a desenvolver um sistema seguro e eficiente para a condenação de frangos de corte, as articulações tibiotársicas de 180 carcaças de um abatedouro comercial foram classificadas visualmente de 0 a 5 com base em seu tamanho e cor. As contagens microbiológicas e a pontuação histológica foram comparadas com essa avaliação visual. O reovírus aviário (12,7%), a Escherichia coli (28,3%), o Staphylococcus aureus (18,3%) e o Streptococcus spp. (12,7%), mas não o Mycoplasma spp. foram cultivados nas articulações avaliadas. Com exceção do grupo 5, os números bacterianos e virais estavam dentro dos limites aceitáveis para a segurança alimentar. A contagem bacteriana do grupo de controle não foi estatisticamente diferente dos outros graus. As lesões histológicas observadas não foram correlacionadas com a presença de bactérias, sugerindo um processo inflamatório estéril que não apresenta risco à saúde pública.

Palavras-chave: artrite, reovírus aviário, problemas locomotores, sinovite, tenossinovite

RESUMEN
Para empezar a desarrollar un sistema seguro y eficaz de condena de pollos de engorde, las articulaciones tibiotarsianas de 180 canales de un matadero comercial se clasificaron visualmente de 0 a 5 en función de su tamaño y color. Los recuentos microbiológicos y la puntuación histológica se compararon con esta evaluación visual. De las articulaciones evaluadas se cultivaron reovirus aviar (12,7%), Escherichia coli (28,3%), Staphylococcus aureus (18,3%) y Streptococcus spp. (12,7%), pero no Mycoplasma spp. Excepto en el grupo 5, las cifras bacterianas y virales estaban dentro de los umbrales aceptables para la seguridad alimentaria. El recuento bacteriano del grupo de control no fue estadísticamente diferente de los demás grados. Las lesiones histológicas observadas no se correlacionaron con la presencia de bacterias, lo que sugiere un proceso inflamatorio estéril que no representa un riesgo para la salud pública.

Palabras clave: artritis, reovirus aviar, problemas locomotores, sinovitis, tenosinovitis

1 INTRODUCTION

Brazil is the largest exporter of chicken meat in the world, and it is the third greatest producer (14.329 million tons produced in 2021 (ABPA, 2022). Some genetic factors, rapid growth, lack of exercise, unbalanced diets, handling failures and microbiological infection, can give rise to birds whose carcasses or parts are condemned in the ante-mortem and post-mortem inspection lines (Xavier et al., 2010).

Several different factors can lead to deformities in the skeleton of broilers that decrease their growth and development (Akyüz; Onbaşilar, 2020; Cook, 2000). Locomotor problems in broilers can be classified as infectious, developmental, or degenerative (HILL et al., 1989; Marcon et al., 2019). Non-infectious joint alterations can be a result of rapid muscle growth which is not accompanied by the development of the broiler's leg joint and skeleton (Kierończyk et al., 2017; Manohar; Omprakash; Kanagaraju, 2015).

A local inflammatory response can be the consequence of trauma that occurs when the articular cartilage and the subchondral bone deform under pressure and the synovial membrane respond by hypertrophy and hyperplasia of villi and lining cells (Marcon et al., 2019). These anomalies can also be caused by the presence of microorganisms such as *Mycoplasma synoviae*, *Salmonella* spp., *Escherichia coli*. and *Staphylococcus aureus*. Avian reovirus can also cause these lesions in addition to weight loss and increased mortality (Raphael Lucio Andreatti Filho et al., 2020; Souza et al., 2018; Swayne et al., 2013).

The most common method used for the evaluation of the leg and joints is visual inspection of the locomotor capacity, which is the determinant criterion for condemnation in poultry slaughterhouses (Kierończyk et al., 2017). Thus, this study aimed to perform macroscopic, histological, and microbiological characterization of nonspecific anomalies in broiler tibiotarsal joints at slaughterhouses.

2 MATERIALS AND METHODS

Source of birds and samples organs

This study was carried out in a federally inspected commercial slaughterhouse with a slaughtering capacity of 120,000 birds/day. The broilers used in this study were of Cobb® and Ross® lineage, with an average slaughter age of 42 days, and an approximate weight of 2.8 kg. The sampling was done in 3 months and the carcasses collected came from 14 producers. A sample of convenience of a total of 180 carcasses was obtained with 30 carcasses classified in each of a six-grade scale based on visual inspection of the tibiotarsal region (see below).

After the carcass classification, birds were taken from the post-mortem inspection line and the legs were disarticulated at the tibiofemoral region with the aid of disinfected knife (70% alcohol). The legs were then packed in sterile bags and kept refrigerated until arrival at the microbiology laboratory. Each sample consisted of a single joint, classified by its macroscopic changes.

Visual classification of broiler leg

To assign a visual (macroscopic) classification score, the principles outlined in the Welfare Quality® Assessment protocol for poultry. Welfare-Quality® Consortium document (Louton et al., 2020; Welfare-Quality, 2009) were adapted for the anatomical region studied. The tibiotarsal joints were graded from 0 (control group) to 5 based on changes in volume and color.

Microbiological examinations of the broiler tibiotarsal joints

For microbiological analyses the external surface of the joint was flamed, and an incision was made in the skin between the tibiofemoral region until the end of the tarsometatarsal region to obtain a representative sample with a sterile swab. The swabs were processed for isolation and quantification *of E. coli, S. aureus* and *Streptococcus* spp. as described in (Quinn et al., 2011). Detection of Mycoplasma spp. and Avian reovirus was done using commercial real-time PCR kits (NewGene MYAmp and NewGene REOAmp (Simbios Biotecnologia, Cachoeirinha, RS, Brazil), according to the manufacturer's instructions.

Histological examination of organs and tissues

Samples of bursa, thymus, spleen, cecal tonsils, trachea, lung, heart, liver, and joints from each tested carcass were harvested in the federal meat inspection service area. Each joint was separated into epidermis, dermis, hypodermis, muscles, tendons, ligaments, tibial cartilage, capsule, and synovial membrane. The organs fragments and joints were kept within 10% formaldehyde and sent to the histological analysis.

Statistical analysis

The results of microbiological quantification were transformed into logarithms and their significance was assessed by the Kruskall Wallis nonparametric test followed by the post hoc pairwise Dunn test to calculate the significance between the means. All tests were performed using the software R-Project for Statistical Computing version 4.0.5 (Wilson A; Norden N, 2015)and

Python for Data Analysis (Jupyter Notebook version 4.1.1) (Mckinney, 2013), with significance of 5% ($p<0.05$)

3 RESULTS

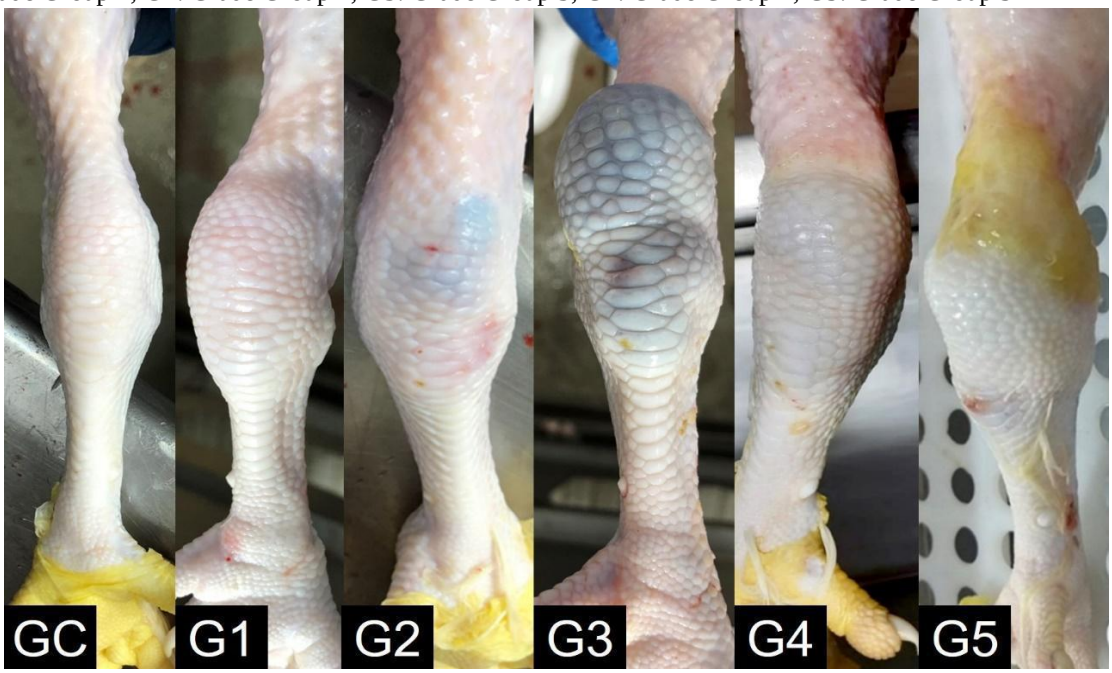
Visual classification scoring of broiler leg abnormalities

Macroscopic grading was done based on the size and color of the tibiotarsal joint (Figure 1). Grade group 0 or control group (GC) had no visible swelling or changes in the color of the joint. Grade group 1 (G1) samples had mild to moderate swelling and no color change. The grade group 2 (G2) had a mild to moderate swelling and a pinkish and/or bluish color. The grade group 3 (G3) had marked swelling and bluish color. The grade group 4 (G4) had marked swelling with a bluish color and the formation of a supra-articular yellow ring. Grade 5 (G5) had slight to moderate swelling, edema with pinkish and/or reddish and/or purplish and/or yellowish coloration or all colors in a single joint.

Microbiological examinations by degrees in the tibiotarsal joints

Mycoplasma spp. was not detected in any of the 180 joints analyzed but *E. coli* was observed in 14.4% (26/180), *S. aureus* in 28.3% (51/180), *Streptococcus* spp. in 18.3% (33/180), and Avian reovirus in 12.7% (23/180).

Figure 1. Visual presentation, by degrees, of the anomalies found in tibiotarsal joints of broilers. GC: Control Group; G1: Grade Group 1; G2: Grade Group 2; G3: Grade Group 3; G4: Grade Group 4; G5: Grade Group 5



Two out of thirty (6.66%) of the CG samples had *E. coli* with a mean count of $7.00x10^1$ CFU/mL; 30% (9/30) had *S. aureus* with a mean of $1.67x10^2$ CFU/mL, 16.6% (5/30) had *Streptococcus* spp. with a mean of $1.10x10^2$ CFU/mL and the presence of Avian reovirus was observed in only one sample (3.33%). In G1 5/30 (16.6%) had *E. coli* with a mean of $2.00x10^1$ CFU/mL; 16.6% (5/30) had *S. aureus*, with a mean of $4.70x10^1$ CFU/mL, 23.3% (7/30) had *Streptococcus* spp. with a mean of $3.40x10^2$ CFU/mL and 10.0% (3/30) had presence of Avian reovirus. One out of thirty (3.33%) of the G2 samples had *E. coli*, with a mean of $3.00x10^1$ UFC/mL; 6.66% (2/30) had *S. aureus* with a mean of $7.00x10^1$ UFC/mL; 13.3% (4/30) had *Streptococcus* spp. with a mean of $1.40x10^2$ CFU/mL, and 13.3% (4/30) had Avian reovirus. In G3, 10.0% (3/30) had *E. coli* with a mean of $1.37x10^2$ CFU/mL, 23.3% (7/30) had *S. aureus* with a mean of $1.27x10^2$ CFU/mL, 16.6% (5/30) had *Streptococcus* spp. with a mean of $7.00x10^1$ CFU/mL and 3.33% (1/30) of the samples had the presence of Avian reovirus. In G4, 10.0% (3/30) had *E. coli* with a mean of $1.30x10^1$ CFU / mL, 43.3% (13/30) had *S. aureus* with a mean of $1.37x10^2$ CFU/mL, 10.0% (3/30) had *Streptococcus* spp. with a mean of $5.00x10^1$ CFU/mL and the presence of Avian reovirus was detected in 36.6% (11/30) of the samples. In G5, 40.0% (12/30) had *E. coli* with a mean of $2.84x10^5$ CFU/mL, 46.6% (14/30) had *S. aureus*, with a mean of $6.90x10^4$ CFU/mL, 26.6% (8/30) had *Streptococcus* spp. with a mean of $3.78x10^3$ CFU/mL and detection of Avian reovirus in 10.0% (3/30).

There was not a significant difference between mean *E. coli*, *S. aureus*, and *Streptococcus* spp. counts in the CG and counts in G1, G2, G3, and G4. As calculated by the Dunn test, a statistically significant difference ($p<0.05$) was detected between the G0 and G5 groups. There was also a statistical significance ($p<0.05$), in the frequency of Avian reovirus in grade 4 (G4) birds (Table 1).

Table 1. Percentage of positive samples by Polymerase Chain Reaction (PCR) to Avian reovirus and mean concentrations of the bacterial species in the quantitative assay, according to the degree of injury of the broiler's tibiotarsal joint.

Grade Group	Microorganisms			
	Avian reovirus (%)	*E. coli* (CFU/ml)	*S. aureus* (CFU/ml)	*Streptococcus* spp. (CFU/ml)
GC	3.33%^{a}	$7.00x10^{1a}$	$1.67x10^{2a}$	$1.10x10^{2a}$
G1	10.0%^{a}	$2.00x10^{1a}$	$4.70x10^{1a}$	$3.40x10^{2a}$
G2	13.3%^{a}	$3.00x10^{1a}$	$7.00x10^{1a}$	$1.40x10^{2a}$
G3	3.33%^{a}	$1.37x10^{2a}$	$1.27x10^{2a}$	$7.00x10^{1a}$
G4	36.6%^{b}	$1.30x10^{1a}$	$1.37x10^{2a}$	$5.00x10^{1a}$
G5	10.0%^{a}	$2.84x10^{5b}$	$6.90x10^{4b}$	$3.78x10^{3a}$
GC	3.33%^{a}	$7.00x10^{1a}$	$1.67x10^{2a}$	$1.10x10^{2a}$

Histological examinations by degrees of the tibiotarsal joints and organs

No significant histological changes in the thymus, spleen, liver, heart, or tonsils were observed. Some of the bursal samples had moderate atrophy consistent with the presence of an immunosuppressive agent. In most of the tracheal samples, moderate tracheitis which is a nonspecific find that can be sequelae of a previous infectious condition or even the consequence of environmental issues (high ammonia concentration and poor air quality) (Table 2).

Table 2. Histological findings, by grade, of the tibiotarsal joints and organs

Lesion	GC	G1	G2	G3	G4	G5
Joint						
Flexor region: connective tissue proliferation at subcutaneous region	++	+++	+++	+++	+++	+++
Flexor region: large vesicles containing polyps of connective tissue or fibrinoid material				X	X	
Synovial membrane: lymphoplasmacytic inflammatory infiltration	+	++	++	++	++	++
Among the tendons: fibrinoid material deposition			X	X	X	X
Liver						
Periportal hepatitis					X	X
Fatty degeneration		+	+			
Trachea						
Chronic tracheitis		++	++	++	++	++
Bursa						
Atrophy			++		++	++

X: condition present; Mild: +; Moderate: ++; Severe: +++.

In all Groups there was evidence of chronic injuries in the joints/tendons; however, the causative factor was difficult to infer. No bacteria were observed in the lesions, not even lesions that suggested prior bacterial infection. The lesions in all groups were similar, varying only in intensity or in the presence or absence of subcutaneous vesicles. Even in the G0 group, it was possible to observe mild lesions. In the G5 group, adipose tissue in was present greater quantity. Although nonspecific, the lesions observed in these joints and tendons were suggestive with sequelae of viral infection by Avian reovirus.

4 DISCUSSION

With the exception of G5, the averages of these microorganisms counts for the GC, G1, G2, G3 and G4 groups were within acceptability thresholds for food security for *E. coli* ($1.00x10^2$ - $5.00x10^3$), *S. aureus* ($1.00x10^2$ - $1.00x10^3$) and *Streptococcus* spp. (aerobic mesophilic; $<1.00x10^5$) in frozen, prepared, minced, marinated and salted chicken meat that have been established by the European Union, Canada, Eurasian Economic Union, China, Singapore, Bolivia and the Gulf

countries (AVA, 2000; BRASIL, 2019; CHINA, 2014; EC, 2005; GSO, 2014; NB, 2017). When comparing the results of the CG group with the other grade groups, no significant differences were found, except for G5 which differed significantly from the control group and with the other grades.

According to our founds and studies conducted by (Andreasen et al., 1991), (Hill et al., 1989) and (Hill et al., 1989), the G5 joints lesions are suggestive of bacterial tenosynovitis and were of a great importance for the comparison of the microbiological and histological results as well as for the visual differentiation.

The microbiological findings were consistent with the histological assessment of the joints in that no significant numbers of bacteria, or alterations that suggested bacterial infection, were observed. The low microbiological numbers of *E. coli*, *S. aureus*, and *Streptococcus* spp. in the GC, G1, G2, G3, and G4 are consistent with findings of (Hammad; Liyanapathirana; Tonge, 2019) and (JIANG et al., 2015), who demonstrated that areas considered as “classically sterile” such as lungs, blood, synovial space and synovial fluid have a resident microbiota. In addition, these tibiotarsal joints pass through the “toilets”, high pressure system (HPS), pre-cooling, and freezing system (Brasil, 1998), which reduce the microbial load in up to 2 Log_{10} (Brasil, 2011; Cavani et al., 2010).

The histological findings suggested that, although non-specific, the observed alterations were consistent with sequelae of Avian reovirus infection. However, the frequency of isolation of this agent was only significant ($p<0.05$) for the G4 group. Thus, the anomalies in the joints could have been due of other causes and that the presence of Avian reovirus does not present a risk for human health (Andreasen et al., 1991; Manohar; Omprakash; Kanagaraju, 2015; Sellers, 2017; Swayne et al., 2013).

The formation of connective tissue and lymphoplasmacytic inflammatory infiltrations, which is suggestive of an inflammatory process in the joints was observed in most samples. In the absence of significant numbers of microbes, the observed alterations may have been due to physiological response of the birds and a part of its innate immune response (HUBER-LANG; Lambris; Ward, 2018; Sharma, 1998). That occurrence can have multiple causes, such as local tissue damage caused by trauma, exercise deficiency, rapid growth of birds, unbalanced diets, management failures, and genetic factors (Marcon et al., 2019).

Current legislation for animal origin products inspection mentions the condemnation of chicken carcasses, their organs and or their parts when they present evidence of an inflammatory process, arthritis or characteristic lesions of arthritis or hypertrophy of the synovial membrane (Brasil, 2020; Marcon et al., 2019). The inflammatory process acts to eliminate the initial cause of the injury, coordinate the reactions of the innate immune system, eliminate the damaged cells and tissues to initiate the tissue repair and to restore function to maintain the body's dynamic homeostasis

(Kaiser, 2010). Inflammation of the synovial membrane can be induced by substances released from damaged articular cartilage, which expose collagen and enzymes that are not found within the joint. Type II collagen stimulates the production of antibodies and once damage to the cartilage has occurred, collagen molecules are released and are immunologically recognized as foreign substances. Phagocytes and their complexes arise and release substances that cause destruction of the cartilage and, consequently, the release of more substances from it, in a self-perpetuating cycle of the inflammatory process (Corr et al., 2003).

In the Brazilian federal meat inspection service, the carcasses are generally considered to have arthritis when they present a swelling, a change in the synovial fluid color and/or a hypertrophic synovial membrane (Brasil, 2020; Raphael Lucio Andreatti Filho et al., 2020). According to the technical statement from (Alberton et al., 2003) with swine non-infectious articular lesions, the synovial fluid that drains when these joints are cut, presents, in addition to its normal constituents, an increased amount of red blood cells and leukocytes that do not represent a risk to human health and therefore there is no need for the carcass condemnation, even the affected member can be consumed.

Arthritis is the inflammation of one or several joints, which can be classified as acute, chronic, infectious, non-infectious, with serous, fibrinous, hemorrhagic, or purulent exudate. It is associated with the decrease and/or natural loss of the amount of cartilage tissue present in the joint. Trauma and overweight can intensify the natural breakdown of cartilage tissue, which in turn, is attacked by the autoimmune system that can affect the synovium resulting in less synovial fluid production. Arthritis manifests itself as redness of the skin around the joint, swelling, pain and mobility difficulties (Sharma, 1998; Swayne et al., 2013). On the other hand, the inflammation of the tendon sheath and its adjacent tendons is called tenosynovitis, which causes swelling, erythema, and fragility of the tendons in the affected joint and then may provoke the occurrence of ruptures in its extension. Thus, this scenario leads to locomotion problems and splayed legs (Mirbagheri; Hosseini; Ghalyanchilangeroudi, 2020; Souza et al., 2018). According to (Corr et al., 2003), the greater production of synovial fluid is associated with synovitis, where the inflammation of the synovial membrane and/or synovium occurs, causing synovial thickening with the presence of more blood vessels in the region and that may result in synovial effusion with or without the presence of blood.

Thus, the inflammatory process, by itself, could not be characterized as a public health concern. There were no histological findings regarding consistent and significant changes in the lymphoid tissues investigated and hypertrophy in the synovial membrane. The anomalies observed

in tibiotarsal joints of grades 1, 2, 3 and 4, according to the literature, do not characterize arthritis (Akyüz; Onbaşilar, 2020; Xavier et al., 2010).

5 CONCLUSIONS

Through of the macroscopic, microbiological, and histological characterization in the anomalies of the tibiotarsal joints, it can be inferred that groups 1, 2, 3 and 4 anomalies are suggestive of an inflammatory process due to the birds innate immune response, which is not caused by bacterial pathogens.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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