

## Effects of age, line and diet on the occurrence of white striping lesions in broiler breast muscle

L.T. Fernandes<sup>1\*</sup>, J.O. Peixoto<sup>1,2</sup>, M.S. Dal Pizzol<sup>3</sup>, A.M.G. Ibelli<sup>1,2</sup>, M.A.Z. Morés<sup>1</sup>, F.C. Tavernari<sup>1,3</sup>, A. Coldebella<sup>1</sup>, M.E. Cantão<sup>1</sup>, M.C. Ledur<sup>1,3</sup>

<sup>1</sup>Embrapa Suínos e Aves, caixa postal 321, 89715-899, Concórdia, Santa Catarina, Brazil; <sup>2</sup>Programa de Pós-Graduação em Ciências Veterinárias, Universidade Estadual do Centro-Oeste, R. Salvatore Renna, 875, 85015-430, Guarapuava Paraná, Brazil; <sup>3</sup>Programa de Pós-Graduação em Zootecnia, UDESC-Oeste, Rua Beloni Trombeta Zanin, 680E, 89815-630 Chapecó, SC, Brazil. \*[lanatf@yahoo.fr](mailto:lanatf@yahoo.fr)

### Abstract

The White Striping (WS) is one of the most prevalent pectoral myopathies in chickens affecting meat quality. Its etiology, as well as the influence of diet, age and line, remains unclear. Therefore, effects of broiler line, nutrition and age were investigated to better understand and identify possible causative mechanisms triggering the onset and the progression of WS myopathy in the breast of broiler chickens. The most relevant macroscopic lesion observed was white striations on the surface of the breasts. For the lesion identified macroscopically, Ross had an odds ratio of 8.43 in relation to TT line, while for the microscopic lesion the odds ratio was 22.52. The high-performance diet had an odds ratio of 1.76 in relation to the regular diet for the macroscopic lesions only. Therefore, we have shown that the presence of WS increases with age even in broiler lines that have slower growth rate than commercial lines.

### Introduction

In recent years, pectoral myopathies are among the main challenges of the poultry industry, since they negatively affect the sensorial and technological quality of meat (Kato et al., 2019). Among the disorders that affect the breast muscles of fast-growing broilers, one of the most prevalent is the White Striping (WS), which consists macroscopically of the presence of superficial striated fat deposition that runs parallel to the muscle fibres. It affects meat quality, increasing the downgrading percentages and condemnation of breast fillets (Kuttappan et al., 2013). The histological features mostly observed are myodegeneration, interstitial inflammation, infiltration of connective tissue and lipidosis (Vanhatalo et al., 2021). The etiology of pectoral myopathies has been associated with several factors, from the rapid growth rate to the high-calorie diets and specific genotypes (Petracci, et al., 2015). Moreover, heritability estimates for WS in different broiler lines suggest a relevant genetic component for the manifestation of WS (Lake et al., 2021). Nowadays, whole transcriptome studies are making it possible to advance in the knowledge of the genetic mechanisms associated with WS (Marchesi et al., 2018). However, a large amount of data from studies evaluating the physiological and histopathological changes in the *Pectoralis major* muscle across ages and lines are essential to achieve a comprehensive picture of the disorder. Hence, effects of broiler line, nutrition and age were investigated to identify possible causative mechanisms triggering the onset and the progression of WS myopathy in the breast of broiler chickens.

### Material and Methods

This study was conducted with two lines of broiler chickens: a paternal broiler line developed by the Embrapa Swine and Poultry National Research Centre called TT and a commercial line

of fast-growing broilers Ross AP 91. The TT pure line has been maintained under multi-trait selection, in open sided poultry houses, since 1992, in Concordia, Santa Catarina State, in the south of Brazil. The main traits under selection in TT pure line are body weight (BW), feed conversion, breast size (mass selection), carcass and cuts yield, abdominal fat (indirect selection based on non-selected sibs), fertility, hatchability of fertile eggs and chick viability (independent culling levels). For more information see Marchesi et al. (2018).

**Experimental design.** In this study, a 2x2 factorial design was used, with two broiler lines (Ross and TT) and two diets (normal and superior). About 2000 male broilers were raised in 60 boxes with 34 animals each. The diets were recommended for regular (2950-3050 kcal ME/kg; 24.4 - 21.2% CP) and high performance (3250 - 3200 kcal ME/kg; 22 - 24% CP) of broilers, according to the Brazilian Tables for Poultry and Swine (Rostagno, 2017).

**Histopathological analysis.** Approximately 30 chickens of each line and diet were weighed and slaughtered by cervical dislocation at 28, 35 and 42 days of age, following the procedures of the Ethics Committee for Animal Use (CEUA) from the Embrapa Swine and Poultry National Research Centre, under protocol # 08/2019. At necropsy, breast of all animals were visually evaluated for presence of superficial medium-to-thick white striations in the cranial part of the fillet or absence of WS (no white striations) (Kuttappan et al., 2013). Subsequently, samples of 2 cm<sup>2</sup> from the centre-cranial part of *pectoralis major* were collected and fixed by immersion in 10% neutral buffered formalin. Fixed tissue sections were dehydrated and embedded in paraffin wax, and transverse and longitudinal fibre segment for each sample were sectioned at 4 mm and stained with hematoxylin and eosin (HE) for morphologic evaluation.

**Statistical analyses.** A logistic regression was applied to analyse WS as a categorical trait (0 - control and 1- affected) with a model considering the fixed effects of age, line, diet and their interactions, which were performed using the Logistic procedure of the Statistical Analysis System<sup>®</sup> (SAS, 2012). This analysis was complemented with the evaluation of the association between the presence of the lesions with broilers body weight at slaughter. We also performed a  $\chi^2$  test for the association between the micro and macroscopic evaluation of lesions.

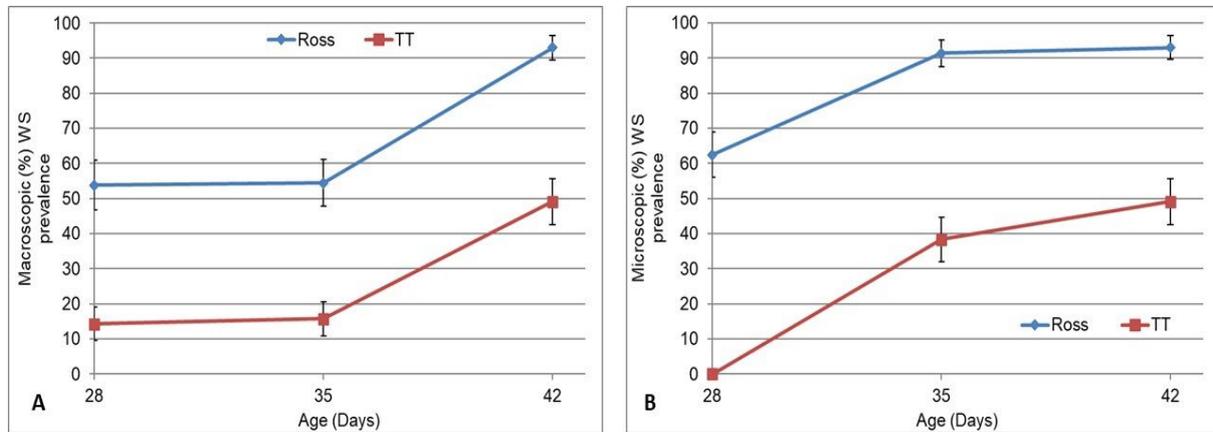
## Results

The observed means for body weight at 42 days of age (BW42) for Ross receiving the high performance diet was 3,349.93 (260.25) and for the regular diet was 3,237.97 (246.46). For the TT line, the observed BW42 was 2,809.23 (173.36) with the high performance diet and 2,806.90 (219.26) with the regular diet.

**Pathology.** The most relevant macroscopic lesion observed was white striations on the surface of the breasts. The main histological features of WS affected breast muscles from both broiler lines were mild to severe degeneration of muscle fibres, moderate to severe degeneration of muscle fibres associated with proliferation of connective tissue and deposition of adipocytes, and mild to severe degeneration of muscle fibres along with mild to severe increased of fat infiltrations (lipidosis).

**Statistical analyses.** The results from the logistic regression showed significant effects of line, diet and age for the presence of macroscopic lesions of WS in the broilers breasts. The microscopic lesions of WS were influenced only by line and age effects. There was an increase in the occurrence of lesions with age and the commercial line Ross showed more WS

lesions than the TT line. Interestingly, at 28 days of age, the TT broilers showed no histological lesions (Figure 1A and 1B). For the lesions identified macroscopically, Ross had an odds ratio of 8.43 in relation to TT line, while for the microscopic lesions the odds ratio was 22.52. The high-performance diet had an odds ratio of 1.76 in relation to the regular diet for the macroscopic lesions only.



**Figure 1. Prevalence and standard error of macroscopic (A) and histologic (B) presence of white striping in TT and Ross broilers breasts by age.**

A negative association between body weight at slaughter and the presence of WS lesions was observed. However, this result was not consistent, since this association was significant for macroscopic lesions only at 42 days of age for both lines and for microscopic lesions only at 28 days for Ross and 42 days for TT (results not shown). There was a positive association ( $p \leq 0.05$ ) between the macro and microscopic detection of WS lesions (Table 1), with an accuracy of 243/335.

**Table 1. Association between macroscopic and microscopic evaluation of lesions.**

| Macroscopic lesions | Microscopic lesions |              | Total |
|---------------------|---------------------|--------------|-------|
|                     | Absent              | Present      |       |
| Absent              | 118 (65.92%)        | 61 (34.08%)  | 179   |
| Present             | 31 (19.87%)         | 125 (80.13%) | 156   |
| Total               | 149                 | 186          | 335   |

## Discussion

In the present study, histopathological changes occurring in WS indicate that the degenerative myopathy observed could be associated with increased growth rate in birds, since both gross and microscopic lesions were significantly influenced by genetic line. Swatland (1990) suggested that fast-growing broilers have muscles that outgrow their life support systems causing muscle damage. Moreover, it was observed an increase in the occurrence of lesions with age. The prevalence of WS lesions in the commercial line was much higher than in the TT in all evaluated ages, although the shape of the curves was similar. Interestingly, at 28 days of age, no microscopic lesion was detected in TT broilers. This result can be explained because the TT line was in its 27<sup>th</sup> generation of annual selection, while the commercial lines are originated from many more generations of continuous selection. The high performance diet was used especially to try to induce TT to develop white striping, as this line is a slower-

growing line compared to Ross. The reason for the high-performance diet had an effect only in the macroscopic lesions of WS is still to be clarified. In our study, the association of WS lesions with body weight was significant only for specific ages, although we have not evaluated this association with breast weight and yield. We also observed a high concordance between the macro and microscopic detection of WS lesions, especially when WS was present.

Selection for breast yield traditionally focus on ages targeting hypertrophy, which occurs normally between 6 and 8 weeks of age, rather than hyperplastic growth. This results in increase in fibre size rather than fibre number, which could favour myopathies occurrence (Orlowski et al., 2021). Therefore, we have shown that the presence of WS increases with age even in broiler lines that have slower growth rate than commercial lines. More physiological and histopathological data from chicken myopathies across different ages and genetic lines can provide essential information to help interpretate and better explore future integrated analyses from the omics methodologies such as RNA-seq, GWAS, exome and epigenome.

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