THE USE OF HEMATOXYLIN AND EOSIN MUSCLE STAINING AND IMAGEJ AS TOOLS TO ASSESS THE INCIDENCE AND SEVERITY OF WHITE STRIPING IN CHICKEN BREASTS

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Abstract

The myopathy known as white striping (WS) is an alteration that increases the deposition of fatty tissue in the breast muscle (*Pectoralis major*) of high yielding broiler chickens. The condition poses a significant threat to the poultry industry as it changes the appearance of important poultry cuts which decreases consumers' willingness to purchase. Macroscopic (objective visual scoring) and microscopic (histological staining) methods have been used to determine the presence and severity of WS. However, a comparison of the effectiveness and usefulness between them needs to be assessed in order to evaluate which one is more adept. Thus, a subsampling from an existing trial evaluating the effects of growth rate (rapid or slow) and L-carnitine supplementation (0 or 100 mg/mL) was used to compare both methodologies. A total of 144 broiler chickens were biopsied on the left cranial ventral region of the *Pectoralis major*. Histological slates were prepared and stained (hematoxylin and eosin; H&E), photographed in triplicates (n=432) using microscope photography technology (Nikon Eclipse TS100), and analyzed using ImageJ software (v. 1.31). Data were analyzed (Proc GLIMMIX, SAS; version 9.3), and the relationship (Proc CORR) between the visual assessment and histological methods were evaluated. No significant interaction between growth rate and L-carnitine supplementation was observed for any of the response variables (P>0.05). Rapid growth rate was seen to increase average cell size (P=0.0315) and percentage adipose tissue relative to muscle cells (P=0.0007), while cell count (P=0.0171) was greater in slow growth birds. Increased incidence and severity of WS was visually observed in birds with rapid growth rate (P<0.0001) and supplementation with 100 mg/mL of L-carnitine (P=0.0348). Also, a significant (P=0.0043) weak correlation (r=0.2375) was found between visual assessment and percentage of adipose tissue relative to muscle cells. Although the study showed that microscopic image analysis with H&E stained was useful in determining presence and severity of WS, it is labor and cost intensive relative to subjective visual assessment which comparatively is more resource efficient.

Introduction

- Poultry seems to fulfill global demand of easy to cook, low caloric, and less expensive meat (FAO, 2010).
- Appearance is a critical consumer criteria in determining quality, and influences acceptance or dismissal of poultry products (Fletcher et al., 2002).
- Carvalho et al. (2021) defines white stripping as the presence of white striations parallel to muscle fibers with distinct degrees of severity which are mostly present in the breast fillet.
- A study that measured consumer acceptance of three degrees of the myopathy concluded that as the severity of the condition increased, there was a decrease in consumer acceptability (Kuttappan et al., 2012).

Objective

Compare two assessment methods of detecting the presence and severity of white striping in broiler breasts of birds with different growth rates (rapid or slow), and different L – carnitine supplementation levels (0 or 100 mg/mL).

Methods and Design

- Broiler chickens' *Pectoralis major* muscle (n=144) were used to determine incidence and severity of WS with two methodologies:
  1. Visual assessment (Bailey et al., 2015)
  2. H&E muscle staining
- Histological slates were developed from biopsies (6mm).
- H&E used to stain muscle and adipose tissue.
- Triplicate images (n=432) were captured using a Nikon TSI 100 microscope and scaled to 100µm using NIS Elements D Software.
- Image analysis with ImageJ software (v 1.31) was performed.
- Cell count, total cell area, average cell size, and percent of muscle area relative to adipose tissue data were obtained.
- Proc CORR and GLIMMIX were done with Tukey adjustment in SAS ($P \leq 0.05$).

Results

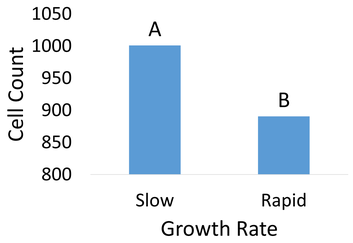


Figure 1. Main effect of growth rate on adipose cell count (P=0.0171).

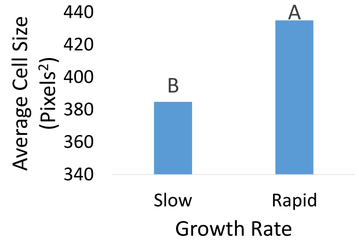


Figure 2. Main effect of growth rate on average adipose cell size (P=0.0315).

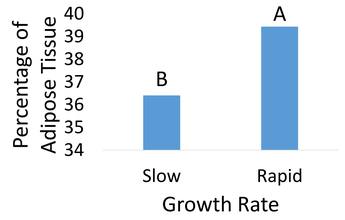
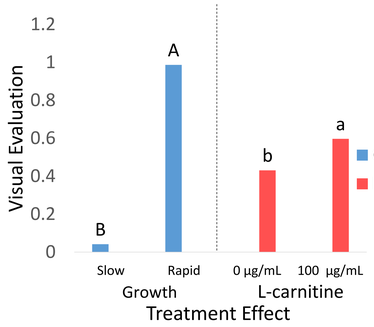


Figure 3. Main effect of growth rate on percentage of adipose tissue (P=0.0007).

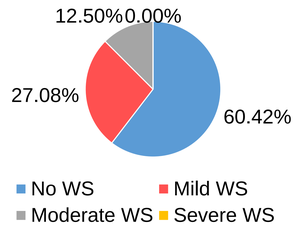
- Birds with slow growth rates showed a higher cell count.
- Average cell size and percentage of adipose tissue was higher in birds with rapid growth rate.



- Birds with rapid growth rate showed greater WS scores.
- When supplemented with 100 µg/mL of L-carnitine, greater WS scores were obtained.

Figure 4. Main effect of growth rate and L-carnitine on visual determination of WS.

[A-B] Significant differences among growth rate (P<0.0001)
[a-b] Significant differences among L-carnitine supplementation (P=0.0348)



- WS was determined with a visual scoring scale from 0 to 3 (0 = No striping; 1 = Mild; 2 = Moderate; 3 = Severe).
- No severe cases of WS were determined via visual assessment.

Figure 5. Visual determination of severity of 144 broiler chickens using a hedonic scale created by Bailey et al. (2015)

- A significant correlation (r=0.2375, P=0.0043) was found between visual assessment and percentage of adipose tissue relative to muscle cells.

Conclusion / Future work

- Image analysis of H&E histology determined presence and severity of WS.
- Visual assessment effectively determined WS and was favored due to its cost effectiveness and reduced labor intensity.
- Oil Red O and Masson's Trichrome staining can serve as a next step to better quantify the change in adipose and connective tissue due to WS.

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