

# Muscle water properties in raw intact broiler breast fillets with the woody breast condition<sup>1</sup>

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**ABSTRACT** The purpose of this study was to investigate the relationships between muscle water properties, water-holding capacity (**WHC**), and woody breast (**WB**) severity in intact raw broiler breast fillets. Broiler pectoralis major deboned at 3 h postmortem was collected from a commercial plant and categorized as normal (**NORM**), moderate WB, or severe WB (**SEV**). Meat drip loss was calculated based on weight loss during overnight storage at 4°C. Water properties of the intact fillets were determined with time domain nuclear magnetic resonance and the T<sub>2</sub> relaxation times were determined using an inverse Laplace algorithm (CONTIN). Three T<sub>2</sub> water components, hydration water (T<sub>2b</sub>), intra-myofibrillar water (T<sub>21</sub>), and extra-myofibrillar water (T<sub>22</sub>), were identified. With increasing WB severity, the time constant of each water component and the relative content of T<sub>22</sub> (P<sub>22</sub>) increased while the relative areas of T<sub>2b</sub> and T<sub>21</sub> (P<sub>2b</sub> and P<sub>21</sub>, respectively) decreased. Spearman

correlation analysis showed that there were significant correlations between the WB condition score and either the time constant or normalized area for each T<sub>2</sub> component. T<sub>22</sub> normalized areas (A<sub>22</sub>) were most strongly correlated with the WB score ( $r = 0.75$ ); however, the weakest correlation was found between the WB score and T<sub>21</sub> areas (A<sub>21</sub>). Pearson correlation analysis revealed that the strongest correlation ( $r = 0.64$ ) was found between A<sub>22</sub> and drip loss; however, there was no correlation between A<sub>21</sub> and drip loss. Within the **NORM** group, drip loss was significantly correlated to the time constants for both T<sub>2b</sub> and T<sub>21</sub>. Within the **SEV** group, only A<sub>22</sub> was significantly correlated to drip loss. These data indicate that the WB condition has a significant impact on the distribution of water within the intact muscle tissue. The content of extra-myofibrillar water in broiler breast fillets may be a key factor responsible for the poor WHC measurements in WB meat.

**Key words:** drip loss, myofibrillar water, NMR T<sub>2</sub> relaxation, pectoralis major, water-holding capacity

2020 Poultry Science 99:4626–4633

<https://doi.org/10.1016/j.psj.2020.05.031>

## INTRODUCTION

The woody breast (**WB**) condition is an emerging myopathy in broiler chickens. Woody breast meat is characterized by palpable hardness and rigidity throughout

the raw pectoralis major (Sihvo et al., 2014; Mudalal et al., 2015) and the exact etiology leading to this defect is not well understood (Petracci et al., 2019). The WB condition has been estimated to cost the industry millions of dollars annually in the United States due to its impacts on meat quality (Kuttappan et al. 2016). In a review, Petracci et al. (2019) pointed out that the characterization of WB meat quality profile and the underlying mechanisms of impaired traits are essential to minimize the negative influence of the myopathy on quality of the end-use chicken products and consumer acceptability.

In addition to having unique texture properties in the raw state, WB meat has also been shown to have altered moisture characteristics and water-holding capacity (**WHC**)/binding capacity when compared to normal (**NORM**) breast meat. WB fillets exhibit greater

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Received November 20, 2019.

Accepted May 22, 2020.

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water/moisture content than NORM fillets (Soglia et al., 2016a, 2016b). During refrigerated storage the WB condition resulted in increased moisture losses compared to NORM fillets (Mudalal et al., 2015; Soglia et al., 2016b; Kuttappan et al., 2017; Bowker et al., 2018; Dalgaard et al., 2018; and Sun et al., 2018). In further processing, WB meat showed poor emulsifying and gelling abilities (Xing et al., 2017; Chen et al., 2018; Petracci et al., 2019) and cook loss of intact broiler fillets with the WB condition can be as much as 10% greater than NORM fillets (Mudalal et al., 2015; Trocino et al., 2015; Zambonelli et al., 2016; Brambila et al. 2017, 2018; Bowker et al., 2018, 2019; Cai et al., 2018).

Time domain nuclear magnetic resonance (TD-NMR) or low-field nuclear magnetic resonance (NMR) has been proposed as an alternative method to investigate muscle water properties and predict WHC in pork and poultry meat (Bertram et al., 2002a; Bianchi et al., 2004). NMR relaxation properties (or transverse relaxation time,  $T_2$ ) provide relevant information about water properties in meat, such as compartmentalization, mobility, and content (Brown et al., 2000). Several published studies have tried using TD-NMR methods to compare water properties between WB and NORM broiler breast meat. Using only a small portion of meat (approximately 600 mg), Soglia et al. (2016a) observed an increase in the proportion and mobility of extra-myofibrillar water in WB meat samples. Tasoniero et al. (2017) reported that the WB condition increased both the proportion of extra-myofibrillar water and the time constant of myofibrillar water in meat strips (1 cm × 1 cm × 4 cm, 5 ± 0.5 g) obtained from the cranial end and medial portion of the fillet. Dalgaard et al. (2018) found that the proportion of extra-myofibrillar water was higher in WB meat cut from the cranial part of the fillet. However, these studies utilized only a small portion of the WB fillet and did not analyze the relationships between the water properties, WHC, and the severity of the WB condition. In addition, use of excised samples may affect the relative proportions of different  $T_2$  time constants or their values.

Thus, the objectives of the present study were to investigate 1) effects of the WB condition on muscle water properties measured with TD-NMR in raw intact broiler breast fillets (pectoralis major), 2) the relationships between the muscle water properties or water property parameters and the severity of the WB condition, and 3) the relationships between the muscle water properties/parameters and WHC measured with drip loss in raw intact broiler breast fillets with varying degrees of the WB condition.

## MATERIALS AND METHODS

### Sample Preparation and Physical Measurements

Boneless and skinless breast fillets (more than 200 and prescreened) were collected from the deboning line of a

commercial processing plant for the large birds at approximately 3 h postmortem. Broiler birds (Ross Line 308) were slaughtered at 8 to 9 wk and processed following the standard operation procedure for retail broiler meat products in the United States, including electrical stunning, soft scalding, immersion chill, automatic evisceration, and hand deboning. Fillets were placed in plastic bags on ice and transported to the U.S. National Poultry Research Center (45 min) where they were trimmed (to remove skin, connective tissue, bone, and mucoid exudate) and weighed. Fillets were scored with a 3-point scale in which, 1 = NORM, 2 = moderate WB (MOD), and 3 = severe WB (SEV) based on palpable hardness and rigidity according to the criteria used by Chatterjee et al. (2016) and Bowker and Zhuang (2019). Fillets were also scored for white striping (WS) with a 3-point scale based on the prevalence and thickness of white striations on the ventral (skin-side) surface of the muscle (Kuttappan et al., 2012). A total of 144 fillets, 48 for each category, were selected for this study.

Breast fillet weight, pH, and color were measured at approximately 6 h postmortem (Zhuang and Bowker, 2018). Muscle pH was measured in the cranial end of each fillet using a Hach H280 GB pH meter equipped with a spear tip probe (EW-5998-20, Cole-Parmer, Vernon Hills, IL). Raw color values (CIE  $L^*a^*b^*$ ) were measured on the dorsal surface (bone side) of each fillet using a Minolta spectrophotometer (VTL CM-700, Konica Minolta, Ramsey, NJ). The spectrophotometer was calibrated by following the manufacturer's instruction for zero (directing the specimen measuring port to midair) and white (directing the port to the white calibration cap) before measurements. Compression force was measured on raw intact breast fillets using a 12-mm diameter probe with a 50 kg loading cell on a texture analyzer (model TA-XT-Plus, Texture Technologies Corp., Hamilton, MA). The trigger force was set at 20 g, and the test speed of the probe was 1 mm/s. For each fillet, 3 compression force measurements were conducted on the middle portion of the ventral side of the muscle. Data for compression force measurements were calculated based on 30% of the fillet height. Similar to the procedure of Sun et al. (2018), the fillets were placed in Ziploc bags and stored at 4°C overnight before fillet weight was measured again (at about 24 h postmortem) for calculating drip loss as a measure of WHC (den Hertog-Meischke et al., 1997). Drip loss was expressed as the percentage of weight change in the breast fillet before and after storage.

### TD-NMR Measurements

A  $^1\text{H}$ -NMR analyzer (Bruker LF90 Proton-NMR Minispec, Bruker Optics, The Woodlands, TX) was used for TD-NMR measurements of muscle water properties in raw intact fillets. Transverse relaxation data ( $T_2$ ) were measured using the Carr-Purcell-Meiboom-Gill pulse sequence (Carr and Purcell, 1954; Meiboom and Gill, 1958) with a  $\tau$ -value (90°–180° pulse separation) of 1 ms. For each fillet sample, whose temperature at

measurements was approximately 4°C, 16 scans were acquired with a total number of 200 echoes. The decay curves were analyzed with the CONTIN regularization algorithm (Provencher, 1982) provided by Minispec (contin-ilt\_mq\_nf, Bruker Biospin GmbH, Rheinstetten, Germany), resulting in the corresponding distributions of the relaxation times and  $T_2$  parameters, hydration water ( $T_{2b}$ ), intra-myofibrillar water ( $T_{21}$ ), extra-myofibrillar water ( $T_{22}$ ), and their time constants, relative proportions, and normalized areas per 100 g of raw meat. The normalized area of  $T_2$  parameters was calculated by the following equation:

Normalized area of  $T_{2x} = 100 \text{ g} \times [\text{the area of a } T_2 \text{ peak/intact fillet weight (g)}]$ .

Like the other peak areas in chemical analysis, the normalized area of the  $T_2$  parameter here is proportional to amount of water that is present in meat or indicates the amount of water that is present in meat.

## Statistical Analysis

Data (including raw meat characteristics and water properties) were subjected to a one-way ANOVA using the PROC MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Significant differences ( $P < 0.05$ ) between means were identified using a Tukey's mean separation method. The relationships between WB score and water properties were analyzed by calculating Spearman correlation coefficients ( $r_s$ ) using the PROC CORR procedure of SAS. Similarly, the relationships between drip loss values and water properties were analyzed with Pearson correlation coefficients ( $r$ ). For the purposes of discussion, in this study the following descriptors were used to describe the relative strength of the correlations: very weak ( $r < 0.20$ ), weak ( $r = 0.20\text{--}0.39$ ), moderate ( $r = 0.40\text{--}0.59$ ), strong ( $r = 0.60\text{--}0.79$ ), and very strong ( $r = 0.80\text{--}0.99$ ) (Bowker and Zhuang, 2019).

## RESULTS

### Fillet Quality Attributes

Table 1 shows that raw intact fillets with the WB (MOD or SEV) condition had greater average weight, drip loss, pH, and WS score compared with NORM fillets ( $P < 0.05$ ). There were no differences in weight, drip loss, and pH value between MOD and SEV samples ( $P > 0.05$ ). SEV fillet samples had greater compression force than NORM samples ( $P < 0.05$ ). For CIE  $L^*a^*b^*$  color measurements in this study, greater  $a^*$  values in SEV fillets and greater  $b^*$  values in SEV and MOD fillets were observed compared with NORM fillets ( $P < 0.05$ ), and there were no differences in  $L^*$  values among the 3 WB conditions ( $P > 0.05$ ).

### TD-NMR Parameters

Figure 1 shows the representative distribution of  $T_2$  relaxation time collected from intact fillets with

**Table 1.** Raw meat characteristics of broiler breast fillets with the WB condition (mean  $\pm$  SD,  $n = 48$ ).

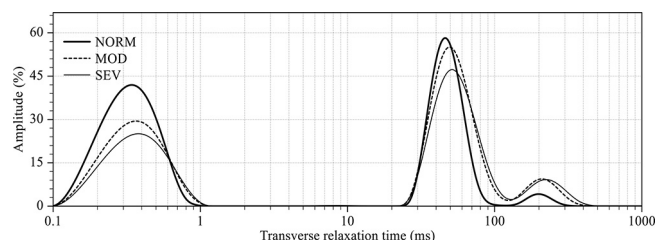
Parameter	WB condition		
	NORM	MOD	SEV
Weight (g)	461 $\pm$ 64 <sup>b</sup>	571 $\pm$ 58 <sup>a</sup>	555 $\pm$ 55 <sup>a</sup>
Drip loss (%)	1.14 $\pm$ 0.53 <sup>b</sup>	1.64 $\pm$ 0.77 <sup>a</sup>	1.84 $\pm$ 0.71 <sup>a</sup>
pH	5.98 $\pm$ 0.13 <sup>b</sup>	6.14 $\pm$ 0.18 <sup>a</sup>	6.11 $\pm$ 0.15 <sup>a</sup>
$L^*$ (dorsal)	60.5 $\pm$ 1.9	62.2 $\pm$ 2.6	60.7 $\pm$ 2.5
$a^*$ (dorsal)	-0.8 $\pm$ 0.5 <sup>b</sup>	-0.1 $\pm$ 0.8 <sup>a,b</sup>	0.4 $\pm$ 0.9 <sup>a</sup>
$b^*$ (dorsal)	11.4 $\pm$ 1.2 <sup>b</sup>	13.8 $\pm$ 1.7 <sup>a</sup>	14.7 $\pm$ 1.6 <sup>a</sup>
WS score <sup>1</sup>	1.28 $\pm$ 0.34 <sup>c</sup>	1.85 $\pm$ 0.44 <sup>b</sup>	2.33 $\pm$ 0.58 <sup>a</sup>
Compression force (N)	19.7 $\pm$ 4.4 <sup>b</sup>	26.9 $\pm$ 6.6 <sup>a,b</sup>	36.4 $\pm$ 9.8 <sup>a</sup>

<sup>a-c</sup>Means within a row lacking a common superscript differ ( $P < 0.05$ ).

Abbreviations: MOD, moderate woody breast; NORM, normal breast; SEV, severe woody breast; WB, woody breast; WS, white striping.

<sup>1</sup>WS score: 1 = no WS; 2 = moderate WS; 3 = severe WS.

CONTIN regularization analysis, where 3 distinct water components were identified despite the WB condition. Table 2 shows the average time constants ( $T_{2b}$ ,  $T_{21}$ , and  $T_{22}$ ), relative areas or proportion ( $P_{2b}$ ,  $P_{21}$ , and  $P_{22}$ ), and normalized areas ( $A_{2b}$ ,  $A_{21}$ , and  $A_{22}$ , normalized to 100 g of meat) of the 3  $T_2$  components. The time constant of  $T_{2b}$  in MOD and SEV fillets was similar (0.43 and 0.44 ms, respectively) ( $P > 0.05$ ), and greater than that in NORM fillets (0.39 ms) ( $P < 0.05$ ). The time constants of  $T_{21}$  and  $T_{22}$  showed significant increases with the severity of the WB conditions. The proportion of  $T_{2b}$  or  $P_{2b}$  in intact broiler breast meat was less than 1% of the total water. In MOD and SEV fillets,  $P_{2b}$  was similar (0.40 and 0.35%, respectively). However,  $P_{2b}$  was greater in NORM fillets (0.79%) than WB fillets ( $P < 0.05$ ). The proportion of  $T_{21}$  or  $P_{21}$  was more than 60% in intact broiler breast meat and was different between the 3 WB conditions ( $P < 0.05$ ).  $P_{21}$  in NORM fillets (81%) was greater than both MOD (65%) and SEV (60%) fillets ( $P < 0.05$ ). The effects of WB on  $P_{22}$  were opposite to those on  $P_{21}$ .  $P_{22}$  was greater in MOD (34%) and SEV (39%) fillets than that in NORM fillets (18%) ( $P < 0.05$ ). The normalized area of  $T_{2b}$  or  $A_{2b}$  in MOD and SEV fillets (2.41 and 2.25 unit/100 g meat) was less than that in NORM fillets (3.65 unit/100 g meat) ( $P < 0.05$ ), whereas  $A_{21}$  was just the opposite.  $A_{21}$  was greater in MOD and SEV fillets (407.63 and 404.47 unit/100 g meat) than that in NORM fillets (385.21 unit/100 g meat) ( $P < 0.05$ ).  $A_{22}$  showed significant increases with the severity of the WB condition.  $A_{22}$  was greater in MOD (223.40 unit/100 g meat) and SEV



**Figure 1.** Representative distribution of  $T_2$  relaxation time for normal breast (NORM), moderate woody breast (MOD), and severe woody breast (SEV) fillets based on CONTIN regularization analysis.

**Table 2.** Effect of the WB condition on water properties time constants (T), relative areas (P), and normalized areas (A) (mean  $\pm$  SD, n = 48).

Parameter	WB condition		
	NORM	MOD	SEV
Time constant (ms)			
T <sub>2b</sub>	0.39 $\pm$ 0.03 <sup>b</sup>	0.43 $\pm$ 0.03 <sup>a</sup>	0.44 $\pm$ 0.02 <sup>a</sup>
T <sub>21</sub>	49.04 $\pm$ 2.91 <sup>c</sup>	54.74 $\pm$ 4.20 <sup>b</sup>	57.21 $\pm$ 4.02 <sup>a</sup>
T <sub>22</sub>	204.88 $\pm$ 19.24 <sup>c</sup>	218.20 $\pm$ 13.60 <sup>b</sup>	234.64 $\pm$ 22.54 <sup>a</sup>
Relative area (%)			
P <sub>2b</sub>	0.79 $\pm$ 0.25 <sup>a</sup>	0.40 $\pm$ 0.18 <sup>b</sup>	0.35 $\pm$ 0.14 <sup>b</sup>
P <sub>21</sub>	81.02 $\pm$ 3.93 <sup>a</sup>	65.48 $\pm$ 8.63 <sup>b</sup>	60.42 $\pm$ 7.55 <sup>c</sup>
P <sub>22</sub>	18.18 $\pm$ 4.08 <sup>c</sup>	34.18 $\pm$ 8.74 <sup>b</sup>	39.23 $\pm$ 7.62 <sup>a</sup>
Normalized area (unit/100 g meat)			
A <sub>2b</sub>	3.65 $\pm$ 0.95 <sup>a</sup>	2.41 $\pm$ 0.75 <sup>b</sup>	2.25 $\pm$ 0.53 <sup>b</sup>
A <sub>21</sub>	385.21 $\pm$ 26.44 <sup>b</sup>	407.63 $\pm$ 34.15 <sup>a</sup>	404.47 $\pm$ 51.13 <sup>a</sup>
A <sub>22</sub>	88.80 $\pm$ 31.06 <sup>c</sup>	223.40 $\pm$ 90.02 <sup>b</sup>	273.36 $\pm$ 95.82 <sup>a</sup>

<sup>a-c</sup>Means within a row lacking a common superscript differ ( $P < 0.05$ ).

Abbreviations: MOD, moderate woody breast; NORM, normal breast; SEV, severe woody breast; WB, woody breast.

(273.36 unit/100 g meat) fillets than that in NORM fillets (88.80 unit/100 g meat) ( $P < 0.05$ ).

in NORM samples ( $r = 0.38$ ); however, it was negatively correlated with purge loss in MOD samples ( $r = -0.29$ ).

### Relationships Between the WB Condition and Water Properties

Table 3 shows the Spearman correlations between the WB score and the water property parameters. There were significant Spearman correlations ( $P < 0.001$ ) between WB scores and the time constants of T<sub>2b</sub>, T<sub>21</sub>, and T<sub>22</sub>, and their corresponding normalized areas A<sub>2b</sub>, A<sub>21</sub>, and A<sub>22</sub>, which were 0.52, 0.68, 0.56,  $-0.58$ , 0.29, and 0.75, respectively. Table 4 shows the Pearson correlations between drip loss or WHC and water property parameters. With the exception of A<sub>21</sub>, there were significant Pearson correlations ( $P < 0.001$ ) between drip loss and the time constants of T<sub>2b</sub>, T<sub>21</sub>, and T<sub>22</sub>, and the normalized areas A<sub>2b</sub> and A<sub>22</sub>, which were 0.38, 0.45, 0.38,  $-0.40$ , and 0.64, respectively. Table 5 shows the Pearson correlations between drip loss and water properties within each WB condition. For time constants, significant Pearson correlations between drip loss and time constants of T<sub>2b</sub> and T<sub>22</sub> were only observed in NORM fillets. For the normalized areas, there were significant correlations between drip loss and normalized areas for all T<sub>2</sub> components in NORM fillets. However, in SEV fillets there was only a significant relationship between drip loss and A<sub>22</sub>. For MOD fillets, significant relationships were noted between drip loss and A<sub>21</sub> or A<sub>22</sub>. In addition, A<sub>21</sub> was positively correlated with purge loss

### DISCUSSION

The raw meat characteristics of broiler breast fillets with the WB condition (Table 1) are well in agreement with data published in previous reports. The average weights of WB fillets were more than that of NORM fillets (Mudalal et al., 2015; Chatterjee et al., 2016; Dalle Zotte et al., 2017; Dalgaard et al., 2018; Sun et al., 2018). The WS scores were positively related to the WB condition (Bowker and Zhuang, 2019). Many studies observed greater drip loss (Mudalal et al., 2015; Soglia et al., 2016b; Kuttappan et al., 2017; Bowker et al., 2018; Sun et al., 2018) and pH values (Mudalal et al., 2015; Brambila et al., 2017; Dalle Zotte et al., 2017; Kuttappan et al., 2017; Bowker et al., 2018; Zhuang and Bowker, 2018; Baldi et al., 2019) in WB meat compared with the NORM ones. So far the CIE L\*a\*b\* color data reported in literature have not shown consistent relationships between the WB condition and CIE L\*a\*b\* color measurements on the dorsal side of fillets. Trocino et al. (2015) and Brambila et al. (2017) found no significant differences between WB fillets and NORM fillets regardless of the CIE L\*a\*b\* parameters. However, Chatterjee et al. (2016) and Zhuang and Bowker (2018) showed the difference in a\* value. Mudalal et al. (2015) found the difference in b\* value. Baldi et al. (2019) reported differences in L\* as well as

**Table 3.** Spearman correlations between WB score (1 = NORM, 2 = MOD, 3 = SEV) and water properties time constants (T) or normalized areas (A).

	Time constant			Normalized area		
	T <sub>2b</sub>	T <sub>21</sub>	T <sub>22</sub>	A <sub>2b</sub>	A <sub>21</sub>	A <sub>22</sub>
WB score	0.52***	0.68***	0.56***	$-0.58$ ***	0.29***	0.75***

\*\*\* $P \leq 0.001$ .

Abbreviations: MOD, moderate woody breast; NORM, normal breast; SEV, severe woody breast; WB, woody breast.

**Table 4.** Pearson correlations between drip loss and water properties time constants (T) or normalized areas (A).

	Time constant			Normalized area		
	T <sub>2b</sub>	T <sub>21</sub>	T <sub>22</sub>	A <sub>2b</sub>	A <sub>21</sub>	A <sub>22</sub>
Drip loss	0.38***	0.45***	0.38***	-0.40***	-0.04	0.64***

\*\*\* $P \leq 0.001$ .

b\* values. Pang et al. (2020) and Sun et al. (2018) demonstrated that compression force increased with the severity of the WB condition. These physical and quality characteristics of NORM and WB fillets confirmed that the broiler breast fillets selected in this study were typical for each WB condition and there were differences between the 3 WB conditions.

Distribution analysis (e.g., inverse Laplace transform) of TD-NMR measurements revealed 3 distinct water components in the fresh intact fillets with T<sub>2</sub> relaxation time constants around 0 to 15, 40 to 80, and 100 to 400 ms (Bertram et al., 2001, 2002a, 2002b). The water component with a T<sub>2</sub> relaxation time constant around 0 to 15 ms is the fastest relaxing component in T<sub>2</sub>. It has been named T<sub>2b</sub> and is hypothesized to reflect hydration water, or water tightly associated to the macromolecules in meat. The water component with a T<sub>2</sub> relaxation time constant around 40 to 80 ms is named T<sub>21</sub> and is hypothesized to reflect the intra-myofibrillar water located within the highly organized myofibrillar lattice space. The slowest relaxing component in T<sub>2</sub> is named T<sub>22</sub> and presumably corresponds to loosely bound extra-myofibrillar water (Bertram et al., 2002b; Hullberg and Bertram, 2005; Tasoniero et al., 2017; Chen et al., 2018). The TD-NMR results collected from intact fillets in this study (Figure 1) reveal a relaxation pattern very similar to that reported in previously published studies collected with raw chicken meat. Three distinct water components (T<sub>2b</sub>, T<sub>21</sub>, and T<sub>22</sub>), from left to right in the T<sub>2</sub> relaxation time distribution, were identified in raw intact broiler breast fillets. Both their time constants and amplitudes appeared to be

influenced by the severity of the WB condition (Figure 1).

The average time constant of T<sub>2b</sub> ranged from 0.39 to 0.44 ms, the average time constant of T<sub>21</sub> from 49.04 to 57.21 ms, and the average time constant of T<sub>22</sub> from 204.88 to 234.64 ms (Table 2). These results are similar to those reported earlier with fresh pork meat (Bertram et al., 2002a, 2002b) and chicken breast meat (Petracci et al., 2012; Soglia et al., 2016a; Xing et al., 2017), where the time constants of T<sub>2b</sub>, T<sub>21</sub>, and T<sub>22</sub> were found centered around 0 to 15, 40 to 80, and 100 to 400 ms, respectively. The differences in T<sub>2</sub> constants between our results and those published by Tasoniero et al. (2017) for T<sub>21</sub> and T<sub>22</sub> might have resulted from the differences in instrumentation, data processing methods, and/or use of intact vs. excised samples. Bertram et al. (2001) also noted differences in T<sub>2</sub> time constants and areas among the published literature and attributed them to magnetic equipment, NMR parameters employed, such as repetition time between succeeding scans and the  $\tau$ -value (time between 90° pulse and 180° pulse), sample temperature, and postmortem time when meat samples were measured.

Our data further showed that with increases in the severity of the WB condition, the means for all T<sub>2</sub> time constants in WB fillets were significantly greater ( $P < 0.05$ ) than those in NORM fillets (Table 2), indicating that the mobility of water in WB fillets is greater than that in NORM fillets. Increased T<sub>2</sub> time constants were also observed in the WB muscle by Soglia et al. (2016a) and Tasoniero et al. (2017). Differences ( $P < 0.05$ ) between NORM and WB samples were also found in both the relative (P) and normalized areas (A) of the 3 water components (Table 2). It should be noted that when data were expressed as the normalized area per 100 g of raw meat, the effects of WB on A<sub>2b</sub> and A<sub>22</sub> were similar to when data were expressed as the relative area P<sub>2b</sub> and P<sub>22</sub>. For A<sub>21</sub>, however, expressing the data as normalized area showed that WB meat contained more intra-myofibrillar water (>400 unit/100 g of meat) than NORM (<390 unit/

**Table 5.** Pearson correlations between drip loss and water properties time constants (T) or normalized areas (A) within the WB condition.

Parameter	WB condition		
	NORM (n = 48)	MOD (n = 48)	SEV (n = 48)
	Drip loss (%)	Drip loss (%)	Drip loss (%)
Time constant (ms)			
T <sub>2b</sub>	0.40**	0.20	-0.07
T <sub>21</sub>	0.49***	0.21	0.18
T <sub>22</sub>	0.19	0.19	0.28
Normalized area (unit/100 g meat)			
A <sub>2b</sub>	-0.32*	-0.16	-0.19
A <sub>21</sub>	0.38***	-0.29*	-0.24
A <sub>22</sub>	0.66***	0.61***	0.50***

\* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ .

Abbreviations: MOD, moderate woody breast; NORM, normal breast; SEV, severe woody breast; WB, woody breast.

100 g of meat). Expressing  $T_{22}$  data as normalized area ( $A_{22}$ ) also suggested that the SEV WB fillets contained approximately 3 times more extra-myofibrillar water than NORM fillets. Using much smaller meat samples (<5 g), [Sogila et al. \(2016a\)](#) and [Tasoniero et al. \(2017\)](#) found that  $P_{2b}$  (hydration) and  $P_{21}$  (intra-myofibrillar) in WB meat were significantly lower than those in NORM meat; however,  $P_{22}$  (extra-myofibrillar) in breast meat with the WB condition was more than 2 times greater than that in NORM. Data in the current study are consistent with published data ([Tasoniero et al., 2017](#); [Chen et al., 2018](#)) and further demonstrate that WB meat contains a greater proportion of extra-myofibrillar water and lower proportion of intra-myofibrillar and hydration water than NORM meat. Furthermore, by expressing data as a function of fillet weight (unit/100 g meat), data in this study suggest that WB meat has a greater abundance of both intra- and extra-myofibrillar water compared to NORM meat. This is likely due to both the overall larger size of the WB fillets compared to the NORM fillets as well as the fact that WB meat has a higher moisture content than NORM meat ([Petracci et al., 2019](#)).

There were significant Spearman correlations ( $P < 0.001$ ) between WB scores and water properties regardless of the  $T_2$  component ( $T_{2b}$ ,  $T_{21}$ , or  $T_{22}$ ) or expression of  $T_2$  components (time constant or normalized area) ([Table 3](#)). These results indicate that water properties such as water mobility and the normalized amount in muscle are associated with the severity of the WB condition in broiler breast muscle. Among the time constants, the strongest correlation was found for  $T_{21}$  ( $r_s = 0.68$ ), followed by  $T_{22}$  ( $r_s = 0.56$ ), and they were all positive. These data suggest that water mobility or water activity may have influences on physical and technological properties of broiler breast meat with the WB myopathy. There were also significant positive relationships ( $P < 0.001$ ) between WB scores and either  $A_{21}$  or  $A_{22}$  ([Table 3](#)). However, the strength of the correlation between  $A_{22}$  and WB scores was approximately 2.5 times higher than between  $A_{21}$  and WB scores and explained more than 50% of the experimental variation. There was also a moderate and negative correlation between WB scores and  $A_{2b}$ . These data demonstrate that the abundance of extra-myofibrillar water is more closely associated with the severity of WB meat. These results also suggest that the greater overall water content and higher amount of extra-myofibrillar water in WB fillets might be at least partially responsible for the physical properties, such as hardness and rigidity, used for scoring WB severity in raw broiler breast meat. Using NMR, [Tasoniero et al. \(2017\)](#) also suggested a connection between the increased muscle hardness observed in WB meat and the longer relaxation times of water trapped within the myofibrillar matrix.

The relationships between the measured water properties and drip loss are shown in [Table 4](#). With the exception of  $A_{21}$ , the water property parameters were significantly correlated to drip loss. The strongest

correlation was found between drip loss and  $A_{22}$  ( $r = 0.64$ ). Moderate correlations were found between drip loss and  $A_{2b}$  ( $r = -0.40$ ,  $P < 0.001$ ) or  $T_{21}$  ( $r = 0.45$ ,  $P < 0.001$ ), whereas, weak correlations were found between drip loss and either  $T_{2b}$  or  $T_{22}$  ( $r = 0.38$ ,  $P < 0.001$ ). These results indicate that changes in WHC, measured with drip loss, of broiler breast meat with the WB condition may be more likely determined by extra-myofibrillar water content.

The correlations between TD-NMR measurements and drip loss were also analyzed within each WB condition to determine if these relationships were influenced by the WB myopathy. The results suggested that the relationship between drip loss and the various water component measurements varies with the WB condition ([Table 5](#)). Similarly, [Bertram et al. \(2001\)](#) found that there were highly significant correlations ( $r > |0.70|$ ) between WHC and either  $T_{21}$  or  $T_{22}$  regardless of the method used for WHC analysis in pork. [Bertram et al. \(2002b\)](#) further demonstrated a strong correlation between the  $T_{22}$  water component and drip loss in pork and concluded that meat WHC was mainly determined by the amount of loosely bound extra-myofibrillar water in the muscle and that the formation of drip loss in NORM pork meat was an ongoing process involving the transfer of water from within myofibrils to the extracellular space. Data in the current study suggest that the mobility of extra-myofibrillar water does not have a significant impact on drip loss in broiler breast meat regardless of the WB condition ([Table 5](#)). In NORM broiler breast meat, however, drip loss was significantly and positively correlated to  $A_{21}$  and  $A_{22}$ , which suggests that drip loss is an ongoing process involving the transfer of water from intra-myofibrillar water components to the extracellular space similar to NORM pork. However, drip loss in SEV WB meat is more likely determined by only the amount of loosely bound extra-myofibrillar water. In meat with the MOD WB condition, the abundance of intra-myofibrillar water, in addition to extra-myofibrillar water, may also be involved in its drip loss over refrigerated storage.

## CONCLUSIONS

TD-NMR has been used to analyze differences in the water properties between NORM and WB meat using whole intact fillets. Experimental data demonstrate that, in agreement with earlier NMR data collected on small muscle subsamples or ground meat, 3 distinct water components (hydration water, intra-myofibrillar water, and extra-myofibrillar water) were identified in raw intact broiler breast fillets. In WB meat, water mobility is greater regardless of the water component as evidenced by a shift of  $T_{2x}$  values to longer times, and there is more relative extra-myofibrillar water and less relative intra-myofibrillar water and hydration water. When expressed as normalized area/100 g of tissue, the abundance of both intra- and extra-myofibrillar water was greater in WB meat compared to NORM breast meat. Data also suggest that greater extra-myofibrillar water

content and intra-myofibrillar water mobility may be responsible for the physical and technological properties of raw WB meat, although this remains to be demonstrated with further studies on their relationships. Among the 3  $T_2$  components in WB meat, the  $T_{22}$  normalized area  $A_{22}$  exhibited the greatest correlation with drip loss indicating that extra-myofibrillar water likely plays a key role in the poor WHC of WB meat. Furthermore, this study reveals that, in addition to the amount of extra-myofibrillar water, both water mobility and the amounts of both hydration water and intra-myofibrillar water affect drip loss in NORM broiler breast meat. However, drip loss may be determined primarily by the amount of extra-myofibrillar water in WB meat.

**Conflict of Interest Statement:** The authors did not provide a conflict of interest statement.

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