WATER-HOLDING CAPACITY AND PROTEIN DENATURATION IN BROILER BREAST MEAT

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Abstract – The aim of this study was to determine the relationship between water-holding capacity (WHC) and protein denaturation in broiler breast meat. Breast fillets were collected at 2 h postmortem and segregated into low- and high-WHC groups based on pH and color. Protein solubility was measured at 6 and 24 h postmortem as an indicator of protein denaturation. Brine uptake and drip loss were measured for WHC. High-WHC fillets had greater brine uptake at 6 and 24 h postmortem and less accumulated drip loss after 2 and 7 days compared to low-WHC fillets. Brine uptake increased with postmortem time of sampling. Myofibrillar protein solubility decreased with postmortem time but was not different between low- and high-WHC fillets. Sarcoplasmic protein solubility increased with postmortem time and was greater in high-WHC fillets. Further analysis showed that brine uptake and drip loss were correlated to the relative abundance of glycogen phosphorylase in both protein fractions, and increased glycogen phosphorylase denaturation was observed in low-WHC fillets. Data suggest that the denaturation of glycogen phosphorylase onto myofibrils may influence WHC but that inherent differences in myofibrillar protein denaturation are not the predominant source of WHC variation in broiler breast fillets.

I. INTRODUCTION

Broiler breast muscle, which is comprised of nearly 100% fast-twitch glycolytic muscle fibers, is highly susceptible to developing inferior water-holding capacity (WHC). The ability of meat to bind water is a complex trait that is influenced by structural and biochemical changes that occur during the transformation of muscle to meat. Muscle pH and protein denaturation are considered the main determinants of WHC in meat [1]. Protein solubility and extractability are often used as measures of protein denaturation within meat. In pork and turkey muscle, low WHC is often associated with excessive postmortem protein denaturation, particularly that of myosin [2, 3]. The underlying mechanisms that control WHC in pale broiler meat are not as well-established. Some reports have shown that myofibrillar proteins from chicken breast muscle are resistant to denaturation [4] and that pale and normal colored fillets have similar protein solubilities [5]. Others have observed lower salt-soluble protein extractability in pale breast meat [6] and increased sarcoplasmic protein denaturation in fillets incubated at elevated temperatures [7]. In broiler breast meat, the effect of postmortem time on the relationship between WHC and protein denaturation is unknown. The objective of this study was to determine muscle protein solubility in broiler breast fillets with widely varying WHC at different times postmortem.

II. MATERIALS AND METHODS

Deboned butterfly breast fillets (n=72) were obtained from a commercial processing line and separated into two groups (low-WHC, high-WHC) based on muscle pH and color values (L*a*b*). At 6 and 24 h postmortem, muscle samples from each butterfly fillet were taken for measurement of water-holding capacity and protein solubility. Brine uptake (%) was measured by homogenization and centrifugation in 0.6 M NaCl buffer [8]. Accumulated drip loss (%) was measured after storage for 2 and 7 days postmortem at 4°C. Protein solubility was measured in 0.25 mM potassium phosphate (pH 7.2) buffer (sarcoplasmic protein solubility) and in 0.1 M potassium phosphate/1.1 M KI buffer (total protein solubility). Myofibrillar solubility was calculated from the difference between total and sarcoplasmic protein solubility.

Table 1. Meat quality measurements (lsmeans) of low-WHC and high-WHC broiler breast fillets.

Measurement:	Low-WHC	High-WHC	SEM
pH 4h	5.99[b]	6.17[a]	0.03
pH 24h	5.83[b]	6.20[a]	0.03
L*	61.3[a]	46.3[b]	0.4
a*	0.4[b]	0.9[a]	0.1
b*	13.0[a]	9.2[b]	0.3

[ab] LSmeans with different letters differ (p<0.05)

At 24 h postmortem, myofibrillar and sarcoplasmic protein fractions were separated by subcellular fractionation [3] in buffer containing 50 mM KCl, 20 mM Tris, pH 7.0, 2 mM EDTA, 4 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 0.1 mM PMSF, and 1% (v/v) Triton X-100, and in rigor buffer (75 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$ 2 mM EGTA, pH 7.0). Protein fractions were loaded onto 4-20% Tris-glycine gels for SDS-PAGE analysis and for transfer to PVDF membrane to detect glycogen phosphorylase in the protein fractions by western blotting. Data were analyzed using PROC MIXED (SAS v. 9.2) models with group (low-WHC, high-WHC) and postmortem time as fixed effects and carcass as a random effect.

III. RESULTS AND DISCUSSION

Selecting broiler breast fillets by color resulted in two distinct groups of samples with regards to meat quality attributes. Expectedly, the pale (low-WHC) fillets had lower pH and higher L* values than dark (high-WHC) fillets (Table 1). At 6 and 24 h postmortem, brine uptake in high-WHC fillets was approximately 3- to 4-fold greater than in low-WHC fillets (Fig. 1).

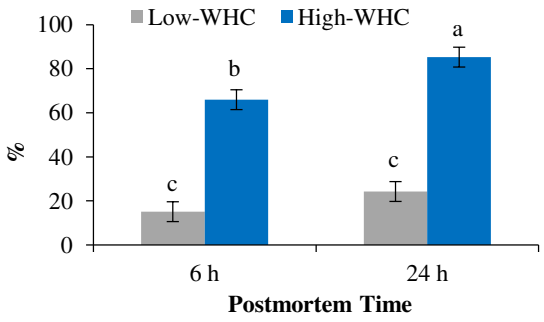

Fig. 1. Brine uptake (%) of broiler breast fillets at 6 and 24 h postmortem.

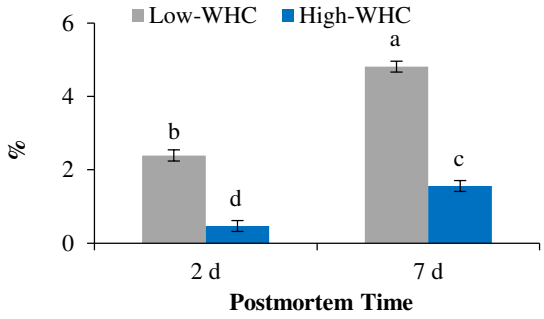

Fig. 2. Accumulated drip loss (%) of broiler breast fillets after 2 and 7 days.

From 6 to 24 h postmortem, brine uptake increased 9% in low-WHC fillets and 20% in high-WHC fillets. Drip loss accumulations were greater in low-WHC fillets after both 2 and 7 days of refrigerated storage (Fig. 2).

Protein solubility was used as an indicator of postmortem protein denaturation within the breast fillets. Myofibrillar protein solubility was similar between low- and high-WHC breast fillets at both 6 and 24 h postmortem (Table 2). Thus, despite the widely divergent WHC attributes between the pale and dark fillets, the overall degree of myofibrillar protein denaturation was not different between the two groups of fillets used in this study. Furthermore, correlation analysis indicated that myofibrillar protein solubility was not related to brine uptake or drip loss (Table 3). These findings suggest that myosin denaturation is not the primary determinant of WHC differences in pale and dark broiler breast fillets and support the idea that myosin from chicken breast meat is resistant to denaturation [4]. Similarly, Van Laack [5] observed only minor differences in total protein solubility between pale and normal colored breast fillets.

Table 2. Protein solubility measurements (lsmeans) of low-WHC and high-WHC broiler breast fillets.

Measurement:		Low-WHC	High-WHC	SEM
Myofibrillar[1]	6 h	121.3[ab]	121.9[a]	2.8
	24 h	114.3[ab]	113.9[b]	
Sarcoplasmic[1]	6 h	79.3[c]	82.7[b]	1.8
	24 h	84.2[b]	88.0[a]	

[ab] LSmeans with different letters within a measurement differ (p<0.05)

[1] Solubility measurements expressed as mg protein/g tissue

Table 3. Correlation coefficients (r) between WHC and protein solubility measurements.

		Brine Uptake		Drip Loss	
Protein Solubility:		6 h	24 h	2 d	7 d
Myofibrillar	6 h	0.13	0.15	-0.14	-0.01
	24 h	0.03	0.09	-0.12	-0.03
Sarcoplasmic	6 h	0.08	0.13	-0.33*	-0.17
	24 h	0.06	0.08	-0.31*	-0.11

* P<0.01

The denaturation of sarcoplasmic proteins is also thought to play a role in determining WHC in meat [9]. In the current study, sarcoplasmic protein solubility was slightly greater in high-WHC breast fillets at both 6 and 24 h postmortem (Table 2). Similar differences in sarcoplasmic protein solubility have also been observed between pale and normal colored broiler breast meat [5].

Other than being weakly related to drip loss accumulation at 2 days, overall sarcoplasmic protein solubility was not correlated to WHC parameters (Table 3). Further analysis showed that within the sarcoplasmic protein fraction of the muscle, glycogen phosphorylase seemed to play a key role in determining WHC. In low-WHC fillets, SDS-PAGE analysis (Fig. 3) indicated that the relative abundance of glycogen phosphorylase was decreased in the sarcoplasmic protein fraction and increased in the myofibrillar fraction compared to high-WHC fillets (Table 4).

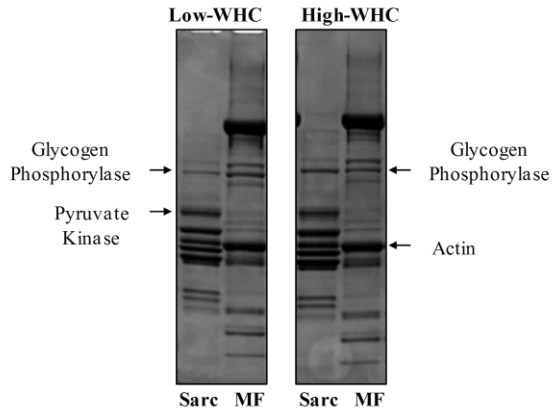

Fig. 3. SDS-PAGE of sarcoplasmic and myofibrillar protein fractions from broiler breast fillets at 24 h postmortem.

Table 4. Relative abundance (lsmeans) of glycogen phosphorylase in myofibrillar and sarcoplasmic protein fractions from broiler breast fillets.

Protein Fraction:	Low-WHC	High-WHC	SEM
Myofibrillar[1]	0.27[a]	0.15[b]	0.01
Sarcoplasmic[2]	0.28[b]	0.52[a]	0.03

[ab] LSmeans with different letters differ (p<0.05)
[1] Glycogen phosphorylase band intensity expressed relative to actin band intensity.
[2] Glycogen phosphorylase band intensity expressed relative to pyruvate kinase band intensity.

This phenomenon was likely due to the early postmortem pH and temperature conditions of the low-WHC fillets causing glycogen phosphorylase to denature and precipitate onto the myofibrils. A similar shift between the sarcoplasmic and myofibrillar protein fractions due to glycogen phosphorylase denaturation has been shown in both turkey and pork [3, 10]. In chicken, incubation of breast muscles at 40°C has been found to induce glycogen phosphorylase denaturation, cause similar shifts in SDS-PAGE banding patterns, and result in higher drip loss [7]. In the current study, the relative abundance of glycogen phosphorylase in both the myofibrillar and sarcoplasmic protein fractions of the breast fillets was strongly related to brine uptake and drip loss measurements (Table 5).

Table 5. Correlation coefficients (r) between WHC and relative abundance of glycogen phosphorylase in myofibrillar and sarcoplasmic protein fractions.

		Brine Uptake		Drip Loss	
Protein Fraction:		6 h	24 h	2 d	7 d
GP-mf[1]	6 h	-0.78***	-0.76***	0.63**	0.66**
	24 h	-0.80***	-0.78***	0.68**	0.72**
GP-sarc[2]	6 h	0.79***	0.80***	-0.64*	-0.67*
	24 h	0.69**	0.70**	-0.58*	-0.61*

* P<0.01, ** P<0.001, *** P<0.0001
[1] Glycogen phosphorylase in myofibrillar protein fraction
[2] Glycogen phosphorylase in sarcoplasmic protein fraction

Aging from 6 to 24 h postmortem, influenced both WHC and overall protein solubility in breast fillets. In general, brine uptake and sarcoplasmic protein solubility increased with aging from 6 to 24 h postmortem and myofibrillar protein solubility decreased (Fig. 1 and Table 2). The relationships between WHC and overall protein solubility measurements

were not influenced by postmortem time of sampling and measurement (Table 3). Correlations between WHC measurements and glycogen phosphorylase abundance were similar at 6 and 24 h postmortem (Table 5).

IV. CONCLUSION

In conclusion, this study demonstrates that overall protein solubility measurements are not closely related to low WHC in broiler breast meat, regardless of postmortem time of measurement (6 or 24 h). These data suggest that myosin denaturation, as measured by myofibrillar protein solubility, is not a distinguishing factor in WHC between pale and dark fillets. Increased glycogen phosphorylase denaturation was observed in fillets with low WHC. This study suggests that while the denaturation of glycogen phosphorylase and its precipitation onto myofibrils may not affect overall myofibrillar protein solubility, it may have a direct impact on WHC attributes. Based on the results of this study, it can be hypothesized that the precipitation of denatured glycogen phosphorylase onto myofilaments may alter their surface interactions with water and lower WHC in broiler breast meat. Further research is needed, however, to determine the mechanism by which sarcoplasmic protein denaturation influences WHC in breast fillets.

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VI. REFERENCES

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