EFFECT OF TRANSPORTATION ON THE EXPRESSION OF HEAT SHOCK PROTEIN 70 AND MEAT QUALITY OF BROILERS DURING SUMMER

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Abstract – The aim of this study was to determine the effects of pre-slaughter transport during summer on stress indicators, heat shock protein 70 (HSP70) expression and meat quality of broilers. We found that short-distance transport under high ambient temperature conditions induced the release of plasma corticosterone (CORT), creatine kinase (CK) and lactate dehydrogenase (LDH), which are significant indicators of stress conditions. The expression of HSP70 in the transport group was significantly lower than in the control group ($P < 0.05$). Moreover, immunofluorescence showed that the distribution of HSP70 shifted from cytoplasm and surface of membranes to extracellular matrix after stress. In addition, meat quality in the transport group showed a pale, soft and exudative (PSE)-like syndrome. Our research suggests that HSP70 may be associated with an unknown mechanism that leads to meat quality deterioration following acute stress.

Key Words – Location, Stress, Water holding capacity

I. INTRODUCTION

Transport and seasonal-type heat stress of broilers has been shown to affect various physiological and metabolic functions which may result in meat quality deterioration. Previous studies have shown that short-distance transport under conditions of heat stress can result in pale, soft and exudative (PSE)-like breast meat [1, 2].

Heat shock proteins (HSPs) are highly conserved and ubiquitously expressed proteins that are synthesized in response to physical, chemical or biological stresses, including pre-slaughter stressors [3]. HSP70 has been widely studied because of its molecular chaperone roles in protein assembly and disassembly, protein folding and unfolding, translocation and interact with damaged proteins under normal and stress conditions [4]. Previous studies have indicated that the content and location of HSP70 in heart, liver, kidney and brain tissues of broilers changed under heat stress or transport stress conditions [5]. However, little is known about the expression and location of HSP70 in skeletal muscle of broilers under conditions of transport stress and its potential relationship with meat quality.

Therefore, the purpose of this study was to evaluate meat quality as well as the expression and location of HSP70 in broilers that had been subjected to short-distance transportation under high ambient temperature.

II. MATERIALS AND METHODS

A total of 84 Arbor Acres broiler chickens were randomly categorized into two treatment groups: a non-transported control and those transported for 0.5 h transport. The ambient temperature was 32-35°C. Each treatment consisted of 6 replicates with 7 birds in each group. All birds (except the control group) were transported according to a designed protocol as previously reported [1]. Blood samples were collected in heparinized anticoagulant tubes and then centrifuged at 4°C for 10 min at 2000 g, and plasma was used for determination of creatine kinase (CK), lactate dehydrogenase (LDH) and corticosterone (CORT). Within 20 min postmortem, 10 g of the proximal region of pectoralis major (PM) muscle was taken and frozen in liquid nitrogen for analysis. Other PM muscles were removed from carcasses, stored at 4°C for assessment of meat quality at 24 h postmortem. Blood and muscle samples were obtained from 8 birds randomly selected from each treatment for determination of plasma and for HSP70 measurements.

Meat quality indicators were measured according to Xing et al. (2015). Plasma parameters were analyzed with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China for CK and LDH; R&D Systems, MN, USA for CORT) in accordance with manufacturer's instructions. The determination of HSP70 was

quantified according to the procedure of Küçükbay et al. [6] with minor modification. A frozen muscle sample was homogenized in buffer (10mM Tris-HCl, 0.1mM NaCl, 0.1mM PMSF, 5μM soybean, pH 7.4). After centrifuged at 17,500 g at 4°C for 20 min, the supernatants were collected and protein concentrations were determined by a BCA protein assay kit (Sigma). For electrophoresis, each homogenate was mixed with an equal amount of 2 × standard sample loading buffer and heated for 5min in a dry heater (100°C). Western-blot was used to semi-quantify HSP70 protein content. For the localization of HSP70 in PM muscle, representative muscle cross sections were cut at 7-μm thickness and air-dried. Muscle sections were blocked for 1 h with normal 10% goat serum. Then, sections were incubated with the primary antibody HSP70 (1:100) diluted in 0.01 M PBS overnight at 4°C. Buffer (0.01 M PBS) without primary antibody was used as negative control. Later, sections were incubated at 37°C for 1 h with an appropriate secondary antibody (Alexa Fluor 488, Molecular Probes). The sections were counterstained with DAPI. The fluorochrome-stained sections were mounted with cover slips. The muscle sections were washed in 0.01M PBS for 3×5 min between each step. Finally, the sections were examined under a confocal laser scanning microscope (LSM700, Carl Zeiss, German) and HSP70 protein content was quantified by image analysis.

Data were analyzed using one-way analysis of variance (ANOVA). Differences were evaluated by using Duncan's multiple-range with the program SAS 9.12 (SAS Institute Inc., Cary, NC, USA, 2003). Differences were considered significant at $P < 0.05$, and the results are presented as mean ± SD.

III. RESULTS AND DISCUSSION

As shown in Table 1, the concentrations of plasma CORT and stress-associated enzymes CK and LDH were significantly higher in the transport group than in the control group ($P < 0.05$). Evidence from animal studies has shown that changes in plasma activities of CK and LDH activities can each be used as indicators of cell muscle damage and muscle fatigue [5]. Plasma CORT was also considered as a sensitive indicator to assess the stress of broilers. The results suggested that pre-slaughter transport under high ambient temperature caused muscle damage and disruption of muscle cell membranes.

Meat quality indicators of breast are presented in Table 1. The breast meat from the transport group had lower $pH_{0.5h}$ and pH_{24h} values compared to the control group ($P < 0.05$). Regarding water holding capacity (WHC), the drip loss and cooking losses of the breast meat from the transport group were higher than in the control group ($P < 0.05$). Moreover, the L^* value (lightness) increased significantly after transportation ($P < 0.05$). In general, these results indicated the transport groups are typical of a PSE-like meat syndrome. Similar findings have been reported in a previous study [1].

Table 1 Effect of transportation on meat quality and plasma parameters of broilers[1]

Item	Control	Transport
CK (U/L)	3.37 ± 1.15[b]	5.84 ± 1.16[a]
LDH (U/L)	1.91 ± 0.51[b]	3.37 ± 0.61[a]
CORT (ng/mL)	137.92 ± 15.73[b]	153.76 ± 12.75[a]
L^*	50.01 ± 1.83[b]	53.21 ± 0.89[a]
a^*	4.12 ± 1.07[a]	4.53 ± 0.80[a]
b^*	8.86 ± 1.71[a]	9.24 ± 2.12[a]
Cooking loss (%)	12.49 ± 2.15[b]	15.59 ± 2.22[a]
Drip loss (%)	2.47 ± 0.49[b]	4.35 ± 1.78[a]
$pH_{0.5h}$	6.46 ± 0.18[b]	6.30 ± 0.08[a]
pH_{24h}	5.82 ± 0.10[b]	5.64 ± 0.24[a]

"a-b": means in the same row with no common superscript differ significantly ($P < 0.05$).
[1]Means ± SD, n=8 in each group for plasma parameters and n=42 in each group for meat quality indicators.

HSP70 levels in muscles of the control and transport group are presented in Fig. 1. There were significant differences between the two groups ($P < 0.05$) with lower values in the transport group. HSP70 has been implied to play a key role in the recovery of normal functions of cell membranes and repairing the denatured proteins [4] following damage. Recently, Di Luca [7] reported significantly negative correlations can be found between the abundance of HSP70 and drip loss in pork. It is presumed that the decreased level of HSP70 may be disadvantageous for the recovery of the normal function of cell membranes and for the

protection of disrupted cell homeostasis which may lead to deterioration in meat quality.

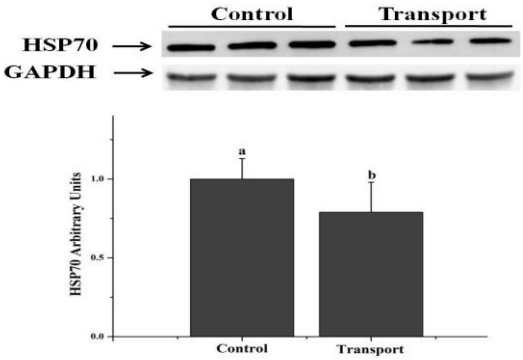

Figure 1. Effects of transportation on the amounts of HSP70. Representative immune-blots of HSP70 and GAPDH and the relative band density of HSP70 after normalizing to GAPDH are shown. Measurements are expressed as the mean ± SD (n = 8 per group) with superscripts (a, b) differing significantly ($P < 0.05$).

Figure. 2 shows that morphologically, the PM muscle of the control group had muscle fibers that almost entirely filled the endomysial spaces and muscle bundles that filled the perimysial spaces. However, muscles from the transport group had enlarged intercellular spaces and showed structural irregularities, as previously reported [8]. However, much of the HSP70 was visualized in the cytoplasm and on surface membranes of PM muscle cells in control group (Fig. 2B). Remarkably, fluorescence spots of HSP70 were discovered on surface membrane and extracellular matrix but were barely visible in the cytoplasm of the transport group (Fig. 2C). It has been shown that the majority of HSP70 is readily diffusible within the cytoplasm in non-stressed muscle fibers, but following various stress interventions, HSP70 primarily binds to cell membranes and stabilizes their structures and functions of the cell membranes [9]. Recently, HSP70 has also been found outside the cell, particularly in various pathological conditions and acts as a danger signal which activates the immune system [10]. Compared with our results, it seems that the different sub-cellular distributions or migration of HSP70 in muscle cells was related to changes in its functional roles. Moreover, the content of HSP70 in the extracellular matrix might reduce as the juice loss increases, which may also explain the decreased HSP70 levels observed here.

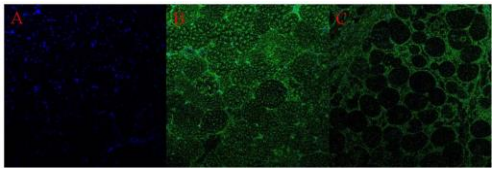

Figure 2. Immunofluorescent staining for HSP70 in broiler PM muscle. A, negative control, B, control group, C, transport group. Magnification 200X.

IV. CONCLUSION

The decreased expression and redistribution of HSP70 in broiler PM muscle after acute stress implies that as molecular chaperone or stress sensor, HSP70 might play a significant role in determining meat quality.

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