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**Screening of heat stress-related biomarkers in chicken serum through label-free quantitative proteomics**

Xiaohui Zhang1\*, Endong Bao2, Qijun Liang3

\*lead presenter

1 995354@hainanu.edu.cn, Key Laboratory of Tropical Animal Breeding and Epidemic Disease Research of Hainan Province, School of Tropical Agriculture and Forestry, Hainan University, Haikou 570100, China

2 Department of Basic Veterinary Medicine, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China

3 Key Laboratory of Tropical Animal Breeding and Epidemic Disease Research of Hainan Province, School of Tropical Agriculture and Forestry, Hainan University, Haikou 570100, China

**Abstract:**

**Background:** Heat stress (HS) can result in sudden death and is one of the most stressful and costly events in chicken. Currently, biomarkers used clinically to detect heat stress state in chickens are not optimal, especially for living ones. Analysis of changes in serum proteins of heat-stressed chickens can help to identify some novel convenient biomarkers for this.

**Methods:** Twenty-four chickens were exposed to HS at 42°C ± 1°C with a relative humidity of 65% for continuous 5 h in a single day, and 10 birds were used as controls (Con). During HS, 15 dead chickens were categorized as heat stress death group (HSD), and 9 surviving ones served as heat stress survivor group (HSS). Label-free quantitative proteomics (LFQP) was used to analyze differentially expressed proteins (DEPs) in serum of tested animals. Candidate proteins associated with HS were validated by enzyme-linked immunosorbent assay (ELISA). Diagnostic value of candidate biomarkers was assessed using receiver operating characteristic (ROC) curve analysis. Source of the selected proteins was analyzed in liver tissues with immunohistochemistry and in cell culture supernatant of primary chicken hepatocytes (PCH) using ELISA.

**Results:** In this study, compared to Con, LFQP identified 123 and 53 significantly different serum proteins in HSD and HSS, respectively. Bioinformatics analysis showed that XDH, POSTN, and HSP90 were potential HS biomarkers in tested chickens, which was similar with results from serum ELISAs and immunohistochemistry in liver tissues. The ROC values of 0.793, 0.752, and 0.779 for XDH, POSTN, and HSP90, respectively, permitted the distinction of heat-stressed chickens from the control. Levels of 3 proteins above in the cell culture supernatant of PCH showed an increasing trend as HS time increased. Therefore, considering that mean concentration of POSTN in serum was higher than that of HSP90, XDH, and POSTN may be optimal biomarkers in serum for detecting HS level in chickens, and mainly secreted from hepatocytes. The former indicates that heat-stressed chickens are in a damaged state, and the latter implies that chickens can repair heat stress damage.

**Conclusion:** In this study, we successfully screened serum proteins XDH, POSTN and HSP90 associated with heat stress in chickens by LFQP and ELISA analysis, determined that XDH and POSTN could be potential biomarkers for detecting the degree of heat stress in live chickens, and found that hepatocytes should be a primary source of serum XDH, POSTN and HSP90 in chickens. This study provides not only information on specific and convenient biomarkers for heat stress in poultry but also novel clues for further researching the injury mechanism of heat stress.

**Keywords:** biomarker; serum; proteomics; heat stress; chicken