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**Proteomics analysis for key molecules in adrenal glands of Wenchang chickens for their resistance to heat stress**

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**Abstract:**

**Background/Objective:** Rising temperatures and intensified agriculture have exacerbated heat stress (HS) challenges in poultry farming, notably causing heat-induced sudden death in chickens. Wenchang chickens, known for their robust HS resistance, offer potential for enhancing poultry farming efficiency. This study aims to investigate key molecules in Wenchang chicken adrenal glands through proteomics to elucidate their HS resistance mechanisms and provide a theoretical basis for poultry farming practices.

**Methods:** 34 healthy Wenchang chickens were selected, 10 as the control group (Con) and the rest exposed to acute HS at 42±1°C and 65% humidity for 5 h. 15 decreased formed the HS death (HSD) group, and remaining 9 formed the HS survival (HSS) group. ELISA measured COR, CORT, EPI, and NE levels. Adrenal gland tissue histopathology was assessed through scoring. Proteomic analysis identified differentially expressed proteins (DEPs), followed by bioinformatics analysis. Results were validated through Western blotting and immunohistochemical staining.

**Results:** ELISA revealed significantly higher (*P < 0.01*) COR and NE levels in HSS, and lowest CORT and EPI in HSD. Histopathology showed major degeneration in HSS cortical and chromaffin cells and extensive necrosis in HSD. Bioinformatics highlighted ER protein processing, especially ERAD, with HSPH1, DNAJA1, HSP90AA1, HSPA8, and HERPUD1 as key regulating molecules. Western blotting confirmed significantly higher (*P < 0.01*) protein levels in both HSS and HSD vs. Con. Immunohistochemistry revealed enhanced signals, strongest (*P < 0.01*) in HSS for HSPH1 and HSP90AA1.

**Conclusion:** HS induces pathological damage in Wenchang chickens’ adrenal glands, affecting hormone secretion, while various HSPs play crucial roles in cellular resistance. These results elucidate the biological basis of HS resistance in Wenchang chickens from the perspective of the adrenal gland and provide research foundations for enhancing broiler performances in high-heat environments and screening drugs for HS treatment.

**Keywords:** Key molecule, Proteomics, Heat-stress, Adrenal gland, Wenchang chicken