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HEAT STRESS AND ITS RELATION WITH EXPRESSION OF HEAT SHOCK PROTEINS IN POULTRY

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Abstract: High temperature coupled with high humidity, impair the thermoregulatory processes of chickens and effectively reduce heat dissipation, this impose severe stress on birds leading to reduced performance in terms of poor growth performance, immune suppression, increased incidence of respiratory diseases and high mortality. When living organisms experience thermal stress, the synthesis of most proteins is delayed, but a group of highly conserved proteins known as heat shock proteins or heat stress proteins is rapidly synthesized and play an important role in the survival of stressed cells and the stabilization of the internal environment. Many of the study in poultry suggested that HSP-70 might be involved in cellular protection in adverse situations, and a relationship between the development between this protein and thermo-tolerance has been established.

Keywords: Heat stress, heat shock protein, expression, poultry.

Introduction

Heat stress is a critical problem in poultry production systems and has a negative effect on poultry health and productivity. In the present scenario of changing climate with increasing environmental temperature, heat stress is considered to be the major cause of loss of production and reduced profit in the poultry production worldwide (Sejian *et al*., 2012), and highly suffering are the tropical and the subtropical zones. Being a tropical country, Indian poultry sector is also under pressure due to the slow and steady increase in ambient temperature. In poultry production; heat stress can be described as acute or chronic. Acute heat stress refers to short and sudden periods of extremely high temperature, whereas chronic heat stress refers to extended periods of elevated temperature. Acute heat stress could also make the broilers engender hyperthermal muscle illness, increase the activity of muscle creatine kinase, change cell membrane integrity in breast muscle glycolytic metabolism, and increase the osmotic effect of the membrane (Bao et al., 2004). There were many reports about the effects of acute heat stress on broilers, which mainly focused on the physiological and biochemical indices, pathological damages of structure and viscera, and the expression of heat shock protein (HSP). High temperature coupled with high humidity, impair the

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thermoregulatory processes of chickens and effectively reduce heat dissipation, this impose severe stress on birds leading to reduced performance in terms of poor growth performance, immune suppression, increased incidence of respiratory diseases and high mortality (Mujahid *et al*., 2005, 2009; Mandal, 2010). Birds are homeo therms, thus have to maintain a near-constant body temperature. To achieve a constant body temperature, heat produced by metabolism must equal heat loss. As the birds do not have sweat glands, non-evaporative cooling (radiation, conduction and convection) is the major route of heat dissipation within thermo-neutral range. Beyond this, majority of heat loss occurs insensible heat loss mode i.e., panting/Gular flutter. Panting is the visible response of poultry during exposure to heat stress associated with considerable energy expenditure. This specialized form of respiration dissipates heat by evaporative cooling at the surface of the mouth and respiratory passage ways. Panting increase the loss of carbon dioxide from the lungs, this leads to a reduction in the partial pressure of carbon dioxide and thus bicarbonate in blood plasma. In turn, the lowered concentration of hydrogen ions causes a rise in plasma pH, a condition generally referred to alkalosis. (Daghir, 2009). Birds also respond by reducing their feed intake and thus metabolizable energy (ME) intake to reduce thermo genesis. If panting (open-mouth breathing) fails to prevent their body temperatures from rising, the birds become listless, comatose and soon die due to respiratory, circulatory or electrolyte imbalances (Saif *et al*., 2003).

Heat shock protein (HSP)

When living organisms experience thermal stress, the synthesis of most proteins is delayed, but a group of highly conserved proteins known as heat shock proteins or heat stress proteins is rapidly synthesized (Al-Aqil and Zulkifli, 2009). These HSP play an important role in the survival of stressed cells and the stabilization of the internal environment (Gabai *et al*., 1997). The first report of HSP appeared in 1962 after Drosophila salivary gland cells were exposed to 37 oC for 30 min and then returned to their normal temperature of 25 ˚C for recovery, a "puffing" of genes was found to have occurred in the chromosome in the recovering cells (Ritossa, 1962). According to the homology and molecular weights, HSP can be classified into 3 main families: HSP90 (~85–90 kDa), HSP70 (~68–73 kDa), and low molecular weight HSP (~16–47 kDa; Basu *et al*., 2002). Among the HSP, HSP 70 is one of the most conserved and important protein families and has been studied extensively (Ming *et al*., 2010). Severe stress is destructive to the cell, causing cell cycle arrest and leading to apoptosis. Adaptation to stress condition caused by mild stress is usually beneficial to cell. Stress disturbs cell

membrane fluidity that may result in activation of different signal transduction pathways, including the Ras single pathways and mitogen activated protein kinases (MAPK), that among others lead to induction of heat shock responses (Park *et al*., 2011).

Expression of Heat shock proteins

The cells, in culture or in vivo, respond to stress by decreasing significantly the synthesis of almost all cellular proteins except a selected group of highly conserved proteins, the heat shock protein -70 (Leandro *et al*., 2004). Exposure of poultry species to mild stressors over a period of time enhances HSP-70 expression, but eventually, the birds become acclimated and no further increase in cellular HSP-70 can be demonstrated (Edens *et al*., 2001). All HSP-70 and related proteins bind ATP with high affinity; many are very abundant in cells and often found in association with other proteins (Lindquist and Craig, 1988). This multifunctional molecule, when under inducible regulatory expression, exhibits a broad range of chaperone functions that respond to both internal and external stresses (Norry and Loeschcke, 2003). It has been suggested that HSP-70 might be involved in cellular protection in adverse situations, and a relationship between the development between this protein and thermotolerance has been established (Givisiez *et al*., 2001). These are induced by a variety of stresses (hyperthermia, ethanol, amino acid analogs, heavy metals, free radicals, etc.) and are believed to play a critical role in protecting cells from these stresses (Van Remmen *et al*., 1996). There are two well defined families of heat shock proteins, molecular chaperones and proteases. Both of them enable cell survival in different ways. Proteases, e.g., Escherichia coli Lon, Clp or HtrA, degrade damaged proteins. Chaperones maintain and control the native structure of proteins by protection and stabilization of stress labile proteins, renaturation of denaturated proteins, and preventing protein aggregation. They are responsible for refolding of misfolded and aggregated protein (Mayer and Bukau, 2005) Protein of all the HSPs families posses chaperone activities; however, the central and most important role is attributed to the HSP-70 family (Zylicz and Wawrzynw, 2001). In all the organisms' expression of heat shock proteins is regulated at the transcription level. 'In vertebrates stress causes activation of specific transcription factors (HSF) primarily HSF-1. The accumulation of denaturated proteins in the cytosol is thought to trigger the HSF-1 as the first step in the stress response. Trimer formation of phosphorylated HSF-1 activates its movement into the nucleus where it binds to stimulate promoter region (heat shock element) and induce HSP gene expression the produced HSPs subsequently bind to the degenerated proteins aggregated proteins, and newly synthesized polypeptides. On the other hand/formation of a HSP-HSF complex suppresses

HSF-1 production in negative feed-back regulatory system (Mayer and Bukau, 2005). The mechanism underlying the cytoprotective provided by HSPs is not fully clear.

Evidence indicates that over expression of inducible HSP-70 diminishes the intracellular Cat‘ response to stress or toxins (Kiang *et al.,*1998) down that regulate the basal enzymatic activities

of protein kinase A (PKA), PKC (Ding *et al*., 1998), c-Jun amino terminal kinase and p38 kinase (Gaibai *et al*., 1997) up regulate the basal enzymatic activities of protein phosphatase-1 and 2A (Liossis *et al*., 1997) and enhances the immunogenicity of tumors cells (Melcher *et al*., 1998). A summary of some of the major physiological signals that activate the inducible form of the 72-kDa heat shock protein (HSP-70) synthesis (top) and a proposed mechanism for increased HSP-70 expression within a cell. Heat shock factors (HSFs), present in the cytosol, are bound by heat shock proteins (HSPs) and maintained in an inactive state. A broad array of physiological stimuli (“stressors”) are thought to activate HSFs, causing them to separate from HSPs. HSFs are phosphorylated (P) by protein kinases and form trimers in the cytosol. These HSF trimer complexes enter the nucleus and bind to heat shock elements (HSE) in the promoter region of HSP-70 mRNA is then transcribed and leaves the nucleus the HSP-70 gene. HSP used mechanisms for the cytosol, where new HSP-70 is synthesized. Proposed mechanisms of cellular protection for HSPs include their functioning as molicular chaperones to assist in the assembly and translocation of newly synthesized proteins within the cell and the repair and refolding of damaged (e.g. stress-denatured) proteins.

Heat shock protein in poultry

The intestinal mucosa is continuously exposed to an immense load of antigens from ingested food, resident bacteria, invading viruses etc. The single-cell epithelial layer lining the gut lumen has conflicting functions, playing a major role in the digestion and absorption of nutrients and at the same time constituting the organism’s most important barrier between the internal and external environments. The continuous epithelial cell layer, interconnected by tight junctions, restricts both transcellular cell layer,and paracellular permeation of molecules, thus constituting the principal component of the intestinal barrier (Johan and Marry, 2001).The heat-stress induced increase in intestinal permeability associated with a massive generalized sloughing of small intestinal epithelial layer from the villus tips and lysis of intestinal epithelial cells, indicating that the increase in intestinal permeability was due to the extensive damage of the epithelial surface Recent findings of Dokladny *et al*. (2006) describe an important protective role of HSP in preventing the heat-induced disruption of

intestinal barrier and suggested that HSP mediated up regulation of occludin protein expression may be an important mechanism involved in the maintenance of intestinal epithelial barrier function during heat stress. Further, inhibition of HSP expression by administration of cycloheximide or quercetin prevented the compensatory upregulation of occludin protein expression and produced a marked disruption in junctional localization of occludin protein and inflicted a drastic increase in intestinal epithelial permeability under heat stress conditions. Sun *et al*. (2007) conducted an experiment on the localization of heat shock protein 70 (HSP-70) and HSP-70 mRNA in heart, liver, lung, kidney, spleen, thymus and bursa of fabricius of 6 hr heat stressed broilers were studied by immunohistochemistry and in situ hybridization. The result showed that the positive signals of HSP-70 mRNA at 6 h heat stress were localized in the liver and lung, especially in the wall of vessel. Tu *et al*. (2010) analyzed the expression of HSP-70 mRNA in Hypothalamic-Pituitary- Thyroid (HPT utilizing real-time) axis under cold stress (12±1°C) by fluorescent quantification reverse transcriptase PCR (FQ-RT-PCR). The expression rule of HSP-70 mRNA was different in Hypothalamic-Pituitary-Thyroid (HPT) axis. Hypothalamus HSP-70 mRNA transcription level was the highest which showed going up trend as a whole. HSP-70 was the up-regulation gene of hypothalamic in cold stress reaction. Pituitary and thyroid HSP-70 mRNA transcription level repressed which showed that it was the down-regulation gene of pituitary and thyroid in cold stress reaction. Tamzil *et al*. (2013) reported increase HSP-70 expression in three chicken lines

(Kampong, Arabic and commercial) when exposed to acute heat stress (40˚C for 0, 0.5, 1.0 and 5h). Strong correlation for HSP-70 only occurred after 3 h of heat stress; however, the strong correlation for HSP70 mRNA occurred from 3 to 10 h of heat stress (Hao *et al.,*2012).

Conclusion

Heat stress is one of the most important environmental stressors challenging poultry production worldwide. The negative effects of heat stress on broilers and laying hens range from reduced growth and egg production to decreased poultry and egg quality and safety. When birds are under heat stress synthesis of most proteins is delayed, but a group of highly conserved proteins known as heat shock proteins is rapidly synthesized and protect the birds under some extent of heat stress. Mechanism how and what extent to protect HSP to the birds are not fully known and still require further research.

References

[1] Al-Aqil, A. and Zulkifli, I. 2009. Changes in heat shock protein 70 expression and blood characteristics in transported broiler chickens as affected by housing and early age feed restriction. Poult. Sci. 88: 1358-1364.

[2] Bao, E. D., Gong, Y.Y., Hartung, J., Fu, X.B., Wang, X. J., Zhang, H.T. and Wang, Z. L. 2004. Relation between pathologic damages and HSP70, changes in acute heatstressed broilers. Sci. Agric. Sinica. 37: 301–305.

[3] Daghir, N. J. 2009. Nutritional Strategies to Reduce Heat stress in Broiler and Broiler Breeders. Lohaman Information. 44(1): 6.

[4] Ding, X. Z., Tsokos, G. C., and Kiang, J. G. 1998. Over expression of HSP-70 inhibits the phosphorylation of HSF1 by activating protein phosphatase and inhibiting protein kinase C activity. FASEB J. 12: 451-459.

[5] Dokladny, K., Moseley, P. L., and Ma, T. Y. 2006. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. Ame. J. Physiol. Gastrointestinal and liver physiol. 290: 204-212.

[6] Edens, F. W., Givisiez, P. E. N., Mahmoud, K. Z. and Sefton, A. E. 2001. Influence of Escherichia coli-challenged chickens. Proceeding for the 50th Western Poultry Disease Conference. University Of California, Devis California.

[7] Gabai, V. L., Meriin, A. B., Mosser, D. D., Caron, A. W., Rits, S., Shifrin, V. I. and Sherman, M. Y. 1997. HSP70 prevents activation of stress kinases. J. Biological Chemist. 272: 18033-18037.

[8] Givisiez, P. E. N., Ferro, M. I. T., Kronka, S. N., Decuypere, E., and Macari, E. 2001. Hepatic concentration of heat shock protein 70 kd (HSP70) in broilers subjected to different thermal treatments. Br. Poult. Sci. 40: 292-296.

[9] Hao, Y., Gu, X. H. and Wang, X. L. 2012. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 1. Intestinal structure and digestive function. Poult. Sci. 91: 781–789.

[10] Johan, D. S., and Mary, H. P. 2001. Stress and the Gastrointestinal Tract 2. Stress and intestinal barrier function. American J. Physiol. Gastrointestinal and liver physiol.280: 7-13.

[11] Kiang, J. G., Gist, I. D. and Tsokos, G. C. 1998. 17b-Estradiol induced increases in glucoseregulated protein 78kD protect human breast cancer T47-D cells from thermal injury. Chinese J. Physiol. 40: 213-219.

[12] Leandro, N. S., Gonzales, M., Ferro, M. I. T., Givisiez, P. E. N. and Macari, M. 2004. Expression of heat shock protein in broiler embryo tissue after acute cold or heat stress. Mole. Reprod. and Develop. 67(2): 172-177.

[13] Lindquist, S., and Craig, E. A. 1988. The heat-shock proteins. Annu. Rev. Genet. 22: 631-677.

[14] Liossis, S. N. C., Ding, X. Z., Kiang, J. G. and Tsokos, G. C. 1997. Over expression of the heat shock protein 70 enhances the TCR/CD3- and Fas/Apo-1/CD95- mediated apoptotic cell death in Jurkat cells. J. Immunol. 158: 5668-5675.

[15] Mandal, A. B. 2010. Dietary modulation of curb impact of changing climate on livestock and poultry. In: International conference on physiological capacity building in livestock under changing climate scenario. 1: 127-136.

[16] Mayer, M. P., and Bukau, B. 2005. HSP 70 chaperons: cellular functions and molecular mechanisms. Cell Mole. Life Sci. 62: 670-684.

[17] Melcher, A., Stephen, T., Nicola, H., Martin, F., Michael, J., and Richard, G. V. 1998. Tumor immunogenicity is determined by the mechanism of cell death via induction of heat shock protein expression. Nat. Medi. 4: 581-587.

[18] Ming, J., Xie, J., Xu, P., Liu, W. B., Ge, X. P., Liu, B., He, Y. J., Cheng, Y. F., Zhou, Q. L. and Pan, L. K. 2010. Molecular cloning and expression of two HSP70 genes in the Wuchang bream (Megalobrama amblycephala Yih). Fish Shellfish Immunol. 28:407–418.

[19] Mujahid, A., Yoshiki, Y., Akiba, Y. and Toyomizu, M. 2005. Superoxide radical production in chicken skeletal muscle induced by acute heat stress. Poult. Sci. 84: 307-314.

[20] Norry, F. M. and Loeschcke, V. 2003. Heat-induced of a molicular chaperone decrease by selecting for long-lived individuals. Exp. Geron. 38: 673-681.

[21] Park, H. S, Jeong, W. J., Kim, E. C., Jung, Y. J., Lim, J. M., Hwang, M. S, Park, E. J., Ha, D. S. and Choi, D.W. 2012. Heat shock protein gene family of the Porphyra seriata and enhancement of heat stress tolerance by PsHSP70 in Chlamydomonas. Mar Biotechnol. 14:332-342.

[22] Saif, Y. M., Barnes, H. J., Gilsson, J. R., Fadly, A. M., McDonald L. R. and Swayne, D.E. 2003. In: Disease of poultry. 11th Ed., p.1056. lowa State Press, A Blackwell Publishing Co. Ames, lowa.

[23] Sejian, V., Naqvi, S. M. K, Ezeji, T., Lakritz, J. and Lal, R. 2012. Introduction. Environmental Stress and Amelioration in Livestock Production. (Eds.). 1–16. Springer-Verlag GMbH Publisher, Germany.

[24] Sun, pei-ming., Liu, Yu-tian., Wang, Qing-hua., Wang, Zhi-liang., Bao, En-dong. 2007 Localization of HSP70 and HSP70 mRNA in the tissues of heat stressed broiler. J. Agr. Biotech. 3: 102-107.

[25] Tamzill, M. H., Noor, R. R., Hardjosworo, P. S, Manalu, W. And Sumantr, C. 2013. Acute Heat Stress Responses of Three Lines of Chickens With Different Heat Shock Protein (HSP-70) Genotypes. Int.J. Poult. Sci., 12(5): 264-272.

[26] Tu, Y., Chen, G., Geng, Z., Su, Y., Wang, K. 2010. The expression of HSP70 mRNA in hypothalamic-pituitary- thyroid (HPT) axis under cold stress. Scientia Agricultura Sinica. 7: 35-71.

[27] Van Remmen, H., Willams, M. D., Heydari, A. R., Takahashi, R., Chung, H.Y., Yu, B. P., and Richardson, A. 1996. Expression of genes coding for antioxidant enzymes and heat shock proteins is altered in primary cultures of rat hepatocytes. J. of Cell physiol. 166: 453-460.

[28] Zyclicz, M., Wawrzynow, A. 2001. Insight into the function of HSP-70 chaperones. IUBMB Life. 51: 283-287.