Journal of Animal and Veterinary Advances 13 (8): 531-537, 2014
ISSN: 1680-5593

Expressions of HSPs mRNA in Different Tissues of Sows During Late Gestation Stage in Thermal Environment

[1]Zhiyong Fan, [1,2]Cimin Long, [1]Yonghui Chen, [1]Jinping Deng and [1,2]Wu Xin
[1]Institute of Animal Nutrition, Engineering Research Center for Feed Safety and Efficient Utilization, Ministry of Education, Hunan Agricultural University, Hunan, China
[2]Key Laboratory of Agro-Ecological Processes in Subtropical Region, Chinese Academy of Sciences, Institute of Subtropical Agriculture, Hunan Provincial Engineering Research Center of Healthy Livestock, Changsha, Hunan, China

Abstract: To study expressions of HSPs mRNA in different tissues of sows during late gestation stage in thermal environment, twelve sows (Landrace x Large White) were selected and randomly assigned to 2 groups. All sows were sacrificed on day 90 and 110 of gestation, respectively and the brain, heart, liver, kidneys, spleen, lung and ovary samples were collected to determine the expression of HSP70 and HSP90 mRNA. The HSP70 mRNA expression abundance in different tissues of sows on day 90 of gestation was as follows: lung>spleen>liver>brain and HSP90 with lung>kidneys>ovary>brain on day 110, HSP70: lung>spleen>liver>brain and HSP90 with lung>ovary>spleen>kidneys. Compared with the day 90, the relative expressions of HSP70 and HSP90 mRNA were lower in lung ($p<0.01$) and HSP90 mRNA was lower in those of day 110 ($p<0.01$). These findings show distinct tissue specificity and the higher expressions of HSP70 and HSP90 mRNA in lung indicated that continuous hot weather may cause stress in lung and this may be important information to prevent heat stress during late gestation in thermal environment.

Key words: Heat shock proteins, sow, tissues, thermal environment, mRNA

INTRODUCTION

In response to either elevated temperatures or several other metabolic insults, cells from all organisms respond by up-regulating Heat Shock Proteins (HSPs) (Welch, 1993). HSPs are a class of functionally related proteins whose expression is upregulated when cells are exposed to elevated temperatures or other stress. As intracellular chaperones, HSPs are an evolutionarily conserved family of proteins in virtually all living organisms from bacteria to humans (Lindquist and Craig, 1988). Despite their designation, most of the HSPs are constitutively expressed and perform essential functions such as protecting organisms against morbidity and mortality (Wischmeyer, 2006; De Maio, 1999). The dramatic up-regulation of HSPs plays a key role in the recovery from stress (Wu *et al*., 2013b). HSPs are named according to their molecular weight. For example, HSP60, HSP70 and HSP90 (the most widely-studied HSPs) refer to families of heat shock proteins on the order of 60, 70 and 90 kDa in size, respectively (Lindquist and Craig, 1988; Hideaki and Yohtalou, 1991). Experimental evidence suggests that some amino acids regulate HSPs expression which is essential in preventing organ dysfunction. HSP70 is the most widely studied of all the heat shock proteins and is reported to have a number of important chaperoning functions. HSP70, the major stress-induced HSP has been found in the extracellular medium and is capable of protecting cells (Beckmann *et al*., 1990). And HSP70 synthesis is tissue specific at high physiological temperatures and may identify a critical target tissue susceptible to early thermal damage (Flanagan *et al*., 1995). HSP90 is an essential component of the signaling pathway (Pratt, 1997).

High environmental temperatures during lactation decrease milk yield, litter growth and reproductive performance (Bloemhof *et al*., 2008; McGlone *et al*., 1988; Black *et al*., 1993). Heat stress resulted that lactating sows reduce the intake obviously. Reduced milk yield muscle growth during heat stress was traditionally thought to result from decreased nutrient intake (Rhoads *et al*., 2011; Prunier *et al*., 1997). Sow mortality associated with high ambient temperatures (D'Allaire *et al*., 1996).

However, few studies have reported the effects of hot weather on the HSPs mRNA expressions in different tissues of sows. The objective of this study was

Corresponding Author: Wu Xin, Institute of Animal Nutrition, Engineering Research Center for Feed Safety and Efficient Utilization, Ministry of Education, Hunan Agricultural University, Hunan, China

to investigate the expressions of HSPs mRNA in different tissues of sows in thermal environment during late gestation stage.

MATERIALS AND METHODS

Animals and experimental design: Twelve sows (Landrace x Large White) with initial Body Weight (BW) of 187±5 kg, parity of 3.2±0.7 and similar reproductive performance in last parity were selected and randomly assigned to two groups with six replicates per group.

All sows were housed individually in gestation crates (2.0×0.6 m, concrete floor) and transferred to individual farrowing crates (2.2×1.5 m) on day 107 of gestation. They were provided 2 kg diet (as-fed basis) daily as 2 equal-sized meals (08:00 and 16:30) during the entire gestation period. All diets were formulated to contain 13.5 MJ metabolizable energy/kg and 14.7 crude protein/kg (as-fed basis). All the sows had free access to drinking water. Rooms were ventilated mechanically, the temperature was kept within the range of 25.5-38.5°C (average temperature: 33.5°C) and the mean average humidity was 75%.

This study was performed in accordance with the Chinese guidelines for animal welfare and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, the Chinese Academy of Sciences.

Samples collection: All sows were sacrificed on day 90 and 110 of gestation, respectively and the brain, heart, liver, kidneys, spleen, lung and ovary samples were collected aseptically over liquid nitrogen and frozen at -80°C (Liu *et al*., 2012).

Then the samples were collected into 1.5 mL micro centrifuge tubes (RNA free) with RNAlater (Applied Biosystems, Valencia, CA, USA) in it and stored at -20°C for RT-PCR analysis.

Real-time PCR analysis: mRNA levels for HSP70 and HSP90 were determined by a standard Real-Time Polymerase Chain Reaction (RT-PCR) Method as previously described (Wu *et al*., 2010, 2013a). Total RNA was extracted from the ileal mucosa using a Guanidinium Isothiocyanate Method (TrizolTM reagent, Gibco BRL, Berlin, Germany) and treated with DNAse according to the manufacturer's instructions. To amplify HSP70, HSP90 and GAPDH cDNA fragments, the following sequences of PCR primer pairs were used: forward 5'-GCCCTGAATCCGCAGAATA-3' reverse 5'-TCCCCACGGTAGGAAACG-3' for HSP70 (152 bp) (NM_001123127); forward 5'-AATCGCCCAGTTGATGTCG-3', reverse 5'-TGTCCACTATCGTGAGGGTCC-3' for HSP90 (206 bp) (NM_213973) and forward 5'-GAAGGTCGGAGTGAACGGAT-3', reverse 5'-ATGGGTAGAATCATACTGGAACA-3' for GAPDH (149 bp) (NM_001206359). PCR amplification was performed in a total volume of 10 μL including Taq DNA polymerase and specific primers with SYBR Premix Ex Taq™ (TaKaRa, Janpan). The RT-PCR conditions were: 30 sec denaturation at 95°C, 30 sec annealing at 60°C and 30 sec extension at 72°C for 40 cycles. The final PCR products were visualized in a 2% agarose gel.

The relative quantification of gene amplification by RT-PCR was performed using Cycle threshold (Ct) values. The comparative Ct Method was employed to quantitate expression levels for genes relative to those for GAPDH.

Statistical analysis: Results were statistically analyzed using one-way ANOVA (SAS Institute, NC, USA) for HSPs variables. The t-test was used to compare differences between the treatment groups. Probability values ≤0.05 were taken to indicate statistical significance. Values are presented as the mean±SEM.

RESULTS

The final RCR products of HSP70 and HSP90 were identified in a 2% agarose gel (Fig. 1).

***HSP70* gene expressions in the tissues:** Real-time PCR analysis results of *HSP70* gene expressionsin different tissues are shown in Fig. 2. HSP70 mRNA expression abundance in different tissues of sows on day 90 of gestation was as follows: lung>spleen>liver>brain>kidney>adrenals ovary>heart and on day 110: lung>spleen>liver>brain>kidney>adrenals ovary>heart.

On 90 day, *HSP70* gene expression level in lung was highest among all the tissues ($p<0.01$) (Fig. 2) its expression levels in spleen, liver and brain were higher than that in heart ($p<0.05$). On 110 day, *HSP70* gene expression levels in lung and spleen were higher than those in other tissues ($p<0.01$) but no differences in levels were found between these two tissues. HSP70 expression levels in kidney, brain and liver were higher than that in heart ($p<0.05$) but there were no differences in its expressions among kidney, brain, liver and adrenals ovary ($p>0.05$).

***HSP90 mRNA* gene expressions in the tissues:** On 90 day, HSP90 mRNA expression abundance in different tissues of sowswas as follows: lung>kidneys>ovary>brain>spleen>liver>heart while on 110 day, lung>ovary>

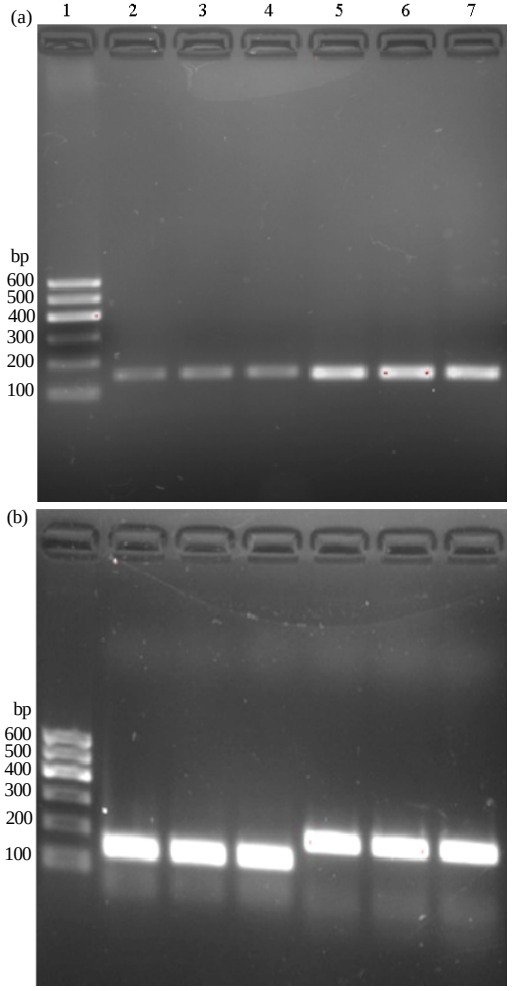

Fig. 1: a) Agarose gel electrophoresis of HSP70 and b) HSP90 PCR products. 1: DNA maker; 2-4: PCR products of GAPDH PCR (149 bp); 5-7: PCR products of HSP70 (152 bp) or HSP90 (206bp)

spleen>kidneys>brain>liver>heart. The relative expression levels of HSP70 and HSP90 mRNA were higher in lung ($p<0.01$).

Similar to that of HSP70, on 90 day, *HSP90* gene expression level in lung was highest among all the tissues ($p<0.01$) but no differences in its expressions levels were found among ovary, spleen, kidney, brain, liver and heart ($p>0.05$) (Fig. 3).

On 110 day, *HSP90* gene expression level in lung was not only higher than those in ovary and spleen ($p<0.05$) but alsohigher than those in kidney, brain, liver and heart ($p<0.01$). No differences in its expression levels were found among ovary, spleen and kidney ($p>0.05$). But HSP90 was expressed higher in ovary than that in brain and liver ($p<0.01$). In addition,

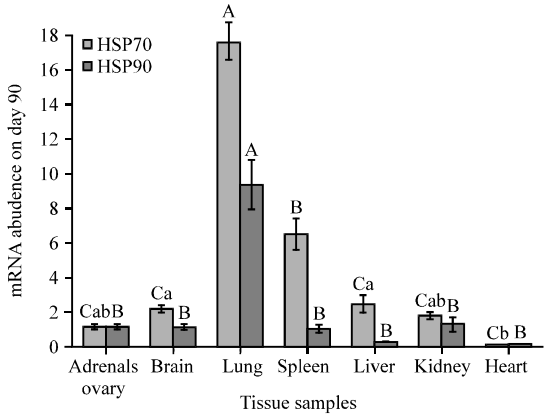

Fig. 2: The expression of HSP70 mRNA in different tissues of gilts at day 90 and 110 of gestation. Values are means±SEM, n = 6. In the same row, values with different small letter superscripts mean significant difference ($p<0.05$) with different capital letter superscripts mean significant difference ($p<0.01$) the same as follows

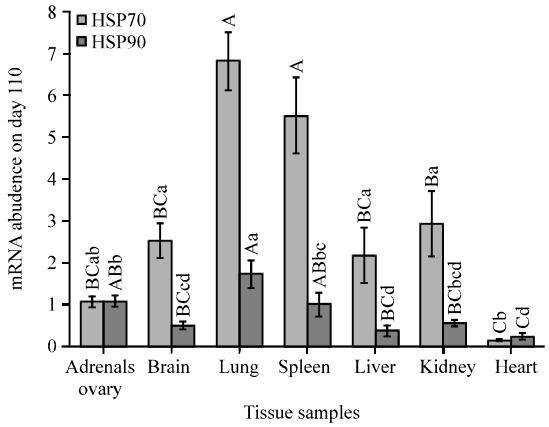

Fig. 3: The expression of HSP90 mRNA in different tissues of gilts at day 90 and 110 of gestation

HSP90 gene expression in spleen and brain were higher than that in liver ($p<0.05$) and heart ($p<0.01$).

The relative expressions of HSP70 and HSP90 mRNA in different tissues of sows at day 90 and 110 of gestation: The relative expressions of HSP70 and HSP90 mRNA in different tissues of sows at day 90 and 110 of gestation are shown in (Fig. 4 and 5).

Compared with the data on 90 day, HSP70 expression level in lung decreased significantly on 110 day ($p<0.05$) while in kidney it tended to be high (Fig. 4). Compared with the day 90, HSP90 mRNA expressions level in lung and brain decreased on day 110 ($p<0.01$) (Fig. 5).

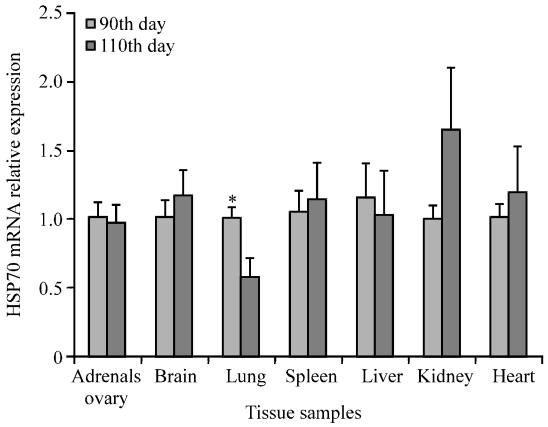

Fig. 4: The relative expressions of HSP70 mRNA in different tissues of sows at day 90th and 110th of gestation. Values are means SEM, n = 6; '*' means significant difference ($p<0.05$)

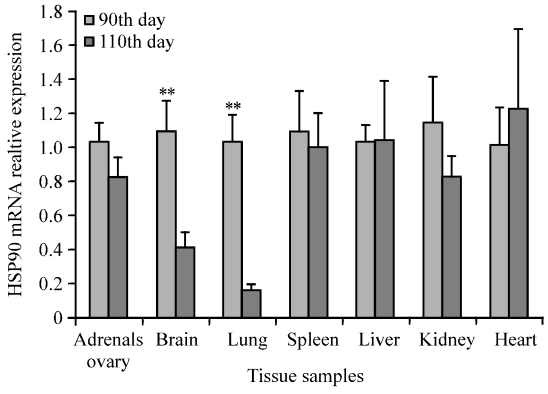

Fig. 5: The relative expressions of HSP90 mRNA in different tissues of sows at day 90th and 110th of gestation; '**' means very significant difference ($p<0.01$)

DISCUSSION

Higher fetal growth rates may require provision of increased nutrients to serve the metabolism in sows and their fetuses. Accordingly, the maternal would trigger many kinds of physiological changes during the late gestation in response to the growth and development of the fetuses. The lactating sows often suffer from heat stress during thermal temperature which may aggravate the physiological changes. Heat stress causes seasonal infertility in sows leading to the decline in reproductive performance (Williams *et al*., 2013).

In response to either elevated temperatures or several other metabolic insults, cells from all organisms respond by up-regulating HSPs expressions (Welch, 1993).

Experimental evidences suggest that some amino acids can regulate HSPs expression which is essential to prevent organ dysfunction. HSP70 is the most widely studied of all the heat shock proteins and is reported to have a number of important chaperoning functions (Wu *et al*., 2013b).

The results from the present study showed that HSP70 and HSP90 mRNA were expressed highest in lung in late gestation which indicated that hot weather in late gestation may cause much stress in the lung. The function of lung would have obvious changes in the respiratory and cardiovascular systems which are essential for meeting the increased metabolic needs of sows and their fetuses. Important respiratory system changes occur in the upper airway, chest wall, static lung volumes and ventilation and gas exchange (Hegewald and Crapo, 2011; Elkus and Popovich, 1992; Fishburne, 1979). It is demonstrated that *in vivo*, donor adenovirus-mediated gene transfer of HSP70 protects rat lung isografts from subsequent ischemia-reperfusion injury (Hiratsuka *et al*., 1999).

Pregnancy induces dynamic changes in the maternal environment including reversible modifications in response to systemic mediators and local signals. Placenta synthesizes and metabolizes key nutrients and hormones including progesterone, Prostaglandin (PG) and estrogen would increase during late gestation which is important in the regulation of maternal and fetal metabolism and the growth and development of the fetus. However, PGs are involved as mediators of inflammation (Ricciotti and FitzGerald, 2011; Aoki and Narumiya, 2012). Since the spleen, the largest secondary immune organ in the body is responsible for initiating immune responses to blood-borne antigens and for filtering foreign substances anddamaged red blood cells from blood, it can be used to determine the effects of pregnancy on multiple cellular populations including those of the erythroid lineage and the immune system (Norton *et al*., 2009). May be these are the reasons why spleen had the second highest HSP70 mRNA expression in the present study.

However, HSP90 mRNA was not expressed highly in spleen in the present study. There are different functional biochemical activities between the two heat stress proteins (Nollen and Morimoto, 2002). HSP70 has essential functions in disaggregation and refolding of non-native proteins under both normal and stress conditions in protein folding and unfolding and provides thermotolerance to cell on exposure to heat stress (Fink, 1999; Martin, 1997). HSP90, unlike HSP70 as a specific component of the cap-binding complex of steroid receptors and an essential component of the signaling pathway (Nollen and Morimoto, 2002;

Brown *et al*., 2007; Pratt, 1997) plays an essential role in the maturation of hormone receptors and protein kinases (Harst *et al*., 2005; Hutchison *et al*., 1992). The HSP90 component showed greater variation (up to about 40 fold) relative to the less variable (up to about 3 fold) HSP70 component of the system (Vamvakopoulos, 1993). This was reported to be in contrast to the untransformed mouse glucocorticoid receptor in L cell cytosol which is associated with HSP90 but not HSP70 (Sanchez *et al*., 1990; Beato and Klug, 2000). Ovarian tissue produces many kinds of hormones (IGF-I, leptin and FSH) which can prevent stress-related changes in HSPs in porcine ovarian tissue (Sirotkin and Bauer, 2011). May be that is why ovary had the second highest HSP90 expression on day 110. This agrees with the recent study with rat as model showing that stress, via the activation of ovarian HSP90 and changes in steroid hormone receptor expression and serum reproductive hormone levels may be involved in the induction of polycystic ovaries in rats (Park *et al*., 2012).

Glucocorticoid hormone (GR) receptor exists in the cytoplasm of target cells in the form of dynamic multiprotein heterocomplexes with heat shock proteins HSP90 and HSP70 and additional components of the molecular chaperone machinery (Filipovic *et al*., 2008; Cvoro and Matic, 2002). However, both of HSP70 and HSP90 were not expressed at high levels in the present study. Previous research also found that acute and chronic stresses are associated with a reversible reduction of HSP90 in the spleen and liver (Vamvakopoulos *et al*., 1993).

Normally, during pregnancy, Renal Plasma Flow (RPE) and Glomerular Filtration Rate (GFR) may be elevated at term which is most pronounced during the last week of gestation (Baylis, 1987; Arthur and Green, 1983). HSP70 and HSP90 appear to participate in the adaptation of medullary cells to high extracellular salt and urea concentrations (Beck *et al*., 2000). HSP70 expression in kidney tended to be high on day 110 in the present research. On the contrary, HSP90 tended to be low on day 110. The constitutive expression of HSP90 in widespread neuronal cell populations suggests a functional role in the physiological molecular program of CNS neurons (Gass *et al*., 1994). However, the results from this present study showed that HSP90 and HSP70 levels were low in brain. Other studies also showed that not only levels of HSP90 decreased some what in certain developing brain regions but basal levels of HSP70 also decreased in the developing and adult brain (D'Souza and Brown, 1998). A normal, uncomplicated pregnancy causes many physiologic cardiovascular changes and symptoms. For example, maternal blood volume, heart rate and cardiac output increase and fatigue, orthopnea and presyncope often occur. In the present study, the relative expressions of HSP70 and HSP90 mRNA in heart were low indicating that heat stress may cause less harm to the heart.

Interestingly, compared with day 90, HSP70 and HSP90 mRNA expressions in lung decreased significantly on day 110. There are multiple paracrine/autocrine events, fetal hormonal changes and overlapping maternal/fetal control mechanisms for the triggering of parturition in maternal in which PG eventually rise (Silver, 1994; Schindler, 2005). As mentioned earlier, PG are involved as mediators of inflammation, therefore the lung appears to be important in regulating arterial levels of endogenous PG clearance (Pitt *et al*., 1981), thus caused the decreased HSP70 and HSP90 expressions in lung.

CONCLUSION

HSP70 and HSP90 mRNA expression showed distinct tissue specificity and the higher expressions of HSP70 and HSP90 mRNA in lung indicated thathot weather may cause much stress in lung on day 90. The available data could provide important information for the target tissue to prevent heat stress during late gestation.

ACKNOWLEDGEMENTS

Supported by grants from the National Key Technology Research and Development Program of the Ministry of Science and Technology of China (2012BAD39B00), the NSFC (31101730, 31072053), A Project Supported by Scientific Research Fund of Hunan Provincial Education Department (09A041) and the program of China Scholarships Council (No. 201204910010).

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