

EXPRESSION OF HSP-70 IN TISSUES OF JAPANESE QUAIL (*Coturnix coturnix japonica*) REARED AT DIFFERENT STOCKING DENSITY

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ABSTRACT

Human pressure on agricultural land resources has lured many poultry farmers to increase the capacity of the poultry pen through increase in the stocking rate despite the high environmental temperature of the tropical environment. This study was carried out to assess the expression of heat shock protein-70 (HSP-70) in tissues of Japanese quails (*Coturnix coturnix japonica*) reared at different stocking densities in the heat period. A total of two hundred and ninety-six Japanese quails were used for the experiment which lasted eight weeks in a completely randomized design with four treatments replicated four times. The birds were divided into four stocking densities of 252.20cm²/bird (11birds), 173.43 cm²/bird (16 birds), 132.10 cm²/bird (21birds), and 106.73 cm²/bird (26 birds) which represented treatments 1-4 respectively adopting a completely randomized design (CRD). The results revealed that the expression of hsp70 gene in quail liver, heart and brain were significantly different ($P \leq 0.05$) and highest in T4 (26 quails) with the mean values of (90.54±0.70; 74.07±1.21 and 86.07±1.17%) respectively. Quails in T3 however had a mild expression of hsp70. However, stocking densities of 173.43cm²/bird and 132.10cm²/bird could be considered for quail production without compromising the health of quails.

Keywords: Heat shock protein; Stocking density; Environmental temperature; Heat stress; Tissues

INTRODUCTION

The extremes of environmental temperature and its attendant heat stress on quails are the most important inhibiting factors to quail production in hot regions. Apparently, quails are susceptible to changes in environmental temperature and thus cannot effectively dissipate the heat produced. Heat stress has been reported to affect various physiological parameters of birds. It increases rectal temperature (Aro *et al.*, 2017) and increases the respiration rate (Inoue *et al.*, 1995). Among various management stressors, stocking density has been known to affect behavioral and physiological indicators such as plasma corticosterone and heat shock protein 70 (HSP-70) in birds (Belmore *et al.*, 2010). Increase urbanization which apparently reduces land available for agricultural purpose has informed poultry farmers to increase the number of birds per unit space. This always results in heat build-up and consequently leads to heat stress (Adebiyi *et al.*, 2011). Heat shock protein (HSP) is induced by high environmental temperature and stress. It is important for the maintenance of a stable internal environment for intracellular proteins (Lindquist and Craig, 1988). HSP assists in protein trans-shipment and damage repair under heat stress, thereby maintaining the integrity of cells (Hendrick and Hartl, 1993). The heat shock protein 70 gene (HSP70) is the most widely studied member of the HSP family. HSP is widely studied among livestock and domestic fowl while data are lacking in Japanese quail. This study seeks to assess the expression of hsp-70 in Japanese quail reared at different stocking density in the upsurge of environmental temperature of transition from rainy period to dry season October to December.

MATERIALS AND METHOD

A total of two hundred and ninety-six day-old sexed and apparently healthy Japanese quails were used for the study for a period of eight weeks. They were divided into four stocking densities of 252.20cm²/bird (11birds); 173.43 cm²/bird (16 birds), 132.10 cm²/bird (21birds), and 106.73 cm²/bird (26 birds) that represented Treatments 1-4 respectively. The treatments were replicated four times adopting a completely randomized design (CRD).

Tissue materials and Sample collection

Three birds per treatment were sacrificed for tissues samples collection. These tissues (brain, heart and liver) samples were harvested into a labeled RNA later (stabilizing buffer) sample tubes and were immediately stored at 4°C and later - 80°C deep freezer to preserve the RNA integrity.

Total RNA isolation

Total RNA was extracted from the tissues using **Quick-RNA™ Miniprep Kit**. The concentration and Purity Isolated RNA was determined using UV/Visible Spectrophotometer.

Complimentary DNA (cDNA) Synthesis

cDNA was synthesized using 1 µg of the isolated RNA within the purity range of 1.8-2.0. Synthesis was carried out in Multigene™ Mini thermal cycler (Labnet Int'l Inc., USA) using the ProtoScript II First Strand cDNA Synthesis Kit (BioLabs, New England) in a 3-step reaction condition: 65°C for 5 min, 42°C for 1 hr and 80°C for 5 min.

Gene expression analysis by real-time quantitative Polymerase Chain Reaction (RT-qPCR)

Real time-quantitative PCR (qPCR) was performed using Luna Universal qPCR Master Mix (BioLabs, New England) on a StepOnePlus Applied Biosystem qPCR System according to the manufacturer's protocols. Primers (Inqaba biotec, Hatfield, SA) to Quail cDNA are listed in Table 1 and were used for the PCR. PCR conditions were as follows: 95°C for 3 min, 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. The b-actin gene was used to normalize the relative expression level of respective gene.

Agarose Gel Electrophoresis Assessment of qPCR Amplicon

The PCR product was run in 0.8% Agarose gel stained with ethidium bromide.

Table 1: Sequence of the Designed Primers Used for the qPCR analysis

S/N	Gene	Sequence	Product Length	Tm	GC%
1.	HSP70	Forward Primer: CCAATGACCAGGGAACAGG	191	60.32	60.00
		Reverse Primer: TGAAGCACTGGCCTTTCACT		59.82	50.00
2.	β-Actin	Forward Primer: TTGTCCACCGCAAATGCTTC	110	59.69	50.00
		Reverse Primer: AAGACGAGCCCTTGTGATGAT		59.44	47.62

HSP70 = Heat Shock Protein 70

RESULTS

Gene expression of Japanese quail tissues reared under different stocking densities.

Table 2 presents the results of expression of HSP70 gene from Japanese quail tissues reared at different stocking densities. The results revealed that the liver of quails in T4 had highest level of HSP70 gene (90.54±0.70%) which was not significantly different ($P \geq 0.05$) from the mean value obtained in T3 and T2 (90.46±0.74 and 89.54±1.09% respectively) while T1 (67.56±2.43%) had the lowest. Quail heart in T4 had the highest level of HSP70 gene (74.07±1.21%) followed by T1 (69.69±0.26%) while the mean value of HSP70 gene expression in the heart in T3 and T2 (61.27±0.76 and 58.68±0.78%) were not different significantly ($P \geq 0.05$). The brain of Japanese quail in T4 had the highest expression of HSP70 gene (86.07±1.17%) while quail brain in T1 had the lowest (70.60±1.41%) expression rate.

Table 2 Expression of HSP70 gene (%) in quail tissues reared at different stocking densities.

Parameters	T1	T2	T3	T4
Liver	67.56±2.43 ^b	89.11±1.09 ^a	90.46±0.74 ^a	90.54±0.70 ^a
Heart	69.69±0.26 ^b	58.68±0.78 ^c	61.27±0.76 ^c	74.07±1.21 ^a
Brain	70.60±1.41 ^c	78.59±0.85 ^b	79.21±0.66 ^b	86.07±1.17 ^a

a, b, c, = Means on the same rows but with different superscripts are statistically ($P < 0.05$) significant. T1 = 252.20cm²/bird (11 birds), T2 = 173.43cm²/bird (16 birds), T3 = 132.10cm²/bird (21 birds) and T4 = 106.73cm²/bird (26 birds).

DISCUSSION

Expression of heat shock protein (HSP-70) gene in Japanese quail tissues reared at different stocking densities

The level of expression of Heat shock protein (HSP-70) in the liver, heart and brain in Table 2 were all significantly high at highest stocking density of 26 quails/m². In the liver tissue, HSP-70 was highest in T4 which diminished with decreasing stocking density. In the heart, the highest expression of HSP-70 was observed in cage density 26 quails/m² while in the brain tissue, highest value of HSP-70 expression was observed also in T4 which decreased down the treatment levels. This indicates a direct relationship between body temperature and HSP synthesis (Yahav *et al.*, 1997). Under the influence of stressful conditions, heat shock factors (HSFs) are responsible for the activation of the heat shock element (HSE), which is an upstream promoter sequence in the heat shock gene (Mizuno *et al.*, 1997). In this study associated heat accompanied by high stocking density increased mRNA expression of HSP-70 in the liver, heart and brain tissues. These were highest in high stocking density and reduced as cage density decreases. In broiler, acute heat stress enhanced HSP-70 activation in the liver, heart, kidneys and lungs (Yu, 2009). In heat- stress chicken, HSP expression is tissue dependent (Rajkumar *et al.*, 2017) and liver tissues are more responsive than muscle tissues. Tissue dependent response may be due to differences in heat-shock factor levels which are responsible for the activation of heat shock elements under stress conditions (Mizuno *et al.*, 1997). In this study, the liver tissue are the most responsive to heat stress out of the three tissues considered, followed by the brain tissue while the heart tissue is the least responsive.

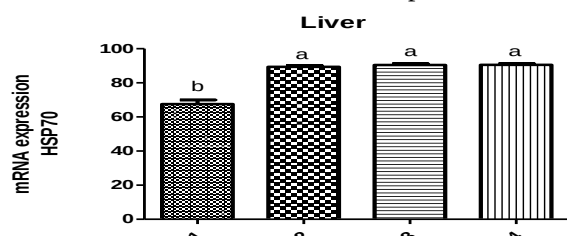


Fig 1 Heat shock Gene Expression in the Liver Tissue of Quail. N=4 Bars are mean $\pm$ SD

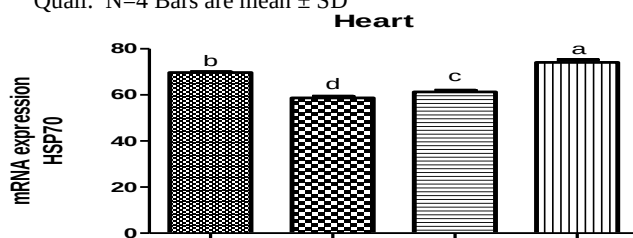


Fig 2 Heat shock Gene Expression in the Heart Tissue of Quail. N=4 Bars are mean $\pm$ SD

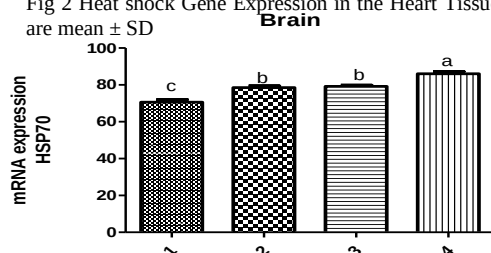
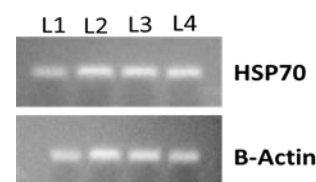
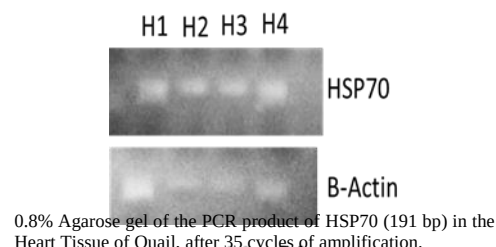


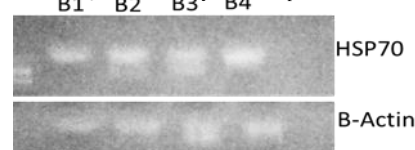
Fig 3 Heat shock Gene Expression in the Brain Tissue of Quail. N=4 Bars are mean $\pm$ SD



0.8% Agarose gel of the PCR product of HSP70 (191 bp) in the Liver Tissue of Quail, after 35 cycles of amplification



0.8% Agarose gel of the PCR product of HSP70 (191 bp) in the Heart Tissue of Quail, after 35 cycles of amplification.



0.8% Agarose gel of the PCR product of HSP70 (191 bp) in the Brain Tissue of Quail, after 35 cycles of amplification.

Conclusion and Recommendation

The study showed that stocking density had effects on the expression of Heat shock protein (HSP70). This was highest in liver, heart and brain tissues of quail stocked at 106.73cm²/bird (26 birds). This indicates that quails are stressed at high stocking density during the upsurge in environmental heat period of rearing in October to December heat period. However, quails in stocking densities of 173.43cm²/bird and 132.10cm²/bird with mild hsp70 expression could be considered for quail production without compromising the health of quails.

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