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RESEARCH ARTICLE
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m-RNA profiling of *HSP-70* under different tropical stress conditions in various broilers

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Abstract

Aim: The present experiment was conducted to study the effect of different tropical stress conditions on haemato-biological traits in various broiler strains during 3 to 4 weeks of age. Introgressing some important major genes likes Naked neck (*Na*) and Frizzle (*F*) into broiler germplasm may substantially improve the heat tolerance.

Materials and Methods: The experiment was designed to evaluate three indigenously developed broilers *viz*. CARIBRO-Tropicana (Naked neck and Frizzle gene bearing), CARIBRO-Mritunjai (Naked neck gene bearing) and CARIBRO-Vishal (Normal plumaged) under different THI (*i.e*. 72, 85 and 91) for 4 hours daily for 7 days. Total 324 broiler chicks (*i.e. 36* chicks in each group) of 3 weeks of age were used in this study.

Results: The m-RNA expression of *HSP-70* gene was observed highest in frizzle plumaged birds and lowest in normal plumaged birds. The CARIBRO-Vishal showed highest stress as compared to other group. Higher the THI more severe was the effect on the traits. During the 7 day of exposure trial birds of all the genetic group exhibited the phenomenon of acclimatization as reveled by the averages of various traits at different days into the exposure. The mRNA expression analysis of *HSP-70* in liver revealed higher expression levels at later days during the exposure trials which indicated the phenomenon of memory (stress memory) and acquired thermotolerance.

Conclusion: Among the three genetic groups, CARIBRO-Tropicana exhibited highest means for *HSP-70* production as well as tolerated the heat stress in a better way; therefore CARIBRO- Tropicana was adjudged to be the best genetic group for production under tropical climate.

Keywords: broiler, HSP-70, mRNA, tropical stress.

Introduction

The center of poultry industry is shifting to sub-tropical countries and this trend is likely to continue. Nearly one third of world–wide broiler stock placement is in Asian countries such as India and china and these countries are emerging as important locations for the production and trade of poultry product, whereas, the major international poultry breeders are located in temperate countries. India has distinctly different seasons with variable temperature and humidity. The high ambient temperature in tropical climate leads to heat stress to poultry in general and to broilers in particular. Heat stress results in poor performance in growth, feed efficiency and meat yield as well as higher mortality. Recent decades have seen significant developments in genetic selection of the meat type fowl, *i.e.* Broilers [1, 2] and Turkeys [3]. The genetic selection has led to rapid growth accompanied by increased feed efficiency and metabolic rate [4], thus providing the poultry industry with heavy domestic fowls in relatively short growth period. Such development in body size logically necessitates increase in the size and efficiency of the cardiovascular and respiratory system. However, relatively inferior development of such major body systems [3] has led to lower ability for balancing energy expenditure and body water content under extreme environment conditions. Thus, acute exposure of chickens to extreme conditions results in major economic losses.

All living organisms possess surveillance and homeostatic mechanisms to adjust the demand of growth, differentiation, environmental stress and ageing. However, under certain circumstances, these mechanisms fail to adequately respond to imbalanced and result in the accumulation of the misfolded proteins inside the cell.

To adapt to these environmental challenges and survive different types of injuries, cells have evolved networks of different responses which detect and control diverse form of stress. One of these responses, known as the heat shock responses (HSR), has attracted a great deal of attention as a universal fundamental mechanism necessary for cell survival under a variety of unfavorable conditions. The heat shock response is transient and lasts only a few hours [5]. This phenomenon of HSR is a very well conserved regulatory network across all eukaryotes and is triggered by the synthesis of a group of proteins [6].

The utilization of major genes for broiler production under tropical conditions exudes much enthusiasm among the poultry breeders. The important major genes are Naked neck (*Na*), Dwarf (*dw*) and Frizzle (*F*). These genes are located on Autosomes and get inherited in Mendelian fashion. Introgressing some important major genes into the poultry germplasm may substantially improve the heat tolerance ability of broilers without reducing their economic performance. Some major genes results in reduction of feather cover while other exerts their effect by decreasing the metabolic heat output [7, 8].

In prokaryotic and eukaryotic cells, the synthesis of specific stress proteins increases under a wide variety of stress conditions. The most extensively investigated stressors is heat stress in which, a sudden increase in temperature induces the synthesis of heat shock proteins (HSPs) [9]. The role of HSPs in the protection of cells from heat stress is well established. An important aspect of HSPs is that organisms which have reversed from previous mild stressful conditions expresses elevated level of stress proteins [10]. HSPs work as molecular Chaperones. These stress proteins can exhibit tolerance against the high levels of the stress causing agents, including heat that would normally cause developmental abnormalities or death [10]. This phenomenon, referred as "Acquired thermotolerance" which is dependent on the increased expression and accumulation of the HSPs [11]. Various families of HSPs are named according to their approximate molecular weight i.e. 8, 28, 58, 72, 90 and 110 KDa, hence referred as *HSP 8, HSP 28, HSP 60, HSP 70, HSP 90* and *HSP 110*. HSPs are required to withstand the toxic effects of extreme temperatures. These proteins also play role in immunity and immunopathology [12]. Heat shock proteins are induced by a variety of agents including stressors. Heat is the most prominent inducer of HSPs. Heat pre-conditioning is related to the expression of HSPs and antioxidant enzymes [21]. *HSP-70* expression levels could be influenced by the cold stress and the expressions levels differed in different tissues in different durations of the cold exposure [22]. Neonatal feed restriction improved *HSP-70* expression in aged birds [23]. Pituitary and thyroid *HSP-70* m RNA transcription level was repressed which showed that it was the down regulation genes of pituitary and thyroid in cold stress reaction [24]. The effect of stresses did not produce any significance effect on mRNA expression of *HSP-70* in ovary and ovarian follicles of Japanese quail at 6 week old age [25]. The response of *HSP-70* was greater in the high fear than the low fear group. Both low fear and high fear showed similar increases in *HSP-70* expressions after crating [26]. Heat stress increases the level of *HSP-70* in brain and ovary [27]. Serum levels of *HSP-70* in broiler chickens also increased after continuous feeding of sodium arsenite in drinking water. This particular observation may be attributed towards systematic inflammation, oxidative stress and hepatocellular injury [28]. Open sided House birds had greater *HSP-70* expression than the environmentally controlled House; the feed restriction chicks showed higher *HSP-70* density than those of *ad libitum* feed groups [29]. The positive signals of *HSP-70* mRNA at 6 hr heat stress were localized in the liver and lung, especially in the walls of vessels. The weak positive signals were seen in the myocardial cells. No significant singles were observed in spleen, thymus and bursa of fabricius [30]. The liver samples for *HSP-70* expression concluded that during stress (40 ºC temperature for 2 hrs) a non-significant ($P > 0.05$) induction of *HSP-70* expression was observed in turkey embryo [31]. The cells with increased HSPs exhibit tolerance against the additional stress hence they are often called as stress markers [32]. Subjected to cold or high incubation temperature, the expression of *HSP-70* in younger embryos had higher *HSP- 70* synthesis than older embryos [17]. The moderate heat stress caused significantly increased *HSP-70* levels compared with the control groups in 51 and 65 weeks laying hens [33]. Resistance to acute heat stress and concentration of *HSP-70* were higher in those birds subjected to more heat stress in broilers [34]. During heat stress (35°C), the increase in both colonic temperature and hepatic *HSP-70* concentration were significantly less in high energy fed broilers [35]. The oxidative stress has been proposed as a key mechanism that mediates HSPs induction [36]. HSPs regulate cellular functions by playing institutive roles in protein folding, unfolding, assembling, disassembling and translocation [37-40].

In view of the above the present investigation was carried out to evaluate the normal, Naked neck and Frizzle plumaged broiler strains developed at Central Avian Research Institute (CARI), Izatnagar under different thermal humidity indices using functional genomics tools in order to throw light on underlying physiological and genetic mechanism in major genes (*Na* or *F* gene) induced heat tolerance in broiler chicken. The present study was under taken with the objectives to undertake mRNA profiling of *HSP-70* under different tropical stress conditions in various broilers strains

Materials and Methods

Ethical approval: Authors followed all the national legislation concerning protection of animal welfare and following the guidelines of the Ethics Committee. This work is approved by the permission of Director, Institutional Animal Ethics Committee, Indian Veterinary Research Institute (IVRI).

Experimental site: The proposed research work was conducted at Experimental Broiler Farm and Disease Genetics & Biotechnology Lab, Division of Avian Genetics and Breeding, CARI, Izatnagar. The trials for exposure of chicks under different THIs were carried out in Psychometric chamber, Veterinary Physiology Division, IVRI.

Germplasm: The CARIBRO-Mritunjai (Necked neck

Table-1. Formula and chemical composition of broiler rations.

Sr. No.	Ingredients	Starter%	Finisher%
1	Maize	58.805	59.5
2	Soyabean	28	26
3	Sunflower meal	5	2.5
4	Fish meal	5	3
5	Limestone	1	0.8
6	Di-calcium phosphate	1.5	1.1
7	Salt	0.2	0.2
8	DL- Methionine	0.06	0.04
9	Trace mineral Premix	0.1	0.1
10	Vitamin premix*	0.15	0.15
11	Vitamin B complex**	0.015	0.015
12	Choline Chloride	0.05	0.05
13	Toxin Binder	0.05	0.05
14	Protexim	0.02	-
15	Coccidiostat	0.05	0.05
16	De-oiled Rise Bran	-	1.42
17	Rape seed meal	-	5
18	Lysine	-	0.02
	Total	100	100
Nutrient Composition			
	Crude Protein(%)	21.66	18.98
	Metabolizable Energy; (Kcal /Kg)***	2843	2850
	Calcium (%)	1.17	1.17
	Available Phosphorus (%)	0.496	0.5
	Lysine (%)	1.24	1.22

* Trace mineral Premix:Mg-300, mn-55,I-0.4, fe-56, Zn-30 and Cu-4kg^{-1}
** Vitamin premix: Vitamin A-8250 IU, Vitamin D3-1200ICU, Vitamin k-1mg, Vitamin E-40 IU, Vitamin B_1-2mg, Vitamin B_2-4mg, Vitamin B_{12}- 10mcg, Percent of values specified by NRC, 1994, *** Calculated

plumaged), CARIBRO-Tropicana (Naked neck and Frizzle plumaged) and CARIBRO-Vishal (normal plumaged) broiler chicks developed at Experimental Broiler Farm, Central Avian Research Institute, Izatnagar, were used in this study.

Reagents: All the chemicals, reagents and kits from standard manufacturers like Fermentas, Quiagen, and Invitrogen and of appropriate grade were used for analyzing various parameters. Molecular biology grade chemicals were used for the preparation of buffers and solutions throughout the experiment in autoclaved distilled/ Milli Q water. The buffers and reagents used in RNA work were prepared in Di-ethyl-pyrocarbonate (DEPC) treated distilled water using nuclease free molecular biology grade chemicals.

Equipment: Cooling micro-centrifuge (3500 Table – top micro refrigerated centrifuge, Cubota Corporation, Tokyo, Japan), Tissue homogenizer (Polytron, Kinematica AG, Switzerland), Micropipettes (Eppendorf AG, Germany), Nano drop-1000 (Thermo Scientific, Singapore), Thermal cyclers (iQ5 Multicolor Real-time PCR Detection System; iCycler, MJ Mini Gradient Thermal Cycler, Bio- Rad laboratories, Inc. Hercules, USA), Gel documentation system (Biometra, UK; Syngene, USA), horizontal submarine electro-phoresis apparatus (Scie-Plas Ltd., Warwickshire, England), Laminar air- flow apparatus (Tanco, India). Auto Gamma counter (COBRA III, Packard Bio Science), Double beam UV-VIS spectrophotometer (ICI, India).

Glass and plastic wares: Glass wares *i.e.* beaker, test tubes, cylinders, and conical flasks, round bottle flasks etc. used throughout the experiment were acquired from Borosil (India). Plastic wares viz. real time PCR tubes and flat caps, microcentrifuge tubes (0.2 ml, 1.5 ml and 2 ml) and tips (20 µl, 200 µl and 1 ml) were procured from axygen (USA). Falcon tubes (15 ml and 50 ml) were purchased from Axygen (India).

Chemicals and kits: TRIzol reagent (M/s Invitrogen, USA), 10X Taq buffer, 10 mM dNTPs mix, Taq DNA polymerase (6U/mL) (M/s Biotools, Spain), Agarose (M/s Amresco, USA), 6x loading dye, molecular size quantitative marker (M/s Fermentas, USA), 5x tris Boric acid EDTA buffer. cDNA synthesis kit (Revert-Aid™ First Strand cDNA synthesis Kit, Fermentas Life Sciences), Real- time SYBR Green qPCR kit (DyNAmo™ HS, M/s Finnzymes, USA.

Hatching of experimental chicks: Fertile eggs were collected at Experimental Broiler Farm for incubation. Total of 36 chicks (three weeks old) of CARIBRO-Mritunjai, CARIBRO-Tropicana and CARIBRO-Vishal each, for each exposure trial were used.

Management of chicks: The chicks were reared up to 3 weeks of age at Experimental Broiler Farm (EBF) under uniform nutrition (Table-1) and management. The chicks were reared at litter with *ad lib* feeding of starter ration (Table-1). At three weeks of age the chicks were shifted to Psychometric chamber, Veterinary Physiology Division, IVRI, Izatnagar, for exposure under particular THI for a period of 7 days. Three weeks old chicks (36) of each germplasm were used in this study in each exposure trial totaling to 108 chicks of each genetic group in the three exposure trials and 324 chicks of all genetic groups in all exposure trials.

Thermal humidity index (THI) exposures for evaluation of broiler stocks: The evaluation of these stocks was carried out at three different THI in three separate trials. The birds of each genetic groups were kept in Psychometric chamber at an age of 3 week and the chamber was set to provide specific temperature and humidity for a particular THI. The desired temperature and humidity at each THI were provided for a

period of 4 hours daily for experimental duration of 7 days.

THI 1 (72): Temperature: 23 ± 1°C and Relative humidity: 40 ± 5% (Zone of comfort)

THI 2 (85): Temperature: 35 ± 1°C and Relative Humidity: 50 ± 5% (Mild stress)

THI 3 (91): Temperature: 41 ± 1°C and Relative Humidity: 45 ± 5% (High stress)

THIs were determined using following formula [13]:

$$THI = T_d - (0.55 - 0.55\ RH)\ (T_d - 58)$$

Where,

T_d = Dry- bulb temperature in °F

RH= Relative Humidity in % (In equation, RH is used as a decimal; in other words, 50% relative humidity is indicated as 0.50.

Organization of exposure trial: The chicks of each genetic group were simultaneously exposed to particular THI and different parameters were recorded. The chicks (3 week old) of each genetic group were housed in battery brooders inside the Psychometric chamber. The battery brooders were consisted of 4 tiers and two compartments in each tier. Each compartment housed 7-8 chicks. Thus, a total of 36 chicks of one genetic group were housed in 5 compartments of battery brooder.

Molecular analysis: Expression profile analysis of HSP-70 in liver tissue at 0^{th}, 3^{rd} and 7^{th} day of experiment.

Sample size: A sample of 4 birds/ genetic groups/ experimental day was slaughtered soon after THI exposure (*i.e*. 4 hours daily on a particular THI) under each THI exposure and liver tissues were collected for *HSP-70* gene expression analysis.

HSP 70 gene expression studies: Total RNA was isolated from the liver following standard TRIZOL method. The purity of RNA was checked before the preparation of first- strand cDNA. Prepared cDNA was stored frozen at -20°C and was used for HSP 70 gene expression studies. Expression of HSP 70 gene was quantified by using specific primer pairs for genes of interest (GOI) in Real–Time PCR. Here β-actin was used as a reference gene and 0^{th} day sample after exposure was taken as control.

Sterilization and inactivation of RNases: Glasswares used for RNA work were sterilized in hot air oven at 180ºc for at least 5 hours to make them RNase free. All the plasticwares used for the same purpose were thoroughly treated with 0.1% Diethyl pyrocarbonate (DEPC) at 37ºC for about 12 hr in order to destroy the RNases and then were sterilized by autoclaving.

Isolation of total RNA: The tissue samples from liver were cut into small pieces and collected in 2 ml RNase free microcentrifuge tubes containing 600 µl TRIzol (M/s Invitrogen, USA) denaturing solution. The tissue sample were homogenized using tissue homogenizer at 2000 rpm perform for about 30 seconds till a uniform suspension is formed. One hundred and twenty microliter (120 µl) of chloroform (molecular biology grade) was added into the tube and was thoroughly mixed. The tissue suspension was centrifuged at 13000 rpm for 15 min at 4ºC in micro-centrifuge (KUBOTA-3500, Japan). The top aqueous phase (containing RNA) was removed and transferred carefully into a fresh 1.5 ml RNase free micro-centrifuge tube without disturbing pellet. Equal volume of isopropanol was added to the aqueous phase and incubated at room temperature (25ºC) for 20 min to precipitate the RNA. The RNA was pelleted by spinning at 10000 rpm for 10 min at 4ºC; thereafter the isopropanol supernatant was decanted carefully without disturbing the pellet. The pellet was washed with 0.5 ml of 70 % ice-cold ethanol and the tube was centrifuged at 10000 rpm for 10 min at 4ºC. The ethanol was decanted and the RNA pellet was air dried in an RNase free environment. The air dried RNA pellet was re-suspended in 50 µl of nuclease free water.

RNA quantification: The purity and concentration of the total RNA was checked using nanodrop (Nano Drop1000, Thermo Scientific, Singapore). The purity of the total RNA was confirmed by considering the ratio of OD values at 260 and 280 nm. The purity was further checked by Agarose gel electrophoresis. The RNA showing any contamination with DNA was incubated with RNase free DNase (Biogene, CA USA) at 37ºC@1U for 1 µl). The DNase was subsequently inactivated by incubation at 65ºC for 10 min. Each DNase treated total RNA sample was computed and all the samples were adjusted to equal concentration (5.0 µg/µl).

First- strand c DNA synthesis: The first-strand cDNA synthesis was carried out using 200 µl PCR microtubes in a thermal block using *Revert Aid*$^{TM\ first}$ strand cDNA synthesis kit (MBI, Fermantas). Eleven micro-liters (11 µl) diluted total RNA (containing 5.0 µg of RNA) was taken in RNase free PCR tube (0.2ml). One micro-liter (1 µl) of random hexamer primer (0.5 µg/ µl) was added to each RNA samples and spinned down for few seconds. Each tube containing 12 µl of reaction mixture was incubated 65 ºC for 5 minutes in thermal cycler. Eight micro liters of master mix containing 5X reaction buffer (4µl), 10mM dNTP mix (2µl), Ribonuclease inhibitor (1µl) and revert Aid^{TM} reverse transcriptase (1µl) was added to each tube and mixed thoroughly. The total reaction mixture was incubated in the thermal cycler according to the manufacturer's instructions i.e. 25ºC for 5min and 42ºC for 60 minutes. Finally, the reaction was stopped by heating at 70ºC for 10 min and the resultant first-strand c-DNA was synthesized and stored at -20ºC till further expression studies.

HSP-70 mRNA quantification: The relative expression of specific gene mRNA was quantified by real-time PCR detection system (IQ5, Bio- Rad laboratories Inc. USA). All reactions were performed in nuclease-free 8

Table-2. Details of the primers used for real-time PCR analysis of HSP-70 gene in liver tissue of broiler chickens during different stress conditions (THIs).

Sr no.	Genes	Primer	Annealing Temperature	Product size (bp)	Accession number
1	HSP- 70	F- GGCACCATCACTGGCTT R-TCCAAGCCATAGGCAATAGCA	56 ºC	74	Hm587997
2	β-actin	F-GGAAGTTACTCGCCTCTG R-AAGACACTTGTTGGGTTAC	58 ?C	114	L08165

F= Forward; R=Revei

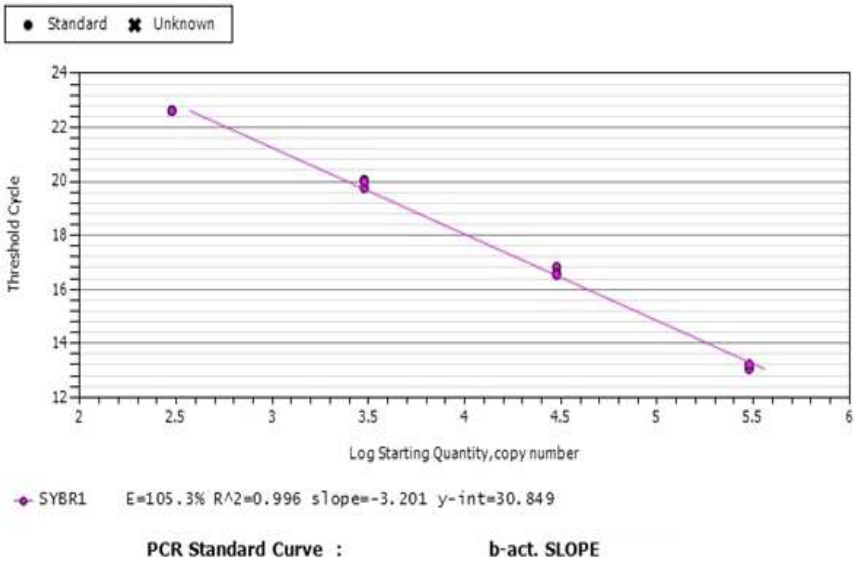


Figure-1. PCR standard curve of β-actin gene slope

tubes with optically clear flat caps (Axygen Scientific, Inc. USA).

Real–time polymerase chain reaction: Twenty micro-liter volume of reaction mixture was used to amplify the gene of interest with final concentration of 1X SYBR Green PCR master mix (2x DyNAmo ™ HS, Finnzymes, USA) which contain SYBR Green 1dye, Meteor *Taq* hot start DNA polymerase, dNTPs including dUTP and $MgCl_2$ with 4M final concentration in optimized buffer components, a 0.05 pM concentration of gene-specific primer (Table-2) and 2.5 µl of diluted (1:10) cDNA template. Real-time PCR cycling conditions were, initial denaturation at 95ºC for 20s, annealing at 56ºC or 58ºC for different gene for 20s and extension 72 ºC for 20s. For each sample a dissociation curve (melt curve) was generated after completion of amplification. A negative control containing all the ingredients except cDNA template (Non-template control; NTC) was set up invariably for each master mix made for conducting the reactions. β-actin was used as reference gene. The results were expressed in terms of the threshold cycle value (Ct). PCR standard curve of β-actin gene is given in fig.-1. Melt peak chart of HSP-70 and β-actin gene is given in fig.-2.

Statistical analysis: The data generated under the present investigation were analysed using SPSS ver 16. The data were classified according to genetic groups and THIs. Analysis of data on these parameters was performed using a fixed model with genetic groups and THI as main effects under each day of experiment. All data were presented in Tables as Mean + Standard error mean. The data recorded as CT values for gene expression analysis of HSP-70 gene was also analysed as per below models.

$$Y_{ijk} = \mu + G_i + T_j + (G \times T)_{(ij)} + e_{ijk}$$

where, Y_{ijk} = Trait recorded under i^{th} genetic group and j^{th} THI on k^{th} individual

μ = Overall mean

G_i = Effect of i^{th} genetic group (i = 1, 2, 3)

T_j = Effect of j^{th} THI (j = 1, 2, 3)

$(G \times T)_{(ij)}$ = Interaction effect between i^{th} genetic group and j^{th} THI

e_{ijk} = Random error distributed with mean '0' and variance'σ^2'

Results

The mean mRNA expression levels of *HSP-70* in liver of birds of three genetic groups at 0^{th}, 3^{rd} and 7^{th} days of experiment under different THIs have been presented in Table-3 and Fig.3. The difference among three genetic groups were significant ($P<0.05$) at 0^{th}, 3^{rd} and 7^{th} day of experiment at THI 72, 85 and 91. The higher mRNA expression levels of *HSP-70* at all the time points under different THIs were exhibited in CARIBRO-Tropicana (1.27±0.01 to 2.51±0.019) as compared to CARIBRO-Vishal (1.037±0.016 to 2.118±0.020) and CARIBRO-Mritunjai (1.164± 0.005 to 2.321± 0.010) (Table-3). CARIBRO-Tropicana (2.379±0.007) exhibited higher expression of HSP-70 on 0^{th} day than CARIBRO-Mritunjai (2.219 ±0.010) and CARIBRO-Vishal (1.993±0.025) at THI 91. CARIBRO-Vishal (1.037±0.016) exhibited lower expression than the CARIBRO-Tropicana (1.270±

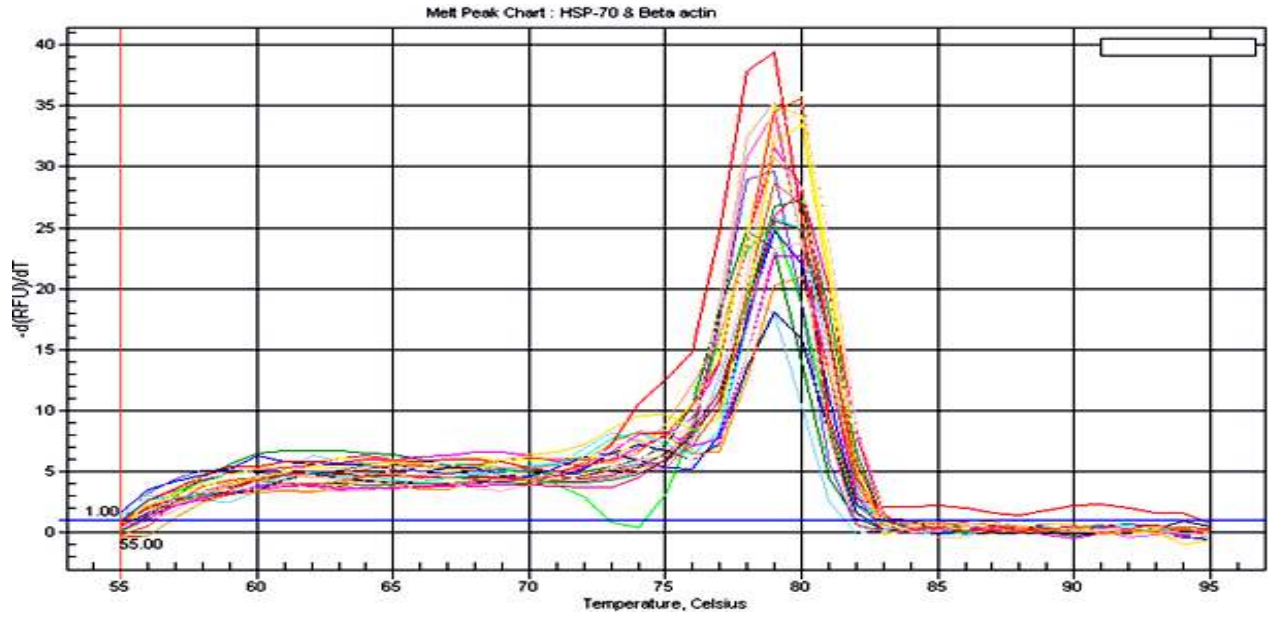


Figure-2. Melt peak chart (Dissociation curve) of HSP-70 and β-actin gene

Table-3. mRNA expression profile of heat shock protein-70 gene in liver of broiler chicken of different genetic groups at different days during experimental period under different THI (Mean±SE)

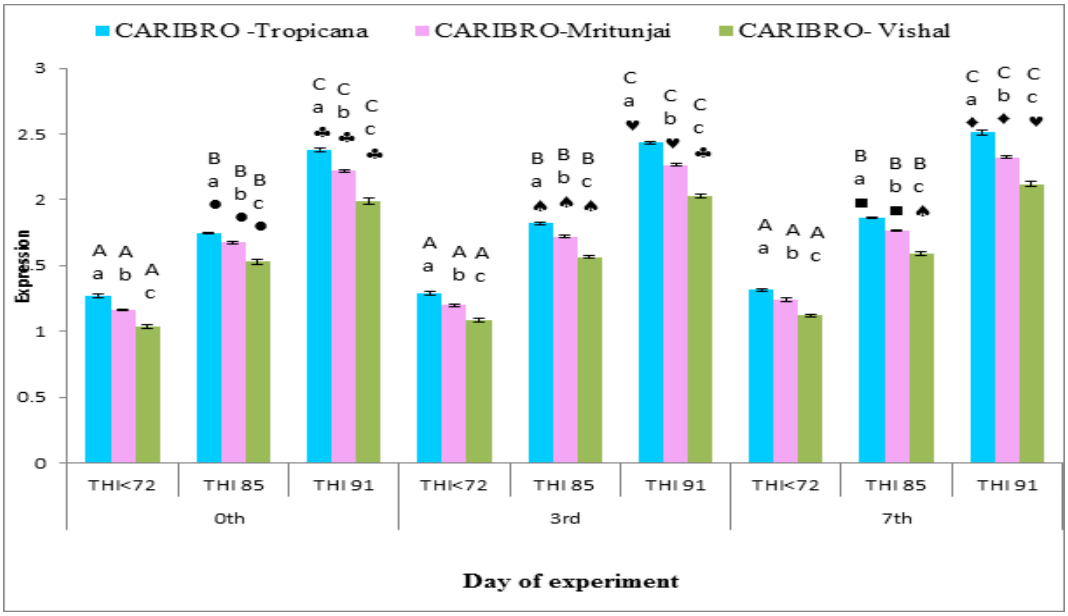
Day of experiment	THI	CARIBRO-Tropicana (T1)	CARIBRO-Mritunjai (T2)	CARIBRO- Vishal (T3)	Overall mean
0th day	THI<72	1.270^{aA}±0.011(4)	1.164^{bA}±0.005(4)	1.037^{cA}±0.016(4)	1.157±0.007(12)
(After Exposure)	THI85	1.744^{aB}±0.006(4)	1.675^{bB}±0.011(4)	1.529^{cB}±0.022(4)	1.649±0.008(12)
	THI91	2.379^{aC}±0.018(4)	2.219^{bC}±0.010(4)	1.993^{cC}±0.025(4)	2.197±0.011(12)
	Overall mean	1.798±0.007(12)	1.686±0.005(12)	1.520±0.012(12)	
3rd day	THI<72	1.290^{aA}±0.015(4)	1.199^{bA}±0.010(4)	1.087^{cA}±0.016(4)	1.192±0.008(12)
	THI85	1.821^{aB}±0.013(4)	1.720^{bB}±0.008(4)	1.564^{cB}±0.008(4)	1.702±0.005(12)
	THI91	2.433^{aC}±0.011(4)	2.266^{bC}±0.008(4)	2.025^{cC}±0.014(4)	2.241±0.009(12)
	Overall mean	1.848±0.007(12)	1.729±0.005(12)	1.559±0.10(12)	
7th day	THI<72	1.315^{aA}±0.012(4)	1.239^{bA}±0.014(4)	1.123^{cA}±0.009(4)	1.226±0.007(12)
	THI85	1.863^{aB}±0.008(4)	1.762^{bB}±0.006(4)	1.591^{cB}±0.014(4)	1.739±0.006(12)
	THI91	2.510^{aC}±0.019(4)	2.321^{bC}±0.010(4)	2.118^{cC}±0.020(4)	2.316±0.010(12)
	Overall mean	1.896±0.008(12)	1.775±0.006(12)	1.611±0.009(12)	

1) Figures in parentheses are the number of observations; 2) Figure bearing same superscript (lower case) in a row and (upper case) in a column within day of experiment do not differ significantly ($P \leq 0.05$); 3) The figures bearing different superscript (symbol) differ significantly in a particular THI during different days in a column.

0.011) and CARIBRO-Mritunjai (1.164±0.005) at THI 72 on 0th day. CARIBRO-Tropicana (1.744±0.006) exhibited higher expression than CARIBRO-Mritunjai (1.675±0.011) and CARIBRO-Vishal (1.529±0.022) on 0th day at THI 85. The effect of THI was also found significant (P<0.05) on mRNA expression of *HSP-70* at different days of experiment in each of the genetic groups (Table-3). The mRNA expression levels of *HSP-70* were significantly (P<0.05) higher at THI 91 than at THI 72 and 85 in each genetic groups. Significantly lower (P<0.05) mRNA expression of *HSP-70* was observed on 0th day (1.744±0.006) than at 3rd day (1.863±0.008) under THI 85 in CARIBRO-Tropicana. mRNA expression of *HSP-70* in CARIBRO-Tropicana under THI 91 was observed significantly lower at 0th day (1.821±0.013) than at 3rd day (2.379±0.018) and 7th day (2.51±0.019). In CARIBRO-Mritunjai, mean mRNA expression of *HSP-70* was observed significantly lower on 0th day (1.675±0.011) than 3rd day (1.720±0.008) and 7th day (1.762±0.006) of experiment under THI 85. Under THI 91, mean mRNA expression of *HSP-70* was observed significantly lower on 0th day (2.219±0.010) than 3rd day (2.266±0.008) and 7th day (2.321±0.010) in CARIBRO-Mritunjai. In CARIBRO-Vishal, mRNA expression of *HSP-70* was observed significantly lower on 0th day (1.529±0.022) than 3rd day (1.564± 0.008) and 7th day (1.591±0.014) of experiment under THI 85. Under THI 91, mRNA expression of *HSP-70* was observed significantly lower on 0th day (1.993± 0.025) than 3rd day (2.025 ±0.014) and 7th day (2.118± 0.020) in CARIBRO-Vishal (Table-3). Under THI-85 and THI-91, the m-RNA expression of *HSP-70* in group was significantly lower at 0th day in comparison to 3rd day and 7th day of experiment (Table-3 and Fig. 3.).

Discussion

mRNA expression of *HSP-70* in different broiler stocks/genotypes under different stress conditions have been reported by [14-19] mRNA expression in CARIBRO-Tropicana (1.23) at 7th day after exposure at 23°C were comparable to those reported by Shoaib



1) Figures bearing same superscript (lower case) do not differ significantly (P≤0.05) among genetic groups within THI.

2) Figures bearing same superscript (upper case) do not differ significantly (P≤0.05)among different THI within genetic groups and within day of experiment.

Figure-3. mRNA expression profile of heat shock proteins-70 gene in liver of broiler chickens of different genetic groups at different days during experimental period under different THI (Mean±SE)

[19], in 3 weeks male of CARIBRO-Vishal at 23°C. In the present investigation, the differences in mRNA expression of *HSP-70* among genetic groups under different THIs for different days were significant. The effect of THI was found significant on mRNA expression of *HSP-70* at different days and different genetic groups. Significant effect at higher ambient temperature on mRNA expression of *HSP-70* in broilers have also been reported in literature [14-19].

Day-wise differences also occurred in CARIBRO-Tropicana, CARIBRO-Mritunjai and CARIBRO-Vishal under THI 85 and 91. Role of HSPs in general and *HSP-70* in particular in thermotolerance/stress and under infectious conditions is well documented in poultry and other animal species [20]. The increment in mRNA expression in broiler chickens under heat stress highlighted the role of *HSP-70* in the protection of cells from heat stress. An important aspect of *HSP-70* is that cells or organisms those have reversed from a previous mild stressful condition express elevated levels of stress proteins. These stress proteins (*HSP-70*) can exhibit tolerance against the high doses of the stress causing agents, including heat that would normally cause developmental abnormalities or death. This phenomenon referred to as acquired thermotolerance has been shown to be dependant on the increased expression and accumulation of the *HSP-70* [11].

Conclusion

The differences due to THI and genetic groups were found significant for *HSP-70* traits studied. During the 7 day exposure trial, birds of all the three genetic groups exhibited the phenomenon of acclimatization as reveled by the averages of various traits at different days into the exposure. Among the three genetic groups, CARIBRO-Tropicana exhibited highest means for *HSP-70* production as well as tolerated the heat stress in a better way than CARIBRO-Mritunjai and CARIBRO-Vishal; therefore CARIBRO-Tropicana was adjudged to be the best genetic group for production under tropical climate.

Authors' contributions

SAA, VKS and SM: Substantially contributed to the conception and design of study. SAA and VKS: Drafted the manuscript, analysed and interpreted the results. SAA and SM: Carried out the laboratory works, revised manuscript for important intellectual content. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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