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Association of HSP70 Gene Polymorphism with Heat Stress Indicators in Different Poultry Genetic Groups

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ABSTRACT

The experiment was conducted during January to December, 2023 at Poultry Research Farm overseen by the Directorate of Livestock Farms, GADVASU, Ludhiana, Punjab, India to study and correlate the allelic variations, if any of different HSP70 genotypes with heat stress indicators viz. average daily gain (ADG) and cloacal temperature in three breeds of poultry birds. 300 birds (100 each from three breeds i.e., Kadaknath, RIR and Punjab Brown) were raised under standard feeding and managemental conditions. Body weight was taken at fortnightly intervals from 0 day upto 26th week of age. Cloacal temperature was recorded weekly twice during 18th to 26th week. Blood samples were collected from 36 representative birds (12 from each breed) at 25th week of age. The DNA was extracted using phenol chloroform method and PCR standardization was done. Punjab Brown birds had significantly ($p \leq 0.01$) higher values for ADG as compared to RIR and Kadaknath at different age groups.785bp HSP70 gene amplicon was sequenced and two genotypes AG and GG were identified with frequencies of 0.6 and 0.4. Distinct alterations in translating amino acid positions, single nucleotide polymorphisms (SNPs), deletions and mutations were observed in all the studied genetic groups, however, the genotypes had no significant effect on average daily gain or cloacal temperature.

KEYWORDS: Poultry, HSP70 gene, sequencing, mutation

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Climate change led to increase in environmental stress affecting productivity, reproductive efficiency and health disorders leading to severe economic losses in livestock species including poultry. Variations in important climatic factors (Temperature, humidity, radiations etc.) could cause potential hazard in the growth and production of different livestock species (Chowlu et al., 2023). Rising temperatures caused by climate change is one of the major environmental concerns affecting chicken farming. Layer and broiler hens are particularly susceptible to the negative effects of heat stress on production performance. A variety of performance like growth, feed intake, electrolyte balance, and immune function are adversely affected by thermal stress which ultimately increase the rate of mortality in both broiler and laying chicken. Because of rapid growth and higher metabolic rates, these birds are severely affected with environmental stress (Kumar et al., 2021). According to Vercese et al. (2012), as temperature increase to 36˚C, there is adverse impact on chicken egg production, quality, and saleable quantity compared to the more comfortable temperature of 21˚C. This temperature sensitivity emphasizes the importance of maintaining optimal conditions for poultry. Heat stress has noticeable effects on birds, leading to less appetite and feed conversion efficiency into energy. As per Yalcin et al. (2001), when broiler stocks were exposed to different levels of heat stress, there was significant reduction in body weight of birds. Heat stress in laying and brood hens lowers their ovulation rates, which reduces their ability to reproduce. This drop is because important reproductive hormones like GnRH, LH, and FSH are not released as much, and parameters like heat shock proteins (HSPs), fatty acid makeup, and antioxidant levels have changed. HSP expression is increased in response to heat stress that leads to a cellular mechanism which protects different protein against damage. Genetic selection, management strategies, and technical advancements must all work together to mitigate the effects of climate change on chicken production in a way that benefits the business as a whole.

The knowledge on molecular mechanisms of heat shock proteins (HSPs), and control of economically significant features are very vital to the current state of poultry production in India. HSPs are chaperone proteins which effectively protect cell organelles and different proteins from stress causing agents. HSP 70 is produced by organism to overcome heat stress (Kiang and Tsokos, 1998). It is related with the heat tolerance trait and plays important role in survival and production performance of chickens. HSP70 genotypes influence the gene expression during heat stress of chicken. As per Liang et al. (2016), chicken having certain HSP70 genotype are found to be heat tolerant and acute heat stress had no negative effect on growth performance and egg production. Several authors have studied the HSP70 gene polymorphism in different chicken breeds (Chen et al., 2016; Duangjinda et al., 2017; Phongkaew et al., 2017; Cedraz et al., 2017; Najafi et al., 2018; Aryani et al., 2019; Habib et al., 2020; Toth et al., 2021; Assi et al., 2023; Budi et al., 2024; Ali et al., 2024). However, very scanty reports for the present birds under investigation are found. Hence, the present study was carried out to find out the association of HSP70 gene polymorphism with average daily gain and cloacal temperature in Kadaknath, RIR and Punjab Brown poultry birds.

2. MATERIALS AND METHODS

This study was performed on three breeds of poultry birds maintained at Directorate Livestock Farm (DLF) of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab, India taken during the year 2023 (January to December).

Ludhiana is located at 30.9˚N 75.85˚E. It has an average elevation of 244 meters (798 ft) meters above mean sea level. It features a humid sub-tropical climate under the Koppen climate classification, with three defined seasons; summer (March to June), monsoon (July to September) and winter (October to February). The average high and low temperatures of the area were 29.8 and 16.7, respectively whereas the average maximum and minimum relative humidity were 82% and 46%, respectively. The district received annual average precipitation of 890 mm (Prabhiyot et al., 2013).

2.1. Experimental materials

Three genetic groups of chickens i.e. Kadaknath, Rhode Island Red (RIR) and one native chicken germplasm (Punjab Brown) maintained under the AICRP on Poultry Breeding Project were included in the study. The data was collected on fortnightly bodyweight (300 birds; 100 from each breed and equally distributed among male and female). The study's data set covered the age span of 0 days to 26 weeks.

2.2. Measurement of variables

Body weights were measured biweekly from 0 day, 2^{nd}, 4^{th}, …..upto 26^{th} weeks of age. Average daily gains (ADG) for the periods 0-2^{nd}, 2^{nd} -4^{th},....., 24-26^{th} weeks were estimated using the formula: $ADG= \frac{W_2-W_1}{N}$ Where, W_2=body weight at the end of period, W_1=body weight at the beginning of period, N=No. of days from previous weight to present weight. The cloacal temperature was taken from 36 representative samples (morning and evening) from 18^{th} to 26^{th} week of age.

2.3. Extraction of genomic DNA

Blood samples were collected from 36 representative random samples (12 from each breed, equal number from each sex). 1.5 to 2.0 ml of venous blood was collected under sterile conditions, from the wing vein of bird in 10 ml polypropylene centrifuge tubes containing 0.2 ml of 0.5M EDTA solution as an anticoagulant. Genomic DNA was extracted using the protocol of Sambrook and Russell (2001). Good quality of genomic DNA corresponding to purity ($A_{260/280}$) of 1.8 to 1.9 was used for the PCR. Permission for sample collection for conducting the study was obtained from Institutional Animal Ethics Committee (GADVASU/2023/IAEC/67/09 dated 14/01/2023).

2.4. PCR standardization of HSP70 gene promoter region

Promoter region, a 785 bp fragment of HSP70 gene was amplified using corresponding set of reported forward and reverse primers (Mazzi et al., 2003; Gan et al., 2015). The final standardized protocol consisted of 35 cycles of denaturation (95°C for 30s), annealing (55°C for 45s), extension (72°C for 45s), final extension (72°C for 7 min) and storage at 4°C.

2.5. Purification and sequencing of amplicons

After checking of amplifications, PCR amplified products and their respective forward and reverse primers were sent for column purification and sequencing. The sequencing of DNA was done by Sanger's method of sequencing at Biologia Research India Pvt. Ltd., Karnal (Haryana). Sequence alignment was performed with the reference chicken HSP70 gene from NCBI Gene Bank using bioinformatics tools, viz. NCBI-BLAST, Clustal Omega Multiple sequence alignment tool (Sievers et al., 2011) for SNP detection.

2.6. Statistical analysis

The gene and genotype frequencies were estimated using chi-square test (Falconer and Mackay, 1996). Least squares constants were estimated and association of genotypes with heat stress indicators viz. average daily gain and cloacal temperature were analyzed using IBM-SPSS 24.0 statistical package.

The following mathematical model was used:

$Y_{ij} = \mu + G_i + e_{ij}$

Where, Y_{ij}=Observation of the trait of the jth individual of ith genotype; μ=population mean; G_i=Fixed effect of i^{th} genotype; e_{ijk}=residual random error associated with the observations. The statistical significance of various fixed effects in the least squares model was determined by 'F' test.

3. RESULTS AND DISCUSSION

3.1. Descriptive statistics of the variables

The average daily gain (gm) and cloacal temperature (0F) of Kadaknath, Rhode Island Red (RIR) and Punjab Brown birds are presented in the Table 1 and Table 2. The Punjab Brown birds had maximum value of ADG as compared to Kadaknath and RIR in both male and females whereas no

Table 1: Average daily gain (ADG) (g day^{-1}) in different groups of poultry birds

Traits	Kadaknath		RIR		Punjab Brown	
	Male (N=50)	Female (N=50)	Male (N=50)	Female (N=50)	Male (N=50)	Female (N=50)
	Mean±SE (g)					
ADG 0-2	1.92*±0.07	2.30*±0.13	4.58±0.25	4.12±0.23	9.51±1.08	9.19±1.15
ADG 2-4	4.41±0.08	4.45±0.09	5.63±0.62	5.89±1.16	10.56±0.84	8.58±1.38
ADG 4-6	14.75*±1.05	10.46*±1.52	8.46±0.46	6.59±0.74	10.07±1.24	6.43±1.51
ADG 6-8	12.26*±1.10	7.45*±1.21	14.75**±0.87	9.76**±1.13	12.69±2.33	10.87±1.69
ADG 8-10	8.94±1.13	8.59±1.69	11.55±0.70	12.76±1.81	13.23±1.05	8.69±2.22
ADG 10-12	13.62±1.14	14.75±1.52	14.90±1.54	12.11±3.08	5.02±1.55	6.67±1.68
ADG 12-14	7.66±1.10	10.37±1.48	10.52*±1.16	16.25*±2.12	14.25±3.28	10.89±1.40
ADG 14-16	5.65±0.33	5.28±0.13	16.62±2.13	13.74±3.17	18.37±2.94	14.11±1.73
ADG 16-18	7.93*±1.29	4.64*±0.28	10.92±1.11	10.90±1.89	10.83±2.07	11.79±2.49
ADG 18-20	4.41±1.10	2.21±0.33	12.43±2.26	9.70±1.64	11.90±1.54	11.90±3.49
ADG 20-22	2.61±0.77	1.87±0.33	16.22**±1.56	6.99**±1.20	16.42±2.79	20.71±3.12
ADG 22-24	1.08±0.15	1.47±0.29	11.51±0.56	13.62±3.70	21.55±3.64	18.09±2.84
ADG 24-26	1.75±0.14	1.34±0.55	12.61±2.72	13.00±3.09	26.42±2.98	20.72±3.43
ADG 0-26	6.69±1.29	5.78±1.18	11.59±1.03	10.42±1.00	13.91±1.56	12.20±1.35

**Highly Significant ($p \leq 0.01$) *Significant ($p \leq 0.05$); within breed between male and female

Table 2: Cloacal Temperature (°F) in different groups of poultry birds

Traits	Kadaknath		RIR		Punjab Brown	
	Male (N=6)	Female(N=6)	Male (N=6)	Female(N=6)	Male (N=6)	Female(N=6)
	Mean ± SE (^{0}F)					
CT 18wk	107.56**±0.19	106.71**±0.09	106.63**±0.14	105.86**±0.14	108.15±0.19	108.11±0.18
CT 19wk	106.75±0.11	106.73±0.13	107.56**±0.08	106.83**±0.11	107.38±0.20	106.97±0.13
CT 20wk	108.08±0.24	107.46±0.21	107.71±0.36	108.14±0.22	106.69±0.12	106.89±0.06
CT 21wk	107.39±0.11	107.04±0.19	107.23±0.21	107.13±0.27	107.57**±0.16	106.88**±0.14
CT 22wk	107.80±0.17	107.54±0.29	107.53±0.18	107.27±0.27	106.96±0.12	106.93±0.13
CT 23wk	107.03±0.16	107.03±0.16	107.23±0.18	106.97±0.27	107.27**±0.16	106.58**±0.14
CT 24wk	107.53*±0.16	107.16*±0.03	107.10±0.12	107.03±0.12	106.89±0.12	107.09±0.06
CT 25wk	107.84±0.11	107.84±0.09	107.40±0.12	107.28±0.15	106.70±0.06	106.76±0.09
CT 26wk	108.04±0.11	108.04±0.09	107.49±0.23	107.69±0.16	106.78±0.15	106.69±0.15
Average CT	107.56±0.15	107.28±0.16	107.32±0.11	107.13±0.21	107.15±0.16	106.99±0.15

**Highly Significant (p≤0.01) *Significant (p≤0.05); within breed between male and female

significant differences in cloacal temperatures were observed in the studied genetic groups of poultry birds.

Chandrashekar et al. (2021) also reported similar values of ADG (8.82±0.14 g) in native chicken during 0 to 12 wks of age whereas Punjab Broiler 2 (PB2) had higher ADG (25.26±0.33 g) during the same period. The findings of cloacal temperatures were in agreement with those of Shanmathy et al. (2017) in Aseel and Kadaknath breeds of chicken.

3.2. Sequence alignment and polymorphism in different poultry genetic groups:

The HSP 70 gene sequence available at Genbank of NCBI (Accession No. LC498496) was used as the reference sequence. All the three genetic groups–Kadaknath, RIR and Punjab Brown - exhibit distinct alterations in translating amino acid positions, single nucleotide polymorphisms (SNPs), deletions, and mutations. Kadaknath poultry birds showed deletion of allele A at 245 bp, G allele having A>G transition mutation at 258 bp, and insertion of G and GC at 203, 208–209 bp position. RIR poultry birds showed deletion of A allele at 246 bp, G allele having A >G transition at 258 bp and G allele having C>G transversion at 598 bp, A>T transversion mutation at 600bp.The Punjab Brown poultry birds showed deletion of allele A & T at 111–112 bp position, C>T transitional mutation at 114 bp, deletion of A allele at 247bp, G allele having A>G transition mutation at 259 bp (Figure 1). Chen et al. (2016) reported SNPs of HSP 70 gene at C.-69A>G 5'-flanking region in White Recessive Rock (WRR) and Lingshan (LS) chickens. Najafi et al. (2018) found SNPs in the coding region at A179C position of HSP70 gene in native breeder chickens.

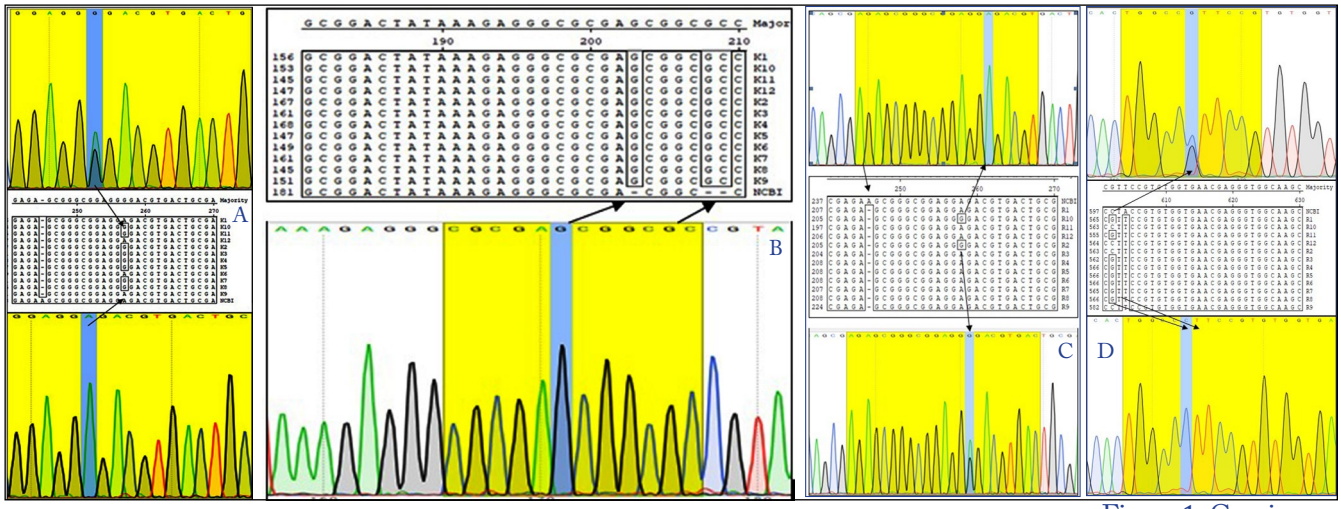

Figure 1: Continue...

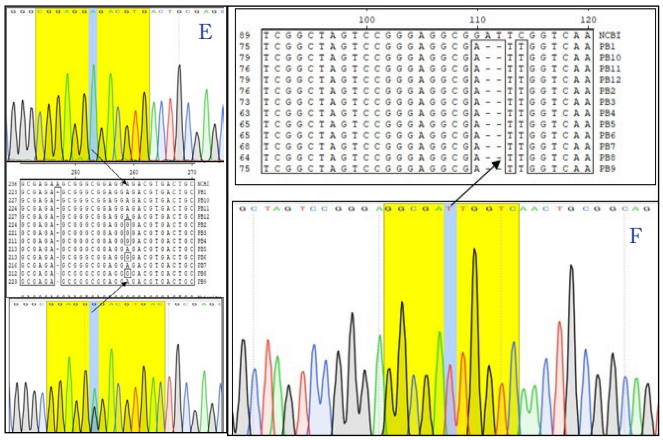

Figure 1: (A) G allele having A>G transition at 258 bp and deletion of allele A at 245 bp position in Kadaknath; (B) Insersion of G and GC at 203, 208–209 bp position in Kadaknath; (C) G allele having A>G transition at 258 bp and A allele deletion at 246 bp position in RIR; (D) G allele having C>G transversion at 598 bp and A>T transversion mutation at 600 bp position in RIR; (E) G allele having A>G transition at 259 bp position and Breed specific deletion of allele A at 247 bp position in Punjab brown; (F) Deletion of allele A & T at 111–112 bp position and C>T transitional mutation at 114 bp position in Punjab brown

Aryani et al. (2019) studied three native Indonesian chicken breeds and found four haplotypes (H1, H2, H3, and H4), mutation at position g.388C>G produced H2, g.370A>G produced H3, g.370A>G and g.388C>G produced H4 however Wild haplotype (H1) was found in KUB and Walik chickens for HSP70 gene.

3.3. Effect of genetic groups and genotypes on heat stress indicators

Two genotypes were observed in our study with 20 samples of AG genotype and 16 of GG genotype. The observed frequency of AG and GG genotypes were 0.6 and 0.4, respectively indicating selection pressure against A allele. The estimates of gene frequency for A and G alleles were 0.28 and 0.72, respectively. The effect of different genotypes on average daily gain (ADG) and cloacal temperature (CT) is presented in Table 3. The genotypes had no significant effect on average daily gain or cloacal temperature through different age groups.

Our observations were in agreement with Liang et al. (2016) who observed no significant association (p>0.05) for respiratory rate or cloacal temperature with HSP70 genotypes in randomly selected Taishu No. 9 native strain of the LRI chicken. Duangjinda et al. (2017) compared commercial broilers with native Thai chickens (Gallus

Table 3: Effect of genotypes on average daily gain (g day^{-1}) and cloacal temperature (°F)

	N=36	ADG 0-2		ADG 2-4		ADG 4-6		ADG 6-8		ADG 8-10		ADG 10-12	
		Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
Geno type	AG (20)	5.61	0.77	6.82	0.67	9.28	0.87	11.10	0.92	9.96	0.88	10.50	1.30
	GG (16)	4.83	0.86	6.29	0.75	9.68	0.98	11.53	1.03	11.45	0.99	12.02	1.45
		CT 18wks		CT 19wks		CT 20wks		CT 21wks		CT 22wks		CT 23wks	
Geno type	AG (20)	107.29	0.20	107.14	0.09	107.38	0.16	107.13	0.10	107.24	0.12	106.98	0.10
	GG (16)	107.00	0.23	106.90	0.10	107.63	0.18	107.29	0.12	107.45	0.14	106.92	0.11

	N=36	ADG 12-14		ADG 14-16		ADG 16-18		ADG 18-20		ADG 20-22		ADG 22-24		ADG 24-26	
		Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
Geno type	AG (20)	12.21	1.16	12.81	1.58	10.68	0.99	10.66	1.28	14.15	1.75	11.51	2.20	14.16	2.43
	GG (16)	10.95	1.30	11.63	1.77	10.02	1.11	10.37	1.43	11.62	1.95	10.22	2.46	10.72	2.72
		CT 24 wks		CT 25 wks		CT 26 wks									
Geno type	AG (20)	107.11	0.07	107.18	0.11	107.30	0.14								
	GG (16)	107.15	0.08	107.45	0.12	107.64	0.16								

domesticus) at 10 week of age and observed no significant (p>0.05) effect of RR (Respiratory rate) and cloacal temperature (CT) on ADG of different genotypes (C1C1, n=38; C1C2, n=38; and C2C2, n=28). However, Najafi et al. (2018) observed significant (p>0.05) association between genotypes and body weight at 28 week of age in native breeder fowls.

4. CONCLUSION

Distinct alterations in translating amino acid positions, single nucleotide polymorphisms (SNPs), deletions and mutations were observed for HSP70 gene w.r.t.

reference sequence LC498496 from NCBI in all the studied genetic groups–Kadaknath, RIR and Punjab Brown. The genotypes had no significant effect on the studied heat stress indicators (average daily gain and cloacal temperature) through different age groups for which small sample size could be one determining factor.

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