Bioinformatics Identification of HSP70 in Chicken (*Gallus gallus domesticus*)

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Abstract: Increasing the ambient temperature beyond the comfortable temperature zone range for chickens causes stress heat in poultry, resulting in a decrease in chicken productivity. The current study assesses of the bioinformatics on the National Center for Biotechnology Information (NCBI) database as well as expected the physicochemical of Heat Shock Protein 70 (HSP70)in chicken (*Gallus gallus domesticus*). Several parameters of physicochemical HSP70-mRNA in chicken (*G. gallus domesticus*) were varied among the genes observed. There is eight HSP70 induced from chicken (*G. gallus domesticus*) deposited in NCBI. The length of the genes was alternated with the genes ascertained. Several lines of coded protein were 273 to 652 amino acid. The mitochondria target peptide value diversified from 0.010 to 0.309, signifying that it is expected to be a presence. The present result indicated the prominence of the variation and role of a physical and chemical characteristic of the distinct amino acids in protein dehydration genes as extreme climate stress in chicken. The NCBI online available overall suite of online resources can be accessed for biological information the data, including the PubMed database and additional NCBI resources, focus on literature for Identification HSP70 Gene in *G. gallus domesticus* results detected in 8 databases. The literature contained, 34 PubMed, 1 RefSeq transcripts, and 1 RefSeq proteins.

1 INTRODUCTION

Stress due to environmental heat is a major problem that often occurs in modern poultry farming, stress due to rising environmental temperatures affecting chickens, especially in the finisher phase, causing chicken deaths and economic losses for chicken farming companies (Lara and Rostagno, 2014). Quails are also like that, and it is challenging to reduce excessive body heat due to the influence of high environmental temperatures. Information about how to control the response of living creatures to stress control genes is very little. Consider this, the research conducted on HSP70 protein in chickens is fundamental (Xie et al., 2014). The primary oligonucleotides used were unique primers for chickens, namely primers with 960-bp from HSP70 amplified. The results show 98% homology with stress proteins HSP70 in *G. gallus* and 99% homology with *Numida meleagris* (Gaviol et al., 2008).

In the breathing mechanism, water vapor brought along with the bloodstream and released while breathing is vital to reduce excessive body heat. However, water that comes out of breathing during prolonged stress can cause a decrease in blood volume and a lack of body fluids or dehydration as a result of changes in venous return and blood circulation. Blood flow will be more dependent on the upper respiratory tract of the body as a result of a decrease in blood volume which ultimately can lead to hyperthermia and lead to the death of chickens. Damage at the cellular level can also occur due to physiological changes due to stress due to the temperature of this environment. During heat stress due to an increase in environmental temperature, there is a drastic increase in HSP70 levels in the chicken's body. Changes that occur at the cellular level during stress that occur due to high ambient temperatures include; membrane liquidity increases, cell viability decreases, cell death matches the intensity of heat stress, cell type, cytoskeleton

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modification, cell cycle stage, membrane component destabilization, stress protein codon formation in the cell nucleus (Velichko et al., 2013).

Stress protein or HSP70 is essential and is present in all cells of organisms or living things. In particular, HSP has a significant role in the complex assembly of multiprotein, cell cycle control and signaling, folding/opening proteins, cell protection against stress/apoptosis and protein transport/sequencing into the correct sub-cellular compartment. It is well known that HSP is involved in antigen presentation with the role of accompanying and transferring antigen peptides to class I and class II molecules from the main histocompatibility complex. Extracellular HSP can also stimulate the best antigen-presenting cells of the immune system which include macrophages and dendritic cells. Based on its molecular weight, HSP can be grouped into HSP10, HSP40, HSP60, HSP70, HSP90, etc. (Srivastava et al., 2006; Mansilla et al., 2012)

In chickens, NTPDase-8 (Nucleoside Triphosphate Diphosphohydrolase 8) is the outer surface of cell nucleotides with a large extracellular domain (ECD) containing the transmembrane domain (TMD) in N-and C-term. The nature of NTPDases-8 is not susceptible to antigens which cause membrane disturbances, in contrast to other cell surfaces which are very vulnerable. cDNA from NTPDase-8 chicken is dissolved, and the protein is purified. The pure soluble ATP-ase activity of chicken results in NTPDase-8 less than 15% of membrane-bound full-length enzymes, the result of ATP, ADP, and P (i) inactivation. Mutants are truncated in the large extracellular domain (ECD) anchored by NTPDase original TMDs from chickens or TMD from NTPDases that are different from NTPDase2 taken from humans. The difference is shown as the timeline for nonlinear ATP hydrolysis (Li et al., 2009).

Selection of various types of chickens for years has been made to choose chickens with higher productivity. The selected chicken is very sensitive and sensitive to extreme environmental changes in its development environment. The global increase in temperature that occurs at this time will greatly affect the productivity of the chicken. Maintenance with an open cage system will make chickens susceptible to changes in environmental temperature from outside the enclosure which will cause chickens to experience stress due to heat. The group of proteins that play an important role in normal cellular physiology and as cell protection against various stressors including heat stress is Heat Shock Protein (HSP). The levels of various HSP groups in chickens increase in almost all tissues to respond to stress due to high ambient temperatures. This increased HSP level will protect cellular proteins to avoid damage due to heat or high temperatures. (Shanmugam et al., 2017)

2 MATERIALS AND METHOD

2.1 Materials

Eight data on HSP70 in chicken *(G. gallus domesticus)* from the NCBI database. The NCBI reference sequence from DNA and amino acids were used in this study:

1. *G. gallus* heat shock protein 70 (HSP70) mRNA, complete cds 1,920 bp linear mRNA Accession: FJ217667.1 GI: 208964709
2. *G. gallus* heat shock protein 70 (HSP70) mRNA, complete cds 2,371 bp linear mRNA Accession: EU747335.1 GI: 190576827
3. *G. gallus* cell membrane ecto-ATPase mRNA, complete cds 2,352 bp linear mRNA. Accession: U74467.1 GI: 1732248
4. Chicken 78-kD glucose-regulated protein, complete cds 2,389 bp linear mRNA. Accession: M27260.1 GI: 211826
5. *G. gallus* ecto-ATP-diphosphohydrolase mRNA, complete cds 1,482 bp linear mRNA Accession: AF426405.1 GI: 16518969
6. *G. gallus* ecto-ATP-diphosphohydrolase mRNA, complete cds 1,570 bp linear mRNA Accession: AF041355.1 GI: 3320414
7. TPA_exp: *G. gallus* RBJ protein (RBJ) mRNA, complete cds 822 bp linear mRNA. Accession: BK001282.1 GI: 42475925
8. *G. gallus* cpsmb7 mRNA for proteasome subunit Z, complete cds 935 bp linear mRNA Accession: AB098728.1 GI: 30268673

2.2 Physical and chemical characteristics of the HSP70 in chicken *(G. gallus domesticus)*

Protparam online (web.expasy.org/protparam/) was used to determine the structure, physicochemical characteristics of HSP70 chicken *(G. gallus domesticus)*. Important factors are calculated for a number of amino acid, molecular weight, a total number of atoms, half-life period, theoretical

isoelectric point values, extinction coefficients, predictable half-life, high average, Grand average of hydropathicity instability and aliphatic index. (Basyuni et al., 2017).

Table 1: Physicochemical characteristic of the HSP70 in chicken (*G. gallus domesticus*)

Variant	X1	X2	X3	X4	X5	X6	X7	X8
Length of genes/bp	1,920	2,371	2,352	2,389	1,482	1,570	822	935
Number of amino acids	634	634	495	652	493	493	273	277
Molecular weight	69814.95	69845.92	54533.71	72018.51	54063.69	54034.79	30778.32	29876.03
Theoretical pI	5.50	5.59	7.86	5.12	5.78	5.95	8.88	5.99
Total number of atoms	9831	9830	7658	10194	7561	7568	4322	4184
Extinction coefficient-ac	35090	35090	77530	29005	88530	88530	30285	18255
Extinction coefficient-ar	34840	34840	76780	28880	87780	87780	29910	17880
Half-life period	30 h	30 h	30 h	30 h	30 h	30 h	30 h	30 h
Instability index	30.97	31.05	34.01	32.95	27.63	27.47	37.49	20.31
Aliphatic index	81.21	79.53	86.73	86.26	87.65	89.23	77.51	86.64
Grand average of hydropathicity	-0.485	-0.522	-0.006	-0.495	0.067	0.088	-0.410	-0.121

Extinction coefficient-ac = assuming all pairs of Cys residues form cystines
Extinction coefficient-ar = assuming all Cys residues are reduced

2.3. Possible peptide transfer and subcellular localization of HSP70. in chicken (*Gallus gallus domesticus*)

Transit peptides can be predicted using the online targetP1.1 server (www.cbs.dtu.dk/services/targetp/). This position is in accordance with the expected existence of Len, mitochondrial targeting peptide (mTP), and Signal peptide of the secretory pathway (SP). Online predictor of subcellular protein localization, PSORT (psort.hgc.jp/form.html), is also used to control the subcellular determination of protein-induced dehydration. (Basyuni and Wati, 2018).

3 RESULTS AND DISCUSSION

3.1 Physicochemical characteristics of the HSP70 in chicken (*G. gallus domesticus*)

Several characters of physicochemical HSP70 induced by Chicken (*G. gallus domesticus* was shown in Table 1. The genes length changes with ascertained genes. Individual lines are coded of protein from 273 to 652 amino acid. Important notes are the theoretical isoelectric point values, heterogeneity of relative molecular weights, extinction coefficients, instability coefficients, total atomic numbers, and general hydropathy rates along the genes analyzed. Despite the availability of a lot of climate pressure related to HSP70 in chickens (*G. gallus domesticus*), quantitative gene expression analysis of these genes is only recently attempted for the identification of candidate genes/factors that are contributing to high-temperature tolerance. With the advent of the qPCR technique, it is easier to quantify each gene and establish its relevance under the given stress situations.

3.2 The promising of potential transit peptide of HSP70 *(G. gallus domesticus)*

Transfer in chickens (*G. gallus domesticus*) can be seen in Table 2. Three reliability was determined: Len, secretory pathway signal peptides, Mitochondrial, Other target peptides, Loc, RC.

Table 2: The promising of potential transit peptide of HSP70 (G. gallus domesticus)

Variant	Reliability					
	Len	Mitochondrial target peptide	Signal peptide of secretory pathway	Other	Loc	RC
X1	634	0.059	0.133	0.870	-	2
X2	634	0.059	0.133	0.870	-	2
X3	495	0.042	0.938	0.028	S	1
X4	652	0.063	0.783	0.248	S	3
X5	493	0.010	0.962	0.115	S	1
X6	493	0.010	0.962	0.115	S	1
X7	273	0.303	0.046	0.787	-	3
X8	277	0.309	0.053	0.611	-	4

It should be noted that the target value of the mitochondrial target diversified from 0.010 to 0.309, indicating that it is expected to be present. This data shows that Heat Shock Protein 70 peptide (HSP70) in Chicken (*G. gallus domesticus*) plays a vital role in the mechanism of tolerance of extreme weather such as high ambient temperatures (Wang et al., 2013).

3.3 Subcellular localization of HSP70 in chicken *(G. gallus domesticus)*

There are four (4) variants (X1, X2, X7, X8) were located in the cytoplasm (cyto), cytoplasm nucleus (cyto-nucl) and mitochondrial inner membrane (mito). One variant (X4) only located in the Golgi membrane (golg). Nearly all variants (X1, X2, X3, X4, X5, X6, and X8) where the HSP70 Peptide is located in peroxisomal (pero) and extracellular (Extr), except the X7 variant is not detected. There are three (3) Variants (X1, X2, and X8) were located in the nucleus (nucl). Only two (2) variants (X1, X2) were located in the cytoskeleton (cysk).

Within the plasma membrane (plas) and the endoplasmic reticulum membrane (ER) there are 3 variants, namely variants X3, X5 and X6 contained in the plasma membrane and variants X4, X5 and X6 are in the endoplasmic reticulum membrane, while in Lysosomes (lyso) there are 4 variants namely X3, X4, X5, and X6. Based on the results of this study, data obtained that HSP70 peptides in almost all variants were found in extracellular (Extr) and proximal (pero), except that the X7 variant was not found in either while the organelles that have the HSP70 peptide-type are the Golgi membranes (golg) which are only possessed by the X4 variant (Ghosh et al., 2014).

Table 3: Subcellular localization of HSP70 in chicken *(G. gallus domesticus*)

Variant	Cyto	cyto-nucl	nucl	pero	cysk	extr	Mito	plas	E.R.	lyso	Golg
X1	19	14	7	2	2	1	1	-	-	-	-
X2	18.5	14	6.5	2	2	2	1	-	-	-	-
X3	-	-	-	6	-	2	-	18	-	6	-
X4	-	-	-	2	-	4	-	-	23	2	**1**
X5	-	-	-	4	-	1	-	22	4	1	-
X6	-	-	-	4	-	1	-	23	3	1	-
X7	5	5.5	-	-	-	-	25	-	-	-	-
X8	11	9	3	4	-	3	11	-	-	-	-

Note: Nucl = nucleus; Cyto = cytoplasm; Cyto-nucl = cytoplasm nucleus, Pero = peroxisomal; Cysk = cytoskeleton; Extr = extracellular; Mito = mitochondrial inner membrane; Plas = plasma membrane; E.R. (m) = endoplas reticulum; Lyso = lysosome; gol = golgi membrane; - : not detected

4 CONCLUSIONS

Eight data were obtained about Heat Shock Protein 70 (HSP70) in chickens (*G. gallus domesticus*) from the NCBI database. The target value of the mitochondrial target is diversified from 0.010 to 0.309. This shows that HSP70 in Chicken (*G. gallus domesticus*) plays an essential role in tolerance mechanisms of extreme weather such as high ambient temperatures. HSP70 peptides in almost all variants were found in extracellular (Extr) and peroxisal (pero), except that the X7 variant was not found in either while the organelles that have the HSP70 peptide-type are the Golgi membranes (golg) which are only possessed by the X4 variant.

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