



(RESEARCH ARTICLE)



In silico genomic analysis of heat shock protein 70 in poultry under tropical conditions

Abayomi Jude Agbana *, Adebayo Omowaye Ete, Roseline Ruth Fanwo, Emmanuel Umakwu Simon and Babatunde Aderemi Yusuf

Department of Animal Health and Production Technology, Kogi State Polytechnic, P.M.B 1101, Itakpe, Kogi State, Nigeria.

GSC Biological and Pharmaceutical Sciences, 2024, 26(03), 037–042

Publication history: Received on 16 December 2023; revised on 04 March 2024; accepted on 07 March 2024

Article DOI: <https://doi.org/10.30574/gscbps.2024.26.3.0032>

Abstract

The negative effect of climate change predisposes farm animals under tropical conditions to heat stress, which limit their productivity. This has necessitated the search for livestock with suitable adaptive genotype. Limited records exist for livestock with high genetic adaptability hence, selection for the most suitable breeding stock remain difficult. This study attempt to use genomic characteristics of heat shock protein 70 (HSP70) in common meat type birds as biomarker for selecting breeding stock of high genetic merit for tolerance. In silico expasy translate tool analysis revealed that heat shock protein 70 gene in chicken (*Gallus gallus*) and Japanese quail (*Cortinix japonica*) are on chromosome 30 and contain 2 and 3 exons linked to 67 and 45 introns, respectively while, in turkey (*Meleagris gallopavo*) and guinea fowl (*Numida meleagris*), HSP70 gene is located on chromosome 24 and 28, respectively. They also contain 2 and 4 exons interspersed by 41 and 38 introns, respectively. The variations in chromosomal locations validates differences observed in the adaptation and geographical spread of studied animals. It may also provide new insight into cellular response to heat stress and adaptive mechanism which is necessary in selection. Functional properties and physiological structures resisting thermal stress needs further investigation.

Keywords: Exons; *In silico*; Heat stress; Adaptive; Heat shock protein 70; Poultry

1. Introduction

The contribution of poultry sector to Nigeria economy is noteworthy as it account for 35% of the National gross domestic product (GDP) and 90% of Animal agriculture [1]. The climate is an important factor influencing agricultural productivity and livestock sector, being a major component of Agriculture. Climate change has a wide range effects on animals productivity and environment such as alterations in temperature, precipitation, atmospheric greenhouse gas concentration which ultimately alters earth's ecosystems. It may also reduce both the quality and quantity of pastures and water supplies, intensifying the outbreak of new pests and diseases during drought periods, and reducing the quality and quantity of products (milk, meat), which limit productivity, reproductive efficiency and health leading to severe economic losses in livestock industry [2].

Animals have adapted to adverse environmental conditions by modifying their phenotypic and genotypic features over long periods [3]. Animals exhibit adaptive mechanisms to survive unfavorable climatic conditions [2]. The adaptivity of animals is assessed in terms of their competence to mate in addition to their ability to survive harsh climatic conditions [4]. In terms of the adaptive ability of animals, this has been reported to involve various mechanisms, such as anatomical, physiological, behavioural, morphological, biochemical, cellular, and molecular attributes, which enable animal to survive in a specific environment [5].

Biological markers or biomarkers, which are substances that indicates the biological state of a change in gene expression or state of protein plays a significant role in assessing the stress adaptation mechanism in livestock. Biochemical

* Corresponding author: Abayomi Jude Agbana; ORCID ID-0009-0006-3265-9653,

markers have been used for the identification of animals with high genetic merit for economic traits in cattle and as reference point in breeding to identify, manipulate and to cross-breed for the improvement of genetic potential in livestock species [6]. Thus, the overall improvement in livestock species is aided by the use of molecular markers to a greater extent.

It has been well documented from a broad studies that heat stress has negative effects on animal productivity such as growth, milk production, feed intake, fertility, and health [1, 4]. Recently, increased concerns have been raised over the effects of heat stress, given growing understanding of the influence of global warming on animal production systems. The selection of thermo-resistant animals is an effective way to improve the productivity of animals under high environmental temperature [7]. Identification and exploitation of genotypes having thermo-tolerance in poultry are a major concern in the changing climate scenario which can have a great impact on livestock productivity [5, 7]. However, how and why stress has negative effects on animals at the cellular and molecular levels have remained unclear. Identifying and understanding heat tolerance genes and the proteins they encode could further be effective for reducing the negative impact of heat stress [6] and could be fundamental to identifying and interpreting the genetic components of complicated adaptive traits.

To maintain homeostasis, stress is essential in all living organisms. However, acclimation, acclimatization and adaptation are the mechanisms through which the animals cope with the stressors [8]. Acclimation includes the phenotypic responses produced by the animal to a specific stressor in the environment, whereas acclimatization is the long-term physiological adjustments as a result of continued exposure to multiple stressors [5, 8]. Acclimation is of less relevance compared to acclimatization as it is harder to find an environment with a single variable changing. However, both of these mechanisms are phenotypic responses as it is induced by the environment and the response goes once the stress is removed. It is usually produced to improve the fitness of the animal to the environment. Animals develop specific adaptive mechanisms if the environment stressors are present for a prolonged period [9]. The mechanism of adaptation involves biochemical changes. Biochemical adaptation to thermal stress involves changes in proteins, membrane lipids and metabolic rate [10]. Genetic markers are of prior importance in the revolutionary development in the field of molecular technology because of its viability as the biochemical adaptation can be utilized in livestock breeding programs compared to the behavioural and physiological responses to stress which is of less relevance. Heat shock proteins is an important biomarker produced in the cellular and tissue defense mechanism whose expression is markedly increased during heat shock [8]. Heat shock protein are group of molecular chaperones that avoid the aggregation of non-specific proteins and help cellular proteins to attain their native structure to maintain cellular homeostasis [4]. HSPs are a large protein family, which allow cells to adapt progressively to the changing environment thereby, significantly impacting on thermal adaptation and stress tolerance [8]. The cellular response to heat stress includes the activation of heat shock factors, improved expression of HSPs, increased levels of amino acid and glucose oxidation, reduced fatty acid metabolism and stimulation of immune and endocrine systems through extracellular secretion of HSPs [11]. HSPs however, are ubiquitous in almost prokaryotic and eukaryotic organisms. Their distribution in various cellular locations shows their crucial roles in cellular homeostasis [12]. Additionally, literatures have shown that HSP gene expression provides accurate and precise facts for assessing heat stress and can be considered a novel indicator of Heat stress [11, 13]. HSP genes may be expediently employed as biomarkers for assessing stress response in poultry and the expression is species and breed-specific [10]. Besides, it has been revealed that the differences in transcripts pattern of heat shock protein 70 family and other HSP genes during various seasons may be mainly significant mechanism for better adaptability in Indian zebu cattle [14]. Comparative genomics of heat shock genes and proteins between closely related animal species provide a chance to understand the evolutionary relationship of Heat Shock Proteins and the selective pressures that control the evolution of these genes.

The problem caused by climate change still has a wide range of effect on the productivity of livestock such as heat stress. Animal breeders therefore, are making efforts towards animal's improvement and developing stock of animals that can exhibit adaptive mechanism. Several work have been done on anatomical, physiological, behavioural, morphological and biochemical status, with lesser work done on molecular and cellular attributes [8, 15]. This can enable animals to survive in a specific environment. Identifying and understanding heat tolerance related genes and the protein they encodes could be effective for selecting thermo-tolerance animals and reducing the negative impact of heat stress. The selection of animals based on their genetic adaptability might improve sustainability in livestock systems in order to secure a food supply that can cope with the increasing global population.

Several attempts have been made at the anatomical level, to identify adaptation of animals to heat stress [4, 7, 15]. However, in terms of molecular adaptation, there is scanty of data on molecular mechanisms behind the adaptation of animals. To obtain a clearer picture of this molecular adaptation, key molecules that play a major role in cellular stress resistance that broadly expressed in many cells and tissues need to be examined. This work is a step towards understanding the genetic polymorphism between some species of birds that have undergone various type of

adaptation to heat stress. Specifically, the study seek to use computer simulation to identify heat tolerance-related HSP 70 genes and proteins that they encodes towards predicting thermo-tolerance and adaptability of birds to heat stress from genomic values.

2. Material and methods.

2.1. Description of Experimental Site

The study was conducted at the Department of Animal Health and Production Technology, Kogi State Polytechnic, Itakpe Campus, which is located in the Guinea Savanna of coordinates Latitude 7.6384 °N and Longitude 6.335 °E. The temperature throughout the year ranges from 18.87 °C to 34.4 °C with an average of 26.64 °C and average annual rainfall of 1280 mm [16].

2.2. Animal Selection

To obtain a comprehensive overview of genomic diversity of Heat shock protein 70 (HSP 70) type for birds in the class of *Phanisianide*, the sequence of this heat shock protein type for Japanese quail (*Corturnix japonica*), Chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*), Guinea fowl (*Numida meleagris*) were obtained from the National Centre for Biotechnological Information (NCBI) database via https://www.ncbi.nlm.nih.gov/genome/annotation_euk/all for bioinformatics analysis [17].

2.3. Genomic characteristics

Information on the genome size, median G.C % and median protein for Japanese quail (*Corturnix japonica*), Chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*), and Guinea fowl (*Numida meleagris*) was obtained from the NCBI Database by selecting “Eukaryotic genome annotated” and then “birds”. Genomic size of some birds was also obtained from <https://www.genomesize.com/search.php>

2.4. Genomic locations of heat shock protein gene

From the NCBI Database, the genomic location of HSP70 for each bird type were determined by choosing the gene ID (gene identity) of the protein for each bird to view the genomic and chromosomal position of the gene that encode it.

2.5. Data analysis

The research employed a FASTA format conversion on Minitab to convert and unify protein sequences. Tabular data obtained was analyzed on Microsoft Excel using SPSS package [18].

3. Results

3.1. Genome characteristics of selected poultry birds (*Phanisianide*)

Data published on the NCBI data base, showed that the genome of chicken (*Gallus gallus*) is 1.27 Mb in size, distributed over 39 pairs of somatic chromosomes and two sex chromosome (ZZ:ZW), with a median protein count of about 54.210 and a median GC of 41.87%. The genome of Japanese quail (*Corturnix japonica*) is 1.35 Mb in size, distributed over 78 (2n) pairs of somatic chromosome and two sex chromosomes, with a median protein count of 42.784 and median GC % of 42.73%. Also, the genome of turkey (*Meleagris gallopavo*) is 1.46 Mb in size, organized in 38 somatic chromosomes and two sex chromosomes, with a median protein count of about 41.653 and a median GC of 40.62%. Similarly, the genome of Guinea fowl (*Numidia meleagris*) is 1.26 Mb in size, distributed over 42 somatic chromosomes and two sex chromosomes with a median protein count of about 40.992 and a median GC % of 42.53 % (Table 1).

Table 1 Genomic Characteristics of Poultry birds as obtained from NCBI database

Birds	Genomic size (Mb)	Median G.C (%)	Median protein count
Chicken (<i>Gallus gallus</i>)	1.27	42.808	54.210
Japanese quail (<i>cortunix</i>)	1.35	42.787	42.784
Turkey (<i>Meleagris gallo</i>)	1.46	41.620	41.653
Guinea fowl (<i>Numidia .m</i>)	1.26	40.534	40.992

GC-Guanine-Cytosine

3.2. Genomic analysis of Heat Shock Protein 70 genes in some selected poultry birds.

The result for genomic locations, number of exons and introns were determined by bioinformatics tools in some poultry birds. The bioinformatics tools revealed that the heat shock protein 70 gene in chicken and Japanese quail, is located on chromosome 30 in both species with 67 and 45 introns, respectively. Turkey and Guinea fowl had HSP 70 gene located on chromosome 24 and 28, respectively while, 67 and 38 introns were recorded, respectively.

Chicken, Japanese quail, Turkey and Guinea fowl recorded 12, 3, 2 and 4 exons, respectively in their chromosomes. The ratio of exons to introns were 3.50, 3.77, 4.30 and 3.00, respectively for chicken, Japanese quail, turkey and guinea fowl. The analysis also showed that HSP 70 proteins differ a little in their molecular weight: 60.27, 60.28, 60.27 and 60.30 kDa in chicken, Japanese quail, turkey and guinea fowl, respectively.

Table 2 Genomic Characterization of Heat shock protein 70 of Poultry birds as obtained from NCBI database

Birds	Protein type	Genomic Locus	Gene ID	Protein weight	molecular	Exons	Introns	Exons:introns
Chicken	HSP 70	Chrom 30	GCF0015778	60.27		2.0	67.0	3.50
J. Quail	HSP 70	Chrom 30	BNID100282	60.28		3.0	45.0	3.77
Turkey	HSP 70	Chrom 24	BNID101411	60.27		2.0	41.0	4.30
G. fowl	HSP 70	Chrom 28	GCF1011320	60.30		4.0	38.0	3.00

HSP-Heat shock protein

4. Discussion

4.1. Genome characteristics of selected poultry birds.

The genomic screening of chicken, Japanese quail, turkey and Guinea fowl revealed that poultry differs in their genomic size, GC content and protein molecular weight. Thus, inferred variable characteristics of genes. In Eukaryotes, GC-rich genome is considered a good biomarker for actively transcribed DNA and thermostability [9, 11]. Our computational analysis recorded high values for GC% across studied bird species though, similar and highest values were recorded in chicken and Japanese quail and lowest value in Guinea fowl. The highest GC-rich genome in both chicken and Japanese quail, suggest better thermostability over turkey and guinea fowl. This may explain the wider spread of chicken and Japanese quail across a wider climatic and geographical conditions compared to turkey and Guinea fowl which are predominant in wet and Swampy climatic Zones [5, 7]. Furthermore, it has been reported that thermostability of genes in animals, increases more slowly with increasing GC content than in random sequences [19]. Thus, thermostability of genes therefore, confer better adaptability to environmental conditions and unique thermo-tolerance on chicken and Japanese quail. This corroborate the findings of Sayed *et al* [2] who reported a higher value for GC content in Zebu cattle compared to sheep and least value recorded in Buffalo.

The variations in molecular weights of protein in studied bird species, suggest differences in structure and sequence of amino acids (especially, glutamic acid and aspartic acid) in cellular DNA and protein network. As obtained in this study, Nikbin *et al.* [20] reported changes in nature of SNPs detected in amino acids of Boer goats where purine base changed to pyrimidine base. The changes observed in amino acid sequences can bring about subsequent changes in protein product and function [13] which will consequently improve the ability of animals to better cope, perform and survive under heat stress.

4.2. Genomic analysis of Heat Shock Protein 70 genes in some selected poultry birds.

It has been revealed that the differences in transcript pattern of HSP 70 genes during various seasons may be mainly significant mechanism for better adaptability [7, 19]. Thus, the observed differences and complete conservative nature of HSP 70 in studied birds may explain the unique characteristics of their adaptability and survivability in endemic environments. This corroborates the reports of earlier findings of Onasanya *et al.* [4] that HSP70 was the most polymorphic and genetically diverse among the HSPs sub-family as their abundant nature confer on animal thermal tolerance advantage to wide range of stressful environmental and physio-thermal variations.

Evolution also, might have promoted the acquisition of genes for thermo-tolerance that created genetic adaptations in species [8, 21]. Test of selection of HSP 70 gene in Nigerian cattle population, as determined by codon based Z test

revealed that at 5% level of significance, non-synonymous mutation (dN) is substantially greater than synonymous mutation (dS) within the amino acid sequence evaluated. Thus, HSP70 gene might have evolved through positive selection ($dN > dS$) within population of four breeds of cattle and this is suggestive of change in protein product which is occasioned by SNPs resulting in possibly positive biological effect of fitness of animals in response to thermo-tolerance at HSP 70 gene loci [22].

Abundance of heat shock protein 70 in eukaryotic cells eliminate or limit adverse environmental conditions and stressors by limiting induced cellular unfolding proteins [15, 16]. The variations observed in the HSP 70 profile for studied bird species might suggest differences in thermo-tolerance, as studies have revealed that HSP 70 is an important molecular chaperone for the development of mammalian cells and protection of cells upon exposure to cold and hot stress; which can denature proteins [14]. The presence of heat shock protein 70 in many chromosomes of the studied bird species may further suggested better innate adaptability potential of birds to survivability under hot tropical weather conditions. This is because, the presence of HSP 70 gene in cells protects the proteins involved in DNA repair and recombination [11]. As such, HSP 70 gene plays a protective role in animal's reaction to hyperthermia as well as other stress conditions by providing a balance between synthesis and degradation of cellular proteins [2, 8]. It also assist in the process of folding, transporting and assembling proteins in the cytoplasm, mitochondria and endoplasmic reticulum [13].

It has been proven from literatures that Japanese quail and guinea fowl have unique thermo-tolerance traits in comparison to other *phanisidiae* [11]. They are known to be hardy and have innate ability to survive in arid areas and can adapt to a high temperature and harsh tropical climatic conditions.

Generally, majority of HSP genes have the absence of non-coding regions or introns. Evgen ev *et al.* [6] describes the molecular mechanism underpinning this as RNA splicing that is affected by various stresses. The transcripts of intron less HSP genes can easily be transported from the nuclear membrane to the cytoplasm without splicing, resulting in selection for intron less genes over the course of evolution [21]. However, some exceptions to this observation has been reported by Le Hir *et al.* [23], who in His study observed the presence of introns in HSP 70 genes in Zebu cattle. These introns allowed greater mRNA accumulation in DNA than their absence. The presence of introns in studied poultry bird species may therefore, reflect self-protection mechanism present in birds under adverse environmental conditions.

5. Conclusion

Changes in Cellular response of animals is one of the primary pathway by which livestock tries to cope up with heat stress challenges. This study identified and characterized heat shock protein type 70 (HSP 70) in Poultry birds. There exist a variation in expression of HSP 70 proteins observed in the most studied birds. The presence or absence of introns in HSP genes, and the GC content in their genomes may correlate with and explain the distribution of some birds in different climatic and geographical conditions or provides an advantage in response to selective pressure imposed by certain environmental conditions. To validate our assertions from the computational analysis, there is the need for more in-depth exploration of the mechanisms of molecular adaptation in these animals by employing whole genomic wide association study in order to enhance genetic breeding to counteract the negative influences of elevated temperature that characterized tropical environmental conditions.

Compliance with ethical standards

Disclosure of conflict of interest

Authors declared no competing interests.

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