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Genetic Polymorphism in the Promoter and 5´ UTR of HSP-70 Gene in Three Strains of Indigenous Tswana Chickens and Commercial Broilers

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ABSTRACT

The current study was conducted to investigate sequence polymorphisms in the promoter, 5´untranslated regions (UTR), and partial exon regions of chicken HSP-70 gene in the normal (n = 24), naked neck (n = 22), and dwarf (n = 12) strains of indigenous Tswana chickens relative to the commercial broiler chickens (n = 20). Genomic DNA extracted from the whole blood of the three strains of indigenous Tswana chickens and the commercial broilers were amplified using PCR and sequenced. The PCR amplicons comprised a 210 bp promoter region, a 112 bp 5´UTR, and a 463 bp partial exon of the chicken HSP-70 gene. Multiple sequence alignments of the partial sequences of chicken HSP-70 gene in indigenous Tswana chickens and the commercial broilers revealed no polymorphisms in the promoter region, two SNPs in the 5´UTR (A303G and G309A) and another two SNPs (G427 and A628G) in the partial exon sequence of chicken HSP-70 gene. The SNP G427A was unique to the normal strain and the other three SNPs were common to all the four chicken strains studied. The identified four SNPs linked up in individual chickens resulted in a total of seven different haplotypes in the studied four chicken populations. A total of seven different haplotypes were found in indigenous Tswana chickens and only two haplotypes were found in the commercial broilers. More nucleotide (4SNPs vs 3 SNPs) and more haplotype diversity (7 haplotypes vs 2) were thus found in indigenous Tswana chickens, compared to the commercial broilers in the partial sequence of HSP-70 gene.

Keywords: HSP-70 gene, Indigenous Tswana chicken, Polymorphism, Sequencing, SNPs

INTRODUCTION

Chickens do not have sweat glands, and thus, body heat dissipation to the environment is not easy and in most cases, chickens are prone to heat stress (Tamzil, 2014; Aryani et al., 2019; Afsal et al., 2021). When the ambient temperature goes beyond the comfort zone of >28°C, then heat stress can crop up anytime in both egg-laying and broiler chickens (Aryani et al., 2019). In chickens, heat stress causes high temperatures that can negatively affect growth and egg quality (Oberheitmann, 2013), and cause decreased feed intake, eggshell quality, egg production, body weight gains, and even death in domestic chickens during summertime (Cahaner et al., 2008; Melesse et al., 2011; Aryani et al., 2019). Cellular mechanisms used to alleviate heat stress in animals may include changes in the functions of cells, including transcription, translation, and protein synthesis (Negri et al., 2013). One of the adaptive mechanisms to increased environmental temperatures in chickens includes the production of heat shock proteins (HSPs) which play an important role in the protection and repair of cells and tissues exposed to high environmental temperatures (Chen et al., 2016). The HSP-70 is one of the heat shock proteins produced by animals in response to extreme heat conditions and it plays an important role in heat tolerance (Zuiderweg et al., 2013).

In chickens, the HSP-70 protein is encoded by the HSP-70 gene which is found in chicken autosome 5 and comprises a single exon, the upstream Promoter and 5` UTR and downstream 3` UTR (Morimoto et al., 1986). According to GenBank: AY143693.1, the entire chicken HSP-70 gene comprises 2594 bp and encodes a protein of 653 amino acids (Junprung et al., 2019). The promoter comprises 210 bp, the 5´UTR comprises 112 bp and the

3´UTR comprises 309 bp. According to Najafi et al. (2018), a total of 35 SNPs have been found in chicken HSP-70 with 25 SNPs occurring in the protein-coding region and the rest in the regulatory regions (Promoter, 5`UTR, and 3`UTR). Most of the studies on chicken HSP-70 gene polymorphism concentrated on the exon or protein-coding region because of its direct effect on the amino acids sequence of the resulting protein (alleles of a gene) (Gan et al., 2015; Najafi et al., 2018). Only a few studies investigated polymorphisms in regulatory regions of the chicken HSP-70 gene. Polymorphisms in the regulatory region of the gene are, however, very important because they influence gene expression (Öner et al., 2017). According to Silver and Noble (2011), pre-transcriptional activation of HSP-70 gene has been thoroughly reviewed while its downstream regulation by 5´UTR and 3´UTR has received less attention. The 5´UTR regions of genes are useful in controlling mRNA expression levels and stability (Basiricó et al., 2011; Sodhi et al., 2013; Öner et al., 2017). The control of translation initiation is mainly under the responsibility of 5′UTRs and gene expression can therefore be influenced by SNPs in the regulatory regions (Araujo et al., 2012; Haimov et al., 2015). Indigenous chickens are known to be more thermotolerant to heat stress compared to exotic chickens (Duangjinda et al., 2017). It is, therefore, hypothesized that the differences in thermotolerance between indigenous Tswana chickens and exotic chickens could be due to sequence variations in the promoter and 5´UTR regions of the chicken HSP-70 gene.

The objectives of the current study were to investigate polymorphism in the promoter and 5` UTR regions of chicken HSP-70 gene in normal, naked neck and dwarf strains of Tswana chickens, compared to the commercial broiler chickens.

MATERIALS AND METHODS

Ethical approval

The keeping of the research birds was approved by the Animal Research Ethics Committee of Botswana University of Agriculture and Natural Resources (Approval No.2020-13), which conforms to the guidelines and the use of research animals.

Experimental animals

Blood samples were collected from a total of 24 normal, 22 naked necks, and 12 dwarf strains of indigenous Tswana chickens. The sampling locations of different strains of Tswana chickens (Table 1). Additional 20 blood samples were collected from Ross broiler chickens at Notwane farm located in Gaborone. The blood samples were collected from the medial metatarsal vein located on the leg of a chicken better suited for puncture using a 23-gauge, 1-in needle. The alternative site for blood collection was the brachial vein on the wings. All blood samples were collected into vacutainer tubes containing EDTA and kept on ice in the field and later stored in the freezer at -20ºC until DNA extraction.

Table 1. Locations of indigenous Tswana chickens and the number of samples per location in Kweneng and Southern districts of Botswana

Sampling location	District	Number of samples per location		
		Normal	Naked neck	Dwarf
Sojwe	Kweneng	4	3	5
Kaudwane	Kweneng	1	1	0
Maboane	Kweneng	2	2	2
Malwelwe	Kweneng	3	3	2
Kweneng	Kweneng	2	2	1
Keng	Southern	3	1	0
Seherelela	Southern	3	2	1
Thankane	Southern	2	4	0
Lerolwane	Southern	2	3	0
Magotshwane	Southern	2	1	1
Total		24	22	12

DNA isolation

Total genomic DNA was extracted from avian blood using QIAGEN DNeasy tissue and blood Kit, California, USA, following the manufacturer's protocol (Qiagen, 2003). Briefly, 200 µl of digestion buffer and 20 µl of proteinase K were added to 5 µl of whole blood. The mixture was vortexed for 15 minutes and then incubated in a water bath at 50°C for 10 minutes. Then, 200 µl of cold 100% ethanol was added to each sample and mixed by vortexing for 15 seconds. The resulting mixture was transferred into a spin column placed inside a collecting tube and centrifuged at 8000 × g for 1 minute. Tubes containing the flow-through were discarded and the spin column was then transferred to new collecting tubes. In the next step, 500 µl of the first washing buffer was added to each spin column and centrifuged at 1000 × g for 1 minute. Again, the collection tubes containing flow-through were discarded and the spin columns were transferred to new collection tubes. Afterward, 500 µl of second washing buffer were added to the spin column and then centrifuged at 14000 × g for 3 minutes. Following centrifugation, spin columns were transferred to clean microcentrifuge tubes. Then, 150 µl of elution buffer was added to the spin columns and incubated at room temperature for 5 minutes. Following the incubation, the microcentrifuge tubes holding the spin columns were centrifuged at 8000 × g for 1 minute to elute the DNA.

DNA quantification

The quantity and quality of extracted DNA were established using a NanoDrop 2000/2000c Spectrophotometer. DNA quality was established by measuring absorbance at 260/280.

PCR amplification and DNA Sequencing

PCR amplifications were performed with a programmable thermal cycler, PTC-100^{TM} (MJ-Research, Inc., Watertown, MA, USA) in a final reaction volume of 50 ng of genomic DNA, 0.2 mM dNTPs, 1.5 mM, $MgCl_2$, 0.6 µM of each primer and 1.5 units *Taq* DNA polymerase. After an initial denaturation step of 94°C for 3 minutes, the reaction mixture was subjected to 34 cycles at 94°C for 45 seconds, 54°C for 30 seconds, and 72°C for 1 minute and 30 seconds, plus a final extension step of 72°C for 10 minutes. Amplification was confirmed by running the PCR products on 2 % agarose gels and visualizing them under ultraviolet rays (Vallone et al., 2008). PCR products were then shipped to Inqaba Biotec-Africa`s Genomics Company, Pretoria, South Africa for sequencing. At Inqaba Biotec PCR amplicons were purified using The PureLink PCR purification kit of Thermo Fisher Scientific, South Africa following the manufacturer's protocol (Vallone et al., 2008). DNA sequences were generated using ABI V3.1 Big dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and the thermocycler GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA). Sequences were generated with both forward and reverse primers (Table 2) and read on ABI3500XL DNA analyzer (Applied Biosystems, Foster City, USA).

Table 2. Primers used on PCR amplification of the chicken 5`UTR of HSP-70 gene

Primer's name	Sequence	Position on the reference sequence
HSP-70-F1	5' GAGTGGCGCAGCGTAGAAAG '3	18
HSP-70-F2	5' GATTGGTCCTTAGCGTTCTGGC '3	208
HSP-70-R1	5' TTCCTCTTGGTCAGTCAGCC '3	382
HSP-70-R3b	5' CTGGGAGTCGTTGAAGTAAGCG '3	856

The letters F and R in primer names refer to their orientation (forward and reverse, respectively). Source: Mazzi et al. (2003).

Sequence analysis

Chromatographs developed from sequencing were processed using ApE, A Plasmid Editor version 2.0.53, (Jorgensen.biology.utah.edu) to verify the sequences and SNPs in the amplified regions of the chicken HSP-70 gene. Furthermore, samples and reference sequences were aligned using the Clustal W multiple sequence alignment program (Simmonds, 2012) to establish the presence of SNPs in the amplified regions (Brocchieri et al., 2008). Nucleotide substitutions at the identified SNP loci were identified from chromatographs generated by the program Chromas Lite (https://technelysium.com.au/wp/). Translation of DNA sequences into protein sequences was done online using the Nucleotide Amino Acid Derived Visualization (NADV) (Abascal et al., 2010).

Statistical analysis

Potential functional motifs in the sequenced promoter region of chicken HSP-70 gene in the three strains of indigenous Tswana chicken and commercial broiler were identified using Proscan software (http.//www-bimass.cit.nih.gov/molbio/proscan/). Allele and genotype frequencies at the identified SNP loci were calculated using Gene Pop program (v 1.2) (Raymond and Rousset, 1995; Lachance, 2008). Haplotypes in the partial sequence of the chicken HSP-70 gene were determined by clustal X (v 1.81) and MEGA (v 4.0) (Tamura et al., 2007). Observed heterozygosity (H_o) and expected heterozygosity (H_e) were estimated using FSTAT (v.2.9.3.2) (Goudet, 2002).

RESULTS AND DISCUSSION

Amplified regions of chicken HSP-70 gene

The two primer pairs used in the sequencing of the chicken HSP-70 gene in the study resulted in a 200 bp promoter region, 112 bp 5´ UTR, and 453 bp fragment of the chickens' HSP-70 gene coding region. The study thus sequenced a 775 bp fragment of the chickens' HSP-70 gene in different strains of indigenous Tswana chickens and the commercial broiler chickens.

Functional motifs in the partial sequence of chicken HSP-70 gene

Functional motifs found in the regulatory regions (Promoter and 5´ UTR) of chicken HSP-70 gene in indigenous Tswana chickens and commercial broiler chickens included CAAT box, specificity protein 1 (SP1), heat shock element (HSE2), and heat shock element 1(HSE1) and a TATA box (Figure 1). All these motifs were previously reported by Aryani et al. (2019) in four different strains of indigenous Indonesian chickens. According to Morimoto et al. (1986) the chicken HSP-70 gene shares 73% and 80% sequence homology with *Drosophila* and human HSP-70 genes respectively,

including common features such as TATA box, CAAT box, SP1, HSE1, and HSE2 in the promoter region. Heat shock elements in the promoter region are a common feature of both mammalian and plant HSP-70 gene. According to Zhao et al. (2020) sequence variations in heat shock elements, the position of the heat shock elements within the promoter and the molecular architecture of the heat shock elements may be responsible for the varying affinity in the Heat shock factors-Heat shock elements interaction which ultimately influences transcription rate of HSP-70 gene and consequently the magnitude of the heat shock response.

SNPs in the partial sequence of chicken HSP-70 gene

SNPs found in the partial sequence of chicken HSP-70 gene in normal, naked neck and dwarf strains of indigenous Tswana chickens (Table 3) and depicted in Figures 1-5. There were no SNPs in the promoter region of chicken HSP-70 gene in normal, naked neck and dwarf strains of indigenous Tswana chickens as well as in the commercial broiler chickens. According to (Öner et al., 2017), SNPs in the promoter region of cattle may have a negative effect on pregnancy, calving rate, spermatogenesis, and embryonic mortality. The monomorphism of the promoter region of the HSP-70 gene in different strains of indigenous Tswana chickens and the commercial broiler may, therefore, be meant to guard against the afore-mentioned negative effects of mutations in the Promoter of chicken HSP-70 gene. Two SNPs (A303G, G309A) were found in the 5´UTR and while the other two SNPs (G427A, A628G) were found in the partial exon sequence of the chicken HSP-70 gene. Gan et al. (2015) found 6 SNPs and 24 SNPS in the 5´UTR and coding region of chicken HSP-70 gene respectively, in Chinese indigenous chickens. More variation has been reported in the 5´UTR of bovine HSP-70 gene with a total of 43 SNPs (Öner et al., 2017). The A303G SNP in the 5´UTR was found in the three strains of indigenous Tswana chickens and the commercial broiler chickens while the G309A SNP was found only in normal and dwarf strains of Tswana chickens. An adenine nucleotide deletion at the 63rd nucleotide (A63 del mutation) was only found in a single chicken of the dwarf strain within what appears like a CAAT box,

According to (Öner et al., 2017) untranslated regions of genes play an important role in terms of gene expression levels and mRNA stability. The 5´UTR controls expression levels of the transcript (Silver and Noble, 2011; Öner et al., 2017), and variations or mutations in the 5´UTR may therefore directly influence the HSP-70 protein expression levels which may result in phenotypic differences in performance. SNP G427A was unique to the normal strain of indigenous Tswana chickens and SNP A628G was found in the three strains of indigenous Tswana chickens and the commercial broiler chickens. More SNPs were thus found in the partial sequence of chicken HSP-70 in indigenous Tswana chickens than in commercial broiler chickens which is consistent with (Öner et al., 2017) who reported more variability in 5´UTR and 3´UTR regions of bovine HSP-70 gene among native Turkish cattle breeds than the exotic Holstein breeds.

All the mutations found in the current study were transitional exchanges and the two SNPs in the coding region were silent (conservative) mutations that do not result in amino acids substitutions in the resulting protein. More transitional mutations (A/G or T/C) and lack of transversional mutations (A/C or T/G) in the chicken HSP-70 gene are consistent with those reported by Vignal et al. (2002) indicating more transitional mutations over transversion mutations in the study of human and mammalian genomes. According to Lamolle et al. (2006), silent mutations in the coding regions of most genes introduce genetic diversity while maintaining protein integrity and functionality.

Table 3. SNPs and the locations in HSP-70 gene sequence in three strains of indigenous Tswana chickens and commercial broiler chickens

Strain	Nucleotide position**	Type	Change	Amino acid identity
Normal, Naked neck, Dwarf and Broiler	5´UTR, 303	Transition	A → g	
Normal and Dwarf	5´UTR, 309	Transition	g → a	
Normal	Exon, 427	Transition	g → a	Same, gt**g** → gt**a** (valine → valine)
Normal, Naked neck, Dwarf and Broiler	Exon, 628	Transition	a → g	Same, tc**a** → tc**g** (serine → serine)

*Altered nucleotides and amino acids are shown in bold. **Nucleotides position is based on GenBank No. AY143693.1

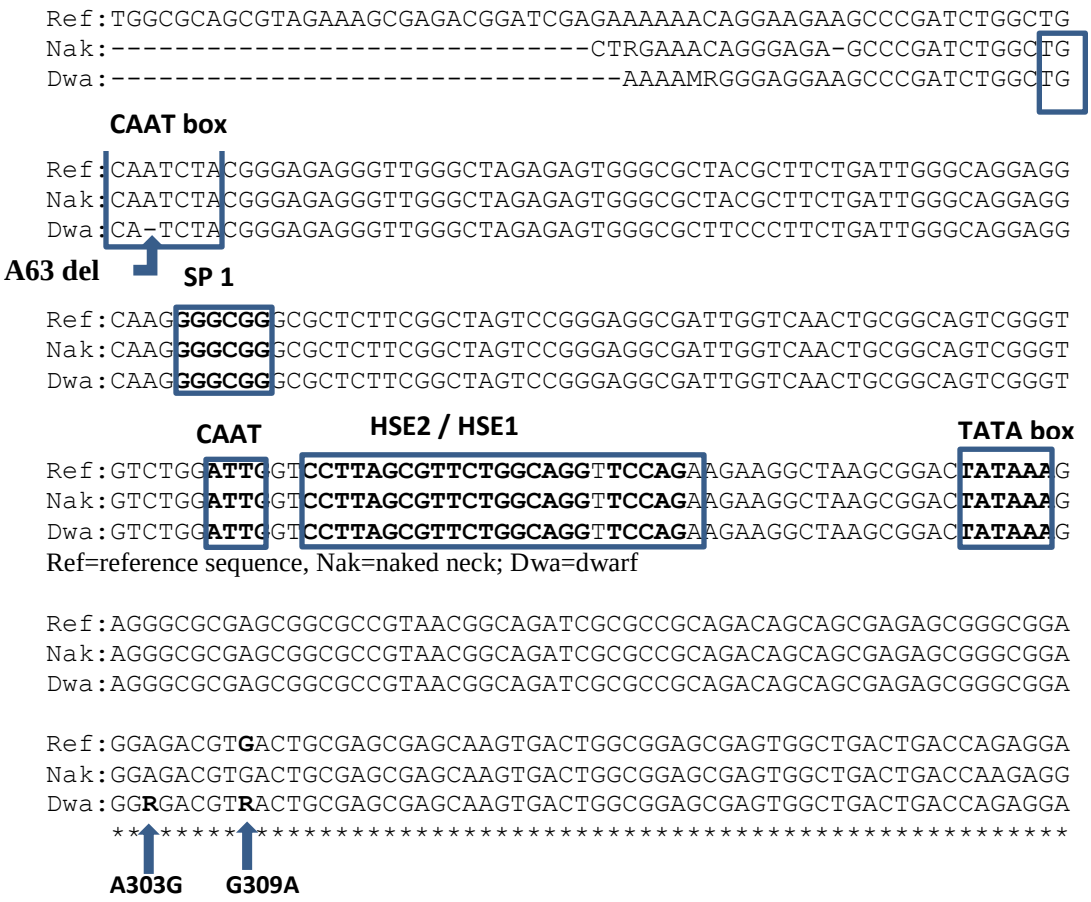


Figure 1. Functional motifs and SNP positions in the Promoter and 5´UTR of indigenous Tswana chicken HSP-70 gene

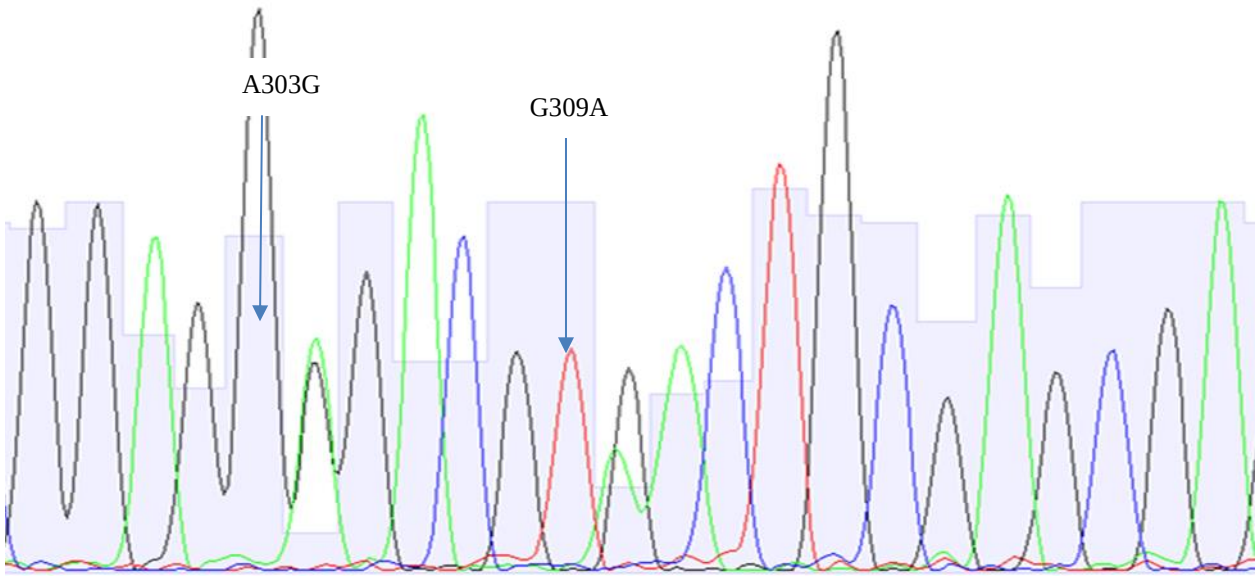
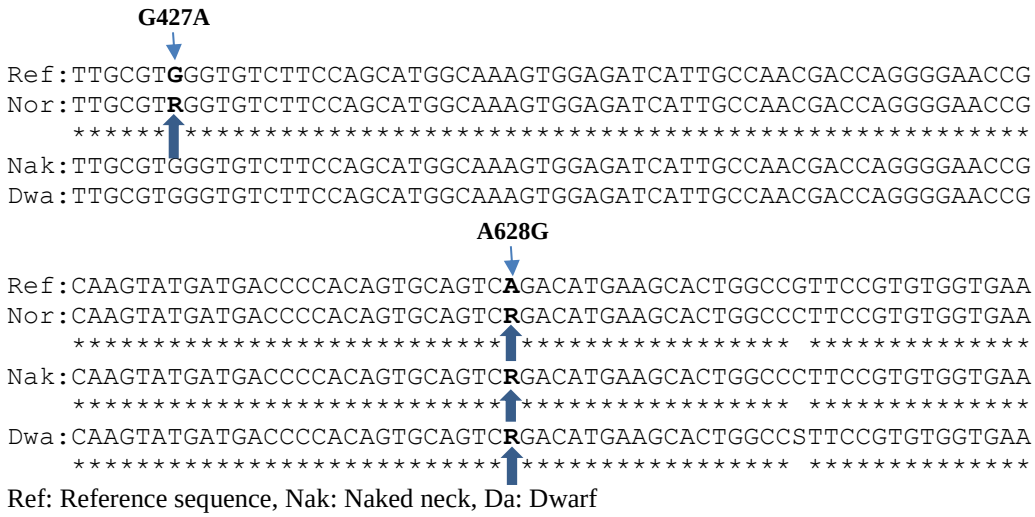


Figure 2. Sequence chromatograph showing SNPs in the 5´UTR of indigenous Tswana chicken HSP-70 gene



Ref: Reference sequence, Nak: Naked neck, Da: Dwarf

Figure 3. SNP positions in the Partial exon sequence of indigenous Tswana chicken HSP-70 gene

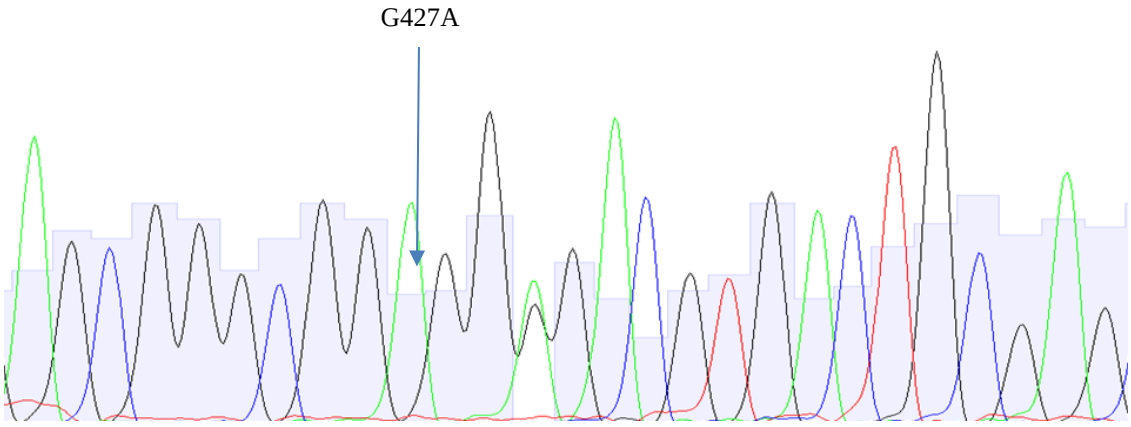


Figure 4. Sequence chromatograph showing the SNP in the partial exon sequence of indigenous Tswana chicken HSP-70 gene

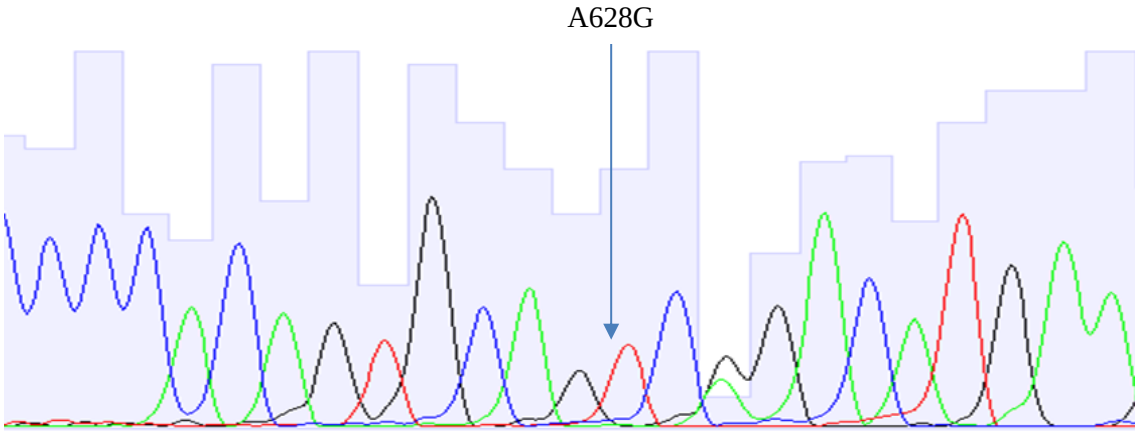


Figure 5. Sequence chromatograph showing the SNP in the partial exon sequence of indigenous Tswana chicken HSP-70 gene

Allele and genotype frequencies at the 5´UTR of chicken HSP70 gene

Allele and genotype frequencies of the identified SNPs at 5´UTR of the chicken HSP-70 gene in different strains of Tswana chickens and the commercial broilers (Table 4). The A- allele at A303G locus was the most frequent in the normal, naked neck, and dwarf strains of indigenous Tswana chickens and the commercial broiler chickens. The G-allele was the least frequent at A303G locus in all the four strains of chickens under the study suggesting that the A-allele was the wild type. At the G309 locus, the G-allele occurred at the highest frequency in all the four strains of the investigated chickens (Table 4). The A-allele at G309A locus occurred at low frequencies in the normal and dwarf strains of indigenous Tswana chickens and was completely absent in the naked neck strain of indigenous Tswana chickens and the commercial broiler chickens suggesting that the A-allele is likely a new variant resulting from very recent mutation.

Allele and genotype frequencies of the identified SNPs in the partial sequence of the coding region of chicken HSP-70 gene in different strains of Tswana chickens and the commercial broiler (Table 4). The A-allele at G427A locus was the only one found in the normal strain of indigenous Tswana chickens and was completely absent in naked neck, dwarf, and commercial broiler chickens. The frequency of G-allele was higher than that of the A-allele at A628G in the normal strain of indigenous Tswana chickens. The A-allele occurred at a higher frequency than the G-allele in normal and dwarf strains of indigenous Tswana of chickens. The A-and G-alleles at A628G locus occurred at equal frequencies in commercial Ross broiler and the A-allele was completely absent in the naked neck strain of indigenous Tswana chickens. The naked neck strain was monomorphic for the G- allele at A628G locus resulting in 100% frequency of the G-allele in the naked neck population of indigenous Tswana chickens.

Table 4. Genotype and allele frequencies across the strains of indigenous Tswana and commercial broiler chickens

		Allele frequency		Genotype frequencies				
Strains	**Loci**	**G**	**A**	**GG**	**GA**	**AA**	**H_o**	**H_e**
Normal	A303G	0.42	0.58	2 (0.08)	16 (0.67)	6 (0.25)	0.67	0.49
	G309A	0.96	0.04	22 (0.92)	2 (0.08)	0	0.08	0.08
	G427A	0.87	0.13	20 (0.83)	2 (0.08)	2 (0.08)	0.08	0.23
	A628G	0.37	0.63	0	18(0.75)	6 (0.25)	0.75	0.47
Naked neck	A303G	0.23	0.77	0	10 (0.46)	12 (0.55)	0.46	0.35
	G309A	1.00	0	22 (1.00)	0	0	0	0.0
	A628G	1.00	0	22(1.00)	0	0	0	0.0
Dwarf	A303G	0.14	0.86	0	4 (0.29)	10 (0.71)	0.29	0.24
	G309A	0.93	0.07	12 (0.86)	2 (0.14)	0	0.14	0.13
	A628G	0.43	0.57	0	12 (0.86)	2 (0.14)	0.86	0.49
Broiler	A303G	0.05	0.95	0	2 (0.10)	18 (0.90)	0.10	0.10
	G309A	1.00	0	20 (1.00)	0	0	0	0.0
	A628G	0.50	0.50	0	20 (1.00)	0	0	0.5

Genotype frequencies at A303G and G309A loci in the 5´UTR of the chicken HSP-70 in different strains of indigenous Tswana and commercial broiler chickens (Table 4). The heterozygous GA genotype at the A303G locus of chicken HSP-70 gene was the most frequent (0.67) followed by homozygous AA genotype, and lastly the GG genotype in the normal strain of indigenous Tswana chickens. Homozygous AA genotype at A303G locus was, however, the most frequent followed by heterozygous GA in the naked neck and dwarf strains of indigenous Tswana chickens and the commercial broilers. The homozygous GG genotype at A303G locus was completely absent in sampled population of naked neck and dwarf strains of indigenous Tswana chickens and commercial broilers. The GG genotype at G309A locus was the most frequent, followed by the GA heterozygotes and lack of AA homozygotes in the sampled population of the normal strain of indigenous chickens. The naked neck strain of indigenous Tswana chickens and the commercial broilers were monomorphic at G309A locus resulting in 100% homozygous GG genotype at that locus. The GG genotype at G309A locus occurred at the highest frequency, followed by GA heterozygotes and lack of homozygous AA genotype in the dwarf strain of indigenous Tswana chickens. The small number of individuals of different strains of indigenous Tswana chickens and the commercial broilers sequenced in the current study might have contributed to absenteeism of some alleles and genotypes (Kgwatalala et al., 2012). The authors observed that selection (both artificial and natural)

also has the potential to change both allele and genotype frequencies in the population (Buffalo and Coop, 2020).

The homozygous GG genotype at the G427A locus of chicken HSP-70 was the most frequent (0.83) and only found in the normal strain of indigenous Tswana chickens. Homozygous AA and heterozygous GA genotypes at G427A locus in the normal strain of indigenous chickens occurred at a similar frequency of 0.08. The GG genotype at A628G locus was the only genotype in the naked neck strain of indigenous Tswana chicken and was completely absent in the dwarf strains of Tswana chickens and the commercial broiler chicken. The heterozygous GA genotype at A628G locus occurred at a relatively higher frequency than the homozygous AA genotype in the dwarf strain of indigenous Tswana chicken and the commercial Ross broiler. All commercial broiler chickens were in fact heterozygous GA at A628G locus.

Genetic diversity measures (observed heterozygosity [H_o] and expected heterozygosity [H_e]) at different SNP loci are presented in Table 4. The highest genetic diversity (measured by H_o) was found at the A628G locus in the normal and dwarf strains at A303G locus in the normal strain of indigenous Tswana chicken. Moderate genetic diversity was only found at A303G locus in the naked neck strain of indigenous Tswana chicken. Low genetic diversity was found at G309A locus in the normal and dwarf strains of Tswana chickens and at G427A locus and at A628G locus in the normal and dwarf strains of Tswana chickens, respectively. There was no genetic diversity at G309A and A628G loci in the naked neck strain of Tswana chicken and commercial broiler. H_o was generally higher than H_e at A303G locus all the four populations under study at A628G in the normal strain, and at G309A and A628G loci in the dwarf strain indicating an excess of heterozygous individuals in the general population and possibly lack of inbreeding at those loci.

Haplotypes found in the partial sequence of chicken HSP70 gene

The SNPs reported in Table 3 linked up in individual chickens to produce haplotypes in the partial sequence of the HSP-70 gene shown in Table 5. The haplotypes are characterized by nucleotides at SNP loci 303, 309, 427, and 628, respectively, with SNP loci numbering according to GenBank: AY143693.1. Seven unique haplotypes were found in the four-chicken population under the study. The H1 haplotype was the most frequent and common to naked neck, normal and dwarf strains of Tswana chickens and the commercial broilers. The H2 haplotype was found in the normal, naked neck and dwarf strains of Tswana chickens and was completely absent in the commercial broilers. The H3, H4, and H5 haplotypes were unique to the normal strain of indigenous Tswana chickens and the H6 haplotype was found only in the naked neck and dwarf strains of indigenous Tswana chickens. The H7 haplotype was found only in the dwarf strain of indigenous Tswana chickens. More haplotype diversity at the HSP-70 locus was thus found in indigenous Tswana chickens, compared to the commercial broiler chickens. This is not surprising as indigenous Tswana chickens have not been subjected to any form of artificial selection while the commercial broilers were subjected to intense selection during development, hence the low genetic diversity in commercial broiler chickens. The high diversity at the HSP-70 locus in indigenous Tswana chickens might also explain their heat tolerance compared to the commercial broilers.

Table 5. Haplotypes and their frequencies (5´UTR and coding region of chicken HSP-70 gene) of indigenous Tswana chicken strains and commercial broiler chickens

Strains	Nucleotide's combinations	Haplotypes	Number	Frequencies
Normal	$A_{303}G_{309}G_{427}A_{628}$	H1	28	0.58
	$G_{303}G_{309}G_{427}G_{628}$	H2	14	0.29
	$G_{303}G_{309}A_{427}G_{628}$	H3	2	0.04
	$G_{303}G_{309}A_{427}A_{628}$	H4	2	0.04
	$G_{303}A_{309}A_{427}G_{628}$	H5	2	0.04
Naked neck	$A_{303}G_{309}G_{427}A_{628}$	H1	24	0.55
	$A_{303}G_{309}G_{427}G_{628}$	H6	10	0.23
	$G_{303}G_{309}G_{427}G_{628}$	H2	10	0.23
Dwarf	$A_{303}G_{309}G_{427}A_{628}$	H1	14	0.50
	$A_{303}G_{309}G_{427}G_{628}$	H6	10	0.36
	$G_{303}G_{309}G_{427}G_{628}$	H2	2	0.07
	$G_{303}A_{309}G_{427}G_{628}$	H7	2	0.07
Broiler	$A_{303}G_{309}G_{427}A_{628}$	H1	20	0.50
	$A_{303}G_{309}G_{427}G_{628}$	H6	20	0.50

CONCLUSION

There were no SNPs in the promoter region of the chicken HSP-70 gene. Two SNPs (A303G and G309A) were found in the 5´UTR (A303G and G309A) and another two SNPs (G427 and A628G) were found in the partial exon sequence of the chicken HSP-70 gene. The SNP (G427A) was unique to the normal strain of indigenous Tswana chicken and the other three SNPs were common to all the four chicken strains studied. The identified four SNPs linked up in individual chickens led to a total of seven different haplotypes in the studied four chicken populations. A total of seven different haplotypes were found in indigenous Tswana chickens and only two haplotypes were found in the commercial broilers.

DECLARATION

Acknowledgments

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions

James Buttie Machete collected samples, designed, and performed the experimental works of this study. James Buttie Machete, Patrick Monametsi Kgwatalala, and Goitseone Malambane l performed the analysis, acquisition, and interpretation of data. James Buttie Machete drafted the manuscript. Patrick Monametsi Kgwatalal, John Cassius Moreki, and Shalaulani James Nsoso revised, edited the manuscript, and approved the final manuscript.

Ethical considerations

All authors approved the final draft of the manuscript for publication. Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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