Characterization of *HSP70* and its expression in tissue: correlation with physiological and immune indices in goose (*Anser cygnoides*) serum

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ABSTRACT. We cloned the goose heat shock protein 70 gene (*HSP70*), to determine its sequence variation and elucidate its mRNA expression. We designed primers to amplify the entire goose *HSP70* sequence. We used 10 commercial Wuzong goslings in a heat-stress experiment. We collected tissue samples for RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR). We analyzed the variation in expression of goose *HSP70* before and after heat stress. We constructed a DNA pool from six different species, for single nucleotide polymorphism (SNP) screening. We detected 18 SNPs and selected three of these SNPs for correlation analysis with biological and immune traits in 200 Wuzong geese. We showed that T+237C was significantly correlated with the serum corticosterone level, whereas T+1122C was significantly correlated with the heterophil to

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lymphocyte ratio. Goose *HSP70* contained no introns. The results of qRT-PCR analysis revealed significant gender differences in the expression of goose *HSP70* at 40°C but not at 25°C; moreover, in general, expression was significantly higher at 40°C than at 25°C. With the exception of the leg muscle and cerebellum, *HSP70* expression was significantly higher in male geese than in female geese. Our results indicate that goose *HSP70* plays an important role in response to severe heat stress.

Key words: Goose; *HSP70* gene; Polymorphism; Heat stress

INTRODUCTION

Environmental temperature has important effects on the performance of poultry (Chen et al., 2004). Heat-shock response is caused by high temperature and causes cryptorrhea, loss of appetite, slow growth, reduced immunity, and disease resistance, thereby leading to increased morbidity and mortality (Mahmoud et al., 1996; Christensen et al., 2000; Zerjal et al., 2013). Heat shock protein (HSP) is induced by high environmental temperature and is important for the maintenance of a stable internal environment for intracellular proteins (Lindquist and Craig, 1988). HSP assists in protein transshipment and damage repair under heat stress, thereby maintaining the integrity of cells (Hendrick and Hartl, 1993). HSPs have been extensively investigated; however, most studies have focused on mammals and chickens, and data regarding other poultry species are lacking. At high temperatures, heat resistance and autoregulation are higher in geese than in mammals (Ajloo et al., 2002). The heat shock protein 70 gene (*HSP70*) is the most widely studied member of the HSP family. Beckham et al. (2008) reported that lack of *HSP70* led to a marked decrease in the heat resistance of cells. Miyabara et al. (2012) showed that in rats suffering from disuse atrophy of skeletal muscle, overexpression of *HSP70* contributed to recovery of the structure and function of skeletal muscle. A few studies have investigated correlations between polymorphism and *HSP70* function in mammals and chickens. Rosenkrans et al. (2010) found that the promoter region of bovine *HSP70* is polymorphic and it may be associated with fertility. Zhen et al. (2006) demonstrated that different genotypes at polymorphism sites (C+276G and A+258G) were correlated with *HSP70* mRNA expression in the liver and leg muscle, and that mRNA expression of heterozygous *HSP70* was significantly higher than that of other genotypes. However, to the best of our knowledge, no previous structural and functional studies of goose *HSP70* have been conducted. In the present study, we investigated the structure of goose *HSP70* and examined its molecular characteristics.

MATERIAL AND METHODS

Sample collection

We obtained 20 commercial goslings (10 male and 10 female) of Wuzong geese (WZG) from Zhiteqi Goose Industry Co. Ltd., Yangjiang City, Guangdong, China. The goslings were raised to 7 days according to Martin et al. (1998). All the goslings were transferred to two manual climatic boxes for 30 min. Next, 10 goslings (5 male and 5 female) were subjected to 1 h of continuous heat stress at 40°C and >70% humidity (treatment group). The remaining 10 goslings (5 male and 5 female) were maintained at 25°C (control group). At the end of the experiment, the goslings

were killed by cervical dislocation. The liver, kidneys, ovaries, testes, spleen, pituitarium, muscular stomach, glandular stomach, lung, uropygial gland, pectoral muscle, leg muscle, hypothalamus, cerebrum, cerebellum, and heart were collected and used in *HSP70* tissue differential expression analysis. After acquisition, samples were immediately stored in liquid nitrogen. All procedures were conducted according to protocols approved by the Committee for the Care and Use of Experimental Animals at South China Agricultural University, Guangzhou, Guangdong, China.

Measurement of serum corticosterone level and heterophil to lymphocyte ratio

We obtained 200 commercial 30-week-old WZG individuals from Zhiteqi Goose Industry Co. Ltd., Yangjiang City, Guangdong, China. We collected blood samples (400 µL) from the wing veins of these geese. We randomly repackaged the obtained blood samples into four different blood collection tubes. We subsequently used the plasma samples for genomic DNA isolation using phenol-chloroform extraction formulation and for index determination of thermotolerance traits. We detected serum corticosterone using an enzyme linked immuno-sorbent assay (Darui Antibody Limited, Guangzhou, Guangdong, China) according to the manufacturer protocol. The heterophil to lymphocyte ratio (H/L) was measured by the People's Liberation Army Hospital 458 (Guangzhou, Guangdong, China).

DNA and RNA isolation and RNA reverse transcription

Genomic DNA of the 20 goslings and 200 WZG was extracted from anticoagulant blood using a standard phenol-chloroform method. Total RNA of the 20 goslings was extracted using an RNA isolation kit (Tiangen, Guangzhou, Guangdong, China) and was purified using DNA Erasol (Tiangen). The RNA was reverse transcribed with a ReverTra Ace-α™ kit (TaKaRa Biotechnology Co., Ltd. Dalian, Liaoning, China). All procedures followed manufacturer protocols.

Polymerase chain reaction, rapid amplification of cDNA ends, and genome walking

We used the chicken *HSP70* sequence (GenBank accession No. AY143693) as a template to design primers A, B, and C (Table 1). The polymerase chain reaction (PCR) mixture contained 1 µL 1 mM goose cDNA, 1 µL 10 mM of each forward and reverse primers, 8 µL 2.5 mM dNTPs, 4.5 µL 10X LA Polymerase Chain Reaction Buffer II (TaKaRa), 0.5 µL 25 mM $MgCl_2$, 5 U/µL LA Taq™ polymerase (TaKaRa), and 33.5 µL ddH_2O (to a final volume of 50 µL). PCR products were detected using electrophoresis in 2% agarose gel containing ethidium bromide.

Rapid amplification of cDNA ends (3'RACE) (TaKaRa) was used to generate the first strand. Two nesting primers -3SP2F and 3SP3F- were designed according to the goose *HSP70* sequence (Table 1). The reaction process included denaturation at 94°C for 3 min; followed by 30 cycles each at 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min; and a final elongation step of 72°C for 10 min.

We amplified untranslated regions (5'UTR) using a Genome Walking kit (TaKaRa). We used the goose *HSP70* sequence (GenBank accession No. EU680475) as a template to design primers SP1R, SP2R, and SP3R (Table 1). The reaction process included denaturation at 94°C for 1 min and 98°C for 1 min; followed by five cycles each at 94°C for 30 s, 65°C for 1 min, and 72°C for 2 min; 15 cycles each at 94°C for 30 s, 65°C for 1 min, 72°C for 2 min, 94°C for 30 s, 65°C for 1 min, 72°C for 2 min, 94°C for 30 s, 44°C for 1 min, and 72°C for 2 min; and a final elongation step of 72°C for 10 min. We designed the primers SP4R, SP5R, and SP6R (Table 1) according to

the results of the final step. The reaction process used to design the primers was the same as that of the final step. All procedures followed manufacturer protocols. We used the genome walking sequence as a template to design verification primer D (Table 1). We used goose genomic DNA and cDNA as templates for PCR amplification and expanded product sequencing.

Quantitative real-time PCR

We analyzed the expression levels of goose *HSP70* in different tissues before and after heat treatment using quantitative real-time PCR (qRT-PCR) with the SYBR Green Real-Time PCR Master Mix (Toyobo, Japan). The qRT-PCR reactions were performed in a final volume of 20 µL according to the manufacturer protocol. The expression level was determined by comparing the *HSP70* mRNA level with the β-actin level. Primer E was used. For each sample, PCR was conducted in triplicate using an ABI7500 Real-Time PCR system (Applied Biosystems, Foster City, USA). The amplification efficiencies of the target *HSP70* and reference β-actin mRNA sequences were approximately equal. The *HSP70* mRNA levels in all samples were normalized using the following formula:

$$\text{Relative quantity of } \textit{HSP70} \text{ mRNA} = 2^{-\Delta\Delta Ct} \quad \text{(Equation 1)}$$

where *ΔΔCt* corresponds to the difference between the ΔCt measured for the mRNA level of each tissue. Here:

$$\Delta Ct = Ct_{\textit{HSP70}} - Ct_{\beta\text{-actin}} \quad \text{(Equation 2)}$$

If the difference in Ct values was >0.04, the two reactions were repeated.

Single nucleotide polymorphism (SNP) screening and genotyping

We randomly selected DNA of 10 individuals from each of six breeds of goose - WZG, Magang Goose, Lion Head Goose, Sichuan White Goose, Landaise Goose, and Yellow-Mane Goose. We used the 60 samples obtained to construct a DNA pool. The DNA pool was prepared by mixing equal amounts (final concentration = 0.02 µg/µL) of DNA from each bird. We used four pairs of primers - G1, G2, G3, and G4 (Table 1) - for PCR amplification; the products were sent for direct sequencing(G1, G2, G3, and G4) and polymorphisms of the *HSP70* were identified. Genotyping of SNP was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) on genomic DNA from 200 WZG, with two pairs of primers - H1 and H2 (Table 1). The PCR-RFLP process was as follows: *Csp*6I, *Hpy*CH4III/*Taa*I, and *Aci*I restriction enzymes (TaKaRa) were tested for genotyping; 10 µL amplified PCR products were digested using the manufacturer protocols; the DNA fragments were analyzed using electrophoresis in 2% agarose gel for 20 min at 120 V; the electrophoresis gel was stained in 0.5X TBE buffer containing 0.5 µg/mL ethidium bromide; and the DNA bands were observed and photographed under UV illumination.

Statistical analysis

We conducted statistical analyses using SAS version 9.0 (SAS Institute Inc., Cary, NC,

USA). We used *t*-tests to test for significant differences. Differences were considered significant at $P < 0.05$. Data are reported as means ± SE.

RESULTS

Goose *HSP70* sequence

We obtained three PCR products of 1715, 435 bp (including a 434-bp 3'UTR obtained using 3'RACE), and 359 bp (a 206-bp product obtained using genome walking included a 97-bp 5'UTR) with primers A, B, and C, respectively. Sequence splicing produced a 2436-bp sequence [subsequently uploaded to the National Center of Biotechnology Information (NCBI) GenBank database (GenBank accession No. EU680475)], which included a 1905-bp open reading frame (ORF), a 97-bp 5'UTR, and a 434-bp 3'UTR. We obtained identical 2103-bp products using PCR amplifications of genomic DNA and cDNA with primer F.

Table 1. Primers used in the experiment.

Primer name		5'→3'	Product (bp)	Annealing temperature (°C)
A	F	CCCCACAGTGCAGTCAGAC	1715	61.5
	R	CGTGCCTTCCCTTTTATCA		
3SP2F		TTGACAAGTGCCGGGAGGTGATCT	447	65
3SP3F		GAAACTCTGCAACCCGATCGTCAC		
B	F	GTGGTGAATGATGCTGGCAGAC	435	61
	R	GCGGTTGTCAAAGTCCTCCC		
C	F	GGACCACGCCGAGCTATGT	359	61
	R	GCGCTGGGAGTCATTGAAGT		
SP1R		CTCAAAGCGGGCACGAGTAATG		
SP2R		ATGCGGTTGTCAAAGTCCTCCCC		
SP3R		GCTGCTGTGGGCTCGTTGATAAT		
SP4R		TAGGCCTCGGCAATCTCCTTC		
SP5R		CACCGTGGGGTCATCGTACTT		
SP6R		TTGCCATGCTGGAAGACGCC		
D	F	CTGCAACGGCAGAACGTG	492	55
	R	GCCTCGGCAATCTCCTTC		
E	F	GTACTCATGCGTGGGCGT	207	52
	R	CACCGTGGGGTCATCGTA		
F	F	CCCCACAGTGCAGTCAGAC	2103	62
	R	GCCTCGGCAATCTCCTTC		
G1	F	CTTCACCGATACTGAGCG3	582	55
	R	GTTGTCAAAGTCCTCCCC		
G2	F	CTGGGGGGGGAGGACTTT	349	53
	R	GGGATACGGGTGGAGCCT		
G3	F	CCAAAAACTACTACAGGA	643	53
	R	TTCCCTTCAGTTTATCAT		
G4	F	GAGGATGATAAACTGAAG	497	54
	R	TTACTTTAGAAGAGACAC		
H1	F	GTGGGCGTCTTCCAGCAT	343	53
	R	TCGGCAATCTCCTTCATCTT		
H2	F	GAGATCGTGCTGGTTGGA	402	55
	R	CTGGAGGGATGCCTGTTA		
β-actin	F	CTCCCCCATGCCATCCTCCGTCTG	150	52
	R	GCTGTGGCCATCTCCTGCTC		

Primers were used as follows: A, for partial goose HSP70 coding region amplification; 3SP2F and 3SP3F, for 3'RACE; B and C, for partial cDNA; SP1R-SP6R, for genome walking; D, for verifying whether the gene contained introns; E, for qRT-PCR; G1-G4, for SNP screening; H1 and H2, for PCR-RFLP; β-actin for internal reference; F for forward primer; R for reverse primer.

Polymorphism of goose *HSP70* and genotyping

We identified 18 SNPs (Table 2) within the goose *HSP70* ORF and 5'UTR, of which three were transversions and 15 were transitions. Amino acid changes were caused by three SNPs - S5, S8, and S9. We genotyped S3, S11, and S13 using PCR-RFLP with *Csp*6I, *Hpy*CH4III/*Taa*I, and *Aci*I, respectively.

Table 2. Polymorphism information regarding the goose *HSP70* gene.

Name	Location	Type	Codon	Amino acid
S1	G-16 A	Transition	-	-
S2	C+6T	Transition	tct-tcc	Ser-Ser
S3	T+237C	Transition	tat-tac	Tyr-Tyr
S4	A+258G	Transition	tca-tcg	Ser-Ser
S5	A+475C	Transversion	acc-ccc	Thr-Pro
S6	C+483T	Transition	gac-gat	Asp-Asp
S7	C+516T	Transition	cgc-cgt	Arg-Arg
S8	A+532C	Transversion	aca-cca	Thr-Pro
S9	A+560C	Transversion	gcc-gac	Ala-Asp
S10	A+876G	Transition	gaa-gag	Glu-Glu
S11	T+1122C	Transition	tac-tat	Tyr-Tyr
S12	C+1146T	Transition	atc-att	Ile-Ile
S13	C+1242T	Transition	acc-act	Thr-Thr
S14	C+1404T	Transition	ccc-cct	Pro-Pro
S15	C+1416T	Transition	cgc-cgt	Arg-Arg
S16	A+1479G	Transition	gcg-gca	Ala-Ala
S17	C+1887T	Transition	act-acc	Thr-Thr
S18	C+1890T	Transition	atc-att	Ile-Ile

Correlation of goose *HSP70* with serum corticosterone level and H/L at different temperatures

We performed association analysis of S3, S11, and S13 of WZG *HSP70* with the serum corticosterone level and H/L at 25° and 40°C (Tables 3 and 4). At 25°C, S3 was significantly associated with the serum corticosterone level ($P < 0.05$); moreover, the serum corticosterone level was higher for TC than for the other two genotypes (Table 4). In contrast, S11 was significantly associated with the H/L ($P < 0.05$); moreover, the H/L was lower for TC than for the other two genotypes (Table 4). At 40°C, none of the three investigated SNPs of WZG *HSP70* were correlated with the serum corticosterone level or H/L (Table 3).

Table 3. Association of S3, S11, and S13 with serum corticosterone level and H/L at 40°C.

Location	Genotype	Corticosterone level (ng/mL)	H/L
S3	TT	12.85 ± 8.93 (16)	0.37 ± 0.03 (18)
	TC	27.65 ± 4.40 (66)	0.37 ± 0.02 (56)
	CC	33.53 ± 3.59 (99)	0.39 ± 0.01 (87)
S11	TT	32.94 ± 3.44 (119)	0.38 ± 0.01 (103)
	TC	26.66 ± 6.17 (37)	0.39 ± 0.02 (32)
	CC	25.86 ± 9.10 (17)	0.36 ± 0.03 (17)
S13	TT	26.75 ± 8.49 (19)	0.38 ± 0.03 (18)
	TC	26.64 ± 5.40 (47)	0.39 ± 0.02 (40)
	CC	32.49 ± 3.48 (113)	0.38 ± 0.01 (100)

Individual number of each genotype is shown in parentheses. Data are reported as means ± SE.

Table 4. Association of S3, S11, and S13 with serum corticosterone level and H/L at 25°C.

Location	Genotype	Corticosterone level (ng/mL)	H/L
S3	TT	11.39 ± 1.70 (14)[b]	0.47 ± 0.05 (15)
	TC	31.53 ± 3.78 (58)[a]	0.41 ± 0.03 (48)
	CC	22.35 ± 3.22 (80)[ab]	0.45 ± 0.02 (66)
S11	TT	27.24 ± 3.03 (99)	0.49 ± 0.05 (81)[a]
	TC	27.51 ± 5.09 (35)	0.36 ± 0.03 (31)[b]
	CC	12.59 ± 8.05 (14)	0.45 ± 0.02 (14)[a]
S13	TT	27.53 ± 7.68 (16)	0.47 ± 0.04 (16)
	TC	27.60 ± 4.74 (42)	0.39 ± 0.03 (35)
	CC	25.07 ± 3.17 (94)	0.47 ± 0.04 (77)

Different superscript letters within the same column indicate significant differences at $P < 0.05$. The individual number of each genotype is shown in parentheses. Data are reported as means ± SE.

Tissue expression levels of goose *HSP70*

The results of qRT-PCR analysis revealed that at 25°C, the expression level of male goose (MG) *HSP70* mRNA was higher in the leg muscle, followed by the hypothalamus, testes, heart, lungs, pectoral muscle, cerebellum, uropygial gland, kidneys, muscular stomach, glandular stomach, pituitarium, cerebrum, liver, and spleen. In contrast, at 40°C, the expression level was higher in pectoral muscle, followed by the lungs, pituitarium, uropygial gland, testes, kidneys, heart, spleen, hypothalamus, liver, glandular stomach, muscular stomach, cerebrum, cerebellum, and leg muscle. With the exception of the leg muscle, the expression levels of *HSP70* mRNA in tissue were significantly higher at 40°C than at 25°C (Table 5).

Table 5. Differences in expression of *HSP70* mRNA in MG tissues at 25° and 40°C.

Tissue	40°C (5)	25°C (5)	P value
Liver	5.84 ± 0.95	0.02 ± 0.004	<0.0001
Kidney	9.09 ± 1.59	0.07 ± 0.01	<0.0001
Testis	9.91 ± 1.32	0.21 ± 0.03	0.0008
Spleen	6.36 ± 0.93	0.02 ± 0.004	<0.0001
Pituitarium	13.34 ± 1.04	0.06 ± 0.01	<0.0001
Muscular stomach	3.41 ± 0.49	0.07 ± 0.02	0.0028
Glandular stomach	4.51 ± 0.49	0.07 ± 0.02	<0.0001
Lung	18.50 ± 4.31	0.16 ± 0.03	<0.0001
Uropygial gland	10.42 ± 1.05	0.08 ± 0.02	0.0008
Pectoral muscle	24.30 ± 2.85	0.11 ± 0.01	<0.0001
Leg muscle	1.32 ± 0.29	0.53 ± 0.14	0.5678
Hypothalamus	6.22 ± 0.60	0.31 ± 0.06	0.0187
Cerebrum	3.05 ± 0.14	0.03 ± 0.006	0.0045
Cerebellum	2.44 ± 0.55	0.11 ± 0.03	0.0043
Heart	6.48 ± 0.93	0.18 ± 0.03	0.0027

Parentheses indicate the number of individuals. Data are reported as means ± SE.

At 25°C, the expression level of female goose (FG) *HSP70* mRNA was higher in the leg muscle, followed by the lungs, cerebellum, hypothalamus, heart, uropygial gland, pituitarium, cerebrum, pectoral muscle, kidney, ovary, spleen, glandular stomach, liver, and muscular stomach. Furthermore, at 40°C, the expression level was also higher in the leg muscle, followed by the lungs, pituitarium, kidneys, uropygial gland, ovaries, hypothalamus, cerebellum, liver, spleen, pectoral muscle, cerebrum, glandular stomach, muscular stomach, and heart. With the exception of the heart, the expression levels of *HSP70* mRNA in tissue were significantly higher at 40°C than at 25°C (Table 6).

Table 6. Differences in expression of HSP70 mRNA in FG tissues at 25°C and 40°C.

Tissue	40°C (5)	25°C (5)	P value
Liver	1.73 ± 0.20	0.02 ± 0.02	0.0158
Kidney	3.79 ± 0.38	0.05 ± 0.02	0.0036
Ovary	3.42 ± 0.87	0.05 ± 0.02	0.0016
Spleen	1.49 ± 0.55	0.03 ± 0.04	0.0096
Pituitarium	4.59 ± 6.21	0.13 ± 0.04	<0.0001
Muscular stomach	0.48 ± 0.22	0.02 ± 0.01	0.0013
Glandular stomach	0.81 ± 0.87	0.03 ± 0.01	0.0006
Lung	7.10 ± 1.64	0.27 ± 0.23	0.0365
Uropygial gland	3.72 ± 1.34	0.16 ± 0.09	0.0093
Pectoral muscle	1.24 ± 1.30	0.06 ± 0.004	<0.0001
Leg muscle	10.07 ± 1.87	0.55 ± 0.49	0.0012
Hypothalamus	2.59 ± 0.84	0.18 ± 0.09	0.0204
Cerebrum	1.18 ± 0.48	0.08 ± 0.05	0.023
Cerebellum	2.50 ± 0.29	0.22 ± 0.19	0.0054
Heart	0.45 ± 0.06	0.18 ± 0.10	0.7935

Parentheses indicate the number of individuals. Data are reported as means ± SE.

At 25°C, the expression levels of *HSP70* mRNA in the pectoral muscle and muscular stomach were significantly higher in MG than in FG (Figure 1).

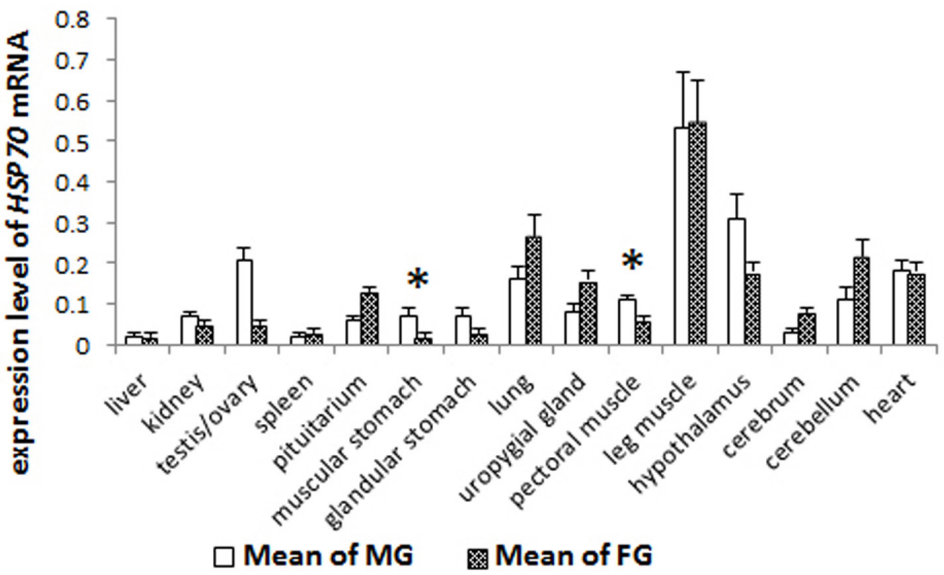

Figure 1. Tissue expression levels of goose *HSP70* mRNA at 25°C. The y-axis indicates the relative expression level of *HSP70*. MG, male goose; FG, female goose; *P < 0.05.

At 40°C, the expression levels of *HSP70* mRNA in all tissues except the leg muscle and cerebellum were significantly higher in MG than in FG (Figure 2). In contrast, the expression level of *HSP70* mRNA in the leg muscle was significantly lower in MG than in FG. The expression level of *HSP70* mRNA in the cerebellum did not differ significantly between MG and FG (Figure 2).

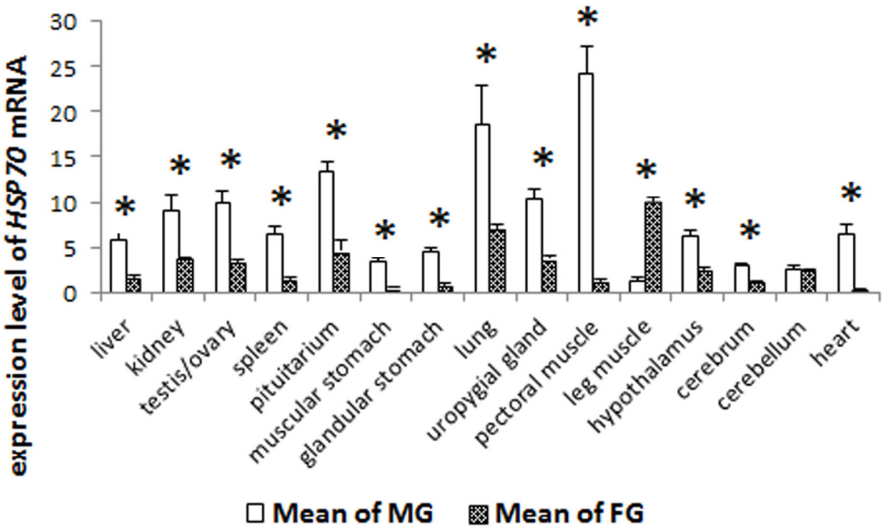

Figure 2. Tissue expression levels of goose *HSP70* mRNA at 40°C. The y-axis indicates the relative expression level of *HSP70*. MG, male goose; FG, female goose; *P < 0.05.

DISCUSSION

The nucleotide sequence of avian *HSP70* is known to be conserved. Gaviol et al. (2008) reported sequence homologies of Japanese quail and red jungle guinea fowl of 98 and 99% respectively. Hence, in the present study, we cloned goose *HSP70* according to chicken *HSP70* sequence. We showed that, similar to other avian species, goose *HSP70* is intronless. This finding implies that *HSP70* is highly conserved. The gene structure may be associated with environmental adaptability.

We sequenced pooled DNA and identified 18 SNPs (one SNP per 106 bp) within the goose *HSP70* ORF and 5'UTR. This polymorphism frequency was higher than that previously found in chicken *HSP70*. Previous studies in our laboratory identified 10 SNPs within the chicken *HSP70* ORF. The SNP frequency was approximately one per 190 bp, which is consistent with the chicken genomic SNP frequency (Wong et al., 2004). However, most of the SNPs did not cause amino acid transformations. In the present study, we found three transformed amino acids among the 18 SNPs, possibly because of the different breeds of geese investigated.

Davis et al. (2008) considered that the low corticosterone level in quail plasma might improve stress resistance and inhibit the expression of *HSP70*. In the present study, SNP S3 of WZG was significantly associated with the serum corticosterone level at 25°C ($P < 0.05$); moreover, TT had a lower serum corticosterone level than the other two genotypes. The serum corticosterone level for TT differed only slightly before and after heat stress, suggesting that the TT genotype exhibits higher heat stress resistance than the other two genotypes. In fowl, strains with low H/Ls show improved heat stress resistance (Chen et al., 2013). SNP S11 of WZG was significantly associated with the H/L ($P < 0.05$); moreover, TC had a lower H/L than the other two genotypes, implying that S11 is related to heat stress resistance.

Goose is a waterfowl species and therefore it may show relatively high sensitivity to heat stress. However, data regarding the mechanisms whereby heat stress affects waterfowl are lacking.

In the present study, we applied acute heat stress and examined the expression level of *HSP70* mRNA. We found that *HSP70* was expressed in all tissues of the goose, and that the expression levels showed gender and tissue specificity. Maak et al. (2003) reported that chicken *HSP70* expression was heat inducible. Zhen et al. (2006) reported heat-induced expression of chicken *HSP70*. These authors showed that under normal growth conditions, expression of *HSP70* in the liver was significantly higher than that in muscle. In contrast, during acute heat stress, expression of *HSP70* was higher in the brain than in the muscle. In our present study, we showed that the expression levels of *HSP70* mRNA in tissue except leg muscle were significantly higher at 40°C than at 25°C, implying that expression of goose *HSP70* mRNA is induced by heat stimulation. After heat treatment, the expression levels of *HSP70* mRNA in each tissue (except the leg muscle and cerebellum) were significantly higher in MG than in FG, indicating that FG may show higher thermal stress resistance than MG.

CONCLUSIONS

In the present study, we cloned goose *HSP70* and showed that this gene contained a 1905-bp ORF and no introns. We found that goose T+237C was significantly correlated with serum corticosterone level, whereas goose T+1122C was significantly correlated with H/L. The expression of goose *HSP70* showed gender differences at 40°C but not at 25°C; moreover, in general, expression was significantly higher at 40°C than at 25°C. Taken together, our findings indicate that goose *HSP70* plays an important role in severe heat stress.

Conflicts of interest

The authors declare no conflict of interest.

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