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INFLUENCE OF BREEDING INTERVALS AND SEASONS ON HEAT SHOCK PROTEIN 70 (Hsp70) EXPRESSION IN HYLA RABBIT IN SOUTHWESTERN NIGERIA

By

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Abstract

The study was conducted to evaluate the effects of seasons and breeding intervals on the gene expressing stress proteins, heat shock protein 70, in Hyla rabbits in southwestern Nigeria. A total of twenty-four healthy adult rabbits, sixteen females and eight males of average 2600g ± 100 g, were used in the study. Does were randomly allotted into the four experimental treatments: T_1 (two weeks), T_2 (four weeks), T_3 (six weeks), and T_4 (eight weeks) breeding intervals with four rabbits per treatment in a Completely Randomized Design under four different seasons (late rain (S_1), early dry (S_2), late dry (S_3), and early rain (S_4)). Blood was collected into EDTA bottles from all does at the middle of each season to quantify the Hsp70 expression. The results showed that RT-PCR analysis indicated Hsp70 mRNAs were expressed in the tested blood from all does in different breeding intervals and seasons. Hsp70 had the higher ($p < 0.05$) gene expression in S_3 and T_4. The interaction between seasons and breeding intervals revealed that the expression of Hsp70 was higher ($p < 0.05$) in S_1 in T_2 and T_3 while higher ($p < 0.05$) in S_3 in T_1 and T_4. Hsp70 can be easily triggered by small increases in temperature and other stressors in response to stress. Environmental stress was very high in the late dry season, which caused high Hsp70 expression. The situation could easily lead to heat stress, which adversely affects the growth, production, and reproduction performance of the does and their kits. Therefore, Hyla rabbit keepers should guard against the excess heat during the late dry season in southwestern Nigeria.

***Key words**: Hyla rabbit, Hsp70, breeding interval, season, RT-PCR, mRNA.*

INTRODUCTION

Across all species, heat shock proteins (HSPs) are among the most widely expressed cellular proteins (Csermely *et al*., 2008). When cells are under stress from high temperatures, HSPs shield them. In cells that are not under stress, they make up 1% to 2% of the total protein. However, the percentage of heat shock proteins rises to 4–6% of cellular proteins under stress (Creve *et al*., 2011). It is widely acknowledged that the production of HSPs is necessary for stress tolerance and that these proteins shield organisms from the harmful effects of heat, cold, and perhaps other stressors such as different chemicals, heavy metals, oxidative stress, and desiccation (Kregel, 2002). They serve as "chaperones," ensuring that the proteins in the cell are positioned and shaped appropriately at the appropriate times.

HSPs are crucial for the function of new or deformed proteins because they aid in their folding into shape. Additionally, they move proteins between compartments and move outdated proteins to the cell's "garbage disposals." It is also thought that heat shock proteins contribute to the appearance of protein fragments (or peptides) on the cell surface, which aids the immune system in identifying sick cells. The molecular weight of heat-shock proteins determines their name. For instance, the most extensively researched HSPs, Hsp60, Hsp70, and Hsp90, are families of heat shock proteins with respective sizes of 60, 70, and 90 kDa (kilodaltons) (Lahvic *et al*., 2013). One of the most abundant HSP families in livestock, the Hsp70, is essential for establishing thermal comfort and managing environmental stress (Gupta *et al*., 2013).

Limited studies have been conducted on the expression of Hsp70 in Hyla rabbits under various breeding intervals and seasonal settings. Nonetheless, research on different breeds of rabbits sheds light on the function of Hsp70 in cellular defense and thermoregulation. Hsp70 was shown to be

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substantially expressed in the testes of male Rex rabbits following a 9-week heat treatment, whereas it was absent in the testes of control rabbits in a study investigating the effects of chronic heat stress on the testicular expression of several heat shock proteins, including Hsp70. The protein's expression levels even partially returned to normal after a 9-week recovery period, but they were still greater than those in the control group (Yangli Pei *et al*., 2011).

Another study looked into the expression of Hsp70 in the retina of rabbits after ischemia-reperfusion injury. The findings showed that, in comparison to normal control, Hsp70 expression rose in the retina 12 hours after reperfusion. Hsp70 was constitutively present in Müller cells, as demonstrated by confocal microscopy, and its expression rose after reperfusion.

These results suggest that the retina's adaptive response to stress may involve Hsp70 on Müller cells (Gohdo *et al*., 2001). Other rabbit breeds' increased expression of Hsp70 in response to heat stress points to a conserved defense mechanism. Under stressful circumstances, Hsp70 probably aids in cellular defense and recovery mechanisms.

Nigeria has a predominantly tropical climate, with two distinct wet and dry seasons and an average temperature between 21 and 35 degrees Celsius (Nigerian Meteorological Agency, 2022b). The wet season, also referred to as the rainy season, has early and late rainy seasons that always take place in the months of April through September (Dennis and Arierhire, 2020). The rainy season is clearly visible on the southeast coast, where annual rainfall is only about 130 inches (330 cm) and temperatures rarely get over 32 °C. The dry season also comprises early and late phases (Adeyefa, 2023). During the late dry season, when dusty northeast breezes are present, midday temperatures can occasionally reach 38 °C (Ojo, 2019). During the dry season, there is less humidity, more sunlight, and less precipitation. According to Dennis and Arierhire (2020), this period is always October through March. Harmattan and a dry spell are frequent during this time (Nigerian Meteorological Agency, 2022a).

Rabbit breeding regimens are frequently based on 7-day intervals for record-keeping convenience. Reducing the breeding interval is a feasible method to boost the quantity of weaned kits generated (Khan *et al*., 2014). Rabbits may mate 24 hours after kindling since they are induced ovulators (Oseni, 2012). However, intensive breeding techniques may lead to an annual increase in the number of does culled due to "burnout." Furthermore, short re-breeding intervals after kiddling may not allow the does' body reserves to fully recuperate. This could lead to a decrease in fertility, milk production, and litter weight at weaning, as well as an increase in kit mortality (Banda and Tanganyika, 2021). Most tropical and underdeveloped countries typically wean kits between 6 and 8 weeks of age, after which rabbit does are re-mated. The number of kits raised annually per doe dropped due to the long re-breeding intervals observed in tropical conditions, which can range from 30 to 60 days or more (Iyeghe-Erakpotobor *et al*., 2005).

Hyla rabbits are outstanding in terms of their high rate of growth and prolificacy (Brahmantiyo *et al*., 2021), and at 70 days of age, their body weight can reach 2160 g (de la Fuente and Rosell, 2012). Their eyes

MATERIALS AND METHODS

Animals and Experimental Design

In this investigation, twenty-four adult rabbits, weighing an average of 2600g ± 100 g and older than six months, were used. There were eight males and sixteen females. In a Completely Randomized Design (CRD), four rabbits per treatment were randomly assigned to four experimental treatments: T_1 (two-week), T_2 (four-week), T_3 (six-week), and T_4 (eight-week) breeding intervals in four distinct seasons of a year (S_1 (late rain—July to September), S_2 (early dry—October to December), S_3 (late dry—January to March), and S_4 (early rain—April to June)). In a 1:1 ratio, bucks were employed to service does, with the remaining bucks set aside for replacement in the event that any of the active bucks are pink, and their coats are pristine white. In Nigeria nowadays, the Hyla breed is the most sought-after for producing meat. Thus, the purpose of this study was to examine how various breeding intervals and seasons affected the expression of Hsp70 by does, as well as how these changes affected the growth and reproductive capabilities of both does and kits. Our research will be useful in providing information on breeding intervals and seasons that result in excess expression of Hsp70, a sign that the rabbits are experiencing excessive stress that may have a detrimental impact on their growth, productivity, and reproduction.died. Throughout the twelve months of the trial, all rabbits received the same concentrate diet (Tables 1 and 2) and were exposed to the same environmental factors. Every recommended management technique was duly followed.

In a closed-door house without environmental control, the animals were kept in galvanized battery cages, each measuring 55 x 65 x 35 cm and elevated 80 cm from the floor. There was always water and feed. In this study, rabbits of the same breed, from separate families, with similar body weights and ages, were kept in the same environment with the same feed. In order to determine the expression of Hsp70 in the blood, blood was drawn from the major artery of the ear in the middle of each season and placed into bottles containing ethylenediamine tetraacetic acid (EDTA).

Table 1: Composition of Formulated pelleted Diet

Ingredients	Kg
Groundnut cake	17.00
Soya bean	2.00
Binder	0.20
Corn bran	15.00
Limestone	2.00
Premix	0.20
Maize	3.00

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Wheat offal	45.40
Palm Kernel cake	15.00
Ensyme	0.20
TOTAL	**100**
Calculated analysis	
CP%	15.21
CF%	13.37
Soluble Carbohydrate%	52.85

*Premix composition (per kg diet): vitamin A (12,000 I.U.), vitamin D3 (2500 I.U.), vitamin K (2 mg), vitamin B1 (2.20 mg), vitamin B2 (6 mg), vitamin B12 (0.015 meg), niacin (40.00mg), pantothenic (15.00mg), folic acid (1.50mg), biotin (0.050 meg), choline chloride (300.00mg), manganese (80.00mg), zinc (50.00mg), iron (20.00mg), copper (5.00mg), iodine (1.00mg), selenium (1.00mg), cobalt (0.50mg), antioxidant (125.00 mg).

Table 2: Proximate Composition of Formulated pelleted Diet

Parameters	(%)
Dry matter	97.99
Moisture content	2.01
Crude protein	15.23
Ash	12.53
Crude fat	10.00
Crude fibre	13.42
Carbohydrate	52.87

Chemical Analyses

RNA extraction and determination of quality

TRIzol (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from the blood of female Hyla rabbits in accordance with the usual procedure. DNase I (TaKaRa, Japan) was applied to the RNA samples for four hours. A NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., San Jose, CA, USA) was used to measure the absorbance at 260 nm in order to determine the purity and concentration of total RNA. By comparing the absorbance at 260 and 280 nm of the samples, which ranged from 1.8 to 2.0, the blood RNA was evaluated. An Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) was used to examine the samples' RNA integrity. Following the recommended procedure, the RevertAid™ First Strand cDNA Synthesis Kit (Ferments) was used to create the first stranded cDNA, which was then kept at -20°C.

Target gene analysis using quantitative real-time reverse transcription polymerase chain reaction (RT-PCR)

Target gene and reference gene (bActin) mRNA levels were ascertained using quantitative real-time RT-PCR, which was created and is presented in Table 3. GenBank mRNA sequences were used to design the PCR primers. 1 µL of RT reaction mix, 10 µL of SYBR® Premix Ex Taq TM (2×) (TaKaRa, China), 0.6 µL of 10 µmol/L primers, and 20 µL of ultrapure water made up the RT-PCR mixture (20 µL). The reactions were conducted on a Bio-Rad fluorescence iCycler. The following were the PCR conditions: 90 seconds at 94°C; 43 cycles of 15 seconds at 95°C, 20 seconds at the primer annealing temperature (Table 1), and 15 seconds at 72°C. Every sample was examined twice. The 2-ΔΔCt method was used to examine the threshold cycle (Ct) from RT-PCR (Livak and Schmittgen, 2001). The geometric mean of the bActin mRNA measurements in the same sample was used to adjust changes in target gene expression.

Reverse transcription polymerase chain reaction (RT-PCR)

The protein-coding sections of rabbit Hsp70 mRNAs were amplified using reverse transcription (RT)-PCR, which was also utilized to find out whether the mRNAs were expressed in the rabbit blood. Following the manufacturer's instructions, one µg of total RNA was reverse-transcribed to cDNA in a 20 µL volume using the Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany). All primers used in this study were synthesized by Sangon (Shanghai, China) and are listed in Table 3. The specific primers of the β-actin (housekeeping gene) and Hsp70 genes were designed using Premier Primer 5 based on the sequences of predicted rabbit Hsp70 (Accession number: NM_001082005) and β-actin (Accession number: NM_236595).

Table 3: Sequences of the primers used for real-time PCR

Gene expression	Gene symbol	Forward primer sequence (5'→ 3')	Reverse primer (5'→ 3')
Hsp70	HSP70A1A	GAGAGGTGCTGGACAAGTGT	CTTGCCGTTCTGGGTTGATG
β-actin	ACTB	GACATGGAGAAGATCTGGCA	ATGCCACAGGATTCCATACC

Statistical Analysis

Using the GLM technique, Luciferase data were examined (SAS Inst. Inc., Cary, NC). n=16 for the mRNA quantification analysis. Means were compared using Tukey's technique. The standard error of the mean, or mean ± SEM, is used to express the results. When $p < 0.05$, differences were deemed statistically significant.

RESULTS AND DISCUSSION

For the Hsp70 gene expression, nucleotide sequences of 150 bp was used to characterize the distribution of Hsp70 in various blood types. The analyzed blood had expressed Hsp70 mRNAs, according to the RT-PCR analysis. Figure 1 demonstrates that during the S_3 (12.28), when temperatures in

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Nigeria are at their highest and relative humidity is at its lowest, the expression of Hsp70 was significantly higher ($P < 0.05$) in the does (Togun *et al*., 2003). In order to offset the effects of any excessive ambient heat the rabbit may experience, the cells will consequently release additional Hsp70. An early heat exposure program may increase Hsp70 expression and may reveal the role of proteins in the stress that heat shock exposure generates in rabbits, according to Morsy (2018) and Sakr *et al*. (2019). This outcome is consistent with what they found.

Figure 2 illustrates that the expression level of Hsp70 in the blood of does in T_4 was considerably ($P < 0.05$) higher (10.16) than in any other blood analyzed from other breeding intervals. This contradicts previous studies that indicated a maximum Hsp expression of 6% during stress (Creve *et al*., 2011). This result, however, might be because the does in T_4 had more time to recuperate following weaning, which led to increased reserves and leptin levels. According to Arias-Álvarez *et al*. (2010), this might have improved fertility and produced larger and heavier litters, which would have raised metabolic processes during breastfeeding or fetus development.

Seasons and breeding intervals interaction on Hsp70 expression in Hyla rabbits (Figure 3) revealed that Hsp70 was significantly ($P < 0.05$) expressed in does in all seasons and breeding intervals. It was higher in T_1 and T_4 in S_3 (12.79 and 35.58, respectively). Hsp70 was, however, expressed higher ($P < 0.05$) in S_1 (2.08) in the does in T_2 and in S_1 (4.83) and S_4 (4.13) in the does in T_3. Hsp70 expression was found to be higher during the peak dry season in both tropical and temperate regions, according to Dangi *et al*. (2012). Higher expression of Hsp70 during heat stress suggests that Hsp70 may be involved in mitigating the negative effects of thermal stress to preserve cellular integrity and homeostasis (Smruti and Tapan, 2014).

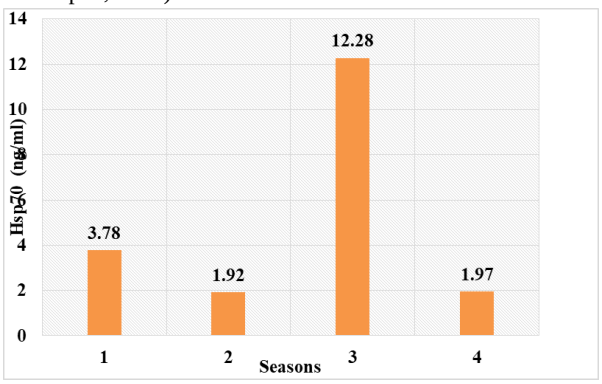

Figure 1: Effect of four varying seasons on the expression of Hsp70 in Hyla rabbit in southwestern Nigeria

1 = Late rain season (S_1); 2 = Early dry season (S_2); 3 = Late dry season (S_3); 4 = Early rain season (S_4).

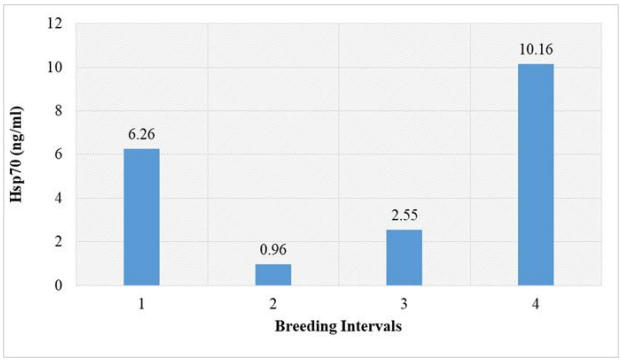

Figure 2: Effect of breeding interval on the expression of Hsp70 in does of Hyla Rabbit in southwestern Nigeria.

Breeding Interval 1: two-week interval; Breeding Interval 2: four-week interval; Breeding Interval 3: six-week interval; Breeding Interval 4: eight-week interval.

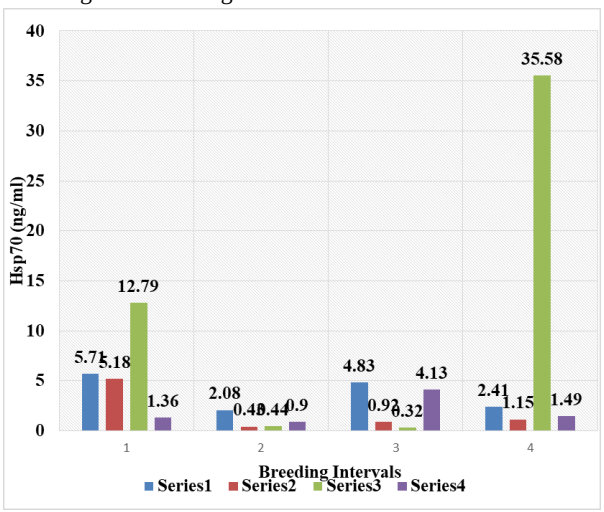

Fig. 3 Effect of interaction between season and breeding interval on expression of Hsp70 in does of Hyla rabbit in southwestern Nigeria

Breeding interval 1: two-week interval; breeding interval 2: four-week interval; breeding interval 3: six-week interval; and breeding interval 4: eight-week interval.

Series 1: season 1 (late rain, July-September); Series 2: season 2 (early dry, October-December); Series 3: season 3 (late dry, January-March); and Series 4: season 4 (early rain, April-June).

CONCLUSIONS

In summary, our study has shown that the genes Hsp70 are encoded in the rabbit genome and that these genes are expressed in the blood of rabbits from different breeding seasons and intervals. Hsp70 expression is easily triggered by an increase in the surrounding temperature. If the consequences of heat stress were more severe than the released Hsp70 could regulate, it would have a detrimental effect on the development, production, and reproductive performance of does and their kits. As a result, during the late dry season, Hyla rabbit breeders and keepers should take precautions against excessive heat. Further research is

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required to determine the precise mechanisms of action of Hsp70 in various stressed pathways.

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