Impacts of Breeding Intervals and Seasons on the Expression of Heat Shock Protein 90 (Hsp90) in Hyla Rabbits in Southwestern Nigeria

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Abstract

The study was carried out to examine the influence of breeding intervals and seasons on the gene expressing stress proteins, heat shock protein 90 (Hsp90), 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: T1 (two-week), T2 (four-week), T3 (six-week), and T4 (eight-week) breeding intervals with four rabbits per treatment in a Completely Randomized Design under four different seasons (late rain (S1), early dry (S2), late dry (S3), and early rain (S4)). Blood was collected into EDTA bottles from all does at the middle of each season to quantify the Hsp90 expression. The results showed that Hsp90 mRNAs were expressed in the tested blood from all does in different breeding intervals and seasons. Hsp90 had the higher ($p < 0.05$) gene expression in S1 and T1. The interaction between breeding intervals and seasons revealed that the expression of Hsp90 was higher ($p < 0.05$) in S1 across T1-T4. Hsp90 rose as stress increased. Higher levels of cold stress in S1 increased the expression of Hsp90, while stress from gestating and lactating simultaneously increased Hsp90 in T1. The development, production, and reproductive performance of does and their kits would be negatively impacted by the impacts of stress that are greater than the released Hsp90 could control. Therefore, Hyla rabbit keepers should guard against the excess cold during the late rainy season and does gestating and lactating at the same time in southwestern Nigeria.

Key words: *Hyla rabbit; Hsp90; Breeding interval; Season.*

INTRODUCTION

Varieties of proteins are induced in livestock when exposed to environmental stress; among them are heat shock proteins (HSPs). HSPs are proteins that provide antioxidant protection in cells as well as thermotolerance (AL-Jaryan *et al*., 2023). They function as chaperones, helping to fold, unfold, and refold stress-denatured proteins. They comprise 1% to 2% of the total protein in cells that are not under stress. Under stress, however, the proportion of heat shock proteins increases to

4–6% of all cellular proteins (Creve *et al*., 2011). Stress tolerance is known to depend on the synthesis of HSPs, which protect organisms against the damaging effects of heat, cold, and maybe other stressors such as various chemicals, heavy metals, oxidative stress, and desiccation (Kregel, 2002). In their capacity as "chaperones," they make sure that the proteins in the cell are oriented and folded correctly at the right periods.

Additionally, they move proteins between compartments and move outdated proteins to the cell's "garbage disposals." 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). Among them, heat-shock protein 90 (Hsp90) stands out as an essential proteostasis hub in eukaryotes, chaperoning hundreds of “clients” (substrates) (Silbermann *et al*., 2023).

Ye *et al*. (2023) examined how seasonal variations affected the expression of Hsp90 in meat rabbits, such as Hyla rabbits, and discovered that summertime Hsp90 levels were noticeably greater than wintertime levels. Although there are few specific studies on how breeding intervals directly affect Hsp90 expression in rabbits, related research suggests that breeding intervals that increase metabolic processes may have an impact on Hsp90 expression, which may have an impact on growth and reproductive performance (Pei *et al*., 2012).

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).

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 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 Hsp90 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 Hsp90, a sign that the rabbits are experiencing excessive stress that may have a detrimental impact on their growth, productivity, and reproduction.

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 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
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 Hsp90 genes were designed using Premier Primer 5 based on the sequences of predicted rabbit Hsp90 (Accession number: NM_00271454) 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')
Hsp90	HSP90AA1	AGGAGGTTGAGGAGGAGGAA	CTTGGTGCTTGATGTCGTGT
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 Hsp90 gene expression, nucleotide sequences of 160 bp were used to characterize the distribution of Hsp90 in various blood types. The analyzed blood had expressed Hsp90 mRNAs, according to the RT-PCR analysis.

Figure 1 shows the seasons' effects on Hsp90 expression in Hyla rabbit does, which indicates that Hsp90 expression in rabbit does in S_1 (38.25) was significantly ($p < 0.05$) higher. This is not in agreement with Yangli *et al*. (2012), who reported that Hsp90 expression only significantly influenced a season when rabbit does were subjected to severe heat stress (S_3), but it is consistent with Yunyan *et al*. (2021), who noted significantly elevated Hsp90 transcriptions in the cysts that were stored in cold and dark conditions. Kim *et al*. (2020) also reported that Hsp90 expression did not rise in heat-stressed conditions; however, this finding supported the findings of Yunyan *et al*. (2021), as they were exposed to cold stress and the atmosphere was typically dark due to high humidity in this season.

Figure 2 shows the impact of various breeding intervals on Hsp90 expression in Hyla rabbit does. Hsp90 expression varied considerably ($p < 0.05$) among does at different breeding intervals. Hsp90 expression is most significantly high (19.34) in T_1 ($p < 0.05$) and lowest (3.99) in T_2. In T_3, the rabbit expressed Hsp90 (12.92), which was significantly ($p < 0.05$) lower than the amount expressed in T_1 and higher than the amount expressed in T_4 (8.75).

The expression of Hsp90 (19.34) was much higher ($p < 0.05$) in T_1. This might be because the does were under stress from breastfeeding and pregnancy at the same time. According to Gupta *et al*. (2013), Hsp90 plays a critical role in the mechanism that protects cells from a wide range of harmful stresses and is essential for an organism's ability to recover and survive. As an essential component of the protective shock response, the fundamental role of Hsp90 is to aid in the correct

folding of nascent proteins and prevent the aggregation of stress-accumulated misfolded proteins, therefore contributing to modifying the protein quality control and protein homeostasis (Biebl and Buchner, 2019, and Zabinsky *et al*., 2019).

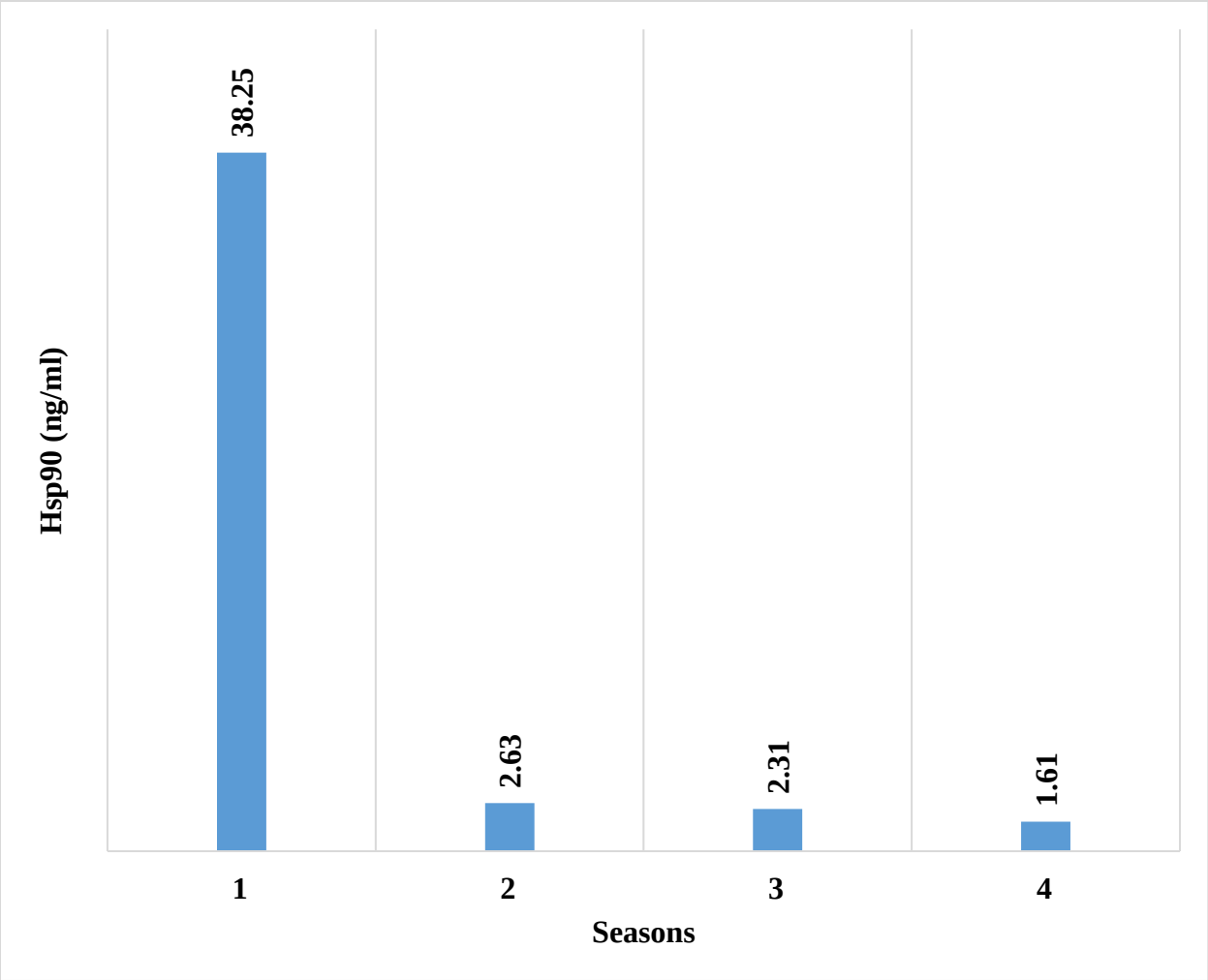

Figure 1. Effect of seasons on the expression of Hsp90 in does of Hyla rabbit in southwestern Nigeria

Season 1: (Late rain, July-September); Season 2: (Early dry, October-December); Season 3: (Late dry, January-March); Season 4: (Early rain, April-June).

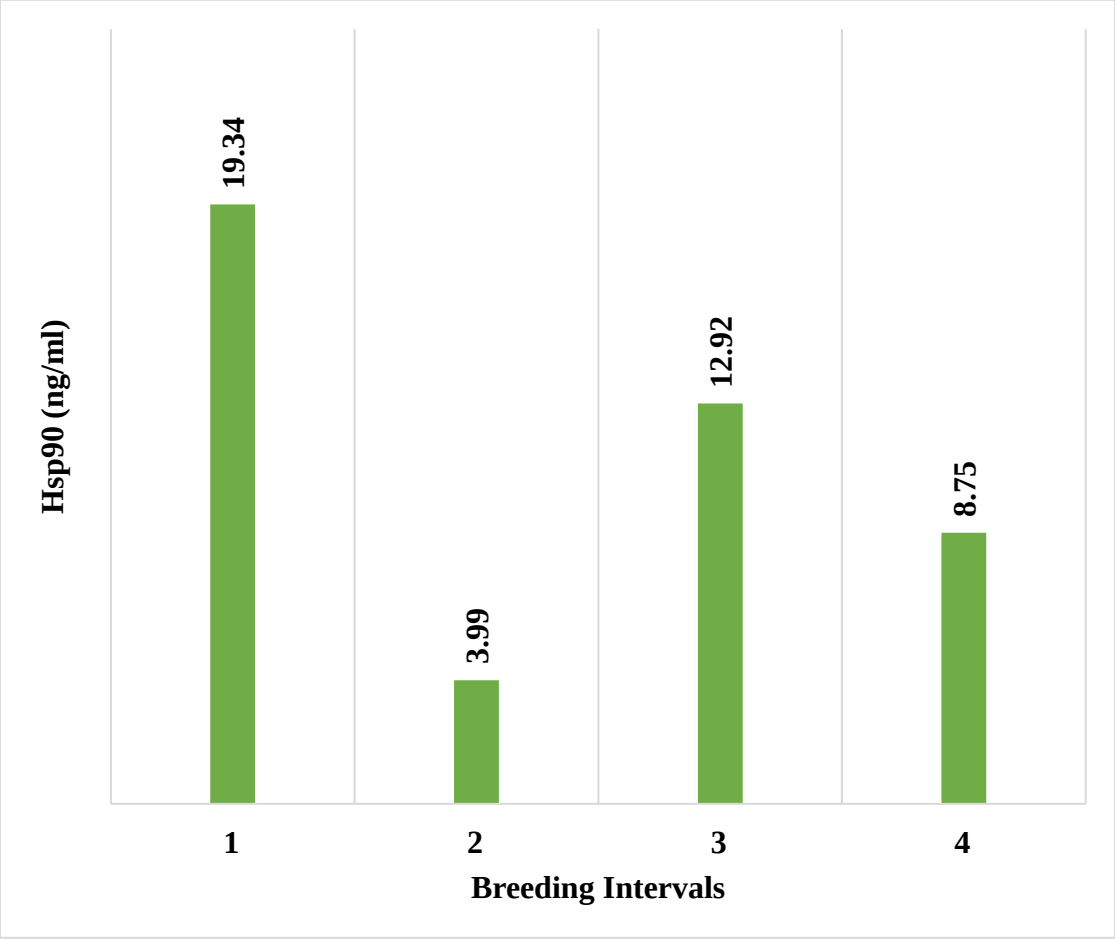

Figure 2: Effect of breeding interval on the expression of Hsp90 in does of Hyla rabbit in southwestern Nigeria
Breeding Interval 1: two-week interval; Breeding Interval 2: four-week interval; Breeding Interval 3: six-week interval, and Breeding Interval 4: eight-week interval.

The effect of the interaction between season and breeding interval on the expression of Hsp90 in does of Hyla rabbits is shown in Figure 3. Hsp90 expression in does in four different breeding intervals varied significantly ($p < 0.05$) throughout the four seasons, and it was significantly ($p < 0.05$) higher in S_1 in all breeding intervals (T_1 to T_4). This could be because of the extra cold that occurs during this season, which is the coolest time of the year in Nigeria. This is consistent with Yunyan *et al*. (2021), who noted significantly higher Hsp90 transcriptions in the cysts that were stored in cold and dark conditions. This finding is in line with that of Rajoriya *et al*. (2014) and Beckam *et al*. (2019), who discovered that summertime Hsp90 mRNA expression was lower than wintertime.

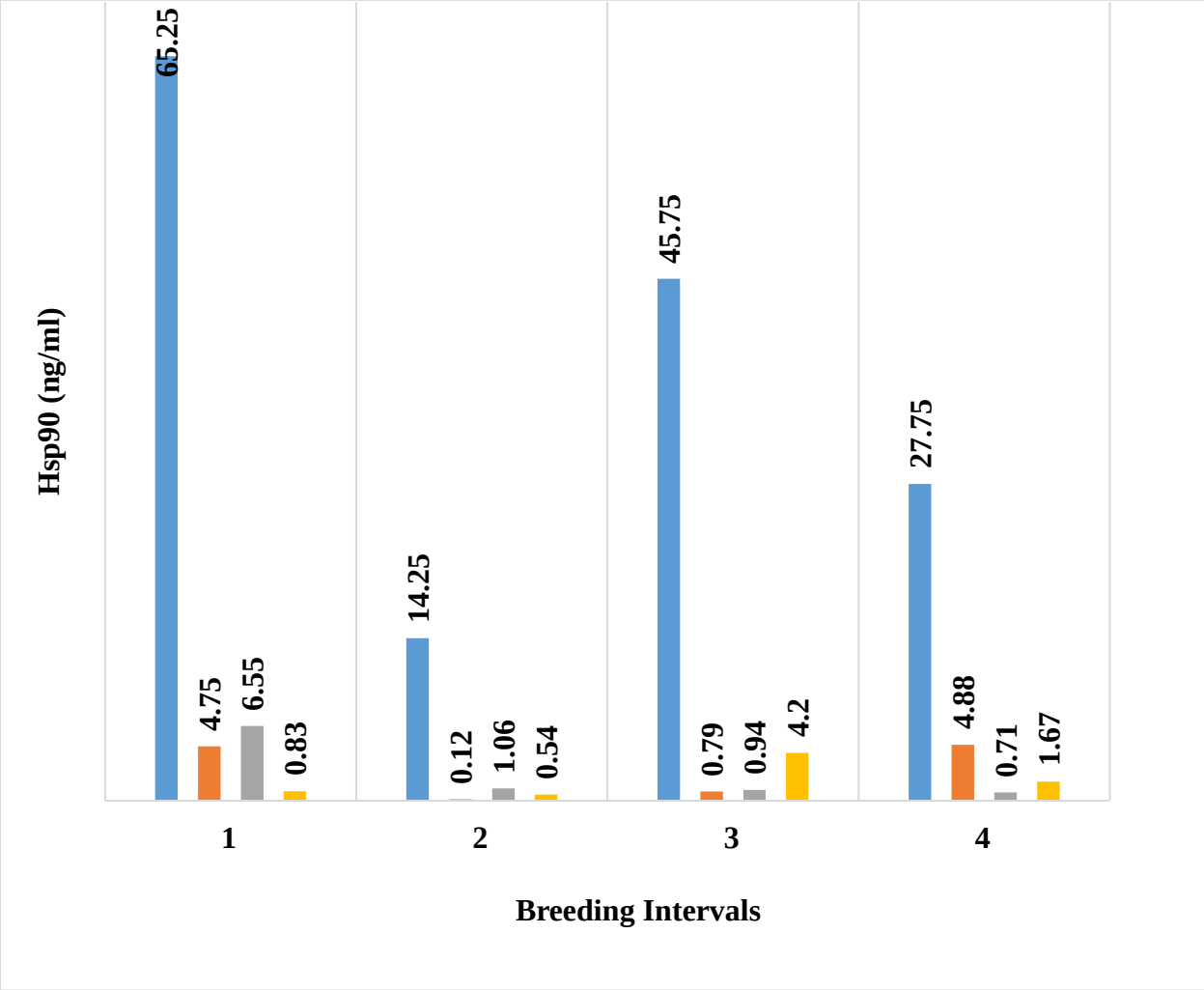

Fig. 3: Effect of interaction between seasons and breeding intervals on expression of Hsp90
Breeding interval 1: two-week interval; **breeding interval 2:** four-week interval; **breeding interval 3:** six-week interval; and **breeding interval 4:** eight-week interval.
Series 1: season 1 (late rain, July-September); **series 2:** season 2 (early dry, October-December); **series 3:** season 3 (late dry, January-March); **series 4:** season 4 (early rain, April-June).

CONCLUSSION

We have concluded that the gene Hsp90 is encoded in the rabbit genome and that it is expressed in the blood of rabbits from different breeding seasons and intervals. An increase in stress, along with a cold environment, may induce the expression of Hsp90. If the combined impacts of heat and cold were more than the released Hsp90 could regulate, stress would have a detrimental effect on the development, production, and reproductive performance of does and their kits. Therefore, breeders and keepers of Hyla rabbits should take precautions against extreme cold in the late rainy season and extreme heat in the late dry season. Further research is required to determine the precise mechanisms of action of Hsp90 in various stressed pathways. Additionally, the study could be conducted on bucks to investigate the variations in outcomes.

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