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COMPARATIVE EVALUATION OF SEASONAL INFLUENCE OF PHOTOPERIOD AND TEMPERATURE ON THE SEMEN QUALITY OF EXOTIC AND INDIGENOUS ROOSTERS

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ABSTRACT

A total number of 40 male birds (10 each of Fayoumi (FAY), Aseel (ASL), Rhode Islam Red (RIR), and Australorp (AUS) of 35±5 weeks of age were randomly selected for trial. An experimental study was conducted at Sindh Institute of Animal Health (SIAH), Karachi for the summer and winter seasons. The semen volume of RIR was significantly (P<0.05) higher as compared to other breeds and for seasons. Semen color was classified as medium white for summer and winter seasons and found significant (P>0.05), however, there was a non-significant (P>0.05) difference among breeds. Moreover, semen pH has a non-significant difference for both seasons and amongst breeds. Sperm motility during summer season was observed significantly (P<0.05) higher in ASL breeds (72.2%) as compared to FAY (71.7%), AUS (64.99%), and RIR (64.65%) breeds. Whereas sperm motility during winter season was observed significantly higher in FAY (84.12%) as compared to AUS (84.10%), ASL (83.03%), and RIR (72.08). Sperm concentration for the winter season was observed significantly (P<0.05) higher in the ASL breed followed by RIR, Australorp, and FAY. Nevertheless, during the summer season, the sperm concentration was notably higher in the RIR breed, followed by AUS, ASL, and FAY, with a significant difference observed (P<0.05). The impact of seasonal variations in photoperiod and temperature on live sperm during summer showed a significantly higher percentage in the ASL breed (74.39%) compared to FAY (73.06%), AUS (65.83%), and RIR (65.2%) (P<0.05). It was concluded that seasonal photoperiod and temperature had significant effects on semen volume, sperm mortality, sperm concentration, and live sperm. In addition, roosters performed better in the winter season as compared to the summer season. Aseel and Fayoumi breeds had better performance as compared to all breeds.

***Keywords*:** Photoperiod, Semen, Exotic and indigenous Roosters

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1. INTRODUCTION

The primary reproductive attributes in a female bird, or hen, encompass fertility, hatchability, embryonic development, semen characteristics, and the age at which the hen lays her first egg. Typically, reproductive ability is evaluated through hatchability, which is subject to the influence of diverse genetic and non-genetic factors (Ren et al. 2021). The most importance is in semen evaluation of poultry breeding is a selection of breeding males (Cock) along with routine monitoring of the male reproductive performance (Cheng et al. 2002). The standard approach for assessing the quality of poultry sperm involves the evaluation of motility, viability, semen morphology, acrosomal integrity and sperm concentration. These can be improved by fertility in breeder flocks through cockerels with high sperm quality. Assisted Reproductive Technologies (ARTs), such as Artificial Insemination (AI), have the potential to enhance poultry production by maximizing the utilization of genetically superior roosters exhibiting high production and reproductive capabilities (Ren et al. 2021; Ye et al. 2022). Consequently, effective application of AI techniques relies on the careful processing, storage, and assessment of semen. The fertilization potential of semen is often determined through parameters like motility, the ratio or percentage of live to dead sperm, and morphological evaluation (Alkan et al. 2002).

The initial essential phase in Artificial Insemination (AI) is the collection of semen, and the achievement of successful collection leads to the acquisition of high-quality semen, maximizing the volume of sperm collected per

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Open Access Journal

ejaculation (Tijjani et al. 2014). The careful selection of males for reproduction holds significant importance in the poultry industry. Consequently, routine monitoring of semen quality traits becomes imperative for evaluating their reproductive capabilities (Banaszewska et al. 2015).

The time of day, length of day, and environment in which the sperm is collected all have an impact on the quality and amount of sperm produced by breeders. Semen production is generally higher in the morning and afternoon when temperatures are lower (Peters et al. 2008), and the breed of chicken also plays a role in the amount of semen produced. The quality of avian sperm has a substantial impact on fertility, which is influenced by the breed (Tabatabaei et al. 2009) and environmental factors (Elagib et al. 2012; Shanmugam et al. 2014). Semen quality can be determined by directly measuring in vitro semen characteristics such as volume, concentration, motility, viability, and morphological defects that affect male fertility (Liu et al. 2008). Fertility is the first and most essential factor in chickens, revealing the reproductive capabilities of males and females demonstrated by their ability to create chicks when paired together. Infertility occurs when an egg fails to show any signs of a developing embryo (Miazi et al. 2012). Hatchability is an economically important feature in the chicken business since it has a considerable influence on chick output (Wolc et al. 2010). Several elements influence it, including egg turning, egg weight, egg size, storage, humidity, shell strength, and genetic factors within the hens kept. The ultimate profitability of chickens is determined by the amount of eggs laid for hatching. Low fertility is thought to be primarily a problem in males, though both females and males contribute to decreased fertility.

The photoperiod is the most important environmental factor influencing reproductive seasonality (Lewis 2009; Shanmugam et al. 2014). In birds, the optimum sperm quality can be observed during long-day photoperiods, when the reproductive activity of many free-range chicken breeds is at its peak (Santiago-Moreno et al. 2012). The influence of photoperiod length on rooster sperm quality is mediated, at least in part, by changes in plasma testosterone concentration (Thurston and Korn 2000). Seasonal variations have an impact on all stages of sperm production in breeder cocks (Ahaotu et al. 2018). Seasonal fluctuation in domestic chicken sperm quality has been recorded (Santiago-Moreno et al. 2012). When broiler male breeders were subjected to a temperature of 32°C, their fertility dropped to 42%, according to Ahaotu et al. (2016). To get a high fertility rate, the quality of the sperm must be outstanding in order to assure the fertilization process. Semen parameters are an effective predictor of reproductive potential (Mothibedi et al. 2016). However, the technique for collecting and analyzing sperm is stressful, time-consuming, and expensive, and these facilities are not available in communities.

Organic farming has recently resurfaced in Pakistan, with the majority of farmers employing Indigenous and Fayoumi varieties. Aseel, a historically indigenous chicken breed, is raised as a game bird in Pakistan (Ahmad et al. 2014). The breed is known for its stamina, pugnacity, majestic gate, and tenacity in battle. Aseel is popular in rural places due to its immunity, resilience to severe circumstances, and capacity to produce organic meat (Mohan et al. 2008). The Fayoumi chicken breed was brought from Egypt as a hardy species that is particularly well suited to hot climes, with the promise of improved output, adaption, and disease resistance (Heinrichs 2007). The scale of these farms could range from 100 to 5000, implying that domestic birds will be subjected to intensive farming. As a result, it is critical to investigate the physiological studies of digestion and muscle building, with a special emphasis on reproductive organs. Male domestic fowls require longer to mature, and no legitimate investigation on domestic fowl sperm features has been recorded so far. Keeping the above mentioned in mind, the current study was aimed to compare the seasonal influence of photoperiod and temperature on the semen quality of foreign and indigenous roosters. Our study's findings are beneficial to farmers.

2. MATERIALS AND METHODS

2.1. Ethical Approval

The experiment was conducted at the Sindh Institute of Animal Health Karachi during the summer and winter seasons. Approval was granted by the Animal Welfare and Ethics Committee of Sindh Agriculture, Tandojam and Sindh Institute of Animal Health, Karachi for conducting the research trial.

2.2. Selection of Male Birds

A total number of 40 male birds (10 each of Fayoumi, Aseel, RIR, and Australorp breeds) of 35+5 weeks of age were selected for this study. For the selection of males following points was considered: Male must already have reached maturity, have no physical defects, and be healthy. The males are sexually active and free from any external parasites. Not terrified when restrained or handled.

2.3. Management Procedure

All the birds were reared in a cage system. Each male bird was kept in an individual cage, provided with a 129gm breeder ration per day per bird and water was provided *ad libitum*. The birds were reared for two seasons (summer and winter). The roosters underwent training for semen collection before the commencement of the initial experiment.

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Open Access Journal

2.4. Semen Collection and Examination

Semen was collected twice a week (Monday and Thursday), early in the morning (6:00 am), for fifteen days (2 weeks) in each season through the abdominal massage technique. Initially, the cloacal region underwent a cleaning process, followed by gentle and rapid stroking of the back and abdominal areas, specifically the tail feathers. This stimulation resulted in the tumescence (erection) of the phallus. Subsequently, semen was released through the application of slight pressure to the inverted cloaca. The collected semen samples were then placed in a sterile glass tube and promptly transported to the laboratory for the assessment of both spermatozoa quality and quantity.

Additionally, meticulous care was taken to protect the semen from cold shocks and direct light. Consistency was maintained in terms of the time, location, and the individual responsible for semen collection throughout the process. The volume of ejaculated semen was measured using a 0.1mL graduated tuberculin syringe. The assessment of semen color was conducted using a visual scoring scale ranging from 1 to 5, where 1 represents watery semen, 2 signifies watery semen with white streaks, 3 indicates medium white semen, 4 reflects thick white semen, and 5 denotes a highly viscous chalky white semen sample.

The pH of each rooster's fresh semen sample was determined utilizing a pH meter strip. Sperm mass motility was assessed on a scale from 0 to 5, with a droplet of semen applied to a microscope slide using a micropipette. The slide was then covered with a glass coverslip to ensure even distribution and prevent drying. Subsequently, the prepared slide was examined under a microscope with a magnification of x40. The motility of the semen sample was quantified as the percentage of sperm cells exhibiting autonomous movement. For semen concentration measurement, the direct cell count method was employed. In this process, a hemocytometer, typically employed for blood cell counting, was utilized. The hemocytometer includes specially designed slides with two counting chambers and two dilution pipettes. Each counting chamber measures 0.1mm and features a ruled area at the bottom, totaling 1.0mm^2, subdivided into 25 smaller squares. Semen and normal saline were diluted at a ratio of 1:19 drops. Using a micropipette, a drop of the diluted mixture was placed at both ends of the hemocytometer and allowed to settle. The loaded hemocytometer was then placed under a microscope with a magnification of 40x. The spermatozoa heads within the subdivided smaller squares at the four edges and center of the hemocytometer were counted. The individuals conducting the assessment exercised judgment to determine the average count per breed. The concentration of sperm/semen was calculated using the following formula:

$C = 50{,}000 \times N \times D$

Where C=Concentration of semen per volume (mL), N=Number of spermatozoa counted, and
D = Dilution rate.

2.5. Live sperm (%)

To determine the live sperm percentage, microscopic observations were conducted using an Eosin-Nigrosine stain. A 10µL drop of fresh semen was combined with 200µL of Eosin-Nigrosine stain, and a smear from this mixture was applied to a slide. The slide was examined under 100X magnification using oil immersion. Utilizing the oil immersion objective lens (100X) of the light microscope, approximately 200 spermatozoa were counted in various fields. The spermatozoa were then categorized as either live (characterized by a white, bright head) or dead (displaying a light or dark pink stained head).

The following parameters were assessed: a) volume of semen (in milliliters), b) color of semen, c) pH of semen, d) percentage of sperm motility, e) sperm concentration (1 billion sperm per milliliter), and f) percentage of live sperm.

2.6. Statistical Analysis

All the data was recorded on selected male birds as per experimental design. The collected data was analyzed statistically on Graph Prism 5 software and student T test was applied to compare the group.

3. RESULTS

This study was structured to comparatively assess the seasonal impact of photoperiod and temperature on the semen characteristics of both exotic and indigenous roosters. A total of 40 male birds, representing Fayoumi, Aseel, RIR, and Australorp breeds, were chosen randomly for the investigation. Parameters such as semen volume, semen color, semen pH, sperm concentration, sperm motility, and the proportions of live and dead sperm were analyzed.

3.1. Semen Volume (mL)

Fig. 1A illustrates the outcomes of the semen volume analysis. Notably, the winter season exhibited a higher semen volume compared to the summer season. Specifically, in the winter, RIR demonstrated the maximum semen volume (0.52), followed by FAY and Australop breeds with values of 0.44 and 0.43, respectively. Conversely, the Aseel breed recorded the minimum semen volume during this season. In the summer season, RIR again displayed the highest

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semen volume (0.41), followed by FAY and Australop breeds with values of 0.38 each. The Aseel breed exhibited the minimum semen volume of 0.38 in the summer. Statistical analysis revealed significant differences in semen volume concerning seasonal variations in photoperiod and temperature, as well as notable variations among breeds.

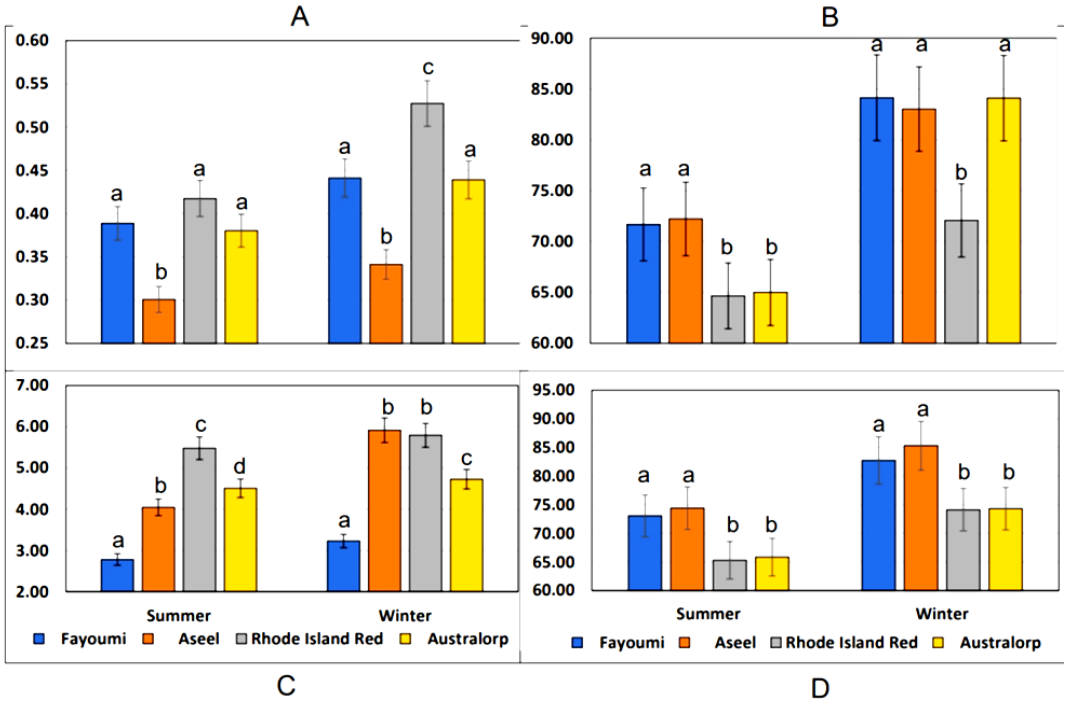

Fig. 1: Effect of seasonal photoperiod and temperature on A) semen volume (mL), B) semen motility (%), C) semen concentration (1x10⁹ sperm/mL), and D) live and dead sperm (%) in Fayoumi, Aseel, Rhode Islam Red, and Australorp chicken breeds. Bars bearing different letter under each season differ significantly (P<0.0001).

3.2. Sperm Motility

The analysis of sperm motility, as presented in Fig. 1B, reveals that the highest sperm motility occurred during the winter season, surpassing the rates observed in the summer season. Specifically, in the winter, FAY and AUS breeds exhibited the maximum sperm motility at 84.12 and 84.10%, respectively, followed closely by ASL with 83.03%. Conversely, RIR breed recorded the lowest sperm motility at 72.08% during the winter season. In the summer season, FAY and ASL breeds demonstrated superior sperm motility at 71.70 and 72.20%, respectively. In contrast, RIR and AUS breeds exhibited the minimum sperm motility at 64.65 and 64.99%, respectively. A comparative analysis of seasonal photoperiods indicates that the winter season is more favorable for all breeds, displaying statistically significant differences.

3.3. Sperm Concentration

The results of the sperm concentration analysis, illustrated in Fig. 1C, indicate that the highest sperm concentration was observed during the winter season, with ASL exhibiting the maximum concentration at 5.91, followed by RIR and AUS with values of 5.79 and 4.72, respectively. Conversely, FAY breed recorded the minimum concentration at 3.23 during the winter season. In the summer season, RIR displayed the maximum sperm concentration at 5.48, followed by AUS and ASL with values of 4.50 and 4.04, respectively. The minimum sperm concentration of 2.78 was observed in FAY during the summer season. The data highlights significant differences between seasonal variations in photoperiod and temperature. In this context, the winter season proved to be more favorable for all breeds in terms of sperm concentration.

3.4. Live Sperm Percentage

The live sperm analysis result is depicted in Fig. 1D and shows the better difference of live sperm (85.30%) observed from Aseel in winter season followed by FAY resulted (82.75%). Whereas the AUS performed live sperms (74.30%) in winter season. While the lowest result was (74.12%) in winter season. In summer season ASL had better live sperm (74.39%) followed by FAY resulted (73.06%), respectively. Whereas live sperm (65%) was observed

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Open Access Journal

from RIR and AUS in summer season respectively. In the comparison of seasonal photoperiod and temperature, winter season was better for all breeds, and a significant effect was influenced by season and temperature.

3.5. Semen Color and pH

The semen color was found medium white color. The highest number was secured by Fayoumi (3.95), followed by Australop, RIR and Aseel (3.93, 3.92 and 3.90), respectively. While higher pH was recorded in Fayoumi (6.99), followed by Australop, RIR and Aseel (6.96, 3.6.90 and 6.90), respectively. Color and pH showed a non-significant difference between seasonal photoperiod, temperature, and breeds.

4. DISCUSSION

The most important environmental factor that controls the season of reproduction is the photoperiod (Lewis 2009). In birds, optimal sperm quality is typically achieved during extended daylight periods, coinciding with elevated reproductive activity in many free-range chickens. The impact of the duration of exposure to light on the quality of rooster sperm is believed to be linked, at least partially, to an elevation in plasma testosterone concentration. Seasonal variations exert an influence on all stages of sperm production in breeding roosters, as highlighted by Ahaotu et al. in 2018. Seasonal variations in the sperm quality of domestic birds have been reported by Santiago-Moreno et al. (2012) and Sabzian-Melei et al. (2022). Semen quality may have differences due to age and decline may occur with increasing age and might be compromised due to high ambient temperature. Further Long et al. (2022) and Du et al. (2021) described that among other causes, a decrease in the weight of testicles and the production of testosterone levels may form a partial explanation for lower sperm production with increasing age. In general, as rooster's age, there is a decline in semen production capacity but to various degrees.

In a study to determine sperm quality results have shown a significant effect of photoperiod. All species produced better sperm in winter compared to summer. These present results have a resemblance with the study of Ahaotu et al. (2018). It has been reported that seasonal changes affect all stages of sperm production in parental cocks. To achieve good results breeding rate, and sperm quality should be very good to ensure a fertilization process. Examination of sperm symptoms provides an excellent indication of their reproductive capacity (Mothibedi et al. 2016). Nevertheless, the procedure of collecting and testing sperm is highly demanding, time-intensive, and costly, with the added challenge that such facilities are not accessible in rural areas. The sperm concentration has a significant effect and increasing age, breed and rooster might compromise due to length of photoperiod (increasing temperature). Such results have resemblance with the results of Sabzian-Melei et al. (2022), he added that the ambient temperature might produce heat stress and affect the semen quality index, fertility rate. Also, he further pointed out that body temperature was also increasing in exposed roosters. Increasing body temperature and exposure of males to high levels of reactive oxygen species (ROS) result in oxidative stress and that stressful condition causes sperm DNA damage. Though damaged sperm cells might be removed through apoptosis during the development and might complete the process but appear as motile sperm with damaged DNA due to a single transient heat stress.

The Improvement in Sperm motility and density may be due to spermatogenesis which is a long-term and highly ordered physiological process related to body weight, testicular size, photoperiod, season, and reproductive performance of breeder (Zhu et al. 2023). The current study found statistically significant results for sperm volume, sperm mortality, and differences between living and dead sperm. According to Wannaratana et al. (2021), the winter season was identified as the optimal period for achieving the highest sperm quality, whereas summer was deemed the least favorable. Sperm quality plays a crucial role in fertility, and various factors such as the type and strain of sperm, age, body weight of the roosters, the collection procedure, and their diet can contribute to variations in sperm levels, as noted by Mosenene (2009). Sperm testing of sperm quality and pregnancy potential can be done using a variety of options, such as live sperm analysis/ mortality percentage and behavioral testing (Lukaszewicz et al. 2008). Previous studies have shown significant differences between converts (Luaakaszewicz and Kruszynski 2003) and have shown the need for male selection in terms of the ability to apply male fertilizer. As defined in another research (Mussa et al. 2021) that the sperm motility of frozen semen was significant ($P<0.05$) for commercial breeds.

Sperm quality will be revealed through its reproductive function. An important management element needed to be monitored regularly to ensure that selected male breeding men were confident of producing the desired results. The volume of chicken pox was small, and the concentration of sperm was high enough. The volume of chicken semen was low because roosters did not have accessory glands such as mammals, so plasma sperm volume was low (Wannaratana et al. 2021). Pimprasert et al. (2023) examined humidity and ambient temperature to calculate the Temperature-Humidity Index (THI) in different seasons. They found that sperm concentration was notably higher in the winter compared to the summer and rainy seasons. Exposure to heat stress, characterized by temperatures exceeding 32±1°C with a humidity range of 55–65%, was observed to induce abnormalities in spermatogenic cells

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in chicken testes and result in decreased testosterone production. Spermatogenesis is reliant on testosterone, and an elevated level of testosterone in chickens is noted during the winter season, potentially contributing to a higher sperm concentration. Similar observations were reported by Wannaratana et al. (2021) regarding increased sperm production during the winter in pigeons, turkeys, and White Leghorns. Hence, it can be inferred that the lower ambient temperature in the winter is associated with an upsurge in sperm production. Furthermore, Wannaratana et al. (2021) observed that the lowest sperm concentration occurred not during the summer (at THI 79–82) but rather in the rainy season, characterized by the highest humidity levels. This suggests a detrimental impact of humidity, which aligns with findings in pigeons where high humidity was also shown to negatively affect sperm concentration. While studies on the influence of humidity on the reproductive performance and physiology of roosters are limited, the adverse effect of high humidity may be explained by its reduction in respiratory efficiency and moisture evaporation. Heat stress prompts panting as a mechanism for heat loss; however, elevated humidity levels hinder moisture evaporation during panting, making roosters more susceptible to heat stress. Consequently, this heightened susceptibility can lead to a decline in semen production.

In their respective studies, Du et al. (2021), Sabzian-Melei et al. (2022) and Long et al. (2022) all noted a linear decline in sperm concentration, semen volume, and the percentage of males involved in semen production with advancing age. They suggested that factors such as the rotation of semen collection, breeder weight, and testicular weight could contribute to a reduction in sperm production, especially under conditions of high ambient temperatures or temperature variations.

Differences in live sperm motility and percentage were observed across various breeds and photoperiods, aligning with findings reported by Du et al. (2021), Sabzian-Melei et al. (2022) and Long et al. (2022). The assessment of functional membrane integrity using the hypo-osmotic swelling test revealed similarity across different ages. Consequently, it appears that the age of the birds does not exert an influence on membrane functionality through alterations in the lipid and fatty acid composition of the sperm membrane.

In this study pH and color had no significant effects on all affected species in winter and summer. Samples with low concentration will appear wet or blurred. The appearance of a pink tinge is an indication of blood pollution. Yellow samples usually have a foul odor. The use of discolored, watery, or contaminated sperm, fluid, or blood will lead to reduced fertility especially if sperm is stored temporarily or for a long time (Ezike 2016).

5. Conclusion

The present study found a significant effect of seasonal photoperiod on semen volume, sperm mortality, sperm concentration, and the difference between live and dead sperm. Among the seasonal photoperiod, breeds performed better in winter season as compared to summer season for semen quality and quantity. Therefore, it is concluded that the Aseel and Fayoumi breed had better results for semen.

Author's Contribution

All the Authors equally contribute and have declared that they have no conflicts of interest to this work.

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ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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