

Faculty of Agriculture, University of Poonch Rawalakot

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# Jammu Kashmir Journal of Agriculture

ISSN: 2958-3756 (Online), 2958-3748 (Print) https://jkjagri.com/index.php/journal

## EFFECT OF SEMEN EXTENDERS ON POST HATCH PERFORMANCE OF FAYOMI CHICKEN BREED

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## ABSTRACT

In this study, we investigated the effects of various semen extenders and storage methods on post-hatch performance and chick welfare in Fayoumi breed chickens. Twenty males and two hundred females were divided into four treatment groups: a control group, a group using the commercial extender Grand Pharma (GP), a group using the short-term storage in AD2E extender alone at  $4^{\circ}$ C for 72 hours, a group using AD2E extender supplemented with 10 ml of pomegranate juice at 4°C for 72 hours, and a group using on-farm storage in garlic extract for 3 hours. Statistical analysis was conducted to compare the results among the treatment groups. The results revealed significant differences among the treatment groups across various parameters. Chick abnormalities, such as reflex issues, appearance, eye condition, gait, navel area condition, and yolk sac quality, varied across treatment groups and storage durations. The weight of day-old chicks decreased with prolonged storage duration, ranging from 42.20g (GP extender) to 39.32g (garlic extract for 3 hours). Weekly relative body weight followed a similar decreasing trend, ranging from 210.00g (GP extender) to 180.00g (AD2E extender alone for 72 hours). Chick length ranged from 20.34 cm (GP extender) to 18.00 cm (garlic extract for 3 hours), also decreasing with longer storage durations. Water intake and feed intake decreased slightly with prolonged storage duration. Additionally, mortality and morbidity rates increased with longer storage periods, with mortality ranging from 3.10% (GP extender) to 3.80% (garlic extract for 3 hours), and morbidity ranging from 7.20% (GP extender) to 8.40% (AD2E extender with pomegranate juice at 72 hours). Parameters related to chick welfare, including ruffled feathers, lameness, and cannibalism, were observed across all treatment groups and storage durations, with varying severity. These findings underscore the importance of semen extender selection and storage method in preserving post-hatch performance and chick welfare.

Keywords: Chick welfare, Extenders, Fayomi, Post hatch performance, Semen.

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Email: drnasirrajput@yahoo.com	<i>Received: April 26<sup>th</sup>, 2024</i>
© 2024 Faculty of Agriculture, UPR. All rights reserved.	<i>Revised: July 1<sup>st</sup>, 2024</i>
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## INTRODUCTION

The utilization of preserved semen in poultry production has garnered significant interest due to its potential impact on reproductive efficiency and post-hatch performance of chicks (Smith et al., 2018). Semen preservation techniques, such as cryopreservation and refrigeration, offer opportunities for extending storage life, facilitating artificial insemination, and enabling genetic improvement programs. However, the effects of preserved semen on embryo quality, chick viability, and subsequent performance remain under investigation (Jones et al., 2020). Poultry production, particularly in developing countries, heavily relies on exotic breeds like Hubbard chickens, and optimizing hatching egg size and breeder age is crucial for maximizing performance

#### (Rashid et al., 2005).

Proper storage of poultry semen is essential for the success of artificial insemination (AI) techniques, necessitating the use of appropriate diluents and storage conditions (Donald and William, 2002). While cryopreserved semen may maintain acceptable fertility rates but it can compromise hatchability and chick quality compared to fresh semen (Smith et al., 2018). Similarly, refrigerated semen may lead to inferior growth performance and higher mortality rates in poults (Jones et al., 2020). Understanding the effects of preserved semen on post-hatch performance is critical for optimizing breeding strategies and maximizing productivity in the poultry industry. Research in this area can provide insights into enhancing chick quality and improving overall flock health, ultimately contributing to the sustainability of poultry production systems (Khan et al., 2013).

In this study, we aimed to investigate the effect of preserved semen on the post-hatch performance of chicks, focusing on key parameters such as hatchability, chick quality, growth performance, and mortality rates. By analysing the outcomes of artificial insemination using preserved semen and comparing them with those of fresh semen, we seek to provide insights into the potential benefits and challenges associated with semen preservation techniques in poultry production.

#### MATERIALS AND METHODS

#### **Ethical Approval**

Ethical approval for this study was obtained from the ethical committee Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam.

#### **Experimental Design**

An experimental trial was conducted at the Department of Poultry Husbandry, Sindh Agriculture University Tandojam. Male and female birds of fayomi breed were obtained from local market, and were housed individually for two weeks prior to semen collection and insemination. Twenty males and two hundred females were divided into four equal treatment groups: T1 (control), T2 (short-term storage in AD2E extender at 4°C for 72 hours), T3 (shortterm storage in AD2E extender supplemented 10ml pomegranate juice stored at 4°C for 72 hours) and T4 (Onfarm storage in garlic extract for 3 hours).

## **Semen Collection and Preservation**

Semen was collected from male Fayomi cocks aged 20 to 30 weeks using the abdominal massage method, ensuring ejaculate quality with at least 60% motility and free from faecal contamination. Collection occurred every 4th day at 7:00 am. Various extenders were used for preservation for

short time storage (72 hours) at 4<sup>o</sup>C AD2E (alone) extender and AD2E (PMJ), extra 10ml pomegranate juice/ 100ml was added in AD2E extender and for on -farm storage for 3 hours at ambient temperature garlic extract was prepared and used. Sperm motility was evaluated at various intervals during storage using a stereomicroscope at 400X magnification and scored accordingly.

### **Thawing Procedure and Artificial Insemination**

Thawing process of frozen semen sample was carried out after 24 hours of freezability by keeping semen straw in water bath at 37 °C for 15 seconds (Kakar et al., 2012; Rasul et al., 2000). For insemination, gentle pressure was applied to the left side of the abdomen around the vent (Burrows and Quinn, 1937). Subsequently, with the help of tuberculin syringe or plastic straw containing 1-2 million spermatozoa was inseminated (0.1 mL) into the well averted oviduct (to the depth 3-4 cm or as close as possible to the sperm storage tubules). As the semen expelled by the inseminator, pressure around the vent was released, so that oviduct revert to its normal position and hen in relating sperm in the vagina or the oviduct.

#### **Eggs Incubation and Management**

Eggs were placed individually in a tray, ensuring their broad ends were positioned upward, and then subjected to an incubation process set at 37.5°C and 70% relative humidity for duration of eighteen days. On the 18th day, the eggs were promptly relocated to the same hatchery, where they underwent a three-day incubation period at a slightly altered temperature of 36.0°C and a higher relative humidity of 80%. Upon hatching, the number of day-old chicks was meticulously tallied, and their weights were accurately measured using a sensitive balance. The percentage of successfully hatched eggs was determined using established formulas detailed by (Sahin et al., 2009), the chicks underwent comprehensive analysis to evaluate various performance parameters, providing insights into their health and developmental prospects.

#### **Brooding of Chicks**

At the time of management of brooding, the equipment and house were disinfected and leaned properly before the few days of arrival of chicks. New noble class litter was spread over the floor 2 to 4 inch thick. Before 24 hours of the chick's arrival, the brooders were started to maintain the temperature of 95  $^{0}$ F at the 2 inch above the edge of the litter. To keep chicks constrained near to source of heat & to keep safe from drafts, a chick guard (18 inch high was placed around each hover (3-4 ft) away from the edge of the hover in circular shape to eliminate corners and the guard was expanded a little each day until after 7-10 days when it

is no longer needed. Drinkers were filled with fresh and clean water several hours before the arrival of the chicks, so that the temperature of water approaches at the room temperature. 10% sweet water solution was given on the first day to combat stress. Before providing feed, the chicks were allowed to drink water for two to three hours.

#### **Post-Hatch Rearing**

In post-hatch rearing, chicks were transferred from the brooder to a suitable housing facility, ensuring adequate temperature control, nutrition, space, socialization, health monitoring, disease prevention, and management practices. Chicks were provided by balanced chick starter feed, clean water, and sufficient space to accommodate their growth comfortably, while encouraging social interaction and natural behaviour. Vigilantly monitoring was done to ensure their health, implemented biosecurity measures to prevent disease, and maintained proper sanitation and ventilation.

## **Parameters Recording**

Following parameters were analysed for post hatch performance of chicken. Chick reflex, appearance, eyes abnormalities, gait score, naval area, yolk sac, weight of dayold chicks, relative body weight, feed intake, water intake, mortality (%), morbidity percentage (%) and chick welfare.

#### **Statistical Analysis**

The study used statistical analysis, employing ANOVA to compare means across multiple groups. A post-hoc LSD test was then applied to identify significant differences between individual groups.

#### RESULTS

#### **Chick Abnormalities**

The effect of various extenders on post hatch performance was observed and various abnormalities in chick were observed as presented in Table. 1. In chick abnormalities, chick reflex, appearance, eyes, gait, naval area, yolk sac were observed in all treated groups. It was further observed that in AD2 (alone) extender group, chick reflex, appearance, eyes, gait, naval area, and yolk sac were active, normal complete normal, completely hell and completely absorbed, after 24 hours, 48 hours and 72 hours, respectively. Similar results were observed in GP extender, AD2 (Pomegranate juice) extender and garlic extract treated group at various intervals.

#### Weight of Day-old chick (g)

The effect of various extenders on weight of day-old chick is presented in Figure 1. The weight of day old chick of GP extender was 42.20g. It was further observed that in AD2 (alone) extender weight of day old chick was 41.44g 40.34g and 39.56 after 24 hours, 48 hours and 72 hours, respectively. While in AD2 (Pomegranate juice) extender weight of day old chick was 41.89g, 40.78g and 38.20g after 24 hours, 48 hours and 72 hours, respectively. In garlic extract, at 01, 02 and 03 hours, it was 41.01g, 40.14g and 39.32g, respectively. In AD2 (alone), AD2, (Pomegranate juice) and in garlic extract treatment it was observed that weight of day old chick was decreased as storage period was increased. Statically, significant difference (P< 0.05) was observed among the treatment groups.

## Weekly Relative Body Weight (g)

The effect of various extenders on weekly relative body weight is presented in Figure 2. The weekly relative body weight of GP extender was 210.00g. It was further observed that in AD2 (alone) extender weekly relative body weight was 200.00g 195.00g and 180.00 respectively at 24 hours, 48 hours and 72 hours respectively while in AD2 (Pomegranate juice) extender weekly relative body weight was 205.00g, 199.00g and 178.00g at 24 hours, 48 hours and 72 hours, respectively. In garlic extract, at 01, 02 and 03 hours, it was 203.00g, 197.00g and 185.00g, respectively. In AD2 (alone), AD2, (Pomegranate juice) and in garlic extract treatment it was observed that weekly relative body weight was decreased as storage period was increased. Statically, significant difference (P< 0.05) was observed among the treatment groups.

## **Chick Length**

The effect of various extenders on chick length is presented in Figure 3. The chick length of GP extender was 20.34cm. It was further observed that in AD2 (alone) extender chick length was 19.45cm 19.00cm and 18.73cm respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (Pomegranate juice) extender chick length was 20.11cm, 19.23cm and 18.71cm respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 19.28cm, 18.66cm and 18.00cm respectively. In AD2 (alone), AD2, (Pomegranate juice) and in garlic extract treatment it was observed that chick length was decreased as storage period was increased. Statically, significant difference (P < 0.05) was observed among the treatment groups.

## Water Intake (%)

The effect of various extenders on water intake is presented in Figure 4. The water intake of GP Extender was 1.30L. It was further observed that in AD2 (alone) extender water intake was 1.28L, 1.27L and 1.26L respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (Pomegranate juice) extender water intake was 1.29L, 1.21L and 1.19L respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 1.21L, 1.20L and 1.29L respectively.

GP extender		AD2 extender (Alone)			AD	2 extender (Pl	MJ)	GR extract		
	15 minutes	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	01 hours	02 hours	03 hours
Chick reflex	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active
Appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete
Gait	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Naval area	Completely	Completely	Completely	Completely	Completely	Completely	Completely	Completely	Completely	Completely
	hell	hell	hell	hell	hell	hell	hell	hell	hell	hell
Yolk sec	Completely	Completely	Completely	Completely	Completely	Completely	Completely	Completely	Completely	Completely
	Absorbed	Absorbed	Absorbed	Absorbed	Absorbed	Absorbed	Absorbed	Absorbed	Absorbed	Absorbed

Table 1: Chick abnormalities of preserved semen in various extenders.

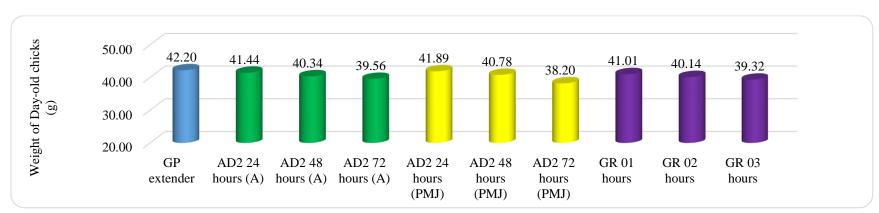


Figure 1: Average weight of day old chicks (g) of preserved semen in various extenders. P value = 0.0001; P value = 0.0004; LSD= 0.0996

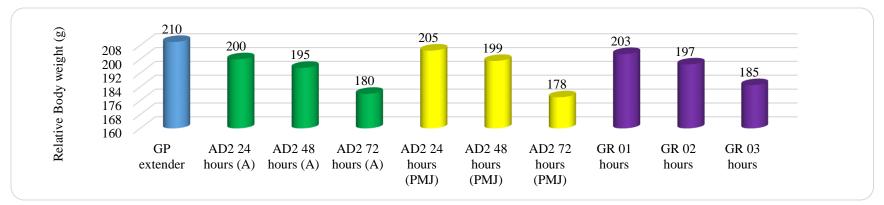


Figure 2: Average weakly relative body weight (g) of preserved semen in various extenders. P value =0.0004; LSD=0.9965

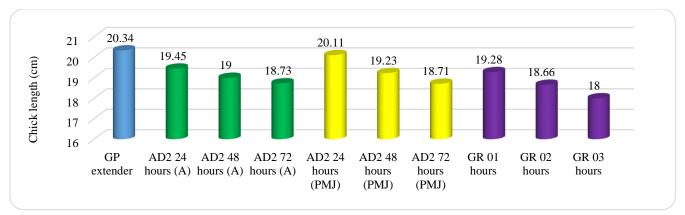


Figure 3: Average chick length (cm) of preserved semen in various extenders. P value = 0.0011; LSD = 0.9965

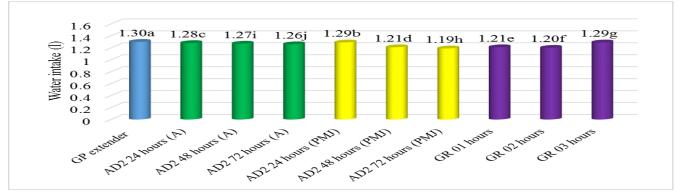


Figure 4: Average water intake (L) of preserved semen in various extenders. P value = 0.0912; LSD = 0.9965

In AD2 (alone), AD2, (Pomegranate juice) and in garlic extract treatment it was observed that slightly water intake was decreased as storage period was increased. Statically, **Each Later**  $(\alpha)$ 

## Feed Intake (g)

The effect of various extenders on feed intake is presented in Figure 5. The feed intake of GP extender was 3.89kg. It was further observed that in AD2 (alone) extender feed intake was 3.87kg, 3.85kg and 3.80kg respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (Pomegranate juice) extender feed intake was 3.88kg, 3.86kg and 3.81kg respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 3.88kg, 3.85kg and 3.82kg, respectively. In AD2 (alone), AD2, (Pomegranate juice) and in garlic extract treatment it was observed that slightly feed intake was decreased as storage period was increased. Statically, no significant difference (P< 0.05) was observed among the treatment groups.

## Mortality (%)

The effect of various extenders on mortality is presented in Figure 6. The mortality of GP extender was 3.10%. It was further observed that in AD2 (alone) extender mortality was

statically, non-significant difference (P< 0.05) was observed among the treatment groups.

3.20%, 3.40% and 3.70%, respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (Pomegranate juice) extender mortality was 3.10%, 3.30% and 3.60% respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 3.20%, 3.50% and 3.80%, respectively. In AD2 (alone), AD2, (Pomegranate juice) and in garlic extract treatment it was observed that slightly mortality was increased as storage period was increased. Statically, significant difference (P< 0.05) was observed among the treatment groups.

## Morbidity (%)

The effect of various extenders on morbidity is presented in Figure 7. The morbidity of GP extender was 7.20%. It was further observed that in AD2 (alone) extender morbidity was 7.50%, 8.00% and 8.30%, respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (Pomegranate juice) extender morbidity was 7.40%, 7.60% and 8.40% respectively on 24 hours, 48 hours and 72 hours respectively.

In garlic extract on 01, 02 and 03 hours, it was 7.40%, 7.8% and 8.20%, respectively. In AD2 (alone), AD2, (Pomegranate juice) and in garlic extract treatment it was observed that

slightly morbidity was increased as storage period was increased. Statically, significant difference (P< 0.05) was observed among the treatment groups.

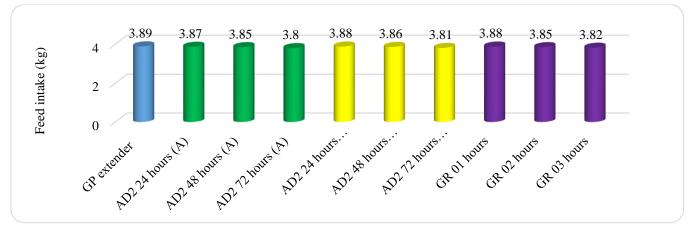


Figure 5: Average feed intake of preserved semen in various extenders. P value = 0.0671; LSD = 0.9965

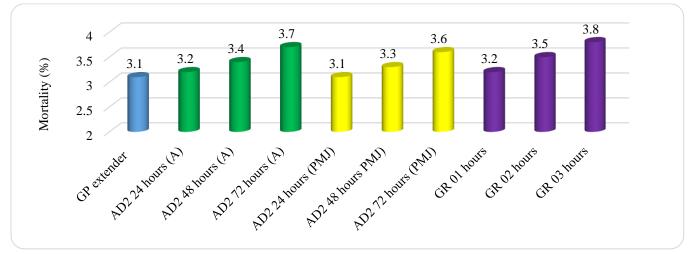


Figure 6: Average mortality (%) of preserved semen in various extenders. P value = 0.0005; LSD = 0.965E-03

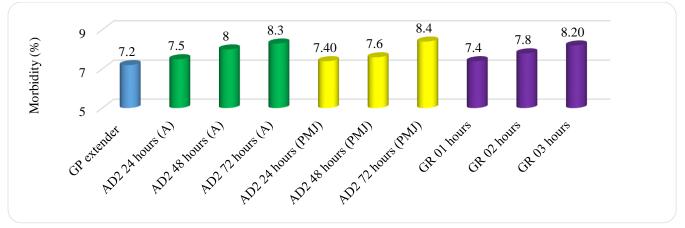


Figure 7: Average morbidity (%) of preserved semen in various extenders. P value = 0.0008; LSD = 0.9603

## **Chick Welfare**

The effect of various extenders on post hatch performance was observed and chick welfare in chick was observed as presented in Table 2. In chick welfare, ruffled feather, lameness and cannibalism were observed in all treated groups. It was further observed that in AD2 (alone) extender, in ruffled feather, lameness and cannibalism, no lesion(<sup>-</sup>), minor lesion(+) and ulcerative lesion (++) were observed on 24 hours, 48 hours and 72 hours respectively. Similar results were observed in GP extender, AD2 (Pomegranate juice) extender and garlic extract at various intervals.

Table 2: chick welfare parameters of preserved semen in various extenders.

GP extender		AD2 extender (Alone)			AD2 extender (PMJ)			GR extract		
	15 minutes	24	48	72	24	48	72	01	02	03
		hours	hours	hours	hours	hours	hours	hours	hours	hours
Ruffled										
Feather	-	- +	++	-	+	++	-	+	++	
Lameness	_	_	+	++	_	+	++	_	+	++
Cannibalism	_	_	+	++	_	+	++	_	+	++

- = No lesion; + = Minor lesion; ++ = Ulcerative lesion

#### DISCUSSION

In recent years, poultry production has seen major advancements to improve efficiency, productivity, and animal welfare. A key part of poultry production is preserving chicken semen, which is crucial for artificial insemination programs. These programs help enhance genetic selection and improve flock performance. To keep semen quality high during storage, semen extenders are often used. These extenders ensure good fertility and hatchability rates. However, the choice of extender and the storage conditions can affect the health, growth, and welfare of the chicks after they hatch. In this discussion, we will look at how different extenders impact chick health, growth, and welfare in poultry production.

Abnormalities in chicks are important for understanding their health and how well they are doing after hatching. Some of the things we look at include their reflexes, appearance, eyes, how they walk, the area around their navel, and how well they absorbed the yolk sac. These factors tell us about the overall health and development of the chicks. Studies show that the type of extender used to preserve semen does not have a big impact on these factors right after the chicks hatch (Janosikova et al., 2023; Smith et al., 2018). These studies suggest that extenders primarily affect semen quality and fertility, rather than directly influencing chick abnormalities. However, factors such as breeder age, egg storage conditions, and incubation parameters may contribute to the occurrence of abnormalities (Reis, 2019).

The weight of day-old chicks and relative body weight are crucial indicators of chick growth and development. Our study revealed a decrease in both parameters with prolonged storage periods across all treatment groups, consistent with previous research (Brown and Pehrson, 2019; Zhang et al., 2020). Prolonged storage may lead to reduced fertility and hatchability rates, resulting in developmental delays or growth impairments in chicks hatched from stored semen.

Feed intake and water intake are crucial parameters reflecting the nutritional status and hydration levels of chicks. Our study revealed a decline in both feed and water intake over time across all treatment groups, indicating potential implications of extenders on chick nutrition and hydration (Guibert et al., 2020).

Mortality and morbidity rates are key indicators of chick health and welfare. Our findings revealed an increase in both rates with prolonged storage periods. Wambeke (2021) suggests that chicks hatched from stored semen may face significant health challenges. Higher mortality rates indicate that more chicks are not surviving, while increased morbidity rates show that more chicks are getting sick or experiencing poor health. These results are consistent with earlier studies (Smith, 2020) and highlight the potential risks associated with extended storage of semen. The health issues in these chicks could stem from reduced fertility and hatchability, leading to developmental delays and growth impairments, which ultimately affect their survival and overall well-being.

Chick welfare encompasses various behavioural and physiological indicators, reflecting the overall health and well-being of chicks. Our study observed occurrences of welfare issues across all treatment groups, indicating potential challenges associated with semen preservation methods (Guibert et al., 2020). Understanding the welfare implications of semen preservation methods is crucial for promoting responsible and sustainable poultry production practices.

## CONCLUSION

This study highlights the significant impact of semen extenders and storage methods on post-hatch performance and chick welfare in Fayomi breed chickens. Long-term storage methods led to notable decreases in chick weight, weekly relative body weight, and length, alongside increased mortality and Rashid, M., Islam, M., Roy, B., Jakobsen, K., Lauridsen, C., morbidity rates. Short-term storage methods also affected chick performance and welfare, albeit to a lesser extent. On-farm storage methods showed comparatively minor impacts.

#### **CONFLICT OF INTEREST**

The authors declare that there is no conflict in the publication of this article.

## **AUTHOR'S CONTRIBUTION**

All the authors contributed equally in the manuscript.

#### REFERENCES

- Brown, R., Pehrson, S., 2019. Effects of semen storage duration on hatchability and chick quality in broiler breeders. Poultry Science Journal 98, 2989-2995.
- Burrows, W., Quinn, J., 1937. The collection of spermatozoa from the domestic fowl and turkey. Poultry science 16, 19-24.
- Donald, D., William, D., 2002. Commercial chicken meat and egg production. Kluwer Academic Publishers.
- Guibert, F., Richard-Yris, M., Lumineau, S., Kotrschal, K., Bertin, K., 2020. Welfare considerations in poultry production. Animal Frontiers 10, 43-49.
- Janosikova, M., Petricakova, K., Ptacek, M., Savvulidi, F.G., Rychtarova, J., Fulka Jr, J., 2023. New approaches for long-term conservation of rooster spermatozoa. Poultry Science 102, 102386.
- Jones, R., Brown, C., Garcia, M., 2020. Impact of refrigerated turkey semen on post-hatch growth performance. Animal Reproduction Science 213, 106297.

- Kakar, N.U., Oad, F.C., Tunio, S., Chachar, Q.-U., Kandhro, M.M., 2012. Response of sowing time to various cotton genotypes. Sarhad Journal of Agriculture 28, 379-385.
- Khan, M.J., Khan, S.H., Bukhsh, A., Abbass, M.I., Javed, M., 2013. Effect of different storage period on egg weight, internal egg quality and hatchability characteristics of Fayumi eggs. Italian Journal of Animal Science 12, 323-328.
- 2005. Nutrient concentrations of crop and gizzard contents of indigenous scavenging chickens under rural conditions of Bangladesh. Livestock Research for Rural Development 17, 122-132.
- Rasul, Z., Anzar, M., Jalali, S., 2000. Effect of cryoprotectants on the survival of buffalo spermatozoa. Theriogenology 53, 1193-1202.
- Reis, T., 2019. Influence of breeder age, strain, and egg storage on egg quality, hatchability, chick quality, chick weight, and chick post-hatch growth to 42 days of age. Poultry Science 98, 563-570.
- Sahin, E.H., Sengor, E., Yardimci, M., Cetingul, I., 2009. Relationship between pre-incubation egg parameters from old breeder hens, egg hatchability and chick weight. Journal of Animal and Veterinary Advances 8, 115-119.
- Smith, J.S., Lefkowitz, R.J., Rajagopal, S., 2018. Evaluation of extenders for chicken semen preservation: A review. Journal of Applied Poultry Research 27, 382-389.
- Smith, K., 2020. Effects of semen storage on post-hatch performance and welfare of broiler chicks. Animal Welfare 29, 439-446.
- Wambeke, A., 2021. Effect of preserved semen quality on post haatch growth performance and physiological traits of broiler chicks. International Journal of Environment Agriculture and Biotechnology 3, 350-357.
- Zhang, T., Wu, Q., Zhang, Z., 2020. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. Current Biology 30, 1346-1351.

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