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Research Article

Impact of Preserved Semen on Fertility and Hatchability of Fayomi Hen Eggs

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Abstract

This study aimed to evaluate the effects of various extenders on fertility, hatchability, and embryonic development in Fayomi breed chickens. Twenty males and two hundred females were divided into four treatment groups: control, grand pharma (gp) commercial based extender, short-term storage in AD2E (alone) extender at 4°C for 72 hours AD2E extender supplemented 10 ml pomegranate juice at 4°C for 72 hours, and on-farm storage in garlic extract for 3 hours. Each treatment group consisted of five males and fifty females. Statistical analysis was conducted to compare the results among treatment groups. Egg production rates ranged from 74% to 78%, with no significant difference observed among treatment groups ($P > 0.05$). Yolk weight, albumin weight, shell weight, and egg weight showed no significant differences among groups ($P > 0.05$). However, fertility rates ranged from 20.05% to 77.43%, with significant differences observed among treatment groups ($P < 0.05$). Similarly, chick hatchability ranged from 17.76% to 78.51%, and embryonic mortality ranged from 21.71% to 81.27%, both showing significant differences among treatment groups ($P < 0.05$). Chick liveability remained high across all groups, with no significant differences observed ($P > 0.05$). Hatch window ranged from 24 to 33 hours, showing no significant differences among groups ($P > 0.05$). Overall, egg quality parameters and chick liveability were unaffected by the extenders, fertility, chick hatchability, and embryonic mortality were significantly influenced by storage methods. Further optimization of preservation methods may be warranted to improve these outcomes in Fayomi breed chickens.

Keywords: *Semen; Fertility; Hatchability; Artificial insemination; Eggs*

Introduction

Artificial insemination (AI) plays a pivotal role in enhancing poultry production by facilitating the widespread use of genetically superior roosters, leading to increased productivity. The advancements in AI techniques have enabled the rapid dissemination

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of genetic material, benefiting genetic enhancement and the control of venereal diseases (Getachew, 2016). Genetic selection and improved nutritional management have resulted in steady increases in poultry production rates, though with some adverse effects on reproduction (Bramwell, 2002). The surge in chicken meat consumption has prompted a focus on boosting layer production, with assisted reproductive technologies (ARTs), including AI, aiding in the broader utilization of genetically superior roosters. AI offers advantages such as efficient utilization of males compared to natural mating, thereby reducing costs (Benoff *et al.*, 1981).

In broiler breeder management, where fertility may decline due to selection for growth and compatibility issues, AI helps address these problems (Reddy, 1995). Increasing fertility is crucial for obtaining more chicks and table eggs, with AI offering advantages over natural mating in terms of settable eggs, overall fertility, and hatchability (Brillard, 2003). Factors crucial for AI success include management of breeder stock, quantity and quality of semen, timing, depth and dosage of semen (King et al., 2002). AI allows for efficient fertilization, with semen from 6-8 males inseminating 150-170 females (Habibullah, 2015). Optimum fertility depends on factors such as insemination interval, number of spermatozoa, semen quality, dosage, and timing (Saleh *et al.*, 2012). Sperm storage duration varies among species, with chickens capable of storing sperm for up to three weeks, while turkeys can maintain sperm for 10 weeks. The adaptive significance of prolonged sperm storage in birds remains unclear (Khillare *et al.*, 2018).

The Fayomi breed of poultry possesses unique characteristics that make it particularly suitable for small-scale and backyard poultry farming systems. Known for its resilience, adaptability to diverse environments, and desirable meat and egg production traits, Fayomi hens are valued assets in sustainable poultry production (King *et al.*, 2002). However, despite its potential, there exists a significant gap in our understanding of how the preservation of semen impacts the fertility and hatchability of Fayomi hen eggs. Despite the significant advancements in poultry breeding, there remains a lack of comprehensive research on the impact of preserved semen on the fertility and hatchability of Fayomi hen eggs. However, this study is design to understand how semen preservation techniques affect fertility rates and hatchability of their eggs.

Methodology

Experimental design and selection of birds

An experimental trial was conducted at the Department of Poultry Husbandry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam. Male and female of fayomi breed for this research were obtained from local market, healthy male and female 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 (grand pharma GP extender), T2 (long-term storage in N-Methyl acetamide extender at -196°C for one month), T3 (short-term storage in AD2E extender alone and short-term storage in AD2E extender at supplemented 10ml pomegranate juice stored 4°C for 72 hours and T4 (on-farm storage in garlic extract for 3 hours).

Semen storage and preservation

Semen was obtained from male Fayomi rooster aged 20 to 30 weeks through abdominal

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massage method, ensuring ejaculate quality with a minimum of 60% motility and devoid of fecal contamination. Collection took place every fourth day at 7:00 am. Various extenders were employed for preservation purposes: AD2E extender for short-term storage (72 hours at 4°C), and AD2E extender supplemented with an extra 10ml of pomegranate juice per 100ml of extender (PMJ) for short-term storage. Additionally, for on-farm storage lasting three hours at ambient temperature, a garlic extract was prepared and utilized.

Thawing procedure 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 (Kaka *et al.*, 2012).For insemination gentle pressure was applied to the left side of the abdomen around the vent (Burrows & 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.

Collection and storage of eggs

After one week of artificial insemination, Eggs were collected daily from each treatment group, marked and stored on paper trays with broad end up. Egg grading was performed to separate best quality eggs. Collected eggs stored in a cool room at 18°C to 20°C and relative humidity of 70% – 85% for a week for days five earlier the eggs were transferred to the incubator in hatchery for incubation.

Fertility assessment

A cabinet type incubator (Khul USA, Model AZYS 800-110) was used to set the eggs from each groups in the hatchery. The eggs were set at 27 - 39°C temperature and 55 – 56% of relative humidity for eighteen days; in incubator through 90° automatically the eggs were also turned**.** To assess the fertility of eggs candling was done on 7th and 14th day. After eighteen days of eggs at setter, eggs were transferred to hatcher for hatching. The 29 - 40°C of the temperature and 70–75% of relative humidity of hatcher was increased from day 19th to time of hatching .After hatching, hatched chicken were analysed for post hatch performance parameters.

Procedure of recording the parameters

Following parameters were analysis for fertility and hatchability

Egg production (%)

Eggs were collected twice in day and stored at 18°C temperature for six days. Eggs production was calculated by following formula**:**

$$\text{Egg production (\%)} = \frac{\text{Total no: of Eggs Production}}{\text{Total no. of birds reared}} \times 100$$

Yolk weight (g)

This was determined by carefully separating albumen from yolk after egg break out and weighed using electronic weighing balance.

Albumen weight (g)

This was obtained by finding the difference of 100 and the sum of percentage yolk weight

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and percentage shell weight.

Albumen weight (g) = [100 – % yolk weight + % shell weight]

Egg shell weight (g)

Egg shell weight was taken after the shell had been air dried and then weighed using electronic weighting balance.

Egg fertility (%)

Fertility is the natural capability to produce offspring. The fertility was calculated by following formula.

$$\text{Fertility (\%)} = \frac{\text{Number of fertile eggs}}{\text{Total no of eggs}} \times 100$$

Chick hatchability (%)

Chick hatchability was calculated by following formula:

$$\text{Hatchability (\%)} = \frac{\text{No of chicks}}{\text{No of hatching Eggs}} \times 100$$

Chick liveability

Chick liveability was calculated by following formula:

$$\text{Livability (\%)} = \frac{\text{No of live birds at specified time}}{\text{Total no. of birds}} \times 100$$

Embryonic mortality percentage

Embryonic mortality percentage was calculated by following formula:

$$\text{Embryonic mortality (\%)} = \frac{\text{No of dead embryo}}{\text{No of hatching embryo}} \times 100$$

Hatch window

Hatch window was calculated by using the time interval from the first to the last chicks hatching in hatcher.

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 group.

Results

Egg production

The effect of various extenders on egg production is presented in Figure 1. The egg production of GP extender was 78.00%. It was further observed that in AD2 (alone) extender egg production was 76.00%, 75.00% and 74.00 respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (PMJ) extender egg production was 77.00%, 76.00% and 75.00% respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 77.00%, 76.00% and 75.00% respectively. In AD2 (alone), AD2, (PMJ) and in garlic extract treatment it was observed that egg production was slightly decreased as storage period was increased. Statically, non-significant difference ($P< 0.05$) was observed among the treatment groups.

Yolk weight

The effect of various extenders on yolk weight is presented in Figure 2. The yolk weight of GP extender it was 13.18%. It was further observed that in AD2 (alone) extender yolk weight was 13.00%, 12.50% and 12.50 respectively on 24 hours, 48 hours and 72 hours

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respectively while in AD2 (PMJ) extender yolk weight was 13.18%, 13.00% and 12.70% respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 12.75%, 12.50% and 12.25% respectively. In AD2 (alone), AD2, (PMJ) and in garlic extract treatment it was observed that yolk weight was slightly decreased as storage period was increased. Statically, among the groups of treatment there was the observation of non-significant difference (P< 0.05).

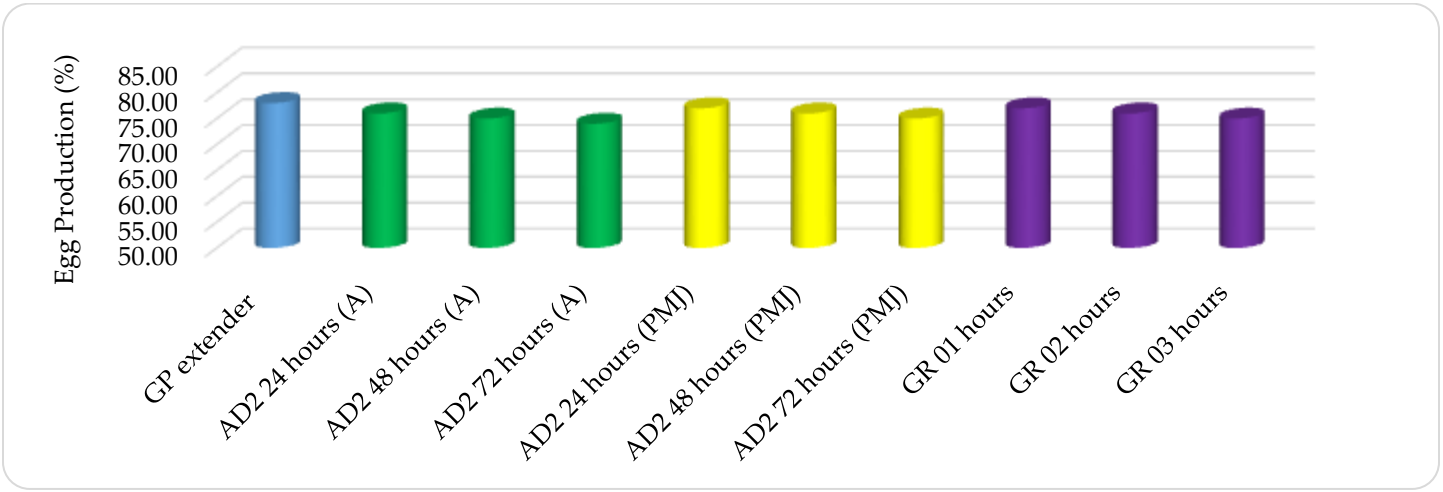

Figure 1. Average egg production of various extenders used in fayomi breed semen.

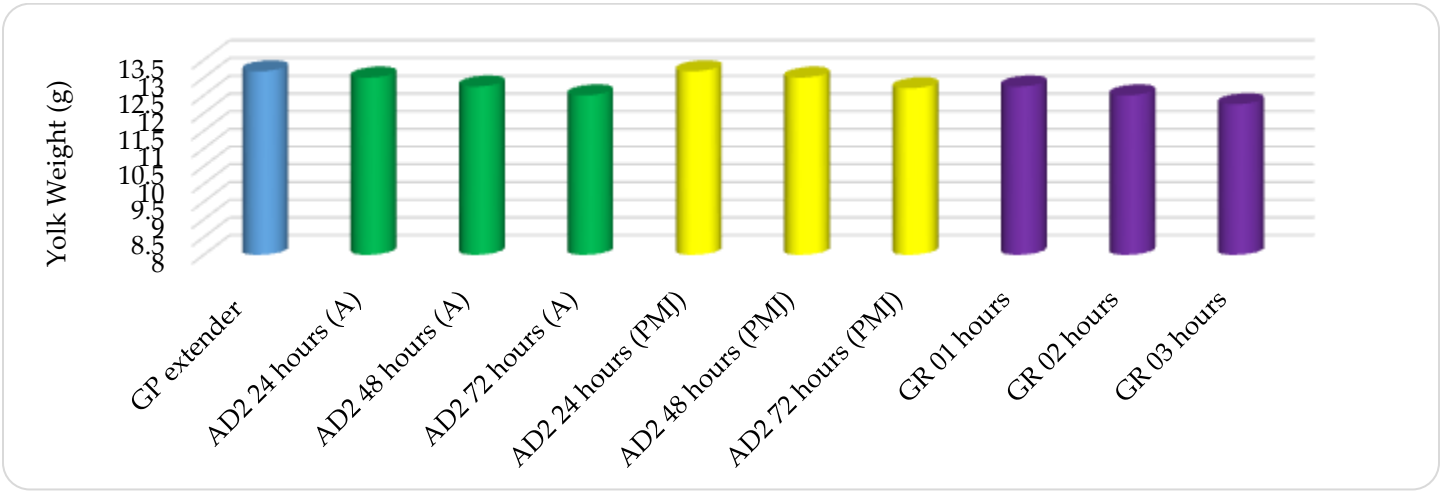

Figure 2. Average Yolk weight of various extenders used in fayomi breed semen.

Albumin weight

The effect of various extenders on albumin weight is presented in Figure 3. The albumin weight of GP extender was 22.25%. It was further observed that in AD2 (alone) extender albumin weight was 20.75%, 20.50% and 20.50% respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (PMJ) extender albumin weight was 21.00%, 21.13% and 20.13% respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 21.25%, 21.25% and 21.00% respectively. In AD2 (alone), AD2, (PMJ) and in garlic extract treatment it was observed that albumin weight was slightly decreased as storage period was increased. Statically, among the groups of

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treatment there was the observation of non-significant difference (P< 0.05).

Shell weight

The effect of various extenders on shell weight is presented in Figure 4. The shell weight of GP extender was 9.55%. It was further observed that in AD2 (alone) extender shell weight was 10.25%, 10.00% and 10.75% respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (PMJ) extender shell weight was 10.35%, 10.13% and 10.90% respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 11.25%, 10.25% and 10.75% respectively. In AD2 (alone), AD2, (PMJ) and in garlic extract treatment it was observed that shell weight was slightly decreased as storage period was increased. Statically, among the groups of treatment there was the observation of non-significant difference (P< 0.05).

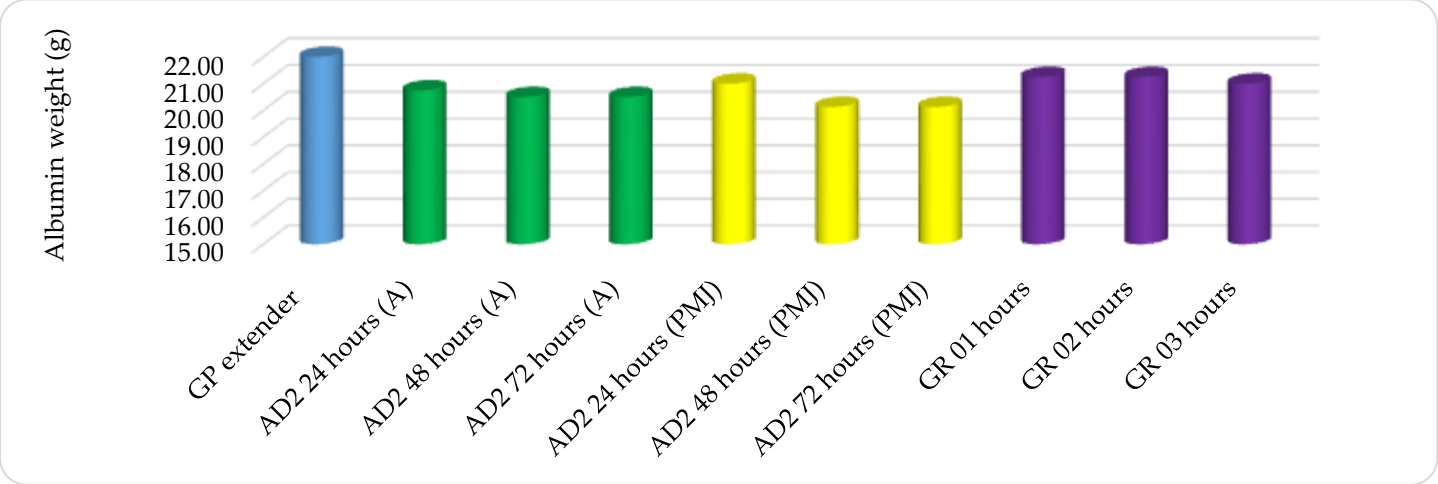

Figure 3. Average albumin weight of various extenders used in fayomi breed semen.

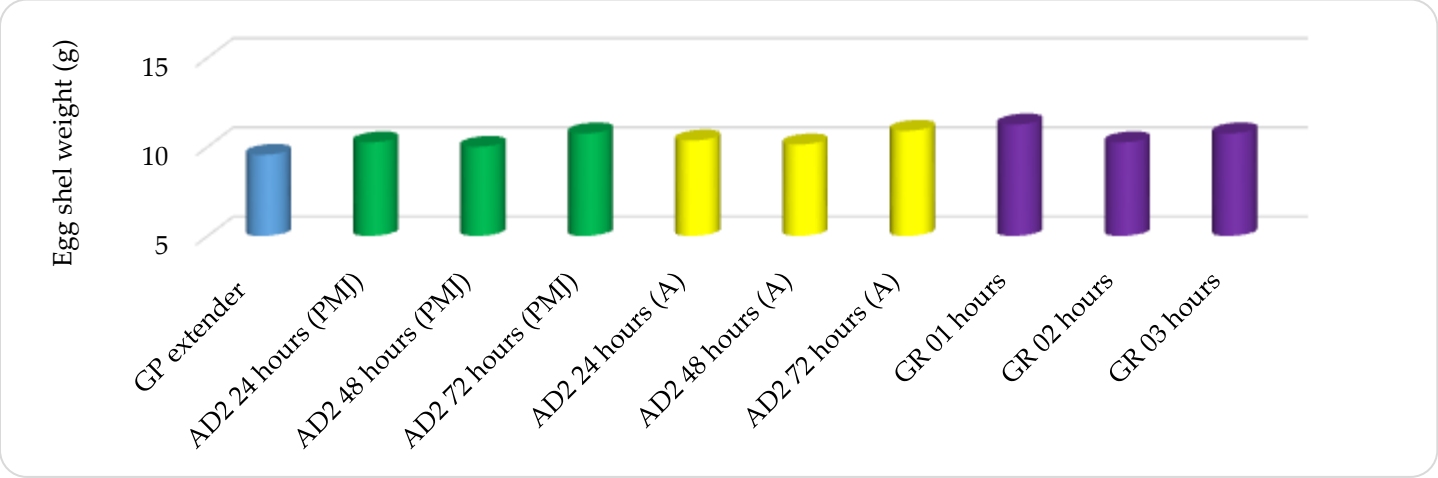

Figure 4. Average shell weight of various extenders used in fayomi breed semen.

Egg weight

The effect of various extenders on egg weight is presented in Figure 5. The egg weight of GP extender was 45.30%. It was further observed that in AD2 (alone) extender egg weight was 44.00%, 43.25% and 43.75% respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (PMJ) extender egg weight was 44.52%, 43.25% and 43.72%

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respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 45.25%, 44.00% and 44.00% respectively. In AD2 (alone), AD2, (PMJ) and in garlic extract treatment it was observed that egg weight was slightly decreased as storage period was increased. Statically, among the groups of treatment there was the observation of non-significant difference (P< 0.05).

Fertility

The effect of various extenders on fertility is presented in Figure 6. The fertility of GP extender was 77.43%. It was further observed that in AD2 (alone) extender fertility was 70.25%, 57.17% and 23.73% respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (PMJ) extender fertility was 76.23%, 68.34% and 39.78% respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 72.95%, 49.66% and 20.05% respectively. In AD2 (alone), AD2, (PMJ) and in garlic extract treatment it was observed that fertility was decreased as storage period was increased. Statically, among the groups of treatment there was the observation of significant difference (P< 0.005).

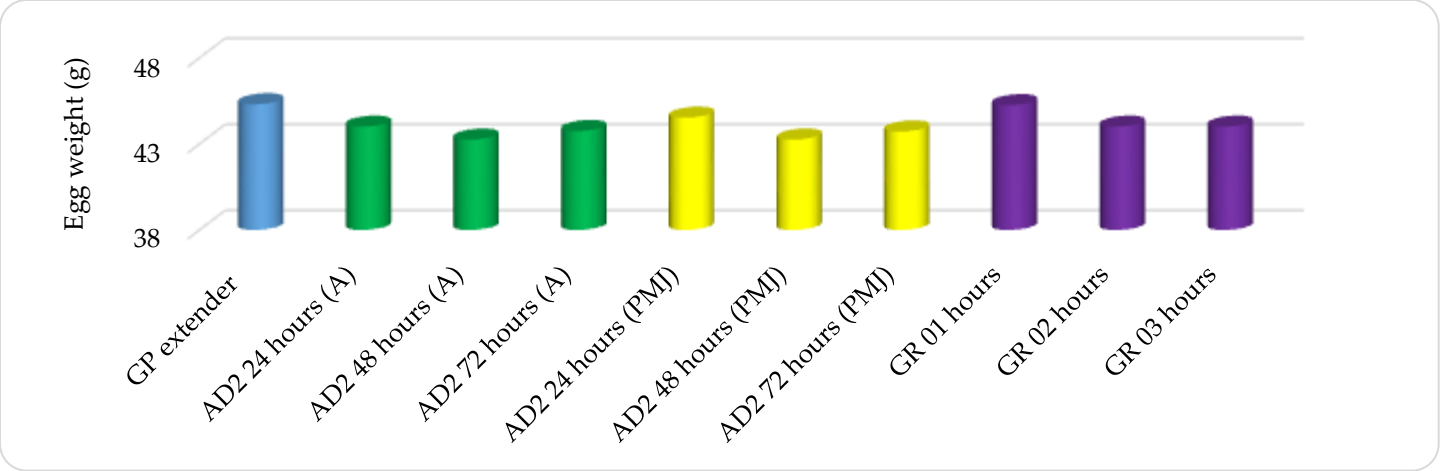

Figure 5. Average egg weight of various extenders used in fayomi breed semen.

Chick hatchability

The effect of various extenders on chick hatchability is presented in Figure 7. The chick hatchability of GP extender was 78.51%. It was further observed that in AD2 (alone) extender chick hatchability was 73.01%, 62.15% and 21.81% respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (PMJ) extender chick hatchability was 76.45%, 68.51% and 37.80% respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 72.95%, 53.35% and 17.76% respectively. In AD2 (alone), AD2, (PMJ) and in garlic extract treatment it was observed that chick hatchability was decreased as storage period was increased. Statically, among the groups of treatment there was the observation of significant difference (P< 0.005).

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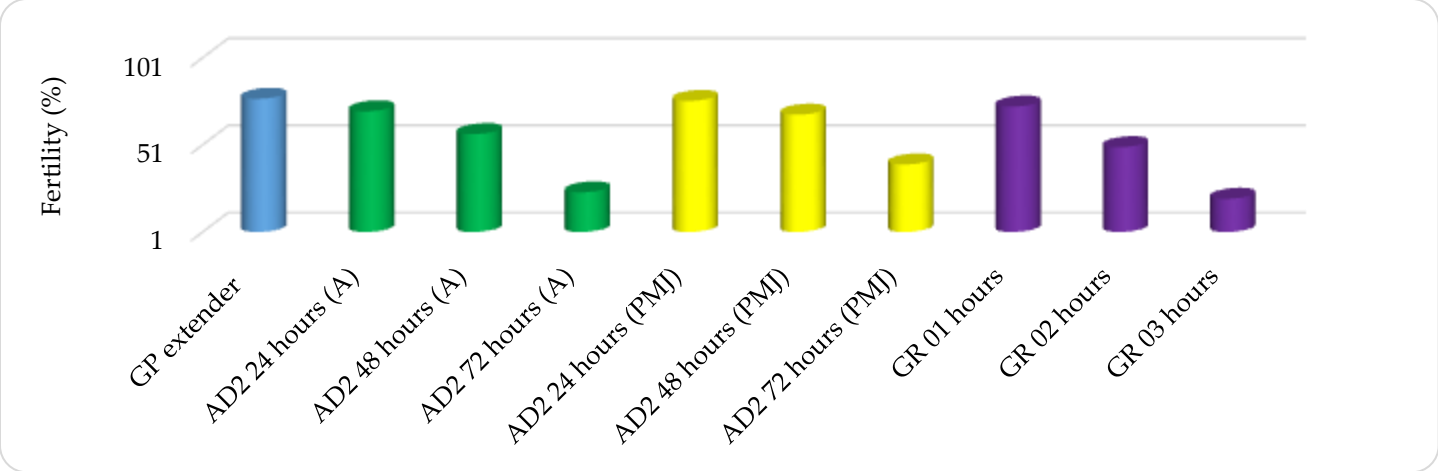

Figure 6. Average fertility of various extenders used in fayomi breed semen.

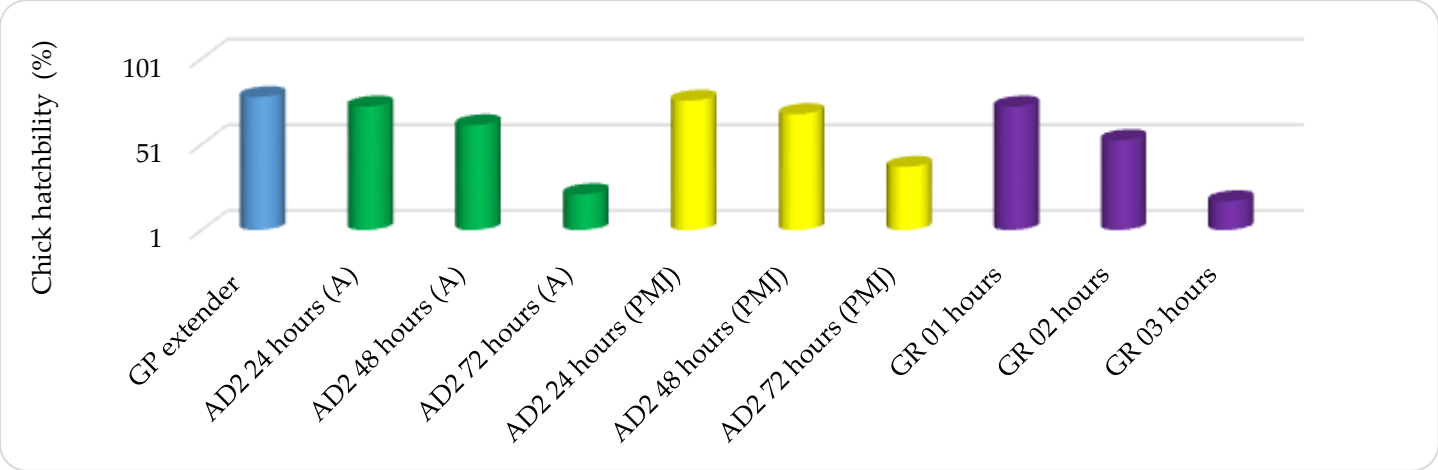

Figure 7. Average chick hatchability of various extenders used in fayomi breed semen.

Chick liveability

The effect of various extenders on chick liveability is presented in Figure 8. The chick liveability of GP extender was 100.00%. It was further observed that in AD2 (alone) extender chick liveability was 100.00%, 97.25% and 95.50% respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (PMJ) extender chick liveability was 100.00%, 100.00% and 98.25% respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 99.25%, 98.00% and 96.50% respectively. In AD2 (alone), AD2, (PMJ) and in garlic extract treatment it was observed that chick liveability was slightly decreased as storage period was increased. Statically, among the groups of treatment there was the observation of non-significant difference ($P< 0.05$).

Embryonic mortality

The effect of various extenders on embryonic mortality is presented in Figure 9. The embryonic mortality of GP extender was 21.71%. It was further observed that in AD2 (alone) extender embryonic mortality was 26.99%, 37.84% and 78.19% respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (PMJ) extender embryonic

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mortality was 23.77%, 31.66% and 60.22% respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 23.12%, 44.12% and 81.27% respectively. In AD2 (alone), AD2, (PMJ) and in garlic extract treatment it was observed that embryonic mortality was increased as storage period was increased. Statically, among the groups of treatment there was the observation of significant difference (P< 0.05).

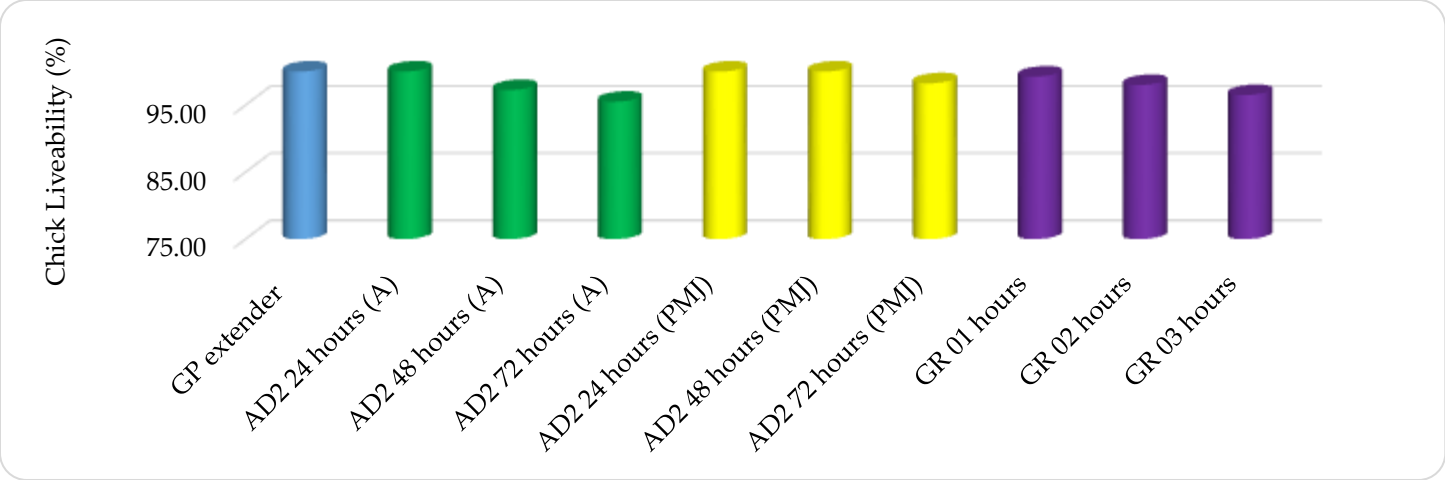

Figure 8. Average chick liveability of various extenders used in fayomi breed semen.

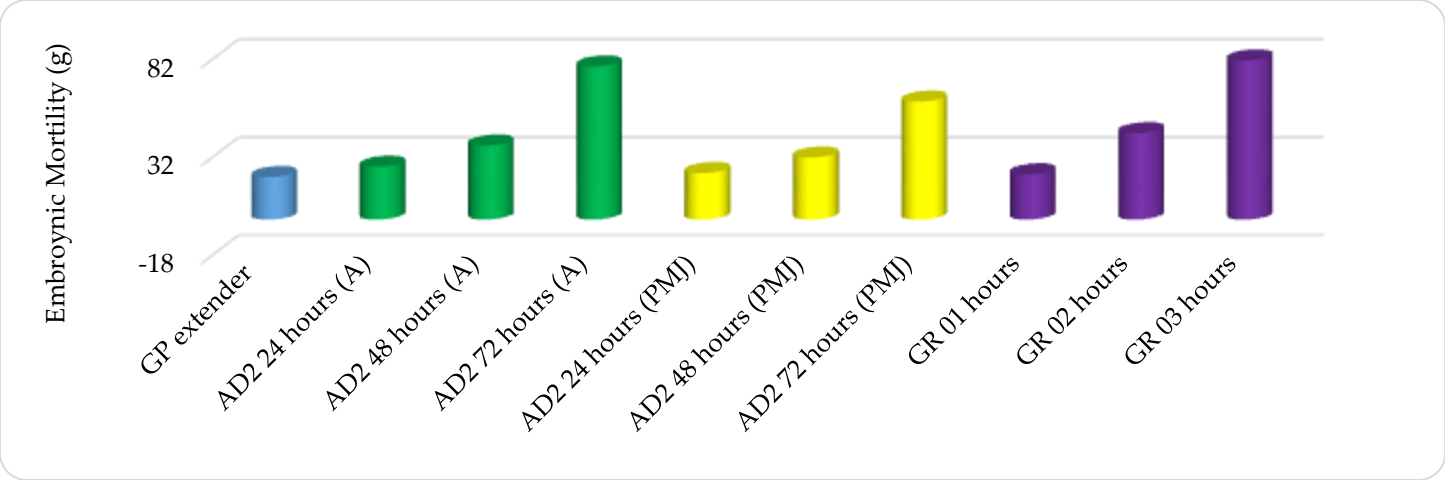

Figure 9. Average embryonic mortality of various extenders used in fayomi breed semen.

Hatch window

The effect of various extenders on hatch window is presented in Figure 10. The hatch window of GP extender was 24.00h. It was further observed that in AD2 (alone) extender hatch window was 27.00h, 29.00h and 32.00h respectively on 24 hours, 48 hours and 72 hours respectively while in AD2 (PMJ) extender hatch window was 26.00h, 29.00h and 31.00h respectively on 24 hours, 48 hours and 72 hours respectively. In garlic extract on 01, 02 and 03 hours, it was 27.00h, 30.00h and 33.00h respectively. In AD2 (alone), AD2, (PMJ) and in garlic extract treatment it was observed that hatch window was increased as storage period was increased. Statically, among the groups of treatment there was the observation of non-significant difference (P< 0.05).

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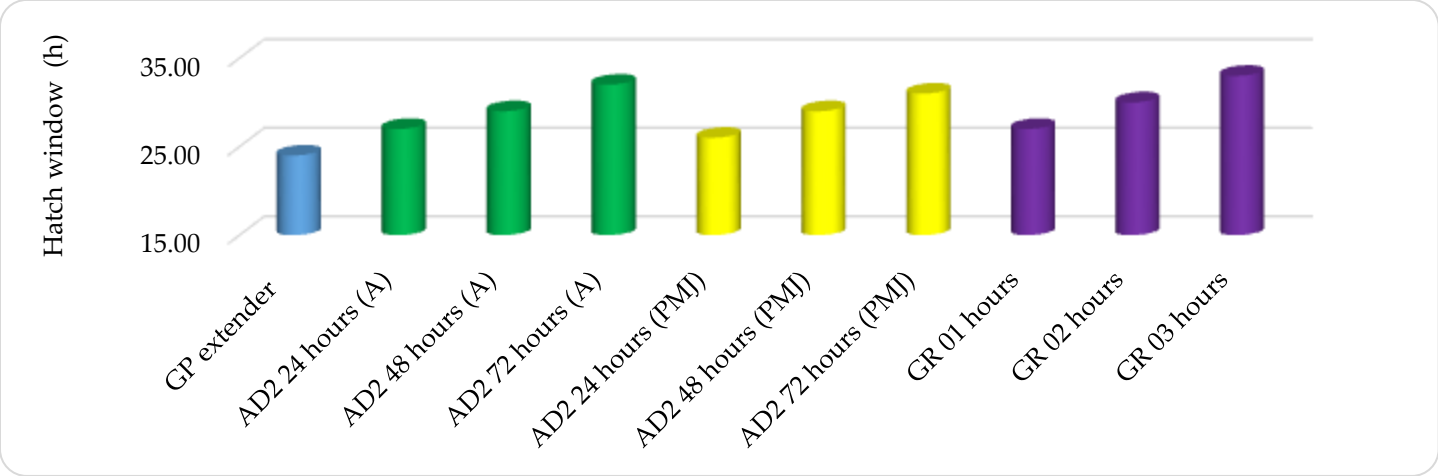

Figure 10. Average hatch window of various extenders used in fayomi breed semen.

Discussion

The study found that preserved semen did not significantly affect egg production in Fayomi chickens, consistent with previous research (El-Sabrout *et al.*, 2022). Similarly, no significant differences were observed in egg production among treatment groups, aligning with previous findings in broiler breeders (Bustani and Baiee, 2021). Despite slight decreases, yolk, albumen, and shell weights remained relatively stable with preserved semen, echoing findings from other studies (Svoradová *et al.*, 2021). These results suggest that semen preservation techniques have limited impact on egg quality parameters. Overall, the study highlights the consistency in egg quality despite variations in semen extenders and storage durations (Hassan *et al.*, 2005).

The decrease in fertility with preserved semen aligns with previous findings by Zong *et al.* (2023), highlighting the sensitivity of sperm quality to preservation techniques. Similarly, reduced chick hatchability observed in this study is consistent with research by Suwimonteerabutr *et al.* (2023), indicating challenges in maintaining optimal hatchability with preserved semen. These declines in fertility and hatchability with increased storage duration are also supported by Wolc *et al.* (2010), emphasizing the vulnerability of stored chicken semen to decreased reproductive success. The importance of timely artificial insemination and storage conditions in optimizing fertility and hatchability is underscored by these findings. Fertility rates following fresh semen insemination align with values reported by Petek and Dikmen (2006) in broiler breeders. Factors such as sperm numbers, hen type, and age can influence sperm storage and subsequently egg fertility in domestic fowl (Tabatabaei *et al.*, 2009). Hatchability is affected by factors including egg fertility and embryonic mortality, with causes of embryonic mortality including prolonged egg storage, season, hen nutritional status, egg size, breeder age, and technical incubation problems (Fairchild *et al.*, 2002).

The slight decrease in chick liveability, albeit non-significant, is in agreement with the work of Shanmugam and Kannaki (2020) and El-Sabrout *et al.* (2022), emphasizing the importance of semen preservation methods in ensuring optimal chick health post-hatch. The significant increase in chick embryonic mortality aligns with studies by Nakage *et al.*

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(2003), highlighting the vulnerability of developing embryos to the effects of semen preservation techniques. While a slight decrease in chick liveability was observed, the lack of statistical significance suggests a potential resilience of chicks to variations in semen storage conditions. This contrasts with the significant increase in embryonic mortality with prolonged storage, a phenomenon noted by researchers such as Bustani and Baiee (2021). The substantial increase in embryonic mortality underscores the sensitivity of developing embryos to the duration of semen storage.

The significant increase in the hatch window is supported by the work of Deines *et al.* (2021) and Dziekońska and Partyka (2022), suggesting that preserved semen may contribute to an extended timeframe for hatching in Fayoumi chickens. The significant increase in the hatch window with extended storage, as observed in this study, resonates with findings by Petričáková *et al.* (2022). This emphasizes that certain semen extenders may contribute to an extended timeframe for hatching, potentially influencing hatchery management practices. In conclusion, the study sheds light on the nuanced effects of different extenders on various reproductive parameters in chicken breeding. These findings emphasize the importance of considering extender choice and storage duration in optimizing artificial insemination protocols for improved reproductive outcomes.

Conclusion

This study emphasizes the critical role of semen preservation methods in determining fertility, hatchability, and embryonic development in Fayomi chickens. While most egg quality parameters and chick liveability remained consistent across extenders, significant variations were observed in fertility rates, chick hatchability, and embryonic mortality among treatment groups. Longer storage periods and specific extenders were linked to reduced fertility, lower chick hatchability, and increased embryonic mortality. These findings emphasize the need for optimized semen preservation techniques to ensure optimal reproductive performance in poultry breeding.

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Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors contributed equally in the manuscript.

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