Bangladesh Journal of Veterinary and Animal Sciences
Journal home page: www.bjvas.com
pISSN 2227-6416
eISSN 2709-2542

Research article

Semen quality and sperm biokinetic parameters of Red Junglefowl (*Gallus gallus*) available in Bangladesh

Md. Kamruzzaman[1], Abdul Gaffar Miah[2*] and Ummay Salma[3]

[1]Department of Dairy and Poultry Science,Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Bangladesh
[2]Department Genetics and Animal Breeding and [3]Department Animal Science and Nutrition, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Bangladesh

ARTICLE INFO

Article history:

Received:20/08/2024

Accepted:18/02/2025

Keywords:

Red Junglefowl, semen collection, semen quality, sperm biokinetic parameters

[*]Corresponding author:
Cell: +8801784973090

E-mail:agmiah@tch.hstu.ac.bd, agmiah2009@gmail.com

ABSTRACT

The present study was conducted to assess the semen quality and sperm biokinetic parameters of Red Junglefowl (RJF) available in Bangladesh. A total of 12 RJF cocks were primarily selected and trained, and finally, 6 cocks were proven suitable for semen collection. Experimental birds were maintained under captive conditions with similar and uniform management. Semen was collected twice a week by the dorso-abdominal massage method and used for the evaluation of pH and concentration of ejaculated fresh semen. Total motility, progressive motility and biokinetic parameters such as curvilinear velocity (VCL), average path velocity (VAP), straight-line velocity (VSL), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) were analyzed by a Computer Assisted Semen Analyzer (CASA). Average volume, pH and sperm concentration of the ejaculated semen of six (6) different proven cocks ranged from 0.35–0.50 ml, 7.0–7.22 and 1.70–3.50 $\times 10^9$/ml, respectively. Total motility and progressive motility were 91–99% and 57–62%, respectively. Among the sperm biokinetic parameters, the highest VCL, VAP, VSL, and STR were 105.50 µm/sec, 68.24 µm/sec, 29.23 µm/sec and 58.77%, respectively. The results of the present study revealed that the quality of semen varies from cock to cock but the average results showed that almost all of the cocks produced good quality semen with standard total motility, progressive motility and biokinetic parameters. Thus, it may be concluded that RJF semen can be collected effectively by abdominal massage method which yields reasonable production in terms of quality and quantity for semen storage and may therefore be used for artificial insemination to increase their population.

To cite this paper:*M. Kamruzzaman, A. G. Miah and U. Salma, 2024.Semen quality and sperm biokinetic parameters of Red Junglefowl (Gallus gallus) available in Bangladesh.Bangladesh Journal of Veterinary and Animal Sciences, 12(2):01-08.*

1.INTRODUCTION

Bangladesh has a rich genetic diversity of indigenous chickens and is one of the homelands of the Red Junglefowl (RJF). The RJF is the ancestor of all modern domestic chickens (Singh et al., 2001; Islam and Nishibori, 2009). It is one of the most vital species for mankind due to its economic and cultural significance. However, the population of RJF has been declining significantly due to habitat destruction, lack of public awareness, and challenges associated with their lower reproductive performance (Mosca et al., 2020; Iorio et al., 2020).

Captive breeding of RJF in Bangladesh is a viable activity that might be helpful in the conservation of this unique species. One key factor in developing any conservation programs is the management of their reproductive capacities. For males, this requires a thorough understanding of gamete production capacities (semen collection and quality characteristics) and fertilization ability, including fertilization after artificial insemination. These studies may lead to the development of semen cryo-preservation programs to manage genetic diversity through artificial insemination. All these studies need to be conducted for RJF in Bangladesh.

The assessment of semen quality characteristics of poultry birds provides an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (Peters et al., 2004). Semen quality is a major factor in determining the breeding value of males because it influences the fertility of females and the reproductive efficiency of their progeny (McGray et al., 2002). Macroscopic and microscopic evaluations of semen are important tools to evaluate male fertility (Peters et al., 2004).

Macroscopic indicators used to evaluate semen quality include volume, color, consistency, and appearance score, while microscopic parameters include sperm concentration, sperm motility, sperm livability, percentage of sperm progressivity, abnormal sperms, and percentage of dead sperms (Moce and Graham, 2008). Semen characteristics of many bird species such as domestic chickens (Tuncer et al., 2006; Malik et al., 2013), turkeys (Burrows and Quinn, 1937), and pheasants (Jalme et al., 2003) have been studied previously. However, successful semen collection from sub-species of Junglefowl has been minimally documented (Malik et al., 2013) and is still lacking for RJF in Bangladesh.

Therefore, this study was designed to evaluate the macroscopic characteristics of semen and the microscopic characteristics of sperm, including total motility, progressive motility, and biokinetic parameters of RJF available in Bangladesh.

2.MATERIALS AND METHODS

Experimental birds and management

Twelve male RJF birds, with an average body weight of 1.8 kg, were used in this study. The study was conducted at the Advanced Avian Research Farm, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The birds were reared under captive conditions. They were offered commercially available layer breeder feed (100 g/day) and were exposed to 16 hours of light per day. Fresh water was provided to the birds throughout the experimental period.

Training of cocks for semen collection

The birds underwent training for semen collection through abdominal massage, as described by Burrows and Quinn (1937). Training was initiated to prepare the birds for semen collection and to ensure a clean ejaculate, free from feces. The training continued until sufficient semen was collected for the planned experiments. Successful ejaculates were obtained after four weeks of training. Semen collection was performed using six cocks that showed the best performance after training.

Semen collection and preparation for analysis

A special type of wooden chair was constructed to lock two legs of the cock. It provided a comfortable seating and massaging arrangement for the semen collector. The primary goal of the semen collection procedure was to obtain a maximum amount of clean, high-quality semen with minimal handling and stress. The testes, located on the dorsum, were stroked and massaged carefully until the cloaca protruded. Semen collection was carried out twice a week at the same time (9:00–10:00 AM) and under consistent conditions to minimize stress and maximize semen quality.

The collected semen was diluted using a previously prepared diluent, known as modified Ringer's solution. Preliminary tests were conducted to determine the optimal dilution ratio for Computer-Assisted Semen Analysis (CASA). The dilution ratio of 1:20 was selected based on better results in preliminary trials. This ratio was used during the final analysis with the CASA analyzer (Miah et al., 2020). For this study, the semen diluent was prepared according to the composition described by Akcay et al. (2006), as presented in Table 1.

Table 1. Composition of the modified Ringer's semen diluents

Ingredients	Amount
Sodium chloride (g)	9.50
Potassium chloride (g)	0.20
Calcium chloride (g)	0.26
Sodium bicarbonate (g)	0.20
Distilled water (ltr.)	1.00
Glucose (g)	1.00

Source:Akcayet al. (2006)

Semen analysis

Macroscopic analysis: Macroscopic parameters such as semen volume, pH, and color were evaluated immediately after semen collection. The semen sample was then transported to the laboratory with minimal delay and incubated in a hot water bath at 37.8°C for 5 minutes before further analysis.

Microscopic analysis: A fresh, clean microscopic slide was prepared. Using a clean and disinfected micropipette, 0.5 µl of diluted semen was placed on the slide. Care was taken to avoid touching the cover slip with hands. Excess semen was removed with a cotton bud tip to prevent floating cells. After resting for 5 seconds, the analysis was performed swiftly to prevent evaporation. Sperm concentration, motility, progressive motility, curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), and beat cross frequency (BCF) were measured using a green filter. The microscope condenser was set for chicken (animal species), i.e., ph-1, and 10X negative phase contrast was used for optimal contrast. The selection program was SCA motility, and at least 500 sperm per sample were counted using CASA.

Statistical analysis

Sperm concentration, motility, progressivity, VCL, VSL, VAP, LIN, STR, WOB, ALH, and BCF were analyzed using one-way ANOVA, based on the Completely Randomized Design (CRD), following the GLM (Generalized Linear Model) procedure of SPSS (2013) version 22.0. The significance of differences among mean sperm values produced by the cocks was compared using Duncan's Multiple Range Test (DMRT). All data were expressed as Mean ± Standard Error of Mean (SEM). A significant difference was considered at P<0.05.

3. RESULTSAND DISCUSSION

Macroscopic characteristics of RJF semen

The semen color was creamy white, showing a normal appearance, with no abnormal color or blood observed. No foreign particles were present in the collected semen. The volume, pH, and sperm concentration of the ejaculated semen from six different proven cocks are shown in Table 2. The ejaculated semen volume was significantly (P<0.05) higher in Cock-3 compared to the other cocks, with the lowest volume observed in Cock-1 and Cock-6. These results are consistent with those of Malik et al. (2013), who reported 0.33 ml/ejaculate semen obtained from RJF. The volume of semen per ejaculate in the present study agrees with the findings of Shanmugam et al. (2014), who observed 0.36 ml in 65-week-old RIR roosters, but it is lower than the volume reported for younger roosters (0.48 ml) at 42 weeks. In general, earlier reports revealed higher values: 0.43 (Kabir et al., 2007), 0.51 (Machal and Krivanek, 2002), and 0.7–0.8 ml (Uysal et al., 2011) in younger RIR roosters aged between 28 and 35 weeks. Sperm concentration also varied significantly (P<0.05) among the cocks, with the highest concentration (3.50×10^9/ml) from Cock-3 and the lowest concentration (1.70×10^9/ml) in Cock-1. The present results are higher than those reported for other adult cocks of different genetic backgrounds ($0.29–0.52\times10^9$/ml) in previous studies (Omeje and Marire, 1990). The average pH of RJF semen in this study was 7.0–7.2, with similar pH values observed in all RJF cocks. This result agrees with that of Malik et al. (2013), who found pH values ranging from 7.0–7.4 in RJF semen. The highest sperm concentration (3.50×10^9/ml) was achieved from Cock-3, while the lowest concentration (1.70×10^9/ml) was found in Cock-1. The average sperm concentration of RJF cocks ranged from 1.70×10^9/ml to 3.50×10^9/ml, which is in line with the findings of Malik et al. (2013), who reported a concentration of

4.44×10^9/ml in RJF semen. Sabra et al. (2017) reported an average sperm concentration of 3.18–4.86×10^9/ml in toms. Similarly, the average semen concentration in RJF and domestic chickens was within the range found in leghorn roosters (1.7–3.5×10^9/ml) in a previous study (Lake, 1966). Das et al. (2015) found similar results (5.30×10^9/ml) in indigenous chickens.

Total motility and progressive motility

The total sperm motility and progressive motility of the cocks are shown in Figure 1 (a and b). The total sperm motility was observed at 93.67, 91.33, 99.28, 94.21, 99.22, and 94.22%, while the progressive motility was 47.70, 49.62, 61.89, 56.67, 61.71, and 55.80% in Cock-1, Cock-2, Cock-3, Cock-4, Cock-5, and Cock-6, respectively. The highest total motility and progressive motility were observed in Cock-3, while the lowest total motility and progressive motility were observed in Cock-2. The total sperm motility and progressive motility in Cock-3 and Cock-5 were significantly (P<0.05) higher than in Cock-1, Cock-2, Cock-4, and Cock-6, respectively, though there was no significant (P>0.05) difference among Cock-1, Cock-2, Cock-4, and Cock-6. The present study revealed that the total sperm motility of the cocks was 91–99%, which is similar to the result obtained (96%) by Malik et al. (2013) in RJF. Mavi et al. (2019) reported sperm motility values of 66.60, 55.38, and 51.95% for RIR, Punjab red, and RIR cross with local chickens, respectively. Mosca et al. (2020) reported a total motility of 87.7% in chicken fresh semen. Progressive motility is an important semen characteristic, also indicating the quality of the semen. The average sperm progressive motility of the cocks was 47–62%. Malik et al. (2013) demonstrated 55% progressive motility in RJF, while Mosca et al. (2020) showed 23.1% progressive motility in fresh chicken semen.

Table 2. Macroscopic evaluation of RJF semen

Proven RJF cock	Semen volume (ml)	pH of semen	Sperm concentration (×10^9/ml)
Cock-1	0.35±0.04^a	7.00±0.12	1.70±0.16^a
Cock-2	0.40±0.06	7.20±0.15	1.80±0.14^a
Cock-3	0.50±0.06^c	7.10±0.16	3.50±0.15^c
Cock-4	0.40±0.05^{ab}	7.14±0.17	2.50±0.13^b
Cock-5	0.45±0.07^{bc}	7.22±0.19	2.00±0.11^{ab}
Cock-6	0.35±0.03^a	7.20±0.10	3.33±0.14^c

Values are Means±SEM; ^{a,bc}Means within a colum without common superscripts differ significantly.

Curvilinear velocity (VCL) and average path velocity (VAP)

The average sperm VCL and VAP for Cock-1, Cock-2, Cock-3, Cock-4, Cock-5, and Cock-6 are presented in Figure 2 (a and b). The average sperm VCL values were 93.36, 83.95, 90.99, 97.93, 105.5, and 85.00 (µm/sec), and VAP values were 57.73, 56.20, 40.71, 62.64, 68.24, and 54.32 (µm/sec), respectively. The VCL and VAP of Cock-4 and Cock-5 were significantly (P<0.05) higher than those of Cock-1, Cock-2, Cock-3, and Cock-6, with the highest VCL and VAP observed in Cock-5, and the lowest VCL in Cock-2 and VAP in Cock-3. However, the highest VCL (105.5 µm/sec) and VAP (68.24 µm/sec) were observed in Cock-5, while the lowest values were observed in Cock-2 (VCL 83.95 µm/sec and VAP 56.20 µm/sec). Mosca et al. (2020) reported a VCL of 55.5 µm/s and a VAP of 36.8 µm/s for fresh chicken semen. Iorio et al. (2020) observed VCL and VAP values of 60.1 and 27.8 µm/sec for tom semen. The VCL and VAP values found in this study were higher than those in these two studies, likely due to differences in genotype, semen quality, or the age of the birds.

Straight line velocity (VSL) and straightness (STR)

The average VSL and STR of sperm from Cock-1, Cock-2, Cock-3, Cock-4, Cock-5, and Cock-6 are shown in Figure 3 (a and b). The VSL values were 37.57, 37.09, 26.34, 37.70, 29.29, and 36.85 (µm/sec), while the STR values were 57.05, 58.77, 49.58, 56.22, 48.94, and 48.22%, respectively. Significantly (P<0.05) higher VSL was found in Cock-1, Cock-2, Cock-4, and Cock-6 compared to Cock-3 and Cock-5, with no significant difference observed among Cock-1, Cock-2, Cock-4, and Cock-6. On the other hand, the average STR was higher (P<0.05) in Cock-1, Cock-2, and Cock-4 compared to Cock-3 and Cock-5. Mosca et al. (2020) reported a VSL of 55.5 µm/s for fresh chicken semen. Iorio et al. (2020) observed a VSL of 41.4 µm/sec and STR of 35.1% in tom semen.

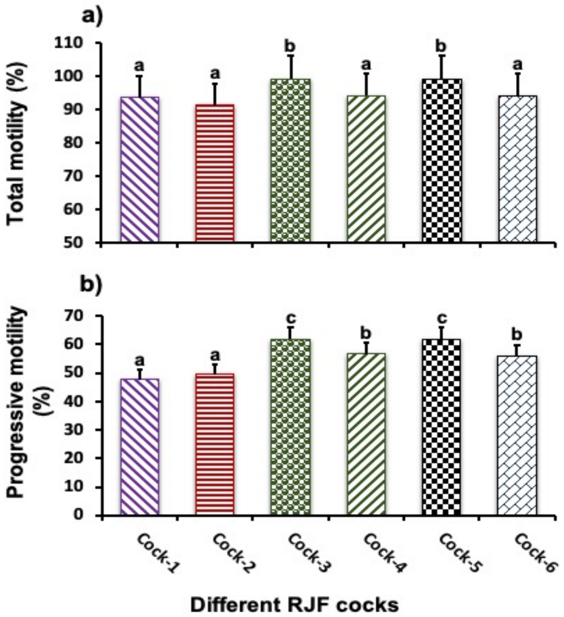

Figure 1. Comparative study on a) Total motility and b) Progressive motility of RJF spermobtained from 6 different proven cocks. Eachbar with error bar represents mean±SEM value.Different letters on error bar indicate significant
differences (P<0.05) among the cocks.

Linearity (LIN) and Wobbles (WOB)

The average sperm LIN and WOB values for the experimental cocks are shown in Figure 4 (a and b). The LIN (%) and WOB (%) values were observed as follows: Cock-1 (38.08 and 63.00), Cock-2 (40.35 and 63.98), Cock-3 (28.29 and 56.57), Cock-4 (37.41 and 61.80), Cock-5 (29.29 and 55.67), and Cock-6 (38.37 and 62.56). The highest LIN and WOB values were found in Cock-2, while the lowest LIN and WOB were found in Cock-3 and Cock-5. For both LIN and WOB, there was a significant (P<0.05) difference between Cock-1, Cock-2, Cock-4, and Cock-6, compared to Cock-3 and Cock-5, with no significant difference was observed among Cock-1, Cock-2, Cock-4, and Cock-6.

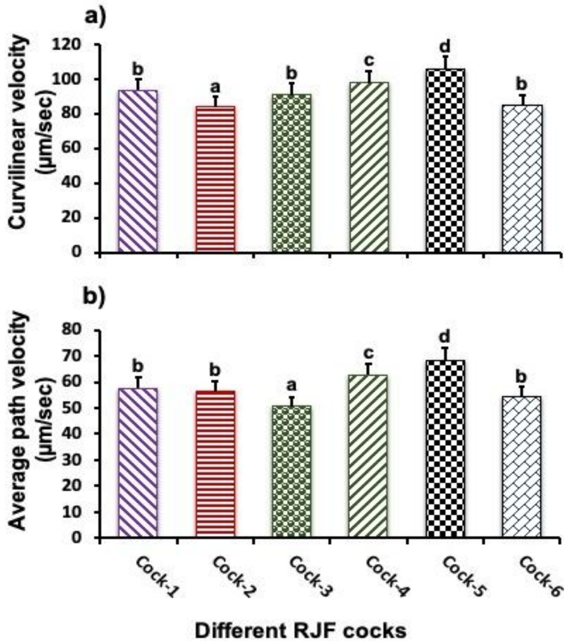

Figure 2. Comparative study on a) Curvilinear velocity and b) Average path velocity of RJF sperm obtained from 6 different proven cocks. Each bar with error bar represents mean±SEM value. Different letters on error bar indicate significant differences (P<0.05) among the cocks.

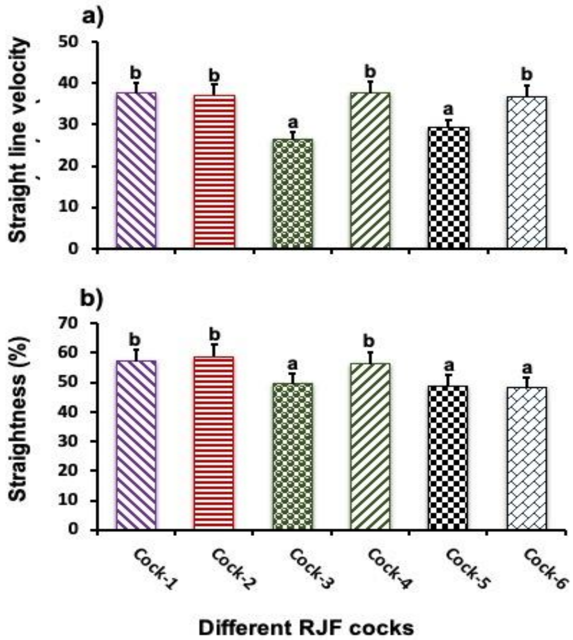

Figure 3. Comparative study on a) Straight line velocity and b) Straightness of RJF sperm obtained from 6 different proven cocks. Each bar with error bar represents mean±SEM value. Different letters on error bar indicate significant differences (P<0.05) among the cocks.

Amplitude lateral head displacement (ALH) and beat cross frequency (BCF)

The ALH and BCF values are shown in Figure 5 (a and b). The ALH values for Cock-1, Cock-2, Cock-3, Cock-4, Cock-5, and Cock-6 were 4.05, 3.66, 4.84, 4.42, 5.38, and 3.87 µm, and the BCF values were 6.09, 5.76, 4.53, 5.63, 4.88, and 5.27 Hz, respectively. The ALH differed significantly (P<0.05) among the cocks, with the highest ALH observed in Cock-5. The BCF also differed significantly (P<0.05) among Cock-1, Cock-2, Cock-4, and Cock-6 compared to Cock-3 and Cock-5, with the highest BCF observed in Cock-1. Iorio et al. (2020) observed ALH and BCF values of 2.8 µm and 4.6 Hz, respectively, for tom semen, with the present study's ALH and BCF values being somewhat similar.

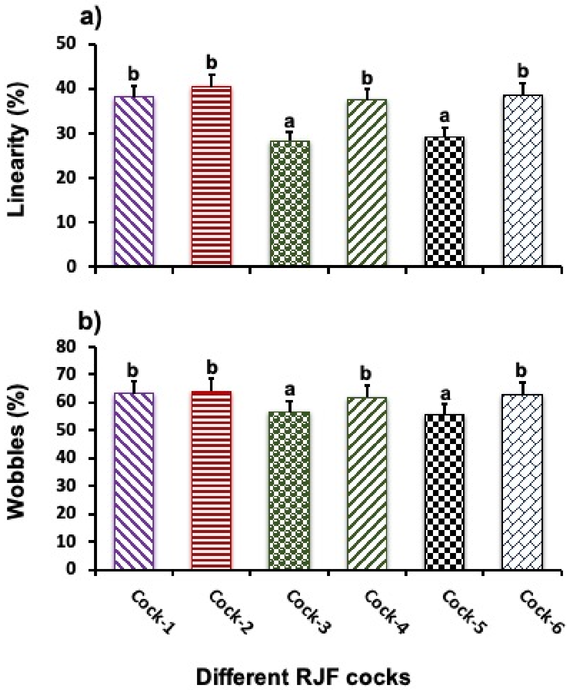

Figure 4. Comparative study on a) Linearity and b) Wobbles of RJF sperm obtained from 4 different proven cocks. Each bar with error bar represents mean±SEM value. Different letters on error bar indicate significant differences (P<0.05) among the cocks.

4. CONCLUSION

The findings of this study underscore the variability in semen quality across different cocks, but overall, the results demonstrate that the majority of the cocks produced high-quality semen with excellent motility and favorable biokinetic characteristics. The total motility, progressive motility, and other sperm parameters were generally well within the desirable range for successful fertilization. This study clearly indicates that the abdominal massage method is an effective and reliable technique for collecting RJF semen. Not only does this method ensure optimal semen quality, but it also provides a sufficient quantity for semen storage and artificial insemination. These advancements offer a promising approach for increasing the population of RJF through more efficient breeding practices.

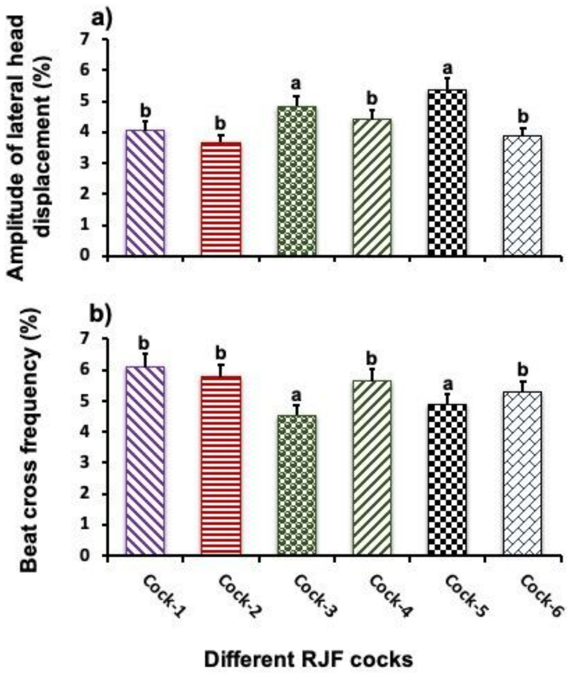

Figure 5. Comparative study on a) Amplitude of lateral head displacement and b) Beat cross frequency of RJF sperm obtained from 6 different proven cocks. Each bar with error bar represents mean±SEM value. Different letters on error bar indicate significant differences (P<0.05) among the cocks.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Bangladesh Bureau of Educational Information and Statistics (BANBEIS), the Ministry of Education, and the Government of the People's Republic of Bangladesh for their financial support of the research project entitled 'Ex-situ Conservation of Red Junglefowl (*Gallus gallus*) as a Native Poultry Genetic Resource of Bangladesh'.

REFERENCES

Akcay, E., Varisli, O., and Tekin, N. 2006. Fertilizing ability of turkey semen diluted with simple sugar-based extenders after cooled storage. Ankara Universitesi Veteriner Fakultesi Dergisi, 44(2): 137–149.

Andersson, L., Knott, S. A., Johansson, M., Andersson-Eklund, L., Edfors-Lilja, I., and Lundstrom, K. 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science, 263(5154): 1771–1774.

Burrows, W. H., and Quinn, J. P. 1937. The collection of spermatozoa from the domestic fowl and turkey. Poultry Science, 26(1): 19–24.

Das, K. K., Ahmed, K., Kalita, N., and Deka, N. 2015. Semen characteristics of Vanaraja and Indigenous chicken of Assam. International Journal of Applied and Pure Science and Agriculture, 1(8): 108–110.

Iorio, M. D., Rusco, G., Iampietro, R., Maiuro, L., Schiavone, A., Cerolini, S., and Iffaldano, N. 2020. Validation of the turkey semen cryopreservation by evaluating the effect of two diluents and the inseminating doses. Animals, 10(8): 1329–1339.

Islam, M. A., and Nishibori, M. 2009. Indigenous naked neck chicken: a valuable genetic resource for Bangladesh. World's Poultry Science Journal, 65(1): 125–138.

Jalme, M., Lecoq, R., Seigneurin, F., Blesbois, E., and Plouzeau, E. 2003. Cryopreservation of semen from endangered pheasants: the first step towards a cryobank for endangered avian species. Theriogenology, 59(4): 875–888.

Kabir, M., Oni, O. O., Akpa, G. N., Adeyinka, I. A., and Rekwot, P. I. 2007. Effects of underfeeding on semen quality of Rhode Island roosters. Pakistani Journal of Biological Science, 11(7): 986–988.

Lake, P. E. 1966. A cytochemical examination of spermatozoa of the domestic fowl. Research in Veterinary Science, 7(2): 121–127.

Machal, L., and Krivanek, I. 2002. Indicators of semen quality of roosters of three parental layer lines and specific conductivity of the semen. Acta Veterinarian Brno, 71(2): 109–116.

Malik, A., Haron, A., Yusoff, W., Bukar, R., Nesa, M., and Kasim, A. 2013. Evaluation of the ejaculate quality of the red Junglefowl, domestic chicken, and bantam chicken in Malaysia. Turkish Journal of Veterinary Animal Science, 37(6): 564–568.

Mavi, G. K., Dubey, R. S., Cheema, S. K., Das, B., and Bansal, B. K. 2019. Comparative analysis of semen quality parameters and their relationship with fertility in different genetic groups of layer chicken. Indian Journal of Animal Research, 53(9): 1269–1274.

McGary, S., Estevez, I., Bakst, M. R., and Pollock, D. L. 2002. Phenotypic traits as reliable indicators of fertility in male broiler breeders. Poultry Science, 81(2): 102–111.

Moce, E., and Graham, J. K. 2008. In vitro evaluation of sperm quality. Animal Reproduction Science, 105(1–2): 104–118.

Mosca, F., Zaniboni, L., Sayed, A. A., Iffaldano, N., Schiavone, D. S. A., and Celolini, S. 2020. Effect of N-Methylacetamide concentration and thawing rate on chicken sperm quality after cryopreservation. Animals, 10(5): 9824.

Miah, A. G., Bathgate, R., Hamano, K., and Salma, U. 2020. Effects of pre-freeze Nigella sativa oil supplementation on cryosurvival of ovine spermatozoa. Reproduction in Domestic Animals, 53(6): 1424.

Omeje, S. S. I., and Marire, B. N. 1990. Evaluation of the semen characteristics of adult cocks of different genetic backgrounds. Theriogenology, 3(1): 1111–1118.

Peters, S. O., Omidiji, E. A., Ikeobi, C. O. N., Ozoje, M. O., and Adebambo, O. A. 2004. Effect of Naked Neck and frizzled genes on egg traits, fertility, and hatchability in local chicken. Self Sufficiency of Animal Protein in Nigeria, 262–264.

Sabra, Z. A. M., El Sawy, M. A., and Ibraheim, S. E. 2017. Estimation of the genetic parameters of semen characteristics in bronze turkey raised in Egypt. Journal of Animal and Poultry Science Production, Mansoura University, 8(8): 311–318.

Shanmugam, M., Vinoth, A., Rajaravindra, K. S., and Rajkumar, U. 2014. Evaluation of semen quality in roosters of different age during hot climatic conditions. Animal Reproduction Science, 145(1): 81–85.

Singh, K. K., Samanta, A. K., and Maity, S. B. 2001. Nutritional evaluation of stylo (Stylosanthes hamata) hay in goats. Indian Journal of Animal Nutrition, 18(1): 96–98.

Tuncer, P. B., Kinet, H., Ozdogan, N., and Demiral, O. O. 2006. Evaluation of some spermatological characteristics in Denizli cocks. Journal of Facultesi Veteriner Universitesi Erciyes, 3(1): 37–42.

Uysal, O., Demir, Z., Cebi, C., Demirtas, S. E., Tirpan, M. B., Tunca, M., and Kamanli, S. 2011. Evaluation of semen quality in pure lines of the brown laying roosters in Poultry Research Institute. Kafkas Universitesi Veteriner Fakultesi Dergisi, 17(5): 655–658.