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Article

Efficiency of natural mating and artificial insemination in turkey (*Meleagris gallopavo*) breeding

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Abstract: This study was conducted to compare the reproductive and economic performance of turkey hen bred by natural mating (NM) and artificial insemination (AI) using fresh and chilled semen. The breeding trial was designed under a completely randomized design (CRD) consisting of three treatment groups (T_1 = NM following ♂1:♀4, T_2 = AI with fresh semen and T_3= AI with chilled semen). A total of 48 turkey hens and 8 toms were used with 4 replicates in each treatment group. Semen collection was performed by using the abdominal massage technique. Semen aliquots was diluted by modified ringer's solution at the ratio of 1:2 and then stored at 3−5^{0}C temperature in refrigerator for 24 hours. Undiluted fresh pooled semen was deposited into the vagina @ 0.02 ml/hen within 30 minutes of collection; and pooled diluted chilled semen was deposited @ 0.2 ml/hen at 4−5 pm in every week. Both NM and AI had no significant effect on egg production, egg weight, hatchability and survivability of poults. Significantly (P<0.01) highest fertility (89.71%) was obtained when the turkey hens were inseminated with fresh semen compared to chilled semen (60.77%) and even though natural mating (59.21%). Late embryonic mortality and dead in shell differed significantly (P<0.05) among the treatment groups but it was not due to use of breeding techniques. Profitability index and rate of return were higher (P<0.05) in the turkey hens inseminated by fresh and chilled semen. Therefore, the results of the study concluded that use of fresh and chilled semen increased fertility and reduced the cost of production.

Keywords: artificial insemination; fertility; natural mating; semen; turkey

1. Introduction

Turkey is a well-known poultry species in western countries but the hobbyist farmers have introduced it recently in Bangladesh. Turkey farming has not started commercially yet in Bangladesh because of poor fertility and lack of experience of the farmers. Artificial insemination (AI) and natural mating (NM) are a well-known technique of breeding for poultry in developed countries. Still its use is not evident among the poultry farmers of Bangladesh. In fact, AI could be a practical solution for poor fertility in turkey because of mating problem. Main reasons of improper mating are heavy weight and presence of large pectoral bone in tom, inactive and

inefficient tom, and non-receptive hen. Field experience showed that average fertility and hatchability of turkey eggs were 50.0 and 32.0%, respectively in Bangladesh (Asaduzzaman *et al.*, 2017). Consequently, farmers are incurring huge loss due to infertility and inadequate technical knowledge about artificial breeding in turkey. Hence, turkey farmers in Bangladesh demands a pragmatic solution to overcome this problem.

The fertility and hatchability are the two main parameters considered with utmost importance to determine the reproductive performance in commercial turkey production. However, these are most sensitive to environmental and genetic influences (Stromberg, 1975). The problem of unfertilized eggs identified as one of the most critical factors limiting the success of turkey breeding ranges from 10.0–98.2% (Mushi *et al.*, 2008; Dzoma and Motshegwa, 2009). Fertility and hatchability were major problems in the turkey industry and loss of eggs from these two factors was about 40.0% of all eggs set for incubation (Stotts and Darrow, 1954). In fact, fertility as well as hatchability are the major determinants of profitability in breeding and hatchery enterprises, and two-thirds of this loss occurred due to infertility or apparent infertility (Peters *et al.*, 2008).

Commercial turkey varieties are primarily selected based on growth rate, feed efficiency and meat yield. It caused a series of negative effects on the reproductive performance, including limited persistence of sexual maturity and decline in egg fertility (Brillard, 2004). Infertility was a critical component of reproduction in turkeys since 1960s. AI was used almost exclusively for commercial turkey production in western countries. Differences in size of tom (large white strains approximately 33 kg) and hen (approximately 9 kg at the onset of laying) resulted in unsuccessful mating and consequently low fertility (Gee *et al.*, 2004). This situation eventually forced to adopt AI for commercial turkey production. Turkey is the only commercial species, which is completely dependent upon AI for fertile egg production (Juliet and Bakst, 2008).

It is well established that AI has relative advantages over natural mating in case of avian species (Surai and Wishart, 1996). It resulted in better fertility than natural mating in poultry. Gee *et al.* (2004) claimed that when even under natural mating 80–85% of eggs become fertile, fertility could be increased by another 5–10% simply by applying AI. Egg yields in turkeys are lower than that of other poultry species. So hatching procedures are very important to increase number of poults (Kaygısız *et al.*, 1994).

Application of AI along with natural mating is widely used in developed countries for turkey breeding. Nevertheless, no initiative has been undertaken yet in Bangladesh to investigate the suitability and efficiency of this technique. It was argued that fresh semen should be inseminated within 15–30 minutes after collection to achieve better fertility (Brillard, 2003). However, within such a short period it is difficult to inseminate a large number of hens. For this reason, in addition to fresh semen, an attempt was made in this experiment, to investigate the performance of chilled semen. Therefore, keeping in mind the above points the experiment was undertaken to compare the fertility and hatchability of eggs of turkey bred by natural mating, and AI using fresh and chilled semen; and investigate the economic feasibility and sustainability of turkey breeding by application of AI.

2. Materials and Methods

2.1. Study site and duration

The study was conducted at the Turkey Research Unit, Advanced Avian Research Farm, under Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh for a period of 21 weeks (26 to 46-week age of turkey; from February to July 2017). The Animal Care and Ethics Committee of HSTU approved all experimental conditions and animal procedures.

2.2. Experimental design

The breeding experiment was performed under a completely randomized design (CRD). A total of 48 turkey hens and 8 toms of 35-week old were randomly assigned into three treatment groups (16 turkey hens were allocated for each group). The breeding pattern was designed as Treatment 1 (T_1) = NM following the mating ratio: ♂1:♀4, so, 16 hens and 4 toms were allocated for this group; Treatment 2 (T_2) = AI with fresh semen (AI-FS) for 16 hens and Treatment 3 (T_3) = AI with chilled semen (AI-CS) for 16 hens. For T_2 and T_3 groups 4 toms were allocated for each in order to semen collection and determining cost effectiveness. More toms were taken to avoid the risk of non-availability of semen at due time. In the early stages of the work, toms were separated from the hens of T_2 and T_3 to avoid any kind of mating which might cause experimental error. Because a hen can store sperm from one or more inseminations for an extended period (up to 14 days), which may result in production of fertilized eggs and distracts the objectives of the study.

2.3. General management

Turkeys were reared in deep litter pens demarcated according to the treatment groups. The turkeys locally collected were crosses of the varieties-Beltsville Small White, Royal Palm, Naragensett, and Board Breasted Bronze. They were maintained in an intensive system under standard management practices following the existing rules and regulations of Bangladesh Veterinary Council regarding animal care and management. All birds were treated equally in all respects, except applying breeding technologies.

Similar diet was supplied to all birds. Feed and water were supplied in plastic feeders and drinkers. Throughout the experimental period *ad libitum* clean drinking water was supplied. Mixture of rice husk and wood shavings were used as litter. Each turkey was marked with colored plastic beads for proper identification. Both natural and mechanical procedures were followed to maintain proper ventilation and illumination in the turkey house. Average environmental temperature was between 21−35°C and light and dark period pattern was 16 h light and 8 h dark. Layer diet was formulated for the experimental turkeys. The diet was formulated with maize, rice polish, soybean meal, animal protein, vitamin-mineral premix, amino acid, salt, toxin binder, and anti-oxidant. The composition of the diet is presented in Table 1.

Table 1. Nutrient composition of experimental diet for the breeder turkeys.

Nutrient	Amount
Metabolizable Energy (kcal/kg)	2780.00
Moisture (%)	12.00
Crude protein (%)	17.00
Crude fat (%)	4.50
Calcium (%)	3.50
Available phosphorus (%)	0.40

2.4. Semen collection

At the age of 26 weeks, all the toms were trained for semen collection using the abdominal massage technique as described by Burrows and Quinn (1937). The training was performed twice a week until the experiment started. By the 30 weeks of age, all the toms became almost equally ready for semen collection. Trial of semen collection from tom and insemination in hen was continued until actual experiment started at the 35 weeks of age.

Figure 1. At a glance sequential activities for semen collection and AI. Here, a) Semen collection syringe and vial, b) Semen collection chair, c) Massaging of Tom d) Collection of semen e) Pooling of semen and f) Insemination of turkey hen.

A special type of wooden chair was made with keeping facilities of locking two legs of tom for easily collection of semen through massaging and for hen for depositing semen. This chair gave comfortable sitting and massaging arrangement for the semen collector. The main goal of the semen collection procedure was to obtain maximum amount of clean and high-quality semen with minimum handling and stress. The testes located at the dorsum were stroked and massaged gently until protrusion of the cloaca. The researcher himself collected semen every week at the same time, and under the same conditions to minimize stress and maximize the quality of semen. Semen was collected once a week in the afternoon (4–5 p.m.) before insemination. The tom was stimulated by stroking the abdomen by right hand and pushing the tail upward and toward the bird's head with left hand. Individual ejaculates were collected into 1 ml microtubes. After each collection, the ejaculates were examined visually as well as microscopically. Special care was taken to avoid contamination of semen with feces, urates, and transparent fluid, which lower the semen quality. Semen was used within 30 minutes of collection. Collected semen was pooled in an equal amount according to required semen volume to eliminate the effect of individual variability of gamete donors. The sequential activities of semen collection from the tom to insemination in the hen are shown in Figure 1.

2.5. Semen dilution, preservation and warming

The dilution of semen was done at the ratio of 1:2 using previously made diluent which was also known as modified Ringer's solution. This process also diluted the sperm concentration to facilitate several insemination doses. The diluted semen was placed in a flask and mixed thoroughly. The flask was then covered with parafilm and stored in a refrigerator for 24 hours at 3–5^{0}C. Chilled semen was kept in a water bath at 37ºC for warming just prior to use for AI. In fact, semen is often diluted in AI practices to provide a suitable medium which sustain and protect spermatozoa and prolongs their fertilizing capacity for insemination (Salisbury *et al.,* 1978). However, for this study semen diluents were used following the composition used by Akcay *et al.* (2006). The composition of the diluents is given in Table 2.

Table 2. Composition of the 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

2.6. Semen analysis

To ensure quality of semen for AI, semen analysis was done for both fresh and chilled semen. The percent of live-dead and abnormal spermatozoa were counted after preparing smears and staining with eosin and nigrosin according to the methods described by Lake and Stewart (1978). Stained spermatozoa were counted as dead. The percent of abnormal spermatozoa was evaluated by observing morphology of a total count of 100 spermatozoa. Sperm motility was assessed by examining a drop of semen (4–5 µl) under the microscope at ×10 magnification and sperm concentrations were determined using a Neubauer haemocytometer.

2.7. AI in turkey hen

The females were inseminated following "Venting" method as described by Hafez (1985). The venting was done by applying pressure to the left side of the abdomen around the vent in such a way that it caused the cloaca to come out and the oviduct to protrude. Then 1 ml plastic syringe without needle with appropriate amount of semen was inserted into the oviduct and semen was delivered at the depth of 1.5 to 2 cm inside the vent. AI was performed once a week between 4–5 p.m. to avoid the presence of a hard-shelled egg in the uterus. It is generally recognized that AI should be carried out when no hard-shelled egg is likely to be present in the uterus or at least not within 3 hours of an oviposition (Giesen *et al.,* 1980). AI was completed within 30 minutes of semen collection. Only the ejaculates with milky appearance, free of fecal material with >70% mass motility were used for AI. Hens were inseminated with the sperm concentration at least 2.7×10^9/ml for both fresh and chilled semen. In case of AI with fresh semen: freshly collected undiluted pooled semen was drawn with a 1 ml syringe and 0.02 ml was deposited into the vagina of each hen; and in case of AI with chilled semen: pooled diluted chilled semen was drawn with a 1 ml syringe and 0.2 ml was deposited into the vagina of each hen. As

the semen was expelled into the vagina, pressure around the vent was released and then the vent area was massaged, which assisted the hen in retaining sperm in the vagina or the oviduct. Rough handling against hen was avoided carefully before, during and after the insemination process. Hens were released gently after insemination to prevent semen regurgitating from the vagina, which might result in lower fertility.

2.8. Data collection

Data on egg production, egg weight, fertile and infertile egg, early embryonic mortality (EEM), late embryonic mortality (LEM), dead in shell (DIS) and survivability of poults were recorded.

Eggs were collected thrice a day and marked according to the treatment groups with date. Eggs were stored in egg crates, placed in a cooler at approximately 15°C temperature and 75% relative humidity prior to incubation. After collection, eggs were sorted out to remove cracks, extra small and large ones. Egg weight was recorded once a week. Egg production and mortality were taken into account to measure egg production percent. The laying rate was calculated as the number of eggs divided by hen days during a production period and expressed as a percent, i.e. hen day egg production (HDEP) method was used to calculate egg production percent. Following formula was used for calculation of egg production.

$$\text{Hen day egg production} = \frac{\text{Total number of eggs laid during the period}}{\text{Total number of hen days in the same period}} \times 100$$

The eggs with standard size were selected for incubation in every week. The eggs were incubated by a forced-air incubator which was built with a fan to circulate the air. The set eggs were candled on the 10^{th} and 25^{th} day of incubation. Proper cleaning, disinfection and fumigation were confirmed before setting of eggs. The total incubation period (28 days) was divided as the setting period (1–25 days) and the hatching period (26–28 days). Temperature and humidity were maintained during the incubation period according to the Table 3.

Table 3. Temperature and humidity maintained during the incubation period.

Incubation period	Days	Temperature (^{0}C)	Relative humidity (%)
Setting	1–25	38	60–65
Hatching	26–28	37	65–70

The eggs were turned five times a day during setting period until the last 3 days before hatch. At 26^{th} day of incubation, eggs were transferred from the setting tray to the hatching tray. During the hatching period, the humidity of the incubator was increased by spraying a small amount of water. The temperature of the added water was maintained same in the incubator so that no stress occurred on the eggs. All the dead embryos were considered as fertile. Hatched poults were collected, counted and weighed using an electronic scale. The fertility levels of each treatment group was calculated as outlined by Sotirov *et al.* (2002) and recorded in percent:

$$\text{Fertility}(\%) = \frac{\text{Number of fertile eggs}}{\text{Number of eggs set}} \times 100$$

The hatchability percent of each treatment was calculated as outlined by Hafez (1985) and Wilson (2008) and recorded in percent:

$$\text{Hatchability}(\%) = \frac{\text{Number of poults hatched}}{\text{Number of fertile eggs at candling}} \times 100$$

2.9. Calculation of economic performance

The calculation for the economic performance was done using market prices of feed ingredients and other necessary items to compare the costs of different treatments. The price of turkey, feed, grass, electricity, labor, medication etc. were taken into account based on the market price during the experimental period in Bangladesh. The financial values of the study were calculated based on the national money unit of Bangladesh. The average exchange rate of Bangladesh Bank over the research period was 1 USD = 80 BDT.

Total variable cost (TVC): TVC is the cost of variable inputs such as feeds, labor and drugs used for production, and it changes directly with the level of production.

Total fixed cost (TFC): TFC is the cost of permanent items, which does not vary when output get changes and therefore have no influence on production decisions in short run.

Total revenue (TR): TR is the total money value of all output produced whether sold, consumed or in stock.

Net farm income (NFI): NFI means difference between total returns and total expenses for production. It was calculated using the following equation:

$$NFI = TR - (TVC + TFC)$$

Where; NFI = Net farm income (NFI), TR = Total revenue, TVC = Total variable cost and TFC = Total fixed cost.

Profitability index (PI): Profitability index (PI) means the net farm income (NFI) per unit of total revenue (TR) and it was calculated using the following equation:

$$PI = \frac{NFI}{TR}$$

Where; PI = Profitability index, NFI = Net farm income and TR = Total revenue

Rate of return on investment (RRI): RRI is the performance measure used to evaluate the efficiency of an investment or to compare the efficiency of different investments. It was calculated using following equation:

$$RRI = \frac{NFI}{TC}$$

Where; RRI = Rate of return on investment, NFI = Net farm income and TC = Total cost.

Capital turnover (CTO): It is the ratio of total revenue to total cost and it was measured using following equation:

$$CTO = \frac{TR}{TC}$$

Where, CTO = Capital turnover, TR = Total revenue and TC = Total cost

Depreciation: To calculate the worth of each fixed cost items, the straight-line method of depreciation was followed using the following equation:

$$\text{Depreciation cost} = \frac{\text{Purchase price}}{\text{Number of useful years of the asset}}$$

2.10. Statistical analysis

Effect of treatment on fertility, hatchability, egg production, egg weight, poult weight, embryonic mortality, survivability and profitability were analyzed using the one-way ANOVA procedure in accordance with the Completely Randomized Design (CRD) following the GLM procedure of SPSS computer software 22.00 (SPSS, 2013). Significance of differences among the means of treatments was compared by using Duncan's Multiple Range Test (DMRT) of the same package. All data were expressed as Mean±Standard Error of Mean (SEM). Significant differences were considered at the levels of $P<0.01$ and $P<0.05$. The linear model : $Y_i = \mu + TR_i + E_i$, was used to summarize the statistics employed to analyze the data; where; Y_i is the dependent variable, μ is the overall mean, TR_i is the treatment effect (the effect due to natural mating and artificial breeding techniques by using fresh and chilled semen) and E_i is the error.

3. Results

The effect of AI on the reproductive as well as economic traits egg production, egg weight, fertility, embryonic mortality, hatchability and survivability are presented in Table 4. There was no significant difference regarding average egg production among T_1 (49.72±4.66%), T_2 (51.14±3.26%) and T_3 (48.52±1.78%) groups. However, weekly egg production differed significantly ($P<0.05$; Figure 2). The egg weight did not differ significantly. The average egg weight was 65 g; and it increased steadily from <60 to >70 g in accordance with the increased age up to 46 weeks (Figure 3).

The fertility differed significantly ($P<0.01$) among the treatment groups. The highest fertility (89.71±4.79%) was found in T_2 group while lower fertility were in T_3 (60.77±1.93%) and T_1 (59.21±3.12%) groups. Hatchability were 88.35±5.33, 88.87±5.06 and 89.21±4.37% for T_1, T_2 and T_3 group, respectively and there was no significant difference among the groups.

Statistically similar EEM was observed in T_1 (3.26±1.40%), T_2 (3.01±1.12%) and T_3 (3.24±1.43%) groups throughout the experimental period. LEM differed significantly ($P<0.05$) and those were 5.28±1.67, 7.11±1.68 and 5.95±1.7% for T_1, T_2 and T_3 groups, respectively. DIS also differed significantly ($P<0.05$) and those were 3.11±1.33, 1.01±0.41 and 1.6±0.67 for T_1, T_2 and T_3 groups, respectively.

The survivability rate and hatched weight of poults did not differ significantly. Survivability rate of hatched poults for the first seven days were 94.91±3.64, 94.82±6.91 and 93.82±5.61% for T_1, T_2, and T_3 groups, respectively. The average weight of the day old poults were 43.83±2.44, 43.33±2.33 and 44.00±3.58 g for T_1, T_2 and T_3 groups, respectively.

To measure the economic efficiency of different breeding techniques, the cost and return items associated with turkey breeding in the study period was calculated in BDT based on market price during the experimental period and presented in Table 5. Variable costs for medication and vitamin-mineral supplement, feed and grass differed significantly ($P<0.05$). Therefore, TVC also differed notably among the treatment groups. In case of fixed costs

depreciation, cost on lab equipment and TFC differed significantly (P<0.05) and it was higher in T_1. Finally, total cost also differed significantly (P<0.05) and it was higher in T_1. Sales of poults, TR and NFI were significantly (P<0.05) higher in T_2 than that of T_1 and T_3. The other profitability variables PI, RRI and CTO differed significantly (P<0.05) among the groups and these were lower in T_1 group.

Table 4. Reproductive performance of turkey hens bred by natural mating and AI with fresh and chilled semen.

Variables	Breeding treatment groups			Level of significance
	T_1	T_2	T_3	
Av. egg production (%)	49.72±4.66	51.14±3.26	48.52±1.78	NS
Av. egg weight (g)	65.11±1.24	65.67±1.20	65.44±1.13	NS
Fertility (%)	59.21±3.12^a	89.71±4.79^b	60.77±1.93^a	**
Early embryonic mortality (%)	3.26±1.40	3.01±1.12	3.24±1.43	NS
Late embryonic mortality (%)	5.28±1.67^a	7.11±1.68^b	5.95±1.78^a	*
Dead in shell (%)	3.11±1.33^b	1.01±0.4^a	1.6±0.67^a	*
Hatchability (%)	88.35±5.33	88.87±5.06	89.21±4.37	NS
Survivability of poult (%)	94.91±3.64	94.82±6.91	93.82±5.61	NS
Day-old poult weight (g)	43.83±2.44	43.33±2.33	44.00±3.58	NS

Values are means±SEM; ^{a,b,c}Means within a row without common superscripts differ significantly; NS = non significant; statistically significant difference is expressed as *(P <0.05) or **(P < 0.01). Here, T_1 = Natural mating, T_2 = AI with fresh semen and T_3 = AI with chilled semen.

Table 5. Economic performance of different breeding techniques.

Variables	Breeding treatment groups			Level of significance
	T_1	T_2	T_3	
A. Variable costs				
Labor	18000	18000	18000	NS
Feeds	14700±102.34^b	13230±108.05^a	13213±101.23^a	*
Grass	2940±203.22^b	2630±195.45^a	2646±195.45^a	*
Medication and vitamin-mineral supplement	2450±112.12^b	1890±150.11^a	1890±150.11^a	*
Water	1000	1000	1000	NS
Electricity	605	605	605	NS
Transportation	1450	1450	1450	NS
Total variable cost (TVC)	41145±203.56^b	38805±207.11^a	38804±219.67^a	*
B. Fixed costs				
Cost of turkey	50000	45000	45000	NS
Depreciation on housing @ 5%	635	635	635	NS
Depreciation on farming equipment@10%	95	95	95	NS
Depreciation on lab equipment @ 10%	2.00±0.001^a	3.00±0.004^b	31.00±3.45^c	*
Total fixed cost (TFC)	50732±507.3^b	45733±607.8^a	45761±705.5^a	*
Total cost	91877±550.5^b	84538±601.9^a	84565±605.3^a	*
C. Revenue				
Sales of poults	170000±110.5^a	178000±134.4^b	171500±103.4^a	*
Sales of litter	700	700	700	NS
Total revenue (TR)	171040±817.2^a	179056±788.3^b	172543±653.5^a	*
Net farm income (NFI)	79163±115.32^a	94518±111.34^c	87978±110.55^b	*
Profitability index (PI)	0.46±0.005^a	0.53±0.004^b	0.51±0.001^b	*
Rate of return on investment (RRI)	86.16±3.11^a	111.81±3.65^b	104.04±4.87^b	*
Capital turnover (CTO)	1.86±0.21^a	2.12±0.32^b	2.04±0.43^b	*

Values are means±SEM; ^{a,b,c}Means within a row without common superscripts differ significantly; NS = non significant; statistically significant difference is expressed as *(P <0.05). Here, T_1 = Natural mating, T_2 = AI with fresh semen and T_3 = AI with chilled semen.

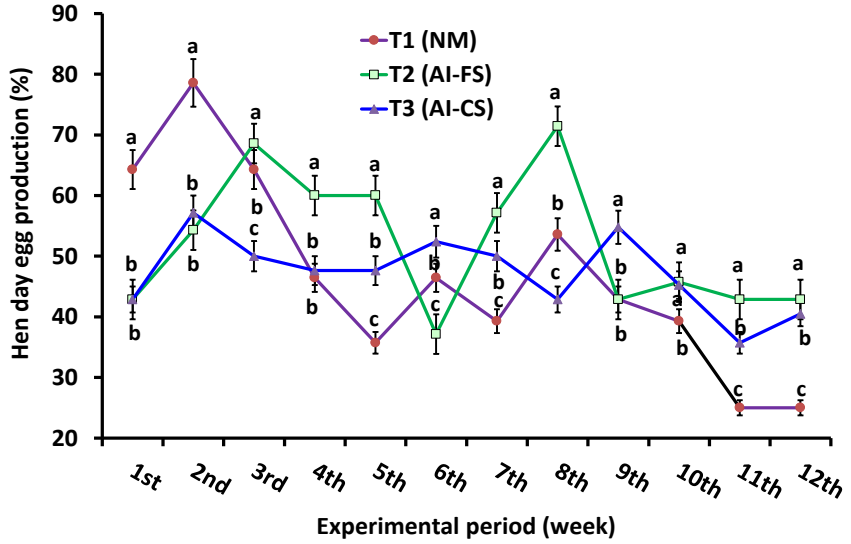

Figure 2. Weekly egg production of turkey hen bred by natural mating and artificial insemination using fresh and chilled semen. Each line with error bar represents the mean±SEM values. Different letters above the error bars indicate statistically significant differences (P<0.05) in the same week. Here, T_1 (NM) = natural mating, T_2 (AI-FS) = artificial insemination with fresh semen and T_3 (AI-CS) = artificial insemination with chilled semen.

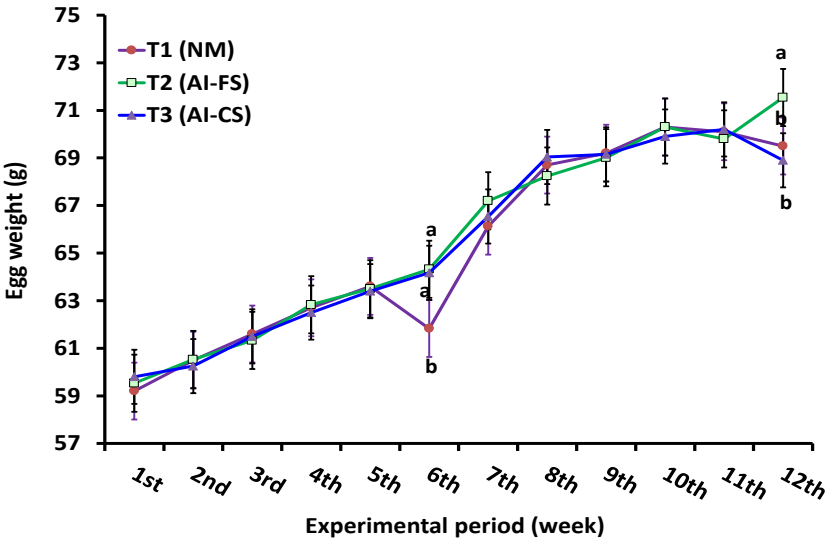

Figure 3. Weekly egg weight pattern of turkey hen bred by natural mating and artificial insemination using fresh and chilled semen. Each line with error bar represents the mean±SEM values. Different letters above the error bars indicate statistically significant differences (P< 0.05) in the same week. Here, T_1 (NM) = natural mating, T_2 (AI-FS) = artificial insemination with fresh semen and T_3 (AI-CS) = artificial insemination with chilled semen.

4. Discussion

4.1. Fertility

This was the first attempt to conduct a study on the artificial breeding for turkey in Bangladesh. The results observed from this study were in general similar with the research works conducted in different countries. It indicates that the use of AI as a supplement or replacement to natural mating obviously improve fertility of turkey hen. The highest percent of fertile eggs (P<0.01) was obtained from the hen inseminated with fresh semen. This result was in agreement with the observations of Mohan *et al.* (2013) and Donoghue (1999) who reported that AI in turkey was more efficient than NM. Similarly, Marire (2011) reported 95.0% success result

from AI and recommended it as only the better method for economic poult production. This experiment further showed that chilled semen could also be used successfully for turkey breeding in Bangladesh. In this experiment, turkey semen was stored in refrigerator for 24 hours and fertility results were consistent with the results of Akcay *et al.* (2006) as well.

AI with fresh semen resulted in the highest fertility, which is in line with the observation of Emilia *et al.* (2010) who experienced the highest fertility up to 98.0%. The experiment was conducted using the turkey aged between 35 to 46 weeks. So good fertility results occurred, which is at par with the observations of Sexton (1977) who argued that the fertility increased to a peak and then gradually declined as turkey hen age increased. This might be occurred due to changes in SST. As the age of hen increases, it might cause fewer sperm at the site of fertilization. Pierson *et al.* (1988) observed decline fertility during late season production, which was related to a reduction of sperm retention in the sperm storage tubule. This fertility results indicated that AI could be a tailor-made sustainable solution for infertility problem of turkey hen in Bangladesh. AI gives opportunity for maximum use of the best toms. Therefore, McDonald (2003) also argued AI as a vital tool for rapid improvement of infertility in turkey. It could be inferred from the results of this experiment that infertility in hen could occur not only from improper mating between tom and hen, but also from improper semen preservation, application of AI technique and semen retaining capacity of hen.

4.2. Hatchability

The hatchability results showed that AI had no effect on hatching of turkey egg. Akcay *et al.* (2006) also found no significant differences on hatchability for semen stored in ringer+glucose-based extender for 24 and 48 h. The hatching rate of this study was lower than the range of 95−100% as reported by Keith (2008); but higher than the range of 22−51% as reported by Machebe *et al.* (2013). Similarly, Ngu *et al.* (2013) and Anandh *et al.* (2012) found 56.25 and 52.85% hatchability, respectively. As egg production in turkeys are lower than other poultry species, so special care should have to be taken during hatching. Camci and Sarica (1991) and Kaygisiz *et al.* (1994) also made similar recommendations with this regard.

4.3. Embryonic mortality

No significant difference was found among the turkey groups for EEM. It indicated that there was no influence of breeding methods on EEM. EEM might be occurred because of rupture of air sac and blood vessels due to poor handling of egg during transportation and setting (Keith, 2008; Bramwell *et al.*, 1996). The results on LEM was lower than those of Emilia (2010) who reported 13.0–23.0% LEM. This result was also lower than that of Khan *et al.* (2013) who reported 7.5, 13.2 and 19.3% deaths as early, mid and late embryonic mortality, respectively. Thick shell of eggs might cause poor oxygen supply for metabolism of embryo. Asphyxiation and retarded development due to insufficient water loss caused LEM (Christensen and McCorkle, 1982). French (1997) reported that because of metabolic heat production by the embryo, the temperature inside the egg rises by 2^0C above the surrounding air temperature, which might have caused death of the embryos due to hyperthermia, which was also observed by Hassan *et al.* (2004).

This result showed that dead in shell increased as the weight of egg increased. Other studies also revealed more dead in shell; for instance, Ngu *et al.* (2013) found 42.75 and 35.16% in local and exotic breeds of turkey, respectively. This might be happened due to difficulty in achieving adequate embryonic temperature at the initial stage and losing embryonic metabolic heat at the later stage of incubation.

4.4. Egg production and egg weight

Similar egg production among the treatment groups revealed that AI had no significant effect on egg production. Weekly egg production (Figure 2) differed significantly ($P<0.05$) might be due to natural egg production pattern of turkey hen. Results also showed that different breeding methods in turkey had no effect on egg weight. The average egg weight (65.40 g) obtained from this study was almost similar to the results of Ozcelik *et al.* (2009). Weekly egg weight trend at different weeks (Figure 3) indicates that weight of egg was increased in accordance with the advancement of age of hen.

4.5. Poult weight and survivability

No significant results of poults weight and survivability rate revealed that the insemination procedure did not have any influence on hatching weight and survivability of poults. The average weight of poults obtained from this study was consistent with the results of Anandh *et al.* (2012). The result of egg weight of the experiment was also in conformity with the observations: hatching weight of poult constitutes 63.5% of egg weight (Shanaway (1987) and 67.0% of the initial egg mass for turkey egg (Rahn *et al.*, 1981). The average poult

survivability rate (94.0%) was also consistent with the result of Anandh *et al.* (2012). In fact, poult mortality occurred owing to inadequate temperature and piling, just after hatching out from the incubator. However, there was no mortality occurred in breeding flock during the experimental period.

4.6. Profitability

The profitability of turkey breeding was calculated from the costs incurred and the returns accrued. The highest variable cost for feed, grass, medication and vitamin-mineral supplement; and TVC was resulted in T_1 group because of rearing more number of toms. The highest depreciation and the lab cost were incurred for T_3 group due to processing of chilled semen. However, total cost was highest for T_1 group due to maintaining more toms for natural mating and treatments of injury occurred during fighting among toms and mating with hen. The T_2 group generated highest TR and NFI because of laying of more fertile eggs. However, PI, RRI and CTO were statistically similar for T_2 and T_3 groups due to lower extent of production pattern.

The profitability indicators, i.e. PI, RRI and CTO were higher for T_2 group. In case of T_2 group, PI 0.53 means each BDT earned as revenue BDT 0.53, which returned to producer as net farm income; RRI 111.81% indicated each taka invested generated 111.81% net income; and CTO 2.12 implied that for each BDT invested returned to producers as revenue BDT 2.12. In fact, higher RRI and capital turnover ratio greater than 1 indicates better success of farm business (Olukosi and Erhabor, 1988) which was resulted in T_2 group. However, due to low fertility, T_1 and T_3 groups resulted in lower number of poults, which ultimately caused lower profitability.

5. Conclusions

From this study, it could be concluded that higher fertility of turkey hen could be obtained by using AI with fresh semen than AI with chilled semen and NM; but fresh as well as chilled semen were cost effective than NM; and different breeding methods had no adverse effect on egg production, egg weight, hatchability and survivability of poults. However, further research is required with this regard to find out the highest level of accuracy of the results.

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Data availability

The data used to support the findings of this study are included within the article.

Conflict of interest

None to declare.

Authors’ contribution

Mohammad Asaduzzaman: as a PhD research fellow conducted this research and wrote the manuscript; Dr. Abdul Gaffar Miah: supervised the research as PhD supervisor; Dr Ummay Salma: supervised the research as PhD co-supervisor; Mst. Shahana Jahan: assisted in data processing and analyzing. All authors have read and approved the final manuscript.

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