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Original article

EFFECTS OF SEMEN DOSE ON EGG FERTILITY, EMBRYO MORTALITY AND HATCHABILITY IN ARTIFICIALLY INSEMINATED GEESE (*ANSER CYGNOIDES)*

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Summary

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The effects of semen dose on egg fertility, hatchability and embryo mortality in geese artificially inseminated with undiluted semen were investigated in a 4-weeks experiment. Twenty-four mature geese (4.0±0.45 kg average weight) were randomly divided into 4 groups of 2 replicates with 3 geese per replicate in a completely randomised design. Fresh semen collected from six ganders (5.2±0.69 kg average weight) was pooled and used to inseminate the geese at 0.05 mL (T1), 0.10 mL (T2), 0.15 mL (T3) and 0.20 mL (T4) respectively at 3-day intervals for 4 weeks. Incubation, candling and transfer of eggs to the hatcher were done using standard procedures and goslings hatched out on day 30. Percentage fertility, early embryo mortality (EEM), mid embryo mortality (MEM), late embryo mortality (LEM), hatch of fertile eggs (HFE) and hatch of set eggs (HSE) were obtained and analysed using descriptive statistics and ANOVA at P = 0.05 and means were separated using Duncan multiple range test. The progressive sperm motility, spermatozoa concentration and spermatozoa liveability of the pooled semen used for insemination were 55.67±9.81%, 203±1.57×10^6 sperm cells/mL and 77±5.29%, respectively. T4 geese had significantly higher fertility (91.07±10.72%) than T1 (56.25±18.48%), T2 (61.67±10.27%) and T3 (64.08±26.25%) groups. The EEM was significantly lower (38.89±11.7%) while MEM was significantly higher (38.89±19.2%) in T4 birds compared to the other groups. The LEM was however higher in T1 birds (37.50±35.3) than in the other groups. Hatch of set eggs was also higher in T3 and T4 geese compared to the other groups. Thus, it can be concluded that egg fertility in geese was semen dose-dependent and that a fresh semen dose of 0.2 mL was sufficient to improve fertility up to 91%.

Key words: artificial insemination, egg evaluation, hatchability, embryo mortality, geese

INTRODUCTION

The low reproductive performance of geese has been a threat to their production over the years (Liu *et al*., 2008). Geese reproduction remains relatively complex due to the physiological and behavioural specificities of the species. The ganders produce a small volume of ejaculate with a low spermatozoa concentration and a

low number of live normal spermatozoa (Lukaszewicz, 2021). Other known causal factors contributing to impaired fertility in these flocks include excessive body weight of ganders, pairing behaviour, male leg disorders, copulatory organ malformation and low efficacy of sperm acceptance in the oviduct of aging females. Hence, the use of artificial insemination to breed geese becomes necessary. However, semen dosage for optimum fertility has not been documented in Nigeria. Besides, the dosage necessary for geese insemination to obtain optimum fertility level is still controversial in the few possible areas where it has been done outside Nigeria.

Reproductive manipulation with artificial insemination has become a common practice in the current animal husbandry, livestock and poultry breeding programmes including geese breeding. It is an effective strategy in improving the reproductive performance and genetics of animals (Lukaszewicz, 2021; Dalton, 2022). It enables the breeder to overcome different breeding problems, preserves the genetic resource of many species threatened by the damage of their natural habitat and reduces the cost of purchasing several males for breeding. It has been used as a powerful alternative to natural mating conditions as it provides a range of ways to control, replace or improve the defective factors causing fertility problems and also prevent the spread of diseases (Rauthan *et al.,* 2022). In the case of natural mating in geese, behavioural problems, such as preferential mating of a male with a single female, may cause alteration to reproductive performance. The use of artificial insemination can help to increase the number of goslings and facilitate the feasibility of performing cross breeding and intergeneric crosses for genetic improvement. Studies conducted in the temperate region have demonstrated the possibilities of artificial insemination to improve the effectiveness of commercial geese production.

Presently, little information has been reported on artificial insemination in geese throughout the world, let alone semen dose requirement and number of spermatozoa necessary for satisfactory and optimum fertility level in geese. Therefore, this study was designed to investigate the effects of semen dose on egg fertility, hatchability and embryo mortality in artificially inseminated geese using undiluted semen.

MATERIALS AND METHODS

Animals, experimental site and experimental layout

This study was carried out at the Poultry unit of the Teaching and Research Farm, University of Ibadan, with a geographical location of latitude 7° 26′ N and longitude 3° 54′ E. Twenty four (24) one-year old crossbred geese and six (6) ganders were randomly selected and used; with an average weight of 5.1±0.4 kg for males and 4.1±0.30 kg for females. The geese were housed separately based on sex and were given feed and water *ad libitum* throughout the period of the experiment. The feed for the animals during the experimental period was commercial layer's mash (brand name: Top Feed) containing crude protein of 16.5%, digestible energy of 2,500 kcal/kg, crude fibre of 6%, crude fat of 5%, calcium of 3.5% and phosphorus of 0.41% as nutrient composition.

Ethical approval

This study was conducted according to the research ethics approved by the committee on research of the University of Ibadan,

Ibadan, Nigeria – UI-ACUREC/19/0115, 10/10/2019.

Experimental design

Twenty four geese were randomly divided into 4 groups of 2 replicates with 3 geese per replicate in a completely randomised design. Semen pooled from 6 ganders was used to inseminate the geese in groups 1 to 4 at 3-day intervals. The groups were as follows: Group 1 – 0.05 mL semen dose, Group 2 – 0.10 mL semen dose, Group 3 – 0.15 mL semen dose, Group 4 – 0.20 mL semen dose.

Semen was collected from the ganders individually using the dorsoabdominal massage method (Johnson, 1954; Lukascewick, 2021). During semen collection, the ganders were carefully captured and restrained by interlocking their wings and were placed on the lap to properly curtail them. They were allowed to rest for some minutes after which they were massaged until their penile organ protruded out as described by Akinbola & Ewuola (2021). A collection tube was placed underneath the carnal to collect semen through the length of the penis. Semen collected from the ganders was pooled together for the insemination process.

Semen parameters evaluated through microscopic examination were semen volume, progressive spermatozoa motility, spermatozoa concentration and ratio of live to dead sperm cells (Abioja *et al.,* 2022). Semen was deposited by finger guided method known as palpation method (Johnson, 1954; Łukaszewicz, 2002). The geese to be inseminated were placed on the lap after capturing and restrained until they were less frightened. The birds were positioned in a way that the head and the neck faced the operator and the neck was allowed to rest underneath the armpit of the operator, to restrict neck twisting. This was done according to the modification of Akinbola & Ewuola (2021). After this, the index finger of the left hand of the operator was inserted into the vent of the bird. Through careful palpation, the opening of the oviduct was located. A syringe with a glass tube attached, containing the semen was inserted into the oviduct of the bird through the vent, and semen was deposited at a depth of about 3–4 cm (FAO, 2002; Łukaszewicz, 2002).

Egg collection, incubation and embryo mortality determination

Eggs from each treatment group were collected daily, marked and stored. Incubation of eggs was done weekly. Candling was also done on day 27 to observe the growth and development of embryo in the fertile eggs and to separate the unfertile ones. The eggs were also transferred to the hatcher on day 27. Goslings hatched out on day 30. Egg collection and incubation was done for 4 weeks. The candle clears were broken after the goslings have been recovered from the hatcher. The eggs were categorised based on the presence or absence of embryonic development in them and the stage of the embryonic development in each egg before death was observed and recorded (early embryo mortality – day 1 to 10, mid embryo mortality – day 11 to 20 and late embryonic mortality – day 21 to 30). The classification was done as per modification by Akinbola & Ewuola (2021). Eggs without embryonic development were classified as infertile eggs.

Data collection and analysis

Data collected included egg fertility (EF), hatch of fertile eggs (HFE), hatch of set eggs (HSE) and embryo mortality (EM)

percentages determined using the equations below:

$$\mathrm{EF}(\%) = \frac{\text{Fertile eggs number}}{\text{Set eggs number}} \times 100$$

$$\mathrm{EM}\ (\%) = \frac{\text{Dead embryos number}}{\text{Fertile eggs number}} \times 100$$

$$\mathrm{HFE}\ (\%) = \frac{\text{Hatched goslings number}}{\text{Fertile eggs number}} \times 100$$

$$\mathrm{HSE}\ (\%) = \frac{\text{Hatched goslings number}}{\text{Set eggs number}} \times 100$$

Data were analysed using descriptive statistics, General Linear Model; means were separated using Duncan Multiple Range Test (SAS, 2003). Values in percentage were log transformed. Differences were considered significant at $P<0.05$.

RESULTS

The semen quality parameters of individual ganders prior to the commencement of the experiment is shown in Table 1. The ganders were about 1-year-old at the time of the assessment. The average semen volume was 0.73 mL while the spermatozoa motility was 56.50% within a range of 45–65%. The spermatozoa concentration was 40.92×10^6 motile sperm cells/mL and the mean live to dead ratio: 80.75% within a range of 53–95% for all animals.

The characteristics of the pooled semen used for inseminating the geese during the experiment is presented in Table 2. The values for spermatozoa concentration, liveability and progressive motility were 203.00×10^6 sperm cells /mL, 77.00% and 55.67%, respectively.

The weekly egg fertility pattern for 4 weeks in geese inseminated with undiluted

Table 1. Semen quality parameters of individual ganders at the pre-experimental period. Values are presented as mean and standard deviation (SD), n=12 and range

S/N	Semen volume (mL)	Spermatozoa motility (%)	Spermatozoa concentration ($\times 10^6$ cells/mL)	Spermatozoa liveability (%)
1	1.10	65.00	21.00	89.00
2	0.50	60.00	36.00	92.00
3	0.60	60.00	43.00	78.00
4	0.70	65.00	33.00	90.00
5	0.60	58.00	8.00	93.00
6	0.90	57.00	31.00	53.00
7	0.80	60.00	6.00	89.00
8	0.40	50.00	190.00	77.00
9	0.90	60.00	57.00	95.00
10	1.00	50.00	3.00	63.00
11	0.50	50.00	22.00	70.00
12	0.71	53.00	41.00	81.00
Mean±SD	0.73±0.22	57.33±5.45	40.92±49.70	80.75±13.23
Range	0.40–1.10	50.00–65.00	3.00–190.00	53.00–95.00

Table 2. Characteristics of pooled semen inseminated at varied semen dosage. Values are presented as mean and standard deviation (SD), n=6 and range

Semen parameters	Mean ± SD	Range
Spermatozoa concentration ($\times 10^6$)	203.00±15.70	71.00 – 377.00
Spermatozoa liveability (%)	77.00±5.29	71.00 – 81.00
Progressive spermatozoa motility (%)	55.67±9.81	50.00 – 67.00

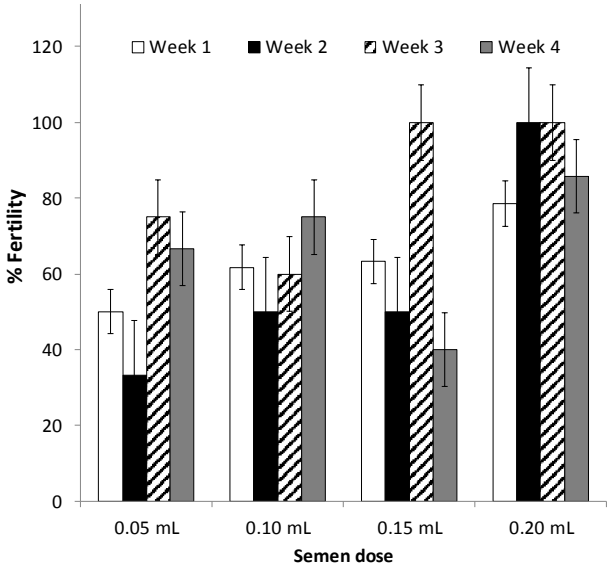

Fig. 1. Weekly egg fertility pattern of geese inseminated with varied undiluted semen doses. Values are presented as mean ± SD (n=24).

semen at varied doses is presented on Fig. 1. There was a sustained and constant high fertility percentage through the 4 weeks duration for geese inseminated at 0.2 mL, as compared with the other groups.

Table 3 shows the effects of semen dose on the overall egg fertility in geese inseminated with undiluted semen. The geese were inseminated with 0.05 mL (T1), 0.10 mL (T2), 0.15 mL (T3) and 0.20 mL (T4) containing 5.65×10^6, 11.30×10^6, 16.95×10^6 and 22.60×10^6 motile sperm cells respectively in this experiment at 3-day intervals for 4 weeks. As presented, the percentage egg fertility

Table 3. Overall egg fertility of geese inseminated with undiluted semen at varied dosages. Values are presented as mean and standard deviation (SD), n = 24

Insemination dose	Egg fertility (%)
0.05 mL (T1)	56.25 ± 18.48^b
0.10 mL (T2)	61.67 ± 10.27^b
0.15 mL (T3)	64.08 ± 26.25^b
0.20 mL (T4)	91.07 ± 10.72^a

Note: Means in the same column with different superscripts (a-b) are significantly different ($P<0.05$).

Table 4. Egg fertility, embryo mortality and hatch parameters of geese inseminated with undiluted semen at varied dosages. Values are presented as mean and standard deviation (SD), n=24

Semen dosage (mL)	Fertility (%)	EEM (%)	MEM (%)	LEM (%)	HFE (%)	HSE (%)
0.05	56.25±18.48^{b}	62.50±28.87^{a}	0.00±0.00^{c}	37.50±35.3^{a}	0.00±0.00^{c}	0.00±0.00^{b}
0.10	61.67±10.27^{b}	61.11±34.6^{a}	27.78±11.7^{b}	11.11±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{b}
0.15	64.08±26.25^{b}	50.00±35.3^{a}	16.67±0.00^{b}	0.00±0.00^{b}	33.33±0.00^{a}	16.67±0.0^{a}
0.20	91.07±10.72^{a}	38.89±11.7^{b}	38.89±19.2^{a}	5.56±0.00^{b}	16.67±0.00^{b}	8.33±0.00^{a}

EEM = early embryo mortality, MEM =mid embryo mortality, LEM = late embryo mortality; HFE = hatch of fertile eggs; HSE = hatch of set eggs; Means in the same column with different superscripts (a-c) are significantly different (P<0.05).

of geese eggs in each dosage group was significantly (P<0.05) different from T4 (0.20 mL), which showed a significantly higher value (91.07%) compared to T1 (56.25%), T2 (61.67%) and T3 (64.08%).

Table 4 presents the fertility, early embryo mortality (EEM), mid embryo mortality (MEM), late embryo mortality (LEM), hatch of fertile eggs (HFE) and hatch of set eggs (HSE) of geese inseminated with different undiluted semen doses. Egg fertility was significantly (P<0.05) higher for geese inseminated with 0.20 mL semen dose. The EEM was significantly lower (38.89±11.7%) while MEM was significantly higher (38.89± 19.2) in T4 birds compared to the other groups. The LEM was however higher in the T1 birds (37.50±35.3) than in the other groups. Hatch of set eggs was also higher in T3 and T4 birds than in the other groups. The EEM, MEM, LEM, HFE and HSE value ranges were 38.89 to 62.50%, 0.00 to 38.89%, 0.00 to 37.50%, 0.00 to 33.33% and 0.00 to 16.67%, respectively.

DISCUSSION

The individual gander semen and their pooled semen quality showed that it had lower spermatozoa motility, concentration and liveability compared to cocks and toms. The values reported were similar to those obtained in geese according to the studies of Łukaszewicz (2021) and Ewuola *et al*. (2023) which showed that ganders produced ejaculate with low spermatozoa concentration and low number of live normal spermatozoa. However, the volume obtained in this study was higher than that reported by Łukaszewicz (2021). This may be due to the differences in the environmental conditions, where the experiments were conducted.

In comparison with males from other poultry species, such as chickens, ducks or turkeys, poor semen quality is often observed in most breeds of ganders. As a consequence, sperm concentrations are relatively low, as are the other main quantitative characteristics of ejaculates (Chełmonska, 1972). The number of live spermatozoa is often similar to that of other poultry species like turkeys and chickens (Łukaszewicz, 2021). However, values above 50% of morphologically normal and viable sperm are only sporadically observed in gander semen (Łukaszewicz, 2002; Liu *et al*., 2008).

The trend of fertility (weekly and overall pattern) obtained at different dosages between 0.05 mL to 0.2 mL may be an indication that higher dosage for geese insemination up to 0.20 mL as compared

to the 0.05 mL recommendation of FAO (2002) may result in increased level of fertility which is necessary for species with low fertility as geese, especially when bred under natural mating process. This however depends on the number of motile sperm cells inseminated per time. The geese in this study were inseminated with 0.05 mL, 0.10 mL, 0.15 mL and 0.20 mL semen containing 5.65×10^6, 11.30×10^6, 16.95×10^6 and 22.60×10^6 motile sperm cells respectively. Fertility percentages of 56.25%, 61.67%, 64.08% and 91.07% respectively were obtained. In the study conducted by Łukaszewicz (2002), 9 million progressively motile, live normal spermatozoa were inseminated weekly with fresh semen resulting in 89% fertility, while the increase in spermatozoa number to 20 million resulted in 95.5% fertile eggs. The insemination with 22.60 million motile sperm cells in this study also resulted to 91.07% fertility that is closely similar to the value obtained in the study of Łukaszewicz (2002): 95.5% when 20 million motile sperm cells were inseminated.

The number of spermatozoa needed by geese for fertilisation to occur in their oviduct is minimal compared to hens and turkeys. Yet, with this, a satisfactory fertility rate can be achieved. A recommendation of 100 million progressively motile spermatozoa dosage to be inseminated weekly has been predicted in the case of domestic hens (Łukaszewicz, 2002), while for geese insemination, 30–40 million spermatozoa every 6 days has been suggested (Davtian & Pimenow, 1970). Fourteen million spermatozoa insemination once or twice a week can result in 54% and 83% of fertile eggs respectively (Grunder & Pawluczuk, 1991). Inseminating not less than 5 million motile spermatozoa could result in a similar fertility level as inseminating 40 million motile spermatozoa (Behr & Hartmann, 1992). According to Borys *et al*. (1978), it is expected that 2.7 million spermatozoa per mL should be present in a fresh gander ejaculate in a day.

Generally in this study, high embryo mortality and low hatchability values observed for all dosage treatments are typical characteristics of the goose species. They are often characterised by low fecundity, low hatchability and high embryo mortality. Hence, the observed high embryo mortality could be due to their longer incubation period (30 to 35 days) as compared to many other poultry species. This was pointed out in the report of Lukaszewicz (2017) who affirmed that for geese egg to hatch, they require longer incubation periods than chickens, turkeys and many other poultry species. Also, geese embryo mortality was reported to be higher due to their extended incubation period and the hatchability rate reported was low in the study of Lukaszewicz (2017). Goose egg production is commonly between 35 and 60 eggs per female in a season and it is very rare to see hatchability rates exceeding 80% (Bednarcysyk & Rosinski, 1999). According to the study of Salamon (2020), hatchability was reported to be 41.12%, 61.94% and 63.77% for goose eggs without manual turning, turned along the long axis and turned along the short axis respectively. Therefore, the probability of obtaining a lower number of hatched goslings yearly is high.

Lower hatchability below these values as observed in this study may also be due to differences in the environmental conditions (temperature and humidity) in temperate and tropical regions since this experiment was carried out at the tropical

region where environmental temperature is higher.

CONCLUSIONS

This study suggests that the egg fertility in geese was semen dose-dependent with 0.2 mL fresh undiluted semen dose (containing 22.60×10^6 motile sperm cells) improving fertility up to 91%. It was evident that the use of artificial insemination improved level of fertility in geese production in Nigeria and it should therefore be encouraged.

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