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Assessment of Fertility and Hatchability of Bhutanese Native Chicken Using Natural Service and Artificial Insemination

Sonam Dorji Layrab[1]*, Tshenden Zangmo[1], and Tshering Gyelpo[1]

Abstract

This study compared fertility and hatchability rates of three different Bhutanese native chicken strains (Seim, Belochem and *Yuebjha Narp*) using the artificial insemination (AI) and natural mating (NM). Ten hens from each strain were paired with two roosters of the same strain in the natural mating group, while ten hens from each strain were artificially inseminated using freshly prepared diluted semen collected from six roosters of the same strain. A total of 228 eggs (AI =111, NM =117) were incubated for 21 days. The eggs from the AI hens showed a lower fertility rate (44.78%) compared to those eggs from NM hens (75.52%) (t (10) = -3.19, p=.01). Within the NM group, NM Seim had the highest fertility rate (89.68%) while NM Yuebjha Narp had the lowest (54.78%). Similarly, in AI group, AI Seim showed the highest fertility (52.78%) while AI Yuebjha Narp showed the lowest (35.75%). Likewise, eggs incubated from AI hens (74.15%) showed a slightly lower hatchability rate compared to NM hens (82.39%), but was not significant (p>.05). Moreover, there were no differences in egg weight, egg shape index, embryonic mortality and day-old chick weight between AI and NM chickens (p>.05). While overall differences exist in fertility rates between AI and NM method, strain-specific variations suggest the need for further in-depth investigations on fertility.

Keywords: Artificial insemination, Fertility, Hatchability, Native chicken, Natural Mating.

Introduction

The poultry industry has emerged as a significant contributor to the global economy due to improved management and efficient breeding programs (Dhama *et al.,* 2014). Among all poultry species, chickens are the most socially and economically important because they provide meat, income, manure, are used for religious offerings and also a source of employment (Dana, 2011; Ngongolo *et al*., 2020). In Bhutan, farmers keep chickens along with other livestock to supplement animal protein and also generate additional income through the sale of meat and eggs (Bhujel *et al.,* 2019). Bhutan has 13 native chicken strains, of which only 10 are recognised by the Domestic Animal Diversity Information System of the Food and Agriculture Organization (FAO) (Dorji & Dorji, 2018). Native chickens are highly adaptable to the local environment and play an integral part in rural livelihoods (Dorji *et al.,* 2012). There is potential for improvement of native chicken production through crossbreeding with commercial chickens as well as with-

[1]Department of Animal Science, College of Natural Resources, Royal University of Bhutan
*Corresponding author: sonamlayrab@gmail,com
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in-breed selection (Padhi, 2016). This would help maintain the native chicken's unique attributes and at the same time avoid genetic erosion and contribute to their conservation (Khan, 2008; Okeno *et al*., 2013).

Poultry breeding involves several reproductive and management technologies, and artificial insemination (AI) is one of the most commonly used methods for breeding (Habibullah *et al.,* 2015). The AI in poultry involves collection of male avian sperm and introducing it to females with the intent of fertilizing eggs (Getachew, 2016). The AI usually requires less than 0.1 ml of semen (100 - 200 x 10^6 viable sperm per insemination) within the hen's vaginal canal (Gordon, 2017), which is a valuable tool because a single sperm ejaculate covers 30 - 100 hens (Das *et al*., 2004), which reduces the cost of production through maintaining a few roosters (Mohan *et al.,* 2018).

Climate change (e.g., global warming) is posing a significant challenge to chicken farming, and so the conservation and/or promotion of native chickens can become critical to supporting the livelihoods of rural people. On the other hand, the introduction of improved chickens has caused loss or dilution of native chicken genetic resources (Batiso, 2020). Additionally, a lack of systematic breeding in rural areas, for example, use of a few roosters for all hens seems to be contributing to inbreeding in Bhutan (Tenzin *et al*., 2023). There is a need to evaluate the effectiveness of AI in native chickens in order to apply in native chicken breeding and also to conserve them. This study aimed to assess fertility and hatchability rates of Bhutanese native chicken strains using AI and NM

Materials and Method

Study area

The experiment was conducted from December to April 2022 at the Native Poultry and Heifer Breeding Center (NPHBC) in Sertsam under Lhuntse dzongkhag and it is located at altitude of 986 meters above sea level. The centre was established to conserve native chickens and cattle breeds (e.g., Nublang) in Bhutan. The centre has 1200 native chickens brought from all over the country, which serve as parent stocks. The centre also distributes native chicken pullets to farmers.

Management practices and selection of chickens

A total of 84 (24 roosters and 60 hens) from 113 37-week-old native chicken strains viz. Seim, Belochem and Yuebjha Narp were selected randomly. Seim (Red Junglefowl-like) is a commonly reared strain in the country that is believed to be an immediate descendent of Red Junglefowl (Nidup *et al.,* 2005) while Yuebjha Narp (Native black / Black plumage) is considered to have medicinal values and Belochem is a crested strain (Dorji *et al.,* 2017). Sixty hens (ten hens of each chicken strain) and six roosters (two roosters of each chicken strain) were selected for NM purpose. Likewise, ten hens and six roosters of each strain were selected for AI purpose. Both roosters and hens were inspected and they were physically viable and free from external parasite infestation. The average body weight of the AI hens was 1.50 ± 0.03 kg and 1.64 ± 0.05 kg for NM hens. Roosters weighed heavier than the hens (AI = 2.28 ± 0.38 kg; NM = 2.28 ± 0.27 kg). There was no significant difference (p>.05) in the body weight among the strains in both hens and roosters. All the selected hens and roosters were kept separately for two weeks to avoid mating.

All roosters and hens were reared under a deep litter system. Each strain of native chickens was reared in different compartments in a single shed. All chickens were provided with layer feed (150g/chicken/day) because there was no breeder feed in the market. Feeding was done twice a day (morning and evening) and water was provided *ad libitum*. Sixteen hours of lighting was provided throughout the experimental period (Jacome *et al.,* 2014). The chickens were provided with a unique numbered leg band.

Collection and evaluation of semen

Six roosters of each strain were trained for semen collection using the abdominal massage method for two weeks (Castillo *et al.*, 2021). The testes were stroked and massaged until copulatory organ become enlarged and the semen was then milked which was collected in a graduated semen collection vial. The semen was collected on alternate days to provide sexual rest period (McDaniel & Sexton, 1977). The semen volume was measured, and color was evaluated in order to ensure that it was translucent milky white which is the normal color in chickens (Asmarawati *et al.*, 2019; Rashid & Khalid, 2023). The color of semen is an indicator of its density and contamination (Peters *et al.*, 2008). There was no significant difference in the average semen volume per ejaculate between the three strains (Seim = 0.23 ± 0.01 ml, Belochem = 0.28 ± 0.02 ml, Yuebjha Narp = 0.24 ± 0.02 ml) at p>.05. A drop of semen with the aid of a micro-pipette was placed on the microscopic slide and was viewed at 100 magnification, the sperm motility was determined as described by Yaman *et al.* (2022). There was no significant difference in the semen motility (Seim = 70%, Belochem = 72 %, Yuebjha Narp = 70 %). Fresh semen from six roosters of each strain was pooled in a clean dry testtube to avoid the effects of individual males on fertilizing ability (Bui *et al.*, 2018). An equal 0.9% NaCl solution was added to testube containing semen, while the temperature was maintained at 38 ^{o}C.

Artificial insemination

Each hen of AI group was inseminated with freshly diluted semen once a day on an alternative day (thrice a week) for 2 weeks between 14:00 - 16:00 hours. The reason for inseminating in the evening is because the presence of hard-shelled eggs in the uterus is minimum, which can affect fertility (Aisha & Zain, 2010). Hens were turned upside down and a light pressure was applied to the left side of the abdomen around the vent until the cloaca everted. A hypodermic syringe (1 ml) containing the diluted semen was inserted into the vaginal orifice to a depth of 2-4 cm and 0.2 ml of semen was dispensed. The inseminated hens showed sexual crouch or squatting which indicates receptivity (Karayat *et al.*, 2016).

Egg collection and incubation

The day on which hens were artificially inseminated was assigned as day 0 (fertilisation of egg). From day 3, eggs were collected for two weeks from both AI and NM groups. Hen day egg production (HDEP) was calculated as the total number of eggs laid per day by number of hens present on that day and multiplied by 100. All eggs collected were weighed by using a digital weighing balance. Also, egg length and width were measured by using a digital vernier calliper to estimate the egg shape index (Reddy *et al.,* 1979). Of 389 eggs collected (AI = 199; NM = 190), 228 eggs (AI = 111; NM =117) were selected and stored at room temperature (17 °C) before transferring to incubator (Hashem *et al*., 2016). One-hundred and sixty-one eggs were rejected eggs because they were either cracked or dirty. The eggs were transferred to the incubator, and eggs from AI and NM hens were arranged separately in the incubator. The average temperature and humidity in the incubators were 37.43 °C and 85.38% respectively. The incubator was equipped with an automatic egg turning systems (eggs turned once per hour).

Fertility, hatchability, embryo mortality and chick weight

On day 10, all eggs were candled to identify the fertile eggs. On day 18, fertile eggs were transferred from setters to hatchers in the hatchery before three days of hatching. All dead embryos (AI= 25; NM = 12) were removed from the hatchers on day 21 of incubation. Fertility rate was calculated as the total number of fertile eggs divided by the total number of eggs set in the incubator. Likewise, hatchability was estimated as the total number of eggs hatched divided by the total number of fertile eggs. The weight of chicks at hatching was weighed by using a digital weighing balance and brooded in a deep litter house from 0 - 8 days of age.

Data analysis

Data was recorded in Microsoft Excel and was exported to International Business Machines- Statistical Package for Social Sciences version 26 for further analysis. Descriptive statistics for fertility, hatchability, embryo mortality, chick weight, HDEP, egg shape index and egg weight were presented in means and standard error of mean (SEM). An independent samples *t*-test was done to check for differences in fertility and hatchability between NM and AI group. One way analysis of variance was used to compare fertility and hatchability among the strains at $p<.05$. Further, a pair-wise comparison was done using a Tukey's honestly significant difference test.

Results and Discussion

Fertility, hatchability rate and day-old chick weight

The hatchability rate in AI hens was similar to There was a significant difference in the fertility rates between AI and NM hens (t (10) = -3.19, p=.01) as shown in Table 1. The fertility rate was lower in AI hens (44.78%) compared to NM (75.52%). A lower fertility rate in hens of AI (32 %) was also reported by Koohpar *et al*. (2010) in Mazandran native hens. Several possible reasons attributed to low fertility in eggs laid by AI hens, such as dilution of semen, inadequate storage of semen in the infundibulum (Amao & Ayorinde, 2000), and hens undergoing stress during insemination procedure (Yeigba *et al.,* 2021; Koohpar *et al.,* 2010; Yeigba *et al.,* 2021). While overall differences of fertility rates exist between AI and NM group, strain-specific variations were also observed, for example, AI Seim hens laid eggs that had the slightly high fertility (52.78 ± 3.93%) than those in NM Yuebjha Narp (35.76%) $p<.05$ (Table 1). The results probably suggest the need for further in-depth investigations on fertility (Table 1).

There was no difference in hatchability rate between AI hens (74.15%) and NM hens (82.39%) ($p>.05$).

Table 1: Fertility, hatchability, embryo mortality and chick weight at hatching of naturally mated and artificially inseminated eggs in Bhutanese native chickens (mean ± standard error of mean)

Traits	Seim		Belochem		Yuebjha Narp	
	AI	*NM*	*AI*	*NM*	*AI*	*NM*
Egg set	53	39	33	38	40	33
Fertile	28	35	16	32	25	15
Infertile	14	4	12	6	25	15
Hatched	23	28	11	28	10	14
Fertility (%)	52.78±3.93^{a}	89.68±1.12^{b}	45.86 ± 24.46^{a}	82.10± 18.88^{b}	35.75± 7.05^{a}	54.78±10.92^{b}
Hatchability (%)	82.30 ± 3.26^{a}	80.10 ± 1.62^{a}	70.83 ± 5.89^{a}	88.31 ± 3.67^{b}	69.32 ± 8.03^{a}	78.75 ± 12.37^{a}
Embryo mortality (%)	17.69 ± 3.26^{a}	19.90 ± 1.63^{a}	29.17 ± 5.89^{a}	11.69 ± 3.67^{b}	30.68 ± 8.03^{a}	21.25 ± 12.37^{b}
Chick weight at Hatching (g)	37.60 ± 3.56^{a}	36.99 ± 3.52^{a}	37.39 ± 2.57^{a}	36.24 ± 2.36^{a}	36.48 ± 1.84^{a}	37.35 ± 3.23^{a}

Values with different superscript letters within each strain indicate significant difference at $p<.05$.

AI - Artificial Insemination, NM - Natural Mating

AI native chickens of Bangladesh (Habibullah *et al.,* 2015). However, the lower fertility rate and higher hatchability rate in AI hens was contrast to Amao & Ayorinde (2000) who reported higher fertility rate but lower hatchability rate. The study attributed this to daily collection of semen which did not allow the successive sperms to mature enough for hatchability. This is because when immature spermatozoa are used for insemination, they fertilize the ova but are not virile enough for hatching (Amao & Ayorinde, 2000; Birkhead *et al*., 2008). Semen was collected on alternate days in the present study providing sexual rest for the roosters.

Embryonic mortality was higher in eggs laid by AI hens (25.84%) compared to those NM hens (17.61%). Among AI groups, the eggs laid by Belochem (29.17%) and Yuebjha Narp (30.68%) showed the highest embryonic mortality while in NM group, eggs laid by Yuebjha Narp (21.25 %) showed the highest and Belochem strain showed the lowest (11.69%). Notably, embryonic mortality in AI Seim was lower than NM Seim and NM Belochem. The average weight of day-old chick also did not differ between the eggs laid by AI (37.30 ± 0.45 g) and NM hens (36.76 ± 0.36 g) at $p<.05$. Similarly, there was no difference in the chick weight at hatching among the strains ($p< .05$) (Table 1).

Hen day egg production, egg weight and egg shape index

The average hen day egg production by AI hens (36.23 ± 2.08 %) was slightly more than the NM hens (34.44 ± 2.24%) but was not significant ($p>.05$) a shown in Table 2. Habibullah *et al.* (2015) also reported slightly higher post insemination production in AI Hubbard native hens of Bangladesh. Among the strains, AI Yuebjha Narp laid more eggs (39.77 ± 4.83%) while AI Belochem laid the lowest (31.05 ± 2.41%) (Table 2). Dolberg (2003) and Padhi (2016) showed that Indian native chickens could lay more eggs with heavier eggs through six generations of selection by

Table 2: Hen Day egg production (HDEP), egg weight, length, width and Egg shape index of naturally mated and artificially inseminated Bhutanese native chickens (mean ± standard error of mean).

Strain	HDEP	Length (mm)	Width (mm)	Weight (g)	Egg shape Index
Seim AI	37.89 ± 3.02	53.24 ± 3.59	40.24 ± 3.77	50.41 ± 7.11	75.60 ± 0.69
Belochem AI	31.06 ± 2.41	52.43 ± 3.46	39.73 ± 3.46	46.98 ± 3.73	76.14 ± 1.19
Yuebjha Narp AI	39.77 ± 4.83	52.37 ± 3.04	39.58 ± 2.94	48.40 ± 5.07	75.59 ± 0.50
Seim NM	36.84 ± 3.60	52.60 ± 3.46	40.83 ± 3.27	50.53 ± 4.21	77.87 ± 1.07
Belochem NM	33.16 ± 4.19	53.44 ± 2.90	39.59 ± 2.50	48.95 ± 4.07	74.21 ± 055
Yuebjha Narp NM	33.33 ± 3.99	52.61 ± 2.92	40.13 ± 1.96	49.09 ± 5.25	76.36 ± 0.42

AI - Artificial Insemination, NM - Natural Mating, HDEP - Hen Day Egg Production using AI. Such breeding approach using artificial insemination technique could also lead to better performance and probably will encourage farmers to rear them.

The eggs laid by NM hens (49.58 ± 0.35 g) were slightly heavier than those eggs laid by AI hens (48.66 ± 0.41 g) but was not significant (p>.05). Likewise, no differences were observed for egg weight among strains at p>.05 (Table 2). The shape index of eggs laid by AI hens (75.77 ± 0.47%) were slightly sharper than the eggs laid by NM hens (76.24 ± 0.46%) though not significant at p>.05. Egg shape index affects hatchability, and embryonic mortality. Hatchability rates are lower but embryonic mortality are higher in sharper eggs (Alasahan & Copur, 2016). This could be another reason for lower hatchability rate and higher embryonic mortality of eggs laid by AI hens.

Conclusion

The study assessed feasibility of artificial insemination in three Bhutanese native chicken strains. The hatchability rates, embryonic mortality, egg weight, egg shape index and chick weight at hatching did not differ significantly between the two methods. While the AI resulted in lower fertility rates compared to NM, comparison among the strains showed variations in fertility. This strain specific variations highlight the need for further in-depth investigation on fertility. The use of AI should be encouraged for large scale production of native chickens as it could help to cut the cost of rearing roosters.

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