Bhutan Journal of Animal Science (BJAS)
Volume 2, Issue 1, Page 7-13, 2018

Full length paper

SEMEN CHARACTERISTICS OF THREE STRAINS OF BHUTANESE INDIGENOUS CHICKEN

SANGAY DORJI[1]* AND NEDUP DORJI[2]

[1]Dzongkhag Livestock, Department of Livestock, Ministry of Agriculture and Forests, Zhemgang, Bhutan.
[2]College of Natural Resources, Royal University of Bhutan, Lobesa, Punakha, Bhutan.

*Author for correspondence: Sangay_d@yahoo.com.

ABSTRACT: The objectives of the study were to compare the semen characteristics of Seim, *Yubjha Naap* and Baylaity chicken and correlate semen characteristics with secondary sexual traits. A total of 12 cocks (4 cocks from each population) were used for semen analyses, which included semen volume, semen pH, semen color, sperm concentration, sperm motility and semen mass activity. Semen was collected by abdominal massage method. There was a significant difference in semen volume, concentration and motility among the chicken populations but there was no significant difference in semen color, mass activity and semen pH. Semen colors were all creamy white. The highest semen volume was recorded in Seim (0.55±0.4 mL) and the lowest in *Yubjha Naap* (0.37±0.02 mL). Seim also had the highest sperm concentration (6.34±0.49 billion mL^{-1}) while *Yubjha Naap* had the lowest (4.45±0.37 billion mL^{-1}). Seim had the highest semen motility (75.00±1.22%) while *Yubjha Naap* (64.25±2.59%) had the lowest motility. The mass activities in Seim, *Yubjha Naap* and Baylaity were 2.75, 2.5 and 3.75, respectively. All chicken populations had an average pH closer to neutral (Seim-7.12±0.12, Yubjha Naap-7.47±0.09 and Baylaity-7.15±0.06). Semen volume, semen concentration, semen mass activity and semen motility were positively correlated with secondary sexual traits. However, semen pH was negatively correlated with all secondary sexual traits. The strongest correlation (r=0.76) was observed between beak length and semen concentration. The semen of all three strains of indigenous Bhutanese chicken were found suitable for preservation and therefore, can be cryopreserved. Moreover, the secondary sexual traits such as body weight, comb size and beak length can be used to predict semen quality of Bhutanese cocks.

Keywords: Chicken; indigenous cocks; semen characteristics; secondary sexual traits.

1. INTRODUCTION

Chicken (*Gallus domesticus*) is an important livestock component in Bhutanese farming community (Nidup and Tshering 2007). Poultry farming is considered as "an entry point for poverty reduction" and "gateway to national food security", particularly in developing countries (Dorji and Gyeltshen 2012). Chickens are kept for egg, meat, feather, manure and social rituals. Poultry farmers seldom sell or slaughter indigenous chicken in Bhutan (Dorji and Dorji 2015). Indigenous chickens are highly adapted to local environment (Brion 2012; FAO 2014). For example, Bhutanese chicken are tolerant to Infectious Bursal Disease (Dorji et al. 2016).

Indigenous chickens are reared under scavenging system and are provided night shelter with little or no feed supplements. However, the hen lays less number of eggs and reaches marketable age at two to three years (Nidup and Tshering 2007). Therefore, the government initiated several development strategies to increase poultry production in Bhutan, such as introduction of commercial layers and subsidies for starting small-scale poultry farms. These initiatives resulted in country achieving 100% self-sufficiency in egg and 47% self-sufficiency in chicken meat since 2012 (Gross National Happiness Commission [GNHC] 2012). On the contrary, the increasing commercial poultry farming may lead to dilution of genetic resource base of indigenous chicken.

Conservation and sustainable utilization of biological diversity is crucial for meeting the food, health and other needs of growing world human population (FAO 2014). More importantly, the climate change has posed threat to extinction of one-fourth of Earth's species (FAO 2015). Global warming causes heat stress to animals and impairs production, reproduction, health and immune response (Nardone et al. 2010). Climate change leads to outbreak of new diseases and parasites (Verge et al. 2009). Therefore, to sustain livestock farming in the face of changing environment, it is important to maintain diverse genetic resources. In Bhutan, the National Biodiversity Center (NBC) has initiated conservation of poultry genetic resources since 2008, through in-situ conservation and gene cryopreservation (Tamang 2011). To conserve good semen quality in the cryobank, it is important to assess the semen characteristics of Bhutanese indigenous chickens. Therefore, this study was conducted with the objective to examine the semen quality and quantity of three strains of indigenous Bhutanese chicken.

2. MATERIALS AND METHODS

2.1 Study area, population and sample size

The study was conducted at NBC, Serbithang, Thimphu. The study area is located at $27^0$25'12.2" N, 089^0 39'04.6" E. It is located at an altitude of 2,487 meters above sea level with an average annual temperature of 11.8°C. It has an average annual relative humidity of 73.9% (NBC 2011).

The sample cocks were sourced from Dagana and Tsirang districts as they have maximum numbers of indigenous chicken (DoL 2014). A total of 12 cocks (4 native black [*Yubjha Naap*], 4 native red [Seim] and 4 short legged [Baylaity]) were used for assessing sperm characteristics. The selected experimental cocks were sexually matured, healthy and free from physical injuries. Five replicates of semen samples at an interval of three days were collected from each cock to obtain accurate data (Tarief et al. 2013).

2.2 Management of experimental birds

Each cock was kept in individual cage 10 days prior to semen collection, in order to prevent fighting and ensure access to feed and water. The battery cage (52 cm × 45 cm × 38 cm) was constructed using locally available materials (Tarif et al. 2013). The hens were also introduced inside the house to induce semen production in cocks. About 16 hrs light was provided both in adaption and experiment period (Bacon et al. 2000). The birds were fed with complete commercial layer poultry feed as feeding layer feed to roosters does not affect semen quality in male (Hubbard 2011). Individual birds were fed twice a day (morning and evening). Clean drinking water was provided throughout the study period. The cocks were de-wormed during adaption period.

2.3 Semen collection

Feathers around the cloacal region were trimmed as per Peters et al. (2008) to prevent sample contamination. Cocks were trained for 10 days prior to semen collection (Tabatabaei 2009). Single ejaculation through abdominal massage technique (Lake 1960) from each cock was collected at specific time, at an interval of three days. Each cock was massaged at the back and stroked close to its tail with slight finger pressure around the base of the tail. The abdomen of the cock beneath the vent was pressed with thumb and it caused immediate release of semen from ducts deferens. The semen was then gently squeezed into the conical graduated collecting tube. The excess squeezing was avoided to prevent bleeding (Lake 1962 as cited by Peters et al. 2008).

2.4 Semen evaluation

The semen evaluation was conducted at room temperature. The Standard Operating Procedure and laboratory protocol of NBC were followed throughout the study period. Using a conical graduated poultry semen collecting tube in millilitre (mL), the per ejaculation volume of semen from each cock was measured fresh, when aspirated from cloacal vent. For semen color, the visual inspection of fresh semen was conducted within three minutes of milking. The color of semen was scored as 1=Creamy white, 2=Between creamy white and opaque and 3=Opaque (Peters et al. 2008). The semen concentration was determined by AccuRead photometer (IMV Germany), which was maintained at 37°C. About 2 mL of 0.9% normal saline (NS) was pipetted into the 15 mL cuvette. The cuvette was then placed in AccuRead photometer and was

calibrated to zero. About 10 µL of fresh semen was added into the cuvette with normal saline. The cuvette was taken out from AccuRead photometer, covered with aluminum foil and then shaken gently until the semen was mixed thoroughly with normal saline. The cuvette with semen and normal saline mixture was placed into AccuRead photometer and pressed "Measure" and recorded semen concentration in billion per mL. A drop of semen was placed on a clean dry preheated slide at 37°C. The semen was covered with a warm glass cover slip to ensure uniform spread of semen and prevent from quick drying. A microscope magnification of – 40X was used to observe the semen motility, which was expressed as percentage of mobile spermatozoa with moderate to rapid progressive movement (Tarif et al. 2013). With the same slide, semen mass activity was scored in the scale: 1=number of perceptible motion, 2=few spermatozoa moving without forming any waves, 3=small with slow moving waves, 4=vigorous movement with moderately rapid wave and eddies and 5=dense, rapidly moving waves and eddies (Tarif et al. 2013).

The pH of semen was determined by using indicator paper strips (INDIKROM PAPERS). About 3-4 drops of fresh semen was added on the indicator paper strips using micro pipette. The semen was gently pressed with the help of glass slide to obtain uniform spread of semen over the indicator paper strip. The paper strip was then air-dried for 1-2 minutes. The visual observation and the reading were recorded by comparing the color obtained with the reference color and pH reading given on the indicator paper strip cover (Kamar et al. 1979). The procedure was repeated three times with different paper strips in each sample to confirm the reading.

2.5 Secondary sexual traits measurements

The secondary sexual traits were measured after completing milking semen from each cock. The traits were measured at the beginning (day 0) and at the end of study (day 12). Electrical weighing balance was used to measure the linear body weight of the cocks. Body weight was measured after semen milking and before feeding. Comb size, comb length and comb height were measured (Dhawale 2012). The comb height was measured from where the comb met the head to the top of the highest spike, while the comb length was measured from back end to front end, using a measuring tape (Navara et al. 2012). Beak length was measured as the distance between the tip of the beak and the base, using measuring tape (Udeh et al. 2011).

2.6 Data Analysis

Data collected was entered in Microsoft Excel sheet and analyzed with SPSS version 21. Analyses of variance (ANOVA) was used to test differences in parameters among strains. Differences were considered significant when p value was less than 5%. Pearson's correlation test was conducted to establish correlation between semen characteristics and secondary sexual traits.

3. RESULTS AND DISCUSSION

3.1 Semen color

All semen colors were creamy white, which was also reported in Nigerian frizzle, naked neck and Nera black chicken (Peters et al. 2008) and RIR rooster in India (Churchil et al. 2014). The similarity could have occurred due to similar feeding management as the semen color is dependent on feed (Dhawale 2012).

3.2 Semen volume

There was no significant difference in semen volume between the populations except for Seim and Baylaity (Figure 1). Seim produced the highest semen volume of 0.55±0.40 mL, followed by Baylaity with 0.45±0.03 mL, and *Yubjha Naap* with 0.37±0.02 mL. The semen volume of all three populations falls within the range of 0.37±0.02 to 0.73±0.01 mL as found for Nigerian local chicken (Peters et al. 2008). The semen volume of Bhutanese chicken was more than the Malaysian domestic chicken (0.33±0.16 mL), Bantham chicken (0.29±0.18 mL) and Red Jungle Fowl

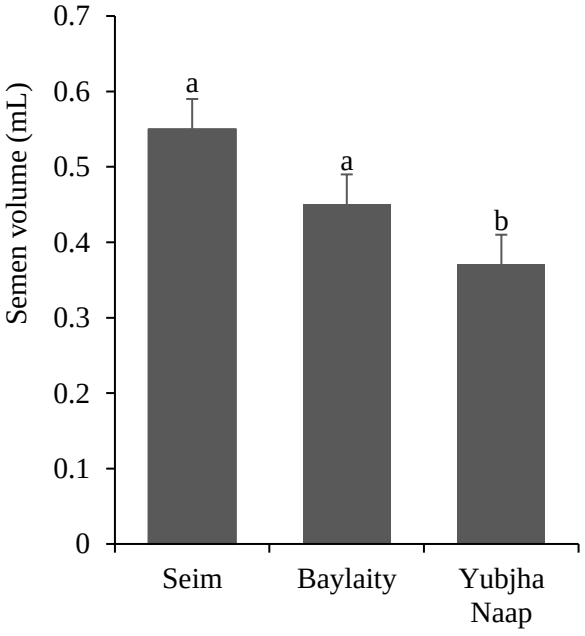

Figure 1: Semen volume of Seim, *Yubjha Naap* and Baylaity (mean±se of mean)**.** Means with different letters differ significantly (p≤0.05).

(0.10±0.10 mL) (Malik et al. 2013). The higher semen volume in this study could be due to differences in body weight (Dhawale 2012). The cocks in Malaysian study were lighter in weight, and semen volume was positively correlated with body weight.

3.3 Semen concentration

There was a significant difference ($p \leq 0.001$) in semen concentration between Seim and *Yubjha Naap*. Semen concentration also differed significantly ($p \leq 0.05$) between Seim and Baylaity. However, no significant difference was observed between *Yubjha Naap* and Baylaity. Highest sperm concentration was produced by Seim population with a mean concentration of 6.34±0.49 billion mL^{-1} and least by *Yubjha Naap* with a mean concentration of 4.45±0.37 billion mL^{-1} (Figure 2). Semen concentration of all three populations fell within the range 1-7 billion sperm cells mL^{-1} as reported by Peter et al. (2008) in Nigerian frizzle, naked neck and Nera black chicken. Differences in semen concentration among the breeds were also observed by Malik et al. (2013) in domestic chicken in Malaysia. However, all cell counts were higher than the Red Jungle Fowl of 4.44×10^9 cells mL^{-1} and Malaysian domestic chicken of 1.83×10^9 cells mL^{-1} (Malik et al. 2013). On the other hand, Zhang et al. (1999) reported no effect of strain on semen concentration in broiler breeds. The variation in semen concentration among breeds indicates the influences of genetic makeup of the birds (Dhawale 2012).

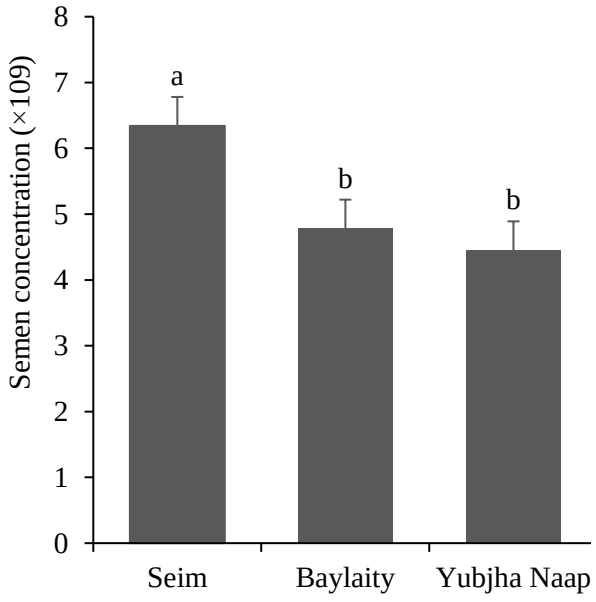

Figure 2: Semen concentration ($\times10^9$) among populations (mean±se of mean). Means with different letters differ significantly ($p \leq 0.05$).

3.4 Semen mass activity

Figure 3 presents the semen mass activity of chicken strains. There was no significant difference in semen mass activity among three populations. The mass activities in Seim, *Yubjha Naap* and Baylaity were 2.75, 2.50 and 3.75, respectively. Semen mass activity was better in Baylaity, followed by Seim and *Yubjha Naap*. It is important to have high semen mass activity for fertility due to long storage of semen in the utero-vaginal junction of female before it reaches fertilization site (Tadondjou et al. 2014). The semen mass activities of all populations were close to Indonesian local chicken of 2.5 and 3 (Almahdi et al. 2014). However, the mass activities of Bhutanese indigenous chickens were lower than the range of 3.80±0.10 to 4.00±0.00 found in four breeds of cocks in Bangladesh (Tarif et al. 2013). The low temperature in the study area as compared to Bangladesh probably contributed to the lower mass activity (Gebriel et al. 2009). There was no variation in mass activities among the populations, which indicates that the semen mass activity was not affected by the genetic makeup, because all the feeding and management practices were same.

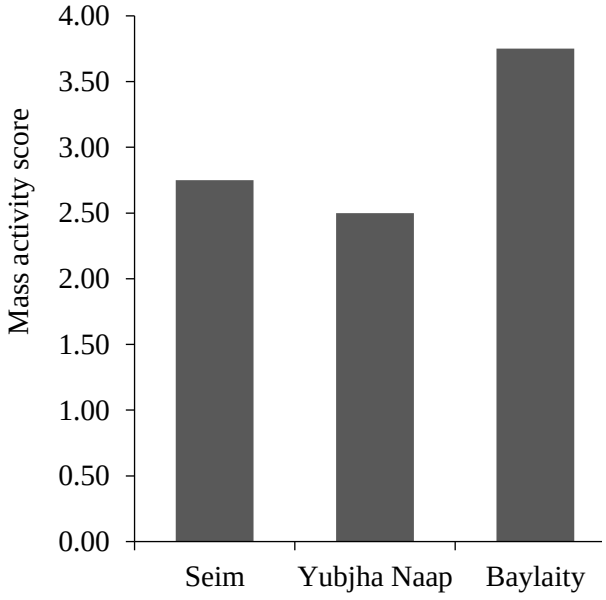

Figure 3: Semen mass activity.

3.5 Semen motility

The semen motility among strains is presented in Figure 4. Semen motility did not differ significantly between Seim and Baylaity. However, the semen motility of Seim and Baylaity was significantly higher ($p \leq 0.00$) than *Yubjha Naap*. Bhutanese chicken semen motility is reported to fall within the

range of 49.40±6.03% - 96.00±2.27% found in Malaysian chicken (Malik et al. 2013) and 62.55±10.55% - 87.35±10.12% found in Nigerian local chicken (Peters et al. 2008). However, the semen motility of indigenous Bhutanese chicken was less than that of Bangkok chicken (84.00±2.23%), Lingnan chicken (84.00±2.23%), Kedu chicken (84.00±2.23%) and Arab chickens (80.00±7.07%) in Indonesia (Almahdi et al. 2014). The lower semen motility in this study could be due to age of the cocks. The best quality semen is produced by the cock at an age of 10-20 months (Nalbandov 1990). The cocks used in this study were all older than 30 weeks, whereas the cocks used by Almahdi et al. (2014) were 10-20 weeks old, which might have affected the semen motility.

3.6 Semen pH

There was no significant difference in pH among the strains (Figure 5). However, *Yubjha Naap* semen was slightly alkaline than others. The pH of semen in Bhutanese chicken matches with that of the Malaysian local chicken (Malik et al. 2013). The pH values of all Bhutanese chicken populations were above the semen pH of Nigerian local chicken (Peters et al. 2008; Ajayi et al. 2011), Bangkok chicken and Arab chickens in Indonesia (Almahdi et al. 2014).

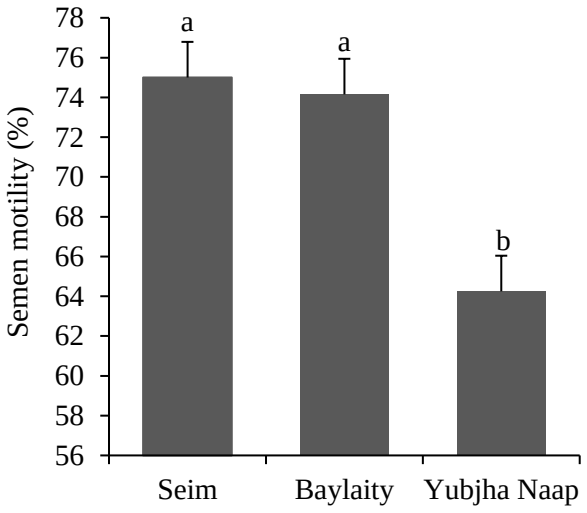

Figure 4: Semen motility (%) among populations (mean±se of mean). Means with different letters differ significantly (p≤0.05).

3.7 Relationship between semen characteristics and secondary sexual traits

The relationships among traits and semen characteristics are shown in Table 1. Semen volume, semen concentration, semen mass activity and semen motility were positively correlated with secondary sexual traits. However, semen pH was negatively correlated with all secondary sexual traits. The strongest correlation was observed between beak length and semen concentration.

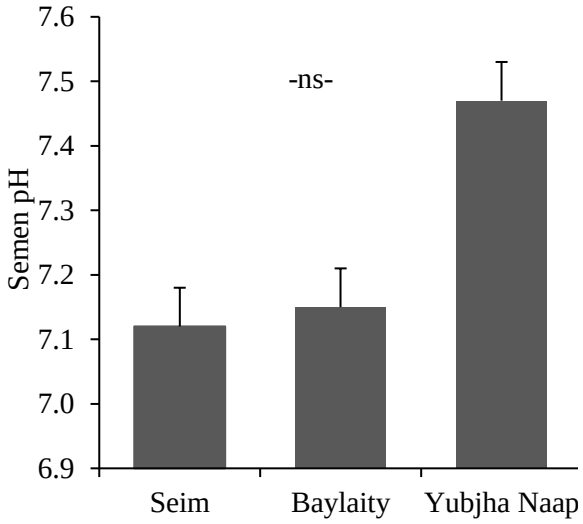

Figure 5: Semen pH among chicken populations (mean±se of mean). Ns-nonsignificant.

4. CONCLUSIONS

Significant variations in semen volume, semen concentration and semen motility exist among all three strains of Bhutanese chicken. Semen color, semen mass activity and semen pH do not differ significantly. All strains have semen suitable for artificial insemination and cryopreservation. All semen characteristics, except semen pH, are positively correlated with secondary sexual traits. The semen color is independent of all secondary

Table 1: Correlations between secondary sexual traits and semen characteristics. Figures inside cells represent Pearson's correlation coefficient.

Traits	Body weight	Comb length	Comb height	Beak length
Semen volume	0.25	0.62*	0.40	0.43
Semen pH	-0.55	-0.30	-0.33	-0.23
Semen mass activity	0.52	0.36	0.17	0.09
Semen concentration	0.60*	0.74**	0.74**	0.76**
Semen motility	0.54	0.75**	0.57	0.52

*p<0.05, **p<0.01

sexual traits in all three strains of Bhutanese cocks. To some extent, the secondary sexual traits may be used to predict the semen quality. Therefore, the cocks with heavier body weight, larger comb size and longer beak length are recommended to be selected as breeder cocks as it has better semen quality. However, for semen cryopreservation, only a cock with bigger comb size and longer beak length may be selected since heavier cock may not be suitable as it has low semen pH.

Acknowledgements

The authors acknowledge the support provided by the National Biodiversity Center in semen collection and laboratory works and college of Natural Resources for supporting rearing of sample birds.

REFERENCES

Ajayi OO, Agaviezor BO, and Ajuogu PK (2011). Semen characteristics of three strains of local cocks in the humid tropical environment of Nigeria. International Journal of Animal and Veterinary Advances, 3 (3): 125-127.

Almahdi AB, Ondho YS, and Sutopo (2014). Comparative studies of semen quality on different breed of chicken in poultry breeding center. International Refereed Journal of Engineering and Science, 3 (2): 94-103.

Bacon WL, Kurginski-Noonan BA, and Yang J (2000). Effects of Environmental Lighting on Early Semen Production and Correlated Hormonal Responses in Turkeys. Journal of Poultry Science, 79: 1669-1678.

Brion ACB (2012). Why choose native breed. Available at: <http://www.bar.gov .ph/chronicle -home/archives-list/42-september-2012-issue/1353-why-choose-native>. (Accessed on 7 September, 2015).

Churchil RR, Praveena PE, and Sharma D (2014). Semen quality parameters, their inter-relationship and post-washing sperm attributes of Rhode Island Red roosters. Veterinary World, 7 (12): 1117-1122.

Dhawale A (2012). The golden secrets of male fertility. Available at: <http://www.worldpoultry.net/Breeders/General/2012/3/The-golden-secrets-of-male-fertility-WP010083W/>. (Accessed on 14 August, 2015).

Dorji J, Dorji T, Tshewang, Dorji TY, Tenzin S, and Gurung RB (2016). Immunological tolerance of Bhutanese native chicken to Infectious Bursal Disease Virus infection. International Journal of Advanced Scientific Research and Management, 1 (2): 46-49.

Dorji N and Dorji P (2015). A country report: Indigenous chicken farming in Bhutan**.** Khon kaen Agrculture Journal, 43 (2): 31-33.

Dorji N and Gyeltshen T (2012). Characterization of family poultry production in Haa and Mongar districts of Bhutan. Livestock Research for Rural Development, 24 (9).

FAO (2014). Family poultry development −issues, opportunities and constraints. Available at: <http://www.fao.org/3/a-i3595e.pdf>. (Accessed on 20 September, 2015).

FAO (2015). The second report on the state of the world's animal genetic resources for food and agriculture. Available at: <http://www.fao.org/3/a-i4787e/index.htmL>. (Accessed on 10 January, 2016).

Gebriel GM, Kalamah MA, El-Fiky AA, and Ali AFA (2009). Some factors affecting semen quality traits in Norfa cocks. Egypt Poultry Science, 29 (2): 677-693.

GNHC (2012). Data collection survey report on food self-sufficiency and food security in the kingdom of Bhutan. Available at: <http://open_jicareport.jica.go.jp/pdf/12080826_01.pdf>. (Accessed 20 September, 2015).

Hubbard (2011). Breeder Nutrition Guide. Available at: <http://www.hubbardbreeders.com/media/breeder_nutrition_guide_final2011__016567500_0945_07012015.pdf >. (Accessed on 30 September, 2015).

Kamar GAR, Obiedah A, Goher NE, and Khalifa MA (1979). Genetical studies on semen characteristics of cocks. Egypt Journal of Animal Production, 19 (1): 101-103.

Lake PE (1960). Studies on the dilution and storage of fowl semen. Journal of Reproduction and Fertility, 5 (1): 30-35.

Malik A, Haron AW, Yusoff R, Nesa M, Bukar M, and Kasim A (2013). Evaluation of the ejaculate quality of the Red Jungle Fowl, domestic chicken and Bantam chicken in Malaysia: Turkish Journal of Veterinary and Animal Sciences, 37 (5): 564-568.

Nardone ABR, Lacetera N, Ranieri S, and Bernabucci U (2010). Effects of climate changes on animal production and sustainability of livestock systems. 10th World Conference on Animal Production (WCAP). Livestock Science, 130 (1-3): 57-69.

Navara KJ, Erin M, Andrison, and Meghan LE (2012). Comb size and color relate to sperm quality: a test of the phenotype-linked fertility hypothesis. Department of Poultry Science, The University of Georgia, 203 Poultry Science Building, Athens, USA.

NBC (2011). Poultry Semen Processing Procedure. Thimphu. Bhutan.

Nidup K and Tshering P (2007). Status of the family poultry production and HPAI in Bhutan. Proceedings of the 8th Asian Pacific Poultry Conference of the World's Poultry Science Association. March 2007, Bangkok, Thailand: 78-83.

Peters SO, Shoyebo OD, Ilori BM, Ozoje MO, Ikeobi CON, and Adebambo OA (2008). Semen Quality Traits of Seven Strain of Chickens Raised in the Humid Tropics. International Journal of Poultry Science, 7 (10): 949-953.

Tabatabaei S, Batavani RA, and Talebi AR (2009). Comparison of semen in indigenous and Ross broiler breeder rooster. Journal of Animal and veterinary advances, 8 (1): 90-93.

Tamang S (2011). Efforts and Initiatives to conserve and promote Indigenous poultry. An.GR (Animal Genetic Resources Conservation Program). National Biodiversity Centre. Indigenous Poultry Conservation Workshop, Thimphu Bhutan.

Tadondjou CDA, Ngoula F, Kana JR, Mube HK, and Teguia A (2014). Characterization of reproduction of local barred male chicken of the western highlands of Cameroon: sexual maturity, fertility and sperm storage term in female. Journal of Physiology and Pharmacology Advances. 4 (2): 323-331.

Tarif AM, Musharraf M, Bhuiyan U, Ferdousy RN, Juyena NS, and Mollah MBR (2013). Evaluation of semen quality among four chicken lines: IOSR Journal of Agriculture and Veterinary Science, 6 (5): 07-13.

Udeh SOC, Ugwu, Ogagifo NL (2011). Predicting semen traits of local and exotic cocks using linear body measurements. Asian Journal of Animal Sciences, 5: 268-276.

Verge XPXC, Dayer JA, Desjardins RL, and Worth D (2009). Long-term trends in greenhouse gas emissions from the Canadian poultry industry. The Journal of Applied Poultry Research, 18 (2): 210- 222.

Zang X, Berry WD, McDaniel GR, Ronald DA, Liu P, Calvert C, and Wilhite R (1999). Body weight and semen production of broiler breeder males as influenced by crude protein levels and feeding regimens during rearing. International Journal of Poultry Science, 78 (2): 190-196.