Original Article

Effects of Environmental Factors, Ages and Breeds on Semen Characteristics in Thai Indigenous Chickens: A One-year Study

Pornjit Sonseeda [1, 2] Thevin Vongpralub [1,2*] Banyat Laopaiboon [1]

Abstract

Several reports on the semen characteristics of domestic fowl have indicated that environmental factors, breed and age significantly affect semen quality. There is, however, a paucity of data on effects of environmental factor, breed and age on semen characteristic in Thai indigenous chickens. The aims of the present study were to investigate the effects of annual ambient temperature and humidity, local breeds (*i.e.* Lueng hang kaow, Pradoo hang dam and Chee), and ages (10 and 18 months) on sperm production and semen quality among Thai native cocks. Thirty-six Thai native cocks were housed under natural environmental conditions, and semen was collected routinely twice a week using the massage method. Sperm production and semen quality were determined every two weeks for one year. Semen was evaluated for volume, mass movement, % total motility, % live normal, sperm concentration/ml and sperm number/ejaculate. Over the 12-month period, ambient temperature and humidity did not significantly affect sperm production and semen quality ($p < 0.05$). Breeds and ages also had no effect on semen characteristics among the Thai native cocks. The result of the present study indicates that the sperm production and semen quality of captive Thai native cocks were not influenced by environmental factors, breeds and ages.

Keywords: age, indigenous chicken, line, season, sperm

[1] *Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand 40002*
[2] *Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand and Agricultural Biotechnology Research Center for Sustainable Economy, KhonKaen University, KhonKaen 40002*
***Corresponding author: E-mail:** vthevi@kku.ac.th*

บทคัดย่อ

ผลกระทบของปัจจัยจากสิ่งแวดล้อม สายพันธุ์ และอายุต่อคุณลักษณะของน้ำเชื้อในไก่พื้นเมืองไทย: การศึกษาในรอบ 1 ปี

พรจิต สอนสีดา [1,2] *เทวินทร์ วงษ์พระลับ* [1,2*] *บัญญัติ เหล่าไพบูลย์* [1]

มีรายงานชี้ให้เห็นถึงผลกระทบของปัจจัยจากสิ่งแวดล้อม สายพันธุ์ และ อายุ ต่อคุณภาพน้ำเชื้อในไก่ แต่ไม่พบรายงานการศึกษาเรื่องผลกระทบของสภาพแวดล้อม สายพันธุ์และอายุ ต่อคุณลักษณะน้ำเชื้อในไก่พื้นเมืองไทย ดังนั้นการวิจัยในครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลกระทบของอุณหภูมิแวดล้อมและความชื้นในรอบ 12 เดือนต่อคุณลักษณะของน้ำเชื้อในไก่พื้นเมืองไทยสามสายพันธุ์ (เหลืองหางขาว ประดู่หางดำ และชี) ที่มีอายุเริ่มต้นแตกต่างกัน 2 ช่วงอายุ คือ 10 และ 18 เดือน ใช้พ่อพันธุ์ไก่พื้นเมืองไทยจำนวนทั้งสิ้น 36 ตัว เลี้ยงในโรงเรือนแบบเปิดภายใต้สภาวะแวดล้อมตามธรรมชาติ รีดเก็บน้ำเชื้อสัปดาห์ละ 2 ครั้งเป็นประจำ และเก็บข้อมูลคุณภาพน้ำเชื้อทุก 2 สัปดาห์ตลอดช่วงระยะเวลา 1 ปี โดยการรีดเก็บน้ำเชื้อใช้วิธีกระตุ้นจากการลูบหลังและบีบที่บริเวณโคนหาง ประเมินคุณภาพน้ำเชื้อโดยการประเมินค่า ปริมาตรของน้ำเชื้อ การเคลื่อนที่แบบกลุ่ม ร้อยละของการเคลื่อนที่รวมของอสุจิ ร้อยละของอสุจิมีชีวิตและรูปร่างปกติ ความเข้มข้นของอสุจิต่อมิลลิลิตร และจำนวนอสุจิทั้งหมดต่อการหลั่ง จากการศึกษาอย่างต่อเนื่องตลอดระยะเวลา 12 เดือนพบว่าอุณหภูมิแวดล้อมและความชื้นสัมพัทธ์ไม่มีผลต่อผลผลิตและคุณภาพน้ำเชื้อในไก่พื้นเมืองไทย สำหรับสายพันธุ์และอายุนั้นพบว่า ไม่มีผลต่อคุณลักษณะของน้ำเชื้อเช่นเดียวกัน ผลการศึกษาครั้งนี้ชี้ให้เห็นว่า ปัจจัยจากสิ่งแวดล้อม สายพันธุ์ และอายุไม่มีผลต่อคุณภาพน้ำเชื้อในไก่พื้นเมืองไทยที่เลี้ยงในโรงเรือนแบบเปิด

คำสำคัญ: อายุ ไก่พื้นเมืองสายพันธุ์ ฤดูกาล อสุจิ

[1] *ภาควิชาสัตวศาสตร์ คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น ขอนแก่น ประเทศไทย 40002*
[2] *ศูนย์ความเป็นเลิศด้านเทคโนโลยีชีวภาพเกษตรสำนักพัฒนาบัณฑิตศึกษาและวิจัยด้านวิทยาศาสตร์และเทคโนโลยีสำนักงานคณะกรรมการการอุดมศึกษา กรุงเทพฯ 10900 และ ศูนย์วิจัยเทคโนโลยี ชีวภาพทางการเกษตรเพื่อเศรษฐกิจที่ยั่งยืน มหาวิทยาลัยขอนแก่น ขอนแก่น 40002*
***ผู้รับผิดชอบบทความ E-mail:** vthevi@kku.ac.th*

Introduction

For best results in AI, poultry breeders need to ensure the highest quality of collected semen (Alkan et al., 2002). The importance of raw semen assessment to identify males of different fertilizing abilities is routinely employed (Wishart, 2009). Avian semen quality significantly affects fertility which is in turn affected by breed (Tabatabaei et al., 2010) and environmental conditions (Elagib et al., 2012).

Seasonal variation in semen quality of domestic fowl has been documented (Santiago-Moreno et al., 2011). McDaniel et al. (1996) reported that an ambient temperature of > 31°C depressed rooster sperm motility, viability and fertilization potential. Even environmental temperature at ejaculation has an important effect on exogenous physiological factors influencing avian sperm motility (Ashizawa and Sano, 1990; Wishart and Wilson, 1999).

There are reports concluding that both breed and age affect the quality of fresh and stored semen (Kelso et al., 1996). Semen from avian species that has been studied. included turkeys, ganders, pheasants, drakes, and cocks (Wishart, 2009). It was found that semen of older birds had significantly lower motility, viability and mass movement than younger birds. They also reported that semen quality of poultry species declined with age (Kelso et al., 1996; Kotloska et al., 2005; Long et al., 2010). Reports on Iranian indigenous broiler (Tabatabaei et al., 2010) and 8 pedigreed lines (Long et al., 2010) indicated that among younger chickens there was greater motility.

Among Thai native chickens, only few have been studied. Therefore, there is a dearth of data on semen quality by season, breed and age. Consequently, the present study was conducted to characterize semen quality by environmental factors, breed and age of Thai native chickens. An understanding of these parameters would allow for design and optimization of semen cryopreservation of Thai indigenous chicken genetic resources.

Materials and Methods

Animals and Semen collection: The experiment was conducted on 36 mature, Thai native cocks kept in individual cages. There were three different breeds of birds (*i.e.* Lueng hang khoa, Pradoo hang dam and Chee) and two different ages (10 and 18 months) at the beginning of the experiment. Cockerels were fed 120-130 g/head/day and water was provided *ad libitum*. The animals were reared under natural environmental conditions, (5˚ 38'N, 105˚ 37'E), where they received a natural light : dark photoperiod (11.14L : 12.46D to 13.01L : 11.59D) throughout the experiment. Semen was collected (using the massage method) from each male twice a week for 1 year (July, 2009 - June, 2010).

Semen evaluation: Semen from an individual cock was collected in a 1.5 ml microtube containing 0.1 ml Schramm diluent comprising2.85 g sodium glutamate, 0.5 g glucose, 0.25 g inositol, 0.5 g potassium acetate, 0.07 g magnesium acetate in 100 ml of double-distilled water. Immediately after collecting, semen characteristics were determined every two weeks.

Volume: Ejaculate volume of semen was determine using 0.1 ml graduated tuberculin syringe (Abd El Ghany, 1997).

Mass movement and motility: Mass movement, aspects of wave motion, was by conducted examining a drop of semen on a warm slide under a microscope at 100x. The score was assigned between 0 (total sperm were motionless) and 5 (wave motion varied rapidly, eddies were present). Motility was assessed on the basis of percentage of sperm showing forward motion (Ax et al., 2000).

% live normal sperm: The percentage of live normal sperm was evaluated according to Ax et al., (2000). Briefly, a drop of well-mixed semen was mixed with a drop of eosin-nigrosin stain on a glass slide. Another glass slide was used to prepare smear film and was, then, evaluated immediately under a microscope. Sperm abnormalities were optimally detected using immersion oil at 1000x and a minimum of 200 cells were evaluated for abnormalities. The unstained and normal morphology spermatozoa were recorded as live normal sperm.

Sperm Concentration: Concentration of the semen sample was assumed by using a hemacytometer (Ax et al., 2000). Briefly, a 1 : 1000 dilution of semen (semen 4 µl : 3,996 µl 4%NaCl) was loaded into a hemacytometer. The count was performed at 200x under bright-field microscopy.

Temperature-humidity index (THI): Temperature and humidity were collected using digital thermometer. The temperature-humidity index (THI) was calculated using a mathematical model (Mader and Davis, 2004).

THI : (0.8 x AT) + [(RH/100) x (AT-14.4)] + 46.4

AT : Ambient Temperature (^{0}C)

RH : Relative Humidity (%)

Statistical analysis: Data collected on semen characteristics were subsequently subjected to a one-way analysis of variance (ANOVA) using SAS (1993).

Results

During 12 months, mean ambient temperature, humidity and THI were 27.6^{0}C (ranging from 20.6-31.2^{0}C), 69.1% (ranging from 53.5-82.2%) and 77.5% (ranging from 67.1-82.1%), respectively. Ambient temperature, humidity and THI did not significantly (p > 0.05) affect sperm production and semen quality of Thai native chicken (Table 1). The average of mass movement, % total motile, volume, %live normal, concentration, and total sperm/ejaculate were 4.3 score, 88.2%, 0.3 ml, 91.1%, 431.9x10^7 cell/ml, and 147.0x10^7 cell/ejaculate, respectively. These data suggest that temperature, humidity and THI have little measurable effect on semen characteristics of the native chickens.

Table 1 Effect of environmental factors on semen quality among Thai native chickens

Annual	Semen quality parameter						Climate		
	Mass movement	Motility %	Volume ml	Live %	Concentration 1x10^{7}cell/ml	Total sperm 1x10^{7}cell/ejac	Temperature ˚C	Humidity %RH	THI %
July	4.28	87.5	0.34	91.7	410.9	151.7	27.9	78.2	79.3
August	4.31	89.5	0.32	92.9	446.8	154.9	27.8	80.3	79.4
September	4.37	88.3	0.32	90.3	458.2	146.8	27.6	82.2	79.3
October	4.31	88.5	0.33	90.8	415.0	140.3	27.5	76.3	78.4
November	4.30	88.8	0.32	91.2	401.1	132.6	25.43	62.7	73.7
December	4.28	88.9	0.34	90.5	437.5	148.0	24.5	62.4	72.3
January	4.31	88.7	0.35	90.6	412.5	146.6	20.6	68.4	67.1
February	4.32	88.5	0.34	91.9	459.8	159.6	27.5	64.7	76.9
March	4.37	87.3	0.33	90.7	420.0	134.5	29.9	53.5	78.6
April	4.35	87.4	0.33	91.4	417.0	140.0	31.2	59.4	81.3
May	4.29	87.5	0.32	90.7	445.9	147.5	30.8	67.7	82.1
June	4.28	87.1	0.35	90.4	458.1	161.3	29.9	72.8	81.6
Mean	4.3	88.2	0.3	91.1	431.9	147.0	27.6	69.1	77.5
SEM	0.05	1.30	0.02	1.33	33.83	15.34	-	-	-

For the effect of age on semen quality, the current study found that the age of Thai native cocks, either between 10 and 22 months (10 months group) or between 18 and 30 months (18 months group) had no significant ($p > 0.05$) influence on semen quality. The average of mass movement, % total motile, volume, % live normal, concentration, and total sperm/ejaculate of the 10-months group were 4.31 score, 88.3%, 0.336 ml, 90.8%, 434.74 x 10^7 cell/ml, and 145.01 x 10^7 cell/ejaculate, respectively. The average of mass movement, % total motile, volume, % live normal, concentration, and total sperm/ejaculate of 18 months group were 4.30 score, 88.08%, 0.335 ml, 91.4%, 429.13 x 10^7 cell/ml, and 149.06 x 10^7 cell/ejaculate, respectively. The result revealed that age had no effect on semen quality between the 10^{th} and 30^{th} month of age among Thai native cocks. The results suggest that Thai native chicken breeds have a long reproductive life time.

The comparative effect of breeds (*i.e.*, Lueng hang kaow, Pradoo hang dam and Chee) on the semen quality of Thai native cocks is presented in Table 2. The average of mass movement, % total motile, volume, % live normal, concentration, and total sperm/ejaculate of Lueng hang kaow, Pradoo hang dam and Chee were 4.30 score, 88.62%, 0.342 ml, 91.2%, 438.43 x 10^7 cell/ml, and 149.71 x 10^7 cell/ejaculate; 4.31 score, 88.06%, 0.328 ml, 90.5%, 413.89 x 10^7 cell/ml, and 137.70 x 10^7 cell/ejaculate; and 4.31 score, 87.96%, 0.336 ml, 91.7%, 434.8 x 10^7 cell/ml, and 147.71 x 10^7 cell/ejaculate, respectively. For the effect of breed on semen quality, the results of this study indicate that there is no statistically significant ($p > 0.05$) influence of breed on semen quality among Thai native cocks.

Discussion

Effect of environmental factor on semen quality can vary considerably over the year in seasonal mammalian species such as sheep, cattle and ibex (D'Alessandro and Martemucci, 2003; Koivisto et al., 2009; Coloma et al., 2010). Several studies on fowl showed that there was a seasonal effect on semen quality. Studies in Sudan (Elagib et al., 2012) and in Sahel region of Nigeria (Bah et al., 2001) on older birds showed a significant reduction in sperm concentration during the summer season, consistent with an earlier report by Elagib (2012) which suggested that high temperatures reduced sperm concentration. However, the abnormal percentage was not harmfully affected during summer months.

Semen quality of indigenous chickens was not significantly different throughout the year (Table 1).These data suggest that temperature, humidity and THI have little measurable effect on semen characteristics of the native chickens. The results of this study are similar to a report by Santiago-Moreno et al. (2011) which showed that the characteristics of fresh semen from native Spanish chicken breeds were not affected by season. In contrast, Bah et al. (2001) found that semen characteristics varied significantly ($p < 0.001$) during the rainy season. The highest sperm concentration was recorded in July, while the greatest volume and highest total sperm count was in August. Similarly, Elagib et al. (2012) reported that one-year-old cocks produced less semen volume during the summer months than during the autumn months. Environmental factors such as temperature and photoperiod housing are thought to play an prominent role in semen quality in many countries (Peltoniemi et al., 1999).The effects of hot ambient temperature on the heterophil/lymphocyte ratio were determined for a comparison of high ambient temperature tolerance between Thai indigenous chickens, cross-bred Thai indigenous chickens and broilers. The Thai native chickens showed higher heat tolerance than cross-bred Thai indigenous chickens and broilers (Aengwanich, 2007). Duangduen (2008) assessed the effect of high environmental temperature in Thai native chickens (Chee) and commercial broilers on productive performance, respiratory rate, and rectal temperature. The results showed that the Thai native chickens (Chee) was more tolerant to high temperature than the broilers. These previous reports suggested the indigenous chickens are fully adapted to high environmental temperature. Floyd and Tyler (2011) determined the effect of different photo stimulation photoperiods (8-18hour) on age of first semen production, testis weight and semen characteristics among male broiler breeders. A large degree of variation was recorded, however, the trend in data suggests that a photoperiod of > 13 hours may be detrimental to testis development and subsequent sperm production. A study in Thailand determined that the average day length (photoperiod) was 12.1 hours (12 hours 7 min, ranging from 11 hour 14 min-13 hour 1 min). The respective photoperiod in the hot, rainy, and cool season were 12.4 hours, 12.5 hour and 11.5 hours (Hydrographic Department, Royal Thai Navy, 2010). The effect of photoperiod in

Table 2 Effect of breeds and age on semen quality of Thai native cocks

		Volume (ml)	Mass movement	Motility %	Live normal%	Concentration 1X10^7cell/ml	Total sperm 1X10^7cell/ejaculate
Breeds	Lueng hang kaoa	0.342	4.30	88.62	91.2	438.43	149.71
	pradoo hang dam	0.328	4.31	88.06	90.5	413.89	137.70
	Chee	0.336	4.31	87.96	91.7	443.8	147.71
Age	10 to 22 months	0.336	4.31	88.3	90.8	434.74	145.01
	18 to 30 months	0.335	4.3	88.08	91.4	429.13	149.06
	Breeds x Age	ns	ns	ns	ns	ns	ns
	SEM	0.012	0.025	0.69	0.67	17.34	7.86

the northeastern region may be less important on the semen quality of Thai native chickens. The data gathered in the current research do not support the influence of seasonality on semen quality; perhaps because of the narrow range of photoperiod, different genetic backgrounds and different location of the chickens.

For the effect of breed on semen quality, the results of this study (Table 2) indicate that there is no statistically significant influence of breed on semen quality among native Thai cocks. A study of four breeds of chicken performed in Poland (Black Minorca, Green-Legged, Italian Partridge Partridge, and White Crested Black Polish) (Siudzinska and Lukaszewiez, 2008) confirmed that breed had no significant affect on semen quality. Furthermore, the volume of ejaculate and sperm concentrations collected from those breeds were not significantly different. Long et al., (2010) investigated fresh semen from 8 pedigreed layer lines and found no differences among poultry lines or strainsvis-à-vissemen quality. The reason for the non-effect of breed on semen quality maybe the close genetic backgrounds (Siudzinska and Lukaszewiez, 2008; Long et al., 2010). Among Thai native chickens, Aumpapron (2010) reported that Lueng hang kaow, Pradoo hang dam and Chee had close genetic backgrounds, which supports the results of the present study. In contrast, Peters et al. (2008) observed differences in strains of Nigerian indigenous cocksvis-à-vissemen volume, concentration and motility. The different results may due to the different genetic backgrounds.

For the effect of age on semen quality, the current study found that the age of Thai native cocks, either between 10 and 22 months or between 18 and30 months, had no significant influence on semen quality. This finding agrees with the results obtained by Chotesangasa (2001) who reported on semen quality of Thai native cocks (between9 months and 2 years old) over a period of 2 months. In contrast, it was reported that ejaculate volume and sperm concentration are dependent on the age of the male (Bakst and Cecil, 1992; Kelso et al., 1996; Kotloska et al., 2005; Long et al., 2010). Moreover, Kotloska et al. (2005) reported that spermatozoa concentration of turkeys declines with age (from 31 to 51 wks). Cerolini et al. (1997) studied the effect of age on semen concentration and found that significantly increased from the 24th wk to the 39th wk of age; but did not vary substantially between the 39th and 54th wk; and was at the lowest concentration in the 72nd wk. The highest rate of sperm motility was observed in the first half of the reproductive period (i.e., in the 24th and 39th wk of life) and it significantly decreased in the second half of the reproductive period (*i.e.*, by 35% at the 39th wk and 42% at the 45th wk). Tabatabaei et al. (2010) reported that age also affected semen quality of the indigenous broiler breeder rooster in Iran which was measured during the 26th to the 45th wk of life. The results of the present study, however, revealed that age had no effect on semen quality between the 10th and 30th month of age among Thai native cocks. The results suggest that Thai native chicken breeds have a longer reproductive lifetime than the commercial breeds and Iranian indigenous chickens previously reported.

Conclusions

The respective semen quality (mass movement, % total motile, volume, % live normal, concentration, and total sperm number/ejaculate) of the cocks within three Thai native breeds (*i.e.*, Lueng hang kaow, Pradoo hang dam and Chee) was not significantly different. Moreover, age and environmental factors had no effect on semen quality. Taken together, the research strongly suggests that between 10 and 30 months of age indigenous cocks have similar quality of semen.

Acknowledgements

This research was partially supported by the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Office of Higher Education Commission, Ministry of Education AG-BIO/PERDO-CHE) and Agricultural Biotechnology Research Center for Sustainable Economy, Khon Kaen University. As well as, a part of research was supported by Research and Development Network Center for Animal Breeding (Native Chicken), Khon Kean University. The authors would like to thank the animal care staff at the Faculty of Agriculture, Khon Kaen University for their assistance and Mr. Bryan Roderick Hamman and Mrs. Janice Helen-Hamman for assistance with the English presentation of the manuscript.

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