Original Article

Boar sperm production in a tropical environment

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Abstract

In a tropical environment, sow productivity is strongly associated with boar sperm production. Regular monitoring of semen quality in boars that produce semen for artificial insemination is important. The objective of the present study was to investigate the boar sperm production in an evaporative cooling system in a tropical environment in association with breed and season. A total of 2878 ejaculates from 68 boars were collected from a boar stud in the western region of Thailand. The breeds consisted of 22 Duroc, 22 Yorkshire, 18 Landrace and six hybrid boars. Seasons were classified as cool, hot or rainy. Semen volume, sperm concentration, total sperm production and extended semen doses per ejaculate were evaluated. The data were analysed by a general linear mixed model procedure. On average, the semen volume, sperm concentration, total sperm production and extended semen doses per ejaculate were 256.2 ± 95.1 mL, 294.6 ± 125.0 × 10^6 sperm/mL, 70.2 ± 28.9 × 10^9 sperm/ejaculate and 16.2 ± 5.8 doses/ejaculate, respectively. In Duroc boars, the semen volume, total sperm production and extended semen doses per ejaculate in the cool season were higher than in the hot and rainy seasons ($P < 0.05$). However, most semen production traits did not differ significantly among season in Landrace, Yorkshire and hybrid boars. In conclusion, seasons influenced the semen traits of Duroc boars kept in an evaporative cooling system in Thailand.

Keywords: artificial insemination, breed, season, sperm, evaporative cooling system

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Introduction

In a tropical environment, sow productivity is strongly associated with boar sperm production. Regular monitoring of semen quality in boars that produce semen for artificial insemination (AI) is important. The fertility of the boars is largely dependent on their semen quality. A recent study clearly demonstrated that heat stress can induce sperm DNA damage in board during the summer in tropical environments (Pena *et al*., 2019). High ambient temperature and inappropriate humidity can be predisposing environmental factors causing heat stress in boars. Heat stress generally occurs when the ambient temperatures rise beyond the pig's thermal comfort zone of about 18–20 °C (Geisert *et al*., 1982; Prunier *et al*., 1997). However, the severity of heat stress on animal production can vary according to contributing factors, e.g., management, nutrition and ventilation.

Studies have demonstrated that boar semen quality can be influenced by several factors, e.g., breed, age, season, temperature and photoperiod (Kozdrowski and Dubiel, 2004; Rivera *et al*., 2005). Earlier studies indicated that season influences the semen parameters in boars (Suriyasomboon *et al*., 2004; Kunavongkrit *et al*., 2005). Photoperiod is one factor that plays a role in sperm production in mammals via the regulation of melatonin (Tast *et al*., 2001). Moreover, temperature and humidity are also important climatic factors affecting boar semen in tropical climates (Kunavongkrit *et al*., 2005). Therefore, the monitoring of sperm parameters and associated factors are important for the pig AI industry (Smital, 2009). The objective of the present study was to investigate seasonal influences on boar sperm production in an evaporative cooling system boar stud in Thailand.

Materials and Methods

Animals: The data used in the present study were collected from the Ratchaburi Artificial Insemination and Biotechnology Research Center, Department of Livestock Development (DLD), Ratchaburi, Thailand. The boar semen produced from this research centre is routinely used for AI in smallholder swine herds surrounding the station and in nearby provinces. The boars were proven sires and were routinely used for semen collection. The age of the boars used for semen collection raged between 1 to 3 years old. The replacement rate was 35% annually. The boars were allocated individual pens and housed in an evaporative cooling housing system to minimise the impact of high ambient temperatures. The semen was collected using the gloved-hand technique. The interval between each semen collection was 5–7 days. The data used in the present study were part of an experiment conducted according to the guidelines of the Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes by the National Research Council of Thailand. The study was approved by the Institutional Animal Care and Use Committee (IACUC) in accordance with the university regulations (animal use protocol no. 1631032).

General management: The boars entered the facility at approximately 8 months of age and were trained to use dummy sows for semen collection before actual collection started at 10–11 months of age onwards. They were kept in pens with a space allowance of 5.0 m^2/ boar. Boars were vaccinated against foot-and-mouth disease and classical swine fever at 8–10 months of age and re-vaccinated every 4 months. They were fed twice per day (about 2.8–3.0 kg of feed/boar/day) with a rice–corn–soybean–fish ration containing 16% crude protein, 3250 kcal/kg metabolisable energy and 1.10% lysine.

Data: Data were collected from the computer recording system of the research centre during the period 2009–2015. In total, 2878 ejaculates of boar semen were collected from 68 boars. The boar breeds were Duroc (n = 22), Yorkshire (n = 22), Landrace (n = 18) and hybrid (Pakchong-5; n = 6). The data included date of the semen collection, boar identity, breed of boar, semen volume (mL), subjective sperm motility (%), pH, sperm concentration (× 10^6 sperm/mL) and number of doses after dilution. The ejaculated semen volume was measured by digital weight scale and the subjective sperm motility determined under a light microscope at 200× magnification. The concentration of spermatozoa was measured by Spermacue® (Minitüb GmbH, Tiefenbach, Germany) and was expressed as the number of spermatozoa (× 10^6) per mL of semen. The total sperm production was calculated by multiplying the semen volume by the sperm concentration. After semen evaluation, the qualified semen was diluted with semen extender to a final of 3000 × 10^6 motile spermatozoa per dose.

Climatic data: Data of the environmental temperature and relative humidity in Ratchaburi province during the period 2009–2015 were collected from an official meteorological station within 60 km of the herds. The data included records of daily 24-h average temperatures, average minimum and maximum daily temperatures and the 24-h average humidity. The temperature–humidity index (THI) was calculated using the following formula (Kelly and Bond, 1971):

$$THI = DB - [0.55 - (0.55 \times RH)] \times (DB - 58)$$

where DB is the average daily temperature and RH is the average daily humidity. The means of environmental temperature, relative humidity and THI during the 7 days before semen collection were calculated and used in the correlation analyses with the semen data. During these years (2009–2015), the means ± standard deviation of the environmental temperature and relative humidity were 27.6 ± 1.9 °C (range 18.2–32.6 °C) and 78.1 ± 7.1%, respectively. The average daily (24-h) minimum and maximum temperature were 23.9 ± 2.2 and 33.1 ± 2.6 °C, respectively.

Statistical analysis: The data were analysed by using general linear mixed model procedure of SAS, version 9.4 (SAS Inst. Inc., Cary, NC, USA). Descriptive statistics (i.e., number of non-missing values, means, standard deviation and range) of the boar semen production were conducted using the MEANS procedure of SAS. Multiple analysis of variance was

carried out to determine the effect of breed, year and season on the semen production traits, including semen volume, sperm concentration, total sperm production and number of doses of extended semen per ejaculate. Seasons were classified as cool (15th Oct–14th Feb), hot (15th Feb–14th Jun) and rainy (15th Jun–14th Oct; Tummaruk *et al*., 2010). Normality of all the outcome variables was determined by evaluating the residual plot and Shapiro–Wilk statistics under the general linear model procedure (PROC GLM) of SAS. The data were analysed by the general linear mixed model procedure using the MIXED procedure of SAS. The statistical models included breed of boar (Landrace, Yorkshire, Duroc or hybrid), year (2009–2015), season of collection (hot, rainy or cool) and interaction between breed and season as fixed effect. The boar identity nested within breed was included in the statistical models as a random effect. Least-squares mean and standard error of the mean (SEM) were obtained from each class of factor and compared among groups by using the least significant difference (LSD) test. In addition, the environmental temperature and relative humidity were compared among seasons by the general linear model procedure of SAS. Moreover, Pearson's correlation was carried out to determine the association between the environmental temperature, relative humidity, THI and all semen traits. The correlation analyses were conducted separately by boar breed. For all analyses, $P < 0.05$ was regarded as statistically significant.

Results

Of all the boar semen data collected, 3.8% (n = 110 samples) were rejected and were not used for semen processing. The reasons for rejection of boar semen included poor semen quality (n = 56), azoospermia (n = 44) and miscellaneous (n = 3). After excluding these ejaculates, the remaining number of ejaculates included in the analyses for each semen trait are presented in Table 1. In our cross-sectional study, the number of ejaculates per boar averaged 42.3 ± 40.9 and ranged from 1 to 194 ejaculates per boar. The average semen volume, sperm concentration, total sperm production and number of extended semen doses per ejaculate are presented in Table 1. Across breed and season, the semen volume, sperm concentration, total sperm production and extended semen doses per ejaculate were 256.2 ± 95.1 mL, 294.6 ± 125.0 × 10^6 sperm/mL, 70.2 ± 28.9 × 10^9 sperm/ejaculate and 16.2 ± 5.8 doses/ejaculate, respectively.

Table 1 Descriptive statistics on the semen production by boars kept in an evaporative cooling system in a boar stud in Thailand

Variables	N	Mean ± SD	Range
Semen volume (mL)	2822	256.2 ± 95.1	50.0–498.0
Sperm concentration (× 10^6 sperm/mL)	2826	294.6 ± 125.0	28.0–809.0
Total sperm production (× 10^9 sperm)	2673	70.2 ± 28.4	5.5–356.3
Extended semen doses per ejaculate	2654	16.2 ± 5.8	1.0–40.0

The environmental temperature, relative humidity and THI in the cool, hot and rainy seasons in Ratchaburi province during 2009 and 2015 are presented in Table 3. The environmental temperature in the cool season was lower than in the hot ($P < 0.001$) and rainy ($P < 0.001$) seasons. Likewise, relative humidity in the cool season was lower than in the rainy season ($P < 0.001$) but higher than in the hot season ($P < 0.001$). The THI in the cool season (76.3 ± 0.08) was lower than in the hot (80.7 ± 0.08, $P < 0.001$) and rainy seasons (79.7 ± 0.08, $P < 0.001$), respectively.

Frequency distributions of the semen parameters are presented in Figure 1. The most common semen volume obtained from the boar ejaculates was 250–300 mL (Figure 1A). Likewise, the most common concentration of boar semen was (250–300) × 10^6 sperm/mL (Figure 1B). Moreover, most of the ejaculates had a total sperm production of (51–60) × 10^9 sperm/ejaculate (Figure 1C). The most common number of doses after dilution per ejaculate was 10–14 doses.

Year, season and interaction between breed and season influenced boar sperm production. Significance levels of these factors in each of the boar sperm production traits are presented in Table 2. As can be seen from the table, year and the interaction between breed and season strongly influenced the semen volume, sperm concentration, total sperm production and the number of doses per ejaculate (Table 2). However, the seasonal effect on all semen traits varied according to the breed of boar, as demonstrated by the significant interaction between breed and season ($P < 0.05$; Table 2). The seasonal effect on boar sperm production differed among breeds. In Duroc boars, the semen volume, total sperm production and number of doses per ejaculate in the cool season was higher than in the hot or rainy seasons ($P < 0.05$). However, most semen production traits did not differ significantly among seasons in Landrace, Yorkshire and hybrid boars (Table 5).

Table 4 shows the average sperm production parameters for each breed of boar in the boar stud. Semen production did not differ significantly among boar breeds (Table 2). However, sperm production differed significantly among years ($P < 0.001$; Table 2). The total sperm production in the boar stud was highest in 2011 and then declined significantly from 2012 to 2015 (Figure 2).

Correlation analyses revealed that the semen volume of Duroc boars was negatively correlated with the average environmental temperature (r = –0.081, $P = 0.026$) and THI (r = –0.098, $P = 0.007$) during the 7 days before semen collection (Table 6). Likewise, the sperm concentration, total sperm production and number of doses per ejaculate was also negatively associated with both the average temperature and THI during the 7 days before semen collection in hybrid boars (Table 6). On the other hand, these associations were not observed in the Landrace and Yorkshire breeds. Interestingly, in Duroc boars, both the semen concentration and the total sperm production was positively correlated with the average relative humidity during the 7 days before semen collection (Table 6).

Table 2 Factors influencing semen traits of boars kept in an evaporative cooling system in a boar stud in Thailand

Variable	Breed	Year	Season	Breed × Season
Semen volume	NS	<0.001	NS	0.001
Sperm concentration	NS	<0.001	NS	<0.001
Total sperm production	NS	<0.001	NS	0.017
Doses per ejaculate	NS	<0.001	0.048	<0.001

NS = $P > 0.05$

Table 3 Climatic data and semen production of boars kept in evaporative cooling system in a boar stud in Thailand in cool, hot and rainy seasons (least-squares mean ± SEM).

Variable	Season		
	Cool	Hot	Rainy
Climatic data			
Temperature (°C)	26.1 ± 0.1[a]	29.0 ± 0.1[b]	27.9 ± 0.1[c]
Humidity (%)	76.8 ± 0.2[a]	75.7 ± 0.2[b]	81.9 ± 0.2[c]
Temperature-humidity index	76.3 ± 0.1[a]	80.7 ± 0.1[b]	79.7 ± 0.1[c]
Number of ejaculates	707	993	919
Semen volume (mL)	258.7 ± 10.5[a]	252.8 ± 10.2[a]	253.3 ± 10.3[a]
Sperm concentration (× 10^6 sperm/mL)	289.1 ± 15.6[a]	296.4 ± 15.3[a]	293.4 ± 15.4[a]
Total sperm production (× 10^9 sperm)	70.3 ± 3.3[a]	69.5 ± 3.2[a]	69.3 ± 3.3[a]
Doses per ejaculate	16.2 ± 0.7[a]	16.1 ± 0.7[ab]	15.7 ± 0.7[b]

[a,b] Different superscript within a row indicates significant difference ($P < 0.05$)

Table 4 Semen production in boars with different breeds kept in an evaporative cooling system in a boar stud in Thailand (least-square means ± SEM).

Variable	Boar breed			
	Duroc	Landrace	Yorkshire	Hybrid
Number of ejaculates	755	689	868	307
Semen volume (mL)	240.1 ± 15.7[a]	267.9 ± 17.1[a]	271.7 ± 15.5[a]	240.0 ± 28.9[a]
Sperm concentration (× 10^6 sperm/mL)	303.3 ± 23.6[a]	270.5 ± 25.8[a]	266.2 ± 23.5[a]	331.9 ± 44.0[a]
Total sperm production (× 10^9 sperm)	68.6 ± 5.0[a]	66.1 ± 5.4[a]	66.5 ± 5.0[a]	77.7 ± 9.1[a]
Doses per ejaculate	15.4 ± 1.0[a]	14.7 ± 1.1[a]	15.5 ± 1.1[a]	18.4 ± 1.9[a]

[a] One letter in common within row do not differ significantly ($P > 0.05$)

Table 5 Semen production of boars kept in evaporative cooling system in a boar stud in Thailand by breed and season (least squares mean ± SEM).

Variable	Season		
	Cool	Hot	Rainy
Duroc (number of ejaculates)	211	271	273
Semen volume (mL)	253.3 ± 16.3[a]	228.7 ± 16.1[b]	238.2 ± 16.1[b]
Sperm concentration (× 10^6 sperm/mL)	289.4 ± 24.2[a]	316.9 ± 24.0[b]	303.6 ± 23.9[a]
Total sperm production (× 10^9 sperm)	72.1 ± 5.2[a]	66.1 ± 5.1[b]	67.7 ± 5.1[b]
Doses per ejaculate	16.4 ± 1.1[a]	15.0 ± 1.1[b]	14.9 ± 1.2[b]
Landrace (number of ejaculates)	194	277	218
Semen volume (mL)	263.5 ± 17.6[a]	273.1 ± 17.4[a]	267.1 ± 17.5[a]
Sperm concentration (× 10^6 sperm/mL)	274.1 ± 26.3[a]	273.8 ± 26.0[a]	263.6 ± 26.1[a]
Total sperm production (× 10^9 sperm)	65.9 ± 5.6[a]	67.8 ± 5.5[a]	64.6 ± 5.5[a]
Doses per ejaculate	14.7 ± 1.2[a]	15.0 ± 1.2[a]	14.5 ± 1.2[a]
Yorkshire (number of ejaculates)	230	336	302
Semen volume (mL)	269.6 ± 16.1[a]	277.9 ± 15.8[a]	267.7 ± 15.9[a]
Sperm concentration (× 10^6 sperm/mL)	263.9 ± 23.9[ab]	258.1 ± 23.7[a]	276.4 ± 23.8[b]
Total sperm production (× 10^9 sperm)	64.0 ± 5.2[a]	67.5 ± 5.1[a]	67.9 ± 5.1[a]
Doses per ejaculate	14.9 ± 1.1[a]	15.9 ± 1.1[b]	15.6 ± 1.1[a]
Hybrid (number of ejaculates)	72	109	126
Semen volume (mL)	248.2 ± 30.0[a]	231.6 ± 29.3[a]	240.2 ± 29.2[a]
Sperm concentration (× 10^6 sperm/mL)	329.1 ± 44.9[a]	336.7 ± 44.3[a]	329.8 ± 44.3[a]
Total sperm production (× 10^9 sperm)	79.3 ± 9.5[a]	76.6 ± 9.3[a]	77.2 ± 9.2[a]
Doses per ejaculate	19.0 ± 2.0[a]	18.5 ± 1.9[ab]	17.7 ± 1.9[b]

[a,b] Different superscript within a row indicates significant difference ($P < 0.05$)

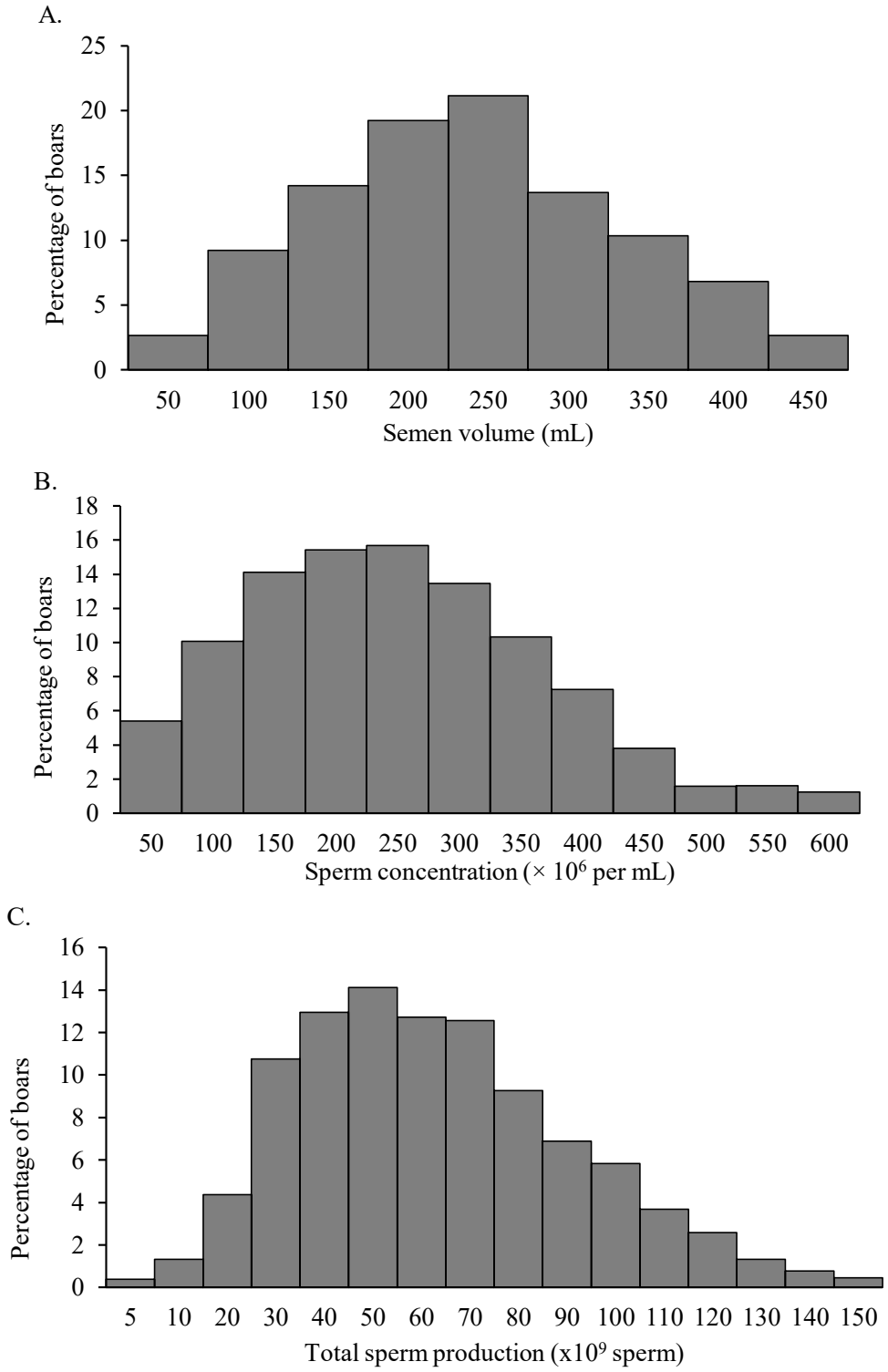

Figure 1 Frequency distribution of semen production of boars kept in an evaporative cooling system in a boar stud in Thailand. A: Semen volume; B: Sperm concentration; C: Total sperm production

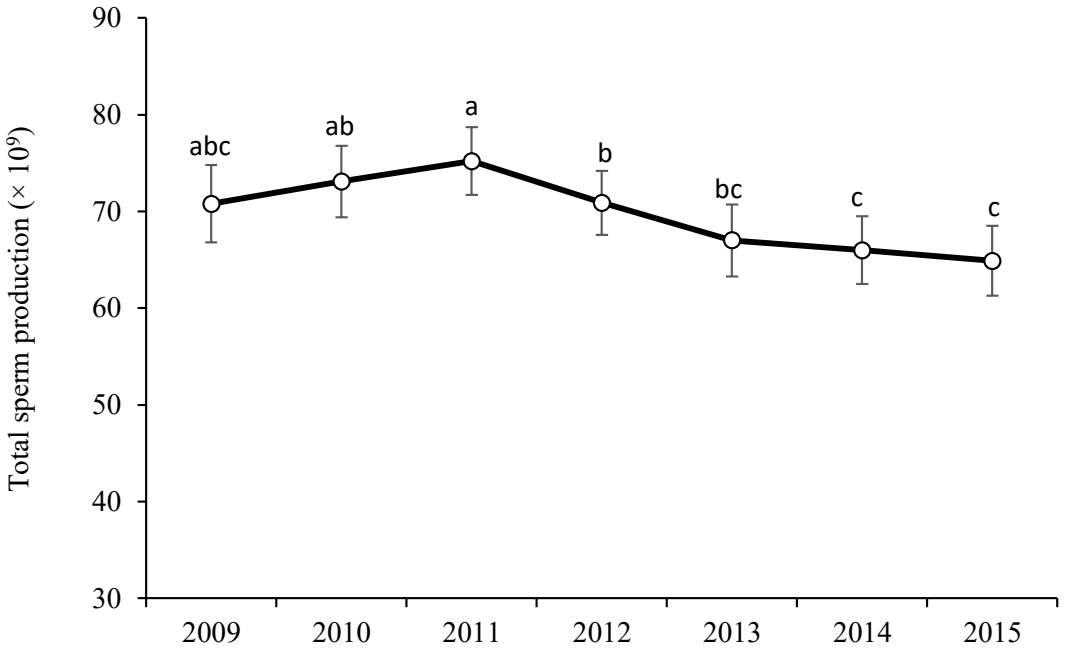

Figure 2 The total sperm production of ejaculated semen from 68 boars kept in evaporative cooling system in a boar stud in Thailand from 2009 to 2015. [a,b,c] Different superscript indicates significant difference ($P < 0.05$)

Table 6 Pearson's correlation among environmental temperature, relative humidity and temperature–humidity index (THI) during the 7-day period before semen collection and the semen traits by breed of boars

Variable	Correlation coefficient		
	Temperature	Humidity	THI
Duroc (n = 805)			
Semen volume	–0.081*	NS	–0.098**
Sperm concentration	NS	0.108**	NS
Total sperm production	NS	0.108**	NS
Doses per ejaculate	NS	0.097**	NS
Landrace (n = 740)			
Semen volume	NS	NS	NS
Sperm concentration	NS	NS	NS
Total sperm production	NS	NS	NS
Doses per ejaculate	NS	NS	NS
Yorkshire (n = 938)			
Semen volume	NS	0.082*	NS
Sperm concentration	NS	NS	NS
Total sperm production	NS	0.068*	NS
Doses per ejaculate	NS	NS	NS
Hybrid (n = 339)			
Semen volume	NS	NS	NS
Sperm concentration	–0.191***	NS	–0.179**
Total sperm production	–0.167**	NS	–0.170**
Doses per ejaculate	–0.158**	NS	–0.181**

Significant levels indicated as * $P < 0.05$, ** $0.05 < P < 0.01$, *** $P < 0.001$ and NS = $P > 0.05$

Discussion

The present study examined sperm production in different boar genotypes in an AI centre in the western region of Thailand. The study showed that the semen volume and total sperm production were lowest during the hot season. This finding agrees with those of earlier studies in Thailand (Suriyasomboon *et al*., 2004; Tretipskul *et al*., 2012). A previous study in a boar stud in Thailand demonstrated that season and breed of boar significantly influence semen production of boars kept in an evaporative cooling system and that total sperm production is highest during cool seasons (Tretipskul *et al*., 2012). Moreover, the seasonal influence on boar semen production was more in evidence in purebred than in crossbred boars (Tretipskul *et al*., 2012). Additionally, high temperature and high humidity also negatively affect sperm morphology (Suriyasomboon *et al*., 2005). However, Suriyasomboon *et al*. (2005) found that boar sperm production in Thailand did not differ significantly between boars kept in an evaporative cooling system and those kept in a conventional open housing system.

The differences between the present and previous studies are that the boar stud in the present study belong to the Thai government (i.e., DLD) and the semen produced from these boars is routinely used for AI in many smallholder farms surrounding the AI station. Therefore, the policy of handling boar sperm production can have a large impact on the reproductive performance of sows in many smallholder pig farms around the country. Moreover, the seasonal effect on semen production in the boar stud handled by the government has never before been comprehensively investigated. Thus, this information could be used as a benchmark for the future improvements in boar stud management policy by the government.

In the USA, the average boar sperm production ranges between 60 and 120 billion sperm per ejaculate and results in 20 to 40 AI doses per ejaculate (Knox, 2016). In the present study, the sperm production averaged 70.2 billion sperm per ejaculate, within the range observed in the USA. However, the diluted semen doses averaged 16.2 doses per ejaculate, lower than that reported in USA (Knox, 2016). In Poland, the semen volumes of Polish Landrace and Yorkshire boars were 251.9 and 250.9 mL, respectively (Knecht *et al*., 2017). In the present study, the semen volumes of Landrace and Yorkshire boars were 267.9 and 271.9 mL, respectively. However, the sperm concentration in the Polish Landrace and Yorkshire boars (393.7 and 397.4 spermatozoa/mL, respectively) were much higher than those in the current study (270.5 and 266.2 spermatozoa/mL, respectively). The relatively poor boar semen production in Thailand may be due partly to high environmental temperature and high humidity (Suriyasomboon *et al*., 2004). In sows, an increased ambient temperature during the hot season results in a reduction in sow reproductive performance (Tummaruk *et al*., 2004[a], 2004[b]; Tummaruk *et al*., 2010). In the boar, when the ambient temperature reaches 29 °C, the integrity of sperm DNA can be affected (Pena *et al*., 2019). However, individual variations among boars have been reported (Cameron and Blackshaw *et al*., 1980). These studies indicate that boar semen production in Thailand needs to be improved in order to achieve an international standard, and one possible approach is to modify the housing conditions and/or management to cope with inappropriate environmental temperatures.

In the present study, the total sperm production and semen volume increased in cool seasons in Duroc boars. Mauget and Boissin (1987) found that the increase in the mass of the testes in wild boars was highest in winter, while in summer it was significantly lower; these changes are correlated with the duration of light period. The increase in the mass of the testes results in an increased endocrine activity of the testes and therefore high testosterone levels in the bloodstream (Mauget and Boissin, 1987, Weiler *et al*., 1996). An increased level of sex hormones during autumn and early winter has also been reported in domestic boars (Claus *et al*., 1985; Borg *et al*., 1993).

To minimise the adverse effect of the season, especially during hot periods, adequate feeding with a high-quality diet is suggested (Quiniou *et al*., 2000). In addition, the management of temperature and humidity using evaporative cooling systems and other techniques that enable AI boar studs to maximise sperm fertility is also recommended (Kunavongkrit *et al*., 2005). In the present study, high humidity was observed during the rainy season (i.e., the relative humidity averaged 81.9%). This high humidity is due to rainfall nearly every day during this period of the year. In the females, high relative humidity (>40%) in the lactation house negatively influences litter size at birth (Suriyasomboon *et al*., 2006). Moreover, Tummaruk *et al*. (2010) demonstrated that high humidity during gestation reduced the total number of piglets born per litter. In addition, a combination of high temperature and high humidity also negatively affected litter size (Suriyasomboon *et al*., 2006). In the present study, THI significantly affect the semen volume in Duroc boars and total sperm concentration, total sperm production per ejaculate and number of doses per ejaculate in hybrid boars. This indicates that a combination of high temperature and high humidity also negatively affects the sperm production of the boar. On the other hand, we observed that humidity was positively correlated with sperm concentration, total sperm production and number of doses per ejaculate in Duroc boars. This is in contrast with a previous report from another Duroc boar stud in Thailand (Suriyasomboon *et al*., 2004). Moreover, it was found that the proportion of proximal cytoplasmic droplets in Duroc boars kept in an evaporative cooling system was significantly increased when the relative humidity inside the house was increased from 75% to 92% (Suriyasomboon *et al*., 2005). The reason behind the impact of humidity on boar sperm production is still unknown and remains to be further investigated. However, these findings indicate that humidity may play an important role for the boars. Therefore, the design of housing for boars in tropical climates should emphasise minimising high environmental temperature and maintain optimal humidity during hot and rainy seasons.

In conclusion: Season influenced the semen production by boars kept in an evaporative cooling system in a tropical environment. In Duroc boars, the semen volume, total sperm production and the number of doses per ejaculate in the cool season were significantly higher than in hot or rainy seasons. However, Landrace, Yorkshire and hybrid boars were more tolerant to the seasonal effect than were Duroc boars. This finding indicated that the housing system for boars currently used in Thailand is not effective in coping with the seasonal influences on semen production. Therefore, other strategies, including improved management practices and extra nutritional approaches for the boars during hot and rainy seasons should be considered in order to minimise the seasonal effects on boar semen production and sperm quality in a tropical environment.

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