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Semen production capacity among boar breeds and effect of organic antioxidant supplementation on sperm characteristics during the hot season in Thailand

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Original Article

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Abstract

The present study was carried out to investigate semen production capacity among boar breeds and measure the efficiency of a new organic antioxidant supplementation on semen production and qualities during the hot season in Thailand. A total of 711 ejaculates from 87 boars (i.e., 26 Duroc, 15 Landrace, 30 Yorkshire and 16 crossbred Landrace × Yorkshire) were included in the study. Semen samples were allocated into two treatments – without organic antioxidant (CONTROL; n = 397) and with organic antioxidant supplementation (TREATMENT; n = 314). The experiment was carried out for three months during the hot season in May (n = 184), June (n = 255) and July (n = 272). Semen volume (VOL), sperm concentration (CONC), total sperm production per ejaculate (TSP), subjective sperm motility (MOL), sperm head with abnormal morphology (AHM) and sperm tail with abnormal morphology (ATM) were evaluated. On average, there was a significant difference ($P < 0.001$) regarding VOL, TSP and MOL among the four breeds of boars, but no difference in the semen parameters compared between the two treatments ($P > 0.05$). Duroc semen volume was lower than Landrace, Yorkshire and Landrace × Yorkshire boars (177.7 ± 66.4 versus 222.1 ± 76.5, 230.0 ± 78.3 and 224.2 ± 73.5 ml, respectively, $P < 0.05$). Similarly, Duroc TSP was lower than Landrace, Yorkshire and Landrace × Yorkshire boars (65.1 ± 28.8 versus 87.5 ± 30.6, 81.6 ± 31.5 and 82.0 ± 33.6 × 10^9 sperm per ejaculate, respectively, $P < 0.05$). Across breeds, TSP of the semen collected in May was higher than those in June and July (86.5 ± 2.8 versus 78.3 ± 2.2 and 74.9 ± 2.0 × 10^9 sperm per ejaculate, respectively, $P < 0.05$). In conclusion, under a tropical climate, semen production of Duroc boars was inferior to other dam line breeds and the semen quality collected in June and July was lower than those collected in May. Supplementation of the current doses of the organic antioxidant cannot improve boar semen production during the hot season.

Keywords: boar, sperm, season, antioxidant

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Introduction

Semen production among commercial boar studs worldwide is controlled by a myriad of factors that result in a considerable variation in semen characteristics, e.g., age, health status, frequency of use, nutrition, housing and season (Suriyasomboon *et al*., 2004; Flowers, 2015; Rungruangsak *et al*., 2021). Besides those factors, breed, nutrition and seasons are always taken into consideration to maximise the breed potential for semen production in the swine industry (Louis *et al*., 1994; Ciereszko *et al*., 2000; Peña *et al*., 2019). The use of boar breeds in swine breeding programmes differs among countries, mostly dependent on country-specific environmental effects such as temperature and humidity. The impact of these factors is associated with the adaptation ability of each breed to the environment (Rungruangsak *et al*., 2021). Latorre *et al*. (2003) indicated that under the climatic environmental conditions in Spain, the purebred Danish Duroc sire line is a good alternative compared to the two hybrid boar lines (Dutch Duroc × Yorkshire and Pietrain × Yorkshire) due to a significantly faster growing rate and better feed conversion ratio. Similarly, the Danish Duroc boar is the common terminal sire used in most commercial swine herds in Thailand (Tretipskul *et al*., 2012; Rungruangsak *et al*., 2021). Moreover, Landrace × Yorkshire crossbred boars in Thailand are also used in some commercial boar studs because of their superior semen production and heat tolerance compared to purebred Duroc and Pietrain boars (Tretipskul *et al*., 2012). However, the comparison of semen production in different boar breeds during the hottest period of the year under commercial conditions has not been evaluated. Moreover, the breed difference concerning the response to the use of an antioxidant compound to reduce the negative impact of heat stress on boar semen production is unknown.

In Thailand – a tropical country, in March–June (summer) and July–October (rainy), the hot days with a maximum temperature of > 25 °C account for more than 20 days a month and the average humidity is in the range of 70–90% all year around (Tretipskul *et al*., 2012; Rungruangsak *et al*., 2021). As a result, oxidative stress occurs and causes negative effects on sperm viability and sperm motility and increases the morphological abnormalities of boar sperm by destroying the sperm plasma membranes (Aitken *et al*., 2007; Sonderman and Luebbe, 2008; Peña *et al*., 2019). Previous studies clearly demonstrated that antioxidant systems of seminal plasma and spermatozoa, including glutathione and ergothioneine, can counteract the harmful effects of reactive oxygen species (ROS) (Strzeżek *et al*., 1999; Taylor, 2001). However, those antioxidant systems could not tackle the excessive amount of ROS when oxidative stress occurs. To reduce these problems, the conventional open-air system has been replaced by the evaporative cooling system in most commercial swine herds in Thailand as well as in south-east Asian countries (ASEAN). This system can decrease the average temperature inside the barn to below 25 °C, but increase the relative humidity in the barn to be as high as 80–90% (Suriyasomboon *et al*., 2004; Tretipskul *et al*., 2012). Nevertheless, the seasonal effect on boar semen production in most commercial swine herds has existed for the past 10 years (Tretipskul *et al*., 2012; Rungruangsak *et al*., 2021). This indicates that the housing approach is inadequate. Thus, to optimise semen production of boars in a tropical environment, an additional approach is required.

Dietary supplementation with organic antioxidants, essential fatty acids and herbal constituents is considered an effective solution to improve porcine reproductive performance in several aspects (Strzeżek *et al*., 2004; Peña *et al*., 2019). For instance, adding curcumin – one of the natural antioxidants extracted from turmeric *(Curcuma longa)* – to the freezing extender at a concentration of 0.25 or 0.5 mmol/l increased progressive motility and acrosome integrity (Chanapiwat and Kaeoket, 2015) or a diet supplemented with omega 3 resulted in positive effects on sperm morphology of Yorkshire and Pietrain boars and spermatic osmotic resistance in the Pietrain breed (Yeste *et al*., 2011). The pharmacological activity of *Withania somnifera* – one type of herbs with biological active chemical components such as alkaloids, steroidal lactones, saponins, glucose, and iron – has been revealed as a hormone precursor, antibiotic, anti-stress constituents, which can help regulate important physiologic processes, prevent inflammation and male sexual dysfunction (Singh *et al*., 2010; Verma and Kumar, 2011). However, no data are available regarding the efficiency of using *Withania somnifera* on boar semen production during hot seasons. Therefore, a small-scale clinical implementation is necessary before a large-scale implementation can be done. The present study aimed to *i.*) investigate the capacity of semen production among boar breeds during the hottest period of the year under a tropical environment and *ii.*) determine the efficiency of using an organic herbal constituent – a new supplemental organic antioxidant product – during hot seasons on semen production in different breeds of boars

Materials and Methods

Animals and general management: The study was carried out in a central isolated boar stud in the eastern part of Thailand from May to July 2020. A total of 711 semen ejaculates including Duroc (D, n = 269), Landrace (L, n = 88), Yorkshire (Y, n = 217) and crossbred Landrace x Yorkshire (LY, n = 137) were collected from 87 boars including D (n = 26), L (n = 15), Y (n = 30) and LY (n = 16). The boars were fed twice a day with a total amount of 2.3–3.2 kg per day of commercial feed as a standard diet (Hygro 568F, Charoen Pokphand Foods Public Company Limited, Bangsaothong, Samutprakarn, Thailand). The boar diet contained 3,000 kcal energy per kg, 14.0% crude protein, 5.8% crude fibre, 5.8% fat, 0.85% calcium, 0.35% phosphorus and 0.65% lysine. Water was provided *ad libitum* via a drinking nipple. The age of the boars ranged between 1–3 years old, nourished in individual pens (9 m^2 per boar) under an evaporative cooling system with an average temperature and humidity around 25 °C and 80%, respectively. All boars included in the present study were proven sires routinely used for artificial insemination. The animals used in this study were part of an experiment conducted under the approval of the Institutional Animal Care and Use Committee (IACUC) in accordance with university regulations, animal use protocol No. 1731012. Ethical Principles and Guidelines for the use of Animals for Scientific Purposes edited by the National Research Council of Thailand were used for review purposes.

Experimental designs: To evaluate the efficiency of a new organic antioxidant supplement, which contained a natural organic compound of selenium, zinc, copper, vitamin C, vitamin E, essential fatty acids and herbal constituents (B-Boost® Swine, *Withania somnifera*, Withanolidse, India), a complete randomised multiple factor (i.e. boar breed, month and treatment effects) experiment with two treatments of with and without the organic antioxidant supplementation was conducted. In total, 711 ejaculates collected from 87 boars were allocated randomly into two groups, control group without supplementary product (CONTROL, n = 397 ejaculates from 58 boars including 15 D, n = 137 ejaculates, 12 L, n = 68 ejaculates, 21 Y, n = 110 ejaculates and 10 LY, n = 82 ejaculates) and treatment group with supplementary organic antioxidant compound (TREATMENT, n = 314 ejaculates from 29 boars including 11 D, 132 ejaculates, 3 L, n = 20 ejaculates, 9 Y, n = 107 ejaculates and 6 LY, n = 55 ejaculates). For boars in the treatment group, the starting dose (0.83 ± 0.1 g) of organic antioxidant compound modified nutritionally based on the doses used for heifers, bulls and roosters was added to their daily feed portion from 1st May until 30th July. After 2 days of adding organic antioxidant compound, the semen was collected and measured to represent for data in the TREATMENT group.

Boar semen production data: In accordance with the duration of the present study, from May to July, the semen ejaculates were collected from 87 boars and allocated in May with 184 ejaculates recruited from CONTROL, n = 110 and TREATMENT, n= 74; June with 255 ejaculates recruited from CONTROL, n= 139 and TREATMENT, n= 116 and July with 272 ejaculates recruited from CONTROL, n = 148 and TREATMENT, n = 124. The different temperature and humidity values for the 3 months associated with the semen parameters were analysed. The boar semen was collected using the gloved-hand method. The semen was routinely collected from all boars every 5–7 days. The routine semen evaluation technique in the boar stud included subjective sperm motility (MOL), semen volume (VOL) and sperm concentration (CONC). The total sperm per ejaculate (TSP) was calculated by multiplying VOL with CONC. The subjective sperm motility was evaluated microscopically at 200× magnification by the same technician. The gel-free semen parameters including VOL in the range of 50–500 ml measured by weight and CONC within the range of 50–800 × 10^6 sperm per ml evaluated by Spermacue® (Minitube, Tiefenbach, Germany).

Boar sperm morphology: Sperm tail with abnormal morphology (ATM) and sperm head with abnormal morphology (AHM) were evaluated by formal saline and William's staining methods, respectively (*Lagerlöf*, 1934). For sperm head morphology, 500 sperm heads were examined under a light microscope (Olympus, Tokyo, Japan) at 1000× with oil immersion. The sperm head abnormalities included narrow head, narrow at the base, pear shape, variable size, abnormal contour, undeveloped, abaxial and loosened heads. Regarding sperm tail morphology, 200 sperm tails were assessed under a phase-contrast microscope (Olympus, Tokyo, Japan) at 400× magnification. The abnormalities of the tail included cytoplasmic droplets, simple bent tail, coil tail, abnormal midpieces, acrosome defects and looseness of the tail (Kaewma *et al*., 2021).

Statistical analysis: The semen production and sperm morphology data were analysed using SAS statistical software, version 9.4 (SAS Inst. Inc., Cary, NC, USA). Descriptive statistics including number of non-missing values, mean, standard deviation and range of the continuous data were calculated using the MEANS procedure of SAS. Multiple analysis of variance was performed to analyse the continuous traits using the general linear model procedure (GLM) of SAS. Variables describing various traits of sperm production, i.e., VOL, CONC and TSP as well as sperm morphology, i.e., ATM and AHM were regarded as dependent variables. The statistical models included the effect of boar breeds (D, L, Y and LY), treatment groups (CONTROL and TREATMENT), months (May, June and July) and interactions between investigated factors (i.e., breed × group, breed × month and group × month). Least square means were obtained from each class of factors and compared using the least-significant difference test. Differences with $P < 0.05$ were considered statistically significant.

Results

Across 711 ejaculates collected from four boar breeds D, L, Y and LY, the average semen VOL, CONC, TSP and MOL of the boars in this study were 208.2 ± 76.5 ml, 319.3 ± 166.6 × 10^6 sperm per ml, 76.2 ± 32.0 × 10^9 sperm per ejaculate and 81.1 ± 4.7%, respectively. The sperm morphology parameters including head abnormality and tail abnormality were 0.4 ± 0.7% and 3.6 ± 4.6% respectively.

Breed difference: The ejaculates of D, L, Y, and YL were recruited from ejaculates allocated in both CONTROL and TREATMENT groups to measure the effect of boar breeds on the semen characteristics. The semen production among boar breeds is presented in Table 1. On average, there was a significant difference ($P < 0.001$) in terms of VOL, TSP and MOL among breeds. The semen VOL in D boars was lower than L, Y and LY boars (177.7 ± 66.4 versus 222.1 ± 76.5, 230.0 ± 78.3 and 224.2 ± 73.5 ml, respectively, $P < 0.001$). The sperm MOL in D boars was lower than L, Y and LY boars (78.5 ± 5.1 versus 83.3 ± 2.8, 83.2 ± 3.3 and 81.6 ± 4.0 %, respectively, $P < 0.001$). Similarly, the TSP in D boars was lower than L, Y and LY boars (65.1 ± 28.8 versus 87.5 ± 30.6, 81.6 ± 31.5 and 82.0 ± 33.6 × 10^9 sperm per ejaculate, respectively, $P < 0.001$). No significant difference in VOL and TSP among L, Y and LY breeds was detected ($P > 0.05$). However, the sperm MOL in

LY (81. 6 ± 4.0%) was lower than Y (82.2 ± 3.3%, $P < 0.001$) and L (83.3 ± 2.8%, $P < 0.001$). Other sperm parameters including CONC and sperm morphology did not differ significantly among the four boar breeds (Table 1).

Table 1 Descriptive statistics (mean ± SD) for boar semen production and the percentage of sperm abnormality in Duroc (D), Landrace (L), Yorkshire (Y) and Landrace × Yorkshire crossbred boars (LY)

Variables	Breed	N	Mean ± SD	*P* value
Volume (ml)	D	269	177.7 ± 66.4 [a]	< 0.001
	L	88	222.1 ± 76.5 [b]	
	Y	217	230.0 ± 78.3 [b]	
	LY	137	224.2 ± 73.5 [b]	
Concentration (×10⁶ sperm per ml)	D	269	392.1 ± 172.9	0.580
	L	88	426.9 ± 174.9	
	Y	217	377.8 ± 154.8	
	LY	137	388.7 ± 165.7	
Total sperm per ejaculate (×10⁹ sperm)	D	269	65.1 ± 28.8[a]	< 0.001
	L	88	87.5 ± 30.6[b]	
	Y	217	81.6 ± 31.5[b]	
	LY	137	82.0 ± 33.6[b]	
Individual motility (%)	D	265	78.5 ± 5.1[a]	< 0.001
	L	86	83.3 ± 2.8[b]	
	Y	217	83.2 ± 3.3[b]	
	LY	137	81.6 ± 4.0[c]	
Abnormal head (%)	D	113	0.38 ± 0.6	0.650
	L	33	0.86 ± 1.6	
	Y	88	0.36 ± 0.2	
	LY	54	0.32 ± 0.2	
Abnormal tail (%)	D	269	3.9 ± 4.9	0.370
	L	88	3.4 ± 4.6	
	Y	217	3.2 ± 4.1	
	LY	137	3.8 ± 4.8	

[a,b,c] Different superscripts in each variable indicate significant differences ($P < 0.001$)

Effect of organic antioxidant supplementation: The effect of organic antioxidant supplementation on the quality of boar semen is presented in Tables 2 and 3. The statistical models revealed that all measured semen parameters were not statistically significant when testing the main effect of organic antioxidants (Table 2). However, the interaction between breed and treatment was significant for VOL and CONC (Table 3). Apart from semen volume of L boars in the TREATMENT group was higher than in the CONTROL group (262.2 ± 16.5 and 214.9 ± 9.7 ml, respectively, $P = 0.010$) (Figure 1) and the sperm concentration of LY boars in the TREATMENT group was higher than in the CONTROL group (446 ± 22.2 × 10^6 and 355.1 ± 18.2 × 10^6 sperm per ml, respectively, $P < 0.01$) (Figure 2), VOL of LY boars in the TREATMENT group was lower than that in the CONTROL group (209 ± 9.9 ml and 236 ± 8.1 ml, respectively, $P < 0.01$) (Figure 1) and the CONC of L boars in the TREATMENT group was lower than that in the CONTROL group (347 ± 37.1 × 10^6 and 443.4 ± 21.8 ×10^6 sperm per ml, respectively, $P < 0.01$) (Figure 2). However, no effect of organic antioxidant supplementation on either VOL or CONC was detected in either D or Y boars ($P > 0.05$) (Figures 1 and 2).

Table 2 Semen characteristics in boars with (TREATMENT) and without (CONTROL) organic antioxidant supplementation (least square means ± SEM)

Semen characteristics	Group		*P* value
	CONTROL	TREATMENT	
Volume (ml)	213.8 ± 3.9	221.8 ± 5.4	0.225
Concentration (×10⁶ sperm per ml)	400.2 ± 8.8	383.9 ± 12.2	0.273
Total sperm per ejaculate (×10⁹ sperm)	80.1 ± 1.6	79.7 ± 2.3	0.892
Individual motility (%)	81.5 ± 0.2	82.0 ± 0.3	0.273
Abnormal head (%)	0.49 ± 0.1	0.31 ± 0.1	0.073
Abnormal tail (%)	3.5 ± 0.2	3.7 ± 0.3	0.564

Table 3 Levels of significance (*P* value) for the main effects and interaction effect of independent variables influencing semen volume (VOL), sperm concentration (CONC), total sperm production (TSP), sperm motility (MOL), abnormal head morphology (AHM) and abnormal tail morphology (ATM)

Independent variables	**Dependent variables**					
	VOL	CONC	TSP	MOL	AHM	ATM
Breed	<0.001	0.580	<0.001	<0.001	0.650	0.370
Treatment	0.230	0.270	0.890	0.270	0.070	0.560
Month	0.041	0.330	0.004	0.036	0.090	0.290
Breed × Treatment	0.004	<0.001	0.290	0.290	0.130	0.600
Treatment × Month	0.630	0.120	0.520	0.170	0.130	0.650
Breed × Month	0.900	0.040	0.630	0.230	0.240	0.700

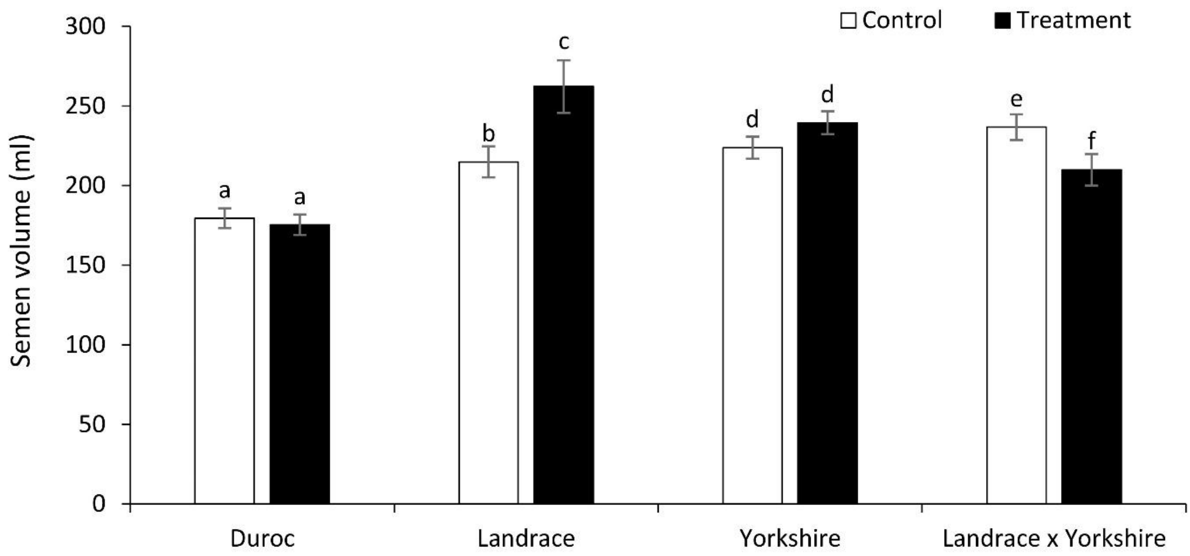

Figure 1 Semen volume (ml) in boars with (TREATMENT) and without (CONTROL) organic antioxidant supplementation by breed. [a,b,c,d,e,f] Different letters in each breed indicate significant differences (*P* < 0.05)

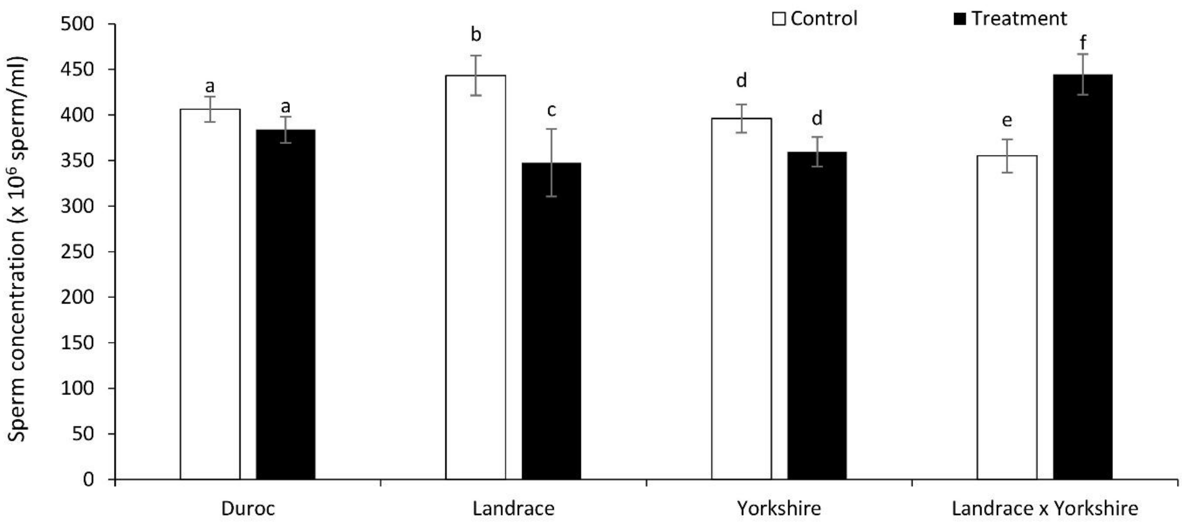

Figure 2 Sperm concentration ($\times 10^6$ sperm/ml) in boars with (TREATMENT) and without (CONTROL) organic antioxidant supplementation by breed. [a,b,c,d,e,f] Different letters in each breed indicate significant differences (*P* < 0.05)

Effect of months: The effect of months within the hot season on boar semen production is presented in Table 4. No significant difference was found in the CONC among the three months (*P* > 0.05). However, the TSP in May was higher than June and July (86.5 ± 2.8 versus 78.3 ± 2.2 and 74.9 ± 2.0 × 10^9 sperm per ejaculate, respectively, *P* < 0.01). Similarly, MOL was higher in May than June and July (82.5 ± 0.4% versus 81.5 ± 0.3% and 81.3 ± 0.3%, respectively, *P* < 0.05). Although no significant difference regarding VOL was detected between in May and June (*P* > 0.05), the VOL in May was higher than in July (226.8 ± 6.7 versus 207.3 ± 4.8 ml, respectively, *P* < 0.05). In addition, there was a significant interaction between breed and month in the

CONC (Table 3). The CONC of D boars in June (366.1 ± 16.5 ×10^6 sperm per ml) and July (371.9 ±17.1 ×10^6 sperm/ml) was lower than that in May (447.4 ± 18.2 ×10^6 sperm/ml) (P = 0.040). However, this seasonal effect was not detected in L (P > 0.05), Y (P > 0.05) and crossbred LY boars (P > 0.05) (Figure 3).

Table 4 Semen characteristics of boars in different months (least square means ± SEM)

Semen characteristics	N	Month			*P* value
		May	June	July	
Volume (ml)	711	226.8 ± 6.7^a	219.1 ± 5.2^{ab}	207.3 ± 4.8^b	0.041
Concentration (×10^6 sperm per ml)	711	408.3 ± 15.2	380.8 ± 11.6	387.1 ± 10.8	0.334
Total sperm per ejaculate (×10^9 sperm)	711	86.5 ± 2.8^a	78.3 ± 2.2^b	74.9 ± 2.0^b	0.004
Individual motility (%)	705	82.5 ± 0.4^a	81.5 ± 0.3^b	81.3 ± 0.3^b	0.036

^{a,b} Different superscripts in each row indicate significant differences

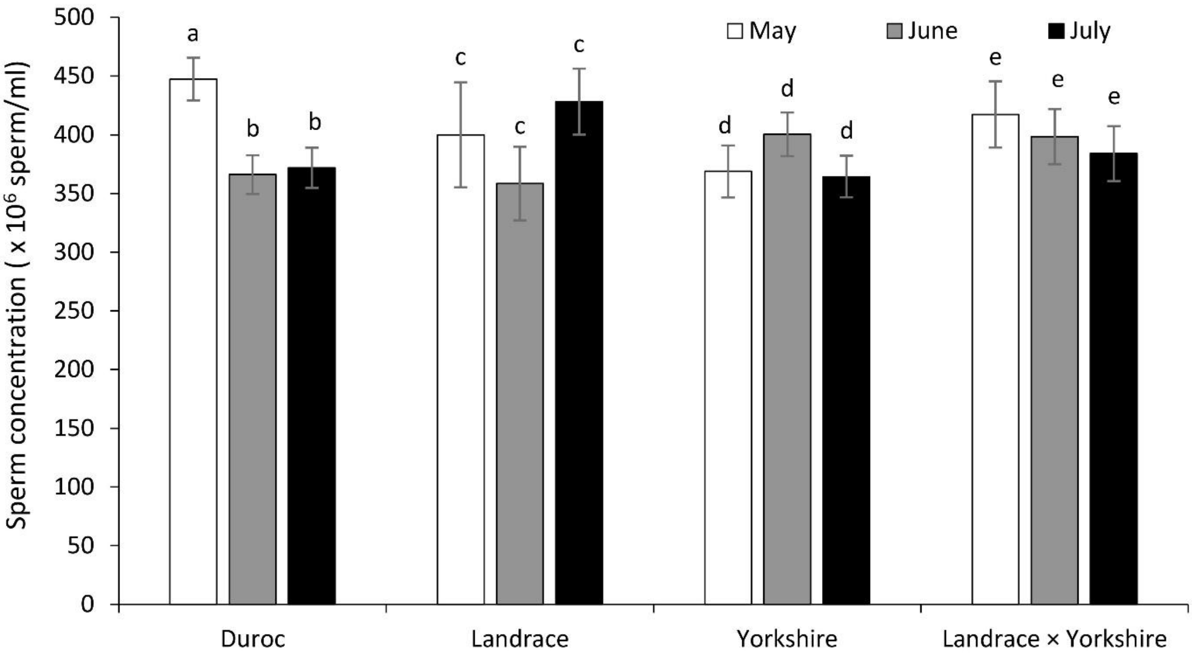

Figure 3 Sperm concentration (×10^6 sperm/ml) in boars in different months by breed. ^{a,b,c,d,e} Different letters in each breed indicate significant differences (P < 0.05)

Discussion

Breed difference: The present study indicates that sperm production differs among boar breeds in which Landrace, Yorkshire and LY crossbred boars showed a significantly higher sperm production than Duroc boars. This result was similar to the records of Tretipskul *et al*. (2012) who revealed that the total sperm per ejaculate of Duroc was lower than that of LY crossbred boars. According to Wolf and Smital (2009), there was a significantly lower semen volume of Duroc compared with other breeds. Similarly, in Korea, Yorkshire boars also had a higher semen volume than Duroc boars (Park and Yi, 2002). The lower semen production in purebred Duroc compared to the other boar breeds could be used as a good example to elaborate the relationship among genotype, phenotype and environment in which the heterosis of crossbred LY would demonstrate a performance superior to Duroc as well as better adaptation to the hot seasons under tropical climates seen in Yorkshire, Landrace and LY boars for semen production.

Looking at the relationship between boar breeds and seasonal effects, and based on the differences in sperm production among boar breeds, this study conformed to that of Rungruangsak *et al*. (2021), in that Yorkshire, Landrace and crossbred LY boars were more tolerant of seasonal effects than Duroc boars. In other words, Duroc boars were more sensitive to fluctuations of temperature and humidity than hybrid boars regarding volume, motility and the total number of sperm per ejaculation (Sonderman and Luebbe, 2008). This is related to differences in genetic lines of boars, which increases the discard rate of ejaculates during hot seasons in purebred Duroc more than that in crossbred lines (Sonderman and Luebbe, 2008). Based on these findings, more attention to the seasonal effect should be paid in Duroc boars than other breeds.

Seasonal variation: The difference in the geographical location, determined based on the equator, results in different characteristics in the climate of each country. Normally, countries in a tropical area such as Thailand will have three seasons (hot: March to June; rainy: July to October; and cool: November to February) while Polar climates with four seasons are found in northern Europe and the USA (Autumn: September to November; Winter: December to February; Spring:

March to May; and Summer: June to August). These differences in season result in seasonal effects on the reproductive performance of animals nourished in different areas (Suriyasomboon *et al*., 2004). In Thailand, the hot seasons, summer and rainy, categorised by more than 20 days with a temperature higher than 25 ℃, have negative effects on sperm output (Tretipskul *et al*., 2012). Peña *et al*. (2019) revealed that when the environment temperature reaches up to 29.0 ℃, it can cause some detrimental effects on the DNA integrity of boar sperm. As other evidence of the seasonal effect on boar semen production, Simital (2008) and Simital (2010) revealed that under climate characteristics and the latitude in the Czech Republic, among three breed groups, the semen volume and total sperm production had low values in the spring and summer but high values in the autumn and winter, in which the highest semen volume and total sperm production was found in November and December. This evidence may be associated with decreasing daylight in autumn and winter causing physiological alternatives and leading to an increase in sperm output (Ciereszko *et al*., 2000; Sancho *et al*., 2004). In the present study, sperm production considerably fluctuated among the three experimental months. Tretipskul *et al*. (2012) demonstrated that the total number of hot days (maximum temperature > 25 °C) in May, June and July accounted for 26–30 days per month. Moreover, the average relative humidity during these 3 months was 85%. This hot and humid climate can cause chronic heat stress in boars and subsequently lead to fluctuations in sperm production. Moreover, the present study was carried in Thailand from May to July, the experimental duration was classified as months of the hot season, but generally the sperm production in May was higher than that in June and July, registered when testing both the effect of individual factor-months and the interaction between Duroc breed and months. This could be explained by the significant variation in sperm production of boar breeds being dependent not only on the total hot days in a month but also on the season transition from summer to rainy season, especially in the Duroc breed.

Antioxidant supplementation: In the modern livestock industry, to tackle the harmful effects of hot seasons, besides the conventional housing system being gradually replaced by the evaporative cooling system, adequate feeding with high-quality nutrition is also a key solution (Quiniou *et al*., 2000; Suriyasomboon *et al*., 2004). Specifically, based on the mechanisms of the free radical scavenger chemical system, supplementary products containing antioxidants and essential fatty acid are used with a view to compensate for the reduction in polyunsaturated fatty acids in the sperm head and tail membranes and supporting the inadequate antioxidant defensive mechanisms of sperm and seminal plasma when exploiting boars under detrimental environment conditions (Surai *et al*., 2000; Yang *et al*., 2011). Kelso *et al*. (1997) stated that there may be a positive correlation in the relationship between the reduction in boar sperm motility and total sperm per ejaculation and the DHA proportion in the sperm phospholipid membranes. Vitamin E supplementation was used to prevent the potential decrease in the proportion of 22:6n-3 polyunsaturates in sperm lipids when lipid peroxidation occurs (Surai *et al*., 2000). The ascorbate and thiol groups have an important role related to protecting spermatozoa from oxidative assault. Their biological functions are as a key chain-breaking antioxidant combating free radicals such as superoxide radicals (O_2 -), hydroxyl radical (OH-) and hydrogen peroxide (H_2O_2) (Strzeżek *et al*., 1999).

The use of any supplementary product for improving semen quality in any animal must be in accordance with the duration of spermatogenesis to maximise the incorporation of supplementary ingredients into the sperm structure and seminal plasma. Calculating based on spermatogenic cycles, the entire spermatogenic process in mammals would be approximately 30–75 days (bull – 60.5 days, goat – 47.7 days and stallion – 54.9 days) (Russell *et al*., 1990; Sharpe, 1994). In boars, each spermatogenic cycle and the total duration of spermatogenesis are 8.6–9.0 and approximately 40 days, respectively (França *et al*., 2005). The sperm maturation through the epididymis transition would take about 10 days and this is similar in most mammals (Swierstra, 1968; França *et al*., 2005). The use of the organic antioxidant in the present experiment was prolonged for three months from 1st May until 30th July with the purpose of following this physiological mechanism. However, the dose of the organic antioxidant applied in the current study may not be sufficient to enhance sperm production in all breeds. Interestingly, a positive effect of the organic antioxidant treatment could be detected in Landrace and crossbred LY but not in Yorkshire and Duroc breeds. Thus, the breed difference in response to the organic antioxidant treatment should be determined.

In a previous study, Strzeżek *et al*. (2004) found that using a 250 g dietary supplementation containing 0.69 mg selenium, 250 mg vitamin E, 187.5 mg vitamin C, mixed with the standard daily diet continuously for 8 weeks changed parameters of boar semen favourably and the increased lipid peroxidation compensated for the high levels of antioxidants. Additionally, a reduction of sperm DNA damage during a peak wet summer was reported after 42 days by using 100 grams daily of boar diets supplemented with a custom-mixed antioxidant compound (Peña *et al*., 2019). Although the positive effects were not seen when testing the effects of individual factors of the organic antioxidant on the measured semen parameters in the present study, there were significant improvements registered for the semen volume of Landrace and sperm concentration of LY boars besides the opposite tendency detected in the sperm concentration of Landrace and semen volume of LY, respectively. However, unlike the total number of sperm per ejaculate, the differences in sperm concentration and semen volume would not be a strong signal to make a final conclusion when comparing semen production capacity among boar breeds due to a deviation related to semen collection, but somehow indicate a promising signal of true effects when increasing the antioxidant supplementation dose. Compared to the previous study (Strzeżek *et al*., 2004; Peña *et al*., 2019), the rationale related to boar spermatogenesis, as well as the different metabolism among breeds during the hot seasons under a tropical climate, it could be explained that the efficiency of using one supplement is not only based on the quality of the product, the period of use following spermatogenesis but also the amount of use as well as the differences in the physiological metabolism among boar breeds.

In conclusion, boar breeds, total hot days in a month and seasonal transition in Thailand could impact variably on boar semen production. When using any supplement to address problems of adverse environmental factors during hot days, it is vital to consider the quantity used and physiological metabolism of boar breeds. This study indicated that the semen production of Duroc boars was significantly lower than Landrace, Yorkshire and crossbred LY boars. The current dose of organic antioxidant supplementation cannot create clear benefits for boar semen production during the hot season. Linking between these findings and the facts regarding the use of sire lines in the swine industry and the tropical climate in Thailand, the Duroc boar is the first candidate used as a terminal sire line due to heterosis advantages to maximize breeding efficiency and pig livestock profitability. Other approaches, such as developing and using high-quality nutritional formulations strategically, improving management practices and Duroc breeding selection should be taken into consideration in order to address adverse environmental effects on Duroc boar semen production in tropical countries.

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