RETROSPECTIVE STUDY ON THE INFLUENCE OF SEASON AND TEMPERATURE HUMIDITY INDEX OF BULGARIAN MURRAH BUFFALO (*Bubalus bubalis*) SEMEN IN LOS BAÑOS, LAGUNA, PHILIPPINES

Kimberly I B. Turaja[1], Renato SA. Vega[1], Thelma A. Saludes[2] and Abraham G. Tandang[2]

ABSTRACT

The study determined the effects of season and temperature humidity index on the mass activity, semen volume, sperm motility and sperm concentration from eight (8) Bulgarian Murrah buffalo bulls raised at the Philippine Carabao Center at the University of the Philippines Los Baños (PCC at UPLB), Laguna from 2017-2018. The data were analyzed using a 2x2 factorial design in CRD, the first factor was the season (rainy and dry), while the second factor was stress condition (mild, 72–78 THI and moderate, 79–88 THI). Results revealed that the semen volume was significantly lower during the dry season and mass activity was significantly lower during 79-88 THI. The interaction between the season and THI did not significantly influence all semen parameters. In conclusion, the changing seasons and different groups of THI did not seem to affect the general semen parameters analyzed in this study, in terms of possible chances of impregnating female buffaloes on estrus. This indicates that the bulls tolerated the changes in the environmental condition of the prevailing area and the management of the bulls employed by the center was effective in helping them to adapt to hot and humid tropical environment.

Keywords: buffalo semen, heat stress, Bulgarian Murrah buffalo, season, temperature humidity index

INTRODUCTION

Heat stress is a condition in which processes are activated to sustain an animal's body thermal balance when subjected to uncomfortable high temperatures (Dash *et al*., 2016). This can be attributed to several environmental factors like temperature, relative humidity and solar radiation (Morell, 2020). The effect is exacerbated when heat stress is followed by high ambient humidity (Marai and Habeeb, 2010a) and with periods of extreme weather (Dash *et al*., 2016).

In countries with a tropical climate, heat stress is generally responsible for the decrease in animal productivity and showed unfavorable effects on the reproductive

[1]Institute of Animal Science (IAS), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), College, Laguna, Philippines 4031; [2]Philippine Carabao Center (PCC) at University of the Philippines Los Baños (UPLB), College, Laguna, Philippines 4031 (email: kbturaja@up.edu.ph).

performance of buffaloes (Sharma *et al*., 2018). It is one of the main factors for the deterioration of fertility of the animals (Ahmad and Tariq, 2010). When elevated temperatures and humidity are present in the environment, it is hard for the animals to dissipate heat resulting in heat stress (Dash *et al*., 2016). Moreover, the normal physiological responses of the animal to regulate body temperature may be overwhelmed when exposed to such environmental conditions (Morell, 2020).

Although buffaloes are well adapted to warm and humid conditions, their reproductive traits are affected by climate changes (Marai and Habeeb 2010b). It was observed that the productive and reproductive performances of buffaloes differ depending on their location (Dash *et al*., 2016). Additionally, the quality of sperm is affected by environmental factors such as temperature, humidity, atmospheric pressure, and day length. An increase in testicular temperature, either by subjecting the animal to elevated ambient temperature and increased body temperature, disrupts spermatogenesis resulting in the abnormality of the sperm leading to low semen quality (Mathevon *et al*., 1998).

Temperature humidity index (THI) is used to estimate the degree of heat stress in animals. The core body temperature of the animal increases when subjected to THI that is above the threshold and prolonged exposure of the animals above the threshold results in greater heat stress. Buffaloes are sensitive to heat stress when the THI level exceeded 75 (Dash *et al*., 2016). Vale (2007) mentioned that THI that is >75 has an adverse effect on the reproductive performances of buffaloes. Since the country is characterized by relatively high temperatures, high humidity and abundant rainfall (PAGASA, 2022), it is of great importance to ascertain the influence of season and THI on Bulgarian Murrah buffalo semen as this environmental condition might predispose the bulls to heat stress affecting their semen quality (mass activity, semen volume, sperm motility and sperm concentration). Hence, this study was conducted.

MATERIALS AND METHODS

A retrospective study was done using the semen production records of eight (8) apparently healthy Bulgarian Murrah buffalo bulls with ages ranging from 3-9 years old, maintained at the institutional herd of the Philippine Carabao Center at the University of the Philippines Los Baños (PCC at UPLB). The data on the semen parameters (mass activity, semen volume, sperm motility and sperm concentration) were extracted from the records of the herd from 2015-2019. Only the data from 2017-2018 were considered in the analysis because the semen production records of the bulls were complete and the method of analysis was consistent.

The bulls were individually confined in pens with enough ventilation and protection against heat during the summer and rainy seasons. Each bull was fed *ad libitum* with good quality forage, such as para grass, with 1-3 kilograms of concentrates and supplemented with mineral blocks.

Two (2) climatic conditions were considered namely: the rainy season (June - November) and the dry season (December-May). The seasons were based on the description set by the Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA, 2022).

The records on the daily dry bulb and wet bulb temperatures covering all seasons were taken from the Agro-meteorological station (AGROMET) of the College of

Engineering and Agro-industrial Technology (CEAT, UPLB) to compute the THI using the following formula: THI = (Tdb + Twb x 0.72 + 40.6) where Tdb and Txb = 0.72 and 40.6 constants by Thom (1959).

The THI groupings were divided into two groups (Group 1: 72-78; mild stress and Group 2: 79-88; moderate stress) since the animals were not exposed to <72 and >89 THI. The study of Dash *et al*. (2016) served as the basis for determining the THI groupings (Table 1) as it reflected the effects of different levels of THI on the performance of the buffaloes.

Semen collection was conducted twice a week, every Tuesday and Friday from 4:00 to 6:00 in the morning. Prior to semen collection, the bull donors were bathed and thoroughly cleaned to remove any dirt that would contaminate the samples. Bull semen was collected by trained bull handlers using an artificial vagina with hot water heated to 45°C. After collection, the ejaculates in the collecting tubes (15 ml capacity) were placed in a pre-heated water bath set at 28–32°C for semen evaluation using a phase contrast microscope (Nikon, Japan; magnification 100x). Each ejaculate was evaluated for its physical characteristics such as mass activity, semen volume, sperm motility and sperm concentration. The first and second ejaculates of bulls were analyzed in this study.

Semen volume was determined using a calibrated collection tube while the appearance of the semen was evaluated through visual inspection. Sperm motility was evaluated using a phase contrast microscope (Nikon®, magnification of 100x). It was estimated by placing a small drop of semen in a glass slide covered with cover slip on a microscope stage set at 36–39°C. Motility was graded using the percentage of motile sperm: 90–100% (excellent motility), 80–85% (very good motility), 70–75% (good motility), 60–65% (fair motility), 5–55% (unfit to use), and 0 (zero motility). Sperm concentration was determined by diluting 40 µl of semen in 3,960 µl saline solution (0.9%). Sperm absorbance was read by using a spectrophotometer IMV® (520 um). Semen was evaluated by a trained technician of the AI center. The mass activity was scored as follows: 1 – with slight movement, 2 – increasing progressive movement, 3 – no swirls formed, excellent progressive movement, 4 – have swirls, good movement and 5 – vigorous movement.

Data on the effects of season and THI on semen parameters were analyzed using 2x2 factorial in Completely Randomized Design (CRD) where factor A was the different seasons and factor B was the different groups of THI. All statistical analyses were conducted using

Table 1. Effects of different levels of THI on the performance of buffalo.

THI	Stress Level	Symptoms in buffalo
<72	None	Optimum productive and reproductive performance.
72 - 78	Mild	Elevation in rectal temperature and respiration rate.
79 - 88	Moderate	Respiration rate is significantly increased. Dry matter intake of buffalo is decreased and ratio of forage to concentrate intake is decreased.
89 - 98	Severe	Excessive panting and restlessness are observed. Rumination and urination are lowered along with a negative impact on reproductive performances in buffaloes.
>98	Danger	Heat stress is extreme and buffaloes may die.

the Statistical Analysis System (SAS version 9.0 software). Comparison of treatment means was analyzed using the Least Significant Difference (LSD) at 5% level of significance.

RESULTS AND DISCUSSION

This study was a retrospective analysis of a dataset collected over a period of 2 years (2017-2018) involving a total of 699 ejaculates from eight (8) Bulgarian Murrah buffalo bulls. These bulls were regularly used by the PCC-UPLB herd as semen donors for nationwide semen production intended for artificial insemination.

Table 2 summarizes the climatic condition to which the bulls were exposed during the period of the study (2017-2018). The data show that the bulls were subjected to the following environmental conditions: temperature (27.94±0.05), relative humidity (83.65±0.26) and THI (78.14±0.07) which fall within the range of the normal environmental condition observed by PAGASA (2022) except for the temperature that is a little higher than the average temperature reported in all weather stations in the country.

According to PAGASA (2022), the country experiences relatively high temperatures, high humidity and abundant rainfall. The high relative humidity is due to high temperatures and surrounding bodies of water. All weather stations recorded a mean annual temperature of 26.60°C excluding Baguio. The coolest months were observed in January (mean=25.5°C) while the warmest months fall in May (mean=28.3°C). Moreover, the average monthly relative humidity varies between 71% in March and 85% in September.

On the other hand, heat stress begins when THI is >72 and animals get seriously affected when it increases to >78. Furthermore, THI which is >82 severely affects the production and reproduction of animals (Hoque *et al*., 2018). Based on these THI values, we conclude that the bulls in the present study were subjected to heat stress having been exposed to higher THI values (78.14±0.07) for the entire period of the study.

The *P*-values of the main effects of season and THI and its interaction effect on the buffalo semen parameters are presented in Table 3. The results of the study reveal that semen volume, sperm motility and sperm concentration were not influenced (*P*>0.05) by the different groups of THI.

There were no interactive effects (*P*>0.05) between season and THI on all semen parameters. However, the main effect of season was significantly (*P*<0.05) observed in mass activity and semen volume. Whereas, the main effect of THI was noted in mass activity.

The effects of season and THI on mass activity are presented in Figure 1. The mass activity in different seasons varied from 2.92±0.02 to 2.78±0.03 with an overall mean of 2.86±0.02. The results indicate that mass activity was significantly influenced (*P*<0.05) by the season. The highest mass activity was recorded during the rainy season (2.92±0.02) while the lowest mass activity was obtained in the dry season (2.78±0.03). This coincides

Table 2. Summary of the climatic condition covering the period of analysis (2017-2018).

Climatic Condition	Rainy	Dry	Overall
Temperature, °C	28.19±0.04	27.59±0.09	27.94±0.05
Relative humidity, %	84.73±0.31	82.11±0.42	83.65±0.26
THI	78.69±0.06	77.36±0.15	78.14±0.07

with the study of Baruti *et al*. (2019) on swamp buffaloes. However, the authors found the highest mass activity during the winter season (3.52) and the lowest was observed in the summer season (3.43). Javed *et al*. (2000) reported similar results but obtained the highest mass activity in autumn (2.97±0.12) and spring (2.87±0.13). The significant decrease in the movement of sperms during the dry season may be attributed to higher temperatures (rainy-28.19±0.04; dry-27.59±0.09) experienced by the bulls during the period of analysis. Comparable results were also obtained by Sarder (2007). He reported that the highest mass activity was found at 25-29°C and with >85% relative humidity. In contrast, Bhakat *et al*. (2014, 2015) indicated that season did not affect (P>0.05) mass activity of Karan bulls and Murrah buffalo semen respectively. Das *et al*. (2016) reported that although mass activity varied during different seasons it was not (P<0.05) significantly affected by season.

There was limited literature on the effect of THI on mass activity. However, the results of the present study clearly show that mass activity was significantly affected (P<0.05) by the different groups of THI. This means that when the bulls are exposed to higher THI values a significant decrease in mass activity can be observed. Dash *et al*. (2016) noted that when the animals are exposed to THI ranging from 79-88, they are subjected to moderate stress exhibiting an increase in respiration rates and their dry matter intake is also decreased.

On the other hand, the mass activity score of the semen should be equally considered important as this is used as a reference in the initial screening of semen prior to semen processing. Additionally, this is very important because without active movement of the

Table 3. *P*-values of the main effects of season and THI and its interaction effect on the buffalo semen parameters.

Semen Parameters	***P*-values**		
	Season[1]	**THI[2]**	**Interaction (THI x Season)**
Mass activity	**0.0028**	**0.0217**	0.8720
Semen volume, ml	**0.0144**	0.4775	0.3213
Sperm motility, %	0.2510	0.5329	0.3366
Sperm concentration, M/ml	0.3471	0.8020	0.2657

[1]Seasons: Rainy (June-November); Dry (December-May)
[2]THI groups: (Group 1: 72-78; Group 2: 79-88)

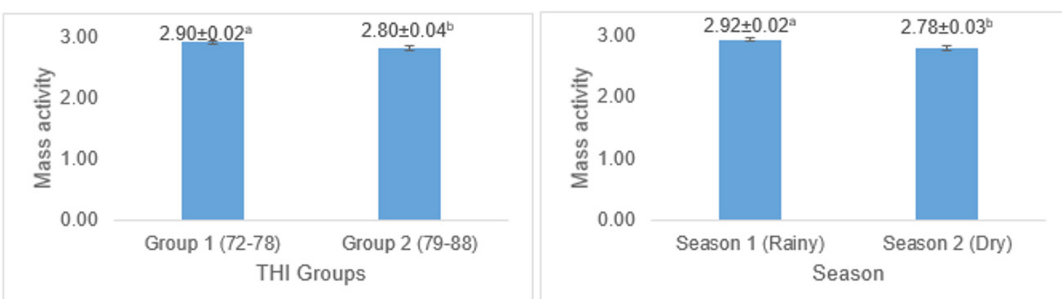

Figure 1. Mass activity of Bulgarian Murrah buffalo bulls in different seasons and THI groups (2017-2018).

[ab]Means with different superscript are significantly different using the Least Significant Difference (LSD) (P<0.05).

sperm it cannot penetrate the ovum and accomplish fertilization (Capitan and Palad, 1999).

The effects of season and THI on semen volume are presented in Figure 2. The season significantly (P<0.05) influenced the semen volume. The overall mean semen volume of Bulgarian Murrah buffalo bulls was found to be at 4.87±0.09 in the rainy season and 5.26 ± 0.14 in the dry season. It can be noted that a higher semen volume was produced during the dry season while the lowest was noted during the rainy season (5.26 vs 4.87). This is in agreement with the study of (Javed *et al*., 2000; Das *et al*., 2016). However, Das *et al*. (2016) obtained the highest semen volume during the monsoon season while Javed *et al*. (2000) recorded the highest semen volume in autumn (5.80±0.81).

Although there were several factors that might affect the semen volume (e.g age, breed), the higher values on the relative humidity (84.73±0.31) and ambient temperature (28.19±0.04) recorded during the year might contribute to the differences in the semen volume. Biniova *et al*. (2017) mentioned that daily average ambient temperatures and daily average humidity have significant effects on semen characteristics. This was supported by Hameed *et al*. (2017) who obtained a decrease in the ejaculatory volume of bulls following an increase in ambient temperature. Furthermore, the volume of semen obtained through an artificial vagina may vary due to breed, age, season and frequency of ejaculation (Mamuad *et al*., 2005).

The season of the year, vigor of the thrust during collection, and disturbance at the time of ejaculation influence the volume of semen (Capitan and Palad, 1999). Also, the bulls in the present study might have adjusted or did not tolerate the changing season of the year as evidenced by lowered semen volume during the rainy season. This is in contrast with the results reported by Koonjaenak *et al*. (2007). Although their study obtained the highest semen volume (3.8±0.2) during summer, semen volume did not differ between seasons. Similar findings were also reported by Hameed *et al*. (2017) in Nili Ravi buffaloes, Tiwari *et al*. (2011), Bhakat *et al*. (2015) and Isnaini *et al*. (2019) in Murrah buffaloes. Sarder (2007) observed that semen volume was not significantly (P>0.05) affected by ambient temperature. He found out that the highest semen volume was recorded at >29°C while the lowest was at 25-29°C. Conflicting results on the effect of season on semen volume might be due to the number of observations and the length of the study period (Javed *et al*., 2000).

There were no significant findings on the effect of THI on semen volume. This is in agreement with the study of Sharma *et al*. (2018). In the present study, the highest semen volume (5.11±0.14) was recorded in Group 2 with THI values ranging from 79-88. However, Sharma *et al*. (2018) recorded the highest semen volume with a lower THI value

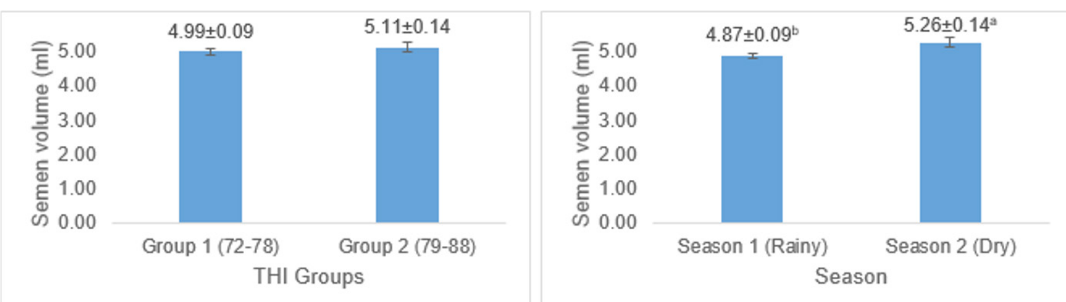

Figure 2. Semen volume (ml) of Bulgarian Murrah buffalo bulls in different seasons and THI groups (2017-2018).

[ab]Means with different superscript are significantly different using the Least Significant Difference (LSD) (P<0.05).

ranging from 64-74. The lowest semen volume was obtained in THI value ranging from 78-84. It is interesting to note that although the buffaloes used in the study were exposed to different groups of THI (72-78; 79-88) which may subject them to mild and moderate stress, the results indicate that THI alone did not (P>0.05) affect the semen volume. This might be due to the management system employed in the herd which includes improved housing facilities and showering the bulls, especially at the warmest time of the day in order for the animals to dissipate heat and adapt to the environment especially when the weather is unfavorable. It is important that males give generous amounts of semen so that many inseminations can be done or many doses can be produced if they are to be extended. However, large volume cannot be translated into high sperm concentration and the relation between the amount of ejaculate and fertility is not established (Capitan and Palad, 1999).

The effects of season and THI on sperm motility are presented in Figure 3. The overall mean of sperm motility of Bulgarian Murrah buffalo bulls was found to be 58.25±0.01 during the rainy season and 56.40±0.01 in the dry season.

The highest percentage of sperm motility was observed during the rainy season. Similar results were also obtained by Koonjaenak *et al*. (2007) and Isnaini *et al*. (2019). Although there were varying values on the relative humidity and ambient temperature in both seasons, the present study shows that that season did not (P>0.05) influence the sperm motility of the bulls. This conforms to the studies of Das *et al*. (2016) and Javed *et al*. (2000) wherein they obtained higher sperm motility in different seasons. They further concluded that sperm motility of buffalo bull semen did not (P>0.05) differ significantly between seasons. Similarly, Hameed *et al*. (2017) mentioned that season did not influence the sperm motility of Nili Ravi buffalo bulls in Pakistan. The lowest individual sperm motility was observed during the winter season and the highest was recorded during the spring season. However, the results are in contrast with the findings of (Mishra *et al*., 2012; Bhakat *et al*., 2014, 2015). Bhakat *et al*. (2014) obtained the highest sperm motility during winter and the lowest in the summer season.

Sperm motility was also not (P>0.05) affected by the different groups of THI. The non-significant effect of season and THI on sperm motility implies that sperm motility could be affected by other factors such as handling and management (Javed *et al*., 2000). Longer exposure of semen to room temperature prior to evaluation may decrease the percentage of sperm motility. This is not in accordance with the previous report of Sharma *et al*. (2018) wherein the highest progressive motility was found at the THI value of 72-78 while the lowest was recorded at the THI value of 78.84. Their results indicate that there was a significant

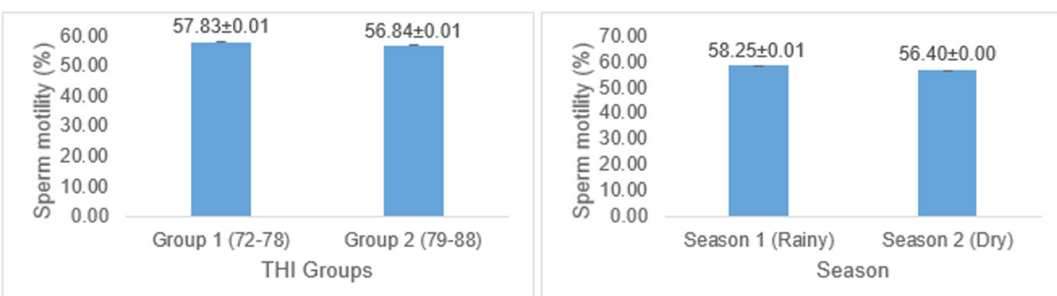

Figure 3. Sperm motility (%) of Bulgarian Murrah buffalo bulls in different seasons and THI groups (2017-2018).

decrease in progressive motility when the THI value increases to 78-84. The non-significant effects of season and THI in sperm motility are due to the immediate submission of collected semen to the laboratory thus preventing the motility of the sperm to decrease in quality caused by longer exposure to room temperature. Like other semen parameters, initial motility is very important because this is one of the best indicators of viability and fertility of semen, although this is not necessarily a sign of normal fertilizing capabilities of the sperm (Capitan and Palad, 1999).

The effects of season and THI on sperm concentration are presented in Figure 4. The number of spermatozoa per unit volume of semen will be used as an important consideration in determining the optimum fertility of the bull (Mamuad *et al*., 2005). The overall mean sperm concentration of Bulgarian Murrah buffalo bulls was found to be 192±0.01 during the rainy season and 202.06±0.02 in the dry season.

It can be noted that a higher sperm concentration was recorded during the dry season. However, results show that season did not (P>0.05) influence the sperm concentration of buffalo semen conforming to the previous results in swamp buffalo bulls in Thailand (Koonjaenak *et al*., 2006, 2007). However, Koonjaenak *et al*. (2007) obtained the highest value in the rainy and winter season. It is more likely that sperm concentration is maintained across seasons, indicating that seasonal changes do not particularly affect testicular production during the year (Koonjaenak *et al*., 2006). Das *et al*. (2016) also mentioned that season did not influence (P>0.05) the sperm concentration in swamp buffaloes in India. This is inconsistent with the findings of (Javed *et al*., 2000; Bhakat *et al*., 2014, 2015; Hameed *et al*., 2017; Isnaini *et al*., 2019). Hameed *et al*. (2017) mentioned that sperm concentration was influenced by season with significantly (P<0.05) higher sperm concentration in autumn followed by spring, dry summer, humid summer and winter. Similar results were obtained by Bhakat *et al*. (2015) but recorded the highest sperm concentration during winter followed by rainy and summer seasons. His findings revealed that a lower concentration of spermatozoa during summer may be due to the significant reduction in feed intake and an increase in dead and abnormal spermatozoa. Isnaini *et al*. (2019) explained that the reduction of sperm concentration in the dry season was possibly associated with the oxidative damage caused by heat stress.

Sperm concentration was not significantly affected (P>0.05) by the different levels of THI although there was a higher sperm concentration observed in Group 2 (197.90±0.01). This is in contrast with the results reported by Sharma *et al*. (2018). He mentioned that sperm concentration varied significantly among 3 groups of THI namely; Group 1<72; Group 2 (72-78) and Group 3 (78-84). A significant decrease in sperm concentration was found at the

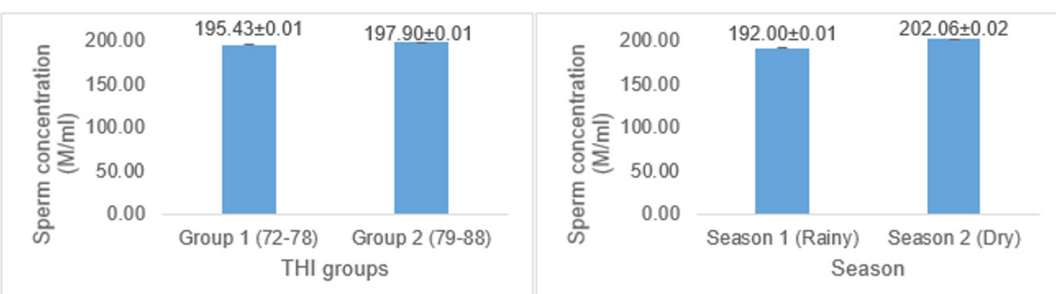

Figure 4. Sperm concentration of Bulgarian Murrah buffalo bulls in different seasons and THI groups (2017-2018).

THI value of 78-84. Additionally, hot-dry or summer seasons where THI values are higher (THI 78-84) affect the various biophysical characteristics of semen in buffalo bulls. In the present study, the bulls were exposed to higher THI values for a long period, however, the sperm concentration was not affected (P>0.05). This means that the buffalo bulls can withstand the environmental condition with higher THI values prevailing in the area. Also, the sperm concentration varies widely, both in the ejaculates of some males and from animal to animal of the same species. Although the relationship between concentration and fertility is not necessarily high, the animals with low sperm count are questionable for their fertility (Capitan and Palad, 1999).

In conclusion, mass activity and semen volume were influenced by the season while mass activity was significantly affected by different groups of THI. The changing seasons and different groups of THI did not seem to affect most of the semen parameters analyzed in this study. This indicates that the bulls tolerated the changes in the environmental condition in the area. Additionally, the management systems employed by the herd such as improved housing facilities, and provision of showers to the bulls during the warmest time of the day were effective in alleviating heat stress and helping the animals adapt to the warm environment.

ACKNOWLEDGEMENT

The authors would like to thank the bull handlers at the institutional herd of the University of the Philippines at the Philippine Carabao Center who facilitated the regular semen collection and the laboratory technicians who analyzed and recorded the data on semen production.

REFERENCES

Ahmad S and Tariq. 2010. Heat stress management in water buffaloes. *Rev Vet* 21:301-313.

Baruti M, Deka BC, Tamuly S, Bhuyan M, Borah P, Das GC, Deka N, Deka R, Biswas RK and Sinha S. 2019. Seasonal effect on seminal constituents of swamp buffalo (*Bubalus carabanensis*) in the foot hill of Eastern Himalaya. *Biol Rhythm Res* 52:603-614.

Bhakat M, Mohanty TK, Gupta AK and Abdullah M. 2014. Effect of season on semen quality of crossbred (Karan Fries) bulls. *Adv Anim Vet Sci* 2:632-637.

Bhakat M, Mohanty TK, Gupta AK, Prasad S, Chakravarty AK and Khan HM. 2015. Effect of season on semen quality parameters in Murrah buffalo bulls. *Buffalo Bulletin* 34:100-112.

Biniova Z, Duchacek K, Stadnik L, Dolezalova M and Hajek R. 2017. Effects of climatic conditions on bovine semen characteristics. *Acta Univ Agric Mendelianae Bruns* 65:17-23.

Capitan SS and Palad OA. 1999. *Manual for artificial breeding of farm animals*. Philippines: College of Agriculture Publications Program. University of the Philippines Los Baños College, Laguna, Philippines.

Das GC, Das PK, Deori S, Mazumdar H, Bhattacharyya BN and Phookan A. 2016. Seasonal variation in the characteristics of the swamp buffalo semen of Northeast India. *Buffalo Bulletin* 36:215-219.

Dash S, Chakravarty AK, Singh A, Updahyay A, Sing M and Yousuf S. 2016. Effect of heat stress on reproductive performances of dairy cattle and buffaloes: A review. *Vet World* 9:235-244.

Hameed S, Masood S, Zaned H, Khan MS, Younis M, Rehman H, Avais M and Khan MUR. 2017. Semen characteristics as influenced by seasonal and climatic variations in Nili-Ravi buffalo breeding bulls. *J Anim Plant Sci* 27:1750-1757.

Hoque MR, Rana MS, Nayan SB, Miraz MFH, Deb GK, Nahar TN, Habib R and Siddiki MSR. 2018. Influence of multiple showering on quality of buffalo semen during hot-humid season. *J Adv Vet Anim Res* 5:12-18.

Isnaini N, Harsi T and Maharani D. 2019. Seasonal effect on semen characteristics of Murrah buffalo bulls raised under tropical climate. *J Kedokt Hewan* 13:73-75.

Javed MT, Khan A and Kausar R. 2000. Effect of age and season on some semen parameters on Nili-Ravi buffalo (*Bubalus bubalis*) bulls. *Vet Arhiv* 70:83-94.

Koonjaenak S. 2006. Semen and Sperm Characteristics of Swamp Buffalo (*Bubalus bubalis*) Bulls for Artificial Insemination in Thailand, in Relation to Season. *Ph.D. Dissertation*. Swedish University Agricultural Sciences.

Koonjaenak S, Chanatinart V, Aiumlamai S, Pinyopumimintr T and Martinez HR. 2007. Seasonal variation in semen quality of swamp buffalo bulls (*Bubalus bubalis*) in Thailand. *Asian J Androl* 9:92-101.

Mamuad FV, Venturina HV, Venturina EV, Morcoso RT, Atabay EC and Kazuhiro K. 2005. *Artificial insemination manual for water buffaloes*. Water Buffaloes and Beef Cattle Improvement Project (WBBCIP).

Marai IFM and Habeeb AAM. 2010a. Buffalo's biological functions as affected by heat stress. A review. *Livest Sci* 127:89-109.

Marai IFM and Habeeb AAM. 2010b. Buffaloe's reproductive and productive traits as affected by heat stress. *Trop Subtrop Agroecosystems* 12:193-217.

Mathevon M, Buhr MM and Dekkers JCM. 1998. Environmental, management, and genetic factors affecting semen production in Holstein bulls. *J Dairy Sci* 81:3321-3330.

Mishra GK, Tiwari R, Rehman SU, Rathore KS, Singh RB, Saxena SK and Siddiqui MU. 2012. Effect of seasons on semen production performance of Jersey bulls. *Indian J Dairy Sci* 65:497-500.

Morell J. 2020. Heat stress and bull fertility. *Theriogenology* 153:62-67.

Philippine Atmospheric, Geophysical and Astronomical Services Administration [PAGASA]. 2022. *Climate of the Philippines*. Retrieved on 2 September 2022 from https://www.pagasa.dost.gov.ph/information/climate.

Sarder JU. 2007. Environment related variations in the semen characteristics of bulls used for artificial insemination (AI) programme in Bangladesh. *Univ J Zool* 26:81-88.

Sharma M, Bhat Y, Sharma N, and Singh A. 2018. Comparative study of seasonal variation in semen characteristics of buffalo bull. *J Entomol Zool Stud* 6:947-951.

Thom EC. 1959. The Discomfort Index. *Weatherwise* 12:56-61.

Tiwari R, Mishra GK, Shukla M, Singh RB, Saxena SK and Siddiqui U. 2011. Seasonal variations in semen production of Murrah buffalo bulls. *Indian J Anim Reprod* 32:5-7.

Vale WG. 2007. Effects of environment on buffalo reproduction. *Ital J Anim Sci* 6:130-142.