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Spermatozoa quality of Landrace Boar in Beltsville thawing solution diluent with various levels of moringa leaf extract

Kualitas spermatozoa Babi Landrace dalam pengencer Beltsville thawing solution dengan berbagai level sari daun kelor

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ABSTRACT

This study aims to find the best level of sun-dried Moringa leaf extract (SDMLE) in Beltsville Thawing Solution (BTS) diluent to preserve the spermatozoa of Landrace boars. Semen from a two-year-old Landrace boar was collected using the massage method. Semen with spermatozoa motility ≥70% and spermatozoa abnormalities <20% was diluted with BTS (P0, control), BTS + 3% SDMLE (P1), BTS + 6% SDMLE (P2), BTS + 9% SDMLE (P3), and BTS + 12% SDMLE (P4). Storage of liquid semen using a cool box with a temperature of 15-20^{o}C, while evaluating the quality of semen every 8 hours for spermatozoa motility, viability, abnormality, and longevity. The results showed that spermatozoa stored for 40 hours in BTS + 3% SDMLE (P1) diluent had higher quality ($P<0.05$) than the other four diluents, namely motility (54.00%), viability (61.16%), abnormality (3.62%), and longevity (45.77%). The results of this study concluded that 3% SDMLE added to BTS diluent was the best level for the preservation of spermatozoa in landrace boars.

Kata kunci:
Beltsville thawing solution
Babi landrace
Sari daun kelor
Spermatozoa

ABSTRAK

Penelitian ini bertujuan untuk menemukan level terbaik sari daun kelor kering matahari (SDKM) dalam pengencer Beltsville Thawing Solution (BTS) untuk preservasi spermatozoa babi Landrace. Materi penelitian berupa semen dari satu ekor babi jantan landrace berumur dua tahun yang ditampung dengan menggunakan metode masase. Semen dengan motilitas spermatozoa ≥ 70% dan abnormalitas spermatozoa < 20% diencerkan dengan BTS (P0, kontrol), BTS+ SDKM 3% (P1), BTS+ SDKM 6% (P2), BTS+ SDKM 9% (P3), BTS+ SDKM 12% (P4). Semen yang telah diencerkan disimpan dalam coolbox pada suhu 15-20^{o}C dan dievaluasi setiap 8 jam terhadap motilitas, viabilitas, abnormalitas dan daya tahan hidup spermatozoa. Hasil penelitian menunjukkan bahwa spermatozoa yang di simpan selama 40 jam dalam pengencer BTS + SDKM 3% (P1) memiliki kualitas yang lebih tinggi (P<0,05) daripada keempat pengencer lainnya, yakni dengan motilitas 54,00%, viabilitas 61,16%, abnormalitas 3,62% dan daya tahan hidup 45,87% jam. Hasil penelitian ini menyimpulkan bahwa SDKM 3% yang ditambahkan ke dalam pengencer BTS merupakan level terbaik untuk preservasi spermatozoa babi landrace.

INTRODUCTION

The quality of spermatozoa is one of the factors determining the success of artificial insemination in pigs. To keep it up for a comparatively long time, spermatozoa must be preserved using a diluent that includes a variety of nutrients that spermatozoa need (Hine et al., 2014). The diluent must contain nutrients as a source of energy for the movement of spermatozoa, not be toxic, be a buffer that can maintain the pH of the diluent according to the needs of spermatozoa, and protect spermatozoa from cold stress (Parera, 2022). Semen diluents

must also be able to prevent the growth of germs and maintain an appropriate electrolyte balance and osmotic pressure (Sari et al., 2015; Bebas et al., 2016).

Beltsville Thawing Solution (BTS) is a commonly used diluent for diluting boar spermatozoa. The diluent can prevent spermatozoa from prematurely capitulating and can maintain swine spermatozoa's motility while stored at cold temperatures (Bebas et al., 2016). The composition of BTS is composed of glucose, sodium citrate, sodium bicarbonate, potassium chloride, penicillin, distilled water, and ethylene diamine tetraacetic acid (EDTA) (Bebas et al., 2016; Lodu et al., 2021). The disadvantage of BTS diluents is that do not contain antioxidant compounds that function to counteract free radicals that are very dangerous for the life of spermatozoa. Free radicals are defined as compounds or molecules that have unpaired electrons (Kamal & Aris, 2021). Excessive production of free radicals during sperm preservation will cause damage to plasma membranes, proteins, and spermatozoa DNA (Candrawati et al., 2014).

Moringa leaves are ingredients that contain various antioxidants (Rizkayanti et al., 2017; Satriyani, 2021). Moringa leaves contain protein, carotene, vitamin C, minerals, amino acids, flavonoids, and phenolic compounds (Suprihartini et al., 2021; Wicaksono & Kalsum, 2023). The addition of vitamin C and flavonoids to the diluent can protect the plasma membrane and increase the viability and motility of spermatozoa during preservation (Fafo et al., 2016; Saryono et al., 2015).

Fafo et al. (2016) were able to preserve the quality of spermatozoa of landrace boars spermatozoa in citrate-egg yolk diluent by adding extracts from Moringa leaves that were dried in an air-conditioned room. This method of drying moringa leaves is impractical and relatively expensive; therefore, this study was designed using sun-dried moringa leaf extract (SDMLE) which is more practical and less costly, but data on the use of SDMLE for preservation of boar spermatozoa is still very limited. The addition of SDMLE to BTS diluent is expected to produce better quality landrace boar spermatozoa. The study aimed to find the best level of SDMLE in BTS diluent for preservation of landrace boar spermatozoa.

MATERIALS AND METHODS

Research Materials

The study material was semen collected from one landrace boar about two years old. The boar was kept in individual pens and given 3 kg of concentrate feed daily, and drinking water was given ad libitum.

Research Methods

The study used a completely randomized design method consisting of five treatments and five replicates. The treatments were P0 (BTS), P1 (BTS + 3% SDMLE), P2 (BTS + 6% SDMLE), P3 (BTS + 9% SDMLE), and P4 (BTS + 12% SDMLE).

Preparation of BTS Diluent

BTS is in the form of a powder composed of a number of components such as glucose, EDTA, sodium citrate, sodium bicarbonate, potassium chloride, penicillin, and streptomycin. 50 grams of BTS should be weighed and dissolved in 1,000 mL of aquabides, then add penicillin 1,000 IU and streptomycin 1,000 mg/mL (Seran et al., 2021). The mixture was placed in an erlenmeyer, and stirred (3.2 rpm, 15 min). The homogenized diluent solution was filled in the erlenmeyer tube and stored in the refrigerator.

Preparation of SDMLE

The SDMLE preparation adopted the method used by Fafo et al. (2016) with slight odifications. Moringa leaves are obtained from dark green moringa leaves, the leaves are separated from the stems, twigs, and dry leaves; then washed thoroughly and dried in the sun for one to two days, while turning until dry. Moringa leaves was blended and sieved to produce flour. Ten grams of moringga flour was weighed and mixed with 60 mL of aquabidest. Stir the mixture using a magnetic stirrer (4.2 rpm, 15 min). Centrifugation of the mixture was carried out for 10 minutes at 3,000 rpm. The supernatant was separated from the sediment, and ready to be used as SDMLE. SDMLE was added to the BTS diluent at 0, 3, 6, 9, and 12% (v/v), based on the SDMLE levels in the five treatments.

Fresh Semen Collection and Evaluation

The collection process and evaluation of fresh semen quality followed the method used by Tamoes et al. (2014). Semen collection was carried out twice per week using the mash

method. The collected semen was then evaluated for macroscopic and microscopic quality.

Macroscopic evaluation includes color, volume, pH, and consistency of semen. Macroscopic evaluation is done visually without using a microscope. Microscopic evaluation was performed using a microscope at 400x magnification, which included: motility, viability, abnormality, and spermatozoa concentration.

The following procedure was used to assess the motility of spermatozoa: fresh semen droplets were placed on an object glass, and the glass was subsequently covered; observed under a microscope at 400x magnification. Motility was calculated subjectively by comparing the number of progressively motile spermatozoa with the total spermatozoa in one microscopic field of view (Feka et al., 2016).

Viability of spermatozoa was evaluated using the eosin-negrosin staining method, with a sequence of procedures: mix one drop of semen with eosin-nigrosin dye on an object glass; after mixing evenly, make a review preparation on a new object glass and heated over a bunsen flame, then observed under a microscope with a magnification of 400x. Live spermatozoa do not absorb dye, while dead ones are purplish red. Viability of spermatozoa was calculated by the formula = (Number of live spermatozoa/Total spermatozoa counted) x 100% (Fafo et al., 2016).

Spermatozoa abnormalities were evaluated in the same way as for viability measurements, but what is calculated is the percentage of abnormal spermatozoa. The formula for calculating spermatozoa abnormality is = (Abnormal spermatozoa count/Total spermatozoa counted) x 100% (Feka et al., 2016).

Spermatozoa concentration was calculated with a hematocytometer (Tamoes et al., 2014). Semen was sucked into the hematocytometer pipette until the 0.5 mark, followed by sucking 3% sodium chloride (NaCl) until the 101 mark. Mix the two liquids until homogeneous and drop one drop on the hematocytometer counting chamber, cover with a cover glass and observe under a microscope with 400x magnification. Count the number of spermatozoa in five counting chambers (four corner chambers and one center chamber. The concentration of spermatozoa per mL was calculated by the formula: Number of spermatozoa counted in five chambers x 10^7.

Semen Dilution and Preservation

Fresh semen that had spermatozoa motility ≥70% and spermatozoa abnormality <20% was diluted with BTS diluent added with SDMLE at various levels according to the treatment. The ratio of semen to diluent was 1:2. Eight sealed 1.5 mL Eppendorf vials, each containing 0.5 mL of diluted semen from each treatment were placed in a cool box and kept at 15-20°C. Evaluation of sperm quality was carried out every eight hours until the percentage of motility decreased to 40% (Hine et al., 2014).

Research Variables

The variables of this study were: spermatozoa motility (%), spermatozoa viability (%), spermatozoa abnormality (%), and spermatozoa longevity (hours). Techniques for measuring motility, viability, and abnormality were the same as those used to evaluate fresh semen, while sperm longevity was calculated based on the length of time spermatozoa remained in storage until their motility decreased to 40%.

Data Analysis

The research data were analyzed using analysis of variance, and continued with Duncan's test if the treatments differed noticeably. Data analysis was carried out with the help of SPSS 26.0 for windows software (Lawa et al., 2021).

RESULTS AND DISCUSSION

Spermatozoa Motility and Viability

The motility and viability of spermatozoa play an important role in determining semen quality during preservation or in vitro storage. This is related to the ability of spermatozoa to fertilize the egg because only spermatozoa that are alive and have progressive active motility are capable fertilize the egg. The discussion of the effect of treatment on these two variables is limited to the point where the percentage of spermatozoa motility decreases to 40 percent. Spermatozoa motility and viability are shown in Tables 1 and 2.

The results from the observations presented in Tables 1 and 2 demonstrate a decline in both the motility and viability of spermatozoa across all treatments as the storage time increases. The decrease in motility is caused by the decrease in energy sources found in the diluent. This can also negatively impact the number of live spermatozoa

Table 1. Motility of landrace boar spermatozoa (%) in BTS diluent supplemented with various levels of SDMLE

Hour	Treatment					
	P0	P1	P2	P3	P4	P-Value
0	84.00± 2.24^{a}	84.00± 2.24^{a}	84.00± 2.24^{a}	84.00± 2.24^{a}	84.00± 2.24^{a}	1.000
8	80.00± 0.00^{b}	84.00± 2.24^{a}	79.00± 2.24^{b}	76.00± 2.24^{c}	76.00± 2.23^{c}	0.000
16	76.00± 2.24^{a}	76.00± 2.24^{a}	73.00± 2.74^{ab}	71.00± 2.24^{b}	70.00± 3.54^{b}	0.004
24	71.00± 2.24^{a}	73.00± 2.74^{a}	65.00± 5.00^{b}	64.00± 4.18^{b}	63.00± 4.47^{b}	0.001
32	62.00± 4.47^{b}	66.00± 5.47^{a}	58.00± 5.70^{bc}	55.00± 7.07^{bc}	53.00± 5.70^{c}	0.013
40	48.00± 4.47^{b}	54.00± 2.24^{a}	44.00± 2.24^{c}	41.00± 2.24^{c}	37.00± 2.73^{d}	0.000
48	33.00± 2.73^{b}	35.00± 0.00^{a}	30.00± 3.53^{b}	26.00± 2.24^{c}	22.00± 2.74^{d}	0.000

Superscripts with different letters on the same line indicate significant differences (P < 0.05). SDMLE = sun dried moringa leaf juice, BTS = Beltsville Thawing Solution, P0= BTS, P1= BTS+SDMLE 3%, P2= BTS+SDMLE 6%, P3= BTS+SDMLE 9%, P4= BTS+SDMLE 12%.

Table 2. Viability of landrace boar spermatozoa (%) in BTS diluent supplemented with various levels of SDMLE

Hour	Treatment					
	P0	P1	P2	P3	P4	P-Value
0	88.63± 0.99^{a}	89.56± 0.51^{a}	87.85± 1.54^{ab}	87.44± 0.55^{ab}	85.91± 2.78^{b}	0.015
8	83.93± 1.09^{b}	86.91± 0.65^{a}	83.08± 2.26^{b}	80.76± 1.75^{c}	79.62± 1.56^{c}	0.000
16	78.92± 2.38^{ab}	80.54± 2.14^{a}	77.24± 2.12^{abc}	75.69± 2.56^{bc}	74.79± 3.08^{c}	0.010
24	74.62± 2.02^{b}	77.61± 1.39^{a}	73.61± 1.92^{bc}	72.36± 2.29^{bc}	71.45± 1.88^{c}	0.001
32	71.23± 1.39^{ab}	75.04± 1.91^{a}	67.36± 2.35^{bc}	64.52± 3.80^{cd}	62.79± 4.42^{d}	0.000
40	56.48± 1.60^{b}	61.16± 4.42^{a}	53.93± 1.22^{bc}	51.56± 1.39^{c}	46.13± 3.78^{d}	0.000
48	42.41± 0.82^{b}	47.76± 2.12^{a}	41.58± 1.23^{b}	38.34± 1.28^{c}	33.77± 1.37^{d}	0.000

Superscripts with different letters on the same line indicate significant differences (P < 0.05). SDMLE = sun dried moringa leaf juice, BTS = Beltsville Thawing Solution, P0= BTS, P1= BTS+SDMLE 3%, P2= BTS+SDMLE 6%, P3= BTS+SDMLE 9%, P4= BTS+SDMLE 12%.

and their ability to move progressively.

The percentage of spermatozoa motility at the 0th hour of storage for all treatments was the same (P>0.05). The decline in motility began to occur at the 8th hour of observation with the highest decline values seen in diluents P4 and P3, followed by treatment P2, P0, and the lowest in treatment P1. The different rates of decline had an impact on the difference in motility observed at the 40th hour of preservation, where the motility of spermatozoa in treatment P1 was significantly higher (P<0.05) than the other four treatments. Similar results were obtained for the variable spermatozoa viability, with the P1 treatment having higher spermatozoa vitality than the other four treatments (P<0.05).

The highest motility and viability of spermatozoa were produced by treatment P1 and the lowest by treatment P4. The high motility and viability of spermatozoa in treatment P1 indicates the positive effect contributed by 3% of SDMLE in BTS diluent. A number of compounds contained in SDMLE, especially antioxidants, play a role in protecting spermatozoa from the negative effects caused by free radicals produced in excess during in vitro storage (Djahilape & Suprijono, 2016; Satriyani, 2021). A number of researchers claim, moringa leaves contain various antioxidants, a molecule whose role is to prevent the oxidation of other molecules, while the oxidation process is a chemical reaction process that produces free radicals, which in turn causes a chain reaction that damages cells. Some of the antioxidants found in moringa include vitamin C, phenols and flavonoids (Wibawa et al., 2020; Kurang et al., 2020; Apriyati et al., 2022; Safnowandi, 2022).

Vitamin C is a potent antioxidant that has the ability to donate one hydrogen atom and form a relatively stable ascorbyl-free radical. Vitamin C can neutralize free radicals and reduce oxidative stress. Vitamin C can protect lipids and proteins in cell membranes from oxidative

damage (Pehlivan, 2017). In addition to vitamin C, phenolic compounds found in moringa leaves also act as antioxidants (Kurang et al., 2020; Rudiana & Indriatmoko, 2021; Toripah, 2014). At low concentrations, phenolics act as antioxidants and protect cells against oxidative stress. Phenolic compounds act as free radical terminators by inhibiting the oxidation process. Phenolic compounds include primary antioxidants that neutralize free radicals, and inhibit lipid oxidation, especially those found in cell membranes (Shahidi & Ambigaipalan, 2015; Zeb, 2020). Moringa leaves also contain flavonoid compounds (Apriyati et al., 2022; Julianawati et al., 2019; Susanty et al., 2019; Warnis et al., 2020). which can prevent cell damage from free radicals and reduce oxidative stress (Shen et al., 2022; Williamson et al., 2020).

On the other hand, increasing the SDMLE level from 6% to 12% caused a more drastic decrease in sperm motility and viability compared to the control treatment and 3% of SDMLE (PM<0.05). This may be related to the increase in vitamin C content as the level of SDMLE in BTS diluent increased. Increased levels of vitamin C can cause a decrease in pH so that the diluent solution becomes acidic and causes a decrease in spermatozoa motility (Fafo et al., 2016; Gena et al., 2021; Parera & Lenda, 2023). According to Bebas et al. (2015), 0.3 mg/mL of vitamin C added to a pig semen diluent reduced sperm motility compared to 0.1 mg/mL. In addition, increased levels of SDMLE in BTS diluent can lead to increased osmotic pressure. High osmotic pressure of the diluent (hypertonic) can disrupt the intra- and extracellular water balance which further impacts on the suppression of spermatozoa motility. In addition, Moringa leaves also contain saponins and tannins (Putra et al., 2016), which have a negative impact on the life of spermatozoa if present in high levels.

Spermatozoa Abnormality

Spermatozoa abnormality is one of the important variables that must be evaluated in a semen sample. This relates to the fertility of the spermatozoa in fertilizing the egg. A semen sample containing spermatozoa with an abnormality percentage higher than 20 percent results in low fertilization and pregnancy rates. Thus, only semen samples that have a spermatozoa abnormality rate below 20% are suitable for artificial insemination (Feugang et al., 2019). Spermatozoa abnormality rates are shown in Table 3.

The data in Table 3 shows that the lowest spermatozoa abnormality was found in the P1 treatment (P<0.05) which was observed from the 0th hour to the 40th hour of storage. This indicates that the addition of 3% SDMLE to the BTS diluent can suppress the rate of increase in spermatozoa abnormality. On the other hand, higher levels of SDMLE (6-12%) caused a higher increase in abnormality. The mechanism of the increase in abnormality at higher levels of SDMLE has yet to be explained. Observations under the microscope showed that spermatozoa abnormalities such as broken tails, heads without tails, and a small number of others showed *cytoplasmic droplets* on the tail. According to Fafo et al. (2016), moringa leaves contain antinutritional substances such

Table 3. Abnormality of landrace boar spermatozoa (%) in BTS Diluent supplemented with various levels of SDMLE

Hour	Treatment					
	P0	P1	P2	P3	P4	P-Value
0	1.92± 0.47^{bc}	1.29± 0.16^{a}	1.75± 0.26^{b}	2.08± 0.28^{bc}	2.36± 0.39^{c}	0.001
8	2.39± 0.31^{bc}	1.85± 0.34^{a}	2.14± 0.29^{ab}	2.44± 0.28^{bc}	2.73± 0.24^{c}	0.000
16	2.85± 0.18^{b}	2.56± 0.09^{a}	2.73± 0.14^{ab}	2.85± 1.12^{b}	3.27± 0.17^{c}	0.000
24	3.14± 0.15^{b}	2.82± 0.26^{a}	3.12± 0.07^{b}	3.36± 0.09^{c}	3.81± 0.08^{d}	0.000
32	3.57± 0.00^{b}	3.13± 0.15^{a}	3.82± 0.05^{c}	3.88± 0.13^{c}	4.28± 0.22^{d}	0.000
40	3.93± 0.06^{b}	3.62± 0.74^{a}	4.27± 0.08^{c}	4.38± 0.07^{cd}	4.42± 0.18^{d}	0.000
48	4.10± 0.08^{b}	3.90± 0.03^{a}	4.70± 0.10^{cd}	4.60± 0.07^{c}	4.78± 0.12^{d}	0.000

Superscripts with different letters on the same line indicate significant differences (P < 0.05). SDMLE = sun dried moringa leaf juice, BTS = Beltsville Thawing Solution, P0= BTS, P1= BTS+SDMLE 3%, P2= BTS+SDMLE 6%, P3= BTS+SDMLE 9%, P4= BTS+SDMLE 12%.

saponins and tannins that, in high concentrations, might be harmful to spermatozoa.

The spermatozoa abnormalities observed were classified as secondary abnormalities characterized by broken tails and heads without tails. Such abnormalities may also be caused by improper treatment during the preparation of preparations for spermatozoa evaluation. The presence of *cytoplasmic droplets* indicates that the spermatozoa are immature (Parrish et al., 2017; Schulze & Waberski, 2022).

In general, the level of abnormality of landrace boar spermatozoa in this study is low because it is still far below the maximum percentage of abnormality that is suitable for artificial insemination, which is 20%.

Spermatozoa Longevity

Spermatozoa longevity is very important to evaluate for each semen sample that is preserved. The longer the spermatozoa survive, the higher the usability of a semen sample and the more efficient its utilization for artificial insemination purposes. A semen sample is still considered suitable for artificial insemination if it has a spermatozoa motility of at least 40%. Thus, the calculation of spermatozoa survival in this study is only based on the percentage of spermatozoa motility decreasing to 40% (Hine et al., 2014). Spermatozoa survival is shown in Figure 1.

The data in Figure 1 shows that the addition of SDMLE significantly affects the longevity of spermatozoa (P<0.05). The addition of SDMLE up to 3% in BTS diluent resulted in higher spermatozoa longevity (P<0.05) than the other four treatments. This illustrates that the compounds contained in SDMLE 3%, especially antioxidants, can support the life and motility of spermatozoa by protecting spermatozoa against adverse effects that can arise during *in vitro* storage.

During in vitro storage, spermatozoa are subjected to cold stress as they are preserved at temperatures much lower than their storage temperature in the epididymis. Compared to ruminant spermatozoa, boar spermatozoa are more sensitive to temperature depletion due to the low cholesterol content that makes up the plasma membrane of spermatozoa, and have phospholipids with a high ratio of unsaturated fatty acids: saturated fatty acids (Yeste, 2018). The high longevity of spermatozoa in the P1 treatment may also be related to the high protein content in Moringa leaves which plays a role in protecting cell membranes against the effects of cold during storage. However, at high concentrations of SDMLE, the survival rate is lower, which may be caused by an increase in osmotic pressure and a decrease in pH. In a hypertonic medium, intracellular water will come out of the cell causing the cells to wrinkle, while a low pH can cause sperm death due to acidic conditions (Mughal et al., 2018; Zhou et al., 2015).

Moringa leaves also contain various antioxidants that can protect spermatozoa from the negative effects of free radicals (Amidi et

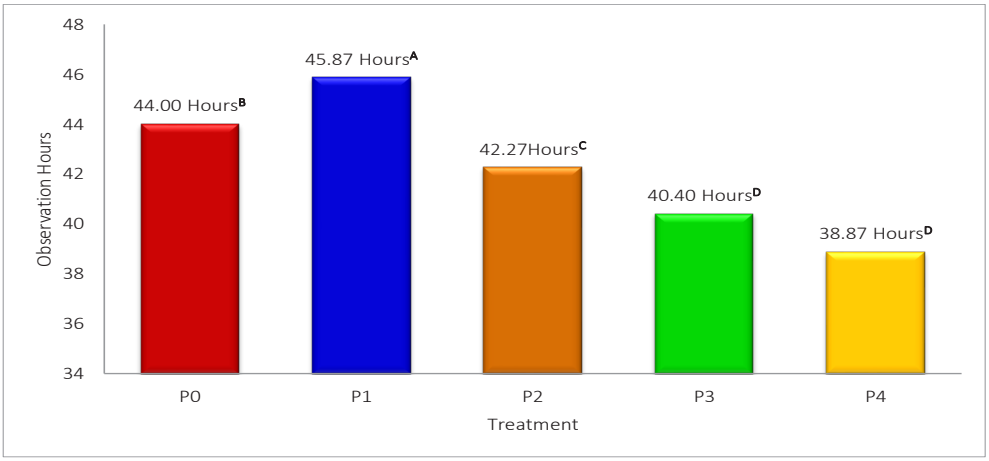

Figure 1. Spermatozoa survival (hours) in BTS diluent added with various levels of SDMLE. Superscripts with different letters in the same row indicate significant differences (P<0.05). SDMLE = sun dried moringa leaf juice, BTS = Beltsville Thawing Solution, P0= BTS, P1= BTS+SDMLE 3%, P2= BTS+SDMLE 6%, P3= BTS+SDMLE 9%, P4= BTS+SDMLE 12%.

al., 2016). A number of anti-oxidants contained in moringa leaves such as vitamin C, phenols and flavonoids play a role in protecting sperm against free radicals. In general, free radicals can come from endogenous sources (mitochondria, peroxisomes, endoplasmic reticulum, phagocytic cells, etc.) and exogenous sources (solvents, heavy metals, and radiation). More than 90 percent of free radicals in eukaryotic cells are generated by mitochondria (Phaniendra et al., 2015). Free radicals can affect various classes of biologically important molecules such as nucleic acids, lipids, and proteins, thereby altering the normal redox status and leading to increased oxidative stress (Phaniendra et al., 2015).

Higher SDMLE levels in BTS diluent caused a decrease in the longevity of spermatozoa. This may be due to the presence of too high vitamin C content in the diluent. Tahir et al. (2016) found that, each gram of Moringa leaves contains 3.31 mg of vitamin C. At higher concentrations, vitamin C acts as a pro-oxidant inducing oxidative stress, either by generating *reactive oxygen species* or by inhibiting the antioxidant system in the presence of iron, which in turn induces lipid peroxidation Chakraborthy et al. (2014). Whether vitamin C has a pro-oxidant or antioxidant effect depends on the concentration gradient and redox state of the cell (Chakraborthy et al., 2014). Similarly, phenols can be pro-oxidant and cytotoxic under certain conditions. The prooxidant activity of phytophenols is manifested under conditions that favor their autooxidation, for example in the presence of oxygen or transition metal ions, and alkaline pH. Many plant phenols are unstable in liquid extracts where they undergo rapid autooxidation (Bayliak et al., 2016).

The low longevity at high SDMLE levels may be due to an increase in osmotic pressure beyond what spermatozoa can tolerate. In general, osmotic pressure varies between spermatozoa diluents, ranging from 240 to 380 mOsm-Kg^{-1}. Porcine spermatozoa can tolerate osmolality ranging from 250 to 390 mOsm-kg^{-1} or can be slightly above the isotonic osmotic pressure of 300 mOsm-kg^{-1} (Yeste, 2018). The results of this study confirmed the positive effect of SDMLE as a supplement ingredient in boar spermatozoa diluent, with the best level of SDMLE in BTS diluent being 3%.

CONCLUSIONS

The results of this study concluded that the best level of SDMLE in BTS diluent for preservation of landrace boar spermatozoa was 3%. The limitation of this study is that it did not assess the spermatozoa membrane's integrity, therefore it is unable to determine the impact of SDMLE on this variable.

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