Sperm Cell Livability and Motility from Boar Semen Extended with Watermelon and (*Citrulluslanatus*)-Egg Yolk Extract

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Abstract. The study was conducted at the University Swine Project and in the Nutrition Laboratory in Cluster Building of College of Agriculture, Central Mindanao University, Bukidnon, Philippines. This study aimed to characterize macroscopic qualities of the extracted boar semen; assess the sperm cell morphology, abnormality and number live and dead sperm cell in the extracted boar's semen; evaluate the initial sperm motility of the extended boar semen with watermelon extract; and determine the longevity of the sperm cells in the extended boar semen with watermelon extract. Semen Extender sustains life of sperm cells even outside of the reproductive tract of the boar. Natural products such as watermelon may prolong the livability of sperm cells considering its lycopene content which is an anti-oxidant. The study was conducted to evaluate the semen volume, color, pH, sperm cell motility and longevity in different treatments (No extender, Commercial Extender and Watermelon extract egg yolk). These were arranged in a Completely Randomize Design (CRD) and replicated five times. Post hoc test was through the Tukey's HSD Test after the analysis of variance for a complete randomized design. The semen macroscopic and microscope characteristics such as volume, color, pH, sperm cell abnormalities, motility and livabilitywere observed and recorded. Results showed 200 ml semen which is milky white in color, pH 8 and an excellent initial motility of 84%. Common sperm cell abnormality was enlarged midpiece (23.08%). Semen without extender has sperm cells alive for only four hours, watermelon extract- yolk extender for 9 hours while the commercial extender allowed sperm cells to live as long as 19 hours after the addition of the extenders. Commercial extenders are better for long-term preservation while watermelon extract- yolk extenders are for short-term only.

Keywords: Semen Extender, Watermelon, Sperm Livability and Motility

Introduction

Pigs are a very popular source of food all through antiquity, from West Asia all the way west to England and east to China, from Scandinavia to North Africa. Pigs care for themselves pretty independently (Carr, 2016). Swine production in the Philippines is a P191-billion industry and is the largest among the livestock and poultry industries of the country and it ranks next to rice with 18.28% contribution to the total value of agricultural production (Livestock Research Division, 2016). The most common problem of swine production is rapidly increasing demand of pork which could be answered through increased pork production .The lack of information about artificial insemination may be a factor. Adequate production of pork is necessary to meet the requirement of our increasing population.

Watermelons have reputed roots in Africa, with the first recorded harvest in Egypt somewhere around 5,000 years ago often oblong and light green in color, they can also be round, spotted, or striped with white bands running from end to end. It has antioxidants, flavonoids, and lycopene content (Mercola, 2017). The bioactive and naturally occurring compounds in watermelon called phytonutrients provide incredible health benefits to the body (Rozeboom et al., 2000). It is also a good dietary source of both citrulline and lycopene, two

very powerful plant compounds (Bjarnadottir, 2015). The bioactive and naturally occurring compounds in watermelon called phytonutrients provide incredible health benefits to the body. In fact, many of these phytonutrients can be found in other fruits and vegetables, as well. Watermelon contains beta-carotene, lycopene, and citrulline which are the potent phytonutrient discovered lately, Citrulline is a kind of phytonutrient that has the ability to relax the blood vessels in the entire body, including those blood vessels found in the penis (Rozeboom et al., 2000).

Table 1. Nutrient Composition of Watermelon

Nutrition Facts of Watermelon	
Amount per 100g	% Daily Value
Calories 30	
Total Fat	0.2% 0%
Saturated Fat	0g 0%
Polyunsaturated Fat	0.1g 0%
Monounsaturated Fat	0g 0%
Cholesterol 0mg	0%
Sodium 1mg	0%
Potassium 112mg	3%
Total Carbohydrates	8g 2%
Dietary Fiber 0.4g	1%
Sugar 6g	0%
Protein 0.6g	1%
Vitamin A	11%
Calcium	0%
Vitamin D	0%
Vitamin B	0%
Vitamin C	13%
Vitamin B6	0%
Magnesium	2%

Source: Bjarnadottir (2015)

The boar semen is a suspension of sperm cells and secretion from the boar reproductive tract (Knox, 2012). Semen quality is a measure of its ability of semen to accomplish fertilization. It is the sperm in the semen that are of importance, and therefore semen quality involves both sperm quantity and quality. Semen extenders contain protective ingredients that permit survival of sperm outside the reproductive tract (Brinsko et al., 2011). Semen extenders purported to maintain sperm viability for up to 10 days are being marketed (Althouse et al., 2010).

According to Blanch et al. (2014), egg yolk LDLs have been proposed to be used in extenders with cryoprotective effects on frozen-thawed bull spermatozoa. The addition of egg yolk LDL has been shown to produce better results (higher sperm motility and better movement characteristics) than the addition of egg yolk. The cryoprotective effect of egg yolk LDLs seems to be related to the ability of the lipoproteins to bind the binder of sperm proteins contained in the seminal plasma of many mammalian ungulates. These proteins modify the sperm membrane by removing cholesterol and phospholipids, which adversely affect the ability of sperm to be preserved. The binding between these proteins and LDL is rapid, specific, and stable even after freeze-thawing of semen.

In this study, the potential effect of watermelon extract in boar semen as an extender was explored.

Methodology

Research Design and Treatments

The collected semen was divided into three equal parts and assigned to the different treatments following the Completely Randomized Design. Five replications were generated per treatment. The replications were the five motility readings taken from each test tube with or without extended semen. The following treatments were used: Treatment 1- Boar's semen without extender, Treatment 2- Boar's semen with commercial extender, Treatment 3- Boar's semen with the yolk- watermelon extract.

The study was conducted to evaluate the semen volume, color, pH, sperm cell motility and longevity in different treatments (No extender, Commercial Extender and Watermelon extract egg yolk).

Research Environment

The study was conducted at the University Swine Project and in the Nutrition Laboratory in Cluster Building of College of Agriculture, Central Mindanao University-Bukidnon, Philippines.

Facilities and Equipment

Semen from a trained boar was collected using a dummy. Materials that were used during the conduct of the study were the following glass or plastic bottle, distilled water, cloth, gloves, haemacytometer, hand tally counter, stirring rod, weighing scale, tissue paper, pentel pen, record notes, ball pen, MS Dilution, eosin negrosin, watermelon extract, medicine dropper, electric microscope, surgical gloves, sterilized vials, test tube, graduated cylinder, beaker, cover slip, semen straw, glass slides and water bath.

Animal and Management

The boar that was used in this study is a matured one that can serve 8- 10 females. The boar is housed in a pen that has a concrete cement floor raised above the ground level. The necessary arrangement for feeding and 11 watering for a sufficient access of fresh air were provided. The boar was fed twice daily with a diet consisting of rations. The general management program including disease prevention in the project was followed. The boar has already been trained to mount on the artificial wooden dummy sow for easy semen collection.

Water Melon and Extender Preparation

One mature ripe watermelon fruit were used in the study. The watermelon extract was separated from the watermelon meat by squeezing the meat using a clean cheese cloth for extraction. The collected watermelon extract was dispensed into a clean sterilized beaker, egg yolk and watermelon extract. The watermelon-yolk extender was poured into clean sterilized beakers. The beakers were kept at 37°C through a water bath. For the commercial extender, the powder form of MS is poured into a beaker containing 1000 ml of distilled water and thoroughly mixed using a stirring rod. This beaker was also placed in the water bath with 30-35°C.

Semen Collection

The collection of boar semen was made through the artificial vagina assembly with optimum pressure and temperature of 37 degree Celsius into a graduated collection tube. The

boar was allowed to mount on the artificial wood dummy sow. After mounting was complete (with seeking movement of the penis), the boar made vigorous upward and forward thrust to the artificial vagina assembly which signify the occurrence of ejaculation. The tube containing fresh collected semen was immediately transferred to the ice box with an appropriate temperature level. The semen was not exposed to any 12 unfavourable condition during or after collection. This activity was done at 7:00 Am.

Table 2. Evaluation of Semen Motility

Evaluation Procedure	Equipment Needed
1a. Visual and olfactory assessment of ejaculation	None
1b. Determine semen volume and sperm concentration	Balance and a hemacytometer
2. Motility	
a. Prepare a 1:10 dilution of semen extender	Small Water Bath
b. Gently rotate the semen	
c. Remove a small sample (5 to 10 ml) and place in a clean glass test tube	
d. If, necessary, warm it to 30 to 37 degrees centrigrade (body temperature)	
e. Place a small drop on a pre-warm slide and gently place a cover slip over the drop.	Slide Warmer
f. Immediately examine the sample at 100x and then at 400x	Self-illuminating microscope capable of 100x, 400x, magnification and glass slides with coverslip.
g. Estimate the percentage of sperm in field that are progressively	
h. Examine several fields and establish an average	
i. Record your estimate to the nearest 5 or 10 percentage units.	Small, disposable plastic pipette
3. Morphology	
a. After the motility estimate is complete, allow the slide to cool. Motility will show or stop and individual sperm cell can be observed.	Self-illuminating microscope capable of 100x and 400x and 1000x (oil) magnification: glass slide and immersion oil.
b. Switch to the 400x objective and observe individuals cell in several fields	
c. Estimate in several fields the percentage of cell that are normal	
4. Acrosome integrity	Self-illuminating phase contrast microscope capable of 100x, 400x, 1000x (oil) magnification

Source: Althouse (2000)

Addition of the Extender to the Collection Semen

Upon arrival in the Nutrition Laboratory, the collected semen in a beaker was placed into the water bath along with the prepared extenders. The water bath was maintained at 30˚C-35˚C. The collected semen was divided into three and place in beakers. The two extenders (watermelon, yolk and commercial) were then mixed in the beaker with semen. The mixing of

the extenders to the semen as well as the different prepared extenders were all placed in the water bath with 30-35˚C to prevent cold shock in the sperm cells.

Motility Evaluation of Extended Semen

Mayayoshi (1968) constructed the criteria for determining motility. With these, characterizing the treatments would be easier. The motility readings in this study were referred to this criteria. Excellent motility- 80% or more spermatozoa are in vigorous motion swirls and "Eddies" caused by movements of the sperm are extremely rapid constant changing. Very good motility- Approximately 70-80 % of the spermatozoa are in vigorous motion. Waves and "Eddies" form a drop rapidly but not s in excellent motility. Good motility- about 50-70% of the spermatozoa is in motion. Motions are vigorous but waves and "Eddies" form slowly across the field. Fair motility- more than 30% of the sperm are in motion. The movement is fairly vigorous. Poor motility- less than 20% of the sperm are in motion. The motion is mostly weak, oscillatory and not progressive. In the semen motility assessment, a drop of semen was taken from the beaker and placed in a glass slide and covered with a cover slip. This was then mounted in the stage of an electric microscope and evaluated for motility with the subjective motility reading based on Mayayashi's criteria. Five replications per treatment were used to check the hourly motility of the three treatments. These motility readings were recorded to serve as the five replications per treatment. If 0% motility is observed in a certain treatment, the evaluation was immediately ended. The liveability of sperm cells for a particular extender treatment was then marked and recorded.

Data Gathered:

1. Date of semen collection
2. Semen quality parameter
    a. Breed
    b. Age of Boar
    c. Color
    d. Initial Motility (%)
    e. pH
    f. volume
3. Sperm cell abnormalities
4. Motility of sperm cells in extenders
5. Livability –the duration of which the extender could support sperm cells motility.

Results and Discussion

Table 3. Physical and Morphological Characteristics of Boar and its Semen

Parameter	
Color	Milky White
Semen volume	200 ml
Total Volume of Commercial Extender	1000 ml
Total Volume of Watermelon Extract with egg yolk	240ml
Breed	Large White
Age	1 year and 2 mons

Macroscopic Quality of the Collected Semen

Table 3 shows the boar from which the semen was with an age of 1 and 2 months. The volume of semen was 200ml with a color. The total volume of commercial extender (MS) that

was used is 1000 ml and the volume of watermelon extract yolk is 240 ml. extender to semen was mixed.

Table 4.Observed Abnormalities in the Sperm Cells of the Boar

Type of Abnormality	Number	Percentage (%)
Head		
Shrunken	1	7.69
Double headed	2	15.39
Enlarged	1	7.69
Midpiece		
Enlarged	3	23.08
Bent	2	15.39
Broken	1	7.69
Tail		
Broken	1	7.69
Long	2	15.39
Total	**13**	**100**

Sperm Cell Abnormalities

Table 4 shows the different abnormalities of sperm cells in the collected semen. Using the electric microscope enlarged midpiece has the highest number and percentage (23.08%) abnormality observed while by the double head (15.39%), bent midpiece (15.39%), long tail (15.39%), shrunken head (7.69%), Enlarged head (7.60%), broken midpiece (7.69%) and lastly the broken tail (7.69%). Sperm cell abnormalities should consider the head 16 shape, tail formation and cytoplasmic droplets, the last of which should not exceed 15% (Rozeboom et al., 2000).

Table 5. Motility (%) of Sperm Cell from the Boar's Semen Extendedwith Watermelon-Yolk Extender (1st reading)

	Replication					
Treatment	1	2	3	4	5	Mean
1	70	70	70	70	67	69.80a
2	83	83	83	83	83	82.40c
3	80	78	80	80	80	79.60b

Note: CV= 1.57% **= highly significant Means with no common letter are significantly different (Tukeys HSD 0.5)

Sperm Cell Motility of Extended Boar's Semen

Table 5 shows the initial motility of the collected semen was 85% which is described as excellent (Akhter et al., 2008). Table 3 shows a highest percentage of motility in Treatment 2 (Commercial Extender) with 82.40% compared to the 17 Treatment 3 (Watermelon Extract with yolk) with 79.60% while the semen without extender (Treatment 1) has the least motility. These difference are highly significant ($p<.01$).

Brinskoet al. (2011) mentioned that extenders permit the sperm to survive outside the reproductive tract and this observation is well supported by many authors as well as the result of this study. Egg yolk is a usual component of many extenders (Moussa et al., 2002). Although a common component in liquid extenders but it is not found in commercial extenders (Forouzanfaret al., 2010). With extenders in semen for freezing or cryopreservation, however; egg yolk is a commonly used. Tris-citric acid yolk extenders (TCAYE) gave the best motility

result in Jermasia and Jermana bucks (Nor-Ashikin& Abdullah, 2011). Egg yolk is a very good protectant for sperm cells against cold shock brought about by differences in temperature during processing (Forouzanfaret al., 2010).

Table 6. Motility (%) of Sperm Cell from the Boar's Semen Extended with Watermelon-Yolk Extender (2nd reading)

Replication						
Treatment	1	2	3	4	5	Mean
1	30	33	30	28	30	30.20[a]
2	82	83	82	80	80	81.60[c]
3	70	70	70	65	65	68.00[b]

Note: CV= 3.47% **= highly significant Means with no common letter are significantly different (Tukeys HSD 0.5)

Second Reading Motility

Table 6 presents the 2nd reading of the study where the commercial extender still had the highest motility rate of 81.60% while the watermelon extract with egg yolk h (68% motility) while a rapid reduction of motility rate in Treatment 1 was readily observed. The observed difference are highly significant (p<.01).

Watermelon contains (among others) lycopene which is a phytonutrient that has an anti-oxidant property (Wiener, 2012). This may explain why sperm cell in Treatment 3 (watermelon extract-yolk extender) has a better motility (68%) than that of Treatment 1 (semen with no extender) as the latter has been thoroughly tested before being commercialized. Kijpoonchaoen et al. (2017) mentioned that the lycopene supplementation in cryopreserved boar semen improved post thawing progressive motility.

Table 7. Motility (%) of Sperm Cell from the Boar's Semen Extended with Watermelon-Yolk Extender (3rd reading)

Replication						
Treatment	1	2	3	4	5	Mean
1	0	0	0	0	0	0.00[a]
2	83	80	80	80	80	80.60[c]
3	70	67	65	65	65	66.40[b]

Note: CV= 3.47% **= highly significant Means with no common letter are significantly different (Tukeys HSD 0.5).

Third Reading Motility

Table 7 shows the highly significant difference (p<.01)3rd reading for the motility where in Treatment 1 sperm cell in boar semen with no extender registered 0% motility already. Those in Treatment 2 and 3 are still having 80.60% and 66.40%. Extenders capacity to extend semen quality is gauged in the sperm cells motility. Karageorgiouet al. (2016) concluded that extenders could short term and long term and that on day 1 to 2 after the addition of the extenders, viability was reduce but the long term extender has better motility.

Table 8. Motility (%) of Sperm Cell from the Boar's Semen Extended with Watermelon-Yolk Extender (4th reading)

Replication						
Treatment	1	2	3	4	5	Mean
1	0	0	0	0	0	0
2	80	78	78	77	77	78
3	60	57	60	55	60	58.4

Note: CV=3.997 % **= highly significant Means with no common letter are significantly different (Tukeys HSD 0.5)

Fourth Reading Motility

Table 6 shows highly significant differences in specifically between the commercial extender (Treatment 2) with 78% while the watermelon extract with egg yolk (Treatment 3) has only 58.45 and that with no extender has 0%. For Treatments 2 and 3, the sperm cell motility is described as good motility. This result gives one idea that boar sperm cells without any extender has only a maximum longevity of five hours under 30-34oC storage (Casas et al., 2015).

Table 9. Motility (%) of Sperm Cell from the Boar's Semen Extended with Watermelon-Yolk Extender (5th reading)

Replication						
Treatment	1	2	3	4	5	Mean
1	0	0	0	0	0	0
2	78	76	77	75	75	76
3	40	35	35	35	35	36

Note: CV=4.77% **= highly significant Means with no common letter are significantly different (Tukeys HSD 0.5)

The 5th reading for the motility is in Table 7 where Treatment 2 has 76% motility (very good motility) while Treatment 3 has only 36% (fair motility). These differences are highly significant (P<.01). Egg yolk is not applicable in the majority of commercial operations (Forouzanfaret al., 2010) for boar's semen considering that in this level, the use of commercial extenders is more utilized.

Table 9.Motility (%) of Sperm Cell from the Boar's Semen Extended with Watermelon-Yolk Extender (5th reading)

Replication						
Treatment	1	2	3	4	5	Mean
1	0	0	0	0	0	0
2	77	75	75	75	73	75
3	20	25	25	25	25	21

Note: CV=4.77% **= highly significant Means with no common letter are significantly different (Tukeys HSD 0.5)

Fifth Reading Motility

Table 9 shows that Treatment 2 has still a very god motility of 75% while Treatment 3 has already a poor motility of 21%. These difference is highly significant (P<.01). Egg yolk reacts with glycoprotein from the bulbourethral secretion that has a triacylglycerol hydrolase

activity which decreases sperm motility by disruption of the cell membrane (Lustyková, 2012), resulting the sperm cells death. Such could be the case in Treatment 3 in this study.

Table 10. Motility (%) of Sperm Cell from the Boar's Semen Extended with Watermelon-Yolk Extender (6th reading)

Replication						
Treatment	1	2	3	4	5	Mean
1	0	0	0	0	0	0
2	75	73	73	73	73	73.4
3	5	0	0	0	0	0

Note: CV= 5.68% **= highly significant Means with no common letter are significantly different (Tukeys HSD 0.5)

Sixth Reading Motility

The 6th reading for the sperm cells shows Treatment 2 still having very good motility with 75% motility with Treatment 3 having only one replication reading (5%) and the rest of the replication has already a 0% motility signalling that the capacity of the watermelon yolk extender is only up to this p oint from boar semen.

Table 11. Commercial Extender Capacity for Extending Sperm Cells Motility of Boar's Semen

Hour	Reading	Average Motility (%)
10	8	72
11	9	70
12	10	70
13	11	67
14	12	63
15	13	61
16	14	60
17	15	58
18	16	52
19	17	38

Table 12 shows the summary % motility readings for treatment 2 (commercial extenders) where progressive reduction was seen from 72.20% (8th reading) to 38% in the 17th reading.

Eight to Seventieth Motility

Readings For the rest of the readings (8th to 17th), it was only the commercial extender which afforded motility of sperm cells. Indeed the result showed the capacity of the preservation commercial extender for such that it is popular and used by commercial swine farms for production of piglets.

Summary, Conclusion and Recommendation

The study was conducted to determine the longevity and motility of boar semen in different treatments. The collected semen through the using of dummy was obtained in the boar from the University Swine Project.

The semen was collected at 11:00 am using the dummy through the collection cup which was kept immediately in a water bath having 32-35o c for further evaluation for macroscopic (volume, color, and pH) and microscopic assessment of sperm cell motility indicates the quality

of semen and longevity. The study was laid out in a Completely Randomized Design with three treatments consisting: Treatment 1 is the (control, no added extender) Treatment 2- commercial extender (MS diluent) and the Treatment 3 is watermelon extract with egg yolk with a ratio of 1:1. Five replications per sampling were made per hourly reading. The analysis of variance for a complete randomized design was used to analyse the data and the Tukey's HSD (0.05) for the comparison of observed significant treatment means.

Results showed that the total volume of semen ejaculated by the boar is 200ml with an excellent motility of 84%, with pH 8 and milky white color without odor. Common abnormalities were enlarged midpiece (23.08%) while the least were shrunken and enlarged head, broken midpiece and broken tail (7.69% each). There were 17 reading performed which started with an initial motility of 85% (excellent motility). For the 1st and 2 nd reading, sperm cells in all treatments were motile with 82% to 69% range, loss in the motility of sperm cells were observed in Treatment 1 while Treatment 2 still had an excellent motility of 80.6% and Treatment 3 had a good motility of 66.4%. The differences among the three treatments means were still highly significant (P<.01).

Seven hours after the first reading, Treatment 2 had 73.4% (very good motility) while Treatment 3 has replication with a poor motility of 5%.

Maximum livability/longevity of sperm cells showed that Treatment 2 (commercial extender) allowed the cells 19 hours after the semen collection, Treatment 1 (semen without extender) for only 5 hours after the semen collection and Treatment 3 with 9 hours after the semen collection.

Based on the results, Treatment 2 (commercial extender) proved that it is preferable to use as a semen extender for artificial insemination purposes. The capacity of the watermelon extract-yolk as an extender is only good for short term (9 hours).

The author would recommend for a further study using lower temperature of 35^{o}C in watermelon extract with egg yolk extender.

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