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Possibility of Tomato (Solanum lycopersicum) fruit juice as Semen Extender Supplemented and its effect on the Quality of Large White Boar (Sus scrofa domesticus) Spermatozoa

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ABSTRACT

This study evaluated the potential of tomato (Solanum lycopersicum) juice extract as a natural semen extender for improving the quality of Large White Boar (Sus scrofa domesticus) spermatozoa and align sustainable development goals. The study aims to determine the proximate composition of tomato Juice extract and assess the potential effect of tomato juice as a semen extender supplement on the pH, color, motility, viability, and morphology of Large White Boar spermatozoa. The Complete Randomized Design (CRD) and post hoc comparison analysis are used for statistical design. Additionally, nonparametric analysis determines the level of significance among the means at 5% level. The treatments in the different levels incorporating tomato juice extract were compared to control and commercial extenders, assessing parameters such as pH, motility, mass activity, viability, color, and morphology. Results indicated that tomato juice significantly improved motility, mass activity, and sperm viability, with Treatment 3 (10% tomato juice) showing the most consistent benefits. However, other parameters, such as color and temperature, were unaffected, while pH stability was better maintained in treatments with tomato juice than in the control. These findings highlight the potential of tomato juice as a cost-effective, antioxidant-rich alternative to synthetic extenders, providing a natural option for enhancing semen preservation and fertility outcomes.

Keywords: Semen Extender, Tomato Juice Extract, Semen quality and Spermatozoa

1. INTRODUCTION

The utilization of tomato juice as a semen extender in the Philippines addresses a variety of SDGs, specifically those providing better methods and practices in agriculture for improved food security and contributing to the economic sustainability of farmers. This application enhances livestock fertility and productivity and supports the development of sustainable agriculture innovations that are important in the country's developmental goals. Giving appropriate guidance to enhance tomato competitiveness as an alternative tomato extender will help local farmers Heiba et al. (2023).

Tomato contains different compounds (e.g. carotenoids, vit. C, flavonoids) that may be responsible for the antioxidant properties(Al-Daraji & Hj, n.d.). A new study has been launched on the significance of a nutrient found in tomatoes to boost fertility after an Indian-American scientist showed that lycopene supplementation could raise sperm count by up to 70%. The rising blood lycopene, the red pigment compound found in tomatoes, improves sperm quality and lifespan (Pti, 2016; Bintara et al., 2022). Moreover, the positive effects of tomato juice are antioxidant-rich products. The semen extender must be selected on the grounds of optimal performance (in terms of fertility and litter size), which, in turn, depends on the conditions of each pig farm. The choice of the extender is crucial given its profound effect on the economic viability of the artificial insemination program (Gadea J. 2003).

Adding tomato juice up to 7 ml per 100 ml of semen diluent significantly increased mass activity and individual motility percentages and decreased dead spermatozoa, abnormal spermatozoa, and acrosomal abnormalities percentages at all storage times when compared with control (Al-Daraji & Hj, n.d.). However, the administration of tomato juice with a concentration of 10% and DMSO was the optimum concentration that showed the highest fertilization rate (Muhiardi et al., 2020).

In the Philippines, local swine raisers considered artificial insemination cost-effective, disease-free, and capable of maintaining accurate breeding records. The weighted values ranged from 3.59 to 3.89, indicating a consensus among respondents that these aspects are highly advantageous. The recognition of cost-effectiveness aligned with the economic considerations of swine breeding, emphasizing the potential for reduced expenses and improved resource allocation through artificial insemination. The results strongly suggest an inclination towards embracing artificial insemination as a preferred breeding technique in the studied community(Tangalin & Malalis, 2024).Moreover, the research and development agenda of both crops Vegetables (e.g. tomato, white potato, mushroom) and livestock is the top priority of the Department of Science and Technology to the Filipino people (HNRDA_2022-2028).

This research gap focuses on the sperm motility and viability effect of tomato juice and addresses how it works. Knowing how the lycopene and other constituents of tomato juice influence sperm health in vitro will help determine how effective tomato juice is as an alternative semen extender in small-scale farmers. Promoting the best quality of tomato juice extract as a semen extender to the farmers that are beneficial in the community.

OBJECTIVE

Determine the proximate composition of tomato Juice extract.

Assessing the potential effect of tomato juice as a semen extender supplement on the pH, color, motility, viability, and morphology of Large White Boar spermatozoa.

METHODOLOGY

The study obtained approval from Institutional Animal Care and Use Committee (IACUC) under protocol number MHAM-032823-08 ensuring that the research adhered to ethical standards and complied with all relevant regulations and guidelines.

Study Area

This study will utilize a senior native boar. This senior boar is currently housed in the Department of Animal Science -Swine project of the University. Furthermore, semen analyses were conducted at the Breeding laboratory of the University from September to October 2024. Before collection, the animal

was conditioned for a week to ensure the boar was fit for the experiment. Additionally, another analysis was conducted at Visayas State University-Central Analytical Service Laboratory.

Semen Collection

Before semen collection, the materials to be utilized were cleaned, sanitized, and dried before use. The collection pen and its surrounding area should avoid distractions that may divert the focus of the boar away from the collection. The procedure of semen collection was based on (Althouse 1998). Trained personnel will collect semen every other day inside a boar pen. The ejaculate will be filtered using a sterile filter paper attached to a sterile beaker. Immediately after collection, freshly collected ejaculates were evaluated for volume, color, and consistency and placed in sterile bottles.

Semen Extender Processing & Preparation

In this study, tomato extract juice was used as an extender in comparison with the commercial extender. The over-ripe tomato waste fruit was utilized in the study at the available market store in Baybay City.

To prepare the tomato extract, it was washed thoroughly with distilled water before cutting into tiny pieces using a sterile knife and will be blended for about five minutes. A clean glass beaker wrapped with a sanitized cotton cloth will serve as a filter, preventing any solid particles from mixing with the extract five times. Lastly, the glass beaker will be covered with aluminum foil to avoid contamination. Right after preparation, the pH of the tomato extract was evaluated using a calibrated pH meter, and all samples were stored at a cool and dry place that were refrigerated before and after the analysis at the Department of Animal Science and will be mixed with Large white Boar semen for further analysis.

Experimental Design and Treatment

There will be five treatments in this study:
T0- Control group (pure large white boar semen)
T1- 50% boar semen + 50% Commercial extender
T2- 50% boar semen + 42% Commercial extender + 8% Tomato juice
T3- 50% boar semen + 40% Commercial extender + 10%Tomato juice
T4- 50% boar semen+ 38% Commercial extender + 12% Tomato juice

Data to be Collected

Volume of Semen

The amount of semen collected can be measured using a graduated tube or small cylinder. The volume of semen may vary on different collection days.

Semen pH

The pH of semen was measured using a pH meter immediately after collection and treatment dilution to avoid the accumulation of lactic acid on semen pH.

Semen Temperature

After collecting the semen, its temperature will be measured using a thermometer and then diluted per treatment.

Semen Color

The color of semen is an important indicator of its quality. It can determine whether the semen sample is in good condition or not. Normally, semen appears to be white and creamy like whole milk. However, if the density of the semen sample is low, it may appear transparent. The color of semen can be evaluated based on a scoring system, which is outlined in (Table 1).

Table 1. Color and consistency grading of extended and unexpended semen.

Score	Color
+++	Thick creamy/thick white/thick yellowish
++	Thick creamy/milky/think yellowish
+	Clear/watery

Sperm Motility

By utilizing the research of Rodriguez (2016) and Capitan and Palad (1999), it can accurately analyze sperm motility. This approach guarantees precision and reliability in data interpretation using the scoring system provided in Table 2.

Table 2. Sperm motility criteria

Sperm Motility Score	Examination Criteria
5	Excellent Motility- 80% or more of the spermatozoa are in a very vigorous motion
4	Very Good Motility- 70% of the spermatozoa are in rapid motion.
3	Good Motility- 50% to 75% of the spermatozoa are in motion
2	Fair Motility- 20% to 50% of the spermatozoa are in motion.
1	Poor Motility- Less than 30% of the spermatozoa are in motion
0	No Motility- Dead or no spermatozoa

Sperm Mass Activity

By utilizing Capitan and Palad's (1999) established criteria, it will be able to conduct a thorough analysis of the mass activity of spermatozoa. The scoring system for mass activity will provide us with accurate and reliable results, as outlined in Table 3.

Table 3. Mass activity criteria

Sperm Motility Score	Examination Criteria
+	With slight movement, individual sperm do not move out of the field
++	Increasing progressive movement
+++	No swirls formed, but have excellent progressive movement
++++	Have swirls and exhibit good movement
+++++	Have vigorous movement, vigorous swirls are present

Sperm Concentration

Total number of spermatozoa per cu.mm. Hemocytometer is a reliable tool for this purpose, spermatozoa will be measured using hemocytometer, calculated by counting the number of spermatozoa in the 80 small squares.

$$\frac{X \times 400}{80} \times \frac{10}{1} \times \frac{200}{1}$$

Live- Dead staining of Spermatozoa. Total number of live and dead sperms will be counted based on the procedures developed by Capitan and Palad (1999).

Percentage of live sperm

%Live Sperm =Total No. of Live Sperms counted x 3 10

Percentage of dead sperm

% Dead Sperm =Total No. of dead Sperms counted x 3 10

Spermatozoa Morphology

Ensuring the quality of sperm cells is crucial for successful breeding. That's why this study follows the Manual for Artificial Breeding of Farm Animals (Capitan & Palad, 1999) to evaluate their morphology and structure. We smear the sample with eosin-nigrosin and let it dry naturally, before inspecting it using high magnification or oil immersion objectives. This rigorous approach ensures that only the best sperm cells are selected for breeding.

Statistical Analysis

The study used the Complete Randomized Design (CRD) and post hoc comparison analysis. The nonparametric analysis determines the level of significance among the means at 5%. All statistical analyses were done using R-studio, statistical analysis software.

Result and Discussion

Effects of Tomato (Solanum lycopersicum) Fruit Juice as Alternative Semen Extender

The effects of various treatments on key semen properties, such as pH levels, sperm motility, sperm color, temperature, mass activity, sperm viability, and morphology. Results showed that the control group had the least stable pH, making it unsuitable for maintaining acidity or alkalinity, while other treatments had a better-balanced pH. Sperm motility varied significantly, with Treatment 3 proving the most effective. Sperm color remained consistent across all treatments, indicating no negative impact on quality, and temperature stability was unaffected. Mass activity significantly improved with some treatments, particularly Treatment 3. For sperm viability, Treatment 3 again led the way, showing higher levels of live sperm. However, morphology changes were minimal across treatments, suggesting small cumulative benefits rather than a single impactful treatment.

Table 4. Proximate analysis of tomato juice extract.

Tomato juice temperature	27.9
pH	4.97
Crude protein	1.619

The proximate analysis of Table 4 showed the tomato juice extract used in the study revealed its key properties. The juice was 27.9°C, close to room temperature, ensuring stability during use. Its pH was measured at 4.97, indicating a slightly acidic nature that could help maintain sperm stability and reduce microbial contamination. The crude protein content was 1.619%, suggesting that the juice provides some nutritional support that may enhance sperm vitality and membrane health. Additionally, tomatoes contain organic acids (like citric acid) that help maintain an ideal pH level, which can lead to greater sperm quality and longevity(Hazim Jabbar Al-Daraji, 2014).

Table 5. Analysis of Variance (ANOVA) for pH levels across different semen treatments, commercial extender, and tomato juice mixtures.

Cases	Sum of Squares	df	Mean Square	F	p
treatment	6.715	4	1.679	28.086	< .001
Residuals	1.494	25	0.060		

Table 5. Analysis of Variance (ANOVA) for pH levels across different semen treatments, commercial extender, and tomato juice mixtures.

Cases	Sum of Squares	df	Mean Square	F	p

Note. Type III Sum of Squares

Table 6. Post Hoc Comparisons of treatments considering their effect on pH

		Mean Difference	SE	t	p_{tukey}
0	1	0.792	0.141	5.609	< .001
	2	1.153	0.141	8.171	< .001
	3	1.133	0.141	8.029	< .001
	4	1.325	0.141	9.387	< .001
1	2	0.362	0.141	2.562	0.109
	3	0.342	0.141	2.421	0.143
	4	0.533	0.141	3.778	0.007
2	3	-0.020	0.141	-0.142	1.000
	4	0.172	0.141	1.216	0.742
3	4	0.192	0.141	1.358	0.659

The Analysis of Variance reported highly statistically significant differences in pH levels across the different treatments (F = 28.086, p-value= <0.001). This indicates that at least one of the treatments has a different impact on the pH of the semen measured. The pH of semen is a critical factor affecting sperm viability and motility(Zhou et al., 2015). Extenders, such as those used commercially, are commonly formulated to stabilize pH and osmolarity. However, the inclusion of natural additives like tomato juice introduces additional buffering capacity and antioxidants, such as lycopene, known for its protective effects against oxidative stress (Agarwal et al., 2005). The control group is shown in Table 6 a significant difference in the pH of the semen measured; this indicates that it is best to avoid the control group as it causes deviation in pH measurements.

Table 7. Kruskal-Wallis Test for Sperm Motility across different treatments of semen, commercial extender, and tomato juice mixtures.

Factor	Statistic	df	p
treatment	19.480	4	< .001

Table 8. Dunn's Post Hoc Comparisons of treatment considering their effect on sperm motility

Comparison	z	W_i	W_j	p	p_{bonf}	p_{holm}
0 - 1	0.817	22.667	18.833	0.414	1.000	1.000
0 - 2	1.989	22.667	13.333	0.047	0.467	0.300

Table 8. Dunn's Post Hoc Comparisons of treatment considering their effect on sperm motility

Comparison	z	W_i	W_j	p	p_{bonf}	p_{holm}
0 - 3	4.014	22.667	3.833	< .001	< .001	< .001*
0 - 4	0.817	22.667	18.833	0.414	1.000	1.000
1 - 2	1.172	18.833	13.333	0.241	1.000	1.000
1 - 3	3.197	18.833	3.833	0.001	0.014	0.012*
1 - 4	0.000	18.833	18.833	1.000	1.000	1.000
2 - 3	2.025	13.333	3.833	0.043	0.429	0.300
2 - 4	-1.172	13.333	18.833	0.241	1.000	1.000
3 - 4	-3.197	3.833	18.833	0.001	0.014	0.012*

The analysis of Variance in Sperm motility showed in Table 7, statistically significant difference in sperm motility across the different treatments (H=19.480, p-value <0.05). This indicates that at least one of the treatments has a different impact on the motility of the semen measured. The use of tomato juice as an extender in goat semen, however in swine semen was not introduced as tomato juice extract as an alternative semen extender. The results indicated that sperm preserved with tomato juice exhibited higher motility and better viability than control semen samples (Liu et al., 2016). Moreover, across all pair-wise comparisons in Table 8, there is a significant difference whenever treatment 3 is applied except when paired with treatment 2. This indicates that treatment 3 has a highly statistically significant effect on the motility of the sperm than the other treatments. However, some factors that influence sperm motility, including morphology, cell damage, and also undergo optimizing semen quality assessment(Van de Hoek et al., 2022).

Table 9. Kruskal-Wallis Test for Color across different treatments of semen, commercial extender, and tomato juice mixtures.

Factor	Statistic	df	p
treatment	5.868	4	0.209

The analysis of Variance shown in Table 9, there is no significant difference in sperm color across the different treatments (H = 5.868, p-value > 0.05). This indicates that the treatments do not affect the color of the sperm. However, commercial extenders are developed to promote sperm survivability and also tomato juice extract but can help to maintain the functionality of the sperm effectively (Liu et al., 2016; Seidel, 2014)

Table 10. Analysis of Variance (ANOVA) for temperature across different treatments of semen, commercial extender, and tomato juice mixtures.

Cases	Sum of Squares	df	Mean Square	F	p
treatment	5.636	4	1.409	1.297	0.298
Residuals	27.158	25	1.086		

Note. Type III Sum of Squares

Table 10 shows there is no significant effect on the temperature of the sperms across the different treatments (F = 1.297, p-value>0.05). This indicates that the treatment levels do not have a significant effect on the temperature of the sperm. The implication is that the interventions used did not make any measurable difference to temperature stabilization, an important aspect of sperm survival and function. Indeed, semen is extremely sensitive to temperature changes, and its mobility, viability, and performance parameters are critically dependent on the environment (Appell et al., 1977). Additionally, environmental conditions and temperature are carefully monitored and must be considered.

Table 11. Kruskal-Wallis Test for mass activity across different treatments of semen, commercial extender, and tomato juice mixtures.

Factor	Statistic	df	p
treatment	25.214	4	< .001

Table 12. Dunn's Post Hoc Comparisons of treatment considering their effect on mass activity.

Comparison	z	W_i	W_j	p	p_{bonf}	p_{holm}
0 - 1	0.000	24.000	24.000	1.000	1.000	1.000
0 - 2	2.482	24.000	12.167	0.013	0.131	0.105
0 - 3	4.125	24.000	4.333	< .001	< .001	< .001
0 - 4	2.307	24.000	13.000	0.021	0.210	0.126
1 - 2	2.482	24.000	12.167	0.013	0.131	0.105
1 - 3	4.125	24.000	4.333	< .001	< .001	< .001
1 - 4	2.307	24.000	13.000	0.021	0.210	0.126
2 - 3	1.643	12.167	4.333	0.100	1.000	0.301
2 - 4	-0.175	12.167	13.000	0.861	1.000	1.000

Table 12. Dunn's Post Hoc Comparisons of treatment considering their effect on mass activity.

Comparison	z	W_i	W_j	p	p_{bonf}	p_{holm}
3 - 4	-1.818	4.333	13.000	0.069	0.691	0.276

The analysis of Variance in mass activity in Table 11, showed a highly statistically significant difference between the treatments (H = 25.214, p-value <0.05). This indicates that at least one of the treatments has a different impact on the mass activity of the semen measured. Furthermore, there is a significant difference in Table 12 between pairs whenever treatment 3 is present except when paired with treatment 2. This indicates that among all treatments, treatment 3 has a statistically significant effect on the mass activity of the sperm. This finding underscores Treatment 3 has a 10% tomato juice extract potential for improving overall sperm performance. However, reported that there was a significant increase in mass activity as the bulls grew beyond 36 and 30 months of age(Mah et al., 1981; Maside et al., 2023).

Table 13. Analysis of Variance (ANOVA) for sperm viability across different treatments of semen, commercial extender, and tomato juice mixtures.

Cases	Sum of Squares	df	Mean Square	F	p
treatment	11.588	4	2.897	14.351	< .001
Residuals	5.047	25	0.202		

Note. Type III Sum of Squares

Table 14. Post Hoc Comparisons of treatments considering their effect on sperm viability

		Mean Difference	SE	t	p_{tukey}
0	1	0.165	0.259	0.636	0.968
	2	0.873	0.259	3.367	0.019
	3	1.648	0.259	6.354	< .001
	4	0.137	0.259	0.527	0.984
1	2	0.708	0.259	2.731	0.077
	3	1.483	0.259	5.718	< .001
	4	-0.028	0.259	-0.109	1.000
2	3	0.775	0.259	2.988	0.045
	4	-0.737	0.259	-2.840	0.062
3	4	-1.512	0.259	-5.828	< .001

The Analysis of Variance reported a highly statistically significant difference in sperm viability levels across the different treatments (F = 14.351, p-value= <0.001). This indicates that at least one of the treatments has a different impact on sperm viability. Additionally, sperm viability can be drastically

reduced if samples are not handled or stored correctly. The optimal storage temperatures (approximately 15-18 °C) is crucial for sustaining sperm viability during transport and storage(Szymanowicz et al., 2019). Moreover, commercial and tomato juice extract can help the nutrients and protective agents that can enhance sperm survivability.

The Analysis of Variance on sperm viability reported a highly statistically significant difference in sperm viability levels across the different treatments (F = 14.351, p-value= <0.001). This indicates that at least one of the treatments has a different impact on sperm viability. Moreover, across all pair-wise comparisons, there is a significant difference whenever treatment 3 is applied. Treatment 3 has a highly statistically significant effect on sperm viability than the other treatments. In contrast, improper handling can lead to viability loss(Rolf et al., 1999)

Table 15. Analysis of Variance for Sperm Morphology across different treatments of semen, commercial extender, and tomato juice mixtures.

Cases	Sum of Squares	df	Mean Square	F	p
treatment	6.133	4	1.533	3.282	0.027
Residuals	11.678	25	0.467		

Note. Type III Sum of Squares

Table 16. Post-hoc comparison of treatments considering their effect on sperm morphology

		Mean Difference	SE	t	p_{tukey}
0	1	0.228	0.395	0.579	0.977
	2	-0.693	0.395	-1.757	0.419
	3	-0.425	0.395	-1.077	0.816
	4	0.573	0.395	1.453	0.601
1	2	-0.922	0.395	-2.336	0.167
	3	-0.653	0.395	-1.656	0.478
	4	0.345	0.395	0.874	0.904
2	3	0.268	0.395	0.680	0.959
	4	1.267	0.395	3.210	0.027
3	4	0.998	0.395	2.530	0.116

The Analysis of Variance in sperm morphology is a statistically significant difference across the different

treatments (F = 3.282, p-value= <0.05). This indicates that at least one of the treatments has a different impact on the sperm morphology. The post-hoc tests show that no pairwise differences between treatments are statistically significant. This suggests that overall differences detected by the Analysis of Variance in Table 16 may be due to a combination of small differences across the different treatments rather than a single strong difference between two specific treatments. The analysis identified subtle morphological differences between the sperm heads from normal and abnormal samples. Sperm heads from normal samples had a higher overall area, greater width, and height, and were less variable in shape compared to those from abnormal samples(Mandawala et al., 2018).

Conclusion

Based on the findings, the analysis highlights that Treatment 3 is consistently effective across multiple semen quality parameters, particularly in motility, mass activity, and viability. The control group, on the other hand, showed significant shortcomings, particularly in pH stability. Additionally, treatments provided moderate benefits, but Treatment 3 remains the most promising. Therefore, tomato juice extract enhances sperm quality as a natural semen extender due to its antioxidants, bioactive properties, and cost-effectiveness. Furthermore, future researchers are encouraged for further studies to be conducted.

Conflict of Interest

The authors declare no conflict of interest.

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