Antioxidative effect of watermelon (*Citrullus lanattus*) juice on fertilizing potential of extended boar semen

¹Oyeniran, J. O., ²Sokunbi, O. A., ³Faniyi, T. O., ¹Nuhu, S. H., ¹Mohammed, A., ¹Sanga, M. J.

¹Department of Animal Health and Production Technology, Federal Polytechnic, Bauchi.

²Department of Animal Science, University of Ibadan, Oyo State

³Department of Crop and Animal Science, Ajayi Crowther University,
Oyo, Oyo-State.

Corresponding author: oojohnson@fptb.edu.ng; +2347037845974

Abstract

Boar semen preservation is associated with the production of reactive oxygen species which leads to reduction of sperm quality and decrease in fertilizing ability. The aim of this study was to replace conventional semen extender with watermelon juice (WMJ) and to ascertain the most effective dilution rate that can maintain the viability of the spermatozoa over a 48-hours extension period. Semen was collected from a boar at the piggery unit of the teaching and research farm University of Ibadan, using the glove hand method. The experiment was divided into five treatments with Beltsville Thawing Solution being replaced by WMJ at the following rate; 0, 12.5, 25, 37.5 and 50% and designated as T1, T2, T3, T4 and T5 respectively. Diluted semen samples were stored at 17°C and were evaluated at 0, 24 and 48 hours for pH, acrosome integrity and lipid peroxidation. The experimental design was a completely randomised Design; acrosome integrity, pH and lipid peroxidation were analysed using descriptive statistics and ANOVA. The data showed that there were significant difference ($p < 0.05$) in acrosome integrity with treatment 1 (0 % WMJ) and treatment 2 (12.5 % WMJ) having the highest mean values. A significant difference was also observed in pH values having treatment 4 (37.5 % WMJ) as the highest mean value) but there was no significance difference in lipid peroxidation. The results showed that 50 % of BTS can be replaced by watermelon juice and it could be concluded that WMJ could be an extender of choice for a reliable short term preservation in swine artificial insemination programme.

Keywords: Boar, Watermelon Juice extension, Semen quality, Spermatozoa.

Effet antioxydant du jus de pastèque (*Citrullus Lanattus*) sur le potentiel fertilisant du sperme de verrat étendu

Résumé

La conservation du sperme de verrat est associée à la production d'espèces réactives de l'oxygène, ce qui entraîne une réduction de la qualité du sperme et une diminution de la capacité de fécondation. Le but de cette étude était de remplacer l'extenseur de sperme conventionnel par du jus de pastèque (JP) et de déterminer le taux de dilution le plus efficace pouvant maintenir la viabilité des spermatozoïdes sur une période d'extension de 48 heures. Le sperme a été collecté sur un verrat dans l'unité de porcherie de la ferme d'enseignement et de recherche de l'Université d'Ibadan, en utilisant la méthode de la main gantée. L'expérience a été divisée en cinq traitements avec la solution de dégel de Beltsville remplacée par JP au taux suivant ; 0, 12,5, 25, 37,5 et 50 % et désignés respectivement par T1, T2, T3, T4 et T5. Des échantillons de sperme dilué ont été conservés à 170°C et ont été évalués à 0, 24 et 48 heures pour le pH, l'intégrité de l'acrosome et la peroxydation lipidique. La conception expérimentale était une conception complètement aléatoire ; l'intégrité de l'acrosome, le pH et la peroxydation lipidique ont été analysés à l'aide de statistiques

descriptives et d'ANOVA. Les données ont montré qu'il y avait une différence significative ($p < 0,05$) dans l'intégrité de l'acroosome avec le traitement 1 (0 % JP) et le traitement 2 (12,5 % JP) ayant les valeurs moyennes les plus élevées. Une différence significative a également été observée dans les valeurs de pH ayant le traitement 4 (37,5 % JP) comme valeur moyenne la plus élevée, mais il n'y avait pas de différence significative dans la peroxydation lipidique. Les résultats ont montré que 50 % du BTS peut être remplacé par du jus de pastèque et on a pu conclure que le JP pourrait être un extenseur de choix pour une conservation fiable à court terme dans le programme d'insémination artificielle porcine.

Mots clés : Sanglier, Extension de jus de pastèque, Qualité de la semence, Spermatozoïdes.

Introduction

Successful swine fertility programs can be achieved using extended semen when compared to those using natural service. Although the former is a routine procedure, its use is limited because sperm cells remain viable for about 3 to 5 days (Gadea *et al.*, 2005). Many studies have been carried out on carotenoids, considered an important group of natural antioxidants (Bast *et al.*, 1998, Møller *et al.*, 2000, Schabath *et al.*, 2004; Rao and Rao, 2007). For over a decade, lycopene, a carotenoid present in tomatoes (*Lycopersicon esculentum*) and several ripe fruits and vegetables, such as watermelon, pink grapefruit and carrots (Nguyen and Schwartz, 1999, Perkins-Veazie *et al.*, 2003 and Tadmor *et al.*, 2005), has been considered in some studies related with human and animal health (Bhuvaneswari and Nagini, 2005, Rao *et al.*, 2006, Avci and Durak, 2007 and Bhom, 2007) and reproductive physiology (Martino *et al.*, 2006, Goyal *et al.*, 2007, Mangiagalli *et al.*, 2007, Turk *et al.*, 2007 and Mendiola *et al.*, 2010). In roosters, in particular, Mangiagalli *et al.* (2010) found positive effects of lycopene addition on fertility and qualitative characteristics of semen. With AI expanding rapidly, the demands for semen increased. The simplest way to meet this demand was to "stretch" each ejaculate further by using fewer spermatozoa per insemination, provided that this could be accomplished without sacrificing fertility. The need to extend and preserve semen has become more evident

due to the increasing use of artificial reproductive technique in animal. Semen extension involves the dilution of high quality semen with a suitable medium. The main objective of semen preservation is to extend the usefulness of genetically superior males for purposes of expanding the genetic base of breed, repopulating/recreating breeds or lines and discovering new gene (Waberski *et al.*, 1994; Purdy *et al.*, 2008). Two major systems (Liquid and Frozen) of storage technologies have been achieved for sperm conservation in the 20th century. For storage of liquid boar semen, the temperature of collection and storage, and the composition of the storage medium are essential (Johnson *et al.*, 2000). The use of liquid to preserve semen for cervical AI in pigs has been widely accepted and accounts for over 80 % of insemination in many regions of the world (Roca *et al.*, 2006b; Bailey *et al.*, 2008). The use of the Tris-egg yolk cryopreservation diluents, such as the one described by Salamon and Ritar (1982) is recommended, as it is easy to use. However, commercial extenders with no biological components have been developed to improve sanitary safety in semen processing (Hinsch *et al.*, 1997; Gil *et al.*, 2003). However, the adoption of these techniques in the third world countries has been hindered due to high cost of these preparations and poor infrastructural development. This has led to the development of local semen extenders from herbal or plant materials in Nigeria (Umesiohi *et al.*, 1998, 2002; Umesiohi

2004). The quality of extended semen, to a great extent, depends on the quality of raw ejaculate, provided that the *in vivo* conditions of the semen are maintained *in vivo*. In other words, poor quality ejaculate cannot give extended semen of high quality. Sperm examination is an important tool in estimating the fertilizing capacity of an ejaculate (Frunza *et al.*, 2008). Although several parameters can be investigated, the actual capacity of the spermatozoa to function at the site of fertilization is difficult to assess. Therefore, assays examining morphological or motility characteristics are most routinely performed (Martin-Rillo *et al.*, 1996). Other parameters that can be measured include concentration, pH and total sperm numbers. However, before one proceeds to do detailed semen evaluation a few parameters can be measured visually at the point of collection, for example, volume, colour, consistency, and density. The most important and well characterised natural antioxidants in the animal body are vitamin E and C. In the body, all antioxidants are working in concert as a team, the (antioxidant system), responsible for prevention of the damaging effects of free radicals and toxic products of their metabolism. However, the antioxidant (team) acts to control levels of free radical formation as a coordinated system where deficiencies in one component impact the efficiency of others (Peter, 2007). Beta carotene is the most widely studied carotenoid (Alam and Sultan, 2004). Carotenoids are widely distributed natural pigments responsible for the yellow, orange, and red colors of fruits, roots, flowers, fish, invertebrates, and birds (Delia, 1997). Beta-Carotene and lycopenes are the major carotenoids which are mainly composed of carbon and hydrogen atoms. In humans and animals, carotenoids play an important role in protection against photooxidative processes by acting as oxygen and peroxy radical scavengers

(Jacques *et al.*, 2008, Lien *et al.*, 2008).

The watermelon fruit has deep green or yellow colored smooth thick exterior rind with gray or light green vertical stripes. Inside the fruit is pink, red or even yellow in color with small black seeds embedded in the middle third of the flesh. Generally, watermelon flesh is the main consumable portion; however, outer rind is also used in some parts of the world (Levi *et al.*, 2001). Watermelon contributes a plethora of nutritional agents as antioxidants (lycopene, beta-carotene etc.) and some specific amino acids (arginine, citrulline etc.). It is a good source of potassium and also contains magnesium, calcium, phosphorus and iron (Quek *et al.*, 2007), the aqueous extract of *Citrullus lanatus* has been known to be a good source of glucose, fibre and also an excellent source of vitamin C, lycopene, and beta carotene (Erhardt *et al.*, 2003). Lycopene scavenging rate is reported to be higher than beta-carotene and tocopherol. Being a reactive carotenoid, lycopene follows three possible routes to interact as adduct formation, transferring electron to free radical and by allelic hydrogen abstraction. It significantly inhibits free radicals invasion at membranes surface and serves as primary defense system. Lycopene combinations with other antioxidants have proven synergistic behavior to scavenge reactive oxygen species. Its synergistic role with vitamins E and C and other carotenoids has affirmative impact on human Crude fibre. For the measurement of crude fibre, fat free health (Huang *et al.*, 2007). It is important to evaluate the effectiveness of natural antioxidants that are readily available, such as watermelon juice in reducing the oxidative stress in post ejaculated boar semen, to evaluate the effectiveness of antioxidant in watermelon juice on the boar semen quality and determine the proportions of *watermelon juice* that is suitable for preserving boar semen quality.

Materials and methods

Location of study

Semen was collected from a boar at the piggery unit of the Teaching and Research Farm, University of Ibadan, Ibadan, located in the tropical rain forest zone in Nigeria on latitude 7°20'N and 3°50'E, with a mean altitude of 277 meters above the sea level. Preparation of watermelon juice and semen analyses were carried out in the animal physiology laboratories of the same institution.

Preparation of watermelon (Citrullus lanattus) juice (WMJ)

Water melon fruits were washed, dried and sliced into small bits. The flesh was cut after the seeds had been removed, then blended, and sieved using muslin cloth. The juice was further filtered using Whitman filter paper (110mm) to remove hidden fibre in the juice, to enhancing visibility of spermatozoa during microscopic evaluation (Gibson *et al.*, 2000).

Preparation of the boar, semen collection and extension

Prior to collection of semen, the boar was thoroughly washed and the preputial pouch was cleaned with water by a milking action, to remove urine and other materials that could contaminate semen during collection.

Semen was collected using the gloved hand method. Once the boar mounts and begins to thrust, the penis was grasped firmly in a gloved hand perpendicular to its body and pressure was applied to the tip of the penis imitating the locking done by the cervix of the sow during natural mating. The grip was sustained until the boar finished ejaculating. Semen was collected into a US bag inserted in a cup such that the pre and post sperm fractions were separated from the sperm rich fraction. Semen and extender were mixed in ratios 1:7, 1:4, 1:3, 1:1.7 and 1:1 as described by Fiser *et al.* (1993). The mixture was refrigerated at 17°C. (Althouse *et al.*, 1998).

Experimental treatments and design

A completely randomized design was utilized for the study, such that diluted semen were allotted to five treatments with three replicates per treatments evaluated at 0, 24 and 48 hours:

Treatment 1: Semen + Beltsville Thawing Solution (BTS) Extender

Treatment 2: Semen + BTS + 12.5 % WMJ

Treatment 3: Semen + BTS + 25 % WMJ

Treatment 4: Semen + BTS + 37.5 % WMJ

Treatment 5: Semen + BTS + 50 % WMJ

The composition of BTS used to dilute collected semen is presented in Table 1

Table 1: Composition of Beltsville Thawing Solution (100mL)

Components	Unit (g/mL)
Glucose	39.1
EDTA	1.32
Sodium bicarbonate	1.3
Potassium chloride	0.79
Penicillin	1.1
Streptomycin	1.1

EDTA = Ethylene diamine tetra acetic acid

Semen evaluation

Semen evaluation was carried out on sperm pH, acrosome integrity and lipid peroxidation at 0, 24 and 48 of liquid preservation.

Semen pH

A pH meter was used to measure the hydrogen ion concentrations produced by sperm cells metabolic activities during the storage period.

Acrosome Integrity

One drop of each semen sample was added

with a drop of eosin-negrosin, mixed together and then stored for 60 seconds under room temperature. The percentage of acrosome abnormality was by counting a total of 100 spermatozoa under microscope at magnification of x 40.

Lipid Peroxidation

The assay method of Hunter *et al*, modified by Gutteridge and Wilkins (1980), was used. Malondialdehyde (MDA), a product of

lipid peroxidation, when heated with thiobabaturic acid (TBA) has a maximum absorbance at 532nm.

Statistical analysis

Data obtained was analysed using the analysis of variance procedure of SAS (2003) and means were separated using the Duncan's Multiple Range Test of the same software.

Results

Table 2: Characteristics of semen collected from boar

Parameters	Value
Volume (ml)	50
Progressive Motility (%)	95
Mass activity	++++
Morphology (%)	95
PH	7.0

Characteristics of semen collected

Table 2 shows the results from the initial evaluation of boar semen before extension

was done. The result reveals that the sample was suitable for extension as the values fall within acceptable ranges for high quality semen.

Table 3: Effect of watermelon juice fortification on spermatozoa fertilizing potential of boar semen at 0 hour (Mean $\pm$ SD)

Parameters (%)	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
PH	6.86 $\pm$ 0.57 ^b	7.00 $\pm$ 0.10 ^{ab}	7.00 $\pm$ 0.00 ^{ab}	7.03 $\pm$ 0.05 ^{ab}	7.16 $\pm$ 0.05 ^a
A.I	94.33 $\pm$ 0.57 ^a	91.00 $\pm$ 0.00 ^b	90.00 $\pm$ 1.00 ^b	7.03 $\pm$ 0.05 ^{ab}	89.7 $\pm$ 1.05 ^b
LP (10 ⁻³)	1.83 $\pm$ 0.002	91.00 $\pm$ 0.00 ^b	1.60 $\pm$ 0.004	1.96 $\pm$ 0.004	1.89 $\pm$ 0.006

abc = mean values in the same row with different superscripts are significantly different (P<0.05); SD = Standard Deviation; A.I. = Acrosome integrity; LP = Lipid peroxidation

Table 3 shows the mean values of acrosome integrity, lipid peroxidation and pH of semen. Significant difference (p>0.05) in acrosome integrity was observed across treatments, with T1 (0 % WMJ) giving the highest mean value. T4 (37.5 % WMJ), though with significant difference between

means gave the closest mean value to T1. However, all treatment gave mean values within acceptable normal range. There were significant difference (P>0.05) in pH. The pH of the semen has the highest mean value in treatment 5 (50 % WMJ) while there was no significant difference observed in lipid peroxidation.

Table 4: Effect of water melon juice fortification on extended boar semen quality at 24 hours (Mean $\pm$ SD)

Parameters	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
PH	6.96 $\pm$ 1.73 ^b	7.16 $\pm$ 0.11 ^{ab}	7.10 $\pm$ 0.00 ^{ab}	7.3 $\pm$ 0.05 ^a	7.2 $\pm$ 0.05 ^{ab}
A.I	90.00 $\pm$ 0.00 ^a	88.00 $\pm$ 2.46 ^{ab}	86.33 $\pm$ 1.53 ^{ab}	85.00 $\pm$ 1.00 ^b	80.67 $\pm$ 1.15 ^c
LP (10 ⁻³)	1.04 $\pm$ 0.001	1.60 $\pm$ 0.007	1.59 $\pm$ 0.004	1.72 $\pm$ 0.000	0.89 $\pm$ 0.006

abc = mean values in the same row with different superscripts are significantly different (P<0.05); SD = Standard Deviation; A.I. = Acrosome integrity; LP = Lipid peroxidation.

Effect of watermelon juice fortification on spermatozoa fertilizing potential of boar semen at 24 hours

Table 4 shows the mean value of lipid peroxidation, acrosome integrity and pH of the semen. There were significant difference in acrosome integrity with

treatment 1 (0 % WMJ) and treatment 2 (12.5 % WMJ) having the highest mean values. A significant difference was also observed in pH values having treatment 4 (37.5 % WMJ) as the highest mean value) but there was no significance difference in lipid peroxidation.

Table 5: Effect of water melon juice fortification on extended boar semen quality at 48 hours (Mean $\pm$ SD)

Parameters	T1(0%)	T2(25%)	T3(50%)	T4(75%)	T5(100)
Ph	7.00 $\pm$ 0.10 ^a	7.06 $\pm$ 0.05 ^a	7.06 $\pm$ 0.05 ^a	7.00 $\pm$ 0.10 ^a	7.06 $\pm$ 0.05 ^a
A.I	86.67 $\pm$ 0.57 ^a	85.33 $\pm$ 4.62 ^a	80.67 $\pm$ 0.58 ^b	81.68 $\pm$ 2.87 ^b	84.33 $\pm$ 3.68 ^a
LP (10 ⁻³)	1.84 $\pm$ 0.006	1.37 $\pm$ 0.005	3.01 $\pm$ 0.002	1.57 $\pm$ 0.004	1.51 $\pm$ 0.0022

abc = mean values in the same row with different superscripts are significantly different (P<0.05); SD = Standard Deviation; A.I. = Acrosome integrity; LP = Lipid peroxidation.

Effect of watermelon juice fortification on spermatozoa fertilizing potential of boar semen at 48 hour

Table 5 shows the mean values of pH, lipid peroxidation and acrosome integrity. Acrosome integrity had lower values (P>0.05) in treatment 3 (25 % WMJ) while no significant differences were observed in pH and lipid peroxidation.

Discussion

Semen pH

In the present study, the result obtained for refrigerated semen (Table 4) showed there was no significant difference in pH of both semen with supplemented extender and control, this may be because pH of both semen and extender were close. This may confirm earlier report by Rangsi *et al.* (2008) that pH changes towards extending medium. Higher mean value of 7.2 was observed in treatment 5 of watermelon juice supplement which still fall within the range at which sperm cell can survive. The semen collected and used for this study could be said to be of very good quality based on the report of Frunza *et al.* (2008) who reported that pH that is higher than 8 denotes poor quality semen. Also, recorded in treatment 4 was a higher pH when compared to other

treatments, but the figure is within the recommended range which can support sperm cells survival. The impact of pH on survival of sperm cells cannot be overlooked because there are some sources of biochemical changes on sperm cells which include: increased concentration of solute, dehydration and changes in temperature (Merryam, 1966; Morris *et al.*, 1987). On the third day there was no significant difference (P>0.05) in the pH across the treatment. This implies that watermelon juice extract does not have a harmful effect on the pH of refrigerated boar semen.

Acrosome integrity

The acrosome integrity in treatment 5 was lower than that of other treatments (T1 –T4), this result indicated that T5 could reduce the acrosome integrity of boar sperm. This is in accordance with the report of Gadea *et al.* (2005) who conclude that reduced glutathione could reduce acrosome integrity, improving the function and fertilizing capacity of frozen boar spermatozoa.

Lipid peroxidation

At 0, 24 and 48 hours of preservation (17°C), the lipid peroxidation level were so low which could be as a result of

antioxidative properties of watermelon juice in the extender. Brzezinka-Slebodzinska *et al* reported that vitamin E reduces glutathione against fatty acid peroxidation in boar semen. Moreover, cerollini *et al.* (2000) reported that alpha-tocopherol in semen extender could help reduce the fatty acid peroxidation in boar semen.

Conclusion

The result showed that supplementation of watermelon juice in semen extender had the potential to reduce the oxidative stress in post ejaculated boar semen over 48 hours of observation during semen storage at 17°C. Therefore, watermelon juice could be used as an antioxidant in alternative to other molecules such as vitamins E and C.

References

- Alam, Z. and M. Sultan, 2004.** Carotenoids contents from various sources and their potential health applications. *Pakistan Journal of Nutrition.* 3(3): 199-204.
- Althouse, G. C., Wilson, M. E., Kuster, C. E. and Parsley, M. 1998.** Characterization of lower temperature storage limitations of fresh-extended porcine semen. *Theriogenology.* 50, 535-543.
- Avci, A. and Durak, I. 2007.** Tomato and cancer. In: Durak I. (Eds.). *Nutraceuticals and natural products in cardiovascular diseases, cancer and hemorrhoids.* 41-46.
- Bailey, J. L., Lessard, C., Jacques, J., Breque, C., Dobrinski, I., Zeng, W. and Galantino-Homer, H. L. 2008.** Cryopreservation of boar semen and its future importance to the industry. *Theriogenology.* 70: 1251-1259.
- Bast, A. Haenan, G. R., Van Den Berg, R., and Van Den Berg H. 1998.** Antioxidant effect of carotenoids. *International journal for vitamin nutrition research,* 68: 399-403.
- Bohm, F., Tinkler, J.H., and Truscott, T.G., 2007.** Carotenoids protect against cell membrane damage by nitrogen dioxide radical. *Natural Medicine.* 1: 98-99.
- Bhuvaneswari, V. and Nagini S. 2005.** Lycopene: a review of its potential as an anticancer agent. *Current Medical Chemistry. Anti-Cancer Agents,* 5: 627-635.
- Delia, B.R., 1997.** Carotenoids and food preparation: The retention of provitamins carotenoids in prepared, processed, and stored foods. *Office Health Nutrition.,* 1: 88-93.
- Erhardt J. G., Meisner C., and Bode C., 2003.** Lycopene, Beta carotene and Colorectal Adenomas. *America Journal of Clinical Nutrition.,* 78(6): 1219-1224.
- Fiser, P.S., Fairfull, R.W., Hansen, C., Panich, P.L., Shrestha, J.N. B. and Underhill, L. 1993.** The effect of warming velocity on motility and acrosomal integrity of boar sperm as influenced by the rate of freezing and glycerol level. *Mol. Reprod. Dev.,* 34: 190-195.
- Frunza, I., Cernescu, H., and Korodi, G., 2008.** Physical and chemical parameters of boar sperm. *Lucraristiintificemedicinaveterinaratimisoara.* 41: 634-640.
- Gadea J, García-Vazquez F, Matás C, Gardón JC, and Cánovas S, 2005.** Cooling and freezing of boar spermatozoa: supplementation of

- the freezing media with reduced glutathione preserves sperm function. *Journal of Andrology* 26: 396-404
- Gagnon C., Iwasaki A., De Lamirande E., and Kovalski N., 1991.** Reactive oxygen species and human spermatozoa. *New York Academy of Science.*; 637: 436-444.
- Gibson D. G., Young L., Chuang R. Y., Venter J. C., Hutchison C. A 3rd, and Smith H.O. 2010.** Enzymatic assembly of DNA molecules up to several hundred kilobases. *Natural Methods.* (5):343-5.
- Gil, J., Rodriguez-Irazaqui, M., Lundeheim, N., Soderquist, L., and Rodriguez-Martinez, H., 2003.** Fertility of ram semen frozen in bioexell and used for cervical artificial insemination. *Theriogenology.* 59:1157-1170
- Goyal A., Chopra M., Lwaleed B.A., Birch B., and Cooper A.J., 2007.** The effects of dietary lycopene supplementation on human seminal plasma. *Bju Int.*, 99: 1456-1460.
- Gutteridge, M.C. and Worthington, H.E.C. 1980.** The in vitro neutralisation characteristics of oleaginous antacid suspensions. *Journal of pharmacy and pharmacology*, 32: 62p-63p.
- Hinsch, E., Hinsch, K.D., Boehm, J.G., Schill, W.B., and Mueller-Schloesser, F., 1997.** Functional parameters and fertilization success of bovine semen cryopreserved in egg yolk free and egg yolk containing extenders. *Reproductive Domestic Animal.* 32:143-149.
- Huang, K. H., Ravindran, V., Li, X., Ravindran, G., and Bryden, W.L., 2007.** Apparent ileal digestibility of amino acids in feed ingredients determined with broilers and layers. *Journal of Science. Food Agriculture*, 87 (1): 47-53.
- Jacques, V.R., J.E. Adriaan, E. Anna-Mart and F.T. Eugene, 2008.** Health benefits of a natural carotenoid rich oil: A proposed mechanism of protection against ischaemia/reperfusion injury. *Asia Pacific. Journal of Clinical Nutrition.*, 17(S1): 316-319.
- Johnson, L.A., Weitze, K.F., Fiser, P. and Maxwell, W.M. C., 2000.** Storage of boar semen. *Animal Reproduction Science.* 62: 143-172.
- Levis, D.G. 2000.** Liquid boar semen production: current extender technology and where do we go from here? Proceedings of iv international conference on boar semen preservation, Betsville, 121-128.
- Lien, A. P. H., Hua and P. Chuong, 2008.** Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science.*, (4)2: 89-96.
- Mangiagalli M.G., Martino P.A., Smajlovic T., Guidobono Cavalchini L., and Marelli S.P., 2010.** Effect of lycopene on semen quality, fertility and native immunity of broiler breeder. *British Poultry Science.*, 51: 152-157.
- Martin Rillo S., Shokouhi V., Garcia Boix E., Hernandez Gil R., Romero L., 1996.** Contamination of semen doses and its possible relationship with the bacterial flora of the prepuce. 15th IPVS Congress. 3, p 60.
- Martino P.A., Mangiagalli M.G., Marelli S.P., Smajlovic T., and**

- GuidobonoCavalchini L. 2006.**Effect of lycopene on semen bacteriological status and native immunity in roosters. *World Poultry Science Journal.*, XII European Poultry Conference, Verona, Italy. 62 Supplement.: 415.
- Mendiola J., Torres-Cantero A.M., Vioque J., Moreno-Grau J.M., Ten J., Roca M., Moreno-Grau S., andBernabeu R. 2010.** A low intake of antioxidant nutrients is associated with poor semen quality in patients attending fertility clinics. *FertilitySterility*, 93: 1128-1133.
- Møller, A.P., Biard C., Blount J.D., Houston D.C., Ninni P., Saino N., and Surai P.F. 2000.**Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian Poultry Biology Review.*, 11: 137-159.
- Nguyen, M.L., and Schwartz S.J. 1999.** Lycopene: chemical and biological properties. *Food Technology*, 53: 38-45
- Perkins-Veazie P., Collins J. K., Edwards A., Wiley E., Clevidence B. 2003.** Watermelon: rich in the antioxidant lycopene. *ActaHorticulture.*, 628: 663-668.
- Peter, F.S., 2007.** Natural Antioxidants in Poultry Nutrition: New Developments. 16th European Symposium on Poultry Nutrition, pp: 669-676. Retrieved from:<http://www.cabi.org/animalscience/uploads/file/animalscience/additionalfiles/wpsastrasbourggaug2007/106.pdf>
- Purdy, P.H.R.,Knox, W., Singleton, K., Spencer,S.F., Spiller, T., Stewart, I., Tharp, N., Welsh, C.S., and Blackburn, H.D.,2008.** Preservatives on cryopreservation, quality control and artificial insemination of frozen-thawed boar sperm in a natural repository. *Symposium proceedings, mid west boar and manager conference*, August 7-8, St Louis, MO, USA.
- Rao A.V., Ray M.R., and Rao L.G. 2006.** Lycopene. *Advanced Food Research.*, 51: 99- 164.
- Rao A.V., and Rao L.G. 2007.** Carotenoids and human health. *Pharmacology Research.*, 55: 207-216.
- Rigau, T., Piedrafita, J., Reverter, J., Canal, M., Rodriguez-Gil, J.E.,1996.** The rate of l- lactate production, A feasible parameter for the fresh diluted boar semen quality analysis *Animal Reproductive Science* 43:161-172.
- Roca, J., Vasquez, J.M., Gil, M.A., Cuello, C., Parrila, I., and Martinez, E.A., 2006.** Challenges in pig artificial insemination. *Reproductive. Domestic. Animal* 41(2):43-53.
- Salamon, S., and Ritar, A.J.,1982.** Deep freezing of Angora goat semen: Effects of diluent composition and method and rate of dilution on survival of spermatozoa. *Australia. Journal of Biology Science* 35:295-303.
- SAS 2003,** Statistical Analysis System Version, 8.1, edition. North Carolina, SAS institute cooperation UK.
- Schabath, M.B., Grossman H.B., Delclos G.L., Hernandez L.M., Sue Day R., Davis B. R., Lerner S.P.,Spitz M.R.,Wu X. 2004.** Dietary carotenoids and genetic instability modify bladder cancer risk. *Journal of Nutrition*, 134: 3362-

- 3369.
- Tadmor Y., King S., Levi A., Davis A., Meir A., Wasserman B., Hirschberg J., Lewinsohn E. 2005.** Comparative fruit colouration in watermelon and tomato. *Food Research International.*, 38:837-841.
- Turk G., Atessahin A., Sonmez M., Yuce A., and Ceribasi, A.O. 2007.** Lycopene protects against cyclosporin A-induced testicular toxicity in rats. *Theriogenology*, 67: 778-785.
- Umesiobi, D.O. 2004.** Functional integrity of boar spermatozoa and sow fertility using raphia (*Raphia hookeri*) palm wine plus nche (*saccoglottis gabonensis*) Urban extender. *Journal of Applied Animal Research*. 26: 13-16.
- Umesiobi, D.O., Illoeje, M.U., and Berepubo, N.A., 1998.** A.I. in Southern Nigeria 2. Locally extended boar semen improves its fertility study. *Journal of Agric Rural Development*. 21: 98-106.
- Umesiobi, D.O., Illoeje, M.U., and Berepubo, N.A., 2002.** Artificial insemination in sows using Guelph and two local semen extenders. *Nigeria Journal of Animal Production*. 29: 121-126.
- Waberski, D. Weitze, K. F., Lietmann, C., Lübbert zur Lage, W., Bortolozzo, F.P., Willmen, T and Petzoldt, R., 1994.** The initial fertilizing capacity of longterm-stored liquid boar semen following pre- and postovulatory insemination, *Theriogenology*, Volume 41, 7, p 1367-1377,

Received: 10th September, 2021

Accepted: 10th December, 2021