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Research Article

The Effect of Different Levels of Lycopene in Egg Yolk Citrate Extenders on Post-Thawed Semen Qualities of Belgian Blue Crossbreed Bull

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Abstract | As many as seven 500-kg-Belgian-Blue-Crossbreed-bulls aged four years old were kept in a mooring cage and served with forage and concentrate (CP 16%) at 10% and 3% to the body weight, respectively. There were also added some egg yolk citrate diluent of 2.9% (v/v), Sodium citrate dihydrate buffer, 100 mL of distilled water, and an egg yolk mixture to a proportion of 20% egg yolk 8% (v/v) glycerol (Merck, Germany), penicillin (1,000 IU/mL), and streptomycin (1,000 mg/mL). Sperm was diluted to 50 million/mL and treated with lycopene extract levels of 0% (control), 1%, 2%, 3%, and 4% in a diluent, equilibrated at the temperature of 5˚C for 4 hours, packed in 0.25 ml straw, and refrozen into liquid nitrogen vapor about 5 cm above the liquid nitrogen surface for 10 minutes. Freezing was run by immersing the forage in liquid nitrogen for 24 hours before the post-thawing examination. Results showed that spermatozoa diluted with egg yolk citrate supplemented with 3% lycopene (P3) had better quality than that found in the other four treatments, namely motility before freezing (70.86%) and post thawing (53.00%); viability before freezing (76.00%) and post thawing (60. 14%); plasma membrane integrity before freezing (71.29%) and post thawing (59.86%); and also showed the lowest abnormality level before freezing and post-thawing, (17.29%) and (24.14%), respectively.

Keywords | Spermatozoa, Belgian blue crossbreed, Egg yolk citrate, Lycopene

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INTRODUCTION

Belgian Blue Crossbreed Bull (BB) is one of the breeds of Bos Taurus that has been developed in Belgium since 1850 (Hartatik et al., 2020). BB bulls have double muscles or carry muscular hypertrophy caused by some deletion of 11 nucleotides in the exon 3 part of the myostatin gene (Bintara et al., 2021). The crossbreed of BB Bull and Friesian Holstein (FH) shows an average weight cut of 533 kg with a carcass percentage of 56.70% (Bintara et al., 2021). Such advantages have made the Ministry of Agriculture of Indonesia choose to develop BB bulls to meet the high demand for beef in the country. The crossbreed was first developed in Indonesia in 2013 by PT KAR using the artificial insemination (AI) technique with Sumba Ongole cattle (SO). A similar program ran at the Livestock Embryo Center (BET) of Cipelang, West Java, and at Gadjah Mada University (Sapi Gama).

The latter crossbred BB with Brahman. The Belgian Blue Cross is more suitable to be developed in Indonesia because the birth weight is not too big. Eventually, artificial insemination (AI) provides superior Simmental seeds (Hartatik et al., 2020).

IB is a technique to insert thawed and pre-processed semen from male cattle into the female genital tract carried out by inseminators (skilled personnel) using insemination guns (Osman et al., 2012). Good sperm quality will increase the success of IB because frozen semen with good quality has a high percentage of motility and viability (Inanc et al., 2017). Its quality, however, can decrease due to such factors as processing, storage in containers, and the distribution of frozen semen. The decrease generally comes from the processing step, especially at the freezing stage. Freezing is a 2-step physical drying process, pre-freezing and freezing. The process gives cold shocks and intracellular changes related to the formation of ice crystals (Hussein et al., 2022).

The quality of semen decreases by about 10-40% during a freezing process (Peruma et al., 2011). The cooling degree is essential in the semen freezing process even though the cooling rate to maintain spermatozoa fertility is still unknown. The Regulation of the Director General of Livestock of the Republic of Indonesia Number: 12207/Hk.060/F/12/2007 has stated that the freezing duration in the pre-freezing stage is 5-9 minutes using N_2 liquid, and freezing takes 9 minutes. Lycopene is one of the dark-yellow to dark-red pigments of the carotenoid group (Collins et al., 2022). The carotenoid compounds bear high antioxidants essential to fight against free radicals caused by pollution and UV radiation. A carotenoid antioxidant is generally catalytic and effective in scavenging free radicals (Assaduzzaman, 2022). Lycopene antioxidants can protect membrane lipids and DNA of several cell types from damage caused by oxidative compounds in vitro (Kim and Park, 2022). The antioxidant content of lycopene can reduce the effect of oxidation that can damage and affect the lipid content in the cell membrane (Meng et al., 2021).

Considering the potential of lycopene as discussed, the researcher came up with an idea to use lycopene to maintain the spermatozoa quality of Belgian Blue Crossbreed Bulls (BB). The antioxidant should help protect spermatozoa from high oxidative stress during the storage process so that its viability, motility, and abnormality can be managed. This work will help increase the success of artificial insemination.

MATERIALS AND METHODS

This research was conducted in the Physiology and Reproductive Laboratory of the Faculty of Animal Science, Gadjah Mada University. The approval of the veterinary ethics committee in carrying out this study is not required because there is no invasive action on animals.

Media preparation

A total of seven Belgian Blue Crossbreed Bulls (Belgian Blue vs. Brahman) of 4 years old with a body weight of 500 kg were used in this study. They were kept in mooring cages and served with forage and concentrate (CP 16%) at 10% and 3% of the body weight, respectively. This research used egg yolk citrate as the sperm extender consisting of a buffer and an egg yolk. The buffer contains 2.9% (v/v) sodium citrate dihydrate and 100 mL distilled water. The buffer and the egg yolk were then mixed with a 20% yolk proportion, 8% (v/v) of glycerol (Merck, Germany), 1,000 IU/mL of penicillin, and 1,000 mg/mL of streptomycin/mL. The sperm was diluted to a final concentration of 50 million/mL.

Semen evaluation and processing

The sperm with extender received a lycopene extract level of 0% (0 mg/ml) (control), 1% (10 mg/ml), 2% (20 mg/ml), 3% (30 mg/ml), and 4% (40 mg/ml) in the egg yolk citrate extender. An equilibration process ran at 5C for 4 hours followed by the sperm packing into 0.25 ml straws. Pre-freezing was conducted by placing the straw in liquid nitrogen vapor about 5 cm above the surface of the liquid nitrogen for 10 minutes Freezing was conducted by immersing the straw in liquid nitrogen for 24 hours, after which post-thawing examination was performed by inserting the straw into the water at 37°C for 30 seconds. To know the effect, the rate of the semen quality was checked before freezing and post-thawing.

RESULTS AND DISCUSSION

The semen quality of the Belgian blue crossbreed before freezing was at the levels of lycopene of 3% and 4%. They were higher ($P<0.05$) than those with the dose of the other level (Table 1). Significant increases ($p<0.05$) were detected in the rate of semen motility, abnormality, viability, and plasma membrane integrity of post-frozen thawed sperm at the lycopene levels of 3% and 4% (Table 2).

The results of this study show a positive effect of the addition of lycopene on the motility and viability of spermatozoa of BB Bull. Lycopene is a lipophilic carotenoid with antioxidant and prooxidant properties (Babaei et al., 2022). The antioxidant activity of carotenoids is generally catalytic and effective at scavenging free radicals. The ability of lycopene has been shown to protect membrane lipids and DNA in several cell types from damage caused by oxidative compounds *in vitro* (Rakha et al., 2022).

Table 1: difference level of lycopene in the egg yolk citrate extender on semen quality motility, viability, abnormality, and integrity of membrane before freezing in Belgian blue crossbreed semen.

Treatment	Motility	Viability	Plasma membrane integrity	Abnormality
P0 (Without lycopene)	64.14±2.91[a]	70.57±1.99[a]	63.29±1.50[a]	18.43±2.44[a]
P1 (1% of lycopene)	65.00±1.63 [a]	72.43±2.07[a]	66.14±1.86[a]	16.43±2.23[a]
P2 (2% of lycopene)	67.00±3.06[a]	72.71±2.29[a]	68.71±1.60[a]	17.57±1.81[a]
P3 (3% of lycopene)	70.86±4.34[b]	76.00±2.65[b]	71.29±1.60[b]	17.29±1.80[b]
P4 (4% of lycopene)	68.00±2.24[b]	75.00±1.73[b]	69.43±1.99[b]	18.43±1.62[b]

Different superscripts along the column indicate the significant differences (P < 0.05).

Table 2: difference level of lycopene in the egg yolk citrate extender on semen quality motility, viability, abnormality, and integrity of membrane post thawing in Belgian blue crossbreed semen.

Treatment	Motility	Viability	Plasma membrane integrity	Abnormality
P0 (Without lycopene)	47.14±1.57[a]	54.43±1.27[b]	55.14±1.77[a]	25.57±2.07[a]
P1 (1% of lycopene)	49.14±2.27 [a]	56.43±2.23[a]	56.86±1.68[a]	21.29±2.69[a]
P2 (2% of lycopene)	51.43±1.27 [a]	58.43±2.37[a]	58.00±1.41[a]	22.86±2.12[a]
P3 (3% of lycopene)	53.00±3.11[b]	60.14±2.48[b]	59.86±1.95[b]	24.14±1.21[b]
P4 (4% of lycopene)	50.57±3.21[b]	58.14±1.95[b]	60.57±1.23[b]	23.00±1.83[b]

Different superscripts along the column indicate the significant differences (P < 0.05).

These results indicate the effect of lycopene on the motility of spermatozoa. It also shows that lycopene can help maintain sperm motility similar to the control conditions. As previously explained, oxidative stress can enter the cytoplasm and affect mitochondrial work in energy metabolism which is needed for the movement of spermatozoa (Khan et al., 2021). The addition of lycopene can offset the accumulation of ROS in spermatozoa to prevent ATP depletion, lipid peroxidation, and adequate-axonemal phosphorylation (Mangiagalli et al., 2010). So that the movement of the tail of the spermatozoa can run well and the motility of the spermatozoa is maintained (Ferramosca and Zara, 2022). Oxidative stress can also interfere with the polymerization and depolymerization of actin-myosin in the tail and this can further impair the movement of spermatozoa (Meng et al., 2021). The addition of lycopene can reduce the influence of oxidative stress so that the process of polymerization and depolymerization of actin-myosin can run well (Rakha et al., 2022). These conditions result in good and normal sperm motility (Zhao et al., 2022).

The results of this study are in line with several studies that used antioxidant compounds as in vitro supplements. Vitamin E can increase spermatozoa motility (Suleiman et al., 1996; Khan et al., 2021). This indicates the potential of using lycopene as an antioxidant additive to improve spermatozoa quality. The lycopene antioxidant compound can be used for therapy and supplements in males with poor sperm motility and males with unclear infertility.

Spermatozoa viability, DNA fragmentation, and abnormality can affect the success of fertilization (Prabowo et al., 2022). Viability and abnormality are parameters of spermatozoa quality (Heidari et al., 2019). A lycopene treatment process before freezing can cause changes in the quality of spermatozoa's viability and abnormalities (Collins et al., 2022). Table 1 shows the viability and abnormalities of spermatozoa from the four treatment groups. The results indicate that the third treatment (P3) with lycopene had the highest viability compared to the other control groups. Besides, the number of abnormalities in P3 was lower than in the other four treatments.

The decrease in spermatozoa viability was detected in the groups treated with lycopene, without lycopene, and in the control group after thawing. That viability decreases over time is a natural condition of spermatozoa (Gungor et al., 2022). Inwati et al. (2022) reported that spermatozoa viability decreased sometime after ejaculation along with the increase of the abnormalities. This is consonant with the results of this study that lycopene can protect spermatozoa cell components to maintain quality when in an environment with oxidative stress. Lycopene can raise the viability of spermatozoa by helping the membrane integrity to resist oxidative stress (Uysal and Bucak, 2007). The lipid membrane in the spermatozoa cell is most susceptible to oxidative stress. The integrity of the phospholipid structure can be disrupted with the presence of oxidative stress (Elsayed et al., 2021). Spermatozoa incubation in lycopene can result in the uptake of lycopene in the spermatozoa, and the highest accumulation occurs in membrane lipids. The accumulation of lycopene in membrane lipids causes a balance between oxidative stress and antioxidants (Inwati et al., 2022).

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NOVELTY STATEMENT

This study found the level of use of lycopene as an anti-oxidant ingredient in Belgian Blue crossbred spermatozoa diluents to protect against high oxidative stress during storage.

AUTHOR'S CONTRIBUTION

SB drafted the manuscript and conducted the literature search. SB conceived, performed the fieldwork, administrated, and helped with the manuscript. AA, PP, RNA conducted data interpretation and edited the manuscript. SB and AA designed and supervised the study. SB and PP performed the statistical analysis and reviewed the manuscript. SB and AA supervised the project. All authors read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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