Supplementation of Red Dragon Fruit Peel Extract (Hylocereus polyrhizus) in CEP-2 Extender on the Qualities of Limousin Bull Chilled Semen

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Abstract. The purpose of this study was to determine the effect of red dragon fruit peel extract (RDFPE) supplementation in CEP-2 extender to the qualities of Limousin semen during 3-5 °C storage. The semen was collected by using artificial vagina twice a week for 5 weeks. The treatments in this research were RDFPE supplementation at 0%, 1%, 3% and 5% on the CEP-2 base extender and the observed variables include semen motility and viability. The results showed that the addition of RDFPE in CEP-2 extender gave a significant effect (P<0.05) to the semen motility and viability. Supplementation of RDFPE at 3% showed better motility and viability than 0, 1 and 5%. The study concludes that the addition of 3% RDFPE was the best treatment in maintaining the quality of Limousin bull semen at cold temperature.

1. Introduction

Artificial Insemination (AI) is a reproductive technology aimed to improve the genetic quality of livestock [1]. Implementation of AI can be done by using whether fresh, chilled and frozen semen. Chilled semen is a more feasible approach to be applied in areas where liquid nitrogen supply is limited and/or liquid nitrogen containers are not available. One of the factors that influence the success of AI implementation is semen quality. Some research showed that the semen processing will reduce its quality [2]. Efforts that can be done to maintain the semen quality during cold storage is to improve the used semen extender quality. One of the developed semen extenders is Cauda Epididymal Plasma-2 (CEP-2). The use of CEP-2 and 20% egg yolk supplementation has shown to maintain the quality of Limousin bull semen [3]. However, the problem faced in semen cold storage is the damage to the spermatozoa plasma membrane due to the lipid peroxidation. In order to minimize the damage, antioxidants can be added to the semen dilution [4].

Red dragon fruit is known to rich of antioxidants, such as flavonoid [5]. Flavonoid compound is one of the antioxidant compounds that has the ability to overcome or neutralize free radicals. The widely used part of red dragon fruit is its flesh, while the peel of fruit, which is about 30-35% of the weight of the fruit is still underutilized, even though the antioxidant compounds are also known to be contained the peel. Moreover, the antioxidant activities in dragon fruit peels is higher than in the flesh of the fruit, so it has the potential to be developed as the source of natural antioxidants [6]. Red dragon fruit peel extract contains anthocyanin [7], a pigment that is classified as flavonoid to counteract free radicals [8]. The present study aims to evaluate the effect of red dragon fruit (Hylocereus polyrhizus) peel extract (RDFPE) supplementation in CEP-2 extender to the quality of Limousin chilled semen.
2. Materials and Method

2.1. Preparation of semen extender
Dragon fruit peel extraction was done by following Putri et al. [8]. The CEP-2 diluent consisted of 15 mmol/L NaCl; 7 mmol/L KCl; 3 mmol/L CaCl\(_2\)(H\(_2\)O); 4 mmol/L MgCl\(_2\)(H\(_2\)O); 11.9 mmol/L NaHCO\(_3\); 8 mmol/L NaH\(_2\)PO\(_4\); 20 mmol/L 3KH\(_2\)PO\(_4\); 55 mmol/L Fructose; 1 g/L Sorbitol; 2 g/L BSA (Sigma); 133.7 mmol/L Tris (Merck); 0.05 g/L Gentamicin; 42.6 mmol/L Citric acid. The ingredients are mixed to produce an osmolarity of 320 mOsm and set the pH at 6.6 [9]. The CEP-2 diluent is added with 20% fresh egg yolk.

2.2. Semen collection and treatment
The Limousin bull semen was collected by using an artificial vagina (Neustadt/Aisch, Müller, Nürnberg, Germany) twice a week for 5 weeks. The semen was diluted at 1:10 ratio for all treatments. After dilution, the was refrigerated at 5 °C and evaluated at 0, 24, 48, 72, 96 and 120 hours. The treatments were as follow: T0 (10 ml CEP-2 without RDFPE supplementation), T1 (10 ml CEP-2 + 0.1 ml RDFPE), T2 (10 ml CEP-2 + 0.3 ml RDFPE), and T3 (10 ml CEP-2 + 0.5 ml RDFPE). Each treatment was replicated for 10 times. The study is done in a completely randomized design.

2.3. Sperm quality assessment
The progressive sperm motility was subjectively rated between 0 to 100% [10]. The viability and morphology evaluation were done by placing a drop of semen on a warm glass slide, and added with one drop of dual-staining Eosin-Negrosin. The solution was then mixed gently and viewed under the microscope at 400x magnification. The dead sperm cells would absorb the stain while live sperm cells would not, and a total of 200 sperm were examined in each sample [11].

2.4. Statistical analysis
The obtained data were analyzed by using the analysis of variance (ANOVA) and followed with Duncan's New Multiple Range Test (DMRT) to determine the variations among different level of RDFPE supplementation. The statistics were done by using SPSS Statistic application version 13.0.

3. Results and discussions
The effect of RDFPE supplementation on the progressive motility (%) of Limousin bull semen is shown in Table 1. At 0-hour, the mean progressive motility for 1% and 5% were not significantly (P>0.05) different from 3%, but significantly different (P<0.05) from 0% RDFPE supplementation. Furthermore, the progressive motility percentage showed a positively correlation to the RDFPE concentration and decreased along with storage time. Previous research also indicated that there was an effect of extender and storage time to the semen progressive motility [12]. Similar with the finding of this research, Muhammad et al. [13] also showed that semen progressive motility was decreased during storage time.

The sperm motility and viability demonstrated a gradual decrease during 120-hours cold storage, which was correlated with the storage time (Table 1 and Table 2). The sperm viability is associated with the intactness of sperm membrane. The addition of RDFPE in CEP-2 base extender might protect the sperm against lipid peroxidation. Spermatozoa motility and fertilization can be improved by antioxidants addition. The antioxidants would neutralize the free radicals and reduce the risk of spermatozoa damage during cryopreservation [14].

The content of dragon fruit peel extract is able to prevent and suppress spermatozoa damage due to the influence of free radicals [5]. Previous studies on the red dragon fruit peel extract [15] showed total phenolic content (31.12 ± 1.56 mg GAE/100 g), inhibitory activity against DPPH (72.94 ± 0.77%), and antioxidant capacity (321.78 ± 6.29 mg EVC/100 g).
Table 1. The effect of RDFPE supplementation on Limousin bull semen progressive motility (%)

| Time (hours) | RDFPE supplementation |
|--------------|-----------------------|
|              | 0%                    | 1%          | 3%          | 5%          |
| 0            | 75.25±5.22<sup>a</sup> | 75.50±5.1<sup>a</sup> | 75.12±6.51<sup>a</sup> | 75.12±2.72<sup>a</sup> |
| 24           | 65.12±5.51<sup>a</sup> | 68.12±5.9<sup>b</sup> | 70.50±2.30<sup>c</sup> | 68.50±1.68<sup>b</sup> |
| 48           | 58.88±5.30<sup>b</sup> | 56.25±3.18<sup>a</sup> | 68.52±5.43<sup>d</sup> | 62.50±2.88<sup>c</sup> |
| 72           | 50.57±3.20<sup>a</sup> | 50.15±2.59<sup>a</sup> | 63.75±4.31<sup>c</sup> | 58.62±1.20<sup>b</sup> |
| 96           | 45.25±2.80<sup>a</sup> | 48.25±2.56<sup>b</sup> | 60.45±3.27<sup>d</sup> | 55.45±3.33<sup>c</sup> |
| 120          | 40.45±2.35<sup>a</sup> | 43.15±2.75<sup>b</sup> | 57.50±3.35<sup>d</sup> | 52.34±2.88<sup>c</sup> |

Means along the same row with different superscripts indicate significant differences (P<0.05)

Figure 1. The effect of RDFPE supplementation on Limousin bull semen progressive motility (%)

Table 2. Effect of RDFPE on viability (%) of Limousin bull semen

| Time (hours) | RDFPE supplementation |
|--------------|-----------------------|
|              | 0%                    | 1%          | 3%          | 5%          |
| 0            | 85.54±3.06            | 83.05±4.70  | 85.61±2.60  | 85.91±3.34  |
| 24           | 80.17±3.39<sup>a</sup>| 79.11±4.94<sup>a</sup> | 83.59±3.27<sup>c</sup> | 80.59±3.34<sup>a</sup> |
| 48           | 70.68±4.51<sup>a</sup>| 68.06±3.55<sup>a</sup> | 78.84±5.65<sup>c</sup> | 74.40±4.38<sup>b</sup> |
| 72           | 66.84±3.37<sup>b</sup>| 60.46±3.62<sup>a</sup> | 73.36±4.62<sup>d</sup> | 69.27±4.75<sup>c</sup> |
| 96           | 60.25±4.41<sup>b</sup>| 57.35±4.54<sup>a</sup> | 68.24±4.31<sup>d</sup> | 63.35±3.89<sup>c</sup> |
| 120          | 52.76±5.23<sup>a</sup>| 53.87±4.55<sup>a</sup> | 63.89±4.89<sup>b</sup> | 53.99±3.79<sup>a</sup> |

Means along the same row with different superscripts indicate significant differences (P<0.05)
Figure 2. The effect of RDFPE supplementation on Limousin bull semen viability (%)

Table 1 and 2 showed that RDFPE supplementation at 1% and 5% had lower semen quality compared to 3% RDFPE supplementation. The antioxidants supplementation at the right dosage would provide effective lipid peroxide inhibition due to the free radical activities in the spermatozoa plasma membrane [16]. Decreased motility and viability of spermatozoa at 5% RDFPE supplementation was allegedly due to the excess of RDFPE supplementation, thus induced other substances such as prooxidant and anti-nutritional substances in the red dragon fruit peel. Research by Ullah et al. [17] also showed similar results, with higher α-tocopherol dosage as an antioxidant is associated with the deleterious impact on sperm viability and functionality during cryopreservation. However, the exact functional mechanism of a high dose of antioxidants in the semen extender with detrimental impact is yet to be clarified.

4. Conclusion
Supplementation of 3% red dragon fruit peel extract in CEP-2 extender showed the best results in maintaining the quality of Limousin bull semen at cold temperature.

Acknowledgements
The authors thank the Balai Besar Inseminasi Buatan Singosari–Malang, Indonesia for supplying the semen samples and providing the facilities of this research.

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