Supplementation of Arabian jujube (*Ziziphus spina Christi*) leaf extracts as extender material on the quality of Bali bull semen

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Abstract. Semen is subject to experience a decrease in the quality including motility of the sperms. Decreasing the quality of semen begins during collecting process and continues at the processing stage. Low quality of semen will have an impact on the low fertilization rate, so that efforts are needed to maintain the quality of semen. This is thought to be caused by oxidative reactions that are triggered by free radical activity. Some active ingredients in the Arabian jujube leaf are thought to function as natural antioxidants. This study aimed to determine the effect of supplementation of Arabian jujube leaf extracts (*Ziziphus spina Christy*) on sperms motility in an effort to maintain the quality of Bali bull fresh semen. This study was conducted at the Laboratory of Animal Reproduction; Semen Processing Unit, Faculty of Animal Science, Hasanuddin University, Makassar. The Arabian jujube leaf extracts was added in the extender at three different treatments; P1 (1%), P2 (3%), P3 (5%) and P0 (control). The results of this study showed that there were different motility of the sperms after supplementing the extender with Arabian jujube leaf extracts. The sperms motility of Bali bull semen at concentration of 5% during five days equilibration at 5ºC had higher than 3%, 1%, and control, respectively (50.0 vs. 48.7, 43.8, and 46.3%). It can be concluded that supplementation of Arabian jujube leaf extract in the extender material could maintain the quality of Bali bull semen.

1. Introduction

Efforts to increase the population of Bali cattle continue to be conducted in various ways, one of them is the application of reproductive technology. Artificial Insemination (AI) is the most widely used reproductive technology in Indonesia. Some things that can affect the success rate of AI include inseminator staff skills, detection of heat, female condition as well as the quality of semen used. Decrease in semen quality of the bulls begins with the collecting process and continues at the processing stage. High sperms mortality in the semen processing according to Herdis [1] is caused by damage to the sperms plasma membrane due to lipid peroxide. Maxwell and Watson [2] also stated that sperms deaths occur due to the spermatozoa membrane contains a lot of unsaturated fats that are susceptible to lipid peroxidation reactions. Lipid peroxide reactions that can damage spermatozoa in the process of processing semen occur because of contact between semen and oxygen (O₂). The process can produce free radicals and hydrogen peroxide. Free radicals when reacting with unsaturated fatty acids will produce lipid peroxide. The highest amount of antioxidants is in foods sourced from vegetables Oguntibeju et al. [3].
The low quality of semen can affect the success of AI, which in turn will cause low fertilization. Thus natural antioxidants are needed in an effort to maintain the quality of semen. Some of the active ingredient in the leaves of Arabic Jujube (Ziziphus spina Christi) is thought to function as a natural antioxidant. This study aimed to determine the effect of supplementation of Arabic Jujube (Ziziphus spina Christi) leaf extract in an effort to maintain the quality of semen in Bali bull.

2. Materials and methods

2.1. Materials
This study was conducted at the Laboratory of Animal Reproduction Semen Processing Unit, Faculty of Animal Science, Hasanuddin University, Makassar. The material used in the study was a five years old Bali bull semen. The other materials included andromed, tris hydroximethyl aminomethane, citric acid, glucose, penicillin, streptomycin, glycerol, aquabidest, egg yolk and the extracts of Arabic Jujube leaf (Ziziphus spina Christy).

2.2. Methods
Before extraction, Arabic Jujube leaf (Ziziphus spina Christy) was firmly cleaning from dirt or dust. Furthermore, it was aerated in open spaces in which avoided from direct sunlight. After the leaves were considered to have dried, the next step was maceration. Macerated samples were then separated the active compounds using 96% ethanol for three times then evaporated.

In the present study, leaf extract supplementation was divided into three treatments in the extender (Tris-egg-yolk). The treatments were: P1 (Tris-egg-yolk + 1% of Arabic Jujube leaf extract), P2 (Tris-egg-yolk + 3% of Arabic Jujube leaf extract), P3 (Tris-egg-yolk + 5% of Arabic Jujube leaf extract) and P0 (Andromed as treatment control) with five replications. The parameters measured in this study were motility of the sperms before and after equilibration at 5ºC. Furthermore, observations of sperms motility were continued on the second to fifth day.

2.3. Statistical analysis
The data obtained were analyzed with a completely randomized design (CRD) factorial pattern of 2×2.

3. Results and discussion

3.1. Characteristics of fresh semen
The results of holding the semen for five replications obtained an average volume of 5 mL. Macro observations show the concentration of thick semen, white colour and pH 6. The quality of fresh semen obtained is generally considered to be quite good. The results obtained are in accordance with the opinion of Hafez [4] that cows cumulate fresh semen with a minimum volume of 4 mL.

3.2. Spermatozoa motility
The motility value obtained at each shelter is quite varied with an average value of 83.8%. This result is in accordance with the opinion of Hafez [4] that the sperms motility value in cattle semen which is considered good, is around 50-80%. Fluctuations in the value of motility obtained at each reservoir may be caused by the quality of the feed provided. Fathul et al. [5] stated that feed is one of the factors that can affect the quality of semen.

Percentage of sperms motility of Bali bull with the addition of Arabic Jujube leaf extract (Ziziphus spina Christy) to Tris Egg yolk extender before and after equilibration is presented in table 1. The results showed that the average value of motility obtained before equilibration was still high enough, as presented in table 1; 83.8%. This means that the condition of the semen is still feasible to be processed to the next stage. After being given supplementation treatment of Arabic Jujube leaf extract (Ziziphus spina Christy) with different concentrations, it was seen that a decreased percentage of spermatozoa motility was different in all treatments and observation time.
Table 1. Average motility of spermatozoa with different equilibration times

| Treatments | Day Motility TO - (%) |
|------------|----------------------|
|            | I        | II       | III      | IV       | V        |
| P0         | 83.8     | 64.0     | 57.3     | 50.9     | 45.3\(^a\) |
| P1         | 83.8     | 61.4     | 55.3     | 48.0     | 43.8\(^a\) |
| P2         | 83.8     | 64.6     | 58.9     | 51.6     | 45.8\(^a\) |
| P3         | 83.8     | 70.4     | 64.5     | 58.1     | 500\(^b\)  |

\(^a-b\) Different letters in the same column show significantly different (P < 0.05).

Analysis of Variance (ANOVA) test results showed that supplementation of Arabic Jujube leaf extract (Ziziphus spina Christi) significantly affected (P <0.05) on the motility of individual sperms. The treatment (P3) showed highest motility after equilibration (50.0%) on the fifth-day observations compared to control, (P0), P1 and P2.

The results of this study are in line with Sabile et al. [6] that the supplementation of noni fruit extract (Morinda citrifolia Linn) in tris egg-yolk extender had a significant effect (P<0.05) on the motility of individual spermatozoa. The results of another study by Wibisono [7] showed that giving vitamin C as an antioxidant up to a dose of 0.20 mg/body weight/day in cattle can reduce the number of sperms that have been damaged by free radicals.

The ability of Arabian jujube extract (Ziziphus spina Christi) in maintaining sperms motility is caused by the presence of antioxidant reactions contain in, in which can inhibits the damage of spermatozoa membrane due to free radicals in the oxidation process during the semen processing. This is in line with the opinion of Herdis [1] that the high spermatozoa mortality in the semen processing is caused by damage to the spermatozoa plasma membrane due to lipid peroxide.

In addition, the effect of Arabian jujube leaf extract supplementation on tris egg-yolk extender may be caused by the presence of nutrients contained in, so that it can meet the energy requirements of spermatozoa and limits the decreased motility of sperms at each phase during semen processing. This is in line with the opinion expressed by Setiadi and Julizar [8] stated that one of the causes in decreasing individual motility in the semen processing is the reduction in energy-producing substances for sperms.

4. Conclusion

The supplementation of Arabian Jujube extracts leaves (Ziziphus spina Christi) with a concentration of 5% in the extender material could maintain the quality of Bali bull semen.

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