

Effect of *Indigofera* sp. leaf extract supplementation on sodium chloride diluter to the semen quality of native rooster

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Abstract

The aim of this research is to determine the sperm quality of native rooster after diluting with sodium chloride supplemented with *Indigofera* sp. leaf extract. Thirty mature native roosters with average body weights of 2.63 ± 0.12 kg and average length of spurs of 0.72 ± 0.03 cm were used in this research. Two treatments were applied, namely: T0 - NaCl Diluter, and T1 - NaCl Diluter supplemented with 1% *Indigofera* sp. leaf extract. Massage technique was used to collect semen. Fresh semen parameters observed were macroscopic (volume, pH, color, viscosity, smell), and microscopic (mass motility, sperm concentration, motility and abnormality). Parameters of diluted semen observed were motility, abnormalities and livability of spermatozoa. Data was analyzed through non-parametric Wilcoxon signed rank. The average volume of fresh semen was 0.24 ± 0.14 ml, with spermin smell, yellowish white colour, viscous, and average pH of 7.25 ± 0.15 . Fresh semen also showed average concentration of $7250.00 \pm 156.25 \times 10^6$, mass motility of 2.00 ± 0.00 , progressive motility of $68.00 \pm 10.01\%$ and sperm abnormality of $8.33 \pm 2.57\%$. After diluting, the livability of spermatozoa was 15 and 75 minutes for T0 and T1, respectively. The motility and abnormality spermatozoa for T0 in 15 minutes were 46.50% and 14.22%, respectively. For T1, in 75 minutes, motility and abnormality were 40.00% and 14.80%, respectively. Supplementation of *Indigofera* sp. leaf extract increased livability of spermatozoa and preserved the motility. Abnormalities in spermatozoa were unchanged.

Keywords: *Indigofera* sp. Leaf extract, semen quality, native rooster

Introduction

Reproduction technology in animal is one of the sectors that rapidly improved. Artificial insemination is one of the reproduction technology applied in animal. Unfortunately, in native chicken artificial insemination mostly used liquid semen. The used of frozen semen during artificial insemination in Native chicken was limited.

The used of liquid semen during artificial insemination need to controlled especially connected with motility, viability and abnormality of the spermatozoa. These parameters are connected with breeding value and fertility rate of Native roosters [1]. The quality of liquid semen is affected by time and also diluter. Longer time semen exposed to the room temperature will affected to the sperm motility, but the sperm quality also supported by quality of diluter. Function of diluter is increasing volume, supporting nutrition for spermatozoa and stabilizes the osmotic pressure of semen. The common diluter used for semen of native roosters is sodium chloride (NaCl). Used of NaCl as diluter showed livability lower than 60 minutes [2]. On other hand, commonly 60 minutes is time needed for artificial insemination on native chicken. Therefore, increasing quality of the diluter is needed to be done.

Research of Ondho et al. [3] used filtrated semen diluent enriched with *Indigofera* sp leaf extract for Etawah grade goat semen showed livability 379 minutes kn room temperature. This research can be applied to the semen of native roosters used NaCl enriched with *Indigofera* sp leaf extract. The aim of this research is to determine motility, viability and abnormality of the spermatozoa after diluted by sodium chloride-*Indigofera* supplementation.

Materials and Methods

Animals

Thirty mature native roosters with average body weight $2.63 \pm 0,12$ kg and average length of spurs $0,72 \pm 0,03$ cm were used in this research. The native roosters were rested for 2 weeks in individual cage before semen collection and fed with concentrate 18% crude protein.

Materials

Individual cage, feeder tray and drinking jar were used for rearing of native roosters. Eppendorf tube and pH universal indicator (range 6.8-8.3 with sensitivity 0.3) were used for semen collecting and macroscopic evaluation of fresh semen. Spuit 1 ml, beaker glass, Haemocytometer pipet, pipet, object glass, cover glass, hand tally counter, Neubauer chamber, eosin 2%, tissue, microscope and sodium chloride 0.9%, were used for microscopic evaluation. *Indigofera* sp leaf extract and sodium chloride 0.9% used as diluter in this research.

Methods

This research consists of 4 stages: 1). *Indigofera* sp. leaf extraction; 2). diluter preparation; 3). semen collecting and 4). data collecting. Three grams of *Indigofera* sp. leaves were added with 100 ml aquabidest and then blend them to become pulp. Centrifuge the pulp on 7,500 rpm for 10 minutes. Collect the supernatant to the tube and centrifuge again until clear. Sucking the *Indigofera* sp. leaf extract used syringe and attached filtration 0,22 μ m on the tip of the syringe. Collect the filtrated *Indigofera* sp. leaf extract to the closed bottle and stored in the refrigerator 3-5 oC. Two treatment was applied in this research namely T0 : Diluter NaCl and T1 : Diluter NaCl supplemented

by 1% *Indigofera* sp. leaf extract. Massage technique were used for semen collection of Native roosters. Parameters observed were macroscopic (volume, pH, color, viscosity, smell) and microscopic (mass motility, sperm concentration, motility and abnormality) of fresh semen. Parameters of liquid semen observed were motility, abnormality and livability of spermatozoa.

Data Analysis

Data was analyzed by statistic non parametric Wilcoxon signed rank. Significance level 5% used in this research. Data analysis was support by use of Statistical Product and Service Solutions 25 (SPSS 25).

Results and Discussions

Fresh Semen Quality

The results of macroscopic and microscopic fresh semen evaluation to the research unit showed in Table 1. Macroscopic and microscopic fresh semen quality showed the normal range for all parameters.

Table 1. Macroscopic and Microscopic Fresh Semen Quality of Native Roosters in The Research

Parameter	Average Value
Macroscopic Semen Quality	
Volume, ml	0.24 ± 0.14
Smell	Spermin
Colour	Yellowish White
Viscosity	Viscous
pH	7.25 ± 0.15
Microscopic Semen Quality	
Sperm Concentration, million/ml	7250.00 ± 156.25
Mass Motility	2.00 ± 0.00
Sperm Motility, %	68.00 ± 10.01
Sperm Abnormality, %	8.33 ± 2.57

Based on the macroscopic and microscopic fresh semen quality of native roosters in this research is feasible for processing of liquid semen. Semen volume minimum for liquid semen production is 0.10 ml [4]. The normal pH semen for production of liquid semen chicken is 7.0-7.5 [5].

Sperm Motility of Native Rooster Diluted by NaCl and NaCl supplemented by 1% *Indigofera* sp. leave extract (ILE)

The motility of Native roosters spermatozoa in this research after diluted by different diluter (NaCl and NaCl +1% ILE) is shown in Figure 1. Sharply declining of native rooster sperm motility was shown on used of NaCl diluter compare with NaCl + 1% ILE.

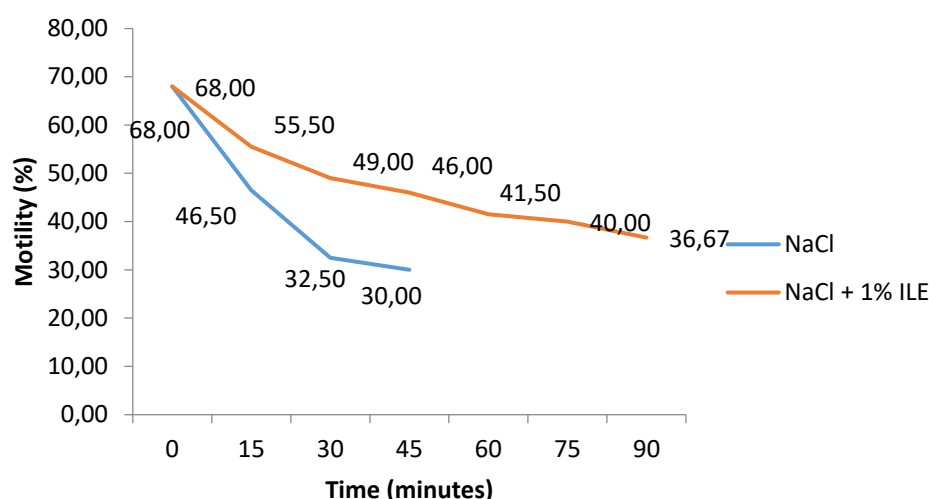


Figure 1. Dynamic of sperm motility of Native rooster diluted by NaCl and NaCl supplemented by 1% *Indigofera* sp. leave extract (ILE)

Non parametric statistic of Wilcoxon signed rank showed significance different ($P < 0.05$) of Native roosters spermatozoa motility on 15, 30 and 45 minutes. The used of diluter NaCl supplemented by 1% of *Indigofera* leaf extract (NaCl + 1% ILE) was able to maintain spermatozoa motility better than diluter NaCl. Diluter NaCl supplemented by 1% of *Indigofera* leaf extract (NaCl + 1% ILE) maintain the sperm motility of native rooster up to 75 minutes (under 40%). Supplementation ILE will support the deceleration peroxidation lipid of spermatozoa. ILE will support antioxidant to reduce the lipid peroxidation. *Indigofera* sp. contains flavanoid as antioxidant to support the sperm motility [6,7]. Sperm motility is needed energy supplied by semen plasm. The reaction of energy used will produce reactive oxygen species (ROS) that increased peroxidative lipid [7]. Supply antioxidant from *Indigofera* sp. will linkage with ROS to reduce peroxidative lipid. Reducing peroxidative lipid will keep the intact of membrane plasma and ensure the sperm motility.

Sperm Abnormality of Native Rooster Diluted by NaCl and NaCl supplemented by 1% *Indigofera* sp. leave extract (ILE)

The sperm abnormality of Native roosters in this research after diluted by different diluter (NaCl and NaCl +1% ILE) is shown in Figure 2. Sharply increasing of native rooster sperm abnormality was shown on used of NaCl diluter compare with NaCl + 1% ILE.

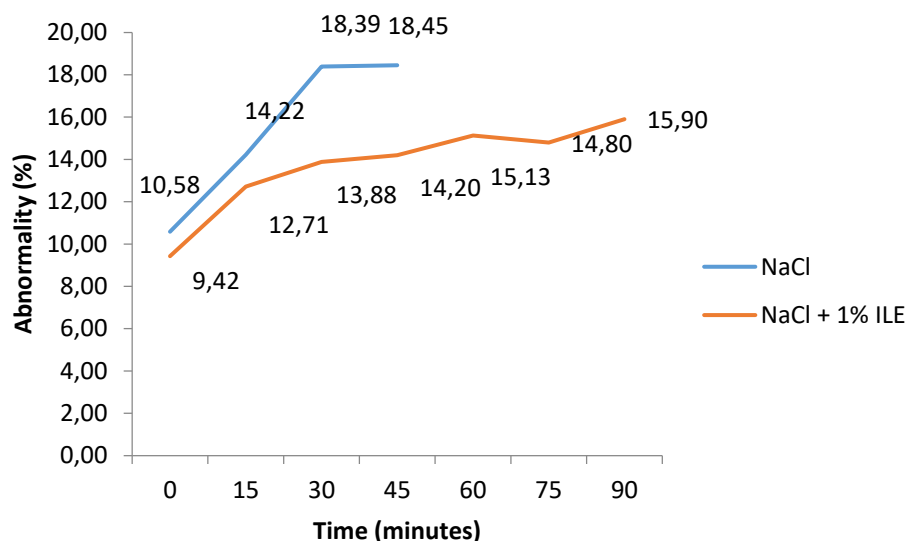


Figure 2. Dynamic of sperm abnormality of Native rooster diluted by NaCl and NaCl supplemented by 1% *Indigofera* sp. leave extract (ILE)

Non parametric statistic of Wilcoxon signed rank showed significance different ($P < 0.05$) of Native roosters spermatozoa abnormality on 15, 30 and 45 minutes. The used of diluter NaCl supplemented by 1% of *Indigofera* leaf extract (NaCl + 1% ILE) was able to maintain spermatozoa abnormality better than diluter NaCl. Supplementation of 1% ILE to the NaCl will maintain the spermatozoa abnormality of native rooster.

Some secondary sperm abnormality found is wrinkles and giant head. The secondary sperm abnormality of wrinkles and giant head is affected by decreasing permeability membranes. Supplementation 1% ILE will support the decreasing peroxidation lipid of spermatozoa. ILE will support antioxidant to reduce the lipid peroxidation. *Indigofera* sp. contains antioxidant to support the intact and permeability membrane [3]. Supply antioxidant from *Indigofera* sp. will linkage with ROS to reduce peroxidative lipid. Reducing peroxidative lipid will keep the intact of membrane plasma and maintain the sperm abnormality.

Sperm Livability of Native Rooster Diluted by NaCl and NaCl supplemented by 1% *Indigofera* sp. leave extract (ILE)

The sperm livability of Native roosters in this research after diluted by different diluter (NaCl and NaCl +1% ILE) is shown in Table 2. Sharply increasing of native rooster sperm motility was shown on used of NaCl diluter compare with NaCl + 1% ILE.

Table 2. Sperm livability of Native Roosters in The Research diluted by NaCl and NaCl supplemented by 1% *Indigofera* sp. leave extract (ILE)

Treatment	Average Value
	 minutes
NaCl	15
NaCl +1% ILE	75

Based on the data of sperm motility and abnormality from this research the sperm livability of native rooster semen with supplementation 1% ILE is higher than NaCl itself. Supplementation of 1% ILE on diluter semen will maintain Sperm livability of native rooster semen up to 75 minutes. This result is higher than the research of Baguio and Capitan [8] that used diluter Tris amino methane for semen of philippine native chicken that showed livability 60 minutes.

Conclusion

Supplementation of *Indigofera* sp. leaf extract increased livability of spermatozoa and preserved the motility. Abnormalities in spermatozoa were unchanged.

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