



## The Spermatozoa Quality of Kampong Chicken Using Various Diluents

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**Abstract :** *Kampong chicken has great potential to be developed into superior breed in an effort to support food security and improve farmer welfare. This study aims to determine the effect of adding lactose to free-range chicken semen macroscopically and microscopically. This research was conducted at the Laboratory of the Center for Artificial Insemination at the Food Security and Animal Husbandry Service of North Sumatra Province in October 2020 - January 2021. This study used a Randomized Block Design method with treatment P0 ( Semen control) P1 (Semen with Ringers diluent), P2 (Semen with diluent lactose), P3 (Semen with Ringers diluent + Lactose), macroscopic test and parameters include color, volume, concentration and pH of semen. To determine the quality of spermatozoa can be seen from the motility test, abnormality and viability. The results showed that the use of Ringer + lactose diluent in chicken semen stored for 8 hours at 40C during the study had a very significant effect on spermatozoa motility with values: P0 (52.88% a), P1 (21.64% bc), P2 (31.39% b), P3 (19.57% c) respectively and had no significant effect on abnormalities of spermatozoa with values: P0 (6.59% tn), P1 (6.11% tn), P2 (7, 36% tn), P3 (4.4% tn) respectively. As conclusions that the use of ringers + lactose diluent can work well to defend spermatozoa from damage to cell membranes resulting from the elements contained in ringers and lactose solutions which can be used as an energy source when the semen is cold shock during the storage period.*

**Keywords:** *chicken, lactose, ringers, semen, spermatozoa*

### 1. Introduction

Maintenance efficiency can be done if the comparison can be minimized and Artificial Insemination (AI) becomes the solution. Maintenance problems also arise when the rooster prefers only one female which results in competition between females causing many unmarried females. [1].

The diluent is used to increase the volume of semen in one ejaculation which can be used for Artificial Insemination of several females. The diluent also serves as storage for some time with the aim of maintaining the quality of spermatozoa to remain good [2].

Efforts to maintain the fertility of spermatozoa can be done in two ways, namely by adding diluents that can guarantee the physical and chemical requirements of spermatozoa and storage at certain conditions and temperatures that can maintain their quality. [3].

Diluents that can be used, such as Ringers' solution or physiological sodium chloride (NaCl), can be used as semen diluents because they can maintain the motility of spermatozoa outside the chicken's body for up to 12 hours after storage, Ringer's solution consists of various mineral salts that have a pH buffering power (buffer) and isotonic which can support the motility of spermatozoa for a longer time. Chicken semen contains electrolyte elements in the form of hydrochloric acid, calcium, potassium, sodium and magnesium. Ringer's lactate solution contains the same Sodium Chloride as electrolyte elements from chicken semen plasma such as sodium, chloride, calcium and magnesium. [4].

Normal spermatozoa cells consist of a head, middle neck (body) and tail that do not deviate. Some forms of deviation from morphology are considered abnormalities, including spermatozoa cells with giant or dwarf heads, double heads, coiled tails, spermatozoa without heads or tails. [5].

Longer storage at a temperature of 5°C to 10°C spermatozoa cells exposed to cold shock will die faster. This is due to the increasing number of damaged and dead spermatozoa due to cold temperatures, the availability of energy in the diluent will decrease. [6].

## **2 Materials and Methods**

### **2.1 Research methods**

The study was carried out experimentally using a randomized block design with 4 treatments and 4 replications. The treatments were as follows:

P0 : Semen control

P1 : Semen with Ringers diluent

P2 : Semen with lactose diluent

P3 : Semen with Ringers diluent + Lactose

### **2.2 Data Analysis Methods**

The data obtained were analyzed based on analysis of variance (Analysis of Variance). If there is an effect of treatment then further testing will be done, if obtained data results that are very real or real then continued with the Duncan test.

## 2.3 Observed Variables

### a. *Spermatozoa Motility*

Is the progressive motility of spermatozoa. Progressive motility of spermatozoa is very important because without good motility, spermatozoa will not succeed in fertilizing the egg.

### b. *Abnormality*

Spermatozoa abnormalities were obtained by counting abnormal spermatozoa by making smear preparations and observed under a microscope with a magnification of 10 x 40. The method for calculating spermatozoa abnormalities was the number of abnormal spermatozoa divided by 200 visible spermatozoa multiplied by one hundred percent.

### c. *Viability*

Is the life force of spermatozoa. Spermatozoa viability examination can be used as an indicator of the structural integrity of spermatozoa cell membranes.

## 3 Results and Discussion

### 3.1 *Fresh Semen Quality*

**Table 1.** The Average Results of the Evaluation of Fresh Semen of KampungChicken

Characteristics of Fresh Semen	Average
Initial Volume (ml)	0,90
Colour	Putih
pH	7,5
Concentration (10 <sup>6</sup> /ml)	3462,5
Mass Movement	++ s/d +++
Motility (%)	86,53
Abnormality (%)	6,82

Based on the results of the Spermatozoa volume obtained from the study as much as 0.90 ml and pH 7.5 included in the normal category. The fresh spermatozoa produced are white with a thick consistency which means the spermatozoa are of good quality [7; 8]. The fresh spermatozoa produced had an average concentration of 3,462.5 x 10<sup>6</sup> cells/ml.

### 3.2 Chicken Spermatozoa Motility Quality Test

Table 2. Evaluation of Spermatozoa Motility Mean Before and After Storage (%)

Treatments	Group				Total	Average
	1	2	3	4		
P0	49,69	51,16	62,72	47,97	211,53	52,88 <sup>a</sup>
P1	15,80	30,26	24,05	16,47	86,58	21,64 <sup>bc</sup>
P2	35,07	40,01	22,14	28,34	125,55	31,39 <sup>b</sup>
P3	16,69	21,56	19,92	20,12	78,28	19,57 <sup>c</sup>
Total	117,25	142,99	128,83	112,90		
Average	29,31	35,74	32,20	28,22		

Based on the results of the study, it was shown that the addition of lactose in Ringers diluent during 8 hours of semen storage at 4°C had a very significant effect on the motility of free-range chicken spermatozoa. Spermatozoa motility in semen that has been stored will decrease in percentage in each treatment. Changes in the percentage yield of fresh semen motility with semen motility after storage ranged from 19.57% - 52.88%.

### 3.3 Spermatozoa Abnormality Quality Test for Chicken Semen

Table 3 . Evaluation of the Average Abnormality of Spermatozoa Before and After Storage (%)

Treatments	Group				Total	Average
	1	2	3	4		
P0	4,68	5,21	10,12	6,38	26,38	6,59 <sup>tn</sup>
P1	6,35	7,81	6,23	4,06	24,43	6,11 <sup>tn</sup>
P2	4,23	8,93	7,97	8,31	29,44	7,36 <sup>tn</sup>
P3	5,32	5,14	2,89	4,27	17,61	4,40 <sup>tn</sup>
Total	20,53	27,09	27,21	23,02		
Average	5,13	6,77	6,80	5,75		

Based on the results of research conducted that spermatozoa abnormalities did not significantly affect the treatment given. The difference in diluent from each treatment after being stored at 4°C for 8 hours did not experience a significant change in spermatozoa abnormalities, this is because during storage it did not result in changes or damage to the initial spermatozoa shape before storage [9]. However, if it is reviewed based on “Table 3”, the semen that was diluted in the treatment changed along with the length of storage at 4°C, it can be seen that the quality of spermatozoa can be maintained at P3 (Ringers' Diluent + Lactose) due to the lowest percentage increase in abnormalities.

### 3.4 Spermatozoa Viability Test for Free-range Chicken

Table 4 . Evaluation of the Average Viability of Spermatozoa Before and After Storage (%)

Treatments	Group				Total	Average
	1	2	3	4		
P0	24,90	21,38	18,19	25,85	90,31	22,58 <sup>a</sup>
P1	8,49	9,14	10,24	10,11	37,97	9,49 <sup>c</sup>
P2	10,64	12,63	17,48	18,75	59,50	14,8 <sup>b</sup>
P3	7,80	8,33	7,94	8,04	32,10	8,03 <sup>c</sup>
Total	20,53	27,09	27,21	23,02		
Average	5,13	6,77	6,80	5,75		

Based on the results of the research conducted, it can be seen that the use of Lactose solution without the addition of other diluents (P2) showed a decrease in the percentage of viability which was higher than (P1) and (P3). This indicates that in the research conducted, lactose can be added to the semen diluent solution as a source of energy during the storage process, but is unable to become a single diluent solution due to the need for diluents that can maintain and replace the electrolyte elements of cell plasma [10]. Based on the results of the data above, a high percentage of spermatozoa viability was found in treatments (P1) and (P3) because the change or decrease from treatment from fresh semen to cold semen showed a lower percentage of change.

### 3.5 .Result Recapitulation

Table 5. Recapitulation of Evaluation of Mean Motility, Viability and Abnormality of Spermatozoa of Free-range Chicken (%)

Treatmenta	Motility Spermatozoa (%)	Abnormality Spermatozoa (%)	Viability Spermatozoa (%)
P0	52,88 <sup>a</sup>	6,59 <sup>tn</sup>	22,58 <sup>a</sup>
P1	21,64 <sup>bc</sup>	6,11 <sup>tn</sup>	9,49 <sup>c</sup>
P2	31,39 <sup>b</sup>	7,36 <sup>tn</sup>	14,87 <sup>b</sup>
P3	19,57 <sup>c</sup>	4,4 <sup>tn</sup>	8,03 <sup>c</sup>

From the recapitulation table, the results showed that the use of Ringer + Lactose diluent in free-range chicken semen which was stored for 8 hours at 4<sup>0</sup>C during the study had a very significant effect on spermatozoa motility and spermatozoa viability, but had no significant effect on spermatozoa abnormalities of native chickens. Based on the results of the data that has been done research on free-range chicken spermatozoa mixed with various kinds of diluents, the Ringers diluent (P1) and Ringer diluent + Lactose (P3) have lower percentage changes compared to (P0) and (P2).

#### 4 Conclusion

The use of Ringers + Lactose diluent in free-range chicken semen stored for 8 hours at 4°C can maintain the motility quality of spermatozoa. From the data obtained, the use of Ringer + Lactose diluent can work well to defend spermatozoa from damage to cell membranes resulting from the elements contained in Ringers' solution and Lactose which can be used as an energy source when the semen undergoes cold shock during the storage period.

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