The Effect of Vitamin E and Selenium Injections on The Semen Quality of Bangkok Roosters (*Gallus domesticus*)

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Abstract. This study aims to determine the effect of vitamin E and selenium injections on the semen quality of male Bangkok chickens. The study used 18 male Bangkok chickens aged 1.5 years from April 10, 2023, to May 26, 2023, consisting of 3 treatments of 6 repeats using Complete Randomized Design, P0 (without injection of vitamin E and selenium), P1 (0.6 ml/head), P2 (0.9 ml/head). Chickens are kept in individual cages and given feed for corn, bran, and concentrates. Chickens are injected once every 2 weeks for 6 weeks. Data analysis used Analysis of Variance (ANOVA), descriptive analysis, and Kruskal Wallis Test followed by Duncans Multiple Ring Test and Mann-Whitney test. The result of observing the color of the injection treatment on Bangkok chickens was milky white, the smell was specific with medium thickness. pH, mass movement, and spermatozoa abnormalities were not significantly different in treatments P0, P1, and P2, the volume of semen obtained was significantly different in treatments P0, P1, and P2, the result of the mean sperm concentration in treatments P0 and P1 showed the significantly different result, however, P0 and P2 with P1 and P2 show no significant difference. It was concluded that the injection of vitamin E and selenium 0.6 ml/head had a better effect on volume and concentration than without injection and injection 0.9 ml/head.

1 Introduction

The poultry industry can be said to play a very important role. This is possible because the poultry industry can produce self-sufficiency in poultry meat and eggs, no less important is that poultry businesses play a role in improving public health and intelligence [1]. One type of poultry is Bangkok chicken. The advantages of Bangkok chickens are that they have a large body, upright posture, dense muscles, and excellent growth performance [2]. Along with the need for protein sources that continue to increase, it is necessary to increase the population of Bangkok chickens. Increasing the productivity of Bangkok chickens can be done with Artificial Insemination (AI) technology. The factor that influences the success of AI is the quality of sperm [3].

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Efforts to improve sperm quality are by meeting the needs of life and providing several contents that can encourage the development and growth of Bangkok chickens, one of which is the injection of vitamin E and selenium. Selenium is a component of the enzyme glutathione peroxidase, which functions to destroy free radicals in the cytoplasm and as an antioxidant for ingredients forming enzymes and body resistance as well as reproduction. Synergistic nutrition with selenium is vitamin E. Vitamin E binds enzymatic free radicals and functions as a lipid antioxidant specifically soluble in cell membranes [4].

The administration of vitamin E and selenium in previous studies for chickens is usually used in feed. Injection treatment mostly occurs in hens as in research according to [5] which states that injection of vitamin E and selenium at doses of 0.6 ml and 0.9 ml can accelerate the time of feather loss periods and improve the chicken production process. With this background, the author innovates by injecting vitamin E and selenium into male Bangkok chickens.

2 Materials and Methods

The research was carried out in Sidorejo RT 03 RW 10, Musuk Village, Musuk District, Boyolali Regency, and at the Poultry Livestock Laboratory and the Reproductive and Animal Health Laboratory of the Yogyakarta Agricultural Development Polytechnic – Magelang Department of Animal Husbandry from April 10, 2023, to May 26, 2023.

The materials used for this study were Bangkok male chickens aged 1.5 years 18 heads, Introvit E – Selen packaging 100 ml, corn 90 kg, concentrate 60 kg, bran 50 kg, NaCl Physiological 0.9% packaging 500 ml, eosin nigrosin solution, formalin solution.

The tools used for this study were individual cages for stud bangkok chickens, syringes or injections of 1 ml, semen storage tubes (microtubes), object glass, cover glass, microscopes, Neubauer chambers, pipettes, beakers, pH paper, feed bins, drinking places, cool boxes, tissues, vaccine needles, ATK equipment.

This study used a Complete Randomized Design (RAL), consisting of 3 treatments and 6 repeats, with a total of 18 roosters used with an age of 1.5 years.

Vitamin E is essential for the successful reproductive process and prevents degeneration of the germinal epithelium in the testes to maintain sperm reproduction and fertility. Selenium is a mineral that is very beneficial for health and includes components of important metabolic pathways, thyroid metabolism, antioxidant defense system, as well as immune function. Selenium with a combination of vitamin E can increase resistance to stress and disease due to increased production and reproductive capacity [6]. The injection is carried out intramuscularly in the breast part of the chicken. The injection is inserted directly into the muscle (musculus), with the aim that vitamin E and selenium can be absorbed by the livestock body quickly compared to subcutaneously [7].

Maintenance is carried out by giving feed in the amount of 150 grams/head/day and given 1 time a day at 07.00 WIB. Injection of vitamin E and selenium is carried out in the morning after intramuscular feeding of chicken breast every two weeks for 6 weeks. Sperm retrieval is done after the last treatment of vitamin E and selenium injections. Semen storage technique with message method on chicken back. Sperm collection is carried out in the morning at 09.00 WIB. The sperm is then accommodated in a microtube placed in a cool box and carried out macroscopic and microscopic examinations in the laboratory.

The treatment given is as follows:

1. P0: Control (without injection of vitamin E and selenium)
2. P1: Injection of vitamin E and selenium dose 0.6 ml/head
3. P2: Injection of vitamin E and selenium dose 0.9 ml/head

The variables observed in this study were color, smell, consistency, pH, volume, mass movement, concentration, and abnormalities of spermatozoa. The data collection is carried out as follows:

2.1 Color

The color of semen can be seen directly after being accommodated in this study conducted by 4 respondents for its assessment where semen is said to be normal if it is milky white. In Wamur's [8] opinion, semen colors are categorized as milky white and watery white or yellowish white.

2.2 Smell

Research Nabilla et al., [9] the smell of fresh semen that has been taken can be assessed by shaking your hand on the sperm storage tube, in this study the assessment of odor was carried out by 4 respondents, where semen is said to be normal if it has a distinctive smell of sperm and is not contaminated with feces.

2.3 Consistency

The fresh semen that has been accommodated is then evaluated for quality by 4 respondents. Consistency is assessed by looking at the speed and remaining sperm returning to the bottom of the tube [9]. In Wamur's [8] opinion, semen viscosity is categorized as viscous, medium consistency, and dilute consistency.

2.4 pH

Evaluation of the quality of fresh semen based on pH can be measured using pH paper. The pH paper that has been provided is then dipped in the sperm that has been accommodated. Then wait for some time until it shows a color change. The value of pH is usually influenced by the concentration of sperm contained therein. The value of normal sperm pH is around 7 [10].

2.5 Volume

Assessment of semen volume by looking at the lines listed on the microtube according to the height of the semen accommodated. Audia's [11] research the volume of chicken semen is between 0.2 – 0.5 ml.

2.6 Mass Movement

Mass movement is seen by dripping fresh sperm on a glass object and then viewed with a magnification of 100 under a microscope. This method can be seen from a group of sperm moving together in one direction counterclockwise to form waves. Assessment of sperm mass movement is carried out based on the thickness of the mass wave and the speed of the mass wave moving places.

2.7 Spermatozoa Concentration

The calculation of spermatozoa concentration was carried out using the help of a Neubauer-type hemocytometer, at a magnification of 450 under a microscope taken from 5 fields of view of each semen. Research by Nabila et al., (2018) said that the fresh sperm of Bangkok chickens has a concentration of 1.25 x 10^9 billion cells/ml to 2.46 x 10^9 billion cells/ml.

2.8 Spermatozoa abnormalities

Spermatozoa abnormalities are seen by dripping 1-2 drops of fresh sperm plus 4-5 drops of eosin nigrosin solution on a glass object. The preparation is viewed as a magnification of 1 x 40 inches under a microscope. Good spermatozoa abnormalities are not more than 15 and if more than 25% it will reduce fertility [12].

$$Abnormality\ (\%) = \frac{\Sigma Abnormal\ sperm}{\Sigma Spermatozoa\ count} X100\ (1)$$

Data analysis in this study used Analysis of Variance (ANOVA), Kruskal Wallis, and descriptive methods. The variables of pH, volume, concentration, and abnormality of spermatozoa were analyzed using Anova Analysis, mass movement variables were analyzed using Kruskal Wallis, and color, odor, and viscosity variables were analyzed using descriptive variables. If there is a significant difference in ANOVA results, it is continued with Duncan's Multiple Range Test (DMRT), while for Kruskal Wallis if there is a significant difference continued with the Mann – Whitney test.

3 Results and discussion

The results of the study of vitamin E and selenium injection on the semen quality of Bangkok roosters are presented in Table 1.

Table 1. Research data

No	Variables	PO	P1	P2
1	Color	Clear white	Milky white	Milky white
2	Smell	Fishy	Fishy	Fishy
3	Consistency	Keep	Thick	Thick
4	pH	7.50 ±0.27^{ns}	7.83±0.27^{ns}	7,75±0,26^{ns}
5	Volume	0,33 ±0,04^{a}	0,57±0,10^{b}	0,45±0,83^{c}
6	Mass Movement	1.50 ±0.55^{ns}	1.83 ±0.41^{ns}	1.67 ±0.52^{ns}
7	Spermatozoa concentration	1.83x10^9 ±0.66x10^{9a}	1.97x10^9 ±0.11x10^{9b}	1.91x10^9 ±0.72x10^{9ab}
8	Spermatozoa abnormalities	13.25 ±1.13^{ns}	12.58 ±1.50^{ns}	12.58 ±1.07^{ns}

Source: Data processed (2023)
Table Description: P0 = No injection of vitamin E and selenium; P1 = injection of vitamin E and selenium 0.6 ml/head; P2 = injection of vitamin E and selenium 0.9 ml/head.

3.1 Color

Table 1. shows the average color results of Bangkok chicken semen observed directly from semen storage tubes or microtubes by 4 respondents. The color produced at P0 is on average clear white, while at P1 and P2 the average is milky white. The best semen color evaluation results in this study were with the injection of vitamin E and selenium at a dose of 0.6 ml/head. The results of color observation from injection treatment in Bangkok chickens show the same average result, namely milky white.

The semen in this study was said to be normal and good because the results of sperm storage were not reddish. The difference in color in semen indicates that the semen is contaminated with blood or feces. In Kusumawati's [13] opinion, poor semen has a color resembling coconut water.

3.2 Smell

Table 1. shows the average results of semen smell assessed by 4 respondents. The smell produced in this study for P0, P1, and P2 is all like in general, which has a distinctive or fishy smell. In general, the smell of chicken semen is accompanied by the characteristic smell of the chicken [8]. Factors that affect the odor of the semen that is accommodated are not contaminated with feces, as well as the presence of infection of the male reproductive tract organs that cause foul odor caused by semen containing pus.

3.3 Consistency

Table 1. shows the average result of consistency of spermatozoa with the same average yield of P1 and P2, namely viscous consistency compared to P0, the average consistency is medium. The best semen viscosity evaluation results in this study were with the injection of vitamin E and selenium at a dose of 0.6 ml/head. The thick consistency shows that the Bangkok chicken semen is good. In Kusumawati's [13] opinion good semen has a viscosity that is almost the same or less viscous than milk, while bad semen has a viscosity resembling coconut water. One of the factors that affect the consistency of sperm is due to the level of stimulation given [14].

3.4 pH

Table 1. shows the average results of pH and results were not significantly different in P0, P1, and P2 treatments. The best semen pH evaluation results in this study were with the injection of vitamin E and selenium at a dose of 0.6 ml/head. The average acidity in this study was 7.6. This is the opinion of Wamur [8] that the acidity of semen in poultry is generally in the range of 7.0 – 7.6. The degree of acidity of semen largely determines the life status of spermatozoa in semen. The semen that has been evaluated in this study can be categorized as suitable for insemination. Storage time can cause the level of pH to decrease is also greater because during storage the metabolic process of spermatozoa continues [14].

3.5 Volume

Table 1. shows the average results of semen volume obtained significantly different results in P0, P1, and P2 treatment. The average volume of semen produced during the study on P1 was better compared to P0 and P2. The volume of semen produced from injections of vitamin E and selenium with a dose of 0.6 ml is better than injections with a dose of 0.9 ml. Dianita et al., [15] stated that the provision of vitamin E and selenium aims to prevent a decrease in

semen volume during the shelter process and can prevent a decrease in libido in chickens. The average volume of semen produced in this study was 0.3 to 0.7 ml. The results of this study show that in the opinion of Wamur [8] that in general, the average semen volume in chickens is between 0.3 to 1.5 ml. The results showed that the volume of Bangkok chicken semen met the standards for artificial insemination in local chickens.

3.6 Mass movement

Table 1. shows the average results of mass movement and obtained results not significantly different in P0, P1, and P2 treatments. At P1 and P2 very well (+++) was seen from large, dark, thick, and active waves resembling black cloud plumes moving around with a percentage of 18 samples that have been taken including 12 samples (+++) and 6 samples (++). The best mass movement evaluation results in this study were with the injection of vitamin E and selenium at a dose of 0.6 ml/head. According to Wamur [8], the movement of spermatozoa will show thick or thin waves and move fast or slow depending on the concentration of sperm living in them. Bangkok chicken semen produced in this study met the criteria for insemination.

3.7 Spermatozoa Concentration

Table 1. shows the average results of sperm concentration, P0 and P1 treatments showed real differences, but P0 and P2 treatments with P1 and P2 showed no real difference. Injection of vitamin E and selenium 0.6 ml/head can affect the concentration of semen produced. The addition of 0.9 ml/head did not affect the resulting concentration but there was an increase when compared to those without vitamin E and selenium injections. Injection of vitamin E and selenium 0.9 ml/head showed a decrease compared to injection of 0.6 ml/head to sperm concentration. Some factors that will affect the concentration in chickens are due to feed, body weight, and chicken type [16].

3.8 Spermatozoa abnormalities

Table 1. shows the average results of spermatozoa abnormalities obtained results are not significantly different in P0, P1, and P2 treatment. These abnormalities include giant or dwarf heads, double heads, circular tails, and headless or tailless. Abnormalities in spermatozoa are divided into two, namely primary abnormalities that occur in the testes and secondary abnormalities that occur in the epididymis during ejaculation.

The average results of spermatozoa abnormality research in this study were P0 of 13.25%, P1 and P2 with an average number of 12.58%. The best abnormal evaluation results in this study were the injection of vitamin E and selenium at a dose of 0.9 ml/head. This result shows that the abnormality of Bangkok chicken spermatozoa is in good condition because it is below 15%. This is the opinion of Hijriyanto [17] who explains that chicken semen to be used for IB should not be more than 15%, and if the abnormality is more than 25%, it will reduce fertility.

Morphological abnormalities of spermatozoa that are often found are large or small heads and broken tails to coil. This study showed that P1 and P2 with injection of vitamin E and selenium can reduce the percentage of spermatozoa abnormalities. This is the opinion of Yulianto et al., [18] which states that the provision of vitamin E and selenium acts as an antioxidant and can protect cell membrane damage and potentially as a protective agent for sperm.

4 Conclusion

Based on the research that has been done, it can be concluded that the injection of vitamin E and selenium affects the volume and concentration of sperm and has no effect on color, smell, consistency, pH, mass movement, and spermatozoa abnormalities. In this study, it can be concluded that the results of vitamin E and selenium injections on the quality of Bangkok rooster semen (*Gallus domesticus*) are best at a dose of 0.6 ml/head.

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References

1. B. P. Widiarso, W. Mubarokah, The effect of Lactobacillus sp-based probiotics on daily weight gain and ND antibody titer in broiler chickens, In Proceedings of the 2021 National Seminar Politeknik Pembangunan Pertanian Yogyakarta Magelang, June **29**, Magelang, Jawa Tengah (2021)
2. T. W. Putra, S. Suharyati, S. Siswanto, M. Hartono, Jurnal Riset dan Inovasi Peternakan **7**, 4 (2023)
3. Fitriani, S. Erlina, A. Gunawan, A. Subhan, W. Nugroho, Jurnal Ilmiah Fillia Cendekia **5**, 2 (2020)
4. F. N. L. Lubis, R. Alfianty, E. Sahara, Jurnal Peternakan Sriwijaya **4**, 1 (2015)
5. N. S. Said, S. Sulmiyati, D. U. Fahrodi, J. Sains dan Teknologi Peternakan **1**, 2 (2020)
6. Siswanto, Budisetyawati, F. Ernawati, Gizi Indonesia **36**, 1 (2013)
7. L. Zalizar, I. D. Rahayu, and A. Mahmud, UMM Press, Malang, (2022)
8. M. F. E. Wamur, A. A. Dethan, C. V. Lisnahan, J. of Animal Science **7**, 2 (2022)
9. A. Nabilla, R. I. Arifiantini, B. Purwantara, J. Veterinary **19**, 2 (2018)
10. S. Sunami, N. Isnaini., S. Wahjuningsih, Ternak Tropika. J **18**, 1 (2017)
11. R. P. Audia, M. A. Salim., N. Isnaini, T. Susilawati, Ternak Tropika. J **18**, 1 (2017)
12. N. M. Ihsan, Universitas Brawijaya Malang (2009)
13. E. D. Kusumawati, H. Leandro, A. T. N. Krisnaningsih, T. Susilawati, N. Isnaini, and R. Widhad, The effect of temperature and shelf life of fresh semen on motility and abnormalities of Etawa Peranakan goat spermatozoa (PE), In Proceedings of the National Seminar on Research Results, August 6, Malang, Jawa Timur (2016).
14. A. S. Dewi, Y.S. Ondho, E. Kurnianto, Animal Agriculture. J **1**, 2 (2012)
15. I. Dianita, S. Hasibuan, S. Syafriadiman, J. Perikanan dan Kelauatan **25**, 1 (2020)
16. Y. Setiawan, N. Ulupi, C. Sumantri, Arifiantini, Acta Veterinaria Indonesiana **9**, 1 (2021)
17. M. Hijriyanto, J. Ilmiah Mahasiswa Veteriner **1**, 1 (2017)
18. R. A. Yulianto, W. Isnaeni, R. Susanti, J. Life Sci **2**, 2 (2013)