

Interaction Effect of Vitamin E-selenium Supplementation and Metabolic Energy on Reproductive Performance of Joper Breeders

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ABSTRAK

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Efek samping dari oksidasi asam lemak saat sintesis lippoprotein adalah pelepasan oksigen dalam jaringan yang disebut reactive oxygen species (ROS). Stress metabolik pada indukan Joper akibat ketidak seimbangan antara ROS dan antioksidan menyebabkan penurunan produksi dan kualitas telur tetas. Oleh sebab itu perlu penelitian untuk meningkatkan performa reproduksi induk Joper dengan mengkombinasikan suplementasi vitamin E-selenium dan energi metabolik. Penelitian ini menggunakan 200 butir telur tetas hasil persilangan antara pejantan Sentul umur 60 minggu dengan ayam petelur strain ISA BROWN umur 35 minggu yang diberi pakan perlakuan. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) Faktorial (2X4). Faktor pertama adalah level energi (2700 dan 2800 kcal/kg) dan faktor kedua dosis suplementasi vitamin E-selenium (0, 25, 50, 75 dan 100ppm). Dosis selenium 1ppm/mg vitamin E. Interaksi antara suplementasi vitamin E-selenium dan energi berpengaruh sangat nyata ($P<0.01$) dalam meningkatkan bobot DOC. Faktor tunggal level energi dan suplementasi vitamin E-selenium berpengaruh nyata ($p<0.05$) dalam menurunkan mortalitas embrio dan meningkatkan daya tetas, sedangkan fertilitas dan kualitas cangkang telur tidak dipengaruhi oleh kedua faktor perlakuan. Kesimpulan dari penelitian ini adalah interaksi antara suplementasi vitamin E-selenium dan level energi dapat meningkatkan bobot DOC Joper, sedangkan faktor tunggal suplementasi vitamin E-selenium dan level energi dapat menurunkan mortalitas *embrio dan meningkatkan daya tetas. Interaksi terbaik pada supelemntasi 100 ppm vitaminE-selenium dan energi 2800 kcal/kg.*

Kata Kunci: Feed supplement, lippoprotein dan indukan Joper

ABSTRACT

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The side effect of fatty acid oxidation during lippoprotein synthesis is the release of oxygen in the tissue called reactive oxygen species (ROS). Metabolic stress in Joper brooders due to an imbalance between ROS and antioxidants causes a decrease in hatching egg production and quality. Therefore, research is needed to improve the reproductive performance of Joper broodstock by combining vitamin E-selenium supplementation and metabolic energy. This study used 200 hatched eggs resulting from a cross between 60 weeks old Sentul males and 35 weeks old ISA BROWN laying hens that had been treated. This study used a Factorial Completely Randomized Design (CRD) (2 x 4). The first factor is the energy level (2700 and 2800 kcal/kg) and the second factor is the dose of vitamin E-selenium supplementation (0, 25, 50, 75 and 100 ppm). Selenium dosage is 1ppm/mg vitamin E. The interaction between vitamin E-selenium supplementation and energy had a very significant effect ($P<0.01$) in increasing DOC weight. The single factor energy level and vitamin E-selenium supplementation significantly ($p<0.05$) in reducing embryo mortality and increasing hatchability, while fertility and eggshell quality were not affected by the two treatment factors. The conclusion of this study is the interaction between vitamin E-selenium supplementation and energy levels can increase the weight of Joper's DOC, while the single factor of vitamin E-selenium supplementation and energy levels can reduce embryo mortality and increase hatchability. The best interaction with 100 ppm vitamin E-selenium supplementation and energy 2800 kcal/kg.

Key Words: Feed supplements, lipoprotein and Joper breeders

INTRODUCTION

The imbalance between demand and supply of native chicken meat in Indonesia is a great opportunity for the development of the native chicken business. In 2019 the demand for native chicken meat was only

supplied by 30% (Immawan et al. 2019). Joper (Jowo-cross) is a crossbreed between domesticated males and laying hens (Muharliien et al. 2020). The main priority in the development of the Joper hatchery industry is improving the quality of hatching eggs. Lipoprotein is one of the key factors in assessing the quality of

hatching eggs because it is the main source of nutrition for embryonic development (Ren et al. 2021; Wang et al. 2021).

Reactive oxygen species (ROS) is one of the triggers for metabolic stress which has a major impact on immunity and regulation of body metabolism. ROS is a by-product of fatty acid oxidation in lipoprotein synthesis (Amevor et al. 2021; Zuidhof et al. 2017). To produce good quality hatching eggs, it is necessary to control the triggers of metabolic stress in breeders. Vitamin E-selenium supplementation and increased metabolic energy are solutions to control metabolic stressors in Joper breeders

Vitamin E is a natural antioxidant that can protect tissues from damage caused by ROS. Amevor et al. (2021) reported that selenium plays an important role in various metabolic processes in the body. Vitamin E works synergistically with selenium in lipid peroxidation (Abd El-Hack et al. 2017; Çelebi 2019). Metabolic energy (ME) is used for maintenance (basal metabolism, regulation of body temperature, immune response and activity, growth and egg production (Hadinia et al. 2019). Surplus energy in the body is stored as body fat mass. Body fat mass plays a role in regulating sexual maturity, egg production, egg yolk composition, maximizing the absorption of vitamin E and reproductive hormone precursors (Heijmans et al. 2021; Ren et al. 2021). Research is needed to determine the interaction between ME and the right dose of vitamin E-selenium supplementation in improving hatching egg quality because there is very little information available..

MATERIALS AND METHODS

Place and time

This research was conducted in February 2020. The research location is in the hatchery of Mr. Manto which is located in Rejotangan Village, Tulung Agung Regency, East Java Province, Indonesia.

Artificial insemination

In this study, artificial insemination was performed intravaginally by inserting semen into the cloaca of the Joper breeder as deep as 3-4 cm. This insemination was applied every 4 days at 4 pm. Semen is collected by massaging the area around the abdomen to the cloaca and collected with a graduated tube to measure its volume. Semen dilution using 0.9% NaCl with the ratio between semen and 0.9% NaCl is 1:10. The dose of inseminated semen is 0.2 ml/hens. The concentration of spermatozoa used for artificial insemination refers to the results of the research by Saleh et al. (2019) of 100 million spermatozoa/hens.

Hatchery management

The incubator was cleaned by wiping the inner and outer surfaces of the incubator using a disinfectant and allowed to dry and continued with fumigation. The fumigation of the incubator was carried out by evaporating formalin into a container containing KMnO₄. Evaporation is done by pouring formalin 40% in a container containing KMnO₄ then the hatching machine is immediately closed and allowed to stand for 24 - 48 hours with the heating condition still on. The fumigation dose per 1m³ of hatchery area is 12-15ml of 40% formalin and 6 g of KMnO₄. Handling hatching eggs before being put into the semi-automatic incubator is cleaned by wiping the dirt on the egg shell. Temperature and humidity settings are regulated according to the needs of the embryonic development period. Day 1 to 18 the temperature is set at 37.5 °C and humidity ranges from 50-60% and on day 19 until hatching the temperature is lowered to 32-33 °C. On day 20 until hatching humidity is set at 80%. Turning eggs every 1 hour so that in a day there are 24 rounds with a slope of 45° (Rahardja et al. 2020). Embryo development was observed every 7 days by candling on the 7th and 14th days after the eggs entered the semi-automatic incubator. Candling is a method used to observe the development of the embryo inside the egg by observing using light.

Breeder Joper and diets

This research is a series of studies from several studies. In previous studies, studies have been carried out on Sentul males to get the best male age which produces good quality semen for artificial insemination where the results of this study are applied to this study. The Joper broodstock used in this study were 400 hens from the ISA BROWN strain and were 35 weeks old with body weight ranging from 1.80-1.85 kg and in good health. These brooders were placed in individual cages with a size of 50 x 40cm and a height of 37cm at the front and 30cm at the back. The hatching eggs of 400 hens were collected and selected, then 200 eggs were taken to be incubated and observed and 200 eggs were used to observe the exterior quality of hatching eggs (egg shell weight and thickness). The total males used were 15 Sentul males aged 60 weeks with body weight ranging from 2.18-2.23 kg and in good health. Sentul roosters are placed separately from the broodstock. These males were placed in individual cages measuring 70 x 50 x 100cm. Sentul rooster was given control feed (E1V0) while Joper broodstock was given treatment feed with the addition of metabolic energy and vitamin E-selenium supplementation according to the treatment. The treatment feed was given 40% at 6 am and 60% at 2 pm. Sentul males and Joper breeders are given drinking water ad libitum.

Table 1. The composition of the experimental feed

Ingredients	Experimental feed									
	E1V0	E1V1	E1V2	E1V3	E1V4	E2V0	E2V1	E2V2	E2V3	E2V4
Corn (%)	48.90	48.90	48.90	48.90	48.90	51.30	51.30	51.30	51.30	51.30
Soy bean meal (%)	21.60	21.60	21.60	21.60	21.60	22.10	22.10	22.10	22.10	22.10
Rice bran (%)	12.20	12.20	12.20	12.20	12.20	12.20	12.20	12.20	12.20	12.20
Meat bone meal (%)	8.00	8.00	8.00	8.00	8.00	8.40	8.40	8.40	8.40	8.40
Grit (%)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Limestone (%)	3.30	3.30	3.30	3.30	3.30	3.10	3.10	3.10	3.10	3.10
Fat powder (%)	0.00	0.00	0.00	0.00	0.00	1.11	1.11	1.11	1.11	1.11
Complete premix (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Monocalcium phosphate(%)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Salt (%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium bicarbonat (%)	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Vitamin E-selenium(ppm)	0	25	50	75	100	0	25	50	75	100

Calculation of feed composition using Brill Formulation software

Table 2. The nutritional content of the experimental feed

Nutrients	Nutritional content									
	E1V0	E1V1	E1V2	E1V3	E1V4	E2V0	E2V1	E2V2	E2V3	E2V4
ME (kcal/kg)	2,701	2,701	2,701	2,701	2,701	2,800	2,800	2,800	2,800	2,800
Crude protein (%)	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00
Crude fat (%)	4.40	4.40	4.40	4.40	4.40	5.06	5.06	5.06	5.06	5.06
Crude fiber (%)	3.60	3.60	3.60	3.60	3.60	3.24	3.24	3.24	3.24	3.24
Lysine (%)	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Methionin (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Methionine + cystine (%)	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87
Threonine (%)	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Triptopane (%)	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Calcium (%)	3.99	3.99	3.99	3.99	3.99	3.91	3.91	3.91	3.91	3.91
Total phosphorus (%)	0.83	0.83	0.83	0.83	0.83	0.78	0.78	0.78	0.78	0.78
Phosphor avail (%)	0.50	0.50	0.50	0.50	0.50	0.49	0.49	0.49	0.49	0.49
Sodium (%)	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13

Calculation of feed composition using Brill Formulation software

ME = Metabolism energy

Parameters measure

The measurement process in a research is very essential because that is where the numbers are obtained to be processed and analyzed so that the answers to the questions in the research are obtained. The procedure used to obtain data based on the variables observed in this study was as follows:

Egg weight, Egg shell weight (ESW) and Egg shell thickness (EST)

Egg weight (EW) was measured by weighing the hatching eggs. Egg weight measurement was carried out

every day and then the average was taken for each week. The weight of the egg shell is one of the determinants of egg quality, especially for hatching eggs. A small eggshell weight indicates a thin eggshell and is not good at hatching. The egg shell weight was measured by breaking the egg and removing the egg white and yolk. Then the weight of the shell was weighed and the results were recorded. The thickness of the egg shell is one of the determinants of the quality of hatching eggs. The thickness of the egg shell is closely related to the ability to hatch. The thickness of the eggshell was measured by breaking the egg, removing the white and yolk and then measuring the thickness of the egg shell using a caliper.

Fertility

Measurement of fertility by calculating the percentage of fertile eggs based on the number of eggs that enter the incubator. Percentage of mortality were calculated based on fertile eggs.

$$\text{Fertility (\%)} = \frac{\text{Number of eggs sett} - \text{Number of clear eggs}}{\text{Number of eggs sett}} \times 100\%$$

Embryo mortality

Observation of the development of this embryo using light emitted on the surface of the egg. Mortality was measured by splitting unhatched eggs and recording the number of embryos that died.

$$\text{Embryo Mortality (\%)} = \frac{\text{Number of fertile eggs} - \text{Number of unhatched eggs}}{\text{Number of fertile eggs}} \times 100\%$$

Hatchability

Hatchability is the result of fertile eggs until they can hatch and are counted at the end of hatching. The hatchability can be calculated as follows. Percentage of hatchability was calculated based on fertile eggs.

$$\text{Hatchability (\%)} = \frac{\text{Number of Eggs that hatch}}{\text{Number of fertile eggs}} \times 100\%$$

Body weight of DOC

Hatch weight is the weight of newly hatched chicks. Hatch weight was measured by weighing the newly hatched chicks.

Experimental design

The data obtained were recorded and tabulated and statistically analyzed using ANOVA with a factorial Completely Randomized Design (CRD) with a 2x4 treatment pattern and each treatment using 5 eggs. The first factor was the ME level (2700 and 2800 kcal/kg) (E) and the second factor was the dose of vitamin E-selenium supplementation (0, 25, 50, 75 and 100 ppm) (V). The dose of selenium is 1 ppm/mg vitamin E. Statistical analysis is continued with Duncan's test if the results obtained provide significant or very significant differences in influence.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where, Y_{ijk} = The results of the observations for the i^{th} level of factor A, j^{th} level of factor B, on the k^{th} test; α_i = General average Effect of factor A at level i ; β_j = Effect of factor B on the j level; $(\alpha\beta)_{ij}$ = Interaction between A and B at factor A level i , factor B level j ; ε_{ijk} = Experimental error for i^{th} level factor A, j^{th} level factor B in the k^{th} replication/group.

RESULTS AND DISCUSSION

Based on the research that has been done, it was found that the effect of metabolic energy level and dose of vitamin E-selenium supplementation in the feed on the reproductive performance of Joper breeders is presented in Table 3 below.

Egg weight

Egg size selection is a selection method that aims to evaluate the feasibility of eggs to be hatched. In this study, interaction between metabolic energy levels with vitamin E-selenium and single factor vitamin E-selenium gave no significant effect ($p>0.05$) on egg weight. The single factor of metabolic energy level had a very significant effect ($p<0.01$) on egg weight. The weight of the eggs before being put into the incubator was 61.13-65.75g. The egg weight obtained in this study is in accordance with the research of Rahman et al. (2016) of 60.95-61.78g and higher than Pagala et al (2020) who got an EW of 56.87g in crosses of bangkok males with laying hens and 47.09g in crosses of broiler breeds with bangkok laying hens. Egg weight based on statistical analysis is known to be influenced by a single factor of ME or vitamin E-selenium supplementation. Statistical analysis showed ME had a more dominant effect on EW than vitamin E-selenium supplementation. The highest EW was obtained from treatment with a ME of 2800kcal/kg and vitamin E-selenium supplementation at a dose of 100 ppm.

Factors that affect EW are the age of laying hens, environmental temperature, strain, feed and body weight. Feed plays an important role in egg production (Pagala et al. 2020). ME factor is the dominant influence on EW, this is because about 99% of the total egg lipid is in the yolk. These lipids are used to supply energy for the developing embryo. The lipid in egg yolk is not free but is in the form of lipoproteins and is derived from the synthesis of ME in feed (Nasr 2015; Liu et al. 2020). Although vitamin E-selenium supplementation did not have a dominant effect on EW, it also had an effect on increasing EW. Slozhenkina et al. (2020) reported that lipids in egg yolk are composed of phospholipids with unsaturated fatty acids that are easily oxidized. Vitamin E-selenium supplementation in this case serves to maintain the integrity of the egg yolk so that it is not damaged by ROS.

Egg shell (weight and thickness)

Interaction between metabolic energy level and single factor energy level and dose of vitamin E-selenium supplementation had no significant effect

Table 3. The average reproductive performance of the Joper broodstock

Treatment	Variable						
	Egg weight (g)	Egg shell weight (g)	Egg shell thickness (mm)	Fertility (%)	Embryo mortality (%)	Hatchability (%)	DOC weight (g)
Metabolic energy level							
E1	62.22±0.05 ^a	8.23 ± 0.08	0.08 ± 0.000	90.50±0.58	27.22±0.76 ^b	72.78±0.76 ^a	42.53±0.19 ^a
E2	64.01±0.02 ^b	8.33 ± 0.06	0.08 ± 0.001	95.00±0.71	21.28±0.69 ^a	78.73±0.69 ^b	44.20±0.37 ^b
Suplementasion vitamin E-selenium							
V0	62.77 ± 0.52	8.19 ± 0.16	0.08 ± 0.001	92.50 ± 2.50	29.19±2.71 ^b	70.81±2.71 ^a	41.50±0.42 ^a
V1	63.20 ± 0.48	7.88 ± 0.13	0.08 ± 0.001	92.50 ± 0.00	24.81±1.00 ^a	75.19±0.00 ^a	42.50±0.17 ^a
V2	63.10 ± 0.41	8.31 ± 0.09	0.08 ± 0.00	93.75 ± 3.13	25.28±0.14 ^{ab}	74.72±0.14 ^a	43.75±0.33 ^b
V3	63.23 ± 0.43	8.63 ± 0.13	0.08 ± 0.00	92.50 ± 1.25	21.49 ± 1.13 ^a	78.51±1.13 ^{ab}	44.00±0.46 ^{bc}
V4	63.25 ± 0.40	8.38 ± 0.19	0.08 ± 0.00	92.50 ± 1.25	20.46±2.45 ^a	79.54±2.45 ^b	45.08±1.04 ^c
Interaction of vitamin E-selenium supplementation and metabolic energy							
V1V0	61.74 ± 0.38	7.88 ± 0.65	0.08 ± 0.00	87.50 ± 8.29	34.61 ± 6.53	65.39 ± 6.53	40.67±0.41 ^a
V1V1	62.25 ± 0.37	7.63 ± 0.41	0.08 ± 0.01	92.50 ± 8.29	26.81 ± 3.34	73.19 ± 3.34	42.83±0.50 ^{ab}
E1V2	62.28 ± 0.36	8.50 ± 0.35	0.08 ± 0.01	87.50 ± 8.29	25.56 ± 2.81	74.44 ± 2.81	43.08±0.64 ^b
E1V3	62.36 ± 0.34	8.38 ± 0.41	0.08 ± 0.00	95.00 ± 8.66	23.75 ± 4.15	76.25 ± 4.15	43.08±0.83 ^b
E1V4	62.45 ± 0.34	8.75 ± 0.25	0.08 ± 0.00	90.00 ± 7.07	25.36 ± 2.86	74.64 ± 2.86	43.00±0.41 ^b
E2V0	63.81 ± 0.39	8.50 ± 0.50	0.08 ± 0.01	97.50 ± 4.33	23.78 ± 7.41	76.22 ± 7.41	42.33±0.24 ^a
E2V1	64.15 ± 0.81	8.13 ± 0.82	0.08 ± 0.00	92.50 ± 8.29	22.81 ± 1.90	77.19 ± 1.90	42.17±0.17 ^a
E2V2	63.93 ± 0.36	8.13 ± 0.22	0.08 ± 0.00	100.0 ± 0.00	25.00 ± 5.00	75.00 ± 5.00	44.42±0.14 ^{bc}
E2V3	64.09 ± 0.20	8.88 ± 0.22	0.08 ± 0.00	90.00 ± 7.07	19.24 ± 3.99	80.76 ± 3.99	44.92±0.28 ^c
E2V4	64.05 ± 0.29	8.00 ± 0.61	0.08 ± 0.00	95.00 ± 5.00	15.56 ± 4.44	84.44 ± 4.44	47.17±0.99 ^d

a, b a, b, ab, c and d values within a column with a very significant effect (p<0.01) in reducing embryo mortality; improve hatchability and increase DOC weight. a very significant effect (p<0.01) in increasing DOC weight. a significant effect (p<0.05) in reducing embryo mortality and improving hatchability. a very significant effect (p<0.01) in increasing DOC weight.

(p>0.05) on EST and EWS. The ESW obtained in this study was 7.63-8.88 g and the EST was 0.76-0.83 mm. The results obtained are higher than some of the literature. Slozhenkina et al (2020) reported the ESW of Sakini chickens in Nigeria was 4.51 g; Barbe et al. (2020) is 0.31 -0.32 mm and Sapkota et al. (2020) is 0.29-0.43 mm.

The quality of the eggshell has an important role during the incubation period. Eggshell quality is related to mineral metabolism, especially Ca²⁺. The main component of eggshell is calcium carbonate (CaCO₃) which is formed by Ca²⁺ and HCO₃ (Darsi & Zhaghari 2021). One of the declines in the quality of hatching eggs is influenced by the quality of the egg shell. The thin egg shell causes the eggs to be easily damaged and cannot be stored for long. Low egg shell quality is associated with a decrease in the intestinal ability to absorb calcium and an increase in egg size with increasing age of the breeder (Nasri et al. 2020). Egg

shell thickness is influenced by heredity and mineral metabolism in the body. Shell thickness is related to calcium metabolism obtained from bone deposits and feed (Sapkota et al. 2020).

Fertility

Fertility is the ability of the breeder to produce day old chick (DOC). Interaction between metabolic energy level and single factor energy level and dose of vitamin E-selenium supplementation had no significant effect (p>0.05) on fertility. The fertility obtained in this study ranged from 87.50-100%. The fertility obtained is similar to Saleh et al (2019) of 73.09 - 90.22% and was higher than the study of Pagala et al. (2020) of 66.67-67.67%. Fertility is one of the parameters to measure the success of insemination. The quality of spermatozoa has a major influence on the success of insemination (Pagala et al. 2020; Tesfay et al. 2020). In general, the

fertility rate is influenced by the ratio of males and females. type of insemination. handling of hatching eggs. environment (Adu-Aboagye et al 2020). abnormal spermatozoa and dead spermatozoa. Good quality spermatozoa if spermatozoa abnormality <17% and dead spermatozoa <10% (Feyisa et al. 2018)

Spermatozoa concentration plays an important role in the success of artificial insemination. In this study, the concentration of spermatozoa used for each treatment was the same, namely 100 million spermatozoa/hens. The same thing was also reported by Saleh et al. (2019) in their research which stated that different concentrations of spermatozoa resulted in different fertility where the best fertility was obtained by artificial insemination using semen with a spermatozoa concentration of 100 million spermatozoa/hens.

Therefore, the selection of males is very important for the hatchery industry. Male selection is done by evaluating the quality of semen to determine its reproductive ability. Evaluation of semen quality and its ability to fertilize can be done by analyzing the percentage of live/dead sperm and morphological evaluation (Tsfay et al. 2020). Spermatozoa quality is influenced by several factors including age of the male, maturity, nutritional adequacy and health status of the male. Spermatozoa concentration that is too high or low actually causes a decrease in fertility.

Embryo mortality

The interaction between vitamin E-selenium supplementation and ME had no effect ($p>0.05$) on embryonic death. Embryo mortality is more influenced by a single factor. ME factors and vitamin E-selenium supplementation factors both have an influence on embryonic death. ME plays more dominant role in reducing embryo mortality than vitamin E-selenium supplementation.

Energy has a dominant role in embryo mortality because ME has an important role in the process of egg yolk formation and egg weight. Egg yolk is a source of nutrition for embryonic development. 75% of the nutrients in egg yolk are lipoproteins (Nasr 2015; Liu et al. 2020). About 99% of the total egg lipid is in the yolk. The lipids in egg yolk are composed of phospholipids with unsaturated fatty acids that are easily oxidized (Slozhenkina et al. 2020). In breeders, surplus energy in the body is stored as body fat mass as a precursor in the synthesis of lipoproteins in egg yolk. The ME also plays a role in the absorption of vitamin E-selenium in the body (Heijmans et al. 2021; Ren et al. 2021). Vitamin E is a natural antioxidant to reduce ROS in egg yolk by reducing lipid peroxidation and oxidation. Oxidation in egg yolks during the hatching process causes the production of toxic malondialdehyde

which can cause embryo death. so vitamin E-selenium supplementation is effective for reducing lipid peroxidation and increasing antioxidant capacity in egg yolks which has an impact on increasing hatchability (Barbe et al. 2020).

Embryo mortality is not only influenced by the quality of the hatching eggs but also by the temperature setting in the incubator. The temperature of the incubator is very influential on the mortality of the embryo. Embryo mortality increases when the incubator temperature exceeds the optimal temperature for embryonic development. This is because the embryonic membrane becomes dry so that the embryo will experience nerve, heart, respiratory and kidney disorders (Pagala et al. 2020).

Hatchability

The interaction between metabolic energy level and the dose of vitamin E-selenium supplementation had no significant effect ($p>0.05$) on hatchability, while the single factor of metabolic energy level had a very significant effect ($p<0.01$) on hatching weight and the single factor of supplementation dose had a significant effect ($p<0.05$) on hatchability. The interaction between metabolic energy level and the dose of vitamin E-selenium supplementation had no significant effect ($p>0.05$) on hatchability. While the single factor of metabolic energy level had a very significant effect ($p<0.01$) on hatching weight and the single factor of supplementation dose had a significant effect ($p<0.05$) on hatchability. Hatchability obtained in this study ranged from 65.39-84.44% almost the same as (Pagala et al. 2020) of 85-93%. Hatchability percentage is affected by a single factor of metabolic energy and vitamin E-selenium supplementation. ME more dominant influence on hatchability improvement compared to vitamin E-selenium supplementation. The increase in ME affects the higher hatchability. Nutrient requirements for embryonic development during incubation are stored in albumen, egg yolk and egg shell. The main source of energy for the development of the embryo is obtained from the yolk sac. The energy in egg yolk for embryo development is obtained from the oxidation of fatty acids in egg yolk. Fatty acid oxidation in egg yolk supplies almost 94% of the total energy requirement of the embryo during development (Nasri et al. 2020).

Slozhenkina et al. (2020) reported that the use of antioxidants in broodstock could increase hatchability. The addition of antioxidants can increase hatchability up to 14.60%. The use of antioxidants will help the metabolism to be normal. Agreeing with this, Barbe et al. (2020) stated that vitamin E is a natural antioxidant to reduce ROS in egg yolk by reducing lipid peroxidation and oxidation. Oxidation in egg yolks

during the hatching process causes the production of toxic malondialdehyde which can cause embryo death. so vitamin E-selenium supplementation is effective for reducing lipid peroxidation and increasing antioxidant capacity in egg yolks which has an impact on increasing hatchability (Barbe et al. 2020).

Adu-Aboagye et al. (2020) and Pagala et al. (2020) explain that there are 3 main factors that affect the percentage of hatchability. The first factor is the quality of hatching eggs. This egg quality includes egg size, egg shape, egg weight and nutrient content. Hatching eggs with low nutrient content will produce a low percentage of hatchability. Nutrients present in eggs are the main source of nutrition for embryonic development. The second factor is the handling of hatching eggs before they are put into the incubator. Handling hatching eggs includes the cleanliness of eggs and the length of storage of hatching eggs before incubation. Eggs that are dirty and stored for a long time will result in low hatchability. The third factor is the hatching process. The hatching process is concerned with setting the incubator during incubation. Setting the temperature, humidity and frequency of egg turning has an important role in determining the success of hatching.

DOC weight

Interaction between vitamin E-selenium supplementation and ME had a very significant effect ($p < 0.01$) on DOC weight. Increasing doses of vitamin E-selenium supplementation and ME had an impact on increasing DOC weight. In this study, the weight of DOC ranged from 40.67-47.17g. The quality of hatching eggs has a major role in the weight of the DOC produced. DOC weight is related to metabolic activity during embryonic development. Availability of nutrients for embryonic development is a key factor in DOC weight. Egg yolk is the main source of energy for embryo development. according to Slozhenkina et al (2020) 99% of the constituents of egg yolk are lipids and lipid oxidation in egg yolk is used to supply energy needs for embryonic development. Hadinia et al. (2019) reported that energy intake in the body of the breeder is metabolized for maintenance, growth, and egg production. Surplus energy is stored as body fat mass. Body fat mass plays a role in egg yolk synthesis.

The metabolic activity of the embryo during incubation has an effect on the rate of embryo development. The increased energy requirements during incubation for embryonic development affect metabolism, especially oxidation reactions. Fatty acid oxidation increases with increasing energy requirements. Increased ROS in egg yolk due to high oxidation causes a low metabolic rate. Nasri et al. (2020) explained that the decrease in metabolism during

incubation caused organ development and embryonic growth to not be optimal. This can cause the resulting DOC weight to be low. Supplementation of vitamin E-selenium in broodstock feed will increase antioxidant levels in hatching eggs. Barbe et al. (2020) reported that antioxidants in egg yolk will control oxidation by reducing or inactivating ROS before working on embryonic tissue.

CONCLUSION

The conclusion of this study is the interaction between vitamin E-selenium supplementation and energy levels can increase the weight of Joper's DOC, while the single factor of vitamin E-selenium supplementation and energy levels can reduce embryo mortality and increase hatchability. The best interaction with 100 ppm vitamin E-selenium supplementation and energy 2800 kcal/kg.

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