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Optimizing Reproductive Performances of Garut Sheep as Superior Local Genetic Resources by Modification of Dietary Grass and Concentrate Balances

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***Abstract*— High-quality Garut rams are important to be selected for Garut sheep development to include improving their semen qualities. The semen quality is highly affected by dietary nutrient intake. This research aimed to conclude the effect of different grass and concentrate balances on performance and semen qualities such as motility, abnormality, the integrity of plasma membrane, and acrosome cap sperms of Garut rams. This research was conducted from August 25th, 2020, to February 2nd, 2021, at Margawati Sheep Breeding Center. A Completely Randomized Design (CRD) was used to compare 3 different grass and concentrate balance treatments (T_1 = 80% grass: 20% concentrate; T_2 = 60% grass: 40% concentrate; and T_3 = 40% grass: 60% concentrate) on performance and semen qualities of Garut rams using 6 replicates (n = 6). The data were analyzed using analysis of variance and Duncan's test. The results showed there was no different among the treatments on average daily gain (ADG, g/head/day) and dry matter intake (DMI, g/head/day) but increasing level of dietary concentrate resulted in increased motility (P<0.01), reduced abnormality (P<0.05), increased integrity of plasma membrane (P<0.001) and increased acrosome cap (P<0.001) of sperms of Garut Rams. The T_2 treatment resulted in an optimal grass (60%) and concentrate (40%) balance. In conclusion, increased dietary concentrate did not significantly increase the daily weight gain, but it improved the Garut ram's semen quality.**

***Keywords*— Concentrate; Garut rams; grass; performance; semen quality.**

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I. INTRODUCTION

Garut sheep is one of the best local breeds in West Java (Decree of Indonesian Agricultural Minister No. 2914/Kpts/OT.140/6/2011). The Indonesian Directorate General of Livestock stated that West Java's total number of sheep is 11.608.559, especially 11.425.574 of the Garut breed [1]. However, this figure differs from Indonesia's populace (257 million people). The disparity troubles can be alleviated by supporting a good diet to improve reproductive factors, including semen quality in Garut rams, and optimizing the reproductive performance of Garut sheep [2]. The role of small ruminants as the most widely cultivated livestock commodity in socioeconomic development and food security in most developing countries cannot be overemphasized. In addition to the preference for other farm animals because of their ability to tolerate extreme environments, sheep productivity is an important part of rural farmers' lives, providing them with a source of animal protein and revenue from sheep trades [3]

Traditionally, West Java's farmers have fed their local sheep with grass as the majority feed using a cut and carry method. Nowadays, farmers feed their sheep with not only grass as the main feed but also concentrate [4]. The typical concentrate ingredients are the by-products of agroindustry such as rice bran, cassava meal, copra meal, palm kernel meal, and so on. The utilization of these waste products as concentrate ingredients are beneficial to not only increase sheep production but also saving the environment from more waste disposal into the landfill [5], [6].

Feed requirements for livestock vary depending on body weight, age, physiological conditions, and type of livestock. Lack of feed in livestock will inhibit reproductive function, one of which is the secretion of hormones that can affect the process of spermatogenesis Nutrition is an important role in maximizing fertility potential. It has a profound effect on the

gonadotropin-releasing hormone that ultimately determines fertility [7].

The nutrient content that affects the reproductive performance of sheep include energy, protein, and fat. The right balance of feed ingredients in the diet of the livestock will produce good quality semen whilst Malnutrition can delay the puberty and inhibit testicular function in adult males [8]. Protein is an important dietary ingredient for regulating many reproductive activities. Good protein intake can increase sperm mass, motility, concentration, and viability [9]. Otherwise, lack of protein intake can causes delayed puberty and decreased fertility [10].

Feed is classified into three, namely: forage, concentrate and feed additive [11]. Nutrient contents that are consumed by livestock are depending on the type of forage provided, one of which is cultivated high quality grass that is intentionally planted. One type of cultivated grasses commonly used is Taiwan Elephant Grass. Taiwan Elephant Grass that cut at 30 days ages contains 15,50% dry matter, 92,01% organic matter, 8,00% ash, 7,50% crude protein, 54,10% Neutral Detergent Fiber (NDF), and 35,30% Acid Detergent Fiber (ADF) [12]. Adding more concentrate into a sheep diet is required especially for an intensive sheep fattening as this will result in significant differences in performance of the sheep compared with those fed only grass, but the sheep still need grass as the main source of fibrous feed. Therefore, to anticipate feed sources deficiency, sheep are given grass and concentrate in optimum balances.

Different balances in grass and concentrate will affect the productive and reproductive performances of sheep [13], [14]. Consuming an appropriate grass and concentrate balance is necessary for rams to meet their nutrient requirements, especially protein and energy which are very influential in male reproduction system. Feeding inadequate protein and energy can cause reproductive disorders due to reduced plasma membrane and acrosome cap integrity of the spermatozoa thereby inhibiting spermatogenesis process.

Spermatogenesis is influenced by the hormone testosterone [15]. Proteins can increase sperm transportation capabilities through hormones mechanism [16]. Treatments of different grass and concentrate balances will affect the motility, abnormality, plasma membrane and acrosome cap integrity of the Garut rams' sperm, because of the protein and energy contents present in each treatment affects the impermeability and cohesiveness of the membrane plasma. The plasma membrane and acrosome conditions greatly influence the process of fertilization. Damaging on the plasma membrane will affect the integrity of the acrosome sperm.

Sperm motility, abnormality, and the integrity of plasma membrane and acrosome cap is quality parameters of spermatozoa that play important role during the fertilization process [7]. The membranes of sperm are composed of 40 lipids (phospholipids, glycolipids, and cholesterol) and 60% protein. The 40% lipids in the sperm membrane consist of 65% phospholipids, 25% cholesterol, and 10% other lipids [17].

Protein requirement in the diet of adult rams is about 14%. Protein supply to the testes will affect the release of Follicle Stimulating Hormone (FSH) as well as inhibin and activin produced by Sertoli cells. Testosterone is produced by Leydig cells and will bind to the Sertoli and secreting Androgen Binding Protein (ABP) [18]. Different grass and concentrate balances will affect the contents of protein and energy in the diet of rams and will influence the formation of spermatogenesis process. High fertility sperms are affected by their plasma membrane and acrosome cap integrity. A proper protein and energy balance can produce high percentages of plasma membrane and acrosome cap integrity and the motility of the sperms.

The main constituents of the plasma membrane and acrosome are lipids and protein. Rams can be categorized to be fertile if they can produce sperms with motility between 60-90% [19]. Jimenez et al. [20] explained that arginine, which contains high-quality protein, can be used to synthesize a variety of components, i.e., H. Sodium (Na+), potassium (K+) and adenosine triphosphate (ATP) are essential. These components regulate the balance of ion moving and ATP supply to maintain the plasma membrane and acrosome cap integrity especially to prevent the sperm head damage [21]. It has also been implicated in improving plasma membrane integrity, permeability, and sperm fertilization ability [22]. Semen is normal if the intact of plasma membrane and acrosome cap reaching 60% or more with the motility more than 70% [19]. The purposes of this exploration were to test the hypotheses that diverse of grass and concentrate balances in the diet of rams will result in different protein and energy consumption where increased concentrate feeding will increase protein and energy intakes leading to better performance and semen quality of Garut rams.

II. Materials and Method

A. Animals and Handling

The objects of this study were 18 Garut rams (mature male sheep) aged 24 months with an average body weight of 44.34 ± 5.09 kg. Each sheep was randomly placed in an individual pen. The experiment was done at Margawati Sheep Breeding Center, Garut Regency, for 103 days. Rams were randomly grouped into three based on the diet treatments of grass and concentrate balances, namely T1 = 80% grass: 20% concentrate; T2 = 60% grass: 40% concentrate; T3 = 40% grass: 60% concentrate. Each ram was fed a corresponding experimental mixed diet three times a day at 07.00 am (25%), 01.00 pm (25%), and 04.00 pm (50%). Each ram was given clean drinking two times, before giving concentrate and after feeding concentrate. Every two weeks, each ram was weighed to determine body weight development.

B. Experimental Diet

The experimental rams were fed Taiwan Elephant Grass and concentrate. The diet was given based on the dry matter requirement of the sheep, as much as 3.3% of body weight. The diet consisted of Elephant Grass cv. Taiwan (*Pennisetum purpureum* cv. Taiwan) given as fed and a mixed concentrate with the ingredients as follows: 11% soybean meal, 33% coconut meal, 1%, corn, 22% rice bran, 25% pollard, 3% molasses, 2% mineral mix, and 3% tapioca flour dregs. The nutrient of this experimental diet is presented in Table 1. Then, based on Table 1, it can be calculated the nutrient contents in each experimental diet (Table 2).

TABLE I
NUTRIENT CONTENT (%) OF FEED INGREDIENTS

Nutrient (%)	Diet	
	Concentrate	Grass
Water	8.11	80.00
Dry matter	91.89	20.00
Crude Protein	16.86	13.05
Crude fiber	17.24	24.77
Lipid	10.90	3.44
Ash	9.48	15.08
Nitrogen free extract	45.52	43.66
Total Digestible Nutrient (TDN)	73.89	62.60

TABLE II
NUTRIENT CONTENT OF EACH FEEDING TREATMENT

Nutrient (%)	Treatment		
	T_1	T_2	T_3
Grass	80	60	40
Concentrate	20	40	60
Water	65.62	51.24	36.87
Dry matter	34.38	48.76	63.13
Crude Protein	13.81	14.57	15.34
Crude fiber	23.26	21.76	20.25
Lipid	4.93	6.42	7.92
Ash	13.96	12.84	11.72
Nitrogen free extract	44.03	44.40	44.78
Total Digestible Nutrient (TDN)	64.86	67.12	69.37

C. Proximate analysis

Each ground sample of grass and concentrate was analyzed using the Association of Official Analytical (AOAC) standard protocol including dry matter (DM), crude protein (CP), ash, ether extract (EE), and crude fiber (CF) [5]. All chemical compounds were expressed as a percentage of dry matter, and the dry matter was the percentage from a fresh sample. Nitrogen-free extract (NFE) and Total digestible nutrients (TDN) were estimated using the following formula [4], [23]:

$$NFE = 100 - (\mathrm{CA} + \mathrm{CP} + \mathrm{CF} + \mathrm{EE})$$

$$\begin{aligned}\text{TDN (concentrate)} &= 70.6 + (0.259 \times \mathrm{CP}) + (1.01 \\ &\times \mathrm{EE}) - (0.76 \times \mathrm{CF}) + (0.0991 \times \mathrm{NFE})\end{aligned}$$

$$\begin{aligned}\text{TDN (grass)} = (-26.685) &+ (1.334 \times \mathrm{CF}) + (6.598 \times \mathrm{EE}) \\ &+ (1.423 \times \mathrm{NFE}) + (0.967 \\ &\times \mathrm{CP}) - (0.002 \times (\mathrm{CF2})) - (0.67 \\ &\times (\mathrm{EE2})) - (0.024 \times (\mathrm{CF} \\ &\times \mathrm{NFE})) - (0.055 \times (\mathrm{EE} \times \mathrm{NFE})) - (0.146 \\ &\times (\mathrm{CF} \times \mathrm{CP})) + (0.039 \times ((\mathrm{CF2}) \times \mathrm{CP}))\end{aligned}$$

D. Data Collection and Measurements

1) Semen Collection: Semen Collection was carried out for six consecutive days from 07.00 – 09.00 am. All experimental rams were divided into six groups, where in one day, the semen collection could be accommodated in three rams (T3, T2, and T1). The method of semen collection used an artificial vaginal device [24]. An ewe was prepared as angler and tied the ewe on the pegs provided. Then, a set of artificial vaginas was prepared and filled with 40°C waters. Each ram was brought close to the angler. Then false mounting was done 2-3 times to increase the male libido. When the ram was riding the angler, the artificial vagina was inserted towards the penis. Then the ram was allowed to ejaculate inside the artificial vagina. Semen was immediately brought to the laboratory for quality evaluation.

2) *Sperm Motility Calculation:* Sperm motility was calculated by calculating the total sperm concentration and the concentration of dead sperm by placing one drop of semen in the Neubauer counting chamber. The method of calculating the concentration of dead sperm was the same as calculating the total sperm concentration, but the solution that was sucked in was physiological NaCl. Then, it was calculated by the formula of Arifiantini [25]:

$$Y = \frac{\sum Total\ Sperm - \sum Dead\ Spermatozoa}{Total\ Spermatozoa}\ x\ 100\% \qquad (1)$$

Y = Sperm Motility (%)

3) *Sperm Abnormalities Evaluation:* Spermatozoa abnormalities were evaluated by semen preparations using eosin-nigrosine dye by one drop of fresh semen on the end of the object glass using an ossicle. A 2% eosin-nigrosine solution was dropped one drop near fresh semen, then both were mixed and covered with an object glass. Fixation of the smear preparation using Bunsen. Observe using a microscope with a magnification of 400x [25]. Spermatozoa that absorb color were declared dead. The number of sperm observed was at least 200 spermatozoa by the formula [26]:

$$Sperm\ Abnormalities = \frac{Abnormal\ Sperm\ Count}{Sperm\ Count\ Observed}\ x\ 100\% \qquad (2)$$

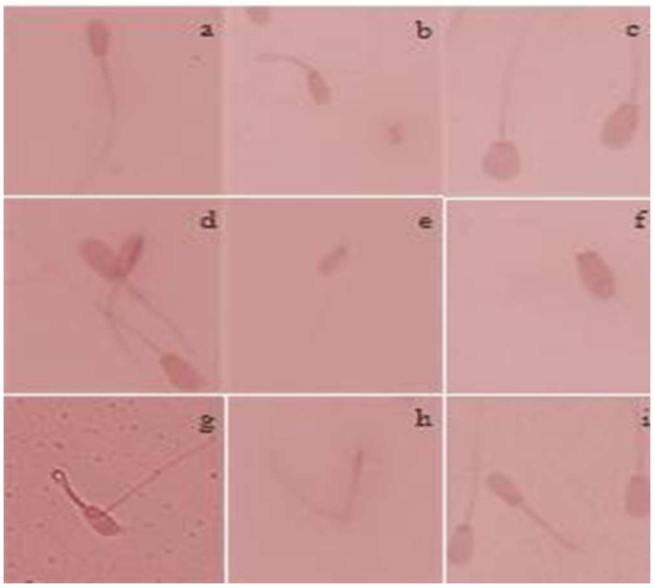

Fig. 1 Normal and Abnormal Spermatozoa Morphology 1000x Magnification with Eosin-Nigrosine Solution. a) Normal Spermatozoa, b) Pear-shaped, c) Macrocephalus, d) Microcephalus, e) Detached Head, f) Head only, g) Circular Tail, h) Tail, and i) Stump Tail. [25].

4) Intact Plasma Membrane Observation: The procedure is carried out to observe the integrity of the plasma membrane according to Sutama [27]. Thirty grams of NaCl was suspended in 100 ml of distilled water as a Hypoosmotic Swelling Test (HOS-test) solution, and HOS-test solution was added in a ratio of 1:6 or 10µl: 60µl. The semen mixed with the HOS-Test solution was then incubated at 38°C for 30 minutes. After incubation, the sample was prepared for review

on an object glass and counted a minimum of 200 spermatozoa. Intact Plasma Membrane (IPM) was marked by a circular or bent tail, while a straight tail indicated that the plasma membrane was incomplete or damaged. The evaluation of the intact plasma membrane could be calculated using the following formula:

$$IPM\ (\%) = \frac{Number\ of\ spermatozoa\ with\ intact\ plasma\ membrane}{Count\ the\ number\ of\ spermatozoa\ (200\ sperm)}\ x\ 100\% \quad (3)$$

5) Observation of the Intact Acrosome Cap: The procedure was carried out to observe the intact of the acrosome cap according to Rizal [28]. A 1% formalin solution was prepared, and 50µl 1% formalin solution was added into 10µl semen and homogenized. Samples were made for review preparations. The sample was observed with as many as 200 spermatozoa with a magnification of 400 times.

$$IAC\ (\%) \frac{Sperm\ count\ with\ intact\ acrosome}{Count\ the\ number\ of\ spermatozoa\ (200\ sperm)}\ \text{x}\ 100\% \quad (4)$$

E. Data analysis

Each chemical content of the diet was averaged from replicates analysis (n = 2). The Completely randomized design was used to compare three different types of grass: concentrate balance treatments. Each treatment was replicated six times. The collected data were analyzed using one-way ANOVA in MINITAB 16 statistic software with Tukey's test used to compare means ($P < 0.05$). The Anderson-Darling normality test was used ($P > 0.05$) to check the normality of residual data.

III. Results and Discussion

A. Results

1) Performance: According to the collected data, T_3 had a higher body weight because they were given a diet with a higher concentrate content than T_1 and T_2 (Table 2). The results showed that ADG and total DMI were not different ($P>0.05$). Increased level concentrate did not increase the average daily increase growth (Table 3).

TABLE III
INITIAL BODY WEIGHT (KG), FINAL BODY WEIGHT (KG), MEAN ADG (G/HEAD/DAY), AND DMI (G/HEAD/DAY)

Measurement	T_1	T_2	T_3	SEM	P-value
Initial Body Weight	40.23	44.40	48.40	1.6344	0.011
Final Body Weight	42.96	47.57	52.66	2.0411	0.015
ADG	26.50	30.7	41.32	11.039	0.629
Total DMI	1709.8	1695.7	1687.7	55.030	0.959

2) Semen quality: The sperm motility of fresh semen of sheep was around 60-80%. As can be seen in Table 4, the treatment was significant ($P<0.05$) on sperm motility of Garut rams. The average motilities of T2 and T3 were higher compared with that of T_1, due to the proportion of T_2 and T_3 rations containing higher TDN and protein than that of T_1 (Table 2.). As it is known that digested nutrients can affect metabolism to produce ATP as energy for sperm motility.

The abnormality of T_3 was significantly ($P<0.05$) lower than that of T_1 and T_2, but the abnormality of T_2 showed the same value as those of T_1 and T_3. The significantly different was due to the proportion of T3 diet had higher protein and TDN than that of T_1 (Table 2). Meanwhile, the proportion of T_2 diet contained TDN and protein, which were not significantly different from those of T_1 and T_3 (Table 2.).

Analysis of variance showed that the balance of grass and concentrate was significant ($P<0.05$) on the plasma membrane integrity. Acrosome Cap Integrity of T_1 had a lesser value ($P<0.05$) related to those of T_2 and T_3. This result was because plasma membranes produced in T1 with T2 and T3 were significantly lower than T_3.

TABLE IIV
AVERAGE PERCENTAGE SPERM MOTILITY (%), SPERM ABNORMALITIES (%), INTEGRITY OF PLASMA MEMBRANE (%), INTEGRITY OF ACROSOME CAP (%) OF GARUT SHEEP

Measurement	Treatment			SEM	P value
	T_1	T_2	T_3		
Sperm motility	76.73^{a}	79.86^{b}	81.40^{b}	0.8417	0.004
Sperm Abnormalities	3.08^{b}	2.00^{ab}	1.67^{a}	0.1363	0.023
Intact of Plasma Membrane	61.33^{a}	2.00^{ab}	73.00^{b}	1.0410	0.000
Intact of Acrosome Cap	61.33^{a}	70.75^{b}	74.92^{b}	1.5103	0.000

Note: Different letters in the column indicate significantly different ($P<0.05$)

B. Discussion

Dietary concentrate is required to improve sheep performance, especially in the fattening phase [14], [29]. However, increased dietary concentrate in the current study did not increase ADG and DMI of the rams. This could happen since the rams used in this current study were about two years old, and the growth of mature sheep is slower than those of lambs below one year old [30]. An increased dietary level of concentrate in this study improves the semen quality of the rams. According to Garner and Hafez [31], sperm motility is categorized as normal in the range of 60-80%. Sperm motility is greatly affected by the nutrient intakes of the animals.

Digested nutrients can affect metabolism to produce ATP as energy for sperm motility [20], [32], [33]. Moreover, the energy for sperm motility came from the breakdown of Adenosine Triphosphate (ATP) in the mitochondrial membrane through a decomposition reaction into Adenosine Diphosphate (ADP) and Adenosine Monophosphate (AMP). In another hand, plasma membrane production has affected intact of the acrosome cap [34], [35]. This is in accordance with the opinion of Herdis and Darmawan [36] that the damaging of the intact plasma membrane is usually accompanied by a breakdown of the acrosome cap, thereby causing the release of enzymes used during the fertilization process.

The protein content in the T1 ration was lower than those in the T2 and T3 rations, so the protein ration as a source of enzymes for sperm motility was still insufficient. Protein is needed as an enzyme for energy needed for sperm motility. This is in line with Rizal and Herdis [37] and McDermott et al. [22], who found that protein is an ATPase enzyme that converts ATP from metabolism into AMP and two inorganic Pi ions. Inorganic Pi ion has a high energy content that makes the microfibrils contract and increases movement in the sperm. Protein in the feed is adequate and able to surges the sperm

amount by boosting Gonadotropin-Releasing Hormone (GnRH) flow which follicle-stimulating hormone (FSH) production to regulate sperm production and luteinizing hormone (LH) to regulate and proliferate Leydig cells. It was possible to increase sperm concentration by stimulating the circulation of testosterone [38]. Testosterone helps the seminiferous tubules produce androgens and help sperm growth through the spermatogenic process, leading to increased sperm concentration [39].

Fat and protein balance in the diet of sheep can affect the spermatogenesis process. Fat deposition in the body cannot be dissolved in the blood, so it must bind to proteins in the form of lipoproteins. This is under Faza et al. [40], who stated that fat was a molecule that was insoluble in water (hydrophobic), which meant that fat could not be dissolved in blood plasma. Fats must bind to proteins called lipoproteins to transport them through the bloodstream. Based on their density, Lipoproteins are divided into four, one of which is the Low Density of Lipid (LDL), which is responsible for transporting cholesterol from the blood circulation to the Leydig cells. This is in line with Syarifuddin et al. [41], who stated that cholesterol as a basic material for testosterone biosynthesis came from blood plasma in the form of LDL and was partially synthesized in Leydig cells. Furthermore, it is supported by Hasbi and Gustina [18] that Leydig cells play a role in producing testosterone which is stimulated by the presence of Luteinizing Hormone (LH). Luteinizing Hormone increases the activity of enzymes that will convert cholesterol into the testosterone hormone. The testosterone hormone will affect the quality of the semen that is ejaculated, so that the higher the protein and fat content in the ration balance, the lower the resulting abnormality of sperm.

Protein in feed affects the nature of permeability of the membrane so that there are molecules that can only pass through the membrane from the inside to the outside of the cell, but there is also the opposite. This is in accord with Gautier and Hinner [42] that the protein and fat on the plasma membrane play a role in the formation of impermeability and membrane cohesiveness. The intact plasma membrane assists the acrosome cap in the fertilization process, and the protein will affect the ability of the fertilization in sheep sperm. This is in accordance with the research of Stzezeck et al. [43], that the high protein content in the feed affects the maintenance of the viability of spermatozoa and the fertility process, and feed that contains a small amount of protein will interfere with membrane stability until capacitation and acrosome reactions. In relation to the viability's membrane that protects sperm and regulates biochemical processes, the plasma membrane integrity is an appropriate structure and helps prevent the destruction to the sperm membrane, thereby improving the regulatory activities inside the sperm.

The low intact plasma membrane is due to the metabolism of protein and fat that are not optimal in the body of livestock. Fat and protein affect the process of spermatogenesis through metabolism, which will increase testosterone hormone production. Decrease in the protein and fat contents in the feed will inhibit the transportation process, causing low levels of testosterone synthesis effect on sperm formation. This is under Zirkin and Papadopoulos [44] that the resulting fat metabolism will help stimulate the Luteinizing Hormone (LH) produced by Leydig cells. Wherein LH will increase the activity of enzymes that will convert the fat into testosterone hormone in the spermatogenesis process. Hasbi and Gustina [18] stated that protein metabolism would secrete Follicle Stimulating Hormone produced by Sertoli cells. Luteinizing Hormone (LH) produced by Leydig cells will produce testosterone, and testosterone will bind to Androgen Receptors (AR) in Sertoli Cells and secrete Androgen Binding Protein (ABP). This Androgen Binding Protein will be used to carry the testosterone. Feeding with high or low protein was due to Total Digestible Nutrients (TDN). This study produced TDN at a balance of 80% grass: 20% was the lowest concentration. This is under the research of Dethan et al. [9] that the lower TDN in the feed resulted in the percentage of the plasma membrane.

The more optimum the protein needed in the sheep; the better quality of the plasma membrane will be better so that the metabolism of sperm is maximized. According to the statement of Husen et al. [45], protein absorbed by the body livestock, namely the amino acid arginine, can protect the plasma membrane of sperm from lipid peroxide damage by increasing nitrite oxidation. Meanwhile, if livestock lacks the amino acid arginine, it can cause a decrease in the plasma membrane quality so that sperm motility will decrease. Lysine is an important amino acid that plays a main role in plasma membrane integrity. Lysine is an L-carnitine formation precursor that is vital in producing metabolic energy by stimulating oxygen intake in mitochondria and improving the plasma membrane integrity [46].

The plasma membrane and the acrosome cap are inseparable units that cannot be separated because both play a role in the fertilization process. Decreasing the average number of sperm with intact acrosome caps is affected by excessive production of Reactive Oxygen Species (ROS) resulting from natural metabolic products [47]. This is because the ROS produced will affect membrane lipids, especially unsaturated fatty acids resulting in lipid peroxide, which will interfere with the plasma membrane, thereby damaging the acrosome cap [48]. Indicators of sperm plasma membrane structure and consistency are influenced by seminal plasma. Seminal plasma stabilizes the sperm environment to defend the structural integrity of the plasma membrane, and it is responsible for transporting sperm through the female reproductive tract and interacting with sperm and oocytes during fertilization [16].

A protein found in seminal plasma plays a function in preserving the structural integrity of sperm [49], and it also defends sperm membranes from reactive oxygen species (ROS), which, when passing through the female, lead to sperm Death reproductive system. Damaging the plasma membrane causes increased permeability cell membrane on the acrosome cap so that many compounds that are not desired can easily enter the cell [50]. This results in changes in the form of swelling and destruction of the acrosome [51].

The acrosome cap that has been damaged causes the hydrolytic enzyme contents contained in the acrosome to come out so that it can reduce the fertility process. Motility is directly connected with the intact plasma membrane and intact acrosome cap. This is evidenced by the results in this study, in which significantly the motility in T1 (balance 80%: 20%) and the results were not significant in T2 and T3. The motility in T2 (79.87%) was higher than that in the research

by Dethan et al. [9] which was 69.88% and the motility in T3 (81.40%) was higher than that of the research by Nurcholis et al. [39] which was 71.25%.

The results of this study are supported by Rumande et al. [52] and that an increase in membrane integrity will greatly affect motility. This increase in motility is due to the influence of fat contained in the plasma membrane in the form of ATP as an energy source. The higher the fat, which was given through the feed, the higher the membrane lipid synthesis in the endoplasmic reticulum, resulting in an increase in the structure of the membrane sperm. In addition, Stefanov et al. [53], Karoui et al. [54], and Rizal [28] stated that sperm motility was needed to reach the site of fertilization and the process of penetration protective barrier of the oocyte, especially the cumulus oophorous. Meanwhile, the acrosomal cap intact is very important because there are enzymes of lysis (acrosin and lysine) that are released during the acrosome reaction. Then these enzymes will be lysed on certain parts of the zona pellucida which will serve as the entry point for sperm into the cytoplasmic oocyte for later form the male pronucleus, and the process is then followed by the occurrence of synaptic and embryo formation. The reactions in the acrosome are influenced by cholesterol [55].

IV. Conclusion

Based on the results, it can be resolved that a balance of dietary grass and concentrate in the ration did not increase ADG and DMI significantly, but it can develop the semen quality of Garut rams. A ratio of 60% grass: 40% concentrate in the diet of rams is the optimal ratio to produce the best sperm quality of Garut sheep.

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