Different reproductive performances of young Garut rams are affected by long-term feeding regimen ratio

Aeni Nurlatifah[1], *Dewi Apri* Astuti[1], *Herdis* Herdis[2*], *Iis* Arifiantini[3], *Didid* Diapari[1], *Dedi Rahmat* Setiadi[3], *Fitra Aji* Pamungkas[2], *Santoso* Santoso[2], *and Pradita Iustitia* Sitaresmi[2]

[1]Faculty of Animal Science, Bogor Agricultural University, 16680 Bogor, Indonesia
[2]Research Center for Animal Husbandry, National Research and Innovation Agency,16915 Jakarta, Indonesia,
[3] Faculty of Veterinary Medicine, Bogor Agricultural University, 16680 Bogor, Indonesia

Abstract. This study aimed to investigate the effect of different feeding regimens in a long-term period on the reproductive performance of young Garut rams. Thirty individuals with an average body weight of 20.12 kg were randomly assigned to two groups. The CF group received 70% concentrate and 30% Pennisetum purpureum, while the FT group was provided with 70% Pennisetum purpureum and 30% tofu waste. The treatments were isoprotein and isoenergy. Rams were fed the same diet consistently starting from weaning age at 2 months. Semen and blood samples were collected at the age of puberty, around 9-10 months old. The results showed that different feeding regimens did not affect the total dry matter intake of young rams or their final body weight. Blood glucose was similar for both groups. Feeding CF resulted in a significantly higher ($P < 0.05$) blood cholesterol. Sperm viability and intact plasm membrane were highest ($P < 0.05$) in the CF group compared to the FT group. Semen volume and testosterone were higher ($P < 0.05$) in the group fed FT compared to the CF group. In conclusion, high concentrates have implications for better sperm membrane and survival, while a higher proportion of forage with tofu waste increases semen volume which is associated with more frozen semen production.

1 Introduction

Reproduction and performance of farm animals are largely dependent on their nutritional status [1]. It has been widely known that appropriate feeding management is crucial for successful mating in flocks [2, 3]. Deficiencies in carbohydrate, protein, and nucleic acid metabolism may impact spermatogenesis, libido, fertility, embryonic development, and survival [4]. There are several studies concerning the relationship between nutrition and fertility. Feed availability is a major environmental factor influencing sperm quality [5].

* Corresponding author: : herd001@brin.go.id

The diet composition changes the quality of the semen and the fertilizing potential of spermatozoa in domestic animals [6]. Garut sheep is one of Indonesia's important germplasms that must be preserved by increasing it is semen quality. Semen quality, testosterone concentration, and testicular size of lamb are positively aligned with nutritional status [7]. In a study on semen production, which different levels of dietary energy on rams showed that progressive motility and mitochondrial membrane of sperm were higher in rams fed control and high-energy diet compared to low energy diet [8]. Supplementing with dietary amino acids improves the quality of sperm in boars, modifies the amino acid composition of seminal plasma in boars, and raises the fertility of boar sperm, all of which are connected to blood hormone levels [9]. Other than energy and protein, Fiber has also been shown to have an effect on sperm quality. Fiber supplementation in pig diets before sexual maturity improves semen quality by changing gut microbiota composition and enhancing short-chain fatty acid production [10].

Most of these reports were carried out during a feed trial period during one cement production cycle or around 60 days. Many studies have also focused on comparing the effects of nutritional deficiencies or super nutritional levels of nutrients on sheep reproduction. Nevertheless, very little is known about the effects of different feeding regimens with different fiber content in isoenergy and isoprotein ration, especially when fed giving in long period, as important factors in productivity in young rams. We hypothesize that in the same amount of total digestible nutrient protein, different proportions of concentrate and protein also different feedstuff are potentially affected ram reproduction. Therefore, the present study aimed to evaluate the effect of different feeding regimens on feed intake, final body weight, testosterone, and semen characteristics in young Garut breed rams reared under intensive conditions.

2 Material and Methode

The experimental work protocols were approved by the Ethical Committee of the National Research and Innovation Agency (BRIN) under approval number 01112023000022. This study was conducted in 2023 at the Cikarawang farm, Bogor, West Java, Indonesia (6°32’38.3” S, 106°43’35,6” E).

2.1 Feed and final weight

Thirty individuals with an average body weight of 20.12 kg were randomly assigned to two groups. The CF group received 70% concentrate and 30% Pennisetum purpureum, while the FT group was provided with 70% Pennisetum purpureum and 30% tofu waste. Lamb fed with total dry matter 3.5% from body weight. Mix ration are given two time a day. The treatments were isoprotein and isoenergy. lambs were fed the same diet consistently starting from weaning age at 2 months. The final weight of Lambs was weighing in the age of 9 months old. Ration formulation and nutrient content are presented in Table 1.

2.2 Semen evaluation

Semen samples were collected in the morning at the age of puberty, around 9-10 months old. Fresh semen was evaluated immediately after semen collection, it was examined for macroscopic quality including volume, color, consistency, pH and microscopic quality including mass movement, concentration, percentage of progressive motility (%M), percentage viable (%V), percentage abnormalities, and percentage of intact plasma membrane (%MPI). Semen volume (mL) was observed by looking at the scale printed on the

collection tube. Color is seen visually. Consistency is observed visually by gently shaking the semen collection tube. The assessment criteria are thick, medium, and thin [7]. The degree of acidity (pH) of semen is measured using pH indicator paper. Microscopic evaluation included mass movement, and individual movement scores, live and dead spermatozoa (viability), and spermatozoa morphology with eosin nigrosin dye using a microscope (Olympus CH21) equipped with a heating table. The spermatozoa concentration was calculated using an SDM 6 photometer.

Table 1. Ration formulation and nutrient content of treatment

Ingredient	CF	FT
Pennisetum purpureum	30	70
Concentrate	70	-
Tofu waste	-	30
Nutrient content	----------%----------	
Crude protein	12,07	12,38
Crude fat	3,61	4,86
Crude fibre	19,35	29,27
Nitrogen free extract	55,81	40,36
TDN	61,22	60,55
Ca	0,46	0,59
P	0,92	0,28

Note: CF = 70% concentrate and 30% Pennisetum purpureum. FT = 70% Pennisetum purpureum and 30% tofu waste.

2.3 Blood metabolite and testosterone

Blood samples were collected one week before semen evaluation at 7.30 a.m. 2 hours after feeding. Blood samples were taken from the jugular vein of the lamb using a 3 mL disposable syringe. The blood was centrifuged at 3000 rpm for 15 minutes. Blood plasma is transferred immediately to a tube and stored at -20 °C for further analysis. Analysis of blood metabolite was done using a spectrophotometer. Analysis of total blood glucose was conducted using Glucose KIT (Cat No. 112191, Greiner, AU), and cholesterol KIT (Cat No. 116392, Greiner, AU). The enzyme-linked immunosorbent assay was used to measure the concentrations of adiponectin and testosterone (ELISA). Testosterone was analyzed using the ELISA kit (Cat. No. BZ-22086709-CPEB, Bioenzy) [8].

2.4 Statistical analysis

A completely randomized design was used in this study. Data were analyzed using analysis of variance. When there was a significant result, the means were compared using Duncan's test. The statistical models were assessed through the use of SPSS ver. 22 analysis software. A significant effect was considered at $P \leq 0.05$. data was presented as (mean ± std)

3 Result and Discussion

3.1 Blood metabolites and final weight

The findings showed that blood glucose was not significantly affected (P>0.05) by the feeding regimen in this study (Table 2). The same blood glucose concentration in this study is probably due to the almost the same energy content or TDN in both feeding regimens. TDN in the CF group is 61.22% and the FT group is 60.55%. Our results are in agreement with the result of lamb fed different kinds of oil with the same level of TDN resulting same blood glucose concentration [11]. Interestingly, the final weight was the same for both of CF and FT groups. Align with the result in blood glucose, the same final weight is probably due to the same energy and protein content in CF and FT treatment. The same finding was also reported in feedlot bull, with similar energy and protein content between treatments resulting same final weight [12].

Table 2. Effect of different feeding regimens on blood metabolite and final weight of young Garut semen

Parameters	CF	FT
Blood glucose	66,85 ± 7,61	64,32 ± 7,56
Blood cholesterol	90,07 ± 18,57 a	71,11 ± 17,56 b
Final weight	23,89 ±2,99	22,17 ± 1,79

Note: CF = 70% concentrate and 30% Pennisetum purpureum. FT = 70% Pennisetum purpureum and 30% tofu waste. Significantly different is indicated with a distinct superscript (P<0.01).

However, blood cholesterol was significantly (P<0.05) increase when lamb was fed with high proportion of concentrate (CF) compared to high proportion of forage (FT) group (table 2). Blood cholesterol in livestock is often associated with the high fat content of the feed [13]. In this study, the fat content in the CF and FT group rations was almost the same, namely 3.61% and 4.81%. The higher cholesterol concentration in the CF treatment when compared to the FT group was probably influenced by the higher fiber content in the FT group compared to the CF group (29.27% vs 19.35, respectively). Studies in growing cattle showed that a lower proportion of roughage will increase serum cholesterol [12]. There is a significant reduction in total cholesterol when animals feed a higher proportion of forage [14]. The mechanism of the decrease in cholesterol levels in blood by fiber is still being studied. Fiber may help lower blood cholesterol levels by reducing gastric emptying, intestinal motility, and fat absorption [15]. Another mechanism probably due to the Fermentation of fibers in the hindgut may reduce endogenous cholesterol production through the absorption of short-chain fatty acids [16].

3.2 Semen quality

The result in macroscopic quality showed that semen volume was significantly (P<0.05) higher in the FT group compared to the CF group. Semen pH, Consistency, and color were not significantly different (P>0.05) for both of CF and FT groups (table 3). The increase in seminal plasma in the FT group may be related to the composition of the feed ingredients used in this treatment. Since the FT group is feeding regimen with high forage combined with the tofu waste, we are looking for the correlation of semen volume with the fiber and active compounds in tofu waste. The effect of fiber on cement quality has not been widely reported.

studies on boar models show that increasing fiber content in feed does not change semen volume [10]. On the other hand, soybeans are often associated with high phytoestrogen content that could affect semen quality [17]. Rams grazing with phytoestrogenic clover reported has higher volume of ejaculates compared to control [18]. Other reported phytoestrogen correlated with decreased sperm production [19]. The mechanism by which these chemicals affect testicular function is not completely understood. Therefore, the reason higher semen volume in this FT group needs to be verified by further studies.

pH, consistency, and color of Garut lamb semen in this study are in agreement with the report in mature Garut rams. Garut rams semen are reported to have pH 6.5-6.7, with medium to thick consistency, and white to milky color [20]. However, semen volume was lower compared to mature rams reported as 0.8-1.2 ml [20]. The volume of lamb ejaculate will likely continue to increase as the lamb ages until it reaches adult ram ages [21]. At the age of 9 months, rams are reported to produce 1.01 ml of ejaculate volume, and this volume continues to increase as the age of the ram increases until it reaches 1.7 ml at the age of 15 months [22].

Table 3. Effect of different feeding regimens on macroscopic quality of young Garut semen

Parameters	CF	FT
Volume (ml)	0,53 ± 0,13 b	0,83 ± 0,33 a
pH (1-14)	6,41 ± 0,22	6,60 ± 0,37
Consistency (1-3)	2,53 ± 0,51	2,21 ± 0,89
Color (1-5)	4,06 ± 0,59	3,71 ± 0,46

Note: CF = 70% concentrate and 30% Pennisetum purpureum. FT = 70% Pennisetum purpureum and 30% tofu waste. [consistency: 1=Dilute, 2=Medium, 3= thick; Color: 1=Yellow, 2=White-Yellow, 3= beige, 4=white-beige, 5=milk white] Significantly different is indicated with a distinct superscript ($P<0.01$).

The findings in microscopic quality in lamb showed that mass movement of sperm was not significantly ($P>0.05$) affected by the feeding regimen (table 4). However, the viability and integrity of the sperm plasm membrane were significantly higher ($P<0.05$) in lamb fed with high concentrate (CF) group compared to high in the forage (FT) group. Abnormality, intact of acrosome, and concentration were not significantly affected ($P>0.05$) by the feeding regimen.

Higher viability and membrane integrity in the CF group align with the result in blood cholesterol levels that are also higher. Sperm viability and integrity of plasm are often correlated with sterols. Cholesterol is a key lipid component of membranes, having a well-known role in regulating lipid order. The main sterols found in mammalian sperm membranes are cholesterol and its immediate precursor, desmosterol [23]. Dietary lipids are critical in sperm quality, and their absence has a negative impact related to the start of oxidative stress and sperm DNA damage. However, feeding overload cholesterol also leads to decreasing sperm quality [24].

Semen quality including mass movement, motility, viability, membrane integrity, abnormality, and concentration of 9-month-old lamb in this study was in agreement with another study. Adult Garut ram reported medium to fast movement, with motility ranging from 77% to 80%, viability 75%, sperm plasm membrane integrity 75-85%, and concentration ranging from 2000-3000 (x 10^6 cell ml^{-1}) [20]. Microscopic parameters reported tend to increase positively from 9-month-old to 15-month-old [22].

Table 4. Effect of different feeding regimens on microscopic quality of young Garut semen.

Parameters	CF	FT
Mass Movement (0-3)	2,26 ± 0,37	2,23 ± 0,70
Motility (%)	78,24 ± 5,08	75,05± 4,91
Viability (%)	86,54 ± 5,00 a	80,39 ± 6,16 b
Integrity of plasma membrane (%)	93,42 ± 1,75 a	86,06 ± 10,11 b
Abnormality (%)	5,85 ± 2,15	8,80 ± 6,91
Intact acrosomes (%)	58,29 ± 9,16	64,79 ± 9,79
Consentration (x 10^6 cell ml^{-1})	2507,93 ± 862,62	2853,200 ± 2110,38

CF = 70% concentrate and 30% Pennisetum purpureum. FT = 70% Pennisetum purpureum and 30% tofu waste. [Mass Movement: 0=Not Moving. 1=Slow. 2=medium. 3=fast]. Significantly different is indicated with a distinct superscript (P<0.01).

3.1 Testosterone

Our finding showed that testosterone was significantly higher (P<0.05) in lamb fed with the FT group compared to the CF group (Fig. 1). FT feeding regimen consists of 70% forage and 30% tofu waste. FT group has higher fiber content compared to the CF group. Higher testosterone concentration was also reported in growing boars fed high-fiber [26]. Increased fiber consumption in growing boars promotes the growth of Leydig cells and increases the levels of testosterone. [26]. Testosterone is a steroid hormone that is usually associated with cholesterol concentration in the blood. Testosterone, a four-ring C18 steroid, is generated from cholesterol by an enzymatic multistep process, principally within the Leydig cells (~95%) located in the testes' interstitium [25]. However, this study shows that increasing cholesterol concentration in the CF group is not associated with higher testosterone in the CF group compared to the FT group. The same finding was reported in sows fed with high fiber has decreased serum cholesterol levels but significantly increased testicular cholesterol [26]. However, the inconsistent between blood serum and cholesterol levels is not known clearly and is worth further investigation.

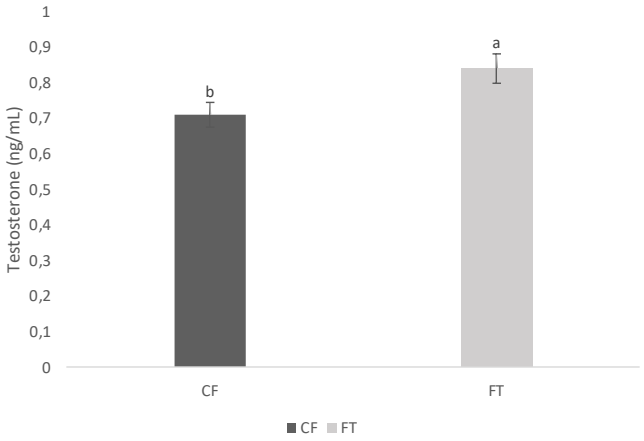

Fig. 1. Effect of different feeding regimens on testosterone of young Garut semen. CF = 70% concentrate and 30% Pennisetum purpureum. FT = 70% Pennisetum purpureum and 30% tofu waste. Significantly different is indicated with a distinct superscript. (P<0.05).

4 Conclusion

The feeding regimen from weaning to puberty affects blood metabolite, semen quality, and testosterone of Garut lamb. High-proportion concentrates have implications for higher blood cholesterol levels with better sperm membrane and survival, while a higher proportion of forage with tofu waste increases semen volume and testosterone.

References

1. F. Ibtisham, A. Nawab, L.G. Li GuangHui, X.M. Xiao Mei, A.L. An LiLong, G. Naseer, Effect of nutrition on reproductive efficiency of dairy animals. (2018)
2. J. Simões, J.A. Abecia, A. Cannas, J.A. Delgadillo, D. Lacasta, K. Voigt, et al, Managing sheep and goats for sustainable high yield production. Animal. **15**:100293. (2021)
3. A. Nurlatifah, L. Khotijah, K. Komalasari, D.A. Astuti, The effect of flushing with fatty acid supplementation in ewes ration on folliculogenesis. In: IOP Conference Series: Earth and Environmental Science. IOP Publishing; (2020)
4. L. Crisóstomo, M.G. Alves, A. Gorga, M. Sousa, M.F. Riera, M.N. Galardo, et al, Molecular Mechanisms and Signaling Pathways Involved in the Nutritional Support of Spermatogenesis by Sertoli Cells. In: Alves MG, Oliveira PF, editors. Sertoli Cells [Internet]. New York, NY: Springer New York; (2018)
5. J.C. Matos, A.R.F De Lucena, B.J.M. Ferreira, W.L. De Souza, G.C. Gois, D.R. Menezes, et al, Feed efficiency improves sperm quality and viability of fertilisation in Dorper sheep. New Zealand Journal of Agricultural Research. **23**;1–11. (2023)
6. M. Adeel, A. Ijaz, M. Aleem, H. Rehman, Ms. Yousaf, M.A. Jabbar, Improvement of liquid and frozen-thawed semen quality of Nili-Ravi buffalo bulls (Bubalus bubalis) through supplementation of fat. Theriogenology. **71**(8):1220–5. (2009)
7. F. Ghorbankhani, M. Souri, M.M. Moeini, R, Mirmahmoudi, Effect of nutritional state on semen characteristics, testicular size and serum testosterone concentration in Sanjabi ram lambs during the natural breeding season. Animal reproduction science. **153**:22–8. (2015)
8. S. Selvaraju, T. Sivasubramani , B.S. Raghavendra, P. Raju, S.B.N. Rao, D. Dineshkumar, et al, Effect of dietary energy on seminal plasma insulin-like growth factor-I (IGF-I), serum IGF-I and testosterone levels, semen quality and fertility in adult rams. Theriogenology.**78**(3):646–55. (2012).
9. H.J. Dong, D. Wu, S.Y. Xu, Q. Li, Z.F. Fang, L.Q. Che, et al, Effect of dietary supplementation with amino acids on boar sperm quality and fertility. Animal reproduction science. **172**:182–9. (2016)
10. Y. Lin, K. Wang, L. Che, Z. Fang , S. Xu, B. Feng, et al. The improvement of semen quality by dietary fiber intake is positively related with gut microbiota and SCFA in a boar model. Frontiers in Microbiology. 13:863315. (2022)
11. A. Nurlatifah, L. Khotijah, R.I. Arifiantini, M.S. Maidin, D.A. Astuti, Colostrum quality of ewe fed flushing diet containing EPA and DHA associated with lamb performance. Tropical Animal Science Journal. **45**(3):348–55. (2022)
12. S. Jeon, S. Jeong, M. Lee, J. Seo, D.K. Kam, J.H. Kim, et al, Effects of reducing inclusion rate of roughages by changing roughage sources and concentrate types on intake, growth, rumen fermentation characteristics, and blood parameters of Hanwoo growing cattle (Bos Taurus coreanae). Asian-Australasian journal of animal sciences. **32**(11):1705. (2019)

13. D.L. Palmquist, T.C. Jenkins, A 100-Year Review: Fat feeding of dairy cows. Journal of dairy science. **100**(12):10061–77. (2017)
14. H.A. Ghasemi, M. Kazemi-Bonchenari, A.H. Khaltabadi-Farahani, M, Khodaei Motlagh. The effect of feeding rations with different ratios of concentrate to alfalfa hay on blood hematological and biochemical parameters of farmed ostriches (Struthio camelus). Tropical animal health and production. **45**:1635–40. (2013)
15. D.K. Lee, S.Y. Park, S. Jang, E.H. Baek, M.J. Kim, S.M. Huh, et al, The combination of mixed lactic acid bacteria and dietary fiber lowers serum cholesterol levels and fecal harmful enzyme activities in rats. Archives of pharmacal research. **34**:23–9. (2011).
16. T. Rideout, Guar gum and similar soluble fibers in the regulation of cholesterol metabolism: Current understandings and future research priorities. VHRM. **4**:1023–33. (2008)
17. C.R. Cederroth, S. Nef, Soy, phytoestrogens and metabolism: A review. Molecular and cellular endocrinology. **304**(1–2):30–42. (2009)
18. K.R. Pool, T.C. Kent, L. Ding, C. Connolly, K.J. Foster, G, Enkhbat, et al. Low-moderate dietary phytoestrogens transiently disrupt spermatogenesis and the seminal plasma proteome in the ram. Reproduction. **165**(4):445–56. (2023)
19. S.L. Mumford, S. Kim, Z. Chen, D.B. Barr, G.M.B. Louis, Urinary phytoestrogens are associated with subtle indicators of semen quality among male partners of couples desiring pregnancy. The Journal of nutrition. **145**(11):2535–41. (2015)
20. K.B. Ariyanto, L. Khotijah, D.A. Astuti, R.I. Arifiantini, J.B. Menassol. Semen quality of garut rams feed by different protein sources and their implementation potential in small farms of West Java. Jurnal Agripet. **20**(1):47–55. (2020)
21. F. Nazari-Zenouz, G.H. Moghaddam, G. Hamidian, J. Ashrafi, S.A. Rafat, B. Qasemi-Panahi. Postnatal testicular development and testosterone changes in Ghezel ram lambs. Small Ruminant Research. **141**:70–6. (2016)
22. N. Maksimovic, S. Hristov, B. Stankovic, M.P. Petrovic, C. Mekic, D. Ruzic-Muslic, et al. Investigation of serum testosterone level, scrotal circumference, body mass, semen characteristics, and their correlations in developing MIS lambs. Turkish Journal of Veterinary & Animal Sciences. **40**(1):53–9. (2016)
23. M. de Las Mercedes Carro, D.A. Peñalva, S.S. Antollini, F.A. Hozbor, J. Buschiazzo. Cholesterol and desmosterol incorporation into ram sperm membrane before cryopreservation: Effects on membrane biophysical properties and sperm quality. Biochimica et Biophysica Acta (BBA)-Biomembranes. **1862**(9):183357. (2020)
24. F. Saez, J.R. Drevet. Dietary cholesterol and lipid overload: impact on male fertility. Oxidative Medicine and Cellular Longevity. (2019).
25. A. Zamir, T. Ben-Zeev, J.R. Hoffman. Manipulation of dietary intake on changes in circulating testosterone concentrations. Nutrients. **13**(10):3375. (2021)
26. Y. Lin, D. Wu, L. Che, Z. Fang, S. Xu, B. Feng, et al, Dietary fibre supplementation improves semen production by increasing leydig cells and testosterone synthesis in a growing boar model. Frontiers in Veterinary Science. **9**:850685. (2022)