ASSOCIATION BETWEEN ENERGY STATUS AND LIPID METABOLISM OF GARUT EWES REARED IN INTENSIVE HOUSING SYSTEM WITH AND WITHOUT FREE RANGE ACCESS

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Abstract

The purpose of this study is to determine the association between energy status and lipid metabolism of Garut ewes reared in intensive housing system with and without free range access. Garut ewes with a total of 20 heads were randomly placed into two treatments. The first group/treatment was intensive housing system without free range access (T1, n=10), while the second group was kept in intensive housing system with free range access 2 hr per day (T2, n=10)). Blood samples were collected in jugular vein at 70 d of experiment. Blood biochemistry analysis was performed at Laboratory of Animal Physiology and Biochemistry Faculty of Animal Husbandry Universitas Padjadjaran. Data were analyzed using a T-test. The results revealed there is no significant different on glucose concentration as energy status and plasma cholesterol and triglyceride as lipid metabolism parameter between treatments. However, ewes reared on intensive housing system with free range access had higher cholesterol and glucose concentration compared to ewes reared on intensive housing system without free range access. Moreover, there is no association between energy status and lipid metabolism parameters. This study shows that mild exercise of 2 hr doing an active behavior such as walking and running in outside area did not reduce energy status and lipid metabolism. In conclusion, intensive housing system with free range access did not give any negative effect on performances supported with normal energy status and lipid metabolism.

Key words: *Rearing system, Garut ewes, Cholesterol, Triglyceride, Glucose*

INTRODUCTION

Garut sheep are ruminant that are reasonably easy to care for and extremely profitable, as well as one commodity that must be protected as a potential genetics resource as a native animal in Indonesia [1]. Garut ewes rarely have horns, and if they do, the size of the horns is minuscule in comparison to Garut ram. Garut ewes can have more than two lambs in a single cycle of delivery [2].

Environment condition in housing system have an impact on livestock productivity and health. The environmental factors such as sunlight radiation, air temperature, air humidity, and wind speed will directly affect livestock [3]. Indonesia is one of the tropical countries with relatively high temperatures and humidity. Tropical environment has a significant impact on animal rearing, including sheep rearing system.

Intensive housing system refers to how sheep are reared inside for whole day with good housing management. By shielding sheep from extreme heat or cold and by providing appropriate nutrition, housing systems may improve the wellbeing and health of sheep. However, poor housing condition will reduce the wellbeing and health of the sheep.

Intensive housing system with free access to external area is a welfare treatment in which controlled livestock rearing adds

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value since the livestock gets more vitamin D from the sun. Furthermore, the muscle in this sheep will be more active, keeping the body in a proportional state. There is also an anticipated advantage that will increase the quality of physiological parameters and sheep health, as evidenced by cholesterol and triglyceride as lipid metabolism. Also, will affect glucose as energy status.

Glucose is needed for body growth, fetal growth (including the growth of the placenta), udder expansion, and general milk production in sheep. Despite being the main metabolic fuel and being necessary for vital organ function, fetal growth, and milk production. Glucose is a sensitive indicator of energy status since it is tightly regulated by homeostasis. Glucose is an important sugar component because it regulates metabolism and energy feed do not meet the requirement, thus plasma glucose levels rise and pancreatic alpha cells release glucagon into the bloodstream. To meet the body's glucose needs, glucagon and epinephrine present in the circulation induce gluconeogenesis in the liver and glycogenolysis in the muscle [4]. Ruminants need blood glucose monitoring because they require protein and glucose in equal amounts throughout their lives [5].

Energy sources in animals depend on metabolism of carbohydrate, protein and lipid. The profile of lipid metabolism in the blood can be affect reproduction, health, and productivity of livestock. Hepatic lipid metabolism is a normal physiological process involving an energy-dependent, complex system that results in the hepatic release of very-low-density lipoproteins (VLDL) into circulation [6]. Lipid metabolism entails the synthesis of structural and functional lipids (such as phospholipids, glycolipids, sphingolipids, cholesterol, prostaglandins, and so on) that are specific to individual tissues, as well as the degradation of lipids to meet the body's metabolic needs (e.g., energy production). Lipid metabolism is in a condition of continual dynamic balance. This means that some lipids are constantly oxidized to supply the metabolic needs of the organism, while others are produced and stored. Changing the activity of lipoprotein lipase and improving glucose metabolism are two ways to modify lipid metabolism [7]. This occurs as a result of the stress experienced by ewes that are not reared, and it is believed that the provision of pens will mitigate this impact.

Triglycerides are a component of the blood fat profile that is widely used to determine the metabolic rate of animals. Triglycerides are a key source of energy for muscle growth and boosting performance, as well as a lipid storage site in the body and bloodstream [8]. The high level of triglycerides in livestock can be produced by a variety of factors, including a high degree of stress in the body, which will increase the body's metabolic rate to compensate for the problem. Providing free access to external area in intensive maintenance might be reduced stress. Therefore, metabolism including lipid metabolism may not disrupt. Normal triglycerides in the ewes' bodies can be maintained and the usage of triglycerides can be more focused on muscle growth and livestock performance.

Cholesterol is a fat component that helps to build skin, muscles, cell membranes, and other internal organ components. Cholesterol plays important role in the body and influence the function of metabolism [9]. Cholesterol serves as a precursor to produce steroid hormones such as progesterone, cortisol, corticosterone, and estradiol. In ewes, normal blood cholesterol levels range from 50 to 140 mg/dL [10].

Cholesterol is required for the formation and maintenance of membrane cells, which allow degrees of distinctive membrane fluids to connect [11]. Garut sheep have higher cholesterol levels in adults than in young and lactating ewes [12]. This is because the higher the amount of activity of animal body parts, the lower the rate of cholesterol [13]. Sheep raised in pens had

lower cholesterol levels as a result of being exposed to heat-dependent sunshine, which turns into vitamin D, which is then converted into cholesterol by an enzyme called 7-dehydrocholesterol reductase (DHCR7) (5).

Previous research found that an intensive housing system had an impact on the energy status and lipid metabolism of sheep. When animals are exposed to the sun during the hot season, their plasma glucose levels rise compared to animals kept in intensive housing system with and without free access [14]. Treatment of Garut sheep with a intensive housing system care approach will involve more exercise than intensive care, where more sheep are exposed to the sun's rays. The objective of this study is to determine the association between energy status and lipid metabolism of Garut ewes reared in intensive housing system with and without free range access.

MATERIAL AND METHOD

Animals, Housing and Design of experiment

Twenty Garut ewes were used in this study. Ewes with ages about 12±2 months old, with an initial average body weight of 19.52 kg were randomly selected and placed into two treatments of housing system. The two-housing system treatment are intensive housing system without free range access (T1, n=10) and intensive housing system with free range access (T2, n=10). Study was carried out for 70 days. Blood samples were collected in jugular vein at 70 d of experiment. The Independent Sample T-test with two treatments and ten replications was employed to assess the study hypothesis. This study is being conducted in the Laboratory of Beef Cattle Production (Ciparanje cage Sheep), Faculty of Animal Husbandry, Universitas Padjadjaran. The individual pen used in this research is a 0.7 m length, 1.5 m wide, and 0.8 m height, with a 200 m^2 wide external pen area.

Diet

The ration arrangement used in this study is a 6:1 ratio of forages and concentrates. Forage was taken from the Ciparanje area and was administered after the grass had been aerated. Pollard, chocolate skin, copra meal, palm oil meal, wafer crust, cassava cake, premix, salt, lime, and corn concentrate make up the concentrate. The nutritional content is shown in Table 1.

Table 1 Content Nutrition of Garut ewes feed

Feed Ingredients	Forage (%)	Concentrate (%)
Water (%)	85,28	9,72
Ash (%)	13,17	6,42
Proteins (%)	13,93	16,17
Fiber (%)	31,48	14,19
Fat (%)	2,29	11,84
Carbs (%)	39,13	51,38
Energy Gross (kcal /kg)	3091	4012
Total Digestible Nutrients (%)	45,48	67,39

Note : Laboratory Ruminant Animal Nutrition and Animal Feed Chemistry (2021)

Blood Analysis

Garut ewes were selected based on their weight and age. Ewes were transported to the research center. Ewes were acclimatation for 14 days and adapted with experimental feed. Feed adaptation is achieved in the first week by supplying forages and concentrates of equivalent quality. At the second week of the trial, both groups started to get the treatment, 10 sheep receiving intensive maintenance and 10 sheep intensive housing system with free access for 2 hr in the morning. Plasma analysis for cholesterol using the Biolabo

kit number catalog 108491, for triglyceride using the Biolabo kit number catalog 80019, and for glucose using the Biolabo kit number catalog 112191, which is then readed by Spectrophotometry.

RESULTS AND DISCUSSION

Energy Status and Lipid Metabolism

Energy status of ewes were represented by plasma glucose concentration. Lipid metabolism were represented by plasma cholesterol and triglyceride concentration. In this research there is no a significant difference ($P<0.05$) on glucose, cholesterol, and triglyceride of ewes from both treatments. This demonstrates that activity within the ewes' pen has no effect on energy status or lipid metabolism.

Table 2 Mean values (mean±SEM) of glucose, cholesterol and triglyceride concentration between ewes reared without free access to external area (T1) and with free access to external area for 2 hr (T2)

Parameter	Treatment		P-value	Sig.
	T1	T2		
Glucose (mg/dL)	69.68±2.56	73.66±1.63	0.207	Not Significant
Triglycerides (mg/dL)	22.19±2.31	22.64±1.79	0.882	Not Significant
Cholesterol (mg/dL)	93.63±6.98	108.77±8.65	0.190	Not Significant

In this study, sheep glucose levels were not influenced by a full in intensive housing system with or without free access to external area. However, the rate of glucose in the blood of the Garut Sheep will be more optimal in intensive housing with free access to external area as compared to the intense treatments without free access to external area. Normal sheep blood glucose levels range from 44 to 81 mg/dL [15]. Glucose concentrations in the present study were in accordance with previous experimental studies that recorded serum glucose levels to be higher in lactation than pregnancy in ewes [14-18]. The increase may reflect the recovery of feed intake and improving energy status of the ewe after rearing.

Our study demonstrates that the influence of housing system with and without free access on the rate of blood glucose is minor or has no genuine difference. According to research by Pankaj [19], there is no significant difference in plasma glucose of Dekani sheep maintained by the system maintenance intense of 54.44 mg/dL, semi-intensive of 58.32 mg/dL, and extensive of 57.11 mg/dL.

A previous study reported that animals managed in a semi-intensive system have lower glucose levels than those kept in an intensive system [20]. There was not much activity that the lamb could do in the intensive care system, which caused an increase in glucose (6.68 mmol/L), whereas the sheep in the semi-intensive care system utilized levels of blood glucose sufficient to meet the demand for energy from activity or exercise (1.72 mmol/L) [21]. According to Sejian in (22) and (23), the hormone cortisol stimulates gluconeogenesis in the heart, which converts non-carbohydrate substances into glucose to supply the energy needs of livestock under stress.

Intensive maintenance with external access resulted in higher plasma glucose levels (73.66 mg/dL) than intensive maintenance without free access (69.69 mg/dL). This is because the ewe's activity treatment was insufficient to complete the exercise, resulting in a greater requirement for glucose and a decline in plasma glucose levels. According to Sejian, Payne, and Wasserman (23-25), the drop rate of glucose in livestock can be supplied by activity that induces energy mobilization and the

existence of decline backup glycogen induced by high require glucose to be energy.

According to Prihatno [26], a lack of glucose can impair the production of follicle stimulating hormone (FSH) and luteinizing hormone (LH), obstructing the formation of follicles, eggs, estrogen, and progesterone. According to Winugroho [27], rate blood glucose when related with the absence of a distinct progesterone hormone profile and there is not yet known an appropriate backup energy that optimum for body transformation from postpartum estrus to normal condition. Maintaining lactating ewes in outdoor enclosures during daytime was beneficial to their behavioural needs, stimulating exploratory and active behaviours. In addition, outdoor-reared ewes produced milk with lower somatic cell counts compared with indoor animals [28].

Our study showed no difference on lipid metabolism between treatments. Plasma triglycerides and cholesterol were similar between treatments. The triglyceride content of the two treatments ranged from 22.19 - 22.64. The triglyceride content in the sheep in this study is the same as the results of Prayitno and Heni's [30] study where the normal triglyceride content in the blood is due to the fact that the body's energy needs have been met. Razali [31] stated that the triglyceride content of goats ranged from 46.67 – 247.67 mg/dl much higher than sheep. Triglycerides are one of the body's energy reserves. Lipid metabolism result in free fatty acid and glycerol. Free fatty acids can be esterified and stored as TAG in liver [32]. Stress affect level of triglycerides in livestock. A previous study showed that stress as indicated with low paraoxonase (PON) were associated with the level of plasma triglycerides [33]. Providing free access to the sheep during intensive management reduced stress.

In this research, cholesterol levels of T2 treatment were 108.77 mg/dL and cholesterol levels of T1 treatment were 93.63 mg/dL, which was thought to be caused by high levels of free fatty acids. This remark is consistent with the findings of Raju [34], who compared treatment intensive and semi-intensive on the biochemistry of sheep blood, yielding treatment intense results of 67.72 mg/dL and semi-intensive results of 46.5 mg/dL. According to study results, cholesterol levels in system grown sheep were relatively the same in intensive without access and intensive systems with free access, due to the presence of activity use the fatty acids.

Cholesterol is synthesized in the small intestine epithelium for the transportation of dietary lipids. Also, cholesterol is the precursor of various steroid hormones. Cholesterol aids in the production of fat components such as skin, muscles, membranes, cells, and other internal organs (9). Table 2 shows cholesterol concentration in ewes's plasma in T1 is lower than T2 (93.63 mg/dL vs 108.77 mg/dL), respectively. The normal cholesterol levels in sheep blood are 50-140 mg/dL [5]. Our study is contradicting with the findings of Karthik [16], who stated that intensive and semi-intensive management had an impact on cholesterol of Nellore sheep. Stress stimulates cortisol hormone to be released, which then activates gluconeogenesis in the heart.

According to Khotijah [17], excessive cholesterol levels in the blood have an impact on Estrogen and progesterone hormones, which have a role in fertility. Hess (18) reported that cattle with high cholesterol levels will have a more difficult time having progeny than cattle with normal cholesterol levels. Plasma cholesterol was lower in the intensive treatment with free access (T2) compared to the intensive treatment without free access (T1). This is due to the presence of higher functional fatty acids in the T2 treatment in lipoprotein metabolism, which reduces the risk of cardiovascular disease [29]. Exercise that attempts to minimize stress did not appear to effect substantial parameter changes by increasing gluconeogenesis liver, on this ewes is capable to fulfilling need energy during stress [23].

Association between Energy Status and Lipid Metabolism

In this study, the Pearson correlation test was performed to examine the relationship between energy status as represented by glucose and lipid metabolism as indicated by triglyceride and cholesterol parameters, the results of which are shown in Tables 3, 4 and 5.

Pearson correlation data demonstrate no significant association between the three parameters between the two treatments. However, it seems there was a negative association between energy status and lipid metabolism (both triglycerides and cholesterol) in the T2 treatment. This suggested the greater the energy status alongside provision for physical activity on external areas in T2 treatment, the smaller the lipid metabolism.

Table 3. Correlation of Energy Status and Lipid Metabolism of ewes reared without free access to external area

		Glucose	Triglycerides	Cholesterol
Glucose	Pearson Correlation	1	.425	.133
	Sig. (2-tailed)		.220	.713
	N	10	10	10
Triglycerides	Pearson Correlation	.425	1	.360
	Sig. (2-tailed)	.220		.307
	N	10	10	10
Cholesterol	Pearson Correlation	.133	.360	1
	Sig. (2-tailed)	.713	.307	
	N	10	10	10

The distribution of pearson correlation graphs of glucose, trigliceride, and cholesterol in Treatment 1 can be viewed as follows :

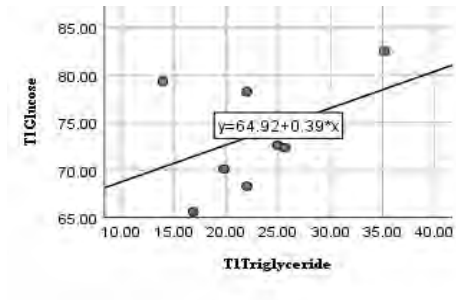

Fig. 1 Simple Scatter Graph with Fit Line of T1-Glucose by T1-Triglyceride

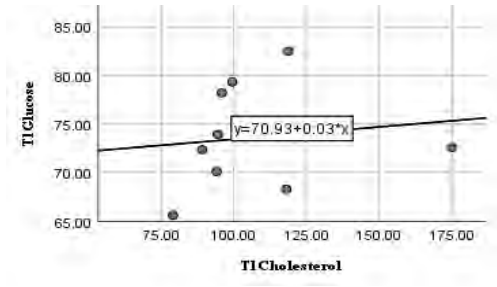

Fig. 2 Simple Scatter Graph with Fit Line of T1-Glucose by T1-Cholesterol

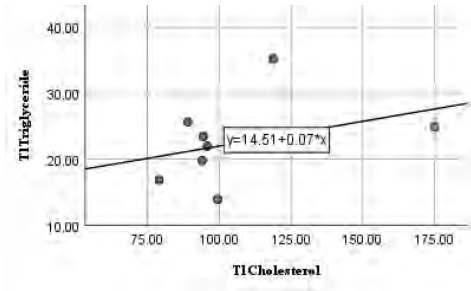

Fig. 3 Simple Scatter Graph with Fit Line of T1-Triglyceride by T1-Cholesterol

Table 4. Correlation of Energy Status and Lipid Metabolism of ewes reared with free access to external area

		Glucose	Triglycerides	Cholesterol
Glucose	Pearson Correlation	1	.080	.193
	Sig. (2-tailed)		.825	.594
	N	10	10	10
Triglycerides	Pearson Correlation	.080	1	-.483
	Sig. (2-tailed)	.825		.158
	N	10	10	10
Cholesterol	Pearson Correlation	.193	-.483	1
	Sig. (2-tailed)	.594	.158	
	N	10	10	10

The distribution of Pearson correlation graphs of glucose, triglyceride, and cholesterol in Treatment 2 can be viewed as follows :

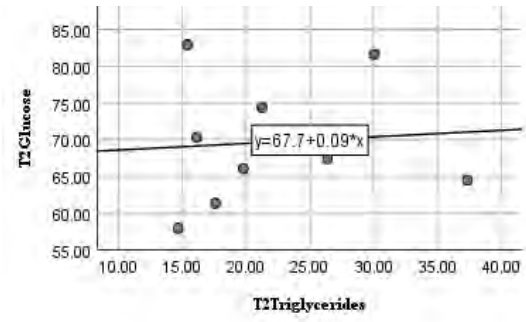

Fig. 4 Simple Scatter Graph with Fit Line of T2-Glucose by T2-Triglyceride

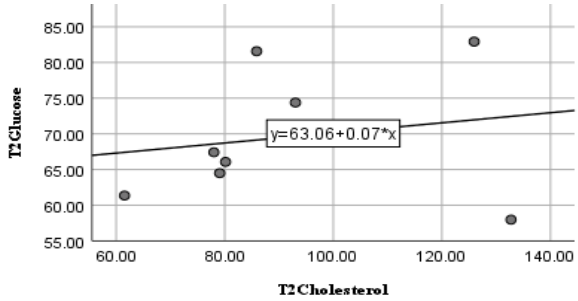

Fig. 5 Simple Scatter Graph with Fit Line of T2-Glucose by T2-Cholesterol

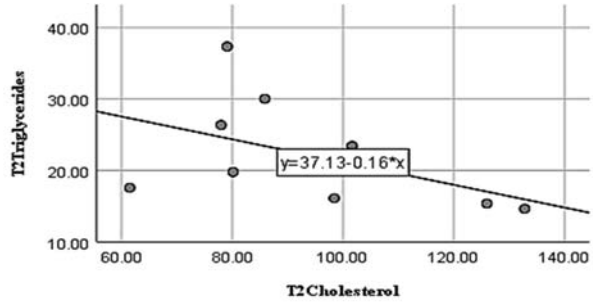

Fig. 6 Simple Scatter Graph with Fit Line of T2-Triglyceride by T2-Cholesterol

Table 5. Correlation of Energy Status and Lipid Metabolism of ewes in all treatment

		Glucose	Triglycerides	Cholesterol
Glucose	Pearson Correlation	1	-.053	.234
	Sig. (2-tailed)		.824	.321
	N	20	20	20
Triglycerides	Pearson Correlation	-.053	1	.195
	Sig. (2-tailed)	.824		.409
	N	20	20	20
Cholesterol	Pearson Correlation	.234	.195	1
	Sig. (2-tailed)	.321	.409	
	N	20	20	20

The distribution of pearson correlation graphs of glucose, trigliceride, and cholesterol in all treatment can be viewed as follows :

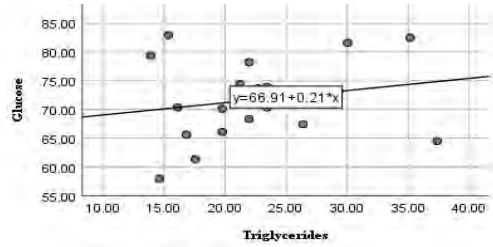

Fig. 7 Simple Scatter Graph with Fit Line of Glucose by Triglyceride

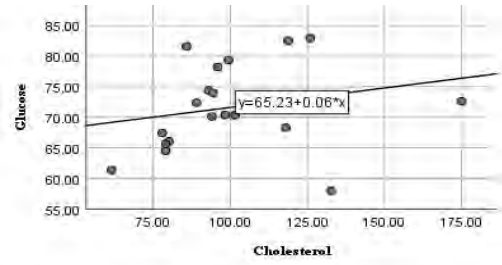

Fig. 8 Simple Scatter Graph with Fit Line of Glucose by Cholesterol

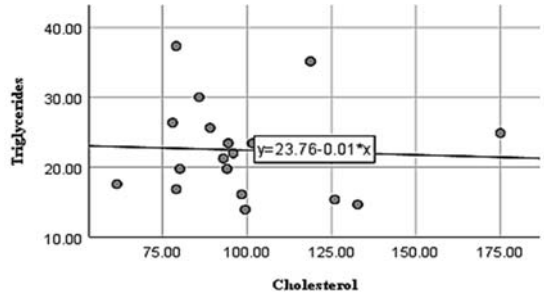

Fig. 9 Simple Scatter Graph with Fit Line of Triglyceride by Cholesterol

CONCLUSIONS

Intensive management with and without provision to pen has the similar effect on concentration of triglyceride, cholesterol and glucose in Garut ewes. Furthermore, intensive housing system with free range resulted in an optimum triglyceride cholesterol and glucose levels that are within the normal range

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REFERENCES

1. Pusparini, A; Indrijani, H; Nurachma, S. Initial Selection of Prospective Performance of Ram and Ewes Sheep at UPTD BPPTD Margawati Garut. Universitas Padjadjaran Press. **2015**.
2. DJPKH (Direktorat Jenderal Peternakan and Kesehatan Hewan). Domba Garut. Kementerian Pertanian Republik Indonesia. **2015**.
3. Made, IN. Environment and Livestock Productivity. Faculty of Animal Husbandry. Universitas Udayana. **2017**.
4. Rahayu, S; Yamin, MC; Sumantri; Astuti, DA. Hematology Profile and Blood Metabolite Status of Garut Sheep Feeding Waste Sprouts in the Morning or Evening. Jurnal Veteriner. **2017**. https://doi.org/10.19087/jveteriner.2017.18.1.38.
5. Vivek, GP; Anuradha, V; Khadilkar; Shashi, A; Chiplonkar; Zulf, MM; Vaman, V; Khadilkar. Varying Relationship between 25-hydroxyvitamin D, High Density Lipoprotein Cholesterol, and Serum 7-dehydrocholesterol Reductase with Sunlight Exposure. Journal of Clinical Lipidology. **2015**. DOI: 10.1016/j.jacl.2015.05.007.
6. Rodríguez, CE; Duque, AMH; Steinberg, J; Woodburn, DB; Chelonia. Pathology of Wildlife and Zoo Animals, 825–854. **2018**. https://doi.org/10.1016/B978-0-12-805306-5.00034-1.
7. Cefalu, WT; Wang, ZQ; Zhang, XH. Oral Chromium Picolinate Improves Carbohydrate and Lipid Metabolism and Enhances Skeletal Muscle Glut-4 Translocation in obese, hyperinsulinemic (JCR-LA corpulent) rats. J Nutr 132(6):1107– 1114. **2002**. DOI: 10.1093/jn/132.6.1107.
8. Adriani, L; Hernawan, E; Hidayat, U. Decreasing Cholesterol And Triglyceride Level On Blood By Adding Orange (Citrus Sinensis) Waste On Padjajaran I Sheep. Science Papers. Series D. Animal Science, 57, 70-73. **2014**.
9. Laurencio, B. Cholesterol A Patient Guide. UK:. J. Med. **2002.**
10. Meyer, DJ; Harvey, JW. Veterinary Laboratory Medicine Interpretation and Diagnosis. Philadelphia Saunders. **2004.**
11. Kartikasari, NI; Syauqy, A. Differences in LDL and HDL Cholesterol Levels Before and After Giving Mung Bean Juice (Phaseolus radiatus linn) in Men with Dyslipidemia. Journal of Nutrition Colleg. **2014**.
12. Ningrum, A. Nutritional Content and

Cholesterol Levels of Garut Lamb during Breastfeeding (Milk Lamb), Young (Lamb), and Adult (Mutton). Bogor: Livestock Products Technology. Faculty of Animal Husbandry. Institut Pertanian Bogor. **2006.**

13. Atsani, FHP. The Effect of Giving The Green Powder (Camellia sinensis) in Rations on Blood Cholesterol and Triglyceride Levels in Priangan Do Sheep. Faculty of Animal Husbandry. Universitas Padjadjaran. Sumedang. **2019**
14. Singh, DN; Wadhwani, KN; Arya, JS; Sarvaiya, NP; Patel, AM. Effect of Housing Systems on Blood Constituents of Ewes During Summer in A Subtropical Climate. Anand Agricultural University.**2008**.
15. Cynthia, MK; Scott, L. The Merck Veterinary Manual. 9th ed. New Jersey (US): Kahn CM Merck & Co Inc. **2005**.
16. Karthik, D; Suresh, J; Reddy, RY; Sharma, GRK; Ramana, JV; Gangaraju, G; Ranga, PR; Kumar, PR; Yasaswini, D; Adegbeye, MJ; Kanth, RR. Adaptive Profiles on Nellore Sheep with Reference to Farming System and Season: Physiological, Hemato-biochemical, Hormonal, Oxidative-enzymatic, and Reproductive Standpoint. Heliyon. **2021**. DOI: 10.1016/j.heliyon.2021.e07117.
17. Khotijah, L; Setiadi, MA; Wiryawan, KG; Astuti, DA. Reproductive Performance, Cholesterol and Progesterone Status of Garut Ewes Fed Ration Containing Different Levels of Sun flower Oil. Pakistan Journal of Nutrition. **2015**. DOI:10.3923/pjn.2015.388.391.
18. Hess, BW; Lake, SL; Scholljegerdes, EJ; Weston, TR; Nayugihugu, V; Molle, JDC; Moss, GE. Nutritional Controls of Beef Cow Reproduction. Jounal of Animal Science. **2005**. https://doi.org/10.2527/-2005.8313_supplE90x.
19. Pankaj, PK; Venkata, RN; Rani, R; Nikhila, M; Ravishankar, K. Biomolecular Expression of Deccani Sheep under Different System of Rearing. Animal Nutrition Association. Bikaner, Rajasthan. **2015.**
20. Onasanya, GO; Oke, FO; Sanni, TM; Muhammad, AI. Parameters Influencing Haematological Serum and Biochemical References in Livestock Animals Under Different Management Systems. Open Journal Of Veterinary Medicine. **2015**. DOI: 10.4236/ojvm.2015.58025.
21. Jimoh; Abubakar, O; Adenekan; Olabisi, O; Jack; Asuquo, A; Umawziozi; Carols, U; Iheijirika. Physiological Variations and Oxidative Status in West African Dwarf Male Sheep and Goat in Semi Intensive Management System. Animal Research International. **2018**.
22. Sejian, V; Vijai, PM; Naqvi, SMK. Effect of Walking Stress on Growth, Physiological Adaptability and Endocrine Responses in Malpura Ewes in a Semi-arid Tropical Environment. Int J Biometeorol. **2012**. DOI: 10.1007/s00484-011-0420-y
23. Sejian, V; Srivastava, RS. Pineal Adrenal Immune System Relationship Under Thermal Stress Effect on Physiological Endocrine and Non Specific Immune Response in Goats. J Physiol Biochem. **2010.** DOI: 10.1007/s13105-010-0040-8**.**
24. Payne, JM; Payne, S. The Metabolic Profile Test. Oxford University. Oxford. **1987**.
25. Wasserman, DH; Williams, PE; Lacy, DB; Goldstein, RE; Sherringtton, AD. Exercise Induced Fall in Insulin and Hepatic Carbohydrate Metabolism During Muscular Work. Am J Physiol. **1989**. DOI: 10.1152/ajpendo.1989.256.4.E500.
26. Prihatno, SA; Kusumawati, A; Karja, NWK; Sumiarto, B. Blood Biochemistry Profile in Dairy Cattle Experiencing Repeated Mating. Jurnal Kedokteran Hewan. **2013**.
27. Winugroho, M. Supplementary Feeding Strategies to Improve the Reproductive Efficiency of Cows. Jurnal Penelitian dan Pengembanran Pertanian Badan Penelitian dan Pengembangan pertanian. Departemen Pertanian. **2017**.
28. Casamassima, D; Sevi, A; Palazzo, M; Ramacciato, R; Colella, GE; Bellitti, A. Effects of two different housing systems on behaviour, physiology and milk yield of Comisana ewes. Small Rumin. Res. 41, 151–161. **2001**. DOI: 10.1016/s0921-4488(01)00201-2.
29. Kasapidou, E; Basdagianni, Z; Papadopiulos, V; Karaiskou, C; Tsiotsias, AKA. Effect on Intensive and Semi-Intensive Production on Sheep Milk Chemical Composition Physicochemical Characteristics Fatty Acid Profile and Nutritional Indices. Jurnal Animal. **2021**. https://doi.org/10.3390/ani11092578.
30. Prayitno, RS; Heni, N. The effect of giving red onion leaves (Allium ascalonicum L.) as an alternative forage on the blood lipid profile of sheep. Journal of Animal Husbandry, 18(1), 19-24. **2021**.

31. Razali, S; Suryaningsih; Sapriani. Blood Serum Triglyceride Values of Male Goats After Giving Infusion of Katuk Leaves (Sauropus androgymus (L.) Merr). Scientific Journal of Biology Education, Educational Biology. 5: 39-42. **2013**.
32. Mayasari, N; Chen, J; Kemp, B; Parmentier, H; Van Knegsel, A. Relationship of Free Fatty Acid and Natural Autoantibodies 2 Weeks Postpartum. In IOP Conference Series: Earth and Environmental Science (Vol. 334, No. 1, p. 012031). IOP Publishing. **2019**. DOI 10.1088/1755-1315/334/1/012031.
33. Mayasari, N; Chen, J; Ferrari, A; Bruckmaier, RM; Kemp, B; Parmentier, HK; Van Knegsel, ATM; Trevisi, E. Effects of dry period length and dietary energy source on inflammatory biomarkers and oxidative stress in dairy cows. Journal of dairy science, 100(6), pp.4961-4975. **2017**. DOI: 10.3168/jds.2016-11857.
34. Raju, VN; Pankaj, PK; Ramana, DBV; Kavitha, V. Intensification in Deccani Sheep Haematological and Biochemical Influences. European Jaournal of Molecular Biologi and Biochemistry. **2015**.