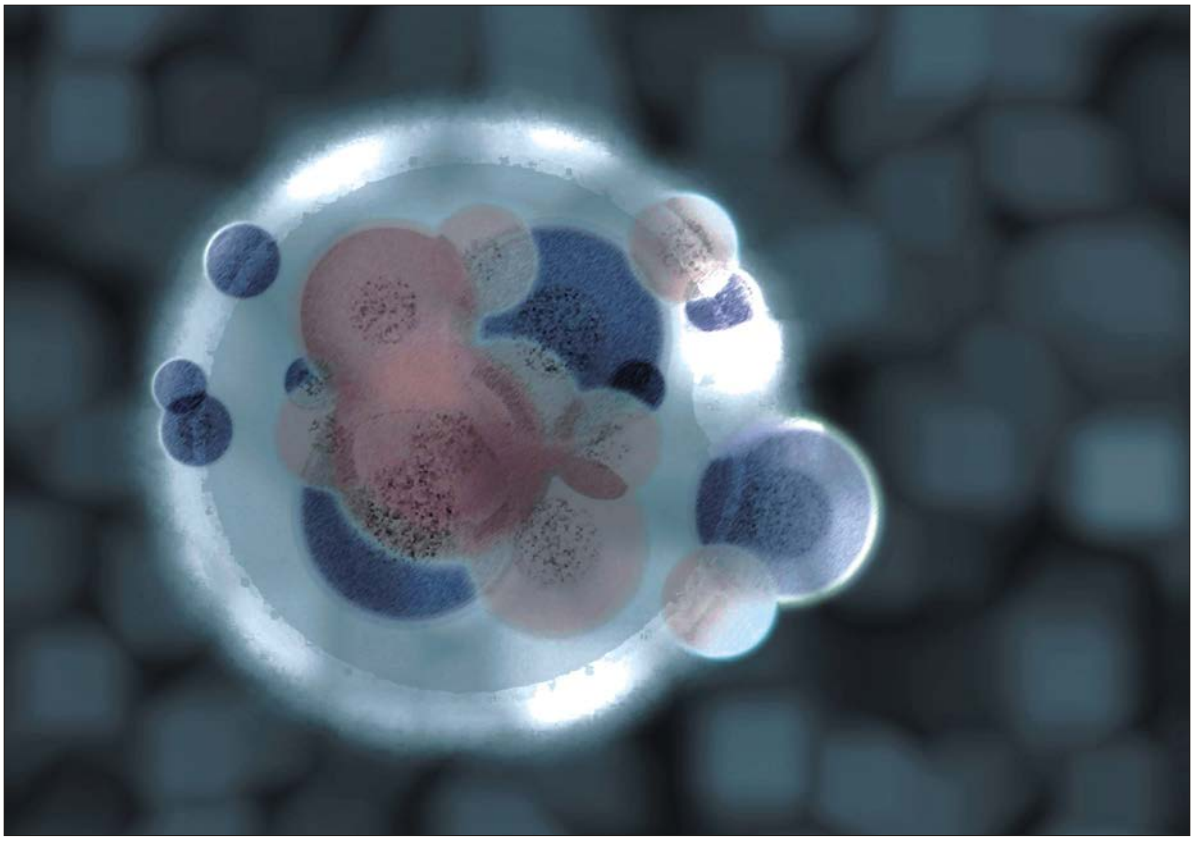

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Research Article
Effects of Protein and Energy Balance in Ration on Physiological and Hematological Status of Garut Sheep

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Abstract

Background and Objective: The quantity and quality of rations play an important role in maintaining livestock performance. This relates to the energy produced which in turn can affect the physiological and haematological status. This study aimed to observe physiological status parameters (respiratory frequency, body temperature, heart rate and pulse) and hematologic parameters (erythrocytes, leukocytes, haemoglobin, haematocrit and platelets) of Garut sheep which are treated by different protein and energy balance. **Materials and Methods:** The study was conducted using 24 weaned Garut sheep, aged 4 months old, using a completely randomized design (CRD). The treatments given are P1 = ration with 12% protein content and 60% total digestible nutrients (TDN), P2 = ration with 12% protein content and 65% TDN, P3 = ration with 14% protein content and 60% TDN, P4 = ration with 14% protein content and TDN 65%, P5 = rations with 16% protein content and 60% TDN and P6 = rations with 16% protein content and 65% TDN. **Results:** The results showed that the energy and protein balance in each ration did not significantly affect the physiological and haematological status, except plasma leukocytes level and haematocrit of sheep. Sheep that provided ration with 14% protein content and TDN 65% (R4) had a significantly higher ($p<0.05$) leucocytes and haematocrit level compared with other treatments. **Conclusion:** The provision of rations with a protein balance of 16% and TDN 60% is sufficient to meet the production needs of sheep in terms of physiological and haematological status in Garut sheep.

Key words: Sheep, production needs, physiological status, blood hematology, plasma, leucocytes. ration protein and energy

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Garut sheep is one type of small ruminant that is widely produced and known by the people of Indonesia, especially people on the island of Java, because of its effortlessness of maintenance and its economical values[1]. A good performance of sheep can be achieved by giving rations in accordance with the maintenance and production requirement of livestock and taking into account the needs of protein and energy in the ration. The performance of livestock can be determined by looking at the physiological and haematological status[2].

In principle, the source of heat in livestock comes from the surrounding environment, food and the results of metabolism. The heat that comes from the surrounding environment is considered the same because livestock are kept in the same environment, so the most dominant one giving different heat is the protein and energy balance in the ration which in turn can affect the final heat product of metabolism.

Protein content both in quantity and quality is needed in sheep growth and meat production[3]. The energy contained in rations is also important to meet the needs of its life (maintenance) and production. The low energy content ultimately affects the overall performance of livestock. A good balance between protein and energy in the ration is needed so that the requirements of ration quality are met[4].

Physiological status and blood tests are needed to determine animal health[5]. Parameters that can be measured in physiological status are body temperature, respiratory frequency, heart rate and pulse[5], while blood tests include examination of erythrocytes, leukocytes, haemoglobin (Hb), haematocrit and thrombocytes (platelets)[6]. The results of the inspection can give an indication that if there is a deviation from the normal state then physiologically it is in an unhealthy condition. Animal physiology is a regulation to maintain the body (homeostasis) in a balanced state[5,7]. Efforts in maintaining the body include maintaining the body temperature from heat stress, livestock will increase the rate of respiration to maintain body temperature in a normal state. The rate of respiration to excrete blood heat also plays a role in homeostasis regulation[7].

Blood is an important factor as an indicator of health status related to nutrition in animals[8]. Haematological parameters are very important parameters for evaluating normal physiological status in animals, as well as being an indicator of nutritional status in animals[2]. Blood consists of red blood cells, white blood cells (leukocytes) and platelets (thrombocytes). Red blood cells, including Hb, play a role in the transport of oxygen (O_2) from the lungs to cells and tissues and transporting carbon dioxide (CO_2) from the tissues. White blood cells play a role in defending the body from foreign substances (antibodies), while platelets play a role in blood clotting to prevent further bleeding, whereas, haematocrit is the proportion of blood cells compared to its plasma and normal haematocrit values are proportional to the number of erythrocytes and haemoglobin levels. Haemoglobin is an indicator of normal red blood cells and a basis for evaluating general health status in livestock[9].

According to our knowledge, the literature regarding the physiological status and haematological profile of Garut sheep is still lacking. This study aimed to determine if the difference of ration protein and energy balance could affect physiological and haematological profile of Garut sheep, which in the end reflects the health status and production performance.

MATERIAL AND METHODS

Study area, animal and housing: This study was conducted from November, 2016 to May, 2017 in the sheep production facility at Sheep Breeding Development Institute (BPPTD), Margawati, located in Sukanegla, Garut Regency. In total, 24 weaned rams (4 months of age) with average body weight of 16 kg with coefficient of variation <10% was involved in this study. Garut sheeps were obtained and maintained at BPPTD-Livestock Breeding Development Center, Margawati. Sheeps were kept in individual cages with feed mats.

Basal ration and measurements: The feed ingredients were purchased from KSU Tandangsari, Sumedang Regency and Kembar Mekar, Bandung. Ration mixing and analysis were conducted in Ruminant Nutrition and Feed Chemistry Laboratory, Faculty of Animal Husbandry, Padjadjaran University. Table 1 shows the nutrient contents in each treatment used in this study.

Table 1: Nutrient contents and total digestible nutrient percentage (TDN) in each treatment

	Treatment					
Nutrient content	R1	R2	R3	R4	R5	R6
Crude protein	12.07	12.11	14.01	14.14	15.92	15.27
Crude fat	6.37	7.31	6.57	7.63	6.64	7.25
Crude fiber	24.16	21.12	24.04	20.52	24.32	22.10
TDN	60.05	65.15	60.22	65.25	60.29	65.41
Ca	0.36	0.31	0.37	0.33	0.33	0.35
P	0.42	0.57	0.42	0.57	0.40	0.52

R1: Basal ration with 12% protein content and 60% TDN, R2: Basal ration with 12% protein content and 65% TDN, R3: Basal ration with 14% protein content and 60% TDN, R4: Basal ration with 14% protein content and 65% TDN, R5: Basal ration with 16% protein content and 60% TDN, R6: Basal ration with 16% protein content and 65% TDN

The animals were first given feed according to their original feed (acclimation period), then gradually for 7 days, mixing adjustments were made for the calculated treatment ration. The ration was given 3 times daily at 7 am, 11 am and 4 pm. Provision of drinking water is done by mixing with complete feed.

Measurement of physiological status (body temperature, respiratory rate, pulse and heart rate) and blood sampling for hematological parameters (erythrocytes, leukocytes, hemoglobin, hematocrit and platelets) were performed at the end of the study.

Experimental design and statistical analyses: The study was conducted with an experimental method using a completely randomized design (CRD) consisting of 6 treatments with increment on protein content and TDN percentage as follows:

- R1 = Ration with 12% protein content and 60% TDN
- R2 = Ration with 12% protein content and 65% TDN
- R3 = Ration with 14% protein content and 60% TDN
- R4 = Ration with 14% protein content and 65% TDN
- R5 = Ration with 16% protein content and 60% TDN
- R6 = Ration with 16% protein content and 65% TDN

Each treatment was repeated 4 times, so there were 24 experimental units. Data generated from the study were tested using analysis of variance to determine the effect of treatment on physiological and haematological status responses[10], while to analyse the differences between treatments, Duncan's multiple range test was performed.

RESULTS AND DISCUSSION

Effects of ration protein and energy balance on physiological status: The effects of different ration protein and energy balance on physiological status is showed in Table 2.

Based on Table 2, it can be seen that the body temperature of sheep in each treatment is in the range of 39.08-39.28°C. The value for each variable in general is in a relatively narrow situation so that after statistical testing using analysis of variance does not show a significant difference. The absence of changes in physiological status is thought to be related to the factors that can influence it are still at the limit that can still be tolerated by sheep in each treatment.

Rectal temperature is a good indicator to describe the internal temperature of the animal's body. Rectal temperature is also a parameter that can show the effect of environmental stress on sheep. The average temperature of research sheep under normal conditions is 39.08-39.28°C. The rectal temperature of sheep in the tropics is in the range[11] of 38.2-40°C. Temperature is a measure to determine the intensity of heat, while the amount of water vapor in the air is called humidity[12]. The sheep's rectal temperature is directly proportional to the ambient temperature. Higher ambient temperature causes sheep's rectal temperature high. Rectal temperature, skin surface temperature and body temperature increase with increasing ambient temperature[13].

Respiration greatly affects the body's needs in certain circumstances, so that the need for food substances, O_2 and heat can be fulfilled and substances that are not needed can be removed[14]. In this study, the respiratory frequency of sheep is between 39.23-43.67 times min^{-1}, which is quite high. Tropical sheep have a respiratory frequency ranging[11] from 15-25 times min^{-1}. It was reported that increasing energy consumption increases the respiration rate[14]. The highest average respiratory frequency was found in treatment P6, 41.98 times min^{-1}. Increased energy and protein consumption will result in increased O_2 demand, due to an increase in metabolism in the animal's body. The increased need for O_2 must be balanced with an increase in breathing so that the body functions normally. The high frequency of breathing is also influenced by the ambient temperature.

The increase in ambient temperature causes the heart rate to increase in an effort by sheep to be able to compensate for the high ambient temperature, so that the body temperature stays within normal limits. The range of normal sheep heart rate[11] is between 70-80 times min^{-1}. this regard, it was reported that heart rate can increase up to more than twice when active in activities. A sharp increase in heart rate occurs when an increase in ambient temperature, movement and muscle activity[15]. In this study, the heart rate of sheep ranged from 62.67-90.25 times min^{-1}. Similar with the heart rate, the results of the pulse of the study sheep was between 69.33-91.00 times min^{-1}.

Sheep is regarded as homeotherm animals which maintain their body temperature in a relatively constant state even if the ambient temperature changes. Basically, the source of heat for livestock aside from the environment can also be sourced from food and metabolic products. There were no significant differences in physiological parameters affected by the treatment given, possibly because livestock are still able to overcome the effects arising from heat from food. This means that the quality of the ration in the form of energy and protein balances is thought to be still within tolerable limits. Physiological status as a physiological indicator is not affected as a result of livestock still having good tolerance.

Table 2: Effects of different ration protein and energy balance on physiological parameters in Garut sheep

	Treatments						
Physiological parameters	R1	R2	R3	R4	R5	R6	Significance
Body temperature (°C)	39.28	39.20	39.08	39.08	38.23	39.23	NS
Respiration rate (times min^{-1})	39.25	41.25	39.50	43.67	40.93	41.98	NS
Heart rate (beat min^{-1})	62.75	71.00	66.75	65.67	62.67	90.25	NS
Pulse (times min^{-1})	81.75	89.00	91.00	81.33	69.33	90.25	NS

R1: Basal ration with 12% protein content and 60% TDN, R2: Basal ration with 12% protein content and 65% TDN, R3: Basal ration with 14% protein content and 60% TDN, R4: Basal ration with 14% protein content and 65% TDN, R5: Basal ration with 16% protein content and 60% TDN, R6: Basal ration with 16% protein content and 65% TDN, NS: Non-significant

Table 3: Effects of different ration protein and energy balance on hematological parameters in Garut sheep

	Treatments						
Hematological parameter	R1	R2	R3	R4	R5	R6	Significance
Erythrocytes (million mm^{-3})	3.83	4.25	3.85	3.89	5.14	3.85	NS
Leukocytes (cells mm^{-3})	10950.00^{ab}	10125.00^{ab}	10925.00^{ab}	16833.00^{b}	9766.67^{a}	9525.00^{a}	$p<0.05$
Haemoglobin (g dL^{-1})	10.33	11.40	11.00	13.93	13.53	10.50	NS
Haematocrit (%)	30.25^{a}	35.00^{ab}	33.50^{ab}	39.00^{b}	36.33^{ab}	30.00^{a}	$p<0.05$
Thrombocyte (cells mm^{-3})	57.750	60.750	56.500	83.333	85.000	48.250	NS

R1: Basal ration with 12% protein content and 60% TDN, R2: Basal ration with 12% protein content and 65% TDN, R3: Basal ration with 14% protein content and 60% TDN, R4: Basal ration with 14% protein content and 65% TDN, R5: Basal ration with 16% protein content and 60% TDN, R6: Basal ration with 16% protein content and 65% TDN, Means with different superscripts within a row indicate significant difference ($p<0.05$), NS: Non-significant

Effects of ration protein and energy balance on blood haematological parameters: The effects of different ration protein and energy balance on blood haematological parameters are showed in Table 3.

From Table 3, it can be seen that the number of erythrocytes is in the range between 3.83-5.14 million cells mm^{-3}, the number of leukocytes between 9,525-16,833 cells mm^{-3}, haemoglobin levels between 10.33-13.93 g dL^{-1}, haematocrit levels between 30.00-39.00% and platelets 48,250-85,000 cells mm^{-3}. To find out the difference statistically, a diversity test was performed and subsequently tested using Duncan's multiple range test.

Even though there is a large difference, but it turns out that after statistical analysis it did not show a real difference in the level of erythrocytes. The same is true for haemoglobin levels and thrombocytes counts. Significant differences found in the number of leukocytes and haematocrit levels ($p<0.05$), in which the number of leukocytes R4 is higher compared with other treatments. Similar with the number of leukocytes, the haematocrit levels in R4 is significantly higher when compared to other treatments ($p<0.05$).The normal haematocrit values of sheep range[9] from 27-45%, therefore, the haematocrit levels in animals in this study is within the normal range. Haematocrit levels were already used as a parameter to detect either anaemia that caused by parasitic diseases[16], nutritional status or immunological status[17]. Furthermore, it was reported that haematocrit values of sheep that susceptible to parasites were lower compared to healthy sheep, suggesting a higher haematocrit value within normal range can be regarded as healthier animal[16].

Low nutritional ration affects animal immunity[18], therefore, it is necessary to protect the body from various disease attacks by increasing leukocytes. Animals will have better endurance in high nutritional content so that leukocytes are not as much as in animals with low nutritional ration.

Haematological profile is basically a physiological condition that is maintained in a relatively constant condition[19], therefore, this is part of the homeostasis process. Relatively long treatment conditions there are several components that can change or experience a shift in the new equilibrium conditions.

Knowledge of the haematological status is basically an important part of studying blood physiology. The finding in this study is in line with earlier study which stated that important parameter to determine the blood physiology is haematological status, including the number of erythrocytes mm^{-3}, haematocrit values and haemoglobin levels[20]. Hematological status is also useful information to determine animal health, the metabolic system, including the physiology of growth and reproduction, also depends on several blood components[21]. The blood component in animals is very dependent on the level of feeding, geographical height, strain (genetic), climate, age, sex, ambient temperature and physiological status[22].

Data on the number of erythrocytes in this study did not show any significant difference in the range of 4 million mm^{-3} and the highest number was achieved in R1 of 5.14 million cells mm^{-3}. It seems that the amount of erythrocytes is maintained in a relatively stable amount because it has more general and basic physiological functions[23], as it is known that the formation of erythrocytes or erythropoiesis is regulated by a glycoprotein hormone called erythropoietin which has a molecular weight of 60,000-70,000 that is stable at high temperatures. Haemoglobin levels also did not show any significant difference, however, the same pattern showed the highest value at R4 with the value of 13.93 g dL^{-1} and the second largest at R5, which was 13.53 g dL^{-1}. Earlier study reported that haemoglobin is influenced by nutrients, especially protein content[24,25].

This study found that the balance of protein and energy in the ration did not directly affect the physiological status and haematological profile. However, it seems indirectly affect the health status and performance because the significant difference of leukocytes was found. As stated early, leukocytes orchestrated the animal's immunity, hence it will affect the general health status in the long run. Although the results seem convincing, further study need to be done on other blood parameters, such as blood metabolites and mineral content to verify if this phenomenon is having the same trends with above-mentioned parameters. As this study tries to explore the ration protein and energy balance effects to Garut sheep, the results are then limited to Garut sheep strain under tropical condition and circumstances.

CONCLUSION

Based on the results of the study it can be concluded that the provision of energy and protein balance in the ration did not have a significant effect on physiological status. The same is true for hematologic blood, except for leukocyte and haematocrit indicators. Giving rations with a 16% protein balance and 60% TDN energy is enough to maintain the performance of the sheep.

SIGNIFICANCE STATEMENT

This study found a possible synergistic effect of protein and energy balance that could be beneficial to maintain the physiological and haematological status of Garut sheep under tropical circumstances. This study will help researchers find the right combination for optimal ration. Thus, new theories about this combination and possibly other combinations, can be explored further.

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