

## Research Article



# Comparison of Morphometry, Physiological Status, and Protein Total in Twin and Single Ewes of Fat-Tail Sheep (Sapudi Indonesian Local Sheep) and Their Crossbred

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**Abstract** | The genetic resources of local Indonesian sheep that must be protected and preserved include the Sapudi sheep and their crosses, which produce meat with highly adaptive and sigmoid fat tails. This study aims to determine the differences in the relationship between physiological status, morphology, and total protein of the single and twin ewe sheep and their crosses. The research method was a field study with quantitative and laboratory analysis, with statistical analysis using ANOVA and LSD with SPSS Statistic 9 software. The research design used descriptive with total sample of 60 heads (morphometry and physiological status) and 24 heads (total protein). There are three breeds of sheep: Sapudi, Dormas (Dormer x Sapudi), and Suffas (Suffolk x Sapudi). Sapudi, Dormas, and Suffas Ewes with only one child each lambing were abbreviated as SAS, DOS, and SUS. The ewe has twin lambs designated by SAT, DOT, and SUT abbreviations. The criteria for the sheep sampled were 2-3 years old, healthy condition (good activity), good appetite with feed given in the form of concentrate (2% of body weight), and forage (10% of body weight). The results showed that morphology, physiology, and total protein did not show a significant relationship ( $p > 0.05$ ) to differentiate broodstock in a single or twin brood in Sapudi sheep or their crosses, except for Suffas sheep. Total protein could show the difference between twin lambs having total protein  $> 6.36$  g/dL and single lambs having total protein  $< 6.36$  g/dL. The conclusion was that there was no relationship between physiological status, morphology, and total protein of the single and twin ewe in Sapudi sheep and their crosses, except for Suffas sheep. The ewes' activity in twin lambing was higher than in single lambing, indicated by the total protein concentration. This research implied that it was necessary to provide feed with a minimum crude protein content of 14.4% - 24.22% since the beginning of pregnancy.

**Keywords** | Fat-tail sheep, Dormer, Suffolk, Crossing, Lambing

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Sheep is one of the small ruminant breeds that can drive the people's economy in various developing countries (Herero et al., 2013; Arunasalam et al., 2019; Shirsat et al., 2019; Offor et al., 2018; Notter and Taylor, 2019; Simoes et al., 2021; Biswal et al., 2021). Indonesia is a developing country with various sheep breeds, including Sapudi Indonesian local sheep (we call Sapudi). Sapudi breed with the same characteristics as fat tail sheep is recognized as one of the local Indonesian sheep breeds with geographic origin in East Java (Indonesian National Standardization Agency, 2018).

Sapudi's fat-tailed sheep advantages include resistance to disease and excellent environmental adaptability in tropical areas in Indonesia (Nasich et al., 2021; Hartoyo et al., 2022). In its development, smallholder farmers have done crossbreeding to increase body size to meet consumer demand for livestock. Offsprings from crossbreeding have been expected to produce a heterosis effect (Ferreira et al., 2015), so many smallholder breeders cross Sapudi sheep with superior livestock imported by the government in the hope that a positive heterosis effect will appear in line with expectations, namely a larger body size than Sapudi.

Exotic breeds used for crossbreeding rams with Sapudi sheep were Dormer and Suffolk rams, superior beef breeds widely used in crossbreeding (Cloete et al., 2003; Notter and Taylor, 2019; Mckibben et al., 2019). Crosses of Dormer rams with Sapudi ewes were called Dormas, while Suffolk with Sapudi was called Suffas breed (Hartoyo et al., 2022).

Sapudi, Dormas, and Suffas sheep are widely grown in the province of East Java, Indonesia, because of their productivity. One factor that affects livestock productivity is the potential for lambing to twins or single lambs. The potential for giving birth to twins or singletons in a small ruminant dam is influenced by prolific genes. Several genes that were very influential in determining prolific traits are GDF 9 (growth differentiation factor), which plays a role in the process of folliculogenesis and prolificacy and triggers progesterone secretion in luteal cells (Mudawamah et al., 2019), BMP 15 (bone morphogenic protein), and ALK 16 (activin receptor-like kinase) (Muneeb and Faiz, 2014). Proteins in the blood are obtained from synthesizing amino acids with gene encoding (Nei, 1978) and can provide an image that characterizes the parent with twins or singletons.

The physiological status of single or twin ewes was expected to give an idea about the health or not stress of livestock. The physiological status could be seen through rectal temperature, respiration, and pulse (Ali et al., 2020;

Protein total was expected to provide an amount of proteins obtained from body secretions, especially the physiological conditions of the body related to biochemical determination and the basis of livestock rearing (Tothova et al., 2016). The protein total was very influential on the body's physiological system, including physiological reproduction (Boland and Lonergan, 2003) and prolificacy trait (twin and single lambing) (Putri et al., 2020; Mudawamah et al., 2021).

Previous research on the identification of total protein as well as the physiological status showed that the ewes with twins were higher than the ewes with single offspring, so it was necessary to increase the feeding of up to 32% in ewes with twins in the early stages of pregnancy (Putri et al., 2020). A specific amount of feed was significant because nutritional deficiencies caused serious health and reproductive problems. Lack of nutrition could affect the fertilization process, affect the development of the embryo and fetus in the uterus so that it can be followed by the death of the embryo and absorption of the embryo by the uterine wall, abortion, or birth of a weak child and neonatal death (Ashworth et al., 2009).

In addition, to increase the accuracy of the selection of prospective ewes, it was necessary to support a comparative study of the morphology of ewes with single or twin offspring as a basis for future selection. Based on this background, this study aims to determine the differences in the relationship between physiological status, morphology, and total protein of the single and twin ewe sheep and their crosses.

## MATERIALS AND METHODS

Blood samples (whole blood) were taken from the jugular vein at 3 ml using a vacutainer tube. This method has been approved by the animal care and uses committee with ethical clearance No.118-KEP-UB-2022.

### TIME AND LOCATION OF STUDY

The research was conducted at the Technical Implementation Unit for Livestock Breeding and Forage for Animal Feed Jember, Animal Husbandry Service of East Java Province. In contrast, the Total Protein and A/G Ratio tests were carried out at the Clinical Laboratory of Prosenda Baru Jember, East Java.

### RESEARCH METHOD AND DESIGN

The research method was a field study with quantitative and laboratory analysis, with statistical analysis using ANOVA and LSD with SPSS Statistic 9 software. The research

design used descriptive with total sample of 60 heads (morphometry and physiological status) and 24 heads (total protein). The samples were ewes 2-3 years old that normal reproduction and healthy condition (good activity and appetite) with single and twin lambing category.

### RESEARCH MATERIALS

The method in this research was a field study with quantitative and laboratory analysis, with statistic analysis using variance and LSD. The samples were three breeds of sheep, namely Sapudi, Dormas (Dormer x Sapudi), and Suffas (Suffolk x Sapudi). Sapudi, Dormas, and Suffas Ewes with single lambing were abbreviated as SAS, DOS, and SUS. Ewes had twin lambs given abbreviated SAT, DOT, and SUT. The criteria for the ewes sampled were 2-3 years old, healthy condition (good activity), good appetite with feed given in the form of concentrate (2% of body weight), and forage (10% of body weight). The breeding activities were carried out with an intensive cage system where the livestock's needs for forage and concentrated feed are fulfilled according to their needs.

The number of samples used for measuring body morphometry and physiological status was 132 heads with details of Sapudi sheep with single and twin lambing 82 and 10 heads, Dormas and Suffas sheep ten heads for single and twin lambing. Measurement of the biochemistry status of each breed of sheep with single birth or twins with four replications.

### THE VARIABLES OBSERVED IN THIS STUDY WERE AS FOLLOWS

**Morphometry:** included body length (BL) was a distance pulled straight from the shoulder joints (*Tubar humerus*) to the lumps of the filter (*Tuber ischiadicus*). Body height (BH) was the highest distance of *Tuber coxae* to the bottom. Chest circumference (CC) from the front of the ribs to just behind the front legs. Ears length (EL) from the range of the ear's base to the tip of the ear. The measurement of morphometry used livestock measuring sticks (BL, BH, and CC), while for EL measurements using livestock measuring tape (Mudawamah et al., 2021).

**Physiology status:** including heart rate (HR), body temperature (BT), and respiration (R). Heart rate (HR) was calculated using a stethoscope placed on the pulse in the sheep's armpit for 1 minute. Respiration calculation (R) was done by placing the palm of our hand on the sheep's chest and counting its movement for 1 minute. Measurement of body temperature (BT) uses an electric thermometer inserted in the anus and allowed to stand until the thermometer beeps (Ali et al., 2020).

**Biochemical status:** protein total. The blood blood was

collected using a sterile syringe and needle size 18 G via the jugular vein ocated lateral to the neck which has been cleaned with a cotton swab given 70% alcohol. The amount of blood drawn 3 ml and put into the tube EDTA, then homogenized. Blood tube stored in a cool box and brought to laboratory to analyse with ethical clearance No.118-KEP-UB-2022 (Ali et al., 2020). Blood protein profile was measured by the Biuret method. The color reaction between the copper reagent and the alkaline peptide CO-NH produces a purple color.

### PROCEDURE OF PROTEIN TOTAL

Blood samples (whole blood) were taken from the jugular vein at 3 ml using a vacutainer tube. Preparation was carried out on whole blood samples to become blood serum by inserting them into a centrifuge at 3000 rpm for 10-15 minutes. Total protein data was obtained through the biuret method with the working principle of the color equation with copper ions, where the protein in serum reacts with copper ions in an alkaline (base) atmosphere, thus forming a purple complex compound whose color intensity was proportional to the protein content in the blood. The stages of testing using the biuret method were as follows: preparing 3 cuvettes (standard absorbance, sample absorbance, blank absorbance); inserting the biuret reagent into each 1000 L cuvette using a micropipette putting 20 L of aqua dest into the blank cuvette, 20 L of standard solution into the standard cuvette, 20 L of blood serum sample into the sample cuvette using a micropipette and homogenize it; incubate at room temperature (37 degrees celsius) for 10 – 30 minutes; reading with a spectrophotometer with a wavelength of 546 nm; perform the interpretation of the results with the following formula:

$$\text{Protein Total} : \frac{\text{Sample Absorbent}}{\text{Standard Absorben}} \times \text{Standard concentration (g/dL)}$$

### STATISTICAL ANALYSIS

Parameters measured include morphometry (body length, body height, chest circumference, ear length), physiological status (heart rate, body temperature, and respiration), and total protein. The data were analyzed by using ANOVA and LSD with SPSS Statistics 19 software.

## RESULTS AND DISCUSSION

### MORPHOLOGY

The mean and variance analysis of the morphological characteristics came from Sapudi Ewes and Crossbreds in single and twin lambing are presented in Tables 1 and 2.

Table 1 showed that ewes with single lambing in BL and CC traits were very significantly different ( $p < 0.01$ ). On the other hand, BH and EL traits were not significantly different ( $p > 0.05$ ) among the three breeds. The results

showed that the morphometric properties of BL and CC could be used as one of the distinguishing criteria between Sapudi and their crosses. The BL and CC traits of Dormas and Suffas crossbred sheep were 10.16%-11.62% and 16.93%-17.54% higher than Sapudi sheep. It meant that there was a superiority of Dormas and Suffas over the breed of Sapudi sheep based on the morphometric traits (BL and CC) ranging from 10.16% - 17.54%. Following the opinion that the crossbreeding program increased sheep productivity (Li et al., 2016; Ferreira et al., 2015). A crossbreeding program is an effective way to increase hybrid vigor or heterosis effect (Petrovic et al., 2019).

Table 2, ewes with twin lambing in BL, CC, and EL traits were significantly different ( $p > 0.05$ ) in the Sapudi breed compared to Dormas and Suffas. By the way, no significant difference was found in BH traits. BL, CC, and EL in Sapudi sheep were 22.55 %-25.81% lower, 13.00%-15.60%, and 13.43%-14.93% compared to crossbreed sheep (Dormas and Suffas). Following the opinion of Bunning et al. (2019), the crossbreeding program produced a heterosis effect that significantly increased the productivity of ruminants.

The results of variance analysis in one breed showed that BL was not significantly different ( $p > 0.05$ ) between single and twin lambing of Sapudi breed, Dormas breed, except for Suffas breed.

Figure 1 showed the morphometry deviation between single and twin lambing in one breed was not too large, a maximum of 10%. The maximum and minimum value range of morphometry also intersects between single and twin lambing so morphometry could not be used to

distinguish between twins and single lambing.

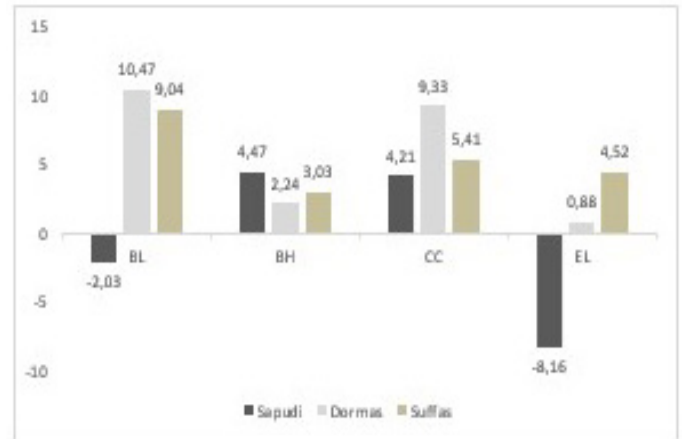


Figure 1: Deviation of morphology between twin and single lambing in Sapudi and their crossbreds (%).

PHYSIOLOGY STATUS

The mean and variance analysis on the physiological status of Sapudi ewes and Crossbreds with single and twin lambing are presented in Tables 4 and 5.

Table 4 shows that ewes with twin lambing in HR and R traits were not significantly different ( $p > 0.05$ ) in the Sapudi and the Crossbreds (Dormas and Suffas), except BT traits, were not significantly different ( $p < 0.05$ ) among the Sapudi breeds and crossbreds.

Table 5 presents that ewes with twin lambing in HR and R traits were not significantly different ( $p > 0.05$ ) in the Sapudi and the Crossbreds (Dormas and Suffas), except BT traits significantly different ( $p < 0.05$ ) among the Sapudi breed and crossbreds.

Table 1: Morphology of sapudi ewes and crossbreds with Single lambing<sup>1</sup>.

Traits	Single lambing			Significant degree
	Sapudi	Dormas	Suffas	
BL	54.74 ± 4.11 <sup>a</sup>	61.10 ± 4.36 <sup>b</sup>	60.30 ± 3.40 <sup>b</sup>	Very significant
BH	57.45 ± 3.60	58.00 ± 2.67	59.40 ± 3.20	No significant
CC	66.02 ± 4.49 <sup>a</sup>	77.20 ± 5.90 <sup>b</sup>	77.60 ± 4.88 <sup>b</sup>	Very significant
EL	10.87 ± 1.27	11.50 ± 1.00	11.05 ± 2.09	No significant

<sup>1</sup>values represent means ± SD; body length (BL); Body height (BH); Chest circumference (CC); Ears length (EL).

Table 2: Morphology of sapudi ewes and crossbred with twin lambing<sup>1</sup>.

Traits	Twin lambing			Significant degree
	Sapudi	Dormas	Suffas	
BL	53.65±3.77 <sup>a</sup>	67.50±9.28 <sup>b</sup>	65.75±5.39 <sup>b</sup>	significant
BH	60.60±3.84	59.30±3.50	61.20±5.01	No significant
CC	68.80±5.90 <sup>a</sup>	84.40±7.47 <sup>b</sup>	81.80±13.93 <sup>b</sup>	significant
EL	10.05±1.23 <sup>a</sup>	11.40±0.94 <sup>b</sup>	11.55±1.46 <sup>b</sup>	significant

<sup>1</sup>values represent means ± SD; body length (BL); Body height (BH); Chest circumference (CC); Ears length (EL)



**Table 4:** Physiology status of sapudi ewes and crossbred with single lambing<sup>1</sup>.

Traits	Single lambing			Significant degree
	Sapudi	Dormas	Suffas	
HR (per minute)	108.16±8.31	114.60±19.68	107.40±12.47	No significant
BT (°C)	38.58±0.82 <sup>a</sup>	39.11±0.47 <sup>b</sup>	39.21±0.46 <sup>b</sup>	significant
R (per minute)	28.40±2.15	27.50±1.72	28.36±2.08	No significant

<sup>1</sup>values represent means ± SD; heart rate (HR), body temperature (BT), and respiration (R).

**Table 5:** Physiology status of sapudi ewes and crossbred with twin lambing<sup>1</sup>.

Traits	Twin lambing			Significant degree
	Sapudi	Dormas	Suffas	
HR (per minute)	112.20± 20.21	117.60±16.78	114.60±14.82	No significant
BT (°C)	38.74±0.66 <sup>a</sup>	39.16±0.46 <sup>ab</sup>	39.55± 0.46 <sup>b</sup>	significant
R (per minute)	28.40±1.65	28.00±2.11	29.10±2.08	No significant

<sup>1</sup>values represent means ± SD; heart rate (HR), body temperature (BT), and respiration (R).

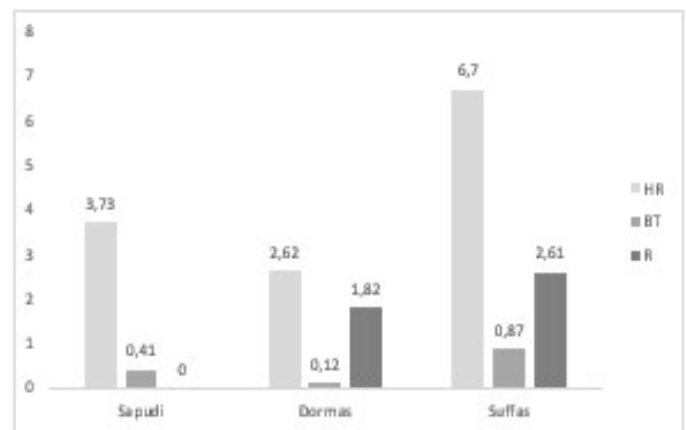
Tables 4 and 5 show that HR in Dormas sheep was higher than in Sapudi and Suffas sheep in singleton and twin births. Heart rate was influenced by several factors: age, sex, body size, activity, feed, and environmental temperature. HR in the data above showed a higher value than the standard value. According to Jackson and Cockroft (2002), the heart rate of sheep in the tropics is 70-90 beats/minute. The increase in the heart rate of livestock is influenced by the increase in feed consumption so that the body's metabolic process also increases, which produces heat. The metabolic process is one of the factors that affect the physiological condition of livestock (Sundrum, 2015).

Tables 4 and 5 show that the temperature of the Dormas and Suffas Sheep is higher than the Sapudi Sheep. Sheep with large body sizes tend to have a high level of feed consumption. High feed consumption causes high metabolism, affecting sheep's high rectal temperature (Elaref et al., 2022). Allen et al. (2015) stated that livestock activity could affect body temperature.

Respiration data in the three types of sheep, both single and twin lambing, in line with Jackson and Cockroft (2002), who revealed that the regular respiration frequency of sheep in the tropics is in the range of 20-30 times/minute. Breathing activity is essential to increase heat loss at high temperatures. Breathing frequency is one of the livestock's efforts to balance body heat. Factors that affect the frequency of breath include the size of the body of the livestock, age, and activity (Allen et al., 2015). Sundrum (2015) said that changes in the frequency of breath could occur when livestock consume nutrients, resulting in increased body metabolic processes, and, in the end, more body heat is produced. So to reduce the body heat received, livestock will increase the frequency of breath.

The results of variance analysis in one breed showed that the

physiology status was not significantly different ( $p>0.05$ ) between single and twin lambing in Sapudi, Dormas, and Suffas breeds.



**Figure 2:** Deviation of physiological status between twin and single lambing in Sapudi and their crossbreds (%).

Figure 2 shows that the difference in the mean physiological status between single and twin lambing was very low, below 4% (0.41%-3.73%), and could not be used as a difference between single and twin lambing. Besides, the low deviation of the mean physiological status also indicates that the sheep's metabolic process was running well. The body's metabolism of livestock was closely related to physiological conditions so that when the metabolism was running well, the heat produced by the body was not so high, which caused the physiological status of livestock to run normally in both ewes that twins and single lambing.

**PROTEIN TOTAL**

Based on the analysis of variance, it showed that the protein total of the Sapudi ewes and crossbreds with single and twin lambing was not significantly different ( $p>0.05$ ) (Table 6).

**Table 6:** Protein Total (g/dL) of sapudi ewes and crossbred with single and twin lambing<sup>1</sup>.

Lambing Breeds	Lambing Breeds			Significant degree
	Sapudi	Dormas	Suffas	
Single	5.40 ± 1.00 (4.40-6.40)	4.93 ± 0.21 (4.72-5.14)	5.63 ± 0.58 (5.05-6.21)	No significant
Twin	6.18 ± 0.76 (5.42-6.94)	6.00 ± 0.98 (5.02-6.98)	7.00 ± 0.64 (6.36-7.64)	No significant

<sup>1</sup>values represent means ± SD.

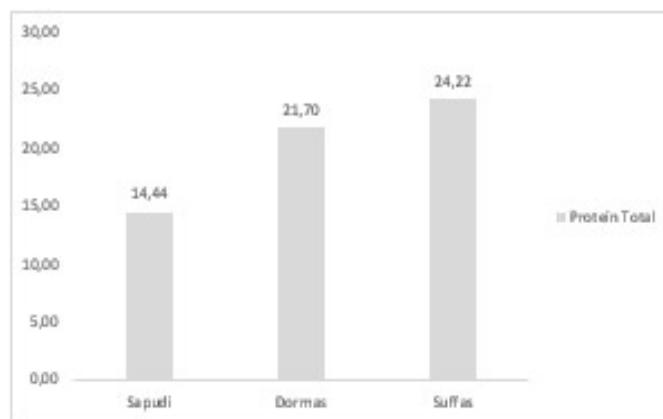
Based on Table 6, the highest average total protein was Suffas ewes, followed by Sapudi, and the lowest was Dormas with twins and singletons, respectively 7.00 g/dL and 5.63 g/dL, Sapudi ewes 6.18 g/dL and 5.40 g/dL and Dormas ewes 6.00 g/dL and 4.93 g/dL. In the results of Masek et al. (2007) the total protein in the Istrian x East Friesian crossbred was 6.23-8.27 g/dL. de Araujo et al. (2014) showed that total protein in Dorper x Santa Inez crossbred lambs ranged from 5.3-6.4 g/dL. In a single calf, the average total protein was below the standard, less than 5.9 g/dL, while twin lambing ewes were still in the normal range of 6.39 g/dL. Standard total protein in sheep ranges from 5.9 to 7.8 g/dL (Kahn and Scott, 2005). The total protein is a collection of blood chemical elements in plasma or serum. Total protein concentration decreation can be caused by hormonal imbalance, low albumin concentration, low globulin concentration, and pregnancy (Abdul et al., 2015).

In Table 6, the range (minimum and maximum values) of total protein in single and intersected twins in Sapudi and Dormas sheep breeds cannot be used to differentiate between single and potential twin ewes. On the other hand, Suffas sheep between single and twin births have a range (minimum and maximum values) that do not coincide so that they have the potential as a basis for early detection markers for single and twin births, namely, single-twinning ewes have a total protein of less than 6.36 g/dL. In comparison, single-born ewes have a total protein of 6.36 g/dL or more.

The results of variance analysis in one breed showed that protein total and A/G were not significantly different (p>0.05) between single and twin lambing of Sapudi breed and Suffas breed, except for Dormas breed had significantly different (p<0.05) between single and twin lambing. Based on the average, the total protein deviation of twin lambing ewes was 14.44%-39.78% higher than those of single lambing ewes (Figure 3).

Figure 3 shows the deviation of protein total between single and twin lambing was more than 10 % (14.44%-24.22%), and could be used as a candidate difference between single and twin lambing. In ewes with twin lambing, there is an

increase in the secretion of synthetic hormones, antibodies, and enzyme activity related to protein metabolism, causing the rate of protein metabolism in the liver to increase so that the total protein level also increases. In this case, the increase in total protein is related to the expression of prolific characteristics of livestock, namely the ability to give birth to more than one child at once in one birth period (Putri et al., 2020). The total protein is very influential on the physiological system of livestock, one of which is physiological reproduction (Boland and Lonergan, 2003).



**Figure 3:** Deviation of protein total between twin and single lambing in Sapudi and their crossbreeds (%).

Suffas, Dormas, and Sapudi sheep were seen from the protein requirements, with the average difference in total protein between single and twin lambing ewes being 24.22%, 21.70%, and 14.44% (Figure 1). With these conditions, it is necessary to pay special attention to the management of maintenance and better nutrition for ewes with twin lambing ewes than single lambing ewes to carry out normal reproduction and growth processes.

Feeding with protein 1.7-2.1 times the main requirement increased the prolific level of sheep. The protein requirement of pregnant ewes was 7.8% – 9.8%, so in this case, it is necessary to feed the ewes of twin lambing with a protein content of 16.38% - 20.58%, or 2.1 times the primary requirement. Lack of nutrients can affect the fertilization process and affect the development of the embryo and fetus in the uterus so that it can be followed by the death of the embryo and absorption of the embryo by the uterine wall, abortion, or the birth of a weak lamb and neonatal death (Grazul-Bilska et al., 2013; Xuan et al., 2018). According to Putri et al. (2020), in ewes of PE goats with twin lambing, it was necessary to increase the feed by at least 32% since the beginning of pregnancy.

## CONCLUSIONS AND RECOMMENDATIONS

There was no relationship between physiological status,

morphology, and total protein of the single and twin ewe in Sapudi sheep and their crosses, except for Suffas sheep. The ewe's activity in twin lambing was higher than in single lambing, which was indicated by the total protein concentration. This research implied that it was necessary to provide feed with a minimum crude protein content of 14.4% - 24.22% since the beginning of pregnancy.

## ACKNOWLEDGMENT

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## NOVELTY STATEMENT

The total protein of ewe's single lambing was < 6.36 g/dL lower than twin lambing >6.36 g/dL for Sapudi Sheep and their Crossbred

## AUTHOR'S CONTRIBUTION

MM planned, designed the research, analyzed all data, and drafted the manuscript. SS, GC, ES, YH and LA provided and help in the research. All authors discussed the results and contributed to the final manuscript.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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