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Blood Biochemical Levels Based on Prolificacy Level in Batur Sheep of Indonesia

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ABSTRACT: The aim of the research was to compare the blood biochemical levels of Batur sheep based on their proliferation rate. The research used 52 Batur ewes having 2 parities from a sheep farming group in Batur District, Banjarnegara, Central Java - Indonesia. This study was conducted based on a survey method designed into 3 groups of prolification. The sheep samples used in this study haveadapted to the local environment fed with basal forage as grass and vegetable waste. Drinking water was given ad libitum. The observed variables include glucose, albumin, blood urea, creatinine, cholesterol and total protein levels in sheep. The data were analyzed using the Kluskal Wallis test. The results of the study showed significant (P<0.01) in albumin, blood urea and creatine levels, significant (P<0.05) in glucose and total protein levels and no significant (P>0.05) in cholesterol levels in the 3 prolificated groups. The highest levels were in glucose at 38.32 mg/dl, albumin 0.74 g/dl, blood urea 36.68 mg/dl, cholesterol 59.29 mg/dl, creatinine 0.083 mg/dl and total protein 14.37 g/dl. Based on the results of the study, it can be concluded that different levels of prolification in sheep have significant differences in blood biochemical levels.

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KEYWORDS: Blood Biochemical, Prolification, Batur Sheep

INTRODUCTION

Sheep are one of the small ruminants that can be utilized as meat producers to help fulfill animal protein needs in Indonesia. Indonesia has several types of local sheep with various characteristics that are not shared by other regions, including fat-tailed sheep, Priangan sheep, Garutsheep, thin-tailed sheep and Batur sheep. Batur sheep is one of Indonesia's local genetic resources (SDG) clumps that developed in Banjarnegara Regency since 1974. Batur sheep is the result of a crossbetween thin-tailed sheep and Merino sheep which is determined based on Ministerial DecreeNumber: 2916/Kpts/OT.140/6/2011 (Muryanto et al. 2020).

Batur sheep have genetic potential with a diversity of specific reproductive characteristics, related to *littersize* or the ability of the ewes to give birth to one to four sheep (Sumaryadi, 2021). Previous research results stated that the prolificacy of sheep was influenced by theFecJ (*Fecundity Java*) gene (Bradford et al., 1991; Elsen et al., 1991; Adjisoedarmo et al., 1997). Increased productivity in Batur sheep carrying the proliferation gene may be due to an increase in thehormone estrogen. The production and secretion of estrogen hormone is initiated through metabolicprocesses to produce energy needed by organs and reproductive glands to work (Andara et al., 2022).The metabolic process requires a good supply of oxygen so that cell oxidation can continue. The oxygensupply is carried by the bloodstream.

Blood in circulation is supported by blood plasma which acts as a supplement in the form of protein as food (Ganong et al., 1999). Good sheep condition is characterized by the balance of biochemicals in the blood, including blood hemoglobin (g/dl), leukocytes (10^3/l), erythrocytes, hematocrit (%), blood glucose (mg/dl), albumin (g/dl), blood urea/urea uv (mg/dl), cholesterol (mg/dl),creatinine (mg/dl) and total protein (g/dl). Blood biochemistry is one of the indicators that determinethe physiological condition and health of livestock. The results of previous studies stated that the bloodglucose levels of sheep in normal conditions ranged from 35-60 mg/dl (Riis, 1983), normal albumin concentrations of 3.30-3.47 g/dl (Pal et al., 2015), blood urea 6, 2015), blood urea 6-36 mg/dl (Wahjuni and Binjati, 2006), blood cholesterol 50-140 mg/dl (Schalm, 2010), creatinine 0.06-0.11 mg/dl (Lendrawati et. al., 2019) and total protein 6.00-7.59 g/dl (Riis, 1983). The better the blood biochemicalconditions in livestock, the better the metabolic performance. The quality of the metabolic processesthat take place in the body of sheep can be determined from the amount of blood components contained in the blood circulation because blood as a carrier component of oxygen and carbon

dioxideneeded in the biochemical process of metabolism so as to produce energy (Andara et al., 2022). Energyplays an important role in initiating reproductive organs to work and stimulating the formation of hormones from the hypothalamus so that it affects prolification. Therefore, further research is neededrelated to blood biochemical levels based on the level of prolification in Batur sheep in Banjarnegara Regency.

MATERIALS AND METHODS

Research Materials

The study used 52 Batur ewes that were more than 2 years old and had 2 parities with prolification one to three (litters 1-3 in a birth) originating from farmer groups in Batur sub-district, Banjarnegara district, Central Java. The tools used were 10 ml *disposable syringe*, EDTA *vacuum tube*, *ice box*, *ice cooler*, *alcohol swab*, 1.5 ml *microcentrifuge* tube, *pipette tips of* various sizes, *micropipette of* various sizes, *centrifuge*, *microplate* and a set of spectrophotometer. Materials used were sheep blood samples, glucose reagent, albumin reagent, uv urea reagent, cholesterol reagent, creatinine reagent and total protein reagent.

Research Methods

The research was conducted by direct observation method (survey) using purposive samplingmethod because the sample was selected specifically based on the purpose of the research, which is to determine the level of blood biochemistry based on the level of prolification in Batur sheep. The study was designed into 3 groups of prolification levels, namely P1 = ewes who always have a record (JAS) 1 or single lambing, P2 = ewes who always have a record of JAS 2, P3 = ewes who always have JAS 3. The sheep samples used have adapted to the local environment both climate and feed given. Feed given in the form of grass and agricultural waste givenad libitum. The observed variables were blood biochemical levels such as blood glucose, albumin, blood urea, blood cholesterol, creatinine levels and total protein.

Research Preparation

Preparation for research by making coded labels to be attached to each EDTA vacuum tube, and ensuring the completeness of tools and other materials for blood sampling such as 10 ml *disposablesyringe,* EDTA *vacuum tube*, *ice box*, *ice cooler,* and *alcohol*. Preparation for blood biochemical testingby preparing the tools and materials needed in the Biotechnology *Integrated Academic Building* (IAB) laboratory.

Research Implementation

Batur sheep are grouped based on the level of prolification, namely P1 as many as 20 heads, P2 as many as 20 heads and P3 as many as 12 heads based on the results of interviews consisting of the number of children born. Sampling of sheep blood is done through the jugular vein taken using a *disposable syringe* 10 ml each 3-5 ml then poured into EDTA *vacuum tube* and then homogenized by shaking to form a figure 8. Furthermore, the blood sample is put into an *ice box* containing *ice cooler* to minimize damage and analysis of blood biochemical tests at the IAB Biotechnology Laboratory.

Blood Biochemical Levels

A. Blood Extraction

B. Blood Glucose (mg/dl)

- Preparation of Standard Solution (homogenized using vortex)
  a. 0 % : 100 µ Aquadest solution
  b. 20 % : 20 µ Glucose Standard Solution + 80 µ Aquadest Solution
  c. 40 % : 40 µ Glucose Standard Solution + 60 µ Aquadest Solution
  d. 60 % : 60 µ Glucose Standard Solution + 40 µ Aquadest Solution
  e. 80 % : 80 µ Glucose Standard Solution + 20 µ Aquadest Solution
  f. 100 % : 100 µ Glucose Standard Solution
- Preparation of Standard Solution Samples
  a. Take 3 µ sample of glucose standard solution
  b. Put into *microplate*
  c. Added 250 µ of glucose reagent solution
- Preparation of Glucose Solution Samples
  a. Taken 3 µ of extracted blood sample
  b. Put into *microplate*
  c. Added 250 µ glucose reagent

After making standard solution samples and glucose solution samples, the microplate wasinserted into a spectrophotometer with a wavelength of 500 nm (Suharti et al., 2017), incubated for 5 minutes at 37 C and *shaken* for 20 seconds with a range of 5 seconds *shake* and 5 seconds *off to* make it homogeneous.

C. Albumin (g/dl)

- Preparation of Standard Solution (homogenized using vortex)
  a. 0 % : 100 µ Aquadest solution
  b. 20 % : 20 µ Albumin Standard Solution + 80 µ Aquadest Solution
  c. 40 % : 40 µ Albumin Standard Solution + 60 µ Aquadest Solution
  d. 60 % : 60 µ Albumin Standard Solution + 40 µ Aquadest Solution
  e. 80 % : 80 µ Albumin Standard Solution + 20 µ Aquadest Solution
  f. 100 % : 100 µ Albumin Standard Solution
- Preparation of Standard Solution Samples
  a. Take 3 µ sample of albumin standard solution
  b. Put into *microplate*
  c. Added 250 µ of albumin reagent solution
- Sample Preparation of Albumin Solution
  a. Taken 3 µ of extracted blood sample
  b. Put into *microplate*
  c. Added 250 µ of albumin reagent

After making standard solution samples and albumin solution samples, the *microplate* wasinserted into a spectrophotometer with a wavelength of 546 nm (Suharti et al., 2017), incubated for 5 minutes at 37 C and *shaken* for 20 seconds with a range of 5 seconds *shake*and 5 seconds *off to* make it homogeneous.

D. Blood Urea/Urea UV (mg/dl)

- Preparation of Standard Solution (homogenized using vortex)
  a. 0 % : 100 µ Aquadest solution
  b. 20 % : 20 µ Blood Ureum Standard Solution + 80 µ Aquadest Solution
  c. 40 % : 40 µ Blood Ureum Standard Solution + 60 µ Aquadest Solution
  d. 60 % : 60 µ Blood Ureum Standard Solution + 40 µ Aquadest Solution
  e. 80 % : 80 µ Blood Ureum Standard Solution + 20 µ Aquadest Solution
  f. 100 % : 100 µ Blood Ureum Standard Solution
- Preparation of Standard Solution Samples
  a. Take 3 µ sample of blood ureum standard solution
  b. Put into *microplate*
  c. Added 200 µ of reagent solution R1
  d. Incubated for 5 minutes
  e. Added 50 µ of R2 reagent solution
  f. Incubated for 2 minutes
- Sample Preparation of Blood Ureum Solution
  a. 3 µ of extracted blood sample was taken.
  b. Put into *microplate*
  c. Added 200 µ of reagent solution R1
  d. Incubated for 5 minutes
  e. Added 50 µ of R2 reagent solution
  f. Incubated for 2 minutes

After making the standard solution sample and the blood ureum solution sample, the *microplate was* inserted into the spectrophotometer with a wavelength of 340 nm, incubated for 5 minutes at 37 C and *shaken* for 20 seconds with a range of 5 seconds *shake*and 5 seconds *off to* make it homogeneous.

E. Blood cholesterol (mg/dl)

- Preparation of Standard Solution (homogenized using vortex)
  a. 0 % : 100 µ Aquadest solution
  b. 20 % : 20 µ Cholesterol Standard Solution + 80 µ Aquadest Solution
  c. 40 % : 40 µ Cholesterol Standard Solution + 60 µ Aquadest Solution
  d. 60 % : 60 µ Cholesterol Standard Solution + 40 µ Aquadest Solution
  e. 80 % : 80 µ Cholesterol Standard Solution + 20 µ Aquadest Solution
  f. 100 % : 100 µ Cholesterol Standard Solution
- Preparation of Standard Solution Samples
  a. Take 3 µ sample of cholesterol standard solution

b. Put into *microplate*
c. Added 250 µ of reagent solution R

- Sample Preparation of Cholesterol Solution
  a. Taken 3 µ of extracted blood sample
  b. Put into *microplate*
  c. Added 250 µ of reagent R

After making the standard solution sample and the albumin solution sample, the *microplate was* inserted into the spectrophotometer with a wavelength of 510 nm, incubated for 5 minutes at 37 C and *shaken* for 20 seconds with a range of 5 seconds *shake*and 5 seconds *off to* make it homogeneous.

F. Creatinine (mg/dl)
- Preparation of Standard Solution (homogenized using vortex)
  a. 0 % : 50 µ Aquadest solution
  b. 0,5 % : 12.5 µ Creatinine Standard Solution + 37.5 µ Aquadest Solution
  c. 1 % : 25 µ Creatinine Standard Solution + 25 µ Aquadest Solution
  d. 1,5 % : 37.5 µ Creatinine Standard Solution + 12.5 µ Aquadest Solution
  e. 2 % : 50 µ Creatinine Standard Solution
- Preparation of reagent solution by mixing reagent 1 and reagent 2 then vortexed to makeit homogeneous. Then the solution is ready to use.
- Preparation of Standard Solution Samples
  a. Take 3 µ sample of creatinine standard solution
  b. Put into *microplate*
  c. Added 250 µ of solution R1 and R2
  d. Incubated for 1 minute at room temperature
- Preparation of Creatinine Solution Samples
  a. Taken 3 µ of extracted blood sample
  b. Put into *microplate*
  c. Added 250 µ of solution R1 and R2
  d. Incubated for 1 minute at room temperature

After making the standard solution sample and the albumin solution sample, the *microplate* was inserted into the spectrophotometer with a wavelength of 520 nm(Lendrawati et al., 2019), incubated for 5 minutes at 37 C and *shaken* for 20 seconds witha range of 5 seconds *shake* and 5 seconds *off to* make it homogeneous.

G. Total Protein (g/dl)
- Preparation of Standard Solution (homogenized using vortex)
  a. 0 % : 100 µ Aquadest solution
  b. 20 % : 20 µ Total Protein Standard Solution + 80 µ Aquadest Solution
  c. 40 % : 40 µ Total Protein Standard Solution + 60 µ Aquadest Solution
  d. 60 % : 60 µ Total Protein Standard Solution + 40 µ Aquadest Solution
  e. 80 % : 80 µ Total Protein Standard Solution + 20 µ Aquadest Solution
  f. 100 % : 100 µ Total Protein Standard Solution
- Preparation of Standard Solution Samples
  a. Take 3 µ sample of total protein standard solution
  b. Put into *microplate*
  c. Added 250 µ of reagent solution R
- Preparation of Total Protein Solution Samples
  a. Taken 3 µ of extracted blood sample
  b. Put into *microplate*
  c. Added 250 µ of reagent R

After making standard solution samples and albumin solution samples, the *microplate* wasinserted into a spectrophotometer with a wavelength of 546 nm (Suharti et al., 2017), incubated for 5 minutes at 37 C and *shaken for* 20 seconds with a range of 5 seconds *shake* and 5 seconds *off to* make it homogeneous.

Analysis

Data collection was conducted after blood samples were analyzed in the laboratory to determine blood glucose levels,

albumin levels, triglyceride levels, blood urea/urea uv levels, blood cholesterol levels, creatinine levels and total protein levels. The data obtained were then entered intodata tabulation and analyzed using the SPSS version 23 application using the Kluskal-Wallis test with the Mann-Whitney follow-up test.

RESULTS AND DISCUSSION

Glucose Level Based on Prolification Level

The metabolic process in the sheep's body plays a role in converting food substances into compounds necessary for the sheep's life processes. Glucose is needed by five tissues of ruminants, namely muscle tissue, nerve tissue, fat tissue, reproductive organs and metabolic processes in the mammary glands (Prakkasi, 1999). The results showed glucose levels based on the level of prolificationpresented in Figure 1.

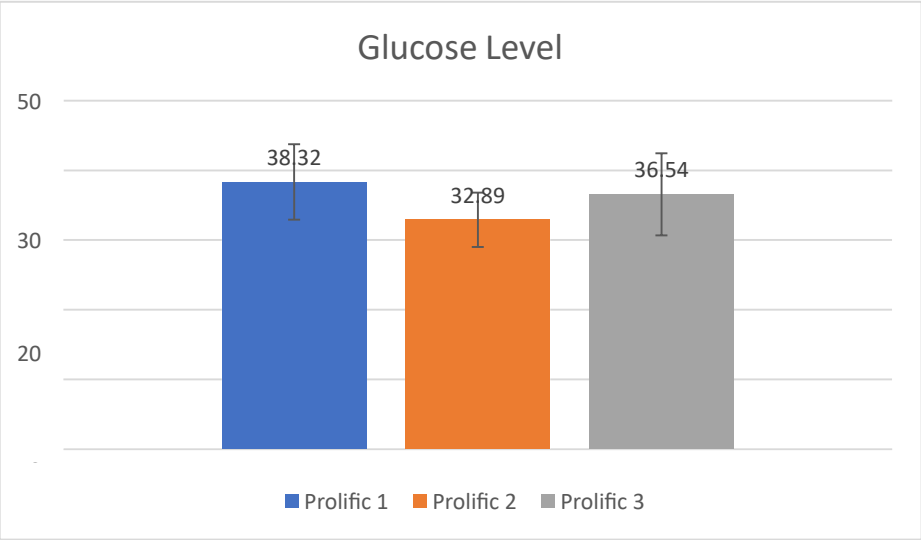

Image 1. Glucose Levels

The results contained in Figure 1 show the average value of glucose levels in Batur sheep. Theresults showed that the Batur sheep with the number of prolific 1 had the highest glucose level of 38.32mg/dl compared to Batur sheep with the number of prolific 2 and 3. The glucose levels of Batur sheepin this study are still in the normal range in accordance with the results of research by Riis (1983) and Schalm (2010) mentioning glucose levels in sheep in normal conditions ranging from 35-60 mg/dl.

According to Riis (1983) Glucose is required in large quantities by several specific tissues including thebrain, adipose tissue, muscle, fetus and mammary glands. This opinion is in line with the results of research by Astuti et al. (2006) that glucose serves as the fastest source of energy to be used as ATP both for major organs such as the brain and nervous system as well as reproductive organs and otherorgans whose role cannot be replaced by other nutrients. According to Khotijah et al. (2020) glucose isthe main metabolic substrate needed for reproduction. The results of statistical analysis showed that the difference in variations in the number of children born, namely the number of births 1, 2 and 3, had a significant effect ($P < 0.01$) on glucose levels in Batur sheep.

Albumin Levels Based on Prolification Level

Albumin is the main protein in blood plasma (Murray et al., 2003). According to Ballmer (2001),albumin concentration is influenced by blood volume and protein intake in the blood plasma. The results showed that albumin levels based on the level of prolification are presented in Figure 2.

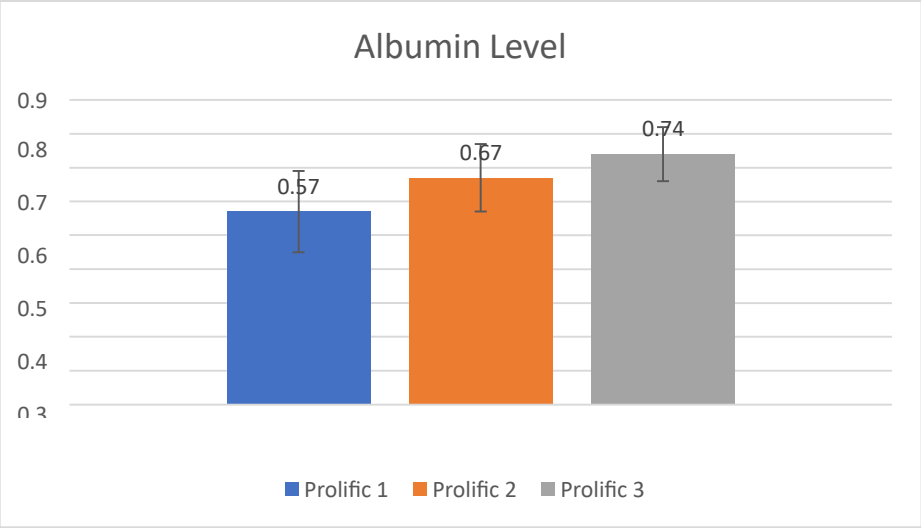

Image 2. Albumin Level

The results contained in Figure 2 show that the average value of albumin levels in Batur sheep. The results show that the Batur sheep with the number of prolific 3 has the highest albumin level of 0.74 g/dl. The albumin levels in this study were lower when compared to the results of research by Baratawidjaja (2006) albumin levels of 2.7-4.55 g/dl. The results of this study are in line with the resultsof research by Pal et al. (2015) that the normal range of albumin levels in sheep is 3.3-3.47 g/dl. According to Kaslow (2010) low albumin levels can cause insufficiency of anabolic hormones or growth hormones.

Blood Ureum Levels by Prolification Level

Urea is the end result of protein metabolism in the body of livestock and secreted through urine, while blood urea comes from rumen ammonia and the rest of amino acid catabolism (Tillman etal., 1998). The results of blood urea levels based on the level of proliferation in Batur sheep are presented in Figure 3.

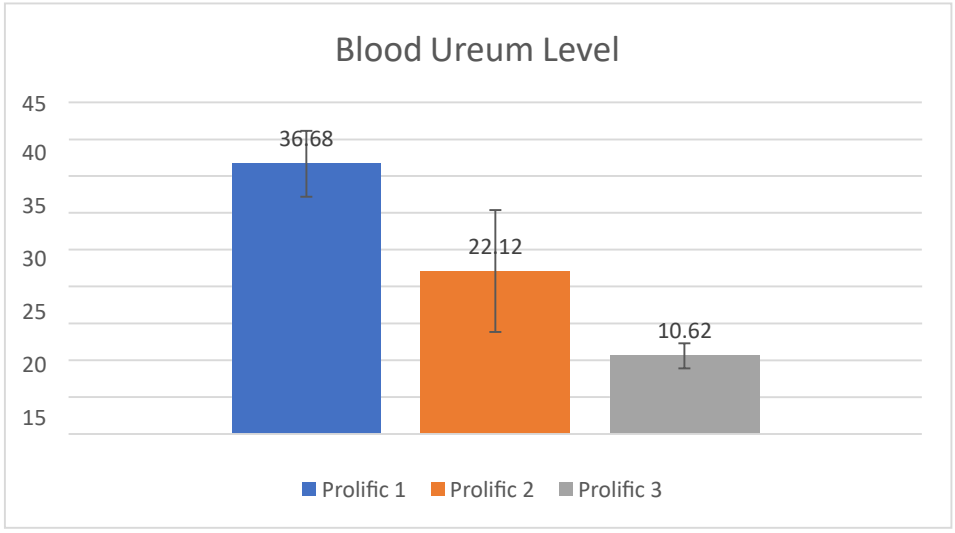

Image 3. Blood Ureum Levels

Based on Figure 3, the blood urea level in sheep with a single prolific number has the highest level of 36.68 mg/dl compared to the number of prolific 2 and 3. The results of the study are still withinnormal limits in accordance with the opinion of Wahyuni and Bijanti (2006) that blood urea levels in sheep range from 6-36 mg/dl. However, the results of this study were lower than the results of researchby Marhaeniyanto and Susanti (2016) that blood urea levels in sheep ranged from 37.25 to 49.18 mg/dl. The results of statistical analysis of blood urea levels showed a significant difference ($P < 0.01$) in prolific1, 2 and 3.

Creatinine Level Based on Prolification Level

Creatinine is the result of the breakdown of creatinine phosphate which is used as a source ofenergy when livestock experience stress (Lendrawati et al., 2019). The results of the study of creatininelevels based on the number of prolifications are presented in Figure 4.

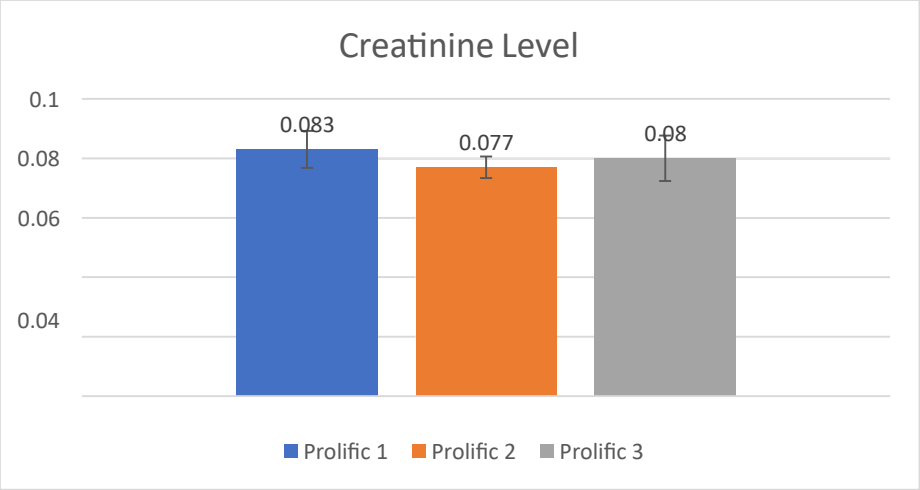

Image 4. Creatinine Level

Based on Figure 4, the results of creatinine levels in prolific 1 are greater than the number of prolific 2 and 3. The results of creatinine levels in prolific 1 were 0.083 mg/dl, prolific 2 were 0.077 mg/dl and prolific 3 were 0.080 mg/dl. The results of this study are still in accordance with the resultsof research by Lendrawati et al., (2019) that the average creatinine levels are 0.06-0.11 mg/dl. However, the results of this study are lower than the results of research by Gopar et al., (2020) that creatinine levels in sheep ranged from 0.008-0.022 mg/dl.

Cholestrol Levels Based on Prolification Levels

Cholesterol is a fat molecule contained in cells and is divided into several parts such as LDL, HDL,total cholesterol and triglycerides (Prayitno and Heni, 2020). The results showed that cholesterol levelsbased on the level of prolification are presented in Figure 5.

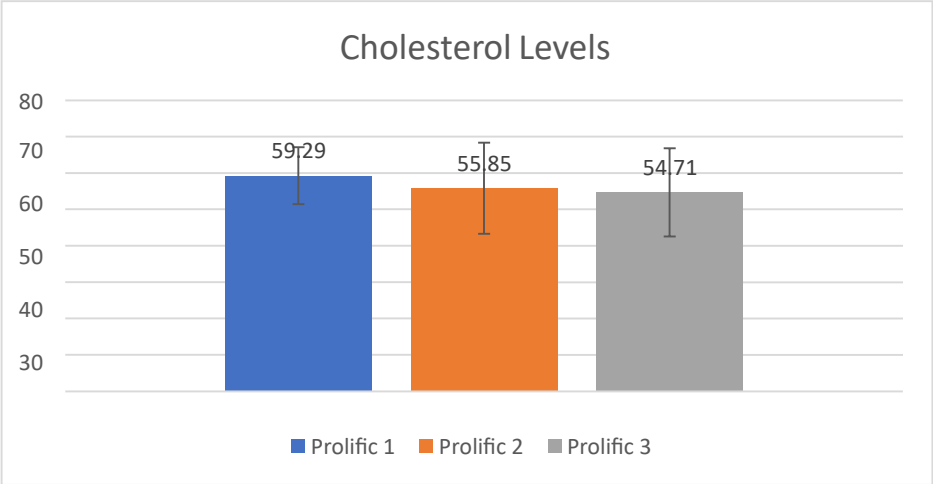

Image 5. Cholesterol Level

The results contained in Figure 5 show the average value in Batur sheep. The results show thatBatur sheep with a single prolific number have the highest cholesterol levels of 59.28 compared to Batur sheep with prolific 2 and 3. Cholesterol levels in this study are still within the normal range in accordance with the results of research by Schalm (2010) ranging from 50-140 mg/dl. However, the results of this study are still higher than the results of research by Khotijah et al. (2020) that the cholesterol levels of sheep given basal feed in the form of *Brachiaria humidicola* grass and concentrateshad cholesterol levels of 51.46 mg/dl.

According to Khotijah et al. (2015) cholesterol levels are needed for the synthesis of steroid hormones that play a role in uterine preparation before mating. Low levels of cholesterol in the bloodcan suppress lust and ovulation or reduce the number of ovulated eggs (Hardjopranjoto, 1995).

The results of statistical analysis showed that the difference in the variation of the number of childrenborn in the number of births 1, 2 and 3 did not have a significant effect (P>0.05) on cholesterol levels in Batur sheep. The results of the study are thought to be due to the content of cholesterol in the bloodis influenced by the feed given. This is in accordance with the statement of Gagah et al. (2016) that thelipid content in the blood of livestock is influenced by the feed intake given. This opinion is supportedby the

results of Prayitno and Heni (2020) research that the high and low content of cholesterol in theblood is influenced by the feed given. Cholesterol is obtained from feed and its biosynthesis occurs in organs such as the intestines and liver (Khotijah et al., 2020).

Total Protein Based on Prolification Level

Sheep have a rumen that is able to digest feed protein and the results of digestion will be partially absorbed by the rumen (Suharti et al., 2017). Feed protein digested in the sheep's body is assisted by rumen microbes. Protein is the last energy reserve that will be broken down to meet the body's energy needs (Cunningham, 2002). The results of total protein research based on the level of prolification are presented in Figure 6.

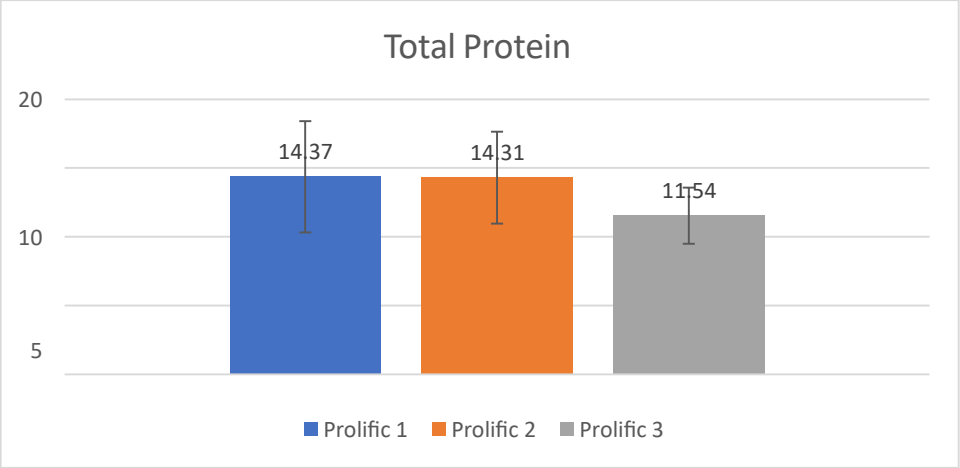

Image 6. Total Protein

Based on Figure 6, the total protein in sheep with a single prolific number has the highest levelof 14.37 g/dl compared to prolific 2 and 3. The results of the study were higher than the results of Marhaeniyanto and Susanti (2016) that the total protein levels ranged from 5.15-6.89 g/dl. The resultsof Marhaeniyanto and Susanti (2016) are in line with the results of Baratawidjaja's research (2006) thattotal protein levels in sheep range from 5.5-8.10 g/dl.

CONCLUSIONS

Based on the results, it can be concluded that different prolification levels in sheep have significant differences in blood biochemical levels.

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