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Hand-Powered Centrifuge Made from Cotton for Assessing Hematocrit

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ABSTRACT

The hematocrit value test serves as a parameter to help reach an established diagnosis. A centrifuge is an equipment that is used to separate the components of blood from each other based on the properties of each constituent substance. This study aims to developing hand-powered centrifuge made from cotton material to assess the hematocrit of blood in pregnant and non-pregnant ewes. To make the Katunfuge, two circles were cut from a piece of cotton cloth that had been impregnated with batik wax. The knitting thread was inserted into the middle part of the cotton disc and each end rope was tied to the handler. The centrifugal speed resulted from Katunfuge was greater than 2,000 rpm, meaning that it is 99% effective for separating blood components in 15 minutes with 14 times of pulling with hands. The hematocrit values of pregnant ewes (33.0 ± 4.1) were lower than those of non-pregnant ewes (38.5 ± 3.4%). This has proven that Katunfuge is highly potential to be used to assess the hematocrit of other animals.

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INTRODUCTION

Local ewes have good reproductive characteristics, such as long calving intervals and high birth rates, as well as good adaptation (Diharjo, 1995). A complete blood count is performed to determine the health status of an animal and support the diagnosis. Changes in blood profile of livestock are influenced by the physiological condition, increase in age, nutritional status, exercise, health, stress, microorganisms, body temperature, changes in environmental temperature, and the reproductive cycle. Regular blood tests include the examination of erythrocyte count, hemoglobin concentration, hematocrit value, total leukocyte count, leukocyte differential count, platelet count, and sedimentation rate (Pusparini, 2005). The examinations of hemoglobin level and hematocrit value are the major parameters to evaluate the condition of circulating red blood cells, as well as to diagnose anemia (Etim et al., 2013).

Low hematocrit value is found in livestock not only when they are pregnant but also when they suffer from anemia. On the other hand, an increase in hematocrit value happens when they suffer from dehydration. Hematocrit can be an indicator of dehydration and haemoconcentration due to decreased fluid volume and increased erythrocytes (Kee, 2008). Diseases with elevated hematocrit levels include severe diarrhea, polycythemia vera, diabetic acidosis, pulmonary emphysema (end-stage), transient ischemic attack (TIA), trauma, surgery, burns (Sutedjo, 2009). Physiologically, hematocrit values in pregnant animals will always be lower than those in non-pregnant animals. The hematocrit values of non-pregnant sheep are 34±3%, while the values in pregnant sheep are 28.60±1.4% (Kozat et al., 2006). This is due to fluid retention, which causes an increase in blood plasma volume (Podymow et al., 2010). Hematocrit is the proportion of blood cells to plasma. The examination of the hematocrit value is generally performed using macro-hematocrit and micro-hematocrit centrifugation techniques (Kiswari, 2014). Hematocrit examination is a particular blood test that can only be conducted in a laboratory using a centrifuge and requires electrical energy (Meyer & Harvey, 2004), as well as needs a relatively high cost. Electric-powered centrifugation is inaccessible under field conditions because it takes a longer diagnostic process, which causes delayed treatment and control of a disease.

A centrifuge is an apparatus that is used for separating the components of a mixture based on the physical properties of the constituent substances. In centrifugation, principally, an object quick move in a circular path will generate a force that can move the object away from the center of the circle (WHO, 2011). Centrifugal force action generates a gravitational field induction in the outward direction relative to the rotation axis and pushes particles or sediment to the bottom of the tube (Sufyan & Sumardiyanto, 2017). Innovative examinations of blood hematocrit values have been developed, one of which is the Paperfuge, inspired by the whirligig toy mechanism. Paperfuge can work with a speed up to 125,000 rpm and it takes 1.5 minutes to separate plasma from red blood cells (RBC) and 15 minutes to isolate buffy coat for malaria diagnosis (Bhamla et al., 2016; Bhamla et al., 2017). However, Paperfuge is not water-resistant and easily broken. These limitations are not suitable for Indonesia's geographical conditions. Indonesia is an agricultural country with a humid tropical climate that receives heavy rainfall and tropical forest areas (Wirjohamidjojo & Swarinoto, 2010). Therefore, innovation needs to be developed. In this study, innovation is made by developing a centrifuge using waterproof material, such as cotton cloth, which can work without electricity. This study aims to make a prototype of a centrifuge using cotton cloth to assess the hematocrit in pregnant ewes. It is expected that the centrifuge resulted in this study can work without electrical energy to monitor the health of animals in the fields away from the laboratory.

MATERIALS AND METHODS

Time and Place

This research was conducted at the Faculty of Veterinary Medicine, Bogor Agricultural University (IPB University). Ewe's blood samples were taken from the Reproduction and Rehabilitation Unit, Division of Reproduction and Obstetrics, Department of Clinics, Reproduction, and Pathology. The production and testing of prototypes were carried out at the Reproductive Surgery Laboratory, Division of Reproduction and Obstetrics, Department of Clinics, Reproduction, and Pathology. The laboratory-standardized hematocrit values were collected and processed with a centrifuge at the Physiology Laboratory, Department of Anatomy, Physiology, and Pharmacology.

Materials and Equipment

A set of hematocrit centrifuge, BIOFUGE Haemo (Heraeus instrument) was used. The prototype of the centrifuge was made using several materials and equipment, including cotton cloth, compact disc (CD), cotton bud, knitting yarn, hot glue gun, alcohol, batik wax, pens (handlers), scissors, ruler, digital tachometer, microcapillary tubes, and clay sealants. Blood samples were taken using a 5 mL syringe and an EDTA blood tube. Eight productive ewes aged 2-3 years old, including pregnant ewes (n=4) and non-pregnant ewes (n=4), were used in this research. Ewe's blood, hot glue stick, and tissue paper were also utilized.

Katunfuge and CDfuge Making

This research produced two hand-powered centrifuges, Katunfuge made from cotton cloth and CDfuge made from plastic of used compact disc (Figure 1). As presented in Figure 1B, Katunfuge was made by making two pieces of squares by cutting the cloth and drawing a circle with a 12 cm diameter on one side of each square. The cloth was dipped in the melted batik wax, and then lifted and let until cooled down. The circles were cut based on the patterns and then affixed to each other using hot glue to create a disc. Two circles with 2 cm diameter were also made and attached to the center of the cotton disc.

The CDfuge was made from a compact disc (Figure 1C), by attaching a small plastic circular disc with a 2 cm diameter to the center of the disc with a 12 cm diameter. Sample tubes for placing microcapillary tubes were made from 3 cm cotton bud sticks. Eight tubes were put on two sides (four tubes on each side) of cotton cloth and CD. Handlers were made from pens with 0.7 cm diameter and 15 cm length. They were used to simplify the centrifugation process. Disc and handler were connected with 2-meter length knitting rope divided into two parts, with each end attached to handler through the central parts of cotton cloth and CD that had been perforated.

Group and Testing

Centrifugations of blood specimens obtained from ewes were carried out in three observation groups. Centrifugations in the first and second groups were performed with Katunfuge and CDfuge, respectively, while the centrifugation in the third group was done by using a centrifuge in the laboratory as the control.

Centrifugation of Blood Specimens using Centrifuge in the Laboratory

The calculation of the hematocrit value of ewe's blood specimens was carried out using microcapillary tubes. The tubes were filled with blood to 1 cm from the top of the tubes and later were clogged with clay sealants. The hematocrit microcapillary tube was centrifuged using a laboratory-standardized centrifuge for 15 minutes at a speed of 2,000 rpm, 3,000 rpm and 12,000 rpm. The hematocrit values were observed with hematocrit reader or ruler (Figure 2).

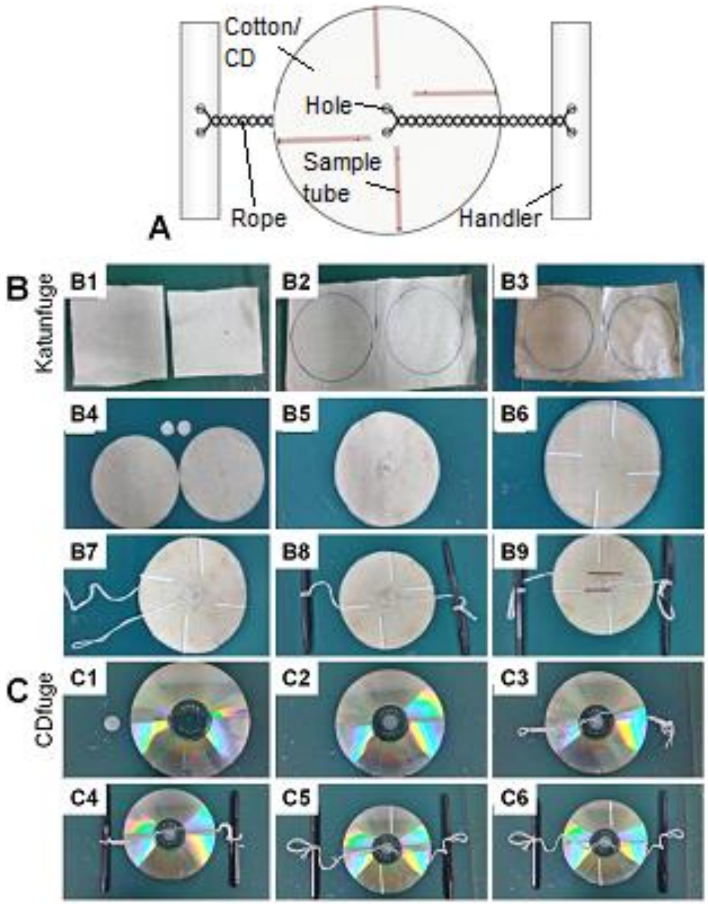

Figure 1. Illustration of centrifuge design (A), Katunfuge making (B): (B1) square cotton cloth, (B2) sketch on cotton cloth, (3) cotton cloth dyed with batik wax, (B4) circular cotton cloth with 12 cm and 2 cm diameters, (B5) pieces of cotton cloth attached, (B6) sample tubes on cotton cloth, (B7) handlers connecting ropes, (B8) handlers on two ends of ropes, and (B9) microcapillary tubes on sample tubes. CDfuge making (C): (C1) CD and plastic circles, (C2) CDfuge, (C3) handlers connecting ropes, (C4) handlers on two ends of ropes, (C5) sample tubes on CD, and (C6) CDfuge ready to be used.

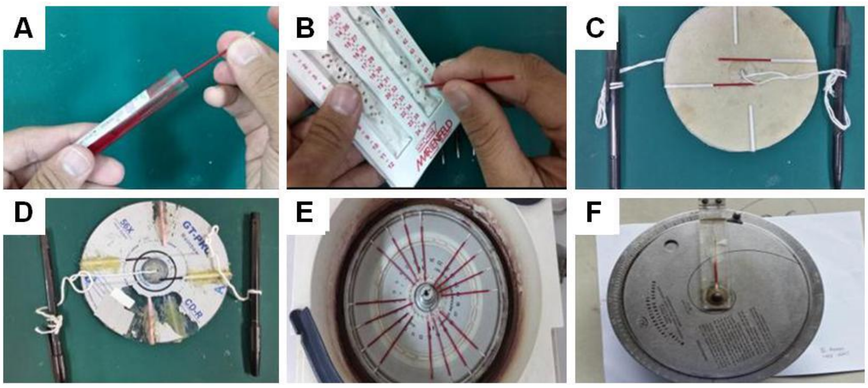

Figure 2. Centrifugation of blood samples using Katunfuge, CDfuge, and laboratory centrifuge. (a) Microcapillary tubes filled with blood, (b) microcapillary tubes clogged with clay sealants, (c) microcapillary tubes on the sample tubes of Katunfuge, (d) microcapillary tubes on the sample tubes of CDfuge, (e) microcapillary tubes on the centrifuge in the laboratory, and (f) the assessment of hematocrit value.

Centrifugation of Blood Specimens using Katunfuge dan Cdfuge

Microcapillary tubes filled with blood and clogged with clay sealants were put into the sample tubes on each side of Katunfuge and CDfuge (Figure 3). The rope was rotated slowly on its axis so that it was twisted until became shorter (the distance between the handlers was getting closer). The handlers were pulled away from each other so that the rotating ropes pulled the handlers back repeatedly, causing the cloth disc or CD to spin. Centrifugations using Katunfuge and CDfuge were carried out for 1, 3, 5, 7, 10, 15, and 20 minutes. The rotational speed was measured using a tachometer for one minute. A tachometer is designed to measure the rotational speed of an object by measuring the revolution per minute (Rana et al. 2006). Hematocrit (%) is the ratio of the volume of packed red blood cells to the total volume of blood in the sample (Silverthorn 2006).

Data Analysis

Data were analyzed using Microsoft Excel 2016 and described narratively.

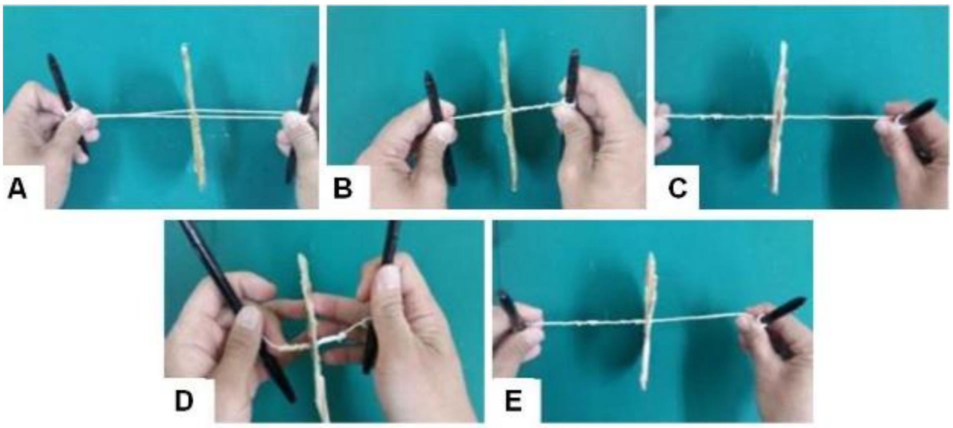

Figure 3. The uses of Katunfuge and CDfuge. (a) Straight rope, (b) twisted rope, (c) handlers pulled away from each other, (d) rope becoming shorter, (e) handlers pulled away from each other repeatedly.

RESULT AND DISCUSSION

Katunfuge and Cdfuge

This study produced simple centrifuges by taking the advantages of cotton cloth and CD to determine the hematocrit values of ewe's blood samples with the principles of centrifugal force. The implementations of these centrifuges are illustrated in Figures 1 and 2. Cotton cloth and CD function as the discs of hand-powered centrifuges. Each side of cotton cloth dyed with batik wax and CD were attached to four sample tubes as the places to put the microcapillary tubes.

Katunfuge and CDfuge were rotated using two pens functioning as the holders and connected using knitting threads inserted through two holes in the middle of the cotton cloth and CD (Figure 3).

Rotational Speed of Katunfuge and Cdfuge

Figure 4 presents the comparison of centrifugation speed between Katunfuge and CDfuge in one minute (n=5). The rotational speeds of Katunfuge and CDfuge were fluctuating. Katunfuge had faster centrifugation speed than CDfuge. The average Katunfuge rotational speed was 2,233±98.2 rpm, while that of CDfuge was 2,035±75.3 rpm.

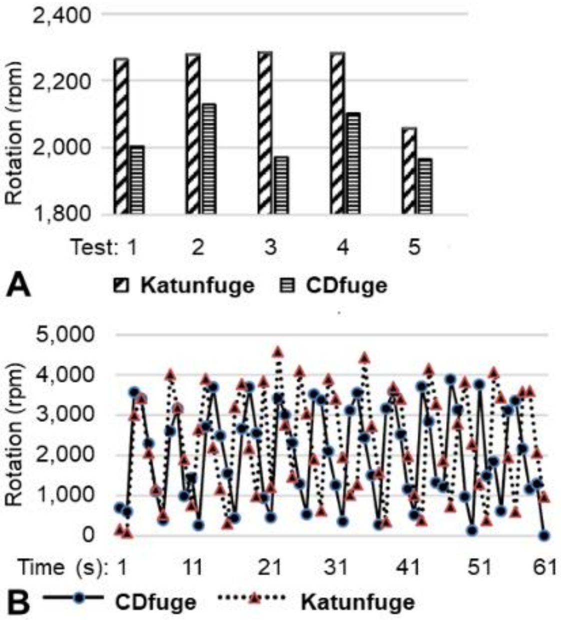

Figure 4. The rotational speed of centrifuges. (A). Average speed and (B) Katunfuge and CDfuge speed per second.

Separation of Whole Blood Components

Table 1 demonstrates the comparison of hematocrit values resulted from centrifugations using Katunfuge, CDfuge, and the centrifuge machine in the laboratory. The average hematocrit values of ewe's blood measured using Katunfuge and CDfuge were constant at minute 15, while the values were constant since minute 3 when centrifugated with a centrifuge machine in the laboratory.

The difference in hematocrit values resulted from Katunfuge, CDfuge, and laboratory centrifuge decreased every minute. The difference remained stable starting from minute 15. Figure 5 summarizes that the hematocrit value resulted from centrifugation with laboratory centrifuge differed 14-15% from that of Katunfuge and 18-19% from that of CDfuge. Figure 6 demonstrates the hematocrit values of blood obtained from pregnant ewes and non-pregnant ewes measured using Katunfuge and a laboratory centrifuge. The blood of pregnant and non-pregnant ewes had the lowest hematocrit values when centrifugated at 3,000 rpm speed and the highest values at 2,000 rpm speed using laboratory centrifuge. When centrifugated with Katunfuge, the hematocrit values of the blood were between in between, 33.0±4.1% for pregnant ewes and 38.5±3.4% for non-pregnant ewes.

Table 1. The hematocrit values of ewes' blood resulted from centrifugations using Katunfuge, CDfuge, and a centrifuge machine in laboratory

Time (minute)	Repetition (n)	Katunfuge (%)	CDfuge (%)	Lab. Centrifuge (%)	The difference with Lab. Centrifuge (%)	
					Katunfuge	CDfuge
1	4	87.7 ± 2.6	85.3 ± 1.2	24.5 ± 0.6	60.8 ± 2.6	63.5±1.2
3	4	66.0 ± 4.0	64.1 ± 2.5	23.0 ± 0.8	43.0 ± 4.0	41.3±2.5
5	4	52.5 ± 9.6	53.6 ± 2.2	23.0 ± 0.8	29.5 ± 9.6	30.6±2.2
10	4	45.2 ± 3.3	42.5 ± 1.3	23.0 ± 0.0	22.2 ± 3.3	19.5±1.3
15	4	37.5 ± 1.7	41.6 ± 6.9	23.0 ± 0.8	14.5 ± 1.7	18.6±6.9
17	4	37.3 ± 6.8	41.9 ± 2.4	23.0 ± 0.5	14.3 ± 6.8	18.9±2.4
20	4	37.1 ± 8.5	41.4 ± 3.4	23.0 ± 0.0	14.1 ± 8.5	18.4±3.4

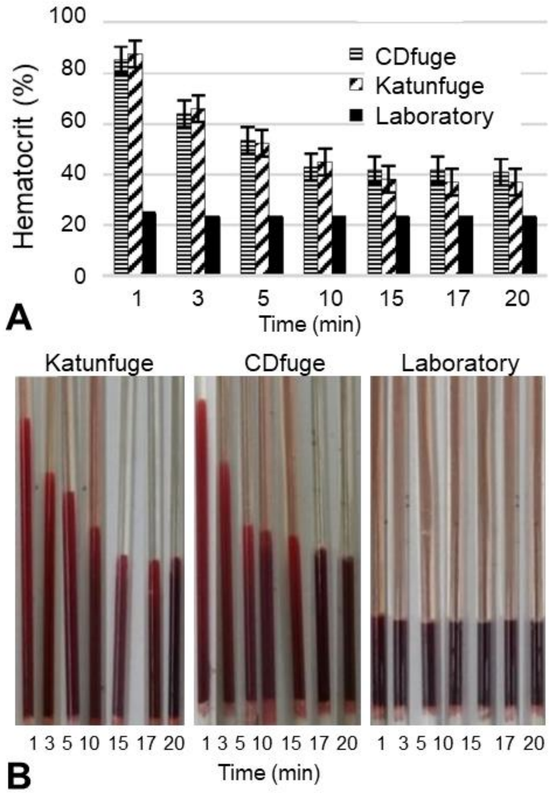

Figure 5. Test for blood component separation. (A) The hematocrit values of ewe's blood centrifugated with Kantunfuge, CDfuge, and laboratory centrifuge at 12,000 rpm speed, and (B) The results of blood component separation.

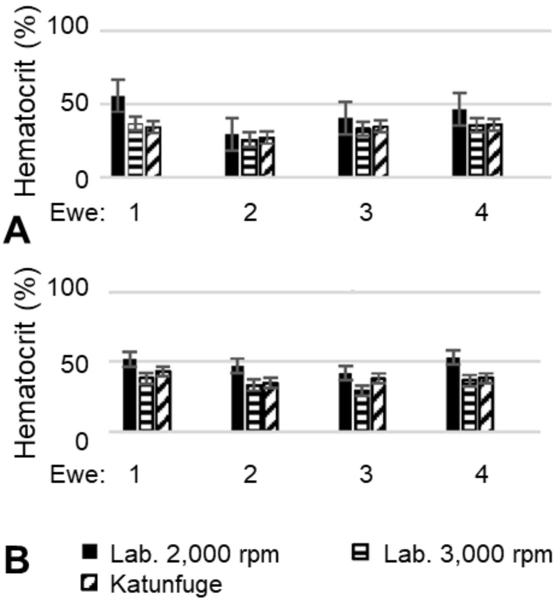

Figure 6. The hematocrit values of the blood of (A) pregnant ewes and (B) non-pregnant ewes centrifugated using Katunfuge and laboratory centrifuge.

As presented in Table 2, Katunfuge had higher average rotational speed, a greater number of pulls required in one minute, and lighter weight than CDfuge. The success rates of plasma separation from whole blood using laboratory centrifuge, Katunfuge, and CDfuge were 100%, 99%, and 92.8%, respectively. The cost for producing a Katunfuge and CDfuge was IDR 1,300 and IDR 3,300, respectively, and the purchase price for laboratory centrifuge was more than IDR 20,000,000.

This study successfully produced and tested centrifuges from cotton cloth and CD with the working principle of the centrifugal force (Figure 6). Centrifugal force causes the induction of gravitational fields in the outward direction relative to the axis of rotation and pushes particles or sediments to the bottom of the tube (Wardani & Pertiwi, 2013). Katunfuge is a simple centrifuge made from cotton cloth. Cotton comes from natural fibers (natural cellulose), which are environmentally friendly, resistant to high iron heat, and resistant to bleach (Poespo, 2005). However, cotton easily absorbs water (Bonita, 2014), and therefore, it is coated with wax, which is hydroponic, to prevent water permeates into the cotton cloth and make it stiffer and stronger. The wax-coated cotton cloth serves as the rotary disc in the centrifugation process. Bhamla et al. (2016) previously created a centrifuge from paper (Paperfuge) but it was easily damaged by water. The present study improved the drawbacks of Paperfuge by substituting the main materials of the centrifuge, from paper to cotton cloth and CD, which functions as a rotary disc. CD is strong, water-resistant, and heat-resistant. However, it is harder and heavier than paper and cloth so that it is easily broken or damaged.

Table 2. The hematocrit values of ewes' blood resulted from centrifugation using Katunfuge, CDfuge, and laboratory centrifuge

Parameter	Katunfuge	CDfuge	Lab. Centrifuge
Speed (rpm)	2233 ± 98.2	2035 ± 75.3	12000
Rotation direction	Vertical 2 directions	Vertical 2 directions	Horizontal one direction
Rotation	Interrupted	Interrupted	Continued
Number of pulls (x/minute)	14	13	None
Weight (g)	6.76	15.08	>5,000
Diameter (cm)	12	12	26
Success (%)	99	92.8	100
Source of energy	Hand-power	Hand-power	Electricity
Price (IDR)	1,300	3,300	>20,000, 000

The average hematocrit values of pregnant ewes were 28.6±1.4% and non-pregnant ewes were 34±3% (Kozat et al., 2003). Centrifugation using Katunfuge was found faster than using CDfuge (Figure 9). CD is made from a combination of polycarbonate plastic, aluminum metal coating, and acrylic resin, which has solid properties (Sharpless, 2003). Acrylic resin has sufficient strength, hardness and stiffness (Anusavice, 2003). CD is heavier than the cotton disc. According to Sumarsono (2009) mass is inversely proportional to the rotational speed of an object, meaning that the greater the mass of an object, the lower the rotational speed will be and vice versa.

The difference in speed is also affected by the difference in rope tension during the centrifugation process using Katunfuge and CDfuge. The rope tension is proportional to speed and mass, and thus, the greater the tension in the rope is, the higher the speed will be (Sumarsono, 2009). Rope tension affects the number of pulls, in which the greater the rope tension is, the greater the rotational speed of the object will be, and thus increasing the number of pulls to rotate. Katunfuge requires 14 times pulls to rotate while CDfuge requires 13 times pulls (Table 2). This causes Katunfuge to have greater average speed than CDfuge. Further, Sumarsono (2009) affirms that rope tension will increase if the rotational speed of an object is made higher to reach the maximum speed.

The success rate of centrifugation using CDfuge (92.8%) was lower than using Katunfuge (99%), while the success rates of centrifugation using both centrifuges were slightly lower than that using laboratory centrifuge (Table 1). This is so for the centrifugation using CDfuge often experiences sliding and microcapillary tube seal using wax is often less tight, which causes blood to come out. Mahode et al. (2011) state that the non-tight closure of the end of the microcapillary tube can cause a tube leak in the centrifugation process.

Centrifugation of pregnant ewe's blood for three minutes using laboratory centrifuge resulted in 23.0±0.8% hematocrit value. All blood components were separated according to the molecular weight of each blood component. Centrifugations using Katunfuge and CDfuge produced higher hematocrit values, compared to the centrifugation using laboratory centrifuge. Blood separation in microcapillary tubes with centrifugation shows that Katunfuge and CDfuge need to be improved (Figure 10). The biggest and most dense particles will settle quickly, followed by less dense and smaller ones (Wardani & Pertiwi, 2013). Budiman (2009) hints that the longer the centrifugation time is, the maximum results obtained will be. The difference in hematocrit values of pregnant and non-pregnant ewes resulted from centrifugation using Katunfuge and laboratory centrifuge at 12,000 rpm ranged between 14% and 15% (Table 1). Centrifugation with Paperfuge at 125,000 rpm (± 5 mm diameter) could separate plasma from whole blood in 1.5 minutes and separate buffy coat to diagnose malaria in 15 minutes (Bhamla et al., 2016, Bhamla et al., 2017).

The hematocrit value of pregnant ewes was lower than that of non-pregnant ewes (Figure 6). According to Podymow et al. (2010), the hematocrit value in pregnant animals is always lower than the value when they are not pregnant because of fluid retention, which causes an increase in blood plasma volume. The hematocrit values of pregnant and non-pregnant ewes obtained using Katunfuge were in the range between the hematocrit values when centrifuged using a laboratory centrifuge with a speed of 2,000 and 3,000 rpm (Figure 6). Nugraha (2015) concludes that the higher the rotational speed is, the greater the produced centrifugal force will be. Budiman (2009) confirms that the higher the centrifuge speed is, the faster the deposition of erythrocytes and vice versa will be. Katunfuge could function as a blood centrifuge with an average speed of 2,233 rpm (Table 2). The average hematocrit value measured with Katunfuge was 33.0±4.1% for pregnant ewes and 38.5±3.4% for non-pregnant ewes (Figure 6). The hematocrit values of pregnant and non-pregnant ewes separated using Katunfuge were close to the range of hematocrit values of pregnant ewes. The hematocrit value of non-pregnant ewe's blood was 34.0 ± 3.0% (Kozat et al., 2003) while pregnant ewes' blood was 28.6 ± 1.4% (Kozat et al., 2006).

CONCLUSION

In conclusion, Katunfuge can be used as a hand-powered centrifuge made of cotton to assess the hematocrit of ewes' blood. This is so because of several pieces of evidence. The hematocrit value of ewe's blood was obtained after 15 minutes of centrifugation with 14x pulls/minute, the rotation speed of 2,000 rpm, and a success rate of 99%. The hematocrit of pregnant ewe's blood was lower than that of non-pregnant ewes. The correction factor applied for the hematocrit value centrifuged using Katunfuge was 14–15% higher than that of the laboratory centrifuge.

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