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## Oxygen saturation (SpO<sub>2</sub>) measurement to predict blood profile in sheep to help diagnose anemia caused by haemonchosis

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### Abstract

*Haemonchus contortus* infection (haemonchosis) often occurs in sheep and cause chronic blood losses. These worms causing symptoms of anemia that can continue in hypoproteinemia or even death. Therefore, early recognition of anemic conditions due to haemonchosis becomes very important. Rapid and accurate detection of the incidence of anemia will help in the determination of appropriate therapy. The purpose of this study was to detect anemia in sheep with easy, fast, and non-invasive way through the SpO<sub>2</sub> examination. A total of 20 sheep were divided into 2 groups, control and the treatment group. Treatment group were sheep with artificial anemia that used as a model for haemonchosis anemia. The results showed that the normal levels of sheep SpO<sub>2</sub> were 90.30±6.98% – 92.80±5.37% which described the number of RBC were 9.78±1.98x10<sup>6</sup>/μl– 10.44±1.5210<sup>6</sup>/μl, Hb 8.42±1.07 g/dl – 9.14±1.03 g/dl and HCT 26.79±3.27% – 29.37±2.94%. When sheep were anemic, SpO<sub>2</sub> values dropped to 81.40±4.09% reflecting RBC levels of 8.18±2.14x10<sup>6</sup>/μl, Hb 6.98±1.28 g/dl and HCT 21.82±3.93%. The strongest relationship of SpO<sub>2</sub> with Hb levels in the equation  $y=0.1334x-3.856$  with a correlation of 85.8%. The coefficient of determination of the equation is 0.74 so that the SpO<sub>2</sub> value can guess the condition of anemia through the approximate hemoglobin value. From these results shows that SpO<sub>2</sub> can be used as an estimator of anemic conditions in sheep. The SPO<sub>2</sub> examination needs to be combined with the results of the EPG examination to confirm whether the anemia that occurs was caused by haemonchosis or due to other causes.

**Keywords:** *Haemonchus contortus*, SpO<sub>2</sub>, Hb, sheep, anemia

### 1. Introduction

Anemia is a condition in which the body lacks red blood cells or described as a reduction in the proportion of the red blood cells (Turner *et al.*, 2022) <sup>[19]</sup>. Anemia can result in decreased physiological function of the body. Clinical symptoms has been seen when animals experience anemia like tachycardia, tachypnea, icterus and mucosal pallor (Katsogiannou *et al.*, 2018) <sup>[11]</sup>. Anemia can appear acutely or chronically and divided into regenerative and nonregenerative forms with their specific characteristics (Johns and Heller, 2021) <sup>[10]</sup>.

The causes of anemia in sheep can vary. One of them is by the infection of the blood-sucking nematode worm *Haemonchus contortus*. This worm can suck as much blood as 0.03 ml/worm/day (Naeem and Iqbal, 2021) <sup>[13]</sup>. Haemonchosis is more common chronically so that the anemia condition experienced by sheep has also been going on for a long time. Therefore, the losses suffered by sheep owners are also not realized. Haemonchosis presents many challenges including fact that infection exists throughout the world, both tropical and subtropic, the presence of strains that are to be resistant to anthelmintic, the difficulty of early diagnosis, and not easy of controlling this disease (Arsenopoulos *et al.*, 2021) <sup>[3]</sup>.

Detection of anemia is usually done by taking a blood sample and then doing a blood test in the laboratory. This technique is considered less efficient because it requires costs and professional staff to do. The development of science related to sensors for acquisition of body physiological data opens up the opportunity to make devices that can predict the anemia status of sheep without taking blood. One of the body's physiological variables that can be examined without being invasive is the peripheral oxygen saturation value (SpO<sub>2</sub>).

Oxygen saturation values have been widely used to determine changes in dissolved oxygen in blood vessels. The peripheral oxygen saturation value (SpO<sub>2</sub>) is the easiest to check among other parameters such as arterial oxygen pressure (PaO<sub>2</sub>) and arterial oxygen saturation (SaO<sub>2</sub>) in assessing tissue oxygenation levels (Röttgering *et al.*, 2021) [15]. Oxygen saturation values are usually checked using an oximeter device although the results are not as good as those of examinations using near-infrared spectroscopy (NIRS) (Cheung *et al.*, 2022) [6].

The oxygen saturation value is checked using a pulse oximetry and then the results are presented in percentage form. The oximeter sensor works in a way to detect the oxygen in the blood so this value describes the amount of oxygenated haemoglobin (ALhyari *et al.*, 2018) [2]. In addition, the oxygen saturation value is reported to have a relationship with the diagnosis anemia because of the changing of tissue deoxygenation rate (Meznar *et al.*, 2009) [12].

In humans, oxygen saturation checks have been common, even in fetuses (Saini *et al.*, 2020) [16]. The value of oxygen saturation has a relationship with blood hemoglobin levels although there needs to be a correction value (Suherlim *et al.*, 2018) [18]. In animals, the estimation of anemia by examining oxygen saturation has never been done. Therefore, this research is needed to determine the relationship between the changes in oxygen saturation values to the incidence of anemia in sheep.

## 2. Materials and Methods

### 2.1 Animal

The animals used in this study were 20 sheep which were divided into 2 groups, namely control and anemia group. The control group was sheep that did not experience anemia. The anemia group was sheep experiencing anemia. The sheep used were male and have an average weight of 16-19 kg. Sheep are kept in a colony pen owned by Mitra Tani Farm located in Tegalwaru Village, Ciampea District, Bogor Regency, Indonesia. Feed is given 3 times a day in the form

of forage and concentrate and drink is given ad libitum.

### 2.2 Artificial anemia method

The treated sheep were artificially anemic sheep. The implementation method for making sheep become anemic has received ethical approval from the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences with number 007/KEH/SKE/VI/2022. Sheep that were made to experience anemia described acute anemia with a total blood loss of 324 mL. The total blood volume is in accordance with the recommendations of the Institutional Animal Care and Use Committee (IACUC) which states that the maximum non-hazardous total blood volume is less than 33% of the total blood volume. The process of bleeding/plebotomy was carried out through the jugular vein and carried out for three consecutive days with each volume of 108 mL per day.

### 2.3 Research variables and data analysis

The variables examined in this study included oxygen saturation values (SpO<sub>2</sub>), Red Blood Cell counts (RBC), Hemoglobin levels (Hb), and Hematocrite values (HCT). In addition, documentation was also carried out on the conjunctival mucosa of the eye and gum mucosa. Examination of the blood sample was carried out on day 0 or before treatment and on day 3 or after treatment of anemia phlebotomy in sheep. The data obtained were analyzed using ANOVA. In addition, a correlation analysis and coefficient of determination was carried out between the SpO<sub>2</sub> value and RBC, Hb, and HCT. Data in the form of photos of the conjunctiva and gum mucosa were compared and analyzed descriptively.

## 3. Results

The sheep in the control and the anemic group had almost the same blood profile and SpO<sub>2</sub> values on day 0. The SpO<sub>2</sub>, RBC, Hb, and HCT values showed no significant difference. The results of measuring the SpO<sub>2</sub> and the red blood profile of the experimental sheep are presented in Table 1.

**Table 1:** The SpO<sub>2</sub> value and the red blood profile of the control and anemic sheep

Group	Day	SpO <sub>2</sub> (%)	RBC (x 10 <sup>6</sup> /μl)	Hb (g/dl)	HCT (%)
Control	0	90.30±6.98 <sup>a</sup>	10.20±1.26 <sup>a</sup>	9.07±0.77 <sup>a</sup>	28.66±2.25 <sup>a</sup>
	3	92.80±5.37 <sup>a</sup>	10.44±1.52 <sup>a</sup>	9.14±1.03 <sup>a</sup>	29.37±2.94 <sup>a</sup>
Anemic	0	91.50±6.42 <sup>a</sup>	9.78±1.98 <sup>a</sup>	8.42±1.07 <sup>a</sup>	26.79±3.27 <sup>a</sup>
	3	81.40±4.09 <sup>b</sup>	8.18±2.14 <sup>b</sup>	6.98±1.28 <sup>b</sup>	21.82±3.93 <sup>b</sup>

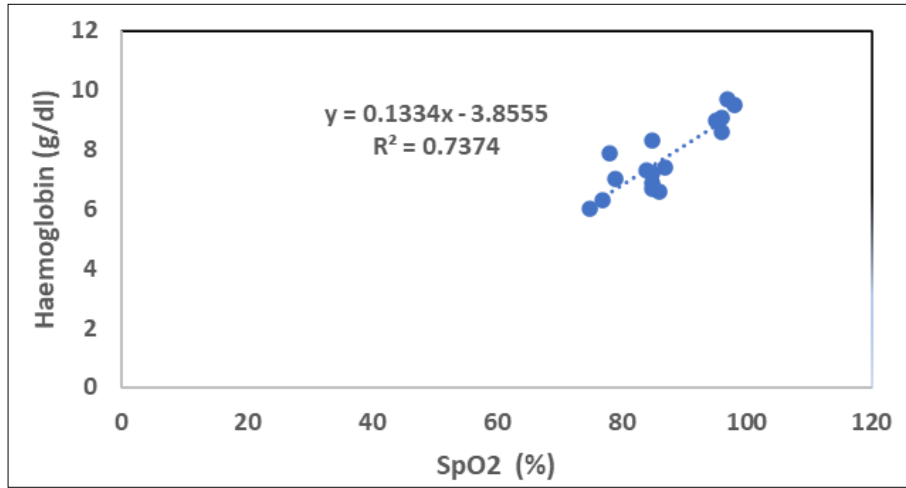
**Note:** Different superscript letters in the same column show significant differences (p<0.05)

On the day 0 or before the phlebotomy, there were no significant differences that showed the conditions of the experimental sheep had the same status. On day 0 in the control and anemic group had SpO<sub>2</sub> values of 90.30±6.98 and 91.50±6.42 respectively. The SpO<sub>2</sub> values were followed by RBC, Hb, and HCT values from the control group respectively 10.20±1.26, 9.07±0.77, and 28.66±2.25. The value did not differ markedly in the treatment group before experiencing anemia, namely 9.78±1.98a, 8.42±1.07a, and 26.79±3.27. The value of these parameters is still included in the normal range of the sheep's blood profile. The normal sheep has erythrocytes, haemoglobin, and hematocrit respectively 10.60±1.01, 9.10±0.70, and 26.00±2.00 (Astuti

and Sudarman, 2015) [4].

On day 3 of sampling, the control group had a stable value for the blood profile. The SpO<sub>2</sub> values were 92.80±5.37 followed by RBC, Hb, and HCT values were 10.44±1.52, 9.14±1.03, and 29.37±2.40, respectively. On the other hand, in the group of sheep that had been made anemic, there was a significant decrease in all blood variables examined. The SpO<sub>2</sub> value of anemic sheep was 81.40±4.09. This value was followed by a decrease in RBC, Hb, and HCT values, there were 8.18±2.14, 6.98±1.28, and 21.82±3.93, respectively.

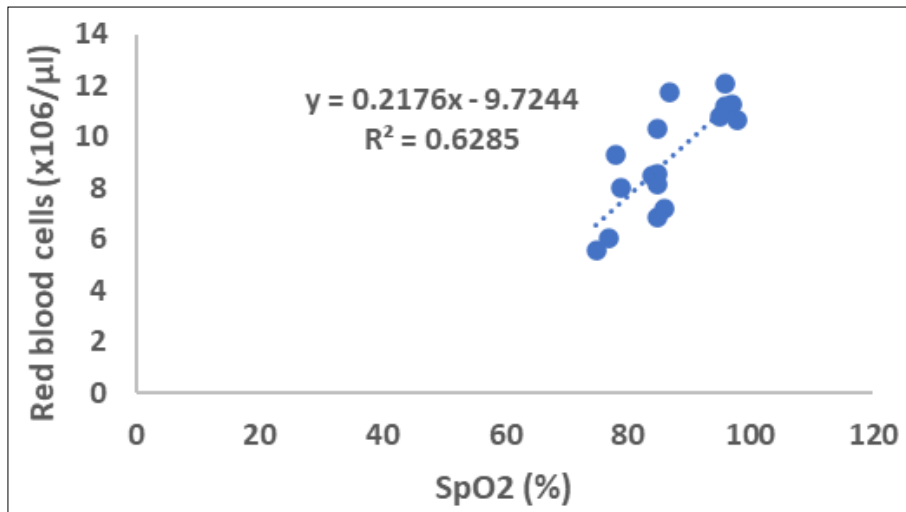
Furthermore, the analysis on the scatter plot of the relationship between SpO<sub>2</sub> and Hb levels is presented in Figure 1.



**Fig 1:** Correlation of SpO<sub>2</sub> values with Hb levels of experimental sheep

The SpO<sub>2</sub> value has a linear correlation with Hb levels in the linear line equation  $y=0.1334x - 3.8555$ . This equation has a coefficient of determination 0.74 which indicates that the SpO<sub>2</sub> value is able to estimate blood haemoglobin levels. A linear relationship was also seen between SpO<sub>2</sub> levels and

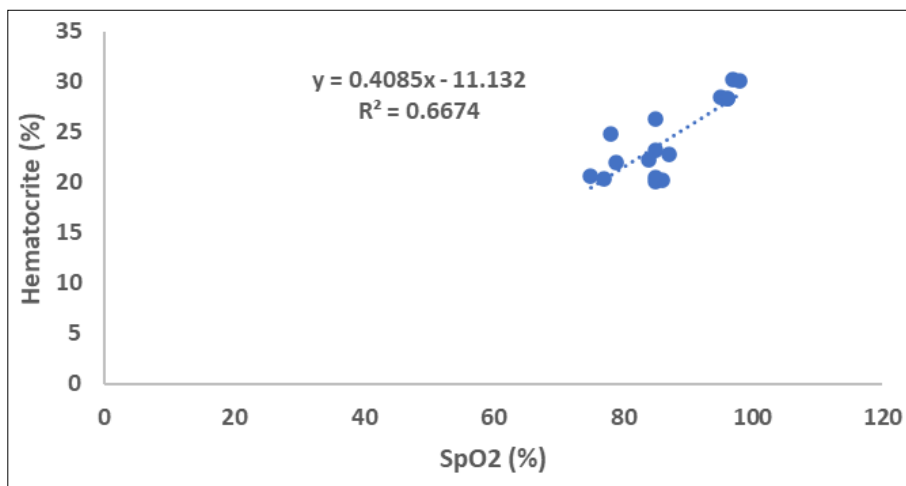
their red blood cell counts. The linear relationship is seen with the equation  $y=0.2176x - 9.7244$  with the value of the coefficient of determination 0.63. In this linear relationship, the SpO<sub>2</sub> value can estimate the number of red blood cells quite well.



**Fig 2:** Relationship of SpO<sub>2</sub> values with red blood cell counts of experimental sheep

Furthermore, the relationship between SpO<sub>2</sub> and HCT value is also seen to have a linear pattern with the equation  $y = 0.4085x - 11.132$  with the value of the coefficient of determination is

0.67. Although not as strong as the relationship with hemoglobin and red blood cell count, the hematocrite value can be sufficiently estimated levels by looking at the SpO<sub>2</sub> value.



**Fig 3:** Show Hematocrite (%)

The results of the examination of SpO<sub>2</sub> level and their blood profile give a clinical appearance in line. The condition of the experimental sheep before the blood draw was seen to have a mucosal color that looked bright red. This condition is a normal condition before the sheep has anemia. The condition

of the normal mucosal color of the conjunctiva and the normal gum mucosa look slightly different. The conjunctival color of the eye normally looks redder than the color of the gums as shown in Figure 4.



**Fig 4:** Conjunctival mucosal color of the eyes and gums before the sheep develops anemia

Furthermore, the color of the mucosa of the eyes and gums of sheep after experiencing a blood draw is presented in Figure 4. The color of the conjunctival mucosa of the eyes of sheep

is noticeably pale pink. The color of the gum mucosa also becomes paler although not too different from the color of the gums before the sheep has anemia.



**Fig 5:** Conjunctival mucosal color of the eyes and gums after the sheep has anemia

#### 4. Discussion

The SpO<sub>2</sub> is a value that indicates the level of oxygen saturation in the blood. The ability to measure SpO<sub>2</sub> values give an advantage because it can be done without invasive action. The SpO<sub>2</sub> value measuring device is pulse oximetry that works through a mechanism to detect the oxygenated haemoglobin use light emitting diodes (LEDs) in specific wavelength. Through this mechanism, the use of an oximeter is enough to be done by attaching a sensor to the surface of the body that has blood vessels close to the surface of the body. The level of oxygen saturation in the blood has a direct relationship with blood conditions including hemoglobin levels, red blood cell counts, and hematocrite values.

The SpO<sub>2</sub> value has the strongest relationship with blood hemoglobin levels so it can theoretically be used to help diagnose anemia. The anemia condition in experimental sheep occurred artificially through blood loss of 108 ml per day. The amount of blood lost is taken into close to the amount of blood loss in conditions of acute haemonchosis. In acute and moderate infections, the number of infecting worms is estimated to be around 400-1500 worm (Alam *et al.*, 2020) [1] and each adult worm is able to suck blood as much as 0.03 ml/worm/day (Naeem and Iqbal 2021) [13]. Haemonchosis significantly lowers red blood cell count, hemoglobin, and hematocrite (Alam *et al.*, 2020) [1]. Therefore, this condition of artificial anemia can give a representative of mucosal color similar to the anemia condition due to haemonchosis.

The pale color of the conjunctival mucosa of the eye is usually detected by examination of the Famacha technique. This technique is carried out by comparing the color of the

conjunctiva with a special measuring chart so that it can be known the degree of paleness that reflects the burden level of *Haemonchus* sp. Infection (van Wyk and Bath, 2002) [21]. This method is practical to use but in some ways must be adapted because the differences condition like conditions of animal husbandry, sheep breeds, and climate in the country.

The amount of blood volume lost through direct blood draw will lower the number of red blood cells. Therefore, hemoglobin levels and hematocrit values also decreased. The number of red blood cells in sheep after blood draw decreased by about 16.36%. The condition directly influences the discoloration of the mucosa. The mucosal color that looks more pale is the color of the conjunctival mucosa of the eyes and the color of the gum mucosa does not change too much. The mucosal color is getting paler because the amount of the red blood cells in the microvascular reduced significantly.

The blood profile parameters in sheep is reported have a wide range and variation. The average of normal red blood cell count is  $7.3 \pm 1.4$  million/cumm (Rahman *et al.*, 2018) [14]. In this study, the number of red blood cells has decreased to about 1.6 million so it can be ascertained that sheep have anemia. This condition is similar in sheep that have haemonchosis or have gastrointestinal parasite infection and show symptoms of anemia that give a blood profile of red blood cell count  $7.3 \pm 1.6 - 8.6 \pm 2.1 \times 10^6/\mu\text{l}$  (Chirkena *et al.*, 2016) [7]. When the experimental sheep has anemia due to the phlebotomy, they show a clinical sign and changing in behavior. Sheep experience a slight weakness and have a slight increase in body temperature. The sheep that had been active before looked more silent after has anemia.

The Hb levels of anemic sheep also decreased significantly. The decrease in hemoglobin levels in anemic sheep was about 17.8%. Normal Hb levels in sheep are about  $10.15 \pm 0.83$  g/dl (Rahman *et al.*, 2018) <sup>[14]</sup>. The function of haemoglobin is to bind oxygen in the blood and carry it to the tissues. Therefore, Hb levels greatly affect the physiological condition of the body. In cases of haemonchosis or gastrointestinal parasite infection, Hb level may decrease to  $6.9 \pm 1.1$  g/dl (Chirkena *et al.*, 2016) <sup>[7]</sup>. At low Hb levels due to haemonchosis, this can cause clinical symptoms including paleness in mucous membranes, especially in conjunctiva with a red-pink appearance to a pale white color (Besier *et al.*, 2016) <sup>[5]</sup>.

The hematocrite value also decreased about 19.2% in anemic sheep. Hematocrite shows the value of the ratio between the volume of red blood cells to a unit of blood volume. The average normal hematocrite value in sheep was  $30.56 \pm 3.66\%$  (Rahman *et al.*, 2018) <sup>[14]</sup> and was reported to be slightly lower in fat tailed sheep at  $33.7 \pm 6.1\%$  (Islam *et al.*, 2018) <sup>[9]</sup>. The hematocrite after the sheep has anemic shows a significant decrease. The results of hematocrit measurements obtained a value of  $21.82 \pm 3.93\%$  which was slightly lower in the condition of sheep who has gastrointestinal parasitic infections reported by Chirkena *et al.* They reported that sheep has gastrointestinal parasitic infection has around  $23.0 \pm 3.7\%$  (Chirkena *et al.*, 2016) <sup>[7]</sup>. The SpO<sub>2</sub> value describes the value of oxygen saturated in the blood and it has the strongest relationship with Hb levels. The ability of the blood to bind oxygen is influenced by the presence of haemoglobin so that these two variables have a strong relationship (ALhyari *et al.*, 2018) <sup>[2]</sup>.

Examination of SpO<sub>2</sub> value for different kinds of animals has the same principle but the practice of laying the sensor can be different. In this study, the sensor was placed on the medial part of the sheep's ears that were not overgrown with hair. The time it takes for the pulse oximetry to be able to measure is 5-10 seconds with the position of the sensor not shifting as long as it is attached to the skin. The SpO<sub>2</sub> examination with the principle of earlobe probes in humans was reported to provide high accuracy values (Seifi *et al.*, 2018) <sup>[17]</sup> so that this study was also carried out in the ear area. The principle of using this oximeter is similar to the conventional pulse oximetry used on the skin. This pulse oximeter monitors oxygen levels in arterial vessels while other types sensor can represent oxygen levels in microvascular (Dixon *et al.*, 2019) <sup>[8]</sup>.

Therefore, when taking measurements, sheep must be handled properly. In dogs and cats, measurements using an oximeter are usually mounted on their lips. In large animals such as cows or horses, oximeter measurements can be used in tongue or in the ear. The results of the linear line equation show that the SpO<sub>2</sub> value can estimate haemoglobin levels with a coefficient of determination of 0.74. Through this estimation, the condition of anemia can be determined without having blood examination in the laboratory.

Furthermore, the application of this estimation is necessary to be connected with the results of the examination of the eggs per gram of faeces (EPG) of *Haemonchus contortus*. This is because the anemia condition is not only caused by haemonchosis, some other causes include piroplasmosis. The incidence of piroplasmosis in sheep is caused by *Babesia* sp. and *Theileria* sp. infections which they are vector borne diseases (Villanueva-saz *et al.*, 2022) <sup>[20]</sup>. With combining the results of SpO<sub>2</sub> measurements to assess the status of anemia conditions and then linked to the results of EPG examination, it can be determined that the anemia occurs is either due to

haemonchosis conditions or by other causes.

## 5. Conclusion

Examination of SpO<sub>2</sub> values can be used to predict the incidence of anemia in sheep. The value of SpO<sub>2</sub> in anemic sheep is around 81% with Hb levels about 6.98 g/dl. The results of the SpO<sub>2</sub> examination need to be linked to the results of the EPG examination to ensure the anemia condition that occurs is caused by haemonchosis.

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