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Original Article

Effect of using protected linseed in rations on sheep blood hematology

Sudiyono Sudiyono, Susi Dwi Widyawati*, Aqni Hanifa, Rendi Fathoni Hadi, Wara Pratitis Sabar Suprayogi, Ari Kusuma Wati, Wari Pawestri

Animal Science Department, Faculty of Agriculture, Sebelas Maret University, Surakarta, 57126, Indonesia
*Correspondence: susidwi@staff.uns.ac.id

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Abstract

Objective: Blood is a body fluid that plays a role in transporting oxygen, nutrients, metabolic waste, and serves as a component of the immune system. Its measurable indices such as hematological profile is affected by the food consumed. This study aimed to examine the linseed use in complete feed on the hematology value of sheep blood.
Methods: This research was conducted at the "Mitra Karya Farm" in Purworejo Village, Gemolong District, Sragen Regency. There were 15 male Fat-Tailed Sheep crossing 1-1.5 years old with an average body weight of 22±2.04 kg as experimental animals. The treatments applied were P1: 100% complete feed, P2: 90% complete feed and 10% linseed, P3: 90% complete feed and 10% linseed meal, P4: 90% complete feed and 10% linseed protected with tannin, and P5: 97% complete feed and 3% linseed oil that are saponified. A one-way, Completely Randomized Design (CRD) was used, and blood hematology was the measured variable, which includes the determination of erythrocytic series (red blood cells, hemoglobin, hematocrit, and erythrocyte index), leukocytes, and platelets.
Results: The results showed that the treatment applied had no significant effect on red blood cells value and erythrocyte index, but had a significant effect on hemoglobin, hematocrit, leukocytes, and platelets value. The highest sheep blood Hb ($P<0.05$) was P3, which did not differ from P4, P1, and P2, while P5 had the lowest Hb level. The highest blood hematocrit was in P1, and this result was not significantly different from P3 and P4, while P2 and P5 produced low hematocrit. The highest leukocytes were achieved by P4 and were not significantly different from P3. Leukocytes were the lowest at P2 and not significantly different from P1. In contrast, P4 sheep platelets were the highest and not significantly different from P3, P1, and P2, while P5 produced the lowest platelets.
Conclusions: This study concludes that the sheep that received complete feed, 90% + 10% linseed meal (P3), had the best hematology.

Keywords: Hematology; Linseed; Protection; Saponification; Sheep

INTRODUCTION

The selection of quality feed ingredients is a top priority in improving livestock products, milk, and meat. Aside from the quality of the feed ingredients, the rations' quality also needs to be considered for the balance and association effects between nutrients. Furthermore, the association effect is complementary to various feed ingredients mixed in the right proportions, resulting in optimal fermentation processes and gas production. Moreover, this interaction causes changes in metabolic pathways in the rumen and the digestive tract.

High digestibility values impact nutrient intake, which will be used for sheep growth [1].

However, the unbalance protein addition with sufficient TDN will not affect increasing diges tibility. Initially, high-fat grains were selected to increase energy density [2]. It was explained that palm oil was the first choice over animal fats and oily seeds, such as kapok seeds, soybeans, and rapeseed. Linseed or flaxseed have high-fat content [3]. Linseed (*Linum usitatissinum*) is an animal feed ingredient that contains biologically active compounds, namely linolenic, linoleic, and lignan fatty acids. These fatty acids have the potential to be transferred from feedstuffs into the rich acid profile of livestock products, such as meat and milk [2]. Benchaar et al. [4] reported that linseed oil can be up to 4% in forage-based rations of dairy cows without adversely affecting rumen function, digestion, and milk production. Concurrently, [5] added that rations containing 5% vegetable oil did not interfere with the consumption, profile, and blood metabolites levels of post-natal adult sheep rations. Linseed oil supplementation in newborn sheep had no significant effect on rations consumption and daily body weight gain. However, it significantly affected the bacterial community compared to those not given linseed oil [6]. Supplementation in a short period also had a rumen microbiome that remained unchanged after the process was stopped.

The physical characteristics of feed ingredients can provide differences in the digestive process regarding palatability, feed flow rate, and degree of degradation by rumen microbes. These factors will affect the consump tion level, the degradation intensity, and the time the feed stays in the rumen. The feed aspect study is significant, considering the highest cost in a business. Currently, animal husbandry has turned into an industrial business, hence, feed formulation aims to improve livestock products quality and quantity. Livestock products in the form of meat are expected to be high in essential fatty acids, especially omega-3 and omega-6, protein, and low in cholesterol.

Besides mixing and matching feed ingredients with physicochemical differences, it is also necessary to consider the goals to be achieved from raising this livestock. The fulfill ment of nutritional needs depends on the optimal performance of the rumen microbial synthesis. Energy synchronization and nitrogen availability in the rumen can potentially increase microbial protein and rumen fermentation efficiency [7]. Protein from rumen microbes can be used to meet the ruminants' protein needs. Furthermore, protein needs will be met by those that escape microbial degradation in the rumen (Rumen Un degradable Protein = RUP). Microbial synthesis is important in ruminants because it can provide 50% of the amino acids needed by the host [7].

The fulfillment of protein needs for ruminants is derived from rumen microbial protein. The rest comes from a protein resistant to rumen microbial degradation, often called a bypass or Rumen Undegraded Protein (RUP). The use of RUP (9.45% of %CP) showed significantly higher nutrient consumption, and digestibility of dry matter (DM) and organic matter (OM) compared to RUP of 7.43 and 8.49% of %CP rations [8].

Tannins often used to protect protein in good quality feed ingredients, making it resistant to degradation by rumen microbes. The use of tannins of 0.75% can increase Undegraded Dietary Protein (UDP) castor seed meal [9]. Meanwhile [10] using a combination of 1% tannins and 0.6% saponins can slow the ammonia release in the rumen and improve the microbes' growth. This study used 1% tannin from linseed dry matter, and saponified linseed oil protects unsaturated fatty acids. Supplementation and protection of kapok seed oil through saponi fication did not affect ruminal fermentation [11]. This research is expected that the use of 3% in the ration does not give an adverse effect in terms of sheep blood hematology.

Linseed, also called flaxseed, is a feed ingredient with a fairly high protein content and good quality [2]. The fat content is high and contains high omega 3 and 6 fatty acids. In addition, it was reported that the use of linseed on PE goat rations, both protected and unprotected, was quite palatable.

The assessment of nutrient balance in the rations can be measured by blood hematology. The provision of complete feeds is expected to meet nutrient needs in quantity and quality. Furthermore, blood hematology is the parameter to determine the nutritional status by measuring red blood cells, hemoglobin, hematocrit, white blood cells, and platelets to detect livestock health. The blood hematocrit aspect can also be used as a benchmark to determine the rations' ability to provide nutrients for livestock in synthesizing blood components. Therefore, this study was designed to examine linseed supplementation, linseed protected with tannins, and oil saponified to form calcium soap.

MATERIALS AND METHODS

Study location

This study was conducted at Mitra Karya Farm, located in Purworejo Village, Gemolong District, Sragen Regency, for 4 months. Meanwhile, the experimental material was analyzed at the Animal Feed and Nutrition Laboratory, Faculty of Agriculture UNS, and Laboratory at the Animal Hospital Prof. Soeparwi, Faculty of Veterinary Medicine UGM, Yogyakarta.

Material

Livestock

About 15 male local sheep were used as study material with an average body weight 22±2,04 kg. The livestock was distributed into 5 treatments and 6 replications based on body weight.

Cage

The cage used was an individual cage equipped with a place to feed and drink.

Ration

The rations consisted of 5 treatments formulated in complete feed and given with a standard of administration based on the need for dry matter (DM), which was 3-6% of body weight. Nutrient requirements for male sheep during the growth period are presented in Table 1.

Table 1. Nutrient requirements for male sheep during the growth period

Nutrient	Total (%)
Crude protein	10.9
TDN	60.00
Ca	0.29
P	0.21

Source: Peraturan Menteri Pertanian Republik Indonesia [12]

Treatment applied

1. P1: Complete Feed + 0% Linseed
2. P2: Complete Feed + 10% Linseed (LS)
3. P3: Complete Feed + 10% Linseed Meal (LSM)
4. P4: Complete Feed + 10% Linseed is Protected by Tannins (LSPT)
5. P5: Complete Feed + 3% Saponified Linseed oil (MLS)

Chemical composition of experimental feed ingredients are presented in Table 2 and composition and nutrient content of treatment rations are presented in Table 3.

Methods

The study was conducted using in vivo-feeding trial technique, and the treatment rations were given at a frequency of 2 times per day. The adaptation period was carried out for 2 weeks and was stopped when the sheep feed consumption was constant. Meanwhile, blood collection was performed at the end of the study and was taken before the feed distribution in the morning. Venoject was injected into the neck, at the jugular vein, then the blood is transferred to a vacutainer with a red and blue cap. The vacutainer was put into a cooling box for sending blood samples to the Animal Hospital Prof. Soeparwi, Faculty of Veterinary Medicine, UGM. The measured variable was blood hematology which includes the determination of red blood cells, hemoglobin (Hb), hematocrit, erythrocytes index, leukocytes, and platelets.

Data analysis

This study used a one-way, Completely Randomized Design (CRD). Duncan's multiple range test was also carried out to determine differences between treatments.

RESULTS

The results showed that the Hb of sheep blood was affected significantly by linseed with various physical characteristics. The highest Hb of sheep blood ($P<0.05$) was in the blood of sheep that received treatment P3, which was 12.23 g/dL and did not differ from P4 (12.00), P1 (12.00), and P2 (11.10), while P5 (10.50) has the lowest level. The hematocrit was affected by linseed in various physical characteristics in sheep rations. The highest hematocrit was produced in treatment P1, which was not significantly different from P3 and P4, while P2 and P5 produced the lowest value but still in normal condition. The results showed that the treatment applied had no significant effect on red blood cells and erythrocytes index.

Table 2. Chemical composition of experimental feed ingredients

Feed Ingredient	DM (%)	CP (%)	CF (%)	EE (%)	Ash (%)	BETN (%)
Complete feed	87.56	10.88	13.51	3.14	10.03	61.94
Linseed	93.33	21.08	10.98	17.98	3.38	46.67

White Blood Cell (WBC) was affected by linseed in various physical characteristics in sheep rations. Linseed supplementation was in the form of seeds (P2), flour (P3), linseed protected with tannins (P4), and saponified linseed oil (P5). Furthermore, treatment P4 produced the highest WBC and was not signi ficantly different from P3. WBC was lowest in treatment P2 and not significantly different from P1 and P5.

The results showed that platelets were affected by linseed in various physical charac-teristics of sheep rations. Platelets produced by P4 sheep were the highest and not significantly different from P3, P1, and P2. The treatment P5 produced the lowest value and the count ranged from 178.50-421.67 thousand/µL. Hematology of experimental sheep blood are presented in Table 4.

DISCUSSION

The blood hematology observation is intended to determine the health problems in the livestock body. Under the function of each blood component such as Red Blood Cell (RBC), hemoglobin (Hb), hematocrit, erythrocytes index, White Blood Cell (WBC), and platelets, health problems can be caused by infection or inflammation. However, this study will examine nutrient balance from complete feed supplemented with linseed of various characteristics. The associative effect between nutrients in food ingredients will be interrelated in meeting the nutritional needs of growing sheep, and this was studied in terms of its hematological value.

The Hb level of sheep blood was between 10.50-12.23 g/dL. Meanwhile, normal Hb levels are 9.0-15.0 g/dL or 11-13 g/100 ml [13]. These results were higher than those obtained in previous studies, where the Hb levels of sheep-fed rations containing bean sprout waste ranged from 8.47±0.29 - 9.77±0.17 g/dL with morning and evening administration [13]. It was explained that the normal Hb level in Garut sheep's blood ranges from 8-16 g/dL. In young sheep, it is 9.2 g/dL, higher than in adults [14]. This study did not decrease protein consumption since blood Hb counts are unaffected. Blood Hb decreased with the use of saponified linseed oil, so it is possible that consumption decreased followed by a decrease in protein consumption. Data protein consumption in treatments P1, P2, P3, P4, P5 were 145.47 grams/head/day; 113.47 grams/head/day; 114.80 grams/head/day; 129,47 grams/head/day, and 123.62 grams/head/day (Widyowati, unpublished).

Hb is a protein in RBC that functions to carry oxygen throughout the body. This situation can be explained by the difference in

Table 3. Composition and Nutrient Content of Treatment Rations

Feed Ingredient	P1	P2	P3	P4	P5
	%				
Complete feed	90	90	90	90	97
LS	0	10	0	0	0
LSM	0	0	10	0	0
LSPT	0	0	0	10	0
MLS	0	0	0	0	3
Nutrient Content					
Crude protein	12.8	13.76	13.76	13.49	13.49
Extract ether	2.38	2.85	2.85	2.80	2.80
Crude fiber	17.63	17.31	17.31	16.97	16.97
Ash	7.62	8.82	8.82	8.65	8.65
BETN	57.99	57.13	57.13	56.03	56.03
TDN	68.99	70.15	70.15	68.80	68.80

Table 4. Hematology of experimental sheep blood

Variable	P1	P2	P3	P4	P5
Hb, g/dL	12.00[ab]	11.10[ab]	12.23[a]	12.00[ab]	10.50[b]
Hematocrit, %	39.70[a]	34.50[bc]	38.60[ab]	37.13[ab]	32.55[c]
RBC, millions/μL	12.61[a]	11.15[a]	13.03[a]	13.22[a]	10.72[a]
MCV (fL)	31.43[a]	31.77[a]	30.00[a]	28.23[a]	31.35[a]
MCH (pg)	9.53[a]	10.13[a]	9.47[a]	9.07[a]	10.00[a]
MCHC (%)	30.43[a]	32.03[a]	31.73[a]	32.30[a]	32.05[a]
RDW (%)	18.37[a]	17.70[a]	17.53[a]	17.27[a]	16.00[a]
WBC, thousands/μL	8.43[bc]	8.13[c]	10.87[ab]	11.53[a]	8.75[b]
Platelets, thousands, μL	303.00[ab]	292.33[ab]	375.33[a]	421.67[a]	178.50[b]

Hb levels from the sheep fed complete feed and supplemented with linseed and different protection technologies, which resulted in differences in the ability of the rations to provide protein for blood Hb protein synthesis. Treatments P1, P2, P3, and P4 showed no significantly different results in producing Hb blood protein. At the same time, P5 uses saponified linseed oil and is not a protein supply. The unsaturated fatty acids supply in linseed oil is saponified, which makes the Hb results significantly (P<0.05) lower than the other treatments.

This situation is reinforced by [15] that supplementation of saponified flaxseed oil at a level of 6% tends to increase VFA production and the propionic acid proportion in the rumen, with the potential as an energy source for beef cattle livestock. The situation indicated that the protected fat addition did not disturb rumen microbial activity in feed fermentation. Benchaar *et al.* [4] stated that linseed oil can be added up to 4% in forage-based rations in dairy cows to enrich the content of n-3 fatty acids without disrupting rumen function, digestion, and milk production.

The dominant Hb is composed of protein compounds derived from feed intake protein and synthesized in the sheep's body [16]. Complete feed protein containing linseed with various characteristics has the same effect on sheep blood Hb levels. It was further explained that Hb could be synthesized from the body's protein reserves when it lacks protein intake. The condition of low sheep rations protein intake results in the degradation of the body's protein reserves for Hb formation, as a result, the animal appears to be emaciated.

The results showed that the treatment applied had no significant effect on the erythrocyte index. The erythrocyte index consists of the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW). Mean corpuscular volume shows the average volume of red blood cells or erythrocytes, and its mean value in fat-tailed sheep given a variety of linseed and complete feed was 30.56 fL. This was still within the normal range of 28-40 fL, in accordance with Weiss and Wardrop [17]. Finally, it indicates that the size of the red blood cells in the circulatory system is normal.

The increased value of MCV is caused by the stress experienced by the sheep during the sampling process [18]. These conditions can decrease after the stress-causing factors disappear [18]. Macrocytic anemia is a condition where the MCV value in livestock increases, indicating that the circulating erythrocytes are large. Furthermore, this is accompanied by abnormal cell nucleus maturation Rapaport [19].

Macrocytic anemia is caused by a deficiency of nutrients such as vitamin B12 and folic acid, as well as chronic diseases of the digestive tract, especially the intestine. These conditions can also be discovered in animals with increased bone marrow activity which can occur due to bleeding, hemolysis, or lack of hematopoietic factors such as B12 and folic acid deficiencies [18]. The decrease in the MCV value is due to the low digestibility of the nutrients provided in the feed [18]. These are discovered in animals with iron and copper deficiency conditions. Similarly, iron deficiency can be caused by chronic infections due to worm infestation in the body or interference with the absorption of this element [20].

Mean corpuscular hemoglobin is the hemoglobin content in each red cell, and the average value in fat-tailed sheep given varying amounts of linseed and complete feed was 9.64 pg. This was within the normal range of 8-12 pg [17]. High MCH values can be caused by animals experiencing stress [18]. This increases and decreases occur when the red blood cells are macrocytosis (increases) and microcytosis and hypochromic (decreases), respectively. Macrocytosis is the production of red blood cells that are larger than normal. Meanwhile, macrocytosis conditions can be caused due to liver disease as well as deficiency of iron and cyanocobalamin [17]. MCH values decrease under conditions of low nutrition due to reduced feed intake [18].

Mean corpuscular hemoglobin concentration (MCHC) is the average concentration of hemoglobin in total erythrocytes, and its average value in fat-tailed sheep given various amounts of linseed and complete feed was 31.71%. This was still within the normal range of 31-34% [17]. The increased MCHC value is caused by the condition of the animal experiencing stress, resulting to a rise in hemoglobin. The higher value of MCHC indicates hemolysis [21]. MCHC values higher than normal are called hyperchromic which can be caused by hemolysis or rupture of red blood cells and hemoglobin in the plasma, leading to hemoglobinemia. This is the detection of free hemoglobin (Hb) in blood plasma, causing the counting of plasma Hb when its concentration is measured. Furthermore, other causative factors are nutritional intake, environmental conditions, and the presence of disease.

Mean corpuscular hemoglobin concentration values decrease in conditions of low nutritional intake. Cattle under stress has low consumption of feed [18]. The high or low levels of MCHC and MCH values in livestock are not the same. Furthermore, differences in these parameters is caused by the amount of iron in the body.

Red blood cell distribution (RDW) width shows the difference in size and area of erythrocytes, and its measurement helps to classify anemia, as it estimates the occurrence of the disease early before the appearance of symptoms [22]. The average value in fat-tailed sheep given varying amounts of linseed and complete feed variety was 17.72%, and it is within the normal range of 16-23 fL [23].

An increased RDW value indicates anemia which can be caused by iron and folic acid deficiencies, as well as cyanocobalamin [24]. The increase is caused by disease in the liver, kidneys, and the presence of inflammation. Furthermore, it indicates anisocytosis [23]. The higher the RDW value, the greater the variation in cell size (anisocytosis). High value of RDW is associated with higher risk of mortality [25].

Variations in the erythrocyte index value can be influenced by the physiological conditions of each animal which is affected by environmental temperature, maintenance management, feed nutrition, and body fluid balance [26]. Nutrients in feed such as iron, copper, protein (amino acids), folic acid, and cyanocobalamin affect the number as well as the shape of erythrocytes. Furthermore, a lack of vitamins causes disturbances in the growth and development of erythrocytes. Changing climate, air pollution, natural pollution, and extreme weather are factors that cause stress and weakness in animals, thereby affecting their blood physiology [18].

Environmental temperature also affects the hematological profile of livestock, and when raised in the highlands, it increases the blood flow rate and hemoglobin levels. This is related to the hemoglobin functions of transporting oxygen into the cells of the body, nutrients throughout the body, and maintaining fluid balance (homeostasis) [27]. Open conditions throughout the day affect hemoglobin levels. This condition allows the animals to get enough oxygen and increases the hemoglobin levels. Therefore, hemoglobin levels increase affect MCHC and MCH. Weiss and Wardrop [17] stated that it is directly proportional to oxygen levels and the number of erythrocytes.

Blood hematocrit is the RBSs percentage in the blood volume, and it is also known as Packed Cell Volume (PCV). The normal hematocrit is 27-45%, hence, the hematocrit of sheep blood given complete feed supplemented with linseed does not interfere with the RBC synthesis in sheep blood. Apart from protein, minerals are also needed for the synthesis process, especially Fe, because this mineral is the core of blood Hb in red blood cells. The use of lemuru fish and canola oil in milk replacers as the fat source had no significant effect on Hb, hematocrit, erythrocyte, and leukocyte levels in sheep [16]. Saponified linseed oil gives the same results as lemuru fish and canola oil.

The treatment applied had no significant effect to RBC. This situation can be interpreted that the protein content in quantity and quality of the five treatments applied can be used to form sheep RBC. Linseed supplementation in the form of seeds (P2), flour (P3), linseed protected with tannins (P4), and saponified linseed oil (P5) was able to supply nitrogen compounds for growth and development of rumen microbes and *bypass protein* in providing amino acids in the small intestine. Treatment P4 produced the highest WBC and was not significantly different from P3. WBC was lowest in treatment P2 and not significantly different from P1.

WBC is an active unit of the body's defense system that protects against infectious agents. An increase or decrease in the WBC level in blood circulation could indicate inflammatory disease agents and allergic reactions. Therefore, it is necessary to know the WBC normal description of each individual. However, white blood cells (WBC) were affected by the use of linseed in the ration. Stuglin and Prasad [28] stated that blood pressure, Hb, RBC, WBC, unaltered after flaxseed diet.

Platelets are blood cells that help the clotting process. Changes in the number of platelets can be influenced by various things, when the number of platelets increases, the condition is known as thrombocytosis, while a decreased number of platelets is also called thrombocytepenia. Platelets produced by P4 sheep were the highest and not significantly different from P3, P1, and P2 (Table 4). The P5 treatment produced the lowest platelets, and the count ranged from 178.50-421.67 thousand/µL. Normal platelet values in sheep are in the range of 250-750 thousand/µL [29].

Platelet values in this study were within the normal range for sheep P1, P2, P3, and P4. Meanwhile, P5 group treatment had platelet values below the normal range (178.50 thousand/µL). Group P5 experienced thrombocytopenia. There was a decrease in the number of platelets affected by the dose given and the administration time was too short so that the effectiveness of saponified linseed oil did not affect much in the circulatory system. These results are in accordance with research conducted by Pradnyani et al. [30] which states that protein plays a role in the blood clotting process related to platelets. Thrombocytopenia occurs due to idiopathic thrombocytopenic purpura, blood clotting disorders throughout the body due to widespread inflammatory infections, or due to inappropriate timing and dosage.

CONCLUSION

In conclusion, the blood hematology of sheep receiving all rations treatments was normal. The sheep receiving P3 treatment were given 90% complete feed supplemented with 10% linseed meal and had the best hematology. It can be concluded that the use of linseed in complete rations does not affect sheep physiology.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest in this research.

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