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Effect of UMMB (urea molasses multi-nutrient block) based on coffee skin on erythrocytes, hematocrit and hemoglobin in transport stress of sheep

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ABSTRACT
Transportation stress in livestock can be caused by distance and travel time, crowding in the transport vehicle, malnutrition during transportation, climatic conditions, lack of availability of food and drink and lack of handling during travel. The aim of providing coffee peel-based UMMB is to overcome nutritional deficiencies and stress in livestock during transportation. This research was carried out experimentally with a non-factorial completely randomized design (CRD) with 4 treatments K0 (without UMMB) as control, K1 (UMMB based on 10% fermented coffee peel), K2 (UMMB based on 20% coffee peel), K3 (UMMB based on fermented coffee peel 30%) and 4 repetitions. The research parameters are erythrocytes, hematocrit and hemoglobin. The results showed that administration of UMMB based on fermented coffee peel had a significant effect ($P<0.05$) on reducing erythrocyte levels (16.58%), decreasing hematocrit levels (18.30%) and decreasing hemoglobin levels (31.72%). The conclusion of this research is that administering coffee peel-based UMMB at a dose of 30% can reduce erythrocyte, hematocrit and hemoglobin levels in sheep transportation stress.
Keywords: Coffee Peel, Erythrocytes, Hematocrit, Haemoglobin, Stress

1. Introduction

Transporting livestock from one place to another, either over short distances or long distances, is very common for various purposes. Transporting livestock over short distances is carried out using land routes and if transportation is carried out over long distances it can involve not only one route but can be via a combination of land, sea and air routes. [1]. Apart from making it easier to mobilize livestock, transportation also has a negative impact on livestock, namely it can cause discomfort felt by livestock during transportation in the form of transportation stress. Stress is a side effect of the environment or management system that forces physiological changes and can result in various things such as reduced body weight and increased levels of cortisol, hematocrit and keratin in the blood [2].

To reduce stress in livestock, it can be done by providing antioxidants. One antioxidant that can be used is coffee peel . Coffee fruit peel contains antioxidant compounds that have the potential to be used as natural antioxidants. Providing antioxidants is an effort to overcome oxidative stress and free radicals during transportation [3]. Apart from containing antioxidants, coffee peel also contains anti-nutritional substances in the form of 2.30% caffeine and 7.8% tannin [4].

Anti-nutritional substances contained in coffee peels must be minimized or eliminated so as not to interfere with the digestion of sheep. One method that can be implemented to eliminate or reduce the antinutrient content in coffee peel is by fermentation. Fermentation is the process of breaking down organic materials through the activities of microorganisms, either aerobic or anaerobic, which can convert complex compounds into compounds that are simpler and easier to digest. Apart from being able to produce biomass, fermentation products can increase or decrease certain substance components, according to their degrading ability. Apart from that, fermentation can provide taste, aroma, and can increase the digestibility value and vitamin and mineral content of feed ingredients [5].

More complex coffee peel preparations can be given in the form of UMMB (urea molasses multi-nutrient block). UMMB (urea molasses multi-nutrient block) is a feed supplement with various kinds of nutrients that have been condensed. UMMB is in solid form and is usually given to ruminants consisting of various food substances such as urea as a provider of NPN, molasses as an energy source, salt, ultra minerals, lime as a supplement and filler and absorbent for molasses such as bran, concentrate, and agricultural waste [6] .

2. Materials and Methods

2.1. Material

16 sheep weighing ± 15 kg obtained from Dompet Dhuafa Farm. fermented coffee peel flour, molasses based on coffee peel, molasses, fine bran, soybean meal, cement, salt, lime, ultra minerals, water and urea as well as multivitamins. Other ingredients used are alcohol, HCl, distilled water. Meanwhile, the tools used were squid, sterile tubes, cool boxes and 1.5 ml Efendorf tubes used for taking blood samples, analytical scales, press tools, containers, rubber bands, polyethylene plastic and paralon pipe measuring 8.5 cm with a height of 5 cm then camera and stationery for documentation and recording research results.

2.2. Research methods

2.2.1. Making Coffee Mole Peel Solution

1. Prepare a fermenter container with a capacity of 2000 ml and 400 grams of coffee peel.
2. Next, add 60 grams of rice bran, 30 grams of brown sugar and 30 grams of molasses.
3. Then add 740 grams of water from rice washing and 740 grams of water from coconuts.
4. Then homogenized and covered using polyethylene plastic and tied with a rubber band.
5. Then fermented for 7 days.
   After these steps, the coffee peel-based local microorganism solution is ready for use.

2.2.2. Making Coffee Peel Fermentation Using MOL

1. Prepare 5 kg plastic is provided as a fermentation medium, chopped coffee fruit peel, MOL and urea solution.
2. Next, 3 kg of coffee peel were weighed for each treatment, then filled with 6% MOL solution, 180 grams of fine bran, 180 grams of molasses and 90 grams of urea and stirred evenly.
3. Then put it in plastic, tie it tightly with a rubber band, and ferment it for 14 days.

2.2.3. Making UMMB Based on Fermented Coffee Peel

1. The ingredients that make up UMMB include fermented coffee peel with MOL, molasses, fine bran, soybean meal, urea, salt, lime, ultra minerals, white cement and multivitamins.
2. First, flour the fermented coffee peel using a grinder, then weigh all the ingredients, namely fermented coffee peel flour, molasses, fine bran, soybean meal, cement, salt, lime, ultra minerals and urea as well as multivitamins according to your needs using an analytical balance.
3. When all the ingredients have been weighed, then mix all the ingredients according to the treatment and after the ingredients are homogeneous, they can be weighed with a mixture weight of 240 grams/block.
4. Then print the block with a mold made from paralon pipe and press it with a block press tool coated with plastic so that it is easy to take the UMMB from the printer.
5. Then the printed dough is placed on a tray and oven at a temperature of 45°-50°C.

2.2.4. Providing UMMB to Transported Sheep

UMMB was given 4 days before transportation in the Dompet Dhuafa farm pen which had been marked for each repetition and treatment. On the first and third days each sheep was given 1 piece of UMMB, on the second and fourth days the remaining UMMB was checked. The fifth day of giving UMMB during transportation. Giving UMMB 240 grams/block.

2.2.5. Blood Sampling

1. Blood samples are taken through the jugular vein. Before taking blood samples, the area around the blood vessels is cleaned using cotton wool soaked in alcohol to determine the blood vessels more clearly.
2. Then, insert the syringe into the jugular vein and withdraw the syringe slowly. Next, take a blood sample of 3 ml from each sheep.

3. Then put the blood into an ethyl diamine tetra acetic acid (EDTA) tube that has been marked with treatment and repeat.
4. Blood samples that have been taken can be taken directly to the Veterinary Laboratory to check hemoglobin, hematocrit and erythrocyte levels. Blood sampling was carried out before and after transportation.

2.3. Research design

The research was carried out experimentally using a completely randomized design (CRD) with 4 treatments and 4 replications, resulting in 16 research units as follows:

K0 = Not given UMMB
K1 = UMMB based on fermented coffee peels 10%
K2 = UMMB based on fermented coffee peels 20%
K3 = UMMB based on fermented coffee peels 30%

3. Results and Discussion

3.1. Erythrocytes

Table 1. Sheep erythrocyte levels before and after transportation

Treatmen	Average erythrocytes (million/mm^3)		Percentage increase/decrease (%)
	Before transportation	After Transportation	
K0	$2{,}56 \pm 1{,}65^{ab}$	$2{,}39 \pm 0{,}51^{a}$	6,64 ↓
K1	$3{,}33 \pm 0{,}07^{b}$	$2{,}82 \pm 0{,}59^{ab}$	15,31 ↓
K2	$1{,}19 \pm 1{,}00^{a}$	$2{,}14 \pm 0{,}39^{a}$	79,83 ↑
K3	$3{,}98 \pm 0{,}89^{b}$	$3{,}32 \pm 0{,}06^{b}$	16,58 ↓

Note: Different superscripts in the same column indicate significant differences (P<0.05)

In Table 1, it can be seen that the average value of erythrocyte levels in sheep before 8 hours of transportation given UMMB with fermented coffee peel UMMB treatment was in the range of 1.19-3.98 million/mm^3. After 8 hours of transportation, sheep erythrocyte levels ranged from 2.14 to 3.32 million/mm^3. Giving coffee peel UMMB up to 30% can reduce erythrocyte levels by up to 16.58%.

Transportation for 8 hours in a pickup truck resulted in erythrocyte levels of 8.14 ± 1.14 million/mm^3 before transportation and 8.11 ± 0.78 million/mm^3 after transportation. The mean erythrocytes of these sheep are all lower than the mean erythrocytes of Girgentana goats [7]. Transportation for 8 hours using a closed truck resulted in erythrocyte levels of 6.45 million/mm^3 before transportation and 8.47 million/mm^3 after transportation [8]. Furthermore, administration of multivitamins and meniran in an open car (Suzuki carry) during 8 hours of transportation resulted in erythrocyte levels of 10.08 ± 1.47 million/mm^3 before transportation and 11.40 ± 0.86 million/mm^3 during transportation [9].

In livestock with the K2 label there was an increase in erythrocytes of 79.83%. This happens because livestock labeled K2 consume the least amount of UMMB while in the pen and traveling. The increase in erythrocytes may be due to sheep experiencing stress, the increase in the number of erythrocytes in order to increase the function of obtaining more oxygen.

Sheep that experience transportation stress will experience an increase in erythrocytes, whereas livestock that do not experience stress will experience a decrease in erythrocytes. Livestock that are exposed to high environmental temperatures will experience stress which increases their efforts to release body heat. This results in increased respiration, body temperature and water consumption and reduces feed consumption. Heat is produced in the body through metabolism which is a chemical reaction in all body cells [10]. Feed is the main source of fuel for metabolism. The body uses energy from feed to operate its various organs, produce heat to maintain a constant body temperature, perform external work, and produce a supply of stored energy (in the form of fat) for needs if needed later [11]. The results of analysis of variance showed that the use of fermented coffee peel UMMB at various doses had a significant effect after transportation (P<0.05) on sheep blood erythrocyte levels. The relationship between treatments can be determined by Duncan's advanced test which shows that the K3 treatment (UMMB containing 30% fermented coffee peels) produces the highest average reduction and is significantly different from K0 (without UMMB).

This happens because the peel of the coffee fruit contains flavonoid and polyphenol antioxidant compounds which have the potential to be used as natural antioxidants against oxidative stress. Coffee peel contains high levels of antioxidant polyphenolic compounds originating from phenolic acids such as caffeine, chlorogenic acid, coumarin, ferulic and sinapic acid [12].

3.2. Hematocrit

Table 2. Hematocrit levels of sheep before and after transportation

Treatmen	Average erythrocytes (million/mm^3)		Percentage increase/decrease (%)
	Before transportation	After transportation	
K0	$11{,}77 \pm 7{,}71^{ab}$	$10{,}95 \pm 2{,}45^{ab}$	6,96 ↓
K1	$15{,}77 \pm 0{,}54^{b}$	$13{,}32 \pm 2{,}70^{bc}$	15,33 ↓
K2	$5{,}42 \pm 4{,}56^{a}$	$9{,}8 \pm 1{,}99^{a}$	80,81 ↑
K3	$18{,}67 \pm 4{,}48^{b}$	$15{,}27 \pm 0{,}46^{c}$	18,21 ↓

Note: Different superscripts in the same column indicate significant differences (P<0.05)

In Table 2, it is known that the average value of hematocrit levels for sheep before transportation that were given UMMB during 8 hours of transportation with UMMB treatment of fermented coffee peels was in the range of 5.42-18.67%. After 8 hours of transportation, sheep erythrocyte levels ranged from 9.8 to 15.27%. Giving coffee peel UMMB up to 30% can reduce hematocrit levels by up to 18.21%. Transportation for 8 hours in a pickup truck resulted in hematocrit levels of 25.70 ± 3.59% before transportation and 24.10 ± 2.60% after transportation. Furthermore, transportation for 8 hours using a closed truck resulted in hematocrit levels of 26.94% before transportation and 28.87% after transportation [13].

In K2, there was an increase in hematocrit to 80.81% because livestock with the K2 label consumed the least amount of UMMB while in the pen and during transportation. The increase in hematocrit values was triggered because sheep were unable to maintain body homeostasis due to dehydration and transportation stress. The increase in hematocrit may be due to sheep experiencing stress, the increase in the number of erythrocytes in order to increase their function to obtain more oxygen.

Sheep that experience transportation stress will experience an increase in hematocrit, whereas livestock that do not experience stress will experience a decrease in hematocrit. An increase in hematocrit values can occur in animals experiencing stress, asphyxia or dehydration. This situation causes the spleen erythrocyte concentration to be high and abnormal. When animals feel afraid, epinephrine increases spleen contractions, which increases the number of red blood cells in the blood circulation and increases the hematocrit value. In conditions of dehydration, fluid loss occurs from all body compartments, resulting in a decrease in plasma fluid volume, resulting in an increased hematocrit value known as hemoconcentration [14].

The results of the analysis of variance showed that the use of fermented coffee peel UMMB at various doses had a significant effect after transportation (P<0.05) on sheep blood hematocrit levels. The relationship between treatments can be determined by Duncan's further test which shows that the K3 treatment (UMMB containing 30% fermented coffee peels) produces the highest average reduction and is significantly different from K0 (without UMMB).

3.3. Haemoglobin

Table 3. Haemoglobin levels before and after transportation

Treatmen	Average Hemoglobin (g/dL)		Percentage increase/decrease (%)
	Before transportation	After transportation	
K0	$7{,}52 \pm 4{,}82^{a}$	$10{,}87 \pm 1{,}78^{a}$	44,54 ↑
K1	$10{,}05 \pm 1{,}57^{ab}$	$8{,}6 \pm 1{,}31^{ab}$	14,42 ↓
K2	$5{,}9 \pm 0{,}76^{a}$	$11{,}3 \pm 0{,}95^{a}$	91,52 ↑
K3	$13{,}65 \pm 0{,}38^{b}$	$9{,}32 \pm 1{,}56^{b}$	31,72 ↓

Note: Different superscripts on the same line indicate significant differences (P<0.05)

In Table 3, it can be seen that the average value of hemoglobin levels of sheep before transportation that were given UMMB during 8 hours of transportation with the treatment of fermented coffee peel UMMB was around 5.9-13.65 g/dL. After 8 hours of transportation, the sheep's hemoglobin levels were around 8. 6-11.3 g/dL. Giving coffee peel UMMB up to 30% can reduce hemoglobin levels by up to 31.72%.

The average hemoglobin of the research sheep both before and after transportation was higher than the average hemoglobin of the sheep at a level of 6.60-6.70 g/dL [15].

In K2 there was an increase in hemoglobin up to 91.52% because sheep with the K2 label consumed the least amount of UMMB in the pen and during transportation. The increase in erythrocytes may be due to sheep experiencing stress, the increase in the number of erythrocytes in order to increase the function of obtaining more oxygen.

Sheep that experience transportation stress will experience an increase in hemoglobin, whereas livestock that do not experience stress will experience a decrease in hemoglobin. Livestock that experience long distance transportation and long travel times can experience transportation stress which causes the number of erythrocytes and hemoglobin levels to decrease due to the large amount of body fluids excreted through sweating, panting (panting) and urination, causing abnormal shape changes in erythrocytes and causing release of bound hemoglobin. The number of erythrocytes and hemoglobin levels can increase at low environmental temperatures and can decrease at high environmental temperatures. An increase in hemoglobin is triggered by the body's need for oxygen due to work or stress which causes an increase in hemoglobin concentration in the plasma. Hemoglobin levels can increase if you live at high altitudes above sea level. However, the increase in hemoglobin levels is triggered by the duration of anoxia and different individual responses. Heavy physical work can trigger an increase in hemoglobin levels. Changes in body posture can result in temporary changes in hemoglobin levels. When standing, hemoglobin levels are higher than when lying down [16]. The results of the analysis of variance showed that the use of fermented coffee husk UMMB at various doses did not have a significant effect after transportation ($P>0.05$) on sheep blood hemoglobin levels. Although the use of UMMB based on coffee skins did not have a significant effect, the percentage reduction in K3 (giving UMMB based on fermented coffee skins 30%) was higher compared to K0 (without giving UMMB).

4. Conclusion

Providing urea molasses multi-nutrient block (UMMB) is able to overcome transportation stress in sheep and the best administration is in the K3 treatment with a dose of 30% fermented coffee peel.

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