Jurnal Sain Peternakan Indonesia
Available at https://ejournal.unib.ac.id/index.php/jspi/index
DOI: https://doi.org/10.31186/jspi.id.16.3.245-250
P-ISSN 1978-3000
E-ISSN 2528-7109
Volume 16 Issue 3 July-September 2021

Analysis of Flavonoids and Saponins Contents in the Sheep Ration Added with Multinutrient Block with Different Level of Papaya Leaves Flour

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ABSTRACT

This study aimed to examine the composition of flavonoids and saponins in sheep rations added with multi-nutrient blocks with different levels of papaya leaves (0%, 2%, 4%, and 6%). The research design used was a completely randomized design with four treatments and three replications. This study used MNB with the addition of different papaya leaf flour (T0: sheep ration plus MNB without papaya leaf flour, T1: lamb ration plus MNB using 2% papaya leaf flour, T2: lamb ration plus MNB using 4% papaya leaf flour, and T3: sheep ration plus MNB using 6% papaya leaf flour). The parameters were flavonoids and saponins. The materials used were sheep ration (forage and concentrate), multi-nutrient blocks, and papaya leaves flour. The study results were that sheep ration added MNB with different levels of papaya leaves flour did not show an increase in flavonoids and saponins. The highest flavonoids content was found at sheep ration added MNB using 4% papaya leaves flour, while the highest saponin content was found at sheep ration added MNB using 6% papaya leaves flour.

Keywords: Sheep rations, Multinutrient block, Flavonoids, and Saponins.

INTRODUCTION

Nutrient requirements of sheep should be fulfilled by following the growth or the level of productivity with the feed material presented is compiled with either becomes rations that are ready to be consumed by livestock. A ration is a mixture of several ingredients balanced feed to be given during the 24 hours used to meet the needs of the sheep to survive and produce. A common problem often faced by farmers is traditional, namely the low-quality feed with high content of crude fiber high. One way to increase the use of low-quality feed ingredients by ruminants, especially sheep, is in the form of a multi-nutrient block formula.

Multinutrien block is a supplement that contains energy, urea, some minerals, and vitamins that can be given to ruminants such as cattle, buffalo, goats, and sheep (Garcia and Restropo, 1995). Multinutrien block is given to livestock to feed the low basal quality (Mohammed et al., 2007). Components multi-nutrient block has its function such as urea as a nitrogen source for the development of the rumen microbes, molasses as a source of carbohydrates that are easily fermented in the rumen, and minerals necessary for structural components antibacterial meet the shortage in the feed material (Turangan et al., 2018). Making a multi-nutrient block mixes all of the ingredients in the feed used into a container until homogeneous and compacted and formed into a block (Mohammed et al., 2007).

Plant papaya (*Carica papaya*) is a plant trunked proper height that reaches 8 – 10 m which uses the leaves to increase appetite, prevent disease and drug worms in livestock animals (Suteky et al., 2020). The use of papaya leaves needs to be processed first as dried and used as flour to lower the influence of antinutrients on livestock (Retnani et al., 2015). Papaya leaves contain several chemical compounds, including papain enzymes, saponins, flavonoids, and tannins (A'yun and Laily 2015).

Flavonoids are one of the compounds which consist of 15 carbon atoms in the plant, which is used as antibacterial and often found in plants (Suteja et al., 2016). Flavonoids in ruminants can manipulate the fermentation of the rumen as well as its products; flavonoids can reduce the number of protozoa in the rumen but can improve the digestibility of the *in vitro* forage, and mineral Zn can trigger the growth of microbes that increase the efficiency of fermentation in the rumen (Mardalena, 2015). But, the content of high flavonoids, if given in livestock, will inhibit the growth of animals and interfere with the thyroid (Hasiib et al., 2015).

Saponin is a compound in glycosides widespread in plants (Minarno, 2016). Saponins have several biological properties, including hemolytic activities, antibacterial, antimouse, antivirus software activity, cytotoxic or anticancer effects hypocholesterolemia, and antiprotozoal (Yanuartono et al., 2017). In ruminants, saponin works to reduce the number of protozoa. The decrease in protozoa can increase microbial protein because protozoa in the rumen use microbial protein as feed (Yanuartono et al., 2019).

This study examines the content of active ingredients such as flavonoids and saponins contained in the ration of sheep that were given multi-nutrient block with the level of papaya leaves is different. This research is beneficial to get more information on the potential of the active ingredients in the ration. They are given a multi-nutrient block with additional flour papaya leaves before giving to cattle.

MATERIALS AND METHODS

The research Study on the Content of Flavonoids and Saponins in the Ration of Sheep Plus the MNB with the Level of Flour Papaya Leaves Different held for four months in April 2019 and August 2019. The collection of material and the making of the ration and the MNB conducted in the Village of Kalisidi, Ungaran, Central Java, and in the Laboratory of Food Nutrition, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang. Testing the content of flavonoids and saponins carried out in the Laboratory of Integrated Research and Testing (LPPT), Universitas Gadjah Mada, Yogyakarta.

Material

This study used forage rations and concentrates prepared with rice bran, cassava, pollard, coconut meal, coffee husk, corn bran, molasses, and VIT R. Multi-nutrient block (MNB) was prepared using; wheat shells, fermented rice straw, molasses, urea, wheat shells, bentonite, and papaya leaves.

Methods

This research was conducted with several stages of manufacture of the ration, making the MNB, and the analysis phase. This research uses experimental design in a completely randomized design (CRD) with four treatments and three replications. The treatments were given, namely:

T0 = Ration of sheep and MNB without the administration of papaya leaf
T1 = Ration of the sheep and the MNB using papaya leaves 2%
T2 = Ration of the sheep and the MNB using papaya leaves 4%
T3 = Ration of the sheep and the MNB using papaya leaves 6%

Stage of Manufacture of Ration

Ration complete sheep consisting of forage and concentrate with a 20: 80. Ration is structured based on the needs of the bodyweight of cattle sheep ± 20 Kg. According to NRC (1985), protein requirements of sheep on the weight of 10 – 20 kg with added weight 200 – 250 g/day is 127 – 167 g/day and the needs of TDN sheep on the weight of 20 kg with an average weight of 100 g/day, i.e., 470 g/day.

Forage used is a mixture of grass Odot (*Pennisetum purpureum* cv Mott) and fermentation excrescence cassava. The Odot grass and fermentation cassava tuber percentage is 50: 50. Odot grass was being used enumerated by using chopped up into small pieces. Prebiotics EM4 dissolved with water and mixed with rice bran, cassava tuber by-product, and a mixture of prebiotics with rice bran and homogenized then stored in barrels closed tightly. The fermentation process excrescence cassava for approximately seven days. The composition of the grass Odot and fermented cassava tuber by-products used can be seen in Table 1.

Table 1. Composition of the forage for sheep ration

Feed ingredient	CP	CF	TDN*
	---------------	%	---------------
Odot grass	8.88	29.87	58.46
Fermented Cassava tuber by-product	3.56	19.82	44.24

Note: the Laboratory Test Animal Drugs and Feed, Center for Veterinary Semarang (2019)
TDN* = calculated according to the formula Hartadi et al. (1980)

Concentrate sheep is created by mixing all the constituent ingredients of the concentrate until homogeneous using a mixer. Mixing materials is done by slightly pouring the elements with the composition of the most up. The concentrate is stored in large vats that have been provided. The composition of the feed ingredients used for the concentrate can be seen in Table 2.

Table 2. Composition of the ingredients

Feed ingredient	CP	CF	TDN*
	--------------- % ---------------		
Rice Bran	5.16	38.86	4.70
Pollard	18.09	10.23	70.22
Dreg	3.66	22.05	76.85
Coconut Cake	18.36	14.61	64.95
Skin Coffee	12.87	31.32	24.98
Molasses	1.02	0.00	67.10
DDGS Corn	28.09	9.14	81.44

Note: the Laboratory Test Animal Drugs and Feed, Center for Veterinary Semarang (2019)
TDN* = calculated according to the formula Hartadi et al. (1980)

Stage of Manufacture Multinutrien Block

Making multi-nutrient blocks (MNB) begins with the manufacture of fermented rice straw. Rice straw is aerated and then smoothed using a *grinder*. Prebiotics EM4 and drops dissolved and mixed with rice bran and rice straw. Mixing is carried out until a homogeneous was deposited and compacted in plastic and sealed vacuum. Fermented rice straw takes approximately 14 days. Papaya leaves were dried under the sunlight and then smoothed using a grinder. The constituent ingredients of the MNB were weighed according to the composition in Table 3.

Table 3. Composition of the multi-nutrient block

Feed ingredient	Treatment			
	T0	T1	T2	T3
	---------- % DM ----------			
Fermented Rice Straw	36	34	32	30
Papaya Leaves	0	2	4	6
Flour Shells	10	10	10	10
Urea	4	4	4	4
Molasses	40	40	40	40
Bentonite	7	7	7	7
Salt	3	3	3	3
Total	100	100	100	100

Note: T0: the ration of sheep and MNB without papaya leaves, T1: ration sheep and MNB papaya leaf level of 2%, T2: the ration of sheep and MNB level of papaya leaves of 4%, and T3: the ration of sheep and MNB level of papaya leaves of 6%

Molasses were heated for 10 minutes at 40 - 50℃ with stirring continuously. Bentonite was mixed into the molasses that had been heated. All the constituent ingredients of the MNB were mixed in a bucket and stirred evenly. The molding process MNB was conducted using pralon. The MNB was stored in a container in the open air without sunlight.

The stage of Mixing Ration with MNB

Mixing ration with the MNB was conducted to analyze the content of nutrients, flavonoids, and saponins. Samples were taken from the ration and the MNB for the analysis of flour. Forage and concentrate that has been provided mixed first up mixed with the homogeneous. MNB formed the boxes are broken down first and then put into a mixture of forages and concentrates and stirred again until homogeneous. The composition of the procedures for changes in feed can be seen in Table 4. After homogeneous, ration smoothed using a blender to become flour. Blender clean after flouring each treatment so that absence mixing between the treatment of the one with the other. Samples were taken of each treatment as much as 200 g per analysis.

Table 4. Composition of the mixture in DM

Feed Ingredient	Treatment			
	T0	T1	T2	T3
	-------- kg DM --------------			
Forage	0.21	0.21	0.21	0.21
Concentrate	0.84	0.84	0.84	0.84
MNB	0.04	0.04	0.04	0.04

Analysis Phase

The parameters observed, among others, were the number of flavonoids and the number of saponins. The analysis phase is performed when ration added MNB with flour papaya leaves homogenized in flour.

The second analysis is done by the testers LPPT at the University of Gajah Mada University, Yogyakarta, where analysis of flavonoids was done with the test method of spectrophotometry with how to 0.05 g of the sample taken and put into a test tube of 10 ml. Sodium nitrite 5% was added to the tube as much as 0.3 ml and left for 10 minutes.

Aluminium chloride 10% plus as much as 0.6 ml and allowed to return for 10 minutes. Sodium hydroxide 1M plus as much as 2 ml and distilled water to a volume of 10 ml with pumpkins measure. The solution was diluted five times and transferred into a cuvette and then at a wavelength of 510 nm absorption to read.

Analysis content of saponins from *Quillaja bark* methods spectophotometry do with how the sample was weighed 50 mg and then plus H2SO4 25% as much as 2 ml fed into the autoclave for 120 minutes at a temperature of 110ºC. Further extracted with Diethyl ether and dried with the filtrate. After that, plus the water as 1 ml and then extracted by vortex for 5 minutes. Then added with 50 µl anisaldehyde and mixed then allowed to stand for 10 minutes. Two ml of sulfuric acid 50% and subsequently heated in a water bath at a temperature of 60ºC for 10 minutes. Water up to 10 ml with Erlenmeyer measurement tube and diluted ten times. Absorption at a wavelength of 435 nm to read.

RESULTS AND DISCUSSION

The content of flavonoids in the ration of sheep plus multi-nutrient block with the level of flour papaya leaves are different at T0, T1, T2, and T3 have the results of the statistical analysis significant effect ($P<0.05$) between the control treatment. The content of flavonoids averages highest in T2, equal to 1,479 %b/b, and the content of flavonoids average lowest in T0 or control, equal to 1,302 %b/b. The test of distance double Duncan showed that the treatment T2 was significantly different ($P<0.05$) with T0 but not significantly different with T4 and T3. Table 5 shows that the content of flavonoids in every treatment is not increased where there is a decrease in T3.

Table 5. The average content of flavonoids and saponins

Treatment	Flavonoids	Saponins
	--------- %w/w---------	
T0	1.302^{b}	1.219
T1	1.436^{ab}	1.436
T2	1.479^{ab}	1.372
T3	1.338^{ab}	1.393

$^{ab,\,b}$: superscript with different letters in the same column indicate a significant difference ($P<0.05$)

Note: T0; sheep ration with MNB without papaya leaves, T1; sheep ration with MNB with papaya leaves a level of 2%, T2; sheep ration with MNB with papaya leaves a level of 4%, and T3; sheep ration with MNB with papaya leaves a level of 6%

Factors that can cause the content of flavonoids in each treatment were not increased, one of which is the solvent used. Nomer et al. (2019) stated that the solvent can be a contributing factor to the content of flavonoids is different because the effectiveness of the extraction is influenced by the degree of solubility of the material with the solvent used. The water content in treatment may also be a factor that causes the content of flavonoids not to increase on treatment. In addition, the water content in each treatment can be a contributing factor to the content of flavonoids was not increased. According to Arifin and Ibrahim (2018) flavonoids dissolve well in polar solvents due to flavonoids bound in the form of glycosides. Robinson (1991) stated that flavonoids are readily soluble in water, especially in glycosides, because the glycosides in flavonoids can be extracted using water as a semi-polar solvent, while flavonoid compounds are slightly soluble in water are semi-polar and can be removed using 80% methanol, ethanol, and acetone as solvents.

If ingested by livestock, especially ruminants, Flavonoids can positively impact livestock, especially on digestibility. Cherdthong et al. (2019) stated that flavonoids could inhibit cell wall synthesis or nucleic acids, affecting the population of protozoa. Some research has shown that the content of flavonoids can help improve the performance of the growth of the cattle, one on the research Balcells et al. (2012) who stated that with the addition of plant extracts containing flavonoids at a concentration of 300 mg/kg was able to increase the growth in cattle. Hasiib et al. (2015) stated that the content of flavonoids is high if it is consumed by livestock was inhibits livestock growth, and interferes with livestock health.

The content of saponins in the ration of sheep plus multi-nutrient block with the level of the leaves of papaya are different at T0, T1, T2, and T3 have the results of the statistical analysis no significant effect ($P>0.05$) between the control treatment. This shows that the content of saponins ration of sheep given multi-nutrient block with different papaya leaf levels is not different from the control. The content of saponins averages highest in T1, equal to 1,436 % w/w, and the content of saponins average lowest in T0 or control, equal to 1,219 % w/w. Table 5. showed that the saponins did not increase every breath.

One of the factors that can lead to the content of saponins is not experiencing an increase in the content of water contained in each treatment. Marpaung and Romelan (2018) stated that saponins have a polar nature that is more soluble in water than other solvents and easily foam when shaken. Saponins are high can be attributed to the absorption value at test identification. Amananti et al. (2017) stated that the higher the content of saponins, the more the colour is inflicted to be more concentrated where many molecules are contained in the sample so that the molecules absorb light at a wavelength of more result in the value of the absorption is high.

Saponin is one of the antinutrients which can result in decreased growth, consumption, and productivity of livestock. Still, if consumed with an amount not too much by way of feed processing, saponins can be suitable for the body farm. Wahyuni et al. (2014) stated that the administration of saponins with the level of 2.5% in the feed could decrease the palatability because it can cause the feed to be bitter. Saponins with a level of 2.5% can be said to be high. Yanuartono et al. (2017) add that saponins can be antinutrients that result in irritation of the digestive tract and lining of the mouth so that cattle can affect the absorption of nutrients when consumed too high. Still, the content of saponins can be derived from the various ways of processing on forage, such as heating or boiling.

CONCLUSION

The research results have been done can be concluded that the content of flavonoids and saponins did not have increased each treatment. The content of flavonoids highest contained at the level of papaya leaves 4% while the content of saponins on the addition of treatment up to 6% still produce saponins are the same.

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