

## **Nutrient, phytochemical, and digestibility evaluation of papaya leaves (*Carica papaya* L.) as an alternative feedstuff for ruminants**

Muhamad Ichsan Haris<sup>1)</sup>, Hamdi Mayulu\*<sup>1)</sup> and Suyadi<sup>2)</sup>

<sup>1)</sup>Animal Sciences Departement of Agricultural Faculty, Mulawarman University, Kampus Gunung Kelua, Jalan Pasir Belengkong, Samarinda, Kalimantan Timur, Indonesia

<sup>2)</sup>Faculty of Animal Husbandry, Brawijaya University, Jalan Veteran Malang, Jawa Timur, 65145, Indonesia

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**ABSTRACT:** Ruminants have a complex digestive system so they can utilize forage nutrients to convert them into food products of high economic value (meat and milk) and papaya leaves have the potential to be an alternative source of new feedstuff. The research was conducted at the Laboratory of Animal Feed Chemistry, Faculty of Animal Husbandry, Hasanuddin University Makassar (for proximate analysis), Laboratory of Animal Nutrition and Feed, Faculty of Animal Husbandry, Gadjah Mada University, Yogyakarta (for quantitative and qualitative analysis). The study was used proximate analysis to determine the nutritional content of papaya leaf, in vitro analysis to measure dry matter digestibility (DMD) and organic matter digestibility (OMD), and Thin-Layer Chromatography (TLC) for quantitative and qualitative tests of alkaloid compounds. The results showed that the DMD values of dry leaf and fresh leaf DMD were 72.74% and 78.44%, respectively. The highest score of rations DMD and OMD was R<sub>2</sub>FPL (69.98%) and R<sub>2</sub>DPL (61.92%), respectively. A qualitative test of papaya leaf bioactive compounds using UV 254 light obtained 3 spots with a detectable R<sub>f</sub> value of 0.72 and a quantitative test with piperine test parameters showed the results of the analysis were "not detected". The results showed that papaya leaves were suitable as an alternative feedstuff for ruminants (cattle, buffalo, goats, and sheep).

**Keywords:** Papaya Leaf; Evaluation; Digestibility; Nutrition; Phytochemicals

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\*Corresponding Author: [mayoeloehsptno@yahoo.com](mailto:mayoeloehsptno@yahoo.com)

## INTRODUCTION

Ruminants have a complex digestive system so they can utilize forage nutrients to convert them into food products of high economic value (meat and milk) (Mayulu, 2019). The feed is an important factor to fulfill livestock growth needs and production to achieve a successful livestock enterprise (Mayulu et al., 2018). The papaya leaves have the potential to be an alternative source of new feedstuff. Papaya leaf belongs to the Caricaceae family allied to the Passifloraceae, and can be found in all tropical countries and many sub-tropical regions of the world (Jafari et al., 2018). Papaya leaf is one of the plant wastes that can be used as local feed ingredients for livestock since it has many benefits such as increasing appetite and improving health. The utilization of local feedstuffs can reduce the feed cost thus it can make a bigger profit for farmers (Mayulu, et al., 2020). Nutrition quality and bioactive compound of papaya leaves contain papain enzyme, antimicrobial, antioxidant, alkaloids (carpaine, pseudocarpaine, dehydrocarpaine I and II) (1569.13 mg/100), vitamin C, vitamin E, choline (Saran and Choudary, 2013), includes,  $\alpha$ -tocopherol (Anjum et al., 2017). Papaya leaves contain glycoside, tannin (310.50 mg/100), flavonoid (kaempferol and myricetin) (866.53 mg/100) (Ugo et al., 2019), steroid, resin (Callixte et al., 2020), flavonoid, sucrose, dextrose, levulose (Khoriyah et al., 2016), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), saponins (898.07 mg/100gr) (Yogiraj et al., 2014; Fauzi'ah and Wakidah, 2019; Ugo et al., 2019; Sharma et al., 2020; Safrida and Sabri, 2020), such as benzyl isothiocyanate,  $\delta$ -tocopherols, glucosinolates,  $\beta$ -cryptoxanthin,  $\beta$ -carotene, carotenoids (Santana et al., 2019), and high chlorophyll (0.3726 mg/L) (Setiyono et al., 2012). Papaya leaf was characterized by low contents of fiber and lignin as well as favorably high in vitro OMD, and high crude protein (Jafari et al., 2018). Using papaya leaves extract increased NH<sub>3</sub> production,

VFA, and microbe protein synthesis in vitro (Ramandhani et al., 2018). Onyimonyi and Ernest (2009) stated that papaya leaf flour contains crude protein (CP) 30.12%, water 10.20%, crude fiber (CF) 5.60%, ether extract (EE) 1.20%, ash 8.45%, and NFE 44.43%. Papaya leaf extract contains moisture 57.01%, CP 6.50 %, ash 2.18%, CF 3.10 %, EE 2.01% and carbohydrate 29.20% (Ugo et al., 2019). The bitter taste and aroma of *Carica papaya* L. are also possible since it contains papain enzyme which is scattered in fruits, stems, and leaves. Its texture and color look like white latex and are sometimes called milky juice (Adachukwu et al., 2013).

Research on papaya leaf informs that it has some pharmacological activities such as antiemetic, trypanocidal activity, antibacterial, and anti-fungal. However, the use of papaya leaf should be done carefully because it contains a lot of alkaloids called carpaine which can cause cardiac contraction (Muljana, 2002). Papaya leaf can be classified as a medicinal crop due to the content of various compounds (alkaloid, proteolytic enzyme, papain, chymopapain, and lysozyme) which are good for the digestion process and facilitate intestines.

Many researchers have been conducted to evaluate the biologically active compound and nutrient content of papaya leaves, such as Ayoola and Adeyeye (2010) were analyzed for the phytochemical composition, phytochemical screening revealed the presence of bioactive compound saponins, cardiac glycoside, alkaloids, and absence of tannins in the green, yellow and brown papaya leaf. Adachukwu (2013) reported that the qualitative phytochemical analysis of *Carica papaya* leaves showed the presence of alkaloid, flavanoid, Saponin, Tannin, and Glycosides. It is obvious from plant secondary metabolites (PSM) (Mirzaei, 2012). The biochemistry of Plant Secondary Metabolites (PSM) has a wide range of biological activities and enormous potential for use in animal production (Makkar et al., 2007).

The analysis of feed material was to observe the chemical composition contained in the feed material using proximate analysis. The resulting data is needed in determining the quality of feed ingredients or the safety level. The objective of this study was to evaluate the phytochemical and nutritional value of papaya leaves as green feeding and evaluate their digestive value in vitro. In vitro is an alternative method to estimate feed degradation in livestock's digestive apparatus without involving the animal (Mayulu et al., 2020).

## **MATERIALS AND METHODS**

The research was conducted in September 2012 at the Laboratory of Animal Feed Chemistry, Faculty of Animal Husbandry, Hasanuddin University Makassar (for proximate analysis), Laboratory of Animal Nutrition and Feed, Faculty of Animal Husbandry, Gadjah Mada University, Yogyakarta (for quantitative and qualitative analysis). The material of this study was used fresh papaya leaf (FPL)/unripe papaya leaf and dry papaya leaf (DPL)/over ripe papaya leaf.

### **Nutritional value evaluation of papaya leaf**

The determination of water content, the determination of total nitrogen using the Kjeldahl method (determination of protein content), the determination of lipid content using *the Soxhlet* method, and the determination of ash content of fresh papaya leaf (green) and dry papaya leaf (brown) was done using the procedure according to AOAC (2005). The determination of fatty acid composition in various feedstuffs has been used to quantify the total lipid content and saturated lipids. Hydrolysis of the sample was performed by adding 10 ml of HCl 6 N and 2 ml ethanol to 2 g of papaya leaf samples. The mixture was hydrolyzed at 70-80°C and shaken for 10 minutes.

After cooling, the mixture was extracted with 100 ml of diethyl ether-petroleum ether (boiling point 30-60°C) (1:1). The collected extracts were dried with anhydrous sodium sulfate, the solvent was removed with nitrogen gas, and the residual solid was dried by a rotary evaporator. After the methylation was done, the sample was dissolved in n-hexane for further analysis using kg.

The separation was performed using a boxed glass column (180 cm x 2.6 mm i.d; 100/120 mesh on GCQ support with 10% silane). The column temperature was 200°C, while the injector and detector temperature were 250°C. The carrier gas was nitrogen (Rohman and Gandjar, 2007).

### **The evaluation of the phytochemical potential of papaya leaf**

Identification of the presence and determination of potentially phytochemical bioactive compounds could be done by quantitative and qualitative analysis of alkaloid content in papaya leaf by TLC (Rohman and Gandjar, 2007). Thin-layer chromatography is generally the first-choice method for chromatographic separation.

### **The extraction and quantitative analysis.**

Papaya leaf was processed to obtain papaya leaf extract. It was then carefully weighed about 0.5 g and then dried in an oven at 45°C for 12 hours, dissolved with ethanol, and added up to 5 ml with a volumetric flask.

Then, it was analyzed using a thin layer of chromatography on a silica-coated aluminum plate. The sample was spotted on the plate using a microsyringe of about 10 µl as well as piperine standard regression. The plates are inserted into a chamber that already contains mobile-phased saturated toluene-ethyl acetate (70:30), eluted until reaching the limit, and air-dried. Piperine spot was positioned at wavelength 334 nm. Calculate the level of piperine.

**Chromatography Resume:**

Stationary phase	:	<i>Silicagel 60 F<sub>254</sub></i>
Mobile phase	:	Toluene-ethyl acetate (70:30)
Spotting volume	:	10 µl
Wavelength	:	334 nm
Distance	:	8 cm
Rf	:	0.50
Standard	:	Piperine 10.1 mg/10 ml
Sample mass	:	0.5052

**Qualitative analysis of alkaloids.**

The papaya leaves were processed into powder (sample) then weighed as much as 100 mg and then added 2 ml ammonia 10%, centrifuged for 2 minutes, 5 ml chloroform was added then re-centrifuged for 2 minutes. Centrifuge for 3 minutes, take chloroform phase and evaporated with nitrogen gas then dissolved in 20 µl chloroform. Spotting the sample as much as 20 µl on the silica gel plate (silicent phase) 60 F254. Put it into the saturated chamber of the mobile phase: ammonia (100: 1.5),

eluted to the limit, then lift and dry. Elution was carried out in a saturated vessel for ±30 minutes at a 10 cm distance. The detection used Sagendorf reagent. The appearance of the spot was seen with a UV lamp 254-365 nm.

**RESULT AND DISCUSSION**

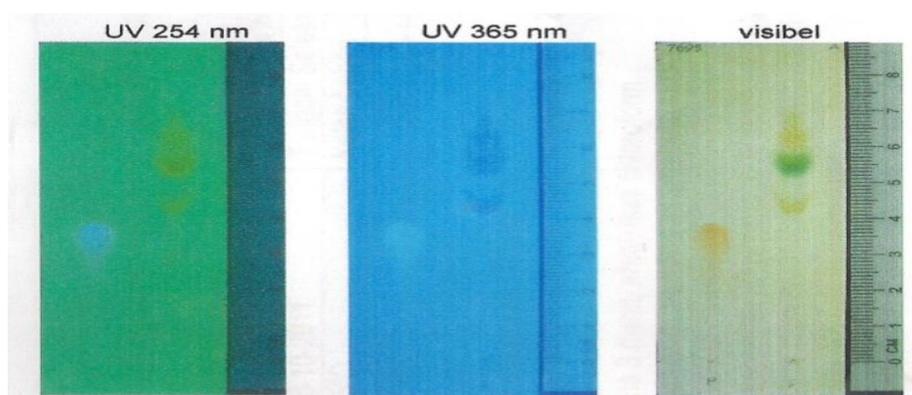
**Qualitative test**

The results of qualitative analysis of bioactive compounds in papaya leaf using thin-layer chromatography showed positive results. It can be seen in Table 1.

**Table 1.** TLC profile of qualitative analysis of alkaloid compound

Testing Parameter: Alkaloid	Qualitative Result: Positive
P = <i>Comparator quinine</i>	Visible spot color of alkaloid: Yellowish orange
S = Papaya leaf	Rf detected alkaloid 0.72
Rf <i>comparator quinine</i> : 0.42	

Description: the results of alkaloid qualitative test of papaya leaves in Integrated Research and Testing Laboratory, UGM, Yogyakarta (2012).



**Figure 1.** TLC profile of Qualitative Test of Alkaloid Compound of Papaya Leaves

P: Comparator Quinine

S: Papaya leaf

Color of alkaloid spotted in visible : Orange yellow

Rf comparator quinine : 0.42

Rf alkaloid detected : 0.72

**Quantitative test**

The results of a quantitative test of bioactive compounds on papaya leaf using thin-layer chromatography method with piperine test as parameter showed “not detected” result, which can be seen in Table 2. Based on detection done with UV light 254 and UV 366, it was shown that the samples of papaya leaf showed samples detected with UV 254 obtained 3 spots with a detected Rf value of 0.72 as shown in

Figure 1. Eleazu et al. (2012) reported that plants containing alkaloid substances have functions such as plant protective toxins from insects and ruminants, the detoxification end products of metabolic activities, growth regulatory factors, and supplier of N elements needed. Alkaloid has insect-repellent compounds and anti-fungus compounds and it is generally believed that the alkaloid compounds are the cause of bitterness.

**Table 2.** Quantitative results of papaya leaf alkaloid with piperine test parameter

Testing parameter: alkaloid piperine (ppm)	Quantitative analysis: not detected (uncontrolled)
Sample = Papaya leaf	Area: -
Sample spotting volume (μ) = 10	Piperine in the sample (ng): -
Number of sample spotting (μg) = 1010.4	Piperinerate (ppm): Not detected
Detection limit: 1.26 ng	

Description: the results of a quantitative test of papaya leaf alkaloid at Integrated Research and Testing Laboratory, UGM, Yogyakarta (2012).

The value of Rf 0.72 when alkaloids were detected showed that papaya leaf samples contained alkaloid compounds. In addition, alkaloids and papain were also able to be used to decrease lipid accumulation in chickens and rats. Analysis of feed material intended to observe the chemical composition contained in the feed material. The chemical composition of feed material can be determined by the proximate analysis method. The resulting data was needed in determining the quality of feed ingredients

or the level of safety. The quality of the feed material and its safety level were mainly determined by moisture content because the moisture content determines the level of the other components. If the water content of a sample is low due to high evaporation, the other component level will be increased, and vice versa (Andarwulan et al., 2011). Therefore, in declaring composition, water content should always be included, or the sample is expressed in free water state or dry.

**Table 3.** The component of proximate analysis of papaya leaf in various physical condition

Sample Physical Condition*	Proximate Analysis Result (%)				
	Water	CP	EE	Ash	CF
Unripe papaya leaf (light green) (fresh) <sup>1</sup>	76.29	20.92	12.40	13.53	13.15
Ripe papaya leaf (dark green) (semi-dry)	9.20 <sup>1</sup>	19.63 <sup>1</sup>	15.13 <sup>1</sup>	12.95 <sup>1</sup>	21.19 <sup>1</sup>
	16.10 <sup>2</sup>	16.04 <sup>2</sup>	9.57 <sup>2</sup>	13.20 <sup>2</sup>	-
Overripe papaya leaf (yellow-brown) (dry)	7.31 <sup>1</sup>	17.39 <sup>1</sup>	13.33 <sup>1</sup>	12.95 <sup>1</sup>	23.92 <sup>1</sup>
	15.71 <sup>2</sup>	14.10 <sup>2</sup>	6.27 <sup>2</sup>	12.08 <sup>2</sup>	-

Description: all fraction is expressed in dry matter, excluding water

\*samples are papaya leaves (*Carica papaya* L.) var California

1. Proximate analysis result in Feed Chemical Laboratory, Unhas.
2. Proximate analysis result in Center for Laboratory Animal Nutrition and Feed, Faculty of Animal Husbandry, UGM.

The results of proximate analysis of unripe, ripe, and overripe papaya leaves can be seen in Table 3. Table 3 above contains data on papaya leaf proximate analysis component in two groups of physical conditions: fresh papaya leaf (unripe papaya leaf) and dry papaya leaf (ripe papaya leaf powder and overripe papaya leaf). It was intended to observe the difference in the nutritional composition of papaya leaf in fresh physical conditions or dry conditions, the difference in nutrient composition is very needed in determining the quality of feed ingredients.

The quality of feed ingredients is often associated with the presence of water in the feed. Therefore, water analysis in feed material is very important both in fresh and dry feed. In dry feed, water content is associated with the stability index, especially during storage. According to Ugo et al. (2019), papaya leaf extract contains moisture 57.01%, CP 6.50 %, ash 2.18%, CF 3.10 %, EE 2.01% and carbohydrate 29.20%. Protein content in papaya leaf varies between fresh papaya leaf and dry papaya leaf which are 14-20% (Table 3). This range shows that the protein content of papaya leaf is high enough and one of the determinants of the quality of a feed ingredient is its protein content because protein is the main source of nutrition, which is the source of amino acids (Utomo et al., 2012).

The result of the evaluation of papaya leaf nutrient determination as shown in Table 3 shows that papaya leaf has potential as a feed ingredient for ruminant livestock mainly small ruminants including goats and sheep based on their nutrient content and, this is supported by the opinion of Agus (2008) and Utomo et al. (2012) that the quality of feed ingredients is determined by their nutrient content or chemical composition, the evaluation of feed ingredients is conducted to determine the nutrient content that determines the quality

of the feed ingredients to provide nutrients for livestock.

Papaya leaf that is fed to livestock based on the source belongs to the "roughage" group. The term "roughage" of papaya leaves given to livestock (in this study) refers to the opinion of Utomo et al. (2012) who stated that fibrous feed material consists of two types based on plant age, which were cut before flowering (forage) and after flowering and seeds or the main results taken for the benefit of humans called roughage. However, Bayer and Bayer (2012) defined "forage" as all parts of vegetation plants that can be eaten by livestock (leaves, flowers, stems/twigs, and roots), so that the leaves are fed as forage which already wilted and dry is considered as forage. Papaya leaf can be an alternative source of forage feed, this is based on the consideration of its relatively high protein content. The result of the proximate analysis showed papaya leaf containing CP 10.71-13.50%; CF 14.68-22.56%; EE 12.03-12.80%; and ash 14.40-17.83%. Research related to papaya leaf nutrient composition summarized from several research results was showed that CP 22.51, CF 16.9, OM 87.78, and DM 18.59 (Khoiriyah et al., 2016), CP 30.12, CF 5.60, NFE 44.43, and EE 1.20 (Onyimonyi and Ernest, 2009).

Fatty acids are the basic structure of lipid, when lipid is hydrolyzed, it will produce glycerol and fatty acids. Based on their saturation, fatty acids can be distinguished into saturated fatty acids and unsaturated fatty acids. The profile of fatty acids contained in papaya leaves consists of 5 saturated fatty acids (capric acid, lauric acid, myristic acid, palmitic acid, and arachidonic acid) and 4 unsaturated fatty acids (oleic acid, linoleic acid,  $\alpha$ -linolenic acid, and erucic acid). Research conducted by Alfonso (2005) summarized some information regarding nutrient compounds contained in papaya leaf in various physical conditions as shown in Table 4.

**Table 4.** Nutrient content of papaya leaf in various physical conditions

Papaya Leaf Condition	Nutrient Analysis Result (%)					
	Water	CP	EE	Ash	CF	NFE
Dry Papaya Leaf	5.90	13.56	12.90	14.45	14.68	38.52
Fresh Papaya Leaf*	75.00	-	2.40	-	-	-
Wet Papaya Leaf (in 100 g) **	-	(8 g)	(2 g)	-	(11.9 g)	-

Note:

\*Fresh papaya leaf content Papain

\*\*Wet Papaya Leaf (in 100 g) content: Vitamin A (18.25 IU); Vitamin B (0.15 mg); Vitamin C (1.4 mg); Fe (0,8 mg) and phospholipid (0,12).

The content of unsaturated fatty acids in papaya leaf is essential fatty acids. Tirtawinata (2006) stated that unsaturated fatty acids are linoleic, linolenic, and arachidonic acids, these unsaturated fatty acids are included in the essential fatty acid group because it is needed by the body. The body cannot produce itself and solely depends on food. The result of fatty acid composition analysis of brown papaya leaf shows there were as many as 9 types of fatty acids found in papaya leaf, and the result can be seen in Table 5.

The profile of fatty acids in papaya leaf obtained a comparison between saturated fatty acids (SAFA) and unsaturated fatty acids (MUFA and PUFA), where the total SAFA was 0.081 per 100 g, the total MUFA 0.072 per 100 g, and total

PUFA was 0.058 per 100 g. The presence of SAFA is represented by lauric acid, myristic acid, and palmitic acid, MUFA is detected with the presence of oleic acid (omega-9) and erucic acid (omega-9), and PUFA is also indicated in papaya leaves after the availability of linoleic acid (omega-6) and  $\alpha$ -linolenic acid (omega-6). EPA, DPA, and DHA as omega-3 groups were undetectable in papaya leaf. The functions of fatty acids, in addition to maintaining body tissues, are as follows: 1) strengthening blood vessel capillaries and cell membrane structures that protect water evaporation from the skin; 2) enable to lower blood cholesterol levels by mixing into cholesterol-ester and playing a role in transport; and 3) prolong the period of blood clotting (Tirtawinata, 2006).

**Table 5.** The result of fatty acid composition analysis (%) of papaya leaf

Analysis Sample	(%) Relative Ester Methyl Fatty Acid
Papaya Leaf (Wilted-Dry Brownish)	C10:0 = 0.1635 <sup>1</sup> (capric/decanoic) <sup>2,3</sup>
	C12:0 = 1.5686 <sup>1</sup> (lauric/dodecanoic) <sup>2</sup>
	C14:0 = 4.1680 <sup>1</sup> (miristic/tetradecanoic) <sup>2</sup>
	C16:0 = 25.2275 <sup>1</sup> (palmitic/hexadecanoic) <sup>2</sup>
	C18:1 = 18.7937 <sup>1</sup> (oleic/octadecanoic) <sup>2</sup>
	C18:2 = 6.0293 <sup>1</sup> (linoleic/octadecadienoic) <sup>2</sup>
	C18:3 = 6.395 <sup>1</sup> ( $\alpha$ -linolenic/octadecatrienoic) <sup>2</sup>
	C20:0 = 0.9747 <sup>1</sup> (arachidonic/eicosanoic) <sup>2</sup>
C22:1 = 3.0469 <sup>1</sup> (erucic)(dokosenoat) <sup>3</sup>	

Description: 1. The result of fatty acid analysis of papaya leaf in Laboratory Animal Nutrition and Feed, Faculty of Animal Husbandry, UGM; 2. Montgomery et al. (1977); and 3. Andarwulan et al. (2011).

The result of the quantitative test of a bioactive compound of papaya leaf with piperine test parameters shows that alkaloid content in papaya leaves is undetected in

part per million (ppm). The results of this quantitative test can be explained by various possibilities, seeing that the presence of alkaloids in plants is mostly present in the

form of salt bonds with organic acids. Alkaloids in the salt form are more soluble in higher-polarized solutions such as ethanol, so the alkaloidal salts are most likely to be found in ethanol, so in the water extract no alkaloids are found.

The result of the quantitative analysis shows that there is no alkaloid detected which can also be caused by processing made by the number of alkaloids contained in extremely dried papaya reduce. The contains of Papaya lives include alkaloids (carpaine, pseudocarpaine, dehydrocarpaine I and II (Saran and Choudary., 2013). The physical and chemical characteristics of the alkaloids are, among other things, crystalline solids, soluble in a relatively non-polar organic solvent, alkaloid in nature so that alkaloids make them particularly susceptible to decomposition by heat and light in the presence of oxygen. Plants contain a large number of biologically active chemicals, some of which can be used to treat various diseases affecting livestock and humans (such as digitoxin, colchicine, and atropine). The presence of certain chemicals in plants is believed to provide some degree of protection from plant predators such as

insects and ruminants. Most of the anti-nutrients are generally obtained from the secondary metabolism of plants. Leaves that are considered to have a bitter taste are proved to have many benefits for the body, some studies say that papaya leaves contain various substances the body needs for fighting disease. Papaya leaf besides containing many useful nutrients also contains other compounds which are useful for phytochemical potential in the pharmacy field and medicine. The compounds are alkaloids, carpaine, caricaksantin, violaxanthin, papain, flavonoid, and polyphenol. Empirical evidence of papaya leaf utilization is treating intestinal parasites (helminth), improving appetite, decreasing dengue fever, as well as overcoming malaria.

The result of statistical analysis showed that the DMD was not significantly different between fresh papaya leaves and dried papaya leaves, although the nominal value of fresh papaya leaves (78.44%) was higher than the digestibility of dry papaya leaves (72.74%) (Table 6). The results of the digestibility analysis of dry and OM papaya leaves can be seen in Table 6.

**Table 6.** The average digestibility value of DM and OM of fresh and dry papaya leaf

In vitro Digestibility (%)	Papaya Leaf Condition	Averages
DM (Dry Matter)	Fresh leaf	78.44±0.87
	Dry leaf	72.74±1.23
	Averages	
OM (Organic Matter)	Fresh leaf	74.67±3.09
	Dry leaf	69.65±1.77
	Average	

*ns: nonsignificant*

Different results are shown from statistical analysis of in vitro OMD evaluation where there was a significant difference between fresh and dry papaya leaf, the average OMD in fresh papaya leaf was 74.67% higher than the average dry papaya leaf digestibility of 69.65%. Higher OMD in fresh papaya leaf can be attributed to the chemical composition of proximate analysis between fresh papaya leaf versus dry papaya leaf (Table 6). It can be seen

from the composition of proximate analysis for fresh papaya leaf shows the average value of protein, EE, and CF were relatively higher than those of dry papaya leaf. The results of statistical analysis from Table 7 shows that in the evaluation of in vitro digestibility value of dry matter there was no significant difference between all group, both in the control group and the treatment groups R<sub>1</sub> and R<sub>2</sub> using fresh papaya leaf and dry papaya leaf.

**Table 7.** The average value of DMD and OMD using fresh and dry papaya

In vitro digestibility (%)	Treatment	Averages
DM (Dry Matter)	R <sub>0</sub>	57.73±0.83
	R <sub>1</sub> DPS	60.14±1.31
	R <sub>2</sub> DPS	69.98±1.58
	R <sub>1</sub> DPK	59.38±1.06
	R <sub>2</sub> DPK	63.58±0.73
	Average <sup>s</sup>	
OM (Organic Matter)	R <sub>0</sub>	56.15±1.57
	R <sub>1</sub> DPS	58.48±1.32
	R <sub>2</sub> DPS	61.81±2.28
	R <sub>1</sub> DPK	58.21±2.01
	R <sub>2</sub> DPK	61.92±0.84
	Average	

ns: nonsignificant

**Description:**

R0: 0% papaya leaf + 60% elephant grass + 40% concentrate

R1DPS: 15% papaya leaf (fresh papaya leaf) + 45% elephant grass + 40% concentrate

R2DPS: 30% papaya leaf (fresh papaya leaf) + 30% elephant grass + 40% concentrate

R1DPK: 15% papaya leaf (dry papaya leaf) + 45% elephant grass + 40% concentrate

R2DPK: 30% papaya leaf (dry papaya leaf) + 30% elephant grass + 40% concentrate

As shown above, nominally dry matter digestibility of feed treatment R<sub>1</sub> and R<sub>2</sub> using fresh papaya leaves shows the highest value among all feed treatment groups where the values obtained were 60.14% and 69.98%, respectively. It can be traced that the increase of papaya leaf concentration in the formula would affect higher dry matter digestibility as well. Different results were shown from statistical analysis of digestibility evaluation in vitro organic matter where there was a significant difference between the feed treatment group of R<sub>1</sub> and R<sub>2</sub> in which feed treatment R<sub>1</sub> was not significantly different.

**CONCLUSIONS**

Protein content in papaya leaves varies between fresh and dry papaya leaves at about 14-20%. A qualitative test of papaya leaf bioactive compounds using UV 254 light obtained 3 spots with a detectable R<sub>f</sub> value of 0.72 and a quantitative test with piperine test parameters showed the results of the analysis were "not detected". the DMD values of dry leaves and fresh leaves DMD were 72.74% and 78.44%,

respectively. The highest score for ration DMD and OMD was R<sub>2</sub>DPS (69.98%) and R<sub>2</sub>DPK (61.92%), respectively.

**SUGGESTION**

It should be done for advanced research which uses the dry papaya leaf as plant wastes that can be used as feed ingredients for livestock.

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