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Evaluation of Digestibility and Rumen Parameters Through *in-vitro* of Concentrate Containing Binahong Flour as Secondary Compound

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ABSTRACT

This study was conducted to determine the effect of different levels of Binahong flour usedin concentrate feed on dry matter digestibility (DMD), organic matter digestibility (OMD), VFA, and NH_3 concentrations *in vitro*. The design used was a completely randomized design with 4 treatments and 4 replications. The treatments studied were: R**0**: concentrate feed without Binahong flour, R**1**: concentrate feed contains 10% Binahong flour, R**2**: concentrate feed containing 20% Binahong flour, R**3**: concentrate feed containing 30% Binahong flour. Based on the results of the study, the averages of DMD were (%) R**0** 73.56±0.75, R**1** 74.50 ±0.61, R**2** 74.98±0.70, R**3** 76.65±0.90, OMD (%) R**0** 69.50±1.10, R**1** 69.82±1.00, R**2** 70.07±0.88, R**3** 72.61±0.99, VFA concentration (mM) R**0** 108.53±8.52, R**1** 110.75±6.51, R**2** 111.40±2.35, R**3** 112.77 ±14.76, NH3 concentration (mM) R**0** 9.74±0.74, R**1** 10.61±0.44, R**2** 11.55±0.41, R**3** 11.55±0.33. The results of the statistical analysis confirmed that the treatment had a very significant effect on the increase in DMD, OMD, NH_3 ($P<0.01$), but not significant on the total VFA concentration. The conclusion of this study is that the use of Binahong flour up to a level of 30% in concentrate feed had an effect on increasing DMD, OMD, VFA, and NH_3 concentrations *in vitro*.

Keywords: Binahong flour, dry matter digestibility, organic matter digestibility, NH_3, total VFA.

INTRODUCTION

In a livestock business, feed is the most important component for livestock. For ruminants, forage feed is the most important component in supporting their production performance. The need for forage in ruminants can reach up to 70 or even 100 percent. However, the provision of forage alone for ruminants is not sufficient to meet the nutritional needs for production, let alone reproduce. It is necessary to add concentrate in the ruminant feed so that the performance of ruminant production can be more optimal. Quality concentrates are concentrates that contain feed ingredients with sufficient nutrition for livestock. Hasanah (2006) in the results of his research explained that concentrates can provide optimal daily body weight gain (DBWG) for ruminants when compared to giving forage alone, but concentrates also have several weaknesses. This means that the crude fiber content is lower than forage and the price is relatively higher. One of the cheap concentrate alternative feed ingredients is Binahong.

Binahong (*Anredera cordifolia* Ten. Steenis) is a leguminous plant that can be used as feed for ruminants. Binahong has the potential as a ruminant feed because of its high carbohydrate and protein content, namely NFE of 37.83% and CP of 20.4 (Widu, 2019). Widodo et al., (2016) reported that Binahong has high nutrient content including dry matter 94.54%, ash 28.70%, crude fiber 8.08%, crude protein 14.80%, crude fat 5.20%, and 2069 metabolic energy (kcal/kg). Quality feed is feed that is formulated according to the nutritional needs of livestock including carbohydrates, protein, fat, fiber, minerals, and vitamins (Usman 2013).

One part of the Binahong plant that is very useful is the leaf because it contains several secondary metabolites that are beneficial to health. Secondary metabolites found in Binahong leaves are phenols, flavonoids, tannins, alkaloids, terpenoids, saponins, and steroids that act as antimicrobials (Astuti et al. 2011; Darsana et al., 2012). Binahong secondary metabolite content is total phenol 85.30 mg/kg, flavonoids 47.40 mg/kg, saponins 66.00 mg/kg, and total alkaloids 2.60 mg/kg. Fauziah et al. (2014) reported that administration of Binahong leaf extract could reduce blood cholesterol levels in male rats. Anggraini and Ali (2017) also showed that the ethanolic extract of Binahong leaves was able to reduce cholesterol levels by 17.7%, 18.0%, and 20.7%, respectively, at concentrations of 200, 400, and 600 ppm.

Hidayah (2016) states that the use of optimal amounts of tannins has a positive impact on ruminants. Tannins can precipitate proteins with functional groups that help form complex bonds that cannot be separated from protein molecules (tannin-phenolic bonds with keto

proteins). This binding means that the protein cannot be broken down by bacteria in the rumen. Stable tannin protein bonds (pH 4-7) will be broken down in the abomasum and then enter the small intestine where they will be digested and absorbed properly by bacteria in the rumen. Other secondary metabolites in Binahong that are beneficial for ruminants are saponins and flavonoids (Aprilliza and Vienna, 2021). The content of saponins and flavonoids in Binahong can also help ruminants in reducing the population of harmful protozoa. Saponins can lyse protozoa by forming complex bonds with sterols so that they can interfere with the growth of protozoa, causing membrane rupture, cell lysis, and finally protozoa death. Hidayah also added that the addition of tannins and saponins at optimal doses can reduce methane (CH4) production, increase total and partial VFA production (especially propionate), rumen the bacterial population, reduce ammonia levels, and most importantly do not interfere with the digestibility of feed ingredients.

This study was conducted to determine the use of the best Binahong flour in concentrate and its effect on nutrient digestibility and rumen parameters in vitro. This study hypothesizes that the use of Binahong flour in concentrate feed affects the digestibility value and rumen parameters in vitro.

MATERIALS AND METHOD

This research was conducted at the Laboratory of Feed Chemistry, Faculty of Animal Husbandry, Nusa Cendana University, Kupang for 4 weeks. The material used in this study was rumen fluid obtained from Slaughterhouses (SH), and the concentrates of feed ingredients presented are presented in Table 1.

Table1. Concentrated feed ingredients

Feed Ingredients	Treatment			
	R**0**	R**1**	R**2**	R**3**
Pollard flour (%)	50	45	40	35
Yellow corn flour (%)	45	40	35	30
Fish flour (%)	5	5	5	5
Binahong flour(%)	0	10	20	30
Total	100	100	100	100
CP (%)	15.11	15.20	15.29	15.38

The method used in this study is an experimental method using a completely randomized design consisting of 4 treatments and 4 replications. The treatments were:

R0 : Concentrated feed without Binahong flour (control);
R1 : Concentrated feed contains 10% Binahong flour;
R2: Concentrated feed contains 20% Binahong flour;
R3: Concentrated feed contains 30% Binahong flour.

Binahong Processing

Binahong used is washed, air-dried, and then chopped to a size of about 1 cm then dried at room temperature until the remaining 10% water content is then ground to make flour.

Measurement of Dry Matter Digestibility Coefficient (DMD_C)

Measurement of $\mathbf{DMD_C}$ and OMDc was carried out based on the formula and method of Tilley and Terry (1963). Fermenting the sample with drops of HgCl for 48 hours, then making the supernatant and precipitate by centrifuging the sample at 3000 rpm for 15 minutes. Furthermore, the supernatant and precipitate were separated and then dissolved with 50 ml of pepsin-HCI solution using a vortex machine. The mixture was then incubated for 48 hours and covered with aluminum foil. After 48 hours, the sample was filtered using Whatman 41 filter paper. The filtered sample was placed in a porcelain dish which was preheated at 105^0C and the weight of the empty cup was known. The sample was put in an oven at 105^0C for 24 hours to get dry matter and the sample was reduced to ashes in an oven for 6 hours at 400-600^0C.

The Dry Matter Digestibility Coefficient (DMDc) and Organic Matter Coefficient (OMDc) are calculated by the formula:

$$\% \text{ DMDc} = \frac{\text{DM sample (g)- DM residue (g)- BK blanko (g)}}{\text{DM sample (g)}} \text{ x } 100\ \%$$

$$\% \text{ OMDc} = \frac{\text{OM sample (g)- OM residue (g)- OM Blanko (g)}}{\text{OM sample (g)}} \text{ x } 100\ \%$$

Measurement of Total VFA Concentration

Volatile Fatty Acid (VFA) was analyzed by steam distillation method (Millar, 1966). The procedure for measuring VFA is to prepare a distillation unit by boiling water and draining the water into a condenser or cooler. A total of 5 ml of sample and 1 ml of 15% H_2SO_4 were put into the distillation unit. The resulting volatile acid was captured in 5 ml of 0.5N hydrochloric acid. The procedure for measuring volatile fatty acids is to prepare a distillation unit with NaOH in an Erlenmeyer flask. After the liquid was maintained to 250 ml, 23 drops of phenolphthalein were added as an indicator and titrated to 0.5 N HCl solution. The total production of volatile fatty acids can be calculated using the formula:

$$\text{mM VFA total} = \frac{\text{(a – b) ml x N HCl x 1000 / 5 ml}}{\text{gram sample x BK sample}}$$

Measurement of Ammonia (NH_3) Concentration

Determination of the concentration of NH_3 using the appropriate Conway micro diffusion method (Millar 1966). The lips of Conway's cup were smeared with Vaseline. The supernatant sample was obtained by centrifugation at 3000 rpm for 15 minutes. Then, 1 ml of the sample was placed on one side of the Conway septum, and 1 ml of saturated Na_2CO_3 solution was placed on the other side of the septum. The position of the Conway dish is tilted so that the two solutions do not mix until the cup is tightly closed. 1 ml of boric acid is located in the middle. The crucible is placed horizontally so that the saturated Na_2CO_3 solution is mixed with the supernatant and the reaction produces ammonia gas. The released ammonia is immediately captured by the boric acid. Titrate boric acid with 0.006M H_2SO_4 until the process is finished after 24 hours and the color changes from blue to pink. Ammonia levels can be calculated using the formula:

$$\text{N } NH_3 \text{ (mM)} = \frac{\text{ml } H_2SO_4 \text{ x N } H_2SO_4 \text{ x } 1000}{\text{gram sample x BK sample}}$$

Data Analysis

The data obtained in this study were calculated and analyzed according to the ANOVA (Analysis of Variance) procedure. If there is a significant effect ($P < 0.05$), then proceed with the Least Significant Difference (LSD) test to determine the difference between the treatments according to Gaspersz (2006) and SPSS software for windows series 20.

RESULT AND DISCUSSION

Ration Nutrient Content

The following presents data on the nutritional content of the treatment rations added with Binahong flour with different levels (0%, 10%, 20%, and 30%).

Table 2. The nutritional content of the treatment ration

Treatment	DM (%)	OM (%DM)	CP (%DM)	EE (%DM)	CF (%DM)	CHO (%DM)	NFE (%DM)	ME (Kcal/kg)
R**0**	90.63	86.30	12.75	5.53	2.36	68.03	65.67	3755
R**1**	89.93	83.96	13.72	5.18	3.46	65.07	61.60	3592
R**2**	90.06	82.91	14.83	4.91	4.37	63.18	58.80	3494
R3	90.69	82.06	15.65	4.63	5.20	61.78	56.58	3409

Source: Results of Analysis of the Undana Faculty of Animal Feed Chemistry Laboratory (2020)

Assessment of feed quality needs to be known by the in-vitro method before being given to livestock. The use of this method is quite effective because it is carried out by imitating the actual state of the livestock rumen so that the results can be used as a reference in the preparation of rations for livestock. Below is the data on the average dry matter digestibility, organic matter digestibility, NH_3 concentration, and the effect of treatment on the total concentrate VFA value.

Table 3. Average Dry Matter Digestibility, Organic Matter Digestibility, NH_3, and Total VFA

Parameter	Treatment				MSE	P-Value
	R_0±SD	R_1±SD	R_2±SD	R_3±SD		
DMDc(%)	$73.56^{a} \pm 0.75$	$74.50^{ab} \pm 0.61$	$74.98^{b} \pm 0.70$	$76.65^{c} \pm 0.90$	6.72	0.001^{**}
OMDc(%)	$69.50^{a} \pm 1.10$	$69.82^{a} \pm 1.00$	$70.07^{a} \pm 0.88$	$72.61^{c} \pm 0.99$	8.15	0.003^{**}
VFA(mM)	108.53 ± 8.52	110.75 ± 6.51	111.40 ± 2.35	112.77 ± 14.76	12.52	0.929^{tn}
NH_3(mM)	$9.74^{a} \pm 0.74$	$10.61^{ab} \pm 0.44$	$11.55^{bc} \pm 0.41$	$11.55^{bc} \pm 0.33$	3.88	0.000^{**}

Different superscripts in the same line showed a very significant effect (P<0.01)

Dry Matter Digestibility (DMD)

Dry matter digestibility (DMD) is one of the criteria to determine feed quality. The higher the dry matter digestibility value, the greater the opportunity for the animal to use the nutrients for growth. Dry matter digestibility due to treatment with concentrates containing Binahong flour with different levels is shown in table 3. The DMD values obtained in this study ranged from 73.56 to 76.65%. The results of the diversity test showed that the treatment had a very significant effect (P<0.01) on the dry matter digestibility value. The results of the Least Significant Difference (LSD) showed that the R3 treatment had a very significant difference (P<0.01) with the R0, R1, and R2 treatments. Treatment R0 was not significantly different (P>0.05) with treatment R1, but significantly different (P<0.01) with treatment R2 and R3. Meanwhile, treatment R2 was not significantly different (P>0.05) with treatment R1, but significantly different (P<0.01) with treatment R0 and R3. Therefore, it can be concluded that the higher the level of Binahong in the concentrate, the higher the dry matter digestibility of the feed. This was because the concentrate from the R3 treatment had a much higher protein content (PK 15.65%) so that it could activate rumen microbes, which could increase deamination and ultimately increase feed digestibility (Wahyuni et al., 2014). Riyanto et al., (2020) explained that high digestibility reflects the large contribution of nutrients, especially protein, to livestock. Proteins are degraded to ammonia by rumen microbes, used as a food source by bacteria, and absorbed for use in metabolic processes. As a result, the concentration of ammonia in the rumen is highly dependent on the protein quality of the feed given to livestock.

Organic Matter Digestibility (OMD)

The data in table 3. shows that the digestibility of organic matter ranges from 69.50 – 72.61%. The results of the diversity test showed that the concentrate treatment with the addition of different levels of Binahong had a very significant effect (P<0.01) on the digestibility of organic matter. The results of the Least Significant Difference (LSD) test showed that the treatment R3 had a very significant difference (P<0.01) with the treatment R0, R1, and R2. Meanwhile, for the treatment of R0, R1, and R2 there was no significant difference (P>0.05). The concentrate added with Binahong flour with a level of 30% (treatment R3), had a higher organic matter digestibility value (P<0.01) compared to the concentrate with the added level of Binahong flour (0%, 10%, 20%). Wahyuni et al., (2014) showed that high-protein diets can activate rumen microbes to increase deamination and ultimately increase dry matter and organic digestibility. The highest organic matter digestibility value was 72.61% in the R3 treatment. This is presumably because the tannins, saponins, and flavonoids contained in Binahong flour inhibit the growth of protozoa to allow microorganisms in the rumen to digest feed optimally (Miah et al., 2004).

Effect of Treatment on Total VFA Levels

Volatile Fatty Acid (VFA) is the final product of carbohydrate fermentation in the rumen. Carbohydrates are the main energy source of rumen fermented feed to produce VFA for rumen microbial growth (Amri and Yurleni, 2014). The formation of VFA begins with

carbohydrate fermentation in the rumen (Susilo et al., 2019). Increased VFA production is an indicator of whether the feed is susceptible or not to fermentation by rumen bacteria. Gumilar (2017) explained that the formation of VFA from fermentable carbohydrates in feed begins with breaking down the composition of complex carbohydrates into simpler forms through hydrolysis, then undergoes a degradation process to convert simple carbohydrates into pyruvate, which is then metabolized into VFAs. The high and low concentration of VFA is influenced by factors such as the physical form of the feed, the type of dissolved carbohydrates, rumen pH, digestibility of feed ingredients, feed control, and the number of additional feed ingredients (Nisa et al., 2017).

The total VFA content according to the results of statistical analysis ranged from 108.53 – 112.77 (mM). This value is still following the standard normal range for total VFA levels, which is 70,150 mM (Mcdonald et al., 2002). From these data, it can be seen that the highest total VFA content, respectively, was concentrated with 30% additional Binahong, followed by concentrate with 20%, 10%, and the lowest concentrate without Binahong (0%). Based on the results of the variance in Table 3, it can be seen that the treatment had no effect ($P > 0.05$) on the VFA concentration due to the addition of Binahong flour at different levels in the concentrate. However, empirically it was observed that there was an increase in the concentration of VFA in the Binahong flour treatment compared to the control. This is caused by the presence of saponins in Binahong flour so that it can reduce the population of protozoa and increase the total VFA concentration. This claim is supported by Hidayah (2016), who reported that the addition of tannins and saponins at optimal doses decreased the protozoan population and increased total and partial VFA production.

Effect of Treatment on NH_3 Concentration

Based on the research data in table 3, it can be seen that the NH_3 content in the concentrate containing various levels of Binahong ranged from 9.74-11.93 (mM). The results of the Variety Test showed that the treatment had a very significant effect ($P<0.01$) on the NH_3 concentration in vitro. The results of the Least Significant Difference (LSD) test showed that the administration of concentrates with Binahong levels up to a level of 30% (treatment R3) resulted in higher NH_3 compared to concentrates at levels of 10%, 20%, and control (without Binahong). The increase in the amount of NH_3 was caused by the high protein content in the treated feed as a result of the addition of Binahong leaf flour.

Therefore, the degradation process of feed protein is faster than the process of microbial protein formation, resulting in the accumulation of NH_3 in the rumen. This is consistent with the findings of McDonald et al (2002), who reported that the high protein content of feed and easily digested protein will increase the concentration of NH_3 in the rumen. McDonald et al further suggested that high NH_3 concentrations resulted in faster degradation of feed protein than microbial protein production, resulting in the accumulation of NH_3 in the rumen. In addition, MacDonald et al. suggested that the optimal concentration of NH_3 in the rumen is between 85-300 mg/L or 6-21 mM. However, the NH_3 obtained in this study ranged from 9.74 to 11.93 (mm). The increase in the amount of N was thought to be caused by the activity of the protease enzyme which helps to break down the protein concentrate which has been fully processed by proteolytic bacteria, undergoing a deamination process to form NH_3.

CONCLUSION

The use of Binahong flour up to a level of 30% in concentrate feed affects increasing DMDc, OMDc, VFA, and NH3 concentrations *in vitro*.

REFERENCES

Amri, U., dan Y. Yurleni. 2014. Efektivitas Pemberian Pakan Yang Mengandung Minyak Ikan dan Olahanya Terhadap Fermentasi Rumen Secara In Vitro. Jurnal Ilmiah Ilmu-Ilmu Peternakan, 17(1), 22-30.

Anggraini, D. I., dan M. M. Ali. 2017. Uji Aktivitas Antikolestrol Ekstrak Etanol Daun Binahong (*Anredera cordifolia* (Ten) Steenis) secara *in vitro*. Jurnal Ilmiah Kesehatan 9(1): 1–6.

Aprilliza, A. M., M. Nuzul, Y. N. Anggraeny, and E. Wina. 2021. The Role of Catechin Compounds and Its Derivates to Mitigate Methane Gas Production in the Rumen Fermentation. Indonesian Bulletin of Animal and Veterinary Sciences 31(1): 13. DOI: http://dx.doi.org/10.14334/wartazoa.v31i1.2548

Astuti, S. M., M. A. Sakinah, R. B. Andayani, and A. Risch. 2011. Determination of saponin compound from Anredera cordifolia (Ten) Steenis plant (Binahong) to potential treatment for several diseases. Journal of agricultural science 3(4) 224-232

Darsana, I. G. O., I. N. K. Besung, dan H. Mahatmi. 2012. Potensi daun binahong (Anredera cordifolia (Tenore) Steenis) dalam menghambat pertumbuhan bakteri Escherichia coli secara in vitro. Indonesia Medicus Veterinus, 1(3): 337-351.

Fauziah, F., H. Arifin., Elisma., N. Agustin. 2014. Pengaruh Pemberian Ekstrak Daun Binahong (*Anredera Cordifolia* (Ten) Steenis) terhadap Kadar Kolesterol Total Darah Mencit Putih Jantan Hiperkolesterolemiaemiaemia. In Prosiding Seminar Nasional Dan Workshop. 212–219.

Gaspersz, V. 2006. Teknik Analisis Dalam Penelitian Percobaan. ke-3. Tarsito.

Gumilar, D. A. K. W. 2017. Konsentrasi Volatile Fatty Acids (VFA), Amonia, (NH3) Dan Produksi Protein Mikroba Cairan Rumen Pada Domba Dengan Pemberian Pakan Siang Dan Malam. In *Skripsi*. Fakultas Peternakan Dan Pertanian. Universitas Diponegoro., Semarang, 1–129.

Hasanah, K. 2006. Penampilan Domba Ekor Tipis Jantan yang Diberi Konsentrat dan Rumput Gajah (*Pennisetum Purpureum*) pada Lama Penggemukan yang Berbeda. In Skripsi. Fakultas Peternakan. Institut Pertanian Bogor, Bogor.

Hidayah, N. 2016a. Pemanfaatan Senyawa Metabolit Sekunder Tanaman (Tanin Dan Saponin) Dalam Mengurangi Emisi Gas Metan Ternak Ruminansia. Jurnal Sain Peternakan Indonesia 11(2): 89–98.

Mcdonald, P., R. A. Edwards., J.F. D. Greenhalgh., dan C. A. Morgan. 2002. Animal Nutrition. 5 th. London: Longman Inc.

Miah, M. Y., M. S. Rahman., M. K. Islam., dan M. M. monir. 2004. Effect of Saponin and L-Carnitine On The Performance Snd Reproductive Fitness of Male Broiler. International Journal of Poultry Science 3 (8): 530–33.

Milllar, R. L. 1966. General Laboratory Procedures. In University of California Press.

Nisa D., J. Achmadi., dan F. Wahyono. 2017. Degradibilitas Bahan Organik Dan Produksi Total Volatille Fatty Acid (VFA) Daun Kelor (*Moringa Oleifera*) dalam Rumen Secara in Vitro. Jurnal Ilmu-Ilmu Peternakan 27(1): 12–17.

Riyanto, J., S. D. Widyawati., dan Sudibya. 2020. Pengaruh Perbedaan Rasio Menir Kedelai Proteksi dan Tanpa Proteksi Terhadap Konsumsi, Kecernaan, dan Nilai Nutrien Pakan Domba Ekor Gemuk. Livestock and Animal Research 18 (3): 240–45.

Susilo, E., L. K. Nuswantara., dan E. Pangestu. 2019. Evaluasi Bahan Pakan Hasil Samping Industri Pertanian Berdasarkan Parameter Fermentabilitas Ruminal Secara In Vitro. Jurnal Sain Peternakan Indonesia 14 (2): 128–36.

Tilley, J. M. A. and R. A. Terry. 1963. A Two-Stage Technique For The In Vitro Digestion Of Forage Crops. Journal Of The British Grassland Society 1 (8): 104–11.

Usman, Y. 2013. Pemberian Pakan Serat Sisa Tanaman Pertanian (Jerami Kacang Tanah, Jerami Jagung, Pucuk Tebu) Terhadap Evolusi PH, N-NH3 Dan VFA Di Dalam Rumen Sapi. Jurnal Agripet 13(2): 53–58.

Wahyuni, I. M. D., A. Muktiani, dan M. Christiyanto. 2014. Kecernaan Bahan Kering Dan Bahan Organik Dan Degrabilitas Serat Pada Pakan Yang Disuplementasi Tanin Dan Saponin. Jurnal Agripet 2(2): 115–24.

Widodo, N., Wihandoyo., N. D. Dono., Suprizal. 2016. Potensi Tepung Binahong (Andredera Cordifolia (Ten.) Steenis) Sebagai Fitobiotik Pada Pakan Ayam Broiler. In Prosiding Seminar Nasional Dan Workshop, Yogyakarta: Fakultas Peternakan, Universitas Gadjah Mada, 165–70.

Widu, A. A. 2019. Pengaruh Penggunaan Pakan Konsentrat Mengandung Tepung Binahong (*Anredera Cordifolia* Ten. Steenis) Terhadap Pemanfaatan Nutrien dan Pertumbuhan Domba Ekor Gemuk di Sabu Raijua. In *Tesis*, Kupang: Universitas Nusa Cendana, 1–84.