Supplementation Effects of Ground Cassava and Cassava Leaves with Different Ratios on *In Vitro* Digestibility of Rice Straw Based-Diet

Muhammad Rizqi Hariz Daulay[1], Dimas Hand Vidya Paradhipta[1,*], Cuk Tri Noviandi[1], Ali Agus[1], Andriyani Astuti[1], Karen Harper[2]

[1]*Faculty of Animal Science, Universitas Gadjah Mada, Jl. Fauna No.3 Bulaksumur, Yogyakarta, 55218, Indonesia*
[2]*School of Agriculture and Food Science, University of Queensland, Gatton Campus, 4343, Australia*
**Corresponding author.Email: dimas.hvp@ugm.ac.id*

ABSTRACT
The present study investigated the supplementation effects of ground cassava (GC) and cassava leaves (CL) with different ratios on digestibility of rice straw (RS) based-diet using *in vitro* technique. Two stages of *in vitro* ruminal digestibility were prepared and applied with different dietary treatments following: CON, consisted of 20% RS and 80% GC; T1, consisted of 20% RS, 70% GC, and 10% CL; T2, consisted of 20% RS, 60% GC, and 20% CL; T3, consisted of 20% RS, 50% GC and 30% CL; and T5, consisted of 20% RS, 40% GC, and 40% CL. Each dietary treatment was prepared in quadruplicate. All dietary treatments were incubated into rumen buffer along with three blanks for analysis of ruminal digestibility (*in vitro* first stage), and then continued into chloride acid solution with pepsin incubation for analysis of total digestibility (*in vitro* first and second stages). Previously, the rumen fluid was collected from two Bali steers that fed *Pennisetum purpureum* and commercial concentrate at 8:2 ratio. Both stages were conducted at 39ºC for 48 h, respectively. In the ruminal digestibility, an increasing ratio of CL was reported to decrease ($P<0.05$) dry matter digestibility (DMD) and organic matter digestibility (OMD) of diet. Digestibility of crude protein (CPD) in the rumen was highest ($P<0.05$) in dietary T3, followed by dietary T2, dietary T1, and then dietary T3 and T4, consecutively. Supported with results of ruminal digestibility, an increasing ratio of CL also decreased ($P<0.05$) DMD and OMD of diet in the total digestibility. Moreover, it also decreased ($P<0.05$) CPD of diet in the total digestibility. Therefore, the present study concluded that dietary treatment consisting of 70% GC and 10% CL with 20% RS presented the most optimum ratio resulting the highest digestibility through *in vitro* technique.

Keywords: *Cassava leaves, Ground cassava, Rice Straw, Supplementation ratio, In vitro digestibility.*

1. INTRODUCTION

The study of nutrient balance in the diet is important to maintain animal growth, especially considering the use of local feeds for animal diet. In Indonesia, rice straw (RS) is commonly used as roughage source for ruminant diet that is cheap and available during a year. In addition, cassava is widely planted and become an alternative to supply the requirement of feed in the field as energy source. Ground cassava (GC) contain crude protein (CP) at 1.86-3.84%, crude fiber at 2.93-3.91% [1] and provide almost 3000 kcal energy [2]. Besides that, cassava leaves (CL) also can be used as animal feed due to containing high nutrient quality. The CL contain CP at 17.7-24%, neutral detergent fiber at 59.6-66.2%, and acid detergent fiber at 41.8-54.6% [3]. With low NDF concentration and High CP concentration, CL can be applied as protein source for animal diet.

The RS based-diet is generally applied by almost of ruminant's farmers in Indonesia. However, RS can not be used as single feed due to poor nutrient content for animal maintenance. The use of RS for animal diet needs the supplementation of another sources, such as energy and protein feed. The information of nutrient balance among GC and CL was in limited study for RS based-diet. Several supplementary ratios among GC and CL were investigated in the present study to know its effect on digestibility using RS based-diet through two stages of *in vitro* technique. Two stage of *in vitro* digestibility

consists of ruminal incubation and pepsin incubation [4], which can help to estimate the total digestibility of diet. Therefore, the present study was aimed to know the supplementation effect of GC an CL on digestibility including dry matter digestibility (DMD), organic matter digestibility (OMD), and crude protein digestibility (CPD) of RS based-diet using two stages of *in vitro* technique.

2. MATERIALS AND METHOD

2.1. In Vitro Incubation

Fresh RS, GC, and CL were dried into 55ºC for 48 h and then ground to pass 1 mm screen according to previous study [5]. Dietary treatments were prepared with different supplementation ratios of GC and CL following: CON, consisted of 20% RS and 80% GC; T1, consisted of 20% RS, 70% GC, and 10% CL; T2, consisted of 20% RS, 60% GC, and 20% CL; T3, consisted of 20% RS, 50% GC and 30% CL; and T5, consisted of 20% RS, 40% GC, and 40% CL. In the first stage of *in vitro* incubation, rumen fluid was obtained from cannulated Bali steer fed *Pennisetum purpureum* and commercial concentrate with 8:2 ratio. Rumen fluid was collected before morning feeding and filtered with PeCap screen. Rumen buffer was prepared by mixture of filtered rumen fluid and McDougall solution at 1:4 ratio. Each dietary treatment at 0.5 gram was placed into incubation bottle (100 mL) and applied 50 mL of rumen buffer with four replications. The incubation bottle was gassed with CO_2 and closed tightly. All incubation bottles along with three blanks were incubated for 48 h at 39ºC. After 48 of incubation, 6 mL of HCL and 2 mL of 5% pepsin were added into incubation bottle for second stage of *in vitro* incubation. In the second stage of *in vitro* incubation, the incubation was also continued for 48 h at 39ºC. The ruminal digestibility (first stage of *in vitro* incubation) dan total digestibility (first and second stages of *in vitro* incubation) were determined in the present study. All procedures for *in vitro* incubation followed the protocol of Tilley and Terry [4].

2.2. Laboratory Analysis

Chemical composition of RC, GC, CL, and all dietary treatments were determined before conducting *in vitro incubation*. Dry matter (DM) was determined by drying 10 g of sample into a forced-draft oven at 105ºC for 24 h (method 934.01) and OM was determined with a muffle furnace at 550ºC for 5 h (method 942.05). The CP were determined by the producer of Kjeldahl (method 984.13) using N analyzer, while EE were determined by the procedure of Soxhlet (method 920.39). The CF was analyzed by boiled sample in acid and basal solution (method 987.10). All protocols to analyze chemical compositions followed AOAC [6]. The DMD, OMD, and CPD of ruminal digestibility and total digestibility were determined and calculated according to protocol of Tilley and Terry [4].

2.3. Statistical Analysis

Data in the present study were analyzed using one-way ANOVA procedure of Software Statistical Product and Service Solution (SPSS, version 16). Mean separation was performed by Duncan's Multiple Range Test. The significant differences were declared at $P<0.05$.

Table 1. Chemical compositions of dietary treatments in the present study

Item	Rice straw	Ground cassava	Cassava leaves	Dietary treatments[1]				
				CON	T1	T2	T3	T4
Dry matter	83.6	81.8	28.6	94.3	92.0	92.6	93.1	92.2
Organic matter	78.0	96.1	91.7	93.4	92.6	91.9	91.2	90.4
Crude protein	4.85	2.21	18.3	5.16	6.34	8.17	8.58	9.87
Ether extract	2.87	2.64	16.0	2.00	2.05	2.90	4.27	5.30
Crude fiber	35.9	2.21	12.4	9.19	10.0	12.0	13.3	15.5
Nitrogen-free extract	34.4	91.7	45.1	77.1	74.1	68.9	65.1	59.8
Total digestible nutrient	18.4	81.0	69.2	61.4	57.9	54.2	51.83	50.37

[1]CON, diet with 80% ground cassava; T1, diet with 70% ground cassava and 10% cassava leaves; T2, diet with 60% ground cassava and 20% cassava leaves; T3, diet with 50% ground cassava and 30% cassava leaves; and T5, diet with 40% ground cassava, and 40% cassava leaves. All diet applied 20% rice straw.

Table 2. Supplementation effects of ground cassava and cassava leaves with different ratios on *in vitro* digestibility

Item	Dietary treatments[1]				
	CON	T1	T2	T3	T4
Ruminal digestibility					
Dry matter	72.61^{a}±1.23	68.15^{b}±1.16	63.81^{c}±0.32	57.50^{d}±0.10	51.15^{e}±0.88
Organic matter	73.54^{a}±0.86	69.03^{b}±1.14	65.36^{c}±0.70	58.88^{d}±1.04	53.24^{e}±0.77
Crude protein	29.57^{c}±0.72	30.85^{b}±0.94	32.64^{a}±0.56	22.58^{d}±0.98	23.60^{d}±1.34
Total digestibility					
Dry matter	82.79^{a}±0.34	76.97^{b}±1.10	73.65^{c}±0.29	69.79^{d}±1.25	65.74^{e}±1.75
Organic matter	83.39^{a}±0.78	78.21^{b}±0.93	73.74^{c}±0.11	69.26^{d}±1.02	64.47^{e}±1.87
Crude protein	67.49^{a}±0.52	60.76^{b}±1.49	61.16^{b}±0.34	56.95^{c}±1.51	54.48^{d}±2.01

[1]CON, diet with 80% ground cassava; T1, diet with 70% ground cassava and 10% cassava leaves; T2, diet with 60% ground cassava and 20% cassava leaves; T3, diet with 50% ground cassava and 30% cassava leaves; and T5, diet with 40% ground cassava, and 40% cassava leaves. All diet applied 20% rice straw.
[a~e]Mean in the same row with different superscript differ significantly (P<0.05).

3. RESULT AND DISCUSSION

An increase of CL ratio in the diet increased (P<0.05) CP, EE, and CF concentrations (Table 1). This could be occurred due to the CL had a higher (P<0.05) CP, EE, and CF concentrations than GC, which also supported with the results of previous studies [1-3]. In addition, the total digestible nutrient (TDN) and nitrogen-free extract (NFE) decreased (P<0.05) by an increase of CL proportion in the diet, which supported with the result of OM. The CL presented lower (P<0.05) OM, NFE, and TDN than GC, which higher ratio of CL in the diet decreased those chemical composition parameters.

In the ruminal digestibility, an increase of CL ratio in the diet was reported to decrease (P<0.05) DMD and OMD concentrations (Table 2). Dietary T2 resulted in the highest (P<0.05) CPD concentration of ruminal digestibility, followed by T1, then T3 and T4. In the total digestibility, an increase of CL ratio in the diet resulted in the decreases (P<0.05) of DMD and OMD concentrations. Dietary T1 and T2 had no difference on CPD concentration of total digestibility. In addition, both dietary T1 and T2 presented higher CPD concentration than dietary T3 and T4. A higher ratio of CL in the diet reduced the concentrations of CP, NFE, and TDN (Table 1), which could decrease the nutrient degradation by animals [5]. High concentration of NFE in diet indicated high concentration of soluble carbohydrate, which was easily to degrade in digestive tract of ruminant [7]. In addition, higher CP concentration in diet also could increase the degradation of protein in the digestive tract of ruminant [8]. Even though had good nutrient content, CL also contained anti-nutritional compound, such as hydrocyanic acid (HCN) [4]. Supported with previous study [4], the high concentration of HCN in the diet could reduce the digestibility, both in the first and second stages of *in vitro* incubations. A higher ratio of CL could increase the concentration of HCN in the diet. It could be a main reason for the decrease of digestibility by the increase of CL ratio in the diet. Moreover, the presence of HCN was a cause for lower digestibility of all supplementary diets compared to CON diet

4. CONCLUSION

The present study concluded that dietary treatment consisting of 70% GS, 10% CL, and 20% RS presented the most optimum ratio that resulted the greatest digestibility compared to another supplementary treatment through *in vitro* technique.

ACKNOWLEDGMENTS

This work has carried out with financial support from the Australian Centre for Indonesian Agricultural Research (ACIAR).

REFERENCES

[1] G.D. Prasetyo, A.D. Kartika, Mashudi, *In vitro* Digestibility of cassava mealfrom various types of cassava plants (*Manihot utilissima*), Jurnal Nutrisi Ternak Tropis 2(2) (2019) 33-37. DOI: https://doi.org/10.21776/ub.jnt.2019.002.02.1

[2] Tilman, A., H. Hartadi, S. Reksohadiprodjo, S.Prawirokusumo, S. Lebdosoekjo. Basic of animal nutrition, Gadjah Mada University Press, Yogyakarta, Indonesia, 1991.

[3] A.O. Oni, C.F.I. Onwuka, O.M., Agribede, U.Y., Anele, O.O. Oduguwa, O.S. Onifade, Z.L. Tan. 2011. Chemical composition and nutritive value of four varieties of cassava leaves grown in South-Western Nigeria. Journal of Animal Physiology and Animal Nutrition 95 (2011) 583-590. DOI: 10.1111/j.1439-0396.2010.01086.x

[4] J.M.A. Tilley, R.A. Terry, A two-stage technique for the *in vitro* digestion of forage crops. Grass Forage Science 18 (1963) 104-111. DOI: 10.1111/j.1365-2494.1963.tb00335

[5] S.S. Lee, H.J. Lee, D.H.V. Paradhipta, Y.H. Joo, S.B. Kim, D.H. Kim, S.C. Kim, Temperature and microbial changes of corn silage during aerobic exposure, Asian-Australasian Journal of Animal Science 32 (2019) 988-995. DOI: 10.5713/ajas.18.0566

[6] Associaton of Official Analytical Chemists, Official methods of analysis, 18th Edition, AOAC International, USA,Washington, 2005

[7] A. Chesson, C.W. Forsberg, Polysaccharide degradation by rumen microorganisms, in: P.N. Hobson, C.S. Stewart (Eds.), The Rumen Microbial Ecosystem, Blackie Academic and Professional, London, UK, 1997, pp 329-381.

[8] R.J. Wallace, R. Onodera, M.A. Cotta, Metabolism of nitrogen-containing compounds, in: P.N. Hobson, C.S. Stewart (Eds.), The Rumen Microbial Ecosystem, Blackie Academic and Professional, London, UK, 1997, pp. 283-328.