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Digestibility Test of Cassava Leaves in Feed Supplement on Buffaloes by *In-vitro*

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Abstract: It has been studied the utilization of cassava leaves as a source of livestock feed supplements by *in-vitro* for buffalo milked that maintain traditionally. The purpose of this research is to evaluation the utilization of Cassava Leaves Flour (CLF) as a food supplement concentrate in pellet form for buffaloes and its influence on rumen characteristics. Rumen liquid taken from the Slaughter House. The research is an experiment using the Completely Randomized Design (CRD) that consist of four treatments and five replications. The treatment were : 1. Feed A+ 0% CLF; 2. Feed B + 10% CLF; 3. Feed C + 20% CLF and 4. Feed D + 30% CLF. The variable was observed the concentration of NH_3, bacteria count, total VFA production, Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) in the rumen. The result of this research indicated that the giving of Cassava Leaves Flour (CLF) as the source of carbon frame which can manipulating rumen with by pass protein is significantly ($p<0.05$) increase NH_3, bacteria count, VFA production, dry matter digestibility and organic matter digestibility of rumen liquid. The Giving CLF up to 10% (treatment B) can be used as source of feed supplement for buffaloes that kept traditionally thus can improve the productivity of livestock.

Key words: Feed supplement, cassava leaves flour, digestibility, rumen, buffalo

INTRODUCTION

The dependence of forage resources in field grass from the environment area with low nutritional quality, have adversely impact to the production and reproduction of buffaloes that kept by farmer. Supplementation of feed with high protein content is necessary to maintain the livestock productivities (Knox and Zahari, 1997). There are many ways that has been done by giving feed supplements with Urea Molasses Multi nutrient Block (UMMB). The use of supplements for buffaloes in India and Pakistan indicated that the adding feed supplement like UMMB can be increase milk production by 8% and the peak production longer (4 vs 2 weeks) than without supplementation (Randhawa *et al*., 2003), However, the inhibit of UMMB today is the limited of urea availability which is commonly used as an agricultural fertilizer in rural areas. The alternative to solution these problems is to use another of protein sources such as cassava leaves.

In Indonesia, cassava is widely grown crop after rice and it is the fifth-largest producer in the worlds after Brazil, Thailand, Nigeria and Zaire or the second largest in Asia after Thailand (FAOSTAT, 2006). This plant can produce a fraction of the leaves as a protein source of feed for ruminants with the potential for production by 1.2-1.9 Ton DM/ha and the protein content of 25-27% (Gomes *et al*., 1984). Wanapat (2009) states that cassava can be available throughout the year with high production and good quality and can contribute to the dairy and beef cattle, especially on small farms. The use of cassava leaves as protein source in dried form or pellet gives good result in ruminant feed.

Cassava leaves (Manihot Esculenta, Crantz) which has been dried (hay) is a source of protein and can be used as a supplement in ruminant nutrition, especially in dairy cows, beef cattle and buffaloes (Wanapat, 1993; Wanapat, 2000ab; Khang *et al*., 2005). The supply can be directly as supplement feed and as protein sources in concentrate form (Wanapat, 2000ab; Hong *et al*., 2003; Kiyothong and Wanapat, 2004ab) or as the component material in blocked feed that has high quality (Wanapat and Khampa, 2006).

Dried cassava leaves contain 19.5% protein dry matter and condensed tannin 4.0% dry matter. The use of dried cassava leaves on swamp buffalo as much as 1 kg dry matter/head/day was improving nutritional status based on the digestibility of dry matter, organic matter, protein, energy consumption and rumen NH_3-N and rumen ecology. According to the high protein content in cassava leaves and the availability of cassava as well as easy to obtain in rural, so its having potential use in feed supplement to replace the limited use of urea as a source of protein. Especially in West Sumatera, buffaloes that kept traditionally that its buffalo milk production is low (1-1.5 l/head/day; Roza and Aritonang, 2009). Usually in West Sumatera buffalo's milk is processed into *"dadih"*. *Dadih* is the traditional food from Minangkabau-West Sumatera by fermentation process of milk that using bamboo tubes.

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Table 1: Composition of the feed supplement material in pellet form with adding cassava leaves flour (kg/10 kg)

	Feed-A		Feed-B		Feed-C		Feed-D	
Bahan	%	Sum/10 kg	%	Sum/10 kg	%	Sum/10 kg	%	Sum/10 kg
Urea	10	1.0	7.5	0.75	5	0.5	2.5	0.25
Palm sugar	20	2.0	20.0	2.00	20	2.0	20.0	2.00
Ricebran	45	4.5	37.5	3.75	30	3.0	22.5	2.25
CLF*	0	0.0	10.0	1.00	20	2.0	30.0	3.00
Cement	10	1.0	10.0	1.00	10	1.0	10.0	1.00
Salt	8	0.8	8.0	0.80	8	8.0	8.0	0.80
Mineral-mix	2	0.2	2.0	0.20	2	0.2	2.0	0.20
Water	5	0.5	5.0	0.50	5	0.5	5.0	0.50
Total	100	10.0	100.0	10.00	100	10.0	100.0	10.00

information: *) Cassava Leaves Flour

Based on the description above then conducted a study to observe the utilization of cassava leaves as a source of feed supplements for buffaloes which kept traditionally. The specifics purposes to be achieved is to evaluate cassava leaves as feed supplement that replace urea in improve the productivity by *in-vitro*.

MATERIALS AND METHODS

The materials used in this research is cassava leaves (*Manihot esculenta*, *Crantz*) is made as a mixture of feed supplements in pellet form with composition : urea, palm sugar, cassava leaves, ricebran, salt, cement, mineral mix and water. Cassava leaves are dried in the oven with the temperature 55-60°C for 3-4 days and then it's ground and mixed homogeneous according with the treatment formula and then molded and dried under the sun bright for 1-3 days. Buffaloes rumen liquid obtained from the Slaughter House.

Experimental method: This research is done by experimentation with Completely Randomized Design (CRD) in four treatments and five replications. The treatment is by giving feed such as : Feed A (R_0) Feed B (R_1), Feed C (R_2), Feed D (R_3), with each compositions was listed in Table 1. The variable observed were nutrient content of feed supplements (proximate analysis : dry matter, organic material, crude protein) and ruminant digestibility by *in-vitro* (NH_3, total of VFA, bacteria count, Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) by Tilley and Terry (1963).

***In-vitro* procedure:** The samples of feed supplements put into the tube which was filled to Erlenmeyer about 5 grams and put the 200 ml of *Mc Doughall's* buffer liquid (temperature 39°C), pH 6.92-7.02 and add 50 ml rumen liquid as a donor of microbes. Flow CO_2 gas among ± 30 sec in order keep the anaerobe condition and cover the tube tidily. And then, put the sample into water bath and incubated for 2 x 24 hrs. After the fermentation ends put the water into the Erlenmeyer tube. Subsequently, all samples were centrifuged at 1200 rpm for 15 min, super nathan was taken to the next measured of NH_3, bacteria count and total of VFA while the precipitate was collected and dried for analysis and DMD and OMD.

RESULTS

The result of the effect CLF giving to rumen liquid characteristics are listed in Table 2. The average production of NH_3 rumen (Table 2) ranged from 11.24-14.32 mg/100 ml. The average concentration of NH_3 contain in the treatments of B is 14.32 mg/100 ml and the lowest is in D treatment about 11.24 mg/100 ml. The result of statistics analysis show that the treatment is very significantly decreases the rumen NH_3, where treatment D is lowest compared to treatment A and treatment B but not significantly different ($p>0.05$) with treatment C.

Microbial activities can be seen from the production of rumen microbes (Table 2). The bacteria count was produced in rumen ranged about 34.45 x 10^9-60.95 x 10^9 CFU/ml. The highest of bacteria count is in treatment B about 60.95 x 10^9 CFU/ml and the lowest is treatment D about 34.45 x 10^9 CFU/ml. The result of DMRT showed that the rumen bacteria count in treatment B were significantly highest but not significantly different with treatments A and C and the lowest rumen bacterias count is in treatment D that giving CLF up to 30%.

From the Table 2 showed that the production of VFA in treatment B (114.75 mM) is highest and the lowest is in treatment A (90.00 mM). The result of statistic analysis shows that the influence of the treatment is very significant ($p<0.01$) on total production VFA, where the total of VFA in treatment B is the highest compared treatment C and treatment D and the lowest is VFA total in treatment A. There are significantly different among the treatment from each other.

The degradation rate of nutrient with CLF feed can be seen from Table 2. The highest digestibility of dry matter is treatment C (63.73%) and the lowest is treatment D about 46.64%. The result of statistic analysis shows that the treatment influence significantly ($p<0.01$) on dry matter digestibility. The result of DMRT shows that dry matter digestibility on treatment C is highly significant ($p<0.01$) highest compared with treatment A and treatment D, but not significantly different ($p>0.05$) with treatment B.

Table 2: The average concentration of NH_3, bacteria count, VFA total, DMD and OMD

Variable	A	B	C	D
NH_3 (mg/100 ml)	13.37^a	14.32^a	11.97^b	11.24^b
Bacteria count (x10^9CFU/ml)	44.42^{ab}	60.95^a	50.95^{ab}	34.45^b
Dry matter digestibility (%)	53.84^b	62.19^c	63.73^c	46.64^a
Organic matter digestibility (%)	64.36^d	67.52^c	66.73^c	62.90^c
VFA total (mM)	90.00^d	114.75^a	106.75^b	99.25^c

^{ab}Means with common superscript do not difference significantly (p>0.05)

Table 3: Proximate analysis of feed supplement

Variable	Pakan A	Pakan B	Pakan C	Pakan D
Moisture (%)	4.77	5.95	7.09	8.99
Ash (%)	27.23	27.74	27.89	29.14
Crude protein (%)	30.44	36.40	24.98	19.78
Fibre (%)	13.41	14.95	15.57	17.66
Fat (%)	5.12	4.16	4.41	4.28
Extract material non nitrogen (%)	17.80	22.75	27.15	29.14
Energy (Kkal/kg)	3698.00	3729.00	3761.00	3718.00

Source: Laboratory of Nutritions and Chemistry Padjajaran University

The highest of organic matter digestibility (Table 2) is treatment B by 67.52% and the lowest is treatment D about 62.90%. The statistic analysis shows that the use of CLF is influence very significant ($p<0.01$) on organic matter digestibility. The result of DMRT shows that the digestibility of organic material in the treatment B is the highest but not significant different with the treatment C as well as the digestibility of dry matter.

DISCUSSION

The high concentration of NH_3 in treatment B caused by the content of Crude Protein (CP) in treatment B is highest than treatment C and treatment D (see Table 3). Crude protein is a precursor in production of NH_3 and with the high supply of protein in the feed formula has increased the activity of proteolytic microbes to produces the NH_3 more in the rumen. This is consistent with the statement of Frannzolin and Alves (2010) that the levels of NH_3 in rumen is an indication of the degradation process and the process of protein synthesis by rumen microbes. If the feed is deficient in protein or the feed is defenses to protein degradation, so the production of NH_3 will be running down and the growth of rumen microbes will to be slowly. Consequently, the digestibility of feed will decrease. Just like in the result of this research, that NH_3 rumen can be increase when the giving of CFL up to 10% (treatment B) as reflected on Table 2. But, when the giving of CFL as much as 20% (treatment C) and 30% (treatment D) the NH_3 production in the rumen decrease significantly. This is caused by crude protein content in the treatment C and D is low so that make a NH_3 that is the main precursor of microbial protein synthesis is low too, whereas the number of NH_3 must be complete. The consequently, there are to be limited of microbes activities, microbial protein synthesis and digestibility rate, so that the energy supply is decrease. The ammonia in rumen is required for growth and development of microbe and for synthesis of microbial protein. Approximately 82% of microbial species has been able to use ammonia as nitrogen source (Orskov, 1982).

The rumen bacteria count within the normal with range is between 10^9 CFU/ml to 10^{12} CFU/ml is corresponding to that presented by Fuller (1992). Treatment D has a negative impact on rumen bacteria, where the bacteria count decreased drastically. The development of bacteria is inhibit because the substitution of urea by the CLF is not utilized properly. So it looks NH_3 decreased and microbial activity also decreased. The giving of CLF as urea substitution is influenced on rumen bacteria count that indication to fermentation rate in rumen. The higher the rumen fermentation activity, the higher the rumen bacteria count. As stated by Czerkawski (1986), fermentation in the rumen suggest the activity and growth of rumen microbes.

The VFA total of rumen liquid increase by giving CFL. This is caused by CFL given will increase the microbes activities in rumen, where the CFL is the source of branched chain fatty acids that used as carbon chain to synthesis the amino acids. VFA is the final product of carbohydrate fermentation and it is used to energy resources for ruminants and for rumen microbial growth. Thus, the high number of protein that contained in feed can increase the bacteria ability to produce VFA. It is accordance with the experiments of Wanapat *et al.* (2010) that NH_3 in buffaloes rumen which give cassava leaves are 11.85 mM and VFA 116 mM.

Dry matter digestibility in treatment B and C are the highest. It caused by the giving CLF up to 20% that can increase the bacteria activities in cellulolytic bacteria making for degradation of carbohydrate into monosaccharides or glucose. But, the higher the CFL giving up to 30% in treatment D can decrease dry matter digestibility. This is caused by CFL giving is too much can decrease the rumen bacteria count, so that the microbe that degradation of carbohydrate fermentatively is decrease and make the dry matter digestibility decrease also.

In this research indicated that treatment B is the best results because bacteria can growth well in connection with the fulfillment of nitrogen that bacteria need. By CFL added can be stimulate the production of cellulolytic bacteria, where CFL is a source of carbon form which manipulating rumen by increasing pH and ecology of rumen through by pass protein (tannin complex protein) and can be increase the dry matter digestibility from low quality food. While tannin that is condensed can improve the absorption in rumen intestines (Makkar *et al*., 1996). The improving of dry matter digestibility by giving CFL caused by the increasing of dry matter consumption and NH_3 production as the effect of CFL.

The increasing of dry matter digestibility is the results of CFL giving and will followed by the increasing organic matter digestibility because dry matter digestibility is directly promotional to organic materials. This is caused by the improving of population and microbes activities in rumen with the availability and the balance of nutrients from CLF. Similar to the research of Preston and Leng (1987), for the growth of the rumen microbes and the using of ammonia as a source of microbes protein forming must be have carbohydrate and energy availability itself. From these results, the best digestibility of organic matter is in treatment B about 67.52%. This results are almost same with digestibility of dry matter that obtained by Foiklang *et al*. (2010) that giving the cassava leaves as a source of protein in feed block about 67.62-68.87%.

Conclusion: The giving of Cassava Leaves Flour (CLF) as the source of carbon frame which can manipulating rumen with by pass protein is significantly ($p<0.05$) increase NH_3, bacteria count, VFA production, dry matter digestibility and organic matter digestibility of rumen liquid. The Giving CLF up to 10% (treatment B) can be used as source of feed supplement for buffaloes that traditionally maintain thus can improve the productivity of livestock.

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REFERENCES

Czerkawski, J.W., 1986. An Introduction to Rumen Studies. Pergamon Press. Oxford, Toronto, Sidney, Frunkfurt. 3-10; 19-27.

FAOSTAT, 2006. Subject: Production quantity, Commodity: Cassava, Year: 2006, Country: World> Diakses 10 January 2010 dari http://faostat.fao.org/DesktopDefault.aspx?PageID=567&lang=en.

Foiklang, S., M. Wanapat and W. Toburan, 2010. Effect of various protein sources in high-quality feed block on dry matter intake, digestibility and rumen fermentation in Swamp Buffalo. J. Anim. Vet. Adv., 9: 2593-2599.

Frannzolin, R. and T.C. Alves, 2010. The Ruminal Physiology in Buflo Compared with Cattle. Proceeding 9th Wold Buffalo Congress, Buenos Aires, Abril 2010.

Fuller, R., 1992. Probiotic 1. The scientific Basic. 1st Edn., Chapman and Hall, London.

Gomes, G., M. Valdiviso, D.de la Cuesta and T.S. Salcedo, 1984. Physico-chemical Make-up of Buffalo Milk in the Standardization of Techniques of Handling Processing and Manufacture of Products. XIX Int. Dairy Congress., B5: 358-377.

Ganguli, N.C., 1974. Physico-chemical make-up of buffalo milk in the standardization of techniques of handling processing and manufacture of products. XIX Int. Dairy Cong., B5: 358-377.

Hong, N.T.T., M. Wanapat, C. Wachirapakorn, P. Pakdee and P. Rowlinson, 2003. Effects of timing of initial cutting and subsequent cutting on yields and chemical compositions of cassava hay and its supplementation on lactating dairy cows. Asian-Aust. J. Anim. Sci., 16: 1763-1769.

Khang, D.N., H. Wiktorsson and T.R. Preston, 2005. Yield and chemical composition of cassava foliage and tuber yield as influenced by harvesting height and cutting interval. Asian-Aust. J. Anim. Sci., 18: 1029-1035.

Kiyothong, K. and M. Wanapat, 2004a. Supplementation of cassava hay and stylo 184 hay to replace concentrate for lactating dairy cows. Asian-Aust. J. Anim. Sci., 17: 670-677.

Kiyothong, K. and M. Wanapat, 2004b. Growth, hay yield and chemical composition of cassava and stylo 184 grown under intercropping. Asian-Aust. J. Anim. Sci. 17: 799-807.

Knox, M. and M.W. Zahari, 1997. Urea molasses blocks for parasite control. Proceeding of a Workshop organized by FAO and the Denish Center for Experimental Parasitology Ipoh, Malaysia.

Makkar, G.S. and S. Saijpaul, 1996. Uromin-Lick - A success story. Progressive Farming, May: 22-23.

Orskov, E.R., 1982. Protein Nutrition in Ruminants. Academic Press, New York.

Preston, T.R. and R.A. Leng, 1987. Maching Ruminant Production System With Available Resource in the Tropic and Subtropics. Penambul Books Armidale. New South Wales. Australia, pp: 21-128.

Randhawa, B., G.R. Pangaonkar, P.S. Brar and A.S. Nanda, 2003. Certain biochemical alterations in relation to the onset of ovarian activity in postpartum buffaloes with supplementary feeding. 4th Asian Buffalo Congress on Buffalo for food security and rural development. New Delhi, India, 25-28 February 2003.

Roza, E. and S.N. Aritonang, 2009. Penerapan Aspek Teknis Pemeliharaan Ternak Kerbau Di Kecamatan Lembah Gumanti Kab. Solok. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner 2010. Puslitbang Peternakan Bogor, pp: 47-55.

Tilley, D.M.A. and R.A. Terry, 1963. A two stage technique for *in vitro* digestion of forage crops. J. Br. Grass. Soc., 18: 104-111.

Wanapat, M., 1993. Utilization of cassava leaf *(Manihot esculenta, Crantz)* in concentrate mixture for swamp buffaloes. In: Ruminant Nutrition Technology Research Project (RUNTERP), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand, pp: 50-59.

Wanapat, M., 2000a. Rumen manipulation to increase the efficient use of local feed resources and productivity of ruminants in the tropics. Asian-Aust. J. Anim. Sci., 13 (Suppl.): 59-67.

Wanapat, M., 2000b. Role of cassava hay as animal feed in the tropics. In: Proc. Interntional Workshop on Current Research and Development in Use of Cassava as Animal Feed. July 23-24, 2001, Khon Kaen University, Thailand, pp: 13-19.

Wanapat, M. and S. Khampa, 2006. Effect of cassava hay in high-quality feed block as anthelmintics in steers grazing on ruzi grass. Asian-Aust. J. Anim. Sci., 19: 695-698.

Wanapat, M., 2009. Potential uses of local feed resources for ruminants. Trop. Anim. Health Prod., 41: 1035-1049.

Wanapat, M., S. Foiklang and W. Toburan, 2010. Effect of various plant protein sources in high-quality feed block on dry matter intake, digestibility and rumen fermentation in swamp buffalo. Khon Kaen University, Thailand. J. Anim. Vet., 20: 2593-2599.