IN VITRO FERMENTABILITY, DEGRADABILITY AND MICROBIAL BIOMASS PRODUCT OF COMPLETE RATION CONTAINING A COMBINATION OF FIELD GRASS, CONCENTRATE AND NUTRIENT RICH SUPPLEMENT

D. S. Wahyuni[1], A. S. Tjakradidjaja[2] and Suharyono[3]

[1] Center for Agricultural Farming Production Technology, Agency for the Assessment and Application of Application of Technology, BPPT Building II, 16th floor, Jl. MH Thamrin No. 8, Jakarta 10340-Indonesia

[2] Department of Nutrition and Feed Technology, Animal Husbandry Faculty, Bogor Agricultural University, Jl. Agatis, Darmaga Campus, Bogor 16680-Indonesia

[3] National Nuclear Energy Assessment, South Jakarta-Indonesia

Corresponding E-mail: dimar_sari_wahyuni@yahoo.com

Received November 02, 2009; Accepted November 30, 2009

ABSTRACT

The objective of this experiment was to obtain an optimum combination between field grass, concentrate and Nutrient Rich Supplement (NRS) based on *in vitro* study using Hohenheim gas test. The experimental diets were: R1 (control diet = 70% field grass + 30% concentrate), R2 (70% field grass + 25% concentrate + 5% NRS), R3 (70% field grass + 20% concentrate + 10% NRS) and R4 (70% field grass + 15% concentrate + 15% NRS). A randomized block design with four treatments and four replications was carried out. Buffalo rumen fluid was taken in different time and was used as block or replication. Data were analyzed by Analysis of Variance (ANOVA). Significant differences among treatments were determined by contrast orthogonal. The results showed that total gas production and total VFA concentration were highest ($P<0.05$) in R4 among the treatments. Addition of 15% NRS in complete ration (R4) increased NH_3 concentration 10.34%. Addition of 10% NRS and 15% NRS in complete ration (R3 and R4) improved the microbial biomass product compared to control and 5% NRS in complete ration (R1 and R2). Dry matter (DM) and organic matter (OM) degradability were significantly higher ($P<0.01$) in 10% NRS and 5% NRS in complete ration (R3 and R2) compared to control and 15% NRS in complete ration (R1 and R4). It was concluded that combination between 70% field grass, 20% concentrate and 10% NRS in complete ration (R3) was more optimal for improving gas production, total VFA, NH_3 concentration, microbial biomass product, dry matter degradability and organic matter degradability, compared to control ration.

Keywords: *complete ration, NRS (Nutrient Rich Supplement), fermentability, degradability, microbial biomass product*

INTRODUCTION

One cause of low productivity of ruminant livestock in Indonesia is the limited supply of grass in the dry season. In addition, the problems were faced by farmers is lower concentrate quality, highly protein price, and nutrient deficiency. Livestock in Indonesia still have nutritional problems, which happened deficient and no good nutrition imbalance of energy, protein and minerals, including vitamins (Suryahadi, 2003). One effort that can be done to overcome the problem of nutritional deficiencies in cattle is to feed the manipulation techniques through the provision of complete ration.

Rations based on agro-industrial waste used in the research is field grass, concentrates and Nutrient Rich Supplement (NRS) which contained bypass protein, defaunation agents, organic minerals and turmeric. The advantages of NRS is quite high at 28.09% crude protein. According to Sutardi *et al*. (1983), the feed rate of degradation which had a high protein needs to be protected so that an increase in the potential availability of amino acids in the small intestine. Defaunation agents were way to control the population of protozoa in the rumen (Erwanto, 1995). Supplementation Zn and Cu minerals in organic form can enhance the absorption of the post-rumen organ than inorganic (Tanuwiria, 2004).

According to Tanuwiria *et al*. (2006), turmeric as an antibacterial and antioxidant more effective when combined with Zn-Cu-organic and organic in the immune system of cattle.

The objective of this experiment was to know the effect of granting complete ration based on the fermentability, degradability and microbial biomass product. Besides that, was to obtain the best combination of grass field, concentrate and NRS in complete rations based on fermentability, degradability and microbial biomass product using the Hohenheim gas test method.

MATERIALS AND METHODS

Complete rations containing raw material feeds from agricultural and food industry by-products consisted of field grass, concentrate and Nutrient Rich Supplement (molasses, cassava waste, bran, soy waste, coconut oilcake, bean curd waste, tea waste, hibiscus leaves, bone meal, limestone, urea, mix mineral, salt, $ZnCl_2$, $CuCl_2$ and turmeric were used in *in vitro* fermentability, degradability and microbial biomass product. One ruminally fistulated *B. bubalis bubalis* buffalo were used as rumen liquor donor. Animal was feed twice daily (07:00 and 16:00 h).

Research Instruments

Research instruments consisted of a set of gas production test equipment, 100 ml Hohenheim glass syringes, Conway dish, a set of distillation equipment and shaker water bath. Nutrient Composition of Field Grass, Concentrate and NRS (DM basis) can be seen in Table 1.

Treatments, Observed Variables and Statistical Analysis

The experimental diets were: R1 (control diet = 70% field grass + 30% concentrate), R2 (70% field grass + 25% concentrate + 5% NRS), R3 (70% field grass + 20% concentrate + 10% NRS) and R4 (70% field grass + 15% concentrate + 15% NRS). Variables were observed in this study consisted of gas production, concentrations of Volatile Fatty Acids (VFA), concentration of ammonia (NH_3), microbial biomass product, dry matter degradability and organic matter degradability. A randomized block design with four treatments and four replications was carried out. Buffalo rumen fluid was taken in different time and was used as replication. Data were analyzed by Analysis of Variance (ANOVA). Significant differences among treatments were determined by contrast orthogonal test (Steel dan Torrie, 1991).

In vitro Rations Measurement

Total VFA concentration measurement was conducted according to method of Kromann *et al*. (1967). NH_3 concentration analysis performed by the method of Conway (1962). Microbial biomass product was measured using techniques that have been done (Blummel *et al*., 1997).

In vitro Gas Production Test

Gas production measurement was conducted according to method of Hohenheim Gas Test

Tabel 1. Nutrient Composition of Field Grass, Concentrate and NRS (DM basis)

Item	Nutrient Composition		
	Field Grass	Concentrate	NRS
Moisture (%)	7.99	8.78	13.25
Dry Matter (%)	92.01	91.22	86.75
Ash (% DM)	7.8	17.47	14.77
Crude Fat (% DM)	6.38	8.44	11.23
Crude Protein (% DM)	7.9	8.54	28.09
Crude Fiber (% DM)	33.47	14.17	15.78
Ether Extract (% DM)	44.45	51.38	30.13
TDN (% DM)	57.31	75.37	74.15
Ca (% DM)	0.26	0.31	0.2
P (% DM)	0.11	0.12	0.02

Source: Result Analysis of Biological Resources Research Center and Biotechnology IPB, 2007

Table 2. *In vitro* Total Gas Production, Total VFA, NH_3 Concentrations, Microbial Biomass Product, Dry and Organic Matter Degradability of Complete Ration after 48 h of Incubation

Variable	Treatments			
	R1	R2	R3	R4
Total Gas Production (ml/200 mg DM)*)	27.62 ± 2.11^{a}	28.04 ± 1.80^{a}	28.54 ± 1.65^{b}	28.77 ± 1.63^{b}
Total VFA Concentration (mM)*)	64.98 ± 7.25^{a}	72.55 ± 5.43^{b}	71.70 ± 3.98^{a}	77.35 ± 6.68^{b}
NH_3 Concentration (mM)**)	20.50 ± 0.36^{A}	21.53 ± 0.67^{B}	22.62 ± 0.89^{Ca}	23.49 ± 0.71^{Cb}
Microbial Biomass Product (mg)	70.78 ± 17.04^{A}	90.36 ± 19.43^{B}	$102,00 \pm 14.00^{C}$	103.83 ± 12.35^{C}
Dry Matter Degradability (%)	42.52 ± 1.51^{A}	47.27 ± 2.46^{B}	49.45 ± 0.92^{B}	43.24 ± 4.30^{A}
Organic Matter Degradability (%)	39.03 ± 2.37^{A}	45.31 ± 3.33^{B}	46.88 ± 1.96^{B}	40.81 ± 4.09^{A}

Values with different superscript in the same row indicate significant differences ($P < 0.05$) ** Values with superscript different capitals in the same row indicate a significant difference ($P < 0.01$) and the superscript lowercase letters differently on the same row indicate significant differences ($P < 0.05$)

(Menke *et al*., 1979; Menke and Steingass, 1988). Samples (0.375 g) were weighed into 100 ml glass syringes (Model Hohenheim). Previously, the sample was dried at a temperature of 600ºC and was mashed with 1 mesh sieve size. Solution and the media were prepared in advance. The media preparation was made by mixing 752.26 ml of distilled water, 0.16 ml solution mikromineral, 501.5 ml of rumen buffer solution, 250.76 ml solution makromineral and 0.68 ml resazurin solution. The media was prepared a day before the rumen fluid, kept inside a large Erlenmeyer flask and sealed with paper to keep the film under anaerobic conditions (CO_2 gas flowed for 5 minutes). Mixed media and 453.42 ml of rumen fluid (temperature 39°C) was stirred with a magnetic stirrer and flowed along with CO_2 gas for 5 minutes, then was added by a solution of reducing as much as 41.22 ml. The medium has changed color from blue to pink and finally colorless. This showed that the reduction process occurs completely. A total of 30 ml of rumen fluid and medium mixture was injected into each Hohenheim syringe containing 0.375 grams of the sample by the silicon tube with a dispenser that had been set volume.

The piston first was covered with petroleum jelly before the sample was put into a syringe. That was done so that the gas did not leak out. Gas bubbles was contained in the syringe out, then covered with a silicon hose clamps, the piston position was read and recorded on the clock to zero (0). Samples were incubated and the resulting gas production was observed in the incubation time interval 0, 3, 6, 9, 12, 24 and 48 hours at a temperature of 39ºC in a water bath incubator. Samples were incubated each duplo. If the position of piston was above 60 ml, this value was recorded and then the clamp was opened and the piston was returned to the position of 30 ml, then the amount of gas previously recorded. Reading was done quickly to avoid changes in temperature. Gas production can be calculated by using the following equation (Menke *et al*., 1979; Menke and Steingass, 1988):

$$GP(ml/200\text{mg } DM\ 48\text{h}) = \frac{(V_{48} - V_0 - Gb_0)\, x200}{B}$$

where
GP = Gas Production
V_{48} = Volume of gas (ml) 48 hours

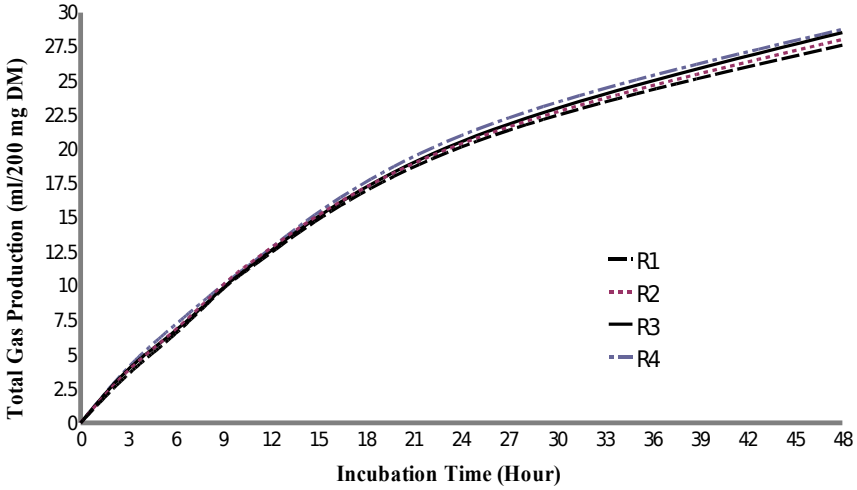

Figure 1. The rate of in vitro gas production (ml/200 mg DM) on the incubation time 0-48 hours

V_0 = Volume of gas (ml) initial incubation
Gb_0 = Blank Average of Gas Production at 48-hour incubation
B = Weight of Test Samples (mg DM)

Dry and Organic Matter Degradability Measurement

After 48 hours of fermentation, rumen microbial fermentation was stopped. Hohenheim glass Syringe was placed on cold water or ice to stop microbial activity, and in turn the contents of a syringe was inserted into a glass beaker and was added with a solution of Neutral Detergent Solution (NDS). The next glass beaker was heated to boil and reflux for 1 hour until brown. Reflux results were filtered with a Crucible glass and put into the 105ºC oven for 24 hours. Digestibility Residues were weighed and put into 105ºC oven to get dry residues (DM). Furthermore, dry residue was inserted into the 550-600ºC furnace to obtain the ashes, and the lost material during the furnace was a residue of organic material (OM residues). That was also done on blank. Blank was the residue of the fermentation without feed samples. Material was from the media and rumen fluid which got the same treatment and then was fermented to take the residuals (Blummel *et al*., 1997). Dry and Organic Matter Degradability were calculated using the formula:

$$DMD(\%) = \frac{DM\ Sample\,(g) - DM\ Residue\,(g) - DM\ Blank\,(g)}{DM\ Sample\,(g)}\ x100\%$$

$$OMD(\%) = \frac{OM\ Sample\,(g) - OM\ Residue\,(g) - OM\ Blank\,(g)}{OM\ Sample\,(g)}\ x100\%$$

where
DM = Dry Matter
OM = Organic Matter

RESULTS AND DISCUSSION

In vitro Total Gas Production, Total VFA, NH3 Concentrations, Microbial Biomass Product, Dry and Organic Matter Degradability of Complete Ration after 48 h of Incubation are presented in Table 2.

Total Gas Production

The results showed that total gas production of R4 was highest ($P<0.05$) among the treatments. Based on orthogonal contrast test results, the total gas production average of treatment R3 and R4 (28.54 and 28.77 ml/200 mg DM) were higher ($P<0.05$) than the total gas production average of R1 and R2 (27, 62 and 28.04 ml/200 mg DM). Treatment of R3 and R4 contain organic material (energy and protein) were higher than R1 and R2 so that the potential in producing higher gas and rations are to be expected as well as potentially easily degraded rations. The rate of in vitro gas production (ml/200 mg DM) on the incubation time 0-48 hours can be seen on Figure 1.

Winugroho *et al*. (1997) stated that the peak of gas production was obtained in the first 24

hours, then decreased until 96 hours and finally reached zero. This sort of thing will happen to all types of feed because if a type of feed was longer in the rumen, protein sources of feed would decrease. It could be converted into NH_3 and was utilized by microorganisms.

Total VFA Concentration

The results showed that total VFA concentration of R1 and R3 treatments were lower ($P<0.05$) than total VFA concentration of R2 and R4 treatments (Table 2). However, the R4 still produces a higher total VFA (77.35 mM) compared to R2 (72.55 mM). Addition of 10% NRS in complete ration (R3) increased total VFA conecentration 10.34% compared to control ration. The increase of VFA concentration reflected protein and carbohydrate sources were easily digested (organic materials) in the treatment R4 complete ration that can be fermented by rumen microbes. This was consistent with results obtained Blummel *et al*. (1993), the increase of easily degraded carbohydrates could increase digested dry matter. Digested dry matter was converted by microbes into VFA and rumen microbial protein increasingly. The addition of protein sources could stimulate rumen microbial growth without the addition of a source of carbohydrates balanced easily degraded.

Amonia (NH_3) Concentration

The NH_3 concentrations average of treatment R3 and R4 (22.62 and 23.49 mM) were higher ($P<0.01$) than the NH_3 concentrations average of R1 and R2 (20,50 and 21,53 mM). Addition of 10% NRS in complete ration (R3) increased NH_3 concentration 10.34% compared to control ration. Rumen fluid NH_3 concentrations that support growth of rumen microorganisms is 4-12 mM and the optimum concentration of NH_3 is 8 mM (Sutardi, 1980).

NH_3 levels produced in this study showed that results were higher than normal range of rumen fluid NH_3, but were still within the normal range of ammonia concentration did not cause poisoning. High concentrations of NH_3 were expected because of feed protein degradation process was faster than the formation of microbial protein, thus resulting ammonia accumulated in the rumen (McDonald *et al*., 2002). This happens because of the concentration of NH_3 measurements performed on 48-hour incubation time. Ammonia which was produced in the incubation time, came from the influence of NH_3 generated from buffer solution and NH_3 production of protein feed that was not absorbed in the *in vitro* system so that the accumulation of NH_3 occurred in the rumen fluid syringe caused high NH_3 concentration (Firsoni, 2005). Complete rations made in this study contains Rich Nutrient Supplements (NRS) which is a source of nutrients (energy, protein and minerals) are most easily degraded. Source of protein contained in soy sauce NRS include soy waste, coconut oilcake, bean curd waste, urea, tea waste and hibiscus leaves. This can be proved that the crude protein content for high NRS (28.09% DM), so that the concentration of ammonia produced in this study high.

Microbial Biomass Product

The results showed that microbial biomass product of R4 was highest ($P<0.01$) among the treatments. Microbial biomass production that had the highest value found in the R4 treatment (103.83 mg). This means that the combination of feed in the ration was the most optimal treatment produced the highest microbial biomass, presumably because of the highest NRS content among other treatments. This was consistent with results obtained by Allen (1996), supplementation strategies could be to maximize microbial protein production in the rumen which aimed to maximize the productivity of livestock. Microbial protein production was highly depended on the available substrate for microbes in the form of easily fermented and degraded organic matter. In addition, increasing levels of NRS on each ration treatment could increase the source of easily degraded carbohydrate (molasses and starch) and NPN sources (urea) also had implications on rumen microbial biomass increased. This statement was supported by Thu and Uden (1994), provision of urea as a non-protein nitrogen sources added to the provision of energy sources could increase rapidly degraded microbial population in the rumen and could increase fermentative digestion by rumen microorganisms.

Dry and Organic Matter Degradability

Dry matter (DM) degradability of 10% NRS and 5% NRS in complete ration treatment (R2 and R3) were significantly higher ($P<0.01$) than control and 15% NRS in complete ration treatment (R1 and R4). Addition of 15% NRS

decreased in the amount of DMD 43.24% (Table 2). As mentioned earlier, rumen microbial activity slowed down because of high crude fiber content (27.92% DM) in the complete ration. Crude fiber were very influential in digestible value, the higher the crude fiber content was lower digestible, because fiber digestion depended on the ability of rumen microbes (McDonald *et al*., 2002).

Organic matter (DM) degradability of 10% NRS and 5% NRS in complete ration treatment (R2 and R3) were significantly higher ($P<0.01$) than control and 15% NRS in complete ration treatment (R1 and R4). In rations traetment, organic matter degradability decreased into 40,81%. According to Makkar (1995), low tannin levls might have potential in improving rumen fermentation and maximizing microbial protein synthesis. Tannins could slow the rate of degradation of feed and could reduce the availability of nutrients, but the available nutrients might provide more proportion to microbial protein synthesis compared to the formation of VFA.

CONCLUSION

In conclusion, R3 (70% field grass + 20% concentrate + 10% NRS) was more optimal treatment in improving total gas production, the concentration of total VFA, the concentration of NH_3, microbial biomass product, dry matter degradability and organic matter degradability compared to the control ration.

ACKNOWLEDGEMENT

The authors are grateful to all researchers team on their collaboration in this research, also thank to the Department of Nutrition and Feed Technology (Animal Husbandry Faculty, Bogor Agricultural University) and Center for Radiation and Isotop Application Technology (National Nuclear Energy Assessment) for financial support and laboratory accommodations.

REFERENCES

Allen, M. S. 1996. Relationship between forage quality and dairy production J. Anim. Feed Sci. Technol. 59 : 51-60.

Biological Resources Research Center and Biotechnology IPB. 2007. Hasil Analisa Rumput Lapang, Konsentrat dan Suplemen Kaya Nutrient. Pusat Antara Universitas, Insitut pertanian Bogor.

Blummel, M. and E. R. Ørskov. 1993. Comparison of *in vitro* gas production and nylon bag degradability roughages in prediction of feed intake in cattle. J. Anim. Feed Sci. Technol. 40 : 109-229.

Blummel, M., H. Steingass and K. Becker. 1997. The relation between *in vitro* gas production, *in vitro* microbial mass yield and ^{15}N incorporation and its implications for the prediction of voluntary feed intake of roughage. Br. J. Nutr. 77 : 911-921.

Conway, E. J. 1962. Microdiffusion Analysis and Volumetric Errors. 5th Edition. Crosby, Lockwood and Son, London.

Erwanto. 1995. Uji banding gamal dan angsana sebagai sumber protein, daun kembang sepatu dan minyak kelapa sebagai agensia defaunasi dan suplementasi, analog hidroksi methionin dan amonium sulfat dalam ransum pertumbuhan sapi perah. Tesis. Program Pascasarjana. Institut Pertanian Bogor, Bogor.

Firsoni. 2005. Manfaat tepung daun kelor (*Moringa oleifera*, Lam.) dan glirisidia (*Gliricidia sepium*, Jacq.) sebagai sumber protein dalam Urea Molases Blok (UMB) terhadap metabolisme pakan secara *in vitro* dan produksi susu sapi perah. Tesis. Program Pascasarjana. Universitas Brawijaya, Malang.

Kromann, R. P., J. H. Meyer and W. J. Stielau. 1967. Steam distillation of volatile fatty acids in rumen ingesta. J. Dairy. Sci. 50 (1) : 73 – 76.

Makkar, H. P. S., M. Blummel and K. Becker. 1995. *In vitro* effects and interactions of tannins and saponins and fate of tannins in rumen. J. Sci. Food Agric. 69 : 481-493.

McDonald, P., R. A. Edward, J. F. D. Greenhalgh and C. A. Morgan. 2002. Animal Nutrition. Sixth Edition. Ashford Colour Press, Gosport.

Menke, K. H. and H. Steingass. 1988. Hohenheim gas test. In: Krishnamoorthy, U. and A. V. H. Fellow. 2001. RCA Training Workshop on *in vitro* Techniques for Feed Evaluation. Department of Livestock Production Management Veterinary College. University of Agricultural Sciences Hebbal, India.

Steel, R. G. D. and J. H. Torrie. 1991. Prinsip dan Prosedur Statistik. Suatu Pendekatan Bio-

metrik. Terjemahan. Edisi Kelima. PT Gramedia Pustaka Utama, Jakarta.

Suryahadi, B. Bakrie, Amrullah, B. V. Lotulong dan R. Laside. 2003. Kajian Teknik Suplementasi Terpadu untuk Meningkatkan Produksi dan Kualitas Susu Sapi Perah di DKI Jakarta. Lembaga Penelitian Institut Pertanian Bogor. Badan Penelitian dan Pengembangan Pertanian Departemen Pertanian, Bogor.

Sutardi, T. 1980. Landasan Ilmu Nutrisi I. Fakultas Peternakan. Institut Pertanian Bogor, Bogor.

Sutardi, T., N. A. Sigit dan T. Toharmat. 1983. Standarisasi mutu protein bahan makanan ruminansia berdasarkan parameter metabolismenya oleh mikroba rumen. Laporan Penelitian Direktorat Pembinaan dan Pengabdian pada Masyarakat. Direktorat Jenderal Pendidikan Tinggi Departemen Pendidikan dan Kebudayaan.

Tanuwiria, U. H. 2004. Suplemen seng dan tembaga organik, dan kompleks kalsium-minyak ikan lemuru dalam ransum berbasis agroindustri untuk pemacu pertumbuhan dan produksi susu pada sapi perah. Disertasi. Program Pasca Sarjana. Institut Pertanian Bogor, Bogor.

Tanuwiria, U. H., E. Harlia, D. S. Tasripin, N. R. Manikam dan P. Indraswari. 2006. Pengaruh suplemen Zn-organik, Cu-organik dan tepung kunyit dalam ransum terhadap daya tahan dan jumlah bakteri susu sapi perah FH. Prosiding Seminar Nasional Sapi Perah. Balai Penelitian Ternak, Badan Penelitian dan Pengembangan Pertanian, Departemen Ciawi, Bogor. 23 November 2006. Halaman 7-12.

Thu, N. V. and P. Uden. 1994. Effect of urea molasses cake supplementation of swamp buffaloes feed rice straw of grasses on rumen environment, feed degradation and intake. Asian-Austr. J. Anim. Sci. 14 (15) : 631-639.

Winugroho, M., S. Hardjosoewignyo, T. R. Wiradaryadan dan A. Ella. 1997. Pengukuran produksi gas dari hasil proses fermentasi beberapa jenis leguminose pakan. Prosiding Seminar Nasional II Ilmu Nutrisi dan Makanan Ternak. Fakultas Peternakan, Institut Pertanian Bogor, Bogor. 15-16 Juli 1997. Page 151-152.