Effect of Feeding Difference Levels of Concentrate

on NNH3, VFA and *In Vitro* Digestibility

Ni Putu Putri Wijayanti1, I Gusti Lanang Oka Cakra2, dan I Gede Mahardika2

1Mahasiswa Program Magister Ilmu Peternakan Universitas Udayana, Denpasar

2Animal Husbandry Faculty, Udayana University, Denpasar

Abstract

The aim of this research was to observe the effect of giving difference levels concentrate on NNH3, VFA production and *in vitro* of dry matter and organic matter digestibility. Completely Randomized Design (CRD) was used in this study with four treatments and four replications, i.e. 75% concentrate + 25% forage (60% king grass + 40% *glyricidia*) (A); 60% concentrate + 40% forage (60% king grass + 40% *glyricidia*) (B); 45% concentrate + 55% forage (60% king grass + 40% *glyricidia*) (C); and 30% concentrate + 70% forage (60% king grass + 40% *glyricidia*) (D). The result of this study showed that the NNH3 concentration was highest in treatment A, which was 13.60 mM. VFA concentration was higher in treatment C, which was 118.40 mM. Dry matter digestibility and organic matter digestibility was higher in treatment A, which were 59.71 and 60.27%. It is concluded that the giving of concentrate with containing urea, lime and cassava from 30 to 75% has non-significant (P>0.05) effect on NNH3 and VFA level in every treatments. Giving higher level of concentrate will be increase dry matter and organic matter digestibility.

Keywords: urea, lime, cassava, in vitro

Introduction

Urea supplement has been widely used in ruminant feed formulation in Indonesia. Urea is cheaper crude protein resources in ruminant feed and it can improve feed efficiency (Galina *et al*., 2000; Ortiz *et al*., 2001; Loest *et al*., 2001). However, it should be careful to use and pay attention to the specific requirement because it can caused poison if ammonia level in rumen too high.

High ammonia level caused by urea when added in feed formulation was rapidly come through hydrolysism to ammonia in rumen. Speed release of ammonia from non-protein nitrogen such as urea is much faster than do use ammonia by rumen microbial, so that when the dose is excessive, in a short time ammonia can reaching toxic level which characterized by tremor, excessive salivation, panting, bloating, and tetany (Stanton and Whittier, 2006). Huntington *et al*. (2006) reported that the urea rapidly hydrolyzed in rumen and ammonia peak production achieved one hour after feeding. Slow release ammonia techniques from urea hydrolysis in rumen considered more efficient and save because it can prevent ammonia poisoning (Gali *et al*., 2003).

Cherdthong *et al*. (2011) found that the addition of mixture of urea-CaSO4 in concentrate with containing 70% cassava can improved rumen ecology and increased protein microbial establishment in beef cattle. Using urea in the feed need to accompany by feed energy source (carbohydrate) which is soluble or available in rumen, due to optimal synthesize protein microbial necessary balance between energy (VFA) and nitrogen in the form of NNH3. Gerpacio *et al*. (1979) reported that the content of cassava starch (48.49%) was higher than cornstarch (45.35%). It showed that the cassava could be use for potential source of energy.

The aim of this research was to observe the effect of giving difference levels concentrate on NNH3, VFA and *in vitro* of dry matter and organic matter digestibility.

Materials and Methods

This research was conducted for 3 month in Animal Nutrition Laboratory, Animal Husbandry Faculty of Udayana University. It is using Completely Randomized Design (CRD) with four treatments and four replications, i.e. 75% concentrate + 25% forage (60% king grass + 40% *glyricidia*) (A); 60% concentrate + 40% forage (60% king grass + 40% *glyricidia*) (B); 45% concentrate + 55% forage (60% king grass + 40% *glyricidia*) (C); and 30% concentrate + 70% forage (60% king grass + 40% *glyricidia*) (D). Concentrate used in this experiment contained 4% urea, 2% lime, and 50% cassava (Table 1). Variables observed in this research are NNH3, VFA, dry matter digestibility, and organic matter digestibility.

Four hours after feeding concentrate and forage, rumen fluid collection was perform by mechanical vacuum pump. As for how making way rumen fluid is as follows: first put the tools in the form of plastic tube size of 3/8 mm with length of 250 cm, the larger tube size ½ mm with 40 cm long, mechanical vacuum pump, Erlenmeyer, 500 ml bottle capacity, sample bottle, and filter. Vacuum pump fitted with Erlenmeyer and from Erlenmeyer fitted tube size 5/14”, which it will enter into goat mouth. Plastic tube was inserted into goat mouth up to reticulorumen with larger tube protected to prevent bites his teeth. Furthermore, vacuum pumps drawn repeatedly so that the rumen fluid was suck out and directly collected with Erlenmeyer. Suction is stopped and plastic tube drawn out after sufficient rumen fluid was obtained. Rumen fluid obtained put into a flask, which it was had been filled with 39oC warm water achieving temperature then immediately taken to the laboratory for tested.

*In vitro* fermentation was using Minson and McLeod Method (1972) technique, which modified by incubation time. It works as follows: samples that have been stramed inserted into the *in vitro* tube of 0.25 g and added 25 ml rumen fluid, Mc Dougall buffer with temperature 40oC, and then incubated in shaker bath at 40oC for 48 hours, removed an centrifuges on 3500 rpm for 10 minutes. Substrates were separated into the sediment at the bottom and the clear supernatant was above. Supernatant was taken for analysis of total NNH3 and VFA. The remaining substrate was used for dry matter digestibility analysis and organic matter digestibility. Centrifuges residue at speed 3500 rpm for 10 minutes was added 25 ml pepsin 1 : 10.000 with 0.2% concentration in HCl 0.1, and then centrifuges again for 48 hours. Furthermore, do the same things like procedure above until it was washing. After the last washing, residue quantitatively transferred into a cup who known of empty weight. Evaporated in forced draught oven temperature 70oC until dry ± 12 hours and moved to dry matter oven temperature 105oC for 9 hours, cooled in desiccators and weighing. Then proceed the furnaces combustion until obtain weight of ash. As a blanco is fermentation residue without substrate. Measurement of dry matter digestibility and organic matter digestibility can be used the formulas:

Table 1. Composition and Nutrient Content of Concentrate

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Feed Ingredient** | **Composition (%)** | **Crude Protein**  **(%)** | **Crude Fat**  **(%)** | **Crude Fiber**  **(%)** | **TDN**  **(%)** |
| Cassava  Soybean skin  Rice bran  Molasses  Urea  Salt  Lime  Mineral and vitamin | 50,00  25,00  14,80  2,00  4,00  2,00  2,00  0,20 | 1,65  5,24  2,04  0,17  1,88  0,00  0,00  0,00 | 0,35  0,31  3,16  0,00  0,00  0,00  0,00  0,00 | 2,65  1,33  2,74  0,00  0,00  0,00  0,00  0,00 | 42,50  17,68  9,55  1,26  0,00  0,00  0,00  0,00 |
| **TOTAL** | **100,00** | **10,98** | **3,82** | **6,71** | **70,99** |

Table 2. Feed Composition

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Composition (%)** | **Treatments** | | | |
| **A** | **B** | **C** | **D** |
| Concentrate  Glyricidia  King grass | 75  10  15 | 60  16  24 | 45  22  33 | 30  28  42 |
| **TOTAL** | **100** | **100** | **100** | **100** |

Table 3. Feed Nutrient Composition

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Nutrient** | **Treatments** | | | |
| **A** | **B** | **C** | **D** |
| Crude protein (%)  Crude fat (%)  Crude fiber (%)  TDN (%) | 12,10  3,51  11,80  68,79 | 12,77  3,32  14,85  67,47 | 13,45  3,14  17,90  64,17 | 14,12  2,95  20,96  64,84 |
| **TOTAL** | **100** | **100** | **100** | **100** |

Source: Proximate Analyzed in Animal Nutrition Laboratory, Animal Husbandry Faculty (2012)

Data were analyzed by analysis of variance, if there was significant different (P<0.05), it was followed by Duncan Multiple Range Test (Steel and Torrie, 1989).

Results and Discussion

NNH3 Concentration

NNH3 concentration in the treatments A, B, C, and D were 13.60; 13.20; 12.78; and 11.50 mM respectively and it was not significant different (P>0.05). This result is still in the normal range to support the optimal growth of rumen bacteria. NNH3 optimum range in rumen is between 85-300 mg/l or 6-21 mM (McDonald *et al*., 1988). According to Sutardi (1980) and Mehrez *et al*. (1977), ammonia concentration was required maximum support rumen microbial growth is 4-12 mM.

NNH3 level was higher in treatment A, because the provision of concentrate at treatment A was higher than the other treatments, which it was 75%, so that it can increase NNH3 concentration. An increase of protein (including NPN) in feed will increase protease derived from rumen microbial by increasing reform of protein into amino acids and ammonia (NNH3), and then NNH3 will reused again by rumen microbial, so that rumen microbial also increased. Soepranianondo (2005) found that the increasing of protein in feed will increased NNH3 rumen because 60% of feed protein converted into N ammonia, whereas 40% of it will digested and absorbed in abomasums and small intestine and finally discharged into the feces.

Ammonia production is affected by time after feeding and generally maximum production was achieve in 2-4 hours after feeding which depends on protein sources and ease of protein degraded. In vitro NNH3 measurements can use for protein degradation estimation and its use by microbe. Higher NNH3 concentration can show that the feed protein degradation process faster than the formation microbial protein, so that ammonia produced accumulates in rumen (McDonald *et al*., 1988).

Table 4. Effect of Giving Concentrate Level Containing Urea, Lime, and Cassava Against NNH3, VFA, and Digestibility *In Vitro*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variables | Treatments | | | | SEM2) |
| A | B | C | D |
| NNH3 (mM) | 13,60a1) | 13,20a | 12,78a | 11,50a | 1,55 |
| VFA Total (mM) | 101,81a | 89,92a | 118,40a | 89,16a | 15,70 |
| Dry Matter Digestibility (%) | 59,71a | 56,38ab | 54,13b | 52,74b | 1,18 |
| Organic Matter Digestibility (%) | 60,27a | 56,83ab | 53,58bc | 52,41c | 1,23 |

1. Means without a common superscript within the same row are significantly different (P<0.05)
2. SEM (*Standard Error of The Treatment Mean*)

VFA Concentration

Volatile Fatty Acids (VFA) concentration in treatment A, B, C, and D are show in table 4. The range of VFA concentration for optimal growth of microbes is between 70-150 mM and the amount influenced by the type of feed given (McDonald *et al*., 1988). Sutardi (1979) found that the range of VFA concentration in normal fermentation is between 80-160 mM. According on it, VFA concentrations in all treatments was still in normal range.

VFA concentration in all treatments of this research was not significant different (P>0.05). It is because VFA concentrations influenced by differences in carbohydrate and protein content. Moreover from forage feed an increase microbe can increase fermentation activity affecting the VFA concentration (Charles, 2008). VFA production of rumen fluid was influence by energy source (Bampidis and Robinson, 2006). In rumen, carbohydrate almost entirely fermented into VFA thus supplying a source of energy for rumen microbial growth (Bergman, 1990). Church and Pond (1988) was explained that fermentation in rumen occurs a process of digestion hydrolytic fermentative substances monomers (carbohydrates fermentation) followed by catabolism into VFA. Tillman *el al*. (1998) stated that the VFA content would increase with increasing feed protein and NPN sources.

Increasing VFA amount indicate feed whether or not easily fermentable by rumen microbial (soluble carbohydrate and protein). If protein in feed has high solubility, then the protein will come through fermentation in rumen and produce VFA and ammonia. On the other hand, if protein in feed have low solubility, then the protein relatively unchanged when through the rumen (by pass) (Widiawati and Thalib, 2008).

Dry Matter Digestibility and Organic Matter Digestibility

Dry matter digestibility was higher in treatment A than other, which was 59.71%. Dry matter digestibility in treatments B, C, and D as statistically was not significant different (P>0.05). Dry matter digestibility in treatment C and D was lower (P<0.05) than treatment A, which were 9.35% and 11.67% lower than treatment A respectively.

According to Anggorodi (1995), the factors that affect dry matter digestibility is temperature, speed through digestive tract, physical form of feed, and the effect of comparison with other substances of feed ingredients. Tillman *et al*. (1998) stated that the factors that affect to feed ingredient digestibility is chemical composition materials, prepared feed (cutting, grinding, cooking, etc), age of cattle, and total feed. The average of dry matter digestibility was lower in treatment D, which was 52.74%, because crude fiber in treatment D was high enough (20.96%) so that making difficult for rumen microbial to perform degradation optimally (McDonald *et al*., 1988).

Dry matter digestibility is one of the indicators to determine feed quality. The higher dry matter digestibility has give the higher chances of nutrients derived for cattle to growth. As well as dry matter digestibility, organic matter digestibility also can be use as benchmarks to assess the feed quality. Tillman *et al*. (1998) stated that most of the organic material is a component of dry matter. If the dry matter digestibility is equal, then the organic matter digestibility is the same.

The calculation result of organic matter digestibility in treatment A and B were not significant different (P>0.05) (Table 4). Organic matter digestibility in treatment A was significant different (P<0.05) higher than in treatment C and D, but in treatment C and D as statistically not significant different (P>0.05). Organic matter digestibility in treatment B was significant different (P<0.05) lower than in treatment D, which was 7.78% lower than treatment B. The average of organic matter digestibility was highest in treatment A, which was 60.27%, because of feed in treatment A has high concentrate level, which it was 75%. Rumen bacterial will increase with giving concentrate more than forage. It is according to Jayanegara *et al*. (2006), giving of high concentrate will activate the rumen microbial and then increase amount of proteolytic bacterial and rising deamination resulting increased organic matter digestibility value.

Conclusion and Suggestion

From the issue in this experiment it can be concluded that:

1. Increasing concentrate level with containing urea, lime and cassava from 30 to 75% has no effect on NNH3 and VFA, which were statistically not significant different (P>0.05).
2. The higher of concentrate level with containing urea, lime and cassava will increase dry matter digestibility and organic matter digestibility.

Suggestion

The in vitro research showed that NNH3 and VFA has no difference result with each treatment. It means urea can be given up to 3% from dry matter with no effect poisoning, but should be balancing with lime and cassava. So, this result should be continued on in vivo experiment to know maximum urea can be given to cross breed goat.

References

Anggorodi. 1995. *Ilmu Makanan Ternak Unggas*. Jakarta: Universitas Indonesia Press.

Bampidis, V.A. and P.H. Robinson. 2006. Citrus by Products as Ruminant Feeds: A review. *Anim*. *Feed Sci*. *Technol*.128: 175-217.

Bergman, E.N. 1990. Energy Contributions of Volatile Fatty Acids from the Gastrointestinal Tract in Various Species. *Physiol*. Rev.70: 567-590.

Charles, 2008. Pengaruh Belerang Sebagai Pupuk Terhadap Kualitas Hijauan Pakan Ternak. [cited 2014 March 13]. Available from: URL: http://www.damandiri.or.id/file/charlesipbbab2.pdf.

Cherdthong, A., M. Wanapat, and C. Wachirapakorn. 2011. Influence of Urea Calcium Mixture Supplementation on Ruminal Fermentation Characteristic of Beef Cattle Fed on Concentrates Containing High Level of Cassava Chips and Rice Straw. *Anim*. *Feed* *Sci*. *Technol*. 163:43-51.

Church, D.C. and W.G. Pond. 1988. *Basic Animal Nutrition and Feeding*. 3th Ed. John Wiley and Sons. New York, Chichester, Brisbane, Toronto, Singapore.

Galina, M.A., C.M. Guerrero, G. Serrano, R. Morales, and G. Haenlein. 2000. Effect of Complex Catalytic Supplementation with Nonprotein Nitrogen on Ruminal Ecosystem of Growing Goats Pasturing Shrub land in Mexico. *Small* *Rum*. *Res*. 36:33-42.

Galo, E., S.M. Emanuele, C.J. Sniffen, J.H. White, and J.R.Knapp. 2003. Effects of a Polymer-Coated Urea Product on Nitrogen Metabolism in Lactating Holstein Dairy Cattle. *J*. *Dairy* *Sci*. 86:2154-2162.

Gerpacio, A.L., F.Sd. Pascual, L.J. Querubin, C.I. Mercado, and C.T. Bechayda. 1979. Evaluation of Tuber Meal as Energy Sources. IV. The Effect of Varying Energy/Protein Feeds on the Feeding Value of Broiler Feeds Containing Tannia (*Xanthosema sp*.) and Pongapong (*Amorphophallus companulatus*) Meals. Phil. *J*. *Vet*. *Animal* *Sci*. V (1):1-13.

Huntington, G.B., D.L.Harmon, N.B.Kristensen, K.C.Hanson, and J.W. Spears. 2006. Effects of a Slow-Release Urea Source on Absorption of Ammonia and Endogenous Production of Urea by Cattle. *Anim*. *Feed* *Sci*. *Technol*. 130:225-241.

Jayanegara, A., A. S. Tjakradidjaja, dan T. Sutardi. 2006. Fermentabilitas dan Kecernaan In Vitro Ransum Limbah Agroindustri yang Disuplementasi Kromium Organik dan Anorganik. *Media Peternakan*. 29(2): 54-62.

Loest, C.A., E.C. Titgemeyer, J.S. Drouillard, B.D. Lambert, and A.M. Trater. 2001. Urea and Biuret as Nonprotein Nitrogen Sources in Cooked Molasses Blocks for Steers Fed Prairie Hay. *Anim*. *Feed* *Sci*. *Technol*. 94:115-126.

McDonald, P., R.A. Edward, dan J.F.D. Greenhalgh. 1988. *Animal Nutrition*. 4th Edition. New York: Longman Scientific and Technical.

Mehrez, Z., E.R. Ørskov and P. Mc Donald. 1977. Rate of Rumen Fermentation in Relation to Ammonia concentration. *J*. *Natur*. 38: 127.

Minson, D.J. and M.N. Mc Leod. 1972*. The In Vitro Technique*. *Its Modification for Estimating Digestibility of Large Number of Tropical Pasture Sample.* Division of Tropical Pasture Technical Paper. Australia: No. 8 Common Welth Scientific and Industrial Research Organization.

Ortiz, R.M.A., G.F.W. Haenlein, and M. Galina. 2001. Effect on Feed Intake and Body Weight Gain When Substituting Maize with Sugarcane in Diet for Zebu Steers Complemented with Slow Release Urea Supplements. Int. *J*. *Anim*. *Sci*. 16:239-245.

Soepranianondo, K. 2005. Dampak Isi Rumen Sapi sebagai Substitusi Rumput Raja terhadap Produk Metabolik pada Kambing Peranakan Etawa. *Media Kedok*. *Hewan*. 21: 94-96.

Stanton, T.L. and J. Whittier. 2006. Urea and NPN for Cattle and Sheep. [cited 2012 Jan 15]. Available from: URL: [http://www.ext.colostate.edu/Pubs/ livestk/01608.html](http://www.ext.colostate.edu/Pubs/%20livestk/01608.html)

Steel, R.G.D. and J.H. Torrie. 1989. *Prinsip dan Prosedur Statistika*. (Bambang Sumantri, Pentj). Jakarta: Gramedia.

Sutardi, T. 1979. Ketahanan Protein Bahan Makanan terhadap Degradasi oleh Mikroba Rumen dan Manfaatnya Bagi Peningkatan Produktivitas Ternak. *Prosiding Seminar Penelitian dan Penunjang Peternakan, LPP*. Bogor. Buku 2. Hal. 91-103.

Sutardi, T. 1980. *Landasan Ilmu Nutrisi*. Bogor: Institut Pertanian Bogor.

Tillman, A.D., H. Hartadi, S. Reksohadiprodjo, S. Prawirokusumo, dan S. Lebdosoekojo. 1998. *Ilmu Makanan Ternak Dasar*. Yogyakarta: Gadjah Mada University Press.

Widiawati, Y. dan A. Thalib. 2008. Comparasi of Fermentation Kinetics (In Vitro) of Grass and Shurb Legume Leaveas: The Pattern of Gas Production, Organic Matter Degradation, pH and NH3 Production. [cited 2014 March 13]. Available from: URL: http://balitnak.litbang.deptan.go.i/index.php?option=comcontent&task=view&id=72& Itemid=56.