Effect of protected crude palm oil on rumen microbial activities and methane production

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ABSTRACT: This study was aimed to determine the effect of CPO with formaldehyde protection on rumen microbial activity and methane gas production. The female sheep were fed by elephant grass and rice bran supplemented by CPO with the level of 0%, 1.5%, 3%, 4.5% and 6% in which protected with formaldehyde as of 0%, 1%, 2% and 3%. The parameters being observed were those of activity of CMC-ase, microbial protein, the number of protozoa and methane production in rumen fluid. The data obtained were analyzed using completely randomized design factorial 4 x 5 (4 levels of formaldehyde as factor A, 5 levels of CPO as factor B). The different on results were tested by Duncan's New Multiple Range Test. The results showed that protecting CPO with formaldehyde had increased of CMC-ase activity, the number of protozoa and methane production, but the decreased microbial protein in the rumen fluid fermentation.

Key words: CMC-ase activity, microbial protein, number of protozoa, methane production

INTRODUCTION

Crude palm oil (CPO) have relatively high polyunsaturated fatty acid (PUFA), such as oleic (C18:1) 43.24%, linoleic (C18:2) 12.15% and linolenic (C18:3) 0.29% (Suharyanto, 2006). Therefore it can be used as the feed material energy resources and unsaturated fatty acid sources for livestock. The use of CPO as a source of unsaturated fatty acid on ruminant diet has some negative effects, namely : (1) The use of CPO containing diet is extremely limited because it will inhibit rumen microbial activity in degrading fiber (Doreau and Chilliard, 1996), so that the digestibility of crude fiber was decreased (Taminga and Doreau, 1991), and thus can control the rumen protozoa, so that it's activity would be disrupted and many would be dead, (2) Fats containing high unsaturated fatty acids would be hydrogenated by rumen microorganisms to become saturated fatty acids (Parakkasi, 1999), so fat of ruminant meat become loaded, the quality of meat decreased and the negative impact on consumer health.

Crude palm oil with protein-formaldehyde (CCPO) which was tested in vitro could reduce the hydrogenation of unsaturated fatty acids in palm oil by rumen microbes (Tiven et al., 2009), thus allowing the negative effects of the limitations of fat could be reduced and relatively increased unsaturated fatty acids in meat. But the passage of fat from rumen degradation process will lead to other problems, namely increasing concentration of methane (CH_4). Methane released by ruminant contributed 15-20% of total production, that caused the global warming (Minami et al., 1992) and 6% of energy intake from livestock lost in the form of methane (Johson and Johnson, 1995).

Many nutritionist have tried to reduce emissions of methane produced by ruminants, such as by the addition of various chemical compounds in livestock diet, e.g unsaturated fatty acids and anthrakinon (Yusiati et al., 2002). Methane production could be inhibited by decreasing the production of hydrogen (H_2) and formic acid (HCOOH) as the main component for the formation of methane, with the addition of reductor compounds as final electron acceptor of the fermentation process (Asamuna et al., 1998), or with the addition of hydrogen binding factor (hydrogen sinks) (Johnson and Johnson, 1995). The efforts to reduce methane gas is not solely because of the role of methane in influencing climate and global warming, but it also because of the large emissions of methane showed inefficient use of feed by ruminants (Yusiati et al., 2002). Therefore this study was conducted to determine the effect of CPO protected by formaldehyde as a source of unsaturated fatty acids on rumen microbial activity and methane production.

MATERIALS AND METHODS

Crude palm oil (CPO) with the level of 0%, 1.5%, 3%, 4.5%, 6% of the died (DM basis), evenly mixed with skim milk with the ratio of 1:2. Formaldehyde 37% pro analysis was added to the mixtures with the level of 0%, 1%, 2% and 3% by weight of the mixture, mixed evenly to form a capsule CCPO (Capsulation Crude Palm Oil). Syringe was inserted into the CCPO (weighed according to treatment), 30 ml rumen fluid of female local sheep was collected with a trocared, and the fermentation solution (mineral solution A and B, buffer, Resazurin and reduction) were prepared in aseptic. Anaerobic conditions was created with the CO_2 gas and close to the piston and clamped. The fermentor syringes were inserted on the incubator at 39 ^oC and gas production was observed during the incubation period of 1, 2, 4, 6, 8, 12, 18, 24, 48 hours. Following the completion of the molecule period fermentor syringe removed from the incubator and the carboxymethyl cellulase activity (CMC-ase), number of protozoa and methane production were tasted.

CMC-ase activity was determined based on the amount of reducing sugar being formed from the substrate CMC (Halliwel et al., 1985), was expressed in specific enzyme activity by measuring the enzyme protein content using Lowry method (Plummer, 1987). The number of protozoa measurement was done by added 0.8 ml of saline formal dehyede solution (37% formaldehyde + 0.9 NaCl% (v/v), with the proportion of 1:9) to 1 ml sample. Furthermore, the protozoa counting was calculated by microscopic techniques, namely by direct calculation using a haemocytometer under a microscope with a magnification of 40 times (Diaz et al., 1993). Measurement of methane production was done by taking gas from fermentation solution as much as 10 ml using a plastic syringe and stored in vaccumtab for analysis of methane concentration using gas chromatography (GC). Methane production was the result of gas production at the 48-hour reading.

Data were analyzed using completely randomized design factorial 4 x 5 (4 levels of formaldehyde and 5 levels CPO). The difference value of the average results were the tested by Duncan's New Multiple Range Test (Gomez and Gomez, 1995).

RESULTS AND DISCUSSION

The Effect of CCPO on CMC-ase Activity

The effect of formaldehyde and CPO level on CMC-ase activity is shown in Figure 1. Statistical analysis indicated that the increasing of CPO levels decreased CMC-ase activity ($P<0.01$), while the increasing of formaldehyde levels increased CMC-ase activity ($P<0.01$). There wasn't any effect of interaction between level of formaldehyde and CPO on CMC-ase activity of fermented rumen fluid.

The decrease of CMC-ase activity is caused by increasing of CPO levels which cover the feed particles and rumen microbes. According to Erwanto (1995), the use of fat could affect the fermentation system because as a non polar compounds, fat was not easy /not immediately soluble in medium of rumen fluid, but tend to associate with feed particles and rumen microbes. The forms of association is physical coating, reducing the access of enzymes microbes on feed particles. The increasing CMC-ase activity was the result as fat an defaunation agent was protected so as not to interfere with cellulolytic bacteria activity. According to Doreau and Chilliard (1996), that by protecting fat caused cellulolytic bacteria would degrade celulosa, which could be seen through the increasing of cellulase activity.

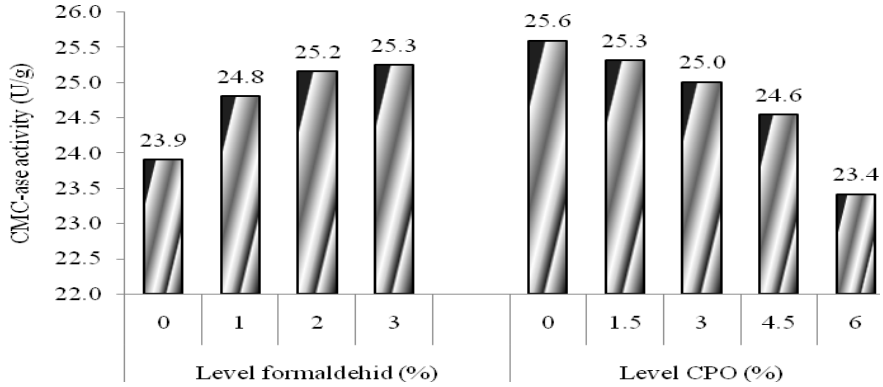

Figure 1. CMC-ase activity (U/g) by in vitro rumen fermentation of king grass-rice bran (2:1) with addition of CPO protected by formaldehyde

The Effect of CCPO on Microbial Protein

The effect of formaldehyde and CPO level on microbial protein was shown in Figure 2. Statistical analysis indicated that the increasing of CPO levels decreased microbial protein ($P<0.05$), while the increasing of formaldehyde levels didn't affect microbial protein being produced. There wasn't any effect of interaction between level of formaldehyde and CPO on microbial protein of fermented rumen fluid.

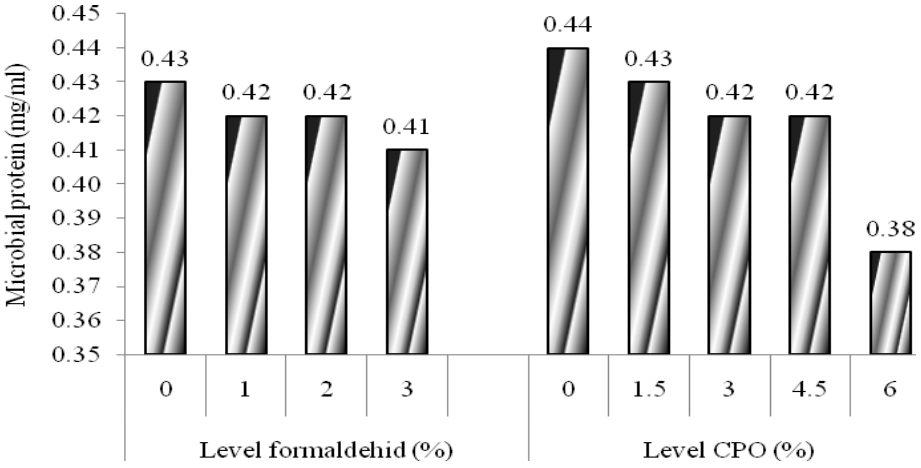

Figure 2. Microbial protein (mg/ml) by in vitro rumen fermentation of king grass-rice bran (2:1) with addition of CPO protected by formaldehyde

The decreasing of microbial protein could be explained by the statement made by Erwanto (1995) that fat covered the particles of feed and rumen microbes (physical coating) would reduce the access of microbial enzymes on feed particles thus allowing the decreasing of total microbial protein. The decreasing of microbial protein due to formaldehyde levels caused the strong bond between formaldehyde and protein, thereby reducing the ability of microbes to degrade proteins. Wachira et al. (1974) stated that the amount of protein would decrease due to the protection of feed by formaldehyde. According to Rachmadi (2003), that the bonds between proteins and formaldehyde would get stronger so difficult to degraded by rumen microbes, when the feed material was protected with formaldehyde was stored for a long time. Microbial protein content was influenced too by the number of protozoa. In this study, higher levels of formaldehyde cause the number of protozoa

increased (Figure 3). According to Jouany (1991), that the decrease in the number of protozoa are predators will provide the opportunity for bacteria to flourish, but an increasing number of protozoa will inhibit bacterial growth so that microbial protein synthesis will decreased.

In vitro fermentation of king grass and bran smooth (80:20) with the addition fish oil of lemuru as much as 2.5% DM had a range of microbial protein content of 0.41 to 0.51 mg/ml (Musyaiddah, 2004), but fermentation king grass with the addition of formal dehide capsulated CPO as much as 7.5% had a range of microbial protein content of at 0.33 to 0.51 mg/ml (Setyawati, 2008). The range of microbial protein obtained in this study is amounted to 0.38 to 0.44 mg / ml.

The Effect of CCPO on Number of Protozoa

The effect of formaldehyde and CPO level on number of protozoa is shown in Figure 3. Statistical analysis indicated that the increasing CPO level had caused the number of protozoa was decreased ($P<0.01$), while increasing levels of formaldehyde had cause the number of protozoa was increased ($P<0.01$). There was interactions affect ($P<0.05$) between level of formaldehyde and CPO on number of protozoa fermentated rumen fluid.

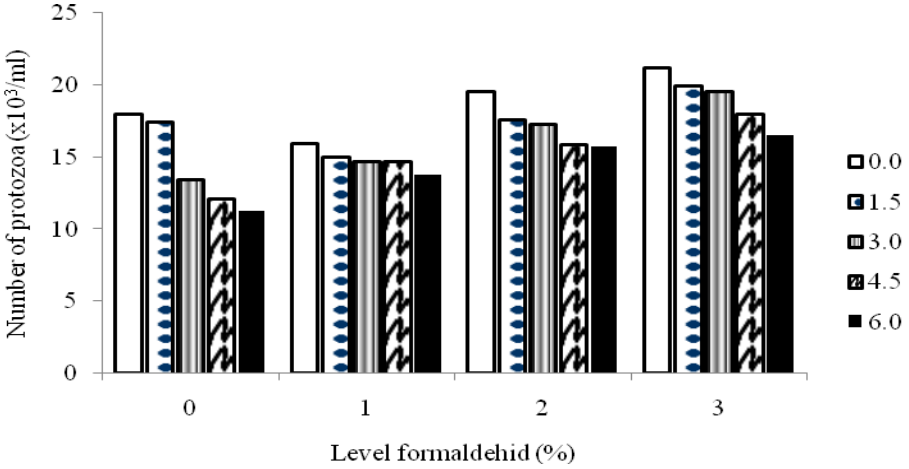

Figure 3. Interactions between level of formaldehyde and CPO on the number of protozoa ($x10^3$/ml) by in vitro rumen fermentation of king grass-rice bran (2:1) with addition of CPO protected by formaldehyde

The decreasing number of protozoa due to the increase of CPO level indicates that the fat was very negative effect on the number of protozoa. Addition of fat which contains unsaturated fatty acids could reduce the number of protozoa in the rumen (Ueda et al., 2003). According to Taminga and Doreau (1991), the fat that was added to the diet could control the population of protozoa, because the protozoa did not have good lipolytic activity as bacteria did so that the fat will be covering the protozoa, resulting its metabolic activity disrupted and many are dead.

The increasing number of protozoa due to the increase of formaldehyde levels indicates that the increased levels of formaldehyde up to 3% relatively had a negative effect on the number of protozoa. The capsulation of CPO with formaldehyde causes fat can not control the protozoa, resulting in an increasing number. Mudrosanto (2006), reported that the number of protozoa on in vitro fermentation of king grass was equal to 17 x 10^3/ml, while the number of protozoa obtained in this study was ranged from 14 to 19 x 10^3/ml. According to Bohatier (1991), that the number of protozoa was influenced among others by the type of substrate, namely a smaller number of protozoa on fermentation of forage compared to the concentrate or mixed forage with concentrates. According to Cieślak et al., (2006), the number of protozoa on rumen fermentation of substrate containing wheat flour and hay with a ratio of 60:40 on in vitro tested was 33.4 x 10^3/ml.

The Effect of CCPO on Methane Production

The effect of formaldehyde and CPO level on methane production is shown in Figure 4. Statistical analysis indicates that the increasing CPO level decrease methane production (P<0.01), whereas the increasing formaldehyde level increase methane production (P<0.01). There is not any effect of interaction between level of formaldehyde and CPO on methane production of fermented rumen fluid.

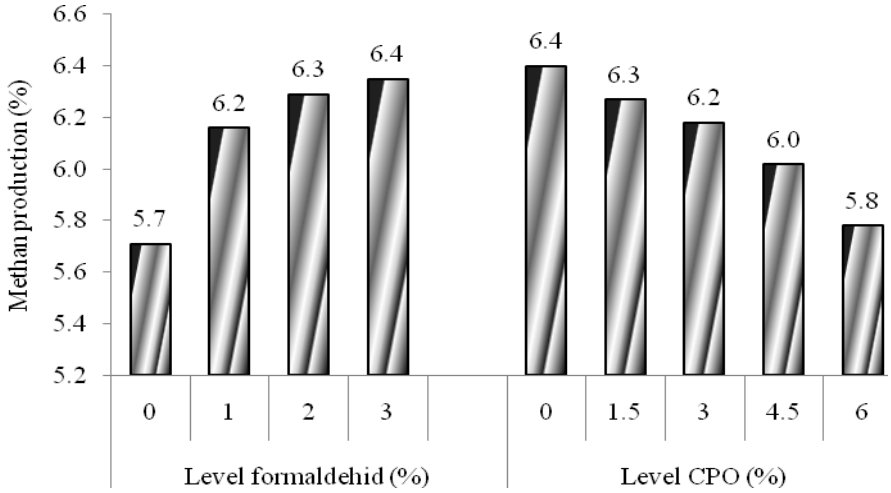

Figure 4. Methan concentration (%) of gas produced by in vitro rumen fermentation of king grass-rice bran (2:1) with addition of CPO protected by formaldehyde

The decreasing and increasing methane production have relation with the increasing and decreasing number of protozoa (Figure 3). As many as 10-20% methanogen was bind with ciliate protozoa (mainly from Methanobacteriaceae families) and was actively in the process of methanogenesis in rumen fluid (Machmüller et al., 2003). Symbiotic relationship between methanogens with ciliate protozoa play an important role in preventing the accumulation of hydrogen (NADH) through the mechanism of hydrogen transfer in interspecies with ciliate protozoa. The increasing amount of methane production caused increasing number of protozoa, which suggests that the increased levels of formaldehyde up to 3% relative had no negative effect on the number of protozoa. The decreasing of methane production due to increased levels of CPO, suggesting that CPO can be a defaunation agent to protozoa. According to Jordan et al. (2006), fat was a natural substance that was used for defaunating, which caused a decline in the mechanism of symbiosis between methanogens with ciliate protozoa, had to only small amount of hydrogen could be converted into methane. The absence of protozoa in the rumen decreasing methane production of 0.13 in a varied diet (Hegarty, 1999).

CONCLUSIONS

From these results it can be concluded that the increasing of CPO level decrease CMC-ase activity, microbial protein, the number of protozoa and methane production, while increasing levels of formaldehyde increase CMC-ase activity, number of protozoa and methane production, but microbial protein decreased. Protection of CPO with formaldehyde was needed to be tested further in vivo to determine its effect on fatty acid composition (rumen fluid, blood, meat), the status of rumen fermentation, microbial activity and production performance and meat quality of local sheep.

LITERATURE CITED

Asamuna, N., Iwamoto and T. Hino. 1998. Offect of the addition of fumarate on methane production by ruminal microorganism in vitro, J. Anim. Sci. Feed and Tech. 69: 576-584.

Bohatier, J. 1991. The rumen protozoa : taxonomy, cytology and feeding behaviour. In : Rumen Microbial Metabolism and Ruminant Digestion. Jouany, J. P. (ed) Institut National de la Recherche Agronomique. INRA. Paris. p. 27-38.

Ciéslak, A., R. Miltko, G.Belźecki, M.Szumacher-Strabel, A.Potkański, E.Kwiatkowska, and T.Michalowski. 2006. Effect of vegetable oils on the methane concentration and population density of the rumen ciliate, Eremoplastron dilobum, grown in vitro. Journal of Animal and Feed Sciences 15 (1): 15-18.

Diaz, A., M. Avendano and A. Escobar. 1993. Evaluation of sapindus saponaria as a defaunating agent and its effect on different ruminal digestion parameters. Livestock Research for Rural Development. vol 5, no. 2.

Doreau, M and Y. Chiliard. 1996. Digestion and metabolism of dietary fat in farm animal. In : An International Conference on Fat in The Diet Of Animals and Man The National Exhibition Centre. Birmingham. United Kingdom.

Erwanto. 1995. Optimalisasi Sistem Fermentasi Rumen Melalui Suplementasi Sulfur, Defaunasi, Reduksi Emisi Metan dan Stimulasi Pertumbuhan Mikroba Pada Ternak Ruminansia. Desertasi. Program Pascasarjana Institut Pertanian Bogor, Bogor.

Gomez, K. A dan A. A. Gomez. Prosedur Statistik Untuk Penelitian Pertanian. Edisi Kedua. UI-Press, Jakarta.

Halliwel, G., N.N.B.A. Wahab, and A.H. Patle. 1985. Chemical composition of endo 1,4-β-glucanase to cellulolitic in trichoderma koningii. J. App. Biochemistry. 7:43-45.

Hegarty, R.S. 1999. Reducing rumen methane emissions through elimination of rumen protozoa. Aust J. Agric. Res. 50:1321-132.

Johnson, K.A. and D.E. Johnson. 1995. Methane emission from cattle. J. Anim. Sci. 73: 2483-2492.

Jordan, E., D. Kenny, M. Hawkins, R. Malone, D. K. Lovett, and F. P. O'Mara. 2006. Effect oy refined soy oil or whole soybeans on intake, methane output, and performance of young bulls. J.Anim.Sci. 84: 2418-2425.

Jouany, J.P. 1991. Rumen Microbial Metabolism and Ruminant Digestion. Institute National de la Recherche Agronomique. Paris. pp. 17.

Machmüller, A., C.R. Soliva, and M. Kreuzer. 2003. Effect of coconut oil and defaunation treatment on methanogenesis in sheep. Reprod.Nutr.Dev. 43: 41-55.

Minami, K., A. Mosier and R. Sass. 1992. CH_4 and CO_2. Global emissions and controls from rice fields and other agricultural and industrial sources. Proceeding of an International Workshop. Tsukuba, Japan.

Mudrosanto, I. 2006. Pengaruh Pemanfaatan Daun Ketepeng Cina (Cassia alata L.) Sebagai Sumber Saponin Terhadap Fermentasi Hijauan dan Konsentrat di Dalam Rumen Secara In Vitro. Skripsi. Fakultas Peternakan UGM, Yogyakarta.

Parakkasi, A. 1999. Ilmu Nutrisi dan Makanan Ternak Ruminan. Universitas Indonesia (UI-Press), Jakarta.

Plummer, D. T. 1987. An Introduction to Practical Biochemistry. Mc. Graw Hill Ltd. Bombay, New Delhi.

Rachmadi, D. 2003. Dampak Pemberian Bungkil Inti Sawit dan Konsentrat Yang Dilindungi Formaldehida pada Domba Terhadap Kinerja dan Kandungan Asam Lemak Poli Takjenuh. Desertasi. Program Pascasarjana IPB, Bogor.

Setyawati, R. 2007. Pengaruh Proteksi Minyak Kelapa Sawit Menggunakan Formaldehid Terhadap Jumlah Protozoa dan Produk Fermentasi Mikrobia Rumen Secara In Vitro. Skripsi. Fakultas Peternakan Universitas Gadjah Mada, Yogyakarta.

Suharyanto., Tri Panji., M.I. Abdullah dan K. Syamsu. 2006. Biokonversi CPO dengan desaturasi amobil sistem kontinyu pada skala semipilot untuk produksi minyak mengandung GLA. Menara Perkebunan. 74(2): 97 – 108.

Taminga, S and M. Doreau. 1991. Lipids and Rumen Digestion. In : J.P. Jouany, editor. Rumen Mikrobia Metabolism and Ruminal Digestion, Paris:INRA.

Tiven, N. C., L. M. Yusiati., Rusman., U. Santoso dan D. Malle. 2009. Asam Lemak Tidak Jenuh Terproteksi Sebagai Aditif Pakan Untuk Meningkatkan Kualitas Daging Domba. Laporan Penelitian Hibah Bersaing. Fakultas Pertanian Universitas Pattimura, Ambon.

Ueda, K., A. Ferlay., J. Chabrot., J. J. Loor., Y. Chilliard and M. Doreau. 2003. Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage : concentrate ratios. J. Dairy Sci. 86:3999-4007.

Wachira, J.D., L.D. Satter., G.P. Brooke and A.L. Pope. 1974. Evaluation of formaldehyde-treated protein for growing lambs and cow. J. Anim. Sci. 39:796.

Yusiati, L. M., Z. Bachrudin., Sugiyanto, Kustantinah, Chusnul Hanim. 2002. Penggunaan senyawa penambat elektron sebagai agensia penghambat methanogenesis, manfaatnya dalam peningkatan efisiensi penggunaan nutrien pakan pada usaha peternakan ramah lingkungan. Laporan Penelitian Hibah Bersaing X/1. Lembaga Penelitian Universitas Gadjah Mada, Yogyakarta.