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# Effect of Defaunation and Supplementation Methionine Hydroxy Analogue and Branched Chain Amino Acid in Growing Sheep Diet Based on Palm Press Fiber Ammoniated

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**Abstract:** This experiment tried to make use the ample supply of palm press fiber (PPF) as the source of roughage. The experiment used in 5x5 Latin Square feeding trial in sheep of 9.4±1.63 kg live weight. The experiment diets composed of 50% elephant grass or 50% PPF and 50% concentrate. The treatment were A = 505% elephant grass, B = 50% PPF, previously treated with 1.5% urea, C = B+1.5% corn oil, D = C+0.1% Ca salt of methionine hydroxy analogue (MHA) and E = D+0.1% val+0.15% leu dan 0.2% ile (Branced Chain Amino Acid = BCAA). Animal on the ammoniated PPF diet (B) had less cultivable rumen bacteria than those on elephant grass 90.88 x $10^{11}$ vs 1.09 x $10^{11}$ colonies/ml). Addition of corn oil (C) reduced the viable rumen protozoa (1.43 x $10^5$ to 1.27 x $10^5$ cell c/ml. The decrease was accompanied by the increase in bacteria to 1.14 x $10^{11}$ colonies/ml and digestibility to 59.4%. Supplementation of MHA (D) increased the bacteria to 1.63 x 10 colonies/ml. The bacterial counts were increased further to 1.89 x $10^{11}$ colinies/ml by the BCAA (E). The later treatment was better in digestibility and N retention than the control but had same effect on growth performance of the animal (104 vs 102 g/d). The live weight gain of the later treatment (E) was significantly better than those on treatment B, C or D.

**Key words:** Palm press fiber, defaunation, MHA, BCCA

## Introduction

Agro-industrial by product like palm press fiber (PPF) are abundant in Indonesia with an annual rate of production exceeding 6 million tones of fresh material and attempts have been made to utilize PPF as feed. But PPF contains high proportion of lignin and its digestibility is very low. Thus, small amount of PPF is being used for feed (15-25 %) in ration. Currently PPF is being utilized as boiler fuel at palm oil mill.

Ammonia treatment of straw has been described in detail by Sundstol (1991). Generally ammonia treatment increase digestibility in the range of 5-15 percentage units, and it also increase the nitrogen content of treated straw. Urea is often used to enhanced digestibility of Fibrous by product through ammoniation (Van Soest, 2006). Ammoniation of crop residues and agro-industrial by product with urea can supply nitrogen to rumen microbe. In our previous experiment were those ammoniation PPF with urea had a little impact to increase the digestibility of PPF. To increase quantity of PPF as ruminant feed, beside the ammoniated treatment we should be increased the population of rumen microbe because the utilization of feed by ruminants depends on microbial fermentative digestion.

Protozoa are now recognized as having an overall negative effect in the rumen, particularly where ruminant are fed forage diets low in true protein (Bird *et al.*, 1990). Protozoa ingest and digest bacteria and reduce the bacterial biomass in the rumen and consequently the protein supply to the animal (Jouany, 1996). In studies with sheep fed straw based diet, it has been found that the apparent digestibility of dry matter was increased by 18% after protozoa had been removed from the rumen (Bird and Leng, 1984).

On most diets based on crop residues and low digestibility forage, the primary limitation the growth of rumen micro-organism is probably the concentration of ammonia in rumen fluid (Leng, 1991). But supplementation non protein N of the diets in ruminant consuming low quality forage commonly result in decreased animal performance compared with supplementation with natural protein. Although ruminal ammonia has been identified as the primary N source obligatory to microbial cellulolytic activity, *in vitro* microbial growth rates and cellulose disappearance were optimized when the ruminal N supply was derived from amino acids (Griswold *et al.*, 1996; Jones *et al.*, 1998) like methionine and branched amino acids (Merchen and Titgemeyer, 1992).

This paper reports the result of experiment in which urea treated palm press fiber that have supplementation with corn oil as defaunating agent, Methionine Hydroxy Analogue and Branched Amino Acid (BCAA) (valin, leucine, isoleucine) was compared to other roughage for the growth of sheep.

## Materials and Methods

**Ammonia treatment of palm press fiber:** In this experiments, palm press fiber was saryed with 4 kg urea in 100 l of water/100 kg dry matter of PPF and stored between sheets of plastic for 3 weeks in an aerobic condition. The ammoniated palm press fiber was aerated prior to feeding.

**Experimental design:** The composition of the feeds, including the concentrate, is given in Table 1. Five sheep were dewormed and randomly allocated in a 5x5 Latin Square Design. One animal was used as the experimental unit. The treatments were:

A. 50% elephant grass+50% concentrate
B. 50% Ammoniated PPF+50% concentrate
C. B+1.5% corn oil
D = C+0.1% MHA
E = D+0.1% Val+0.15% Leu+0.2% Ile

Feed was offered twice daily at 0800 h and 1300 h and intake of roughage was measured each day throughout the trial. Fed was offered at 3% LW. Each experimental periods consisted of 21 d: 14 d for diet adaptation and 7 d for total collection fecal and urine. Fecal sub samples (10%) were dried partially at 50°C for 48 h. Urine Aliquots (10%). Volatilization of ammonia N prevented by adding 10 ml of 6 N HCl to urine collection vessels. Samples of feed, feces and urine, composite by animal across the 7 d collection period, were analyzed for DM, OM and N by standard procedures (AOAC, 1984). Rumen samples were collected from each sheep 4 h post feeding initially and were used to determined bacterial and protozoa population (Suryahadi, 1990), $NH_3$ concentration (microdifusi conway method), VFA concentration (Chromatography gas) dan pH rumen fluid.

Data were analyzed by ANOVA. Difference between the control treatment and each of enzyme treatment were analyzed by Orthogonal contrast (Steel and Torrie, 1980)

## Results and Discussion

PH, $NH_3$ and VFA concentration, and population of rumen bacteria and protozoa of sheep with experimental diets indicated in Table 2. This table showed that there were significant difference ($P< 0.05$) were found in the $NH_3$, VFA concentration, population of rumen bacteria and protozoa between experimental diet but no significant different was found in pH.

Ammoniated PPF had a concentration of $NH_3$ higher than other treatment but could not increase the population of rumen bacteria. Defaunation with corn oil decreases the number of protozoa and may lead to an increase the number of bacteria and allantoic urine were similar to those of Santa and Karim, 2002; Machmuller *et al.*, 2003.

Digestion on feed in the rumen is influenced by the rumen population. Supplementation with corn oil, MHA and BCAA can increased population of the rumen microba. The result showed that bacterial growth in rumen animal fed ammoniated PPF was limited by the available of amino acids. This phenomena is an agreement with the report of McCracken *et al.* (1993). showing the growth of rumen microbe was increased by supplementation of methionine to low quality feed.

In this study number of rumen bacteria was higher in fed with supplementation BCAA. These result showed that BCCA give the positive effect to rumen bacterial growth because BCCA was needed to promotes growth of cellulolytic bacteria (Baldwin and Allison, 1983). It also has been demonstrated before that BCAA were increased the number of rumen bacteria (Jones *et al.*, 1998). Russel and Sniffen (1984) also found greater synthesis of cell protein when branched chain fatty acids and trypticase were added together. Rumen bacteria can thrive on ammonia as sole nitrogen source but grow at higher rates when performed amino acids are also present in media (Bryant, 1973).

Table 1: Ingredient composition and Nutrient of experimental diet

| Ingredient/ nutrient (%BK) | Diet A | B | C | D | E |
|---|---|---|---|---|---|
| Elephant grass | 50.00 | - | - | - | - |
| Ammoniated PPF | - | 50.00 | 50.00 | 50.00 | 50.00 |
| Coconut cake | 23.50 | 23.50 | 23.50 | 23.50 | 23.50 |
| Pollard | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| NaCl | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Lime stone | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Urea | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Vitamin and mineral | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Supplementation | | | | | |
| Corn oil | - | - | 1.50 | 1.50 | 1.50 |
| MHA | - | - | - | 0.10 | 0.10 |
| Valin | | | | | 0.10 |
| Leucine | | | | | 0.15 |
| Isoleucine | | | | | 0.20 |
| Nutrient (%) | | | | | |
| Protein | 16.18 | 15.99 | 16.06 | 16.21 | 16.64 |
| Fat | 3.24 | 5.26 | 6.24 | 6.26 | 6.26 |
| Acid Detergent Fiber | 31.74 | 37.35 | 39.08 | 40.25 | 40.25 |
| Calcium | 0.73 | 0.65 | 0.62 | 0.62 | 0.62 |
| Phosphor | 0.56 | 0.47 | 0.45 | 0.45 | 0.45 |
| Gross Energy (MJ/kgDM) | 15.85 | 13.12 | 16.67 | 16.67 | 16.67 |

Table 2 also showed that supplementation of MHA and BCAA could improve ferment ability ammoniated PPF that can be seen at increased of VFA like as reported of Mir *et al.* (1991) and change the VFA individual profile where there were increase production of propionate. Propionic acid is mayor source to produce glucose for ruminant. More than 50% glucose in ruminant were produced from propionic acid. Hence supplementation of MHA and BCAA that increased the propionic acid could be available of energy to production process. Beside that fermentation system that produced more propionic acid would be increase the diet efficiency because the methan production decreased (Orskov and Ryle, 1990).

Supplementation of BCCA significantly increased the iso acid in rumen fluid which is in agreement with McCollum

Table 2: Rumen characteristics of sheep feed the experimental diets

| Item | Diet A | B | C | D | E | SE |
|---|---|---|---|---|---|---|
| pH rumen fluid | 6.63 | 6.52 | 6.53 | 6.48 | 6.46 | 0.17 |
| $NH_3$ (mM) | 7.21[a] | 11.04[d] | 9.86[c] | 8.94b | 7.48[a] | 0.75 |
| Bacteria x $10^{10}$ colonies/ml | 10.90[b] | 8.80[a] | 11.40[b] | 16.28[c] | 18.88[d] | 0.08 |
| Protozoa x $10^{5}$ cells/ml | 1.35[b] | 1.45[b] | 1.28[a] | 1.27[a] | 1.26[a] | 0.05 |
| Allantoin urine(mg/d) | 56[a] | 49[a] | 57[a] | 65[b] | 73[b] | 11.59 |
| Total VFA (mM) | 115.87[c] | 102.60[a] | 104.70[a] | 110.95[b] | 120.62[c] | 14.96 |
| Acetate (mM) | 75.36[c] | 66.73[a] | 68.06[a] | 72.24[b] | 78.21[c] | 5.68 |
| Propionat (mM) | 27.82[b] | 22.18[a] | 23.36[a] | 25.29[a] | 28.65[b] | 2.56 |
| Butirat (mM) | 6.66[a] | 8.42[b] | 7.76[b] | 7.04[a] | 5.96[a] | 1.14 |
| Isoacids (mM) | 6.02[a] | 5.28[a] | 5.01[a] | 6.38[b] | 7.82[b] | 1.25 |

A = Elephant grass + concentrate, B = ammoniated PPF + concentrate, C = B + Corn oil, D = C + MHA, E = D + BCAA
Values with different superscript are significantly different (P<0.05)

Table 3: Dry matter intake and digestibility, N retention and live weight gain of sheep with experimental diets

| Item | Diet A | B | C | D | E | SE |
|---|---|---|---|---|---|---|
| Dry matter intake (g/d) | 615[b] | 552[a] | 555[a] | 593[b] | 632[b] | 37.56 |
| Dry matter digestibility (%) | 65.36[c] | 56.47[a] | 59.38[b] | 64.59[c] | 69.37[d] | 1.94 |
| Organik matter digestibility (%) | 65.36[c] | 57.84[a] | 60.81[b] | 66.00[c] | 70.34[d] | 1.92 |
| N retention (g/d) | 8.56[c] | 5.64[a] | 6.82[b] | 7.55[b] | 10.37[d] | 0.94 |
| Liveweight gain (g/d) | 102[c] | 88[a] | 89[a] | 94[b] | 104[c] | 3.94 |

A = Elephant grass + concentrate, B = ammoniated PPF + concentrate, C= B + Corn oil, D = C + MHA, E = D + BCAA
Values with different superscript are significantly different (P<0.05)

*et al.* (1987); Hefner *et al.* (1985). This acid is needed to promote the growth of rumen microbe that indicated increasing rumen microbe population (Table 2).

Dry matter intake, Dry matter digestibility, N retention and live weight gain of sheep are given in Table 3. Dry matter intake of ammoniated PPF was lower than other treatment, but after supplemented with MHA and BCAA, dry matter intake increased. This result showed that corn oil, MHA and BCCA could improve population of rumen microbe, so digestibility was also increased and could be increase the rate of passage in rumen. It also has been demonstrated before that BCAA could improve the digestibility (Mir *et al.,* 1991). The improvements in digestibility and intake of poor quality roughages supplemented with protein (Berger *et al.*, 1980; Orskov *et al.*, 1980) have, at least in part, been attributed to the supply of branched chain fatty acids (BCFA) derived from deamination of BCAA.

Digestibility nutrient in diet with dalam press fiber ammoniated lower than other treatment although $NH_3$ concentration higher than other. It is indicated that rumen microbe cannot grow with ammonia as sole nitrogen source. Supplementation corn oil, MHA and BCCA improved number of rumen bacteria and increased the digestibility and intake of ammoniated PPF.

This increased was accompanied by the increased in N retention and live weight gain of sheep.

**Conclusion:** Palm press fiber could be used as source roughage in diet and had same effect with elephant grass on growth performance of ruminant after ammoniated with urea and supplemented with corn oil, MHA and BCAA.

## References

AOAC, 1984. Official Methods of Analysis, Association of Official Analytical Chemists. Washington, DC.

Baldwin, R.L. and M.J. Allison, 1983. Rumen metabolism. J. Anim. Sci., 57: 461.

Berger, L.L., T. Klopfenstein and R.A. Britton, 1980. Effect of sodium hydroxide treatment on rate of passage and rate of ruminal fiber digestion. J. Anim. Sci., 50: 745-749.

Bird, S.H. and R.A. Leng, 1984. Further studies on the effect of defaunation of presence or absence of protozoa in the rumen on live weight gain and wool growth of sheep. Br. J. Nutr., 52: 607-611.

Bird, S.H., J.V. Nolan and R.A. Leng, 1990. The Nutritional significance of rumen protozoa, In The Rumen Eco. system, The Microbial Metabolism and its Regulation, (S. Hoshino, R. Onodera, H. Minato and H. Itibashi, editors. Tokyo.

Bryant, M.P., 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. Fed Proc., 32: 1809-1812.

Griswold, K.E., W.H. Hoover, T.K. Miller and W.V. Thayn, 1996. Effect of form of nitrogen on growth of ruminal microbes in continous culture. J. Anim. Sci., 74: 483-481.

Hefner, D., L.L. Berger and G.C. Fahey Jr., 1985. Branched-chain fatty acid supplementation of corn corp. residue diets. J. Anim. Sci., 61: 1264.

Jones, D.F., W.H. Hoover and W.T.K. Miller, 1998. Effect of concentrations of peptides on microbial metabolism in continous culture. J. Anim. Sci., 76: 611-616.

Jouany, J.P., 1996. Effect of rumen protozoa on nitrogen utilization by ruminant. J. Nutr., on Vol 126 No.4 Suppl. April 1335-1346.

Leng, R.A., 1991. Application of Biotechnology to Nutrition of Animal in Developing countries. FAO Animal Production and Health Paper.

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

McCollum, F.T., Y.K. Kim and F.N. Owens, 1987. Influence supplemental four and five-carbon volatile fatty acids on forage intake and utilization by steers. J. Anim. Sci., 65: 1674-1679.

McCracken, B.A., M.B. Judkins, L.J. Krysl, D.W. Holcombe and K.K. Park, 1993. Supplemental methionine and time of supplementation effect on ruminal fermentation, digesta kinetic and in situ dry matter and neutral detergent fiber disappearance in cattle. J. Anim. Sci., 71: 1932-1939.

Merchen, N.R. and E.C. Titgemeyer, 1992. Manipulation of amino acids supply to the growing ruminant. J. Anim. Sci., 70: 3238.

Mir, P.S., Z. Mir and B.M. Pink, 1991. *In vitro* digestibility of forage supplemented with cellulase (filter paper) and branched chain fatty acids or amino acids. Can. J. Anim. Sci., 72: 1149.

Orskov, E.V. and M. Ryle, 1990. Energy Nutr. in Ruminant. Elsevier Appl. Sci., London.

Orskov, E.R., F.D. Hovell and F.D. Mould, 1980. The nylon bag technique for nutritional studies in ruminants. Trop. Anim. Prod., 5: 195-205.

Russel, J.B. and C.J. Sniffen, 1984. Effect of carbon 4 and carbon 5 volatile fatty acids on growth of mixed rumen bacteria in vitro. J. Dairy Sci., 7: 987.

Santa, A. and S.A. Karim, 2002. Influence of ciliata protozoa on biochemical changes and hydrolytic enzyme profile in the rumen ecosystem. J. Appl. Microbial., 92: 801-811.

Steel, R.G.D. and J.H. Torri, 1980. Principles and Procedure of Statistics. McGraw-Hill Book Co. Inc., New York.

Sundstol, F., 1991. Large Scale Utilization of Straw for Ruminant Production Systems, In Recent Advances on The Nutr. of Herbivora Ed. Y.W. Ho. H.K. Wong, N. Abdullah and Z.A. Tajuddin. Malaysia Society of Animal Nutr.

Suryahadi, 1990. Penuntun praktikum Ilmu Nutrisi Ruminansia, Pusat Antar Universitas Ilmu Hayat. IPB. Bogor.

Van Soest, P.J., 2006. Rice straw the role of silica and treatment to improve quality. J. Anim. Feed. Sci. and Tech., 130: 137-171.