Increasing Rumen Microbial Protein Synthesis with Additional Dietary Substrate of *Saccharomyces cerevisiae* and Soybean Oil

W Suryapratama* and FM Suhartati

Faculty of Animal Science, Jenderal Soedirman University, Jl. Dr. Suparno 60, PO Box 110, Purwokerto 53123
Telephone +62-281-638792, Central Java, Indonesia
***Corresponding author email: mas_eo@yahoo.com**

Absract. A study with the purpose to increase microbial protein synthesis was carried out using in vitro experiment with 2x2 factorial. Completely Randomized Design were used in this study. The first factor was with or without substrate of *Saccharomyces cerevisiae*. The second factor was the addition of 2 levels of soybean oil, 0% and 3%. There were 4 treatments, each treatment was replicated 5 times so that there were 20 experimental units. The materials of this research were Napier grass, concentrates and substrate of *Saccharomyces cerevisiae*, consisted of a mixture of 30% rice bran, 20% tapioca by-product, 20% coconut meal, 20% corn, 9% pollard, 1% mineral, and soybean oil. The rumen fluid as a source of rumen inoculum was taken immediately after a cattle was slaughtered in the slaughterhouse. The variables measured were N-NH_3 concentration and the product of rumen microbial protein synthesis. Data were analyzed using analysis of variance, followed by a test of Honestly Significant Differences (HSD). The conclusion was that the use of substrate of *Saccharomyces cerevisiae* increased rumen microbial protein synthesis as much as 9.42% and the use of 3% of soybean oil increased rumen microbial protein synthesis as much as 18.64%.

Keywords: rumen microbe, *Saccharomyces cerevisiae*, soybean oil

Abstrak. Sebuah studi dengan tujuan untuk meningkatkan sintesis protein mikroba dilakukan dengan menggunakan percobaan in vitro dengan pola faktorial 2x2. Rancangan Acak Lengkap digunakan dalam penelitian. Faktor pertama adalah dengan atau tanpa substrat *Saccharomyces cerevisiae*. Faktor kedua adalah penambahan 2 level minyak kedelai, 0 % dan 3 %. Penelitian melibatkan 4 perlakuan dan setiap perlakuan diulang 5 kali sehingga ada 20 unit percobaan. Materi penelitian ini adalah rumput gajah, konsentrat dan substrat *Saccharomyces cerevisiae*, terdiri dari campuran 30 % dedak padi, 20 % produk samping tapioka, 20 % bungkil kelapa, 20% jagung, 9% pollard, 1% mineral, dan minyak kedelai. Cairan rumen sebagai sumber inokulum rumen diambil sesaat setelah ternak disembelih di rumah potong. Variabel yang diukur adalah konsentrasi N-NH_3 dan produk dari sintesis protein mikroba. Data dianalisis dengan menggunakan analisis variansi, dilanjutkan dengan uji Beda Nyata Jujur (HSD). Kesimpulannya adalah bahwa penggunaan substrat *Saccharomyces cerevisiae* meningkatkan sintesis protein mikroba sebesar 9,42% dan penggunaan 3% minyak kedelai meningkatkan sintesis protein mikroba rumen sebesar 18,64%.

Kata kunci : mikroba rumen, *Saccharomyces cerevisiae*, minyak kedelai

Introduction

The growth rate and productivity of rumen microorganisms determine the performance of ruminants. Rumen microorganisms play an important role in the process of fermentation in the rumen, and are important sources of protein for the animal host. The low growth rate of rumen microorganisms may inhibit the rate of fermentation of feed in the rumen (Bohnert et al., 2002), can further reduce rumen fermentation products. The lower rate of growth of rumen microorganisms affects fiber digestibility of feed and the availability of the microbial protein for the host.

The amount of microbial biomass protein supply to the small intestine reaches 50-80% of total absorbed protein (Bach et al., 2005), while the microbial nitrogen entering to small intestine of lactation cows may reach 251-292 g/head/day (Ipharraguerre et al., 2005). This phenomenon confirms that microbial protein has an important role for the ruminant. Dietary manipulation to increase microbial protein synthesis is necessary to be done. One among the many ways that can be done is by using yeast as an additive of feed materials, such as *Saccharomyces cerevisiae*. To be able to grow and develop, *Saccharomyces cerevisiae* requires substrate as the energy source (Paryad and Rashidi, 2009). The sources of energy that may be used among others, are rice bran and tapioca by-products, which are easily available, inexpensive and is not compete with human needs.

Beside protein, the rumen microbes also require essential fatty acids that may be derived from vegetable oils, such as soybean oil (Ivanov et al., 2010). The information of the influence of soybean oil on rumen fermentation products is still very limited. The objective of this research was to study the use of substrates of *Saccharomyces cerevisiae* and soybean oil in the diet; its influence on the rumen microbial protein synthesis.

Materials and Methods

As a materials of this research were Napier grass, concentrates, and *Saccharomyces cerevisiae* substrate consisted of a mixture of 30% rice bran, 20% tapioca by-product, and soybean oil. The rumen fluid as a source of rumen inoculant was taken immediately after the cattle was slaughtered in the slaughterhouse. The research was carried out experimentally by in vitro and a 2x2 factorials CRD were used. The first factor was with or without substrate of *Saccharomyces cerevisiae*. The second factor was the addition of soybean oil with the levels of 0%, and 3%. There were 4 treatments, each treatment was replicated 5 times so that there were 20 experimental units. The four treatments were: T1: without the substrate of *Saccharomyces cerevisiae*, without soybean oil; T2: without the substrate of *Saccharomyces cerevisiae,* with 3% of soybean oil; T3: with the substrate of *Saccharomyces cerevisiae*, without soybean oil; T4: with the substrate of *Saccharomyces cerevisiae*, with 3% of soybean oil.

The feed consisted of napier grass and concentrate with a ratio of 60%:40%. The concentrate consisted of 50% substrate of *Saccharomyces cerevisiae* (mixture of 30% rice bran and 20% tapioca by-product), 20% coconut meal, 20% corn, 9% pollard and 1% minerals. The nutrient content of diets was listed in Table 1.

The variables measured included: 1) the concentration of N-NH_3, using the Conway method, 2) the rumen microbial protein synthesis using the method of Zinn and Owens (1995). The data were analyzed using analysis of variance followed by a Honestly Significant Difference (HSD) test (Steel and Torrie, 1993).

Table 1. The nutrient content of Napier grass and concentrate

Item	DM (%)	DM Basis (%)				
		CP	Extract Ether	CF	Ash	NFE
Napier grass	12.80	9.27	2.84	33.58	11.96	42.35
Concentrate without *Saccharomyces cerevisiae*	88.56	11.43	5.90	12.81	4.90	54.16
Concentrate with *Saccharomyces cerevisiae*	70.37	13.88	6.26	12.29	4.37	59.06

DM = Dry Matter; CP = Crude Protein, CF = Crude Fiber; NFE = Nitrogen Free Extract

Results and Discussion

Observation from the average values obtained, the concentration of N-NH_3 in rumen fluid fed concentrates diets containing no substrates of *Saccharomyces cerevisiae* was in the range that was ideal for the growth of rumen microbes, while the given feed of concentrates that contained substrate of *Saccharomyces cerevisiae* was far less than ideal for the growth of rumen microbes (Table 2).

According to Boucher et al. (2007), the concentration of ammonia (N-NH_3) which was ideal for rumen microbials growth was 2-13 mg/dL. The function of N-NH_3 in the rumen is as a source of nitrogen for rumen microbes, primarily for cellulolytic bacteria (Valkeners et al., 2006). This point of view suggested that the low concentration of N-NH_3 of rumen fluid that was fed dietary concentrate that contained substrate of *Saccharomyces cerevisiae*; because the N-NH_3 was used by rumen microbes for protein synthesis, not due to a lower production.

There was interaction between the use of *Saccharomyces cerevisiae* with the addition of soybean oil that affected concentration of N-NH_3 in rumen fluid. The rumen fluid with additional dietary concentrate which contained a mixture unfermented of rice bran (30%) + tapioca by-product (20%), without soybean oil (T1), 34.58% was higher (P<0.01) compared to that received concentrate feed which contains a mixture unfermented of rice bran (30%) + tapioca by-product (20%), added 3% soybean oil (T2), 145.91% higher (P<0.01) compared to that received concentrate which contained a mixture of *Saccharomyces cerevisiae* fermented of rice bran (30%) + tapioca by-product (20%), without soybean oil (T3) and the 125% higher (P<0.01) compared to that received concentrate that contained a mixture of *Saccharomyces cerevisiae* fermented of rice bran (30%) + tapioca by-product (20%), added 3% soybean oil (T4), T2 is higher 82.73% (P <0.01) compared to T3 and the higher 93.27% compared to T4, whereas between T3 and T4 there was no significant difference (P>0.05).

The high concentration of N-NH_3 in rumen fluid at T1, most probably was not used optimally by rumen microbes, whereas low concentration of N-NH_3 at T4 was used by rumen microbes to its protein synthesis. It happened because microbial protein synthesis requires a balance between the availability of nitrogen and energy. Pathak (2008) reported that microbial protein synthesis depended on the balance between nitrogen and carbohydrate sources. This statement was in accordance with the statement of Ginting (2005), that the transformation process of nutrients into microbial protein requires optimum environment conditions for rumen microbial growth. The condition includes the availability of various nutrients in the amount, composition and appropriate time. Nitrogen compounds, carbohydrates, vitamins, minerals, co-factors and the various growth factors are the elements of rumen microbial growth, however, nitrogen compound and carbohydrate are needed in the largest amount. The nutrients should be available simultaneously to encourage the growth of

Table 2. Mean concentration of N-NH_3 of rumen fluid (mM)

Type of concentrate	The addition of soybean oil	
	0%	3%
Without substrate of *Saccharomyces cerevisiae*	5.41±0.85[a]	4.02±0.57[b]
Contain the substrate of *Saccharomyces cerevisiae*	2.20±0.33[c]	2.08±0.27[c]

[a,b,c,] Different superscript in the same column and row indicate there were differences at P<0.01

microbes rapidly. In other term, there must be synchronization among the degradation of protein (nitrogen) and energy which can be derived from soybean oil.

Understanding synchronization can be associated with the relationship; positive-associative that is the utilization of a nutrient increases when it is combined with other nutrients at the right time and amount. The oil seeds when added to ruminant feed will increase the energy density. When metabolized, its fatty acids produce more energy than another organic compounds. Energy value of fat is twice as greater or more than seeds (Hess et al., 2008). Thereby the addition of 3% soybean oil in the concentrate feed that contained substrate of *Saccharomyces cerevisiae* causes an increase in energy supply for rumen microbes, which can be used for microbial protein synthesis by utilizing the $N\text{-}NH_3$, as a result, the concentration of $N\text{-}NH_3$ rumen fluid decreased.

The usage of substrate of *Saccharomyces cerevisiae* increased microbial protein synthesis by 9.42% ($P<0.05$). The fact indicated that the use of substrate of *Saccharomyces cerevisiae* increased the protein content of feed ingredients (Table 1). Feed protein was then fermented by rumen microbes to produce $N\text{-}NH_3$. As described previously that $N\text{-}NH_3$ in the rumen was used as a source of nitrogen for rumen microbes, primarily by cellulolytic bacteria. Research results of Doležal et al. (2005) showed that the addition of *Saccharomyces cerevisiae* increased the use of ammonia than the control. There by $N\text{-}NH_3$ produced by rumen fluid with additional concentrate that contained substrate of *Saccharomyces cerevisiae* was more available, resulting in improved microbial protein synthesis.

The addition of 3% soybean oil increased rumen microbial protein synthesis by 18.64% ($P<0.01$) (Table 3). This was due to an increase in the supply of energy source for rumen microbes. As previously described, transformation process of nutrients into microbial protein requires optimum environment and conditions for rumen microbial growth, therefore, there must be synchronization between the degradation of proteins (nitrogen) and energy, so that the microbe is more capable of utilizing $N\text{-}NH_3$ for microbial protein synthesis. In addition, according to Oldick and Firkins (2000) the increase in the efficiency of microbial protein synthesis it associated with an increase of degree of unsaturated fat in diet. Soybean oil contains a lot of unsaturated fatty acids. Therefore, the addition of 3% soybean oil could improve rumen microbial protein synthesis.

The research results also proved that there was a negative relationship between the concentration of $N\text{-}NH_3$ with microbial protein synthesis by the equation $Y = 61.11 – 2.380\ X$ ($r^2 = 0.30$) (Figure 1). The derived equation illustrated that decreasing concentration of $N\text{-}NH_3$ resulted in microbial protein synthesis increase, because $N\text{-}NH_3$ had been utilized by rumen microbes.

Table 3. Mean of Rumen Microbial Protein Synthesis (mg/ml)

Type of concentrate	Soybean Oil		Mean ± SE
	0%	3%	
Without substrate *Saccharomyces cerevisiae*	44.31 ± 2.18	56.95 ± 6.68	50.53 ± 9.71[a]
Contain the substrate of *Saccharomyces cerevisiae*	52.48 ± 3.69	58.09 ± 2.77	55.29 ± 6.08[b]
Mean ± SE	48.40 ± 5.79[c]	57.42 ± 7.43[d]	

[a,b] Different superscript in the same column indicate there were differences at $P<0.05$.
[c,d] Different superscript in the same row indicate there were differences at $P<0.01$.

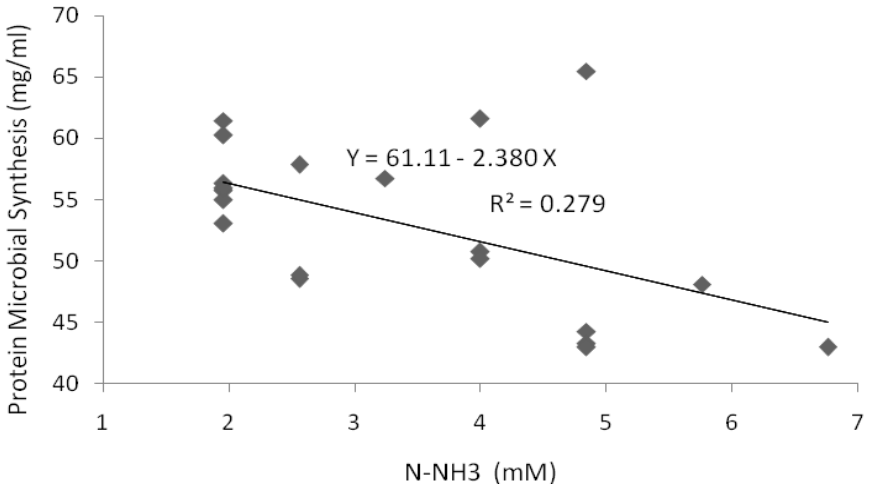

Figure 1. Relationship between concentration of N-NH_3 and the rumen microbial protein synthesis

Conclusions

The use of substrate of *Saccharomyces cerevisiae* increases microbial protein synthesis as much as 9.42%. The use of 3% soybean oil can improve rumen microbial protein synthesis as much as 18.64%.

References

Bach A, S Calsamiglia and MD Stern. 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88 (E. Suppl.):E9-E21.

Bohnert DW, CS Schauer, SJ Falck and T DelCurto, 2002. Influence of rumen protein degradability and supplementation frequency on steer consuming low-quality forage. II. Ruminal fermentation characteristics. J. Anim. Sci. 80:2978-2988.

Boucher SE, RS Ordway, NL Whitehouse, FP Lundy, PJ Kononoff and CG Schwab. 2007. Effect of incremental urea supplementation of a conventional corn silage-based diet on ruminal ammonia concentration and synthesis of microbial protein. J. Dairy Sci. 90(12):5619-5633.

Doležal P, J Doležal and J Třináctý. 2005. The effect of *Saccharomyces cerevisiae* on ruminal fermentation in dairy cows. Czech J. Anim. Sci. 50(11):503–510.

Ginting SP, R Krisnan and K Simanihuruk. 2005. Kulit Nenas sebagai pakan dasar pada kambing persilangan Boer X Kacang sedang tumbuh . JITV 12(3):195-201.

Hess BW, GE Moss and DC Rule. 2008. A decade of developments in the area of fat supplementation research with beef cattle and sheep. J. Anim. Sci. 86 (E.Suppl.):E188–E204.

Ipharraguerre IR, JH Clark and DE Freeman. 2005. Varying protein and starch in the diet of dairy cows. 1. Effects on ruminal fermentation and intestinal supply of nutrient. J. Dairy Sci. 88(7):2537-2555.

Ivanov DS, JD Lević and SA Sredanović. 2010. Fatty acid composition of various soybean products. Food and Feed Research 2:65-70.

Oldick BS and JL Firkins. 2000. Effects of degree of fat saturation on fiber digestion and microbial protein synthesis when diets are fed twelve times daily. J. Anim. Sci. 78:2412–2420.

Paryad A and M Rashidi. 2009. Effect of yeast (*Saccharomyces cerevisiae*) on apparent digestibility and nitrogen retention of tomato pomace in sheep. Pakistan J. Nutrition 8(3):273-278.

Pathak AK. 2008. Various factors affecting microbial protein synthesis in the rumen. Veterinary World 1(6):186-189.

Steel RGD and JH Torrie. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc. New York.

Valkeners D, A The´wis, S Amant and Y Beckers. 2006. Effect of various levels of imbalance between energy and nitrogen release in the rumen on microbial protein synthesis and nitrogen metabolism in growing Double-Muscled Belgian Blue bulls fed a corn silage-based diet. J. Anim. Sci. 84:877–885.

Zinn RA and FV Owens. 1995. A rapid procedure purine measurement and its use for estimating net ruminant protein synthesis. Can. J. Anim. Sci. 66:157-166.