

**METAGENOMIK MIKROBA RUMEN DAN KECERNAAN *IN VITRO* PROTEIN-LEMAK DENGAN METODE PROTEKSI BERBEDA SEBAGAI SUPLEMEN PAKAN TERNAK RUMINANSIA**

**INTISARI**

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Penelitian ini bertujuan untuk mengevaluasi penggunaan campuran proteksi *crude palm oil* (CPO), bungkil kedelai, dan *premix* mineral *Agromix* sebagai substitusi ransum terhadap nilai kecernaan, karakteristik fermentasi rumen, dan keberagaman serta kelimpahan mikroba rumen. Penelitian ini dilaksanakan dalam tiga tahap. Penelitian tahap I yaitu evaluasi metode proteksi bungkil kedelai dan CPO terhadap kecernaan nutrisi dan karakteristik fermentasi cairan rumen. Bungkil kedelai diproteksi dengan dua metode yaitu proteksi formaldehid pada level 0,6, 0,8, 1,0, dan 1,2% kemudian dilakukan analisis kecernaan secara *in vitro* dan *in sacco*, dan pemanasan dengan suhu 60, 80, 100, dan 120°C selama 10, 20, 30, dan 40 menit kemudian dilakukan analisis kecernaan secara *in vitro*. Proteksi CPO dilakukan dengan metode saponifikasi konsentrasi NaOH 3, 5, dan 10% kemudian dilakukan analisis kecernaan secara *in vitro*. Data hasil proteksi protein dengan pemanasan dianalisis dengan ANOVA pola factorial 4x4, sedangkan data hasil proteksi protein dengan formaldehid dan proteksi CPO dianalisis dengan rancangan acak lengkap pola searah. Penelitian tahap II yaitu pengaruh ransum dengan substitusi pakan terproteksi (dengan level substitusi 0; 4,4; dan 7,6% BK ransum) terhadap kecernaan, karakteristik fermentasi rumen, dan profil asam lemak secara *in vitro*. Data yang diperoleh dianalisis dengan rancangan acak lengkap pola searah. Penelitian tahap III yaitu pengaruh ransum dengan substitusi pakan terproteksi (F1 = tanpa substitusi pakan terproteksi, F2 = level substitusi 4,4% BK ransum dan F3 = level substitusi 7,6% BK ransum) terhadap keberagaman dan kelimpahan mikroba rumen menggunakan analisis *next generation sequencing* (NGS). Data kuantitatif keragaman dan kelimpahan mikroba rumen dianalisis secara deskriptif. Hasil penelitian tahap I menunjukkan bahwa proteksi bungkil kedelai dengan 0,8% formaldehid menurunkan kecernaan nutrisi dalam rumen baik secara *in vitro* maupun *in sacco* ( $P < 0,05$ ) jika dibandingkan dengan proteksi pemanasan. Proteksi CPO dengan 5% NaOH menunjukkan peningkatan kecernaan BK dan BO rumput pangola sebagai substrat, hal ini menunjukkan bahwa proteksi CPO mengurangi efek negatif lemak terhadap kecernaan pakan. Hasil penelitian tahap II yaitu substitusi pakan terproteksi dalam ransum menurunkan kecernaan nutrisi dan karakteristik fermentasi rumen ( $P < 0,05$ ) namun masih dalam kisaran normal. Selain itu juga memperbaiki profil PUFA dalam cairan rumen, dengan meningkatnya konsentrasi PUFA dalam cairan rumen dan menurunkan konsentrasi asam lemak jenuh cairan rumen. Hasil penelitian tahap III menunjukkan bahwa substitusi pakan terproteksi dalam ransum mempengaruhi kelimpahan *archaea* namun menurunkan kelimpahan relatif *bacteria* rumen. Terdapat 2 kingdom yang muncul yaitu *Archaea* dan *Bacteria*. Hasil penelitian menunjukkan kelimpahan relatif *Methanobacteriaceae* pada perlakuan F1, F2, dan F3 berturut-turut adalah 11, 21, dan 23% dari total sequen hasil amplifikasi NGS. Kelimpahan relatif bakteri dominan pada perlakuan F1, F2, dan F3 berturut-turut

adalah filum *Bacteroidetes* (51, 45, dan 26% dari total bakteri), *Firmicutes* (29, 24, dan 22% dari total bakteri), dan *Proteobacteria* (21, 18, dan 11% dari total bakteri cairan rumen).

Kata kunci: Substitusi pakan, Protein dan lemak terproteksi, *In vitro*, Asam lemak, Metagenomik.

RUMEN MICROBES METAGENOMIC AND IN VITRO DIGESTIBILITY OF  
PROTEIN-FAT PROTECTED BY DIFFERENT METHODS AS FEED  
SUPPLEMENT FOR RUMINANTS

**ABSTRACT**

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This study aimed to evaluate the use of a protective mixture of crude palm oil (CPO), soybean meal, and Agromix mineral premix as a ration substitution for digestibility values, rumen fermentation characteristics, and rumen microbial diversity and abundance. This research was conducted in three stages. The first stage of research was evaluating the protection methods of soybean meal and CPO on nutrient digestibility and fermentation characteristics of rumen fluids. Soybean meal was protected by two methods, namely formaldehyde protection at the levels of 0.6, 0.8, 1.0, and 1.2%, then in vitro and in sacco digestibility analysis was carried out, and heating with a temperature of 60, 80, 100, and 120 °C for 10, 20, 30, and 40 minutes then an in vitro digestibility analysis was performed. CPO protection was carried out by saponification methods with NaOH concentrations of 3, 5, and 10%, then in vitro digestibility analysis was performed. Data from protein protection by heating were analyzed using ANOVA with a 4x4 factorial pattern, while data on protein protection with formaldehyde and CPO protection were analyzed using a unidirectional completely randomized design. The second stage of research was the effect of rations with substitution of protected feed (with substitution levels of 0; 4,4; and 7,6% DM of rations) on digestibility, rumen fermentation characteristics, and fatty acid profile in vitro. The data obtained were analyzed with a unidirectional completely randomized design. Phase III research is the effect of ration with protected feed substitution (F1 = without protected feed substitution, F2 = substitution level of 4,4% DM of ration and F3 = substitution level of 7,6% DM of ration) on the diversity and abundance of rumen microbes using next generation sequencing (NGS) analysis. Quantitative data on the diversity and abundance of rumen microbes were analyzed descriptively. The results of the study I showed that the protection of soybean meal with 0.8% formaldehyde decreased nutrient digestibility in the rumen both in vitro and in sacco ( $P < 0.05$ ) when compared to heating protection. CPO protection with 5% NaOH showed an increase in the digestibility of BK and BO pangola grass as a substrate, this indicates that CPO protection reduced the negative effect of fat on feed digestibility. The results of the study II showed that the substitution of protected feed in the ration reduced nutrient digestibility and rumen fermentation characteristics ( $P < 0.05$ ) but was still in the normal range. In addition, it also improves the PUFA profile in rumen fluid, by increasing the PUFA concentration in the rumen fluid and decreasing the concentration of saturated fatty acids in the rumen fluid. The results of the study III showed that substitution of protected feed in the ration affected archaea abundance but decreased the relative abundance of rumen bacteria. There are 2 kingdoms that emerged, namely *Archaea* and *Bacteria*. The results showed that the relative abundance of Methanobacteriaceae in the F1, F2, and F3 treatments were 11, 21, and 23% of the total sequences resulting from the NGS amplification, respectively. The relative abundance of dominant bacteria in the F1, F2, and F3 treatments, respectively, are the phylum *Bacteroidetes* (51, 45, and

26% of total bacteria), *Firmicutes* (29, 24, and 22% of total bacteria), and *Proteobacteria* (21, 18, and 11% of the total rumen fluid bacteria).

Keywords: Feed substitution, Protected protein and fat, In vitro, Fatty acids, Metagenomics