

## The Protein-Energy Synchronization Index of The Tropical Legumes for Ruminants

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• Diterima: 22 Desember 2021 • Direvisi: 14 Februari 2022 • Disetujui: 21 Februari 2022

**ABSTRACT.** The study was aimed to assess protein-energy synchronization (PES) index of the tropical legumes as feed for ruminants. The research was carried out experimentally in two stages. The first stage was measured the nutritional value of legumes using the proximate method. All types of legumes had been dried (at 60°C for 3 days) and grinded. The second stage is to calculate the PES index based on the average degradation of g protein and Kg of organic matter (OM) per hour for each legume. Protein degradation and OM were measured at 2, 4, 6, 8, 12, 24, 48, and 72 hours of observation. The results of the degradation observations at each hour were analyzed by linear regression to obtain the average degradation per hour, then the results were included in the calculation formula of the PES index. The PES index of each legume was discussed descriptively. The analysis showed that legume is a source of fiber with an average crude fiber of 18% and a high protein content of 23.23%. Based on the calculation of the PES index *Leucaena leucocephala* (0.34) and *Calliandra calothyrsus* (0.31) are at a low level, while *Indigofera* (0.48), *Sesbania grandiflora* (0.42), and *Gliricidia sepium* (0.47) are at the medium level. This difference was caused by differences in the rate of degradation of protein and organic matter of each legume, besides the presence of antinutrients greatly affected the resulting PES index. The research concluded that the tropical legumes in ruminant feed has the PES indexes at low to medium levels.

**Keywords:** Protein-energy synchronization index, legumes, tropics, ruminant

**ABSTRAK.** Penelitian bertujuan untuk mengkaji indeks sinkronisasi protein-energi (SPE) leguminosa dari daerah tropis sebagai pakan ternak ruminansia. Penelitian dilaksanakan secara eksperimental dalam dua tahapan. Tahap pertama yaitu mengukur nilai nutrisi leguminosa menggunakan metode proksimat. Seluruh leguminosa yang digunakan telah dikeringkan (suhu 60°C selama 3 hari) dan dihaluskan. Tahap kedua yaitu menghitung indeks SPE berdasarkan rata-rata degradasi g protein dan Kg bahan organik per jam pada masing-masing leguminosa. Degradasi protein dan BO diukur pada waktu pengamatan jam ke 2, 4, 6, 8, 12, 24, 48, dan 72. Hasil pengamatan degradasi pada tiap jam dianalisis dengan regresi linier untuk mendapatkan rata-rata degradasi per jam, lalu hasilnya dimasukkan dalam rumus perhitungan indeks SPE. Indeks SPE masing-masing leguminosa dibahas secara deskriptif. Hasil analisis menunjukkan bahwa leguminosa merupakan sumber serat dengan rata-rata serat kasar sebesar 18% dan memiliki kandungan protein yang tinggi dengan rata-rata sebesar 23,23%. Berdasarkan perhitungan indeks SPE lamtoro (0,34) dan kaliandra (0,31) berada pada level rendah, sedangkan indigofera (0,48), turi (0,42), dan gamal (0,47) berada pada level medium. Perbedaan hasil ini disebabkan karena adanya perbedaan laju degradasi protein dan bahan organik pada masing-masing jenis leguminosa, selain itu keberadaan antinutrisi juga sangat mempengaruhi hasil indeks SPE. Penelitian menyimpulkan bahwa leguminosa dari daerah tropis bagi pakan ruminansia memiliki indeks SPE pada level rendah hingga medium.

**Kata kunci:** Indeks sinkronisasi protein-energi, leguminosa, tropis, ruminansia

### INTRODUCTIONS

Feeds are the most important part that must be considered in the livestock business. Adequacy of feed for livestock must be considered because it is needed to meet the basic needs of life, growth, production, and reproduction. Animal productivity will be

optimal if the feed given is sufficient and in accordance with the needs of the livestock. Forage is the main feed given to ruminants because it contains the fiber needed so that the physiological functions of the rumen can function properly. Elephant grass is a forage commonly given to ruminants, but the crude protein content of elephant grass is quite low.

Nurhayu and Pasambe (2016) reported that the crude protein content of elephant grass is 6.46% so it requires additional feed as a protein source.

Legumes are a forage commonly used as a feed ingredient for ruminants. Legumes has a high protein which is necessary for the productivity and growth of ruminants. The advantage of legumes are a high protein compared to forages while the crude fiber content is lower than forages. Legumes commonly used as animal feed are Indigofera (*Indigofera tinctoria*), river tamarind (*Leucaena leucocephala*), calliandra (*Calliandra calothyrsus*), vegetable hummingbird (*Sesbania grandiflora*), and gliricida (*Gliricidia sepium*). It group has a high protein content. The crude protein content of sesbania, gliricida, and calliandra reached 23.76%, 22.92%, and 21.15% (Hadi et al., 2011). The crude protein content of indigofera reached 27.97% and leucaena reached 27% (Argadyastro et al., 2015). However, legumes just can be used up to 30% of the total fiber sources, because they generally have antinutrients.

The difference in chemical composition in each legume caused differences in the concentration on animal feed. The percentage in ration depends on the nutritional quality of the legume used. Indigofera can be used as feed without causing a negative impact on ruminants reached 75%, gliricida leaf reached 70%, and leucaena leaf reached 50% each one of the total feed dry matter (Simanihuruk and Sirait, 2009; Paga et al., 2001; Suardin et al., 2014). Chemical composition differences of the legumes affect sensitivity and will also affect the balance of degradation rates in the rumen, so information on the nutritional value of legumes is needed in the preparation of ruminant rations.

The preparation of ruminant rations must be take an attention to the nutrient value of the feed ingredients, besides that, it must also attention to the microbial activity in the rumen. Rumen microbes have a very important role in the digestion process of ruminants because the feed consumed will first be fermented by rumen

microbes. Therefore, microbial growth in the rumen must be optimized so that the rumen microbial protein synthesis process can run optimally. The microbial protein synthesis (MPS) process requires an optimal rumen environment for microbial growth, namely the availability of nutrients needed by microbes in sufficient quantities and at the right time. The advantage of using legume as feed is to increase the amount of protein provided without reducing the amount of fiber intake for livestock (Wina, 2010; Syamsi et al., 2017).

The optimal environment for the rumen, namely the availability of nitrogen (N) compounds must be balanced simultaneously with the availability of energy. A feed with a good protein and energy synchronization (PES) index will increase microbial protein synthesis (MPS) efficiency, a high MPS level will maximize feed utilization in the rumen fermentation process and support optimal livestock productivity. According to Widyobroto et al. (2007), the degradation kinetics of organic matter (OM) must match with the degradation of the protein. The equilibrium degradation kinematics of the two nutrients will supply N and energy synchronously for MPS of the rumen. The proper degradation kinetics of the two nutrients will provide a simultaneous (synchronous) supply of energy and protein to MPS.

The PES index data for local feed ingredients have not been inventoried, even though it plays an important role in the preparation of ruminant livestock rations (Syamsi et al., 2019). Inventory of the PES index of feed ingredients (including legumes) can be carried out through a series of degradability tests for protein and organic matter through in vivo techniques, however, this method is limited by the need for fistulated livestock and the high cost. The PES index of feed ingredients can be measured in vitro (da Silva et al., 2013; Syamsi et al., 2020; Syamsi et al., 2021). This study was aimed to assess the potential of legume as feed for ruminants based on nutritional value and protein-energy synchronization (PES) index.

## MATERIALS AND METHODS

### Experimental Design

The research was conducted experimental methods at the Laboratory of Animal Feed and Nutrition, Faculty of Animal Science, Jenderal Soedirman University. The material used was rumen fluid from three slaughtered goats which were taken immediately after slaughtering at the Kalibogor Slaughterhouse, West Purwokerto, Banyumas Regency. The study used 5 main materials, namely *Indigofera tinctoria*, *Leucaena leucocephala*, *Sesbania grandiflora*, *Calliandra calothyrsus*, and *Gliricidia sepium*. The legumes obtained from forage fields of BBPTU-HPT Baturraden. The tested legumes were fresh legumes > 1 year old which were obtained after harvesting was carried out. All legumes were dried in oven 60°C for three days before being used as research samples.

The research is divided into 2 stages. The first stage carried out a proximate analysis of several types of legume based on (AOAC, 2005). It needs to do to get information about the protein and organic matter content of each legume before we measure the PES index. The variables measured at this stage were the content of dry matter (DM), organic matter (OM), ash, crude protein (CP), extract ether (EE), crude fiber (CF), nitrogen free extract (NFE), and total digestible nutrient (TDN). Protein and organic matter are the main variables needed. The next step was measuring the degradation of protein and OM of each legume using in vitro techniques (Tilley and Terry, 1963). The variables measured at this stage were protein degradation, OM degradation, and protein-energy synchronization (PES) index.

### Variable Analysis

#### Proximate analysis of legumes

Proximate analysis of legumes was carried out using the AOAC (2005) to determine the nutrient levels of each legume. The DM levels

were obtained by baking 2 g of legume samples at 105°C for 8 hours or until the sample weight was stable. The OM levels were obtained by measuring ash content. The two g of legume samples was burned at 600°C for 12 hours, then OM is obtained from 100% DM minus the ash content. The fat content of feed ingredients was obtained by extracting 2 g of legume sample in Soxhlet with ether as a solvent. The crude fiber content of feed ingredients was obtained by washing 1 g of legume sample with several chemical solutions (H<sub>2</sub>SO<sub>4</sub>, NaOH, acetone, and aquadest). The protein content of the feed ingredients was obtained by distilling 0.1 g of legume samples and the results of the distillation were then titrated with HCl solution. Determination of the NFE content of the feed ingredients is achieved by the following formulation,  $NFE = 100\% - \text{content of } (CP + EE + CF + \text{ash})$ . The TDN levels are calculated based on Hartadi et al. (1990) with the formula,  $TDN = (70.60 + 0.259 CP + 1.01 EE) - (0.76 CF + 0.0991 NFE)$ .

#### Measuring the legumes degradation of protein and organic matter

Measurement of protein and OM degradation of legumes was carried out using in vitro techniques based on da Silva et al. (2013). In vitro digestion was carried out using a 250 ml Erlenmeyer tube filled with 4 g of sample, each feed ingredient was added with 32 ml of rumen fluid and 48 ml of McDougall's solution then put into a shaker water bath with a temperature of 39°C, the Erlenmeyer was shaken with CO<sub>2</sub> for 30 seconds, with a pH of 6.5-6.9 and then covered with a ventilated rubber, and fermented for different times. The time used to measure the degradation of each legume followed Orskov and McDonald (1979), namely 2, 4, 6, 8, 12, 24, 48, and 72 hours. The degradation of protein and OM at each time used were calculated based on the equation made by Tilley and Terry (1963) are as follows.

$$\begin{aligned} \text{Organic Mater (OM) Digestibility} \\ &= \frac{\text{OM Sample} - \text{OM Residue} - \text{OM Blank}}{\text{OM Sample}} \\ \text{Crude Protein (CP) Digestibility} \\ &= \frac{\text{CP Sample} - \text{CP Residue} - \text{CP Blank}}{\text{CP Sample}} \end{aligned}$$

**Measuring protein-energy synchronization (PES) index of legumes**

The PES index of legume was measured according to Syamsi et al. (2017) through in vitro measurement of protein and OM degradation of feed ingredients. The time intervals used were 2, 4, 6, 8, 12, 24, 48, and 72 hours. The degradation rate of protein and OM at each time interval were then analyzed in regression to obtain the degradation rate of grams of N and kilograms of OM per hour. After that, it is used to calculate the PES index of each legume which is then used as the basis for preparing the ration with the following equation.

$$\text{PES index} = \frac{20 - \sum_{1-24}^n \sqrt{\frac{(20 - \frac{N}{OM} \text{ per hour})^2}{24}}}{20}$$

Note: n: observation time, N / OM per hour: the rate of protein degradation compared to the rate

of OM degradation every hour (Hermon et al., 2008).

**Data Analysis**

The data of protein and organic matter degradation were regression analyzed. The rate of degradation of protein and organic matter obtained from the regression then was calculated using the SPE index formula. The results of the PES index are discussed descriptively.

**RESULTS AND DISCUSSION**

Legumes are a type of animal feed plant that contains high protein which is needed for the growth and productivity of livestock. The use of legumes as animal feed has been carried out by breeders by giving it directly or with prior treatment. The use of legume as animal feed has the advantage that it can increase feed protein and fiber as well. This is in accordance with Syamsi et al. (2018) which stated that the use of legume as feed can increase feed protein intake without reducing crude fiber consumed by livestock. The nutritional values of some of the legumes proximate analysis results are presented in Table 1.

Table 1. The results of the proximate analysis of the legume

Nama Bahan Pakan	DM (%)	OM (%)	Ash (%)	CP (%)	EE (%)	CF (%)	NFE (%)	TDN (%)
Indigofera	28.29	91.01	8.99	26.2	3.65	18.6	42.56	62.72
Leucaena	30.27	94.73	5.27	22.7	7.63	21.49	42.91	63.60
Sesbania	30.45	91.83	8.17	24.7	6.39	17.84	42.90	65.64
Calliandra	32.59	94.16	5.84	20.8	3.91	15.71	53.74	62.67
Gliricida	28.30	92.51	7.49	21.75	3.38	16.29	51.09	62.20

Note: DM: dry matter, OM: organic matter, CP: crude protein, EE: extract ether, CF: crude fiber, NFE: nitrogen free extract, TDN: total digestible nutrients; NFE calculation = 100 - (% CP + % EE + % CF + % NFE), TDN calculation = (70.60 + 0.259 CP + 1.01 EE) - (0.76 CF + 0.0991 NFE) [14].

The proximate analysis results showed that the average of crude protein in the legume was 20.8-26.2%. The crude protein values is higher than grass or gramineae. The report of Nurhayu and Pasambe (2016) showed that the crude protein content of elephant grass was 6.46%. Based on the proximate analysis, it was found that the highest crude protein content was

Indigofera 26.2%, then Sebania 24.7%, Leucaena 7.63%, Gliricida 21.75%, and the lowest Calliandra 20.8%. The crude protein analysis is not much different with Hadi et al. (2011), which reported that the crude protein content of Leucaena, Gliricida, and Calliandra were 23.76%, 23.70%, and 21.16%. The research of Mayasari and Ismiraj (2019) reported that

Indigofera crude protein content was 27.9%. The high crude protein content in the legume has quite potential to be used as a forage in feed. The crude protein content is important for the growth and development of rumen microbes and livestock, but it must be balanced with the energy content of the feed, so that energy and protein synchronization occurs. On different occasions, Mariani et al. (2015) stated that the balance of protein and ration energy greatly determines the quality of nutrients in feed ingredients that affect livestock productivity.

The quality of the feed ingredients is determined based on the nutrient content of the feed ingredients. Dry matter serves to avoid decomposition of feed ingredients or changes in nutrient content due to water activity. Based on the proximate analysis, it was found that the average value of the dry matter in the five legumes was at 28.29-32.59%. The highest dry matter in calliandra was 32.59%, followed by sesbania 32.59%, leucaena 30.27%, gliricida 28.30%, and indigofera 28.29%. This value is different from with Hadi et al. (2011) which reported levels DM of Sesbania, Gliricida, and Calliandra were 20.43%, 22.92%, 35.23%, this was due to differences in plant origin. Thence, Rusdy (2012) states that the difference in the dry matter content of a plant is due to differences in the temperature of the plant's environmental conditions.

The proximate analysis showed that the OM value has a negative correlation with ash content. Generally, fresh forages contain high organic matter and low ash. The organic matter and ash from the five legumes were at 91.01-94.73% and 5.84- 8.99%. Legumes have a high value of organic matter because they contain the nutrients needed by livestock. Utomo (2012) confirms that legumes are proteinaceous roughages, namely plants with a high protein source of fiber, reaching 22% per gram of dry matter. Observation of other nutrients, the five legumes had fat content varying between 3.38-7.63%. The fat content is needed by ruminants as

a source of energy that is easily degraded in the rumen. In other hand, Wina and Susana (2013) stated that fat functions to provide energy sources with high density and produces high energy compared to carbohydrates.

The results of the proximate analysis also showed that the average of crude fiber in several types of legume was 15.71 - 21.49% of DM. The content of crude fiber in feed functions to maintain rumen microbial activity so that rumen pH is maintained. Utomo (2012) confirms that the high and low levels of CF content would affect the penetration of rumen microbes in digesting feed nutrients. In other side, NFE and TDN which are indicators of the digestibility of a feed ingredient. The value of NFE is influenced by other nutrient components, based on proximate analysis of the NFE values in some of these legumes which are almost equivalent. The low silica and lignin content caused the legume NFE and TDN content to be higher than the gramineae type. According to Syamsi et al., (2019), the high silica and lignin content in straw caused the NFE and TDN content to be low.

The information of the nutritional value of each legume is needed in the preparation of ruminant rations, however, until now there is not much information on energy and protein synchronization. The OM and protein content are the main information needed to determine the PES index of a feed ingredient (Syamsi et al. 2017). The degradation of both will be measured by the PES index formula to determine the synchronization indication of N and energy supply with a range between 0 and 1. The PES index number of a feed ingredient which is getting closer to 1, shows the ability to supply N and energy that is getting more harmonious (synchronous). The degradation equation and the average degradation of protein and OM, as well as the PES index for each legume are presented in Table 2.

The results of the degradation regression analysis of g of protein and Kg OM in the time range 2, 4, 6, 8, 12, 24, 48, and 72 hour for each

type of legume showed different equations. This was due to the different levels and levels of OM and protein digestibility in each legume. The digestibility of organic matter describes the availability of feed ingredients nutrients, so that different feed ingredients will produce different

levels of degradation. This is in accordance with Syamsi et al. (2017) and Walidi et al. (2017) who reported the results of research on energy and protein synchronization showed the same pattern in testing different feed ingredients.

Table 2. Protein-energy synchronization index in each legume

Feedstufs	Eq protein	Ave g protein / hour	Eq OM	Ave kg OM/ hour	PES Index
Indigofera	y = 2.5287x + 729.63 R <sup>2</sup> = 0.9503	911.70	y = 0.0195x + 1.2882 R <sup>2</sup> = 0.9724	2.69	0.48
Leucaena	y = 1.3452x + 381.22 R <sup>2</sup> = 0.5842	478.07	y = 0.0064x + 1.1313 R <sup>2</sup> = 0.5842	1.59	0.34
Sesbania	y = 1.257x + 634.68 R <sup>2</sup> = 0.7176	724.68	y = 0.0182x + 0.7499 R <sup>2</sup> = 0.8141	2.06	0.42
Calliandra	y = 2.8068x + 270.45 R <sup>2</sup> = 0.9234	472.54	y = 0.0145x + 0.7157 R <sup>2</sup> = 0.9213	1.76	0.31
Gliricida	y = 2.5989x + 427.8 R <sup>2</sup> = 0.685	614.92	y = 0.0131x + 1.3041 R <sup>2</sup> = 0.685	2.25	0.47

Note: Eq protein: The equation of protein degradation; Ave g protein / hour: The average of g protein degradation/hour; Eq OM: The equation of organic matter degradation; Ave kg OM/ hour: The average of kg organic matter degradation/hour; PES Index: protein-energy synchronization index.

The results of the coefficient of determination for each feed varied in calliandra and indigofera, the results of which were close to 100%. This is because digestibility is greatly influenced by the organic matter of feed. The feed digestibility is greatly influenced by the chemical composition of the feed, namely crude fiber and crude protein content (Tillman et al., 1998). The results of the proximate analysis showed that indigofera crude proteins that is greater than other legumes and is balanced with sufficient crude fiber so that the level of feed degradation is high, whereas in leucaena, the crude protein content is high but the crude fiber is also high resulting in lower degradation compared to indigofera and calliandra. Another factor that causes differences in the coefficient of determination is the existence of a different degradation pattern over time. The high fermentability of feed ingredients at 4 hours in the rumen and will subsequently be variously influenced by the availability of nutrients in the rumen. The diversity of the degradation feed pattern as evidenced by the formation of a cubic graph. The difference in degradation which is

significant over time causes the coefficient of determination to be smaller (Syamsi et al., 2017).

Based on Table 2, the results of the calculation of the synchronization index for each legume varied. This difference is due to the difference in the level of degradation of each legume as shown in Table 2. The results of the calculation of the energy and protein synchronization index for low levels of leucaena (0.34) and calliandra (0.31); medium levels in indigofera (0.48), sesbania (0.42), and gliricida (0.47). Indigofera has the highest synchronization index because it has the highest digestibility compared to other legumes, besides that calliandra also has the highest organic matter content compared to other legumes. The legume with the lowest synchronization index was calliandra and leucaena, this can be seen at the rate of g protein and Kg OM digested per hour which was quite lame compared to other legumes. This shows that protein degradation is faster than the degradation of feed organic matter. In other hand, Yang et al. (2010) stated that each feed ingredient has different protein-energy synchronization characteristics because it

is influenced by the nutritional value and digestibility of the feed material itself.

Another factor that can be observed is the legume crude fiber content (Table 1). *Leucaena* has the highest crude fiber content (21.49 %) compared to another legume. The crude fiber content affects the feed degradation process in the rumen. The higher crude fiber content would be the lower digestibility in feed ingredients. The optimal ratio of protein degradation and OM in the rumen for forage originating from tropical/local areas is 20 g N / kg OM fermented with high rumen MPS efficiency and high ration efficiency, and it is used as standard value in determining the index synchronization of protein and OM degradation of feed ingredients in the rumen. This figure is lower when compared to the forages of sub-tropical areas (Hermon et al., 2008).

The study of the PES index is very important concerning the preparation of ruminant rations. Studies regarding the preparation of a ration plan to conserve protein-energy have proven to have a positive impact on the productivity of ruminants. It proved by Syamsi et al. (2017) that the preparation of rations with the PES index ended 1, linearly increasing microbial protein synthesis. Besides, Syamsi et al. (2018) added that the existence of an energy and protein program can improve energy efficiency based on the production of volatile fatty acids in the ration based on the PES index, and Yang et al. (2010) added that protein-energy synchronization is very possible in increasing livestock productivity. Increased microbial protein synthesis caused the PES index, which will increase digestibility and rumen fermentation products. Increasing rumen fermentation products, especially VFA, will increase livestock productivity. Therefore, PES index inventory on various types of feed ingredients is needed. The results of the PES index calculation on 5 types of legume can be used as a starting point in developing ruminant ration based on the PES index in Indonesia.

## CONCLUSIONS

The research concluded that the tropical legumes in ruminant feed has the PES indexes at low to medium levels.

## CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

## ACKNOWLEDGMENT

The authors thanks to the Lembaga Penelitian dan Pengabdian Kepada Masyarakat (LPPM) Jenderal Soedirman University which has fully funded our research through BLU funds in the Riset Peningkatan Kompetensi (RPK) Scheme in 2021.

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