Effect of Heat Treatment on Rumen Degradability of *Gliricia sepium* leaves

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**Abstract.** An experiment with the objective to investigate the effect of different temperature regimes during heat treatment of *Gliricidia sepium* leaves was conducted following a completely randomized design with 4 treatments and 3 replications. Gliricidia leaves were exposed to three temperature regimes namely oven heating at 60°C (T<sub>60</sub>), 90°C (T<sub>90</sub>), and 120°C (T<sub>120</sub>) respectively and followed by sun-drying compared to sun drying as control (Con). The dry matter and protein degradation were measured using *In Sacco* method in three fistulated Bali cows fed a standard diet. Protein solubility (\(a\)) and the insoluble but potentially degradable fraction (\(b\)) were linearly reduced (P<0.001) by the increased temperature in the oven as compared to sun drying. The degradation rate (\(c\)) of protein in the rumen was increased at all heating treatments. The effective dry matter and protein degradation of gliricidia leaf was significantly (P<0.01) reduced by heat treatment in T<sub>90</sub> and no further reduction in T<sub>120</sub>. The solubility fraction of DM was significantly reduced (P<0.05) when heated in the oven at 120°C, meanwhile the b value for DM linearly decline with the increasing heating temperature. Heating also increased the lag time required for the degradation to commence. It, therefore, can be concluded that oven drying at 90°C is a simple effective means to reduce ruminal protein degradation of gliricidia leaves.

**Keywords:** heat treatment, solubility, protein degradation, gliricidia leaf

1. Introduction

*Gliricidia* sepium, tropical tree forages, has been used as high protein supplement for ruminants, and has significantly contributed to the improvement of ruminants in many tropical countries [1]. However, studies reported that animal fed *G. sepium* legumes had widely varies in intake and weight gain [2]. This is due to the variation of high degradability of *G. sepium* which can reach more than 75% [3]. High response to the use of *G. sepium* leaves as a supplement, with this characteristic, is obtained when it is offered to animal consuming low quality feed. In contrast, when *G. sepium* leaves are used as supplement in animal particularly for high-producing purpose such as fattening and lactation, *G. sepium* leaves generally had lower impact on animal performance compared to other tree legumes such as *Leucaena leucocephala* [2]. This is caused by insufficient total flow of amino acids out of the rumen, which is highly dependent on feed portion that is not degraded in the rumen and is digested and absorbed in the small intestine (bypass protein). Therefore, reducing degradability level of *G. sepium* leaves when it used as a protein supplement for animal is necessary important.

Heating to a certain temperature is a method which can reduce protein degradability of feed [4][5]. Protein will form with other complex substrates, especially carbohydrates (maillard reaction) and resistant to microbial degradation in the rumen but can be digested in the small intestine[6][7]. However, most of the studies regarding heating to reduce protein degradability of feed were on grains and legumes, and there is limited information on similar strategy to reduce protein degradability of forages. Each feed
has an optimal heating temperature and depending on the protein and carbohydrate content of the feed, moisture content and other factors [8]. There is no information, however up to date, regarding the effective heating temperature required to reduce protein degradability of *G. sepium* leaves without decreasing the dry matter. Thus, this study was aim to assess the effective heating temperature in the oven to reduce protein degradability of *G. sepium* leaves without decreasing the dry matter.

2. Methods

Fresh *G. sepium* leaves were harvested in March. The leaves were chopped in 2-3 cm before divided into 12 parts. Each part was randomly assigned to 4 treatments and 3 replications following a complete randomized design. The heating treatment was T₀: leaves were sun dried (control), T₆₀: leaves were oven for 4 hours at 60°C and then sun dried, T₉₀: leaves were oven for 4 hours at 90°C and then sun dried, and T₁₂₀: leaves were oven for 4 hours at 120°C and then sun dried.

*In sacco* method was used in this study to determine the degradability of dry matter and protein in the rumen of cows. Three Bali cows were fitted with permanent rumen cannula. The animals were offered a standard feed 1/3 of concentrate (CP = 12%; corn meal, rice bran, fish meal, coconut meal) and 2/3 of good quality grass according to the maintenance energy requirements of cow.

The dried *G. sepium* leaves (DM: 92%) was then ground through a 1.5 mm and about 1 gram of feed was weighed and put into 7.5 x 10 cm nylon bag (pore size of 37 x 37 µm²). 21 nylon bags were prepared for each feed to evaluate protein degradability in the rumen. Bags plus the sample was then incubated into the rumen of fistulated cows for 0, 4, 8, 16, 24, 48 and 96 hours. The bags were immediately frozen after removal from the rumen, and then washed under running tap water for one hour until clear. Feed residue in the bag was then transferred to nitrogen-free filter paper and dried for at least 20 hours at 105°C. The disappearance values of incubated (dried feed) samples were then calculated as the difference between weight (dry matter) before and after incubation was assumed to be degraded in the rumen at a certain incubation time (Yᵣ). Meanwhile, protein degradability was assumed as the difference between weight of protein before and after incubation at a certain time. Protein content was analysed using the Kjeldahl method.

The obtained degradability data was then fixed with the exponential formula using a simultaneous model [9]:

\[
Y(t) = \begin{cases} 
  a & \text{if } t \leq t_0 \\
  a + b \left(1 - e^{-c(t-t_0)}\right) & \text{for } t > t_0
\end{cases}
\]

Where:
- Y(t): the degraded part after incubating for time t
- a: the dissolved part
- b: part is not dissolved but has the potential to be degraded
- c: degradation rate constant (per hour)
- t: incubation time (hours)
- t₀: time delay

2.1. Statistical Analysis

All data obtained in this study were statistically analysed using Proc. Anova of SPSS 24.

3. Results and Discussion

3.1. Effect of heating on protein degradability of *G. sepium* leaves

The efficiency of feed protein supplementation utilisation is highly dependent on the synchronization between the supply rate and the place where the protein is required. In ruminants, some of dietary protein consumed will be degraded into ammonia by rumen as cell protein. The efficiency of this transformation is highly dependent on the rate of supply and the rate of utilization by microbes. Generally, the rate of ammonia supply exceeds the rate of utilization, especially 2-3 hours after meal [10], and results in the accumulation of ammonia in the rumen which then will be absorbed and converted to urea before
excreted in the urine. Thus, dietary protein with high level of degradability, such as *G. sepium* leaves, must be protected from being degraded in the rumen, by heating. The result of reducing degradability level of *G. sepium* leaves in this study is presented in Table 1.

Table 1. Effects of heating on solubility (a), degradation potential (b), degradation rate (c), lag time (LT) and effective degradability at feed flow rates of 2% (DE2) and 4% (DE4) of *G. sepium*.

| Degradability Parameters | Treatment | SEM | P value |
|--------------------------|-----------|-----|---------|
|                          | Control   | T60 | T90     | T120     |        |
| a (%)                    | 31.83a    | 30.28b | 26.06c | 25.07d   | 0.021  | <0.001 |
| b (%)                    | 54.05a    | 43.34b | 39.06c | 39.90c   | 0.437  | <0.001 |
| c (%/h)                  | 8.57a     | 14.04b | 13.09b | 13.00b   | 1.596  | 0.028  |
| LT (h)                   | 2.10abc   | 2.74a  | 1.49bc | 0.68c    | 0.289  | 0.005  |
| DE2 (%)                  | 83.53a    | 71.25b | 63.93c | 64.37c   | 0.570  | <0.001 |
| DE4 (%)                  | 81.30a    | 69.02b | 62.78c | 63.79c   | 0.759  | <0.001 |

* Means within a row different superscript differ (P<0.05).

The value of protein part of *G. sepium* leaves soluble in water (a) in this study was rapidly fermented into amino acids and then into ammonia. The protein dissolved included NPN and soluble protein. Unlike in the division of the feed protein fraction in Cornel Net Carbohydrate Protein System (CNCPS) where the soluble protein is separated between NPN and easily dissolved protein [11], in this study the two protein fractions were not separated. The results indicated that protein of *G. sepium* leaves was relatively high, as much as 31.8% for sundried *G. sepium* leaves. The result of this study is not differ from the results reported by [12] who found the protein solubility of *G. sepium* leaves was between 31.39% to 32.88% of the samples collected during the dry and wet seasons. Compared to other tree legumes, the solubility value in this study is equivalent to that of *Leucaena leucocephala* leaves but much lower than that of *Sesbania grandiflora* leaves. [13] indicated that the solubility of *Sesbania grandiflora* leaves reached as high as 49.33%. High solubility is often not benefits the animal in terms of protein utilization, because when ammonia concentration in the rumen exceeds the minimum threshold for rumen microbial growth and activity, then ammonia will be absorbed and excreted through urine after converted to urea in the liver. Therefore, reducing protein solubility through heating will have a significant impact on the utilization of *G. sepium* leaves and at the same time stimulate an increase in amino acid uptake from the small intestine.

Increasing heating temperature in this study decreased (P <0.001) protein solubility of *G. sepium* leaves. This finding confirms the results of previous studies where protein solubility can be reduced by heating. The effect heating on decreasing protein solubility has been reported mostly in grains or cereals. [14] reported a decrease in protein solubility by using *in sacco* from 25% to 22% in barley. [11] also reported a significant reduction in protein solubility due to heating from 22.3% to 1.7% in corn, 28.4 to 10.1% in wheat and 26.2 to 2.0% in barley, respectively. Meanwhile, the heating effect of forage protein was reported by [8] who noted a decrease in the solubility of clover and grass protein.

The potentially degradable fraction (b) of protein of *G. sepium* leaves which is insoluble in water but can be degraded in the rumen also decreases with increasing heating temperature (P <0.001) to 90°C and there was no further decreases when the heating temperature was increased to 120°C. Several studies have also reported that heating, especially steam heating can reduce the potential of protein degradation. [14] reported that the b value of protein of barley decreased from 72% to 52% when heated at a temperature of 120°C.

The solubility reduction mechanism and potential degradation are generally related to the *maillard* reaction that occurs during heating. The *maillard* reaction or glycation reaction occurs between reducing sugars and amino acids, especially those bound to fibers [15]. Maillard reaction produces furosine (N-ε-2-furoylmethyl-L-lysine), N-ε- (carboxymethyl) lysine (CML), 2-amino-6- (2-formyl-5-hydroxymethyl-1- pyrrolyl) -hexanoic acid (pyrraline) and arginine derivative N-δ- (5-hydro-5-methyl-4-imidazolon-2-yl) -ornithine (MG-H1) which relatively undegradable in the rumen but can be hydrolyzed in the small
intestine [16][17]. Factors affecting maillard reaction include the structure and protein binding in the feed, the proportion of protein fraction, concentration and reactivity of free sugars [18] and moisture content [16]. The difference in response of various types of forage to heat treatment is largely explained by one or more of these factors.

The above discussion partially explained an overview of the utilization pattern of *G. sepium* leaves as a protein source in ruminants. However, due to strong interaction between parameters, the effective degradability parameter is generally used to measure of the possible fermentation rate of *G. sepium* leaves protein in the rumen, though with different assumption of feed flow rates. In this study, the assumptions of feed flow rates were 2% and 4%, which are the typical feed flow rates for forage-based livestock. Increasing heating of *G. sepium* leaves at different temperatures (60°C to 120°C) had the same decreasing degradability pattern. Effective degradability decreased (P < 0.001) sharply (about 10% each) when *G. sepium* leaves were heated at 60°C and 90°C before sundried. However, a further degradability decrease was not detected when *G. sepium* leaves heated at 120°C. The results of this study indicate that the effective temperature to reduce the protein degradability of *G. sepium* leaves is heating in an oven with a temperature of 90°C for 4 hours followed by sundried.

Findings from the present study are expected to contribute to the utilization of *G. sepium* leaves as strategies to increase ruminant production in tropical areas including East Nusa Tenggara of Indonesia where feed availability and quality are often shorten during dry season. Higher rumen degradable protein level is required for ruminant animals consuming low-quality forage as basic diet, thereby heating at higher temperature is not required compared to sundried. In contrast, the animal that RDP has been fulfilled and offered *G. sepium* leaves heated temperature of at 90°C is expected to increase the supply of amino acids that are absorbed in the small intestine. Medium and high production animals required this condition where supply of amino acids from the microbial cells of the rumen is only sufficient for maintenance and moderate production [19]. Meanwhile, for higher production animal required a higher proportion of bypass-protein [20].

### 3.2. Effect of heating on degradability of *G. sepium* leaves dry matter

Degradability parameters of the dry matter of *G. sepium* leaves dried at different temperatures is presented in Table 2. In contrast to protein degradability, a decrease in dry matter degradability of *G. sepium* leaves is not expected. The energy sources for animal are not only derive from protein, but also from carbohydrates and fats. In forages, most of carbohydrates are in the form of fiber. In contrast to proteins where the escape protein is digested in the small intestine at a high rate, fiber is mostly digested in the rumen. Thus, if the dry matter degradability decreases, the energy supply from *G. sepium* leaves will decrease. Consequently, a decrease in dry matter degradability will cause reduction in animal production if *G. sepium* leaves supply most of the ration i.e. as a basal feed. In addition to the fermentation rate, reduction of degradability can directly lesser feed consumption [21] and thus lowers the energy supply for animal. *G. sepium* leaves have sufficient energy content and can be used as basal feed.

| Degradability Parameters | Treatment | P value |
|--------------------------|-----------|---------|
|                          | Control   | T60     | T90     | T120    | <0.001 |
| A (%)                    | 32.64a    | 32.33b  | 33.06c  | 40.05d  | <0.001 |
| B (%)                    | 51.15a    | 47.17b  | 45.63b  | 36.34c  | <0.001 |
| C (%)                    | 18.52     | 12.32   | 13.09   | 12.37   | 0.252  |
| LT (h)                   | 1.11a     | 2.57b   | 2.79b   | 3.49b   | 0.015  |
| DE2 (%)                  | 82.63a    | 77.08b  | 76.15bc | 73.88c  | <0.001 |
| DE4 (%)                  | 81.52a    | 74.78b  | 73.76bc | 71.56c  | <0.001 |

* Means within a row different superscript differ (P<0.01).
Increasing heating temperature in this study decreased (P < 0.001) dry matter degradability of G. sepium leaves. The effective degradability assumed for flow rate of 2 and 4% / hr decreased by about 12 and 9%, respectively, when oven at 120°C. In contrast to the decrease that occurred in protein, degradability reduction was mainly due to a sharp decrease in the insoluble fraction but potentially degraded (b value). This indicates that the existing fibers interact with protein to form fibers that are relatively difficult to ferment in the rumen. Similar finding was reported by [22] who noted a decrease in b2 and b3 fractions of the feed that has the potential degradation between moderate and difficult. Furthermore, the increasing lag time (LT) with increasing heating temperature also shows that microbes were unable to access fiber. Lag time is the time required by microbes to initiate fermentation activity and the length of time required can indicate resistance to these fibers.

Heating is known to decrease not only protein and fiber degradability in the rumen, buts also fermentation or biohydrogenation of fat in the rumen [23]. Protein matrix denatured by heating can block fat droplets from biohydrogenation activity by rumen microbes to escape from the rumen and are then digested in the small intestine [24]. Biohydrogenation reduction benefits ruminants because saturated fat blocks microbes from accessing fiber so that it has the potential to reduce fiber degradation in the rumen. The following advantages are polyunsaturated fatty acids (PUFA) which escape from biohydrogenation in the rumen and are absorbed in the small intestine, improving the quality of milk and meat [25]. However, in this study it was unknown whether the reduction in fat biohydrogenation in the rumen contributed significantly to the decrease in dry matter degradability. Although there was an impact, the possibility is quite small due to the fat content in G. sepium leaves is relatively low.

4. Conclusion

Heating G. sepium leaves in an oven at 90°C for 4 hours followed by sundried effectively reduces protein degradability. This mainly due to reduction of both protein solubility and insoluble but potentially degraded protein fraction. In addition, heating at the same temperature also reduces the degradability of dry matter which can reduce the potential energy supply to ruminants.

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