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# Tannin treatment for protecting feed protein degradation in the rumen *in vitro*

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**Abstract.** This experiment was conducted to evaluate the effect of tannin extract addition for protecting feed protein from microbial degradation in the rumen *in vitro*. Design of experiment employed a factorial  $5 \times 2$  randomized complete block design with 3 replicates. The first factor was tannin extract addition, i.e., control without tannin (T0), addition of 2% tannic acid (T1), addition of 2% tannin chestnut (T2), addition of 2% tannin extract from *Calliandra calothyrsus* (T3), and addition of 2% tannin extract from *Clidemia hirta* (T4). The second factor was two feed ingredients rich in protein contents, consisted of soybean meal and *Indigofera zollingeriana* forage. Results showed that there was no interaction between tannin treatment and different protein sources. Tannin addition from various sources significantly decreased degradation of dry matter and rumen degradable protein ( $P < 0.05$ ), but had no significant effects on pH, dry matter digestibility and crude protein digestibility. Comparing among different tannin sources, the best tannin to protect protein degradation was chestnut tannin. *Indigofera zollingeriana* forage had higher undegradable protein than soybean meal after being added with tannin ( $P < 0.05$ ). It can be concluded that tannin treatment is able to reduce feed protein degradation.

## 1. Introduction

Protein is necessary to increase the production of ruminant livestock [1]. Excessive protein degradation reduces the benefit of protein [2]. Tannin provides beneficial effects for ruminant by mitigating methane emission, protecting protein degradation in the rumen (bypass) and improving productivity [2,3]. With regard to the latter, high-quality protein can be protected by tannin and therefore it is more readily available in the post-rumen gastrointestinal tract. The protein-tannin complex may release at low pH in the abomasum and small intestine [4]. This makes tannin as a substance to manipulate the level of protein degradation in the rumen. However, tannin may also act as an anti-nutritional compound which can reduce feed intake and nutrient digestibility [5]. Supplementation of tannin source therefore should be appropriate to prevent negative effect on ruminant. Tannin from different sources may differently affect nutrient availability and utility, even when used at similar concentration, due to the very heterogeneous of its chemical structure [6] and thus its biological activity [7]. This experiment aimed to evaluate the effect of tannin extract addition from different sources for protecting feed protein degradation in the rumen *in vitro*.



## 2. Materials and method

### 2.1. Sample collection and preparation

Leaves from *Calliandra calothyrsus* and *Clidemia hirta* were collected from the Field Laboratory of Agrostology, Faculty of Animal Science, Bogor Agricultural University. The leaves were air-dried at room temperature until dry and ground to pass a 0.5 mm screen. The ground leaves were extracted for their phenolic compounds. This study also used commercial tannin sources, i.e., tannic acid and chestnut tannin. Soybean meal and *Indigofera zollingeriana* forage were used as protein sources. These samples were ground to pass 1 mm screen for further analyses.

### 2.2. Tannin extraction and quantification

Extraction of tannin was performed in an ultrasonic water bath. An amount of 200 mg of each tannin source was mixed with 10 ml of 70% aqueous acetone and put in the ultrasonic water bath for 25 min at room temperature [8]. The extraction was done twice of each sample, and both supernatants were pooled and centrifuged at 6,000 g for 10 min at 4°C. Total phenols were determined with the Folin-Ciocalteu reagent and detected at 725 nm [8]. A calibration curve was prepared using tannic acid (Merck Chemicals, Madrid, Spain) as the reference standard.

### 2.3. In vitro fermentation

In vitro fermentation technique was based on Theodorou [9]. An amount of 0,5 g substrate and treatment (2% crude tannin extract) were inserted into incubation bottle and mixed with 50 ml buffer solution and rumen liquor. The rumen fluid (containing solid material) was sampled through the fistula from three different Ongole crossbred cattle before morning feeding and filtered through a nylon net. All bottles were sealed with butyl rubber stoppers and aluminium crimp seals. Samples were incubated in water bath at 39°C for 48 h under anaerobic condition. After 48 h incubation, rubber cap was opened and the content was centrifuged at 6,000 rpm for 10 min. The precipitate was analyzed for dry matter degradability and followed by a crude protein analysis to determine the value of crude protein degradation. For measurement of dry matter digestibility, the residue obtained after centrifugation was added with 50 ml of 0.2% pepsin-HCl and incubated for another 48 h [10,11]. The precipitate was analyzed again to obtain dry matter digestibility and crude protein digestibility values.

## 3. Results and discussion

Almost all ingredients used had high protein contents (table 1). Soybean meal had the highest protein content whereas *Calliandra calothyrsus* showed a high value for total phenol.

**Table 1.** Chemical composition of feed ingredients (% dry matter).

Feed	Composition						
	DM	Ash	CP	EE	NDF	ADF	TEP
Soybean meal	90.09	7.28	51.20	1.33	27.61	9.02	na
<i>Indigofera zollingeriana</i>	91.58	11.33	34.44	2.97	35.98	24.06	na
<i>Calliandra calothyrsus</i>	90.87	5.39	24.65	3.60	44.38	33.15	24.29
<i>Clidemia hirta</i>	88.45	6.38	12.97	2.19	27.89	15.63	12.08

DM=dry matter; CP=crude protein; EE=ether extract; NDF=neutral detergent fiber; ADF=acid detergent fiber; TEP=total extractable phenolic; na=not analyzed.

Dietary tannin had no significant effect on ruminal pH (table 2). The rumen fluid pH value in this study was 6.69-6.95. The pH value indicates the optimal range for the growth of rumen microbes. The ideal pH of rumen microorganisms for breeding is 6-7 [12].

**Table 2.** Degree of acidity (pH) of rumen fluid.

Parameter	Protein source	Tannin source				
		T0	T1	T2	T3	T4
pH	<i>Indigofera zollingeriana</i>	6.69 ± 0.18	6.75 ± 0.24	6.85 ± 0.25	6.91 ± 0.27	6.93 ± 0.25
	Soybean meal	6.86 ± 0.29	6.94 ± 0.28	6.91 ± 0.31	6.95 ± 0.29	6.87 ± 0.30

T0=control without tannin; T1=addition 2% tannic acid; T2=addition 2% tannin chestnut; T3=addition 2% tannin extract from *Calliandra calothyrsus*; T4=addition 2% tannin extract from *Clidemia hirta*.

Tannin treatment generally decreased DM degradation in the rumen ( $P < 0.05$ ; Table 3). It was evident that T1-T3 had lower RDP values as compared to T0 ( $P < 0.05$ ; Table 4), apparently due to protein protection effect of tannin from rumen degradation. The RDP value of SBM was higher than that of *Indigofera zollingeriana* ( $P < 0.05$ ).

**Table 3.** Degradation of dry matter (DDM) and dry matter digestibility (DMD).

Item	Protein source	Tannin source					average
		T0	T1	T2	T3	T4	
DDM	IZ	47.13±5.29	42.16±3.77	41.07±3.43	45.38±2.35	49.96±2.65	45.14 <sup>a</sup> ±1.63
	SBM	81.61±3.19	68.99±2.94	64.40±3.27	73.18±1.29	72.72±1.50	72.18 <sup>b</sup> ±1.80
	average	64.37 <sup>c</sup> ±8.19	55.57 <sup>ab</sup> ±6.36	52.73 <sup>a</sup> ±5.63	59.28 <sup>abc</sup> ±6.33	61.34 <sup>bc</sup> ±5.26	
DMD	IZ	77.95±1.97	73.49±2.62	72.25±2.31	65.61±8.22	70.40±1.69	71.94 <sup>a</sup> ±1.90
	SBM	92.49±2.33	85.75±7.79	85.01±7.88	90.46±2.98	86.58±4.15	88.06 <sup>b</sup> ±2.23

Different superscripts in the same row or column indicate significant differences ( $P < 0.05$ ).

T0=control without tannin; T1=addition 2% tannic acid; T2=addition 2% tannin chestnut; T3=addition 2% tannin extract from *Calliandra calothyrsus*; T4=addition 2% tannin extract from *Clidemia hirta*; SBM=soybean meal; IZ=*Indigofera zollingeriana*.

**Table 4.** Rumen degradable protein (RDP), crude protein digestibility (CPD) and rumen undegradable protein (RUP).

Item	Protein source	Tannin source					average
		T0	T1	T2	T3	T4	
RDP	IZ	52.92±4.49	48.27±6.29	45.29±2.87	47.95±3.54	50.62±5.64	49.01 <sup>a</sup> ± 1.92
	SBM	81.13±2.88	67.38±3.12	67.92±2.45	74.22±2.31	76.87±3.20	73.50 <sup>b</sup> ± 1.76
	average	67.02 <sup>c</sup> ±6.74	57.83 <sup>a</sup> ±5.30	56.60 <sup>a</sup> ±5.33	61.08 <sup>ab</sup> ±6.17	63.75 <sup>bc</sup> ±6.54	
CPD	IZ	84.45±0.85	84.55±1.49	83.51±1.55	79.71±2.48	81.07±0.96	82.66 <sup>a</sup> ±0.78
	SBM	95.39±0.94	93.86±2.18	92.60±1.75	95.41±0.07	92.52±1.29	93.96 <sup>b</sup> ±0.64
RUP	IZ	31.53±6.82	36.27±8.58	38.22±2.30	31.76±6.88	30.44±8.28	33.64 <sup>b</sup> ±6.66
	SBM	14.25±5.33	26.48±8.64	24.68±3.83	21.19±3.97	15.64±3.47	19.45 <sup>a</sup> ±5.75
	average	22.89 <sup>a</sup> ±10.93	30.76 <sup>b</sup> ±10.35	31.45 <sup>b</sup> ±7.93	26.47 <sup>a</sup> ±7.66	23.04 <sup>a</sup> ±9.89	

Different superscripts in the same row or column indicate significant differences ( $P < 0.05$ ).

T0=control without tannin; T1=addition 2% tannic acid; T2=addition 2% tannin chestnut; T3=addition 2% tannin extract from *Calliandra calothyrsus*; T4=addition 2% tannin extract from *Clidemia hirta*; SBM=soybean meal; IZ=*Indigofera zollingeriana*.

Nutritional models for feeding protein to dairy cattle have evolved from basic CP [13] to more complex system based on rumen-degradable and undegradable protein [14]. The basic structure of all models are similar with N input is provided by dietary, recycled, and endogenous N. Dietary protein is divided into rumen-degradable and undegradable protein with RDP composed of nonprotein and true protein N. True protein is degraded to peptides and AA and eventually deaminated into ammonia N or incorporated into microbial protein. Non protein N composed of N present in DNA, RNA, ammonia, AA and small peptides with the N from peptides, AA and ammonia being used for microbial growth [15].

The addition of tannin decreased rumen degradable protein, meaning that tannin is generally active in protein protection either by the mechanism of protein complex or by the inhibitory mechanism for microbial protein degradation. Such decrease of RDP was accompanied with an increase of RUP value both in SBM and IZ substrates. Treatment with addition of 2% tannin did not significantly affect crude protein digestibility, indicating that tannin is released post-ruminally. Protein structure apparently explains the difference of protein degradability and digestibility between SBM and IZ. Protein molecular structural characteristics had been shown to strongly correlated with protein solubility, ruminal degradation and intestinal digestibility [16].

#### 4. Conclusion

The best tannin to protect protein degradation from soybean meal and *Indigofera zollingeriana* forage was chestnut tannin. *Indigofera zollingeriana* had a higher undegradable protein than that of soybean meal after being added with tannin.

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