Research Article

Influence of Ensiling and Tannins Addition on Rumen Degradation Kinetics of Soy Sauce Residues

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Abstract | Condensed tannins (CT) and hydrolyzable tannins (HT) are secondary metabolites substances that are widely proposed as safe additives to improve protein utilization in ruminants. This study aimed to investigate the effect of tannins extracted from acacia and chestnut on the degradation kinetics of ensiled and non-ensiled soy sauce residue (SSR) *in sacco*. Two fistulated beef cattle (425±25 kg) fed a diet consisting of 80% *Pennisetum purpureum* and 20% concentrate was used to incubate the SSR samples (ensiled and non-ensiled) using a 6×11 cm standard nylon bag. Each SSR sample was added with 2% CT and HT, respectively. Soy sauce residue without tannins served as control, giving a total of six experimental units as the combined result of 2 types of the ensilage process and 3 levels of tannin addition treatments. The experiment was arranged following a 2×3 factorial design with four replications. Degradability parameters were measured at 0, 4, 12, 24, 48, and 72 h of incubation time. Results showed that adding either CT or HT on soy sauce residue did not affect the degradation kinetics as represented by the values of the soluble fraction (*a*), degradation rate (*c*), and effective degradability (ED) value. However, there was a significant decrease in the value of potentially degradable fraction (*b*) and *a+b* (potential degradation) of dry matter (DM) and organic matter (OM) ($p<0.05$) for the SSR with or without fermentation. In conclusion, extracted CT and HT from acacia and chestnut showed a protective effect on the potentially degradable nutrients from soy sauce residue and their effects were similar either on ensiled SSR or non-ensiled SSR.

Keywords | Acacia, Chestnut, Nutrient degradation, Soy sauce residue, Tannins

Received | September 22, 2021; **Accepted** | November 25, 2021; **Published** | January 05, 2022
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Citation | Sadarman, Irawan A, Ridla M, Jayanegara A, Nahrowi, Ridwan R, Sofyan A, Herdian H, Darma ING, Wahyono T, Febrina D, Harahap RP, Nurfitriani RA, Adli DN (2022). Influence of ensiling and tannins addition on rumen degradation kinetics of soy sauce residues. Adv. Anim. Vet. Sci. 10(2): 270-276.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.2.270.276
ISSN (Online) | 2307-8316

INTRODUCTION

In Asian countries, a large amount of soy sauce residue (SSR) is produced during soy sauce manufacturing because soy sauce is a popular condiment. However, this residue is considered as a by-product and has not been optimally utilized. This residue is rich in protein and antioxidant contents and therefore can be used as a feedstuff for ruminants. Optimizing the use of industrial byproducts as a sustainable feed resource has a long been research interest for researchers in Indonesia dealing with more limited resources of high-quality feed ingredients. Previous experiments have reported that the SSR contained 30.8-34.5% crude protein (CP) and isoflavones with high palatability (Chen et al., 2014; Sadarman et al., 2020). Excessive protein degradation by rumen microbes is one major limitation for high protein-containing feedstuffs which restrict protein utilization by ruminant animals (Irawan et al., 2020). One approach to optimizing such constrain is to protect the protein from ruminal degradation.

Tannins have been reported to protect the dietary protein from ruminal degradation due to their ability to form tannins-protein complexes. Among natural polymers, the affinity of tannins was reported to be highest on protein polymers. This protective effect led to suppress enteric methane formation and ruminal biohydrogenation rate and to increase nitrogen use efficiency and productive performance of ruminants. Thus, dietary tannins are proposed as an effective strategy to sustain the ruminant production system through minimizing the environmental impacts from a livestock operation. There are two types of tannins according to their molecular structure: condensed tannins (CT) and hydrolyzable tannins (HT), which have a distinguished role in rumen fermentation. For instance, Jayanegara et al. (2019) suggested that levels of HT and CT presence in the ensilaged materials had different activities to preserve protein from degradation as indicated from NH3 production. On the other hand, Mezzemo et al. (2011) demonstrated that CT addition in soybean meal concentrate-based diet of beef cattle increased CP utilization without detrimental effect on rumen fermentation. Buccioni et al. (2017) also suggested that CT and HT had similar effectivity to modulate rumen biohydrogenation. Such evidence indicates that CT and HT may act differently on protein digestion depending on the type of diet.

Several experiments have demonstrated the dietary inclusion effects of either CT and HT on nutrient utilization and animal performance (Buccioni et al., 2017; Rivera-Méndez et al., 2016; Rufino-Moya et al., 2019). Several studies also reported on their effects on soybean meal digestion in the rumen. While there has been reporting evidence on poultry, the use of SSR for ruminant diets. However, limited information is available regarding the interaction effect between chestnut and acacia tannins to modulate rumen fermentation and nutrient degradability. To our best knowledge, there is no study reporting tannins' effect on degradation kinetics of soy sauce residue in the rumen, in particular when the SSR is ensiled. Therefore, our study aimed to investigate the interaction effects between tannins form and ensiled or non-ensiled soy sauce residue on rumen degradation kinetics using *in sacco* study. Acacia plant and chestnut were used as the source of CT and HT, respectively.

MATERIALS AND METHODS

Ethical approval

All procedures were carried out in accordance with the standard of the ethical procedure followed by the Faculty of Animal Science, IPB University, Indonesia (Aproved Protocol number 150-2019 IPB).

Site of experiment

This *in sacco* experiment was conducted at Research Unit for Natural Product Technology, Indonesian Institute of Sciences (BPTBA LIPI) Gunungkidul, Yogyakarta, Indonesia. Two fistulated cattle (425±25 kg) were used as experimental animals. The animals were managed according to the ethical standard of animal welfare, the Indonesian Institute of Sciences (LIPI). They were kept in a 5×3 m individual barn and were fed 80% forage and 20% concentrate twice a day. The nutrient composition of the basal diet was following the Indonesian National Standard of feed for beef cattle.

Sample preparation

The soy sauce residue was obtained from the soy sauce industry (PT. Zebra) in Bogor, Indonesia. Acacia bark (*Acacia mangium* Wild.) was obtained from the fiberboard industry of PT. Indonesia Fiberboard Industry is located in Palembang, Indonesia. The acacia bark was oven-dried in the PAU laboratory, IPB University, and was extracted using hot water extraction at 120°C with a pressure of 2 Bar for 3 h. Maltodextrin was added to the liquid tannins extracted from the acacia bark to form condensed water using an atomizer. The condensed form of the sample was heated to form a dried-powder sample. In addition, chestnut tannin was purchased commercially.

The SSR was prepared for the ensiling following the method of Kondo et al. (2016). Briefly, the sample was air-dried and was evaluated for dry matter (DM) determination. The sample was weighed at 1.3kg, mixed with additive, and was homogenized. The sample was ensiled in an anaerobic condition in a laboratory silo for 30 d at room temperature (25-27°C). The silos were opened at 30 d ensiling and the samples were weighted, oven-dried at 60°C for 24 h, and then ground to pass 4-1 mm siever following chemical analysis.

In sacco procedure

In sacco incubation procedure the previous method Ørskov and McDonald (1979). A 6×11 cm nylon bag with 45 µm porosity was used to incubate 5 g soy sauce residue samples. Duplicate sample bags were incubated in two fistulated cattle for 0, 4, 12, 24, 48, and 72 h, giving 4 samples for each incubation time. Total number of samples collected are 24 sample for each incubation time. The experiment animals were kept in well ventilated and hygienic individual stalls with fresh and clean adlibitum water. All animals were drenched with antelminth and sprayed with an accaricide to eradicate internal and external parasites respectively. Animals were fed twice a day on 80% native grass and 20% concentrate diet. On removal the nylon bags were thoroughly washed for 10 min and rinsed, air-dried, and then oven-dried at 50°C for 48 h. Following this, the residual samples were weighted and analyzed for DM and OM determination. The DM and OM analyses were conducted on 110°C ovens and 550°C furnaces, respectively (AOAC, 2016).

Calculation

The potential degradation of the samples was determined using Neway Excel Program ver.6 (Chen, 1997) before effective degradability calculation. The DM and OM potential degradation (PDDM and PDOM) profiles were calculated following the non-linear equation Ørskov and McDonald (1979) as follow:

$$PD = a+b\ [1-e^{-cxt)}]$$

Where; *PD* is a potential degradation of DM and OM; *a* is a soluble fraction; *b* is a potentially degradable insoluble component (mL); *a+b* = potential degradation, including % substrate disappeared from nylon bag; and *c* is the degradation rate (% per h) of *PD*. In addition, the effective degradability (*ED*) of DM and OM (*EDDM* and *EDOM*) was estimated at:

$$ED=a+[bxc/(c+k)]\times e^{-k\times t}$$

Where k was the particulate passage rate and was given at 0.058 per hour.

Data analysis

Soy sauce residue (with or without ensiled) and the addition of CT and HT tannins were treated as experimental treatments. The experiment was designed as a completely randomized block design according to two-ways ANOVA following a 2×3 factorial arrangement. The first factor was the Soy sauce residue (ensiled= ESSR and not ensiled = SSR) where the second factor was the type of tannins (CT and HT) given at 2%. The statistical model used to analyze the data was:

$$Y_{ijk}= \mu+A_i+B_j+AB_{ij}+\varepsilon_{k(ij)}$$

Where Y_{ijkl} = response variable; μ = mean effect or intercept; A_i = effect of type of soy sauce residue; B_j = effect of type of tannins addition; AB_{ij} = interaction effect between the type of soy sauce residue and type of tannins addition; and ε_{ijk} = unexplained errors. Treatment means were compared by Tukey's HSD test for multiple comparisons means when at $p<0.05$. Data results are reported as least square means with their associated standard error of means (SEM). All statistical calculations are calculated using CoStat version 6.400 (2008) Statistical CoHort Software.

RESULTS AND DISCUSSION

Dry matter and organic matter degradability

Degradation rates of DM and OM during 72-h incubation are presented in Figures 1 and 2, respectively. There were significant interactions between the type of SSR and type of tannins used in the experiment at 48 and 72 h whereas ensiled SSR showed higher degradability with CT decreased the DM degradability on both types of SSR ($p<0.01$). In this study, no effect was found on HT treatments either NE or E types. Similar patterns were also shown on OM degradability where the type of tannins had detrimental effects on both SSR types ($p<0.05$). No effect was found on DM and OM degradability when observed before 48 h of incubation.

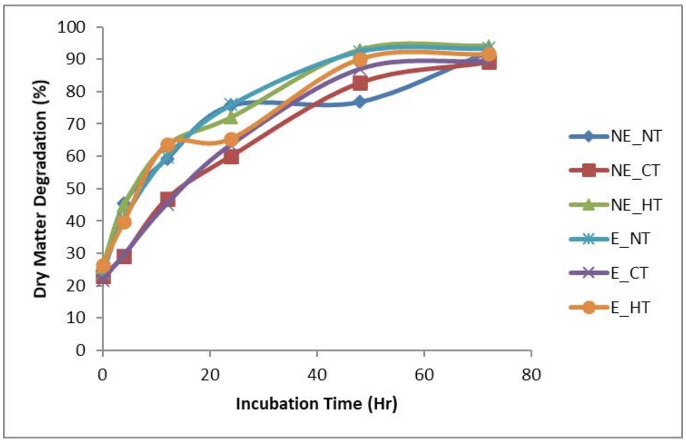

Figure 1: Dry matter degradation percentage of incubated samples as influenced by tannins addition.

Degradation kinetics

DM and OM degradation kinetics are presented in Tables 1 and 2, respectively. Interaction effects between dietary tannins and type of SSR were found to be significant on *b* and *a+b* values of DM degradation kinetics ($p<0.01$). *b* value represents an insoluble fraction with potential degradation while *a+b* represents the optimal degradation of the DM fraction. For degradation rate (*c* value), the type of tannins was found to be significant ($p<0.05$) while no difference was found between types of SSR. No interaction was observed on the effective degradability (ED) index. The ensiled SSR added with tannins extracted from acacia (E+CT) showed the highest b value.

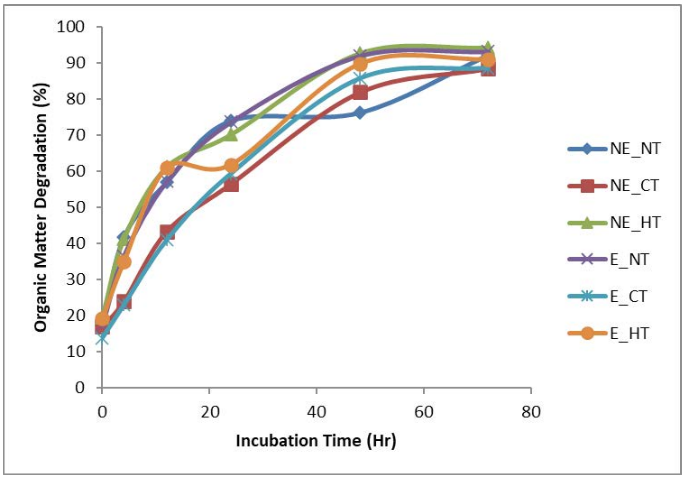

Figure 2: Organic matter degradation percentage of incubated samples as influenced by tannins addition.

Decreasing DM and OM degradability on SSR after CT addition indicated that a protective mechanism might occur on the nutrient content of SSR especially protein. Indeed, CT has been suggested to protect CP from rumen bacterial degradation and their reaction has been well-explained through several mechanisms, i.e., via hydrogen bonds of peptides and phenolic groups or hydrophobic mechanisms. However, varying responses were reported and given the fact that CT effects on rumen fermentation and digestion depend on the source, type, molecular weight, type of diets, as well as animal species (Jayanegara et al., 2019; Patra and Saxena, 2010).

In the present experiment, HT addition did not have any negative effect on the degradability, reflecting that CT and HT act differently in SSR-based diets. Similar results were reported from previous studies whereas dietary tannins source did not affect DMI on soybean meal (SBM) based diets as well as did not affect CP digestion (Dentinho et al., 2014; Jolazadeh et al., 2015). Nevertheless, a dietary concentrate containing 203 g/kg CT decreased nutrient digestibility (Gerlach et al., 2018). Tannins are being the most explored subject in ruminant animals during the last few decades and inconsistent results have been reported. Levels of tannins are suggested to be the most important factor explaining their effect on metabolism. Several researchers have suggested different recommendations regarding the effective effect of tannins. Min et al. (2003) suggested a maximum of 55 g/kg DM of CT could be safely used to promote beneficial effects. Using meta-analysis, Jayanegara et al. (2012) noted that under 100 g/kg DM, tannins were effective to decrease methane emission without any effects on nutrient digestibility and animal performance, with emphasis depending on the type of tannins.

On the other hand, the present study also found that HT did not alter ruminal nutrient degradation as shown by the similar value of treated or non-treated HT. Chestnut tannins rich in HT did not impair production and nutrient utilization in dairy cows (Liu et al., 2013; Aguerre et al., 2016). They concluded that HT might also have decreasing effects when fed given >3% DM. As this study used 2% HT, our findings supported previous reports that incorporating 2% chestnut tannins on SSR diets is safe. Rivera-Méndez et al. (2016) suggested that chestnut did not affect DM intake and average daily gain of finishing beef steers because HT can be fractionated into a simple substance that might be digested by rumen microbes (Saminathan et al., 2016). Another reason is that chestnut was suggested to suppress lactic acid concentration. Therefore, organic matter present in the SSR was not optimally protected thus could be more degradable (Deaville et al., 2010). In addition, our study found that higher DM and OM degradability were found on ensiled SSR when compared to SSR without ensiling process. This might be attributed to the fact that during ensiling, some fiber degrading bacteria might degrade complex structural carbohydrates into more easily degradable nutrients (Kondo et al., 2016).

Findings on DM and OM degradation kinetics in this study agreed with some previous study and suggesting that acacia tannins showed a higher degradation rate although added to the diet with slowly degradable fractions. This might be attributed to the rumen microbes that have adapted to the antinutritional substances. A more recent study supported the reason that rumen residing microbes would adapt to condensed tannin to avoid negative effects from the antinutritional factors (Rivera-Méndez et al., 2016; Rufino-Moya et al., 2019). Though, the adaptability mechanism has not been clearly explained in the available literature. Some argued that changes in the rumen microbial population due to dietary tannins might be occurred (Dentinho et al., 2014). On the other hand, ensiled SSR added with chestnut tannins showed the highest ED index (63.5%, Table 1). This may be due to hydrolyzable tannins can catalyze ether-bound or complex tannins compounds. This process did not affect carbon-hydrogen bound thus allowing rumen microbes to degrade nutritional fractions of the diet (Pawelek et al., 2008).

Table 2 shows that the types of SSR affected the percentage of organic matter degradation kinetics in the insoluble fraction (*b*) and optimal degradation (*a*+*b*), but did not affect the percentage of the soluble fraction (*a*) and the effective degradation (ED) index. Tannins types affected the percentage of organic matter degradation kinetics in the soluble (*a*) and insoluble fractions (*b*), optimal degradation, and the rate of degradation in the soluble fraction (*c*), as well as affected the ED index. The addition of tannins from acacia and chestnuts had a significant effect ($p<0.05$) on the values of *b* and *a+b* on either ensiled or non-ensiled SSR. This could be associated with higher fiber fractions of

NDF and ADF on SSR with no ensiled process (Sadarman et al., 2019; Sadarman et al., 2020), resulting in lower degradability to this treatment. Meanwhile, ensiled SSR had a higher degradation potential because inoculants used in ensiling produced fibrolytic enzymes helped to reduce the NDF and ADF fraction (Irawan et al., 2021).

Table 1: Dry matter degradation kinetics of soy sauce residue as influenced by tannins addition.

Factor A	Factor B	Degradation kinetics (%)				Effective degradability (%)
		a	*b*	*a+b*	*c*	
NE	NT	25.5	59.2ab	84.8b	0.08	60.7
	CT	21.7	73.6a	94.7a	0.03	51.1
	HT	28.5	67.1a	95.7a	0.06	63.5
E	NT	24.8	70.8a	95.6a	0.06	62.3
	CT	20.1	74.7a	95.5a	0.04	52.1
	HT	28.0	65.7a	93.7a	0.05	60.4
SEM		0.77	1.34	1.02	<0.01	1.15
p-value						
A		0.34	0.05	0.02	0.14	0.85
B		<0.01	<0.01	<0.01	<0.01	<0.01
A × B		0.87	0.01	<0.01	0.16	0.13

a is soluble fraction; *b* is potentially degradable insoluble component (%); *a+b* = potential degradation, including % substrate disappeared from nylon bag; and *c* is the degradation rate (% per h); NE: Non-ensiled SSR; E: ensiled SSR; NT; without tannins addition; CT: +2% condensed tannins from acacia bark; HT: +2% hydrolizable tannins from chestnut tannins; SEM: standar error of mean. A: p-value of type of SSR effect; B: p-value of the effect of tannins addition; A × B: p-value from the interaction effects between A and B. [a-c]: means within a row without a common superscript letter differ significantly at $p<0.05$.

The addition of tannins from acacia and chestnuts did not affect the OM degradation kinetics of ensiled and non-ensiled SSR (Table 2). The interaction between the type of fermentation and the type of tannin affected the percentage of organic matter degradation kinetics in the insoluble fraction but did not affect the percentage of effective degradation. The lowest fraction value was obtained from the NE+CT treatment (15.7%) and the highest from the NE+HT treatment (22.6%). The use of tannins from acacia as a silage additive resulted in less soluble fraction loss compared to the other treatments. The lowest b fraction value in this study was obtained from the NE without tannins addition (63.9%) and the highest was found on NE+CT (79.3%). The difference in the degradation values of DM and OM, as well as the degradation kinetics, could be attributed to many factors, including the dimensions of the bag used, the size of the bag pores, the weight of the feed incubated, the maximum incubation time, the cattle feed used during the study, the feed tested, the protein of the feed that can be degraded. and the time and temperature used during postincubation bag washing (Rakhmani et al., 2005; Anele et al., 2009). Condensed tannins were reported to disturb ruminal fermentation and nutrient degradation kinetics in the rumen (Piñeiro-Vázquez et al., 2018; Castro-Montoya et al., 2018; Irawan et al., 2020; Noviandi et al., 2021).

Table 2: Organic matter degradation kinetics of soy sauce residue as influenced by tannins addition.

Factor A	Factor B	Degradation kinetics (%)				Effective degradability (%)
		a	*b*	*a+b*	*c*	
NE	NT	19.9	63.9ab	83.8b	0.08	58.2
	CT	15.7	79.3a	94.1a	0.03	47.4
	HT	22.6	73.0a	95.6a	0.06	61.0
E	NT	18.7	76.7a	95.3a	0.06	59.5
	CT	12.5	63.9a	94.3a	0.04	47.5
	HT	21.5	79.3a	93.2a	0.05	56.9
SEM		0.89	1.45	5.79	<0.01	1.28
p-value						
A		0.14	0.03	0.03	0.12	0.37
B		<0.01	<0.01	0.01	<0.01	<0.01
A × B		0.18	0.01	<0.01	0.16	0.11

a is soluble fraction; *b* is potentially degradable insoluble component (%); *a+b*: potential degradation, including % substrate disappeared from nylon bag; and *c* is the degradation rate (% per h); NE: Non-ensiled SSR; E: ensiled SSR; NT: without tannins addition; CT: +2% condensed tannins from acacia bark; HT: +2% hydrolizable tannins from chestnut tannins; SEM: standard error of mean; A: p-value of type of SSR effect; B: p-value of the effect of tannins addition; A × B: p-value from the interaction effects between A and B. [a-c]: means within a row without a common superscript letter differ significantly at $p<0.05$.

CONCLUSIONS AND RECOMMENDATIONS

Condensed tannin from acacia bark and HT from chestnut tannins had different abilities to protect nutrient degradability during 72-h rumen fermentation. CT had a more protective effect on the organic matter of soy sauce residue from rumen degradation as shown from lower DM and OM ruminal degradability. In addition, ensiled process resulted in higher DM and OM rumen degradation due to the inoculant ability to decrease fiber fraction of SSR during ensiling.

ACKNOWLEDGEMENTS

The authors thank the Indonesian Ministry of Research, Technology, and Higher Education through "Hibah Dasar berbasis Kompetensi (HIKOM)" year 2019, contract number 3/E1/KP.PTNBH/2019 for providing research funds and allowing us to conduct the experiment.

NOVELTY STATEMENT

Our novelty besides in the point of view of the interaction effect between tannins form and ensiled treatments on rumen degradation kinetics. Our study report the different abilities from HT and CT to protect nutrient degradability in soy sauce residue silage. Furthermore, ensiled process on soy sauce residue could increase DM and OM degradation.

AUTHOR'S CONTRIBUTION

SS, AJ, MR, and NN participated in research design and conceptualization. SS, AS, HH, IYGD, TW, DF, RPH, and RAN are involved in *in sacco* study and laboratory analyses. SS and AI drafted the manuscript. AJ, RR, MR, and NN reviewed the manuscript. All authors contributed in giving input and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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