

Anaerobic Fermentation Effectively Reduces Concentration of Total Tannins in *Chromolaena odorata*

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ABSTRAK

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Chromolaena odorata merupakan sumber pakan alternatif potensial, namun penggunaannya terkendala oleh kandungan berbagai senyawa metabolik sekunder dalam jaringan tumbuhan ini. Salah satu kelompok senyawa tersebut adalah tannin. Penelitian ini bertujuan untuk mengevaluasi pengaruh berbagai metode perlakuan awal terhadap total konsentrasi tannin dan daya cerna bahan kering dan bahan organik yang diukur secara *in vitro* dan konsentrasi produk fermentasi rumen. Rancangan acak lengkap (8 x 3) digunakan untuk menguji perbedaan 8 jenis perlakuan yaitu daun *chromolaena* segar sebagai kontrol (*Fresh*), dijemur selama 3 x 24 jam (*Sun-dried*), dikeringkan pada suhu 60°C selama 24 jam (*Oven-dried*), direbus dalam air selama 5 menit (*Boiled*), direndam dalam air biasa selama 4 jam (*RenWater*), direndam dalam NaOH selama 4 jam (*RenNaOH*), direndam dalam HCl selama 4 jam (*RenHCl*), atau difermentasi secara anaerobik selama 21 hari (*Fermented*). Parameter yang diukur adalah konsentrasi tannin total dan kandungan nutrisi dalam setiap bahan yang mendapat perlakuan tertentu. Hasil penelitian menunjukkan bahwa perlakuan Jemur, Rebus, RenAir, dan fermentasi secara nyata menurunkan total tannin sehingga besar 43% hingga 62% dibanding kontrol. Penurunan terbesar (62%) ditunjukkan oleh perlakuan Fermentasi. Sebaliknya, penggunaan panas tinggi (dioven) atau bahan kimia (HCl dan NaOH) tidak nyata menurunkan konsentrasi tannin. Kandungan protein kasar meningkat sebesar 60% dan serat kasar menurun sebesar 32% pada perlakuan fermentasi dibanding kontrol. Disimpulkan bahwa metode fermentasi anaerobik dapat digunakan sebagai strategi efektif untuk menurunkan konsentrasi tannin dalam tumbuhan semak bunga putih (*Chromolaena odorata*) tanpa mengurangi nilai nutrisinya sebagai bahan pakan.

Kata Kunci: *Chromolaena odorata*, Tannin, Daya Cerna, NH₃, VFA

ABSTRACT

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Chromolaena odorata is a potential feed source but its usage is hampered by presence of various secondary metabolic compounds in plant's tissues. One group of them is tannin. This experiment was aimed to evaluate various pre-treatment methods on total tannin concentration and *in vitro* digestibility of dry- and organic-matter. An 8 x 3 completely randomized experimental design was employed to test 8 different treatments. The treatments were: Fresh = freshly-chopped chromolaena leaves as control, Sun-dried = sun-dried (3x 24 hours) chromolaena leaves, Oven-dried = oven-dried (60°C for 24 hours) chromolaena leaves, Boiled = water-boiled (5 minutes) chromolaena leaves, RenWater = water-soaked (4 hours) chromolaena leaves, RenNaOH = NaOH-soaked (4 hours) chromolaena leaves, RenHCl = HCl-soaked (4 hours) chromolaena leaves, and Fermented = anaerobically-fermented (21 days) chromolaena leaves. Parameters measured were concentration of total tannins and nutrient content. The results showed that application of low heat (Sun-dried), hot water (Boiled), water soaking (RenWater), or anaerobic fermentation technique significantly reduced total tannin by 43% into 62% compared to control. The highest suppression (62%) was achieved by Fermented treatment. In the contrary, medium heat application (oven-dried at 60°C) or chemical treatments (HCl or NaOH) had no effect. Protein content of chromolaena was improved by 60% and crude fiber was reduced by 32% in Fermented treatment compared to the control. It could be concluded that anaerobic fermentation can be used an effective strategy to reduce tannin concentration in *Chromolaena odorata* without affecting its feeding value.

Key Words: *Chromolaena odorata*, Tannins, Digestibility, NH₃, VFA

INTRODUCTION

Siam weed (*Chromolaena odorata*) is a pasture weed for Eastern Indonesia rangelands which have extremely high biomass production (up to 70 ton

DM/ha/year) with crude protein content of about 21-36% (Mullik 2002) yet contains various anti-nutrient agents in the form of secondary metabolic compounds. Some of these secondary metabolic properties are tannins, anti-trypsin, haemagglutinin, saponin,

oxalate, pitate acid, alkaloids, steroids, terpenoids, and flavonoids (Akinmoladun et al. 2010; Onkaramurthy et al. 2013). The presence of these compounds lower palatability index of *C. odorata* (Hai et al. 2012) due to strong mint odour and relatively bitter taste. Ruminants rarely consume *C. odorata* in a fresh form. Therefore, pre-treatment is required to overcome these nutritional and intake limitations. The pre-treatments should be directed to reduce or eliminate anti-nutrient compounds but maintain nutrient quality and safe for animals and environment.

Tannin group is a dominant anti-nutrient compound in *C. odorata* (Onkaramurthy et al. 2013). Reducing or eliminating tannins is likely to increase palatability and feeding value of this plant. Provision of feedstuff with high tannin content as single diet to livestock could suppress feed intake, palatability, daily weight gain (Wina 2010), protein degradation in rumen due to tannin-protein binding effects (Patra & Saxena 2011), and toxic for rumen microbes (Bhatta et al. 2009). Tannins also have the potency to disrupt digestive tract function (Makkar 2003) due to an inhibition in the activities of digestive enzymes such as proteases, lipases, and glycosidases (Hagerman 1992).

Physical, chemical and biological treatments are existing pre-treatment methods used to reduce or eliminate tannins in feedstuff (Roger et al. 2015). Physical treatments (chopping, milling, pelleting), chemical treatments (heating, soaking in water or acid or alkali solution), and biological treatment (microbial fermentation) have been adopted worldwide in feed processing. Thus, they could be used to treat *C. odorata* to reduce its anti-nutrient properties. However, various researchers have shown that response of tannins to treatment is not consistent among feed sources. As an example, Hue et al. (2010) reported that withering and drying significantly reduce tannin concentration in cassava leaves, yet Wina et al. (2000) found an increase in tannins in aerobically-dried *Calliandra calothyrsus* leaves. This inconsistency could be related to high variability in chemical structures of tannins among plants and plant materials (Patra & Saxena 2011; Gemede & Hassen 2015).

Due to the inconsistency in tannin response to processing, it is not clear, what is the effective pre-treatment method to reduce tannin concentration in *C. odorata*. Two latest studies (Mullik et al. 2014; Bira et al. 2015) showed that a serial pre-treatments (sun-drying, milling, and pelleting) still not able to guarantee that *C. odorata* can be used as a safe feed source for cattle. These researchers (Mullik et al. 2014; Bira et al. 2015) found that total feed intake, digestibility and rumen fermentation begins to decrease as inclusion of chromolaena meal in the diet increased from 30% to 40%. Based on these findings, the present study was designed to test effects of sun-drying, oven-drying,

water-boiling, soaking in water or HCl and NaOH solution, and fermentation on total tannins and nutrient content of *C. odorata*.

MATERIALS AND METHODS

This study was carried out in August-December 2014. Tannin evaluation and analysis of nutrition content of *C. odorata* was conducted at Animal Feed Technology Laboratory (ITP) of Faculty of Animal Science, Bogor Agricultural University. *C. odorata* leaves used in this study were obtained from Kupang, East Nusa Tenggara. The leaves were harvested by pruning the plants at a height of ± 50 cm from the ground. The leaves were then separated from the rod and assigned to the treatments.

This study used 8 treatments which were arranged in a 8 x 3 Completely Randomized Design. The treatments were:

- Fresh = Fresh *C. odorata* as control
- Sun-dried = *C. odorata* was sun-dried for 3 x 24 hours
- Oven-dried = *C. odorata* was 60°C oven-dried for 24 hours
- Boiled = *C. odorata* was boiled for 5 minutes
- RenWater = *C. odorata* was soaked in the water for 4 hours
- RenNaOH = *C. odorata* was soaked in NaOH 0.1N 10% for 4 hours
- RenHCl = *C. odorata* was soaked in HCl 0.1 N 10% for 4 hours
- Fermented = *C. odorata* was fermented without additive ingredient for 21 days

Sampling and handling procedures

For the first treatment (Fresh), *C. odorata* leaves were chopped as fine as possible and then used for analysis. For the sun-dried treatment, *C. odorata* leaves were spread on top of a tarp, dried under sun light for 3 days, finely grounded and then analyzed. For oven-dried treatment, *C. odorata* leaves were placed in a tray and fed into oven at 60°C for 24 hours. Dried materials was then grounded, and analyzed. For boiled treatment, *C. odorata* leaves were boiled in water (with a ratio of 100 g leaves/150 mL water) for 5 minutes using medium heat. The boiled materials was then filtered, wind-dried, finely chopped, and then analyzed for chemical composition. For the RenWater treatment, *C. odorata* leaves was soaked in plain water (a ratio of 200 g leaves/1000 mL water) at room temperature for 4 hours. For RenNaOH and RenHCl treatment procedures were same with the RenWater, but the solution used was 0.1N NaOH or 0.1N HCl (Ratio: 200 g leaves/1000 mL of 10% NaOH or 10% HCl). After soaking, the

samples were washed with fresh water to reduce negative effect of NaOH and HCl, filtered, wind-dried, finely ground, and analyzed. For fermented treatment, fresh leaves were chopped, arranged into 1 liter jar, densified, air-tight sealed, and fermented for 21 days. After fermentation, the feedstuff was unpacked, wind-dried, then finely ground prior laboratory analyzes for chemical composition. All chemical analyzes were done at Animal Feed Technology Laboratory (ITP) of Faculty of Animal Science, Bogor Agricultural University.

Parameters and measurements

Total tannins content

Quantification of total tannins was done by analyzing samples from each treatment. Sample (1.2 g) was extracted with aquades in 100 mL volumetric flask for 4 hours at room temperature, filtered using whatman paper number 40. About 10 mL supernatant was pipetted into 500 mL volumetric flask where 10 mL *indigo carmine* solution and 300 mL aquades were then added. This solution was then distilled using 0.1N KMnO₄ until the colour of the solution change from blue to green. The titration process continued until the colour of the solution turned into golden yellow. Standar solution of *indigo carmine* was made by dissolving 3 g of *indigo carmine* in 250 mL hot aquades and then cooled. After colling, 25 mL H₂SO₄ was added and diluted into 500 mL aquades. The mixture was then allowed to cool down before filtered. The blank standard was made of 10 mL *indigo carmine* solution and 300 mL aquades.

Total tannin concentration was obtained by using titrimetric method according to Atanassova & Christova-Bagdassarian (2009) modified from The International Pharmacopoeia (2003) and AOAC (1965).

$$T (\%) = \frac{(V - V_o) \times 0.004157 \times 250 \times 100}{g \times 25}$$

where:

V = volume of 0.1N KMnO₄ for sample titration (mL)

V_o = volume of 0.1N KMnO₄ for blanco sample titration (ml)

0.004157= tannin was equivalent in 1 mL 0.1N KMnO₄

G = sample mass used in the analysis (g)

250 = volume of volumetric flask (mL)

25 = volume of *Indigo carmine* (mL)

100 = percentage (%)

Nutrition content

Nutrition content determination in each sample was derived by chemical analysis in a dry matter basis

(DM). The nutrients were ash, crude protein (CP), crude fat (CFat), crude Fiber (CF). The method employed was AOAC (2005). The results were used to compute nutrient content in the basis of g/kg DM or percentage (%).

Data analysis

Data were analyzed by general linear model for Completely Randomized Design with configuration of 8 treatments and 3 replicates. Treatment differences was set at α value of 0.05%. Data were analysed using SPSS version 23. Normalization of data was performed using transformation technique.

RESULT AND DISCUSSION

Total tannins

Total tannin concentration (Table 1) showed a decline trend for all treatments. The highest reduction (62%) was obtained in Fermented treatment (0.94%) compared with control (2.17%). A medium decline in tannin concentration (43-53%) was shown by sun-drying (1.17%), water boiling (1.40%), and water soaking (2.31%). Lowest respons (4-9%) was detected in Oven-dried (2.23%), RenNaOH (2.31%), and RenHCl (2.36%). Total tannin concentration detected in the current experiment fit to the range of 1.3-17.2% reported by Gemedé & Hassen (2015) in various tropical shrub forages in South Africa.

Table 1. Total tannins of *Chromolaena odorata* subjected to various pre-treatment methods

Treatment	Total tannins (% DM)*
Fresh	2.47 ^b
Sun-dried	1.17 ^a
Oven-dried	2.23 ^b
Boiled	1.40 ^a
RenWater	1.31 ^a
RenNaOH	2.31 ^b
RenHCl	2.36 ^b
Fermented	0.94 ^a
SEM	0.033
P-value	<0.001

*Different superscript in the same column shows significant difference at alpha 5%

Analysis variance showed that there were 4 treatments which significantly reduced tannin concentration by 43-62% compared with control. The treatments were Fermented, Boiled, RenWater, and

Sun-dried. Other treatments (Oven-dried, RenNaOH, and RenHCl) had no significant effect ($P>0.05$) compared with control. These results are in line with Roger et al. (2015) who showed that fermentation reduces total tannin concentration. Conclusive explanation about treatment effects on tannin concentration could not be established in the present experiment since types of tannins were not characterized or have not been published elsewhere. However, reduction in tannin concentration in Fermented treatment could be the effect of chemical activities from enzymes produced by various fermentative microbes. These enzymes might damage tannin-enzyme and protein-tannin complex to release protein from tannin in the residual solution (Taylor & Duodu 2014). Furthermore, there was a possibility of continuing hydrolysis of soluble tannins by microbial enzymes to form other compounds during anaerobic fermentation.

A significant decreased in tannin concentration for water-boiled and water-soaked treatments could be related to dissolution of soluble tannin compounds (*gallotannin* group) in the water. Unknown tannin composition (types of tannins) in the present study results in unknown proportion of soluble tannins compared with other three groups (*ellagitannins*, *complex tannins* dan *condensed tannins*). Dissolution of tannins in the water in the current study could be possible since most gallotannins have polyol residues derived from D-glucose (Khanbabaee & van Ree 2001). Khanbabaee & van Ree (2001) reported that gallotannins type *2,3,4,6-tetra-O-galloyl-D-glucopyranose* and *1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose* are intermediary key compounds which have a crucial role in biosynthesis of almost all phenolic compounds in plants. Available water would be a good medium for chemical reactions related to tannin hydrolysis.

Low heat application (sun-dried) also significantly reduced tannin concentration by about 53% in *C. odorata*. This is clearly different from medium heat application (oven-dried at 60°C) which showed an insignificant effect (only 9%) compared with control. Lesser tannin reduction (3.4%) was also reported by Rakić et al. (2004) who oven-drying oak skin at 60°C temperature. Djordjevic et al. (1995) proposed that important reactions occur due to heating are hydrolysis, oxidation, polymerization, and interaction of composition and decomposition processes.

Different response of tannins to heat application could be explained by using statement of Hagerman (2002) that tannins reactions is highly affected by temperature. High temperature stimulate condensation reaction of tannins to form complex bond with other compounds. Slow drying at a low temperature, such as

in Sun-dried treatment, might allow hydrolysis and decomposition of tannins (Makkar & Becker 1996). As tannins have hydrophobic bonds, it will form a strong ionic bond at high temperature (Haslam 1989). This might be the explanation to the insignificant reduction in Oven-dried treatment.

No different in NaOH or HCl treatment with control showed that tannins are less reactive to strong acid or basic solution. Lack of tannin response to strong acid (2M HCl) and base (2M NaOH) was also reported by Osawa & Walsh (1993) for tannic acid. This might related to ionization in hydroxyl phenol group in tannins (Hagerman 2002) hence protecting it from hydrolysis processes.

Nutrition content

The effects of various treatments on nutrient content of *C. odorata* is shown in Table 2. There are three essential nutrient variables determining biological value of *C. odorata* which were affected by treatment i.e organic matter, crude protein, and crude fiber. Data in Table 2 shows that the highest organic matter was obtained in RenHCl treatment (922 g/kg DM) and the lowest one was in Fermented treatment (879 g/kg DM). Organic matter usually has high hydrogen thus increase opportunity for tannins to bind to other organic materials such as cellulose and hemicellulose (McSweeney et al. 2001). Theoretically, soaking of feed materials in acid solution, such as HCl, could cause hydrolysis of complex chemical bonds in organic compounds including tannin-protein or tannin-other organic materials. However, acid or basic affect in the present study was very low compared with other treatments. Data in Table 1 showed that HCl ability to reduce tannins in *C. odorata* was much lower than Fermented treatment. This possibly related to mode of hydrolysis between the two treatments. For RenHCl treatment, the only mode of hydrolysis was by acid effect since most of fermentative microbes might have died in strong acid solution. In contrast, hydrolysis processes in Fermented treatment is mostly done by a variety of fermentative microbes. Released organic matter in HCl treatment might not undergo further decomposition. In the Fermented treatment, even though it has a high ability to reduce tannins causing high amount of organic matter released from the complex, this organic matter might undergo further hydrolysis by microbe during fermentation hence reduces its proportion in the residu. In such a condition, addition of soluble carbohydrates (SCs) is strongly recommended. The SCs are important in fermentation processes as it will improve nutrient value and reduce

Table 2. Nutrient content of *C. odorata* due to treatments

Treatment	Nutrient content					
	Dry material	Organic material	Crude protein	Crude Fat	Crude Fiber	BETN
	----- g/kg DM -----					
Fresh	266 ^b	908 ^c	174 ^f	30 ^e	127 ^c	578 ^a
Sun-dried	839 ^a	866 ^f	258 ^b	48 ^d	108 ^b	452 ^d
Oven-dried	863 ^a	888 ^d	250 ^c	67 ^c	99 ^b	471 ^{bc}
Boiled	245 ^b	914 ^b	196 ^e	106 ^a	135 ^c	477 ^b
RenWater	271 ^b	914 ^b	228 ^c	109 ^a	145 ^d	433 ^e
RenNaOH	247 ^b	867 ^f	199 ^{de}	62 ^c	130 ^c	474 ^b
RenHCl	183 ^c	922 ^a	202 ^d	107 ^a	151 ^d	462 ^{cd}
Fermented	243 ^b	879 ^e	290 ^a	75 ^b	86 ^a	429 ^e
SEM	0.002	0.002	0.012	0.006	0.006	0.002
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*Different superscript in the same column show significant different at alpha value of 5%

dry material losses (McDonald et al. 1991). The strategy is very crucial in silage making particularly for feedstuff with low C : N ratio.

The highest crude protein content (290 g/kg DM) was shown in Fermented treatment and the lowest (174 g/kg DM) was in control (Fresh). Data in Table 2 shows that all treatments increased protein content above control. This might be explained by data in Table 1 that all treatments were able to decrease tannin concentration hence an increase in tannin free proteins. Fermentation increase crude protein of *C. odorata* due to its capability disrupt tannin-protein complex. Besides, chemical activity of fermentative microbes and lysis of tannin-enzyme and tannin-protein complexes (Taylor & Duodu 2014). It is presumed that an increase in protein content in the Fermented treatment is likely due to contribution of microbial proteins synthesized during fermentation process. Crude protein content in water-boiled treatment (196 g/kg DM) was higher than control but lower than other treatments. This possibly related to dissolution of tannin-protein complex. Heat from boiling likely to cause denaturation of protein hence releases proteins from protein-tannin linkage in *C. odorata*.

Lowest crude fiber (86 g/kg DM) was found in fermented treatment and the highest (145 g/kg DM) content was in RenWater treatment. A decline of crude fibre in Fermented treatment likely to be caused by fiber-digested bacterial activities during fermentation. Among cellulotic species, there are fiber digested bacteria to digest cellulose, hemicellulose, and starch (Kana Hau et al. 1005). Water soaking (RenWater) had a very poor capability to reduce crude fiber content. Fiber consists of cellulose, hemicellulose, and lignine.

Usually, cellulose and hemicellulose would be degraded with the help of cellulose and hemicellulose enzymes, whereas water has no direct effect on crude fiber degradation.

Crude fat of fresh *C. odorata* was 30 g/kg DM and tended to increase after treatment. Highest fat content detected in RenWater treatment (109 g/kg DM). This increase might be caused by a significant loss in non-fat compounds, particularly carbohydrates due to fermentation and other processes. Reduction in non-fat organic compounds will automatically increase proportion of crude fat in feed materials.

BETN consist of highly digestible carbohydrate compounds. BETN content of fresh *C. odorata* was 578 g/kg DM. Lowest BETN content (429 g/kg DM) was in the Fermented treatment. BETN content in fresh *C. odorata* was very high since all soluble carbohydrates have not been degraded, whereas in the Fermented treatment, soluble carbohydrates have been degraded by microbes during the fermentation processes. Provision of additives such as soluble carbohydrate is highly recommended in fermentation (McDonald et al. 1991).

CONCLUSION

Fermentation was the most effective technique to reduce tannin concentration yet improves nutrient value of *C. odorata*. Fermentation decreased total tannin concentration by 62%, reduced crude fiber from 127 g/kg DM into 86 g/kg DM (32% improvement), and increased crude protein from content from 175 g/kg DM to 290 g/kg DM (60% improvement). This study showed that anaerobic fermentation is the best method

to treat *C. odorata* to improve its biological value as a potential animal feed.

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