Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: http://www.tandfonline.com/loi/taar20

Effects of glycerol and chestnut tannin addition in cassava leaves (Manihot esculenta Crantz) on silage quality and in vitro rumen fermentation profiles

Theo Mahiseta Syahniar, Muhammad Ridla, Anuraga Jayanegara & Anjas Asmara Samsudin

To cite this article: Theo Mahiseta Syahniar, Muhammad Ridla, Anuraga Jayanegara & Anjas Asmara Samsudin (2018) Effects of glycerol and chestnut tannin addition in cassava leaves (Manihot esculenta Crantz) on silage quality and in vitro rumen fermentation profiles, Journal of Applied Animal Research, 46:1, 1207-1213, DOI: 10.1080/09712119.2018.1485568

To link to this article: https://doi.org/10.1080/09712119.2018.1485568

Published online: 08 Jun 2018.

JOURNAL OF APPLIED ANIMAL RESEARCH
2018, VOL. 46, NO. 1, 1207–1213
https://doi.org/10.1080/09712119.2018.1485568

Taylor & Francis
Taylor & Francis Group

Effects of glycerol and chestnut tannin addition in cassava leaves (*Manihot esculenta* Crantz) on silage quality and *in vitro* rumen fermentation profiles

Theo Mahiseta Syahniar[a,d], Muhammad Ridla[b], Anuraga Jayanegara[b] and Anjas Asmara Samsudin[c]

[a]Graduate School of Nutrition and Feed Science, Faculty of Animal Science, Bogor Agricultural University, Bogor, Indonesia; [b]Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor, Indonesia; [c]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Malaysia; [d]Study Program of Animal Production, Polytechnic of Agriculture and Animal Husbandry MAPENA, Tuban, Indonesia

ABSTRACT

The effects of glycerol and chestnut tannin addition on non-ensiled and ensiled cassava leaves which were then incubated in an *in vitro* rumen system were investigated to evaluate the ensiling characteristics and fermentation profiles, respectively. Both non-ensiled and ensiled cassava leaves were prepared using laboratory-scale minisilos with 4 different treatment types of additives. The treatments were Control (cassava leaves without additive [S0]), Cassava leaves + 3% dry matter (DM) of glycerol (SG), Cassava leaves + 3% DM chestnut tannin (ST), and Cassava leaves + 3% DM glycerol + 3% DM chestnut tannin (SGT). The cassava leaves silage recorded with high concentration of lactic acid, negligible amount of butyric acid, and low concentration of ammonia-N, demonstrates a well-preserved silage quality after 4 weeks fermentation. The *in vitro* total gas production and volatile fatty acid (VFA) concentration did not differ between the treatment groups, but the *in vitro* dry matter digestibility (IVDMD) and the *in vitro* organic matter digestibility (IVOMD) increased after the ensiling process. The addition of glycerol alone or in combination with chestnut tannin showed propiogenic property in the non-ensiled cassava leaves. Chestnut tannin, when added singly or in combination with glycerol, reduced the ammonia-N and iso-VFA concentration in the ensiled cassava leaves. Furthermore, the addition of glycerol and/or chestnut tannin improved some of the silage quality and showed no detrimental effects on the *in vitro* rumen fermentation profiles.

ARTICLE HISTORY
Received 6 November 2017
Accepted 30 May 2018

KEYWORDS
Cassava leaves; glycerol; tannin; *in vitro*; rumen fermentation

1. Introduction

Cassava leaves (*Manihot esculenta* Crantz) are a valuable protein supplement for ruminants and have been used in a number of countries, particularly in the tropics including Indonesia (Marjuki et al. 2008) and Malaysia (Sharif et al. 2015). Generally, cassava is harvested for its roots while the leaves can be used as ruminant feed, and therefore it can be implied that the increase of cassava crop production also increases the cassava leaves production (Widodo et al. 2015). However, due to the abundant availability during production, cassava leaves require the use of storage technology to maintain their nutritional characteristics and to avoid deterioration. Ensiling has been widely applied in many countries and proved to be able to conserve the nutrient quality and prevent too much decline from harvesting quality. The principle of the ensilage mechanism is to ferment materials in an anaerobic condition so that lactic acid bacteria (LAB) proliferate and produce an acidic environment at a pH of 4 or lower to suppress and even stop the activity of proteolytic enzymes and harmful microorganisms (McDonald et al. 1991; Napasirth et al. 2015). The ensiling process of cassava leaves is challenging due to low water soluble carbohydrate (WSC) and high protein contents; such characteristics may lower the ensiling quality of cassava leaves; thus, they need to be addressed with proper additives. The additives used in this present study were glycerol and chestnut tannin.

The use of glycerol is associated with a tremendous increase of glycerol production in the biodiesel industry in order to meet the increasing global demand for renewable and sustainable energy. Generally, this industry produces crude glycerol which makes up as much as 10%–20% of the total volume of biodiesel produced (Quispe et al. 2013). Glycerol has been safely used for animal feed (FDA 2006), and many countries in the European Union, USA, Australia and even Thailand have begun to adapt glycerol as a feed substitution for energy source. Some published studies revealed that glycerol supplementation in ruminants did not cause any adverse effect on rumen fermentation characteristics (Lee et al. 2011), cattle performance and carcass characteristics (Ramos and Kerley 2012) as well as the production and composition of milk (Donkin et al. 2009). Glycerol supplementation is generally used by mixing it with the ration, and to our knowledge, glycerol has never been used as a silage additive. Therefore, to expand the utilization of glycerol, which is not only used as a feed supplement but also as a substitute of energy sources in the ration, this current study attempted to utilize glycerol as a silage additive. Chestnut tannin is a type of hydrolyzable tannin (Jayanegara et al. 2015) and has been used either as an additive

CONTACT Anjas Asmara Samsudin anjas@upm.edu.my Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, UPM 43400Serdang, Selangor, Malaysia

in ration or in silage. Tannins are able to form complexes with protein and are resistant to proteolytic activity of microbes either in silos (Salawu et al. 1999) or in the rumen (Messman et al. 1996). Tabacco et al. (2006) used chestnut tannin as an additive in alfalfa silage and proved its ability to reduce protein degradation during storage. The objective of this study is to evaluate the non-ensiled and ensiled characteristics of cassava leaves with additions of glycerol and/or chestnut tannin and their fermentation profiles when incubated in an *in vitro* rumen system. The use of both types of additives, i.e. glycerol and/or chestnut tannin, on cassava leaves is hypothesized to improve the silage quality as well as maintain its nutrient value during storage. In addition, it is expected that the influence of the additives on the non-ensiled and ensiled cassava leaves will not cause adverse effects on ruminal fermentation done by *in vitro* incubation.

2. Materials and methods

2.1. The making of non-ensiled and ensiled cassava leaves

The cassava leaves (*Manihot esculenta* Crantz) used in this study were obtained from the Selangor Wholesale Market, Malaysia. They were grown locally and collected from various farms in the Selangor state. The non-ensiled and ensiled cassava leaves were prepared by separating the leaves from the stems, and then the leaves were chopped manually into the length of 4–5 cm. Subsequently, the chopped cassava leaves were mixed thoroughly and divided into four parts, with each part consisting two kg of fresh cassava leaves. Each part of the cassava leaves was sprayed with or without silage additives using a mini hand-sprayer according to the following treatments: control (cassava leaves without additives [S0]), cassava leaves + 3% DM glycerol (Glycerol C0347-91552409, GmbH Chemicals [SG]), cassava leaves + 3% DM chestnut tannin (MAMA989 Sintotan, Sintofarm, Italy [ST]), and cassava leaves + 3% DM glycerol + 3% DM chestnut tannin (SGT). The additives were dissolved first in distilled water and the solutions were added at a level of 20 ml/kg fresh leaves. These procedures were repeated three times on different days and served as repetitions. The cassava leaves were ensiled for 28 days in mini polyethylene silos with a capacity of 450 ml and stored in a room at a temperature of 28–31°C. After 28 days of incubation, the silos were opened and a fraction of the silos' content was oven-dried at 60°C for 48 hours or until constant weight had been achieved to reduce the moisture content. Later, it was pass through a 1 mm screen mini-grinder (Hung Chuan Machinery Enterprise Co. Ltd., China) to be ground and used for the *in vitro* gas production assays and analysis of the nutrient composition. Supernatant produced from the ensiled samples was used to measure the silage quality. The non-ensiled cassava leaves were prepared as above sprayed with or without silage additives according to the 4 treatments as previously described except that the samples were not incubated in mini polyethylene silos.

2.2. In vitro *gas production*

As much as 200 mg DM of non-ensiled and ensiled ground cassava leaves was used for *in vitro* gas production assay based on the Hohenheim gas test method (Menke et al. 1979). The inoculum for the *in vitro* incubation was obtained from a ruminally fistulated bull fed with rice straw and palm kernel cake pellets (60:40 w/w). Rumen fluid was collected before the morning feeding, filtered through two layers of cheesecloth and immediately kept in an airtight thermos flask, before being transported transported to the laboratory. The *in vitro* assay was conducted in three runs (repetitions) at different retrieval times of rumen fluid. Each replicate consisted of four syringes per treatment in which two syringes were used for measurement of fermentation characteristics and the two other syringes were used for determination of gas production kinetics.

Solid and supernatant samples were taken at 24 hours after incubation to measure fermentation characteristics and digestibility, i.e. *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD). Solid samples were collected by filtration using a sintered glass crucible and then oven-dried overnight to constant weight for IVDMD calculation. After that, the samples were burned in the furnace at 520°C for 3 hours. The IVOMD was calculated using the following formula:

$$\text{IVOMD} = ((\text{OM incubated [g]} - \text{OM residue [g]})/\text{OM incubated [g]}) \times 100\%. \quad (1)$$

Meanwhile, the pH for the supernatant samples were immediately checked by using a digital portable pH meter. The samples were then added with 2–3 drops of 10% sulphuric acid and kept frozen at −20°C to preserve the remaining supernatant samples. The gas production kinetics were observed at 0, 1, 2, 4, 8, 12, 18, 24, 48, 72 and 96 hours of incubation and then calculated based on Gompertz equation (Beuvink and Kogut 1993) in which μ0/D was replaced by the simplified factor B using the non-linear regression procedure of SPSS software version 16.0. Gompertz equation was:

$$Y = Ae^{-Be^{-Ct}} \quad (2)$$

where: Y is the cumulative total gas production (ml) at time t; A is the total gas production (asymptotic value, ml/g), B is the latency period before gas production starts (lag phase, h) and C is the specific gas production rate (slope, ml/h).

2.3. Chemical analysis and calculation

The supernatants of non-ensiled and ensiled cassava leaves samples were measured for pH, volatile fatty acid (VFA) composition, lactic acid and ammonia-N concentration. The supernatants were extracted according to the Ridla and Uchida (1998) method. Firstly, wet silage (25 g) was blended with 100 ml distilled water then filtered manually using the Whatman filter paper No. 1. Then the pH value of the supernatant was measured immediately with a glass electrode pH meter (Mettler Toledo). The remaining supernantant was preserved with 2 - 3 drops of 10% sulphuric acid and kept frozen at −20°C for VFA, lactic acid and ammonia-N analyses. The VFA and lactic acid concentrations were measured using gas chromatography (GC, Agilent 69890N Series Gas Chromatography System from Agilent Technologies, USA) equipped with a flame ionization detector as described by Cottyn and

Boucque (1968). The ammonia-N analysis was conducted according to the modified phenate method from Parsons et al. (1984) using a spectrophotometer (Genesys 20 Monochromator Init, Thermo Spectronic) at the wave length of 640 nm, and the ammonia-N concentration was calculated by regression equation of the standard.

Next, the oven-dried samples of non-ensiled and ensiled cassava leaves were analyzed for nutrient composition according to AOAC (2005). Dry matter (DM) content was obtained by oven-drying at 105°C for 24 hours to constant weight. The organic matter (OM) was calculated as the weight loss upon ashing, and the crude ash was obtained by burning at 600°C for 4 hours. The ether extract (EE) was measured by petroleum ether extraction which uses a Soxhlet apparatus (2050 Soxtec, FOSS Tecator, Denmark). Also, the crude protein (CP) was analyzed using a Dumatherm nitrogen-determination system run under the combustion method (Gerhardt Malaysia, Kuala Lumpur, Malaysia). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using the the method by Van Soest et al. (1991).

The determination of VFA and ammonia-N concentrations in fermentation fluid of the *in vitro* assay was achieved with a procedure similar to that of silage supernatant. Methane concentration was estimated using the VFA stoichiometry model according to Moss et al. (2000) as follows:

$$CH_4 = 0.45\,C_2 - 0.275\,C_3 + 0.40\,C_4 \quad (3)$$

where: CH_4 is methane, C_2 is acetate, C_3 is propionate, and C_4 is butyrate.

Methane concentration in mmol/l was converted as a proportion of total VFA.

2.4. Parameters and statistical analysis

The parameters of this current study were divided into silage fermentation characteristics and *in vitro* assays of non-ensiled and ensiled cassava leaves with the addition of glycerol and chestnut tannin. The silage fermentation characteristics, which consisted of lactic acid, acetic acid, propionic acid, butyric acid, pH value, ammonia-N and also nutrient compositions (DM, OM, CP, EE, NDF, and ADF), were evaluated to measure the non-ensilage and ensilage quality. Meanwhile, the parameters on the *in vitro* assays consisted of the *in vitro* gas production kinetics, the digestibility (IVDMD and IVOMD), and the *in vitro* rumen fermentation profiles (pH value, total VFA, acetate, propionate, iso-butyrate, butyrate, iso-valerate, valerate, acetate-propionate ratio, methane and ammonia-N). They were studied to evaluate the effects of the additives and the ensiling treatments on the *in vitro* ruminal fermentation.

The data for the silage fermentation characteristics, *in vitro* assays and nutrient composition of non-ensiled and ensiled cassava leaves were analyzed using the analysis of variance (ANOVA) according to a factorial randomized complete block design with the following statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{(ij)} + \tau_k + \varepsilon_{ijk} \quad (4)$$

where: Y_{ijk} is the dependent variable, μ is the grand mean, α_i is the glycerol and/or tannin effect ($n = 4$), β_j is the ensiling effect ($n = 2$), $\alpha\beta_{(ij)}$ is the treatment interaction effect, τ_k is the block effect ($n = 3$), and ε_{ijk} is the random error.

Outliers were removed by using the standardized residual method; data were considered as outliers when their standardized residuals were lower than −2.0 or more than 2.0. When a parameter showed significantly different at $P < 0.05$, the treatments were separated by Duncan's multiple range tests. All the statistical analyses were performed by using SPSS software version 16.0.

3. Result

3.1. Chemical composition and silage fermentation characteristics of non-ensiled and ensiled cassava leaves

The chemical composition and silage fermentation characteristics of the non-ensiled and the ensiled cassava leaves are presented in Table 1 and Table 2, respectively. The DM contents of cassava leaves were affected by the additive and the ensiling (both at $P < 0.001$) but showed no interaction (Table 1); they were in the standard range of good quality silage. The result revealed that the ensiling reduced the OM contents by a relatively small percentage, i.e. around 0.2% ($P < 0.001$). However, neither the additive nor the ensiling affected the CP contents of cassava leaves. Other than that, the ether extract contents were found to have increase due to the ensiling ($P < 0.001$).

Table 1. Chemical composition (g/kg dry matter) of non-ensiled and ensiled cassava leaves.

		Additive					*P*-value		
Parameter	Ensiling	S0	SG	ST	SGT	SEM	Additive	Ensiling	A*E
Dry matter	No	291^{a}	303^{b}	313^{c}	310^{c}	1.03	<0.001	<0.001	0.491
	Yes	303^{b}	322de	327^{e}	317^{cd}				
Organic matter	No	926^{cd}	927^{d}	926b^{cd}	925^{bcd}	0.24	0.026	<0.001	0.114
	Yes	925^{bcd}	924^{bc}	922^{a}	924^{ab}				
Crude protein	No	334	335	335	331	1.11	0.206	0.209	0.805
	Yes	334	333	329	326				
Ether extract	No	5.02^{a}	4.75^{a}	4.68^{a}	4.93^{a}	0.57	0.599	<0.001	0.533
	Yes	5.76^{b}	5.64^{b}	5.78^{b}	5.55^{b}				
NDF	No	451^{b}	427^{b}	432^{b}	433^{b}	2.85	0.327	<0.001	0.404
	Yes	389^{a}	384^{a}	397^{a}	389^{a}				
ADF	No	237^{c}	226^{bc}	226^{bc}	222^{abc}	1.74	0.125	<0.001	0.431
	Yes	217^{ab}	221^{ab}	210^{a}	210^{a}				

$^{a–e}$Means values with different superscripts within the same parameter are different at $P < 0.05$.

A*E means interaction between additive and ensiling level. S0 = control (without additive); SG = S0 + 30 g/kg DM glycerol; ST = 30 g/kg DM chestnut tannin; SGT = S0 + 30 g/kg DM glycerol + 30 g/kg DM chestnut tannin; ADF = acid detergent fiber; NDF = neutral detergent fiber; SEM = standard error of the mean.

Table 2. Silage fermentation characteristics of non-ensiled and ensiled cassava leaves.

Parameter	Ensiling	Additive				SEM	*P*-value		
		S0	SG	ST	SGT		Additive	Ensiling	A*E
Lactic acid	No	nd	nd	nd	nd	6.12	0.960	na	na
(g/kg DM)	Yes	41.0	37.2	31.6	38.4				
Acetic acid	No	1.30^{a}	2.96^{ab}	1.32^{a}	1.44^{a}	0.99	0.711	0.006	0.712
(g/kg DM)	Yes	10.36^{b}	6.42^{ab}	5.36^{ab}	6.45^{ab}				
Propionic acid	No	nd	nd	nd	nd	0.04	0.178	na	na
(g/kg DM)	Yes	0.32	0.24	0.54	0.29				
Butyric acid	No	nd	nd	nd	nd	na	na	na	na
(g/kg DM)	Yes	nd	nd	nd	nd				
pH value	No	6.07^{d}	5.88^{c}	5.96^{c}	5.97^{cd}	0.07	<0.001	<0.001	0.396
	Yes	4.86^{b}	4.70^{a}	4.82^{ab}	4.79^{ab}				
Ammonia-N	No	0.97^{a}	1.46^{a}	1.47^{a}	1.49^{a}	1.14	0.992	<0.001	0.984
(g/kg TN)	Yes	15.0^{b}	14.7^{b}	14.4^{b}	14.0^{b}				

$^{a–d}$Means values with different superscripts within the same parameter are different at $P < 0.05$.

A*E means interaction between additive and ensiling level. na = not applicable; nd = not detected.S0 = control (without additive); SG = S0 + 30 g/kg DM glycerol; ST means 30 g/kg DM chestnut tannin; SGT = S0 + 30 g/kg DM glycerol + 30 g/kg DM chestnut tannin; DM = dry matter; TN = total nitrogen; SEM = standard error of the mean.

Furthermore, the ensiling ($P < 0.001$) caused both NDF and ADF contents to decrease, but no changes were observed among different the additive treatments.

The ensiled cassava leaves generally showed a well-preserved quality after four weeks of fermentation. The main characteristics were shown by the high proportion of lactic acid, negligible amount of butyric acid and low concentration of ammonia-N (Table 2). Lactic and propionic acids were not detected in the non-ensiled cassava leaves while their concentrations became prominent after four weeks fermentation; the concentrations did not differ among additive treatments which were around 37 and 0.35 g/kg DM, respectively. As expected, the pH value of each batch of cassava leaves decreased after the ensiling ($P < 0.001$). The result also revealed that the addition of glycerol decreased the pH of both non-ensiled and ensiled cassava leaves more than that of the control group ($P < 0.05$) while the addition of chestnut tannin did not. The Ammonia-N concentration was not influenced by different additives, but its concentration increased considerably after the ensiling ($P < 0.001$).

3.2. In vitro *gas production kinetics, digestibility and fermentation characteristics of non-ensiled and ensiled cassava leaves*

The total gas production (A) and gas production rate (C) were not affected by the ensiling, the additives and their interaction (Table 3). The results revealed that the latency period (B) of cassava leaves increased after the ensiling ($P < 0.001$). Moreover, the *in vitro* digestibility of cassava leaves, both IVDMD and IVOMD, showed no difference among different additives, but the values increased after ensiling ($P < 0.001$).

The addition of glycerol or chestnut tannin to the cassava leaves showed a decrease in the pH of the *in vitro* rumen fermentation compared to that of the control ($P < 0.05$; Table 4). However, the response became insignificant for the ensiled cassava leaves. The difference of pH value between the additives showed a relatively small variation ranging from 0.05 to –0.08. The concentration of the total VFA showed no difference between the additives or the ensiling, but the proportion of individual VFA has changed. The addition of glycerol alone or in combination with chestnut tannin decreased the acetate compared to control ($P < 0.05$), but the effect was insignificant in the ensiled cassava leaves. An interaction between the additives and the ensiling occurred for a propionate proportion ($P < 0.05$) as shown by its increase due to the glycerol addition to the non-ensiled cassava leaves, but there was no difference found for the ensiled leaves. Such acetate and propionate changes were reflected in the acetate to propionate ratio. In addition, butyrate showed the highest value ($P < 0.05$) in both the non-ensiled and ensiled cassava leaves compared to the others. Furthermore, valerate was insignificant among the additives, but its value increased in the ensiled cassava leaves ($P < 0.05$). The proportion of iso-VFA (isobutyrate and isovalerate)

Table 3. *In vitro* gas production kinetics and digestibility of non-ensiled and ensiled cassava leaves.

Parameter	Ensiling	Additive				SEM	*P*-value		
		S0	SG	ST	SGT		Additive	Ensiling	A*E
A (ml)	No	140	152	147	153	16.20	0.315	0.502	0.343
	Yes	162	162	163	151				
B (h)	No	4.12^{ab}	3.90^{a}	3.83^{a}	3.89^{a}	0.055	0.398	<0.001	0.145
	Yes	4.23^{ab}	4.57^{b}	4.19^{ab}	4.60^{b}				
C (ml/h)	No	0.108	0.100	0.106	0.103	0.001	0.687	0.172	0.064
	Yes	0.096	0.103	0.096	0.108				
IVDMD (g/kg)	No	348^{a}	461^{bc}	359^{a}	421^{ab}	11.10	0.552	<0.001	0.080
	Yes	551^{cd}	528^{cd}	600^{d}	558^{cd}				
IVOMD (g/kg)	No	423^{a}	502^{bcd}	446^{ab}	475^{abc}	8.53	0.443	<0.001	0.145
	Yes	558de	544^{cde}	609^{e}	565de				

$^{a–e}$Means values with different superscripts within the same parameter are different at $P < 0.05$.

A*E means interaction between additive and ensiling level. S0 = control (without additive); SG means S0 + 30 g/kg DM glycerol; ST = 30 g/kg DM chestnut tannin; SGT = S0 + 30 g/kg DM glycerol + 30 g/kg DM chestnut tannin. A = total gas production; B = latency period; C = gas production rate. IVDMD = *in vitro* dry matter digestibility. IVOMD = *in vitro* organic matter digestibility. SEM = standard error of the mean.

Table 4. *In vitro* rumen fermentation characteristics of non-ensiled and ensiled cassava leaves incubation.

		Additive					*P*-value		
Parameter	Ensiling	S0	SG	ST	SGT	SEM	Additive	Ensiling	A*E
pH value	No	6.78^{c}	6.70^{a}	6.70^{a}	6.75^{bc}	0.005	0.025	0.572	0.012
	Yes	6.75^{bc}	6.73^{ab}	6.73^{ab}	6.73^{ab}				
Total VFA (mmol/l)	No	52.4	62.2	58.5	58.3	1.920	0.513	0.257	0.789
	Yes	55.0	57.3	65.5	66.0				
Acetate	No	71.2^{b}	69.3^{a}	71.7^{b}	69.6^{a}	0.159	<0.001	0.302	0.817
(g/100 g VFA)	Yes	70.4^{ab}	68.9^{a}	71.2^{b}	69.5^{a}				
Propionate	No	18.0^{a}	20.8^{b}	19.0^{a}	20.5^{b}	0.147	0.001	0.035	0.019
(g/100 g VFA)	Yes	18.9^{a}	19.1^{a}	18.5^{a}	19.1^{a}				
Iso-butyrate	No	1.00^{b}	0.83^{a}	0.82^{a}	0.84^{a}	0.018	0.001	<0.001	0.005
(g/100 g VFA)	Yes	1.14^{b}	1.33^{c}	1.00^{b}	0.99^{b}				
Butyrate	No	7.13^{ab}	7.36^{bc}	6.74^{a}	7.34^{abc}	0.066	0.019	0.845	0.263
(g/100 g VFA)	Yes	6.90^{ab}	7.88^{c}	6.99a^{b}	7.05^{ab}				
Iso-valerate	No	1.27^{b}	0.92^{a}	0.92^{a}	0.94^{a}	0.030	0.001	<0.001	0.045
(g/100 g VFA)	Yes	1.78^{c}	1.92^{c}	1.50^{b}	1.42^{b}				
Valerate	No	0.97^{abc}	0.88^{ab}	0.84^{a}	0.85^{ab}	0.018	0.271	<0.001	0.429
(g/100 g VFA)	Yes	1.06^{cd}	1.17^{d}	1.04^{cd}	1.01^{bcd}				
Acetate/Propionate	No	3.89^{b}	3.34^{a}	3.78^{b}	3.40^{a}	0.034	0.001	0.101	0.081
	Yes	3.73^{b}	3.70^{b}	3.86^{b}	3.59^{ab}				
Methane	No	29.8^{c}	28.4^{a}	29.7^{c}	28.6^{a}	0.086	<0.001	0.517	0.549
(g/100 g VFA)	Yes	29.5^{bc}	28.7^{a}	29.8^{c}	29.0^{ab}				
Ammonia-N	No	8.90^{b}	8.23^{ab}	8.80^{b}	7.59^{a}	0.089	<0.001	<0.001	<0.001
(mg/dl)	Yes	12.5^{d}	13.6^{e}	10.7^{c}	11.3^{c}				

$^{a-e}$Means values with different superscripts within the same parameter are different at $P < 0.05$.
A*E means interaction between additive and ensiling level. S0 = control (without additive); SG = S0 + 30 g/kg DM glycerol; ST = 30 g/kg DM chestnut tannin; SGT = S0 + 30 g/kg DM glycerol + 30 g/kg DM chestnut tannin. VFA = volatile fatty acid. SEM = standard error of the mean.

in the cassava leaves decreased in comparison to the control ($P < 0.05$) due to the chestnut tannin addition. Also, the estimated methane concentration decreased due to glycerol addition in both the non-ensiled and ensiled cassava leaves ($P < 0.05$). However, the addition of the chestnut tannin did not change the concentration of methane. Ammonia-N concentration was reduced due to the chestnut tannin addition, both when added singly or in combination with glycerol, especially in the ensiled cassava leaves compared to that of the control ($P < 0.05$).

4. Discussion

The protein content of cassava leaf silages was in standard range of 29.3%–38.6% DM according to Yeoh and Chew (1976). The decrease of the NDF in the cassava leaves after ensiling is similar to the study of Man and Wiktorsson (2002) in which the NDF content was reduced to about 8% DM from fresh matter. Such NDF reduction is associated with hydrolysis of fiber components, particularly hemicelluloses by acid formed during fermentation (Jatkauskas and Vrotniakiene 2006) so that the carbon chain could be used as an energy source for LAB growth. It was shown that hemicelluloses were solubilized during a 150–day ensiling period (Morrison 1979). More than half of hemicelluloses contained in the feed materials can be degraded either by hemicellulase from plant origin, hemicellulase from bacteria or through hydrolysis by organic acids produced during fermentation (McDonald et al. 1991).

The ensiled cassava leaves with or without the addition of glycerol and/or chestnut tannin exhibited a well-preserved quality as shown by high concentration of lactic acid, negligible amount of butyric acid and low concentration of ammonia-N. Generally, the silage quality is determined by the fermentation characteristics at the end of the ensiling period in which the main indicators are pH value, organic acids and ammonia-N concentration. The quality of the ensiled cassava leaves in the present study is aligned with the standards of well-preserved quality of high protein silage, i.e. pH 4.3–4.5, lactic acid proportion 65%–70% of total acids (Kung 2010; Ward and de Ondarza 2008), lactic acid concentration 30–60 g/kg DM (Seglar 2003), acetic acid <30 g/kg DM, propionic acid <5 g/kg DM, butyric <1 g/kg DM (Ward and de Ondarza 2008) and ammonia-N concentration <8 g/kg TN (McDonald et al. 1991).

The lactic acid concentration reached an average of 37 g/kg DM or 80%–85% of total acids in the ensiled cassava leaves, which was higher than the lactic acid concentration in the cassava leaf silage with or without molasses as reported by Man and Wiktorsson (2002) which was about 9.5–9.9 g/kg DM. The difference may be attributed to the different varieties of cassava leaves and the different processes in making silage. The high concentration of lactic acid shows a rapid LAB growth, which is able to suppress and dominate undesirable microorganisms (McDonald et al. 1991) during fermentation. Such good fermentation characteristics are confirmed by the negligible butyric acid concentration and low concentrations of acetic and propionic acids. Butyric acid indicates nutritional damage due to secondary fermentation of clostridial activity (McDonald et al. 1991). Acetic and propionic acid indicate the fermentation did not achieve the standard quality when they are produced beyond 3% since generally their function is to maintain aerobic phase stability (Seglar 2003).

The high production of lactic acid was not able to significantly decrease the pH value. The lower pH value on ensiled SG may be due to glycerol utilization by LAB to produce organic acids. This is supported by Santos et al. (2015) who stated that glycerol is a rich source of energy for anaerobic microorganisms. It may lead to produce more acids as primary microbes' metabolites which decrease the pH value in ensilages. The relatively high (>4.5) pH value of ensiled cassava leaves is apparently due to the high buffering capacity

since the materials contained high protein. The fermentation of protein leads to the formation of ammonia-N as a result of proteolysis and breakdown of amino acids. Proteolysis is inhibited by deactivation of proteolytic enzymes due to high organic acids produced in the silage. The addition of chestnut tannin (ST and SGT treatments) helps to reduce proteolysis through its ability to bind protein and to form stable complexes at pH 3.5–7.5 (Barry and McNabb 1999). This result is consistent with that of a previous study which showed that the addition of 4% DM chestnut tannin in alfalfa silage reduced proteolysis activity (Tabacco et al. 2006).

Total gas production, and gas production rate were unchanged by the ensiling and the additives. It is generally agreed that increasing the level of glycerol supplementation in the diet (Meale et al. 2013) and the addition of different purified tannins at low levels (Jayanegara et al. 2009, 2015) have no effect on the gas production kinetics or cumulative gas production after 24 hours. Different from the finding of this study, other authors reported that gas production increased linearly when glycerol was added to alfalfa hay (Krueger et al. 2010) or, on the contrary, a reduction in gas production occurred when glycerol was substituted for alfalfa hay or corn grain (Lee et al. 2011). This study revealed that the different additives had no detrimental effect on IVDMD and IVOMD of both non-ensiled and ensiled cassava leaves. These results were in agreement with both of the previous meta-analysis *in vitro* study in which glycerol supplementation was used as an energy source for ruminants (Syahniar et al. 2016) and the addition of low level tannin showed a lower adverse effect on digestibility (Jayanegara and Palupi 2010). On the contrary, the present study indicated that ensiling significantly increases the percentage of IVDMD and IVOMD as shown by lower NDF and ADF contents of the cassava leaves after ensiling. It has been shown that NDF and ADF have a negative correlation with feed digestibility (Riaz et al. 2014; Laconi and Jayanegara 2015).

The concentration of total VFA was unchanged by the different additives or ensiling, but the proportion of individual VFA, especially for non-ensiled cassava leaves, indicated a propiogenic property due to the addition of glycerol. Propiogenic property was shown by an increase of propionate and a decrease of acetate to propionate ratio. Such propiogenic property of glycerol was also observed in other studies (Rémond et al. 1993; Avila-Stagno et al. 2014). Bergner et al. (1995) demonstrated that most of 14C labelled glycerol was found in propionate form, and rumen microbes used glycerol selectively for producing propionate rather than acetate (Ramos and Kerley 2012). Since propionate serves as a hydrogen sink, which is a main substrate for methanogenesis, in the rumen, the addition of glycerol decreased the emission of methane (Lee et al. 2011). However, the propiogenic property of glycerol became negligible when cassava leaves were ensiled, probably because of its utilization by LAB that prevented rumen microbes from metabolizing the substance.

The proportion of iso-VFA and ammonia-N concentration was lower than the control, especially in the ensiled cassava leaves with the addition of chestnut tannin (when added singly or in combination with glycerol), apparently due to a lower extent of proteolysis and deamination. Such results confirm the ability of tannin to bind protein molecules. Hassanat and Benchaar (2013) also observed that chestnut tannin at the level of 20–200 g/kg decreased ammonia and iso-VFA concentration. The low level of proteolysis and deamination in the rumen are expected to increase the protein by-pass and improve protein utilization of cassava leaf silage in the small intestine.

5. Conclusion

The study revealed that the addition of glycerol lowered the pH of cassava leaf silage only marginally and that chestnut tannin tended to decrease ammonia-N of the silage. Apparently, such effects are small due to the already good silage quality of the control group (no additive) as indicated by the high lactic acid concentration and the negligible amount of butyric acid. The additives might have resulted in greater effects on fermentation characteristics of silage if the silage quality is low. Glycerol addition shifted rumen fermentation profiles towards more propionate production at the expense of acetate and led to lower methane emission. The addition of chestnut tannin may strategically be used to increase the amount of rumen by-pass protein as it lowered the proteolysis and deamination processes in the rumen without causing adverse effects on extent and rate of gas production, *in vitro* digestibility and total VFA production. The combination between glycerol and chestnut tannin either in silage fermentation or in rumen fermentation system apparently did not reveal any synergistic nor antagonistic effects. An *in vivo* experiment to evaluate on the effects of glycerol and chestnut tannin addition on cassava leaf silage warrants further investigation.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The research was co-financed by Direktorat Jenderal Pendidikan Tinggi BPPDN DIKTI 2013 and UPM Research grant number FRGS 5524272. Ministry of Higher Education Malaysia.

References

[AOAC] Association of Official Analytical Chemists. 2005. Official methods of analysis. 18th ed. Gaithersburg, Maryland, USA.

Avila-Stagno J, Chaves AV, Ribeiro GOJr, Ungerfeld EM, McAllister TA. 2014. Inclusion of glycerol in forage diets increases methane production in a rumen simulation technique system. Br J Nutr. 111:829–835.

Barry T, McNabb WC. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Br J Nutr. 81:263–272.

Bergner H, Kijora C, Ceresnakova Z, Szakacs J. 1995. *In vitro* investigation on the glycerol transformation rumen microbes. Archiv für Tierernähr. 48:245–256.

Beuvink JMW, Kogut J. 1993. Modeling gas production kinetics of grass silages incubated with buffered ruminal fluid. J Anim Sci. 71:1041–1046.

Cottyn BG, Boucque CV. 1968. Rapid method for gas-chromatographic determination of volatile fatty acids in rumen fluid. J Agric Food Chem. 16:105–107.

Donkin SS, Koser SL, White HM, Doane PH, Cecava MJ. 2009. Feeding value of glycerol as a replacement for corn grain in rations fed to lactating dairy cows. J Dairy Sci. 92:5111–5119.

[FDA] Food and Drug Administration. 2006. Food and drug administration, code of federal regulations, 21CFR582.1320, Title 21, Vol. 6, [accessed 2015 Feb 11]. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=582.1320.

Hassanat F, Benchaar C. 2013. Assessment of the effect of condensed (acacia and quebracho) and hydrolysable (chestnut and valonea) tannins on rumen fermentation and methane production *in vitro*. J Sci Food Agric. 93:332–339.

Jatkauskas J, Vrotniakiene V. 2006. Effects of silage fermentation quality on ruminal fluid parameters. Biologija. 4:65–71.

Jayanegara A, Goel G, Makkar HPS, Becker K. 2015. Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population *in vitro*. Anim Feed Sci Technol. 209:60–68.

Jayanegara A, Makkar HPS, Becker K. 2009. Emisi metana dan fermentasi rumen *in vitro* ransum hay yang mengandung tanin murni pada konsentrasi rendah [*In vitro* methane emission and rumen fermentation of hay diet contained purified tannins at low concentration]. Media Peternak. 32:85–195.

Jayanegara A, Palupi P. 2010. Condensed tannin effects on nitrogen digestion in ruminants: a meta-analysis from *in vitro* and *in vivo* studies. Media Peternak. 33:176–181.

Krueger NA, Anderson RC, Tedeschi LO, Callaway TR, Edrington TS, Nisbet DJ. 2010. Evaluation of feeding glycerol on free-fatty acid production and fermentation kinetics of mixed ruminal microbes *in vitro*. Bioresour Technol. 101:8469–8472.

Kung LJr. 2010. Understanding the biology of silage preservation to maximize quality and protect the environment. Proceedings of the 40th California Alfalfa and Forage Symposium and Corn/Cereal Silage Conference; 30 Nov–2 Dec; Davis, CA, USA: Department of Agronomy and Range Science Extension, University of California. pp. 41–54. http://alfalfa.ucdavis.edu/+symposium/2010/files/talks/CAS05_KungBiologyOfSilage.pdf

Laconi EB, Jayanegara A. 2015. Improving nutritional quality of cocoa pod (*Theobroma cacao*) through chemical and biological treatments for ruminant feeding: *in vitro* and *in vivo* evaluation. Asian Australas J Anim Sci. 28:343–350.

Lee SY, Lee SM, Cho YB, Kam DK, Lee SC, Kim CH, Seo S. 2011. Glycerol as a feed supplement for ruminants: in vitro fermentation characteristics and methane production. Anim Feed Sci Technol. 166-167:269–274.

Man NV, Wiktorsson H. 2002. Effect of molasses on nutritional quality of cassava and gliricidia tops silage. Asian Australas J Anim Sci. 15:1294–1299.

Marjuki, Sulistyo HE, Rini DW, Artharini I, Soebarinoto, Howeler R. 2008. The use of cassava leaf silage as a feed supplement in diets for ruminants and its introduction to smallholder farmers. LRRD. 20(6). http://www.lrrd.org/lrrd20/6/marj20093.htm

McDonald P, Henderson AR, Heron SJE. 1991. The biochemistry of silage. 2nd ed. Kingston, Kent: Chalcombe Publications.

Meale SJ, Chaves AV, Ding S, Bush RD, McAllister TA. 2013. Effects of crude glycerin supplementation on wool production, feeding behavior, and body condition of merino ewes. J Anim Sci. 91:878–885.

Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. J Agric Sci. 93:217–222.

Messman MA, Weiss W, Albrecht KA. 1996. *In situ* disappearance of individual proteins and nitrogen from legume forages containing varying amounts of tannins. J Dairy Sci. 79:1430–1435.

Morrison IM. 1979. Changes in the cell wall components of laboratory silages and the effect of various additives on these changes. J Agric Sci Camb. 93:581–586.

Moss AR, Jouany JP, Newbold J. 2000. Methane production by ruminants: its contribution to global warming. Annales de Zootechnie. 49:231–253.

Napasirth V, Napasirth P, Sulinthone T, Phommachanh K, Cai Y. 2015. Microbial population, chemical composition and silage fermentation of cassava residues. Anim Sci J. 86:842–848.

Parsons TR, Maita Y, Lalli CM. 1984. A manual of chemical and biological methods for seawater analysis. Elmsford, NY: Pergamon Press.

Quispe CAG, Coronado CJR, Carvalho JAJr. 2013. Glycerol: production, consumption, prices, characterization and new trends in combustion. Renew Sustain Energy Rev. 27:475–493.

Ramos MH, Kerley MS. 2012. Effect of dietary crude glycerol level on ruminal fermentation in continues culture and growth performance of beef calves. J Anim Sci. 90:892–899.

Rémond B, Souday E, Jouany JP. 1993. *In vitro* and *in vivo* fermentation of glycerol by rumen microbes. Anim Feed Sci Technol. 41:121–132.

Riaz MQ, Sudekum KH, Clauss M, Jayanegara A. 2014. Voluntary feed intake and digestibility of four domestic ruminant species as influenced by dietary constituents: a meta-analysis. Livest Sci. 162:76–85.

Ridla M, Uchida S. 1998. Effects of combined treatment of lactic acid bacteria and cell wall degrading enzymes on fermentation and composition of Rhodegrass (*Chloris gayana* Kunth.) silage. Asian-Australas J Anim Sci. 11:522–529.

Salawu MB, Acamovic T, Stewart CS, Hvelplund T, Weisbjerg MR. 1999. The use of tannins as silage additives: effects on silage composition and mobile bag disappearance of dry matter and protein. Anim Feed Sci Technol. 82:243–259.

Santos WP, Carvalho BF, Avila CLS, Junior GSD, Pereira MN, Schwan RF. 2015. Glycerin as an additive for sugarcane silage. Annals of Microbiol. 65:1547–1556.

Seglar B. 2003. Fermentation analysis and silage quality testing. Proceedings of the Minnesota Dairy Health Conference; 20 May; St. Paul, MN, USA: College of Veterinary Medicine, University of Minnesota, pp. 119–136. http://conservancy.umn.edu/bitstream/handle/11299/108997/Seglar.pdf?sequence=1&isAllowed=y.

Sharif NFA, Razak SIA, Nayan NHM, Yahya MY, Rahman WAWA. 2015. Poly (lactic acid) coated cassava leaves biocomposite packaging sheets. Cellulose Chem Technol. 49:659–665.

Syahniar TM, Ridla M, Samsudin AA, Jayanegara A. 2016. Glycerol as an energy source for ruminants: a meta-analysis of *in vitro* experiments. Media Peternak. 39:189–194.

Tabacco E, Borreani G, Crovetto GM, Galassi G, Colombo D, Cavallarin L. 2006. Effect of chestnut tannin on fermentation quality, proteolysis, and protein rumen degradability of alfalfa silage. J Dairy Sci. 89:4736–4746.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci. 74:3583–3597.

Ward RT, de Ondarza MB. 2008. Fermentation analysis of silage: use and interpretation. [accessed 29 May 2015]. http://www.foragelab.com/Media/Fermentation-Silage-NFMP-Oct-2008.pdf.

Widodo Y, Wahyuningsih S, Newby J. 2015. Fuelling cassava development to meet the greater demand for food and biofuel in Indonesia. Energy Procedia. 65:386–394.

Yeoh HH, Chew MY. 1976. Protein content and amino acid composition of cassava leaf. Phytochemistry. 15:1597–1599.