



Ensiling characteristics of silages of Stylo legume (*Stylosanthes guianensis*), Guinea grass (*Panicum maximum*) and their mixture, treated with fermented juice of lactic bacteria, and feed intake and digestibility in goats of rations based on these silages

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ABSTRACT

The aim of the current study was to evaluate the ensiling characteristics of silages prepared from Guinea grass, Stylo legume or Stylo legume mixed with Guinea grass (50:50 w/w). Guinea grass and Stylo legume were harvested 45 and 60 days after regrowth, respectively and treated with a fermented juice of lactic acid bacteria (FJLB) prior to being ensiled. After 45 days, selected ensiling characteristics were determined. The nutritive value of rations based on the experimental silages was evaluated using six male, rumen cannulated crossbred Anglo Nubian × Native goats in a replicated 3 × 3 Latin square design study. Concentrate was provided at 0.9% body weight and experimental silages ad libitum. Apparent fecal macronutrient digestibility was determined. The pH values, NH₃-N and lactic acid contents were not different between the silages but greater contents ($P < 0.05$) of acetic and butyric acid were found in the silage prepared from Guinea grass. Voluntary silage intake was similar for the three silages. Digestibility of dry and organic matter did not differ between treatments. However, the digestibility of crude protein, neutral detergent fiber and acid detergent fiber was greater ($P < 0.05$) when the rations contained silage from Stylo legume instead of Guinea grass. In conclusion, the process of fermentation of silages is similar for Stylo legume and Guinea grass. The feeding of rations based on silage from Stylo legume versus Guinea grass can enhance animal production due to its higher nutritive value.

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1. Introduction

Stylo (*Stylosanthes guianensis* CIAT184) is a tropical legume that combines a high potential dry matter (DM) yield with a good resistance to anthracnose (Noble et al., 2000; Phaikaew and Hare, 2005). As fresh Stylo legume cannot be dried during rainy season, ensiling may be a practical way to preserve this forage (McDonald et al., 1991; Arbabi and Ghoorchi, 2008; Bureenok et al., 2011). However, Liu et al. (2011, 2012) reported high pH and NH₃-N values in ensiled Stylo, indicating an unsuccessful process of fermentation. As legumes have a relatively low concentration of water soluble carbohydrates (WSC) and a high buffering capacity after harvesting, the process of fermentation is more complicated compared to grasses (McDonald et al., 1991; Yahaya et al., 2004).

Besides Stylo legume, Guinea grass (*Panicum maximum* TD58) is also a widely available forage for ruminant production in tropical areas (Aganga and Tshwenyane, 2004). However, both the DM content and the concentration of WSC are considered too low for successful ensiling. This consideration is corroborated by Bureenok et al. (2005a) who reported high pH, NH₃-N and butyric acid values in silage from Guinea grass. However, addition of fermented juice of lactic acid bacteria (FJLB) prior to ensiling appears to be an effective method to improve the silage quality of unwilted forages with a low DM content (Ohshima et al., 1997; Bureenok et al., 2005b; Wang et al., 2009). As such, treatment of Stylo legume with FJLB prior to ensiling may increase the success of achieving a stable, nutritious silage. The first objective of this study was to assess the quality of FJLB treated silages from either Guinea grass or Stylo legume alone or Stylo legume mixed with Guinea grass. The silage prepared from Guinea grass alone served as a control. In general, silage making and the feeding of silage from Stylo legume is not customary in tropical regions. Consequently, there is limited infor-

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Table 1

Chemical composition of fresh Guinea grass and Stylo legume and the experimental silages ($n = 3$) after an ensiling period of 45 days in plastic pouches containing an initial amount of 100 g forage.

	Before ensiling		Experimental silages			SEM ¹	P-value
	Guinea grass	Stylo legume	Guinea grass	Stylo legume	Guinea grass + Stylo legume		
Dry matter (DM, g/kg)	204	272	188 ^c	289 ^a	235 ^b	4.7	<0.01
Epiphytic LAB ² (log cfu/g)	5.0	4.7	NA ³	NA	NA		
Buffering capacity (mequiv./g DM)	180	260	NA	NA	NA		
NH ₃ -N (g/kg total N)	NA	NA	79	69	70	3.5	0.20
pH	5.72	5.64	4.43	4.43	4.45	0.02	0.62
g/kg DM							
Water soluble carbohydrates	11	41	NA	NA	NA		
Crude ash	75	84	77	81	80	1.1	0.15
Crude protein	69	106	67 ^c	101 ^a	97 ^b	0.9	<0.01
Neutral detergent fiber	725	615	737 ^a	690 ^b	686 ^b	6.5	<0.01
Acid detergent fiber	424	442	379 ^b	435 ^a	458 ^a	6.0	<0.01
Hemicellulose	301	173	358 ^a	255 ^b	228 ^b	7.9	<0.01
Lactic acid	NA	NA	77	60	58	6.9	0.60
Acetic acid	NA	NA	70 ^a	45 ^b	40 ^b	2.8	<0.01
Butyric acid	NA	NA	5.0 ^a	0.9 ^b	0.0 ^b	0.6	<0.01

Values within the same row with different superscripts were significantly different ($P < 0.05$).

¹ Standard error of mean.

² Lactic acid bacteria.

³ Not analyzed.

mation on the feeding value of whole rations based on silage from Stylo legume or Stylo legume mixed with Guinea grass. Therefore, the experimental silages described above, were used to formulate three rations to evaluate the effects of silage type on feed intake, selected indices of rumen fermentation and apparent digestibility of macro-nutrients in goats.

2. Materials and methods

2.1. Preparation of FJLB

Fermented juice of lactic acid bacteria was prepared according to the method described by Bureenok et al. (2005b). Briefly, 200 g fresh Guinea grass or Stylo legume was macerated in 1000 ml sterilized distilled water in a blender. Then, the forage specific content of the blender was filtered over a double layer of cheesecloth into a glass bottle and 2% glucose added. The bottles were capped and stored under anaerobic conditions at 30 °C for 2 days. This procedure yielded forage specific FJLB of Guinea grass and Stylo legume containing 5.50 log₁₀ and 5.67 log₁₀ colony-forming units (cfu)/g of lactic acid bacteria (LAB), respectively. The FJLB used for the mixture of Guinea grass and Stylo legume, was prepared by mixing the individual forage specific FJLB in a 1:1 ratio (v/v).

2.2. Silage preparation

Guinea grass and Stylo legume were harvested on the same day, 45 and 60 days after regrowth, respectively and subsequently chopped with a forage cutter to 2–4 cm. Guinea grass and Stylo legume used to assess ensiling characteristics, was sampled immediately after harvesting. The nutrient composition of the fresh forages is shown in Table 1. The crops were not wilted and the three experimental forages were prepared immediately after cutting, i.e., 100% Guinea grass, 100% Stylo legume and a mixture of Guinea grass and Stylo legume (50:50 w/w). Thereafter, 1% (fresh weight) of forage specific FJLB was added to each experimental forage and subsequently tightly packed in either oxygen impermeable plastic pouches (100 g in 20.32 × 33 cm pouches, 120 μ thickness; M-PLASPACK, Bangkok, Thailand) or 100 l plastic drums with clamp lid (60 kg of forage as fed). Air was withdrawn from the plastic pouches by means of a vacuum sealer while the forages were manually compacted when the forages were stored in the drums. Three

pouches for each experimental forage were prepared to assess the ensiling characteristics and nutrient composition after ensiling and the pouches were stored for 45 days at ambient temperatures (27–30 °C). The experimental forages were ensiled for 30 days in the plastic drums before being used to assess feed intake, digestibility and selected indices of rumen fermentation.

2.3. Animals and feeding

All procedures were approved by the Ethical Principles for the Use of Animals for Scientific Purposes of the National Research Council of Thailand. Six, male Anglo Nubian × Thai native, rumen cannulated, crossbred goats with a mean body weight (BW) of 40 ± 5.0 kg were used. Prior to the experiment, the goats were dewormed by means of Ivomec F plus, (Bangkok, Thailand) and injected with vitamin A (500,000 I.U.), D₃ (75,000 I.U.) and E (50 I.U.) (Biotecnochem, Dallas, USA). The goats were individually housed in pens (60 × 120 × 90 cm) and water was available at all times. The trial had a replicated 3 × 3 Latin square design (Cochran and Cox, 1957). The goats were randomly assigned to each sequence of feeding on the three experimental silages, i.e., 100% Guinea grass, 100% Stylo legume and a the mixture (50/50, as fed) of Guinea grass and Stylo legume. The silages were provided ad libitum to the goats. Next to the experimental silage, goats were offered concentrates (Table 2) at a level of 0.9% of body weight. Each experimental period lasted 21 days with a 14-day adaptation followed by a 7-day collection period. The experimental rations were provided three times per day in equal portions at 08.00, 12.00 and 16.00 h. The goats were weighed before the morning feeding at the beginning and end of each experimental period.

2.4. Collection of samples

Samples taken at 45 day after closure of the plastic pouches were subsamples (50 g fresh material), macerated with 150 ml of distilled water and stored in a refrigerator at 4 °C for 12 h (Bureenok et al., 2006). Then, the material was filtered (filter paper no. 5; Whatman, England) and the pH of the filtrate recorded (SI analytics, Mainz, Germany) before being stored at –20 °C until analysis of lactic acid, volatile fatty acids (VFAs) and ammonia-N (NH₃-N). Samples from the center of the plastic drums were taken immediately after opening the drums. Samples from both types of ensiling

Table 2
Ingredient composition of the concentrate.

Ingredient	(g/kg)
Cassava chips	545
Rice bran	251
Soybean meal	40
Whole cottonseed	80
Molasses	44
Urea	18
Salt	9
Oyster shells	4
Dicalcium phosphate	4
Sulfer	1
Premix ^a	4
Chemical composition	
DM (g/kg)	902
Ash (g/kg DM)	70
CP (g/kg DM)	142
NDF (g/kg DM)	179
ADF (g/kg DM)	55

^a The premix consisted of (units/kg dry matter) 44,000 IU of vitamin A, 60,000 IU of vitamin D3, 30,000 IU of vitamin E, 11.6 g of Fe, 0.03 g of Co, 5.3 g of Mn, 5.6 g of Cu, 11.6 g of Zn, 0.07 g of I, 15.0 g of P, 10.0 g of Mg and 0.06 g of Se.

container were dried for 48 h at 60 °C, ground and stored in an air-dry form in sealed jars at room temperature until the analysis for macronutrient composition.

At the end of each experimental period, rumen fluid samples were collected from the rumen cannulated goats immediately before and 4 h after the morning feeding. The rumen fluid was immediately filtered over 2 layers of cheesecloth and the pH of the filtrates measured with a glass electrode pH meter (SI analytics GmbH, Mainz, Germany). The filtrates were divided into three portions. The first portion was used to determine the NH₃-N and VFAs for which 90-ml filtrate samples were acidified with 10 ml of 1 M H₂SO₄, centrifuged at 16,000 × g for 15 min, and the supernatant was stored at –40 °C until analysis. The second portion was mixed with a sterile 0.9% (m/v) saline solution containing 10% (v/v) formalin and bacteria, protozoa, and fungal zoospores in rumen fluid were counted by the method of Galyean (1989) using a haemocytometer (Boeco, Hamburg, Germany). The third portion was diluted with sterilized distilled water for identification of bacterial groups (e.g., cellulolytic, proteolytic, amylolytic) and total count of viable bacteria using the roll-tube technique as described by Hungate (1966).

During the last 7 day of each experimental period, feces were collected quantitatively from each goat in the morning and afternoon. The daily feces production of each goat was mixed thoroughly, and 3% of the wet weight was stored at –20 °C. At the end of each collection period, the stored feces fractions from each goat were combined, mixed thoroughly and sampled. Then, samples were dried (48 h at 60 °C), ground and stored in air-dry form in sealed jars at ambient temperature until analysis.

2.5. Chemical analyses

The macronutrient composition of the feedstuffs and feces were determined by the same methods. The ash content was determined by combustion at 550 °C for 16 h (AOAC, 1995) and the nitrogen (N) contents were determined by the macro Kjeldahl method (AOAC, 1995). A factor of 6.25 was used to convert N into crude protein (CP). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to the method of Van Soest et al. (1991) and expressed inclusive of residual ash. Buffering capacity and WSC content were determined according to the method as described by Playne and McDonald (1966) and Dubois et al. (1956), respectively.

Lactic acid and VFA in both rumen fluid and silage extracts were measured by HPLC (Aminex[®] HPX-87H, 300 mm × 7.8 mm i.d.; column temperature, 40 °C flow rate, 0.60 ml/min, Shimadzu Ltd., Kyoto, Japan). Lactic acid bacteria in forage specific FJLB, fresh Guinea grass and fresh Stylo legume were enumerated on MRS agar and plates were incubated at 35 °C for 48 h. The NH₃-N content of silage extract and rumen fluid was determined using a steam distillation technique (Cai, 2004).

2.6. Statistical analyses

The data related to silage macronutrient composition and quality were subjected to one-way analyses of variance (ANOVA, Statistical Analysis System, 1994). Data obtained in the digestibility trial were subjected to ANOVA with goat, experimental period and experimental ration as factors (Statistical Analysis System, 1994). Treatment means were compared using Tukey's *t* test (Statistical Analysis System, 1994). Throughout, the level of significance was set at *P* < 0.05.

3. Results

3.1. Quality of silage ensiled in plastic pouches

The lactic acid content, NH₃-N contents and pH was similar in all silages (Table 1). In contrast, the contents of both acetic and butyric acid were greater (*P* < 0.01) in the silage from Guinea grass. The differences in both the DM and CP contents of Guinea grass and Stylo legume were mirrored by the differences in DM and CP of the experimental silages. The NDF contents of fresh Guinea grass and Stylo legume were lower than that of the respective silages while the ADF contents of the fresh crops were greater than the respective silages. The NDF and hemicellulose contents were greater (*P* < 0.01) in the silage from Guinea grass while the ADF content was lower (*P* < 0.01) in this silage.

3.2. Quality of silage ensiled in plastic drums

The DM content of the silages were similar between treatments but the crude ash contents differed considerable (*P* < 0.05) between the experimental silages (Table 3). Consequently, CP contents and that of ADF were different (*P* < 0.05) between the experimental silages. The NDF content of the silage prepared from Guinea grass was greater (*P* < 0.05) than that of the silages prepared from either Stylo legume alone, or Stylo legume mixed with Guinea grass.

3.3. Feed intake, nutrient intake and digestibility

Throughout the experiment, the goats fully consumed the offered amount of concentrates. Orts from silage were collected and quantified before the morning feeding and used to correct feed intake. On average, 5% Orts were collected of the amount offered during the collection 7 day period.

The intake of silage was similar for the three experimental silages (Table 4) and the mean intake across treatments (*n* = 3) was 1.34% (SE = 0.015) of BW. Because the goats were fed a fixed amount of concentrate, total DM intake was also similar between the experimental rations. The CP content of the Stylo legume containing rations was greater and this was reflected by a greater intake of CP (*P* < 0.01) than in the pure grass treatment. Total intake of organic matter (OM), NDF and ADF was not different for the three experimental rations. Digestibility of DM and OM was not different between the experimental rations. In contrast, the digestibility of CP, NDF and ADF was greater (*P* < 0.01) in goats fed the silages

Table 3
Chemical composition of the experimental silages ($n=3$) after an ensiling period of 60 days in 100 l plastic drums.

	Experimental silages			SEM ¹	P-value
	Guinea grass	Stylo legume	Guinea grass + Stylo legume		
Dry matter (DM, g/kg)	282	293	282	7.7	0.17
g/kg DM					
Crude ash	137 ^a	87 ^c	105 ^b	4.1	<0.01
Crude protein	55 ^c	98 ^a	85 ^b	1.6	<0.01
Neutral detergent fibre	682 ^a	645 ^b	662 ^b	8.3	<0.01
Acid detergent fibre	453 ^a	421 ^c	440 ^b	4.0	<0.01

Values within the same row with different superscripts were significantly different ($P<0.05$).

¹ SEM = standard error of mean.

Table 4
Feed and nutrient intake and apparent digestibility's of selected nutrients after feeding the experimental rations.

	Experimental rations			SEM ¹	P-value
	Guinea grass	Stylo legume	Guinea grass + Stylo legume		
Dry matter intake, g/day					
Silage	520	542	526	12.9	0.79
Total	886	907	891	12.7	0.79
Total nutrient intake, g/day					
Organic matter	789	835	810	11.7	0.38
Crude protein	81 ^b	105 ^a	97 ^b	1.9	<0.01
Neutral detergent fiber	420	415	414	5.9	0.67
Acid detergent fiber	256	248	252	3.8	0.94
Apparent fecal digestibility (% of intake)					
Dry matter	82.2	82.3	81.8	0.86	0.92
Organic matter	82.8	83.1	83.1	0.73	0.92
Crude protein	74.5 ^b	76.2 ^a	76.5 ^a	0.22	<0.01
Neutral detergent fiber	64.1 ^b	66.1 ^a	66.3 ^a	0.24	<0.01
Acid detergent fiber	51.7 ^b	53.6 ^a	54.4 ^a	0.33	<0.01

Values within the same row with different superscripts were significantly different ($P<0.05$).

¹ Standard error of mean.

from either Stylo legume alone, or Stylo legume mixed with Guinea grass.

3.4. Selected indices of rumen fermentation and rumen microbes

Before the morning feeding as well as postprandial, pH, total VFA and the proportions of acetic-, propionic-, and butyric acid in the rumen were not affected by the experimental rations (Table 5). However, compared to 4 h after feeding, greater $\text{NH}_3\text{-N}$ values ($P<0.05$) were found in goats before the morning feeding of the silages from either Stylo legume alone, or Stylo legume mixed with Guinea grass.

Bacteria and protozoa counts did not differ between the experimental rations. Postprandial bacteria and protozoa counts (log cfu/ml) were not affected by silage type ($P>0.05$), the mean values for the combined treatments ($n=3$) was 13.2 (SE = 0.02) and 5.8 (SE = 0.03), respectively. Likewise, the experimental diets affected the rumen cellulolytic and amylolytic bacteria only minorly, the average values (log cells/ml) across the treatments being 7.5 (SE = 0.13) and 5.8 (SE = 0.01), respectively. In contrast, the rumen content of proteolytic bacteria was 2% higher ($P<0.01$) when the rations contained silage from either Stylo legume alone or the mixture of Stylo legume and Guinea grass instead of Guinea grass alone; the values were found to be 5.4 (SE = 0.01, $n=2$) and 5.3 log cells/ml, respectively.

4. Discussion

4.1. Silage quality and chemical composition

It is generally accepted that in well preserved silages, pH values should be <4.5, lactate >30 g/kg DM (McDonald et al., 1991), $\text{NH}_3\text{-N}$

<100 g/kg total N (Umana et al., 1991) and butyric acid <10% of total short chain fatty acids. Clearly, all the experimental silages met these criteria. These data presented here on the ensiling characteristics of the three silages are in line with that of Ohshima et al. (1997) who reported that the addition of FJLB prior to ensiling, effectively inhibited the growth of Clostridia in alfalfa with a low DM content. The current results are not in line with those found by Liu et al. (2011, 2012,) who reported high pH and $\text{NH}_3\text{-N}$ values in silage from Stylo legume, indicating an unsuccessful process of fermentation. However, in contrast to Liu et al. (2011, 2012), all experimental silages were treated with FJLB prior to ensiling. Probably, the addition of FJLB stimulated the growth of LAB, thereby, increasing the lactic acid content of the silages and subsequently lowering the pH (Bureenok et al., 2012). Furthermore, the observed low pH in the Stylo legume containing silages may have prevented protein degradation and thus high $\text{NH}_3\text{-N}$ values (McDonald et al., 1991).

The microbial composition of FJLB used in the present study was not described in detail. Thus, it is unknown which species of LAB in FJLB are responsible for the ensiling process. Nevertheless, the beneficial effect of FJLB on the process of ensiling is well documented (Tamada et al., 1999; Masuko et al., 2002; Wang et al., 2009; Bureenok et al., 2005b, 2012).

In the current study, the proportion of acetic acid (% of total short chain fatty acids) was >40%. This observation is in line with the high moisture content of Guinea grass and Stylo legume because such high moisture contents may have prevented the rapid production of lactate and stimulated the production of acetic acid (Nishino et al., 2012). Furthermore, the WSC content of Guinea grass and Stylo legume were <4.2% (DM basis) and it can be speculated that the WSC content dropped rapidly during the early phase of the fermentation process. Possibly, part of the lactate was converted into acetic acid

Table 5
Selected indices of rumen fermentation before and 4 h after feeding the experimental rations.

	Experimental rations			SEM ¹	P-value
	Guinea grass	Stylo legume	Guinea grass + Stylo legume		
Before feeding					
pH	6.92	6.43	6.52	0.13	0.17
Total VFA ² (mmol/l)	95.4	104.4	110.6	1.69	0.46
Acetic acid (mol/100ml)	60.7	64.5	59.4	7.02	0.41
Propionic acid (mol/100ml)	24.1	22.5	26.9	2.16	0.34
Butyric acid (mol/100ml)	15.2	13.1	13.6	1.63	0.71
NH ₃ -N (mmol/l)	7.3 ^b	10.6 ^a	10.0 ^a	0.18	0.03
4 h after feeding					
pH	6.28	5.78	6.40	0.20	0.27
Total VFA (mmol/l)	111.2	118.2	122.2	0.19	0.70
Acetic acid (mol/100ml)	66.4	66.5	62.2	2.86	0.26
Propionic acid (mol/100ml)	24.8	23.4	24.8	0.49	0.81
Butyric acid (mol/100ml)	8.7	10.1	13.1	0.55	0.06
NH ₃ -N (mmol/l)	12.5	15.0	13.9	0.24	0.10

Values within the same row with different superscripts were significantly different ($P < 0.05$).

¹ Standard error of mean.

² Volatile fatty acids.

by *Lactobacillus buchneri* under these conditions (Driehuis et al., 1999). A high acetic acid (>50 g/kg DM) content of a silage is beneficial because it has the potential to inhibit the growth of yeast and molds, thereby, increasing the aerobic stability of a silage (Danner et al., 2003; Li and Nishino, 2013). In any event, voluntary intake of silage was not affected by the origin of the silage, indicating that the silages prepared from Guinea grass and Stylo legume were equal in terms of palatability.

The NDF contents of Guinea grass and Stylo legume were affected by the process of fermentation. The increase in NDF content observed in all experimental silages may be explained by plant respiration during the process of fermentation or loss of soluble components during ensiling which results in increased fiber concentrations (Bolsen, 1995). In contrast to the silage prepared from Stylo legume, the ADF contents of the silage prepared from Guinea grass were lower. This observation may be explained by a difference in respiration rate between grasses and legumes (Muck and Pitt, 1993). A higher respiration rate is associated with heat production (Muck et al., 2003) and it cannot be excluded that reducing sugars were bound to amino acids (i.e., Maillard reaction), thereby, affecting the analyzed content of ADF because glycated amino acids are detected as ADF (Bolsen, 1995).

4.2. Voluntary feed intake and nutrient digestibility

Despite the fact that apparent fecal digestibility of both NDF and ADF were higher after the feeding of the rations containing silage from either Stylo legume alone, or Stylo legume mixed with Guinea grass, voluntary feed intake of silage was similar for the three experimental silages. It is well known that the digestibility of NDF is an important determinant of voluntary feed intake (Stensig et al., 1993; Qiu et al., 2003; Kendall et al., 2009). Apparently, the observed increase in NDF digestibility was not high enough to effectively increase the dry matter intake from the silage.

The greater apparent fecal digestibility of CP after the feeding of the rations containing silage from Stylo legume corroborates data by Mustafa et al. (2000) who demonstrated that CP originating from legumes have a higher apparent digestibility compared to that of grasses. However, the underlying reason of the higher apparent CP digestibility is not exactly clear but it might be related to the level of CP intake. It can be speculated that, at least in part, the contribution of endogenous CP in feces was relatively lower at higher CP intakes resulting in a somewhat higher apparent digestibility of CP.

The ash contents of the silages ensiled in the drums were greater than those ensiled in the pouches, i.e., 77.9, 7.4 and 31.3% for Guinea

grass, Stylo legume or Guinea grass mixed with Stylo legume, respectively. Obviously, a greater content of ash results in a lower CP, NDF and ADF content. However, when CP, NDF and ADF contents were expressed as g/kg OM, it appeared that the CP and ADF contents of only the Guinea grass silage differed more than 10%. In the light of fore mentioned reasoning on endogenous CP losses, it can be speculated that the apparent CP digestibility of the Guinea grass silage produced in the pouches is somewhat greater compared to the silage from the drums. Furthermore, it is well known that the ADF contents of forages are negatively related to digestibility (Njidda and Nasiru, 2010). Therefore, the ADF digestibility of Guinea grass silage from the pouches was most likely greater than that from the drums.

Under the assumption that the concentrate had a DM digestibility of 90%, the DM digestibility's of the individual silages from Guinea grass and Stylo legume were calculated to be 76.7 and 77.1%, respectively. Given the high NDF and ADF contents of both forages (Table 3), the calculated DM digestibility's can be considered high (Bamikole et al., 2001). However, it can be suggested that at least partially, the difference in level of feed intake of the goats explains the discrepancy in DM digestibility between the current study and that reported by Bamikole et al. (2001), i.e., 30 and 13 g/kg BW, respectively. It is well known that a greater level of feed intake depresses DM digestibility (Mertens, 2007; McDonald et al., 2010).

4.3. Rumen fermentation

Both total VFA concentrations and the proportions of acetic, propionic- and butyric acid were found to be within the ranges reported by France and Siddons (1983) and Hristov et al. (2001). Furthermore, all rumen NH₃-N concentrations were >2.94 mmol/l which is considered the minimum concentration of NH₃-N required to supply the rumen microbes with sufficient N for protein synthesis (Satter and Slyter, 1974). Rumen NH₃-N levels were, at least numerically, greater when the ration contained silage from Stylo legume (Table 5). The difference in rumen NH₃-N levels of goats fed silage of Stylo legume or Guinea grass did not reach statistical difference which was most likely due to a high inter-individual variation. Nevertheless, the observed higher rumen NH₃-N levels are in line with the observed greater proteolytic activity in the rumen. Indeed, it was shown by Hung et al. (2013) that increased intake of CP stimulates the growth of proteolytic bacteria.

Bacteria and protozoa counts were similar for all three experimental rations which suggest that all three rations supplied similar amounts of substrate to the rumen microbes (Leng, 1990). This

reasoning is corroborated by the observation that both the OM digestibility (Table 4) and selected indices of rumen fermentation (Table 5) also were similar for the three experimental rations.

5. Conclusion

The fermentation characteristics of silages prepared from FJLB treated Stylo legume are similar to that of silages prepared from FJLB treated Guinea grass. However, the digestibility of the protein and fiber fraction of Stylo legume versus Guinea grass based rations is greater and is, therefore, of interest to enhance goat production.

Conflict of interest

The authors declare no conflict of interest.

References

- Aganga, A.A., Tshwenyane, S., 2004. Potentials of guinea grass as forage crop in livestock production. *Pak. J. Nutr.* 3, 1–4.
- AOAC, 1995. *Official Methods of Analysis*, 12th ed. Association of Official Analysis Chemists, Washington, DC, USA.
- Arbabi, S., Ghoorch, T., 2008. The effect of different levels of molasses as silage additives on fermentation quality of Foxtail Millet (*Setaria italica*) silage. *Asian J. Anim. Sci.* 2, 43–50.
- Bamikole, M.A., Ezenwab, I., Akinsoyina, A.O., Arigbedec, M.O., Babayemi, O.J., 2001. Performance of West African dwarf goats fed guinea grass-verano stylo mixture, N-fertilized and unfertilized guinea grass. *Small Rumin. Res.* 39, 145–152.
- Bolsen, K.K., 1995. Silage: basic principles. In: Barnes, R.F., Miller, D.A., Nelson, J.C. (Eds.), *Forages: The Science of Grassland Agriculture*, vol. 2. Iowa State University Press, Ames, IA, pp. 163–176.
- Bureenok, S., Namihira, T., Kawamoto, Y., Nakada, T., 2005a. Additive effects of fermented juice of epiphytic lactic acid bacteria on the fermentative quality of guinea grass (*Panicum maximum* Jacq.) silage. *Grassl. Sci.* 51, 243–248.
- Bureenok, S., Namihira, T., Tamaki, M., Mizumachi, M., Kawamoto, Y., Nakada, T., 2005b. Fermentative quality of guineagrass silage by using fermented juice of the epiphytic lactic acid bacteria (FJLB) as a silage additive. *Asian-Aust. J. Anim. Sci.* 18, 807–811.
- Bureenok, S., Namihira, T., Mizumachi, S., Kawamoto, Y., Nakada, T., 2006. The effect of epiphytic lactic acid bacteria with or without different byproduct from defatted rice bran and green tea waste on napiergrass (*Pennisetum purpureum* Schumacher) silage. *J. Sci. Food Agric.* 86, 1073–1077.
- Bureenok, S., Suksoybat, W., Kawamoto, Y., 2011. Effects of the fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses on digestibility and rumen fermentation characteristics of ruzigrass (*Brachiaria ruziziensis*) silages. *Livest. Sci.* 138, 266–271.
- Bureenok, S., Yuangklang, C., Vasupen, K., Schonewille, J.T., Kawamoto, Y., 2012. The Effects of additives in Napier grass silages on chemical composition, feed intake, nutrient digestibility and rumen fermentation. *Asian-Aust. J. Anim. Sci.* 25, 1248–1254.
- Cai, Y., 2004. Methods for feed evaluation of forages: silage analyses. In: *Field and Laboratory Methods for Grassland Science*. Japan Livestock Technology Association, Tokyo, pp. 279–282.
- Cochran, W.G., Cox, G.M., 1957. *Experimental Design*, 2nd ed. John Wiley & Sons, Inc., Oxford, England.
- Danner, H., Holzer, M., Mayrhuber, E., Braun, R., 2003. Acetic acid increases stability of silage under aerobic conditions. *Appl. Environ. Microbiol.* 69, 562–567.
- Driehuis, F., Oude Elferink, S.J.W.H., Spoelstra, S.F., 1999. Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with *Lactobacillus buchneri* inhibits yeast growth and improves aerobic stability. *J. Appl. Microbiol.* 87, 583–594.
- Dubois, M., Giles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28, 350–356.
- France, J., Siddons, R.C., 1983. Volatile fatty acid production. In: Forbes, J.M., France, J. (Eds.), *Quantitative Aspects Ruminant Digestion and Metabolism*. CAB International, Willingford, pp. 107–122.
- Galjean, M., 1989. *Laboratory Procedure in Animal Nutrition Research*. Department of Animal and Range Sciences. New Mexico State University, New Mexico.
- Hristov, A.N., Ivan, M., Rode, L.M., McAllister, T.A., 2001. Fermentation characteristics and ruminal ciliate protozoal populations in cattle fed medium- or high-concentrate barley-based diets. *J. Anim. Sci.* 79, 515–524.
- Hung, L.V., Wanapat, M., Cherdthong, A., 2013. Effects of Leucaena leaf pellet on bacterial diversity and microbial protein synthesis in swamp buffalo fed on rice straw. *Livest. Sci.* 151, 188–197.
- Hungate, R.E., 1966. *The Rumen and its Microbes*. Academic Press, New York, USA.
- Kendall, C., Leonardi, C., Hoffman, P.C., Combs, D.K., 2009. Intake and milk production of cows fed diets that differed in dietary neutral detergent fibre and neutral detergent fibre digestibility. *J. Dairy Sci.* 92, 313–323.
- Leng, R.A., 1990. Factors affecting the utilization of poor quality forages by ruminants particularly under tropical conditions. *Nutr. Res. Rev.* 3, 277–303.
- Li, Y., Nishino, N., 2013. Effects of ensiling fermentation and aerobic deterioration on the bacterial community in Italian Ryegrass, Guinea grass, and whole-crop maize silages stored at high moisture content. *Asian-Aust. J. Anim. Sci.* 26, 1304–1312.
- Liu, Q., Chen, M., Zhang, J., Shi, S., Cai, Y., 2012. Characteristics of isolated lactic acid bacteria and their effectiveness to improve stylo (*Stylosanthes guianensis* Sw.) silage quality at various temperatures. *Anim. Sci. J.* 83, 128–135.
- Liu, Q., Zhang, J., Shi, S., Sun, Q., 2011. The effects of wilting and storage temperature quality and aerobic stability of stylo silage. *Anim. Sci. J.* 82, 549–553.
- Masuko, T., Hariyama, Y., Takahashi, Y., Cao, L., Goto, M., Ohshima, M., 2002. Effect of addition of fermented juice of epiphytic lactic acid bacteria prepared from timothy and orchardgrass on fermentation quality of silage. *Grassl. Sci.* 48, 120–125.
- McDonald, P., Henderson, A.R., Heron, S.J.E., 1991. *The Biochemistry of Silage*, second ed. Chalcombe Publications, Marlow, Buckinghamshire, UK, pp. 109.
- McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A., Wilkinson, R.G., 2010. Voluntary intake of food. In: *Animal Nutrition*, seventh ed., pp. 461–477 (Chapter 17).
- Mertens, D.R., 2007. Digestibility and intake. In: Barnes, R.F., Nelson, C.J., Moore, K.J., Collins, M. (Eds.), *Forage: The Science of Grassland Agriculture*, vol. 2, sixth ed. Blackwell Publishing, Ames, Iowa, USA, pp. 487–507.
- Muck, R.E., Moser, L.E., Pitt, R.E., 2003. Postharvest factors affecting ensiling. In: Buxton, D.R., Muck, R.E., Harrison, J.H. (Eds.), *Silage Science and Technology*. American Society of Agronomy, Madison, WI, pp. 251–304.
- Muck, R.E., Pitt, R.E., 1993. Ensiling and its effect on crop quality. Silage production from seed to animal. NRAES-67. Proc. Nat. Silage Prod. Conf., Syracuse, NY, 23–25 February 1993. Northeast Reg. Agric. Eng. Serv., Ithaca, NY, pp. 57–66.
- Mustafa, A.F., Christensen, D.A., Mckinnont, J.J., 2000. Effects of pea, barley, and alfalfa silage on ruminal nutrient degradability and performance of dairy cows. *J. Dairy Sci.* 83, 2859–2865.
- Nishino, N., Li, Y., Wang, C., Parvin, S., 2012. Effects of wilting and molasses addition on fermentation and bacterial community in guinea grass silage. *Lett. Appl. Microbiol.* 54, 175–181.
- Njidda, A.A., Nasiru, A., 2010. In vitro gas production and dry matter digestibility of tannin-containing forages of semi-arid region of North-eastern Nigeria. *Pak. J. Nutr.* 9, 60–66.
- Noble, A.D., Orr, D.M., Middleton, C.H., Rogers, L.G., 2000. Legumes in native pasture—asset or liability? A case history with stylo. *Trop. Grassl.* 34, 199–206.
- Ohshima, M., Cao, L., Kimura, E., Ohshima, Y., Yokota, H., 1997. Influence of addition of previously fermented juice to alfalfa ensiled at different moisture contents. *Grassl. Sci.* 43, 56–58.
- Phaikaew, C., Hare, M.D., 2005. Stylo adoption in Thailand: three decades of progress. *Trop. Grassl.* 39, 216.
- Playne, M.J., McDonald, P., 1966. The buffering constituents of herbage and of silage. *J. Sci. Food Agric.* 17, 264–268.
- Qiu, X., Eastridge, M.L., Wang, Z., 2003. Effects of corn silage hybrid and dietary concentration of forage NDF on digestibility and performance by dairy cows. *J. Dairy Sci.* 86, 3667–3674.
- SAS (Statistical Analysis System), 1994. *Users Guide*, Fourth edition. SAS Institute Inc., Cary, NC, USA, Version 6.
- Satter, L.D., Slyter, L.L., 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. *Br. J. Nutr.* 32, 194–208.
- Stensig, T., Weisbjerg, M.R., Madsen, J., Hvelplund, T., 1993. Estimation of voluntary feed intake from in sacco degradation and rate of passage of DM or NDF. *Livest. Sci.* 39, 49–52.
- Tamada, J., Yokota, H., Ohshima, M., Tamaki, M., 1999. Effect of additives, storage temperature and regional difference of ensiling on the fermentation quality of napier grass (*Pennisetum purpureum* Schum.) silage. *Asian-Aust. J. Anim. Sci.* 12, 28–35.
- Umana, R., Staples, C.R., Bates, D.B., Wilcox, C.J., Mahanna, W.C., 1991. Effects of a microbial inoculant and (or) sugarcane molasses on the fermentation, aerobic stability, and digestibility of bermudagrass ensiled at two moisture contents. *J. Anim. Sci.* 69, 4588–4601.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597.
- Wang, J., Wang, J.Q., Zhou, H., Feng, T., 2009. Effects of addition of previously fermented juice prepared from alfalfa on fermentation quality and protein degradation of alfalfa silage. *Anim. Feed Sci. Technol.* 151, 280–290.
- Yahaya, M.S., Goto, M., Yimuti, W., Gamo, Y., Kim, W., Karita, S., Smerjai, B., Kawamoto, Y., Ogawa, S., 2004. Epiphytic microbiota on tropical Tinaroo legume (*Neonotonia wightii*) as revealed by denaturing gradient gel electrophoresis (DGGE) and scanning electron microscopy (SEM) and their effects on silage fermentation and ruminal degradability. *J. Anim. Vet. Adv.* 3, 339–347.