PAPER

The effects of adding lactic acid bacteria and cellulase in oil palm (*Elais guineensis* Jacq.) frond silages on fermentation quality, chemical composition and *in vitro* digestibility

Mahdi Ebrahimi,[1] Mohamed Ali Rajion,[1] Yong Meng Goh,[1] Abdoreza Soleimani Farjam,[2] Awis Qurni Sazili,[3] Jan Thomas Schonewille[4]

[1]Department of Veterinary Preclinical Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[2]Institute of Tropical Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[3]Department of Animal Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[4]Department of Farm Animal Health, Utrecht University, The Netherlands

Abstract

The main objective of the current study was to evaluate whether oil palm frond (OPF) can be successfully ensiled without or with the additives cellulase or lactic acid bacteria (LAB). Thus, fresh OPF was ensiled either without additives or with cellulase or LAB or their combination. Ensiling was carried out by storing 2 kg samples in airtight glass jars at 25-30°C for 12 weeks. Thereafter, the silage samples were subjected to proximate analyses, an *in vitro* digestibility assay and measures on selected indices of fermentation. Fermentation of OPF without additives appeared to be unsuccessful as both pH and ammonia content were too high (4.9 and 9.9%, respectively). In contrast, the use of cellulase or LAB resulted in silages with a pH<4.5 and ammonia fractions <8.4%, but the lowest values were found when both cellulase and LAB were used, *i.e.* pH=4.1 and ammonia fraction=6.7%. *In vitro* digestibility of dry matter was significantly higher in the cellulase treated silages. The process of ensiling was associated with both a significant decrease of the fat content of OPF and a significant change of the fatty acid profile. However, the proportions of major fatty acids (C16:0 and C18:2n-6) were not affected by the process of ensiling. In conclusion, the use of cellulase additive appears a practical tool to safeguard the process of fermentation. Using a cellulase enzyme or its combination with LAB improves the fermentation profile and increases the nutritional value of the OPF silage.

Corresponding author: Prof. Mohamed Ali Rajion, Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Tel. +603.8609.3411 - Fax: +603.8947.1973.
E-mail: mohdali@upm.edu.my

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Introduction

An adequate supply of roughage to ruminants is essential for optimum rumen function and thus important in relation to the animals' health and production. However, the availability of roughage for ruminant nutrition may vary from day to day in Malaysia mainly because farmers usually own only a limited amount of land to grow forages. However, the oil palm frond (OPF), a byproduct of the oil palm tree (Sumathi *et al*., 2008), is widely available in Malaysia throughout the year (Goh *et al*., 2010). Thus, the use of OPF may provide a continuous source of roughage for the Malaysian ruminant livestock industry. In practice, the OPF is harvested periodically usually after harvesting of the fruits. Consequently, the freshly harvested OPF has to be preserved to ensure the continuity of roughage supply to the animals. For obvious reasons, sun drying can be used to preserve OPF but adequate drying of fresh OPF (>85% DM) is not possible during periods of heavy rainfall. Wilting of grasses up to a dry matter (DM) content of least 35% is commonly practiced to prevent high levels of NH_3 and butyric acid in silage (Kung and Ranjit, 2001). However, fresh OPF usually has a DM content of 45%. Thus, it can be speculated that the DM content of fresh OPF is already high enough to ensure an uncomplicated process of fermentation but this is yet not known. Furthermore, the concentration of water-soluble carbohydrates (WSC) in OPF (approximately 1% DM) may be too low for successful ensiling (McDonald *et al*., 1991). Addition of cellulase, potentially increases the amount of substrate for lactic acid bacteria (LAB) and thus may be a practical tool to enhance the process of ensiling. It is also not known whether the numbers of epiphytic LAB in OPF are high enough to ensure sufficient conversion of sugars into lactate to attain a pH <4.5. Thus, the main objective of the current study was to evaluate the effects of adding cellulase and LAB or the combination of the two on the process of fermentation of OPF. Oil palm frond is rich in both linoleic and α-linolenic acid and values are typically in the order of 12.8 and 24.5 g/100 g fatty acids, respectively (Ebrahimi *et al*., 2012). However, to the authors' knowledge, there is yet no information on the fate of linoleic and α-linolenic acid during the process of ensiling. Therefore, the fatty acid composition of OPF both before and after ensiling was assessed as well.

Materials and methods

Preparation of the experimental silages

Fresh OPF was harvested from the fields of the Malaysian Agricultural Research and Development Institute (MARDI), Kuala Lumpur, Malaysia. The OPF was chopped into 2-3 cm and immediately transferred to the laboratory after harvesting. The freshly chopped OPF was either not treated with additives or treated with LAB (*Lactobacillus plantarum* MTD1; Ecosyl, Stokesley, UK) or cellulase (Onozuka R-10; Yakult Ltd., Tokyo, Japan) or a combination of LAB and cellulase. Oil palm frond treated with LAB contained at least 1×10^6 colonies forming units (CFU) per gram fresh weight and cellulase was added at a level of 2 g/kg fresh weight. The LAB and cellulase were dissolved in sterile water and then

sprayed on the OPF by means of a water sprayer. The control treatment was sprayed with sterile water alone to adjust the moisture content of the experimental forages. Thereafter, the experimental OPF were tightly packed in three glass jars for each treatment until the jars were completely filled. Each jar was then sealed with a lid and the joint was covered with parafilm to prevent the entry of air. The jars were stored at ambient temperatures ranging from 25 to 30°C. The silages in triplicates were opened after 12 weeks for chemical analysis.

Collection and preparation of samples

After 12 weeks of ensiling, the top 5 cm of the OPF silage was removed and from the remaining silage, a subsample of 20 g of silage was taken and mixed for 2 min with 180 g sterile water by means of a laboratory blender (Waring, Torrington, CT, USA). Then, the extract was filtered through four layers of gauze and No. 1 filter paper (Whatman Inc., Maidstone, UK) and the pH of the filtrate was recorded (Mettler-Toledo Ltd., Leicester, UK). The filtrate was stored at -20°C until the analysis of lactic acid, NH_3-N, WSC, volatile fatty acids (VFA), ethanol and LAB.

Samples of fresh and ensiled OPF samples were dried at 55°C for 48 h, ground to pass a 1 mm screen and stored at -80°C until analysed for analysis for ash, crude protein (CP), ether extract (EE), acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL).

A triplicate portion (0.25 g) of dried, ground non-ensilaged and ensilaged OPF was taken to determine the *in vitro* digestibility of DM. The OPF was incubated in gas-tight 100 mL, plastic syringes containing 20 mL of a phosphate-bicarbonate buffer adjusted to a pH of 6.8 (Fievez *et al*., 2005) and 5 mL of rumen content. Then, all air was expelled from the syringes, after which their tips were closed. Syringes were placed in an incubator at 39°C for 24 h. The rumen contents were obtained from four adult, rumen fistulated, Kacang crossbred male goats that were fed a ration consisting of 30% fresh OPF and 70% commercial concentrate (W/W). Rumen contents were transferred into pre-warmed thermos flasks which were flushed with CO_2 during transport to the laboratory. Prior to the incubations, rumen contents were filtered through four layers of cheesecloth under continuous flushing with CO_2. After incubation, the content of the gas syringes for all treatments including the blank, was quantitatively transferred into pre-dried beakers and subjected to digestion as described by Tilley and Terry (1963). Briefly, at the end of 24 h incubation, rumen fluid samples were centrifuged at 378 g for 10 min and the precipitated sample was washed by distilled water thrice. In the next step, washed samples were mixed with 50 mL pepsin-HCl solution (containing 2 g/L pepsin and 17.8 mL/L HCl) in 100 mL serum bottles and incubated at 39°C for 24 h. After incubation, the samples were centrifuged and the precipitated feed was dried at 100°C for 48 h. The in vitro dry matter disappearance (IVDMD) was calculated according to the following formula:

$$\text{IVDMD (\%)} = \left(\frac{\text{initial sample (g) - residue sample (g)}}{\text{initial sample (g)}}\right) \times 100$$

After the process of digestion, the content of the beakers was dried at 100°C until a constant weight.

Chemical analysis

Lactic acid, VFA and ethanol were determined using gas-liquid chromatography (Quadrex Corporation Bethany, CT, USA) equipped with a flame ionisation detector. In the case of lactic acid and VFA, a silica capillary column of 15 m 0.32 mm ID 0.25 µm film thickness (Agilent Technologies, Santa Clara, CA, USA) was used. The injector/detector temperature was programmed at 220/230°C respectively. The column temperature was set in the range of 70 to 150°C with a temperature increment at the rate of 7°C/min. Peaks for VFA were identified by comparison with authentic standards of acetic, propionic, butyric, isobutyric, valeric, isovaleric and 4-methyl-n-valeric acids (Sigma Aldrich, St. Louis, MO, USA). The internal standard used for VFA was 4-methyl-n-valeric acids and the internal standard for lactic acid determination was fumaric acid (Sigma Aldrich). Peaks for lactic acid were identified by comparison with an authentic standard of lactic acid (Sigma Aldrich). In the case of ethanol a HP-1 capillary column of 30 m 0.25 mm ID 0.25 µm film thickness (J&W Scientific, Folsom, CA, USA) was used. The flow rates of H_2 and air were set at 30 and 300 mL/min, respectively. The injector/detector temperature was programmed at 225/285°C respectively. The column temperature was set in the range of 45 to 245°C with a temperature increment at the rate of 45°C/min. The volume that was injected was limited to 1 µL. Water-soluble carbohydrates were determined by the modified phenol sulfuric acid method as described by Guiragossian *et al*. (1977) and the concentrations of NH_3-N were determined in fresh silage with the use of colorimetric method as described by Solorzano (1969).

The total number of LAB in the silage was determined on MRS Rogosa agar (Oxoid CM 627; Oxoid Ltd., Basingstoke, UK). Agar plates were incubated at 37°C for 72 h. The numbers of LAB were measured by the plate count method and the number of CFU was expressed as $\log_{10}$ per gram of DM OPF.

The DM of silage samples was analysed by drying at 55°C for 48 h. Ash was determined by combustion at 525°C for 6 h (method 923.03; AOAC, 1990). Nitrogen was determined by the Kjeldahl method (method 978.02; AOAC, 1990), and the CP content was calculated as N×6.25. Crude fat was extracted with petroleum ether (Soxtec 2050; Foss Analytical, Hillerød, Denmark). The contents of NDF, ADF and ADL were determined according to Van Soest *et al*. (1991). Heat stable amylase and sodium sulphite were used in the procedure of determining NDF and the results of NDF and ADF were expressed on an ash-free basis.

The total fatty acids were extracted from fresh and ensiled OPF based on the method of Folch *et al*. (1957) with some modifications by Rajion *et al*. (1985). The methylated fatty acids were separated by Agilent 7890A gas chromatography (Agilent Technologies) as described by Ebrahimi *et al*. (2013).

Statistical analysis

All data were checked for normality using the UNIVARIATE procedure of the SAS rev. 9.1., and LAB data were normalised using the $\log_{10}$-transformation. Then, all data were subjected to ANOVA, using the MIXED procedure of the SAS software package, version 9.1 (SAS, 2007). The statistical model used the following equation:

$$Y_{ijk}=\mu+T_i+F_j+e_{ijk}$$

where µ is the overall mean, T_i is effect of treatment (i=1 to 4), F_j is the random effect of replicate (j=1 to 3) and e_{ij} was the residual error. When the influence of treatment reached statistical significance, Tukey's *t* test was used to identify treatments with different effects on the variable involved. Throughout, the level of statistical significance was set at $P<0.05$.

Results and discussion

Fermentation quality

The LAB count was raised after 12 weeks of ensiling in all experimental silages but the LAB counts were only significantly higher in the silages treated with additives (Table 1).

The rise in LAB count was associated with a significant decrease of the WSC content and a concomitant increase of the lactic acid content of the silages. The highest lactic acid content was found in the silage treated with both LAB and cellulase (Table 1). The increase in the lactic acid was associated with a decrease of the pH and the lowest pH values were found in the silages treated with additives. The NH_3-N fraction and the concentrations of ethanol, acetic-, propionic- and butyric acid were significantly increased after 12 weeks of ensiling compared to fresh OPF. The highest ethanol concentrations were found in the silage treated with both LAB and cellulase, while the highest concentrations of acetic- and butyric acid and that of NH_3-N were found in the silage without additives (Table 1).

Parameters such as silage pH, short chain fatty acids and ammonia content are commonly used as indicators of silage quality. It is generally accepted that in well preserved silages, pH values should be <4.5 (McDonald *et al*., 1991) and ammonia levels <100 g/kg total nitrogen. Clearly, these criteria were not met by the OPF that was ensiled without additives. Thus, it seems that the osmotic pressure of fresh OPF is not high enough to ensure an uncomplicated process of fermentation (Muck, 1988). The relative low osmotic pressure can be explained by the low WSC content (approximately 1% DM) in OPF which is considered too low for successful ensiling (McDonald *et al*., 1991). This reasoning is in line with the observation that the addition of cellulase significantly increased lactate concentrations with a concomitant decrease in pH. Thus, it can be suggested that the addition of cellulase effectively provided more substrate for fermentation by the LAB (Stokes, 1992; Ridla and Uchida, 1993; Sheperd *et al*., 1995). Furthermore, the addition of LAB instead of cellulase significantly increased the lactic acid content, suggesting that the numbers of epiphytic LAB in OPF may limit the conversion of sugars into lactate to attain a pH <4.5. Consequently, the addition of both cellulase and LAB resulted in the highest lactate concentrations (Table 1). However, the relevancy of this high lactate concentration is not exactly clear because both silage pH and NH_3-N concentrations in the silages with additives were not significantly different between silages and met the criteria as indicated earlier.

Interestingly, the highest lactate concentrations found in the current study, were approximately 33% lower than the threshold of sufficient preservation (McDonald *et al*., 1991). Thus, the required pH for well-preserved silages was attained at relatively low lactate concentrations. This result suggests that OPF has a relatively low buffer capacity. It is well known that the buffer capacity of forage is positively related to the CP content (McDonald Henderson, 1962). Because OPF has a low CP content (4 to 5%, DM basis), it can be suggested that OPF also has a low buffering capacity. Finally, in all silages the acetic acid/total fermentation acids ratio was found to be >0.57 which is considerable higher than the recommended value, *i.e.* <0.20 (Lima *et al*., 2011). Therefore, it may be speculated that the aerobic stability (Weinberg *et al*., 1993; Kung and Ranjit, 2001; Danner *et al*., 2003) of all silages can be disputed. The relative high proportions of acetic acid are difficult to explain but they may be related to a relative lack of rapid fermentable carbohydrates in OPF. Indeed, the use of appropriate amounts of molasses as an additive has been shown to produce silages with low proportions of acetic acid in combination with high proportions of lactic acid (Bureenok *et al*., 2012; Lima *et al*., 2011).

Chemical composition and digestibility

Both the DM and EE contents were significantly decreased due to the process of ensiling but there was no significant difference between DM in the ensiled OPF. The CP content of the OPF silages was significantly higher after 12 weeks of ensiling (Table 2). In all silages, the NDF content was numerically lower than in the fresh OPF, but the difference only reached statistical significance in the two silages treated with cellulase. Likewise, the ADF and cellulose contents of the silages were lower compared to the fresh OPF and the differences were most pronounced when the silages were treated with both cellulase and

Table 1. Selected indexes of fermentation of oil palm frond, before and after an ensiling period of 12 weeks.

	Before ensiling	Experimental silages				SEM	P
		No additive	LAB	Cellulase	LAB+cellulase		
LAB, log10 cfu/g	6.05	6.28^{b}	7.22^{a}	6.92^{a}	7.02^{a}	0.09	0.001
WSC, g/kg DM	10.57	3.18^{d}	4.67^{c}	6.10^{b}	7.56^{a}	0.57	0.001
Lactic acid, g/kg DM	nd	9.19^{d}	15.05^{b}	12.02^{c}	19.68^{a}	0.67	0.001
pH	6.12	4.88^{a}	4.18^{b}	4.28^{b}	4.09^{c}	0.11	0.001
NH3-N, g/100 g total N	1.58	9.9^{a}	8.3^{b}	7.9^{b}	6.7^{b}	0.55	0.017
Ethanol, g/kg DM	4.72	7.58^{c}	9.01^{bc}	10.41^{b}	12.50^{a}	0.36	0.001
Acetic acid, g/kg DM	2.46	32.74^{a}	18.72^{b}	21.18^{b}	23.30^{ab}	1.74	0.001
Propionic acid, g/kg DM	0.51	1.66^{b}	3.16^{a}	3.13^{a}	3.35^{a}	0.18	0.001
Butyric acid, g/kg DM	0.03	1.10^{a}	0.62^{b}	0.67^{b}	0.58^{b}	0.09	0.001
Acetic acid:propionic acid	4.80	19.72^{a}	5.92^{b}	6.76^{b}	6.96^{b}	0.39	0.001

LAB, lactic acid bacteria; WSC, water soluble carbohydrates; nd, not detected (zero value was used in statistical analysis); DM, dry matter. $^{a-d}$Means within the same rows with different superscripts are significantly different at P<0.05.

Table 2. Chemical composition and *in vitro* dry matter digestibility of oil palm frond before and after an ensiling period of 12 weeks.

	Before ensiling	Experimental silages				SEM	P
		No additive	LAB	Cellulase	LAB+cellulase		
DM, g/kg	448	389	385	375	374	4.88	0.653
Digestibility, %	50.0	50.5^{b}	52.0^{ab}	54.0^{a}	55.1^{a}	1.01	0.031
Ash, g/kg DM	68.1	72.4	75.3	75.1	75.9	1.85	0.659
CP, g/kg DM	43.5	44.6^{b}	47.8^{a}	47.8^{a}	51.0^{a}	0.92	0.031
EE, g/kg DM	31.4	27.3^{b}	28.5^{ab}	29.7^{a}	29.5^{a}	0.30	0.025
NDF, g/kg DM	741	729^{a}	726^{a}	693^{b}	674^{b}	8.86	0.027
ADF, g/kg DM	517	503^{a}	494^{ab}	460^{b}	456^{b}	6.55	0.007
Cellulose, g/kg DM	335	328^{a}	315^{a}	294^{ab}	284^{b}	6.61	0.034
Hemicellulose, g/kg DM	223	226	232	233	218	10.6	0.813
ADL, g/kg DM	182	175	179	166	172	2.54	0.325

LAB, lactic acid bacteria; DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; cellulose, ADF-ADL; hemicellulose, NDF-ADF; ADL, acid detergent lignin. ^{a,b}Means within the same rows with different superscripts are significantly different at P<0.05.

LAB. The process of ensiling did not significantly affect the hemicellulose, ADL and ash content of OPF (Table 2). Ensiling without additives influence the digestibility of OPF, especially when cellulase was used as an additive, the *in vitro* digestibility of the ensiled OPF increased by 8 to 10% compared to the fresh OPF (Table 2).

The fermentation of OPF was associated with an overall loss of 15% of DM (Table 2). The underlying reason is not exactly clear but it may be, at least partly, related to the formation of volatile end-products of fermentation. This reasoning is corroborated by Pedroso *et al*. (2008) who showed a similar loss of DM due to the evaporation of volatile end-products during drying. Next to the loss of DM an 8% loss of EE also occurred during the process of ensiling. The loss of EE during the ensiling process is corroborated by several other studies (Dewhurst and King, 1998; Elgersma *et al*., 2003; Van Ranst *et al*., 2009) and can be explained by the oxidation of fatty acids (Dewhurst and King, 1998).

In the current study, an increase of 12% of the CP content was observed in the ensiled OPF with additives. This observation is in line with that of Zahiroddini *et al*. (2004) and Bureenok *et al*. (2012) who also observed an increase in the CP content of silages from whole-crop barley and Napier grass, respectively. The observed increases of the CP content of the ensiled OPF is most likely related to the observed decrease of the NDF and to a lesser extent of the EE content of the silages. Clearly, the loss of NDF and EE increases the relative proportion of CP, expressed in g/kg DM.

Compared to fresh OPF, the addition of cellulase alone or in combination with LAB, caused a significant decrease of the NDF and ADF content of the ensiled OPF. It appears that cellulase effectively degraded at least partly, structural carbohydrates such as cellulose (Ren *et al*., 2007). Indeed, the lowest cellulose contents were found in the OPF silages treated with cellulase. This result is corroborated by Ridla and Uchida (1993) who also showed that the addition of cellulase decreases the content of cell wall carbohydrates associated with the NDF and ADF content. It appears that the additional cellulase stimulated the conversion of cellulose to WSC thereby rendering glucose available for LAB and potential subsequent conversion to lactate (Stokes, 1992; Ridla and Uchida, 1993; Sheperd *et al*., 1995). The effective degradation of NDF and ADF by cellulase is also reflected by the improvement of DM digestibility. It appeared that the *in vitro* digestibility of OPF ensiled with cellulase was at least 6.9% greater than the silage without additives.

Fatty acid composition

The process of ensiling was associated with a significant decrease of the total fatty acid content of OPF and a significant effect on the fatty acid profile of the fat fraction. The proportion of C18:1trans-11 was significantly higher in the experimental silages with cellulase additives (Table 3). There were no significant effects on the proportions of C15:0, C15:1 and C18:3n-3 compared to no additive OPF. The proportions of the two major fatty acids, *i.e.* C16:0 and C18:2n-6, were not significantly affected by process of ensiling. The ratio of n-6: n-3 fatty acids remained unchanged after ensiling (Table 3).

The loss of EE is in line with that of fatty acids (Table 3). Some studies report a decrease in the total fatty acid content of silages compared to that of fresh products (Dewhurst and King, 1998; Elgersma *et al*., 2003; Van Ranst *et al*., 2009; Han and Zhou, 2013). The decrease in proportions of C15:0, C15:1 and C18:3n-3 in ensiled OPF compared to fresh OPF are probably related to the oxidation of these fatty acids by bacteria. Indeed, it has been reported by Elgersma *et al*. (2003) that ensiling of grass decreased the concentrations of most fatty acids, especially C18:3n-3. This observation is corroborated by Lee *et al*. (2006), who also reported a reduced proportion of C18:3n-3 when grass was ensiled in combination with *Lactobacillus plantarum*. Clearly, the current study did not provide clues with respect to the underlying mechanism for the reduced proportions of C18:3n-3, but it was shown by Lee *et al*. (2008) that lipoxygenases from plants play a role in the oxidation of C18:3n-3 during the process of ensiling.

In the current study, the proportions C18:1trans-11 was significantly higher in OPF silage. This outcome is in line with the observations of Lough and Anderson (1973) and Vanhatalo *et al.* (2007) who reported increased proportions of C18:1trans-11 in grass and red clover silages. The exact origin of C18:1trans-11 in silage is not clear but it was shown by Ogawa *et al*. (2005) and Kishino *et al*. (2009) that LAB can produce this fatty acid. Kishino *et al*. (2009) demonstrated that LAB can isomerise C18:2n-6 into conjugated C18:2 and subsequently hydrogenate this fatty acid into C18:1trans-11. However, this explanation is not in line with the results of the current study

Table 3. Total fatty acid content (mg/g DM) and fatty acid composition (g/100 g of total identified fatty acids) of oil palm frond before and after ensiling for 12 weeks.

	Before ensiling	Experimental silages				SEM	P
		No additive	LAB	Cellulase	LAB+cellulase		
TFA	36.70	31.44	32.58	31.61	30.69	0.21	0.193
C12:0	1.97	2.36	2.56	2.52	2.65	0.05	0.281
C14:0	3.97	3.40	3.66	3.73	3.65	0.06	0.186
C15:0	0.62	0.21	0.39	0.38	0.32	0.03	0.061
C15:1	3.44	1.73	2.53	2.26	2.38	0.09	0.864
C16:0	35.14	35.80	35.19	35.48	34.93	0.35	0.061
C16:1	0.90	1.24	1.21	1.54	1.52	0.06	0.140
C17:0	2.10	2.46	2.69	2.51	2.74	0.08	0.531
C17:1	0.62	0.57	0.54	0.50	0.51	0.02	0.734
C18:0	7.44	6.11	5.93	6.27	6.01	0.25	0.061
C18:1n-9	6.58	8.98^{a}	7.98^{b}	8.33^{ab}	7.80^{b}	0.16	0.034
C18l:1trans-11	0.00	0.36^{b}	2.10a	1.75a	2.15a	0.20	0.001
C18:2n-6	12.76	12.44	12.23	12.61	12.49	0.28	0.975
C18:3n-3	24.47	22.36	22.98	22.12	22.83	0.29	0.738
Total SFA°	51.23	52.33	50.43	50.89	50.31	0.33	0.101
Total MUFA#	11.54	12.88^{b}	14.36^{a}	14.39^{a}	14.37^{a}	0.33	0.034
Total n-3PUFA§	24.47	22.36	22.98	22.12	22.83	0.24	0.738
Total n-6PUFA^	12.76	12.44	12.23	12.61	12.49	0.29	0.975
n-6:n-3$	0.52	0.56	0.53	0.57	0.55	0.02	0.912
UFA:SFA	0.95	0.91	0.98	0.97	0.99	0.01	0.116
PUFA:SFA	0.73	0.67	0.70	0.68	0.70	0.01	0.116

LAB, lactic acid bacteria; TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids. °Total SFA, sum of C12:0, C14:0, C15:0, C16:0, C17:0, C18:0; #total MUFA, sum of C15:1, C16:1, C17:1, C18:1n-9; §total n-3PUFA, C18:3n-3; ^total n-6PUFA, C18:2n-6; $n-6: n-3, total PUFA n-6 (C18:2n-6):total PUFA n-3 (C18:3n-3). ^{a,b}Means within the same rows with different superscripts are significantly different at P<0.05.

because the proportion of C18:2n-6 was not affected by the process of ensiling (Table 3). Likewise, it can be suggested that C18:3n-3 acted as a precursor of C18:1trans-11 during the process of ensiling but, to the authors' knowledge, this is currently not known.

Next to C18:1trans-11, the proportion of C18:1n-9 also was increased in the experimental silages. Theoretically, this result can be explained by the conversion of C18:0 into C18:1n-9 mediated by a Δ9 desaturase, which is in line the observed decrease in the proportion of C18:0. Unfortunately, to the authors' knowledge, the activity of Δ9 desaturase in silage is not yet reported in the literature. Furthermore, when C18:1n-9 was expressed as g/kg DM (data not shown), the contents of the ensiled OPF was similar to that of the fresh OPF indicating that the observed proportional increase of C18:1n-9 after ensiling was, at least partly, due to the loss of other fatty acids such as C15:0, C15:1 and C18:3n-3. Clearly, the issue on the alteration of the fatty acids profile associated with ensiling is not yet settled (Glasser *et al*., 2013).

Conclusions

The quality of silage derived from fresh OPF is below standards. Addition with LAB or cellulase or the combination of LAB and cellulase significantly improves the quality of the OPF silage. Cellulase appeared to be the most effective additive in relation to silage quality. The results of the current study implicate that OPF can be well preserved by ensiling and thereby represents a continuous source of roughage for ruminant livestock diets in Malaysia.

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