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

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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 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 an uncomplicated process of fermentation but this is yet not unknown. Furthermore, the concentration of water-soluble carbohydrates (WSC) in OPF is not possible during periods of heavy rainfall. Wilting of grasses up to a dry matter (DM) content of at least 35% is commonly practiced to prevent high levels of NH3 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. 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 at least 35% is commonly practiced to prevent high levels of NH3 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 is not possible during periods of heavy rainfall. Wilting of grasses up to a dry matter (DM) content of at least 35% is commonly practiced to prevent high levels of NH3 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. 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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., 2008). 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 at least 35% is commonly practiced to prevent high levels of NH3 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

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; Ecospyl, 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 1×10⁸ 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

[Ital J Anim Sci vol.13:2014] [page 557]
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 paraffilm 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 (Metter-Toledo Ltd., Leicester, UK). The filtrate was stored at -20°C until the analysis of lactic acid, NH₃-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 ensiled 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 (WW). Rumen contents were transferred into pre-warmed thermos flasks which were flushed with CO₂ during transport to the laboratory. Prior to the incubations, rumen contents were filtered through four layers of cheesecloth under continuous flushing with CO₂. After incubation, the content of the gas syringes for all treatments including the blank, was quantitatively transferred into predried 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 mM/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:

\[
\text{IVDMD} (\%) = \left( \frac{\text{initial sample (g) - residual sample (g)}}{\text{initial sample (g)}} \right) 
\]  

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). Peaks for lactic acid 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 standard internal 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 H₂ 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 NH₃-N were determined in fresh silage with the use of colorimetric method as described by Solorzana (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 log₁₀ 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 (Sextec 2050; Foss Analytical, Hillerod, 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 Folkh et al. (1957) with some modifications by Rajon 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 log₁₀ 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, Tᵢ is effect of treatment (i=1 to 4), Fⱼ is the random effect of replicate (j=1 to 3) and eᵢⱼ 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).
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 lactic acid 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; Shepherd 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 NH3-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 considerably 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).

### 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<sup>b</sup>     | 7.22<sup>c</sup> | 6.92<sup>c</sup> | 7.02<sup>c</sup> | 0.09 | 0.001 |
| WSC, g/kg DM        | 10.57          | 3.18<sup>b</sup>     | 4.67<sup>c</sup> | 6.10<sup>c</sup> | 7.56<sup>c</sup> | 0.57 | 0.001 |
| Lactic acid, g/kg DM| nd             | 9.19<sup>c</sup>     | 15.05<sup>c</sup> | 12.02<sup>c</sup> | 19.68<sup>c</sup> | 0.67 | 0.001 |
| pH                  | 6.12           | 4.88<sup>b</sup>     | 4.18<sup>c</sup> | 4.28<sup>c</sup> | 4.09<sup>c</sup> | 0.11 | 0.001 |
| NH3-N, g/100 g total| 1.58           | 9.9<sup>c</sup>      | 8.3<sup>b</sup>  | 7.9<sup>b</sup>  | 6.7<sup>b</sup>  | 0.55 | 0.017 |
| Ethanol, g/kg DM    | 4.72           | 7.58<sup>c</sup>     | 9.01<sup>c</sup> | 10.41<sup>c</sup> | 12.50<sup>c</sup> | 0.36 | 0.001 |
| Acetic acid, g/kg DM| 2.46           | 32.74<sup>c</sup>    | 18.72<sup>c</sup> | 21.18<sup>b</sup> | 23.30<sup>b</sup> | 1.74 | 0.001 |
| Propionic acid, g/kg DM| 0.51           | 1.66<sup>b</sup>     | 3.16<sup>a</sup> | 3.13<sup>a</sup> | 3.35<sup>a</sup> | 0.18 | 0.001 |
| Butyric acid, g/kg DM| 0.03           | 1.10<sup>a</sup>     | 0.62<sup>a</sup> | 0.67<sup>a</sup> | 0.58<sup>a</sup> | 0.09 | 0.001 |
| Acetic acid:propionic acid | 4.80           | 19.72<sup>c</sup> | 5.92<sup>c</sup> | 6.76<sup>b</sup> | 6.96<sup>c</sup> | 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. *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 |
| LAB, lactacid 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. *Means within the same rows with different superscripts are significantly different at P<0.05.

| Parameter           | Before ensiling | Experimental silages | SEM  | P   |
|---------------------|----------------|----------------------|------|-----|
| DM, g/kg            | 448            | 389                  | 385  | 375 | 374 | 4.88 | 0.653 |
| Digestibility, %    | 50.0           | 50.5                 | 51.2<sup>a</sup> | 51.0<sup>a</sup> | 51.1<sup>b</sup> | 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                 | 47.8  | 47.8 | 51.0<sup>b</sup> | 0.92 | 0.031 |
| EE, g/kg DM         | 31.4           | 27.3                 | 28.5<sup>a</sup> | 28.7<sup>a</sup> | 29.5<sup>c</sup> | 0.30 | 0.025 |
| NDF, g/kg DM        | 741            | 729                  | 726   | 693<sup>b</sup> | 674<sup>c</sup> | 8.86 | 0.027 |
| ADF, g/kg DM        | 517            | 503<sup>c</sup>      | 494<sup>a</sup> | 460<sup>b</sup> | 456<sup>c</sup> | 6.55 | 0.007 |
| Cellulose, g/kg DM  | 335            | 328<sup>b</sup>      | 315<sup>b</sup> | 294<sup>a</sup> | 284<sup>a</sup> | 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 | 0.225 |
LAB. The process of ensiling did not significantly affect the hemicellulose, ADL and ash content of OPF (Table 2). Ensiling without additives influences 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 Pedrero 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 Zabirollidini 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.

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.

| Fatty Acid | 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         | 2.56 | 2.52 | 2.65 | 0.05 | 0.281 |
| C14:0     | 3.97         | 3.66 | 3.73 | 3.65 | 0.06 | 0.186 |
| C15:0     | 0.62         | 0.39 | 0.38 | 0.32 | 0.03 | 0.061 |
| C15:1     | 3.44         | 2.53 | 2.26 | 2.38 | 0.09 | 0.064 |
| C16:0     | 35.14        | 35.80 | 35.19 | 34.48 | 34.93 | 0.35 | 0.051 |
| C16:1     | 0.90         | 1.24 | 1.21 | 1.54 | 0.16 | 0.049 |
| C17:0     | 2.10         | 2.69 | 2.51 | 2.74 | 0.08 | 0.531 |
| C17:1     | 0.62         | 0.54 | 0.50 | 0.51 | 0.02 | 0.734 |
| C18:0     | 7.44         | 6.11 | 5.93 | 6.27 | 0.25 | 0.061 |
| C18:1n-9 | 6.58         | 8.88b | 7.98b | 8.33b | 7.80b | 0.16 | 0.034 |
| C18:1trans-11 | 0.00 | 0.36b | 2.10a | 1.75a | 2.15a | 0.20 | 0.002 |
| C18:2n-6 | 12.76        | 14.44 | 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 | 50.69 | 50.31 | 0.33 | 0.101 |
| Total MUFA | 11.54 | 12.68 | 14.56 | 14.39 | 14.37 | 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 | 14.44 | 12.23 | 12.61 | 12.49 | 0.29 | 0.975 |
| n-3/n-6 | 0.52 | 0.56 | 0.53 | 0.57 | 0.55 | 0.02 | 0.912 |
| UFAsFAs | 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; UFAs, unsaturated fatty acids. *Total SFA, sum of C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C18:1n-9, C18:2n-6, C18:3n-3; total MUFA, sum of C15:1, C16:1, C17:1, C18:1n-9, C18:2n-6; total n-3PUFA, C18:2n-6; total n-6PUFA, sum of C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C18:1n-9, C18:2n-6, C18:3n-3.

Table 3 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 A9 desaturase, which is in line the observed decrease in the proportion of C18:0. Unfortunately, to the authors’ knowledge, the activity of A9 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 indicating that the observed proportional decrease in syringes: a tool to estimate short chain fatty acid production that requires minimal laboratory facilities. Anim. Feed Sci. Tech. 123:197-210. Folch, J., Lees, M., Sloane Stanely, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509. Glasser, F., Doreau, M., Maxim, G., Baumont, R., 2013. Fat and fatty acid content and composition of forages: a meta-analysis. Anim. Feed Sci. Tech. 185:19-34. Koh, C.S., Tan, K.T., Lee, K.T., Bhatia, S., 2010. Bio-ethanol from lignocellulose: status, perspectives and challenges in Malaysia. Bioresearch Technol. 101:4834-4841. Guiragossian, V.Y., 1977. Chemical and biological methods for grain and forage sorghum. Department of Agronomy, International Programs in Agriculture, Purdue University Publ., Purdue, IN, USA. Han, L., Zhou, H., 2013. Effects of ensiling processes and antioxidants on fatty acid concentrations and compositions in corn silages. J. Anim. Sci. Biotechnol. 4:48. Kishino, S., Ogawa, J., Yokozeuki, K., Shimizu, S., 2009. Metabolic diversity in bihydrogenation of polyunsaturated fatty acids by lactic acid bacteria involving conjugated fatty acid production. Appl. Microbiol. Biot. 84:87-97. Kung, L. Jr., Ranjit, N.K., 2001. The effect of Lactobacillus buchneri and other additives on the fermentation and aerobic stability of barley silage. J. Dairy Sci. 84:1149-1155. Lee, M.R., Scott, M.B., Tweed, J.K., Minchin, F.R., Davies, D.R., 2008. Effects of polyphenol oxidase on lipolysis and proteolysis of red clover silage with and without a silage inoculant (Lactobacillus plantarum L54). Anim. Feed Sci. Tech. 144:125-136. Lee, S.W., Chouinard, Y., Binh Nguyen, V., 2006. Effect of some factors on the concentration of linolenic acid of forages. Asian Austral. J. Anim. 19:1148-1158. Lima, R., Diaz, R.F., Castro, A., Hoedtke, S., Fievez, V., 2011. Multifactorial models to asses responses to sorghum proportion, molasses and bacterial inoculant in invitro quality of sorghum-soybean silages. Anim. Feed Sci. Tech. 164:161-173. Lough, A.K., Anderson, L.J., 1973. Effect of ensiling on the lipids of pasture grasses. Proc. Nutr. Soc. 32:61A-62A. McDonald, P., Henderson, N., Heron, S., 1991. The biochemistry of silage. Chalcombe Publ., Cambrian Printers, Limited Merlow, Bucks, Abeystwyth, UK. McDonald Henderson, P.A.R., 1962. Buffering capacity of herbage samples as a factor in ensilage. J. Sci. Food Agr. 13:395-400. Muck, R.E., 1998. Preparing high quality alfalfa silage. pp 1-11 in Int. Seminar of Conservation of High Quality Flora, Los Angeles, Chile. Ogawa, J., Kirishino, S., Aodo, A., Sugimoto, S., Mihara, K., Shimizu, S., 2005. Production of conjugated fatty acids by lactic acid bacteria. J. Biosci. Bioeng. 100:355-364. Pedroso, A., Russio, L.G., Loures, D.R.S., Paziani, S., Ribeiro, J.L., Mari, L.J., Zoppollato, M., Schmidt, P., Mattos, W.R.S., Horii, J., 2008. Fermentation, losses, and aerobic stability of sugarcane silages treated with chemical or bacterial additives. Sci. Agr. 65:389-394. Rajion, M., McLean, J., Cahill, R., 1985. Essential fatty acids in the fetal and newborn lamb. Aust. J. Biol. Sci. 38:33-40. Ren, H., Richard, T.L., Moore, K.J., 2007. The impact of enzyme characteristics on corn stover fiber degradation and acid production during ensiled storage. Appl. Biochem. Biotech. 137-140:221-238. Ridla, M., Uchida, S., 1993. The effect of cellulase addition on nutritional and fermentation quality of barley straw silage. Asian Austral. J. Anim. 6:383-388. SAS, 2007. SAS/STAT users guide: version 9.1. SAS Inst. Inc., Cary, NC, USA. Shepard, A.C., Maslanka, M., Quinn, D., Kung, L. Jr., 1995. Additives containing bacteria and enzymes for alfalfa silage. J. Dairy Sci. 78:565-572. Solorzano, L., 1969. Determination of ammonia in natural waters by the phenol-hypochlorite method. Limnol. Oceanogr. 14:799-801.
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