Animal Feed Science and Technology 242 (2018) 31–40

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Proposal and validation of new indexes to evaluate maize silage fermentative quality in lab-scale ensiling conditions through the use of a receiver operating characteristic analysis

Igino Andrighetto[a], Lorenzo Serva[a], Matteo Gazziero[a], Sandro Tenti[a], Massimo Mirisola[a], Elisabetta Garbin[a], Barbara Contiero[a], Daniel Grandis[b], Giorgio Marchesini[a,*]

[a] Department of Animal Medicine, Production and Health, University of Padova, Viale dell'Università, 16, 35020 Legnaro (PD), Italy
[b] KWS italia S.p.A, via Lombardia, 28, 35043, Monselice (PD), Italy

ARTICLE INFO

Keywords:
Maize silage
Fermentative quality index
Flieg-Zimmer's score
Roc analysis
Lab-scale silages

ABSTRACT

In the context of dairy cow feeding, it is increasingly important to know the quality of the maize silage used in the ration and therefore, it appears to be crucial optimizing the techniques necessary to assess it. The aim of this study was to evaluate whether some reference indexes, like the Flieg-Zimmer's (FZS), the German agricultural society's (DLG) and Vanbelle's scores, could properly estimate the quality of fermentations of maize silage made in a lab-scale ensiling system, and to calculate and validate new quality indexes suitable for lab-scale fermentations. The experimental dataset was obtained by analysing through near-infrared spectroscopy 522 samples of whole maize crop ensiled immediately after the harvest, using the vacuum-packing technique. The six (I1 – I6) new indexes were calculated on the basis of chemical and physical parameters as: pH, organic acids, ethanol, etc. All the indexes were tested for normality with the Shapiro–Wilk test. In order to define the accuracy with which the new indexes ranked the maize silage on the basis of its fermentation quality, a receiver operating characteristic (ROC) analysis was performed, using the FZS as gold standard test and dichotomizing the FZS in two levels according to a cut-off (FZS $<$ 80, non-excellent vs. FZS $\geq$ 80, excellent). Accuracy was determined through the value of the area under the curve (AUC). Finally, a one-way ANOVA was used to compare the quality of maize silage with low ($<$ 320 g/kg), medium (320–360 g/kg) and high ($>$ 360 g/kg) dry matter (DM). In the lab-scale silages the new indexes were normally distributed, whereas the reference indexes were not. The new indexes showed values of AUC ranging between 0.76 and 0.89, with the I5 index showing the best combination of sensitivity (0.87) and specificity (0.77) in discriminating between good and poor quality silage. The cut-off of the new indexes ranged between 45.0 and 57.4 points. The lab-scale silages were all excellent, no matter the category of DM. However, while FZS and DLG did not differ among the 3 categories, I1 – I6 were significantly higher in silages with low DM ($P < 0.001$). Silages with low DM had the highest concentrations of lactic acid (56.4 g/kg DM, $P < 0.001$), ammonia (61.4 g/kg DM, $P < 0.001$) and butyric acid

Abbreviations: ADF, ADF expressed inclusive of residual ash; aNDF, NDF assayed with a heat stable amylase and expressed inclusive of residual ash; AUC, area under the curve; CP, crude protein; DLG, the German agricultural society score; DM, dry matter; EE, ether extract; FZS, Flieg-Zimmer's score; I1 – I6, new quality indexes to evaluate maize silage; R2, coefficient of determination; ROC, receiver operating characteristic; SD, standard deviation; SEC, standard error of calibration; SECV, standard error of cross-validation; SEM, standard error of means; VBS, Vanbelle's score; 1-VR, Coefficient of determination in cross-validation

* Corresponding author.
E-mail address: giorgio.marchesini@unipd.it (G. Marchesini).

https://doi.org/10.1016/j.anifeedsci.2018.05.009
Received 23 January 2018; Received in revised form 21 May 2018; Accepted 24 May 2018

(0.62 g/kg DM, P < 0.001) as well. Data confirmed that the new proposed indexes are promising in describing the fermentation quality of maize silage in lab-scale conditions.

1. Introduction

Maize (*Zea mays*, L.) silage is one of the most widely used feed in cattle rations in great part of the world (Erdman et al., 2011; Marchesini et al., 2017), especially in temperate areas, since it is a very productive crop, characterized by an excellent nutritional profile and it is suitable to be preserved through ensiling (Khan et al., 2015). Although the nutritional composition of silage, expressed in terms of content in dry matter (DM), crude protein (CP), starch, fibre and nutrients digestibility is of primary importance to optimize animal performance (Kuehn et al., 1999; Addah et al., 2011; Krämer-Schmid et al., 2016), several authors stated that the quality of the fermentation during the ensiling process and its aerobic stability are important as well (Woolford, 1984; McDonald et al., 1991; Oude Elferink et al., 2000). In fact, a silage that has undergone an abnormal fermentation has a lower nutritional value, and is often rejected by animals, leading to reduced dry matter intake and lower performance (McDonald et al., 2011). The quality of fermentations occurring during the ensiling process can be determined through the measure of pH and the analysis of the concentration of a wide range of fermentation products such as: lactate, acetate, propionate, butyrate, isobutyrate, ethanol, mannitol and ammonia (Cherney et al., 2004; Nishino et al., 2004; Johnson et al., 2005). The pH, which in a maize silage should range approximately from 3.7 to 4.2 (Johnson et al., 2002; Cherney et al., 2004; Romero et al., 2017), is the result of the concentration of acids, urea and ammonia produced by microorganisms and the buffer capacity of the substrate. Among organic acids, lactate should be the most present acid, as it contributes most to the decline in pH and is associated with a lower DM and energy loss during storage (McDonald et al., 2011), whereas the high concentration of acetate (> 30 g/kg DM) or the presence of propionate, butyrate and isobutyrate are associated with a higher loss of DM (Muck, 1988; McDonald et al., 2011). In addition, acetic acid, typically produced during heterolactic fermentations, although having antimycotic properties seems to interfere with cattle dry matter intake (McDonald et al., 1991; Nishino et al., 2004) over a certain concentration (> 60 g/kg DM). Furthermore, butyrate suggests the presence of clostridia (Hoedtke and Zeyner, 2011; McDonald et al., 2011) which are undesirable microorganisms, as they degrade proteins and produce ammonia, amines and other substances that compromise the palatability of silage (Muck, 1988). Other compounds, such as ethanol and mannitol, are mainly produced in secondary fermentations by yeasts or heterofermentative bacteria (Pahlow et al., 2003; Nishino et al., 2004). Each of these parameters give information only on a certain aspect of fermentation, indicating for example in the case of butyrate, the presence or absence of fermentations by clostridia, but in order to be able to say whether a fermentation was qualitatively better than another, we need an index that takes into account and weighs each of these parameters. In this regard, there are scores, such as the Flieg-Zimmer's score (FZS) described by Woolford (1984), that are calculated on the basis of the concentration of lactic, acetic and butyric acids, whereas others take into account the concentration of lactate, volatile fatty acids and ammonia, such as the Vanbelle's (VBS) score (Vanbelle and Bertin, 1985), or consider the pH and the concentration of acetic and butyric acids, such as the German agricultural society (DLG) score (DLG et al., 2006; Gallo et al., 2016a). These scores are still used in assessing the quality of silage in field trials, but they sometime show poor capability in discriminating between well- or poorly-preserved silages (Gallo et al., 2016a), especially in conditions of limited variability, as those found in laboratory-scale ensiling systems, where the ensiling conditions are strictly controlled (Cherney et al., 2004; Hoedtke and Zeyner, 2011). These lab-scale ensiling systems are often used, because they are suitable for experimental designs which involve numerous variables, allow a more complete control of the ensiling conditions (Johnson et al., 2005; Romero et al., 2017) and are less costly and labour intensive than farm-scale silos, while still achieving a fermentation reasonably similar to that taking place in field-scale silos (Cherney and Cherney, 2003).

The aims of this study were to evaluate the ability of FZS, VBS and DLG to estimate the quality of fermentations of maize silage made in a lab-scale ensiling system and to calculate and validate new indexes of maize silage fermentation quality, suitable for lab-scale fermentations.

2. Materials and methods

2.1. Sample collection, preparation and analysis

Samples of whole maize crop (n = 522) belonging to early (variety E, FAO class 200, n = 14) and late (variety L, FAO class 600–700, n = 15) ripening cultivars were harvested and ensiled during the summer season 2016. In order to have samples representative of the wide variability in maize silage, plants belonging to 29 cultivars were grown in areas with different pedoclimatic conditions (from ideal to very stressful) and cropped at different ripening phases (from 5 days before, to 5 days after the 33% milk line stage). Two samples (500 ± 50 *g*) for each freshly harvested whole maize crop (n = 1044) were chopped immediately after the harvest and ensiled in vacuum-packed bags (Orved 2633040, Orved SpA, Musile di Piave, VE, Italy). Samples were treated according to the procedures recommended by Johnson et al. (2005), especially to avoid bloating (Hoedtke and Zeyner, 2011) and they were stored at 23 °C for 60 days, before being opened for analysis. Bags (300 × 400 mm) were 90 µm thick, were made of polyamide and polyethylene (PA/PE) and had a gas permeability at 23 °C ± 2 of 65, 15 and 200 $cm^3\ m^{-2}\ day^{-1}\ atm^{-1}$ to oxygen, nitrogen and CO_2, respectively. Vacuum-packing was performed using a vacuum-packing machine (Cuisson 41, Orved SpA, Musile di Piave, VE, Italy) drawing 25 m^3 of air per hour for 12 s. Bags were then automatically sealed after air extraction. After 60 days of ensiling, the content