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Modelling a quantitative ensilability index adapted to forages from wet temperate areas

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Abstract

Forage ensilability mainly depends on dry matter (DM), water soluble carbohydrates (WSC) and buffer capacity (BC) values at harvest time. According to these parameters, and based on a collection of 208 forages of known ensilability characteristics including short and long term meadows for grazing, italian ryegrass, maize, triticale, soybean, faba bean crops, and samples coming from cereal-legume associations, the objective of this study has been to define a quantitative ensilability index (EI) based on a relationship between DM, WSC and BC contents at harvest date, adapted to the characteristics of fodder from wet temperate areas. For this purpose, a discriminant procedure was used to define this EI based on a linear combination of DM, WSC and BC of forages at harvest time. The quantitative calculated indexes distinguish five successive ranges of ensilability: high ensilability (EI > +28), medium high ensilability (+9 < EI ≤ +28), medium ensilability (–28 < EI ≤ +9), medium low ensilability (–47 ≤ EI ≤ –28) and low ensilability (EI < –47). This quantitative index was externally evaluated and 100% of samples were successfully classified.

Additional key words: buffer capacity; dry matter; silage; water soluble carbohydrates.

Introduction

In some countries, the small land surface available on farms, and the seasonality of forage production are some of the structural factors limiting the profitability of the agricultural and livestock sector. Moreover, the increased size of herds involves the need for more quantity of food stored to meet the nutritional needs of animals during winter and drought periods.

Forage quality influences the economic efficiency of the milk and meat production. Nowadays, the technology of using green food from fields during vegetation growth has been abandoned and replaced by inside feeding using diets of similar composition throughout the year, which includes large amounts of high quality silage (Dinic *et al.*, 2010a).

The weather, season, management, grazing intensity, chemical and botanical composition of forages to be ensiled and the phenological stage, etc., are factors affecting the epiphytic microflora of forage and their ensilability characteristics (Woolford, 1984; McDonald *et al.,* 1991). For a successful conservation of forages, it is necessary to know the content of water soluble carbohydrates (WSC) and buffer capacity (BC), because the amount of WSC is related with the potential to resist changes in pH also called BC. Haigh (1990) demonstrated that sunshine and rainfall data might be used to predict WSC of herbage cut for ensiling and subsequent silage dry matter. In addition, researches conducted by Martínez Fernández (2003), have confirmed previous results related by Muck *et al.* (1991) establishing that WSC content is not dependent

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Abbreviations used: BC (buffer capacity); DISCRIM (discriminant analysis); DM (dry matter); EI (ensilability index); FC (fermentability coefficient); HE (high ensilability); HEI (high ensilability index); LAB (lactic acid bacteria); LE (low ensilability); LEI (low ensilability index); SERIDA (Regional Institute of Agro-food, Research and Development); WSC (water soluble carbohydrates).

of phenological stage, whereas BC decreases when increasing maturity stage.

According these previous considerations, Piñeiro & Pérez (1992) carried out studies to evaluate differences in ensilability between several species of grasses and legumes in pasture mixtures usually used in wet areas of north of Spain. These authors have remarked the good ensilability of grasses compared with legumes due to the higher amount of WSC.

The WSC content of herbage ensiled is often regarded as a useful indicator of herbage quality for ensiling. A concentration of 25-35 g kg^{-1} in fresh herbage is desirable for silages without additive, although a higher value of 30-35 g kg^{-1} has also been suggested (Haigh & Parker, 1985).

The BC in forages depends on the species and its phenological state, with a wide variability between 200 and up to 600 meq NaOH kg^{-1} DM (McDonald *et al*., 1991; Jaster, 1995). The BC of ryegrasses is ranged between 250 and 400 meq NaOH kg^{-1} DM, whereas for legumes (clover, lucerne, soybean) BC goes up 500-600 meq NaOH kg^{-1} DM (Playne & McDonald, 1966; Tobía *et al*., 2008). The BC in maize has the lowest values, ranged between 148 and 351 meq NaOH kg^{-1} DM according to Kaiser & Piltz (2002).

Therefore, to characterize the acidification potential of a crop, the ratio WSC/BC is used. For a good fermentation process to obtain a good quality silage without presence of butyric acid, this ratio must be 3.0 or higher (Weissbach, 1999; Dinic *et al.,* 2010a). This ratio (WSC/BC) also depends on the DM content in ensiling material. If the DM content of a plant material is too low, a minor nutrient concentration and an insufficient availability of carbohydrates for the activity of lactic acid bacteria (LAB) in silages is indicated (Pries, 2004). Low DM content requires a more intense acidifying process in order to prevent forming of butyric acid and the proportion of WSC/BC has to be higher in order to provide a stable pH values (Dinic *et al.,* 2010b). By contrast, in high DM silages with reduced water availability, the presence of suitable, osmo-tolerant LAB could become a limiting factor in the ensiling process. It has been shown that these bacteria represent only a small percentage of the indigenous microflora on forage crops (Pahlow & Weissbach, 1996). Forages with DM content above 50% are considered difficult to ensile (Staudacher *et al*., 1999).

In this sense, Weissbach (1999) stated that, the minimum DM content for a good fermentation process increases when WSC/BC ratio decreases and can be calculated as DMmin (%) = 45-8WSC/BC. Poor silage fermentation can be efficiently suppressed by pre-wilting the herbage despite of a low WSC/BC ratio.

However, the poor fermentation due to conditioning, compaction or silo closure, contamination with soil or manure, human or mechanical failures of any kind, has not solution and it only can be avoided by making a good silage process. But, assuming good harvesting and ensiling techniques, initial silage fermentation can still be sub-optimal. This can be due to a lack of suitable LAB and/or WSC. The minimum number of LAB required to inhibit clostridial activity was found to be at least 10^5 colony-forming units per gram of fresh crop (Weissbach & Honig, 1996; Kaiser & Weiss, 1997).

Summarizing all previous information, the amount of WSC necessary to obtain adequate fermentation depends on the DM content and the BC of the crop. Weissbach & Honig (1996) characterized the relationship between these factors as follows,

$$\text{Fermentation coefficient (FC)} = \text{DM (\%)} + 8\text{WSC} / \text{BC} \quad [1]$$

The Eq. [1] does not apply for crops with low nitrate content, such as extensively managed grasses and immature whole-crop cereals, because these crops are more liable to clostridial fermentations than crops with moderate nitrate content (Spoelstra, 1983, 1985).

The aim of this study was to develop a general predictive simulation ensilability index using DM, WSC and BC of different grass and crops. Such a model could be useful to designing keys for better understanding of the ensilage process and for making decisions before ensiling.

Material and methods

Population of forages and analytical methods

This study was conducted on the experimental farm of the Regional Institute for Agro-food, Research and Development (SERIDA) located in the North of Spain (43°28’50”N, 5°26’27”W, 10 m asl, Asturias) and with grasses and crops collected between 2000 and 2010.

A total of 65 samples (Set 1) of maize (*Zea mays* L.) (N = 20), triticale (× *Triticosecale* Wittm.) (N = 27), and soybean (*Glycine max* L. Merr.) (N = 18) were used to develop a quantitative ensilability index. A Set 2 with 143 samples including Italian ryegrass alternative and no alternative monocultures (*Lolium multi-*

florum L.) (N = 20), faba bean (*Vicia faba* L.) (N = 33) and associations such as triticale-faba bean (N = 12) and sown meadows of Italian ryegrass-red clover (*Lolium multiflorum* L. and *Trifolium pratense* L.) (N = 28) and of perennial ryegrass-white clover (*Lolium perenne* L. and *Trifolium repens* L.) (N = 50) were used to establish different ensilability quantitative categories.

Finally, the developed EI was applied to an external sample set (Set 3) including 16 forages and crops from temperate areas, with a wide range of DM, WSC and BC content ranged from low to high ensilability. The reference classification (low, medium or high) for each sample was established according to the range of values summarized in Table 1 (Playne & McDonald, 1966; Haigh, 1990).

In forages and crops the parameters that define the ensilability characteristics, DM, WSC and BC, were determined. For each sample, two representative fresh forage and crops subsamples were taken and later each one was divided in three parts: (i) the first one was dried in an air-forced oven at 102°C for 24 h to determine DM content; (ii) for WSC analysis, the second subsample was dried at 60°C for 24h and after that milled through a 0.75 mm sieve; and (iii) the third one was kept as fresh material to estimate BC. All samples (dried and fresh) were analyzed in duplicate.

WSC were extracted in hot water with stirring and determined by reduction ferrocyanide with UV-VIS detection at 540 nm according Hoffman (1937). For BC the chopped fresh mass was first macerated with distilled water. The pH of the macerate was recorded and the BC was measured by electrometric titration using a Radiometer pHmeter. The macerate was titrated first to pH < 3 with 0.1 mol L^{-1} HCl in order to release bicarbonate as CO_2 and then was titrated to pH = 6 with 0.1 mol L^{-1} NaOH. BC was expressed as mmol required changing the pH from 4 to 6 per kg of DM (Playne & McDonald, 1966).

Description of the model

This predictive model was developed in three steps:

— The first step was to group separately the forages and crops with high and low ensilability (Set 1, Table 2). The lowest WSC and highest BC were observed for soybean (forage with low ensilability - LE), whereas highest WSC with lowest BC were observed for maize and triticale (forages with high ensilability - HE). These criteria are guaranteed by their respective conditions of legumes and grasses. We can see the wide range of variations for DM, WSC and BC parameters in all considered forages. The forages and crops, different to maize, triticale and soybean, could not be classified *a priori*, as HE, or LE.

— The second step of this approach was to establish a linear correlation between the quantitative variables that define ensilability, DM, WSC and BC. The coefficients of the correlation matrix were fitted to same pattern for the samples of triticale, maize and soybean, showing a positive correlation ($p < 0.001$) between DM and WSC and negative between DM and BC ($p < 0.001$) and between WSC and BC ($p < 0.001$) (see Table 3).

— The third step was to develop a classification model using a DISCRIM procedure from SAS (1999) based on a linear combination of the DM, WSC and BC values. It will allow classifying each observation into one of the groups previously established (HE and LE). This procedure develops a discriminate function or classification criterion using a measure of generali-

Table 1. Reference ranges of dry matter (DM), water soluble carbohydrates (WSC) and buffer capacity (BC) to define qualitative ensilability categories of forages and crops

Ensilability categories	DM ($g\ kg^{-1}$)	WSC ($g\ kg^{-1}DM$)	BC (mmol NaOH $kg^{-1}DM$)
High	> 250	> 150	< 250
Medium	200-250	80-150	250-350
Low	< 200	< 80	> 350

Table 2. Ranges of dry matter (DM), water soluble carbohydrates (WSC) and buffer capacity (BC) of soybean, maize and triticale

Forage	DM ($g\ kg^{-1}$)	WSC ($g\ kg^{-1}DM$)	BC (mmol NaOH $kg^{-1}DM$)
Soybean (N = 18)	177.3-274.1	38.6-109.0	411.3-565.0
Maize (N = 20)	206.7-351.5	118.3-253.0	116.5-194.0
Triticale (N = 27)	206.4-383.3	146.3-313.4	74.4-256.1

Table 3. Correlation matrix between dry matter (DM), water soluble carbohydrates (WSC) and buffer capacity (BC) of crops with high (triticale and maize) and low ensilability (soybean)

	DM	WSC	BC
DM		0.7216***	–0.6566***
WSC			–0.7932***
BC			

***: $p < 0.001$.

zed squared distance assuming that each class has a multivariate normal distribution. The classification criterion is based on either the individual within-group covariance matrices or the pooled covariance matrix. It also takes into account the prior probabilities of the classes. With this procedure, each observation is placed in the class from which it has the smallest generalized squared distance.

Results

The linear correlation between the quantitative variables, DM, WSC and BC, calculated following Step 2, has made possible to establish two linear discriminant functions to define the index for high ensilability (HEI) and low ensilability (LEI):

$$HEI = -61.5 + 0.276\ DM + 0.017\ WSC + 0.312\ BC \qquad [2]$$

$$LEI = -213.8 + 0.473\ DM - 0.068\ WSC + 0.687\ BC \qquad [3]$$

where parameters are expressed as $[DM] = g\ kg^{-1}$, $[WSC] = g\ kg^{-1}$ DM, $[BC] = mmol\ NaOH\ kg^{-1}$ DM.

Taking into account these two functions, when a sample has a high value of HEI and low LEI, it will be classified like HE sample, and a greater difference between both indexes (HEI-LEI) for a forage will indicate a higher ensilability. For low ensilability forages the reasoning will be the opposite. These linear functions allow us to classify 100% samples of Set 1 correctly, soybean as LE samples (N = 18) and maize and triticale as HE samples (N = 47) (Fig. 1a).

The Set 2, including forages and crops such as short and long term meadows for grazing, italian ryegrass, faba bean crops, and samples coming from cereal-legume associations, cannot be classified a priori as high, or low ensilability. To classify all samples we studied the possibility to obtain a rating to discriminate HE and LE forages and crops, and also to establish an intermediate ensilability group between both categories (HE and LE).

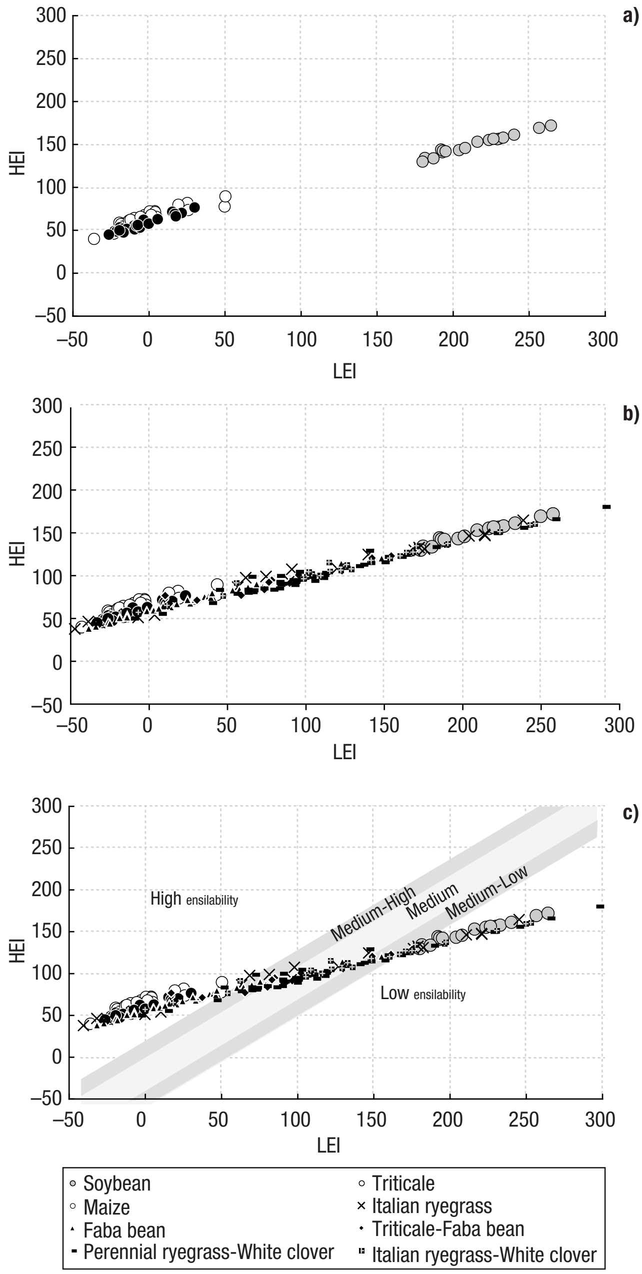
Both functions, HEI and LEI, were combined to establish a new general quantitative index named ensilability index (EI), calculated as:

$$EI = HEI - LEI$$

with the following expression:

$$EI = 152.29 - 0.197\ DM + 0.085\ WSC - 0.375\ BC \qquad [4]$$

Applying Eq. [4] over samples included in Set 1, positive values of maize and triticale will indicate HE and negative values for soybean will be related with LE.



<**Figure 1.** Classification of forages and crops populations according the ensilability indexes established in the discriminant analysis: (a) Set 1, N = 65; (b) Sets 1 + 2, N = 208; (c) intervals defined for grass and crops used for silage in extensively farms located in humid temperate areas. HEI: high ensilability index; LEI: low ensilability index.

After that, the Eq. [4] was applied to Sets 1 and 2 together (N = 65 + 143), and the obtained values were ordered from lowest to highest ensilability index values, to examine the possibility of establish three quantitative ensilability categories, high, low and intermediate. The ensilability parameters of Set 2

Table 4. Ranges of dry matter (DM), water soluble carbohydrates (WSC) and buffer capacity (BC) for the complementary forages involved in the study (N = 143)

Forages	DM (g kg^{-1})	WSC (g kg^{-1}DM)	BC (mmol NaOH kg^{-1}DM)
Italian ryegrass monocultures (N = 20)	114.7-231.9	64.0-339.6	183.7-562.0
Italian ryegrass-Red clover (N = 28)	111.2-269.1	33.6-223.1	201.3-599.3
Perennial ryegrass-White clover (N = 50)	102.2-429.4	31.1-175.4	128.0-659.6
Triticale-Faba bean intercrop (N = 12)	151.2-339.7	29.6-251.3	127.4-433.3
Faba bean monoculture (N = 33)	141.8-251.1	93.7-234.0	153.6-450.0

Table 5. Variation of ensilability index (EI) of forages and crops used as silage in extensive farms located in humid temperate coastal areas of Spain

Forages	Ensilability index		Classification interval
	Minimum	Maximum	
Soybean	–92.16	–47.11	Low ensilability
Italian ryegrass monocultures[1]	–81.40	77.67	From low to high ensilability
Italian ryegrass-Red clover	–92.47	56.37	From medium low to high ensilability
Triticale-Faba bean intercrop	–46.64	58.95	From medium low to high ensilability
Faba bean monoculture	–35.51	69.15	From medium low to high ensilability
Maize	46.48	72.52	High ensilability
Triticale	28.04	78.33	High ensilability

[1] Italian ryegrass Westerwold.

(N = 143) are detailed in Table 4. As can be seen in Table 4, the complementary fodder included in this study (different to those selected to define the ensilability indexes as maize, triticale and soybean), also show a great variability into the parameters that define the ensilability. For better understanding, Fig. 1b includes all samples in the global population (N = 208) by representing their high and low ensilability indexes (HEI and LEI). It can be seen that most of the samples show intermediate values between the highest ensilability values for maize and triticale and the lowest for soybeans.

For maize and triticale, EI always was > 28, whereas for soybeans, it was < –47, obtaining intermediate values for the other forages and crops tested (see Table 5). This range between –47 and +28 is too large to express medium ensilability, so that intermediate values need to be established to adequately characterize the ensilability of different forages. Dividing this medium segment in four equal parts we could define three categories by including 25%, 50% and 25% of samples (Fig. 1c), which were denominated medium-high, medium and medium-low ensilability, respectively.

The classification criterion for the developed ensilability index, as can be seen in Fig. 1c, is: (i) high ensilability when EI > +28, (ii) medium-high ensilability +9 < EI ≤ +28, (iii) medium ensilability –28 < EI ≤ 9), (iv) medium-low ensilability –47 ≤ EI ≤ –28 and (v) low ensilability always when EI < –47.

Finally the modeled index was externally evaluated applying the Eq. [4] to an independent sample population (Set 3, N = 16). Classification results attending ensilability intervals are shown in Table 6. The EI values obtained are in agreement with those expected according to DM, WSC and BC parameters (Table 1).

Discussion

The forage and crops ensilability characteristics included in this study have a wide variability range depending on their DM content and WSC/BC ratio. In this sense forage legumes are difficult for ensiling. Their low sugar content, high BC and extremely unfavourable WSC/BC ratio indicate unsuitability for ensiling. By contrast perennial grasses are much more suitable for ensiling due to their favourable WSC/BC ratio (Dinic *et al.,* 2010b).

According to Eq. [1] (FC index defined by Weissbach & Honig, 1996), when the DM content is too low, or there are not enough WSC available, the FC

Table 6. Ensilability index (EI) calculated to classify the external evaluation set

Forages	DM (g kg^{-1})	WSC (g kg^{-1} DM)	BC (mmol NaOH kg^{-1} DM)	EI	Low	Medium-low	Medium	Medium-high	High
Faba bean	173	93.5	304	12.65				√	
Faba bean	185	149	222	45.17					√
Maize	352	121	125	46.49					√
Maize	249	237	136	72.24					√
Italian ryegrass	116	94.2	562	–73.38	√				
Italian ryegrass	169	240	297	28.09					√
Italian ryegrass-Red clover	123	86.8	423	–23.30			√		
Italian ryegrass-Red clover	143	140	419	–21.17			√		
Soybean	183	81.9	500	–62.57	√				
Soybean	206	68.2	469	–58.50	√				
Triticale	363	268	110	62.29					√
Triticale	310	308	120	72.30					√
Sown meadow	133	134	383	–6.32			√		
Sown meadow	252	101	404	–40.20		√			
Meadow	161	84.8	250	34.22					√
Meadow	254	96.7	272	8.38			√		

will be < 35 (Oude Elferink *et al*., 2000) and thus the plant material will be difficult to ensile. In these cases, adequate fermentation can only be achieved when the WSC is increased, either by adding sugars directly (*e.g.* molasses) or by adding enzymes that release extra sugars from the crop. In forages with a FC > 35, sufficient fermentable substrates are available. In this situation, adding suitable LAB can accelerate and improve the ensiling process. With a FC > 45, a stable fermentation can be guaranteed.

Applying Eq. [1] to the forages included in Set 1 we obtained the following results: (i) all soybean samples were correctly classified as forages with low ensilability (Oude Elferink *et al*., 2000), due to their insufficient fermentable substrate or too low DM content (FC < 35); and (ii) 60% of maize and triticale were assigned to HE group (FC > 35), moreover, applying this criterion only a stable fermentation process for 33% of maize and triticale samples (FC > 45) could be expected.

In the same way, when we applied the ratio WSC/BC to maize and triticale (both considered as high ensilability crops), only 2% showed values above 3.0, that according to Weissbach (1999) and Dinic *et al*. (2010a) ensures good fermentative quality without presence of butyric acid. This misclassification for high ensilability crops justifies the development of a new ensilability index better adapted to the characteristics of fodder in wet temperate areas.

The new established EI classify without penalties 100% of samples pertained in Set 3 improving those obtained when applying the FC (Eq. [1]).

The developed EI allow us to design keys to avoid and prevent fermentation problems, due to high BC and/or insufficient fermentable carbohydrates, or low DM content and to take decisions such as: (i) prewilting forage, that increases the level of DM in silo mass and reduces losses of nutritive substances, obtaining good quality silage without the presence of butyric acid (Dinic *et al*., 2010a; Martínez-Fernández *et al.,* 2010), (ii) using absorbents in the form of ground grain or dried sugar beet pulp, which reduce effluent losses (Haigh, 1999) and decrease pH value and the acetic acid content of silages, and elevate the concentration of lactic acid and WSC in the silage; and (iii) using ensilage additives in the form of fermentation inhibitors or stimulants (McDonald *et al.*, 1991) as unique supplementation or associated with absorbents (Pys *et al.*, 2002). Inoculants that increase lactic acid fermentation might be useful to inhibit clostridia activity.

All these alternatives may even minimize the effects of excessive moisture, to help in case of shortage of lactic acid and butyric spore presence and even improve digestive and metabolic utilization of silage (Meeske *et al*., 2002).

The results obtained have allowed us to develop and to establish a quantitative ensilability index (EI) to classify a wide variety of forages and crops harvested in wet temperate coastal areas to make silage, based on a linear combination of the ensilability parameters DM, WSC and BC. This index ranks the forages and crops into five successive categories from low to high ensilability, with three intermediate categories (medium-low, medium and medium-high). This classification is a useful tool to design some keys for better understanding the ensilage process and for making decisions before ensiling concerning to prewilting forages and/or using additives.

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