SCAP
SOCIEDADE DE CIÊNCIAS AGRÁRIAS DE PORTUGAL

Carbohydrate fractionation, fermentation and aerobic stability of silages with different maize hybrids†

Fracionamento de hidratos de carbono, fermentação e estabilidade aeróbia da silagem de diferentes híbridos de milho

Egon H. Horst[1,*,†], Valter H. Bumbieris Junior[1], Mikal Neumann[2], André M. Souza[2], Edelmir S. Stadler Junior[2] and André Dochowat[2]

[1] Department of Animal Science, State University of Londrina (UEL), 86057-970, Londrina, PR, Brazil
[2] Department of Veterinary Medicine, State University of the Middle-West (UNICENTRO), 85040-080, Guarapuava, PR, Brazil
† Part of first author's thesis
*(*E-mail: egonhh@yahoo.com.br)*

https://doi.org/10.19084/rca.17723
Received/recebido: 2019.04.25
Accepted/aceite: 2019.09.26

ABSTRACT

The objective of this study was to evaluate the chemical composition and the fermentative variables of different maize hybrids and their interference in the aerobic stability of silage. The hybrids used were the Maximus VIP3, Defender VIP and Feroz VIP, being simple, triple and double hybrids, respectively. The chemical composition and the aerobic stability were evaluated 60 days after the silos were sealed. One kilo of sample from each replicate was placed in polypropylene containers lined with plastic bags and transferred to a climatic chamber at 25 ± 2°C, and the silage temperatures were measured three times a day for seven days. In the chemical composition, only the fraction A of the non-fibrous carbohydrates presented a difference between the treatments. The Maximus VIP3 hybrid was superior in relation to the others for acetic acid production and did not differ from the Defender VIP hybrid in lactic acid concentration. A longer time was required for the Maximus VIP3 silage temperature to increase by 2°C (125.3 hours), while the other silages did not differ significantly (53.3 and 45.3 hours for Defender VIP and Feroz VIP). The hybrid Maximus VIP3 is recommended for silage production due to its characteristics, including a high concentration of sugars and a greater aerobic stability.

Keywords: bromatology, deterioration, organic acids, temperature, *Zea mays* L.

RESUMO

O objetivo do trabalho foi avaliar a composição química e variáveis fermentativas de diferentes híbridos de milho e sua interferência na estabilidade aeróbia da silagem. Os híbridos utilizados foram o Maximus VIP3, Defender VIP e Feroz VIP, sendo híbridos simples, triplo e duplo, respectivamente. A composição química e a estabilidade aeróbia foi avaliada 60 dias após a vedação dos silos. Amostras de um quilo foram colocadas em recipientes de polipropileno revestido com saco plástico e transferidos para câmara climática à temperatura de 25 ± 2 °C, e as temperaturas das silagens foram medidas três vezes ao dia durante sete dias. Na composição química, apenas a fração A dos hidratos de carbono não fibrosos apresentou diferença entre os tratamentos. O híbrido Maximus VIP3 foi superior em relação aos demais para produção de ácido acético, e não diferiu do híbrido Defender VIP na concentração de ácido lático. Tempo significativamente mais longo foi necessário para que a temperatura da silagem de Maximus VIP3 aumentasse em 2°C (125,3 horas), enquanto as outras silagens não diferiram entre si (53,3 e 45,3 horas para Defender VIP e Feroz VIP). O híbrido Maximus VIP3 apresentou alta concentração de açucares e maior estabilidade aeróbia, sendo recomendado para produção de silagem pelas suas características.

Palavras-chave: ácidos orgânicos, bromatologia, deterioração, temperatura, *Zea mays* L.

INTRODUCTION

The use of deteriorated silage in ruminant feed can result in decreased dry matter intake and animal performance (Dolci *et al*., 2011), which compromises production. The most commonly observed indicators of deterioration are the development of molds, loss of dry matter, increase of pH, ammonia nitrogen and fiber concentrations and reduction in nutrient digestibility, as well as spontaneous heating, which is translated to a loss of aerobic stability.

By definition, aerobic stability is the resistance that the ensiled mass gives to deterioration after being exposed to air (Tres *et al*., 2014a), and according to the same authors, several factors interfere with this stability and, consequently, the maintenance of the quality of silage, thereby emphasizing the forage species, the dry matter and soluble sugars content, the concentration and species of aerobic and anaerobic microorganisms, as well as the concentration of organic acids.

The organic acids that were produced, which were predominantly lactic acid and acetic acid, are dependent on the genre of epiphytic bacteria that is present in the plant (Aoki *et al*., 2013), knowing that the predominance of homofermentative bacteria results in the low aerobic stability of silage, and those rich in heterofermentative bacteria generate relatively stable silages (Holzer *et al*., 2003).

Another factor of importance is related to the chemical quality of the plant, mainly due to the concentrations of soluble carbohydrates through which these organic acids are produced during the initial fermentation process, which contribute to a reduction of the pH values of silage (Santos *et al*., 2010).

Regarding the fermentation characteristics, the maize crop can be considered ideal because it presents high concentrations of soluble carbohydrates and low buffering power (Bernardes *et al*., 2012). However, Santos *et al*. (2013) indicate that the composition of a microbiological community of silage directly affects its fermentation standard and its aerobic stability. Liu *et al*. (2012) stated that different maize genotypes have different microorganisms coexisting in plants and that the nutritional composition is mainly responsible for this distinction.

Diver studies have been conducted to compare the potential of different maize genotypes (Emygdio *et al*., 2010), but these studies are still scarce in the field of silage production. Therefore, this study compares different hybrid maize genotypes through aerobic stability assessments.

MATERIALS AND METHODS

The experiment was conducted at the Animal Production Center (NUPRAN) of the Agrarian and Environmental Sciences Sector of the State University of the Middle-West (UNICENTRO, Guarapuava – PR), in partnership with the State University of Londrina (UEL, Londrina – PR).

The hybrids Maximus VIP3, Defender VIP and Feroz VIP (Syngenta®) were single, triple and double hybrids, respectively, and were implanted with a density of 65,000 plants ha^{-1} under the recommendations of soil analysis (CQFS RS/SC, 2004). The harvesting of silage occurred when the plants reached approximately 35% dry matter. Subsequently, the silage was ensiled in experimental PVC tubes that were 20 cm in diameter and 40 cm in height and then were compressed to obtain compaction of 600 kg fresh matter m^{-3}.

The silos were stored in a covered area for a period of 60 days, and after opening, a 10-cm layer was discarded. A 500-g sample of each replicate was collected, which were weighed and pre-dried in a forced air oven at 55°C until a constant weight for determination of the dry matter (DM), according to AOAC (1995). Afterwards, the samples were milled in a "Wiley" type mill with a 1-mm mesh sieve.

The total dry matter was determined in an oven at 105°C, the crude protein (CP) by the micro Kjeldahl method, mineral matter (MM) and ethereal extract (EE), according to AOAC (1995). The neutral detergent fiber (NDF) contents were determined using thermostable α-amylase (Termamyl 120 L, Novozymes Latin America Ltda.) according to Van Soest *et al*. (1991), and the acid detergent fiber (ADF) contents were determined according to Goering and Van Soest (1970). The non-fibrous carbohydrates were obtained through the equation proposed by Sniffen *et al*. (1992). The soluble sugar contents were determined according to Hall (2000), and the organic acids were determined according to Silva and Queiroz (2009). The determination of the starch content was performed according to the methodology described by Walter *et al*. (2005). The total digestible nutrient contents (NDT) were obtained from the equation of Bolsen *et al*. (1992). The determination of ammoniacal nitrogen ($NH\text{-}_3N$) was obtained by using the methodology described by Bolsen *et al*. (1992).

Immediately after opening the silos, one kilo of samples were deposited in polypropylene containers lined with plastic bags and transferred to a climatic chamber at a temperature of 25 ± 2°C. Silage temperatures were measured three times a day for seven days, with a thermometer inserted at 10 cm in the center of the mass. The loss of aerobic stability was defined as the time required for the silage to show an elevation of 2°C relative to the ambient temperature (Taylor and Kung Jr., 2002). As a physical measure, the aerobic dry matter loss was evaluated at the end of the seven days.

Another group of containers with one kilo of silage each was transferred to the climatic chamber with the objective to determine the pH changes according to Silva and Queiroz (2009), with measurements taken every eight hours.

The experimental design was completely randomized, with three replications. The data were submitted to the Shapiro-Wilk and Bartlett tests in order to verify the assumptions of normality and the homogeneity of variance, respectively. The F test was applied to a 5% probability of confidence using an analysis of variance (ANOVA) and then the Tukey test of the comparison of multiples means at 5% of significance. For the pH data of the aerobic stability, a polynomial regression analysis was also performed through the "proc reg" procedure of the statistical SAS program (v. 9.2; SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

The methodology used in selecting a favorable harvesting time for dry matter contents was similar between the treatments, according to Rabelo *et al*. (2014), which is ideal, because there is no interference of the different osmotic pressures. From this, it was observed that the silages stabilized their fermentation process with very close pH values.

Table 1 shows the values of chemical composition and the fermentation products of silages with different maize hybrids. In general, the data presented values that were similar to those described by Tres *et al*. (2014b), which highlighted the high productive potential of simple hybrids in comparison to double and triple hybrids, but without significant differences in chemical composition. Emygdio *et al*. (2010) emphasized the trend of superiority in the grain production of the simple hybrids, which may explain the starch values found in the present study ($P = 0.0620$), with 37.27% for the Maximus VIP3 hybrid, compared to 34.33% and 34.09% for the Defender VIP and Feroz VIP hybrids, respectively.

In the chemical composition, only fraction A of non-fibrous carbohydrates, which are composed of soluble sugars and organic acids, presented a difference between the silages ($P < 0.05$). After the silo fence, the lactic acid bacteria preferentially consume glucose and fructose, converting them to organic acids; with higher concentrations of these sugars, there is a greater the tendency to produce lactic and acetic acid (Bernardes *et al*., 2012). This affirmation is confirmed by the fact that the Maximus VIP3 hybrid had the highest value of soluble sugars and, consequently, a higher value of organic acids (4.55% and 6.25%, respectively).

The Maximus VIP3 hybrid was superior ($P < 0.05$) in relation to the others for acetic acid production; however, it did not differ statistically from the Defender VIP hybrid in lactic acid concentration, which, according to Liu *et al*. (2012), may be due to the profiles of epiphytic microbiota for each material. Nishino *et al*. (2012) corroborate that the microbiological community standard of silage also provides different ratios of acids. It is worth adding that the value of acetic acid that was obtained for this hybrid resembles the findings by Szucs *et al*. (2012) in silages inoculated with *Lactobacillus buchneri* ($3x10^5$ cfu g FM^{-1}), and other treatments that were approximate to those found by the same authors in their control treatment.

The ratio of lactic acid: acetic acid was 0.94, 1.77 and 1.52 for Maximus VIP3, Defender VIP and

Table 1 - Effect of maize hybrid on chemical composition of silage and fermentative quality

		Maximus VIP3	Defender VIP	Feroz VIP	P
		Chemical composition, % DM			
Dry matter, % NM		34,30	34,07	34,27	0,8453
Ash, % DM		3,01	2,51	2,58	0,3651
Crude protein, % DM		9,14	8,75	8,98	0,2696
Lipids, % DM		3,44	2,90	3,12	0,1985
NDF, % DM		43,71	49,07	45,54	0,1020
ADF, % DM		26,54	29,23	25,85	0,1651
Lignin, % DM		4,97	5,12	4,94	0,3696
Total Carbohydrates, % DM		81,66	85,21	84,54	0,2985
Non-fibrous carbohydrates, % DM		46,03	41,96	40,03	0,0965
Fraction A	Soluble sugars	4,55[a]	4,05[b]	3,93[b]	0,0321
	Organic acids	6,25[a]	5,06[b]	4,61[b]	0,0229
Fraction B1	Soluble fiber	3,67	4,97	5,16	0,1665
	Starch	37,27	34,33	34,09	0,0620
Fraction B2	Hemicellulose	17,17	19,84	19,69	0,8527
	Cellulose	21,57	24,10	20,91	0,4693
Fraction C		11,93	12,29	11,86	0,1576
pH, index		3,90	3,97	3,96	0,8466
Lactic acid, % DM		3,03[a]	3,23[a]	2,80[b]	0,0220
Acetic Acid, % DM		3,22[a]	1,83[b]	1,84[b]	<0,0001
Acetic acid:lactic acid ratio		1,06[a]	0,57[b]	0,66[b]	<0,0001
Butyric acid, % DM		ND	ND	0,01	0,8577
NH_3-N, % TN		2,29	2,10	2,17	0,3572

Averages, followed by different lowercase letters in the line, differ from each other by the Tukey Test at 5%. NM: Natural matter; DM: Dry matter; TN: Total nitrogen; ND: Not Detected

Feroz VIP silages, respectively (Table 1). These results suggest that not only higher concentrations of acetic acid, but lower lactic acid: acetic ratios may be related to the higher aerobic stability of silage (Table 2; Figure 1).

Table 2 summarizes the parameters related to the aerobic stability of maize silages. Significantly longer time was required for the Maximus VIP3 silage temperature to increase by 2°C (125.3 hours), which is a value similar to that described by Szucs *et al*. (2012). The other silages did not differ significantly among themselves (53.3 and 45.3 hours for Defender VIP and Feroz VIP).

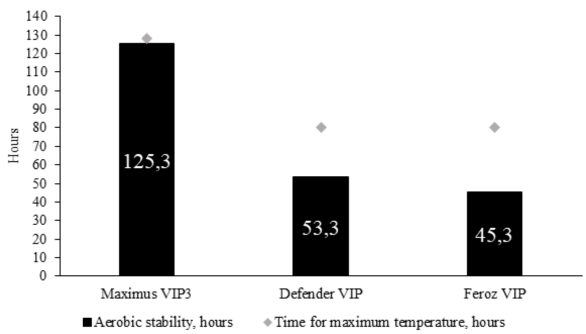

Figura 1 - Aerobic stability of different corn hybrids silage.

Table 2 - Values related to the aerobic stability of silage of different maize hybrids

	Hybrid			P
	Maximus VIP3	Defender VIP	Feroz VIP	
Aerobic stability, hours	125,3[a]	53,3[b]	45,3[b]	<0,0001
Time for maximum temperature, hours	50,7	88,0	80,0	0,7229
pH after stability test	5,1[b]	7,0[a]	7,1[a]	0,0034
Dry matter loss in aerobiosis,%	13,98	14,81	15,66	0,0923

Averages, followed by different lowercase letters in the line, differ from each other by the Tukey Test at 5%.

The long aerobic stabilities highlighted here might not occur in field conditions due to unavoidable environmental contaminations; tests performed under laboratory conditions may not provide accurate aerobic stability values in practice, but it is an appropriate procedure for comparing different treatments in a controlled environment.

The pH values after aerobic stability test, which lasted 152 hours, were different ($P < 0.05$), and the silages with a loss of faster stability ended up with the highest pH values (7.0 and 7.1 to Defend VIP and Feroz VIP). Similar values were described by Rabelo *et al*. (2012). Balieiro Neto *et al*. (2009) already verified higher pH values in sugarcane silage, approximately 8.3 after 152 hours of exposure to oxygen, probably due to the different characteristics of the materials studied by the authors.

These results allow us to affirm that silages with good aerobic stability do not necessarily result from silages that present a more adequate fermentation profile. According to criteria suggested by Santos *et al*. (2010), the Maximus VIP hybrid silage was classified as good (85), while the Defender VIP and Feroz VIP hybrids silages were excellent (90), because the authors took into account only the maleic effects of acetic acid, such as their correlation with dry matter and energy losses.

Figure 2 shows the behavior of the pH values of silages during the aerobic stability test. Maximus VIP3 silage, which had the highest aerobic stability, showed a lower increase in pH values with an hourly advance (0.0009 pH points $hour^{-1}$), while the silages of Defender VIP and Feroz VIP hybrids added 0.0249 and 0.0263 pH points $hour^{-1}$, respectively.

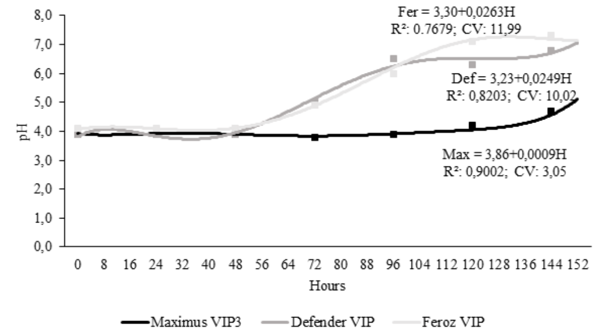

Figure 2 - Behavior of pH values during the aerobic stability test.

It is possible to observe that the pH curves, for all the silages, beginning with their advances being near to those that registered a loss stability for temperature. Similar behavior can be observed in the study of Silva *et al*. (2016), with high moisture grain silage indicating that a pH should be considered as a complement to the tests on the aerobic stability of the temperature increase.

CONCLUSION

The variation of the maize hybrid and the genotype can affect the chemical composition of silage, the fermentation profile and the aerobic stability. Among the evaluated materials, the Maximus VIP hybrid is recommended because of its quality and high aerobic stability.

ACKNOWLEDGMENTS

To the Coordination of Improvement of Higher Education Personnel (CAPES).

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