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Nutritional composition and aerobic stability of winter cereal silage at different storage times

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ABSTRACT. The objective of the experiment was to evaluate the nutritional composition, dry matter (DM) recovery and aerobic stability of winter cereal silages under different storage periods. The materials used were wheat (*Triticum aestivum* cv. BRS Gralha Azul), barley (*Hordeum vulgare* cv. BRS Brau), white oat (*Avena sativa* cv. URS Guará), black oat (*Avena strigosa* cv. Embrapa 139) and triticale (*X Triticosecale* IPR 11), in three storage periods: 60, 120 and 180 days. The experiment was conducted in a 5x3 factorial, completely randomized design, consisting of five forage species and three storage periods, with five replicates. In nutritional assessment, barley silage presented low values of acid detergent fiber (ADF; 331.2, 355.2 and 378.5 g kg DM^{-1} for 60, 120, 180 days, respectively), high total digestible nutrients (TDN; 558.2, 544.7 and 531.6 g kg DM^{-1} for 60, 120, 180 days, respectively), high DM recovery and aerobic stability. Wheat and triticale showed a decrease in DM recovery with the increase in storage length, although showed high aerobic stability. The storage period had a different effect on forages; however, storage period above 60 days provided no benefits for the variables evaluated.

Keywords: storage, aerobic deterioration, DM recovery.

Composição nutricional e estabilidade aeróbia de silagens de cereais de inverno com diferentes tempos de estocagem

RESUMO. O objetivo do experimento foi avaliar a composição nutricional, a recuperação de matéria seca (MS) e a estabilidade aeróbia de silagens de cereais de inverno submetidas a diferentes tempos de estocagem. Os materiais utilizados foram o trigo (*Triticum aestivum* cv. BRS Gralha Azul), cevada (*Hordeum vulgare* cv. BRS Brau), aveia branca (*Avena sativa* cv. URS Guará), aveia preta (*Avena strigosa* cv. Embrapa 139) e triticale (*X Triticosecale* cv. IPR 11), em três tempos de estocagem: 60, 120 e 180 dias. O delineamento experimental foi inteiramente casualizado em arranjo fatorial 5x3. Na avaliação nutricional, a silagem de cevada apresentou baixos teores de fibra em detergente ácido (FDA; 331; 355 e 378 g kg MS^{-1}, para 60, 120 e 180 dias, respectivamente) e altos de nutrientes digestíveis totais (NDT; 558; 544 e 531 g kg MS^{-1} para 60, 120 e 180 dias, respectivamente), além de alta recuperação de MS e estabilidade aeróbia. O trigo e o triticale apresentaram decréscimo na recuperação de MS com o aumento dos dias de estocagem, embora tenham apresentado alta estabilidade aeróbia. O tempo de estocagem influenciou de maneira distinta as forrageiras, no entanto, tempos de estocagem acima de 60 dias não proporcionaram benefícios para as variáveis avaliadas.

Palavras-chave: armazenamento, deterioração aeróbia, recuperação de MS.

Introduction

As any fermentation process, ensiling is a set of biochemical reactions, including oxireductions which are intrinsically mediated by enzymes and/or other metabolic products of microorganisms (Jobim, Nussio, Reis, & Schmidt, 2007). In this sense, the basic assumption of this fermentation is the use of carbohydrates and later formation of organic acids by microorganisms like lactic acid bacteria, thus causing a rapid drop in pH, which consequently leads to conservation of the material by means of inhibition of spoilage microorganisms, for instance, yeast and enterobacteria (Muck, 2010).

In this way, the fermentative stability of ensiling begins after the ensiled mass reaches the pH required to cause a reduction in microbiological activity (Muck, 2010). However, there is evidence of alterations in the nutritional quality of the food

during conservation, since there is also the presence of pH-tolerant enzymes that can perform partial hydrolysis of fiber particles, contributing to the pool of soluble carbohydrates in the forage (Der Bedrosian, Nestor Jr., & Kung Jr., 2012). Thus, the period of storage, in turn, with respect to the quality of silages, assumes great relevance.

Biochemically, during the storage period it is prudent to state that the main alterations highlighted in the literature are the hydrolysis of prolamins and hemicellulose. Prolamins, a protein matrix that covers starch granules, can be solubilized by organic acids present in the silage, increasing the exposure and consequently the digestibility of the starch (Huntington, 1997). The possible hydrolysis of the hemicellulose fraction, in turn, increases the carbohydrate pool in the material and can generate gains in the food value of silages (Weinberg & Chen, 2013).

Nevertheless, the focus of research on this topic has been mainly on corn (Der Bedrosian et al., 2012, Young, Lim, Der Bedrosian, & Kung Jr., 2012), and for winter forages, there is a lack of researches reported in literature, especially in the evaluation of winter crops for this purpose under the Brazilian conditions. It is worth noting that these cereals are of great importance for their nutritional value, digestibility and ease of cultivation in the southern region of the country, which allow to be an alternative for periods of forage shortage (Fontaneli et al., 2009, Lehmen, Fontaneli, Fontaneli, & Santos, 2014).

Given the above, the goal of this work was to evaluate the silages of five winter cereals subjected to different storage periods and their effects on nutritional quality, dry matter losses and aerobic stability.

Material and methods

The experiment was conducted at the Animal Production Center (Nupran) belonging to the Sector of Agrarian and Environmental Sciences of the Midwest State University (Unicentro), in the municipality of Guarapuava, state of Paraná, located in subtropical area, at the geographical coordinates 25º 23' 02" South latitude, 51º 29' 43" West longitude and 1,026 m altitude.

The climate of the region, according to the classification of Köppen, is Cfb (subtropical mesothermal humid), with mild summers and moderate winter, with no defined dry season and with severe frosts. The average annual rainfall is 1,944 mm, mean annual minimum temperature is 12.7°C, mean annual maximum temperature is 23.5°C and relative humidity is 77.9%.

The soil of the experimental area is classified as Latossolo Bruno Típico (Pott, Müller, & Bertelli, 2007) and before planting, it presented the following chemical characteristics (0-20 cm layer): pH 4.7; 0.01M $CaCl_2$: 4.7; P: 1.1 mg dm^{-3}; K^+: 0.2 $cmol_c$ dm^{-3}; OM: 2.62%; Al^{3+}: 0.0 $cmol_c$ dm^{-3}; $H^+ + Al^{3+}$: 5.2 $cmol_c$ dm^{-3}; Ca^{2+}: 5.0 $cmol_c$ dm^{-3}; Mg^{2+}: 5.0 $cmol_c$ dm^{-3} and base saturation: 67.3%.

Experimental material was wheat (*Triticum aestivum* cv. BRS Gralha Azul), barley (*Hordeum vulgare* cv. BRS Brau), white oat (*Avena sativa* cv. URS Guará), black oat (*Avena strigosa* cv. Embrapa 139) and triticale (*X Triticosecale* cv. IPR 11). The experimental field consisted of a total area of 225 m^2, distributed in five plots of 45 m^2 for each cereal.

Cereals were sown on June 3rd, 2014, in a no-tillage system using Semeato SHM 15/17 planter, uniformly (same density) for all crops. The row spacing was 0.17 m, with a sowing depth of 0.04 m, with a distribution of 400 seeds per m^2.

Upon planting, the basal fertilization was performed with 300 kg ha^{-1} of the formulate fertilizer 08-30-20 (N N-P_2O_5-K_2O), respecting the recommendations of the soil fertility commission of Santa Catarina and Rio Grande do Sul. Topdressing nitrogen fertilization was done at once, 30 days after planting, with 196 kg ha^{-1} urea (46-00-00), which totaled 90 kg ha^{-1} nitrogen. The silages, for each crop, were produced when the plants reached the stage of soft dough grain, indicated for ensiling, according to Fontaneli et al. (2009). In this context, wheat, triticale and barley silages were produced at 115 days after planting (September 27th, 2014) and silage of white oat and black oat at 121 days after planting (October 4th, 2014).

At the period of ensiling, plants of each plot were harvested at 8 cm from the ground, according to Fontaneli et al. (2009), and later the materials were processed in stationary forage chopper (Nogueira® EM 6400) to an average particle size of 3.7 cm, according to the methodology proposed by Jobim, Nussio, Reis, and Schmidt (2007).

After this process, samples of about 1.0 kg of each cereal were vacuum ensiled in plastic bags (mini bags) with welds (nylon poly, 150 microns, 25 cm wide x 35 cm long) using vacuum packer (TM-280 Tecmaq) for the removal of oxygen and adequate sealing of the experimental silos. The treatments consisted of five cereals and different storage periods (storage for 60, 120 and 180 days). The experimental design was completely randomized, in a 5x3 factorial arrangement of five forage species and three storage periods, with five replications each, totaling 75 experimental silos. Each silo represented an experimental unit.

After ensiling, the silos were stored in a roofed area. After opening the silos, a 200 g sample of each treatment/replication was oven-dried for the determination of DM and then sent to the chemical analysis.

In the evaluation of losses in silage according to the storage period, the dry matter recovery index (DMRI) was calculated through the methodology proposed by Jobim et al. (2007), with the Equation 1:

$$DMRI = (FMO \star DMO) / (FMS \star DMS) \quad (1)$$

where:
FMO: Forage mass at opening;
DMO: Dry matter at opening;
FMS: Forage mass in silage;
DMS: Dry matter in silage.

The evaluations of aerobic stability were obtained by means of temperature and pH measurements, which started after opening the silos. In each silo, the silage was decompressed to facilitate exposure of the ensiled material to air, as described by Kung Jr., Rosinloon, and Ranjit (2000), and a sample of approximately 400 g of the material was placed in buckets with a capacity of 4.0 kg. The experimental period for aerobic stability lasted 168 hours (7 days after opening the silos).

The buckets were placed in an environment with temperature control, at 25°C, throughout the period. To determine the aerobic stability of the silages, the temperature of the silages was read directly in the buckets, using a long rod digital thermometer Gulterm 1001 inserted at the center of the forage mass. The temperature and pH readings were taken daily at 6, 12 and 18 hours. The pH readings were made using a digital potentiometer, according to Jobim et al. (2007).

Aerobic stability break was considered when the temperature of the ensiled material exceeded the temperature of the environment by 2ºC, as recommended by Taylor and Kung Jr. (2002), or when the pH increased above 0.5 units in up to five days, as mentioned by Weinberg, Chen, and Solomon (2008).

Pre-dried samples of the winter cereal silages were ground at 1 mm in a Wiley mill, for subsequent determination of the total dry matter in an oven at 105°C for 12 hours, crude protein (CP) by the micro Kjeldahl method and mineral matter (MM) by incineration at 550ºC (4 hours). Neutral detergent fiber (NDF) content was determined according to Van Soest, Roberttson, and Lewis (1991), using thermostable α-amylase (Termamyl 120 L, Novozymes Latin America Ltda.), acid detergent fiber (ADF) and hemicellulose contents were determined by difference (Hemicellulose = NDF - ADF).

The total digestible nutrient (TDN) content in forage was estimated according to the Equation 2:

$$TDN = 74.49 - 0.5635 \star ADF \ (r^2 = 0.84) \quad (2)$$

Described by Cappelle, Valadares Filho, Silva, and Cecon (2001) for forages.

The results were tested by analysis of variance and compared using the Tukey's test at 5% level of significance, using SAS statistical software.

Results and discussion

In the evaluation of the nutritional composition of the silages (Table 1), it was observed that the barley presented the lowest content of ADF among the cereals evaluated and, consequently, the highest estimates of TDN. In contrast, black oat showed high values of NDF, ADF and MM, and low content of TDN.

Table 1. Nutritional composition of winter cereal silages ensiled at the soft dough stage and subjected to different storage periods.

Winter cereal	Storage period			
	60 days	120 days	180 days	Mean
	Neutral detergent fiber (g kg DM^{-1})			
Wheat	666^{ab}	663^{bc}	666^{bc}	665^{B}
Triticale	670^{ab}	674^{b}	707^{b}	683^{B}
Barley	614^{b}	627^{c}	642^{c}	628^{C}
White oat	680^{abB}	660^{bcB}	711^{bA}	683^{B}
Black oat	709^{aB}	785^{aA}	799^{aA}	764^{A}
	Acid detergent fiber (g kg DM^{-1})			
Wheat	420^{a}	434^{b}	419^{bc}	422^{B}
Triticale	416^{a}	438^{b}	446^{b}	433^{B}
Barley	331^{b}	355^{c}	378^{c}	354^{C}
White oat	424^{a}	417^{b}	459^{b}	433^{B}
Black oat	443^{aB}	507^{aA}	520^{aA}	490^{A}
	Hemicellulose (g kg DM^{-1})			
Wheat	246	228^{b}	246^{c}	240^{C}
Triticale	254^{AB}	236^{bB}	261^{abcA}	250^{B}
Barley	283	271^{a}	264^{ab}	272^{A}
White oat	255	243^{b}	252^{bc}	250^{B}
Black oat	265	277^{a}	279^{a}	273^{A}
	Crude protein (g kg DM^{-1})			
Wheat	55^{bC}	70^{B}	95^{abA}	73^{B}
Triticale	54^{b}	59	74^{bc}	62^{C}
Barley	87^{a}	86	99^{a}	90^{A}
White oat	54^{b}	78	66^{c}	66^{B}
Black oat	43^{bB}	60^{A}	58^{cA}	53^{C}
	Mineral matter (g kg DM^{-1})			
Wheat	41^{cB}	48^{bcA}	45^{bAB}	44^{D}
Triticale	44^{c}	45^{c}	52^{ab}	47^{CD}
Barley	50^{b}	53^{ab}	48^{ab}	50^{BC}
White oat	54^{ab}	53^{ab}	50^{ab}	52^{B}
Black oat	58^{a}	57^{a}	55^{a}	56^{A}
	Total digestible nutrients (g kg DM^{-1})			
Wheat	508^{b}	499^{b}	508^{ab}	505^{B}
Triticale	510^{b}	497^{b}	493^{b}	500^{B}
Barley	558^{a}	544^{a}	531^{a}	544^{A}
White oat	505^{b}	509^{b}	485^{b}	499^{B}
Black oat	494^{bA}	458^{cB}	451^{cB}	467^{C}

Mean values followed by different lowercase letters, in the same column, and uppercase letters, in the same row, are significantly different by Tukey's test at 5%.

Lehmen, Fontaneli, Fontaneli, and Santos (2014) analyzed two distinct black oat genotypes and obtained a mean NDF of 74.5%, which is 4.9% higher than our results for the storage period of 60 days and 5.1 and 6.8% lower than those demonstrated for the storage period of 120 and 180 days, respectively. Meinerz et al. (2011), in turn, examined three black oat genotypes and reported a mean NDF of 69.6%, which is lower than the results of the present study, regardless of the storage period.

In general, all cereals evaluated showed NDF content above 600 g kg^{-1} DM. High concentrations of NDF are undesirable because it may impact gastrointestinal fullness and may limit the intake of ruminant animals, which may limit production potential (Mertens, 1997). In addition, the decrease of soluble carbohydrates results in a decrease in NDT content.

In relation to ADF content, it can be observed that barley silage presented the lowest values, regardless of the storage period. This is explained by the higher proportion of grains in the forage mass. Also, according to Huuskonen (2013), the low content of lignified material provides barley with a high digestion potential.

Hemicellulose, on the other hand, among the components of NDF, is the one with the highest digestion potential (Mason & Stuckey, 2016). Besides that, this fraction has an important role in the fermentation profile, since there is evidence of solubilization during the process, increasing the soluble carbohydrate pool (Muck, 2010), which would increase the nutritional value of the material stored for a longer period. For corn silage, Der Bedrosian, Nestor Jr., and Kung Jr. (2012) observed partial solubilization of hemicellulose, generating an increase in nutritional value. However, in the present study this effect was not observed for cereals.

With respect to the protein content, barley silage presented higher content than other winter cereals, regardless of the storage period. Compared with data available in the literature, the results found for this cereal are lower than the 101 g kg^{-1} DM found by Huuskonen (2013) and higher than the 74 g kg^{-1} DM reported by Meinerz et al. (2011). Lehmen et al. (2014) verified a value very close to the findings of the present study (84 g kg^{-1} DM). The great variation in CP contents of barley silages can be explained by the genetic material used, soil and climatic conditions and management factors.

With the increase in storage period, there was an increase in protein content for wheat and black oat. According to Der Bedrosian et al. (2012), this increase could be a consequence of the greater amount of soluble nitrogen, resulting from the intense proteolysis that occurred during the fermentation period. In agreement with these authors, even under low pH conditions during storage, some proteolytic enzymes of the microorganisms of the ensiled mass are still active, degrading the material, especially the prolamins coating the grains, and increasing the amount of ammonia nitrogen ($N\text{-}NH_3$). Young, Lim, Der Bedrosian, and Kung Jr. (2012) also registered this same tendency in corn silages stored for long periods.

However, the amount of prolamins is lower in winter cereals than in corn. In this way, the most acceptable justification for the increase in protein content would be the concentration of this nutrient, with a view to lower DM recovery.

Importantly, the crude protein content of a food is related to the buffering capacity of the ensiled material, which can be understood as a resistance of the forage mass to the lowering of pH inside the silo (Muck, 2010). This fact could reflect a larger proliferation of microorganisms, which would substantially increase DM losses.

The DM recovery index of the silages was not different between winter cereals for storage during 60 days (Table 2). However, at 120 and 180 days of storage, there was a difference ($p < 0.05$), in which barley (92.2 and 90.2%) showed the highest indices, and black oat (74.7 and 77.2%) showed the lowest values, respectively, although it was not different from triticale and white oat.

Table 2. Dry matter recovery index of winter cereal silages ensiled at the soft dough stage and subjected to different storage periods.

Treatments	DM recovery (%)			
	60 days	120 days	180 days	Mean
Wheat	89.8^{A}	80.4^{abB}	78.1^{bB}	82.8^{B}
Triticale	83.6^{A}	83.9^{abA}	80.9^{abB}	82.8^{B}
Barley	92.5	92.2^{a}	90.2^{a}	91.6^{A}
White oat	82.0	85.7^{ab}	80.1^{ab}	82.6^{B}
Black oat	82.3	74.7^{b}	77.2^{b}	78.0^{C}

Mean values followed by different lowercase letters, in the same column, and uppercase letters, in the same row, are significantly different by Tukey's test at 5%.

Wheat and triticale showed a decrease in DM recovery with the increase in storage days. Meanperiod, for these cereals, high aerobic stability was also observed (Figure 1). The justification could be precisely in the epiphytic flora of these cereals. In wheat, for example, Li, Wang, Cai, and Pang (2015) detected a high number of heterofermentative microorganisms, contributing with 66.7% of the total lactic acid bacteria; these microorganisms have a distinct fermentation pattern, leading to the

formation of different compounds, such as acetic acid and 1,2-propanediol, which result in higher losses of DM compared to the homolactic pathway, which could justify the greatest losses found (Muck, 2010).

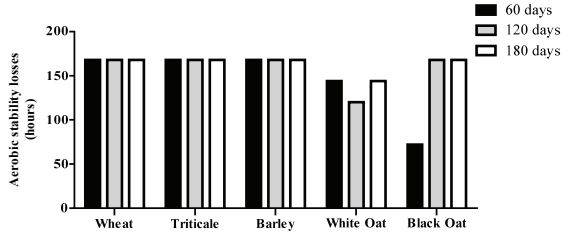

Figure 1. Period (hours) required for loss of aerobic stability of winter grain silages subjected to different storage periods.

Kleinschmit and Kung Jr. (2006) argued that these microorganisms, even under low pH, can remain active, performing the previously mentioned biochemical cycle, contributing to the losses of DM. Triticale can also have the same justification, since this material has its genetic matrix originated from wheat (McGoverin et al., 2011).

Further, the epiphytic flora composed of hetero fermentative microorganisms implies a higher production of acetic acid and 1,2-propanediol, which is later transformed into propionic acid (Muck, 2010). The higher stability of wheat and triticale silages (Figure 1), in this way, may be associated with a possible higher concentration of organic acids, such as acetic and propionic, although not determined in the present study. These compounds have an antifungal effect, acting on yeast, the main spoilage microorganisms of the silage, thus increasing aerobic stability (Muck, 2010).

It is worth mentioning that barley also presented high aerobic stability, associated with a high recovery of DM.

Conclusion

The storage period had a different influence on forages, however storage periods over 60 days did not provide benefits for the evaluated variables.

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