Modification of lipid fraction in ensiled high moisture corn

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Abstract

The aim of this work is to study the changes of the lipidic fraction of ensiled high moisture corn (HMC). 11 maize hybrids were used, ensiled each in 3 mini experimental silos of 100 litres. For each hybrid 1 sample of fresh high moisture corn was obtained immediately after milling and 3 samples of ensiling HMC were kept after 2, 7 and 12 months. All samples were analysed for pH, dry matter, lactic acid, ammonia-N, ether extract, fatty acid composition and volatile fatty acids (VFAs). Ether extract of fresh high moisture corn was 35.7 g/kg dry matter (DM) and increased after 2 and 7 months of storage up to 39.4 g/kg DM (P≤0.01); after 12 months it decreased to 38.1 g/kg DM (P≤0.01). Both saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) percentages decreased from 18.23% to 15.47% and from 24.84% to 23.57% respectively (before ensiling vs 12 months P≤0.01). Linoleic acid percentage increased from 55.34% to 59.44% (before ensiling vs 12 months P≤0.01). The linoleic acid content (g/kg of DM) increased on average from 19.1 g/kg DM to 22.5 after 12 months of ensiling. These differences may affect the linoleic acid content of heavy pig diets when maize is used as HMC instead of corn meal.

Introduction

In the north of Italy farmers use stored high moisture corn (HMC) more frequently for swine feeding. The benefits of this feed include a reduction in costs due to not having to dry the maize, less loss of product during harvest (usually brought forward by 15 days) and good corporate management and mechanization of the production (Wood, 1981). Engelke et al. (1984) suggest that HMC stored under anaerobic condition has equal feeding value to dried corn for growing-finishings swine when compared on equal dry matter basis. Some studies have investigated changes in HMC during ensiling. The modifications induced by the ensilage of corn were studied regarding carbohydrates and proteins. The prolonged storage of HMC is possible thanks to anaerobic microbial fermentation that lowers the pH during ensiling. This reduction in pH is primarily due to the fermentation of water-soluble carbohydrates with production of lactic acid and acetic acid (Engelke et al., 1984). Dure (1960) and Abrams et al. (1976) suggested that, because of a partial germination process that occurs during ensiling, there is an increase in protein solubility; Niven et al. (2007) pointed out that stored HMC contains more soluble phosphorus than fresh HMC and Young et al. (1977) found that the level of alfa tocopherol in HMC decreases during storage. Little is known about the changes in the lipidic fraction of HMC.

In Italy, the production of the Protected Designation of Origin (PDO) ham (e.g., Parma Ham and San Daniele Ham), the most important product derived from heavy pig carcass, is headed by Regulations (Prosciutto di Parma; Protected Designation of Origin) (European Commission, 1992) that govern every production step. In order to avoid soft and oily ham subcutaneous fat, which may turn rancid during the 12-18 month curing process, hams whose subcutaneous fat shows a high unsaturation level (more than 15% linoleic acid on total fatty acids and iodine value higher than 70) are not suitable for PDO raw ham production. Therefore, the regulations severely restrict the use of added fats and high-fat feedstuff in finishing heavy pig diet, stating that the linoleic acid content must not exceed 2% of the diet (on dry matter basis). Due to the fact that the heavy pig is traditionally fed diets based on cereals, among which maize is the most important, this tradition has been codified by the Regulations (European Commission, 1992). In diets for pigs from 80 kg liveweight to slaughtering, cereals must represent at least 55% of DM while maize or high moisture maize (HMC) can represent up to 55% of DM. Therefore in formulating a diet for heavy pigs, it is crucial to know the linoleic acid content of the maize used, because differences of only 0.3% (linoleic acid on dietary DM) can lead to significant changes in fatty acids composition of deposited fats (Della Casa et al., 2010). The fat content and the fatty acid profile of different maize genotypes show wide differences (Dunlap et al., 1995a, 1995b; Cheesbrough et al., 1997). Corn varieties with high percentages of oleic acid and low linoleic acid have been selected as suitable for heavy pig feeding (Della Casa et al., 2010).

Hence, the purpose of this study is to analyse changes in lipid fraction during ensiling of different maize hybrids to determine whether any benefits gained due to genetic improvement of maize might be lost, or whether the contrary silage causes such a reduction in linoleic acid content that it is less necessary to choose hybrids low in linoleic acid.

Materials and methods

Maize samples and silage preparation

We used 11 different maize hybrids to study changes in lipid composition: Corona, DK6309, Duende, Eleonora, Hellen, Kalibo, Kermess, Lolita, PR33A46, PR33L24 and Sancia. These hybrids were chosen because they have a wide range of oil and linoleic acid content. All hybrids were grown in the same place (Sant’Angelo Lodigiano, LO, Italy), harvested separately at about 29% moisture, and ground by the same mill to a homogeneous mixture. Immediately after grinding the high moisture corn (HMC) was wrapped in a plastic bag and compacted as much as possible in 100 L bins of

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Key words: Ensiled high moisture corn; Lipid fraction; Linoleic acid; Heavy pig; Fat quality.

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PVC (poly-vinyl chloride). There were 33 bins, three replicates for each maize hybrid. Bins were stored in the dark at ambient temperature in a closed room to avoid sudden changes in temperature or possible light effects. Eleven samples of fresh high moisture corn were obtained immediately after grinding and a sample from each bin of stored HMC was taken out after 2, 7 and 12 months of storage. These sampling times were chosen because usually the start of silage use occurs after 2 months from ensiling, the end of the use is after 12 months. Seven months was chosen as intermediate time.

Chemical and fatty acids analyses

Chemical analyses were performed in duplicate and are presented on a DM basis. All samples were subjected to dry matter, pH, lactic acid, ammonia-N, ether extract and fatty acid composition analysis. Ensiled HMC samples were also subjected to volatile fatty acids (VFA) analysis. DM of the samples was determined at 60°C in a forced-air oven for 48 h. Ashes of the oven dried samples were obtained after 3 h at 550°C. The ether extract (991.36), crude fibre (978.10), crude protein (984.13), ammonia-N (941.04) and pH (943.02) of the HMC were analysed according to AOAC (1995). Silage juice was extracted by blending 20 g of silage in 200 mL of distilled water for 30 s and filtered through Whatman 54 filter paper (Clifton, NJ, USA). The water extract was used to measure fermentation acids. Volatile fatty acids (acetic, propionic, butyric and iso-butyric acids) and lactic acid concentrations were determined using a 8000 Top Fisons gas chromatograph (Thermo Electron Corporation) equipped with a Supelco 80/120 Carbopack B-DA 4% Carbowax 20M column and flame ionization detector as described by Abes et al. (2011). The gas chromatograph oven was programmed as follows: 140°C for 1 min, 10°C increase/min to 175°C and a final holding time of 26 min; the injector and detector temperature was 250°C. Chrom-Card for Trace software for Windows (ver. 2.3, Thermo Electron Corporation) was used for data analysis.

The fat for fatty acids analysis was extracted with the chloroform/methanol method of Folch et al. (1957). The preparation of methyl esters was performed following the method of Stoffel et al. (1959); 100 mg of fat were dissolved in methanol/HCl (5 mL) and placed at 100°C for 40 min and 2 mL of water and 2 mL of hexane were added after cooling. The organic phase was filtered through anhydrous sodium sulphate and injected into a Trace Ultra gas chromatograph (Thermo Electron Corporation) equipped with a flame ionisation detector and an automatic injection system (S 2000 Thermo Electron Corporation). The column was a SP 2380 fused-silica capillary column (30 m length, 0.25 mm i.d. 0.20 μm film thickness). The initial column temperature was 160°C raised to 250°C in 20 min; the injector and detector temperature was 250°C. The Helium carrier gas pressure was 70 kPa and 1 μL of sample solution was injected (split ratio 1:40).

Chrome-Card for Trace software for Windows (ver. 2.3, Thermo Electron Corporation) was used for data analysis. Identification was accomplished by comparing the retention time of unknown fatty acids methyl esters (FAME) with those of known FAME standard mixtures (Supelco, Inc.; Alltech Associated, Inc., Deerfield, IL, USA).

Statistical analysis

The data for the ensiling time, were analysed using the procedure Mixed Linear Model according to the following model:

\[ Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + C_k + E_{ijkl} \]

Y = the dependent variable considered, μ = overall mean, A_i = fixed effect of replicate (i = 1, ..., 3); B_j = fixed effect time ensiling (j = 1, ..., 4); (AB)_{ij} = effect of the interaction (replicate, time ensiling);

Table 1. Analytical composition of high moisture corn before and after ensiling.

|                | Before ensiling | 2 months | 7 months | 12 months | SE     |
|----------------|----------------|----------|----------|-----------|--------|
| pH             | 6.48^a         | 4.26^b   | 4.28^a   | 4.19^c   | 0.029  |
| Dry matter, g/kg | 711.0^c     | 704.1^b  | 701.3^c  | 704.0^b  | 0.606  |
| Crude protein, % | 8.09^a       | 8.57^b   | 8.35^b   | 8.47^c   | 0.173  |
| Crude fibre, %  | 2.12^a       | 1.89^b   | 2.24^c   | 2.08^b   | 0.01   |
| Ammonia-N, mg/kg DM | 88.0^a     | 73.3^c   | 84.5^b   | 103^c   | 24.835 |
| Ash, %         | 1.32          | 1.31     | 1.20     | 1.21     | 0.015  |
| Short chain fatty acids and lactate composition g/100g DM |         |          |          |          |        |
| Acetate        | nd            | 0.30^b   | 0.27^c   | 0.33^a   | 0.011  |
| Propionate     | nd            | tr       | tr       | tr       |        |
| Isobutyrate    | nd            | 0.12     | 0.12     | 0.12     | 0.014  |
| Butyrate       | nd            | 0.01^b   | 0.01^b   | 0.02^a   | 0.003  |
| Lactate        | nd            | 3.59^b   | 3.98^b   | 4.42^b   | 0.32   |

DM, dry matter; nd, not detectable; tr, traces. ^a,bRow means within group with different superscript letters differ significantly at P<0.01; ^cRow means within group with different superscript letters differ significantly at P<0.05.

Table 2. Fat and fatty acids composition of high moisture corn before and after ensiling.

|                | Before ensiling | 2 months | 7 months | 12 months | SE     |
|----------------|----------------|----------|----------|-----------|--------|
| Ether extract, g/kg DM | 35.7^c      | 39.4^b   | 39.3^c   | 38.1^b   | 1.22   |
| Linoleic acid, g/kg DM | 18.1^c     | 21.8^a   | 22.5^b   | 22.3^b   | 0.71   |
| Fatty acid composition (g/100 g total fatty acids) |         |          |          |          |        |
| C10:0          | 0.18^a       | tr       | 0.84^a   | 0.84^a   | 0.004  |
| C12:0          | 0.52^a       | 0.20^b   | 0.12^c   | 0.05^c   | 0.014  |
| C14:0          | 0.06^a       | 0.05^b   | 0.03^c   | 0.02^c   | 0.003  |
| C16:0          | 15.07^a      | 15.40^a  | 14.42^c  | 13.39^b  | 0.322  |
| C16:1          | 0.14^b       | 0.16^c   | 0.10^e   | 0.07^e   | 0.006  |
| C18:0          | 2.32^a       | 2.18^b   | 2.03^c   | 1.91^b   | 0.098  |
| C18:1n9        | 23.78^a      | 23.05^b  | 23.71^c  | 23.25^a  | 0.524  |
| C18:1n7        | 0.60^m       | 0.64^n   | tr       | tr       |        |
| C18:2n6        | 55.34^c      | 56.12^c  | 57.28^e  | 59.44^e  | 0.578  |
| C18:3n3        | 1.58^a       | 1.71^d   | 1.50^e   | 1.52^e   | 0.032  |
| C20:1n9        | 0.25^a       | 0.32^b   | 0.25^d   | 0.25^d   | 0.012  |
| Total SFA       | 18.23^a      | 17.92^a  | 16.61^b  | 15.47^b  | 0.283  |
| Total MUFA      | 24.84^a      | 24.25^a  | 24.12^b  | 23.57^a  | 0.514  |
| Total PUFA      | 56.93^c      | 57.83^c  | 59.27^d  | 60.95^c  | 0.583  |

DM, dry matter; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; tr, traces. ^a,bRow means within group with different superscript letters differ significantly at P<0.01. ^c,dRow means within group with different superscript letters differ significantly at P<0.05.
### Results

The analytical composition of HMC induced by silage is presented in Table 1. The ensiling induced a drop in pH, an increase in ammonia, and the appearance of volatile fatty acids and lactate. The DM lost 0.7% during the first two months and was stable thereafter.

The fat and fatty acid compositions of the HMC before and after ensiling is shown in Table 2. After 12 months of ensiling, ether extract increased by 2.4 g/kg and linoleic acid increased by 3.4 g/kg. The variations of fatty acids percentage differed according to the number of double bonds. In the same period the saturated fatty acid (total SFA) percentage decreased by 2.76%; the palmitic acid (C16:0), the main SFA, decreased by 1.68%. Monounsaturated fatty acids (total MUFA) percentage decreased by 1.27% and the oleic acid (C18:1n9), the main MUFA, decreased by 0.53%. Polysaturated fatty acids (total PUFA) percentage increased by 4.02% and linoleic acid (C18:2n6), the main PUFA, increased by 4.10%.

The ether extract content of each maize hybrid is presented in Table 3. The percentage of linoleic acid increased from 2 to 12 months after ensiling and reached the maximum after 7-12 months; different hybrids showed different variations of linoleic acid percentage from +1.9% (DK6309) to +6.79% (Kalibo).

### Discussion

#### Chemical composition and silage quality

The data regarding pH, ammonia, volatile fatty acids and lactate confirm that HMC silages were all well preserved, as several authors have shown laboratory silos are an accurate and reliable experimentation unit (O’Kiely and Wilson, 1991); different sizes of mini-silos are used to study fermentation characteristics of corn forage (Burmeister et al., 1965; Wilson and Wilkins, 1972; Cherney et al., 2004; Alves et al., 2011). Hoffman and Muck (1999) stated that the optimum harvest moisture for good fermentation of HMC is 28 to 32% and, in a proper fermentation process, the pH of HMC should drop quickly to 4.0-4.3; moreover a rapid decrease in pH inhibits the growth of undesirable anaerobic microorganisms such as *enterobacteria* and *clostridia* and helps to limit the breakdown of protein by inactivating plant proteases (Kung and Shaver, 2001). Lactic acid were the primary acid produced, as highlighted by Kung and Shaver (2001) and Taylor and Kung (2002). High concentrations of ammonia-N are indicative of excessive protein breakdown during fermentation; after 12 months of ensiling our HMC achieved 1037 mg/Kg ammonia-N, a value below the limit...
(ammonia-N of <10% CP) set by Kung and Shaver (2001). DM losses can result from (aerobic) respiration, (anaerobic) fermentation, or effluent losses, and depending on the type of silage they can be very high (McDonald and Whittenbury, 1973; Alves et al., 2011). Effluent losses are sugars, soluble nitrogenous compounds, fermentation acids and minerals (Alves et al., 2011). Under our experimental conditions no effluent was produced. According to Haigh (1999) and McDonald and Whittenbury (1973) the volume of effluent produced during silage is influenced mainly by the DM content of the ensiled crop; they pointed out that with a DM over 300g/Kg the effluent production is minimal or absent. However, in well-compacted and sealed silos, anaerobic conditions are established very quickly, so these losses can be expected to be small (Dewhurst and King, 1998). A high DM recovery connected to a high percentage of lactic acid in silage can be interpreted as a signal of a good homolactic fermentation process (Kung and Shaver, 2001). DM losses in the present study were comparable with those shown by Taylor and Kung (2002) and Burmeister (1965). In our HMC the ashes were stable throughout the year of ensiling.

Effect of ensiling on fat and fatty acids composition of silage

The good results of ensilage, the optimal anaerobic conditions, and the pH value all suggest that HMC was well compacted, preventing losses from oxidative degradation of fat and fatty acids. The increase of total fat in silo could be explained by a decrease of DM due to a loss of carbohydrates and proteins through anaerobic degradation or effluent losses; according to Mackie et al. (1991) fat and fatty acids anaerobic degradation is improbable. In this experiment the DM content decreased during the first two months by 6.9 g/kg and the ether extract increased in the same period by 3.7 g/kg. The absence of effluent losses and the good preservation of the silage corroborate the hypothesis that DM losses were the major determinant of EE increase.

Alves et al. (2011) reported a decrease of C18:2-n6 and C18:3-n3 percentage in whole plant corn silage compared to fresh whole plant. They suggested that losses of PUFA were probably due to oxidation by lipoxigenase, which converts C18:2-n6 and C18:3-n3 into PUFA hydroperoyds that are catabolized into volatile compounds. In our experiment the linoleic acid C18:2-n6 content of HMC increased as a percentage during ensilage at the expense of MUFA and SFA in particular. The variation of fatty acids took place after ensiling, at which time, according to Burmeister et al. (1965) the bacteria population increased from 10 to 100 fold. One possible explanation is that these two variations could be linked. Some authors have reported that ensiling increases the concentration of linoleic acid in grass silage (Boufaied et al., 2003; Alves et al., 2011), but the causes are not completely understood. Further studies are necessary to clarify this topic.

The trend in ether extract content and linoleic acid percentage was similar for each maize hybrid. According to literature an increase in dietary linoleic acid caused a higher deposition in this fatty acid in pig backfat (Della Casa et al., 1990; Pantaleo et al., 2000; Motrot, 2001; Avergette Galitin et al., 2002; Nugyen et al., 2003; Wood et al., 2008; Della Casa et al., 2010). Therefore, heavy pigs diets prepared with high percentage of corn (up to 55%), without taking into account the linoleic acid content of the hybrid used, can lead to quite differing amounts of this fatty acid being fed to the pigs and consequently to fat deposits of unequal suitability for raw ham curing. Moreover using HMC, in which silage causes an increase of linoleic acid content (of up to 6.79% in our trial) can aggravate this kind of problem.

Conclusions

Ensilated HMC is often used instead of maize meal as the main component in heavy pig diet. The 11 maize hybrids showed an increase of both ether extract and linoleic acid percentages up to 12 months of ensiling. Linoleic acid and ether extract contents in the heavy pig diet are the key factors that define the percentage of linoleic acid in DM that, according to Parma Ham Rules, must not exceed 2% of the diet for heavy pigs. In order not to exceed this limit, it is necessary to check the DM linoleic acid content of ensiled HMC in particular, if this feed-stuff is used in heavy pig diets at the higher allowed levels.

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