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ANIMAL RESEARCH PAPER

Influence of cultivar, sowing date and maturity at harvest on yield, digestibility, rumen fermentation kinetics and estimated feeding value of maize silage

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SUMMARY

Two maize hybrid cultivars, early (Cisko FAO 300) and late maturing (Arma FAO 700), were sown on three different dates (March, April or May) and harvested at two stages of maturity (kernel milk line (ML) scores of 1/4 < ML < 1/3 or 1/2 < ML < 2/3) in the western Po plain (Italy) in 2008. Yield, chemical composition and *in vitro* digestibility and fermentation kinetics of pre-ensiled whole-crop maize and of silage were measured. Cultivar and sowing date influenced the dry matter (DM) yield of whole-plant maize, with DM yield being 40% higher in Arma than in Cisko, and DM yield decreasing with later sowing dates. Later maturity increased DM concentration at harvest for both hybrids, due to differences in kernel development. The neutral detergent fibre (NDF) content declined and starch increased as plants matured. Digestibility was estimated *in vitro* following the Ankom procedure. Rate and extent of ruminal degradation were estimated from gas production (GP) profiles during incubation in diluted rumen fluid. Cultivar, planting date and maturity had no effect on *in vitro* DM digestibility of pre-ensiled whole-crop maize, but following 240 days of ensiling significant differences between cultivars in digestibility were detected. GP kinetic parameters differed between cultivars for pre-ensiled whole-crop maize, with Cisko having higher asymptotic GP but lower fractional fermentation rates and longer lag times than the Arma hybrid. GP volumes were greater as sowing or harvest dates were delayed. Energy value and milk production were estimated using the Milk2006 Model. With the pre-ensiled whole-crop maize, a 38% greater milk yield/ha was expected with Arma than with Cisko, matching the 40% greater DM yield. The same trend was observed in maize silage, where cultivar and planting date affected milk production/ha, with greater values for Arma than for Cisko and lower values for the latest planting date. Optimal management practices, including decision making on planting and harvest time and hybrid cultivar selection, can influence the yield and nutritive value of maize silage.

INTRODUCTION

Maize (*Zea mays* L.) silage has high yield potential and suitable ensiling properties; it is a valuable source of energy and nutrients, and incorporation into total mixed rations (TMR) is feasible. Its use is therefore widespread as a forage for dairy cows (Johnson *et al.* 2002*a*). Factors such as total dry matter (DM) produced and amount of grain obtained were initially considered as main target indicators for selecting a maize hybrid (Cox *et al.* 1994). In recent years, other attributes related to nutritional quality of the feedstuff and subsequent livestock performance (e.g. high fibre and starch digestibility) have gained increasing interest, in an attempt to maximize the amount of milk produced/ha or /kg of silage (Barriere *et al.* 1995; Neylon & Kung 2003). One of the factors affecting the nutritive value and digestibility of silage is the stage of maturity at harvest (Johnson *et al.* 1999), which is in turn affected by management practices such as harvest date or hybrid selection (Xu *et al.* 1995). Harvesting too early can be unfavourable, not only due to effluent

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losses of nutrients from the silo, but also because the energy content of the feed is lower due to incomplete starch accumulation in kernels (Wiersma *et al.* 1993). In contrast, late harvesting at the black layer (BL) stage may result in reduced starch and fibre digestibility (Wiersma *et al.* 1993). Bal *et al.* (1997) and Johnson *et al.* (2002a) have suggested that 2/3 milk line (ML) is the optimum maturity stage to harvest maize for silage to be fed to lactating dairy cows. In their review, Johnson *et al.* (1999) highlighted the negative interaction between grain development and stover lignin content associated with maturity of maize. The decline in digestibility of the stover with progressive maturity is associated with decreasing non-structural carbohydrates and increasing fibre and lignin concentrations (Bal *et al.* 1997). Neutral detergent fibre (NDF) in whole crop maize decreases as maturity advances from early ML (1/3 ML) to 2/3 ML, then remains constant until the BL stage of maturity (Bal *et al.* 1997). The *in situ* degradability of whole crop maize decreases progressively from early to late maturity stages despite the decline in NDF content (Johnson *et al.* 1999). Xu *et al.* (1995) suggested a strategy to improve maize silage quality through the selection of maize hybrids that maintained stover quality with advancing maturity. High levels of dietary fibre can limit intake through ruminoreticular fill (Cox *et al.* 1994), thus affecting performance adversely. When rumen fill becomes a limiting factor, one approach for increasing dry matter intake (DMI) is to increase NDF digestibility. Oba & Allen (1999) reported that a one-unit increase in forage NDF digestibility was associated with a 0·17 kg increase in DMI and a 0·25 kg increase in 4% fat-corrected milk yield.

Laboratory methods have been developed and refined to provide information on forage quality and to obtain accurate predictions of DMI and digestibility (Damiran *et al.* 2008). The Ankom filter bag technique for determining *in vitro* digestibility allows a large number of samples to be analysed in a short time and reliable estimates of digestibility to be obtained (Damiran *et al.* 2008). Undersander *et al.* (1993) proposed an index of forage quality based on acid detergent fibre (ADF) and NDF analyses to predict the milk yield/kg of forage DM. This index was modified for maize silage by Schwab & Shaver (2001) using modified National Research Council energy values (NRC 2001), improving predictions of DMI and NDF digestibility. An updated version of this index, Milk2006 (Shaver *et al.* 2006), has become a key tool for maize hybrid breeding programmes based on predictions of milk produced/kg of maize silage. In addition to *in vitro* digestibility, rumen fermentation kinetics assessed with the gas production (GP) technique can be used to estimate rate and extent of feed degradation in the rumen (Mauricio *et al.* 1999). This technique has been used to evaluate the effects of variety, growing site and cereal species (Opatpatanakit *et al.* 1994), and to compare maize grains and methods of processing (DePeters *et al.* 2007). The assessment of differences within hybrids characterized by different FAO maturity class can also be of significant importance.

The aim of the current study was to compare two FAO class maize hybrid cultivars, and to examine the effects of sowing date and maturity stage at harvest, and their interactions with hybrid class, on yield, chemical composition, *in vitro* digestibility and rumen fermentation kinetics and estimated net energy for lactation (NE_L) of maize silage and the corresponding whole-crop maize before ensiling.

MATERIALS AND METHODS

Experimental procedure

The trial was conducted at the experimental farm of the University of Turin in the western Po plain, Italy (44°50′N, 7°40′E, 232 m asl), which is characterized by sandy loam soil, with a sub-alkaline pH. The climatic patterns (1978–2008) were characterized by mean daily temperature ranging from 0·6 °C in January to 22·3 °C in July, and an average maximum July temperature of 28·8 °C. The monthly and annual average air temperatures and accumulated rainfall from January to December are reported in Table 1.

The experimental field was divided into three blocks to give three replications per treatment (one in each block). Each block was split into three (90 × 12 m) plots, which were then assigned randomly to one of three sowing dates. Within each plot, maize variety was assigned as the sub-plot factor and the harvest date was assigned as the sub-sub-plot factor within each sub-plot. Maize hybrid cultivars (Cisko and Arma, NK Syngenta Seeds S.p.A., Madignano (CR), Italy) were sown on 13 March, 9 April and 14 May 2008 at an intended planting density of 74 000 seeds/ha. Cisko and Arma are dual purpose hybrid cultivars with floury starch that can be harvested either for grain or for silage (usually high grain and forage yields). Both are included in the European catalogue of varieties of agricultural plant species (European Commission 2011)

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Table 1. *Monthly and annual mean temperatures and accumulated precipitation for the study period and the long-term average (1978–2008)*

	Temperature (°C)		Rain (mm)	
Month	2008	Mean 1978–2008	2008	Mean 1978–2008
January	2·4	0·6	46	35
February	3·9	2·9	9	32
March	8·5	7·6	2	48
April	11·3	11·2	118	92
May	16·7	16·2	109	103
June	21·0	20·1	171	68
July	22·4	22·3	83	36
August	22·0	21·6	44	58
September	16·7	17·4	56	69
October	12·7	12·2	4	85
November	6·4	5·7	54	56
December	0·7	1·7	7	36
Annual	*12·1*	*11·6*	*703*	*719*

as simple hybrids and maturity ratings FAO 300 (Cisko hybrid) and FAO 700 (Arma hybrid). The FAO rating is the maize relative maturity system adopted in Europe, calculated on the basis of the number of days required for grain to contain <0·20 g moisture/g grain and developed to aid breeders and growers to place genotypes into the correct adaptation zones (Troyer 2000). The two FAO classes (FAO 300 and FAO 700 for early and late maturing maize, respectively) were chosen to represent the minimum and maximum maturity ratings of maize hybrids used for silage in the Po plain (Italy). Fertilizer was applied at the rate of 40 kg P_2O_5/ha and 55 kg K_2O/ha immediately before planting. An additional 160 kg N/ha as urea was top-dressed at the six leaves stage. Irrigation was provided by a sprinkler system on 17 July, at a rate of 500 m^3 water/ha. Thirty whole plants per plot (randomly sampled) were harvested and ensiled at two different stages of maturity, the first with ML between 1/4 and 1/3 ML, the second stage ranging from 1/2 to 2/3 ML for both hybrid cultivars. The forage was chopped to *c.* 12 mm. The kernel ML was measured on five plants randomly sampled from each plot following Afuakwa & Crookston (1984). The chopped forage from each plot was sampled for chemical analysis and ensiled in 30-litre plastic containers equipped with a lid that allowed gas release. The silos were maintained at 20 °C and opened after 240 d. Upon opening the silos, weight of silage was recorded, a layer of *c.* 100 mm from the top of each silo was discarded, and the remaining silage sampled for analysis. At each sampling time, 10 additional plants were randomly harvested and separated by hand into grain, stalk, leaves, tassel, husk and cob. Each plant part was dried separately and the ratio between grain and whole plant was calculated on a DM basis.

Sample preparation and chemical analysis

Samples were assayed in duplicate according to AOAC (2000). The pre-ensiled whole-crop maize and the silage were oven-dried at 60 °C for 72 h, weighed to determine DM content and ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass through a 1 mm screen. Samples were analysed for crude protein (CP), ash, ether extract (EE) and starch concentration according to the AOAC methods 954·01, 942·05, 920·39 and 920·40, respectively (AOAC 2000). The NDF, ADF and lignin were determined using an Ankom fibre analyser (Ankom Technology Corp., Fairport, NY, USA), following the procedure of Van Soest *et al.* (1991). The NDF was analysed with the addition of sodium sulphite and heat stable amylase to the solution. Silage sample preparation and analyses were performed as described in detail by Tabacco *et al.* (2011). The silage pH was determined in a water extract, using a pH meter (Mettler Toledo, Novate Milanese, Italy) with a glass electrode. The water extract was obtained by mixing 30 g of silage with 270 ml of distilled water in a Stomacher blender (Seward Ltd, UK) for 4 min. The lactic and short-chain fatty acids (acetic, propionic and butyric acids) were determined by high performance liquid chromatography (HPLC; Agilent Technologies Italia, Cernusco sul Naviglio, Italy) in an aqueous acid extract obtained after blending (for 4 min) 50 g of fresh silage mixed with 250 ml of H_2SO_4 0·05 M.

Animals and rumen fluid collection

Six rumen-fistulated Merino sheep were used as donors of ruminal inoculum for the *in vitro* assays. Animals were fed good-quality alfalfa hay and had free access to clean water. A sample of ruminal contents was collected before the morning feeding in thermos flasks and taken to the laboratory where it was strained through two layers of cheesecloth, kept at 39 °C under CO_2 atmosphere and diluted (1:4, v/v) with a culture medium containing macro and micro mineral solutions, resazurin and a bicarbonate buffer solution, prepared as described by Menke & Steingass (1988).

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Oxygen was reduced by the addition of a solution containing cysteine hydrochloride and Na_2S.

Animal handling followed the recommendations of European Council Directive 86/609/EEC for protection of animals used for experimental and other scientific purposes, and experimental procedures were approved by the University of León (Spain) Institutional Animal Care and Use Committee.

In vitro GP

In vitro GP measurements were conducted using a pressure transducer (Delta Ohm, Caselle di Selvazzano, Italy) as described by Theodorou *et al.* (1994), in which 0·50 ± 0·01 g of sample was incubated in a 120 ml serum bottle containing 50 ml of diluted rumen fluid. Blanks were used to compensate for GP in the absence of added substrate. Once filled, bottles were sealed with rubber stoppers and aluminium seals, shaken and placed in the incubator (Shel Lab, Sheldon Manufacturing, Inc., Cornelius, OR, USA) at 39 °C. The head-space gas pressure released upon fermentation of feed was measured manually by inserting a sterile needle connected to the pressure transducer after incubation for 3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 and 144 h. Gas volume was estimated from pressure measurements using the equation proposed by López *et al.* (2007). Two incubation runs were performed in different weeks, using two bottles per sample and three bottles containing only medium as blanks in each run. The incubation residue after 144 h of fermentation was determined by filtration to estimate the potential DM disappearance (D144). In order to assess the parameters of fermentation kinetics, the exponential model proposed by France *et al.* (2000) was fitted to GP profiles:

$$G = A[1 - e^{-c(t-L)}]$$

where G (ml/g DM incubated) is the cumulative GP at time t (h), A (ml/g DM) is the asymptotic GP, c (/h) is the fractional rate of fermentation and L (h) is the lag time.

Volume of gas (ml/g DM) produced after 24 h of incubation (G24) was used as an index of digestibility and energy feed value, as suggested by Menke & Steingass (1988). The extent of degradation (ED) in the rumen, for a rate of passage (k) of 0·033/h (characteristic of forage at maintenance level of intake (Carro *et al.* 1991)), was estimated using the equation suggested by France *et al.* (2000):

$$ED = \frac{c\ D144\ e^{-kL}}{c + k}$$

In vitro digestibility

In vitro dry matter digestibility (IVDMD) was determined using the Ankom–Daisy procedure following the approach proposed by Van Soest *et al.* (1966). Samples (0·25 ± 0·01 g) were weighed into F57 Ankom bags (Ankom Technology Corp., Fairport, NY, USA) with a pore size of 25 µm, heat-sealed and then placed into an incubation vessel. Each vessel was a 5-litre glass container with a plastic lid provided with a single-way valve, which prevents the accumulation of fermentation gases, and was filled with 2 litres of buffered rumen fluid in anaerobic conditions, then placed into the DaisyII Incubator (Ankom Technology Corp., Fairport, NY, USA). Temperature was maintained at 39 °C in the incubator with continuous rotation. After 48 h of incubation the vessels were emptied and the bags were gently rinsed under tap water and dried in an oven at 60 °C. Bags were then washed with a neutral detergent solution at 100 °C for 1 h and rinsed with distilled water into the fibre analyser, so as to remove bacterial debris. *In vitro* NDF degradation (IVNDFD) was estimated from the amount of NDF incubated. Each sample was replicated in four incubation runs carried out in different weeks.

Statistical analysis

The model Milk2006 (Shaver *et al.* 2006) was used to estimate energy values (NE_L, NRC 2001) of maize fodder and silage, and to make predictions of probable milk yield/kg DM of each forage and per ha based on its chemical composition and digestibility. Milk2006 uses updated information and user-defined input flexibility for these predictions.

Chemical composition, GP parameters, *in vitro* digestibility, estimated energy value and milk production data of pre-ensiled whole-crop maize and of silage were analysed by ANOVA according to a split–split–plot design with the whole plots arranged in a randomized complete-block design, and involving three experimental factors (fixed effects), namely planting date, maize cultivar and harvest date. Planting date was the whole-plot factor, maize cultivar the sub-plot factor (within each whole plot), and harvest date the sub-sub-plot factor (within each sub-plot). Random effects were: planting date × block as the whole-plot error to test planting date effects, planting date × cultivar × block as the sub-plot error to test variety effects and cultivar × planting date interaction, and the pooled residual error to test maturity effects and its

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Table 2. *Yield and grain to crop ratio of whole crop maize*

Cultivar	Planting date	Maturity at harvest	Yield (t/ha)		Grain/whole crop (on DM basis)
			Fresh crop yield	DM yield	
Cisko (FAO 300)	1st planting	1/4 < ML < 1/3	64·4	17·7	0·366
		1/2 < ML < 2/3	57·2	17·9	0·511
	2nd planting	1/4 < ML < 1/3	65·0	20·0	0·471
		1/2 < ML < 2/3	50·9	19·1	0·525
	3rd planting	1/4 < ML < 1/3	54·5	17·1	0·480
		1/2 < ML < 2/3	44·5	16·9	0·509
Arma (FAO 700)	1st planting	1/4 < ML < 1/3	88·7	25·3	0·380
		1/2 < ML < 2/3	80·8	27·0	0·450
	2nd planting	1/4 < ML < 1/3	92·7	26·7	0·395
		1/2 < ML < 2/3	86·3	26·0	0·447
	3rd planting	1/4 < ML < 1/3	73·1	23·2	0·406
		1/2 < ML < 2/3	71·5	23·1	0·486
Planting date (P)			0·001	0·002	0·004
Cultivar (C)			< 0·001	< 0·001	0·001
Maturity (M)			< 0·001	0·945	< 0·001
P × C			0·078	0·228	0·085
P × M			0·091	0·034	0·001
C × M			0·005	0·223	0·385
P × C × M			0·054	0·521	0·001
S.E.M.					
P			0·99	0·34	0·0056
C			0·81	0·28	0·0045
M			0·68	0·23	0·0040
P × C × M			1·68	0·57	0·0097

interactions with planting date and cultivar effects. Analyses were carried out using the PROC MIXED procedure of SAS (SAS Institute 2004). Mean values of each parameter and S.E.M. are reported in the Tables.

RESULTS

Whole crop maize production

Whole-crop maize yield (fresh crop and DM) and grain:whole crop ratio are presented in Table 2. Whole crop yield (fresh or DM) was 40% greater ($P<0·001$) with Arma (82·2 t whole-crop and 25·2 t DM/ha) than with Cisko (56·1 t whole-crop and 18·1 t DM/ha). There was a slight increase in DM yield from the first to the second planting date for the Cisko hybrid, and a decrease on the third sowing date for both hybrids. Harvest maturity of maize had an impact ($P<0·001$) on fresh silage yield/ha, decreasing as maturity advanced for both hybrids (73·1 and 65·2 t whole-crop maize/ha for early and late harvest, respectively), but DM yield was not affected by maturity.

Cultivar, sowing date and harvesting maturity affected ($P<0·01$) grain:whole crop ratio. Cisko (0·48) showed a higher grain proportion than Arma (0·43), with larger differences between hybrids for the second and third planting dates. The contribution of the grain to the whole crop maize yield increased from 1/4 < ML < 1/3 (0·42) to 1/2 < ML < 2/3 (0·49), with greater differences between maturity stages for the Cisko hybrid in the first planting date.

Chemical composition

The chemical composition of pre-ensiled whole-crop maize is presented in Table 3. The DM content for both cultivars increased ($P<0·001$) with later maturity stage. The ash content was similar across cultivars, planting and harvest dates. The CP, NDF, lignin and starch concentrations of the pre-ensiled whole-crop maize were affected by cultivar ($P<0·05$), with Arma having higher values of CP (68·7 *v.* 60·3 g/kg DM), NDF (468 *v.* 444 g/kg DM) and lignin (43·3 *v.* 35·9 g/kg DM), and lower values of starch (278 *v.* 310 g/kg DM) than the Cisko hybrid. Maturity had an effect ($P<0·05$) on CP and starch concentrations of the pre-ensiled whole-crop maize, with decreasing CP (66·5 *v.* 62·6 g/kg DM)

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Table 3. *Chemical composition of pre-ensiled whole-crop maize (g/kg DM, except DM)*

Cultivar	Planting date	Maturity at harvest	DM (g/kg crop maize)	Ash	CP	EE	NDF	ADF	Lignin	Starch
Cisko (FAO 300)	1st planting	1/4<ML<1/3	278	44	64	29	455	252	33	296
		1/2<ML<2/3	316	39	57	29	434	240	31	318
	2nd planting	1/4<ML<1/3	311	43	61	28	457	249	39	298
		1/2<ML<2/3	374	39	59	29	433	239	34	316
	3rd planting	1/4<ML<1/3	315	39	61	28	437	237	39	319
		1/2<ML<2/3	378	40	60	27	446	244	39	313
Arma (FAO 700)	1st planting	1/4<ML<1/3	282	45	70	27	461	248	40	279
		1/2<ML<2/3	334	44	68	29	452	241	46	299
	2nd planting	1/4<ML<1/3	291	48	72	27	478	261	43	262
		1/2<ML<2/3	302	45	68	27	465	251	44	281
	3rd planting	1/4<ML<1/3	316	44	71	26	485	266	45	259
		1/2<ML<2/3	370	40	63	26	470	257	42	286
Planting date (P)			<0·001	0·478	0·438	0·370	0·795	0·737	0·222	0·575
Cultivar (C)			0·039	0·044	<0·001	0·145	0·033	0·104	0·004	0·001
Maturity (M)			<0·001	0·142	0·004	0·351	0·180	0·200	0·783	0·016
P×C			0·007	0·629	0·329	0·699	0·577	0·316	0·423	0·233
P×M			0·071	0·856	0·887	0·370	0·745	0·708	0·653	0·771
C×M			0·043	1·000	0·503	0·600	0·964	0·685	0·247	0·416
P×C×M			0·007	0·458	0·115	0·861	0·640	0·695	0·468	0·467
S.E.M.										
P			3·5	1·6	0·9	0·8	11·3	6·7	1·5	6·8
C			2·9	1·2	0·8	0·6	8·8	5·7	1·2	6·1
M			2·8	1·2	0·8	0·6	8·7	5·6	1·2	6·1
P×C×M			6·5	2·9	1·8	1·2	17·3	10·3	2·9	11·1

and increased starch (285 *v.* 302 g/kg DM) contents for early (1/4<ML<2/3) compared with mid-late (1/2<ML<2/3) maturity. The chemical composition of maize silage is presented in Table 4. All the silages had undergone an apparently good fermentation with pH values ranging from 3·58 to 4·02 and negligible butyric acid concentrations detected in all the experimental silos (data not shown). Maize silage obtained from Arma had a higher ($P<0·05$) NDF content than that from Cisko (457 *v.* 437 g/kg DM, respectively). The NDF (460 *v.* 433 g/kg DM) and ADF (275 *v.* 257 g/kg DM) content declined ($P<0·05$), whereas starch (318 *v.* 343 g/kg DM) content increased ($P<0·05$), from the early (1/4<ML<1/3) to mid-late (1/2<ML<2/3) maturity. In general, maize silages showed a similar chemical composition to the corresponding pre-ensiled whole-crop maize, although DM and starch were in many cases slightly greater in the silage.

In vitro digestibility and parameters of GP kinetics

Treatment (cultivar and planting date) and interaction effects on GP kinetics and *in vitro* digestibility for pre-ensiled whole-crop maize and for silage are presented in Tables 5 and 6, respectively.

In pre-ensiled whole-crop maize, all GP parameters were significantly increased ($P<0·05$) with maturity at harvest. Cisko maize showed on average greater asymptotic GP (358 *v.* 349 ml/g DM), slower GP rates (0·051 *v.* 0·053/h) and longer lag times (3·9 *v.* 3·5 h) than Arma ($P<0·01$). As a result, effective degradability estimated from these parameters was greater ($P<0·01$) for Arma (0·450) than for Cisko (0·438) maize. Planting date only affected G24 values ($P<0·05$), which tended to increase as planting date was delayed. Estimated IVDMD and IVNDFD at 48 h of incubation showed an average value of 0·782 g/g DM and 0·522 g/g DM, respectively, and were not affected by any of the treatments, with only a significant ($P<0·05$) cultivar×planting date interaction.

Treatment effects were less consistent in silages. There was a significant effect of cultivar on G24 (238 *v.* 229 ml/g DM) and *A* (351 *v.* 334 ml/g DM) parameters and on IVDMD (0·783 *v.* 0·769) and effective degradability (0·457 *v.* 0·450), which were greater ($P<0·05$) in Cisko than in Arma silage.

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Table 4. *Chemical composition of maize silage (g/kg DM, except DM)*

Cultivar	Planting date	Maturity at harvest	DM (g/kg silage)	Ash	CP	EE	NDF	ADF	Lignin	Starch
Cisko (FAO 300)	1st planting	1/4 < ML < 1/3	300	51	80	26	471	276	32	298
		1/2 < ML < 2/3	336	46	75	28	443	265	31	344
	2nd planting	1/4 < ML < 1/3	331	39	72	29	426	250	25	351
		1/2 < ML < 2/3	398	40	69	28	422	248	22	339
	3rd planting	1/4 < ML < 1/3	342	37	67	26	450	274	27	338
		1/2 < ML < 2/3	400	38	69	29	412	244	26	366
Arma (FAO 700)	1st planting	1/4 < ML < 1/3	292	47	71	24	474	285	31	302
		1/2 < ML < 2/3	344	34	68	28	424	250	26	356
	2nd planting	1/4 < ML < 1/3	322	38	69	25	476	291	29	304
		1/2 < ML < 2/3	331	38	68	26	442	266	28	333
	3rd planting	1/4 < ML < 1/3	334	37	69	24	471	285	30	313
		1/2 < ML < 2/3	360	35	70	25	456	268	27	322
Planting date (P)			0·004	0·100	0·432	0·512	0·268	0·329	0·277	0·319
Cultivar (C)			0·001	0·238	0·487	0·100	0·026	0·046	0·846	0·053
Maturity (M)			< 0·001	0·170	0·441	0·082	0·005	0·011	0·081	0·015
P × C			0·013	0·684	0·382	0·660	0·103	0·161	0·256	0·166
P × M			0·752	0·335	0·711	0·703	0·652	0·951	0·853	0·305
C × M			0·003	0·505	0·825	0·916	0·825	0·613	0·906	0·490
P × C × M			0·002	0·862	0·773	0·277	0·194	0·228	0·628	0·546
S.E.M.										
P			5·3	2·1	2·1	1·0	10·8	7·7	1·5	8·0
C			4·7	1·8	1·7	0·9	9·3	7·3	1·2	7·3
M			4·7	1·6	1·6	0·9	9·1	7·2	1·0	7·1
P × C × M			7·4	4·1	4·2	1·5	15·0	11·2	2·7	13·9

Estimated energy value and milk production

Estimated net energy content and predicted potential milk yield from pre-ensiled whole-crop maize and from silage are reported in Tables 7 and 8, respectively. Cultivar affected ($P < 0·001$) milk yield/ha (t/ha) of pre-ensiled whole-crop maize, with Arma showing a 38% higher value than Cisko. The NE_L and the potential milk production (kg/kg forage DM) were similar for the two hybrids and across planting dates, and they were slightly greater ($P < 0·05$) for the earlier maturity stage. The sowing date affected ($P < 0·01$) the milk production/ha, with greater values at the first two planting dates.

The potential milk yield/ha of the maize silage was affected ($P < 0·01$) both by cultivar and sowing date, with greater values for Arma than for Cisko (33·4 *v.* 24·3 t milk/ha) and lower values for the third planting date, whereas the NE_L and the potential milk production (kg/kg silage DM) were not affected ($P > 0·05$) by any of the treatments.

DISCUSSION

Chemical composition

Changing management practices can be a strategy to influence the nutritive value of maize silage. The proper selection of the hybrid used for producing whole-crop silage is a key issue to obtain high forage yield and favourable nutritional characteristics. The hybrid should be of the proper maturity rating for the area in which it is grown in order to maximize the full growing season available in terms of growing degree days. In the environmental conditions of the experimental site for the current study, the late maturing hybrid (Arma FAO 700) showed the potential of producing >23 t DM/ha, up to 38% more than the early maturity hybrid (Cisko FAO 300). DM yields of whole-crop maize were within the expected range for maize hybrids grown in the Mediterranean area (Tabacco *et al.* 2011), with similar observed differences between early (FAO 300) and late (FAO 700)

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Table 5. *Rumen fermentation kinetics and in vitro digestibility (g digested/g incubated) of pre-ensiled whole-crop maize*

			Kinetics of GP				*In vitro* digestibility			
Cultivar	Planting date	Maturity at harvest	G24	*A*	*c* (/h)	*L* (h)	IVDMD	IVNDFD	D144	ED
Cisko (FAO 300)	1st planting	1/4 < ML < 1/3	223	352	0·050	3·62	0·781	0·519	0·809	0·432
		1/2 < ML < 2/3	222	356	0·049	4·14	0·772	0·472	0·819	0·428
	2nd planting	1/4 < ML < 1/3	227	359	0·050	3·95	0·791	0·543	0·823	0·435
		1/2 < ML < 2/3	230	363	0·050	3·90	0·811	0·562	0·824	0·436
	3rd planting	1/4 < ML < 1/3	233	360	0·052	3·96	0·787	0·512	0·831	0·447
		1/2 < ML < 2/3	237	359	0·054	3·81	0·772	0·487	0·827	0·451
Arma (FAO 700)	1st planting	1/4 < ML < 1/3	226	350	0·051	3·59	0·793	0·551	0·819	0·442
		1/2 < ML < 2/3	232	353	0·054	4·08	0·786	0·526	0·834	0·452
	2nd planting	1/4 < ML < 1/3	216	335	0·050	3·33	0·770	0·511	0·808	0·436
		1/2 < ML < 2/3	229	343	0·054	3·65	0·768	0·506	0·818	0·451
	3rd planting	1/4 < ML < 1/3	233	348	0·053	3·08	0·778	0·542	0·821	0·458
		1/2 < ML < 2/3	248	364	0·056	3·46	0·780	0·532	0·820	0·460
Planting date (P)			0·028	0·103	0·058	0·258	0·601	0·611	0·668	0·065
Cultivar (C)			0·419	0·006	0·004	0·003	0·164	0·232	0·637	0·006
Maturity (M)			0·048	0·036	0·031	0·010	0·669	0·105	0·274	0·264
P × C			0·073	0·023	0·552	0·048	0·009	0·013	0·118	0·425
P × M			0·613	0·855	0·867	0·139	0·369	0·168	0·438	0·877
C × M			0·146	0·231	0·087	0·113	0·942	0·785	0·529	0·332
P × C × M			0·958	0·276	0·769	0·403	0·345	0·521	0·953	0·666
S.E.M.										
P			2·7	2·3	0·0007	0·098	0·0052	0·0090	0·0062	0·0044
C			2·3	2·0	0·0006	0·069	0·0047	0·0069	0·0050	0·0038
M			2·3	2·0	0·0006	0·069	0·0047	0·0067	0·0050	0·0038
P × C × M			4·8	4·2	0·0013	0·155	0·0089	0·0159	0·0090	0·0071

G24, cumulative GP at 24 h of incubation (ml/g DM); *A*, asymptotic GP (ml/g DM); *c*, fractional rate of GP; *L*, lag time; D144, DM disappearance after 144 h of incubation.

Table 6. *Rumen fermentation kinetics and in vitro digestibility (g digested /g incubated) of maize silage*

			Kinetics of GP				*In vitro* digestibility			
Cultivar	Planting date	Maturity at harvest	G24	*A*	*c* (/h)	*L* (h)	IVDMD	IVNDFD	D144	ED
Cisko (FAO 300)	1st planting	1/4 < ML < 1/3	229	341	0·051	2·12	0·767	0·508	0·786	0·445
		1/2 < ML < 2/3	247	357	0·056	2·88	0·790	0·528	0·821	0·470
	2nd planting	1/4 < ML < 1/3	234	351	0·052	2·68	0·783	0·488	0·810	0·454
		1/2 < ML < 2/3	240	355	0·052	2·07	0·794	0·543	0·815	0·464
	3rd planting	1/4 < ML < 1/3	240	353	0·053	2·33	0·784	0·508	0·801	0·458
		1/2 < ML < 2/3	237	350	0·053	2·57	0·780	0·465	0·793	0·449
Arma (FAO 700)	1st planting	1/4 < ML < 1/3	229	335	0·053	2·01	0·780	0·537	0·797	0·458
		1/2 < ML < 2/3	229	338	0·054	2·96	0·782	0·490	0·788	0·444
	2nd planting	1/4 < ML < 1/3	231	333	0·055	2·18	0·765	0·512	0·774	0·448
		1/2 < ML < 2/3	223	329	0·052	2·27	0·765	0·461	0·783	0·446
	3rd planting	1/4 < ML < 1/3	229	334	0·054	2·34	0·751	0·470	0·785	0·451
		1/2 < ML < 2/3	229	336	0·054	2·78	0·773	0·506	0·794	0·450
Planting date (P)			0·859	0·926	0·595	0·265	0·282	0·301	0·512	0·722
Cultivar (C)			0·001	< 0·001	0·158	0·825	0·011	0·668	0·000	0·002
Maturity (M)			0·509	0·192	0·524	0·012	0·101	0·287	0·293	0·772
P × C			0·976	0·109	0·373	0·450	0·066	0·630	0·007	0·089
P × M			0·270	0·123	0·159	0·004	0·824	0·750	0·674	0·705
C × M			0·141	0·225	0·453	0·110	0·845	0·473	0·498	0·189
P × C × M			0·335	0·279	0·604	0·535	0·164	0·018	0·118	0·207
S.E.M.										
P			2·9	2·0	0·0011	0·192	0·0055	0·0141	0·0070	0·0053
C			2·3	1·5	0·0010	0·186	0·0051	0·0119	0·0066	0·0049
M			2·3	1·5	0·0010	0·186	0·0051	0·0118	0·0066	0·0049
P × C × M			4·8	3·5	0·0016	0·241	0·0080	0·0221	0·0099	0·0080

G24, cumulative GP at 24 h of incubation (ml/g DM); *A*, asymptotic GP (ml/g DM); *c*, fractional rate of GP; *L*, lag time; D144, DM disappearance after 144 h of incubation.

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Table 7. *Estimated energy value and milk production of pre-ensiled whole-crop maize (Milk 2006 model)*

Cultivar	Planting date	Maturity at harvest	$NE_{L-3\times}$ MJ/kg DM	kg milk/kg maize DM	t milk/ha
Cisko (FAO 300)	1st planting	1/4 < ML < 1/3	6·18	1·38	24·4
		1/2 < ML < 2/3	6·21	1·38	24·7
	2nd planting	1/4 < ML < 1/3	6·23	1·40	28·1
		1/2 < ML < 2/3	6·04	1·35	25·8
	3rd planting	1/4 < ML < 1/3	6·31	1·42	24·3
		1/2 < ML < 2/3	5·81	1·25	21·2
Arma (FAO 700)	1st planting	1/4 < ML < 1/3	6·21	1·40	35·3
		1/2 < ML < 2/3	6·16	1·38	37·1
	2nd planting	1/4 < ML < 1/3	5·97	1·31	35·0
		1/2 < ML < 2/3	6·07	1·34	34·8
	3rd planting	1/4 < ML < 1/3	6·07	1·35	31·3
		1/2 < ML < 2/3	5·88	1·29	29·8
Planting date (P)			0·131	0·106	0·003
Cultivar (C)			0·160	0·223	< 0·001
Maturity (M)			0·043	0·037	0·147
P × C			0·654	0·289	0·149
P × M			0·078	0·100	0·080
C × M			0·169	0·184	0·128
P × C × M			0·351	0·439	0·972
S.E.M.					
P			0·059	0·019	0·67
C			0·053	0·017	0·58
M			0·053	0·017	0·55
P × C × M			0·101	0·033	1·11

Table 8. *Estimated energy value and milk production of maize silage (Milk 2006 model)*

Cultivar	Planting date	Maturity at harvest	$NE_{L-3\times}$ MJ/kg DM	kg milk/kg silage DM	t milk/ha
Cisko (FAO 300)	1st planting	1/4 < ML < 1/3	5·95	1·30	23·1
		1/2 < ML < 2/3	6·13	1·37	24·4
	2nd planting	1/4 < ML < 1/3	6·26	1·39	28·0
		1/2 < ML < 2/3	5·96	1·32	25·3
	3rd planting	1/4 < ML < 1/3	6·16	1·37	23·7
		1/2 < ML < 2/3	5·75	1·23	20·7
Arma (FAO 700)	1st planting	1/4 < ML < 1/3	6·01	1·33	34·1
		1/2 < ML < 2/3	6·19	1·38	37·7
	2nd planting	1/4 < ML < 1/3	6·06	1·34	35·2
		1/2 < ML < 2/3	6·12	1·35	34·1
	3rd planting	1/4 < ML < 1/3	5·93	1·29	30·4
		1/2 < ML < 2/3	5·95	1·30	30·1
Planting date (P)			0·178	0·190	0·007
Cultivar (C)			0·772	0·937	< 0·001
Maturity (M)			0·661	0·672	0·333
P × C			0·908	0·837	0·035
P × M			0·099	0·180	0·024
C × M			0·034	0·060	0·023
P × C × M			0·437	0·446	0·863
S.E.M.					
P			0·107	0·039	1·30
C			0·103	0·038	1·28
M			0·094	0·036	1·27
P × C × M			0·142	0·050	1·49

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maturity classes (Masoero *et al.* 2011; Randjelovic *et al.* 2011). Ensiling at the correct DM concentration and optimum stage of maturity is also critical to achieving good quality composition of the resulting silage.

In the current study, advancing the stage of maturity of whole-plant maize at harvest from 1/4 < ML < 1/3 to 1/2 < ML < 2/3 increased the DM concentration for both cultivars, in agreement with other reported results (Hunt *et al.* 1989; Xu *et al.* 1995; Johnson *et al.* 2002*a*, 2003). This effect can be related to differences in kernel development (Argillier *et al.* 1995). Furthermore, maturity had an effect on fibre content, which declined with later maturity for both cultivars. Similar trends have been reported by Hunt *et al.* (1989) and Johnson *et al.* (2002*a*) who observed a decrease in NDF and ADF concentrations in maize silage from 1/3 ML to 2/3 ML maturity stages. This decrease was related to a higher proportion of grain in mature whole-plant maize, and the results are consistent with those of Argillier *et al.* (1995) who stated that although the fibre content of the stover increases with maturity, the fibre content of whole-plant maize decreases due to an increasing proportion of grain. In the present experiment the changes in the starch content with grain maturity showed an opposite trend to that observed for fibre. The increase in starch should be attributed to the greater proportion of grain in the more mature whole-plant maize, in agreement with other authors (Bal *et al.* 1997; Johnson *et al.* 2002*a*).

In vitro digestibility and parameters of GP kinetics

Digestibility of maize silage was determined by the conventional *in vitro* technique with Ankom procedure following the approach proposed by Van Soest *et al.* (1966). Rate and extent of ruminal degradation were estimated from GP profiles derived from *in vitro* incubation of maize fodder and silage samples in buffered rumen fluid (Theodorou *et al.* 1994). This method has been accepted as a sensitive and reliable tool in feed evaluation (López 2005), because GP correlates well with *in vivo* and *in vitro* digestibility (Khazaal *et al.* 1993; López 2005) and with microbial protein synthesis (Blümmel *et al.* 1997). This technique has already been used by DePeters *et al.* (2007) to compare maize hybrids and processing methods.

In the present study, the IVDMD and IVNDFD of pre-ensiled whole-crop maize were not affected by cultivar, planting date and maturity. In contrast, other authors (Johnson *et al.* 2002*b*, 2003; Lewis *et al.* 2004) found that DM and NDF digestibility were lower at later maturity stages. An explanation for this discrepancy would be the later stage of maturity of the maize used by the above authors, with a greater ML than the mature stage used in the current study (1/2–2/3 ML). Different response of cultivars to sowing date may be related to the differences in the climatic conditions influencing the growth and development of each hybrid, such as temperature and growing degree days during the spring months. In silage, observed differences in DM digestibility can be partially explained by the different NDF and lignin contents in maize of each hybrid. According to Bal *et al.* (2000) and Johnson *et al.* (2003) the DM digestibility in maize silage is greater as NDF is lower, as a result of the increased content of digestible starch. Differences in digestibility are primarily associated with the chemical composition of the silage, especially to their cell wall and lignin content (Ivan *et al.* 2005). The cell wall fraction may have a negative influence on digestibility as described in conventional feedstuffs by Van Soest (1994). Cell contents (starch) are readily and completely digested, whereas cell walls are slowly digested and only to a certain extent, depending on their degree of lignification.

In general, cultivar, sowing date and maturity effects on rumen fermentation kinetics followed a trend similar to that observed for chemical composition and *in vitro* digestibility of maize and silage. Hetta *et al.* (2012) used the GP technique to assess the digestibility and rate of degradation in the rumen of maize silage and concluded that the technique was valuable to evaluate hybrid and maturity effects on the nutritional value of maize silage.

The apparent differences between the pre-ensiled whole-crop and the maize silage were of little biological significance, and could be attributed to the fermentation of water-soluble carbohydrates contained in the pre-ensiled material.

Estimated energy value and milk production

Yield and nutrient analysis data of maize silage were used as inputs for Milk2006 (Shaver *et al.* 2006) to estimate the net energy for lactation at 3× maintenance ($NE_{L-3\times}$), milk production/kg DM and per ha (t milk/ha). *In vitro* NDF digestibility at 48 h of incubation was used as the cell wall digestibility value required by the model for the calculations. Expected silage energy value and animal responses to feeding of maize silage calculated from Milk2006 were within

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the range reported by other authors using the same model (Schwab *et al.* 2003; Tabacco *et al.* 2011). Given the lack of cultivar, sowing date and maturity effects on NDF digestibility, no differences in energy value or expected milk production from maize silage were expected. This finding is in agreement with Oba & Allen (1999), who concluded that the expected animal responses to feeding of maize silage depend mainly on the digestibility of the cell wall. Likewise, Johnson *et al.* (1999) observed that maize maturity had a limited effect on animal performance in dairy cows fed maize silage of increasing maturity.

The 38% higher milk yield/ha observed for Arma is in agreement with the fodder yield, since this hybrid produced *c.* 40% more t milk/ha than Cisko. Regarding other variables, there were no differences between cultivars or maturity stage in energy value and potential milk yield/kg DM. Reduced values of milk production/ha for the latest planting date mainly reflected the differences observed in DM yield.

In the present experiment, when maize was harvested at $1/4 < ML < 1/3$, NE_L values (6·1 *v.* 6·0 MJ/kg DM) and milk production (1·34 *v.* 1·32 kg/kg silage DM) were greater than those predicted for maize harvested at $1/2 < ML < 2/3$. However, these differences seem to be of little biological significance, and no differences were observed for the potential milk yield/ha (t/ha). In silage, cultivar and planting date affected milk production/ha (t/ha), with greater values for Arma than for Cisko and reduced values for the latest planting date, as observed in pre-ensiled whole-crop maize.

CONCLUSIONS

The late maturing maize hybrid (Arma FAO 700) yields more fodder and silage than the early maturing maize hybrid (Cisko FAO 300), with no differences between cultivars in the chemical composition and *in vitro* digestibility of maize and silage. Estimated energy and expected milk production calculated with Milk2006 emphasized that milk yield/ha will be greater with Arma than with Cisko maize silage due to the differences in DM yield/ha. The stage of maturity has a limited effect on the nutritional value of maize silage, with no substantial change in silage feeding value within a range in maturity between 1/4 and 2/3 ML.

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