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PAPER

Response on yield and nutritive value of two commercial maize hybrids as a consequence of a water irrigation reduction

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Abstract

The present study investigated in a practical farm condition the response of two commercial maize hybrids (maturity class FAO rating 700) grown for silage production on chemical composition and digestibility of whole maize plant as consequence of a severe water irrigation reduction. Based on different irrigation applications, water restricted (WR) and fully irrigated (FI) plots received 50 and 200 mm of irrigation water, respectively. A split-plot factorial arrangement in a randomised complete block design with two main plots (WR and FI) and two sub-plots (hybrid A and B) with 12 replications/treatment was performed. Studied parameters were dry matter (DM) yield, harvest index (HI), chemical composition, rumen *in situ* DM and neutral detergent fibre disappearance (DMD and NDFD, respectively), indigestible NDF (iNDF), 7h *in vitro* starch degradability (7hIVSD) and net energy (NE) for lactation content. Total DM and grain yields, HI and chemical composition differed ($P<0.05$) between FI and WR crops and only slight differences were recorded between hybrids. When compared to FI plants, WR had lower starch and higher fibre contents ($P<0.05$). Higher DMD (59.2 *vs* 56.4% DM) and NDFD (61.0 *vs* 58.4% NDF_{OM}) were measured for FI with respect to WR crops, whereas iNDF was about 36% higher ($P<0.05$) in WR than FI. Lastly, WR plants had a lower NE content than FI plants ($P<0.05$). Our research showed that a drastic reduction in water irrigation negatively affected whole plant yield, chemical composition and nutrient availability of forage maize.

Introduction

Dairy farming in Europe is becoming more intensive and specialised with an increase in average herd size and milk yield per cow. Intensive dairy farming systems have been blamed of negative impact on environment and landscape, contributing to reduction of plant biodiversity, alterations of soil integrity, excessive use of fertilisers and water for irrigation and pollution (European Commission, 2000). Even if agricultural water demand in Europe varies considerably depending on climatic conditions, for most Mediterranean countries irrigation water for agricultural purposes exceeds 50% of the total national water requirement, particularly during summer (Wriedt *et al.,* 2008). Global climate change and competition among agricultural, domestic and industrial water users will unavoidably intensify problems of water scarcity in the Mediterranean regions (Goubanova and Li, 2007; IPCC, 2007; Rodriguez Diaz *et al.,* 2007).

In the current scenario, maize represents the main forage crop cultivated in intensive dairy farming, having high production performance and high concentration of energetic nutrients such as starch (Neylon and Kung, 2003; Borreani and Tabacco, 2010; Opsi *et al.*, 2013). To guarantee the maximum yield and quality production high amounts of inputs as fertilisers, herbicides and irrigation water are routinely used (Masoero *et al.,* 2010, 2011; Islam *et al.,* 2012). Furthermore, other management practices, such as plant maturity at harvest or class hybrid selection, should be considered to optimise corn crop yield (Opsi *et al*., 2013), as well as chemical composition and digestibility (Masoero *et al*., 2011; Opsi *et al*., 2013). However, in support to the European-wide policy development requiring a sustainable use of water resources (Wriedt *et al.,* 2008), several improvements are currently needed in terms of use of available water. In addition, the cost water for irrigation and the limited sources might force farmers to reduce irrigation application (Çakir, 2004).

Advancement in water irrigation usage could be the adoption of different water-saving irrigation schedules as opposed to the irrigation regime routinely used by farmers for maize growing for silage production. The aim is to minimise yield loss while maintaining the forage nutritive value in restricted irrigation water condition. Accordingly, Masoero *et al.* (2013) recently observed that reducing irrigation water by 26% in specific growth stages [VT, R1 and R2; classification proposed by Hill (2007)] could be suitable for new commercial maize hybrids while preserving similar whole-plant yield and forage nutritive value. On the contrary, Islam *et al.* (2012), studying the effect of four irrigation levels (0, 153, 305 and 480 mm of total water) in maize, reported decreased levels of irrigation water causing a linear decrease in yield, crude protein (CP) and water-soluble carbohydrate contents along with an increase in fibrous fractions. Similarly, Simsek *et al.* (2011), testing different amounts of irrigation water (on average 451 to 975 mm of water), reported a modification in chemical composition of maize grown in semi-arid conditions. Due to these equivocal results on the effect of irrigation water on nutritive value of maize silage and in a view to reducing irrigation water applied to maize, it appears crucial to investigate the response of maize grown under severe reduction of water irrigation in terms of chemical composition, rumen dry matter (DM) and fibre digestibility. The present study was therefore designed to evaluate in a practical farm condition the response of two commercial maize hybrids grown for silage production on chemical composition and digestibility of whole maize plant as consequence of a severe reduction of water irrigation. Results are important for nutritionists

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and stakeholders when feeding ruminants with maize silages made from planned or unexpected water stressed maize.

Materials and methods

Maize hybrids, field site and treatments

Two commercial maize hybrids (Dekalb-Monsanto; Agricoltura S.p.A., Lodi, Italy) belonging to the same maturity class (FAO rating 700) and selected for whole-plant silage production (hybrids DKC 6854 and DKC 6666 respectively for hybrids A and B) were used. The plant phenological growth stages were divided into vegetative stages, from VE (emergence) to VT (tasseling), and reproductive stages, from R1 (silking) to R6 (physiological maturity) as previously reported (Hill, 2007).

The experiment was carried out during the 2013 growing season in a climate location of the Po valley of Northern Italy (Cremona, Italy; N 45°9’, E 9°51’). Agronomical practices were in agreement with the code of the good agricultural practice (European Commission, 1991) and are summarised in Table 1. Irrigation water was provided by a drip sprinkler irrigation system and five irrigations were applied. The treatment consisted of different amounts of water supplied to the crop during vegetative growth stages. In particular, the reduction of irrigation water applied to water restricted (WR) irrigation regime plots with respect to fully irrigated (FI) plots were 50% on 20 June, 78% on 7 July, 80% on 15 July, 72% on 28 July, and 80% on 7 August. Based on these applications, WR and FI plots received 50 and 200 mm of water by irrigation, respectively. A graphical description of rainfall and irrigation schedule applied to WR and FI plots is given in Figure 1. The total cumulative amount of water (irrigation plus rainfall) supplied to WR and FI plots was 290 and 440 mm, respectively.

A split-plot factorial arrangement in a randomised complete block design with two main plots (WR and FI) and two sub-plots, consisting of the two maize hybrids (A and B) with 12 replications/treatment, was performed. The individual large-scale plots (5.25 m width and 133.5 m length) consisted of 8 rows of plants seeded at 70 and 21 cm inter and within rows distances, respectively. A 4 m space between WR and FI plots was planned to avoid osmotic effect of irrigation.

All plants were harvested on 15 October, with FI crops at 3/4 milk line stage of maturity of kernels (corresponding to an average whole plant DM content of 355.0±27.3 g/kg), and chopped to a particle length of about 1.8 cm with a harvest machine equipped with a 98D Series Maize Header (New Holland, Turin, Italy). Representative chopped samples (about 800 g) were collected from each plot, immediately dried at 55°C for 48 h in a ventilated oven before being stored until analysis. Three whole plants were also randomly selected from each experimental plot to estimate the whole plant and grain yields. Collected plants were used to measure harvest index (HI) calculated as the ratio between average dry grain yield and average total dry biological yield of plant (Hay, 1995).

Chemical analyses

Dry samples were ground (1-mm screen) using a laboratory mill (Thomas-Wiley; Arthur H. Thomas Co., Swedesboro, NJ, USA). Samples were assayed according to the AOAC (2000) for DM, CP (N×6.25), crude lipid (CL) and ash contents (methods 930.15, 976.05,

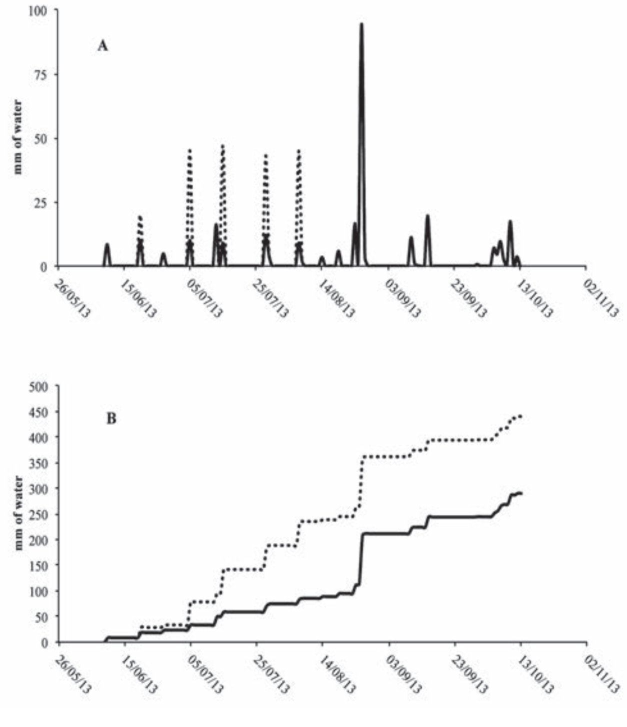

Figure 1. Absolute (A) and cumulative (B) total irrigation water plus rainfall applied to fully irrigated (dashed line) and water restricted regime (continuous line) plots.

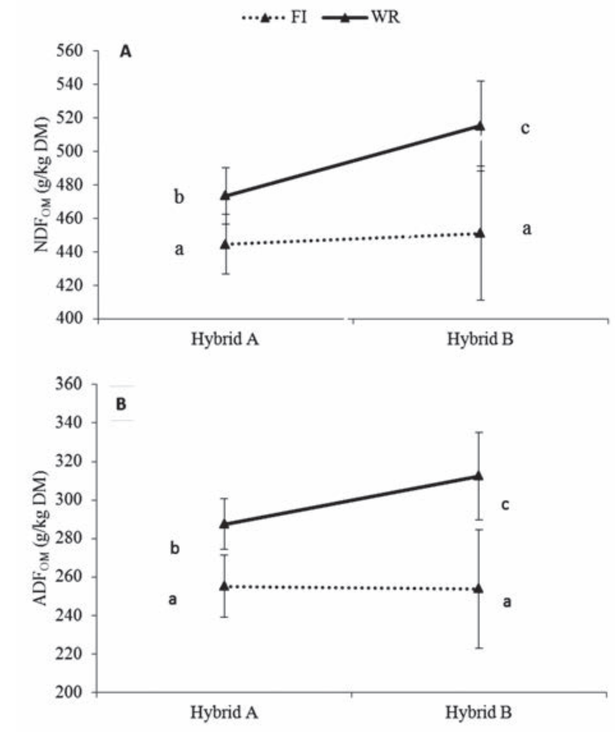

Figure 2. Means and standard deviation of NDF$_{OM}$ (A) and ADF$_{OM}$ (B) contents of two hybrids (A and B) grown under two tested irrigation regimes: fully irrigated (FI; dashed line) or water restricted (WR; continuous line). Means with different letters differ for P<0.05.

954.02 without acid hydrolysis and 942.05, respectively). Total starch content was determined by an enzymatic method (Masoero *et al.,* 2010). The neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (lignin) contents (Van Soest *et al.,* 1991) were determined using Ankom F57 filter bags in an Ankom200 fibre analyser (Ankom Technology, Macedon, NY, USA). A heat stable amylase (Sigma A-3306; Sigma-Aldrich® Co., St. Louis, MO, USA) and sodium sulfite (Carlo Erba 483257; Carlo Erba® Reagenti SpA, Milan, Italy) were used for NDF determination. The sulphuric acid (sa) method was used to analyse lignin [lignin(sa)]. Both NDF and ADF were expressed exclusive of residual ash and defined as NDF_{OM} and ADF_{OM} respectively, where OM stands for organic matter. All samples were analysed in duplicates.

In situ disappearances measurements

Rumen *in situ* disappearance at 48 h of rumen incubation of DM and NDF (DMD and NDFD, respectively) and indigestible NDF (iNDF) after 288 h of rumen incubation were determined in two rumen-fistulated dry cows. Animals were kept in tie stall under controlled environment and fed at maintenance (National Research Council, 2001) a total mixed ration (120 g/kg CP and 550 g/kg NDF_{OM}) composed by grass hay, maize silage and a protein-vitamin supplement (700, 200 and 100 g/kg DM, respectively). Research protocol and animal care were in agreement with European Commission Council Directive guidelines (European Commission, 1986). Milled samples (about 300 mg) were weighed into filter bags (Ankom F57, two bags *per* sample), introduced into a plastic net and

Table 1. Type of field soil, agronomical practices and characteristics of the current experiment.

Type of field soil	Silt-loam
Agronomical practices	
Previous crop	Italian ryegrass
Planting date	9 June 2013
Planting rate, plants/ha	≈68000
Planting depth, cm	4
Within-row spacing, cm	21
Inter-row spacing, cm	70
Fertiliser	100 kg N/ha as cow manure (4 March 2013); 160 kg N/ha (provided by 343 kg/ha of urea, 20 May 2013)
Herbicide°	4 kg/ha Lumax™ (13 June 2013)
Insecticide[#]	18 kg/ha Force™ (9 June 2013)
Water provided, mm	
From irrigations	FI=200; WR=50
From rainfalls	240
Total	FI=440; WR=290
Harvest date	15 October 2013

FI, fully irrigated regime; WR, water-restricted regime. °From Syngenta crop protection SpA (Milan, Italy). Lumax™ active ingredients are: terbutilazin, 169 g/kg; S-metolachlor, 282 g/kg; mesotrione, 34 g/kg. [#]From Syngenta crop protection SpA (Milan, Italy). Force™ active ingredient is: tefluthrin, 5 g/kg.

Table 2. Effect of water restriction on yield and chemical, biological and energy characterisations of whole maize plants.

	FI		WR°		$\sqrt{\text{MSE}}$	Main effects (P)			
	A	B	A	B		Block	H	T	H×T
Yield, t DM/ha	24.7	23.9	18.7	16.9	1.76	ns	ns	<0.05	ns
Grain yield, t DM/ha	12.5	12.6	9.1	8.2	0.81	ns	ns	<0.05	ns
DM, g/kg	345	364	445	469	4.9	ns	ns	<0.05	ns
Harvest index[#]	0.505	0.529	0.485	16.9	0.0320	ns	ns	<0.05	ns
Chemical composition, g/kg DM									
Starch	273	293	235	234	20.7	ns	ns	<0.05	ns
CP	67	70	68	68	3.4	ns	ns	ns	ns
CL	19	19	19	19	2.7	ns	ns	ns	ns
Ash	40	39	39	39	1.7	ns	ns	ns	ns
NDF_{OM}	444	451	473	515	26.2	ns	<0.05	<0.05	<0.05
ADF_{OM}	255	253	287	318	20.3	<0.05	ns	<0.05	<0.05
Lignin(sa)	36	38	44	54	16.2	ns	ns	<0.05	ns
Digestibility parameters									
DMD, % DM	57.0	61.3	54.5	58.3	1.08	ns	<0.05	<0.05	ns
NDFD, % NDF_{OM}	58.6	63.4	56.2	60.6	1.94	ns	<0.05	<0.05	ns
iNDF, % DM	9.5	9.5	14.5	15.5	0.10	ns	ns	<0.05	ns
7hIVSD, % starch	82.5	81.2	82.8	81.0	14.12	ns	ns	ns	ns
Energy evaluation[§]									
$\text{NE}_{\text{L3x-ADL}}$, Mcal/kg	1.40	1.46	1.35	1.28	0.151	ns	ns	<0.05	ns
$\text{NEL}_{\text{3x-48h}}$, Mcal/kg	1.46	1.52	1.41	1.42	0.126	ns	ns	<0.05	ns

FI, fully irrigated plots; WR, water restricted plots; $\sqrt{\text{MSE}}$, root mean square error; A, hybrid A; B, hybrid B; H, hybrid; T, water treatment; DM, dry matter; CP, crude protein; CL, crude lipid; NDF_{OM}, ash-corrected neutral detergent fibre; ADF_{OM}, ash-corrected acid detergent fibre; lignin(sa), lignin analysed with sulphuric acid method; DMD, rumen *in situ* DM disappearance; NDFD, neutral detergent fibre disappearance; iNDF, indigestible NDF; 7hIVSD, 7h *in vitro* starch degradability; NE, net energy; ns, not significant at P>0.05. °The total amount of irrigation water supplied to FI and WR plots was 200 and 50 mm, respectively. [#]Calculated as the ratio between average dry grain yield and average total dry biological yield of plant. [§]Net energy values for lactation were estimated according to National Research Council (2001) and based either on chemical parameters ($\text{NE}_{\text{L3x-ADL}}$) or both chemical parameters and NDFD ($\text{NE}_{\text{L3x-48}}$h).

then incubated (Gallo *et al.,* 2013). Then, bags were removed from rumens after 48 and 288 h and carefully washed by hand under tap water until the washing water remained clear before being dried in a forced-air oven to constant weight at 60°C for 48 h. Dried bags were weighed to determine the residual DMD. The residual content of bags was then analysed for NDF_{OM} and the NDFD was calculated and expressed as a proportion of NDF_{OM}, whereas iNDF was expressed as a proportion of DM content.

In vitro starch degradability

The 7h *in vitro* starch degradability evaluation (7hIVSD) was performed with the *in vitro* method as proposed by Sveinbjörnsson *et al.* (2007). Rumen fluid was collected from the same two fistulated dry cows used for the *in situ* study and 6 h after the morning feeding. Collected rumen liquors were combined, carefully filtered through two layers of cheesecloth, maintained in a warm insulated flask filled with CO_2 and used within 20 min. About 200 mg of starch *per* each sample were incubated (in a shaking water bath at 39°C with 50 rpm) with 30 ml buffered rumen fluid solution (buffer to rumen ratio of 2:1 v/v; Sveinbjörnsson *et al.,* 2007) into 125 mL-glass bottles equipped with rubber stoppers. An anaerobic environment was maintained throughout the experiment by continuous CO_2 flushing. Blanks (three bottles with only the diluted rumen fluid) and internal standard (Gelose 80 maize starch; Penford Food Ingredients Co., Centennial, CO, USA) were included. After 7h of incubation, samples were collected from bottles and the remaining starch was enzymatically determined (Sveinbjörnsson *et al.,* 2007). Within each bottle, the 7hIVSD was calculated as the ratio between the amount of starch disappeared and the amount of starch in the sample before incubation, after correction for blanks. Samples were analysed in duplicate in 3 separate runs, and bottles within runs were considered repetitions, whereas bottles between runs as replicates.

Net energy calculation

The net energy (NE) content of collected samples was calculated in agreement to National Research Council (2001). In particular, two energy estimations, one based on chemical parameters (*i.e.*, $NE_{L3x\text{-}ADL}$) and the other based on both chemical and NDFD digestibility parameters (*i.e.*, $NE_{L3x\text{-}48h}$), were calculated. The neutral and acid detergent-insoluble CP fractions were obtained from tabular data (National Research Council, 2001). Details on specific equations used to compute NE calculation have been extensively reported by Gallo *et al.* (2013).

Statistical analyses

Data were tested for normality distribution with the Shapiro-Wilk test and analysed by MIXED procedure of SAS (2003) according to a split-plot factorial arrangement in a randomised complete block design. The fixed effects in the model were water treatment (n=2, whole plot), maize hybrid (n=2, sub-plot) and associated first order interaction (water treatment×maize hybrid). The block effect entered the model as random and the sub-plot as experimental unit. Means were declared different for $P \leq 0.05$.

Results

Total DM and grain yields differed ($P<0.05$) between FI and WR crops, being on average 24.3 *vs* 17.8 t DM/ha and 12.5 *vs* 8.6 t DM/ha, respectively. The HI was lower in WR than FI, being on average 0.486 *vs* 0.517, respectively ($P<0.05$), whereas similar yield performance values were reported considering hybrids. On average, the WR plants showed a DM content 22.4% higher ($P<0.05$) than FI at harvest time. No effect of hybrid was measured for performance parameters.

Overall, chemical composition was markedly influenced by the applied irrigation regime and slightly differed between the two hybrids. On average, when compared to FI plants, WR had lower (17.1%; $P<0.05$) starch content and higher ($P<0.05$) fibre contents. In particular NDF_{OM}, ADF_{OM} and lignin(sa) contents increased ($P<0.05$) by 9.4, 16.0 and 24.5% in WR plants when compared to FI. Effects were numerically higher in hybrid B than hybrid A (hybrid water treatment interaction $P<0.05$; Figure 2), except for lignin(sa). Digestibility parameters differed ($P<0.05$) both between hybrids and irrigation treatments. In particular, higher ($P<0.05$) DMD (59.2 *vs* 56.4% DM) and NDFD (61.0 *vs* 58.4% NDF_{OM}) values were respectively measured in FI and WR crops, whereas iNDF resulted about 36% higher ($P<0.05$) in WR than FI. The 7hIVSD was similar between irrigation regimes and hybrids.

Lastly, the $NE_{L3x\text{-}ADL}$ of whole maize plants was similar between hybrids, whereas the WR plants had a lower ($P<0.05$) energy content than FI plants. In particular, $NE_{L3x\text{-}ADL}$ and $NE_{L3x\text{-}48h}$ were 1.43 *vs* 1.32 Mcal/kg and 1.49 *vs* 1.42 Mcal/kg, respectively for FI and WR plots ($P<0.05$).

Discussion

In our previous experiment (Masoero *et al.,* 2013) four commercial maize hybrids were grown under two imposed water irrigation regimes: FI and WR. In particular, the water saving schedule reduced by 26% the irrigation water by skipping irrigations during specific plant growth stages (VT, R1 and R2). The observed lack of differences on yield and nutritive value between the two irrigation schedules was explained in a good aptitude of tested hybrids in using residual water from soil along with two other conditions, such as amount of water saved and specific growth stages in which water was reduced. However, other authors (Simsek *et al.,* 2011; Islam *et al.,* 2012) reported negative effects on yield, chemical composition and nutrient digestibility of whole maize crops subjected to water restriction regimes. Taking into account that more severe water shortages were tested in these aforementioned experiments, the intent of this study was to reduce irrigation water even more drastically. Consequently, irrigation water was reduced by 75% in WR compared to FI plots. However, the meteorological conditions of 2013 growing season were characterised by frequent rain events, especially during the reproductive growth stage of maize. Thus, water restriction was imposed mainly during the vegetative phase, the water from rains being 240 mm and representing about 50% of total water requirement for the crop.

In agreement with previous findings (Kamara *et al.,* 2003; Yazar *et al.,* 2009; Nelson and Al-Kaisi, 2011), the limited water available for crops reduced both whole-plant and grain yields. Correspondingly, several researchers reported a negative relationship between crop yield performances and water availability (Çakir, 2004; Payero *et al.,* 2006). In addition, the extent of yield reduction depends not only on the severity of water shortage, but also on the stage of the plant development (Çakir, 2004). Accordingly, previous works indicated that water restriction during vegetative grown stages caused several undesirable effects in crops. In particular, plant stand damage, decreased number, growth and expansion of leaves, a minor stem cell expansion, a lower number of kernels for ear, a general lower rate of photosynthesis and changes in the metabolite composition have been reported, thus concurring to reduce plant development, nutrient production and mobilisation to kernels (Guelloubi *et al.,* 2005; Rusere *et al.,* 2012; Witt *et al.,* 2012).

The main focus of our research was to verify the effect of irrigation shortage on chemical composition and nutrient digestibility of whole maize plant, as well as their energy content (Table 2). Concerning the nutritional aspects, few and contrasting results were reported, probably related to specific experimental conditions (growth stage, water deficiency level and environmental changes during water drought) and to the adaptability of tested plants. Recently, Islam *et al.* (2012) observed a linear decrease in CP and in water-soluble carbohydrate contents and a linear increase in DM and NDF when increasing the supply of irrigation water. Contrarily, Ali *et al.* (2010) reported water stress reduced the kernel sugar, protein and moisture and increased ash and fibre contents, without affecting the kernel starch amount. Data from Ge *et al*. (2010) indicated crops grown under moderate (soil relative water content of 60%) or severe (soil relative water content of 40%) stress conditions reduced grain yield from 60 to 80% and starch content from 8 to 33% with respect to the full water supply regime (soil relative water content of 80%). Lastly, Schittenhelm (2010) reported a similar chemical composition of maize grown under different water restriction regimes (60 to 80%, 40 to 50% and 15 to 30% plant-available soil water respectively for high, medium and low water supply). Overall, findings of the current trial indicated that plants belonging to the WR regime were characterised by a higher DM and fibre contents and a lower amount of starch compared to FI. Likewise, Curran (2002) indicated that water stressed maize might yield lower starch content silages, since the presence of less grain in silage, as confirmed by the lower HI measured in WR with respect to FI. Presumably, the lower water supply during the vegetative grown stages in WR crops could have altered enzyme activities and therefore affecting the metabolism of tested plants with changes in translocation and accumulation of assimilates (Bruce *et al.,* 2002; Ali *et al.,* 2010), thus resulting in a different chemical composition. Accordingly, Beckles and Thitisaksakul (2013) indicated that drought-stressed cereals showed a reduced starch accumulation by up to 40%, due to alteration in starch biosynthetic enzyme activity. In addition, having all crops being harvested concomitantly, the WR showed higher amount of DM at harvest compared to FI crops, since a probable advanced stage of maturity due to a reduced maize growth stage in the WR crops could have occurred.

Considering nutrient digestibility parameters, our findings are in agreement with Simsek *et al.* (2011), where a decrease of *in vitro* DMD with decreasing amount of irrigation water in maize has been reported. In our condition, the higher amount of $\mathrm{ADF_{OM}}$ and lignin(sa) could have reduced the potential digestibility of WR maize, being iNDF highly correlated to ADF and lignin(sa) more than NDF fractions (Gallo *et al.*, 2013). In addition, the lack of differences in the 7hIVSD between hybrids and water treatment could be imputed, at least in part, to the high variability of the used *in vitro* method (Giuberti *et al.,* 2014) rather than the effect of water treatment *per se*. From the foregoing results and evidences reported in aforementioned literature, it could be retained that a drastic water shortage during corn crop grown can negatively impact not only crop yield, but also chemical composition and nutrient digestibility. However, further studies should be carried out on several sites or over different years to confirm present findings.

Conclusions

In conclusion, present findings indicate that a drastic reduction in water irrigation compromises maize whole plant yield, chemical composition and nutrient availability of maize in terms of starch and fibre, thus resulting in an energy loss and a lower nutritional value. As a consequence, when feeding such type of maize silage, diets for lactating dairy cows should be reformulated to cover milk yield requirements and rumen functionality. However, considering contrasting data reported in literature, further investigations are needed to improve knowledge concerning the response of whole plant maize under different water irrigation treatments.

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