

Chemical composition and *in vitro* digestibility of whole-crop maize fertilized with synthetic fertilizer or digestate and harvested at two maturity stages in Boreal growing conditions

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Maize cultivation for silage could be a sustainable option in Boreal conditions, especially when combined with nutrient recycling. Effects of digestate (sludge from biogas of domestic origin) application in comparison with synthetic fertilizer and two maturity stages on chemical composition and *in vitro* digestibility of whole-crop maize were investigated. Starch, neutral detergent fiber, water soluble carbohydrate (WSC) and digestible organic matter (DOM) contents of maize did not differ in response to the two fertilizer treatments. However, starch, DOM and metabolizable energy of maize increased, while ash, crude protein and WSC contents decreased with increasing maize maturity. Heavy metals in maize fertilized with digestate remained low. The results indicate that whole-crop maize fertilized with digestate and harvested at 150 days after sowing is a promising feed and has good nutritive value, even in Boreal conditions.

Key words: digestate, heavy metals, *in vitro* digestibility, nutritive value, *Zea mays* L.

Introduction

Whole-crop maize (*Zea mays* L.) is an important and widely investigated forage for ruminants (Bal et al. 1997, Jensen et al. 2005, Cone et al. 2008, Khan et al. 2015), and is also used as an energy crop (Ericsson and Nilsson 2006, Seleiman et al. 2013). It has been used to produce silage because of its high nutritive value and potential for high yield in a single harvest (e.g. Phipps et al. 2000, Kirkland et al. 2005, Njoka et al. 2005). The growth of maize typically benefits from high temperatures and short days which has limited its use as feed in Boreal conditions. Although early maturing varieties have become available that are better adapted to the climate of northern Europe (Jensen et al. 2005, Mussadiq et al. 2012) the implementation of maize as feed requires more knowledge on factors affecting the development pattern and nutritional characteristics of maize grown in long day conditions.

Maturity is an important preharvest factor that affects nutrient content, digestibility and ensilability of forages (Mussadiq et al. 2012, Lynch et al. 2013). Advancing maturity is known to increase dry matter (DM) and starch contents and decrease neutral detergent fiber (NDF) and crude protein (CP) contents of maize, and the DM content in particular has been used as an indicator of optimum harvest time associated with ensiling and feeding quality (Johnson et al. 2002). Dry matter content of ensiled crop higher than 250 g kg⁻¹ is desirable to avoid effluent and nutrient losses and to improve crop ensilability characteristics (McDonald et al. 1991).

The positive responses of replacing high-quality grass silage with moderate quality (DM 257 g kg⁻¹, starch 219 g kg⁻¹ DM) maize silage (O'Mara et al. 1998) support growing maize even in zones where maize does not get its thermal requirement, and consequently does not mature optimally or reach optimal DM content. In addition, maize can be grown with organic fertilizers to reduce the demand of synthetic fertilizers and to recycle nutrients from the organic sources (Pain et al. 1989, Jarvis 1993).

Anaerobic digestion has become an alternative option for recycling nutrients available in organic residues from e.g. domestic, agricultural and industrial sources (Odlare et al. 2008, Massé et al. 2011, Albuquerque et al. 2012, Insam et al. 2015, Gómez-Brandón et al. 2016). The major advantages of anaerobic digestion are renewable energy production (biogas), conservation of natural resources through using the end-product (i.e. digestate) as fertilizer and soil amendment (Stinner et al. 2008, Möller and Stinner 2009). Digestate contains high level of phosphorus (P)

and nitrogen (N) (Seleiman et al. 2012). N applied into soil through the application of digestate is rapidly available for plant growth during short-term (Albuquerque et al. 2012). However, a slow rate of microbial activities owing to the low temperature in the soil might reduce the capacity of digestate as fertilizer (Albuquerque et al. 2012). Digestate application improves chemical and microbiological properties of the soil (Odlare et al. 2008, Albuquerque et al. 2012), plant growth and quality (Albuquerque et al. 2012, Kouřimská et al. 2012, Seleiman et al. 2012).

The objective of the current study was to investigate the chemical composition and *in vitro* digestibility of whole-crop maize fertilized with synthetic fertilizer or digestate and harvested at two maturity stages.

Materials and methods

Plant material and experimental design

The plant material originates from a previously published investigation on suitability of forage maize for bioenergy in Boreal conditions (Seleiman et al. 2013). Three field experiments were conducted at the Viikki Experimental Farm (60° 13' N, 25° 10' E, 3 m a.s.l., average length of growing season 175 days), University of Helsinki, Finland. Maize (cv. Ronaldinio, intermediate maturity class FAO 240, KWS SAAT, Einbeck, Germany) was developed for the majority of mainstream sites (270–310 g kg⁻¹ DM) and is categorized as a fodder cultivar.

Soil samples were collected annually prior to sowing from topsoil (0–20 cm depth), and were analyzed at Viljavuuspalvelu Oy, Mikkeli (2009–2010) and Suomen Ympäristöpalvelu Oy, Oulu (2011), Finland (Table 1). According to the WRB system (IUSS Working Group) the fields are typically Luvic Gleysols or Luvic Stagnosols (Michéli et al. 2006). In 2009 and 2010, the plots were sown on organic medium clay, while in 2011 they were sown on organic sandy clay.

Fertilization treatments, synthetic fertilizer and digestate (sludge from biogas of domestic origin), were estimated on the basis of total N needed for maize (i.e. 120 kg N ha⁻¹) taking into account the soil fertility. The 100% digestate (3.87 t digestate ha⁻¹) contained 11.60 kg ammonium (NH₄⁺), 0.02 kg nitrate (NO₃) and 108.38 kg organic N. The 100% synthetic fertilizer (120 kg N ha⁻¹, N-P-K:28-3-5, Cemagro Oy, Lohja, Finland) contained 58.50 kg NO₃ and 61.50 kg NH₄⁺. The amount of digestate was calculated based on the total N content (Table 1). Both fertilizers were applied prior to seeding. The digestate was tilled to a depth of 7 cm. Sowing dates were 13 May in 2009, 16 May in 2010 and 15 May in 2011. The sowing density of maize was about 95200 plants ha⁻¹, and distance between rows was 70 cm and plant spacing was 15 cm. Weeds were controlled using 2.3 kg ha⁻¹ Lentagran WP (pyridate 450 g kg⁻¹, Belchim Crop Protection) at 25 days after sowing (DAS) (BBCH stage 13, Meier 2001). There was no supplementary irrigation during the growing seasons. The precipitation level during the growing seasons of 2009, 2010 and 2011 was 343, 288 and 393 mm; while the average temperature during those growing seasons was 14.5, 15.7 and 15.8 °C, respectively (Table 2). The field experiment was performed in a randomized complete block design with four replicates. Plot size was 20 m² (8 x 2.5 m).

Table 1. Chemical properties of soil and digestate used during the three years (2009–2011)

Parameter	Soil properties			Digestate
	2009	2010	2011	
pH (1:5)	6.40	6.30	6.20	7.20
N	20.4 ^b	14.1 ^b	14.6 ^b	31.0 ^c
P	17 ^b	19 ^b	1650 ^a	26 ^c
K	300 ^b	300 ^b	8050 ^a	2 ^c
Mn	9 ^b	4 ^b	270 ^a	220 ^a
Cd	0.3 ^a	0.6 ^a	0.5 ^a	0.4 ^a
Cr	35 ^a	48 ^a	72 ^a	30 ^a
Cu	53 ^a	77 ^a	91 ^a	270 ^a
Pb	30 ^a	74 ^a	97 ^a	20 ^a
Zn	96 ^a	180 ^a	90 ^a	470 ^a
As	7.1 ^a	7.8 ^a	9.6 ^a	5.0 ^a

a = mg kg⁻¹; b = mg l⁻¹; c = g kg⁻¹

N, P, K and Mn for soil are presented in soluble form, whereas all elements for digestate are presented in total concentration.

Table 2. Long-term (1971–2000; FMI 2011) and monthly means for temperature and precipitation during three growing seasons at Kaisaniemi, located near Viikki, Finland

Month	Temperature (°C)				Precipitation (mm)			
	2009	2010	2011	1971–2000	2009	2010	2011	1971–2000
April	4.5	4.7	6.0	3.3	8	42	29	36
May	11.0	11.9	10.8	10.0	45	59	27	32
June	14.1	15.1	17.4	14.6	77	33	49	49
July	17.5	22.4	20.9	16.9	131	49	56	62
August	17.0	17.7	16.9	15.3	50	97	173	78
September	13.1	11.5	12.9	10.1	40	50	88	66
October	4.3	5.1	7.6	5.2	90	29	69	73

Measurements and analyses

The whole-crop maize samples of 1.0 m² from each plot during the three growing seasons were manually harvested twice during ripening at 120 DAS (BBCH stage 83, early dough stage: kernel content soft; Meier 2001) and 150 DAS (BBCH stage 85, dough stage: kernels yellowish to yellow) on 15th of September and 15th of October, respectively. The plants were cut at ground level and weighed. Then plant samples were cut into small pieces and dried at +105 °C for 72 h and weighed (dry weight) to obtain dry matter content. Dried samples were ground (ZM 200, Retsch GmbH, Haan, Germany) into a fine powder (0.5 mm mesh size) and stored at room temperature for further analysis.

The ash content of whole-crop maize was analyzed from 1 g subsamples by complete combustion in a muffle furnace (LV 15/11/P320, Nabertherm GmbH, Bremen, Germany) as described by Seleiman et al. (2012). The total N content of whole-crop maize was analyzed (Seleiman et al. 2013) from 200 mg subsamples using the Dumas combustion method (VarioMAX CN, Elementar Analysensysteme GmbH, Hanau, Germany). N content was multiplied by 6.25 to obtain the crude protein content for each sample (Horwitz 1980).

Starch content of a 100 mg ground sample (0.5 mm) was measured with an assay kit (K-TSTA-50A/K-TSTA-100A, Megazyme, Wicklow, Ireland) and protocol supplied by the manufacturer using the UV-spectrophotometer (Model UV-1800 240V IVDD, Shimadzu Inc., Kyoto, Japan).

Water-soluble carbohydrates were analyzed at 630 nm in a spectrophotometer (Model UV-1800 240V IVDD, Shimadzu Inc., Kyoto, Japan) using a method based on anthrone assay (Yem and Willis 1954) with modifications related to sample size, which was decreased to 30 mg.

Neutral detergent fiber (aNDFom) was analyzed using a Tecator Fibertec system (1020 Hot extractor and 1021 Cold extractor, FOSS, Hillerød, Denmark) according to Van Soest et al. (1991) with sodium-sulfite and α -amylase and was expressed exclusive of residual ash.

Digestible organic matter content in DM (DOMD) was analyzed using a method based on pepsin-cellulase solubility (Friedel 1990) with the modifications of Nousiainen et al. (2003). The results were calculated using a correction equation to convert pepsin-cellulase solubility to *in vivo* OM digestibility (Huhtanen et al. 2006). Metabolizable energy content (ME) was calculated according to Luke (2015) as $ME (MJ) = 0.0155 \times DOMD$.

For determination of heavy metals and metalloids (i.e. As, Cd, Cr, Cu, Pb and Zn), subsamples of 300 mg were digested in nitric acid with microwave heating, filtered and analyzed in an Inductively Coupled Plasma-Optical Emission Spectrometer (iCAP 6200, Thermo Fisher Scientific Inc., Cambridge, United Kingdom) as described by Seleiman et al. (2012), with every 20th sample a standard. All glassware was acid-washed to avoid chromium contamination.

Statistical analysis

The effects of harvest dates, fertilizer treatments, years and their interactions as fixed effects on dry matter content, chemical composition and *in vitro* digestibility of whole-crop maize were tested with split split-plot analysis of variance (ANOVA) using Predictive Analytics Software (PASW) statistics 20.0 (IBM Inc., Chicago, IL, United States of America). The standard errors of the means (SEM) were determined using PASW. Statistical significance was established at $p \leq 0.05$ for all analyses.

Results

No statistically significant interactions for fertilizer x harvest date x year were recorded (data not shown) while the significant two-factor interactions are presented in Table 3 (fertilizer x harvest date) and Figs. 1 and 2 (year x harvest date). There were no effects of fertilizer treatments (synthetic fertilizer and digestate application), harvest dates (120 and 150 DAS) or their interactions on the whole-crop maize DM content. However, in 2010 DM content of whole-crop maize was lower at 150 DAS compared to 120 DAS while in 2009 and 2011 it was higher or the same (interaction year x harvest date $p < 0.001$) (Fig. 1).

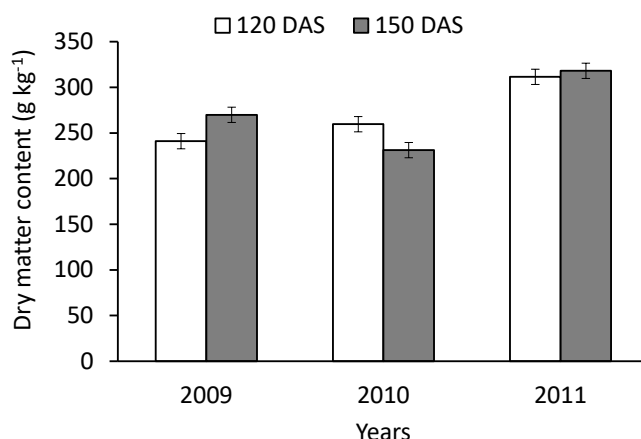


Fig. 1. The interaction between harvest date and year for dry matter content of whole-crop maize. Data shown are means \pm standard errors of means.

Delaying harvest from 120 DAS to 150 DAS decreased ($p < 0.001$) ash content (Table 3). However, the ash content was lower with digestate than with synthetic fertilizer at 120 DAS while it was higher with digestate at 150 (interaction $p < 0.01$). Digestate application resulted in lower ($p < 0.01$) CP content in whole-crop maize compared with fertilizing maize with synthetic fertilizer. Also, CP content obtained from whole-crop maize harvested at 150 DAS was lower ($p < 0.01$) than that harvested at 120 DAS (Table 3).

Table 3. Chemical composition, *in vitro* digestibility, DOMD and ME content at 120 and 150 DAS for whole-crop maize fertilized with synthetic fertilizer or digestate averaged over three years

Harvest dates	Fertilizer	Ash	CP	Starch	WSC	aNDFom	DOMD	OMD	ME
		(g kg ⁻¹ DM)					(MJ kg ⁻¹ DM)		
120 DAS	Synthetic fertilizer	61.3	88.9	107	126	573	654	686	10.1
	Digestate	58.6	82.1	114	121	563	654	687	10.1
150 DAS	Synthetic fertilizer	45.3	82.4	220	67	565	681	714	10.6
	Digestate	47.2	73.6	207	60	554	700	732	10.8
SEM		0.83	2.18	11.3	4.9	14.0	3.4	3.4	0.05
Significance (p)	Harvest dates (H)	<0.001	<0.01	<0.001	<0.001	0.520	<0.001	<0.001	<0.001
	Fertilizer (F)	0.629	<0.01	0.821	0.209	0.459	0.336	0.330	0.211
	H x F	<0.01	0.633	0.340	0.913	0.981	0.326	0.321	0.200

DAS = Days after sowing; WSC = Water soluble carbohydrate; CP = Crude protein; DMOD = Digestible organic matter content in dry matter; OMD = Organic matter digestibility; ME = metabolizable energy; aNDFom = Neutral detergent fiber; SEM = Standard error of mean

Digestate application resulted in similar starch contents in whole-crop maize as for synthetic fertilizer at 120 or at 150 DAS (Table 3). However, whole-crop maize harvested at 150 DAS had higher ($p < 0.001$) starch content than that harvested at 120 DAS. Overall, starch content in whole-crop maize was increased by 113 g kg⁻¹ DM with synthetic fertilizer and by 93 g kg⁻¹ DM with digestate when the harvest of maize was delayed until 150 DAS. However, at 150 DAS the lowest starch content (146 g kg⁻¹ DM) of whole-crop maize was recorded in 2010, while much higher starch contents (242 and 252 g kg⁻¹ DM) were recorded in 2009 and 2011, indicating interaction between harvest date and year factors ($p < 0.001$) (Fig. 2).

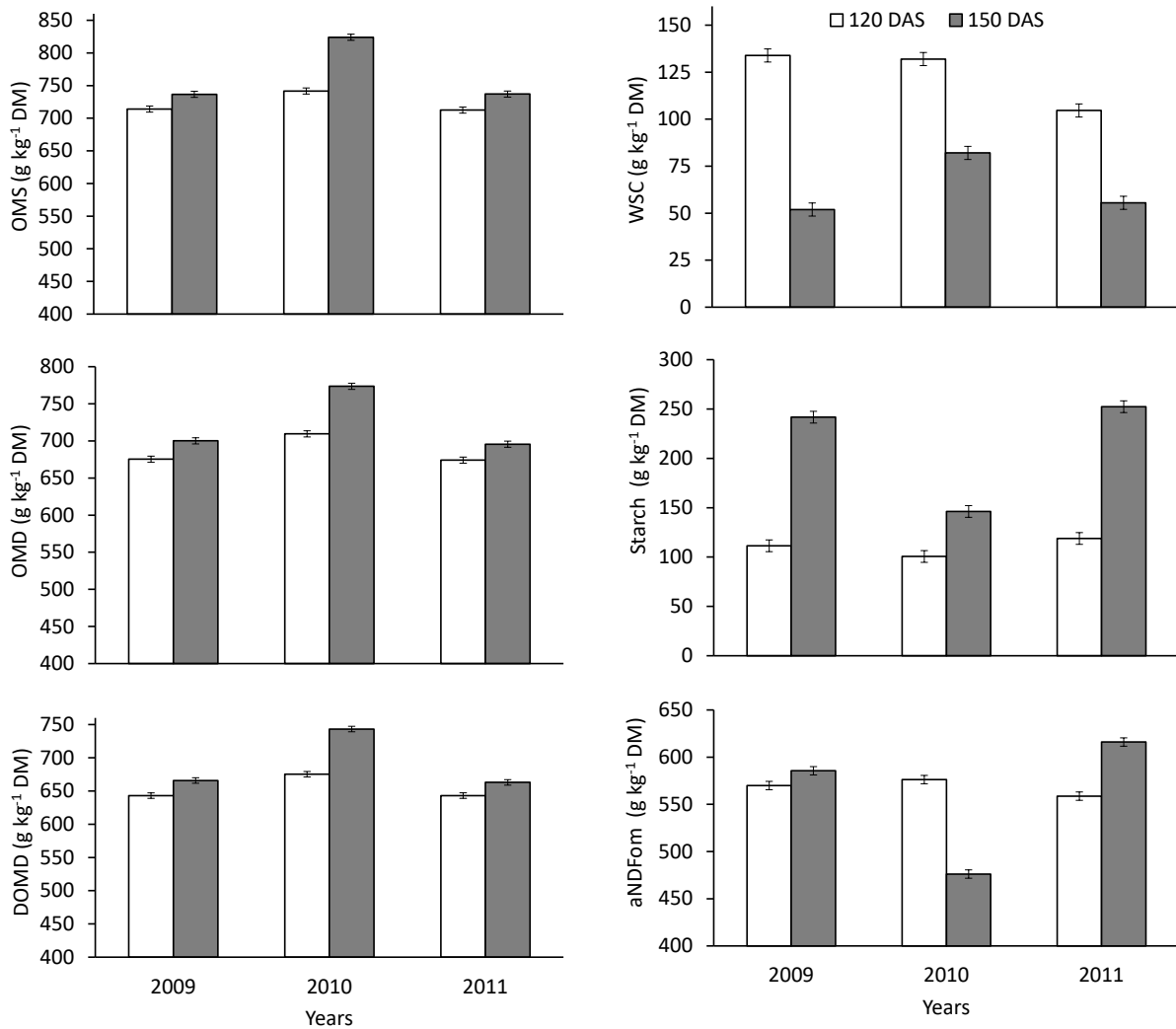


Fig. 2. The interaction between harvest date and year on the chemical composition, organic matter solubility (OMS) and digestibility (OMD) and digestible organic matter in dry matter (DOMD) of whole-crop maize. Data shown are means ± standard errors of means.

There were no differences between the fertilizer treatments on the WSC content of maize. The WSC content of whole-crop maize harvested at 120 DAS was higher ($p < 0.001$) than for that harvested at 150 DAS, while the differences for WSC of whole-crop maize between harvest dates was higher in 2009 than in 2010 and 2011 (interaction $p < 0.05$) (Fig. 2). The content of aNDFom in whole-crop maize was not affected by the fertilizer treatment (Table 3). In 2010, aNDFom content was clearly lower at 150 DAS than at 120 DAS, while in 2009 and 2011 the content was higher at 150 DAS than 120 DAS (interaction $p < 0.05$) (Fig. 2). The highest aNDFom content (616 g kg⁻¹ DM) in whole-crop maize was recorded at 150 DAS during 2011.

Organic matter digestibility, DOMD and ME content in whole-crop maize were not affected by the fertilizer treatments. However, they were higher ($p < 0.001$) in maize harvested at 150 DAS than when harvested at 120 DAS (Table 3). Markedly higher values of DOMD and ME for whole-crop maize were recorded when harvested at 150 DAS during 2010 in comparison with maize harvested during 2009 or 2011 (interaction $p < 0.05$) (Fig. 2).

Digestate application resulted in a slightly higher content of heavy metals and metalloids in whole-crop maize compared with that fertilized with synthetic fertilizer, but the increase was not significant ($p > 0.05$) (Fig. 3). Whole-crop maize fertilized with digestate contained As 0.71, Cd 0.10, Cr 7.33, Zn 62.00 mg kg⁻¹ DM, while maize fertilized with synthetic fertilizer contained As 0.56, Cd 0.09, Cr 6.42, Zn 58.00 mg kg⁻¹ DM.

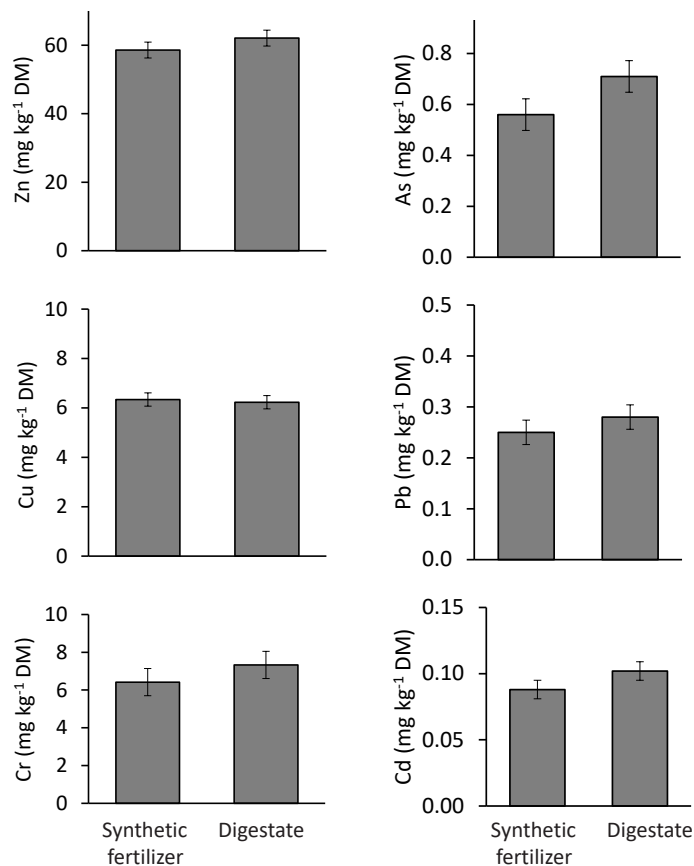


Fig. 3. Micro-element (mg kg⁻¹ DM) content at 150 DAS in whole maize plants fertilized with synthetic fertilizer and digestate. Data shown are means averaged over three years \pm standard error of means.

Discussion

The dry matter content did not differ when maize was fertilized with synthetic fertilizer or digestate. Neither was there an effect of harvest date on the average DM content. This was a result of the lower DM content at 150 DAS than at 120 DAS in 2010. The minor increase in DM content between the harvest dates in 2009 and 2011 was comparable with the results of Lynch et al. (2013) who reported pre-ensilage chemical composition of different maize hybrids harvested in September and October in Ireland. However, in the present experiment the advancing maturity between 120 DAS and 150 DAS was evidenced by doubled starch content. Thus, the DM content of whole plant is not necessarily an accurate indicator of the maturity stage. The importance of interactions between environmental and genetic factors on the development of forage maize was shown in the investigation of Mussadiq et al. (2012). In their two year study at northern latitudes (56°N–59°N) the DM content of three forage maize hybrids varied between 160–370 g kg⁻¹ at approximately 150 DAS. Khan et al. (2015) concluded that the DM intake, milk yield and milk protein content increased with advancing maturity, reaching an optimum level for maize ensiled at DM contents of 300–350 g kg⁻¹, and then declined slightly at further maturity beyond 350 g kg⁻¹. It should be noted that in the present experiment samples were taken at harvest, and that the effect of DM content on the success of ensiling process may modify the feeding value of maize silage.

Crude protein content in whole crop maize decreased at 150 DAS (78.0 g kg⁻¹ on average) in comparison with harvest at 120 DAS (85.5 g kg⁻¹ on average). The results are consistent with findings of other studies showing CP content decrease from 84.7 to 82.2 g kg⁻¹ (Johnson et al. 2002), and from 99.1 to 90.5 g kg⁻¹ (Lynch et al. 2012) with increasing maturity. The average CP content of maize was higher with synthetic fertilizer (85.7 g kg⁻¹ DM) than with digestate treatment (77.9 g kg⁻¹ DM). This might be due to the slightly higher DM yield of maize fertilized with digestate in comparison with maize fertilized with synthetic fertilizer (Seleiman et al. 2013).

The increase in starch content of whole-crop maize and the parallel reduction in the content of water-soluble carbohydrate, with the later harvest, were in accord with Hunt et al. (1989), Filya (2004) and Lynch et al. (2012). This can be related to the increased contribution of cob to the whole-crop DM yield and accumulation of starch in

the grains during the maturation period (Cone et al. 2008, Lynch et al. 2012). The starch content of maize reached at 150 DAS in 2009 and 2011 ($250 \text{ g kg}^{-1} \text{ DM}$) was comparable with starch contents in early maturing hybrids in Ireland with later harvest dates of 174 DAS in 2008 and 168 DAS in 2009 (Lynch et al. 2012, 2013). Silage made from whole-crop maize necessitates high starch content and consequently a high fraction of well-matured cob because sufficient digestibility of stover components will only sustain levels of animal performance that can be attained by using average quality of grass silage (O’Kiely and Moloney 1995). The reduction of starch in whole-crop maize at 150 DAS during 2010 was due to the reduction of the DM yield (Seleiman et al. 2013), which resulted from bird damage to the developing ears.

Cutting at high stubble height (45.7 cm) resulted in lower aNDFom and N content and higher starch content of whole-crop maize compared with cutting at 12.7 cm (Neylon and Kung 2003). Thus cutting at ground level in the current experiment probably slightly overestimated the aNDFom content and underestimated DOMD value of the maize samples compared with potential values at a higher cutting height. The study of Kruse et al. (2008) showed that most of the variation in aNDFom content was explained by harvest maturity followed by maturity type and environmental conditions.

The DOMD value and consequently ME value of the whole-crop maize increased with delaying harvest from 120 DAS to 150 DAS. This agrees with the *in vitro* results of Lynch et al. (2012) between 126 DAS and 153 DAS while the further delay of harvest to 174 DAS decreased digestibility. Many investigations have revealed the negative effects of increasing maize maturity on digestibility, mainly when maize is harvested with high DM content ($\leq 350 \text{ g DM kg}^{-1}$; Khan et al. 2015). This indicates changes in the cob/stover ratio and changes in the digestibility of the nutrient components while the profile of digestibility changes differ among hybrids (Lynch et al. 2012, 2013, Khan et al. 2015). However, no reduction in OM digestibility was recorded when four maize silages containing between 220 and 380 g DM kg^{-1} were fed in dairy cow diets comprising $420 \text{ g maize silage kg}^{-1}$ total DM intake (Sutton et al. 2000).

The increase in DOMD of the whole-crop maize with the delay in harvest was consistent with lessening NDF and increasing starch content. The considerable rise in starch content of grains reduced the content of NDF in the whole crop maize due to the simultaneous increase in the ratio of grains to the whole-crop maize DM (Bal et al. 2000). The reduction of CP and water-soluble carbohydrate, and the stability of NDF by delaying harvest, might be due to the dilution that occurred with the increasing starch content (Deaville and Givens 2001).

With respect to heavy metal and metalloid content in whole-crop maize, digestate application resulted in a higher content of As and Cd, by 0.15 mg kg^{-1} and 0.014 mg kg^{-1} , respectively, than application of synthetic fertilizer. However, digestate did not result in critical contents of all studied heavy metals and metalloids in whole-crop maize according to the indices of animal health, and they were below the maximum tolerable levels for cattle feed (As 30 mg kg^{-1} , Pb 100 mg kg^{-1} , Cr 100 mg kg^{-1} , Cu 40 mg kg^{-1} , Zn 500 mg kg^{-1} , Cd 10 mg kg^{-1} [NRC 2005]). This indicates that whole-crop maize fertilized with digestate, based on the total N needed by maize, can be used safely as ruminant feedstuff.

Conclusion

Chemical composition and *in vitro* OM digestibility of whole-crop maize did not differ between fertilizer and digestate treatments. The DM ($231\text{--}318 \text{ g kg}^{-1}$) and starch ($146\text{--}252 \text{ g kg}^{-1} \text{ DM}$) contents that were reached 150 days after sowing, at the beginning of October, suggest that it is possible to harvest at least moderate quality maize forage even in Boreal conditions. Further, the contents of heavy metals in whole-crop maize fertilized with digestate were comparable to those with synthetic fertilizer, thus digestate can be recycled safely as fertilizer for silage maize production.

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