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Chemical Composition, Crude Protein Fractions and Ruminal Degradation of Maize Silage Produced in Isfahan

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Abstract: The objective of present study was to evaluate the chemical composition, crude protein fractions and *in situ* ruminal digestibility of Maize Silages (MS) produced in Isfahan. The MS samples were taken from bunker silos from 20 farms across Isfahan province. The crude protein was fractionated based on the Cornell Net Carbohydrate and Protein Model. Ruminal digestion kinetics of Dry Matter (DM) was assessed using two ruminally fistulated Naeini rams. Chemical compositions were analyzed in a completely randomized design. Degradability data were analyzed in a randomized complete block design and means were compared using Duncan's test. Results revealed a lower DM (23.4 vs. 28-32%), lower nonfiber carbohydrates (24.5 vs.>30-35%), lower effective ruminal degradation of DM (EDDM, 48.4 vs.>55%), higher neutral detergent fiber (NDF, 58.5 vs.<50-52%) and higher acid detergent fiber (35 vs.<30%) than the optimum ranges. Regression analysis demonstrated a strong negative relationship between EDDM and NDF content of MS. The harvest of Maize Crop (MC) at a greater DM content than 24-28% when the kernels are more developed is suggested to maximize benefits from the current progressive growth in MC production in Iran. Wilting the MC to increase its DM content could be an alternative, when short day-length or unpredictable climate do not allow for a timely MC harvest.

Key words: Maize crop, ensilage, crude protein fraction, harvest time, ruminal degradability

Introduction

Total Maize Crop (MC) produced in Iran in 2003 mounted to 1,800,000 tone. This amount is 13.8 and 1.4 times greater than those in 1990 and 2000 (FAO, 2004). As Maize Silage (MS) consists a major portion of commercial rations for ruminants, such MC yield growth may not be realized maximally until its timely harvest is fully appreciated. The MC ensiled in Iran is usually planted in July as a second crop. As a result, the insufficient time to achieve the optimum maturity and Dry Matter (DM) can reduce the resulting silage quality. Besides, the common use of bunker silos by local farmers makes a well-conserved ensilage more difficult to maintain, particularly with high-moisture MC (McDonald *et al*., 1995). Thus, harvesting the MC at optimal DM needs to be clearly communicated among scientists, extensionists and farmers.

Inverse relationship between the DM of MC and the stage of maturity at harvest has well been documented (McDonald *et al*., 1995, Johonson *et al*., 2002). Ensiling the forage may decrease Crude Protein (CP) degradability by increasing unavailable N (Chalupa and Sniffen, 1996). Bal *et al.* (1997) reported a continuous rise in DM and starch in MC as maturity advanced from early-dent to quarter-milkline, two-third-milkline and black-layer; whereas NDF and ADF were highest at early-dent. Interestingly, the greatest total tract digestibility of ADF, CP and starch were observed at early-dent. However, milk yield was lowest at early-dent and highest at two-third-milkline. Such dependence of MS quality on grain development and stover fibrousness highlights the unfavorable impacts of harvesting the MC at too-early or too-late maturity on animal productivity (Johnson *et al*., 1999).

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Data are scarce on nutritional quality of MS produced in Iran. Also, it is crucial to uncover the nutrient composition of MS along emphasizing a timely MC harvest. The objectives of present study were, therefore, to evaluate the chemical composition, fractionate the CP and estimate the ruminal digestion kinetics of MS produced in Isfahan. An additional objective included attributing such criteria to MC maturity at harvest.

Materials and Methods

The maize silage samples from twenty dairy farms across the central Iranian province of Isfahan were collected during fall, 2002. The silages were from unknown varieties and were all grown and ensiled in 2002, except for sample No. 19 ensiled in 2001. At each farm, about 1.5 kg of silage was sampled from 10 different sites of the bunker silos in a depth of 30 to 40 cm. The samples were immediately preserved, labeled and transported to the Nutrition Lab at Isfahan University of Technology and stored at -20°C until analyses. For DM determination, the individual MS samples were dried at 55°C in a forced-air oven for 48 h and ground through a 1 mm screen (Wiley's pulverizer for laboratory, Ogaw Seiki Co., Ltd., Tokyo, Japan) before chemical analysis. The MS were analyzed for NDF (Van Soest *et al*., 1991), ADF (Proc. ID. 973.18), CP (Proc. ID. 984.13), ether extract (Proc. ID. 920.39) and ash (Proc. ID. 942.05) based on AOAC (1990). The values for ADF and NDF are with residual ash. The CP fractions of A, B_1, B_2, B_3 and C, respectively representing NPN, easily degradable true protein, moderately degradable true protein, slowly degradable true protein and unavailable protein, were determined based on the Cornell Net Carbohydrate and Protein Model (Sniffen *et al*., 1992) and as analytically described by Licitra *et al*. (1996). Nonfiber carbohydrate (NFC) was calculated by difference [NFC (g kg^{-1}) = 1000 – (NDF + CP + EE + ASH) in g kg^{-1}]. The pH of MS was determined in wet samples with a pH electrode (HI 8314 membrane pH meter, Villafranca, Italy) as described by Bal *et al*. (2000).

Two ruminally fistulated Naeini rams (39±1 kg BW) were used to determine the *in situ* DM degradability of MS. The sheep were adapted for 3 week to a diet of 41% MS, 29% wheat straw, 21.8% barley grain, 6.9% cottonseed and 1.3% mineral supplement (on a DM basis) and fed twice daily at 0900 and 1800 h. Polyester nylon bags (8×10 cm; pore size 52 μm; ANKOM Co, Fairport, NY) containing 2 g of dry ground (2 mm screen) silage were incubated in the rumen for 8, 12, 16, 18, 24, 48 and 72 h. There were four measurements (two sheep x two replicates) for each sample at each incubation time. The bags were soaked in 39°C water instead of rumen to estimate the soluble fraction of DM. Upon removal from the rumen, nylon bags were rinsed under running tap-water until the effluent was clear. Bags and residues were dried in a 55°C forced-air oven for 48 h to measure DM disappearances. The DM disappeared at all incubation times including time 0 were fitted to a logarithmic model initially proposed by Ørskov and McDonald (1979) to determine the quickly (a) and potentially degradable (b) DM fractions and the fractional disappearance rate of b (c, %/h). The ruminal effective degradability of DM was then estimated using this equation: EDDM (%) = $a + [(b \times c / c + k) (1- e^{-(c+k)t})]$, where EDDM = the effective ruminal DM degradability (%), k = the rumen outflow rate (5% h^{-1}) and t = incubation time.

The descriptive statistics were obtained using Univariate Procedure of SAS Institute (1996). A completely randomized design was used to analyze the chemical compositions and CP fractions. *In situ* data were analyzed as a randomized complete block design with sheep as the block. Means were compared using Duncan's test. Correlation procedure of SAS was used to determine the Pearson's correlation coefficients between pairs of variables (n = 20). Relationships between NDF content of MS and EDDM were developed using the linear regression Procedure of SAS (1996).

Results and Discussion

Nutrient Composition and pH

The DM of most MS in the present study was way below 35% (Table 1), recommended for optimum MC yield and productivity of lactating cows (Bal *et al.*, 2000). A negative correlation existed between DM and NDF of MS (r = -0.41, p = 0.07), which agrees with Johnson *et al.* (2003b) who reported a decline in NDF as DM rose. However, such negative relationship is usually observed as maturity advances from early to mid stages of maturity. The NDF content seems, however, to rise as DM rises in later stages of maturity (Bal *et al.*, 2000, Johnson *et al.*, 2003b). The higher NDF of MC was associated with a significant decrease in ruminal DM degradability (Fig. 1). This result concurs with Bal *et al.* (2000) who reported the greater ruminal DM and starch disappearances at mid than at early MC maturity. Thus, harvesting MC for ensilage appears unreasonable when MC DM is less than 25-28%, most likely due to difficulties in proper preservation (McDonald *et al.*, 1995).

The ranges in NDF and ADF were greater (Table 1, p<0.01) than those in the literature (39.7-42.1 and 27.8-29.6%, Johnson *et al.*, 2003b; 40.5-52 and 23.9-32%, Bal *et al.*, 1997; 40.5-54.6 and 23.9-38.5%, Johnson *et al.*, 1999; 34.9-45.4% NDF, Cherney *et al.*, 2004). A positive strong correlation was found between NDF (r = 0.64, p<0.01) and ADF (r = 0.8, p<0.0001) with

Table 1: Chemical composition and CP fractions of maize silages (g kg^{-1}) sampled across Isfahan farms (DM basis)[1]

Item (g kg^{-1})	DM	pH	NDF	ADF	EE	ASH	NFC[2]
Mean±SE	234.1± 21.6	3.71±0.14	584.8±31.1	349.0±24.4	23.9±3.4	76.0±11.7	245±42
Maximum	279.8	4.01	511.9	389.6	29.3	100.5	337
Minimum	205.3	3.41	629.3	294.7	14.5	61.3	181
Quant, 25	216.5	3.64	557.3	340.5	226	66.7	219
Quant, 75	244.4	3.8	607	362.7	25.5	82.2	279
N fractions[3] (g kg^{-1})	CP	A	B_1	B_2	B_3	C	A/CP
Mean±SE	89.0±13.5	48.4±12.2	2.4±1.6	21.7±13.7	3.7± 2.7	1.38±0.23	0.53±0.06
Maximum	118.1	70.8	5.5	30.2	10	18.2	0.63
Minimum	73.2	30	0.3	16	1	8.7	0.4
Quant, 25%	76	40.4	1.3	18.6	2.1	12.9	0.50
Quant, 75%	93.3	52.7	3.2	24.1	5.1	15.2	0.58

[1]n = 20. Quant 25, 25% of observations are lower than this value; Quant 75, 75% of observations are lower than this value. Chemical composition and CP fractions were significantly different across samples (p<0.01). SE, Standard Error. [2]Non fibrous carbohydrates = 1000 – (NDF + CP + EE + ASH) in g kg^{-1}. [3]A, B_1, B_2, B_3 and C are expressed as % of DM and represent NPN, easily degradable CP, moderately degradable CP and slowly degradable CP, respectively (Licitra *et al.*, 1996)

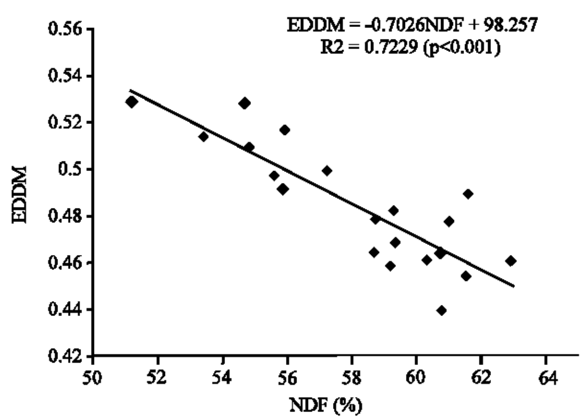

Fig. 1: The relationships between NDF (a) of maize silages and effective ruminal DM degradability (EDDM). p-value is for the regression coefficient

pH of MS, postulating the critical impact of slowly-degradable carbohydrates on initial fall in the pH of ensilage. The ranges in DM and NDF of MS observed in our study could indicate that most farmers may have harvested the MC at too early maturity when the moisture was too high. As farmers had used large bales of straw at the bottom of the bunkers to absorb the effluent and thereby, to enrich the straw nutritive value, no considerable impact of seepage waste on silage DM were expected.

The NFC range of MS was considerably lower than others' reports (Johnson *et al*., 2003a, b). Johnson *et al.* (2002) speculated that NFC in MS increases as maturity advances because kernels develop and their starch content rises. The NFC was negatively correlated with NDF ($r = -0.88$, $p<0.0001$) and positively correlated ($r = 0.33$, $p<0.15$) with DM in the present trial. Also, the pH of MS decreased dramatically ($r = -0.7$, $p<0.001$) as NFC level enhanced. These relationships suggest that MS with higher DM could be richer in grain or NFC. Of note, the impacts of cultivar (Johnson *et al.,* 1999) and hybrid (Kung and Shaver, 2001) are also determining in silage quality i.e., DM content. In the current study, the maize hybrids were unknown, which might help to explain the lower correlation between NFC and DM.

The silage pH in this study was less than 4.2, suggested as an index for good silage quality (Kung and Shaver, 2001). The low final pH, however, does not necessarily mean a desired fermentation throughout ensilage when the rate of pH decline was unknown. When pH of ensilage falls slowly, the lactic acid producing bacteria establish slowly and clostridia are more likely to proliferate (Kung and Shaver, 2001). The study used the ratio of NPN (A fraction) to total CP to estimate the rate of pH drop across bunkers (Table 1). A high correlation existed between CP and NPN ($r = 0.89$, $p<0.0001$) and between CP and NPN/CP ($r = 0.5$, $p<0.05$). As DM and CP were not correlated, the above correlations may indicate that MC was harvested at almost the same stage of maturity across farms.

Crude Protein Fractions

Fraction B_1 of CP in MS was negligible (Table 1), which agrees with the basic silage biochemistry that ensilage decreases the less quickly degradable fractions (i.e., true proteins found in B_1 and B_2) of forage CP and instead increase NPN (McDonald *et al*., 1995, 1991). The B_2 fraction of CP of MS was positively correlated with NDF ($r = 0.5$, $p<0.05$) and ADF ($r = 0.45$, $p<0.05$) and negatively correlated with NFC ($r = -0.64$, $p<0.01$). These could suggest that B_2 fraction of protein is rather associated with non-grain portions of MC. Thus, lower grains and greater non-grain, non-lignified parts (e.g. the newly developed leaves) in young MC could have contributed to the higher B_2 in this study. The term B_3 represents the rumen-escape protein such as zein, typically found in corn kernels (Sniffen *et al*., 1992). The B_3 fraction of CP in the present study was lower than that listed by Sniffen *et al.* (1992). The low B_3 could offer further evidence for deficient kernel development and thus, for the too-early harvest of MC by Isfahani farmers.

The fraction C in the current study was wider in range but similar on average to that reported by Johnson *et al.* (2002). Fraction C basically represents the protein associated with lignin, tannins and maillard reaction products (Sniffen *et al*., 1992). Hence, it can be used to estimate serious heat damage during unwell-preserved ensilage. The greater ADF (Table 1) of MS in our trial compared with that in Johnson *et al.* (2002) may explain the discrepancy in C between the two studies.

Ruminal Degradation Kinetics

The macro *in situ* technique has recently been used (Johnson *et al*., 2003b) to incubate wet, unground MS samples. This method accounts for the effects of hybrid and physical properties of MS on its ruminal digestion kinetics. Nonetheless, we decided to dry and grind MS. Grinding was indeed to minimize the confounding from the above-mentioned factors and to highlight the main effect of harvest time on degradation kinetics of MS. The principal goal of this study was to document the

Table 2: Ruminal degradation kinetics of maize silages across Isfahan farms (DM basis)[1]

DM degradability[2]	8 h	12 h	16 h	24 h	48 h	72 h
Mean±SD	0.28±0.03	0.39±0.036	0.45±0.038	0.51±0.04	0.57±0.048	0.68±.034
Maximum	0.37	0.46	0.53	0.59	0.66	0.72
Minimum	0.23	0.33	0.37	0.44	0.50	0.59
Quant, 25	0.25	0.36	0.42	0.47	0.53	0.67
Quant, 75	0.31	0.41	0.47	0.54	0.59	0.70
Ruminal digestion kinetics of DM[3]		a (%)	b (%)	c (% h^{-1})	EDDM	
Mean±SE		27.09±3.7	48.95±5.37	4±1	0.48±0.02	
Maximum		35.9	62.33	5.9	0.53	
Minimum		21.5	40.83	2.3	0.44	
Quant, 25		24.85	44.95	3	0.46	
Quant, 75		29.3	52.11	4.7	0.50	

[1]n = 20. Quant 25, 25% of observations are lower than this value; Quant 75, 75% of observations are lower than this value. Degradability of DM in all incubation times and ruminal digestion kinetics of DM were significantly different across samples (p<0.01). SD, Standard Deviation; SE, Standard Error. [2]Coefficients of ruminal DM degradation of maize silages at different incubation times. [3]a = quickly degradable fraction of DM; b = potentially or slowly degradable fraction of DM; c = degradation rate of b in %/h; *EDDM* = effective ruminal degradability of DM

nutrient composition, CP fractions and the concern on too-early MC harvest rather than to compare the MS management policies among farms. The significant differences in particle size distribution of fresh MS across farms (data not shown) would have otherwise caused major differences in digestion kinetics of MS if wet and unground.

A significant negative correlation (r = -0.71, p<0.001) existed between soluble DM and NDF in MS. Simply put, samples with highest b had the slower degradation rate (r = -0.59, p<0.01) (Table 2). Conceptually similar, Bal *et al.* (2000) reported that the degradability of MS decreased as maturity advanced. Fraction b (slowly degradable DM) varied considerably across farms, probably indicating the differences in MC hybrids used by different farmes, as addressed earlier by Bal *et al.* (2000). The 24 h ruminal DM disappearance of MS at early and mid maturity was greater (p<0.05) in their study than that in this study. The DM of MS in the study of Bal *et al.* (2000) was also greater (>30%, p<0.01) than that in our study. However, Johnson *et al.* (2003b) reported a similar ruminal disappearance of DM at early maturity (52.9 vs. 50.7%). Putting the reports together, the rise in DM and NDF degradability at the earlier maturity may not compensate for the high NFC and low NDF at mid maturity (Bal *et al.* 2000). This is because the net amount of available starch and fiber per crop unit would be still greater at mid maturity. Additionally, total DM yield of MC would be lower at early maturity when kernels are not adequately developed (Johnson *et al*., 1999; Bal *et al*., 1997).

The soluble fraction (a) and effective degradability of DM (EDDM) of MS (Table 2) were lower in our trial than that found by Von Keyserlingk *et al.* (1996). The lower EDDM (p<0.01) could be due to the lower soluble DM that was indeed because of the greater (p<0.01) NDF and ADF in MS in the current trial. The high fiber content may then explain the lower disappearance rate of the potentially degradable DM in this study, when compared to the reports by Von Keyserlingk *et al.* (1996). The NDF content of MS was negatively correlated (-0.82, p<0.0001) with EDDM (Fig. 1). Noteworthy, this negative relationship stands solely for ruminal degradation and should, hence, not be compared with total tract digestibility studies (Bal *et al*., 1997) or macro *in situ* trials (Johnson *et al*., 2003b) unless cautiously. Importantly, NDF explained more variations in EDDM than did DM of MS. These results suggest that even in low-DM MS, higher NDF content may significantly reduce MS fermentability, highlighting the inter-hybrid differences in the link between MC degradability and stage of maturity.

Conclusions

The DM content and effective ruminal DM degradation were lower and cell-wall fibers were greater for MS ensiled in bunker silos across Isfahan, when compared to several ranges reported and recommended by the literature. The too-early maturity of the maize crop at harvest (mostly with DM <24%) may have contributed to such nutritional and digestion characteristics. Future studies using larger numbers of farms will further uncover and strengthen the recommendation as to when to harvest the MC for optimal crop yield, silage quality and animal productivity. If so, the effects of hybrid and cultivar need full attention. To imply, it seems reasonable to harvest the MC when DM rises at least to about 28-30% and kernels (high-energy portions) are more developed. Where the climate and short days do not allow for a timely harvest, wilting the harvested MC could potentially contribute to a well-preserved, pro-lactate ensilage.

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