



## AGRARIAN SCIENCES

# Effects of Different Nitrogen Dose and Sources as Top-Dressing on Yield and Silage Quality Attributes of Silage Maize

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**Abstract:** Effects of different nitrogen doses and sources applied as top-dressing on dry matter yield and quality of maize silage were investigated in this study. Along with  $7.5 \text{ kg N da}^{-1}$  application as starter at sowing in the form of 15-15-0+Zn, nitrogen doses of 0, 7.5, 15 and  $22.5 \text{ kg da}^{-1}$  were applied as top-dressing at 6-leaf stage of plants in the forms of ammonium nitrate, urea, DMPP blended ammonium sulphate nitrate and urea with NBPT urease inhibitor. Experiments were conducted in randomized blocks-factorial experimental design with 3 replicates in 2013 and 2014. The greatest dry matter yield were obtained from 15 and  $22.5 \text{ kg N da}^{-1}$  in 2013 and from  $22.5 \text{ kg N da}^{-1}$  in 2014. Nitrogen sources had also significant effects on dry matter yield. According to the average of two years, both DMPP blended ammonium sulphate nitrate and urea with NBPT urease inhibitor increased yield respectively by 7 and 3% as compared to ammonium nitrate and urea fertilizers. Nitrogen doses significantly improved the silage quality attributes. Nitrogen treatments increased silage protein ratio and decreased ADF and NDF ratios of silage samples. The greatest relative feed value was obtained from  $15 \text{ kg N da}^{-1}$  treatment. It was concluded based on present findings that besides the nitrogen doses, nitrogen sources also significantly improved yield.

**Key words:** nitrogen fertilizer, nitrification and urease inhibitors, forage yield and quality.

## INTRODUCTION

Maize is one of the most significant roughage sources of animals. It is used either in fresh forms or as silage in animal feeding. Maize is quite available for machine-culture, has high unit area yields, can be cultured as a second crop in several regions, has high digestibility levels, provides quality and palatable source of silage and is ensiled without any needs for additives. Therefore, it is among the mostly preferred feed source both in Turkey and throughout the world (Celebi et al. 2010, Ileri et al. 2018). Maize is a C4-plant, thus has quite high dry matter productions. Maize uptakes much more

nutrients from the soil than the other plants. Therefore, proper and well-balanced fertilization should be practiced over maize fields to get desired yield levels. Nitrogen is among the most important nutrients influencing maize yield and feed quality (Carpıcı et al. 2017, Fallah & Neisani 2017). Maize plants get into a rapid growth phase at 6-leaf stage and thus significant increases are observed in water and nitrogen uses in this stage of growth (Rozas et al. 1999, Girma et al. 2011). Rapid nitrogen uptake is generally observed between V10-V14 stages (Mueller et al. 2017). Depending on environmental factors and genotypes, maize plants get about 65-70% of total nitrogen content at vegetative growth

stage and get the rest from the soils after tassel formation (Gallais & Coque 2005, Mueller & Vyn 2016). Thus, sufficient nitrogen levels should be available in soils throughout the vegetative growth stages until tassel formation. However, nitrogen efficiency in maize culture is at quite low levels (<50%) (Gagnon et al. 2012). Majority of supplied nitrogen is lost through leaching, denitrification, volatilization, immobilization and soil erosion.

Special fertilizers are produced by the industry to reduce or prevent such losses. Stabilized fertilizers are among the most important ones of these special fertilizers (Trenkel 2010). N-(n-butyl) thiophosphoric triamide (NBPT) treated urea is one type of stabilized fertilizers. NBPT urease inhibitors control urease enzyme activity transforming urea into ammonium. They reduce hydrolysis of the urea, prevent abrupt increases in pH around the fertilizer granules and ultimately decrease  $\text{NH}_3$  volatilization losses (Rozas et al. 1999). 3,4-dimethylpyrazole phosphate (DMPP) treated fertilizer is another type of stabilized fertilizers. DMPP nitrification inhibitor has quite low solubility in water and does not have any phytotoxic impacts on plants (Zerulla et al. 2001). This nitrification inhibitor even at small quantities may inhibit nitrification for about 6-8 weeks based on soil temperature and bacteria-static impacts on *Nitrosomanos* bacteria (Zerulla et al. 2001, Villar & Guillaumes 2010). So, DMPP nitrification inhibitor is commonly used in nitrogenous fertilizers. Different researchers indicated that DMPP inhibitor significantly diminished nitrogen losses through denitrification and nitrate leaching (Wu et al. 2007, Liu et al. 2013, Martinez-Alcantara et al. 2013).

Various researches have been conducted about the effects of different nitrogen sources and stabilized fertilizers on maize yield and

quality (Gagnon et al. 2012, Motavalli et al. 2013, Abalos et al. 2014, Halvorson & Bartolo 2014, Mota et al. 2015). However, results varied largely based on climate, soil factors and management practices. Therefore, in this study, effects of different nitrogen doses and nitrogen sources applied as top-dressing at 6-leaf stage on silage maize yield and silage quality were investigated. In this way, efficiency of increasing doses of different nitrogen sources applied at a certain growth stage with the greatest need for nitrogen was compared.

## MATERIALS AND METHODS

### Experimental site

Experiments were conducted over the experimental fields of Agricultural Research and Implementation Center of Erciyes University-Kayseri located between 39° 48' North latitudes and 38° 73' East longitudes and at an altitude of 1053 m in the years 2013 and 2014. According to long-term averages for climate parameters of the experimental site, annual average total precipitation is 391 mm and annual average temperature is 10.6 °C. Monthly minimum, average and maximum temperatures, monthly total precipitation and monthly average relative humidity values throughout the vegetation periods of the experimental years are given in Table I. Experimental soils were sandy-loam in texture. Soils were almost neutral in pH (7.6) and unsaline ( $\text{EC}=0.183 \text{ dS m}^{-1}$ ). Organic matter content was 0.5%, lime content was 1.27% and available phosphorus was 10.159 kg  $\text{P}_2\text{O}_5/\text{da}$ .

### Experiment

Experiments were conducted in randomized blocks-factorial experimental design with 3 replicates. NK Atria dent corn variety of Syngenta was used as the plant material of the study. As top-dressing, 33% ammonium nitrate (AN), 46%

**Table I. Precipitation, temperature and relative humidity values of the years 2013 and 2014.**

Years	Measurements	Months				
		May	June	July	August	September
2013	Monthly average temperature, °C	17.5	20.3	21.7	21.7	16.8
	Monthly maximum temperature, °C	30.2	36.8	33.9	34.9	35.0
	Monthly minimum temperature, °C	6.1	7.5	9.1	9.8	2.4
	Monthly relative humidity, %	48.6	42.9	41.4	39.5	47.3
	Monthly total precipitation, mm	31.3	12.6	3.4	0.8	10.3
2014	Monthly average temperature, °C	16.1	19.2	24.4	24.4	18.8
	Monthly maximum temperature, °C	31.0	32.9	37.3	37.2	34.8
	Monthly minimum temperature, °C	3.4	7.0	10.9	10.4	1.4
	Monthly relative humidity, %	54.5	50.1	37.4	41.5	54.4
	Monthly total precipitation, mm	39.7	52.9	---	47.4	85.4

urea (U), DMPP blended ammonium sulphate nitrate (DMPP+ASN, trade name ENTEC 26) and NBPT blended urea (NBPT+Urea, Trade name UTEC 46) fertilizers were used. Before sowing, 7.5 kg N da<sup>-1</sup> was applied to all plots in the form of 15-15-0+Zn and were incorporated into soil with a cultivator. Sowing was performed on 09 May 2013 and 1 May 2014. About 70 cm spacing was provided between the plots and 1 m spacing was provided between the blocks. Plot lengths were arranged as 3 m. Six rows were opened with a hand marker adjusted at 70 cm. Sowing was performed as to have two seeds in every 20 cm and thinning was performed after emergence. Sprinkler irrigation was performed for homogeneous emergence. In addition to 7.5 kg da<sup>-1</sup> starter nitrogen, 0, 7.5, 15 and 22.5 kg da<sup>-1</sup> (N0, N7.5, N15, N22.5) nitrogen were supplied in the above-specified forms 35-40 days after sowing through scatter in between the rows as top-dressing and experimental site was irrigated with drip irrigation. Then based on plant moisture status, irrigations were performed in 10-day intervals.

Harvest was performed manually at 50% milk-dough stage of the kernels on 28 August 2013 and 27 August 2014. Two rows from each side and 50 cm sections from the top and

bottom of each plot were omitted as to consider side effects and 10 plants were harvested from the remaining sections of each plots. Harvested plants were weighed to get green herbage yield of the plots. Two plants were selected from each plot as to represent the plot; they were freshly weighed; separated into stem, leaf and ear; wilted for a while; dried at 65 °C until a constant weight and weighed again. Leaf, stem and ear ratio of dry herbage were determined. Green herbage yields were multiplied by dry matter ratios to get dry matter yields. Fresh plants of each plot were chopped into 2-3 cm pieces and ensiled into 3 kg vacuum bags. Silages were kept at room temperature for 60 days. They were opened, emptied into appropriate containers, 150 g samples were taken from each silage bag and samples were dried at 65 °C until a constant weight to get dry matter ratios. Another 40 g samples from each silage bag were shaken in 360 ml distilled water, filtered through and pH of filtrates was measured with a pH meter. With the aid of dry matter ratios and pH values of silage samples, Flieg points were calculated by using the equations provided in Oten et al. (2016) derived from Kılıç (1986). Dried silage samples were ground to pass through 1 mm sieve. Nitrogen content of ground samples was

determined with Kjeldahl method and resultant value was multiplied by 6.25 to get crude protein ratios. NDF (neutral detergent fiber) and ADF (acid detergent fiber) analyses were performed in accordance with the method specified by Van Soest & Wine (1967) and Van Soest (1963) respectively by using an Ankom 200 Fiber Analyzer device. Feed quality parameters of dry matter intake (DMI), digestible dry matter (DDM) and relative feed value (RFV) were calculated by using the equations provided in Başaran et al. (2011) derived from Rohweder et al. (1978).

### Statistical analysis

Experimental data were subjected to variance analysis with "SPSS for Windows" software in accordance with randomized blocks-factorial experimental design. Significant means were identified with F-test and means were grouped with the aid of Duncan's multiple range test. Initial variance analysis was performed on combined years, and then variance analysis was performed separately for each year since the years were found to be significant in initial analysis.

## RESULTS AND DISCUSSION

Variance analyses revealed that fertilizer sources, nitrogen doses and source x dose interactions had significant effects on dry matter yields in 2013 and nitrogen dose and source had significant effects on dry matter yields in 2014. Increasing dry matter yields were observed with increasing nitrogen doses. In the first year of the experiments, as compared to the control treatment, N7.5 treatment increased dry matter yields by 51.64%, N15 treatment by 86.68% and N22.5 treatment by 82.24%. In the second year of the experiments, nitrogen treatments increased dry matter yields by 42.92,

58.50 and 68.00%, respectively (Table II). The rate of increase decreased with increasing nitrogen doses. Similarly, Greer & Pittelkow (2018) indicated significant responds of maize kernel yields to nitrogenous fertilization and reported as compared to the control treatment that 179 kg N ha<sup>-1</sup> nitrogen treatment increased yields significantly, but there were not any distinctive increases in yields between 179 and 269 kg N ha<sup>-1</sup> treatments. Carpıcı et al. (2010) carried out a study with silage maize and reported 16% increase in yields between 100 and 200 kg N da<sup>-1</sup> doses and 4% yield increase between 200 and 300 kg N da<sup>-1</sup> doses.

Present dry matter yields varied with the nitrogen doses. The greatest dry matter yields were obtained from N15 and N22.5 treatments (2270 and 2216 kg da<sup>-1</sup>) in 2013 and from N22.5 treatment (2469 kg da<sup>-1</sup>) in 2014 (Table II). Dhital & Raun (2016) indicated that yield and nitrogen respond of maize might vary in the same location from year to year and such variations were mostly attributed to unexpected changes in environmental conditions.

As compared to the classical urea, NBPT+urea and DMPP+ASN fertilizers increased dry matter yields respectively by 1.6 and 4.3% in the first year and by 5 and 2.5% in the second year; as compared to ammonium nitrate, NBPT+urea and DMPP+ASN fertilizers increased dry matter yields respectively by 8.78 and 11.67% in the first year and by 6.44 and 3.84% in the second year. In a previous study carried out with maize, Pasda et al. (2001) reported that DMPP+ASN treatments increased kernel yield by 2.6% as compared to ASN. Gagnon et al. (2012) reported that urea, polymer-coated urea and DMPP inhibitor urea effected kernel yields differently in different years. Abalos et al. (2014) carried out a meta-analysis and reported that inhibitor fertilizers (DMPP, DCD and NBPT) increased yields by about 7.5%, fertilizer efficiency varied based

**Table II. Effects of different nitrogen sources and doses on dry matter yield and distribution.**

Treatment	Dry matter yield (kg da <sup>-1</sup> )		Leaf percentage (%)		Stem percentage (%)		Ear percentage (%)	
	2013	2014	2013	2014	2013	2014	2013	2014
<b>Fertilizer source</b>								
AN	1765 b	2033 b	16.57 a	17.20	41.06 a	31.27	42.39 c	51.53
Urea	1890 a	2061 b	16.48 a	17.35	39.98 ab	31.52	43.55 bc	51.13
DMPP+ASN	1971 a	2111 ab	15.58 b	17.13	38.49 b	31.61	45.93 a	51.26
NBPT+Urea	1920 a	2164 a	16.51 a	18.68	39.10 b	30.77	44.39 ab	50.55
<b>Nitrogen doses</b>								
N0	1216 c	1470 d	16.55 a	17.08	47.32 a	36.20 a	36.12 c	46.72 b
N7.5	1844 b	2101 c	16.92 a	17.57	38.88 b	29.34 b	44.19 b	53.11 a
N15	2270 a	2330 b	15.84 b	18.44	35.49 c	29.34 b	48.67 a	52.22 a
N22.5	2216 a	2469 a	15.81 b	17.27	36.93 c	30.31 b	47.26 a	52.42 a
<b>ANOVA</b>								
Source (S)	**	*	*	ns	**	ns	**	ns
N dose (N)	**	**	**	ns	**	**	**	**
S x N	*	ns	ns	ns	ns	ns	ns	ns

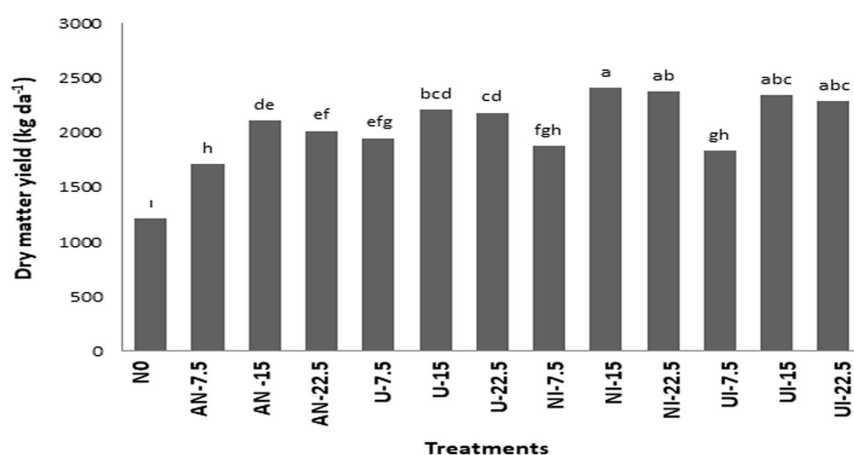
Values within the same column followed by different letters are significantly different at  $P < 0.05$  of Duncan's multiple range test. \*: Significant at 0.05 probability level, \*\*: Significant at 0.01 probability level, ns: not significant.

on environmental and management factors and was greater in coarse-textured soil and irrigated conditions. Pasda et al. (2001) indicated that yield increase achieved with nitrification inhibitor was not resulted solely from prevention of nitrogen losses, but also from ammonium nutrition. Huffman (1989) indicated that  $\text{NH}_4^+$  and  $\text{NO}_3^-$  forms of nitrogen could be used in maize culture, the plant response to combined ammonium and nitrate nutrition was better than the plant response to nitrate alone and in this sense, nitrification inhibitors were significant in prolonging ammonium nutrition durations.

When the classical fertilizers were compared within themselves, Urea showed significantly higher yield over AN in 2013. The difference between Urea and AN was not significant in 2014. Present findings comply with the results of Biswas & Ma (2016) who reported an inconsistent

effect of N source on yield and N use efficiency indices of maize.

With regard to dry matter yields, fertilizer source x dose interaction was found to be significant ( $P < 0.05$ ) in the first year and insignificant in the second year. The greatest dry matter yield was obtained from N15 dose of DMPP+ASN fertilizer and the lowest dry matter yield was obtained from N7.5 dose of AN fertilizer (Figure 1). While stabilized fertilizers had greater yields at high N doses, urea fertilizers had high yield at N7.5 treatment. Regression equations revealed that in 2013, the maximum yield in AN treatments (2083 kg da<sup>-1</sup>) was obtained from 18.33 kg N da<sup>-1</sup> dose, in urea treatments (2261 kg da<sup>-1</sup>) from 17.50 kg N da<sup>-1</sup>, in DMPP+ASN treatments (2419 kg da<sup>-1</sup>) from 19.86 kg N da<sup>-1</sup> and in NBPT+urea (2336 kg da<sup>-1</sup>) from 19.56 kg N da<sup>-1</sup> dose. In 2014, the maximum yield in AN treatments (2428 kg da<sup>-1</sup>) was obtained from 25.21 kg N da<sup>-1</sup> dose, in urea



**Figure 1. Source x dose interactions for dry matter yields in 2013. Values within the same column followed by different letters are significantly different at  $P < 0.05$  of Duncan's multiple range test. (AN: Ammonium nitrate; U: Urea; NI: DMPP+ASN; UI: NBPT+Urea).**

treatments ( $2386 \text{ kg da}^{-1}$ ) from  $18.01 \text{ kg N da}^{-1}$ , in DMPP-ASN treatments ( $2513 \text{ kg da}^{-1}$ ) from  $23.25 \text{ kg N da}^{-1}$  and urease inhibitor treatments ( $2566 \text{ kg da}^{-1}$ ) from  $20.48 \text{ kg N da}^{-1}$  dose (Figure 2).

Leaf ratio of the years 2013 and 2014 was respectively measured as 16.28 and 17.59%. The greatest stem ratios were observed in N0 treatments (47.32 and 36.20%) and nitrogen treatments reduced stem ratios. The greatest ear ratio was observed in DMPP treatment (45.93%) in the first year and the differences in ear ratios of nitrogen sources were not significant in the second year. The lowest ear ratio was observed in N0 treatments of both years and nitrogen treatments increased ear ratios as compared to the control. In silage maize culture, majority of herbage yield and nutritional values come from the ears (Coors et al. 1997). Therefore, high ear ratios are desired at harvest. In present study, stem ratios decreased and ear ratios increased with nitrogen treatments. Celebi et al. (2010) reported the greatest stem ratio and lowest ear ratio for the control plots without any nitrogen treatments. Akar et al. (2014) indicated that maize plants exhibited a normal growth with sufficient nitrogen levels and such levels speeded up ear kernel formation, increased ear ratio and thus reduced stem ratio, but plants were forced to bloom earlier, had shorter growth periods and

thus quite less number of kernels formed at the end of ears under deficit nitrogen conditions.

The greatest protein ratios in both vegetation periods were observed in N22.5 treatments (6.98 and 8.66%) and the lowest ratios were observed in N0 treatments. Protein ratios increased with increasing nitrogen doses (Table III). Present findings on protein ratios were similar with the findings of Islam et al. (2012) and Safdarian et al. (2014).

NDF ratios of maize silage varied between 38.91 - 52.91%, ADF ratios varied between 22.62 - 31.85%, DMI values varied between 2.27 - 3.09%, DDM values varied between 64.09 - 71.28% and RFV values varied between 112.87 - 170.86. NDF and ADF are commonly used to estimate feed intake and digestibility (Tekce & Gül 2014). Increasing NDF and ADF ratios reduce feed digestibility, give animals the sense of fullness, thus, limit animal feed consumptions (Canpolat & Karaman 2009). As compared to the control, nitrogen treatments reduced ADF and NDF ratios. Such a case then increased dry matter consumption and digestible dry matter quantities. Similarly, decreasing ADF and NDF ratios were reported with nitrogen treatments (Lamprey et al. 2018, Safdarian et al. 2014, Kaplan et al. 2016). Nazlı et al. (2014) reported significant decreases in fiber content of silage maize with



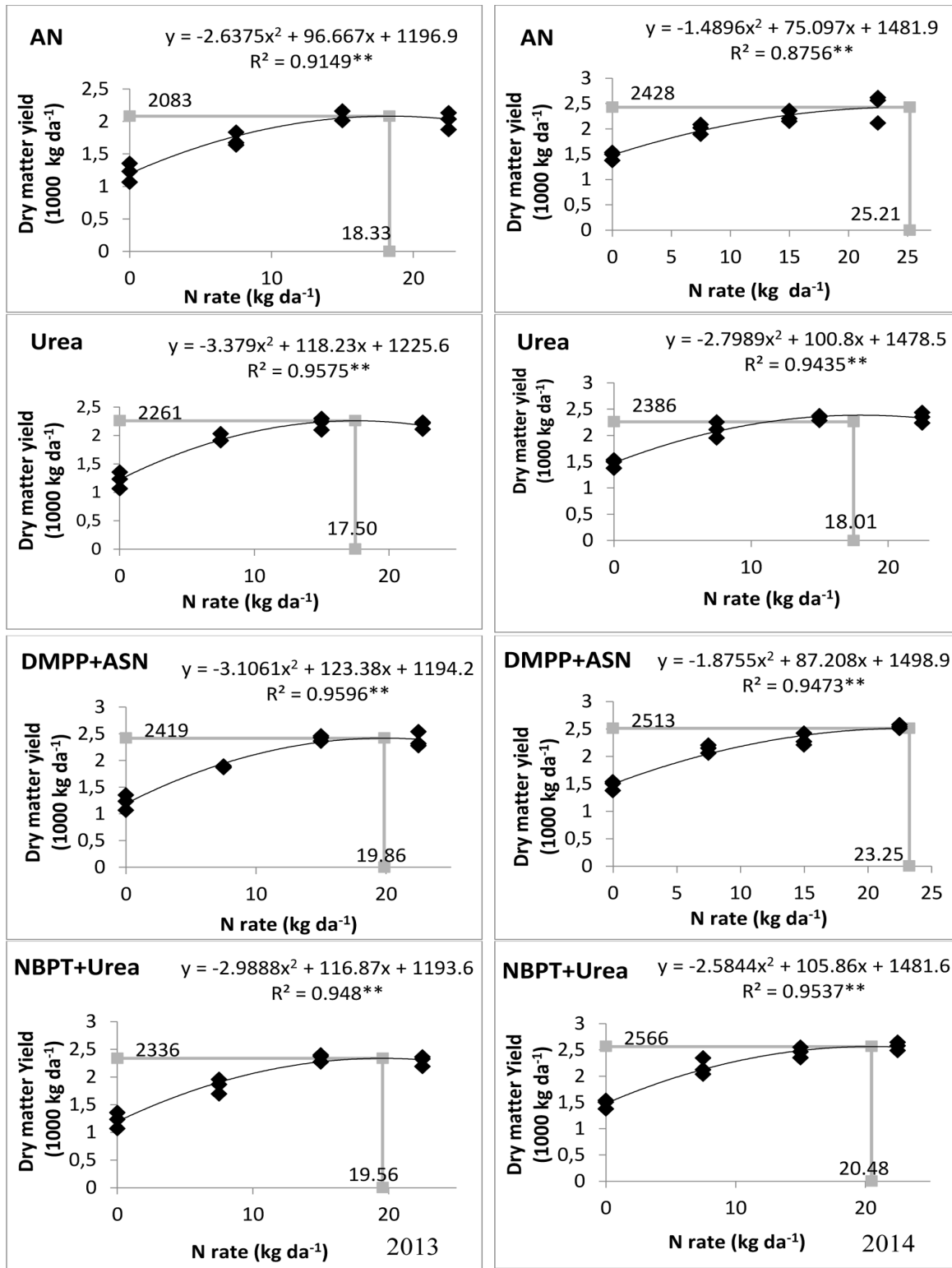


Figure 2. Dry matter yield response of N fertilizer doses and sources of silage maize in 2013-2014, \*\* P < 0.01.

**Table III. Effects of different nitrogen doses on silage quality.**

N doses	Crude protein (%)		ADF (%)		NDF (%)	
	2013	2014	2013	2014	2013	2014
N0	4.75 d	4.95 d	28.73 a	31.85 a	49.86 a	52.91 a
N7.5	5.65 c	6.97 c	25.33 b	26.55 b	42.80 b	41.94 b
N15	6.53 b	7.93 b	22.62 c	26.97 b	38.91 c	40.54 b
N22.5	6.98 a	8.66 a	22.81 c	26.61 b	40.65 c	40.84 b
N doses	DMI (%)		DDM (%)		RFV	
	2013	2014	2013	2014	2013	2014
N0	2.41 d	2.27 c	66.52 c	64.09 b	124.58 c	112.87 b
N7.5	2.81 c	2.87 ab	69.17 b	68.22 a	150.89 b	151.52 a
N15	3.09 a	2.96 a	71.28 a	67.89 a	170.86 a	155.94 a
N22.5	2.96 b	2.94 ab	71.13 a	68.17 a	163.59 a	155.45 a
N doses	DM (%)		pH		Flieg point	
	2013	2014	2013	2014	2013	2014
N0	26.46 c	31.57 b	3.94	3.91	100.18 c	111.88 b
N7.5	28.36 b	33.78 a	3.97	3.84	102.82 b	118.90 a
N15	29.56 a	34.38 a	3.96	3.89	105.82 a	118.36 a
N22.5	29.52 a	34.16 a	3.96	3.86	105.53 a	118.85 a

Values within the same column followed by different letters are significantly different at  $P < 0.05$  of Duncan's multiple range test.

increasing nitrogen doses or increasing nitrogen content of plant tissues. RFV is commonly used to estimate feed intake and energy values and it is a compounded index of ADF and NDF. Feed RFV values are classified under 6 categories and the feeds with  $RFV > 151$  are classified as prime class feeds (Rohweder et al. 1978). Nitrogen doses significantly influenced relative feed values and the greatest RFV was obtained from N15 treatments of both years (170.86, 155.94). Silage pH values varied between 3.84 - 3.97% and both the treatments and the years did not have any significant effects on pH values. Flieg point is used as an easy way of assessment for silage quality attributes. Flieg point is calculated based on pH and dry matter ratio and all factors effecting pH and dry matter ratio thus directly influence Flieg points (Oten et al. 2016). As compared to the control, nitrogen treatments increased Flieg point of silage samples.

## CONCLUSIONS

Considering the entire findings together, it was concluded that DMPP+ASN and NBPT+Urea fertilizers applied as top-dressing increased dry matter yields as compared to classical fertilizers, but fertilizer sources did not have any significant effects on investigated silage quality parameters. As compared to the control, stem ratios decreased and ear ratios increased with increasing nitrogen doses. Nitrogen treatments increased dry matter yield, silage protein ratios and decreased ADF and NDF ratios of silage samples. It was finally concluded based on present findings for yields and quality attributes that  $7.5 \text{ kg N da}^{-1}$  as starter fertilizer at sowing and  $15\text{-}22.5 \text{ kg N da}^{-1}$  as top-dressing at 6-leaf stage of plants significantly improved yield and quality and stabilized fertilizers as the source of nitrogen significantly improved yields.



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Sati Uzun: literature review, design of methodology, data collection, data analysis and article writing. Hamdi Özaktan and Oğuzhan Uzun: design of methodology, data collection and data analysis.

