

Research Article

# Effects of Nitrogen Fertilization on Forage Production and Nutritive Value of Geukdong 6, Teosinte Hybrid [*Zea mays* L. subsp. *mexicana* (Schrad.) H. H. Iltis]

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## ABSTRACT

This study was conducted to evaluate the effects of different nitrogen levels on the yield and nutrient quality of a newly developed domestic hybrid of Teosinte, Geukdong 6 [*Zea mays* L. subsp. *mexicana* (Schrad.) H. H. Iltis]. The field experiment was conducted in a randomized block design with three replicates and consisted of four nitrogen (N) application rates, T1 (200 kg/ha), T2 (300 kg/ha), T3 (400 kg/ha), and T4 (500 kg/ha). No differences were found in plant length, leaf length, leaf width, leaf number, dead leaves, stem hardness, tiller number, and fresh yield ( $p>0.05$ ). The T3 showed significantly greater dry matter yield at harvest (heading stage) compared to the other treatments ( $p<0.05$ ). The crude protein content of T4 (10.49%) was higher than those of T1 and T2 ( $p<0.05$ ). However, there was no significant difference between T4 (10.49%) and T3 (9.63%). The effects on crude fat, crude ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude fiber were not significant ( $p>0.05$ ). The sugar content was higher in the T2 treatment than the other treatments ( $p<0.05$ ). For Ca, T3 showed significantly greater content ( $p<0.05$ ). However, no significant effects were found in the contents of Cu, Fe, K, Mg, Mn, Mo, and Zn ( $p>0.05$ ). Na content was higher in order of T2 > T4 > T3 > T1 ( $p<0.05$ ). Total mineral contents were not significantly different among the treatments ( $p>0.05$ ). Given these results, we recommend the amount of nitrogen fertilization necessary for “Geukdong 6” to be around 400 kg per ha (T3), when considering, high fresh yield, dry matter yield, number of leaves and content of crude protein.

**(Key words:** Dry matter yield, Geukdong 6, Nitrogen level, Teosinte hybrid)

## I. INTRODUCTION

Based on statistical data of the Korea Rural Economic Institute (KREI), as of December 2018, the number of Hanwoo (Korean native cattle) and beef cattle has reached 3,090 thousand (KREI, 2019a), and the number of dairy cows have reached 408 thousand (KREI, 2019b). The number of Hanwoo and beef cattle has been steadily increasing since 2015. Therefore, the demand for forage crops is expected to increase more than the capability of domestic forage supply. One of the solutions is to tillage high yielding forage crops. ‘Geukdong 6’ is a hybrid of Teosinte, which has recently been developed for high forage yields due to high tiller producing ability. In addition, Geukdong 6 has more leaf to stem ratio, so palatability is good (Sagan et al., 1993). However, research results reported mostly about yield (fresh, dry matter, total digestible nutrients), feed value, and seeding dates (Cui et al., 2016; Kim and Lee, 2019). Determination of

nitrogen fertilization levels is very important, particularly for newly developed highly producing forage crops. The optimum nitrogen level can result in high yield and good quality forage crops. Nitrogen positively affects leaf area development and maintenance of growth, photosynthetic efficiency (Arduini et al., 2006), and dry matter partitioning to reproductive organs (Prystupa et al., 2004). However, the levels of nitrogen in the soil are insufficient to meet the needs of high yield and good quality crops (Wira et al., 2013; Che et al., 1987).

Fertilization helps to translocate a higher amount of dry matter yield, which is directly related to nitrogen (Wira et al., 2013), and the amount of applied nitrogen can determine the yield and quality of forage crops. When the amount of nitrogen is not sufficient, plant growth and tiller numbers generation are reduced, therefore, the total biomass declines (Sagan et al., 1993; Vos and Biemond, 1992). However, too much nitrogen application can elevate the content of nitrate, which is known to be responsible

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for plant disease (grass tetany and nitrate toxicity) and reductions in plant quality (Barker et al., 1971; Yoon, 1996). The optimum levels of nitrogen fertilizer can promote the growth of forage crops, and can improve the utilization efficiency of N fertilizer with lower environmental pollutant (Anjana and Iqbal, 2006; Dordas and Sioulas, 2008).

The present study was conducted to determine optimum nitrogen fertilization levels of a newly developed Teosinte hybrid, “Geukdong 6” for forage production.

## II. MATERIALS AND METHODS

### 1. Experimental site and treatment

The experiment was conducted at the Kyungpook National University experimental farm, South Korea (Sangju, 36.3783 N, 128.1458 E) from May 20<sup>th</sup> to September 24<sup>th</sup> of 2016 (harvested on the heading stage). A randomized complete block design with four factors and three replicates were used. The four factors were nitrogen application levels : 200 (T1), 300 (T2), 400 (T3), and 500 kg/ha (T4). At this time, the fertilizer used in the experiment was urea<sup>®</sup>. The test plot area was 15 m<sup>2</sup> (3mx5m). The variety of seed was a *Geukdon 6* of Teosinte hybrid [*Zea mays* L. subsp. *mexicana* (Schrad.) H. H. Iltis].

### 2. Crop Establishment and field management

The seeding method used was 75 cm in row spacing, and 30 cm in seed spacing (4 rows per plot). The fertilizer was applied

as nitrogen and potassium (150 kg/ha), with 60% as the basal fertilizer, and 40% as the additional fertilizer. The phosphorus (200 kg/ha) used the entire amount as the basal fertilizer. The time of additional fertilizer application was performed when crop growth was at the six leaves stage (Table 1).

The soil used in the experiment was similar to normal soil for pH, total nitrogen and exchangeable cation, but was slightly lower in organic matter (Table 2). And, monthly meteorological data during the experimental period are presented in Table 3.

### 3. Field Data Collection and Laboratory Analysis

Growth characteristics (plant length, leaf length, leaf width, leaf number, dead leaves, stem hardness, and tiller number) were measured from 10 plants randomly selected from each plot in the experimental field. These characteristics were measured just prior to harvesting. The fresh yield was calculated after cutting the two central rows. Dry matter concentration was determined by drying forage samples in an forced air drying oven at 65°C for more than four days. Dried samples were ground and stored under vacuum until use for analysis.

Chemical compositions were analyzed for crude protein (CP), ether extract (EE), crude ash (CA), and crude fiber (CF) according to the methods of the Association of Official Analytical Chemists (AOAC, 1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the Goering and Van Soest method (1970). Total digestible nutrients (TDN) were calculated using the formula [(TDN% = 88.9 - (0.79 x ADF)] by Holland et al. (1990). The brix degree content was measured using a PR-101

Table 1. Experimental design and method of fertilizer application

Treatments	Nitrogen level (kg/ha)	Potassium (kg/ha)	Phosphorus (kg/ha)	Method of fertilizer application
T1	200	150	200	1) Nitrogen and potassium were applied two times (Basal fertilizer: additional fertilizer, 6:4) 2) Phosphorus used the entire amount as the basal fertilizer
T2	300	150	200	
T3	400	150	200	
T4	500	150	200	

Table 2. Chemical properties of the soil before experiments

pH	OM <sup>1)</sup> (%)	T-N <sup>2)</sup> (%)	AvP <sub>2</sub> O <sub>5</sub> (mg/kg)	Exchangeable cation (cmol <sup>+</sup> /kg)				CEC <sup>3)</sup> (cmol <sup>+</sup> /kg)
				K	Na	Ca	Mg	
5.45	1.81	0.08	135	0.62	0.15	4.31	1.03	10.12

OM<sup>1)</sup>; organic matter, T-N<sup>2)</sup>; total nitrogen, CEC<sup>3)</sup>; cation exchange capacity

Table 3. Monthly meteorological data during the experimental period

Month	Mean temp (°C)	Precipitation (mm)	Days of precipitation (day)	Duration of sunshine (hr)
May	19.8	52.8	10	104.8
June	23.7	47.0	9	204.2
July	25.9	313.6	12	165.6
August	23.9	116.3	7	249.6
September	21.3	162.0	17	113.2

sugar degree meter (Atago Co. Ltd., Tokyo, Japan). PAL-14S, PAL-15S, PAL-12S, PAL-16S, and PAL-18S of refractometer (Atago Co. Ltd., Tokyo, Japan) were used for the content of fructose, glucose, dextran, isomerase, and inverted sugar, respectively. These measurements were carried out with the juice after compressing the plants. At this time, the compressing point was about 10 cm from the cutting part of the plant. The measurement of mineral contents of the extracted solution based on AOAC (1995) method. The solution were analyzed by ICP mass spectrometry (Inductively Coupled Plasma, Iris Intrepid, Thermo Elemental Co., UK). The analysis conditions were as follows: approximate RF power of 1,150 W, pump rate of 100 RPM, and sprayer pressure observation height set to 30 PSI every 15 mm.

#### 4. Statistical analysis

The results were subjected to one-way analysis of variance with nitrogen level as a main effect. The mean values and standard deviations of the experimental results were obtained using SAS (Statistics Analytical System, USA. 2002) program. Duncan's multiple comparison test was used to identify differences among the treatments, which was considered significant when  $p < 0.05$ .

### III. Results and Discussion

#### 1. Effects of different nitrogen levels on growth characteristics and yields

The results of the effects of different nitrogen levels on growth characteristics and yields are shown in Table 4. Plant length, leaf length, leaf width, number of leaves, stem diameter, dead leaves, stem hardness, number of tillers, and fresh yield were not significantly different according to nitrogen fertilization level ( $p < 0.05$ ). There was no significant difference between the treatment

levels, even though the T3 treatment (400 kg/ha) showed the highest mean values in leaf width, leaf number, number of tillers, and fresh yield. It seemed that the considerable variations in individual plants might cause non-significant differences in T3, which showed the greatest mean values. In a study by Lim et al. (2014), plant height was not significantly influenced by nitrogen fertilization levels in cultivated corn, and stem diameter increased almost linearly from 0 kg/ha to 180 kg/ha but decreased at the excessive nitrogen fertilization level of 360 kg/ha.

Dry matter yield increased significantly ( $p < 0.05$ ) as the nitrogen fertilization level increased from 200 kg/ha (T1) to 400 kg/ha (T3). However, in the T4 treatment fertilized at the highest nitrogen level, there was no significant difference compared to T2 or T3, but the dry matter yield decreased. Lee and Seo (1988) reported that the yield of dry matter increased as the nitrogen fertilization level increased from 0 kg/ha to 400 kg/ha. Redillas et al. (2011) reported that excess nitrogen fertilization can cause lodging, lower yield, and increases susceptibility to pest attacks. On the other hand, nitrogen deficiency in plants leads to growth retardation, lower yield, and death (Ammann and Armengaud, 2009)

The greatest total digestible nutrients (TDN) yield was found in T3 (11,373 kg/ha), followed by T4 (9,292 kg/ha), T2 (9,117 kg/ha), and T1 (8,385 kg/ha) ( $p < 0.05$ ).

As shown in the above results, there were no significant differences in growth characteristics (plant length, leaf length, leaf width, leaf number, stem diameter, dead leaves, hardness, number of tillers) because the cultivation period was short. According to the report of Kim and Lee (2019), the new variety "Geukdong 6" of Teosinte develops to the maximum inherited character when the cultivation period is over 160 days. Therefore, investigations of the level of nitrogen fertilizer applied according to the purpose of use (such as soiling and silage) and dependence on the cultivation period should be explored in further studies.

Table 4. Growth characteristics and yield of *Geukdong 6*

Items	Treatments			
	T1	T2	T3	T4
Plant length (cm)	284.4±2.3 <sup>ns</sup>	285.1±6.8	286.8±1.8	284.9±6.9
Leaf length (cm)	116.9±2.2 <sup>ns</sup>	114.0±4.0	115.3±3.2	115.9±2.3
Leaf width (cm)	5.4±0.3 <sup>ns</sup>	5.4±0.2	5.7±0.1	5.7±0.1
Number of leaves (No.)	12.4±1.0 <sup>ns</sup>	13.3±1.1	15.0±4.3	11.9±1.9
Stem diameter (mm)	18.3±0.7 <sup>ns</sup>	18.6±0.5	18.9±0.3	18.7±1.2
Dead leaves (No.)	3.2±0.6 <sup>ns</sup>	3.3±0.9	3.6±0.9	3.6±0.9
Stem hardness (km/cm <sup>2</sup> )	0.11±0.02 <sup>ns</sup>	0.12±0.00	0.11±0.11	0.11±0.11
Number of tillers (No.)	12.3±0.6 <sup>ns</sup>	13.7±1.4	16.2±4.4	12.9±1.5
Fresh yield (kg/ha)	86,595±5898 <sup>ns</sup>	87,070±5288	91,164±4585	90,106±3601
Dry matter yield (kg/ha)	13,485±819 <sup>b</sup>	14,697±1,032 <sup>a</sup>	17,998±2,035 <sup>a</sup>	15,253±727 <sup>a</sup>
TDN yield(kg/ha) <sup>1)</sup>	8,385±509 <sup>b</sup>	9,117±640 <sup>b</sup>	11,373±1,286 <sup>a</sup>	9,292±443 <sup>b</sup>

<sup>1)</sup>TDN: total digestible nutrition, ns: not significant, <sup>a,b</sup>Means with different superscripts in the same row are significantly different ( $p<0.05$ ).

## 2. Effects of different nitrogen levels on chemical compositions

The effects of the different nitrogen levels on chemical compositions are shown in Table 5. Crude protein showed the highest content in the T4 treatment (10.49%), while T1 was the lowest (7.53%) ( $p<0.05$ ), but there was no significant difference between T4 and T3. These results were somewhat higher than those reported by Cui et al. (2016) and Kim and Lee (2019). Lee and Seo (1988) showed that higher content of crude protein of a Sorghum x Sudangrass hybrid, pearl millet, and Teosinte were detected by increasing nitrogen fertilization levels from 0 kg/ha to 400 kg/ha. This coincides with the present study. Mahmud et al. (2003) reported that application of nitrogen increased crude

protein in forage. They also mentioned that plant nutrition may improve the quality of forage in terms of its protein content. Also, Mullins et al. (1988) reported that nitrogen fertilizer increased forage protein content in corn. Many investigators found that the protein content of forage maize could be raised by increasing levels of nitrogen (Ganguar and Karia, 1988; Cox et al., 1993). When nitrogen levels are limited and deficient, the photosynthesis is not fully used during organic nitrogen compound synthesis (Karic et al., 2005) Crude fat was highest in the T3 treatment, followed by T1, T4, and T2 treatments, but there were no significant differences among the treatments. Also, crude ash was the highest in the T1 treatment compared to the other treatments, but this difference was not significant. Crude fiber and neutral

Table 5. Chemical compositions of *Geukdong 6* (DM %)

Items	Treatments			
	T1	T2	T3	T4
Crude protein (%)	7.53±0.18 <sup>b</sup>	7.85±0.81 <sup>b</sup>	9.63±0.31 <sup>a</sup>	10.49±1.23 <sup>a</sup>
Crude fat (%)	1.64±0.09 <sup>ns</sup>	1.40±0.29	1.77±0.52	1.59±0.22
Crude ash (%)	12.97±3.07 <sup>ns</sup>	11.55±1.57	10.62±1.05	11.42±0.60
Crude fiber (%)	28.65±2.32 <sup>ns</sup>	28.93±0.99	30.21±0.49	30.93±1.71
NDF <sup>1)</sup> (%)	62.23±1.73 <sup>ns</sup>	62.67±1.28	62.31±3.50	64.57±0.93
ADF <sup>2)</sup> (%)	34.30±1.03 <sup>ns</sup>	34.49±2.42	33.01±1.89	35.92±2.36
TDN <sup>3)</sup> (%)	62.18±1.15 <sup>ns</sup>	62.03±2.70	63.19±2.11	60.92±2.64

NDF<sup>1)</sup>: neutral detergent fiber, ADF<sup>2)</sup>: acid detergent fiber: TDN<sup>3)</sup>: total digestible nutrition, ns: not significant, <sup>a,b</sup>Means with different superscripts in the same row are significantly different ( $p<0.05$ ).

detergent fiber (NDF) content were higher in T4, which had the highest nitrogen fertilization level, but lower in T1, which had the lowest fertilization level. However, there were no significant differences between these treatments. This result is different from study such as Almodares et al. (2009), in which the crude fiber content decreased as the nitrogen fertilization level increased. However, Eltelib et al. (2006) reported that higher levels of nitrogen showed little effect on the fiber content of forage maize. The ADF content ranged between 33.01% and 35.92% according to the nitrogen fertilization level, but this difference was not significant. Similar results were obtained for NDF.

The TDN contents were the lowest in the T4 treatment with high NDF and ADF content, but there was no significant difference among the treatments. These results are consistent with the finding of Kim and Lee (2019) and Kim et al. (2015), who reported that high NDF and ADF content reduced TDN content.

### 3. Effects of different nitrogen levels on sugar contents

The effects of the different nitrogen levels on sugar contents are summarized in Table 6. The Brix degree was significantly higher in the T2 treatment (4.84%) than in the other treatments, whereas it was the lowest in the T3 treatment (3.66%) ( $p<0.05$ ). Fructose was highest in the T2 treatment, followed by T1, T4, and T3 ( $p<0.05$ ). Glucose content was the highest in the T2 treatment (4.93%), while the T3 treatment (3.63%) showed the lowest glucose content. However, there was no significant difference between these treatments. Dextran and inverted sugar contents were significantly higher in the T2 treatment than in the others ( $p<0.05$ ). However, there was no significant difference in inverted sugar content among the T1, T3, and T4 treatments. According to these results, the T2 treatment showed

the highest sugar content represented as brix degrees, fructose, glucose, dextran, isomerase, and inverted sugar.

Cui et al. (2016) reported that brix degrees, fructose, glucose, dextran, isomerase, and inverted sugar were 4.90%, 5.19%, 4.78%, 4.26%, 5.16%, and 5.42%, respectively. Therefore, compared to the results of this experiment, the T2 group showed a similar tendency, while the T1, T2, and T3 groups decreased. Generally, the sugar contents of the forage crops vary depending on the seeding time, growing period, fertilization level, climatic conditions, and soil conditions (Do et al., 2012). Sugar content in the silage are very important because it affects the fermentation quality of the silage ingredients (Smith, 1972; Hwang and Lee, 2014).

### 4. Effects of different nitrogen levels on mineral contents

The effects of the different nitrogen levels on mineral contents are shown in Table 7. The accumulation of macro nutrients in the “Geukdong 6” forage crop grown under different nitrogen fertilization levels was greatest for K, followed by Ca, Mg, and Fe, and the accumulation of micro nutrients was greatest for Na, followed by Zn, Mn, Cu, Mo, and Co. This is consistent with the experimental results from Cui et al. (2016). The Ca content linearly increased from T1 to T3 but decreased at T4, as it was only significantly different in the T3 treatments, just state that it was highest in T3.

The contents of Cu, Co, Fe, K, Mg, Mn, Mo, and Zn showed no significant difference according to nitrogen fertilization level. The Na content was highest in the T2 treatment, but was only significantly lower in the T1 treatment ( $p<0.05$ ). Total mineral contents were highest in the T4 treatment (23,211.0), followed by T3 (22,138.6), T1 (21,922.3), and T2 (21,239.6 mg/kg) treatment, but there were no significant differences between the treatments.

Table 6. Sugar contents of *Geukdong 6* (% fresh content)

Items	Treatments			
	T1	T2	T3	T4
Brix degree (%)	4.18±0.02 <sup>b</sup>	4.84±0.12 <sup>a</sup>	3.66±0.16 <sup>c</sup>	4.59±0.58 <sup>b</sup>
Fructose (%)	4.10±0.09 <sup>b</sup>	5.25±0.43 <sup>a</sup>	3.77±0.11 <sup>b</sup>	3.90±0.23 <sup>b</sup>
Glucose (%)	4.00±0.3 <sup>ns</sup>	4.93±0.67	3.63±0.14	3.98±0.21
Dextran (%)	3.18±0.18 <sup>b</sup>	4.15±0.23 <sup>a</sup>	3.14±0.14 <sup>b</sup>	3.05±0.26 <sup>b</sup>
Isomerase (%)	2.44±0.2 <sup>c</sup>	5.47±0.71 <sup>a</sup>	2.82±0.14 <sup>c</sup>	4.22±0.16 <sup>b</sup>
Inverted sugar (%)	4.60±0.05 <sup>b</sup>	5.56±0.40 <sup>a</sup>	4.35±0.09 <sup>b</sup>	4.25±0.16 <sup>b</sup>

ns: not significant, <sup>a,b,c</sup>Means with different superscripts in the same row are significantly different ( $p<0.05$ ).

Table 7. Mineral contents of *Geukdong 6* (DM mg/kg)

Items	Treatments			
	T1	T2	T3	T4
Ca	1,693.1±371.8 <sup>b</sup>	1,816.8±377.2 <sup>b</sup>	2,068.0±227.5 <sup>a</sup>	1,774.8±268.7 <sup>b</sup>
Cu	5.11±0.65 <sup>ns</sup>	5.69±1.19	8.55±0.88	4.78±0.49
Co	0.08±0.02 <sup>ns</sup>	0.09±0.01	0.08±0.02	0.09±0.01
Fe	148.50±18.87 <sup>ns</sup>	184.68±31.44	175.64±1.11	154.61±35.24
K	19,143.2±277.9 <sup>ns</sup>	17,993.2±1109.2	18,658.4±938.8	19,894.7±1565.7
Mg	789.5±27.6 <sup>ns</sup>	1,074.8±128.4	1,062.6±220.8	1,228.3±212.5
Mn	31.95±5.02 <sup>ns</sup>	38.19±6.95	49.95±0.28	38.00±18.07
Mo	0.80±0.26 <sup>ns</sup>	0.85±0.01	0.92±0.28	2.10±2.04
Na	56.87±2.26 <sup>b</sup>	72.35±2.43 <sup>a</sup>	66.81±2.60 <sup>a</sup>	69.13±6.22 <sup>a</sup>
Zn	53.16±1.90 <sup>ns</sup>	52.97±12.19	47.67±9.03	44.44±10.07
Total	21,922.3±572.7 <sup>ns</sup>	21,239.6±911.7	22,138.6±1329.3	23,211.0±1883.7

ns: not significant, <sup>a,b</sup>Means with different superscripts in the same row are significantly different ( $p<0.05$ ).

The total mineral content in this experiment was lower than that reported by Cui et al. (2016) in all treatments. Many researchers reported that the concentrations of minerals in plants depend on the cultivar, climatic conditions, water conditions, fertilization conditions, harvest date, and rootstock condition in particular (Jaroszewska, 2011; Jeon et al., 2012; Nergiz and Yýldýz, 1997)

#### IV. Conclusion

Geukdong 6, a new variety of Teosinte, is a forage crop with a high demand for nitrogen, a high tiller ability, and high dry matter yield. However, it was found that low and high levels of nitrogen fertilization have adverse effects on growth and yield. Therefore, adequate nitrogen fertilization is very important for the cultivation of Geukdong 6. From this experiment, we determined that considering the growth characteristics, the feed value, the dry matter yield, and total digestible nutrition yield, the level of nitrogen fertilization is properly judged to be about 300 to 400 kg per ha.

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(Received : March 9, 2020 | Revised : March 13, 2020 | Accepted : March 13, 2020)