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Botanical Composition and *In Situ* Dry Matter Degradability of Legume- Grass Mixture Pasture Fertilized with Different Amounts of Nitrogen

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ABSTRACT

This study was conducted to determine the effects of different nitrogen (N) fertilizer levels on botanical composition, dry matter (DM) yield, chemical composition, *in situ* ruminal DM and NDF degradability and net energy lactation (NEL) of a native legume-grass mixture pasture. Field and degradability trials were arranged in a Complete Randomized Block Design with three replicates. Pasture was fertilized at six levels of N fertilizer (ammonium nitrate, N0, N50, N100, N150, N200 and N250 kg/ha). Three rumen cannulated Holstein Friesian heifers (3-4 year old) were used. Feed samples were incubated for 0, 8, 12, 24, 36, 48, 72 and 96 h. Application of nitrogen fertilizer results indicated that nitrogen fertilizer decreased the legumes ratio (LR) of pasture, approximately 22.4%, Although N fertilizer increased the grass ratio (GR) of pasture approximately 55.8%. Dry matter (DM), Net energy lactation (NEL, Mcal/kg) and ash contents of pasture were increased by increasing N fertilizer level. However, when N fertilizer level increased acid detergent fiber (ADF) concentration of pasture decreased. Application of the different rate of nitrogen fertilizer had no effect on *in situ* rumen degradability of DM and NDF of pasture. It was also found that there was the significant positive relationship between effective NDF degradability at 48 h. rumen incubation period and different level of N fertilizer. In addition, it was determined that there was a linear and quadratic positive relationship between DM yield and different nitrogen doses. In conclusion, different rates of nitrogen application changed botanical composition, decreased ADF content and increased NEL and effective rumen degradability of neutral detergent fiber ($ED_{NDF\ 48\ h.}$) of pasture.

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Introduction

Native pasture originates mostly grass and legumes which are the main source of herbage for feeding ruminants. Therefore, pasture must be managed correctly to increase the herbage quality. One of the important management practise and topic in intercropping systems is nitrogen fertilizer. Some research results have shown that nitrogen fertilizer positively affects the grasses, on the other hand, negatively affects the legumes in intercropping systems (Acikgöz, 2001). Naturally grown grass species give the high quality herbage by applying of N fertilizer (Khan et al., 1999). Farmers want to produce the more quality forage from grassland for their animals. Therefore, N fertilizer is used on grassland for contributing to the pasture productivity in terms of forage yield and quality. Increasing of the amount of nitrogen fertilizer applications decreased ADF and NDF concentration of rye grass (Zhang et al., 1995**)**. So that, nutritive value of grassland can be increased with increasing levels of nitrogen application (Valk et al., 2000). Besides, same researchers reported that increasing nitrogen fertilizer enhanced the grass rate, ruminal DM degradability of grass and herbage yield of grass- legume pasture. On the other hand, nitrogen fertilizer decreases legume ratio and NDF content of grass- legume pasture (Arvas et al., 2011). Bleanger et al. (1998) pointed out that nitrogen deficiency reduces *in vitro* true DM and cell wall digestibilities**.** Besides, nitrogen fertilizer increased DM digestibility of some tropical grasses by 9% (Carver et al., 1975). Forage quality in terms of chemical composition, ruminal DM degradation characteristics and

ME are big indicators for feed evaluation. These effects of fertilizer on animal feed intake and animal productivity by converting plant material to animal tissue are important for animal feeding (Zimmerman, 1980). Some important features of cell-wall structure and development may be related to degradability of the cell wall by ruminal microorganisms (Jung and Allen, 1995). Recently, researchers have shown that nitrogen fertilizer application is beneficial to production in terms of DM digestibility. Little information on fertilizer management of native legume- grass mixtures is available (Dong et al., 2005).

The hypothesis tested in this study was that application of nitrogen on pasture would significantly change botanical composition, chemical composition, *in situ* ruminal dry matter degradability characteristics and NEL content of a native grass-legume mixture pasture.

Materials and Methods

Location and Climate Condition

The trial was conducted in a native grass-legume mixture pasture in Ceyhan, Adana, Turkey in 2010 during the growing season. Experimental area was estabilished at latitude and longitude 37º 00' 64" N and 35º 44' 87" E, altitude is 100 m above sea level. The soil of the experimental area was slightly salty and clay. The enviromental conditions and dates were collected from the meteorological service of Turkish state. During the growing period, average temperature and annual precipitation of experimental area were 13.9ºC and 629.9 mm.

Agronomic Practices

At the beginning of this trial, experimental area contained the high percentage of legume species as *Trifolium fragiferum, Trifolium nigrescens, Trifolium lappaceum, Medicago hispida, Medicago orbicularis* and *Scorpirus subvillosus* and as grasses *Hordeum bulbosum, Lollium italicum, Koeleria phloides, Alopecirus myososroides, Phalaris canariensis* and *Polypogon mospeliensis.* Six levels of N fertilizer (N0, N50, N100, N150, N200 and N250 kg/ha as ammonium nitrate) were applied in 4 m x 20 m plots and replicated three times with complete randomized block design. After harvested herbage sample taken from pasture was separated by hand as grass, legume and other species in order to determine botanical composition. On the other hand, the ratio of other species was very lower than grass and legume plants in the pasture.

Sample Preparation

Forage samples were collected with a harvester at 50% flowering stage of plants from the each plot. An area of 1 m² was hand clipped from each of the 3 replicated plots for estimation of forage production. The forage samples of each treatment were taken as three replicates and then herbage samples were brought to the laboratory. Samples were dried at 70ºC for 48 h. and ground in mill 1 mm for chemical analysis and 2.5 mm screen for in situ DM degradability analysis. Dry matter content of forage samples was determined by drying at 105ºC over night then their ash content was determined by burning the samples in muffle furnace at 525ºC for 8 h. (AOAC, 1990). Additionally, ADF and NDF contents of forage samples were determined (Van Soest et al., 1991) by using ANKOM Fiber Analyzer (F220/220 Operator's Manual, Ankom tech.).

In Situ Incubations

Holstein Friesian heifers (3-4-year-old) were cannulated with large rumen cannula (10 cm internal diameter; Diamond Inc.). Heifers with an average body weight (BW) of 450±30 kg, were used to evaluate forages by using *in situ* nylon bag method. Besides heifers were housed in individual pens and allowed to adapt to the experimental conditions during the 3 weeks period. Animals were fed on alfalfa (70%) and grass forage (30%) based diet as recommended by NRC (2001) and mineral supplement (One kilogram of premix contains the following: 400 g limestone, 100 g Calcium perphosphate, 200 g salt, MgO 90 g, Vit A 320.000 IU, Vit D 75.000 IU, Vit E 165 mg/kg, Fe, 1.500 mg, Cu 685 mg, Zn 2.500 mg, Mn 1.500 mg, Se 80 mg, I30 mg, Co 25 mg), salt and free access to fresh water twice daily in equal portions at 08.30 and 16.30 h. The herbage samples taken from the pasture were dried and ground to 2.5 mm screen then, 5 g subsamples were transfered to nylon bags. (bag made of polyester and 7.5 cm x 15.5 cm, 40 micron pore size, Bar Diamond Inc.). Nylon bags were put into the rumen for incubations of 0, 24, 36, 48, 72 and 96 h. Heifers was used as block in complete randomized block design and the experimental groups was three replicates. After incubation, the samples were withdrawn from the rumen and bags were washed in cold water. Zero time disappearances was obtained by washing unincubated bags in similar fashion, and then bags were dried at 55ºC in an oven for 48 hours (Lai and Thu Huong, 1999). *In situ* rumen DM degradability for each incubation period was calculated by using the equation below,

DM degradability (DM, g/kg) = (Inital weight– final weight) / (Inital weight) ×100.

Degradation characteristics of DM were calculated by the equation given by Ørskov and McDonald (1979) below,

$$D= a+b\ (1-e^{-ct}),$$

Where;

D = the actual degradation after " t" hours,
a = the portion of DM solubilized at the beginning of incubation (zero time),
b = slowly degraded portion of DM in the rumen,
c = rate constant for the degradation of "b",
t = time of incubation.

Degradation characteristics; a, b, c and effective ruminal degradability of DM was calculated by Ørskov and McDonald (1979).

The effective dry matter and NDF degradability were calculated by using equation,

$$ED = a+ (bc / c+k).$$

Where:

k = ruminial out flow rate, k_1 = 0.02/h, k_2=0.05/h, for dry matter and NDF.

The $NEL_{(Mcal/kg)}$ was calculated by the equation given by NRC, (2001)

$$NEL_{Mcal/kg} = 2.149-(0.0223 \times ADF)$$

Statistical Analysis

Data were calculated by the general linear model procedures (SPSS, 2007) as a completely randomized design with heifers as block. One–way analysis of variance (ANOVA) was used to determine the effects of N fertilizer on the chemical composition, $NEL_{Mcal/kg}$ and rumen degradation characteristics of forage samples. Significance between the treatments means was identified using Duncan multiple range test**.** Prediction equations for effective degradability (ED) and forage yield based on chemical composition were calculated by linear and quadratic multiple regressions.

Results

Botanical composition of pasture was significantly changed after application of N fertilizer. Legume and grass rates of pasture were summarized in Table 1. Although nitrogen application from 0 to 250 kg per hectare steadily decreased ratio of legumes from 85 to 66%, N application increased grass ratio of pasture from 15 to 34% (P<0.05**)**. DM yield of pasture was presented in Table 1. Dry matter yield of pasture ranged from 5592 to 7195 kg/ha, the lowest and the higest forage yield was obtained from control and N200 application groups respectively. But, there was no significant difference between different rates of N fertilizer applications. In contrast, N application on pasture significantly increased DM content of pasture from 90.4 to 92.1%, respectively. Likewise, ADF content of pasture was significantly decreased with increasing N application (P<0.01).

NEL values of pasture were seen in Table 2. Application of N fertilizer significantly increased $NEL_{(Mcal/kg)}$ content of pasture (P<0.01). The highest NEL values were obtained from 150 kg/ha and 250 kg/ha nitrogen application groups

Ash content of pasture herbage was increased up to 4.3% with increasing the level of nitrogen from 0 to 250, except on N50 group. Besides, NDF content of grass-clover mixed swards ranged from 56.3 to 58.5%, was not significantly affected by N treatments (Table 2).

Ruminal DM and NDF degradability and ruminal degradation characteristics of pasture were summarized in Table 3 and in Figure 1. Nitrogen fertilizer application did not changed DM and NDF degradability and ruminal degradation characteristics of native pasture for other rumen incubation times. However, the effective rumen degradability of NDF of pasture was significantly affected in 48 h period of rumen incubation. As shown in Table 3.

The equations for prediction of forage yield and ED by using multiple regression were given in Table 4 and Relationship between ADF content and grass ratio was shown in Figure 2. As shown on regression equation, the forage yield was increased by increasing rate of nitrogen fertilizer. As a result, there was a significant linear and quadratic positive relationship between forage yield and application of nitrogen fertilizer on pasture (P<0.05, R^2 =0.248). As indicated in this equation (Forage yield $_{(kg/ha)}$ = 5846.048+ 5.946 N), each kilogram of N increased in a 5.946 unite of forage yield.

The ADF concentration of the pasture was affected by ratio of legume and grass of the pasture. As illustrated in Figure 2 and Table 4, there was a strong positive relationship between legume ratio and ADF content of mixture swards. In contrast, there was a very significant negative relationship between grass ratio and ADF content of pasture in terms of pasture composition (P<0.01, R^2 = 0.457). Besides, there was a significant linear and quadratic positive relationship between N application and effective rumen degradability of NDF at the 48h. rumen incubation period (P<0.05, R^2=22; P<0.05, R^2=0.328).

Table 1 Botanical composition and dry matter yield of pasture

Parameter	Nitrogen fertilizer (kg/ha)						SEM	Sig.
	H0	H50	H100	H150	H200	H250		
Legume (%)	85.0 ^{a}	77.0 ^{abc}	80.0 ^{bc}	76.0 ^{abc}	67.0 ^{ab}	66.0 ^{c}	3.70	*
Grass (%)	15.0 ^{a}	23.0 ^{abc}	20.0 ^{ab}	24.0 ^{abc}	33.0 ^{bc}	34.0 ^{c}	3.70	*
Forage yield $_{(DM\ kg/ha)}$	5592	6077	6709	7028	7195	6938	247.15	ns

*Means with different subscripts within the same rows are significant (P<0.05); ns, Non significant; SEM, Standard error of mean

Table 2 Chemical composition of fertilized pasture

Parameter %	Nitrogen fertilizer (kg/ha)						SEM	Sig.
	N0	N50	N100	N150	N200	N250		
DM	90.4 ^{a}	91.0 ^{ab}	92.4 ^{c}	91.7 ^{bc}	91.6 ^{bc}	92.1 ^{bc}	0.30	*
NDF	58.5	57.4	57.3	56.3	57.2	57.2	0.84	ns
ADF	47.7 ^{c}	47.0 ^{c}	46.4 ^{bc}	44.0 ^{a}	44.3 ^{ab}	44.0 ^{a}	0.58	**
Ash	3.1 ^{ab}	2.5 ^{a}	2.7 ^{ab}	4.0 ^{bc}	4.1 ^{bc}	4.3 ^{c}	0.64	*
$NEL_{(Mcal/kg)}$	1.085 ^{c}	1.101 ^{c}	1.116 ^{bc}	1.168 ^{a}	1.156 ^{ab}	1.168 ^{a}	0.0095	**

DM, Dry matter; NDF, Neutral detergent fiber; ADF, Acid detergent fiber; Means within rows by different letters differ *(P<0.05), ** (P<0.01); ns, Non significant; SEM, Standard error of mean.

Table 3 Ruminal degradation characteristics of fertilized pasture

Item	Nitrogen fertilizer (kg/ha)						SEM	Sig.
	N0	N50	N100	N150	N200	N250		
DM, (%)								
a	30.16	28.52	29.33	31.26	29.76	27.27	0.80	ns
b	38.70	39.08	39.88	40.10	40.46	42.11	2.16	ns
a+b	69.09	67.60	69.21	70.90	70.21	69.38	4.22	ns
$k_{1\ (0.02/h)}$	0.291	0.298	0.289	0.283	0.285	0.289	0.092	ns
$k_{2\ (0.05/h)}$	0.727	0.745	0.723	0.708	0.713	0.721	0.023	ns
$ED_{1(k=0.02/\ h)}$	41.6	40.5	41.3	42.9	42.0	40.6	0.80	ns
$ED_{2(k=0.05/h)}$	35.7	34.4	35.2	36.9	35.7	33.9	0.80	ns
NDF, (%)								
b	27.75	24.67	21.76	22.65	23.22	19.07	2.98	ns
a+b	-1.97	-4.62	-11.32	-13.50	-10.16	-17.46	4.58	ns
c (h^{-1})	0.0069	0.0064	0.0054	0.0051	0.0056	0.0045	0.0005	ns
$k_{1\ (k=0.02/h)}$	0.349	0.373	0.365	0.341	0.356	0.362	0.115	ns
$k_{2\ (k=0.05/h)}$	0.871	0.931	0.913	0.852	0.889	0.903	0.265	ns
$ED_{1(k=0.02/h)}$	-29.17	-28.87	-32.75	-35.08	-32.94	-36.27	1.98	ns
$ED_{2(k=0.05/h)}$	-29.50	-29.12	-32.95	-36.01	-33.20	-36.43	1.94	ns

ME, metabolizable energy; ED, effective degradability; DM, dry matter; NDF, neutral detergent fiber; SEM, standard error of mean; ns, Non significant.

Table 4 Prediction of some parameter of pasture by using multiple regresion

Equations	Significance	R^2	RMSE
Forage yield (kg/ha) = 5846.048+ 5.946 N	*	0.248	937
Forage yield (kg/ha) = 5514,679 + 15,887 N - 0,04 N^2	*	0.215	928
ADF = 48.871-13.178 GR	**	0.457	1.37
ADF = 35.693+13.178 LR	**	0.457	1.37
$ED_{(NDF\ deg\ 48h)}$ = 41.122+0.233 N	*	0.220	3.98
$ED_{(NDF\ deg\ 48h)}$ =42.983 -0.032 N +0.0002 N^2	*	0.328	3.81

N, nitrogen; ADF, acid detergent fiber; ED, effective degradability; LR: Legume rate, GR: grass rate; *(P< 0.05), ** (P<0.01); RMSE, root mean square error

Discussion

Botanical Composition

Nitrogen fertilizer increased grass ratio approximately 55.8% and decreased legume ratio of clover-grass pasture approximately 22.4%. It is reported that this reaction of N fertilizer depended on grass species of pasture (Peyraud et al., 1998; Mosquera et al., 2004). Wilman and Fisher (1996) reported that N fertilizer response of grass-clover mixed swards was lower than pure graminaeus swards due to the sensitivity of clover to nitrogen fertilizer. Because high nitrogen application negatively affects nodulation (Silva et al., 1993; Yolcu et al 2010) and symbiotic fixation (Fan et al., 2006) in legumes, the findings of this study about botanical composition were similar this results. Increasing N doses increased forage yield, on the other hand, were not significant statistically. Mosquero et al. (2004) reported that using 30 kg N ha^{-1} fertilizer increased annual forage yield from pasture in the first year, followed by a decline in the second and third years and no response in the fourth year.

Chemical Composition

Nitrogen fertilizer influenced the dry matter content of pasture. 100 kg N/ ha fertilizer increased approximately 2.21% the dry matter content of clover-grass pasture. Mosquero et al. (2004) pointed out that fertilizer increased DM content of grass-clover mixed swards in the first and fourth year. Similiar results have been reported by Salalün et al. (1999) for rye grass, Hatipoglu et al. (2001) for clover-grass pasture, Budakli et al. (2012) for barley and hairy vetch. NDF content of grass was not markedly influenced by different amounts of nitrogen fertilizer. In the present study, the findings concur with Valk et al. (1996) and Salaün et al. (1999).

Acid detergent fiber (ADF) concentration of native clover-grass pasture was decreased t al. (1996) found that ADF content of pasture with 150 N fertilizer treatments was higher tsignificantly approximately 7.76% with increasing N fertilizer from 0 to 250 kg ha. These findings are agreement with the results of Zhang et al. (1995) for *rye grass*. In addition, Valk ehan of 450 N grass in the two periods. Besides, Hassan et al (2015) reported that the acid detergent fiber content of Panicum maximum was significantly influenced by nitrogen fertilization and increased nitrogen application significantly decreased ADF content. Moreover, Belanger et al. (1998) expressed that N deficiency would increase lignification. Hatipoğlu et al. (2001) reported that when the average environmental temperature at the time of growth stage of the plants was lower than the average temperature of that region and the rain falls more. The rate of pasture lignin was lower. However, same researchers found that N fertilizer had little effect on crude fiber or ADF of pasture (Peyraud et al., 1998). In contrast, some researchers found that N fertilizer increased ADF content of timothy (Messman et al., 1992), mileno grass (Campos et al., 2013), forage rape (Islam et al., 2012). $NEL_{(Mcal/kg)}$ content of pasture increased up to 8.3% by increasing amount of nitrogen application. NEL content depends on ADF concentration of pasture.

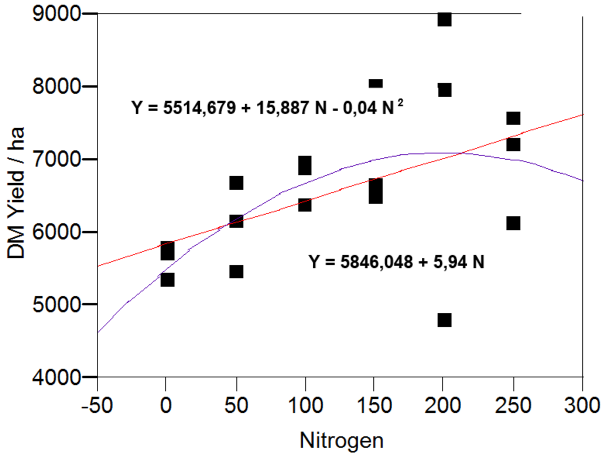

Figure 1 Relationships between nitrogen fertilizer and dry matter yield of pasture

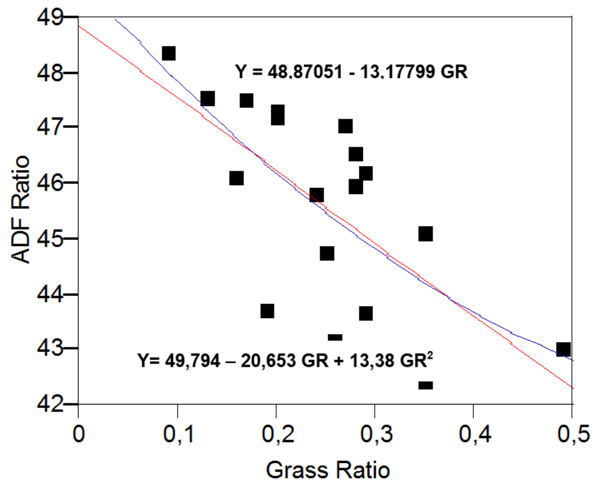

Figure 2 Relationships between acid detergent fiber content and grass ratio

Ash co of clover-grass sward increased up to 4.3% by increasing the level of nitrogen from 0 to 250, except on 50 kg/ha nitrogen doses. In the present study, this result is agreement with the findings of Hunt **(**1973). Onyeonagu et al. **(**2012) showed that increasing N fertilizer resulted in high uptake of nitrogen and potassium (K) from plant root and with perennial ryegrass, raising the level of nitrogen fertilizer from 59 to 111 kg/ha significantly increased K, P, Ca, Mg and Na minerals in dry matter. Linear and quadratic regression indicated a significant relationship between N fertilizer rate and forage DM yield for clover – grass mixture. Similar findings results were reported by Dong et al. (2005) for perennial grass mixture. Besides, Malhi et al. (1986) reported that annual fertilizer N applications sustained more dry matter production than single N applications for bromegrass. In present study, relationship between ADF content of mixture sward and grass ratio was showed in Figure 2. Acid detergent fiber content of pasture was decreased linearly by using different N levels on pasture field. These results concur with the findings of Valk et al. (1996) for *Lolium perenne.*

In Situ Degradability

In the present study, DM and NDF degradability were not affected by using N fertilizer. Similar findings were stated by Reid et al. (1966) for *Orchardgrass*, Van Vuuren et al. (1991) for perennial rye grass, Messman et al. (1992) for brome grass, Dong et al. (2005) for perenniel grass mixtures. In contrast, Calder and Mcleod (1968) reported that nitrogen applying in amounts of 52 and 103 kg/ha to permanent pasture resulted in a decrease of digestibility by 2.15 and 2.23%, respectively. Conversely, Valk et al. (1996) reported that higher degradation rate of cell walls with increased levels of N fertilization. There are a lot of different results about cell wall degradability. Viken and Volden (2009) expressed that NDF degradation characteristics are probably caused by interaction between N fertilization and climatic factors like temperature and water availability. Applied N fertilizer on pasture increased effective degradability of NDF in the 48-h degradation period. Similar results were pointed out by Valk et al. (1996) who found that the degradation rate of potentially degradable NDF was significantly increased at higher N application levels.

In conclusion, it was observed that applied 150 kg/ha of nitrogen increased NEL content by 8.3% and dry matter by 13% of pasture. Even if not statistically significant, the forage yield of pasture has increased by 28%. It is thought that more pasture management studies should be done in order to get more benefit from pasture in Turkey. In addition to, legumes cause bloating when freshly consumed by ruminant. During the spring period, the bloating losses are increased in the grazing animals. In the legume dominant native pasture, Decrease of the legume ratio by the increase of applied nitrogen doses can reduce animal losses.

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