Anadolu Tarım Bilimleri Dergisi
Anadolu Journal of Agricultural Sciences
http://dergipark.ulakbim.gov.tr/omuanajas

Araştırma/Research

Anadolu Tarım Bilim. Derg./Anadolu J Agr Sci, 30 (2015) 160-168
ISSN: 1308-8750 (Print) 1308-8769 (Online)
doi: 10.7161/anajas.2015.30.2.160-168

Changes in nitrate (NO_3-N) and macro mineral content of different forage sources affected by increasing nitrogen doses

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Geliş/Received 09/03/2015 Kabul/Accepted 20/06/2015

ABSTRACT
Nitrate toxicity due to excessive nitrogen (N) fertilization in forage crops is one of the most important nutritional concerns. The aims of this study were to evaluate the effects of increasing rates of N fertilizer on the NO_3-N content and K/(Ca+Mg) ratio of some forage crops such as pasture (Experiment 1) (Exp 1), vetch (*Vicia pannonica* Crantz.) and barley (*Hordeum vulgare* L.) mixture (2/1, Exp 2) and corn (*Zea mays* L.) (Exp 3) and the effect of silage on the NO_3-N contents of forage corn (Exp 4). These experiments, except for Exp 4 were carried out under dryland field conditions by applying increasing rates of N (0, 50, 100, 150 and 200 kg ha^{-1}) in a randomized plot with three replicates. Dry matter yield, crude protein and some mineral (calcium (Ca), phosphorus (P), potassium (K) and magnesium (Mg)) contents and ratio amoung some minerals and NO_3-N content of forage from each experiments were determined. The results showed that the NO_3-N contents and K/(Ca+Mg) ratio of the forages increased up to toxic levels in accordance with increasing rates of N fertilizer independing of type of forage.

Keywords:
Concentration
Forages
Macro mineral
Nitrate accumulation
Nitrite
Quality

Artan azot dozlarına göre farklı kaba yemlerin nitrat (NO_3-N) ve makro element içeriğindeki değişimler

ÖZET
Aşırı nitrojen gübrelemesinden dolayı, kaba yemlerde görülen nitrat toksisitesi, en önemli besinsel konulardan biridir. Bu çalışmanın amacı; mera (Deneme 1), macar fiğ (*Vicia pannonica* Crantz.) + arpa (*Hordeum vulgare* L.) karışık ekimi (2/1 Deneme 2) ve mısır (Deneme 3) gibi bazı kaba yemlerin NO_3-N ve K/(Ca+Mg) oranı üzerine nitrojenin artan oranlarının etkisini ve mısır kaba yeminin NO_3-N içeriği üzerine silajlamanın (Deneme 4) etkisini değerlendirmektir. Dördüncü deneme hariç, diğer denemeler 3 tekerrürlü olarak Tesadüf Blokları deneme deseninde yürütülmüştür. Denemelerde nitrojenin artan oranları (0, 50, 100, 150 ve 200 kg ha^{-1}) kurak şartlarda uygulanmıştır. Her bir denemeden elde edilen kaba yemin kuru madde verimi, ham protein ve bazı mineral içerikleri (Ca, P, K, Mg) ile bazı mineraller arasındaki oran (K/(Ca+Mg)) ve NO_3-N içeriği belirlenmiştir. Sonuçlar, yemin tipine bağlı olmaksızın, artan nitrojen gübrelemesinin kaba yemin NO_3-N içeriğini ve K/(Ca+Mg) oranını toksisite seviyesine yükselttiğini göstermiştir.

Anahtar Sözcükler:
Konsantrasyon
Kaba yem
Makro mineral
Nitrat birikimi
Nitrit
Kalite

1. Introduction

In order to meet the feeding demand of 11 million cattle and 29 million sheep, approximately an extra 15 million ton forage production is required in Turkey. The most pratical and effective method to increase dry matter production in forage crops is fertilization (Frame, 1992; Aydin and Uzun, 2005). Fertilization, especially with N can increase dry matter production up to three fold in forage crops, especially grasses, depending on the annual rainfall or irrigation practices (Elliott and Abbott, 2003). Forage quality can be described as the conversation of consumed forage to animal product. One of the main criterion is the crude protein concentration in the forage (Gillen and Berg, 1998). In general, forage quality increase under suitable fertilization application conditions but over use of fertilizer, especially N, cause significantly decreases in forage quality. The most critical problem arising from fertilization, especially N, in the forage production is occurrence of nitrate poisoning and tetany risk as a result of unbalanced mineral substances in dry matter.

Although, nitrogen fertilizations increase the yield and protein content of plants, the application of excessive amounts of N can cause the accumulation of toxic levels of nitrate NO_3-N in plants (Nesic et al., 2008; Gulmezoglu et al., 2010). Nitrate toxicity can cause chronically or acute health problem in livestock. The common results of the chronic NO_3-N poisoning are a decrease in the rate of weight gain or milk production. Abnormal growing conditions such as drought, frost or prolonged cool temperatures, hail, shade, disease, high levels of soil nitrogen, soil mineral deficiencies or herbicide damage can cause high nitrate accumulation in forages (Ridder, 1974).

Most commonly, nitrate poisoning occurs in cattle and sheep. In ruminants, nitrate is reduced by microbial reductases to nitrite. The rumen microbes utilize this nitrite by converting it into ammonia as a nitrogenous source. However, excessive nitrite gets accumulated in rumen, from where it is readily absorbed into blood stream and combines with ferrous ion of hemoglobin (Hb) to form met-hemoglobin (met-Hb). The met-Hb is a poor transporter of oxygen in the body and the animal suffers from oxygen deficiency.

Nitrate intake is only one of many factors influencing nitrite accumulation in the rumen, with critical concentration levels of NO_3-N in dietary components changing according to the age, genus and reproductive conditions of animals. For this reason, NO_3-N concentration in forage is still being investigated to determine levels. Cash et al., (2006) reported that forages containing <350 ppm NO_3-N is generally safe for all conditions and livestock, 350-1130 ppm NO_3-N is generally safe for nonpregnant livestock. They also reported that usage of 350-1130 ppm NO_3-N should be limited for bred animals to 50% of the total ration while this range of NO_3-N sould be limited to 25-50% of ration for nonpregnant livestock if the NO_3-N content of forage was 1130-2260 ppm and pregnand animals should not be fed with this amount of NO_3-N since it might cause abortions, weak calves and reduced milk production.

Nitrate contents of the plants changes widely depending on plant species (Sidhu et al., 2011). Nitrate toxicity is most likely to occur when livestock are grazed or fed green-chop. Silage is the least hazardous feed. Ensiling forage usually lowers the nitrate levels by 10 to 60 percent. The nitrate level in hay usually remains constant or declines slightly in storage.

Grass tetany or hypomagnesemic tetany in cattle is caused by an imbalance of K, Ca, and Mg in the diet. Mineral imbalances, deficiencies or excess and low bio-availability of essential minerals result in negative economic impacts when animal performance and health are compromised (Van Soest, 1983). Environmental factors incidence grass tetany. Grass tetany occurs during periods of cool weather or when cool weather is followed by warmer weather that causes rapid forage growth. Therefore, tetany may be a risk in the spring season in Mediterranean climatic countries. The risk of grass tetany dramatically increases when the K/(Ca+Mg) ratio, calculated based on equivalent weight, of forage exceeds 2.2, especially for cows during early lactation (Georgievskii, 1982; Jefferson et al., 2001). Lactating cows grazing in native rangelands require forage containing 1.7–2.0 g kg^{-1} Mg, 2.8 g kg^{-1} Ca and 7.0 g kg^{-1} K in dry matter (NRC, 1996). When forage Mg concentration is below 0.2% in dry matter, the balance between Mg, Ca and K in the animal is not adequate and grass tetany risk can be occured (Grunes and Welch, 1989). In addition to Mg, other forage mineral concentration limits have been related to the hypomagnesaemia disease. These include a forage Ca concentration lower than 0.4% and a forage K level above 3.0% in dry matter (Wilkinson and Mayland, 1997).

Weather, water and soil conditions also affect tetany risk in grazing cattle on rangelands (Mayland, 1986). Mediterranean climatic conditions are characterized by warm dry summers and cool or cold wet winters. In most areas where grassland is the natural vegetation (e.g. Turkey), lack of rainfall in summer and low temperature in winter restricts the grazing period. The experimental area of the present study in the Black Sea Region has favoring ecological conditions for tetany and nitrat accumulation. In the spring period, rainy and cloudy days are highly prevailing and there are limited number of sunny days. It is expected that there is theoricaly a high risk tetany related with nitrate poisioning. Therefore, aims of the present study were to evaluate the effects of N fertilizer on the NO_3-N content and mineral content realted to grass tetany risk of some forage resources such as natural pasture, Hungarian vetch (*Vicia pannonica* Crantz.) and barley mixture and corn (*Zea mays* L.).

2. Materials and Methods

2.1. The experimental area

In order to determine the effects of N-fertilizers on forage quality, three different experiment (one in rangeland and the others in the field area) were carried out between 2010 and 2011. Experiments were conducted at Research Stations, Samsun, located on The Blacksea coast of Turkey (41° 21’ N, 36° 15’E, elevation 120 m) between 2000-2001. The main factor restricting productivity of forage production is short and irregular precipitation throughout the year in the Mediterranean climatic region which include Turkey. A climatic diagramof the experimental year of the study areas are presented in Figure 1. Soil characteristics of experiments areas were determined following the Rowell, (1996) method were found to be as follows; the soil texture are clay; organic matter were 1.68-2.88%; extractable P by 0.5N $NaHCO_3$ extractionare were 2-6 mg kg^{-1}; extraction K by 1N ammoniumacetate extraction are 34-45 mg kg^{-1}; pH are 5.88-7.45 in soil saturation extract.

Before beginning of the rangeland experiment, botanical composition of rangeland area based on weight was determined in seven quadrates, each equals 1 m^2 in May 2011. Botanical composition of experimental area consist of 27% legumes, 45% grasses, and 28% other families. The common legume species in the botanical composition were burr medic (*Medicago hispida* Gaerth.) and subterranean clover (*Trifolium subrerraneum* L.), and red clover

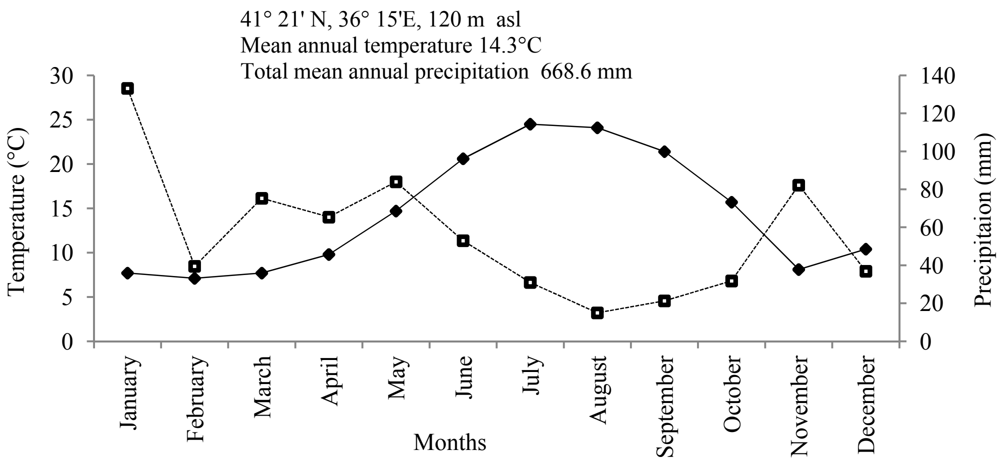

Figure 1. Climatic diagram of Samsun (□) montly means of precipitaion (♦) and temperature in 2011

(*Trifolium pretense* L.); grass species were downy brome (*Bromus tectorum* L.) and perennial ryegrass (*Lolium perenne* L.) and others families species were narrowleaf plantain (*Plantago lanceolata* L.) and smail sorrel (*Rumex acetosella* L.).

2.2. Experimental procedures

Phosphorus (40 kg ha^{-1}) and potassium (80 kg ha^{-1}) were applied all experimental areas as standart in December. The experimental design consisted of completely randomized block with three replications. Nitrogen were applied in three experiment areas with rates of 0, 50, 100, 150 and 200 N ha^{-1}. The fertilizers were applied by hand in the form of ammonium sulphate (N), potassium sulphate (K_2O) and triple superphosfate (P_2O_5).

Experiment 1

Each plot was 4 m × 7 m with a distance of 1 m between plots. Half of the N-fertilizer was applied in the end of February and the other part was applied in the end of March. Herbaceous vegetation was harvested within 9 m^2 area after ignoring 0.5 m^2 area from all sides of the plots when grass plants reached full flowering state in the beginning of June. And then, green forage production per 9 m^2 area was recorded. Plants within 1 m^2 quadrate in each plot were classified as legumes, grasses and the others as well as determining the dry weight ratio each group in the botanical composition. Sample taken from 1 m^2 area of each plot within each group were oven-dried at 60 °C and dry weight ratio of each sample was calculated. Dry matter production of each plot was calculated through the value of green forage production and dry-weight percentage for each family group. Crude protein of harvested legumes, grasses and the others were analyzed by micro-Kjeldhal to determine crude protein concentration of each plot. All plant samples were sieved into less than 0.50 mm and digested with 3:1 (v/v) HNO_3;$HClO_4$ wet digestion methot (Ryan et al., 2001). Ca, Mg and K concentrations were determined by atomicabsorbtion spectrophotometers (PerkinElmer, Model 2280 (using emission (flame) mode according to Johnson and Ulrich (1959)). The three macro nutrients (Ca, Mg, and K) were expressed as g kg^{-1} of dry matter and ratio of K/(Ca+Mg) was calculated on a milliequivalent basis.

While, mineral composition were calculated in the forage legume, grass and other plant species were analized separately. Mineral composition of the plots were calculated concerning legume, grass and other species percentage in samples. NO_3-N contents of plant samples were measured potentiometrically using a NO_3-N electrode according to EPA, (1996).

Experiment 2

Hungarian vetch (*Vicia pannonica* Crantz.) and barley (*Hordeum vulgare* L.) were sown in October in a ratio of 2/1 with a 20 cm of distance between rows by sowing 120 kg ha^{-1}. Each plot was 2 m × 5 m with a distance of 1 m between plots. Half of the N-fertilizer was applied during sowing and the other part was applied in the end of March. The plots were harvested in the beginning of June from 6.4 m^2 area. After harvesting, plants were separated by hand and classified as Hungarian vetch and barley there after the plants were oven-dried at 60 °C and dry weight ratio of each sample was calculated.

Experiment 3

Maize (*Zea mays* L.) was sown in four rows, each 5.0 m long, with 0.15 m spacing within and 0.75 m between rows. in May. Each plot was 4.2 m × 5 m with a distance of 1 m between plots. The plots were harvested in the beginning of September from 11.2 m^2. Dry matter contents of the plants for each plot were determined after drying the samples at 60 ^{o}C.

Experiment 4

Maize samples from different plots were copped to a length of 1-2 cm and were silajed in specially prepared iron containers holding 2 kg of the samples. In Exp. 2, 3 and 4, nitrate levels, crude protein concentrations, mineral matter concentrations and the ratio of K/(Ca+Mg) were determined as in Exp. 1. All data were subjected to analysis of variance based on general linear models for a split plot arrangement of treatments using the SPSS statistical package. Means were separated using Least Significant Difference (LSD) Test.

3. Results

3.1. Dry matter production

The effects of N applications on dry matter production were significant in all experiments. In Exp. 1, while dry matter production in the control plot was 2577 kg ha^{-1}, it increased in line with applied N doses and it ranged from 3407 to 4320 kg ha^{-1}. In Exp. 2, dry matter production in the control plot was 1807 kg ha^{-1}, it increased in line with N application and it ranged from 2253 to 2857 kg ha^{-1}. In the Exp. 3, dry matter production in the control plot was 4197 kg ha^{-1} and it increased in line with increased N application and it ranged from 6703 to 9643 kg ha^{-1} (Table 1).

In Exp.1, it was observed that dry weight ratio of grasses increased, while dry weight ratio of legumes decreased drastically in response to N application (Figure 2). In control plot, dry weight ratio of grasses was 28.2% and it increased up to 88.7% and 92.5% in plots received 100 and 150 N kg ha^{-1}, respectively. However, dry weight ratio of legumes was 65.4% in control plot, it decreased markedly in plots treated with N, which was found to be between 2.8% and 5.7% (Figure 2).

3.2. Crude protein concentration

N fertilization decreased the crude protein concentration in Exp. 1 and Exp 2. In Exp 1, the crude protein content in control plot was 137.0 g kg^{-1}, and it decreased 88.7 to g kg^{-1} in plots by 150 kg N ha^{-1} application. In Exp. 2, while the crude protein concentration was 141.0 g kg^{-1}, it changed from 100.7 g kg^{-1} to 133.7 g kg^{-1} in plots applied with N application. In Exp. 3 and Exp. 4, N application did not affect the protein concentration. The protein concentration changed between 40.5 g kg^{-1} and 79.5 g kg^{-1} in plots of these experiments (Table 1).

In Exp.1, the crude protein concentration of legumes and other families were between 132 g kg^{-1} and 182 g kg^{-1}. In term of protein concentration, grasses was more poor than legumes and other families. The protein concentration of grasses changed from 67 to 91 g kg^{-1} in plots applied N (Figure2).

3.3. K, Ca, Mg and P concentration

N application did not affect K concentration in the forage dry matter all treatments. K concentration was between 24.45 and 27.95 g kg^{-1} in Exp.1 and it was between 14.35 and 21.21 g kg^{-1} in Exp. 2. K concentration changed between 6.29 and 8.95 g kg^{-1}, in maize samples and between 2.50 and 3.97 g kg^{-1} in maize silage samples (Table 2). With regard to K concentrations, the ratio of the families was found to be similar. It changed between 24.40 – 31.63 g kg^{-1} (Figure 2) in all samples.

In Exp. 1 and Exp. 2, Ca content of samples decreased with N fertilization. In Exp. 1, Ca content of the control samples was 9.96 g kg^{-1}, it decreased 6.91 g kg^{-1} applied with 50 kg N ha^{-1}. Ca content of samples changed from 4.00 to 4.23 g kg^{-1} applied with 100, 150 and 200 kg N ha^{-1}. In Exp.2, Ca content of the control samples was 9.66 g kg^{-1}, it ranged from 9.16 to 9.33 g kg^{-1} applied with 50 and 100 kg N ha^{-1}. It decreased 7.63 to 7.83 applied with 150 and 200 kg N ha^{-1}. N fertilization did not change Ca content of samples in Exp. 3 (5.71-6.06 g kg^{-1}) and Exp 4 (3.07-3.77 g kg^{-1}) (Table 2). In samples of rangeland, legumes (11.90-13.80 g kg^{-1}) and other plants (11.97-14.40 g kg^{-1}) was richer than grasses (2.93-3.67 g kg^{-1}) with respect to Ca content (Figure 2).

Exp 1 and Exp 2, N application caused a decreases in Mg content of the forages. In Exp. 1, while Mg content was 2.47 g kg^{-1} in control samples, it decreased 1.98 g kg^{-1} applied 50 kg N ha^{-1}. Mg content was between 1.31 and 1.41 g kg^{-1} other N applications. In Exp. 2, Mg content was 2.33 g kg^{-1} in control samples. It decreased 1.84 g kg^{-1} applied with 200 kg ha^{-1}. N fertilization did not change Mg content of samples in Exp. 3 and 4 (Table 2). Mg content in samples of maize and silage found to be between 2.02 and 3.22 g kg^{-1}. In samples of rangeland, legumes (3.10-3.50 g kg^{-1}) and other plants (2.93-3.83 g kg^{-1}) was richer than grasses (1.0-1.5 g kg^{-1}) as Mg concentration (Figure 2).

N fertilization slighty decreased P content only in Exp. 2. While P content was 3.70 g kg^{-1} in control samples, it changed between 3.31 and 3.37 g kg^{-1} in plots received 100, 150 and 200 kg N ha^{-1}. P content ranged from 3.42 to 3.58 g kg^{-1} in Exp. 1. It was found to be between 2.49 and 2.96 g kg^{-1} in Exp. 3 and changed from 1.30 to 1.50 g kg^{-1} in Exp. 4 (Table 2). With regard to P concentrations, the families was found to be similar in Exp. 1. It changed between 2.93 – 3.73 g kg^{-1} (Figure 2).

3.4. K/(Ca+Mg) ratio

K/(Ca+Mg) ratio increased by the N fertilization only in Exp. 1. K/(Ca+Mg) ratio in the control samples was 1.07 and it was not different from that in plot treated with 50 kg N ha^{-1}. An evident increase was observed in K/(Ca+Mg) ratio were between 2.00 and 2.20 for plots received 100, 150 and 200 kg N ha^{-1}. K/(Ca+Mg) ratios of Exp. 2, Exp. 3 and Exp.4 was between 0.60 and 0.82, 0.15 and 0.45 and 0.15 and 0.29, respectively (Figure 3). K/(Ca+Mg) ratio of grasses was remarkable higher than legumes and other plants. K/(Ca+Mg) ratio of grasses was between 1.67 and 2.33 K/(Ca+Mg) ratio of legumes and other plants was between 0.81 and 0.65 and 0.69 and 1.24, respectively in whole experimental samples (Figure 2).

3.5. Nitrate accumulation

NO_3-N in samples increased by with N fertilization in all Exp. In Exp. 1, NO_3-N was 510 ppm not different from that in plot treated with 50 and 100 kg N ha^{-1}. Nitrate content in samples increased 1338 and 1991 ppm received 150 and 200 kg N ha^{-1}. In Exp. 2, NO_3-N was 217 ppm in control plot, and it increased up to 695 ppm in plot applied 150 kg N ha^{-1}. Nitrate content remarkable increased as 1087 ppm in plot received 200 kg N ha^{-1}. In Exp. 3, nitrate content was 351 ppm in control plot, it increased 638 ppm and 1198 ppm received 150 and 200 kg N ha^{-1} (Figure 3).

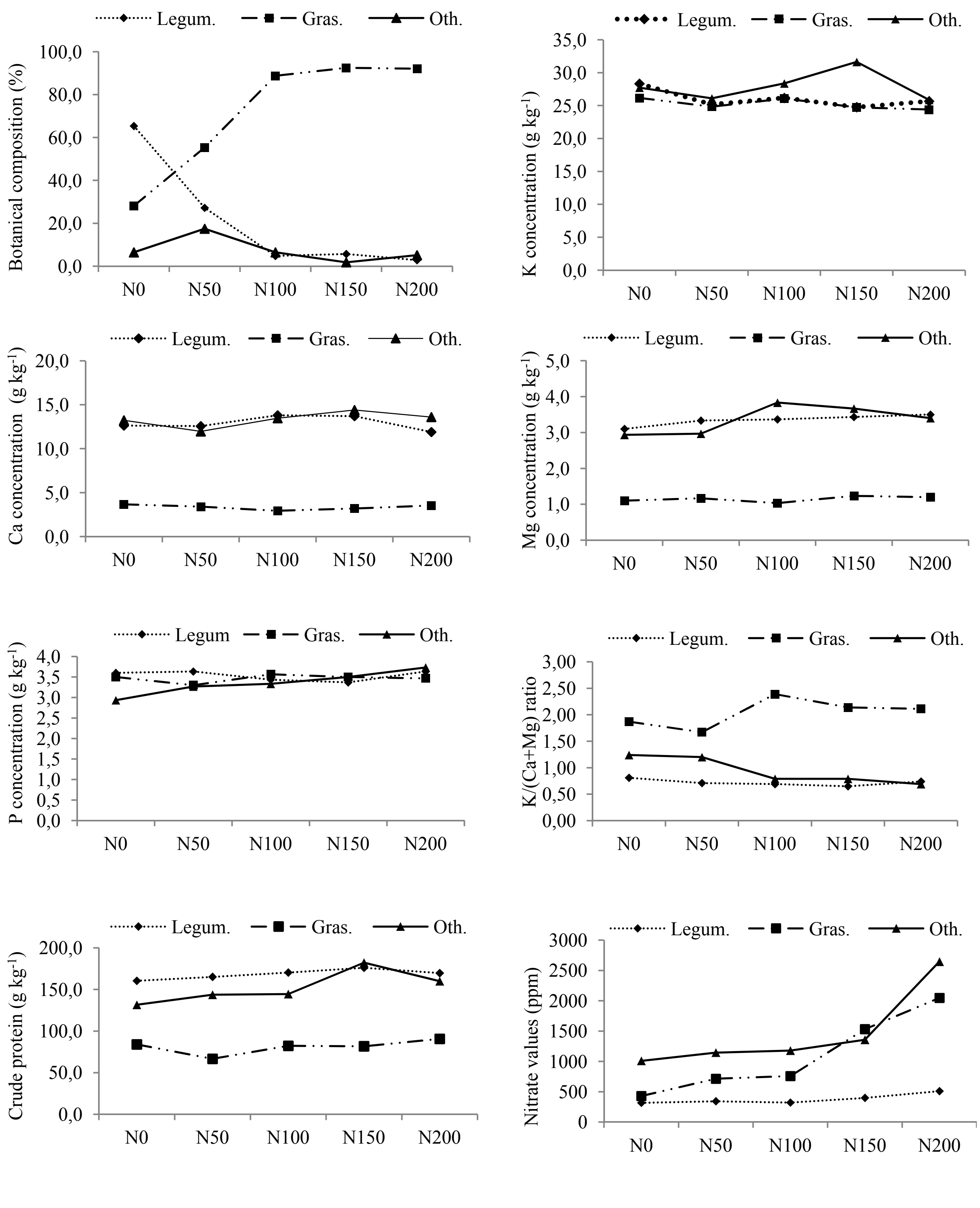

Figure 2. K, Ca, Mg, P concentrations, crude protein, nitrate values, K/(Ca+Mg) ratio and botanical composition in rangeland

NO_3-N was 206 ppm in control plot of Exp. 4. It increased to 881 ppm applied with 200 N kg ha^{-1}.

In rangeland, NO_3-N in samples of legumes (400-600 ppm) did not changed with N application. NO_3-N content was 428 ppm in control plot of grasses, it slight increased with applied 50 and 100 kg N kg^{-1}. NO_3-N content in grasses increased up to 1531 and 2046 ppm applied with 150 and 200 kg N ha^{-1}, respectively. NO_3-N content of the other plants was 1008 ppm in control plot and it increased to 1356 ppm applied with 150 kg N ha^{-1}. NO_3-N content of the other plants remarkable increased (2644 ppm) with applied 200 kg N ha^{-1} (Figure 2).

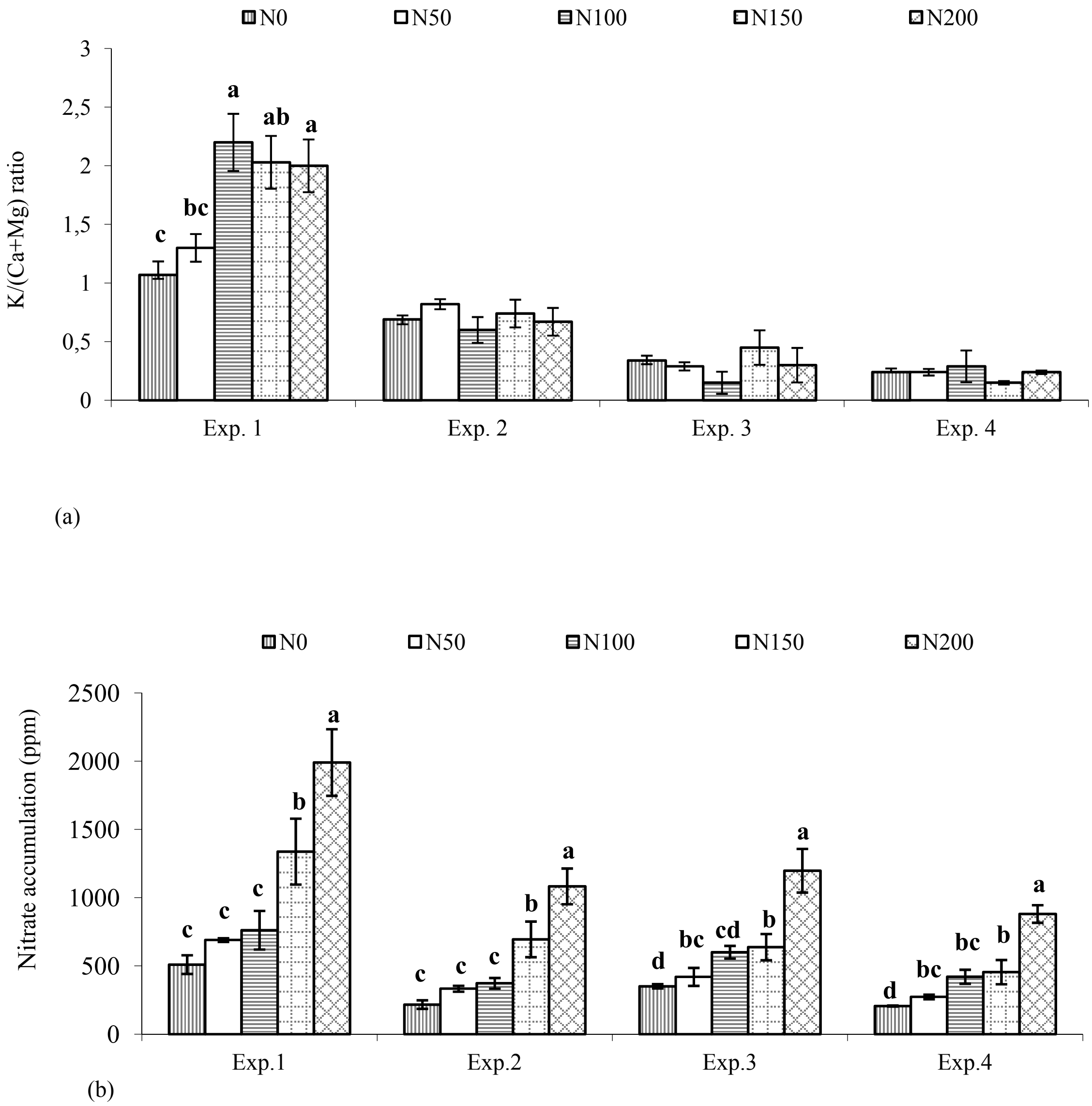

Figure 3. K/(Ca+Mg) (a) and nitrate accumulation (b) on a rangeland with different fertilization treatments as mean of the four experiments. Values with different letters within colums differ significantly at the level of $P < 0.01$. Error bars indicate S.D.

Table 1. Dry matter production and crude protein concentration

	Dry matter production (kg ha^{-1})			Crude protein concentration (g kg^{-1})			
N doses	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0	2577 b	1807 c	4197 c	137.0 a	141.0 a	66.9	46.9
50	3407 ab	2253 b	6703 b	104.3 b	133.7 a	77.5	46.6
100	4187 a	2500 ab	9643 a	91.3 b	113.0 b	61.5	40.5
150	4320 a	2857 a	8863 a	88.7 b	100.7 c	74.3	53.0
200	3798 a	2750 a	8927 a	95.7 b	104.7 bc	79.5	53.4

Values within columns with different letters differ significantly ($P < 0.01$)

Table 2. K, Ca, Mg and P concentrations of the experiments

	K concentration (g kg^{-1})				Ca concentration (g kg^{-1})			
N doses	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0	27.95	18.53	7.21	3.47	9.96 a	9.66 a	5.71	3.27
50	25.54	21.21	6.30	3.53	6.91 b	9.33 a	5.84	3.43
100	26.22	15.19	7.25	3.97	4.08 c	9.16 a	6.02	3.07
150	24.91	15.92	8.95	2.50	4.00 c	7.63 b	5.97	3.60
200	24.45	14.35	6.29	2.83	4.23 c	7.83 b	6.06	3.77
	Mg concentration (g kg^{-1})				P concentration (g kg^{-1})			
N doses	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0	2.47 a	2.33 a	3.06	2.40	3.57	3.70 ab	2.69	1.50
50	1.98 b	2.24 a	2.02	2.53	3.42	3.78 a	2.82	1.50
100	1.31 c	2.15 a	3.22	2.37	3.58	3.37 bc	2.49	1.30
150	1.41 c	1.91 b	2.75	2.67	3.50	3.31 c	2.96	1.37
200	1.36 c	1.84 b	2.94	2.63	3.48	3.33 bc	2.74	1.37

Values within columns with different letters differ significantly ($P < 0.01$)

4. Discussion

Mediterranean-climate ecosystems are characterized by warm dry summer and cool or wet winter. In most area where covered natural rangelands (e.g. Turkey), lack of rainfall in summer and low temperature in winter restricts forage producition. Environmental factors are associated with the quality of forage. This case also affects the health of the animals fed with these forages. It is clear that fertilization of a forage resources with N can increase forage production, but the effect of fertilization on forage quality is often not considered (Tsutomu et al., 1985).

Fertilization, especially with N can increase dry matter production up to two-to three-fold in rangelands depending on the annual rainfall and moisture in the region (Elliott and Abbott, 2003). But, in the our 3 experiments (rangeland, forage (barley and wetch) and maize), nitrogen application increased the dry matter production of the plots by about 50-100%. As seen in Figure 1, because of insufficient rainfall during late spring and summer months was the main reason that yield did not increase in parallel with the increase in N-doses.

Several researchers indicated that N-fertilizers increased the crude protein content in forage. In fact, the case is not valid all the time. For instance, it was found in the present study that N-fertilizer application reduced the ratio of crude protein in both Exp.1 and Exp. 2. In fact, the reason of this result was due to a sharp decline of legumes in botanical composition.

As seen in Figure 2, the effect of N-fertilizer on crude protein content of different families was not clear. Legume crops was found to be two-fold rich compared to grasses in terms of crude protein content. Fertilization may enhance not only dry matter production, but also effects botanical composition in rangelands (Kalmbacher and Martin, 1996). The significant effect of fertilization on forage mineral content is due to the different mineral content of legumes, grasses and the other families. The variation in the ratios of legume and grass having different levels of mineral content was the main reason of changes in the levels of mineral content of the samples in plot.

In this study, Ca and Mg concentrations in samples decreased by N application in Exp. 1 and Exp. 2 (Table 2). The main reason for the decrease in Ca and Mg content in response to N treatment might be the negative effect of N application on legume percentage in botanical composition (Figure 2). Decreasing legume percentage in dry matter having much more Ca and Mg compared to grasses resulted in, naturally, a decrease in Ca and Mg content in forage.

Legumes and other plants had tree fold hinher Ca and Mg content then grasses (Figure 2). Ca content of all samples had richer than the recommended daily (2.8 g kg^{-1}) for beef cattle demand (NRC, 1996). In samples of rangeland, Mg content had lower than (1.7-2.0 g kg^{-1}) for beef cattle demand (NRC, 1996). Mg concentration was sufficient other in samples of all Exp.

The samples of rangeland and forage (barley+vetch) had higher K content than the recommended daily (7.0 g kg^{-1}) for beef cattle (NRC, 1996) (Table 2). K concentration in samples of maize was sufficient for beef cattle demant (NRC, 1996). But, the samples of silage had K content half of the recommended daily for beef cattle (NRC, 1996). Plant K requirements and the content in plant tissues have usually exceeded the animal requirements (Fontenot et al., 1989). In the study, K content of legumes, grasses and the other families exceeded requirements for beef cattle about three- to four-fold.

In the present study, NO_3-N concentration in dry matter of all samples increased 3-4 fold with N fertilization (Figure 3). It was clear that there was a marked difference between the effects of N 150 and N 200 doses on nitrate accumulation.

In Exp. 1, NO_3-N content of all the samples was over 350 ppm. Suggested that forage containing NO_3-N between 350-1130 ppm should not be included to the ration of the pregnant animals more than 50%. NO_3-N content in the dry matter of control samples was 510 ppm while it was 1350 ppm which was over 1130 ppm being considered as critical level of danger to pregnant animals. With the application of 200 kg ha^{-1} N-fertizer, NO_3-N levels of the samples reached up to 1990 ppm. This NO_3-N level was very close to 2260 ppm which must not be found in forage for pregnant animals.

It was also found that NO_3-N content was over 350 ppm for all the samples of the mixture of wetch + barley in both control and the plots applied with 50 kg N ha^{-1}. For the highest N-fertilizer application, NO_3-N content of the samples reached 1083 ppm considered not to be harmful to pregnant animals but should not exceed 50% in animal rations.

NO_3-N content in green maize samples were found to be more than 350 ppm. It was also determined that NO_3-N content of the samples from plots applied with N-fertilizer up to 150 kg ha^{-1} was less than critical threshold of 1130 ppm being harmful to pregnant animals. On the other hand, NO_3-N content of green maize samples from the plots applied with 200 kg N ha^{-1} was 1198 ppm which was over the critical level for pregnant animals (1130 ppm) reported by Cash et al. (2006).

NO_3-N content of silaged maize samples from the plots applied with 50 kg N ha^{-1} was less than 350 ppm while it was 881 ppm in the samples of silaged maize from the plots applied with 200 kg N ha^{-1}. Some researchers reported that NO_3-N content of the silaged maize declined in comparison to green maize samples. According to present study results it was suggested that the percentage of silaged maize obtained from higher N applied plots should not be more than 50% in rations of animals during the getation period.

Grasses and other plants had two fold NO_3-N then legumes in plot of non-fertilizer. N fertilization did not affect nitrate accumulation in legumes. Grass and legumes had 1500 ppm NO_3-N in plots of applied with 150 kg N ha^{-1} and it increased up to 2100 ppm in samples applied 200 kg N ha^{-1}. When taking the fact that N-fertilizer increased grass in botanical composition into account, this case seems to be important in terms of NO_3-N poisoning. Having legume crops in vegetation could be considered as an insurance to NO_3-N poisoning.

K/(Ca+Mg) ratio was not affected by N fertilization in Exp 2, 3 and 4. There was no tetany risk in samples of these experiments. But, N application increased K/(Ca+Mg) ratio in samples of rangelands. While K/(Ca+Mg) ratio was 1.07 in control plot, it increased 2 fold in the plots applied with 100, 150 and 200 kg N ha^{-1}. The ratio of K/(Ca +Mg) (1.07) resulted from N-fertilizer applications was found very close to the critical value of 2.2 related to tetany risk.

There was a marked difference between grasses and legumes regarding K/(Ca+Mg) ratio. Over all N-fertilizer applications, while K/(Ca+Mg) ratio of legumes was found to be 0.7-0.8, it was 2.4 (more than the critical value of 2.2 for tetany risk) for grass. For this reason, it was very important to include legumes into botanical composition.

Another important part of understanding the causation of grass tetany concerns the way in which fertilization practices affect the K, Mg and Ca content of the plants in the present study, legumes contained higher amounts of Ca and Mg (Figure 2) than grasses, as reported by Kidambi et al., (1989). For this reason, the decrease in legume percentage in the botanical composition resulted in an increase of K/(Ca+Mg) ratio in dry matter. Although rainy, humid ecological conditions are prevalent, and there are limited numbers of sunny days in the experimental area of the present study, there was no tetany risk in non-fertilized rangelands (Table 1).

5. Conclusions

The nitrate concentration and K/(Ca+Mg) ratio of the plants may increase up to the harmful levels especially in rangelands by nitrogen fertilizers. Therefore, it can be concluded that fertilization programmes avoiding decrease legume content in the botanical compositon of rangelands may be useful to prevent the tetany risk and nitrate poisoning risk.

Acknowledgment

Financial support for this project was provided by the Ondokuz Mayıs University project management office (Project no: ZRT.1904.11.009).

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