Nutritional evaluation of berseem
2- Effect of nitrogen fertilizer on berseem fed as silage to goats

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Abstract: Eighteen male goats were used to determine the effect of nitrogen fertilizer rates (0, 23.8 and 47.6 kg N ha^{-1}) on intake, nutritive values, nitrogen balance and blood hematology of silages made from berseem of the 1st and 2nd cuts with sugar can bagasse (70:30 on DM basis). The contents of DM, OM, CF and NFE decreased, but CP, EE and ash contents of fresh berseem increased with increasing the rate of N fertilizer. The pH value decreased, however TVFA's and NH_3-N concentrations increased with increasing the rate of N fertilizer for both the 1st and 2nd cuts of berseem silages. The contents of DM, OM, CF and NFE decreased, but CP, EE and ash contents of silages increased with increasing the rate of N fertilizer. The digestibility coefficients of DM, OM, CP, EE and NFE and TDN and DCP values of berseem silages by goats increased significantly ($P<0.05$), but CF digestibility decreased significantly ($P<0.05$) with increasing the rate of N fertilizer. Average daily DM, TDN and DCP intake by goats increased significantly ($P<0.05$) with increasing the rate of N fertilizer. The pH value decreased significantly ($P<0.05$), however the concentrations of TVFA's and NH_3-N increased significantly ($P<0.05$) in rumen liquor with increasing the rate of N fertilizer. The nitrogen (N) intake, digested excretion in urine and retained by goats increased significantly ($P<0.05$) with increasing the rate of N fertilizer. While, N excretion in feces did not significantly affected by the rate of N fertilizer ($P>0.05$). The counts of red blood cells (RBC) and white blood cells (WBC) and hemoglobin concentration in blood of goats fed the different silages increased significantly ($P<0.05$) with increasing the rate of N fertilizer. The DM intake and nitrogen excretion in feces were higher, however, ruminal NH_3-N, nitrogen excretion in urine and red blood cells were lower when goats fed silages of the 2nd cut compared with feeding 1st cut silage.

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1. Introduction

In intensive grassland management systems, high nitrogen (N) application increases grass growth and as a consequence grass can be harvested, either by grazing or by cutting, in earlier stages of maturity (vegetative, pre- and early bloom) giving high nutritive values and maximum voluntary intake of grass (Minson, 1990).

Making silage of surplus fodders during their abundant growth period will not only help overcome the irregular fodder supply pattern, one of the major culprits for lower ruminant productivity, but it well also enhance fodder yield of multicut fodder crops by increasing their number of cuts. Moisture content of leguminous fodder could be reduced either by field wilting or by the addition of some absorbent. But field wilting is not desirable due to higher labor costs. Dry roughages high in DM and low in nitrogen content could be added to improve the DM of berseem before ensilation (Fransen and Strubi, 1998).

A chief part of berseem clover (*Trifolium alexandrinum* L.) is ensiled to preserve its quality and nutritional value. It is possible through moisture and temperature reduction of silage and the use of additive materials such as chemicals and plant materials to silage contents. Among the plant materials, it can be referred to milled grains such as maize, oat and barley, also grain stubble and beet molasses (Fairchild, 1992; Kennedy and Mackie, 1995). Successful silage crop production is based on a number of key factors that include species and variety selection, soil type, soil pH, fertilizer regime, pest control, optimal harvest date and storage losses (O'Donvan et al., 2000; Ross et al., 2004a&b; King, 2007).

The quality of ensiled material depends on the composition and nutritional value of fodder, type of ensiled fodder, variety and operations of harvesting and silage preparation. Indices such as pH, lactic acid, ammonia, color, scent and taste of ensiled materials are used to determine the quality of silage (Edwards, 1997).

The objective of this study was to investigate the effect of nitrogen fertilizer of berseem on silage quality and composition, feed intake, digestibility, rumen activity and blood hematology of goats.

2. Materials and methods

The current work was carried out at the Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University to investigate the effect of nitrogen fertilization on quality and composition of berseem (*Trifolium alexandrinum L.*) silage, digestibility, nitrogen balance, rumen activity and blood hematology of goats.

The soil texture of the experimental site was clay loam. Rate of seeding was 47.6 kg ha^{-1}. Seeds were of local berseem from Kafrelsheikh Governorate. Berseem was planted during the middle of October in the two seasons. Four hundred and seventy six kg of superphosphate (15.5% P2O5) ha^{-1} was added during the land preparation. Ammonium sulphate (20% N) was added before the first irrigation at rates of 0, 119 and 238 kg ha^{-1} to give the N fertilization rate of 0, 23.8 and 47.6 kg N ha^{-1}, respectively. The first two cuts of berseem were taken during the experiment, 1^{st} cut at 28-29^{th} of December and 2^{nd} cut at 13-14^{th} of February.

Silage was made from berseem of 1^{st} and 2^{nd} cuts and bagasse. Berseem wilted in the field to decrease moisture content to about 75%. Sugar cane bagasse was collected from shops of sugar can juice and sun dried by spreading bagasse on plastic sheets in layers of 10 cm thickness for two days with shuffled up side-down daily to reach approximately 90% dry matter content. Bagasse was chopped into 20-25 cm pieces and mixed with berseem (70% berseem + 30% bagasse, on DM basis) in bunker one ton capacity on stack and pressed, covered by plastic sheets and 20 cm clay layer and ensiled for eight weeks. After open the silos were examined for texture, odor and color. Silage extract was obtained by extracting 20 g of wet silage in a blender with 100 ml of distilled water. The extract was filtered through a filter paper (Whatman No. 40) and the filtrate used in determination of pH directly using Beckman pH meter, ammonia–nitrogen (NH_3-N) concentration was determined by using magnesium oxide (MgO) as described by AOAC (1995) and total volatile fatly acids (TVFA's) concentration was determined by steam distillation method as described by Warner (1964).

Six digestibility trials were conducted using three male Angora goats (Bucks) with average live body weight of 38 kg and aged three years in each. Goats were fed different silages *ad libtum* for 15 days preliminary period followed by 7 day collection period. Each buck kept individually in metabolic cage during the entire experiment period and fed in two equal parts daily at 8 a.m. and 4 p.m. water was available freely in buckets to the animals. The amount of feed intake, feces and urine were recorded daily and representative samples were taken for analyses. Samples of feed and feces were dried in a forced air oven at 65 ^{o}C for 48 hours, ground and thoroughly mixed for each animal. The contents of crude protein (CP), ether extract (EE), crude fiber (CF), ash and NFE (by difference) were determined in feed and feces and nitrogen in urine according to AOAC (1995).

Rumen liquor samples were taken from each buck at the end of digestibility trials using stomach tube at three hours post the morning feeding. The rumen liquor was filtered through a double layer of cheese cloth into plastic bottles. The pH value, ammonia–nitrogen (NH_3-N) and total volatile fatly acids (TVFA's) concentrations were determined as shown previously in silage filtrate. Blood samples were taken from the Jugular vein for each buck at the same time of rumen liquor samples in sterile tube containing ethylene diamine tetra acetate (EDTA) as anticoagulant. Total red blood cells (RBC) and white blood cells (WBC) were counted using haemocytometer. Hemoglobin was determined according to Zijlstra (1960).

The obtained data were statistically analyzed using factorial models procedure adapted by SPSS (2008) for N fertilizer and cuts. The Duncan multiple range test was used to compare difference between means.

3. Results and discussion

Chemical composition of 1^{st} and 2^{nd} cuts fresh berseem presented in Table (1) revealed that the contents of DM, OM, CF and NFE decreased, but CP, EE and ash contents increased with increasing the rate of N fertilizer. The quadratic response of DM, OM, CP, CF, EE, NFE and ash in fresh berseem of 1^{st} and 2^{nd} cuts for the N fertilization were R^2 = 0.13, 0.05, 0.85, 0.47, 0.74, 0.47 and 0.05, respectively. Moreover, N fertilizer positively correlated with CP, EE and ash contents being r = 0.92, 0.86 and 0.22 and negatively correlated with DM, OM, CF and NFE contents being r = - 0.35, - 0.22, - 0.68 and - 0.69, respectively. These results reflect the differences in leaves to stems ratio as affected by N fertilizer. There were little differences in composition among the 1^{st} and 2^{nd} cuts, which CP and ash contents tended to decrease, but DM, OM, CF and NFE contents tended to increase from 1^{st} to 2^{nd} cut. These results are in accordance with those obtained by Vuckovic et al. (2005) who found strong positive effect of N fertilizer on crude protein, ash and fat contents of meadows, while, the negative on crude fiber content. Vuckovic et al. (2006) reported that increasing N rates increased the contents of protein, ash and fat and reduced the contents of cellulose. Almodares et al. (2009) found that treatment of 200 kg/ha urea had the highest protein content and the lowest soluble carbohydrates and fiber contents. Chemical composition of sugar can bagasse obtained here was similar to those obtained by Helali et al. (1992) and Farghaly (1993).

The quality characteristics of different silages are shown in Table (2). The pH value decreased, however

TVFA's and NH_3-N concentrations increased with increasing the rate of N fertilizer for both the 1st and 2nd cuts of berseem silages. Silage quality was nearly similar for both the 1st and 2nd cuts of berseem silages. The quadratic effect of N fertilizer on pH vale, TVFA's and NH_3-N concentrations of different silages were R^2 = 0.48, 0.74 and 0. 98, respectively. The pH value negatively correlated (r = - 0.69), but TVFA's and NH_3-N concentrations positively correlated (r = 0.85 and 0.96) with N fertilizer. These results indicated a good quality silage. Good quality silage should have pH value 4.2 or less, ammonia-N concentration less than 10% of total-N and more concentration of TVFA's (McDonald et al., 1995).

Chemical composition of different silages made from berseem and bagasse (70:30) for both 1st and 2nd cuts are shown in Table (2). Composition of different silages revealed similar trend to the composition of fresh berseem. The contents of DM, OM, CF and NFE decreased, but CP, EE and ash contents increased with increasing the rate of N fertilizer. There were little differences in the mean of silage composition among the 1st and 2nd cuts, which CP and ash contents tended to decrease, but OM, CF and NFE contents tended to increase from 1st to 2nd cut silage. The quadratic response of DM, OM, CP, CF, EE, NFE and ash contents in different silages for the N fertilization were R^2 = 0.30, 0.05, 0.85, 0.47, 0.75, 0.47 and 0.48, respectively. Moreover, N fertilizer positively correlated with CP, EE and ash contents being r = 0.92, 0.86 and 0.69 and negatively correlated with DM, OM, CF and NFE contents being r = - 0.55, - 0.21, - 0.69 and - 0.69, respectively. The differences in chemical composition of silages reflect the differences in composition of fresh berseem.

Digestibility coefficients and nutritive values of different silages in Table (3) showed that the digestibility coefficients of DM, OM, CP, EE and NFE and TDN and DCP values by goats increased significantly ($P<0.05$), but CF digestibility decreased significantly ($P<0.05$) with increasing the rate of N fertilizer. Moreover, there were no significant differences in the digestibility coefficients and nutritive values among both the 1st and 2nd cuts of berseem silage. The quadratic effect of N fertilizer on the digestibilities of DM, OM, CP, CF, EE and NFE and TDN and DCP values being R^2 = 0.80, 0.79, 0.77, 0.90, 0.80, 0.85, 0.73 and 0.82, respectively. The correlations between N fertilizer and the digestibilities of DM, OM, CP, CF, EE and NFE and TDN and DCP values being r = 0.89, 0.89, 0.88, - 0.95, 0.90, 0.92, 0.86 and 0.90, respectively. These results agree with those obtained by Nour et al. (1987) who found that nitrogen fertilizer increased total digestible nutrients. High nitrogen (N) application increases grass growth and as a consequence grass can be harvested in earlier stages of maturity giving high nutritive values (Minson, 1990). Reducing fertilizer N decreased in-vitro digestibility (Valk et al., 2000).

Results in Table (4) revealed that the average daily DM, TDN and DCP intake by goats increased significantly ($P<0.05$) with increasing the rate of N fertilizer. These results may be attributed to decrease fiber content and increase TDN and DCP values with increasing the rate of N fertilizer. The mean DM intake was significantly higher ($P<0.05$) for 2nd cut than that of 1st cut silage, while the mean intake of TDN and DCP were nearly similar for both 1st and 2nd cuts of berseem silage. The quadratic effect of N fertilizer on DM, TDN and DCP intake by goats were R^2 = 0.70, 0.75 and 0.99, respectively. The correlations between N fertilizer and DM, TDN and DCP intake were r = 0.83, 0.86 and 0.99, respectively. The High nitrogen (N) application increases grass growth and as a consequence grass can be harvested in earlier stages of maturity giving maximum voluntary intake of grass (Minson, 1990). Valk et al. (2000) found that a reduction in N fertilization from 450 to 150 kg/ha per year did not affect grass intake.

The pH value decreased significantly ($P<0.05$), however the concentrations of TVFA's and NH_3-N increased significantly ($P<0.05$) in rumen liquor with increasing the rate of N fertilizer (Table 4). The mean pH value and TVFA's concentration in rumen liquor were nearly similar for silages of both 1st and 2nd berseem cuts, while the mean of NH_3-N concentration was significantly higher ($P<0.05$) with feeding the silage of the 1st berseem cut compared with feeding 2nd cut berseem silage. The pH value, TVFA's and NH_3-N concentrations affected quadraticlly by N fertilizer (R^2 = 0.82, 0.90 and 0.32, respectively). The correlations between N fertilizer and pH value, TVFA's and NH_3-N concentrations being r=-0.91, 0.93 and 0.56, respectively). These results agree with those obtained by Zhang (1995) who found that ruminal pH value tended to lower when lambs fed diets containing fertilized ryegrass.

Nitrogen balance in Table (5) showed that the nitrogen (N) intake, digested, excretion in urine and retained by goats increased significantly ($P<0.05$) with increasing the rate of N fertilizer. While, N excretion in feces did not significantly affected by the rate of N fertilizer ($P>0.05$). Moreover, the mean of N intake, excretion in feces and urine, digested and retained were nearly similar for both 1st and 2nd cuts of berseem silage. The nitrogen balance was positive for the different silages. Nitrogen intake, excretion in feces, digested, excretion in urine and retained affected quadraticlly by N fertilizer (R^2 = 0.95, 0.07, 0.99, 0.74 and 0.97, respectively). The correlations between N fertilizer and N intake, excretion in feces, digested, excretion in urine and retained were r = 0.97, 0.09, 0.99, 0.86 and 0.99, respectively. These results are in accordance with those obtained by Zhang (1995) who reported that lambs excreted more urinary N when fed diets included fertilized ryegrass silage. Astigarraga et al. (2002) found that N fertilization is efficient mean to manipulate N balance in grazing dairy cows.

Results in Table (6) showed that the counts of red blood cells (RBC) and white blood cells (WBC) and hemoglobin concentration in blood of goats fed the different silages increased significantly (P<0.05) with increasing the rate of N fertilizer. Moreover, RBC count was significantly higher (P<0.05) with feeding the silage of 1st berseem cut than that with feeding the silage of 2nd berseem cut. While, WBC count and hemoglobin concentration were nearly similar for feeding the silage of both 1st and 2nd berseem cuts. The quadratic response of RBC and WBC counts and hemoglobin concentration in blood of goats to N fertilizer of berseem were R^2 = 0.55, 0.86 and 0.56, respectively. The correlations between N fertilizer and RBC and WBC counts and hemoglobin concentration were r = 0.74, 0.92 and 0.75, respectively.

4. Conclusions

Form this study it could be occluded that nitrogen fertilizer at the rate of 47.6 kg N ha^{-1} showed the best results concerning silage quality and composition, digestibility, feed intake, rumen activity, nitrogen balance and blood hematology and little differences exist between 1st and 2nd cuts berseem silage.

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Table 1. Chemical composition of fresh berseem and sugar can bagasse.

Item	1st cut				2nd cut				Sugar can bagasse
	N fertilizer (kg ha^{-1})				N fertilizer (kg ha^{-1})				
	0	23.8	47.6	Mean	0	23.8	47.6	Mean	
DM %	11.6	10.8	10.0	10.8	12.8	12.0	11.1	12.0	91.0
Composition of DM %									
OM	85.1	84.6	83.9	84.5	85.77	85.4	84.8	85.3	97.7
CP	17.6	18.9	20.4	18.9	16.93	18.7	19.5	18.4	4.9
CF	26.5	25.4	24.2	25.4	27.32	26.4	25.5	26.4	42.8
EE	3.16	3.35	3.58	3.36	3.11	3.28	3.45	3.28	3.90
NFE	37.9	37.0	35.7	36.9	38.41	37.0	36.3	37.2	46.1
Ash	14.9	15.4	16.1	15.5	14.23	14.6	15.2	14.7	2.26

Table 2. Quality characteristics and chemical composition of different silages.

Item	1st cut				2nd cut			
	N fertilizer (kg ha^{-1})				N fertilizer (kg ha^{-1})			
	0	23.8	47.6	Mean	0	23.8	47.6	Mean
Quality characteristics								
pH value	4.27	4.21	4.18	4.22	4.25	4.13	4.07	4.15
TVFA's % of DM	1.91	2.20	2.32	2.14	2.05	2.14	2.29	2.16
NH_3-N % of total N	6.31	7.77	7.99	7.36	6.40	7.68	8.03	7.37
Chemical composition								
DM %	35.4	34.8	34.3	34.9	36.3	35.7	35.1	35.7
Composition of DM %								
OM	88.7	88.3	87.8	88.2	89.1	88.8	88.4	88.8
CP	14.0	15.0	16.0	15.0	13.6	14.8	15.4	14.6
CF	31.1	30.3	29.4	30.3	31.7	31.0	30.4	31.0
EE	3.37	3.50	3.67	3.51	3.33	3.45	3.58	3.45
NFE	40.2	39.6	38.6	39.5	40.6	39.5	39.0	39.7
Ash	11.3	11.7	12.2	11.8	10.9	11.2	11.6	11.2

Table 3. Digestibility coefficients and nutritive values of different silages.

Item	1st cut				2nd cut			
	N fertilizer (kg ha^{-1})				N fertilizer (kg ha^{-1})			
	0	**23.8**	**47.6**	**Mean**	**0**	**23.8**	**47.6**	**Mean**
Digestibility coefficients %								
DM	66.2^c	70.6^b	75.2^a	70.7	63.6^c	67.4^b	72.2^a	67.9
OM	67.8^c	71.8^b	76.6^a	72.1	65.1^c	69.0^b	73.6^a	69.2
CP	68.7^c	73.1^b	77.4^a	73.1	66.0^c	70.2^b	74.3^a	70.2
CF	60.4^a	57.9^b	55.9^c	58.1	60.9^a	58.4^b	56.4^c	58.6
EE	68.1^c	73.0^b	77.4^a	72.8	65.4^c	70.1^b	74.4^a	70.0
NFE	68.8^c	73.2^b	77.2^a	73.1	66.0^c	70.3^b	74.2^a	70.2
Nutritive values %								
TDN	61.2^c	63.2^b	65.1^a	63.2	59.9^c	61.8^b	63.5^a	61.7
DCP	9.65^c	10.9^b	12.4^a	11.0	8.96^c	10.4^b	11.5^a	10.3

a, b, c: Values in the same row for each cut with different superscripts differ significantly at 5% level.
There were no significant differences between means at 5% level.

Table 4. Feed intake and rumen activity of goats fed different silages.

Item	1st cut				2nd cut			
	N fertilizer (kg ha^{-1})				N fertilizer (kg ha^{-1})			
	0	**23.8**	**47.6**	**Mean**	**0**	**23.8**	**47.6**	**Mean**
Feed intake (g/head/day)								
DM	791^c	863^b	908^a	854^B	845^c	921^b	970^a	912^A
TDN	484^b	545^a	591^a	540	507^b	569^a	615^a	564
DCP	76^c	94^b	113^a	94	76^c	96^b	111^a	94
Rumen activity								
pH value	6.55^a	6.23^b	5.99^b	6.26	6.49^a	6.17^b	5.93^b	6.20
TVFA's mM/dl	8.39^c	10.6^b	11.8^a	10.3	7.7^c	9.78^b	10.8^a	9.43
NH_3-N mg/dl	12.8^c	13.7^b	14.5^a	13.7^A	11.1^c	11.9^b	12.6^a	11.9^B

a, b, c: Values in the same row for each cut with different superscripts differ significantly at 5% level.
A, B: Means in the same row with different superscripts differ significantly at 5% level.

Table 5. Nitrogen balance (g/day) by goats fed different silages.

Item	1st cut				2nd cut			
	N fertilizer (kg ha^{-1})				N fertilizer (kg ha^{-1})			
	0	**23.8**	**47.6**	**Mean**	**0**	**23.8**	**47.6**	**Mean**
N-intake	22.2^c	26.0^b	29.6^a	25.9	22.9^c	27.5^b	30.3^a	26.9
N-feces	6.95^{ab}	7.01^a	6.70^b	6.88^B	7.78^b	8.21^a	7.79^b	7.93^A
N-digested	15.3^c	19.0^b	22.9^a	19.1	15.1^c	19.3^b	22.5^a	19.0
N-urine	9.68^c	12.1^b	14.0^a	11.9^A	8.45^c	11.1^b	13.3^a	11.0^B
N-retained	5.61^c	6.96^b	8.92^a	7.16	6.65^c	8.22^b	9.19^a	8.02

a, b, c: Values in the same row for each cut with different superscripts differ significantly at 5% level.
A, B: Means in the same row with different superscripts differ significantly at 5% level.

Table 6. Blood hematology of goats fed different silages.

Item	1st cut				2nd cut			
	N fertilizer (kg ha^{-1})				N fertilizer (kg ha^{-1})			
	0	**23.8**	**47.6**	**Mean**	**0**	**23.8**	**47.6**	**Mean**
RBC x10^6/ml	12.7^c	14.0^b	14.8^a	13.8^A	11.4^c	12.5^b	13.3^a	12.4^B
WBC x10^3/ml	4.88^c	5.26^b	5.51^a	5.22	4.76^c	5.14^b	5.39^a	5.10
Hemoglobin g/dl	12.6^b	12.9^{ab}	13.3^a	12.9	12.8^b	13.2^{ab}	13.6^a	13.2

a, b, c: Values in the same row for each cut with different superscripts differ significantly at 5% level.
A, B: Means in the same row with different superscripts differ significantly at 5% level.

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