

Nutritional evaluation of berseem. 3. Effect of nitrogen fertilizer on berseem fed as hay to goats

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SUMMARY

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, blood haematology, calcium and phosphorus balance of 3rd and 4th cuts berseem hay. The contents of DM, OM, CF and NFE decreased, but CP, EE, ash Ca and P contents increased with increasing the rate of N fertilizer. There were little differences in the mean composition of hay among the 3rd and 4th cuts, which CP and ash contents tended to decrease, but CF content tended to increase from 3rd to 4th cut.

The digestibility coefficients of DM, OM, CP, EE and NFE and TDN and DCP values and the intake of DM, TDN and DCP by goats increased significantly ($P < 0.05$), but CF digestibility decreased significantly ($P < 0.05$) with increasing the rate of N fertilizer. Moreover, the digestibility coefficients of DM, OM, CP, EE and NFE and DCP value and the intake of DM, TDN and DCP were significantly higher ($P < 0.05$), but CF digestibility was significantly lower ($P < 0.05$) for 3rd cut compared to 4th cut berseem hay. While, TDN value tended to higher in 3rd cut than 4th cut berseem hay.

The pH value decreased significantly ($P < 0.05$), however the concentrations of TVFA's and NH₃-N and nitrogen intake, digested, excretion in urine and retained increased significantly ($P < 0.05$) in rumen liquor with increasing the rate of N fertilizer. The mean pH value and NH₃-N concentration in rumen liquor and N excretion in feces were nearly similar for hay of both 3rd and 4th berseem cuts, while the mean of TVFA's concentration and N intake, excretion in feces and urine,

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digested and retained were significantly higher ($P < 0.05$) with feeding the 3rd cut hay compared with feeding 4th cut berseem hay.

The counts of red blood cells (RBC) and white blood cells (WBC) and hemoglobin concentration in blood of goats and the intake, excretion in feces, absorption, excretion in urine and retention of calcium and phosphorus increased significantly ($P < 0.05$) with increasing the rate of N fertilizer. Moreover, RBC count was significantly higher ($P < 0.05$), but phosphorus balance was significantly lower ($P < 0.05$) with feeding the 4th cut than that with feeding 3rd cut berseem hay. While, WBC count and haemoglobin concentration and calcium balance were nearly similar for feeding both 3rd and 4th cuts berseem hay.

Keywords: feed intake, digestibility, nitrogen balance, rumen activity, blood haematology, calcium and phosphorus balance.

INTRODUCTION

Berseem clover (*Trifolium alexandrinum* L.) is an erect, cool-season annual legume, which is native to the Mediterranean region (Knight, 1985). This species has the advantage over other annual species, including annual medics and peas, of providing multiple harvests during the growing season. Quality of hay is affected by stage of maturity because the chemical analysis and digestibility change from early to late stage of maturity, which is reflected on the nutritive value of the plants. The later cuts of berseem had higher values when calculated as fed, but in fact they were poorer in digestible protein than earlier cuts on DM basis (Saleh, 1986).

Chauhan et al. (1980) found that the DM and CF content of berseem increased, but the CP content (on DM basis) decreased with the progress of cut. Phillip (1996) reported that nutritive values of berseem hay as a sole feed for sheep was 56.52% TDN and 9.88% DCP. Berseem clover is an annual legume used in crop rotations (Evers et al., 1993) and contains high concentrations of digestible DM and CP (Hattab and Harb, 1994). Information on the nutritive value of berseem clover for ruminants is limited. Karsli et al. (1999) reported that the effects of berseem clover hay supplementation on intake and digestibility of corn residues by sheep are similar to those of alfalfa hay.

The objective of this study was to investigate the effect of nitrogen fertilizer on composition, feed intake, digestibility, rumen activity, blood haematology and calcium and phosphorus balance of berseem hay by goats.

MATERIAL 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.) hay, digestibility, nitrogen balance, rumen activity, blood haematology and calcium and phosphorus balance of goats.

The soil texture of the experimental site was clay loam. Rate of seeding was 47.6 kg per hectare. 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 rate of 0, 119 and 238 kg ha⁻¹ to give the N fertilization rates of 0, 23.8 and 47.6 kg N ha⁻¹, respectively. The two cuts of berseem were taken during the experiment, 3rd cut on 1st -2nd of April and 4th cut on 13-14th of May. Berseem of 3rd and 4th cuts were cut and spread on ground in rows and layers of range 10-25 cm height. Berseem was turned up side down every day at 9 a.m. after dew disappearance until being cured. After that hay was collected and stored as bales until used in feeding goats.

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 *ad libitum* 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 °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 (NH₃-N) concentration was determined by using magnesium oxide (MgO) as described by AOAC (1995) and total volatile fatty acids (TVFA's) concentration was determined by steam distillation method as described by Warner (1964). 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. Haemoglobin was determined according to Zijlstra (1960). Calcium was determined by Atomic Absorption Spectrophotometer (Perkin Elmer 2380). Phosphorus was determined using Spectrophotometer (Milton Roy Company Spectronic 20 D).

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.

RESULTS AND DISCUSSION

Chemical composition of berseem hay for 3rd and 4th cuts is shown in Table 1. The contents of DM, OM, CF and NFE decreased, but CP, EE, ash Ca and P contents increased with increasing the rate of N fertilizer. There were little differences in the mean composition of hay among the 3rd and 4th cuts, which CP and ash contents tended to decrease, but CF content tended to increase from 3rd to 4th cut. The quadratic response of DM, OM, CP, CF, EE, NFE, ash, Ca and P contents in different hays for the N fertilization were $R^2 = 0.04, 0.12, 0.73, 0.73, 0.43, 0.12, 0.67, 0.89$ and 0.92 , respectively. Moreover, N fertilizer positively correlated with CP, EE, ash, Ca and P contents being $r = 0.85, 0.66, 0.82, 0.94$ and 0.96 and negatively correlated with DM, OM, CF and NFE contents being $r = -0.07, -0.34, -0.86$ and -0.35 , respectively. These results revealed that N fertilizer had high effect on the contents of CP, CF, EE, ash, Ca and P of berseem hay. These results agree with those obtained by Ferri et al. (2004) who reported that N fertilization affects chemical composition of forage from rye pasture. Vuckovic et al. (2005) use different rates of nitrogen fertilizer (0, 40, 80, 120 and 160 kg ha⁻¹) on natural *cynosurtum cristati* type meadows and found that nitrogen fertilizer had positive effect on increasing crude protein, fat and ash contents and negative effect on crude fiber content. Polat et al. (2007) stated that nitrogen fertilization significantly influenced the composition of plants.

Table 1 Chemical composition of berseem hay.

Item	3 rd cut				4 th cut			
	N fertilizer (kg ha ⁻¹)				N fertilizer (kg ha ⁻¹)			
	0	23.8	47.6	Mean	0	23.8	47.6	Mean
DM	87.09	86.99	86.69	86.92	87.82	87.50	87.29	87.54
Composition of DM %								
OM	85.50	85.18	84.23	84.97	85.94	85.62	84.70	85.42
CP	14.34	15.12	16.10	15.19	13.40	14.23	15.52	14.38
CF	25.24	23.95	22.56	23.92	26.25	25.21	23.46	24.97
EE	2.45	2.54	2.65	2.55	2.25	2.36	2.48	2.36
NFE	43.47	43.57	42.92	43.32	44.04	43.82	43.25	43.70
Ash	14.50	14.82	15.77	15.03	14.07	14.38	15.30	14.58

Ca	2.30	2.60	2.80	2.57	2.50	2.60	2.80	2.63
P	0.26	0.27	0.29	0.27	0.26	0.27	0.28	0.27

Digestibility coefficients and nutritive values of different berseem hays in Table 2 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, the digestibility coefficients of DM, OM, CP, EE and NFE and DCP value were significantly higher ($P < 0.05$), but CF digestibility was significantly lower ($P < 0.05$) for 3rd cut compared to 4th cut berseem hay. While, TDN value tended to higher in 3rd cut than 4th cut berseem hay. The quadratic effect of N fertilizer on the digestibility of DM, OM, CP, CF, EE and NFE and TDN and DCP values being $R^2 = 0.69, 0.66, 0.65, 0.59, 0.69, 0.64, 0.27$ and 0.76 , respectively. The correlations between N fertilizer and the digestibility of DM, OM, CP, CF, EE and NFE and TDN and DCP values being $r = 0.83, 0.82, 0.80, -0.77, 0.83, 0.80, 0.52$ and 0.87 , respectively. These results are in accordance with those obtained by Ferri et al. (2004) who found that N fertilization affects rumen digestion of forage from rye pasture.

Table 2 Digestibility coefficients and nutritive values of berseem hay by goats.

Item	3 rd cut				4 th 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	62.69 ^c	65.80 ^b	68.97 ^a	65.82 ^A	60.21 ^c	63.19 ^b	66.24 ^a	63.21 ^B
OM	64.21 ^b	66.90 ^b	70.25 ^a	67.12 ^A	61.67 ^b	64.24 ^b	67.47 ^a	64.46 ^B
CP	65.07 ^c	68.06 ^b	70.93 ^a	68.02 ^A	62.49 ^c	65.36 ^b	68.11 ^a	65.32 ^B
CF	58.30 ^a	55.98 ^b	54.35 ^b	56.21 ^B	60.21 ^a	58.38 ^a	56.30 ^b	58.30 ^A
EE	64.47 ^c	67.98 ^b	70.98 ^a	67.81 ^A	61.92 ^c	65.29 ^b	68.17 ^a	65.12 ^B
NFE	65.09 ^b	68.15 ^a	70.83 ^a	68.02 ^A	62.51 ^b	65.45 ^a	68.01 ^a	65.32 ^B
Nutritive values %								
TDN	55.90 ^b	57.29 ^{ab}	58.32 ^a	57.17	54.85 ^b	56.17 ^{ab}	57.00 ^a	56.01
DCP	9.33 ^c	10.29 ^b	11.42 ^a	10.35 ^A	8.37 ^c	9.30 ^b	10.57 ^a	9.42 ^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.

The average daily DM, TDN and DCP intake by goats increased significantly ($P < 0.05$) with increasing the rate of N fertilizer (Table 3). The mean of DM, TDN and DCP intake were significantly higher ($P < 0.05$) for 3rd cut than that of 4th cut hay. These results reflect the differences in chemical composition and TDN and DCP values of different hays. The quadratic effect of N fertilizer on DM, TDN and DCP intake by goats were $R^2 = 0.49, 0.46$ and 0.69 , respectively. The correlations between N fertilizer and DM, TDN and DCP intake were $r =$

0.68, 0.66 and 0.83, respectively. There are several reports which show a close relationship between plant composition and forage quality in rangelands (Aydin and Uzun, 2005). Ferri et al. (2004) reported that N fertilization affects voluntary intake of forage from rye pasture.

Table 3 Feed intake and rumen activity of goats fed different hay.

Item	3 rd cut				4 th 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	760 ^b	787 ^b	827 ^a	791 ^A	668 ^b	756 ^a	766 ^a	730 ^B
TDN	425 ^b	451 ^{ab}	483 ^a	453 ^A	367 ^b	425 ^a	437 ^a	409 ^B
DCP	71 ^c	81 ^b	94 ^a	82 ^A	56 ^c	70 ^b	81 ^a	69 ^B
Rumen activity								
pH value	6.65 ^a	6.20 ^b	5.98 ^b	6.28	6.59 ^a	6.20 ^b	5.93 ^c	6.24
TVFA's mM/dl	12.31 _b	12.68 _b	14.40 _a	13.13 ^A	11.86 _b	12.17 _b	13.05 _a	12.36 ^B
NH ₃ -N mg/dl	11.00 _c	11.90 _b	12.65 _a	11.85	10.10 _c	12.10 _b	13.00 _a	11.73

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.

The pH value decreased significantly ($P < 0.05$), however the concentrations of TVFA's and NH₃-N increased significantly ($P < 0.05$) in rumen liquor with increasing the rate of N fertilizer (Table 3). The mean pH value and NH₃-N concentration in rumen liquor were nearly similar for hay of both 3rd and 4th berseem cuts, while the mean of TVFA's concentration was significantly higher ($P < 0.05$) with feeding the 3rd cut hay compared with feeding 4th cut berseem hay. The pH value, TVFA's and NH₃-N concentrations were affected quadratically by N fertilizer ($R^2 = 0.87, 0.68$ and 0.88 , respectively). The correlations between N fertilizer and pH value, TVFA's and NH₃-N concentrations being $r = -0.92, 0.78$ and 0.93 , respectively). These results agree with those obtained by Ferri et al. (2004) who found that the rumen concentrations of NH₃ increased with N fertilization of forage from rye pasture.

Nitrogen balance in Table 4 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 faeces was not significantly affected by the rate of N fertilizer ($P > 0.05$). Moreover, the mean of N intake, excretion in faeces and urine, digested and retained were significantly higher ($P < 0.05$) for 3rd cut compared with 4th cuts berseem hay. The nitrogen balance was positive for the different hay. Nitrogen intake, excretion in faeces, digested, excretion in urine and retained were affected quadratically by N fertilizer ($R^2 = 0.66, 0.34, 0.69, 0.72$ and 0.65 , respectively). The correlations between N fertilizer

and N intake, excretion in faeces, digested, excretion in urine and retained were $r = 0.81, 0.56, 0.83, 0.85$ and 0.81 , respectively. These results are in accordance with those obtained by Archibeque et al. (2001) who found that N fertilizer levels affected N metabolism of steers, which N digested and N retention increased as N level increased. Astigarraga et al. (2002) reported that N fertilization and N supplementation are efficient means to manipulate N balance in grazing dairy cows.

Table 4 Nitrogen balance (g/day) by goats fed berseem hay.

Item	3 rd cut				4 th 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	17.44 _c	19.04 _b	21.30 _a	19.26 ^A	14.32 _c	17.21 _b	19.02 _a	16.85 _B
N-feces	6.09	6.08	6.19	6.12 ^A	5.37 ^b	5.96 ^a	6.07 ^a	5.80 ^B
N-digested	11.35 _c	12.96 _b	15.11 _a	13.14 ^A	8.95 ^c	11.25 _b	12.96 _a	11.05 _B
N-urine	6.41 ^c	7.16 ^b	8.12 ^a	7.23 ^A	5.14 ^c	6.43 ^b	7.30 ^a	6.29 ^B
N-retained	4.94 ^c	5.80 ^b	6.99 ^a	5.91 ^A	3.81 ^c	4.82 ^b	5.65 ^a	4.76 ^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.

Results in Table 5 showed that the counts of red blood cells (RBC) and white blood cells (WBC) and haemoglobin concentration in blood of goats fed the different hay kinds increased significantly ($P < 0.05$) with increasing the rate of N fertilizer. Moreover, RBC count was significantly higher ($P < 0.05$) with feeding the 4th cut than that with feeding 3rd cut berseem hay. While, WBC count and haemoglobin concentration were nearly similar for feeding both 3rd and 4th cuts berseem hay. The quadratic response of RBC and WBC counts and haemoglobin concentration in blood of goats to N fertilizer of berseem were $R^2 = 0.15, 0.86$ and 0.60 , respectively. The correlations between N fertilizer and RBC and WBC counts and haemoglobin concentration were $r = 0.37, 0.92$ and 0.76 , respectively.

Table 5 Blood hematology of goats fed berseem hay.

Item	3 rd cut				4 th 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	12.87 ^b	13.02 ^b	14.69 ^a	13.53 ^B	16.16 ^c	17.16 ^b	17.92 ^a	17.08 ^A
WBC x10 ³ /ml	5.21 ^c	5.69 ^b	6.21 ^a	5.70	5.42 ^b	6.04 ^a	6.22 ^a	5.89
Hemoglobin g/dl	12.35 ^b	13.05 ^a	13.09 ^a	12.83	12.70 ^b	13.20 ^b	13.77 ^a	13.22

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.

The intake, excretion in faeces, absorption, excretion in urine and retention of both calcium and phosphorus by goats fed berseem hay increased significantly ($P < 0.05$) with increasing the rate of N fertilizer (Table 6). Moreover, calcium intake, excretion in faeces, absorption, excretion in urine and retention were nearly similar for both the 3rd and 4th cuts berseem hay. However, phosphorus intake, excretion in faeces, absorption, excretion in urine and retention were significantly higher ($P < 0.05$) for the 3rd cut compared with 4th cut berseem hay. The quadratic effect of N fertilizer on calcium intake, excretion in faeces, absorption, excretion in urine and retention were $R^2 = 0.91, 0.92, 0.90, 0.92$ and 0.87 , respectively. The corresponding values for phosphorus were $0.71, 0.76, 0.67, 0.78$ and 0.61 , respectively. The correlations between N fertilizer and calcium intake, excretion in faeces, absorption, excretion in urine and retention were $r = 0.95, 0.96, 0.94, 0.96$ and 0.92 , respectively. The corresponding values for phosphorus were $0.84, 0.87, 0.82, 0.88$ and 0.78 , respectively. These results agreed with those obtained by Bamikole (2003) who found that applying nitrogen fertilizer to grass could be used to achieve better utilization of more macro-minerals in forages.

Table 6 Calcium and phosphorus balance (g/day) by goats fed berseem hay.

Item	3 rd cut				4 th cut			
	N fertilizer (kg ha ⁻¹)				N fertilizer (kg ha ⁻¹)			
	0	23.8	47.6	Mean	0	23.8	47.6	Mean
Calcium								
Intake	17.48 ^c	20.46 ^b	23.16 ^a	20.37	16.70 ^c	19.66 ^b	21.45 ^a	19.27
Excretion in feces	6.99 ^c	8.18 ^b	9.26 ^a	8.15	6.68 ^c	7.86 ^b	8.58 ^a	7.71
Absorption	10.49 ^c	12.28 ^b	13.89 ^a	12.22	10.02 ^c	11.79 ^b	12.87 ^a	11.56
Excretion in urine	3.85 ^c	4.50 ^b	5.09 ^a	4.48	3.67 ^c	4.32 ^b	4.72 ^a	4.24
Retention	6.64 ^c	7.78 ^b	8.80 ^a	7.74	6.35 ^c	7.47 ^b	8.15 ^a	7.32
Phosphorus								
Intake	1.98 ^c	2.12 ^b	2.40 ^a	2.17 ^A	1.74 ^c	2.04 ^b	2.14 ^a	1.97 ^B
Excretion in feces	0.69 ^c	0.74 ^b	0.84 ^a	0.76 ^A	0.61 ^c	0.71 ^b	0.75 ^a	0.69 ^B
Absorption	1.28 ^c	1.38 ^b	1.56 ^a	1.41 ^A	1.13 ^c	1.33 ^b	1.39 ^a	1.28 ^B
Excretion in urine	0.38 ^c	0.40 ^b	0.46 ^a	0.41 ^A	0.33 ^c	0.39 ^b	0.41 ^a	0.38 ^B
Retention	0.91 ^c	0.98 ^b	1.10 ^a	1.00 ^A	0.80 ^c	0.94 ^b	0.99 ^a	0.91 ^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.

CONCLUSIONS

From this study it could be concluded that nitrogen fertilizer at the rate of $47.6 \text{ kg N ha}^{-1}$ showed the best results concerning composition, digestibility, feed intake, rumen activity, nitrogen balance, blood haematology and calcium and phosphorus balance of berseem hay by

goats and some differences exist between the 3rd and 4th cuts berseem hay.

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