Nutritional evaluation of berseem
4- Effect of phosphorus fertilizer on berseem fed as hay to goats

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Abstract: Twelve buck goats were used to determine the effect of phosphorus fertilizer (47.6 kg P 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 and NFE tended to decrease, however CP, CF, EE, ash, Ca and P tended to increase with phosphorus fertilizer. Moreover, the contents of DM, OM, CF, NFE, Ca and P tended to be lower, but CP, EE and ash tended to be higher in 3rd cut compared with 4th cut berseem hay. All nutrients digestibility and nutritive values for 3rd and 4th cuts berseem hay increased significantly (P<0.05) with phosphorus fertilizer. Moreover, the digestibilities of DM, OM, CP, EE and NFE and TDN and DCP values were significantly higher (P<0.05), however, CF digestibility was significantly lower (P<0.05) for 3rd cut compared to 4th cut berseem hay. The average daily DM, TDN and DCP intake increased significantly (P<0.05) with phosphorus fertilizer and were significantly higher (P<0.05) for 3rd cut than those of 4th cut berseem hay. The ruminal pH value and NH_3-N concentration decreased significantly (P<0.05), but TVFA's concentration increased significantly (P<0.05) in rumen liquor with phosphorus fertilizer. The pH value and TVFA's concentration were nearly similar for both hay cuts, while, NH_3-N concentration was significantly higher (P<0.05) with feeding 3rd cut than that with feeding 4th cut berseem hay. The nitrogen intake, excretion in feces, digested and retained by goats fed both 3rd and 4th cuts berseem hay did not significantly (P>0.05) affected by phosphorus fertilizer. While, the nitrogen excretion in urine increased significantly (P<0.05) with phosphorus fertilizer. Moreover, nitrogen intake, excretion in feces and urine, digested and retained were significantly higher (P<0.05) for 3rd cut compared with 4th cut berseem hay. The count of white blood cells (WBC) and hemoglobin concentration in blood of goats fed 3rd cut berseem hay and the counts of red blood cells (RBC) and WBC in blood of goats fed 4th cut berseem hay increased significantly (P<0.05) with phosphorus fertilizer. All blood haematology were nearly similar with feeding 3rd and 4th cuts berseem hay. Calcium intake, excretion in feces and urine increased significantly (P<0.05), however, Ca absorption and retention decreased significantly (P<0.05) with phosphorus fertilizer for 3rd and 4th cuts berseem hay. While, phosphorus intake, excretion in feces and urine, absorption and retention increased significantly (P<0.05) with phosphorus fertilizer for 3rd and 4th cuts berseem hay. Moreover, only Ca intake was significantly higher (P<0.05) and P excretion in feces was significantly lower (P<0.05) for 4th cut compared with 3rd cut berseem hay.

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1. Introduction

Berseem (*Trifolium alexandrinum* L.), the most common winter and spring fodder in Egypt, is notoriously low in phosphorus contents (0.14%) and abundant in calcium (1.44%). Owing to a wide Ca:P ratio, animals fed predominantly on berseem, unless supplemented with some rich source of phosphorus, may suffer from phosphorus deficiency syndromes. Being berseem crop, the phosphorus application would greatly enhance the forage yield (Ranjhan, 1993).

In terms of animal production, the quality of forage is more important than the quantity. Forage quality is determined by the content of different nutrients such as minerals, crude protein and fiber component. The concentration of chemical components in the forage varied depending on many variables such as plant species (Ramirez et al. 2004), harvesting date (Ball et al. 2001), fertilization (Türk et al. 2007), soil properties and other environmental variables (Kulik, 2009).

Phosphate fertilizer applications are also often beneficial on hay meadows. The soils are usually shallow and have a high pH. Both factors limit the amount of phosphorous available to plants. When phosphorous is needed, producers should apply three parts nitrogen to one part phosphorous. Percent crude protein (CP) and total digestible nutrients (TDN) are often used as indicators of hay quality. As the

percentages of CP and TDN increase, hay quality also rises. The crude protein percentages represent nitrogen compounds in forage and are lumped together when computing rations for livestock. TDN is a calculated figure representing the sum of all the digestible organic nutrients in the feed (Torell, et al., 2005).

The objective of this study was to investigate the effect of phosphorus fertilizer on composition, feed intake, digestibility, rumen activity, blood hematology and calcium and phosphorus balance of berseem hay by 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.*) hay, nutrients digestibility, nitrogen balance, rumen activity, blood hematology and calcium and phosphorus balance 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. Three hundred and ten kg of superphosphate (15.5% P_2O_5) to give phosphorus rate of 47.6 kg ha^{-1} was added during the land preparation. 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.

Four digestibility trials were conducted using three buck Angora goats (Bucks) with average live body weight of 38 kg and aged three years. Goats were fed *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) 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). 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). 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 P fertilizer and cuts. The Duncan multiple range test was used to compare difference between means.

3. Results and discussion

The chemical composition of 3rd and 4th cuts berseem hay as affected by phosphorus fertilizer is presented in Table (1). The contents of DM, OM and NFE tended to decrease, however CP, CF, EE, ash, Ca and P tended to increase with phosphorus fertilizer. Moreover, the contents of DM, OM, CF, NFE, Ca and P tended to be lower, but CP, EE and ash tended to be higher in 3rd cut compared with 4th cut berseem hay. The organic constituents, such as ADF, NDF, TDN and crude protein in plant tissue varies due to cultural practices such as fertilization, irrigation, cutting stage (Ball et al. 2001; Türk et al. 2007).

Results in Table (2) revealed that all nutrients digestibility and nutritive values for 3rd and 4th cuts berseem hay increased significantly ($P<0.05$) with phosphorus fertilizer. Moreover, the digestibilities of DM, OM, CP, EE and NFE and TDN and DCP values were significantly higher ($P<0.05$), however, CF digestibility was significantly lower ($P<0.05$) for 3rd cut compared to 4th cut berseem hay. Phosphorus fertilizer was effective in stimulating nutrients digestibility, which the increase of nutrients digestibility and nutritive values of berseem hay with P fertilizer demonstrate that P could develops the rumen bacterial growth, resulting in greater rate of digestibility of fibrous feedstuffs. These results agree with those obtained by Breves and Schroder (1991) who reported that phosphorus is required by ruminal microorganisms for digestion of cellulose and synthesis of microbial protein. Durand and Komisarczuk et al. (1987) stated that available phosphorus within the rumen should be at least 5 g/kg of organic matter digested to optimize degradation of cell walls from feeds by microbs. Zain et al. (2010) found that phosphorus supplementation led to

significant increase in nutrients digestibility by Pesisir calves vs. control.

The average daily DM, TDN and DCP intake by goats fed 3rd and 4th cuts berseem hay increased significantly (P<0.05) with phosphorus fertilizer as shown in Table (3). Moreover, the average daily intake of DM, TDN and DCP were significantly higher (P<0.05) for 3rd cut than those of 4th cut berseem hay. These results may be due to that P fertilizer could improve population of rumen microbe than will be increase the rate of passage in rumen. These results are in accordance with those obtained by Ishaq et al. (2006) and Zain et al. (2010) who reported that DM intake by Sahiwal and Pesisir calves increased significantly with phosphorus supplementation.

The pH value and NH_3-N concentration decreased significantly (P<0.05), but TVFA's concentration increased significantly (P<0.05) in rumen liquor with phosphorus fertilizer as shown in Table (3). The pH value and TVFA's concentration were nearly similar for both 3rd and 4th cuts berseem hay. While, ruminal NH_3-N concentration was significantly higher (P<0.05) with feeding 3rd cut than that with feeding 4th cut berseem hay. The depression of ruminal pH value and ammonia-N concentration with the rise in VFA concentration with phosphorus supplementation may be attributed to that phosphorus essential for proper functioning of rumen microorganisms, which is required by ruminal microorganisms for fermentation and utilization of ammonia-N to synthesis microbial protein as reported by Breves and Schroder (1991). Gaafar et al. (2004) reported that dietary phosphorus supplementation resulted in significant increase in TVFA's concentration accompanied by a reduction in pH value and progressively fell in ammonia-N.

Nitrogen balance presented in Table (4) showed that nitrogen intake, excretion in feces, digested and retained by goats fed both 3rd and 4th cuts berseem hay did not significantly (P>0.05) affected by phosphorus fertilizer. While, the nitrogen excretion in urine increased significantly (P<0.05) with phosphorus fertilizer. Moreover, nitrogen intake, excretion in feces, digested, excretion in urine and retained were significantly higher (P<0.05) for 3rd cut compared with 4th cut berseem hay. These results agree with those obtained by Shanklin (2001) who found that phosphorus supplementation not revealed any significant effect on nitrogen intake, absorption and retention by lambs.

Results of blood haematology in Table (5) showed that the count of white blood cells (WBC) and hemoglobin concentration in blood of goats fed 3rd cut berseem hay and the counts of red blood cells (RBC) and WBC in blood of goats fed 4th cut berseem hay increased significantly (P<0.05) with phosphorus fertilizer. All blood haematology were nearly similar with feeding 3rd and 4th cuts berseem hay. The values of blood haematology obtained in this study were in agreement with those obtained by Daramola et al. (2005) who reported that blood haematology of adult buck Dwarf goats were 9.6±0.5 g/dl for hemoglobin, 11.8±2.8 x10^6/dl for RBCs and 15.26±3.5[a] x103/dl for Total WBCs.

Results in Table (6) revealed that Ca intake, excretion in feces and urine increased significantly (P<0.05), however, Ca absorption and retention decreased significantly (P<0.05) with phosphorus fertilizer for 3rd and 4th cuts berseem hay. While, phosphorus intake, excretion in feces and urine, absorption and retention increased significantly (P<0.05) with phosphorus fertilizer for 3rd and 4th cuts berseem hay. Moreover, only Ca intake was significantly higher (P<0.05) and P excretion in feces was significantly lower (P<0.05) for 4th cut compared with 3rd cut berseem hay. These results agree with those obtained by Tani et al. (2007) who found that urinary and fecal phosphorus excretions and intestinal net phosphorus absorption were significantly increased, but intestinal net calcium absorption was significantly decreased by the high phosphorus diets, dependent on the amount of dietary phosphorus.

Table 1. Chemical composition of berseem hay.

Item	3rd cut			4th cut		
	P fertilizer (kg ha^{-1})			P fertilizer (kg ha^{-1})		
	0	47.6	Mean	0	47.6	Mean
DM	87.11	87.01	87.06	87.84	87.52	87.68
Composition of DM %						
OM	85.50	85.18	85.34	85.94	85.62	85.78
CP	14.34	14.44	14.39	13.40	13.49	13.45
CF	25.24	25.42	25.33	26.25	26.43	26.34
EE	2.45	2.47	2.46	2.25	2.27	2.26
NFE	43.47	42.86	43.16	44.04	43.43	43.73
Ash	14.50	14.82	14.66	14.06	14.38	14.22
Ca	2.30	2.35	2.33	2.50	2.60	2.55
P	0.26	0.33	0.30	0.27	0.35	0.31
Ca: P ratio	8.85	7.12	7.98	9.26	7.43	8.34

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

Item	3rd cut				4th cut			
	P fertilizer (kg ha^{-1})				P fertilizer (kg ha^{-1})			
	0	47.6	Mean	P-value	0	47.6	Mean	P-value
Digestibility coefficients %								
DM	62.70	64.80	63.75*	0.016	60.21	63.19	61.70	0.004
OM	64.21	65.90	65.06*	0.034	61.67	64.24	62.96	0.007
CP	65.07	67.73	66.40*	0.008	62.49	65.00	63.75	0.008
CF	58.30	60.67	59.49	0.008	60.21	62.65	61.43*	0.009
EE	64.47	66.98	65.73*	0.010	61.92	64.29	63.10	0.011
NFE	65.09	68.33	66.71*	0.004	62.51	65.60	64.05	0.005
Nutritive values %								
TDN	56.34	58.64	57.49*	0.017	54.85	57.11	55.98	0.016
DCP	9.33	9.78	9.56*	0.016	8.37	8.77	8.57	0.038

* Means in the same row differ significantly (P<0.05).

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

Item	3rd cut				4th cut			
	P fertilizer (kg ha^{-1})				P fertilizer (kg ha^{-1})			
	0	47.6	Mean	P-value	0	47.6	Mean	P-value
Feed intake (g/head/day)								
DM	750	787	768*	0.043	718	756	737	0.030
TDN	422	462	442*	0.030	394	432	413	0.023
DCP	70	77	73*	0.028	60	66	63	0.034
Rumen activity								
pH value	6.65	6.20	6.43	0.013	6.59	6.20	6.40	0.020
TVFA's mM/dl	12.31	13.68	13.00	0.002	11.86	13.17	12.52	0.003
NH_3-N mg/dl	11.00	9.85	10.43*	0.006	10.10	8.75	9.43	0.003

* Means in the same row differ significantly (P<0.05).

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

Item	3rd cut				4th cut			
	P fertilizer (kg ha^{-1})				P fertilizer (kg ha^{-1})			
	0	47.6	Mean	P-value	0	47.6	Mean	P-value
Intake	17.21	18.19	17.70*	0.167	15.40	16.33	15.86	0.141
Fecal excretion	6.01	5.87	5.94*	0.233	5.77	5.71	5.74	0.567
Digested	11.20	12.32	11.76*	0.081	9.62	10.62	10.12	0.071
Urinary excretion	6.41	7.16	6.78*	0.002	5.14	6.43	5.79	0.001
Retention	4.79	5.16	4.98*	0.376	4.48	4.19	4.33	0.398

* Means in the same row differ significantly (P<0.05).

Table 5. Blood hematology of goats fed berseem hay.

Item	3rd cut				4th cut			
	P fertilizer (kg ha^{-1})				P fertilizer (kg ha^{-1})			
	0	47.6	Mean	P-value	0	47.6	Mean	P-value
RBC x10^6/ml	11.87	12.02	11.95	0.517	11.16	12.19	11.67	0.026
WBC x10^3/ml	15.21	15.69	15.45	0.006	15.42	16.04	15.73	0.003
Hemoglobin g/dl	10.35	11.05	10.70	0.028	10.70	11.20	10.95	0.077

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

Item	3rd cut				4th cut			
	P fertilizer (kg ha-1)				P fertilizer (kg ha-1)			
	0	47.6	Mean	P-value	0	47.6	Mean	P-value
Calcium								
Intake	17.24	18.49	17.87	0.013	17.95	19.66	18.80*	0.004
Excretion in feces	6.99	9.27	8.13	0.001	6.68	9.84	8.26	0.001
Absorption	10.25	9.22	9.74	0.004	11.27	9.82	10.54	0.002
Excretion in urine	3.85	4.55	4.20	0.001	3.67	4.37	4.02	0.001
Retention	6.40	4.67	5.54	0.002	7.60	5.45	6.52	0.001
phosphorus								
Intake	1.95	2.60	2.27	0.001	1.94	2.65	2.29	0.001
Excretion in feces	0.69	0.75	0.72*	0.007	0.61	0.68	0.64	0.006
Absorption	1.26	1.85	1.55	0.001	1.33	1.97	1.65	0.001
Excretion in urine	0.38	0.41	0.39	0.005	0.33	0.40	0.36	0.002
Retention	0.88	1.44	1.16	0.001	1.00	1.57	1.28	0.001

* Means in the same row differ significantly (P<0.05).

4. Conclusions

Form this study it could be concluded that phosphorus fertilizer at the rate of 47.6 kg P ha^{-1} showed the best results concerning composition, digestibility, feed intake, rumen activity, nitrogen balance, blood hematology and phosphorus balance of berseem hay by goats and some differences exist between the 3rd and 4th cuts berseem hay.

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