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EFFECT OF PHOSPHORUS FERTILIZATION ON SILAGE QUALITY OF PERSIAN CLOVER (*TRIFOLIUM RESUPINATUM* L.)

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Abstract
The aim of the research was to determine the effect of phosphorus fertilization (0, 30, 45, 60, 75 and 90 kg ha^{-1}) on silage yield and quality traits of Persian clover (Trifolium resupinatum L.). The experiment was conducted at Yoruk village of Malkara-Tekirdag with three replications in randomized block design in 2021. Silage yield (t ha^{-1}), dry matter (%), crude protein (%), crude ash (%), acid detergent fiber (ADF, %), neutral detergent fiber (NDF, %), P (%), K (%), Ca (%), Mg (%), digestible dry matter (DDM, %), dry matter intake (DMI, %), relative feed value (RFV), total digestible nutrients (TDN), net energy-lactation (NEl), net energy-maintenance (NEm), net energy-gain (NEg) and flieg score were determined. Silage yield, dry matter, pH, crude protein, ADF, NDF, P, Mg, DDM, DMI, RFV, TDN, NEg and Flieg Score were determined statistically significant at P<0.01. The highest silage yield (72.83 t ha^{-1}) and ADF ratio (25.36 %) were obtained from 30 kg P ha^{-1}. The highest dry matter (39.92 %), DDM (70.30 %), DMI (3.22 %) RFV (175.056) and TDN (68.90) were found at application of 90 kg P ha^{-1}. The highest flieg score was determined at application of 60 kg P ha^{-1}. The highest crude protein (15.50-15.51 %) was found at application of 45 and 60 kg P ha^{-1}.

Keywords: Persian clover, phosphorus doses, silage, quality properties

1. INTRODUCTION

Grazing is the lowest-cost in animal feeding. However, in most regions seasonal shortages limits the productivity of rangelands. The limited range and the low yield level of pasture areas increases the importance of silage production, which is considered the closest to fresh forage. Silage is the ideal method for the high quality forage conservation. In periods when fresh forage is not available, silage, which is harvested in certain periods and fermented in an airless environment, is important in terms of providing cheap roughage.

Legumes are used for forage, silage, food, ornament, soil improvement, pollen, nectar resources, and grasslands. Among plant species, annual forage legumes are considered as the major source of nutrients for livestock and are grown worldwide (Ates et al., 2010). They are a very important feed source for ruminants and can be used as hay, silage or grazing (Fernandes et al., 2013). Clovers are in the tribe *Trifolieae* of the subfamily *Papilionoideae*, family *Fabaceae*, *Trifolium* L. The genus contains approximately 250-300 species. Clover species are herbaceous annuals, perennials, often prostrate, and rarely more than 100 cm tall. Persian clover (*T. resupinatum* L.) and Persian clover-annual grasses mixtures are commonly used for herbage, hay and grazing in the Mediterranean region and in temperate areas of world. It is a semi-erect annual legume, that is reseeded as winter forage crop and can survive at -18 °C under the snow (Ates, 2013). There are three main subspecies

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of Persian clover: ssp. *majus* Boiss., ssp. *typicum* Fiori et Paol. and ssp. *resupinatum* Gib. These are a native of western Asia, central and southern Europe, Mediterranean countries. Originating in Anatolia and northern Iran, it was cultivated for forage during the 18th century in Italy and France (Tekeli and Ates, 2011). Persian clover is adapted to a wide range of soil types, but it is best suited for low-lying areas with well-drained, clay-loam and airy textured soils of pH from 5 to 8 (Hoveland and Evers, 1995; Ate and Servet, 2004; Ates and Tekeli, 2007). It has been successfully grown in areas that receive between 450 and 600 mm annual rainfall (Ates and Tekeli, 2001).
The cultural practices such as fertilization and growth stages of forage crops affect the forage yield, palatability, digestibility and chemical composition cell wall components, protein, mineral and vitamin contents (Tenikecier and Ates, 2018). Fertilizer management has a vital role in the success or failure of the production of forage and other crops. It is an important factor for obtaining higher yields of forage crops. Application of balanced fertilizer increases vegetative growth and improves yield and quality of herbage/hay in forage crops.
Phosphorus (P) is classified as a major essential nutrient even though its content in forage crops is much lower than contents of nitrogen (N) and potassium (K), and for field pea and other forage legumes particularly, lower than that of calcium (Ca). Supplying these elements in forage legumes and grasses fields could be increase forage yield and quality properties (Tenikecier and Ates, 2021). Positive effects of N, P and K applications on yield and quality traits of forage crops reported by Wolday et al. (2015). Phosphorus fertilization affects dry matter yield and chemical composition of forage crops (Dasci et al., 2010; Yuksel and Turk, 2019). For forage crops to be of superior quality, they must be high in four factors: (a) nutrients, (b) digestibility, (c) palatability, and (d) efficiency of utilization.
The aim of the research was to determine the effect of different phosphorus doses (0, 30, 45, 60, 75 and 90 kg P ha^{-1}) on silage quality of Persian clover (*T. resupinatum* ssp. *majus* Boiss.).

2. MATERIALS AND METHODS

The experiment was conducted at Yörük village of Malkara-Tekirdag, Turkey with three replications in randomized block design in 2020-2021 growing season. The experimental area's soil properties were given in Table 1.

Table 1. Soil properties of the experimental area

	Quantity	
pH		7.40
Lime	%	11.05
Organic Matter	%	3.57
N	%	0.18
P	(ppm)	96.83
K	(ppm)	506.64
Ca	(ppm)	8110.27
Magnesium (Mg)	(ppm)	376.66
Iron (Fe)	(ppm)	7.10
Copper (Cu)	(ppm)	1.64
Zinc (Zn)	(ppm)	2.17
Manganese (Mn)	(ppm)	19.68

The total precipitation of the experimental area was 421.7 mm on average and an annual overall temperature of 10.9 °C. Demet-82 variety was used as seed material. Seeds were sown in to 8 rows

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with 5 m long plots by hand with 0.25 m inter-row space at 20 kg ha^{-1} (Tekeli and Ates, 2011) seeding rate on 08 November 2020. The six different phosphorus doses, 0 (control)-30-45-60-75-90 kg ha^{-1}, were applied with TSP (triple superphosphate (TSP), P 43%) to plots at sowing. Besides, a basal fertilizer containing urea (50 kg N ha^{-1}) was incorporated into the soil at the time of seedbed preparation. The plots of the Persian clover were harvested on at full-bloom stage from each plot for determine the silage yield (t ha^{-1}), dry matter (%), crude protein (%), crude ash (%), ADF (%), NDF (%), P (%), K (%), Ca (%), Mg (%), digestible dry matter (DDM) (%), dry matter intake (DMI) (%), relative feed value (RFV), total digestible nutrients (TDN), net energy-lactation (NEl), net energy-maintenance (NEm), net energy-gain (NEg) and Flieg score. The fresh samples were chopped mechanically and left to wither for 2 hours, weighed to calculate the silage yield, and the samples without additives were vacuumed in to the 18x22 cm plastic bags. The bags were kept in storage without light for app. 45 days for fermentation at ambient temperature (15-28°C) (Can et al., 2019; Jia et al., 2021). Oven drying for feed analysis at temperatures above 60°C can result in heat damaged protein and elevated fiber and lignin values (Reed and Van Soest, 1984). For this reason, maturated silage samples were dried at 60 °C for 48 hour followed by storage for a further day at room temperature, ground to small (≤1 mm) pieces and used for the analyses (Ates and Tenikecier, 2022; Tenikecier and Ates, 2022). The samples were analyzed for N using procedures of the Association of Official Analytical Chemists (AOAC, 2019). Crude protein (CP) content (%) of the samples were calculated by multiplying N contents by a coefficient of 6.25. The samples were wet-fired with nitric-perchloric acid, and P content (%) was determined spectrophotometrically, while K (%), Ca (%), and Mg (%) contents were obtained using an atomic absorption spectrophotometer (ICP- OES, inductively coupled plasma-optical emission spectrometer) (Isaac and Johnson JR, 1998). The crude ash (CA), acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents (%) were determined by Weende and Van Soest methods (AOAC, 2019; Van Soest et al., 1991). All samples were analyzed in duplicate. The digestible dry matter (DDM, %), dry matter intake (DMI, %), relative feed value (RFV, %), total digestible nutrients (TDN), net energy lactation (NEl), net energy-maintenance (NEm) and net energy-gain (NEg) were calculated according to the equations adapted from common formulas for forages (Schroeder, 1994). The Fleig scores were calculated based on the formula presented by Kilic (1984). All data were analyzed statistically by analysis of variance using TARIST software (Acikgoz et al., 2004) and means of treatments were compared using least significant difference (LSD) test with MSTAT-C software (Duzgunes et al., 1987).

3. RESULTS AND DISCUSSIONS

Means of the silage yields (t ha^{-1}) of the different phosphorus doses applied Persian clover and the dry matter (%), crude protein (%), crude ash (%), ADF (%), NDF (%), P (%), K (%), Ca (%), Mg (%), digestible dry matter (DDM) (%), dry matter intake (DMI) (%), relative feed value (RFV), total digestible nutrients (TDN), net energy-lactation (NEl), net energy-maintenance (NEm), net energy-gain (NEg) and Flieg score means of the ensiled Persian clover were given in Table 1 and 2.
Silage yield, dry matter, crude protein, ADF, NDF, P, DDM, DMI, RFV, TDN, NEg and Flieg Score were determined statistically significant at $P<0.01$ and crude ash, K, Ca, Mg, net energy lactation and net energy-maintenance were not affected from different phosphorus applications. Silage yields of the Persian clovers under different phosphorus doses were varied between 62.18-72.38 t ha^{-1} and highest was determined at application of 30 kg P ha^{-1} (Table 2). The requirement of a 500 kg beef cattle (*Bos taurus* L.) of superior milking ability nursing a calf the first 3 to 4 months

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postpartum is a minimum of 28.6 Mcal of digestible energy (NRC, 2001). These requirements can be satisfied with 11.8 kg of DM (Essig, 1985) from forage legumes. Daily intake of digestible DM is more closely correlated with DM intake than with DM digestibility. In most forage crops, the cell wall components account for 55-85 percent of DM. These components of forage crop species are affected by the many factors mentioned above (Tenikecier and Ates, 2018). Dry matter is an indicator of the amount of nutrients that are available to the animal in a particular feed. Dry matter of the ensiled Persian clover were varied between 39.66 % and 39.92 %. The highest dry matter percentage was obtained from 60 kg P ha^{-1}. The highest crude protein ratios were found at applications of 45 and 60 kg P ha^{-1} (15.50 and 15.60 %), and the lowest was at 30 kg P ha^{-1} (Table 2). In order to digest cellulose and hemicellulose, which are known as water-insoluble carbohydrates in the cell wall of plants, at a certain rate, ruminant animals physically break down the cell wall by ruminating and slowly ferment with the help of cellulotic bacteria in their rumen. Feed intake is also affected because roughage with a high fiber content takes up space in the rumen for a long time. Since the ADF ratio in the forage plants grown gives an idea about the quality of the feed, it is absolutely necessary to know the ADF ratio in the roughage before the ration preparation. The NDF value of the grass produced from the forage grasses gives an idea about the volume of the grass. The high NDF value of the plant produced indicates that the grass volume is high (Atalay and Ates, 2020). The lowest ADF was determined at application of 75 kg P ha^{-1} and the lowest NDF was at application of 90 kg P ha^{-1}.

Table 2. Silage Yield, Dry Matter, pH, CP, CA, ADF, NDF, P, K, Ca and Mg of the ensiled Persian clover

	Phosphorus doses (kg ha^{-1})							
Characteristics	*0*	*30*	*45*	*60*	*75*	*90*	**Mean**	**LSD**
Silage Yield (t ha^{-1})	65.48bc	72.83a	62.18c	71.76ab	70.58ab	70.51ab	68.89	6.356**
Dry Matter (%)	39.66b	39.69b	39.75ab	39.77ab	39.64b	39.92a	39.74	0.194**
pH	3.83b	4.01a	3.79bc	3.70c	3.99a	4.03a	3.89	0.102**
Crude Protein (%)	15.38b	14.87d	15.50a	15.51a	15.24c	15.21c	15.28	0.111**
Crude Ash (%)	9.26	9.26	9.25	9.25	9.25	9.25	9.25	ns
ADF (%)	25.05b	25.36a	24.42c	24.82b	23.46e	23.88d	24.50	0.267**
NDF (%)	39.47a	39.49a	38.08b	38.06b	38.09b	37.36c	38.42	0.078**
P (%)	0.48ab	0.47b	0.49a	0.49a	0.47b	0.49a	0.48	0.018*
K (%)	1.51	1.50	1.51	1.50	1.51	1.50	1.50	ns
Ca (%)	1.23	1.23	1.23	1.23	1.23	1.23	1.23	ns
Mg (%)	0.27	0.27	0.26	0.26	0.27	0.26	0.26	ns

Mineral balance is very important to keep animal healthy. Deficiency of one mineral element in the diet cannot be balanced by the others. These elements should be in certain ratio. For example, Ca and P are closely related to animal health and metabolism. It is very important to keep a proper balance of Ca and P in relation to vitamin D (Tekeli and Ates, 2005). Skeleton is containing approximately 68-73% of the Mg in the total Mg content of animal body. The content of P in the rumen is also important, with higher levels of P favoring magnesium absorption. Cows grazing P-deficient pastures might have low concentrations of P in the rumen, and Mg absorption might be further impaired. The Ca content in the blood also plays a role in (Ates, 2017). The highest phosphorus content of the ensiled Persian clover were determined at applications of 45, 60 and 90 kg P ha^{-1} (0.49 %) (Table 2).

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DDM, DMI, TDN of the ensiled Persian clover varied between 69.12 and 70.30 %, 3.04 and 3.22 %, 67.16 and 68.90. The highest DDM, DMI, TDN were determined at application of 90 kg P ha^{-1}. Additionally, the highest NEg was obtained from 45 and 90 kg P ha^{-1} doses (Table 3).

Table 3. DDM, DMI, TDN, Nel, NEm, NEg, RFV and Fleig Score of the ensiled Persian Clover

	Phosphorus Treatments (kg ha^{-1})							
Characteristics	*0*	*30*	*45*	*60*	*75*	*90*	**Mean**	**LSD**
DDM (%)	69.37bc	69.12c	69.88ab	69.57bc	69.59bc	70.30a	*69.64*	0.570**
DMI (%)	3.04b	3.04b	3.15ab	3.15ab	3.13ab	3.22a	*3.12*	0.114**
TDN	67.54d	67.16e	68.27b	67.87c	67.84cd	68.90a	*67.93*	0.300**
NEl	0.70	0.69	0.70	0.70	0.70	0.71	*0.70*	ns
NEm	0.76	0.75	0.77	0.76	0.76	0.78	*0.76*	ns
NEg	1.69bc	1.68c	1.72a	1.70b	1.70b	1.73a	*1.70*	0.019**
RFV	163.54c	162.83c	170.87b	170.17b	170.10b	175.06a	*168.76*	3.780**
Flieg Score	131.25b	123.97c	133.03ab	136.40a	127.67c	123.77c	*129.35*	3.778**

Rohweder et al. (1978) determined the limit values of quality standards according to crude protein, ADF and NDF ratios of forages. They report that the relative feed value is considered 100 when the ADF and NDF ratios are 41% and 53% respectively, and if the RFV is greater than 151, the feed is the best quality. The RFV of the Persian clover under different doses of phosphorus application were varied between 162.83-175.06 and the highest RFV was determined at 90 kg ha^{-1}. Silage quality is classified based on Fleig scores as follows: very good for Fleig scores of 85–100; good for Fleig scores of 60–84; moderate for Fleig scores of 40–59; satisfactory for Fleig scores of 20–39; worthless for Fleig scores of <20 (Kilic, 1984). The Fleig score of the Persian clover silages were varied between 123.97-136.40 and the highest was determined at 60 kg ha^{-1}. Balazadeh et. al., (2021) stated that the Persian clover verities which used in the study has 144.5 g kg^{-1} CP, 368.34 g kg^{-1} ADF, 417.5 g kg^{-1} NDF, 602.02 g kg^{-1} DDM, 2.9 % DMI and 134.2 % RFV at under irrigated conditions. Tekeli et al. (2003), Ate and Servet (2004) and Tekeli and Ates (2006) found a CP of 15.01-24.4 % and a CF of 11.2-21.2 % for Persian clover. P content of 0.31 % was found by Essig (1985), whereas Tekeli et al. (2003), Ate and Servet (2004), Tekeli and Ates (2006) reported 0.24-0.51 % P from Persian clover. Lanyon and Smith (1985) and Robinson (1985) found a K of 10 g kg^{-1} DM for white clover (*T. repens* L.) and strawberry clover (*T. fragiferum* L.). Kim et. al. (2004) determined the DM 10.2 %, 17.0 % CP, 35 % NDF, 25.0 % ADF, 185 RFV and 69.2 % TDN of Persian clover hay, and stated that the Persian clover is the superior in forage quality among legumes.

4. CONCLUSIONS

According to results of the ensiled Persian clover under the effect of the different phosphorus doses, the highest silage yield was determined at application of 30 kg P ha^{-1}. But the highest dry matter was found at application of 90 kg P ha^{-1}. In addition, the highest crude protein ratios were obtained from 45 and 60 kg P ha^{-1} doses. The lowest ADF was determined at application of 75 kg P ha^{-1} and the lowest NDF was at 90 kg P ha^{-1}. The highest phosphorus content were obtained from 45, 60 and 90 kg P ha^{-1} doses. The highest RFV, DDM, DMI and TDN were found at application of 90 kg P ha^{-1}. It can be recommended from the results, 30 kg P ha^{-1} dose for the purpose of the high yield.

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But for the high feeding quality of Persian clover silage, 90 kg ha^{-1} phosphorus application can be preferred.

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