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Research article

Response of shaftal (*Trifolium resupinatum* L. var. *majus* Boiss.) to phosphorus fertilization: implications for forage yield and quality

Hazım Serkan Tenikecier*

Tekirdağ Namık Kemal University, Agriculture Faculty, Field Crops Department, Türkiye
*Corresponding author email: hstenikecier@nku.edu.tr

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Abstract

This study evaluated the effects of different phosphorus (P) doses (0-30-45-60-75-90 kg P ha^{-1}) on morpho-agronomic traits, yield, and forage quality of shaftal. The research was conducted in the Thrace region of southeastern Europe (Türkiye) from 2019 to 2021 for two years. The plant height was varied between 101.17 to 114.54 cm, leaf length 21.44 to 25.45 cm, leaflet length 3.97 to 4.48 cm, leaflet width 2.37 to 2.89 cm number of leaves per plant 9.23 to 10.19 pcs, stem diameter 6.89 to 7.93 mm, green fodder yield 56.49 to 83.65 t ha^{-1}, dry matter yield 7.30 to 10.72 t ha^{-1}, crude protein 17.13 to 20.37%, crude fiber 24.23 to 27.74%, NDF 34.33 to 36.53%, ADF 21.92 to 24.54%, RFV 177.74 to 194.64%, Ca 24.12 to 29.10 g kg^{-1}, Mg 2.45 to 2.71 g kg^{-1}, P 2.62 to 2.88 g kg^{-1}, K 24.86 to 28.54 g kg^{-1}, tetany ratio 0.89 to 0.97, β caroten 249.02 to 357.91 mg kg^{-1}, α-tocopherol 116.00 to 125.49 µg kg^{-1}, DDM 69.78 to 71.82%, DMI 3.28 to 3.50%, TDN 68.13 to 71.14%, NEl 0.70 to 0.74 Mcal lb^{-1}, NEm 0.77 to 0.81 Mcal lb^{-1}, NEg 0.44 to 0.48 Mcal lb^{-1} at P levels, respectively. P application significantly affected plant growth characteristics, green fodder and dry matter yields, chemical composition, fiber fractions, mineral content, vitamin concentrations, digestibility indices, and net energy values. Increasing P doses generally enhanced biomass production, crude protein content, digestibility parameters, mineral composition, and antioxidant vitamin levels, while reducing structural fiber fractions. The most consistent improvements in both yield and forage nutritive value were observed at an application rate of approximately 60 kg P ha^{-1}, whereas higher rates resulted in diminishing returns. These findings indicate that balanced P fertilization is critical for optimizing productivity and forage quality in the shaftal.

Keywords: Clovers, Digestibility indices, Fodder yield, Forage legumes, Phosphorus nutrition, Nutritive value, Vitamin.

Introduction

Clovers (*Trifolium* L.) include economically valuable species that are extensively cultivated worldwide and are widely used in forage production systems for grazing, hay, silage, and soil improvement (Tekeli and Ates, 2003; Sood *et al*., 2016). They serve as a crucial source of nutrients for livestock and are cultivated across various agroecological regions worldwide. Ruminant animals can convert forage biomass into economically valuable products such as meat, milk, and wool, which are central to livestock production systems. Given the importance of clovers in animal feeding, maintaining high forage quality alongside low levels of anti-nutritional compounds is a key objective. Forage quality in clovers is largely determined by nutritive value, which is reflected in animal performance indicators such as milk yield, live weight gain, and product quality. Depending on animal species, physiological status, and seasonal conditions, clovers may supply approximately 15 to 100% of dietary protein requirements and 20 to 100% of energy demands (Essig, 1985).

Although clovers generally contain lower concentrations of cell wall components than grasses (*Poaceae* Barnhart), their cell walls tend to be more highly lignified, which can limit digestibility relative to grass species. The nutritive value and mineral composition of clovers, as well as their effects on forage digestibility and voluntary intake, are influenced by several interacting factors. These include climatic conditions, stage of maturity, harvest timing, leaf-to-stem ratio, disease and insect pressure, weed infestation, and inherent soil properties (Tekeli and Ates, 2006).

T. resupinatum L., commonly known as shaftal or Persian clover, is an annual forage legume cultivated primarily in Mediterranean-type environments due to its high biomass production, favorable forage quality, and adaptability to cool-season conditions. Its tolerance to waterlogging and a range of soil textures makes shaftal a valuable component of livestock-based farming systems, particularly in regions with autumn-winter rainfall that supports vegetative growth. The species is characterized by relatively high crude protein content, a favorable fiber composition, and the presence of bioactive compounds such as β-carotene and α-tocopherol, which contribute to its nutritional value for ruminants (Ate and Servet, 2004). However, fertilization practices that consistently support both high yield and forage quality across diverse environmental and soil conditions remain insufficiently defined.

The primary role of phosphorus (P) in plants is storage and transfer of energy produced by photosynthesis for growth and reproductive processes (NRCS, 2014). P is a critical macronutrient influencing legume growth, root development, nodulation, and biological nitrogen (N) fixation. Leguminous species typically exhibit high P requirements due to the energy-intensive nature of symbiotic N fixation. Limited P availability can restrict vegetative growth, leaf development, stem elongation, and biomass accumulation (Ates and Tenikecier, 2022a; 2022b). In addition, P nutrition affects forage nutritive value through its influence on crude protein (CP) content, fiber fractions, mineral composition, and energy-related attributes that collectively determine digestibility and intake potential. Mouazen and Kuang (2016) and Granada *et al*. (2018) reported that P suitable for optimal plant production is limited to approximately 5.7 billion hectares worldwide. In addition, even though P is abundant in soil, nearly 40% of agricultural soils are deficient in P due to its immobilization and the slow uptake of P ions by plant roots (Solangi *et al*., 2023). In natural ecosystems, P losses from the soil-plant cycling system are replenished through the slow process of rock weathering (Bouwman *et al*., 2009), while in human-managed systems these losses are offset by fertilizer inputs (animal waste, human excreta, or non-renewable geological P sources) (Alewell *et al*., 2020). Considering the widespread occurrence of P deficiency in agricultural soils, appropriate P management is an important consideration for improving forage productivity and quality in shaftal-based systems. Although P fertilization has been widely studied in forage legumes, information regarding its effects on shaftal remains is limited. In particular, responses of comprehensive nutritive value parameters: such as relative feed value (RFV), digestible dry matter (DDM), dry matter intake (DMI), total digestible nutrients (TDN), net energy for lactation (NEl), net energy for maintenance (NEm), and net energy for gain (NEg)-to different P application rates have not been fully documented for this species. Improved understanding of these responses may assist in the development of P management practices suited to specific soil and climatic conditions.

This study looked at how six different P application rates affected the growth, yield, mineral content, bioactive compounds, and nutritional value of shaftal in the Thrace region of southeastern Europe (Türkiye). The results aim to help improve P management and support better forage production in similar environments.

Materials and Methods

Study area: The experiment was conducted in Yörük Village (40°55′33″N, 27°03′45″E), Malkara District, Tekirdağ Province, Türkiye, during the 2019-2021 growing seasons, using a randomized complete block design with three replications. Soil samples collected during the 2019-2020 growing season were analyzed to determine their physicochemical properties. The soil exhibited a slightly alkaline pH (7.40) and low salinity (0.04%), with a calcareous structure (11.05% $CaCO_3$). The organic matter content was 3.57%, and total N was measured at 0.18%. Available P and potassium (K) concentrations were 10.83 ppm and 506.64 ppm, respectively. Calcium (Ca) and magnesium (Mg) contents were 8,110.27 ppm and 376.66 ppm. Micronutrient contents, including iron (7.10 ppm), copper (1.64 ppm), zinc (2.17 ppm), and manganese (19.68 ppm), were quantified in the soil samples. Climatic conditions during the study period were characterized by total precipitation of 271.6 mm in 2019-2020 and 426.8 mm in 2020-2021, with mean air temperatures of 10.4°C and 9.5°C, respectively.

Crop management and measurements: The Demet-82 variety was used as the seed material. Sowing was carried out manually on 14 November 2019 and 08 November 2020. Plots consisted of eight rows, each 5 m long, with an inter-row spacing of 0.25 m, and seeds were sown at a rate of 20 kg ha^{-1} (Tekeli and Ateş, 2011). Six P application doses, 0 (control), 30, 45, 60, 75, and 90 kg ha^{-1}, were applied at sowing as triple superphosphate (TSP, triple superphosphate 43% P_2O_5). N was supplied as urea at a rate of 50 kg N ha^{-1} and incorporated into the soil during seedbed preparation.

Harvesting was performed once annually at the full-bloom stage on 07 June 2020 and 06 June 2021 by cutting plants at a stubble height of 3 cm. No irrigation or fertilizer application was carried out following harvest. The number of leaves per plant (pcs), plant height (cm) and stem diameter (mm) were measured on ten randomly selected plants per plot. Stem diameter was measured at the third or fourth node. For the same plants, leaf length (cm) was measured from leaves located at these corresponding nodes. Subsequently, from these leaves, the length (cm) and width (cm) of the middle leaflet were determined (Ates, 2011a; 2011b).

Fresh forage samples were weighed immediately after harvest to determine green fodder yield (GFY, t ha^{-1}). Approximately 500 g herbage samples were then oven-dried at 60°C (Reed and Van Soest, 1984) for 48 h, equilibrated at room temperature for 24 hours, and reweighed to calculate dry matter yield (DMY, t ha^{-1} (Ates, 2015). Subsequently, the samples were ground to pass a 1-mm sieve and used for laboratory analyses (Ates and Tenikecier, 2022a; Tenikecier and Ates, 2022).

Chemical analyses: The N content of the samples was determined according to the procedures of the Association of Official Analytical Chemists (AOAC, 2023), and CP (%) was calculated by multiplying the N content by 6.25. Crude fiber (CF), acid detergent fiber (ADF), and neutral detergent fiber (NDF) contents (%) were determined using the Weende and Van Soest methods (AOAC, 2023; Van Soest *et al*., 1991).

For mineral analysis, samples were wet-digested with a nitric–perchloric acid mixture. P (g kg^{-1}) content was determined spectrophotometrically, whereas K (g kg^{-1}), Ca (g kg^{-1}), and Mg (g kg^{-1}) contents were measured using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Isaac and Johnson Jr., 1998). The tetany ratio [K/(Ca+Mg)] was subsequently calculated (Cherney *et al*., 2002). The β-carotene (mg kg^{-1}) and α-tocopherol (mg kg^{-1}) contents of dried samples were determined following slightly modified procedures described by Ates *et al*. (2020) and Ates (2021). All chemical analyses were performed in duplicate. DDM (%), DMI (%), RFV(%), TDN (%), and NEl (Mcal lb^{-1}), NEm (Mcal lb^{-1}) and NEg (Mcal lb^{-1}) were calculated using equations adapted from commonly accepted forage evaluation formulas (Schroeder, 1994)

Statistical analyses: Statistical analyses were performed using the TarPopGen-Tarist software package (Anonymous, 2025) from the means of the two years. All data were subjected to analysis of variance (ANOVA), and treatment means were compared using Fisher's least significant difference test (LSD). The regression equations were calculated and the curves were selected using Yurtsever (1984). The curves were plotted in Microsoft Excel, P doses were used as the independent variable, and the relationships between the observed and measured characters were examined as the dependent variables.

Results and Discussion

Significant (p <0.01) differences in plant height, leaf length, leaflet length and width, number of leaves per plant, stem diameter, green fodder yield, DM yield, CP, CF, NDF, ADF, RFV, mineral contents (Ca, Mg, P, and K), tetany ratio, vitamin concentrations (β-carotene and α-tocopherol), DMI, TDN, and net energy values (NEl, NEm, and NEg) were observed in response to P application doses. The results are presented in Tables 1 to 3. Response of morpho-agronomic characteristics and yield performance, chemical composition, fiber fractions and mineral profile, forage quality indices, vitamin content and net energy values of shaftal to P fertilization were given in Figures 1 to 3.

Morpho-agronomic characteristics and yield performance: The plant heights were determined to be highest at 75 and 90 kg P ha^{-1} (114.54 and 112.35 cm). Leaf length ranged from 21.44 to 25.45 cm, with the lowest recorded at the 75 kg ha^{-1} application rate. Leaflet lengths were determined to be highest at 60, 90, and 0 kg P ha^{-1} (4.48, 4.45, and 4.41 cm) doses. Leaflet width decreased from 2.86 to 2.37 cm, and the highest values were determined at 90 kg P ha^{-1}, control, and 30 kg P ha^{-1} rates. The number of leaves per plant varied significantly across treatments, ranging from 9.23 to 10.19, with the highest count obtained at 45 and 30 kg P ha^{-1} doses. Stem diameter ranged between 6.89 mm and 7.93 mm, reaching its maximum at the 90 kg ha^{-1} P dose. The GFY increased from 56.49 t ha^{-1} to a peak of 83.65 t ha^{-1} and the highest yield was obtained at 60 kg P ha^{-1}. A parallel trend was observed for DMY, which varied from 7.30 to 10.72 t ha^{-1}, with the highest yield achieved at the 60 kg ha^{-1} P dose (Table 1; Fig 1).

P is a vital component of plant metabolic pathways, participating in energy transfer, photosynthesis, and nucleic acid synthesis, thereby playing a key role in regulating vegetative growth and biomass accumulation (Lambers and Plaxton, 2015). The results of the present study demonstrated that P fertilization significantly improved morphological characteristics and yield components of shaftal. This finding is consistent with numerous studies reporting that P is a critical nutrient that enhances plant growth and yield performance. According to Rasheed (2025), P application at increasing doses has a positive effect on berseem clover for PH, GFY, DMY and CP. Sakar and Açıkbaş (2025) determined increases in PH, GFY, and DMY of berseem clover (*T. alexandrinum* L.) at increasing P applications. The observed increase in plant height of shaftal with increasing P application aligns with previous research that showed P fertilization enhances plant stature by stimulating cell division and elongation mechanisms, thereby promoting stem and shoot growth (Ashraf *et al*., 2025). Kale and Takawale (2025) observed an increase in the PH of berseem clover at increasing P levels. Tekeli *et al*. (2003) reported that there are three known varieties of shaftal, one of which is *T. resupinatum* L. var. *majus* Boiss., which can grow to a height of 1.8 m. Similarly, studies on legumes and other crops have reported increased plant height, leaf number, and yield components under adequate P nutrition compared to unfertilized controls (Eken and Türk, 2021). The significant positive effect of P on leaf-related traits observed in this study may be linked to its influence on photosynthetic capacity and nutrient uptake. Adequate

Table 1. Morphological characteristics and yield as affected by P fertilization

Characteristics	P doses (kg ha^{-1})						Average	LSD (P=0.01)
	0	30	45	60	75	90		
Plant height (cm)	101.17c	111.20b	102.09c	108.91b	114.54a	112.35ab	*108.38*	4.450
Leaf length (cm)	23.97ab	21.72bc	23.18abc	23.82abc	21.44c	25.45a	*23.26*	2.430
Leaflet length (cm)	4.41ab	4.18bc	4.01c	4.48a	3.97c	4.45ab	*4.25*	0.291
Leaflet width (cm)	2.86a	2.74ab	2.56bc	2.37d	2.43cd	2.89a	*2.64*	0.193
Number of leaves per plant (pcs)	9.23c	9.94ab	10.19a	9.71b	9.79b	9.77b	*9.77*	0.348
Stem diameter (mm)	7.63b	6.89e	7.25cd	7.39bc	7.10de	7.93a	*7.37*	0.251
GFY (t ha^{-1})	61.40c	61.23c	56.49c	83.65a	76.92b	74.12b	*68.97*	6.398
DMY (t ha^{-1})	7.47d	7.46d	7.30d	10.72a	9.63b	9.33c	*8.65*	0.296

Letters that differ within row indicate values that are significantly different at P < 0.01. GFY: Green Fodder Yield, DMY: Dry Matter Yield

P nutrition has been shown to enhance photosynthetic efficiency by promoting P-based metabolic reactions and improving leaf area development, which increases the overall photosynthetic surface area available for carbon assimilation (Duan *et al*., 2022). Yield parameters, including green fodder yield and dry matter yield, were markedly improved by P fertilization, particularly at intermediate application rates. This trend is consistent with findings from forage research showing that P enhances forage yield by increasing shoot growth rate and mass per shoot, likely through improved nutrient flow from roots to above-ground biomass (Wang *et al*., 2024). However, similar to other studies, there appears to be an optimum threshold beyond which additional P does not result in further yield benefits, suggesting the importance of balanced nutrient management to maximize nutrient use efficiency and crop productivity (Duan *et al*., 2022). The observed morpho-agronomic traits and yield parameters are consistent with the findings of Tekeli and Ateş (2002), Tekeli and Ates (2003) and Tekeli *et al*. (2003) on shaftal. In addition, Erdemli *et al*. (2007) reported an average dry hay yield of 5.47 t ha^{-1} for shaftal, whereas Açıkgöz (2021) stated that this yield could reach as high as 10 to 12 t ha^{-1}. Zamanian *et al*. (2024a) reported 2.76 Mg ha^{-1} dry forage yield, 40.88cm PH, whereas Zamanian *et al*. (2024b) reported 4.43 t ha $^{-1}$ dry forage yield from the two-year means.

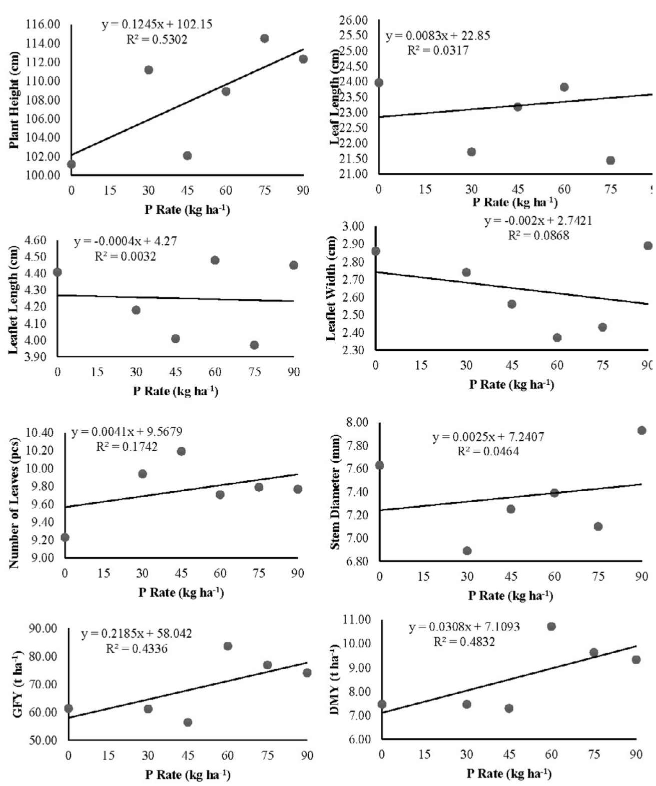

Fig 1. Responses of morphological characteristics to P fertilization

Chemical composition, fiber fractions and mineral profile: The CP contents ranged between 17.13 and 20.37%, the highest CP ratio was observed at 60 kg P ha^{-1}. The CF content declined significantly with increasing P doses, with the lowest values observed at moderate to high application doses (60, 75 and 90 kg ha^{-1}, 24.24, 24.23 and 24.63%, respectively). Similarly, NDF and ADF concentrations decreased significantly as P doses increased, with minimum values recorded at 60, 75 and 90 kg ha^{-1} P rates (NDF: 34.33, 34.33 and 34.363%, ADF: 21.94, 21.92 and 21.92% respectively). RFV values were the highest at 75 kg ha^{-1}, 60 kg ha^{-1} and 90 kg ha^{-1} P application rates (194.64, 194.55 and 194.64%, respectively). The highest Ca contents were determined at 75 and 90 kg ha^{-1} P rates (29.10 g kg^{-1}). The Mg contents were varied between 2.45 and 2.71 g kg^{-1} and the lowest values were determined at 0 kg ha^{-1} and 30 kg ha^{-1} (2.45 and 2.46 g kg^{-1}). The P content of shaftal increased with increasing P doses and reached its highest values (2.88 and 2.86 g kg^{-1}) at 75 and 60 kg P ha^{-1}. K contents were determined

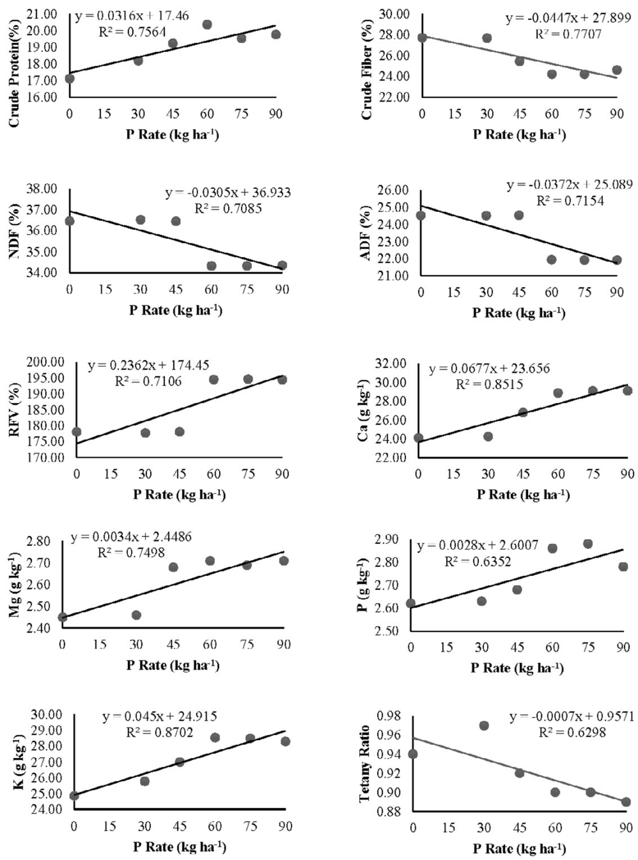

Fig 2. Responses of chemical composition, fiber fractions and mineral profile to P fertilization

to be highest in (28.30–28.54 g kg^{-1}) at 60, 75 and 90 kg ha^{-1} P rates. The P also modified the tetany ratio, which varied from 0.89 to 0.97 across treatments and the lowest ratios were determined at 60, 75 and 90 kg ha^{-1} P rates (Table 2, Fig 2).

The response of CP to P doses indicates that adequate P availability enhances N utilization and protein synthesis in forage crops. Comparable increases in CP content, along with improvements in TDN and RFV and concurrent reductions in ADF and NDF, have been reported in forage legumes subjected to P fertilization (Yıldız and Türk, 2015). Krolov *et al.* (2004) reported a positive correlation on CP, P and Mg content in shaftal and subterraneum clover (*T. subterraneum* L.) with increased P application. Zamanian *et al.* (2024a) determined the following values under the effect of 150 kg ha^{-1} P application: CP 17.66%, NDF 41.85%, ADF 32.44 and RFV 141.82%. Zamanian *et al.* (2024b) also reported the following values for the shaftal genotypes over two years: CP 16.70%, ADF 34.40%, NDF 43.45%, RFV 133.61%, P 0.26 and K 1.32%. The decline in CF content at increasing P doses is consistent with findings reported for pea (*Pisum sativum* L.), where P fertilization significantly reduced ADF and NDF concentrations while increasing DMY and RFV (Yuksel and Türk, 2019; Tenikecier and Ates, 2021). These results are consistent with previous findings, emphasizing the role of P in reducing cell wall components that hinder feed digestibility. Reductions in these structural carbohydrate fractions are closely associated with improved forage quality, as lower NDF and ADF contents enhance rumen fermentation efficiency and voluntary feed intake in ruminants by reducing cell wall constraints on digestibility (Van Soest, 1994; NRC, 2001). Accordingly, RFV values increased markedly from approximately 178.09 in the control treatment to over 194.46-194.64 at P application doses of 60 to 90 kg ha^{-1}, reflecting the combined effects of improved digestibility and intake potential. Overall, these findings demonstrate that P fertilization substantially enhances forage feeding value, particularly at intermediate application doses. Maintaining mineral balance is crucial for animal

Table 2. Chemical composition, fiber fractions and mineral profile of forage as affected by P fertilization

Characteristics	P doses (kg ha^{-1})						Average	LSD (P=0.01)
	0	30	45	60	75	90		
CP (%)	17.13f	18.20e	19.24d	20.37a	19.54c	19.76b	*19.04*	0.097
CF (%)	27.74a	27.69ab	25.45abc	24.24c	24.23c	24.63bc	*25.66*	3.072
NDF (%)	36.46a	36.53a	36.45a	34.33b	34.33b	34.36b	*35.41*	0.082
ADF (%)	24.53a	24.52a	24.54a	21.94b	21.92b	21.92b	*23.53*	0.058
RFV (%)	178.09b	177.74b	178.09b	194.55a	194.64a	194.46a	*186.26*	0.421
Ca (g kg^{-1})	24.12e	24.25d	26.79c	28.88b	29.10a	29.10a	*27.04*	0.058
Mg (g kg^{-1})	2.45b	2.46b	2.68a	2.71a	2.69a	2.71a	*2.62*	0.058
P (g kg^{-1})	2.62d	2.63cd	2.68c	2.86a	2.88a	2.78b	*2.74*	0.058
K (g kg^{-1})	24.86d	25.78c	27.01b	28.54a	28.50a	28.30a	*27.16*	0.443
Tetany Ratio (K/Ca+Mg)	0.94b	0.97a	0.92c	0.90d	0.90d	0.89d	*0.92*	0.012

Letters that differ within row indicate values that are significantly different at P < 0.01. CP: Crude protein, CF: Crude fiber, NDF: Neutral detergent fiber ADF: Acid detergent fiber, RFV: Relative feed value, Ca: Calcium, Mg: Magnesium, P: Phosphorus, K: Potassium

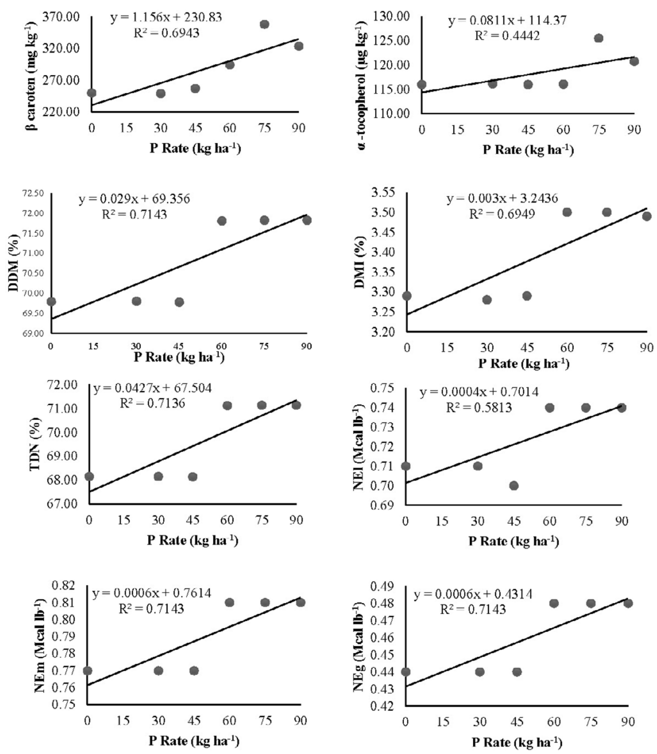

Fig 3. Responses of forage quality indices, vitamin content and energy values to P fertilization

health. The presence of others cannot compensate for a deficiency in one mineral, and these elements need to be present in specific proportions (Tenikecier, 2021). The skeleton contains approximately 68 to 73% of the total Mg in an animal's body. The P levels in the rumen are also significant, as higher P concentrations can enhance Mg absorption. In P-deficient pastures, rumen P levels may be low, potentially worsening Mg absorption. Additionally, blood Ca levels influence the onset of grass tetany. When blood Ca drops, Mg levels in the cerebrospinal fluid decrease more rapidly if blood Mg is already low due to insufficient absorption (Ates, 2017). The elements K, Ca, P, Mg, sulfur (S), sodium (Na), and chlorine (Cl) are present in the animal body at concentrations equal to or greater than 400 mg kg^{-1} of body weight, thus they are referred to as macro-minerals (Wu, 2018). Similar alterations in macro element composition following P fertilization have been reported in pasture-based systems, although the extent of these changes has been shown to depend on species-specific responses and soil nutrient status (Çaçan and Kökten, 2023). The observed decline in tetany ratio at higher P doses suggests a shift toward a more favorable balance between K and divalent cations, particularly Ca and Mg. Such changes in mineral balance are considered important for maintaining metabolic stability and reducing the risk of grass tetany in grazing ruminants (Van Soest, 1994; NRC, 2001). Taken together, the present findings indicate that P fertilization improves forage nutritive value not only through enhanced protein and mineral concentrations but also via reductions in structural fiber components that constrain digestibility. The most pronounced improvements were generally observed at intermediate P application doses (approximately 60 kg ha^{-1}), underscoring the importance of optimizing P inputs to achieve high-quality forage production while minimizing the risk of nutrient imbalance. Similar to shaftal in this research, tetany ratio of berseem (Sakar and Açıkbaş, 2025) and hairy vetch (*Vicia villosa* Roth.) (Türk *et al.*, 2009) decreased at increasing P doses.

Forage quality indices, vitamin content and net energy values: The concentration of β-carotene increased markedly with increasing P doses, reaching a maximum level of 357.91 mg kg^{-1} at a P dose of 75 kg ha^{-1}. A similar trend was observed for α-tocopherol, which also peaked at 125.49 mg kg^{-1} at the same P dose. DDM ratios were determined to be highest at 75, 90 and 60 kg ha^{-1} P rates (71.82, 71.82 and 71.81%). DMI contents were found to be highest at 60, 75 and 90 kg ha^{-1} P rates (3.50, 3.50 and 3.49%). TDN contents were obtained highest at 75, 90 and 60 kg ha^{-1} P rates (71.14, 71.14 and 71.12%). NEl (0.74 Mcal lb^{-1}), NEm (0.81 Mcal lb^{-1}) and NEg (0.48 Mcal lb^{-1}) of the shaftal were determined to be highest at 60, 75 and 90 kg ha^{-1} P application rates (Table 3, Figure 3).

Applying P increased the accumulation of antioxidant vitamins in forage tissues. The enhanced accumulation of these antioxidant compounds under P fertilization is likely to be associated with improved photosynthetic activity and greater development of metabolically active leaf tissues. Both β-carotene and α-tocopherol play essential roles in chloroplast membranes, contributing to photoprotection and the mitigation of oxidative stress generated during photosynthesis (Havaux, 1998; Munné-Bosch, 2005). Plants generate the antioxidants α-tocopherol and β-carotene in their fresh foliage. Their main role is to protect plant and animal cells from oxidation and the harmful by-products of the oxidation process (Muhonen, 2018). The vitamin content of forage crops is influenced by many factors, including growth stage and leaf/stem ratio, as well as climatic and edaphic factors such as geographic location, topographic properties, seasonal and yearly variation, illuminance-associated diurnal variation and soil traits. Other factors include biotic damage, the conservation methods used for herbage/hay (e.g., dehydration, ensiling and drying) and storage conditions (Ateş and Tenikecier, 2024; Ateş, 2025). Danielsson *et al.* (2008) observed that the vitamin content of autumn-harvested herbage was higher than that of herbage harvested at other times of the year. This suggests that climate may influence the vitamin content of harvested herbage. However, the findings presented here differ from those previously reported. The improvements of moderate to high P application doses

Table 3. Effects of P doses on forage quality indices, vitamin content and energy values

Characteristics	P doses (kg ha^{-1})						Average	LSD (P=0.01)
	0	30	45	60	75	90		
β caroten (mg kg^{-1})	249.93e	249.02e	257.23d	294.07c	357.91a	323.59b	*288.63*	1.222
α-tocopherol (mg kg^{-1})	116.00c	116.16c	116.01c	116.09c	125.49a	120.77b	*118.42*	0.276
DDM (%)	69.79b	69.80b	69.78b	71.81a	71.82a	71.82a	*70.80*	0.045
DMI (%)	3.29b	3.28b	3.29b	3.50a	3.50a	3.49a	*3.39*	0.030
TDN (%)	68.15b	68.15b	68.13b	71.12a	71.14a	71.14a	*69.64*	0.067
NEl (Mcal lb^{-1})	0.71ab	0.71ab	0.70b	0.74a	0.74a	0.74a	*0.72*	0.036
NEm (Mcal lb^{-1})	0.77b	0.77b	0.77b	0.81a	0.81a	0.81a	*0.79*	0.036
NEg (Mcal lb^{-1})	0.44b	0.44b	0.44b	0.48a	0.48a	0.48a	*0.46*	0.035

Letters that differ within row indicate values that are significantly different at $P < 0.01$. DDM: Digestible dry matter, DMI: Dry matter intake, TDN: Total digestible nutrients, NEl: net energy for lactation, NEm: Net energy for maintenance, NEg: and net energy for gain

on DDM, DMI, and TDN are closely associated with the concurrent reduction in structural fiber fractions, which enhance rumen degradation, voluntary intake, and overall nutrient utilization in ruminants (Van Soest, 1994; NRC, 2001). The parallel increase in TDN further reflects an improvement in forage energy concentration as P availability increased. Net energy values, including NEl, NEm, and NEg, were also significantly affected by P fertilization. All net energy indices increased with increasing P application, with the highest values generally observed at 60 to 90 kg P ha^{-1}. These responses are consistent with improvements in digestibility and energy-related quality indices, since net energy values are derived directly from forage digestibility and total digestible nutrients (TDN) content (Weiss *et al*., 1992; NRC, 2001). The DM is an indicator of the amount of nutrients that are available to the animal in a particular feed (Ates and Tenikecier, 2022b). The daily consumption of digestible DM is more closely associated with DM intake than with its digestibility (Tenikecier and Ates, 2018). The results of the research were higher than those of Zamanian *et al.* (2024a) and Zamanian *et al.* (2024b), who declared TDN 59.47 and 56,94%, DMI 2.87 and 2,77%, DDM 62.10%, NEl 1.45 and 1.39% in shaftal, respectively.

Conclusion

P fertilization significantly improved growth, yield, and forage quality of shaftal. Increasing P application enhanced biomass production, CP content, digestibility indices, mineral composition, and net energy values, while reducing structural fiber fractions. Based on the conditions of this study, a P application dose of 60 kg P ha^{-1} appeared to provide the most consistent improvements in yield and forage nutritive value. Higher P rates did not result in proportional gains, indicating diminishing returns beyond this level. Accordingly, this rate may be considered a suitable P recommendation for shaftal production under comparable soil and climatic conditions, although further multi-location and multi-season studies are warranted to validate its broader applicability.

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