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Aims and Scope

Turkish Journal of Range and Forage Science is the official publication of Society of Rangeland and Forage Science. The Journal is dedicated to publishing quality original material that advances rangeland management and forage crops production.

Turkish Journal of Range and Forage Science is a peer-reviewed, international, electronic journal covering all aspects of range, forage crops and turfgrass management, including the ecophysiology and biogeochemistry of rangelands and pastures, terrestrial plant–herbivore interactions, rangeland assessment and monitoring, effects of climate change on rangelands and forage crops, rangeland rehabilitation, rangeland improvement strategies, conservation and biodiversity goals. The journal serves the professions related to the management of crops, forages and grazinglands, and turfgrass by publishing research, briefs, reviews, perspectives, and diagnostic and management guides that are beneficial to researchers, practitioners, educators, and industry representatives.

Publication Date and Subscription Information

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TURKISH JOURNAL OF RANGE AND FORAGE SCIENCE

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After favorable opinions of reviewers, Editorial Board is made the final evaluation. The articles accepted for publication by Editorial Board are placed in an issue sequence.

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The peer review process that has long time is an important problem. Naturally, the author(s) wish to take an answer about their submissions. Turkish Journal of Range and Forage Science aims to complete the all peer review process within 8 weeks after submission (one week for initial evaluation, 6 weeks for reviewer evaluation and one week for final evaluation).

The author(s) that submit an article to the Turkish Journal of Range and Forage Science consider accepting of these peer review conditions and procedures.

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Research

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A Preliminary Study of USDA 110 (*Bradyrhizobium diazoefficiens*) Strain Nodulation Performance and Soybean Growth Under Water Scarcity Conditions

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A R T I C L E   I N F O

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A B S T R A C T

Nitrogen fixation is one of the key benefits of the economic and environmentally sustainable approach that legumes contribute to crop production. With the fruitful cooperation of legume-rhizobia symbiosis, soybean cultivation contributes to this sustainability while drought threatens this sustainable agricultural system. Thus, this study aimed to verify the influence of water deficit on the soybean nodulating performance concerning different inoculants, crop growth and quality. A field experiment was conducted to determine the effects of irrigated and water scarcity conditions (full: WHC 100% and deficit: WHC 50%) on soybean yield and quality and also to test the nodulation performance of two different inoculants USDA 110 (*Bradyrhizobium diazoefficiens*) and Azotek (*Rhizobium* spp.) applied to 3 different soybean cultivars (Umut-2002, Cinsoy and Altınay). For this purpose, plant height (cm), first pod height (cm), number of pods per plant, 1000 seed weight (g), seed yield (kg ha^{-1}), SPAD chlorophyll content, leaf area (cm^2), crude protein and oil content (%) traits were measured. According to the field and root observations, no nodulation history was observed in both Rhizobia strains under irrigated and water scarcity conditions. Water limitation resulted with the negative impact on soybean yield (≈35% less) and yield formation. In addition to yield reduction, water scarcity caused a significant decrease in SPAD chlorophyll content in the reproductive stages and leaf area of the plant. As a result of this preliminary study, water scarcity has irreversible effects on soybean plant physiology and yield formation in the hot climate conditions of Aydın province. Further field studies are needed to observe the nodulation performance of soybean plants in the region which has not been observed in the field studies so far.

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the prominent crops that ensures an important source of protein for nourishment and livestock. It enriches the chemical and biological structure of the soil through its deep root system and fixes atmospheric N by biological nitrogen fixation. It is widely used as an industrial raw material around the world due to its wide adaptation and high nutrition values (Yüzbaşı, 2021). Soybean exemplifies the most significant and cultivated food legume in crop rotation. It comes to the forefront of fixing atmospheric nitrogen into the

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soil by *Bradyrhizobium japonicum* strain leads to effective and higher usage of nitrogen in sustainable agriculture with minimal input requirements (Islam et al. 2022). Also, it has the potential as a forage crop and there is an increasing trend in adopting soybean silage for animal feeding in the last years (Sürmen and Kara, 2017).

Climate change affects crop productivity through extreme weather conditions (etc. high temperature, drought and irregular rainfall). Climate change will affect crop development timing, and the exact changes will depend on variations in agronomic properties that cause yield and quality reductions in soybean response to extreme weather conditions. According to the future precipitation climate projection based on the HadGEM2-ES (Global Circulation Model) during the period (2016-2040) there would be an increase of about 10-40% in precipitation during the winter period in the coastal regions of Aegean, Central Black Sea, and East Anatolia regions it is expected to decrease about 20% in the spring precipitation amount in a large part of Türkiye. In terms of precipitation, it is predicted that the amount of precipitation tends to increase in the winter season in both RCP4.5 and RCP8.5 scenarios, will not be in the form of heavy snow and precipitation, and thus will not contribute to the water budget of the summer season (Demircan et al. 2017).

A challenging area in the field of soybean crop development is that it is produced in many arid, semi-arid and sub-humid regions where water resources are limited, and spring precipitation has preliminary importance for water storage of the soil. Although soybean production areas have increased, restricted agro-climatic conditions are not ideal for widespread soybean cultivation, especially in Europe. Long periods of high temperatures and extremely heat in semi-arid regions reveal the scarcity of water that is more common in Mediterranean climate conditions. (Koca et al. 2015). Soybean can cope with drought conditions that occur in the early vegetative stages without considerable yield reduction, but irrigation is vital for soybean plants in the reproductive stages (beginning of flowering to pod development) and until maturity. These reproductive stages are mainly observed during the summer period when stress conditions (high temperature and water deficiency) have significant reverse impacts on the crop development of soybean (Matoša Kočar et al. 2023).

Stress factors not only reduce agricultural productivity but also limit and prevent land use for agricultural purposes. Abiotic stress conditions such as light, temperature, water (drought) salt, and heavy metal stress cause physiological and metabolic changes in soybean, negatively affecting plant growth and development as well as result in quality reductions (Korkmaz & Durmaz, 2017). Drought stress can be very effective, especially during flowering, pod formation, and seed-filling periods. The stress experienced during the grain filling period causes a decrease in grain size and low yield. Decreases in grain size are attributed to shorter seed-filling periods (Brevedan and Egli, 2003). It has been stated that high temperature and drought stress also significantly reduce plant growth and development and thus grain yield, particularly the number of pods in the plant, which is one of the main yield components (Hu & Wiatrale, 2012). Water scarcity particularly occurs during the generative phases (the flowering and pod development stages) and is the most significant stress factor affecting seed yield because it reduces the flowering rate and, as a result, formation of the number of pods in the plant (He et al. 2017). Water stress adversely reduces the synthesis and breakdown of metabolites that contribute to yield energy and inhibits the function of the structure serving as primary support for photosynthetic metabolism. Drought causes a reduction in photosynthesis by restricting stomatal operations. The plants exposed to water stress have also significantly lower leaf areas compared to non-stressed plants (Mangena, 2018). Drought stress reduced chlorophyll content (SPAD) and relative water content in soybean at each growth stage. As the duration of stress increases, a dramatic decrease in these parameters is observed. Severe stress duration (10 days) caused a decrease in SPAD chlorophyll content (from 46.20 to 36.22) compared to adequate water supply conditions and the lowest SPAD values observed both seedling and seed-filling severe drought conditions (Dong et al. 2019).

Symbiotic nitrogen fixation has an indispensable property for the global nitrogen cycle and agricultural practices. To understand the mechanisms of symbiosis on plant physiology, ecology, and genetics of rhizobia have been studied to achieve better knowledge about rhizobia. In this context *Bradyrhizobium diazoefficiens* strain USDA 110 was originally isolated from a soybean nodule in Florida, USA in 1957 has been widely used for molecular genetics, plant physiology and ecology (Kaneko, 2002). The rhizobia inoculation of soybean is a sustainable practice to promote nitrogen fixation and subsequently improve crop productivity and soil fertility. Different environmental factors such as temperature, pH, salinity, genotype, and soil nitrogen content affect the legume, rhizobia symbiosis and nodulation performance of soybean (Yuan et al. 2020). Commercial inoculants of *Bradyrhizobium japonicum* strain are used in Türkiye however even after no legume history and only a small part of nodules was observed in Aydın province according to the conducted previous field experiments. This situation may be linked to heat and drought stress conditions occur during soybean growth period in the area has typical Mediterranean climate conditions. We aimed to observe the performance of USDA 110 strain in deficit and irrigated conditions representative of these climatic conditions. Within the scope of the present study, it is aimed to investigate soybean growth (yield, chlorophyll and quality) and nodulation performance under water scarcity conditions in hot climate conditions.

2. Material and Method

The study was conducted in Aydın/Türkiye ecological conditions located about 33 m (37°45´22´´N 27°45´36´´E) above sea level with Mediterranean climate conditions during the 2019 growing season. The climate is described as temperate, dry and hot summer (Csa) according to Köppen-Geiger climate classification. Three soybean cultivars (Cinsoy, Altınay, Umut-2002) described growth habit as middle-early maturing cultivars were used as genetic material obtained from Aegean Agricultural Research Institute, İzmir. As follows soybean nodulating Rhizobia applications: the commercial ~~soybean~~ inoculant (*Rhizobium spp.*-Azotek) obtained from Soil, Fertilizer, Water Resources Central Research Institute, Ankara and USDA 110 containing *Bradyrhizobium diazoefficiens* obtained from Germany were used for inoculation of soybean seeds. Full (Water Holding Capacity: 100%: FI) and deficit (Water Holding Capacity 50%: WS) irrigation doses were calculated based on cumulative evaporation amount from the class A evaporation container (US Weather Bureau Class A Pan) by different coefficients (Kanber, 1984) and drip irrigation was applied every week according to the equation given below;

I=Kpc.Ep.P.A [I: amount of irrigation to be applied to the plot, Kpc: evaporation container coefficient 100%, Ep: cumulative evaporation amount (mm), P: plant cover (%), A: Plot area (m^2)]

Irrigation was applied when the first open flower (flowering: BBCH 51) is observed on the main stem regardless of where the flower is located (Munger et al. 1997). A total of 242 mm of irrigation water was applied to the 50% irrigation dose during the growth and development period, while a total of 485 mm was given to the 100% full irrigated plots (Figure 1). The experimental layout was set up as a split-split plot design with three replications. There was 5.0 m separation between each plot to ensure minimal water movement among treatments. Each experiment plot was 5m x 2.8 m (4 rows per plot) and had a total area of 14 m^2 at sowing. The soil of the trial area categorizes as sandy loam texture with a slightly alkaline reaction. The organic matter content was low (1.7%) while phosphorus (10.7 ppm), potassium (305 ppm), calcium (1745 ppm) magnesium were high level in the experimental soil.

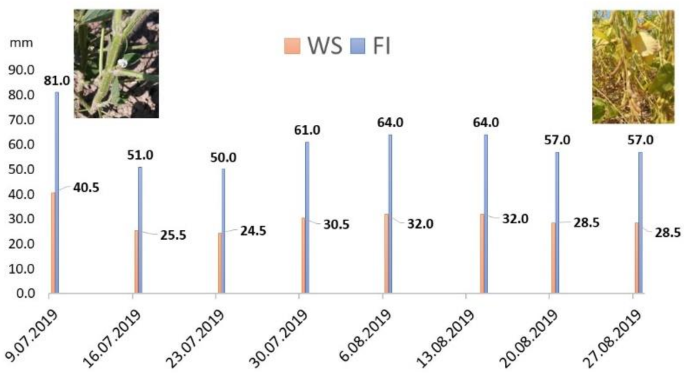

Figure 1. Irrigation amounts based on evaporation applied to plots (WS: water scarcity 50% and FI: Full irrigation 100%)

Considering the long-term climate values, mean temperature values were favorable (except in June) during soybean growth periods. There was sufficient precipitation in April before the 24th (sowing date), when the experiment was established but the precipitation amount decreased in May causing an adverse effect the on vegetative growth of soybean. The amount of precipitation required during the vegetative growth period of soybean plants was observed to shift towards July and a high amount of rainfall was observed about 97.7 mm in one month. Rainfall was almost non-existent in July and August when irrigation started due to water scarcity and full irrigation conditions, so soybean plants responded optimally to irrigation during the generative growth periods (Figure 2). Before sowing, seeds were inoculated with Rhizobium bacteria inoculants. Weed control was made by hand as a mechanical control method. Disease and pest control was performed at the required locations in addition Twospotted spider mites (*Tetranychus urticae* Koch) were controlled by Oberon Bayer® spiromesifen 240 SC (22.9%, w/w) insecticide application.

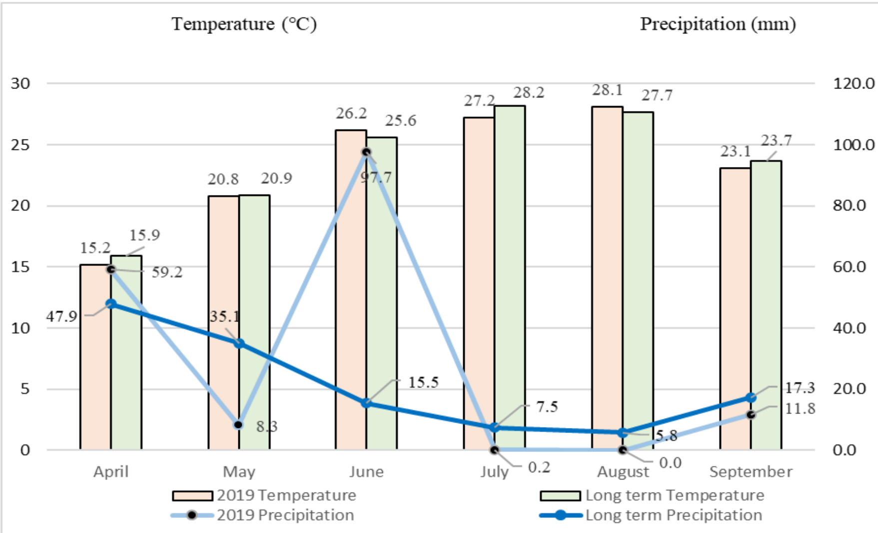

Figure 2. Monthly and long-term (1985-2019) temperature and precipitation values (Turkish State Meteorological Service, Station: Koçarlı)

For this study, agronomical, physiological and quality traits such as plant height (cm), first pod height (cm), number of pods per plant, 1000 seed weight (g), seed yield (kg ha^{-1}), SPAD chlorophyll content, leaf area (cm^2), crude protein and oil content (%) were investigated. Nodulation performance was observed from carefully dug out roots at randomly selected plants from each plot. Soil Plant Analysis Development (SPAD) chlorophyll content was measured at the last fully developed leaf in three generative growing periods; $SPAD_{FL}$: the beginning of flowering, about 10% flowers open (BBCH 61), $SPAD_{PD}$: about 50% of pods have reached final length (BBCH 75), $SPAD_{RS}$: ripening of seeds, advanced ripening (BBCH 85) using chlorophyll meter (SPAD-502 Konica Minolta, Japan). The leaf area measurement was performed by using LICOR (Lincoln, NE, USA) LI-3000C portable leaf area meter when the vegetative growth was accomplished (BBCH 49) (Figure 3). The Near Infrared Reflected Spectroscopy (NIRS) method was used to analyze crude oil and protein content of soybean seed flour using Bruker MPA^{TM} (Bruker, Ettlingen, Germany).

Figure 3. Location and general view of field experiment (© Google Earth Pro)

The analysis of variance (ANOVA) was conducted to compare the means and the LSD multiple comparison method was conducted to indicate statistically different means using TARİST software. Boxplots was created in MS Excel with the calculation of standard deviation values.

3. Results and Discussion

According to our results, irrigation caused statistically significant changes ($p \leq 0.01$) in the evaluated parameters such as plant height, first pod height, number of pods per plant, 1000 seed weight, seed yield, SPAD chlorophyll content in generative periods, and leaf area except for quality parameters (protein and oil content).

Rhizobia strains applied in the study caused no significant changes in almost all parameters and this situation can be explained because no nodule (no nodules detected in roots) history was observed in the field experiment by dug out roots.

According to the variance analysis results, the cultivar caused significant changes in all evaluated parameters except for crude oil content. The interaction between bacteria, cultivar, and irrigation (B*C*IR) showed significant differences just for plant height ($p \leq 0.05$), number of pods per plant ($p \leq 0.05$), and $SPAD_{PD}$ ($p \leq 0.05$) parameters (Table 1 and 2). Seed quality parameters did not show a clear response as there were significant differences between applications. For the mean square values of crude protein content evaluated in relation to bacteria, cultivar, and irrigation applications, only cultivar caused a statistically significant difference ($p \leq 0.05$) while no statistically differences were observed in crude oil content (Table 2).

Table 1. Analysis of variance (mean square values) for plant height, first pod height, number of pods, 1000-seed weight, and seed yield parameters.

Source	Df	Plant Height (cm)	First Pod Height (cm)	Number of pods (pods plant^{-1})	1000-seed weight (g)	Seed Yield (kg ha^{-1})
Bacteria (B)	1	476.69 ns	31.17 ns	30.61 ns	976.66*	2589.7 ns
Error-1	2	35.80	6.57	6.67	51.26	167.2
Cultivar (C)	2	1237.75**	162.04**	80.75**	414.37*	2933.9*
B*C	2	242.19 ns	25.00 ns	134.01**	307.22 ns	2333.2*
Error-2	8	57.07	17.64	6.52	74.21	428.7
Irrigation (IR)	1	5715.36**	1016.54**	529.00**	2018.55**	16571.8**
B*IR	1	5872.66**	24.83 ns	74.53**	352.37*	1587.3*
C*IR	2	268.03**	21.34 ns	102.52**	34.59 ns	917.9 ns
B*C*IR	2	120.96*	12.59 ns	17.51*	78.05 ns	437.0 ns
Combined Error	12	29.98	22.09	3.13	38.40	269.7
Corrected Total	35	477.66	55.56	40.63	193.95	1181.1
ns: non-significant; *: significant at 0.05 level; **: significant at 0.01 level						

Table 2. Analysis of variance (mean square values) for chlorophyll content (SPAD), leaf area and quality parameters.

Source	Df	$SPAD_{FL}$	$SPAD_{PD}$	$SPAD_{RS}$	Leaf Area (cm^2 $plant^{-1}$)	Crude Protein (%)	Crude Oil (%)
Bacteria (B)	1	36.44*	50.17 ns	163.62 ns	49443.36*	0.63 ns	0.15 ns
Error-1	2	0.53	5.41	14.09	10532.71	0.77	0.08
Cultivar (C)	2	26.29**	31.63*	14.08**	222220.03*	27.71*	1.11 ns
B*C	2	31.58**	0.61 ns	25.75**	18234.90 ns	1.92 ns	0.13 ns
Error-2	8	1.81	4.97	1.44	29772.04	4.14	0.64
Irrigation (IR)	1	29.52**	93.83**	470.38**	2872347.04**	5.14 ns	1.42 ns
B*IR	1	26.01**	13.49*	14.50 ns	318336.68*	5.05 ns	0.01 ns
C*IR	2	7.58 ns	55.49**	24.83**	796725.53 ns	4.56 ns	0.09 ns
B*C*IR	2	2.77 ns	10.53*	2.92 ns	28742.69 ns	0.55 ns	0.20 ns
Combined Error	12	1.46	2.31	3.54	53172.37	3.29	0.62
Corrected Total	35	7.81	12.53	24.81	151052.26	4.69	0.53
ns: non-significant; *: significant at 0.05 level; **: significant at 0.01 level							

3.1. Plant height (cm)

Plant height is morphological characteristic reflects growth and development of crops and observed easily in field conditions; studying these plant characteristics help researchers to observe drought stress effectively (Dong et al. 2019). While irrigation and cultivar caused significant changes in plant height, *Rhizobia* strain applications showed non-significant changes (Table 1). According to the obtained mean values, the lowest plant height was measured in the application of USDA 110 at water scarcity conditions in Cinsoy cultivar, and the highest plant height value was obtained from the Umut-2002 cultivar with the application of USDA 110 and fully irrigated condition. Regarding cultivars, Umut-2002 had the highest plant height (88.5 cm) and Cinsoy had the lowest value (68.4 cm). Soybean plant height was interrupted by water scarcity stress and reduced plant height by approx. 27% compared to full irrigation (Table 3).

The plant height values have been found to be typical of Gaweda (2017) reported that they obtained plant height values between 73.70 cm and 135.40 cm, İlker et al. (2010) stated that soybean plant height values varied between 63.1 cm and 125.4 cm in the Mediterranean climate and Kars and Ekberli (2021) found the results varied between 88.33 cm and 127.77 cm in their studies. Drought stress caused by the lack of irrigation water for a long period leads to a reduced water supply to the upper soil layer consequently reducing the water use efficiency resulting in crops coupled with an excessive evaporation demand furthermore, drought stress can damage photosynthetic organs and reduce soybean seed germination rate, plant height, pod number and therefore yield (Wang et al. 2022).

Table 3. Average plant height (cm) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	62.5 fg	80.4 de	124.5 a	86.5 cd	88.5 A
Cinsoy	53.4 h	57.2 gh	98.0 b	65.1 f	68.4 C
Altınay	59.9 fgh	90.0 c	102.5 b	74.9 e	81.1 B
Mean Irrigation	66.7 B		91.9 A		
Mean USDA 110	82.9				
Mean Azotek	74.7				
Lsd C:7.1; Lsd IR: 3.9; Lsd B*IR: 5.6; Lsd C*IR: 6.8; Lsd B*C*IR: 9.7					

3.2. First pod height (cm)

The height of the first pod is positively related to plant height, but negatively related to seed weight, number of seeds per pod and number of pods per plant (Oz et al., 2009). According to Ramteke et al. (2012) stated in their study that the height of the first pod is positively related to plant height, number of nodes and stem diameter. However, it has been reported that high first pod height values may cause lower values in the number of pods per plant, and because the plant height is directly proportional to the first pod height, it may be negatively related to seed yield (Ghodrati, 2013). The first pod height is a genetic feature that minimizes harvest losses and the highest harvest-effective cultivars should have the traits that attach the first pod to the soil surface from a higher level (İlker et al. 2010). Irrigation and cultivar have been found statistically significant (*p*≤0.01) for the first pod height while bacteria applications caused non-significant differences (Table 1). As expected the results show that fully irrigated condition triggered higher plant height and higher first pod height values (21.5 cm) while water scarcity condition had the lowest first pod height value (20.9 cm).

Both Umut-2002 and Altınay cultivars had the highest first pod height values (28.5 and 28.1 cm) and Cinsoy had the lowest (22.0 cm) first pod height values as shown in Table 4.

On the other hand, there were no significant differences in the first pod height between *Rhizobia* applications and USDA 110 resulted in 27.1 cm while Azotek resulted in 25.3 cm values. Overall, applications (B*C*IR) have not been found statistically significant for the first pod height. The first pod height values ranged from 17.3 cm (Water scarcity, Cinsoy, Azotek) to 37.1 cm (Full irrigated, Altınay, USDA 110).

Table 4. Average first pod height (cm) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	21.1	26.1	34.8	32.1	28.5 A
Cinsoy	18.2	17.3	27.9	24.4	22.0 B
Altınay	23.7	18.9	37.1	32.7	28.1 A
Mean Irrigation	20.9 B		21.5 A		
Mean USDA 110	27.1				
Mean Azotek	25.3				
Lsd C:3.9; Lsd IR: 3.4					

In the study, the obtained values correlate favorably with the previous studies but higher first pod height values were obtained than the previous studies. It was stated that the first pod height values varied between 12.4 cm and 22.1 cm and yield characteristics were investigated under main crop conditions (Yetkin & Arıoğlu, 2010). Tayyar and Gül (2007) also reported in their study that the first pod height values of soybean varieties ranged from 13.1 cm to 20.6 cm.

3.3.Number of pods (pods plant^{-1})

Stress conditions at reproductive phases have irrevocable effects and hence result in severe loss of soybean productivity. The occurrence of drought and high-temperature conditions are considered to be major limiting environmental factors that affect pollen viability and increase flower abortion resulting in less productivity of soybean (Onat et al. 2017; Jumrani and Bhatia, 2018). The number of pods per plant was significantly affected by water scarcity (*p*≤0.01), cultivar (*p*≤0.01) and interaction (B*C*IR) (*p*≤0.05) imposed at the reproductive stages of soybean while bacteria applications had no significant effects on pod number (Table 1).

The number of pods per plant decreased as water scarcity conditions occurred (20.5 pods plant^{-1}) and as expected in irrigated condition pod number enhanced approx.. 27.0% compared to deficit irrigation with the value of 28.1 (pods plant^{-1}). Decreases in pod number appeared to be slightly higher under water deficit condition and the lowest pod number (17.2 and 16.4 pods plant^{-1}) was obtained from 50% WS, Altınay, USDA 110 and 50% WS, Umut-2002, Azotek applications, respectively. Full irrigated condition attributed to getting higher pod numbers almost for all cultivars except Cinsoy (21.0 and 22.7 pods plant^{-1}) plus 100% FI, Umut-2002 and USDA 110 application had the highest pod number value per plant. The statistical differences were observed between cultivars on the number of pods per plant. While the highest average pod number was obtained from both Umut-2002 (26.0) and Altınay (26.6) cultivars per plant, Cinsoy cultivar had the lowest pod number value (21.3) per plant (Table 5).

Rhizobia applications did not differ significantly, and mean values ranged from 23.4 to 25.8 pods plant^{-1}. Overall, water stress condition occurs in the beginning of flowering resulted with lower pod number per plant and well-watered conditions caused a significant increase in the pod number, which greatly contributes to yield. The number of pods and grain size are decreased because of the stress encountered following the onset of flowering and throughout the whole flowering period. When water stress occurs, grain yield and components suffer greatly (Korte et al., 1983). These values have been found to be typical of Boydak et al. (2018) and Yamika and Ikawoti (2012) with the values 19.46-35.80 and 42.90, respectively. Shamima and Farid (2005) investigated the effects of irrigation practices on yield parameters in soybean and reported that the number of pods varied between 35.4 and 46.9 per plant, and these values were found to be consistent with the pod number values we obtained in our study.

Table 5. Average number of pods (pods plant^{-1}) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	23.4 def	16.4 h	38.2 a	26.1 cd	26.0 A
Cinsoy	19.3 gh	22.3 efg	21.0 fg	22.7 ef	21.3 B
Altınay	17.2 h	24.3 de	32.4 b	28.5 c	25.6 A
Mean Irrigation	20.5 B		28.1 A		
Mean USDA 110	25.8				
Mean Azotek	23.4				
Lsd C:2.4; Lsd B*C: 3.4; Lsd IR: 1.2; Lsd B*IR: 1.8; Lsd C*IR: 2.2; Lsd B*C*IR: 3.1					

3.4.1000 seed weight (g)

Drought stress causes irreversible effects especially during flowering, seed formation and seed filling periods. The stress experienced during the seed-filling period causes a decrease in grain size and yield (Desclaux et al., 2000). Decreases in seed size are attributed to shorter seed-filling periods and earlier onset of ripening.

The ANOVA results of thousand seed weights of soybean varieties with different irrigation doses and *Rhizobia* treatments are presented in Table 3. According to the results of this analysis of variance, irrigation treatments were found to be statistically significant at 0.01 level, and bacteria*cultivar and bacteria*water dose interactions were found to be statistically significant at 0.05 level. There was no statistically significant effect of cultivar and *Rhizobia* treatments on kernel weight of soybean (Table 1).

The mean values of thousand-grain weight of soybean varieties in the experiment are given in Table 10. According to this average table, thousand-grain weight values varied between 103.75 g and 147.00 g in soybean varieties. In the experiment, the lowest thousand-grain weight was observed in USDA 110 bacteria application in Cinsoy cultivar at 50% irrigation dose, while the highest thousand-grain weight value was observed in USDA 110 bacteria application in Umut cultivar at 100% irrigation dose. Significant effects of irrigation doses on thousand-grain weight were determined.

Depending on the irrigation doses, thousand-grain weight averages were obtained at 50% irrigation dose with 113.86 g and at 100% irrigation dose with 128.83 g. Cultivar mean values on thousand-grain weight were close to each other. Umut cultivar had the highest thousand-grain weight with 126.15 g, followed by Altınay cultivar with 123.09 g and Cinsoy cultivar with 114.79 g (Table 6).

The effect of *Rhizobia* treatments on thousand seed weight was found significant at 0.05 level. The mean thousand grain weight of bacterial treatments varied between 116.13 g (Azotek) and 126.55 g (USDA 110). Karakaya & Ödemiş (2019) reported that the increase in irrigation levels positively affected yield, 1000 grain weight and protein ratio. The values obtained in our study are consistent with the results of previous studies (Kobraee et al., 2011; Onat et al., 2017; Yıldırım et al., 2022).

Table 6. Average 1000 seed weight (g) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	126.80	110.60	147.00	120.20	126.15 A
Cinsoy	103.75	104.43	133.93	114.07	114.79 B
Altınay	117.26	117.30	130.58	127.21	123.09 A
Mean Irrigation	113.86 B		128.83 A		
Mean USDA 110	126,55 A				
Mean Azotek	116,13 B				
Lsd B: 10.26; Lsd C: 8.11; Lsd IR: 4.50; Lsd B*IR: 6.36					

3.5.Seed Yield (kg ha^{-1})

Water deficiency has strongly negative influences on productivity, physiological and biochemical traits of soybean plants. The occurrence of immature pod opening may be one of the dysfunctions caused by the decrease in cell turgor result in yield losses (Moura et al. 2023). Although water deficit during vegetative development can cause developmental retardation, the most sensitive periods to drought are flowering and pod-filling periods. To achieve high yield, it is important to avoid water restrictions during these periods. During flowering and early pod-filling periods, yield and quality can be negatively affected by drought. (Poudel et al. 2023). The results of the study indicate that irrigation ($p \leq 0.01$) and cultivar ($p \leq 0.05$) are statistically significant factors influencing seed yield (kg/ha). Conversely, *Rhizobia* applications did not demonstrate a significant effect because of no nodulation history in soybean plants (Table 1).

Water scarcity resulted in approximately 35% less grain yield value (2520 kg ha^{-1}) compared to fully irrigated (3880 kg ha^{-1}) condition. Delice (2017) reported that soybean grain yield values varied between 202 kg/da and 439/da kg in different irrigation doses (25%, 50%, 75%, 100%, 125%) and investigated the yield losses under deficit irrigation conditions at the same level of our study results. Karakaya & Ödemiş (2019) investigated the effects of five different irrigation dose applications (25%, 50%, 75%, 100%, 125%) on yield parameters in soybean. Their findings revealed that grain yields ranged from 198.5 to 518 kg/da, with an average of 201.54-807.12 mm of irrigation during the growing season. The yield differences observed were considerable, with the greatest yields obtained under the highest irrigation levels. As in other observed parameters (plant height, first pod height, number of pods and seed weight), Cinsoy cultivar had the lowest average value in terms of grain yield compared to other cultivars (Table 7). It is important to mention that summer crop yields (soybean, maize, cotton etc.) are highly dependent on irrigation compared to rainfed production of winter crops. It is clear that a reduction in irrigation levels will result in a notable decline in yield, particularly in the context of climate change. The combination of rising temperatures and increased evaporation results in a depletion of the soil's water budget. When this occurs concurrently with deficit irrigation, it becomes inevitable that yield reductions will occur in the future. Another explanation for the overall low yield may be the absence of nodule formation. It can be postulated that plants are unable to reach their full yield potential without effective nitrogen fixation. Conversely, with effective nodulation, it is hypothesized that there may be an increase in yield potential for the region.

Table 7. Average seed yield (kg ha^{-1}) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	3280	1940	5270	3510	3500 A
Cinsoy	2140	2380	3070	2930	2630 B
Altınay	2320	3070	4730	3760	3470 A
Mean Irrigation	2520 B		3880 A		
Mean USDA 110	3470				
Mean Azotek	2930				
Lsd C: 610; Lsd B*C: 870; Lsd IR: 370; Lsd B*IR: 530					

3.6.Leaf Area (cm^2 plant^{-1})

Leaf area construction and development is a primary factor that affects the amount of solar radiation intercepted. The development of leaf area contributes to plant growth in photosynthetic metabolism, but it can be reduced by water stress conditions as a result of the function of the number and size of leaves (Gutiérrez-Boem and Thomas, 2001). As it clear in the obtained results; the leaf area of soybean plants in water scarcity condition caused decreases in the leaf area (approx. to -59% level). The soybean plants had a chance to grow higher amount of canopy with the full irrigated water supply.

Among the cultivars, Umut-2002 had higher leaf area (1236.69 cm^2/plant) amount compared to Cinsoy and Altınay (Table 8). In the previos study conducted by Herliana et al. (2019); who investigated the effects of *Rhizobium* bacteria and different doses of nitrogen fertilizer applications on yield and growth of black soybean, the highest value (138.75 cm^2) was obtained from the combination of *Rhizobium* isolate (*R.* nepotum) and 50% supplied nitrogen fertilizer application.

Table 8. Average leaf area (cm^2 plant^{-1}) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	925.69	925.08	1782.25	1313.76	1236.69 A
Cinsoy	742.58	505.98	1509.46	1121.51	969.88 B
Altınay	817.16	915.41	1452.73	1041.78	1056.77 B
Mean Irrigation	805.32 B		1370.25 A		
Mean USDA 110	1204.98 A				
Mean Azotek	970.59 B				
Lsd B: 147.79; Lsd C: 162.50; Lsd IR: 167.55; Lsd B*IR: 236.94					

3.7.SPAD Chlorophyll Changes in Reproductive Stages

The SPAD Chlorophyll Meter is used to determine the nitrogen status of plants and provides important information on chlorophyll and photosynthetic status with its ease of use and rapid measurement under field conditions, especially under water stress conditions. (Ahmed et al., 2010; Wicharuck et al. 2024). SPAD Chlorophyll meter measurements showed a significant correlation result with yield prediction during grain filling period under high temperature and water scarcity conditions in soybean (Ergo et al., 2018). With in the light of this information, our study results showed that a notable decline in SPAD chlorophyll content values was observed under conditions of restricted irrigation also this decline was particularly pronounced during the ripening of seeds (BBCH 85; SPAD$_{RS}$) period (Figure 4).

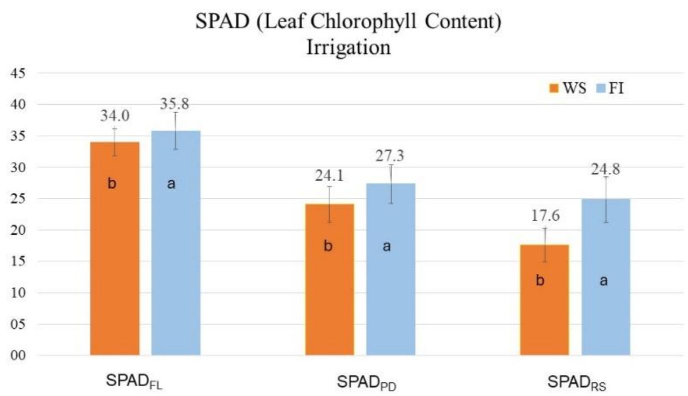

Figure 4. The changes of SPAD values observed during the flowering (SPAD$_{FL}$: BBCH 61), pod development (SPAD$_{PD}$: BBCH 75) and ripening of seeds (SPAD$_{RS}$: BBCH 85) stages under different water regimes

The highest SPAD values were observed at the beginning of flowering stage and showed a greater decline in the development and ripening of seeds stages. Plants showed a decreasing trend for SPAD values during reproductive stages, and this can be linked to senescence of plants according to maturity. Considering that water scarcity is the main factor that reduces soybean growth including yield and photosynthesis. (Felisberto et al. 2023). While both cultivars (Cinsoy and Altınay) had the lowest SPAD values in $SPAD_{RS}$ stage, Umut-2002 had the highest value. The leaf greenness and nitrogen status stayed longer in the ripening of seeds and remained more stable in Umut-2002 than the other cultivars (Figure 5). The obtained SPAD values are consistent with the previous soybean studies (Erbil and Gür, 2017; Tunçtürk et al. 2021).

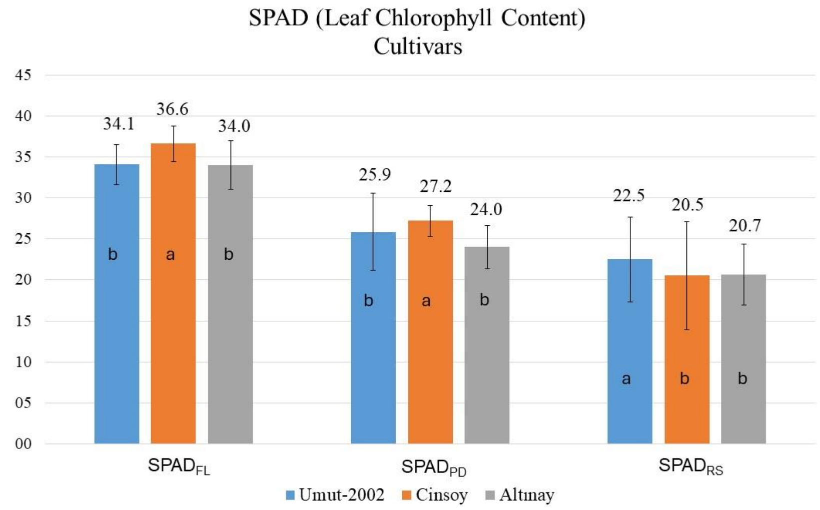

Figure 5. The changes of cultivar SPAD values observed during the flowering ($SPAD_{FL}$: BBCH 61), pod development ($SPAD_{PD}$: BBCH 75) and ripening of seeds ($SPAD_{RS}$: BBCH 85) stages

3.8.Crude Oil and Protein Content (%)

Soybean is of great interest worldwide due to its high protein content, which makes it one of the most important protein sources in the food and feed industry. Drought and water scarcity conditions not only affect yield formation of soybean, but also negatively influence the quality composition. With the reduced nitrogen fixation, the biosynthesis of protein is also affected by drought conditions (Poudel et al., 2023). However, the consistency of protein and oil content of soybean is varied in drought conditions. These quality traits are mainly controlled by genes as well as environmental constraints (Krisnawati and Adie, 2017). Considering the crude protein and oil content results, only crude protein content was affected by the cultivar in our study (Table 1). This result is supported by genetic factors that are potentially effective on the protein content of soybean (Table 9.)

Table 9. Average crude protein content (%) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	28.7	28.0	30.2	30.6	29.4 B
Cinsoy	32.4	31.8	32.6	32.8	32.4 A
Altınay	31.1	31.0	29.4	31.9	30.8 AB
Mean Irrigation	30.5		31.2		
Mean USDA 110	30.7				
Mean Azotek	31.0				
Lsd C: 1,9					

According to both quality traits, there were no statistically significant differences observed in *Rhizobia* and irrigation treatments (Table 1). Crude oil content (%) showed no statistically significant results in all treatments (Table 1).

Kırnak et al. (2010) examined the effects of different water stress conditions (0, 25, 50, 75, 100%) on soybean and reported that the highest protein value was obtained under full irrigation (100%) conditions while the highest oil yield was obtained under no irrigated (rainfed) conditions. Gök (2021) reported that the oil content of soybean grain varied between 10.76% and 22.18%, and Gaweda et al. (2017) had crude oil content results between 17.20 and 18.60% in their study. According to the previous studies, higher oil content values (19.9-21.2%) were obtained without being affected significantly by any treatments applied in our study (Table 10).

Table 10. Average crude oil content (%) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	20.7	21.0	21.2	21.0	21.0
Cinsoy	20.2	20.1	20.8	20.7	20.5
Altınay	20.6	19.9	20.6	20.5	20.4
Mean Irrigation	20.4		20.8		
Mean USDA 110	20.7				
Mean Azotek	20.6				

4. Conclusion

In this one-year experiment conducted under Mediterranean climate conditions, the effects of full irrigation and water scarcity applications on the yield and quality of soybean varieties and the nodule formation performance of *Rhizobia* bacteria applications on soybean roots were determined. In the initial study conducted in the Aydın province, the performance of the USDA 110 bacterial strain was evaluated under local ecological conditions. The objective was to identify a solution to the lack of nodule formation observed in soybean crops in the region. According to the results of the study, no nodule formation was observed in soybean plants even if in full irrigated condition and this may be linked to high ambient and soil temperature conditions in the growing season. As a result of the study, it was determined that irrigated condition (100% WHC) had positive effects on yield formation and SPAD chlorophyll content of soybean compared to water scarcity (50%, WHC) condition. Among the soybean cultivars grown in the experiment, Umut-2002 had higher yield values compared to Cinsoy and Altınay varieties used in the experiment. The experiment demonstrated that *Rhizobia* bacteria applications, which had not previously been investigated in the region under different water regimes, did not have any discernible effect on the properties examined. Introducing or developing more newly adapted inoculants may improve soybean yield potential which is important for soybean cultivation potential in Aydın province. Consequently, it was concluded that further field trials should be conducted over multiple years to observe the USDA 110 nodulation performance in the future.

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Production Performance and Chemical Composition of Various Hydroponic Fodder Species

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A B S T R A C T

The traditional agricultural system is highly dependent on the soil and the natural environment. It is encountering significant challenges from climate change, soil degradation, and water scarcity. Hydroponic fodder production offers as an alternative solution to traditional agricultural system of fodder cultivation which does not rely on soil and can be produced in controlled environment while yielding highly nutritious fodder. This study assesses biomass production, plant height, primary root length, chlorophyll index, nutritional content and economic feasibility of five hydroponic fodder species which includes maize (*Zea mays*), wheat (*Triticum aestivum*), oat (*Avena sativa*), sorghum (*Sorghum bicolor*), and cowpea (*Vigna unguiculata*). The research was conducted at Dr. Purnendu Gain field laboratory and Animal Husbandry laboratory at Khulna University, Bangladesh. Experimental design was completely randomized design (CRD). There were five repetition and, in each repetitions consisted of four replications for each species. Seeds were carefully selected, prepared, and grown in a controlled environment. It was harvested at 11th day after germination. Results indicated that oat consistently achieved the highest biomass yield, peaking at 1254.22g ± 249.98 from 250 g seeds on day 11, followed by cowpea at 1045.22 g ± 71.57 from same quantity of seeds. Oat also maintained the highest plant height reaching up to 19.81 cm ± 1.34 by day 11. Maize showed the longest root length, measuring of 28.59 cm ± 0.120. Cowpea demonstrated the highest chlorophyll levels across all days. Wheat was proved to be the most cost-effective option. Highest dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), total ash (TA) and nitrogen-free extract (NFE) was found in wheat (26.62% ± 2.91), cowpea (25.80% ± 0.48), oat (19.31% ± 1.62), maize (3.59% ± 0.17), cowpea (9.61% ± 0.36) and maize (54.15% ± 2.48), respectively. The results demonstrated the potential of hydroponic fodder production as a viable, sustainable solution for livestock farming, particularly in regions where traditional fodder cultivation is constrained.

1. Introduction

Population estimates from the UN indicate that there will be 10.1 billion people on the globe in 2100, up from 7.7 billion people today, and 9.3 billion people in 2050 (Lee, 2011). Migration and reclassification put agriculture in competition with thriving urban areas for soil, water, and labor, and require it to fight climate change on all fronts maintaining biodiversity, preserving habitats, and producing more food with fewer workers and less land. It seems that agriculture, as the hub of the food chain, is facing a significant challenge. Despite the thousands of acres treated with chemical pesticides and fertilizers that are no longer suitable for farming because of soil

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degradation, water scarcity, and climate change, open-field agriculture is still widely practiced worldwide (Zárate, 2014). The majority of today's farming practices rely on soil and water, making them very susceptible to disasters. Therefore, it is vital that current economic policies of farming systems must be changed (Gashgari et al., 2018). Several climate changes impacts; declining soil fertility, water availability, and competition between cereal crops and fodder have made this situation even worse (El-Morsy et al., 2013).

Researchers have focused their emphasis on investigating more effective alternative methods of producing fodder in considering the limitations associated with the traditional method and the substantial gap between the availability and demand for green fodder (Girma & Gebremariam, 2018; Naik et al., 2015). Hydroponics is one of the soilless culture methods. The Greek terms "hydro" (meaning water) and "ponos" (meaning work) are the origin of the term "hydroponics" (Ani & Gopalakirishnan, 2020). Hydroponic forage is grown without the need of soil and with water. Nutrient-rich liquids can be used in greenhouses for brief periods of time. The feed, which consists of roots, seeds, and plants, resembles a mat and is likely 20 to 30 cm tall. Animals find it to be extremely tasty, easily digested, and nutrient-dense. When hydroponic fodder is used, milk production increases by 8–13%. In locations where the production of conventional green fodder is restricted, this is the ideal substitute technique for use with inexpensive resources for dairy animals (Naik et al., 2015).

A viable substitute technique for sustainable livestock farming is hydroponic fodder production (Girma & Gebremariam, 2019). A several varieties of fodder crops, including barley, oat, wheat, sorghum, alfalfa, cowpea, and maize, can be grown using hydroponic technology (Al-Karaki & Al-Hashimi, 2012; Brown et al., 2018; Farghaly et al., 2019; Guerrero-Cervantes et al., 2016; Kide et al., 2015;). Cereal green fodder is cultivated hydroponically over a period of 7-9 days (Farghaly et al., 2019; Fazaeli et al., 2011; Wang et al., 2019). The choice of hydroponic fodder varieties is dependent upon the particular geographic and agroclimatic conditions, in addition to the availability of seeds. Furthermore, the economic viability of hydroponic fodder production can be facilitated through the incorporation of wheat (*Triticum aestivum* L.) (Guerrero-Cervantes et al., 2016; Tayade & Chavan, 2018).

Hydroponic fodder production has become popular for its advantages in enhancing livestock well-being. Hydroponics fodder is known for its added palatability, digestibility and valuable nutritional value, which contributes to the well-being of livestock (Naik et al., 2015). Hydroponic growing systems can achieve a larger harvest of livestock feed, all the while utilizing substantially less space when compared with traditional methods (Schoenian, 2013). Fodder seeds are grown using tap water or nutrient-enriched solutions without soil which makes hydroponic fodder a feasible alternative for fresh feed (Bakshi et al., 2017). In terms of ether extract, nitrogen-free extracts, organic content, and crude protein, hydroponic fodder performs better than conventional non-leguminous fodder. However, the total digestible nutrient content, metabolizable energy, and gross energy all decrease during sprouting. This results from the plant's energy intake during respiration (Ajmi et al., 2009; Fazaeli et al., 2011). This study aims to estimate the comparative performance of five different hydroponic fodders by assessing their biomass production, height, primary root length, chlorophyll level, their nutritional content through proximate analysis, and determining their recurring production costs.

2. Materials and Methods

2.1. Site selection

The research took place at the Dr. Purnendu Gain Field Laboratory and Animal Husbandry Laboratory of the Agrotechnology Discipline at Khulna University. The research unit is situated on 22º80' N and 89º53' E latitude and longitude.

2.2. Design of the study

The research was conducted using a completely randomized design (CRD). The five species examined including maize (*Zea mays*), wheat (Triticum aestivum), oat (*Avena sativa*), sorghum (*Sorghum bicolor*) and cowpea (*Vigna unguiculata*) crops. Each species represented as a treatment group. No control group was considered as the research focus on comparative analysis among the treatment groups. There were five repetitions. Each repetition had four replications for each treatment group (species).

2.3. Collection and selection of seeds

Fodder seeds were collected from local markets of same local variety for each species. To ensure high-quality hydroponic fodder production, good quality seeds free from damage and disease were carefully selected.

2.4. Preparation of growing area

The growing area and trays were thoroughly cleaned and disinfected by using 0.3 % chlorhexidine gluconate + 3% cetrimide solution then again rinsed to establish an aseptic environment. This proactive measure effectively prevented the proliferation of bacteria or disease-causing organisms. Also, proper drainage system was ensured to avoid the issue of waterlogging. Arrangement of the research unit of hydroponic fodder growing area is shown in Figure 1. Photographs of oat hydroponic fodder on harvest day, sorghum hydroponic fodder on harvest day and measurements of chlorophyll levels of oat hydroponic fodder are shown in Figures 2, 3 and 4, respectively.

Figure 1. Arrangement of the research unit of hydroponic fodder growing area

Figure 2. Oat hydroponic fodder on the day of harvest

Figure 3. Sorghum hydroponic fodder on the day of harvest

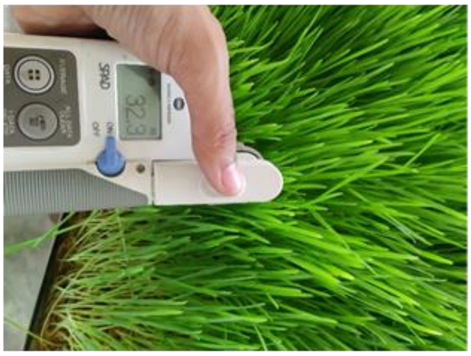

Figure 4. Measuring of chlorophyll level of oat hydroponic fodder

2.5. Preparation and placement of seeds

The seeds were rinsed under running water to remove impurities and debris. After thorough rinsing, the seeds were soaked in water for 12 hours to initiate germination. After draining the water, the seeds were kept in a gunny bag for 24 hours in a dark environment until they sprouted. For each species and each replication, 250 grams of seeds were taken throughout the five repetitions of the experiment. The seeds were evenly spread on the trays after they sprouted. The trays were placed in the rack which held the trays in a 1-inch tilted position to facilitate excess water drainage.

2.6. Uniformity of the environment

The research was conducted in winter season of Bangladesh. The average ambient temperature ranged from 21.02°C ± 0.10 to 25.91°C ± 0.36 during the study. Temperature was recorded four times for each day. Recorded temperatures during the study period are shown in Table 1. After germination, seeds were spread on the trays and trays were covered with cloth and curtain was also used to maintain a dark environment for the first three days. From day four to harvest both natural and artificial lighting was provided. Two 15watt white color LED lights were used in the research unit and was lit for 12 hours each day (6AM-6PM).

Table 1. Average temperatures (°C) throughout the experiment

Day	Repetition 1 (December)	Repetition 2 (December - January)	Repetition 3 (February)	Repetition 4 (February)	Repetition 5 (March)
1	20.50	22.50	21.30	26.75	24.75
2	20.40	21.50	23.15	26.50	24.60
3	21.00	22.25	23.30	26.25	24.00
4	21.25	23.50	24.25	23.60	26.00
5	21.00	22.25	23.45	24.25	24.65
6	21.10	22.75	22.00	25.25	26.65
7	21.10	23.75	24.50	25.05	27.10
8	21.40	22.00	23.85	24.25	26.60
9	21.40	20.15	24.25	25.20	26.25
10	21.00	17.25	25.65	25.30	27.00
11	21.10	18.00	25.05	26.25	27.45
MEAN	21.02	21.45	23.70	25.33	25.91
SEM	0.10	0.64	0.38	0.31	0.36

Mitigation of potential biasness was implemented and the randomization of the experimental setup was ensured by a systematic shuffling of the trays containing each species throughout the duration of the study. By periodically rearranging the trays, variations in factors such as light exposure, temperature, humidity, and other microclimate were evenly distributed across all treatment groups (species). Artificial lighting during daytime was also provided along with natural lighting to ensure uniformity. Exposure to natural light was also controlled with the help of curtain to eliminate excess heating and dehydration as it can damage the seeds or hydroponic fodder.

2.7. Supplementation of water

Seeds were watered regularly 3-4 times daily manually. Only tap water was used from the same source. No nutrient solution was use with tap water.

2.8. Harvesting

The hydroponic fodder was harvested at 11th day after germination.

2.9 Cleaning and repeating

The growing area and trays were cleaned thoroughly to eliminate any residue. The process was repeated by soaking and preparing new seeds then again spreading them evenly on the trays to initiate a new cycle of hydroponic fodder production. The experiment was repeated five times with four replications for each treatment group.

2.10. Biomass yield

Biomass of different hydroponic green fodder was measured using a weighing balance. The quantification and record keeping of biomass production took place everyday morning before watering. Seeds weight after they were germinated (Germination weight) was also recorded on the very first day before spreading the seeds on the trays.

2.11. Plant height

Three plants were randomly chosen from each tray for measurement of height. Every plant was measured for height from the tray floor to the top leaf, and the average height of the plants was noted.

2.12. Primary root length

Three plants were selected at random from every tray to measure the height of their primary roots. Root length of various hydroponic fodder species was measured between day 4 and day 11.

2.13. Chlorophyll index

The chlorophyll levels of various hydroponic fodder species were assessed using an SPAD meter from day 7 to day 11.

2.14. Determination and comparison of production cost

The production costs associated with each hydroponic fodder species were analyzed and determined. The hydroponic fodder with the highest economic feasibility and profitability was determined, considering both biomass yield and production costs based on per kg seed for comparison.

2.15. Proximate analysis

The chemical composition of dried fodder samples was determined at the Animal Husbandry Laboratory, Agrotechnology Discipline, Khulna University, Khulna. The dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and total ash (TA) contents of hydroponic fodder samples were estimated according to AOAC (1990). The samples used in this analysis contained the full part of the hydroponic fodder including the leafy portion, seeds and roots.

2.16. Data analysis

For analysis, a one-way ANOVA was employed. Descriptive statistical tools, including the calculation of averages and standard errors were applied using the tabular technique. The analysis was conducted utilizing SPSS software (version 26.0).

3. Results

3.1. Biomass yield

Biomass yields of different hydroponic fodder species measured over 11 days are shown in Table 2. Germination weights from 250g seed showed that cowpea (514.72g ± 4.95) had the highest values, followed by oat (399.89g ± 5.38), wheat (363.81g ± 1.62), maize (337.28g ± 3.34) and sorghum (332.20g ± 4.12) where the mean difference was significant ($p<0.001$). Oat (360.40g ± 64.10) and cowpea (344.15g ± 38.15) had the highest biomass yields on day 2 followed by wheat (277.95g ± 13.48) and sorghum (272.47g ± 15.73) while maize (229.03g ± 24.08) had the lowest biomass. Oat and cowpea had significantly higher biomass yields compared to sorghum and maize at day 5. Biomass of wheat did not significantly differ from any of the other species on the same day. Oat (602.57g ± 55.09) had the highest biomass yield on day 5, followed by cowpea (562.86g ± 61.86), wheat (446.93g ± 21.78), maize (397.21g ± 25.34) while sorghum (384.77g ± 21.65) had the lowest biomass yield. Oat (1240.89g ± 281.56) continued to show the highest yield on day 10, with cowpea (800.93g ± 86.65), wheat (704.54g ± 45.99), maize (694.18g ± 61.96) were followed and sorghum (664.02g ± 42.68) showed lowest yield. Oat had a significantly higher yield compared to all other species during day 8 to 10. Cowpea, wheat, maize, and sorghum during this time did not show a significant difference from each other for biomass yield. Oat (1254.22g ± 249.98) and cowpea (1045.22g ± 71.57) had the highest biomass yields at day 11. Wheat (732.85g ± 46.78), sorghum (720.44g ± 53.58) were followed and maize (707.79g ± 56.81) had the lowest yield.

Table 2. Biomass yields (g) of different species of hydroponic fodders from 250g of seeds

Day	Sorghum (*Sorghum bicolor*)	Cowpea (*Vigna unguiculata*)	Wheat (*Triticum aestivum*)	Maize (*Zea mays*)	Oat (*Avena sativa*)	F-value	Sig.
Germination	$332.20^{d} \pm 4.12$	$514.72^{a} \pm 4.95$	$363.81^{c} \pm 1.62$	$337.28^{d} \pm 3.34$	$399.89^{b} \pm 5.38$	410.31	***
2	$272.47^{ab} \pm 15.73$	$344.15^{a} \pm 38.15$	$277.95^{ab} \pm 13.48$	$229.03^{b} \pm 24.08$	$360.40^{a} \pm 64.10$	2.94	**
3	$347.54^{abc} \pm 21.49$	$472.63^{a} \pm 54.01$	$323.21^{bc} \pm 14.36$	$290.89^{c} \pm 27.42$	$433.81^{ab} \pm 63.81$	4.23	**
4	$370.13^{b} \pm 22.75$	$548.25^{a} \pm 68.83$	$405.88^{ab} \pm 24.07$	$348.79^{b} \pm 29.75$	$558.69^{a} \pm 89.65$	3.82	*
5	$384.77^{c} \pm 21.65$	$562.86^{ab} \pm 61.86$	$446.93^{bc} \pm 21.78$	$397.21^{c} \pm 25.34$	$602.57^{a} \pm 55.09$	4.40	**
6	$424.74^{c} \pm 24.56$	$605.85^{b} \pm 63.68$	$513.97^{bc} \pm 33.29$	$459.83^{bc} \pm 31.04$	$816.84^{a} \pm 144.87$	5.48	***
7	$465.06^{c} \pm 27.44$	$676.91^{b} \pm 75.10$	$567.15^{bc} \pm 34.85$	$523.50^{bc} \pm 34.78$	$942.29^{a} \pm 162.69$	5.89	***
8	$531.96^{b} \pm 32.62$	$733.86^{b} \pm 83.53$	$630.47^{b} \pm 39.76$	$584.62^{b} \pm 40.36$	$1099.35^{a} \pm 214.29$	5.77	***
9	$595.79^{b} \pm 38.36$	$773.59^{b} \pm 91.13$	$679.10^{b} \pm 42.07$	$637.89^{b} \pm 46.30$	$1168.87^{a} \pm 224.25$	4.877	**

10	$664.02^{b} \pm 42.68$	$800.93^{b} \pm 86.65$	$704.54^{b} \pm 45.99$	$694.18^{b} \pm 61.96$	$1240.89^{a} \pm 281.56$	4.29	**
11	$720.44^{b} \pm 53.58$	$1045.22^{a} \pm 71.57$	$732.85^{b} \pm 46.78$	$707.79^{b} \pm 56.81$	$1254.22^{a} \pm 249.98$	8.07	***

*** p < 0.001, ** p < 0.01, * p < 0.05; Means with uncommon superscripts in a row differed significantly (p<0.05).

3.2. Plant height

Height of different hydroponic fodder species was measured during day 4 to day 11 and are shown in Table 3. On day 4, wheat (3.23 cm ± 0.62) and oat (3.23 cm ± 1.22) had the highest height while sorghum (1.40 cm ± 0.15) showed lowest height. Wheat and oat significantly differed from sorghum, while the heights of maize, and cowpea were not significantly different. On day 11, oat (19.81 cm ± 1.34) followed by maize (19.04 cm ± 1.40) consistently remained the tallest among all the days and cowpea (16.02 cm ± 0.69), wheat (13.97 cm ± 0.81) and sorghum (13.74 cm ± 0.54) showed the shorter height throughout the cultivation period. Oat significantly differed from sorghum, cowpea, and wheat, while the height of maize was not significantly different with oat on day 8, 9, 10 and 11. Statistical analysis unveiled significant difference in height among different species, with varying degrees of significance recorded across different days (***p < 0.001, **p < 0.01, *p < 0.05).

Table 3. Heights (cm) of different species of hydroponic fodders at different stage of growth (d)

Day	Sorghum (*Sorghum bicolor*)	Cowpea (*Vigna unguiculata*)	Wheat (*Triticum aestivum*)	Maize (*Zea mays*)	Oat (*Avena sativa*)	F-value	Significance
4	$1.40^{b} \pm 0.15$	$2.23^{ab} \pm 0.23$	$3.23^{a} \pm 0.62$	$2.51^{ab} \pm 0.27$	$3.23^{a} \pm 1.22$	3.271	**
5	$2.26^{c} \pm 0.33$	$3.37^{bc} \pm 0.39$	$5.23^{ab} \pm 0.87$	$4.91^{ab} \pm 0.50$	$6.06^{a} \pm 2$	4.819	**
6	$4.68^{b} \pm 0.48$	$6.59^{b} \pm 0.71$	$7.10^{b} \pm 0.83$	$6.96^{b} \pm 0.43$	$10.17^{a} \pm 1.17$	4.654	**
7	$6.01^{c} \pm 0.55$	$8.54^{b} \pm 0.75$	$8.62^{b} \pm 0.81$	$8.99^{b} \pm 0.44$	$12.32^{a} \pm 0.65$	6.146	***
8	$8.37^{c} \pm 0.31$	$10.78^{b} \pm 0.78$	$10.47^{bc} \pm 0.66$	$12.30^{ab} \pm 0.68$	$13.67^{a} \pm 0.35$	6.614	***
9	$10.51^{c} \pm 0.50$	$12.07^{bc} \pm 0.74$	$12.12^{bc} \pm 0.67$	$14.26^{ab} \pm 0.68$	$16.32^{a} \pm 1.43$	6.347	***
10	$11.96^{b} \pm 0.34$	$13.56^{b} \pm 0.68$	$13.22^{b} \pm 0.75$	$17.31^{a} \pm 1.17$	$18.06^{a} \pm 1.38$	8.635	***
11	$13.74^{b} \pm 0.54$	$16.02^{b} \pm 0.69$	$13.97^{b} \pm 0.81$	$19.04^{a} \pm 1.40$	$19.81^{a} \pm 1.34$	7.459	***

*** p < 0.001, ** p < 0.01,; Means with uncommon superscripts in a row differed significantly (p<0.05).

3.3. Root length

Root length of different hydroponic fodder species was measured during day 6 to day 11 and are shown in Table 4. Maize (7.90 cm ± 0.12) had the longest root on day 6, followed by oat (3.99 cm ± 0.11), cowpea (3.61 cm ± 0.05), sorghum (2.74 cm ± 0.09), and wheat (2.59 cm ± 0.05). Root length of maize was significantly differed from all other species. Maize (28.59 cm ± 0.12) had the longest root length at day 11 which was consistently from day 6 to day 11 after germination, followed by oat (12.60 cm ± 0.06), wheat (8.18 cm ± 0.05), sorghum (6.30 cm ± 0.04), and cowpea (6.09 cm ± 0.05). Shortest root length was found in cowpea from day 9 to day 11 after germination. Significant difference was not found between sorghum and cowpea but they were significantly different from wheat, maize and oat at the day of harvest.

Table 4. Average primary root length (cm) of different species of hydroponic fodders at different stage of growth (d)

Day	Sorghum (*Sorghum bicolor*)	Cowpea (*Vigna unguiculata*)	Wheat (*Triticum aestivum*)	Maize (*Zea mays*)	Oat (*Avena sativa*)	F-value	Level of significance
6	$2.74^{d} \pm 0.09$	$3.61^{c} \pm 0.05$	$2.59^{d} \pm 0.05$	$7.90^{a} \pm 0.12$	$3.99^{b} \pm 0.11$	728.3	***
7	$4.47^{c} \pm 0.12$	$4.30^{c} \pm 0.06$	$3.34^{d} \pm 0.04$	$8.40^{a} \pm 0.11$	$5.82^{b} \pm 0.10$	451.6	***
8	$5.54^{c} \pm 0.12$	$4.94^{d} \pm 0.06$	$4.29^{e} \pm 0.12$	$10.10^{a} \pm 0.10$	$7.26^{b} \pm 0.03$	478.4	***
9	$5.81^{c} \pm 0.13$	$5.25^{d} \pm 0.09$	$5.27^{d} \pm 0.11$	$19.25^{a} \pm 0.09$	$9.61^{b} \pm 0.06$	2936.8	***
10	$6.13^{d} \pm 0.07$	$5.58^{e} \pm 0.07$	$7.07^{c} \pm 0.09$	$19.55^{a} \pm 0.08$	$12.16^{b} \pm 0.09$	5037.2	***
11	$6.30^{d} \pm 0.04$	$6.09^{d} \pm 0.05$	$8.18^{c} \pm 0.05$	$28.59^{a} \pm 0.12$	$12.60^{b} \pm 0.06$	18780.5	***

*** $p < 0.001$, Means with uncommon superscripts in a row differed significantly ($p<0.05$).

3.4. Chlorophyll index (SPAD reading)

Chlorophyll level of different hydroponic fodder species was measured during day 7 to day 11 which are shown in Table 5. During day 7 cowpea (36.12 ± 0.44) had the highest chlorophyll level, followed by oat (31.34 ± 0.45), wheat (28.82 ± 0.54), maize (28.59 ± 0.41), and sorghum (24.38 ± 0.29). Chlorophyll level of cowpea was significantly different from that of all other species. Chlorophyll level of cowpea remained consistently high throughout the experiment and also at the day of harvest, where sorghum (21.81 ± 0.24) consistently had the lowest chlorophyll content throughout the days when it was recorded. Chlorophyll level of cowpea differed significantly from all other species.

Table 5. Chlorophyll level of different species of hydroponic fodders at different stage of growth (d)

Day	Sorghum (*Sorghum bicolor*)	Cowpea (*Vigna unguiculata*)	Wheat (*Triticum aestivum*)	Maize (*Zea mays*)	Oat (*Avena sativa*)	F-value	Level of significance
7	$24.38^{d} \pm 0.29$	$36.12^{a} \pm 0.44$	$28.82^{c} \pm 0.54$	$28.59^{c} \pm 0.41$	$31.34^{b} \pm 0.45$	106.8	***
8	$23.63^{d} \pm 0.13$	$34.73^{a} \pm 0.75$	$32.82^{b} \pm 0.43$	$29.55^{c} \pm 0.27$	$32.47^{b} \pm 0.44$	80.5	***
9	$23.01^{d} \pm 0.15$	$39.03^{a} \pm 0.63$	$29.70^{b} \pm 0.37$	$26.20^{c} \pm 0.34$	$29.47^{b} \pm 0.19$	214.3	***
10	$22.02^{e} \pm 0.10$	$40.20^{a} \pm 0.47$	$33.77^{b} \pm 0.39$	$24.33^{d} \pm 0.34$	$31.11^{c} \pm 0.30$	436.9	***
11	$21.81^{e} \pm 0.24$	$39.17^{a} \pm 0.69$	31.96^{b} b± 0.43	$26.31^{d} \pm 0.30$	$28.64^{c} \pm 0.07$	203.1	***

*** $p < 0.001$, Means with uncommon superscripts in a row differed significantly ($p<0.05$).

3.5. Cost analysis

Production costs per kilogram (in Bangladeshi Taka, BDT, 1 USD is equivalent to 118 BDT) for different hydroponic fodder species is shown in Table 6. Sorghum (51.16 BDT/kg ± 3.90) had the highest production cost, closely followed by cowpea (50.52 BDT/kg ± 3.99) followed by Oat (39.25 BDT/kg ± 8.60) which had a moderate production cost, while maize (33.01 BDT/kg ± 2.22) showed a lower production cost. Wheat (21.12 BDT/kg ± 1.17) had the lowest production cost among the hydroponic fodder species. Sorghum and cowpea were not significantly different from each other for production costs. Wheat had a significantly less production cost compared to all other species.

Table 6. Production cost of different hydroponic fodder species

Species	Production cost (BDT#. kg^{-1})
Sorghum (*Sorghum bicolor*)	$51.16^{a} \pm 3.90$
Cowpea (*Vigna unguiculata*)	$50.52^{a} \pm 3.99$
Wheat (*Triticum aestivum*)	$21.12^{c} \pm 1.17$
Maize (*Zea mays*)	$33.01^{b} \pm 2.22$
Oat (*Avena sativa*)	$39.25^{ab} \pm 8.60$
F-value	14.957
Level of significance	***

*** p < 0.001# 1 USD is equivalent to 118 BDT (approx.)

3.6. Chemical composition

Chemical composition of different hydroponic fodder species is presented in Table 7. Wheat fodder (26.62% ± 2.91) had the highest dry matter (DM) content, with cowpea (9.87% ± 0.22) having the lowest DM content. Wheat DM was significantly different from sorghum, cowpea, oat, and maize. Cowpea (25.80% ± 0.48) showed highest crude protein (CP) content, where maize (11.38% ± 0.26) had the lowest CP content. Cowpea was found significantly different from sorghum, oat, wheat, and maize for their CP contents. In term of crude fiber, oat (19.31% ± 1.62) had the highest content, and wheat (8.00% ± 0.07) had the lowest CF content. Regarding ether extract (EE), maize (3.59% ± 0.17) had the highest content, where cowpea (2.39% ± 0.13) had the lowest EE content. Maize was significantly different from sorghum, oat, wheat, and cowpea for EE content. Cowpea (9.61% ± 0.36) had the highest total ash (TA) content, where oat (3.39% ± 0.06) showed lowest TA content. Cowpea was noticed significantly different from sorghum, oat, wheat, and maize. In case of nitrogen-free extract (NFE), maize (54.15% ± 2.48) showed highest result, followed by sorghum (53.05% ± 0.92), oat (47.11% ± 2.41) and wheat (39.76% ± 2.59), while cowpea (35.26% ± 0.92) had the lowest NFE content.

Table 7. Chemical composition (%) of different species of hydroponic fodders

Fodder species	Chemical composition (%)					
	Dry matter (DM)	Crude Protein (CP)	Crude Fiber (CF)	Ether extract (EE)	Total ash (TA)	Nitrogen Free Extract (NFE)
Sorghum	$18.11^{b} \pm 0.69$	$13.12^{b} \pm 0.37$	$8.16^{b} \pm 0.10$	$3.13^{b} \pm 0.17$	$4.42^{b} \pm 0.22$	$53.05^{c} \pm 0.92$
Cowpea	$9.87^{bc} \pm 0.22$	$25.80^{a} \pm 0.48$	$17.08^{bc} \pm 0.23$	$2.39^{bc} \pm 0.13$	$9.61^{a} \pm 0.36$	$35.26^{b} \pm 0.92$
Wheat	$26.62^{a} \pm 2.91$	$18.78b^{c} \pm 0.30$	$8.00^{b} \pm 0.07$	$3.35^{c} \pm 0.05$	$3.49^{bc} \pm 0.13$	$39.76^{b} \pm 2.59$
Maize	$17.52^{b} \pm 2.51$	$11.38^{bd} \pm 0.26$	$9.75^{b} \pm 0.10$	$3.59^{a} \pm 0.17$	$3.61^{bd} \pm 0.17$	$54.15^{a} \pm 2.48$
Oat	$13.20^{b} \pm 1.47$	$13.80^{b} \pm 0.52$	$19.31^{a} \pm 1.62$	$3.18^{d} \pm 0.10$	$3.39^{be} \pm 0.06$	$47.11^{bc} \pm 2.41$
Total	16.86 ± 1.29	16.60 ± 1.05	12.38 ± 0.94	3.12 ± 0.10	5.01 ± 0.48	46.04 ± 1.66
F-value	11.701	225.646	64.809	11.274	146.998	18.853
Significance	***	***	***	***	***	***

*** p<0.001; Means with uncommon superscripts in a row differed significantly (p<0.05).

3.7. Correlation matrix

Table 8 presents correlations between various parameters related to plant growth and development, including germination weight, biomass yield, height, root length, and chlorophyll level, all measured on the 11th day after germination. Table contains a Pearson correlation coefficient, indicating the strength and direction of the linear relationship between two variables. Chlorophyll level strongly correlates with both germination weight and biomass yield, suggesting that plants with higher chlorophyll content tend to have heavier seeds and greater biomass. However, there is a significant negative correlation between germination weight and root length (-0.398**), suggesting that heavier seeds may produce plants with shorter roots.

Table 8. Correlation matrix (r) among germination weight, biomass weight, height, root length and chlorophyll levels of hydroponic fodders at day 11.

	Germination wt. (g)	**Biomass wt. at day 11 (g)**	**Height at day 11 (cm)**	**Root length at day 11 (cm)**	**Chlorophyll level at day 11**
Germination Wt. (g)	1				
Biomass yield at day 11 (g)	0.525**	1			
Height at day 11 (cm)	0.115	0.112	1		
Root length at day 11 (cm)	-0.398**	-0.204	0.511**	1	
Chlorophyll level at day 11	0.869**	0.381*	0.005	-0.293	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

4.1. Biomass yields

In an experiment it was noticed that biomass yield from 500g seed of red sorghum was 900g ± 53.24 harvested at 7th day after germination which is higher than our current findings (Akinmutimi et al., 2022). In case of wheat Bari et al. (2022) revealed that, biomass yield was 6.27kg ± 0.15 from 450g of seed harvested on day 8 which was also higher than our current findings. Another study revealed that 3.5kg of wheat fodder can be hydroponically grown from 1kg of seed when harvested at 8th day which is higher than current study (Rahman et al., 2020). Another study noticed biomass yield of maize hydroponic fodder at 8th day was 8.00kg ± 0.36 from 500g seed which does not correspond to the present study and present study showed lower biomass yield in maize hydroponic fodder (Kide et al., 2015). Maize production in hydroponic technology was found lower in the current investigation than some other studies (Rahman et al., 2020; Upreti et al., 2022). Hiller and Perry (1969) observed that, from 100g of oat seed 550g of oat hydroponic fodder can be produced when harvested at 6th day which is higher than present study. According to Upreti et al. (2022), when 1 kg oat seeds were used it was found that it produced 7.96 kg of hydroponically grown oat fodder at 11th day which is higher than present findings. In another different investigation oat was found to produce 3.02 kg to 3.35 kg of hydroponically grown fodder from 1 kg of seed at 8th day which is higher than the current study. Cowpea hydroponic fodder found to 4.12 kg to 4.29 kg at 8th day which is also higher present finding (Jolad et al., 2018). In a separate experiment, researchers maintained a temperature of 24°C and a relative humidity of 50-73% to examine the output of barley fodder. They utilized growing trays measuring 45 cm x 25 cm x 8 cm and hydroponic fodder was harvested 9th day. The yield of barley fodder was 25.0 kg/m2 in fresh weight and 4.1 kg/m2 in dry weight (Al-Karaki, 2011). Gebremedhin (2015) illustrated, barley and maize had fresh yield values of 52.9 kg/m2 and 47.1 kg/m2, respectively. According to Shit (2019), different hydroponic fodder production methods may result in variations in biomass yield. Some factors such as design of hydroponic trays and the types of nutrient solutions used can affect growth and yield of the fodder. No nutrient solution was used in the present study, that may be the cause for lower biomass production than that of other researchers. Biomass yield of hydroponic fodder can also be influenced by environmental conditions such as humidity, temperature, and light intensity (Shit, 2019). Assefa et al. (2020) noticed that, seed rate can also affect the biomass yield. Some experiments suggest in situations with limited sunlight, artificial or supplemental lighting is utilized to compensate for the insufficient light needed for photosynthesis. The use of additional lighting has significantly increased plant productivity (Hao et al., 2018; Rahman et al., 2021).

4.2. Plant height

A study found plant height of maize, oat and wheat were 27.77 cm, 27.11 cm and 25.03 cm respectively which is higher than our findings (Upreti et al., 2022). In another investigation by Murthy et al. (2017) revealed that, at 5th day height of maize, sorghum and cowpea were 18.1 cm, 10.8 cm and 25.33 cm respectively which is higher than the current findings. According to Bari et al. (2022), height of wheat hydroponic fodder at 11th day was 14.10 cm ± 0.51 which is also higher than present investigation. Growth of plants can be affected by the production methods employed in hydroponic systems and based on the use of nutrient solutions. Use of nutrient solution instead of tap water as nutrient source can significantly increase plant height (Dung et al., 2010).

4.3. Root length

Bari et al. (2022) illustrated that, the root lengths on day 6, day 7, and day 8 were 7.10cm ± 0.22, 8.56cm ± 0.21, and 9.72cm ± 0.32, correspondingly, indicating higher root length compared to the results of the present investigation. Another study demonstrated root lengths of yellow maize on the sixth, seventh, and eighth days were 13.9cm ± 0.181, 14.5cm ± 0.331 and 17.5cm ± 0.26, respectively, which exceeded the outcomes of the present study. In case of sorghum (Jowar) recorded root lengths were 16.6cm ± 0.38, 17.9cm ± 0.22 and 20.3cm ± 0.35 in day 6, day 7 and day 8 respectively which is significantly higher than the current findings (Jemimah et al., 2018). Jolad et al. (2018) found that root lengths at 8th day of fodder maize, wheat, oat and fodder cowpea were found 22.71 cm, 15.70 cm, 15.67 cm and 18.57 cm, respectively which is higher than the present investigation. Difference in the root length may be attributed to several factors including the use of tap water instead of nutrient solution, pH level of the supplied water, temperature, humidity, available oxygen, type of hydroponic system implemented.

4.4. Chlorophyll index (SPAD reading)

Experiment conducted by Jolad et al. (2020) showed hydroponic fodder of maize, wheat, oat, and cowpea at seventh day chlorophyll index was found to be 32.18, 31.89, 31.84, and 38.11, respectively. On the eighth day, the chlorophyll index for fodder maize, wheat, oat, and fodder cowpea were recorded as 33.78, 32.80, 32.76, and 39.10, respectively. Chlorophyll index at 7th day for fodder maze fodder, wheat and fodder cowpea was higher than the current findings but oat is in accordance with the present study. During eighth day, wheat and oat were in accordance with current findings but in case of fodder maize and fodder cowpea it was higher. The chlorophyll levels in hydroponic fodder vary due to factors such as light intensity and quality, nutrient availability, water quality, environmental stresses, and genetic differences among plant species.

4.5. Cost analysis

Bari et al. (2022) noticed, cost of production of per kg wheat hydroponic fodder was 4.97BDT. ± 0.12 (1 USD is equivalent to 118 BDT) which lower than our findings. Another investigation revealed that, hydroponic maize fodder, oat fodder and wheat fodder required total cost of 20.64, 24.67 and 18.76 Nepalese rupee for one kg of hydroponic fodder production, respectively, which is lower than our current findings (Upreti et al., 2022). Another experiment demonstrated the costs to produce 1kg hydroponic fodder for yellow maize, cowpea, sorghum were 3.20, 10.90, 7.90 Indian Rupee, respectively, which is significantly lower than the present study (Jemimah et al., 2018). According to Jolad et al. (2020), costs of fodder maize, wheat, oat, and cowpea were 22.14, 40.14, 52.14, 72.14 Indian Rupee per kg seed and cost of fodder maize was found similar to our study but wheat, oat and cowpea fodder showed lower production cost in the current study. The cost difference can be caused by high costing of the seed and lower biomass yield.

4.6. Chemical composition

Bari et al. (2022) noticed that, when harvested at day eight CP, EE, CF, NFE and ash content per 100g DM for wheat were 19.83g ± 0.35, 2.70g ± 0.03, 4.68g ± 0.03, 69.82g ± 0.33, 2.96g ± 0.01, respectively, where CP content was similar to our findings but EE, CF, Ash contents were higher in the present study and NFE content was lower. Another study demonstrated that when harvested at 11th day, DM and CP contents for maize hydroponic fodder were 12.55% ± 2.05 and 12.51% ± 0.3, respectively. In case of oat, it was 14.13% ± 0.71 and 13.96% ± 2.08, respectively, and in case of wheat it was 14.49% ± 1.18 and 16.16% ± 1.59. Crude protein content of the oat is in accordance with our findings but CP content of maize was lower and for wheat it was higher in our study and DM contents of oat was lower and for maize and wheat was higher in the present investigation (Upreti et al., 2022). According to Jemimah et al. (2018), CP, CF, EE, TA and NFE of yellow maize hydroponic fodder were 10.55%, 5.51%, 6.42%, 1.80% and 77.52%, respectively, where CP, CF and TA contents of the present study were higher and EE and NFE were lower. In case of cowpea those components were 27.84%, 6.51 %, 1.93%, 4.88% and 58.84%, respectively, and CP, EE and TA content were higher in the current findings but CP and NFE contents were lower. For sorghum CP, CF, EE, TA and NFE were, 13.27% 13.39%, 4.99%, 2.98% and 65.37% where CP content were similar to our findings but TA content in the present study was higher but CF, EE and NFE were lower.

Jolad et al. (2020) showed that, total protein contents of fodder maize, wheat, oat and fodder cowpea were 14.58%, 12.75%, 12.38% and 16.06%, respectively, and current study found higher CP content in wheat, oat, cowpea but lower in maize hydroponic fodder. Crude fat was found 7.20%, 6.11% and 6.07% for fodder maize, wheat and oat, respectively. Crude fat of all of species of hydroponic fodder was higher than the current study. In a separate study conducted on maize hydroponic fodder found that CP, EE, CF, NFE and TA contents were 13.57%, 3.49%, 14.07%, 66.72% and 3.84%, respectively, when harvested at seventh day (Naik et al., 2015). Present study found higher EE content and lower CP, CF, NFE and TA contents than that of Naik et al. (2015). In another observation maize hydroponic fodder had lower DM, ash and higher CP, EE, CF, NFE contents than the present study. In their observation by Kide et al. (2015), it was found that DM, CP, EE, CF, NFE and Ash were 18.25% ± 0.12, 14.56% ± 0.29, 4.67% ± 0.19, 10.00% ± 0.17, 68.47% ± 1.63 and 2.83% ± 0.03, respectively. In a different study it was observed that DM, CP, EE, CF, TA and NFE contents were higher in case of maize when harvested at 14th day. In case of wheat, CP and CF contents were similar but it had increased NFE content and lower DM, EE and TA contents than the present findings. Average DM, CP, EE, CF, TA and NFE contents of maize were 20.15% ± 0.40, 17.43% ± 0.24, 4.85% ± 0.05, 18.39% ± 0.12, 3.94% ± 0.01, 55.39% ± 0.019, respectively, and wheat had 14.64% ± 0.16, 18.94% ± 0.01, 3.13% ± 0.06, 8.10% ± 0.22, 3.38% ± 0.09 and 66.46% ± 0.18, respectively (Mahale et al., 2020). It was demonstrated by Akinmutimi et al. (2022) that, significantly higher DM with higher CP and ash contents, lower EE content and similar CF and NFE content in maize hydroponic fodder which was allowed to grow to 7 days. In case of red sorghum that DM was significantly higher, lower CP contents, higher EE, CF and ash contents and similar NFE contents. They found average DM, CP, EE, CF, NFE, ash contents for maize hydroponic fodder were 87.63%, 12.84%, 3.14%, 9.65%, 57.35%, 4.67% and for red sorghum hydroponic fodder were 88.09%, 15.33%, 3.36%, 11.55%, 53.05%, 4.81%, respectively. Hillier & Perry (1969) observed higher DM, NFE and EE contents with lower CP, CF and ash contents in hydroponically grown oat fodder with growing period of 6 days. Average DM, CP, CF, NFE, EE and ash contents were 89.7%, 12.3%, 10.1%, 69.5%, 4.9% and 3.2%, respectively. The proximate composition of hydroponic fodder may vary due to factors such as implemented hydroponic techniques, composition of nutrient solution, plant species and varieties, environmental conditions, water quality, growth stage, stress factors and harvesting time.

5. Conclusion

This study highlights feasibility of hydroponic technology when implemented on various fodder species in tropical region like Bangladesh. This technology can be utilized if traditional agricultural system faces significant challenges. The comparative analysis of biomass yield, plant height, root length, and chlorophyll content of maize, wheat, oat, sorghum, and cowpea indicate that hydroponic cultivation can effectively be used for fodder cultivation and higher nutrient contents of these hydroponic fodder can supply sufficient nutrients. Oat and cowpea demonstrated high biomass production. This experiment represents potential of hydroponic technology in the tropical climate of southwestern region of Bangladesh and it can be used to mitigate fodder shortages and improve livestock nutrition sustainability. For supplementation of nutrients to the animals, it can also be combined with cultivated fodder. It offers a promising solution to meet the rising demand for animal feed and supply them with nutrients. The adoption of this technology could benefit farmers in increasing agricultural productivity and sustainability in fodder production when there is a lack of cultivable land or traditional agricultural system face challenges.

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Research

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Research on Heavy Metal Content of Fattening and Dairy Feeds

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A B S T R A C T

Heavy metal pollution for the world is reaching more alarming dimensions every day. Soil, water and air are polluted due to industrial developments, industrial wastes and heavy metals are included in the food chain through crop production and pose a risk to all living things. Feed is one of the most important links in this chain. In this study, 25 feed samples, including 18 fattening feeds and 7 dairy feeds, which were on commercial sale in different provinces in Türkiye in 2023, were obtained and As, Cd, Cr, Cu, Fe, Ni and Pb concentrations were determined. As content varied between 0.00-0.06 mg kg^{-1}, Cd content ≤0.01 mg kg^{-1}, Cr content 0.00-0.74 mg kg^{-1}, Cu content 2.29-30.79 mg kg^{-1}, Fe content 13.16-43.99 mg kg^{-1}, Ni content 0.39-1.88 mg kg^{-1} and Pb metal was not detected. None of the heavy metal concentrations exceeded the permissible limit values. Although Fe and Cu concentrations did not exceed the permissible limit values, they were found to be even lower than the recommended amounts in feeds. No heavy metal contamination was found in the 25 feed samples examined, but it would be appropriate to check the heavy metal levels of feeds at regular intervals due to the rapid increase in environmental pollution and the risk of contamination of crop production and the food chain, particularly in regions where traditional fodder cultivation is constrained.

1. Introduction

Metals with a density exceeding 5 g cm^{-3} are defined as heavy metals (Gao et al., 2024). While trace elements such as manganese, copper and zinc are essential for the life of living organisms, heavy metals such as lead, cadmium, chromium, arsenic and nickel are not essential and do not play a vital role in the biological processes of living organisms. Heavy metals can naturally occur in trace amounts in soil and their excessive accumulation can degrade soil quality and harm to plants. Cr, As, Ni, Cd, Pb, Cu and Zn are recognized as priority toxic pollutants by the United States Environmental Protection Agency (USEPA) (Cheng et al., 2023). Heavy metals such as lead, cadmium, arsenic and mercury are released into the environment in large quantities by the developing industry and activities of various industrial branches and pose a danger to living organisms, including humans (Dinakar et al., 2008; Kusvuran et al., 2016; Alzahrani et al., 2018). At the same time, fertilizers used in agricultural production have long-term effects on heavy metal accumulation in soils. Some inorganic fertilizers contain a certain amount of heavy metal contamination and long-term application of fertilizers can lead to heavy metal accumulation in

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soils (Carnelo et al., 1997; Deng, 2024). Due to the intensive production methods applied in agriculture and the rapidly developing industry, it is stated that the problems related to heavy metal, aflatoxin, and pesticide contamination in the feedstuffs produced and their final product, compound feed, are increasing and need to be eliminated (Dagasan and Ozen, 2011). When animals consume contaminated feeds, harmful components such as heavy metals and pesticides can reach levels that threaten human health by passing into final products such as meat, eggs, and milk. In order to prevent animal foods from becoming harmful, the feeds used should be kept under control (Kurtoglu and Coskun 2001).

Merako (2010) reported that arsenic (As) was found in 11%, lead (Pb) in 22%, cadmium (Cd) in 78%, copper (Cu) and zinc (Zn) in 100% of 27 feed samples and that lead (Pb) content in two of the samples was above the limit values required in feeds. Dagasan and Ozen (2011) analyzed some heavy metals in milk, beef fattening, lamb fattening, egg cage, and broiler feed samples taken from compound feed factories in 5 regions where animal husbandry and compound feed production is the most intensive. They reported that the amount of Hg in two of the feed samples and Pb in one of them exceeded the maximum values allowed in the regulation.

There are different commercial feeds available in the market for ovine, bovine and poultry farming. The content of these feeds is under the influence of many factors. Heavy metal concentrations in feeds vary depending on the plant species, the heavy metal level of the soil in which the plant grows, the properties of feed additives and many other reasons.

As a result of the literature review, it was observed that similar studies on dairy and fattening feeds were conducted in 2011 and before. Due to the increasing environmental pollution and the increasing heavy metal contamination, there is a need for heavy metal screening of commercial feeds in the market and obtaining up-to-date data.

In this research, it was aimed to investigate the heavy metal contents of commercially available dairy and fattening feeds, to determine whether the results obtained exceed the permissible limit values in our country and in different countries and to evaluate the heavy metal risk in these feeds.

2. Materials and Method

This study investigated dairy and fattening feeds of 25 different companies in the market in 2023. In the study, 18 different fattening feeds and 7 different dairy feeds were used. The website of the Ministry of Agriculture and Forestry contains a list of feed facilities active in Türkiye (Anonymous, 2024). Feed samples were tried to be obtained by taking care to take one sample from each province from the regions where the enterprises are concentrated. In addition, care was taken to select the sampled firms from the largest establishments operating in that province.

The label information of a general dairy feed and a general fattening feed is presented in Table 1 for informative purposes.

Table 1. Nutrient content information of fattening and dairy feeds in general

	Cattle fattening feed				Cattle dairy feed		
Crude protein	16%	Fe	50 mg kg^{-1}	Crude protein	21%	Fe	50 mg kg^{-1}
C. cellulose	11%	I	0.8 mg kg^{-1}	C. cellulose	8.50%	I	0.8 mg kg^{-1}
Ash	10%	Co	0.15 mg kg^{-1}	Ash	9.50%	Co	0.1 mg kg^{-1}
C. Oil	2.40%	Cu	20 mg kg^{-1}	C. Oil	3%	Cu	10 mg kg^{-1}
Sodium	0.45%	Mn	50 mg kg^{-1}	Sodium	0.40%	Mn	50 mg kg^{-1}
Vitamin D_3	2000 IU kg^{-1}	Zn	50 mg kg^{-1}	Vitamin D_3	3000 IU kg^{-1}	Zn	100 mg kg^{-1}
Vitamin E	20 mg kg^{-1}	Se	0.15 mg kg^{-1}	Vitamin E	40 mg kg^{-1}	Se	0.3 mg kg^{-1}
Vitamin A	7000 IU kg^{-1}			Vitamin A	9000 IU kg^{-1}		

Note: Compiled from the label information of private companies for informational purposes.

The provinces where the firms are located are given in Table 2 and shown in Figure 1. Approximately 100 g samples were taken from the feeds and dried in an oven at 70 °C and the dry weight feed samples were ground to achieve homogeneity. The dried and ground samples were subjected to microwave digestion (Miller, 1998) and then metal concentrations were determined by ICP-MS (Inductively Coupled Plasma Mass Spectrometry).

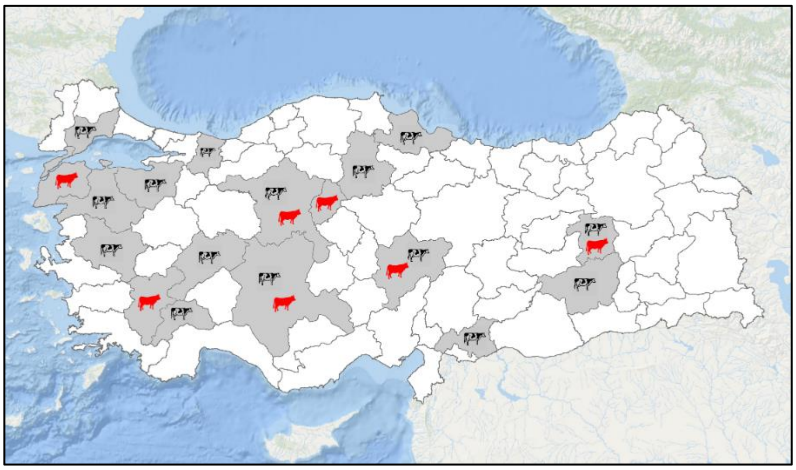

Figure 1. Map view of the provinces where fattening and dairy feed companies/factories are located (Red color represents dairy feed and black color represents fattening feed)

Table 2. Provinces where fattening and dairy feed companies/factories are located

Fattening feed / Province where the firm is located		Dairy feed/ Province where the firm is located
Afyon	Gaziantep	Ankara
Ankara	Gaziantep	Bingöl
Balıkesir	Kayseri	Çanakkale
Bingöl	Kayseri	Denizli
Burdur	Konya	Kayseri
Bursa	Manisa	Kırıkkale
Çorum	Sakarya	Konya
Diyarbakır	Samsun	
Gaziantep	Tekirdağ	

Microwave digestion: The digestion process was modified according to the method described in the literature (Miller, 1998). After the feed samples were dried and ground, they were weighed 1 g and transferred to the vessels of the microwave device (CEM-MARS 6) and 10 ml of nitric acid (HNO3) was added. Adjustments were made for the microwave digestion process. After appropriate dilution and filtration, the samples were measured by ICP-MS (Inductively coupled plasma mass spectrometry).

- Calculation process: Total As, Cd, Cr, Cu, Fe, Ni, Ni, Pb in feed (mg kg-1) = It x F (1)

- It = the measurement value of the feed solution adjusted according to the sample solution

- F= dilution factor/ sample amount

As a result of the necessary calculation procedures, heavy metals in feeds were determined as mg kg^{-1}.

3. Results

3.1.Heavy metal concentrations in fattening feeds (As, Cd, Cr, Cu, Fe, Ni and Pb mg kg^{-1})

As, Cd, Cr, Cu, Fe, Ni and Pb contents of fattening feeds obtained from eighteen (18) different companies were analyzed and shown in Table 3. The lowest As concentration was 0.00 mg kg^{-1} in feed sample number 15 from Manisa province, the highest As concentration was 0.06 mg kg^{-1} in feed samples from 9 provinces and the average As concentration was 0.05 mg kg^{-1} in all feed samples. Cadmium (Cd) concentration was ≤0.01 mg kg^{-1} in all feed samples. The lowest Cr concentration of 0.00 mg kg-1 was determined in feed samples from Bingöl, Kayseri and Tekirdağ provinces, while the highest Cr concentration of 0.74 mg kg^{-1} was determined in sample number 10 from Gaziantep province. The average Cr content of all feed samples was 0.31 mg kg^{-1}. The lowest Cu concentration of 2.63 mg kg^{-1} was determined in feed sample number 4 from Bingöl and the highest Cu concentration of 30.79 mg kg^{-1} was determined in sample number 7 from Çorum.

The average Cu content of all feed samples was 7.60 mg kg^{-1}. The lowest Fe concentration was determined as 13.16 mg kg^{-1} in feed sample number 3 from Balıkesir province and the highest Fe concentration was determined as 43.99 mg kg^{-1} in sample number 10 from Gaziantep province. The average Fe content of all feed samples was 22.72 mg kg^{-1}. The lowest Ni concentration of 0.39 mg kg^{-1} was determined in feed sample number 13 from Kayseri province and the highest concentration of 1.88 mg kg^{-1} was determined in sample number 10 from Gaziantep province. The average Ni content of all feed samples was 0.94 mg kg^{-1}. Pb metal was not detected in the analyzed fattening feed samples.

Table 3. Heavy metal concentrations of fattening feeds (mg kg^{-1})

		Concentrations (mg kg^{-1})						
Sample No.	**Province**	**As**	**Cd**	**Cr**	**Cu**	**Fe**	**Ni**	**Pb**
1	Afyon	0.05	0.01	0.32	11.32	20.04	1.16	ND
2	Ankara	0.05	0.01	0.30	8.20	16.74	0.81	ND
3	Balıkesir	0.05	0.01	0.30	6.50	13.16	0.92	ND
4	Bingöl	0.05	0.01	0.00	2.63	18.09	0.44	ND
5	Burdur	0.05	0.01	0.30	3.58	32.41	0.46	ND
6	Bursa	0.06	0.01	0.36	4.86	21.37	1.10	ND
7	Çorum	0.06	0.01	0.34	30.79	22.17	1.40	ND
8	Diyarbakır	0.06	0.01	0.66	2.78	35.04	1.14	ND
9	Gaziantep	0.06	0.01	0.42	4.74	13.62	0.97	ND
10	Gaziantep	0.06	0.01	0.74	3.08	43.99	1.88	ND
11	Gaziantep	0.06	0.01	0.52	4.54	31.24	1.12	ND
12	Kayseri	0.06	0.01	0.00	6.68	15.78	0.81	ND
13	Kayseri	0.05	0.01	0.00	4.28	15.84	0.39	ND
14	Konya	0.06	0.01	0.45	7.63	30.56	0.87	ND
15	Manisa	0.00	0.01	0.35	7.85	24.57	0.74	ND
16	Sakarya	0.05	0.01	0.27	7.34	19.46	0.94	ND
17	Samsun	0.06	0.01	0.28	6.04	19.01	0.72	ND
18	Tekirdağ	0.05	0.01	0.00	14.05	15.81	0.99	ND
	Minimum	0.00	0.01	0.00	2.63	13.16	0.39	
	Maximum	0.06	0.01	0.74	30.79	43.99	1.88	
	Mean	0.05	0.01	0.31	7.60	22.72	0.94	
	Standard Dev.	0.00	0.00	0.21	6.32	8.31	0.35	
Requirement for mineral nutrition (mg kg^{-1}) (Hejna ve ark., 2018)		nr	nr	-	10	50	-	nr
Maximum tolerable levels (MFAL,Notification, 2014/11) (mg kg^{-1})		2	1	-	-	-	-	10
Maximum tolerable level of trace elements (NRC, 2005) (mg kg^{-1})		30	10	100	40	500	100	100

ND:Not Detected, nr:not required

3.2. Heavy metal concentrations in dairy feeds (As, Cd, Cr, Cu, Fe, Ni and Pb mg kg^{-1})

The As, Cd, Cr, Cu, Cu, Fe, Ni and Pb contents of dairy feeds obtained from seven different companies were analyzed and shown in Table 4. The lowest As concentration was 0.05 mg kg^{-1} and the highest 0.06 mg kg^{-1} and the average As concentration was 0.06 mg kg^{-1} in all feed samples. Cadmium (Cd) concentration was ≤0.01 mg kg^{-1} in all feed samples. The lowest Cr concentration of 0.00 mg kg^{-1} was determined in feed samples from Bingöl, Çanakkale and Kayseri provinces, while the highest concentration of 0.39 mg kg^{-1} was determined in sample number 6 from Kırıkkale province. The average Cr content of all feed samples was 0.20 mg kg^{-1}. The lowest Cu concentration of 2.29 mg kg^{-1} was determined in feed sample number 2 from Bingöl and the highest Cu concentration of 23.42 mg kg-1 was determined in sample number 5 from Kayseri province. The average Cu content of all feed samples was 8.38 mg kg^{-1}. The lowest Fe concentration was 13.35 mg kg^{-1} in feed sample number 5 from Kayseri province and the highest Fe concentration was 25.24 mg kg^{-1} in sample number 7 from Konya province. The average Fe content of all feed samples was 18.36 mg kg^{-1}. The lowest Ni concentration was 0.56 mg kg^{-1} in the feed sample number 2 from Bingöl province and the highest was 1.41 mg kg^{-1} in the sample number 6 from Kırıkkale province. The average Ni content of all feed samples was 0.88 mg kg^{-1}. Pb metal was not detected in the analyzed dairy feed samples.

Table 4. Heavy metal concentrations of dairy feeds (mg kg^{-1})

		Concentrations (mg kg^{-1})						
Sample No.	**Province**	**As**	**Cd**	**Cr**	**Cu**	**Fe**	**Ni**	**Pb**
1	Ankara	0.06	0.01	0.34	6.86	19.72	1.09	ND
2	Bingöl	0.05	0.01	0.00	2.29	18.00	0.56	ND
3	Çanakkale	0.06	0.01	0.00	3.59	18.74	0.64	ND
4	Denizli	0.06	0.01	0.35	6.02	13.82	0.86	ND
5	Kayseri	0.06	0.01	0.00	23.42	13.35	0.76	ND
6	Kırıkkale	0.06	0.01	0.39	6.61	19.68	1.41	ND
7	Konya	0.06	0.01	0.30	9.90	25.24	0.86	ND
	Minimum	0.05	0.01	0.00	2.29	13.35	0.56	
	Maximum	0.06	0.01	0.39	23.42	25.24	1.41	
	Mean	0.06	0.01	0.20	8.38	18.36	0.88	
	Standard Dev.	0.00	0.00	0.17	6.54	3.72	0.27	
Requirement for mineral nutrition (mg kg^{-1}) (Hejna ve ark., 2018)		nr	nr	-	10	50	-	nr
Maximum tolerable levels (MFAL,Notification, 2014/11) (mg kg^{-1})		2	1	-	-	-	-	10
Maximum tolerable level of trace elements (NRC, 2005) (mg kg^{-1})		30	10	100	40	500	100	100

ND:Not Detected, nr:not required

The average heavy metal contents of fattening and dairy feeds are given in Figure 2. As seen in Figure 2, Fe was the most detected heavy metal, followed by Cu, Ni and Cr. The lowest values in fattening and dairy feeds were Cd and As. Pb was not detected in fattening and dairy feeds. As can be seen from the graph, Cr, Fe and Ni concentrations were higher in fattening feeds than in dairy feeds.

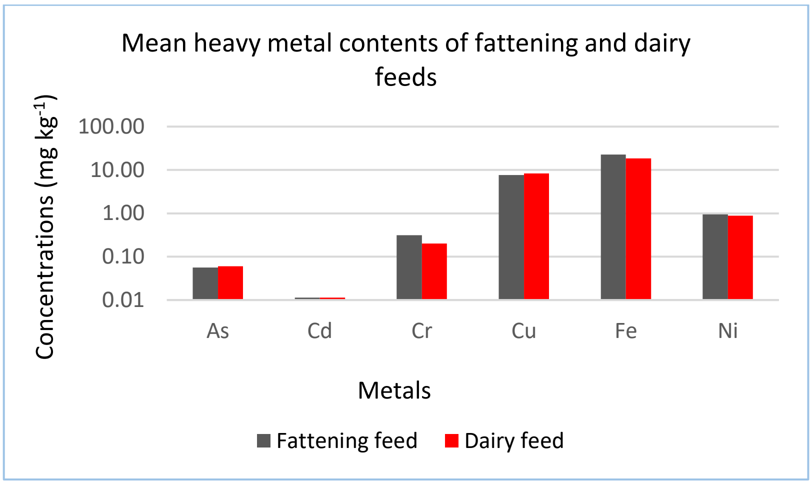

Figure 2. Graph of average heavy metal contents of fattening and dairy feeds

4. Discussion

Besides heavy metals (Cd, Pb, Hg, As, Cr), which are undesirable in animal nutrition, metals that are considered essential microelements (Fe, I, Co, Cu, Mn, Mo, Se) can also have direct potential negative effects on livestock. These metals can also enter the food chain through the consumption of animal products and thus pose a risk to humans (Järup, 2003). The As content in the 25 different dairy and fattening feed samples examined varied between 0.00-0.06 mg kg^{-1}. In the notification published by the Ministry of Food, Agriculture and Livestock (MFAL) (Regulation on Undesirable Substances in Feed, 2014/11), the maximum acceptable amount for As was reported as 2 mg kg-1 and the maximum tolerable As level was reported as 30 mg kg-1 according to NRC (2005) and none of the feed samples examined exceeded these permitted limit values. In 100 different dairy, fattening, egg and broiler feeds collected from different regions of Türkiye, As levels exceeded the permissible limit in only 2 samples (2.45 and 2.98 mg kg^{-1}) (Dagasan and Ozen, 2011). The average As content of fattening feeds produced in Texas between 2012 and 2015 was 0.15 mg kg^{-1} (Dai et al., 2016).

Cd content of the feeds was determined as ≤0.01 mg kg^{-1}. According to MFAL (2014/11), the maximum acceptable level of Cd was reported as 1 mg kg^{-1} and according to NRC (2005), the maximum tolerable level of Cd was reported as 10 mg kg^{-1} and none of the feed samples examined exceeded these permitted limit values. In previous studies, Cd levels in dairy feeds were determined as 0.005-0.082 mg kg^{-1} by Dai et al. (2016) in USA and 0.00-23.25 mg kg^{-1} by Zhang et al. (2012) in China.

Cr concentrations of dairy and fattening feeds examined varied between 0.00-0.74 mg kg^{-1}. According to MFAL (2014/11), the maximum acceptable amount of Cr was not stated, but according to NRC (2005), the maximum tolerable level of chromium (Cr^{3+}) was reported as 100 mg kg^{-1} and none of the feed samples examined exceeded these permitted limit values. In a study by Dai et al. (2016), the average Cr concentration in 13 cattle feeds was 4.91 mg kg^{-1}. Besides the toxic and adverse effects of Cr(VI), there are studies showing that supplementation of Cr(III) at certain levels is beneficial. The US Food and Drug Administration Center for Veterinary Medicine has allowed Cr propionate (Cr Prop) to be used to supplement cattle diets up to 0.5 mg kg^{-1} DM. Chromium supplementation from Cr Prop was found to improve insulin sensitivity in growing cattle (Spears et al., 2012). Recent studies have shown that Cr Prop supplementation can improve the performance and health of calves under stress (Bernhard et al., 2012) and increase milk production in dairy cows (Vargas-Rodriguez et al., 2014; Rockwell and Allen, 2016). Spears et al. (2017) found Cr concentration below 0.05 mg kg^{-1} in all samples examined in 103 feed stuffs.

Cu is another important mineral closely related to animal production. When added to the diet of some animals, this trace element causes faster growth and better feed conversion rate (Polen and Voia, 2015) and prevents anemia in animals (Suleiman et al., 2015). However, high amounts of Cu are an environmental concern and can enter the human food chain through the consumption of contaminated products of animal food source (Alfthan et al., 2015). Cu concentrations of dairy and fattening feeds varied between 2.29-30.79 mg kg^{-1}. According to MFAL (2014/11), the maximum acceptable amount of Cu was not specified, while according to NRC (2005), the maximum tolerable Cu level was reported as 40 mg kg^{-1} and none of the feed samples examined exceeded these permitted limit values. However, the requirement of 10 mg kg^{-1} Cu in feeds in mineral nutrition has been reported (Hejna et al., 2018). While Cu content was <10 mg kg^{-1} in 15 of the 18 fattening feeds examined, Cu content was below 10 mg kg^{-1} in 6 of the 7 different dairy feeds examined. Among the 25 different dairy and fattening feeds produced in Türkiye, only 4 of them reached the recommended level for mineral nutrition, while 19 feeds were deficient in terms of Cu content. Tufan (2008) reported that Cu content in 30 feed raw materials in Tekirdağ province varied between 2.21-4.50 mg kg^{-1}. According to a study conducted by Wang et al. (2013) in China, Cu content in feeds was found to be 15.7 mg kg^{-1}. Fe concentrations of the feeds varied between 13.16-43.99 mg kg^{-1}. According to MFAL (2014/11), the maximum acceptable amount of Fe was not specified, but according to NRC (2005), the maximum tolerable Fe level was reported as 500 mg kg^{-1} and none of the feed samples examined exceeded these permissible limit values. However, it has been reported that feeds should contain 50 mg kg^{-1} Fe in mineral nutrition (Hejna et al., 2018). In 25 different fattening and dairy feeds examined, no toxic level of Fe content was found, but the recommended Fe content in feeds was not determined and only 1 fattening feed was found to have Fe concentration close to this recommended value. In Fe deficiency in animals, growth suppression and decreased blood levels are observed (Rincker et al., 2004). Again, iron deficiency in animals can lead to decreased animal performance, loss of appetite and weight, breathing spasms and ultimately death (Underwood, 2012; Byrne and Murphy, 2022). Fe content was determined as 0.6-6.2 mg kg^{-1} in feed samples taken as pallet feed, straw and barley mash (Bilgucu, 2010). Tufan (2008) examined wheat, barley and sunflower as feed raw materials and reported Fe contents as 29.11-109.13 mg kg^{-1}.

Fe and Cu concentrations in feeds were generally below the recommended level. The reason why Fe and Cu concentrations in the feeds were not at the recommended level is thought to be due to the limited uptake of Fe and Cu in the soils where the plants in the feed content grow. The pH of most of the soils in Türkiye is above 7 and the lime content is high (Ucgun et al., 2019). Microelement uptake is low in soils with high pH levels. The high pH value, low organic matter and moisture value of the soils of our country reduce the availability of microelements present in the soil to plants (Eraslan et al., 2010).

In Europe, Regulation 2002/32/EC sets limits for undesirable substances such as As, Cd, Pb and Hg in animal feed. Although maximum limits are set for As, Cd, Pb and Hg, animal feed can be contaminated with other heavy metals such as nickel (Ni) and Cr due to the production process. For example, Ni has been reported to be immunotoxic and neurotoxic and may be carcinogenic (ATSDR, 2011). The Ni concentrations of the feeds varied between 0.39-1.88 mg kg^{-1}. According to MFAL (2014/11), the maximum acceptable amount of Ni was not specified, but according to NRC (2005), the maximum tolerable Ni level was reported as 100 mg kg^{-1} and none of the feed samples examined exceeded these permitted limit values. The average Ni content of 154 fattening feeds was reported to be 2.81 mg kg^{-1} (Dai et al., 2016). Ni concentration levels have been studied in different feeds in Europe, England and Wales (Nicholson et al., 1999) and Bulgaria (Alexieva et al., 2007). These levels ranged from 0.1-11.2 mg kg^{-1} for dairy feed and 0.2-8.3 mg kg^{-1} for fattening feed. Notably, the highest Ni concentrations were measured in oats and barley (Nicholson et al., 1999). Alexieva et al. (2007) observed Ni levels of up to 16 mg kg^{-1} in other feed ingredients and found the highest levels in wheat (up to 14 mg kg^{-1}) in cereal feed (EFSA, 2019).

Exposure to high levels of Pb in animals causes harmful effects on many organs, as well as decreased feed consumption and growth ratio (Taha et al. 2019). Pb was not detected at ppm (mg kg^{-1}) level in all fattening and dairy feeds examined. Dagasan and Ozen (2011) stated that out of 100 different feed samples they examined, only 1 feed sample exceeded the permissible limit value. In earlier studies, it was found that lead levels measured in plants and soils growing on roadsides with high traffic density often exceeded safety limits. The decrease over the years is thought to be related to the use of unleaded petrol after 2004 (Ogutucu et al.,2021).

5. Conclusion

Twenty-five fattening and dairy feeds sold in the market in different provinces were examined in terms of some heavy metals and compared according to the limit values permitted by the institutions. Among the elements As, Cd, Cr, Cu, Fe, Ni, and Pb, Pb was not detected in the feeds, while As, Cd, Cr, and Ni were found much below the limit values. Although Fe and Cu contents are below the maximum permitted values, they are recommended to be used in animal nutrition at certain levels, but none of the 25 different feed samples examined had the recommended Fe content and 19 did not have the recommended Cu content. No heavy metal toxicity was found in the fattening and dairy feeds examined, but increasing environmental pollution causes an increase in heavy metals in soil, water, and air. There will always be a risk of heavy metals contaminating animal feed through crop production. In future studies, it is recommended to include other feed types in the study, to examine a much larger number of feed samples, to compare the results of this study with new data, and to take measures according to the predicted contamination rate.

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The Effect of the Roughage Production Project on Forage Crops Agriculture in Kırşehir

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This study aimed to determine the effects of the Roughage Production Project on forage crop agriculture, which was carried out to introduce and extend forage crops to the region's producers and show how to produce cheap and high-quality roughage. In the research, a mixture of 70% Hungarian vetch and 30% triticale was grown on the lands of farmers selected from the region's producers, especially those engaged in animal production, during growing seasons between 2018 and 2024. Because forage crop agriculture is not sufficiently developed in the region, in the production carried out within the project's scope, one-on-one application was made to the producers in the field in all processes from planting to harvest. During the project, 1363 tons of quality roughage was produced on the areas of 6270 decares with 67 producers in seven production seasons. The Roughage Production Project practices have contributed not only to seed and fertilizer support for the producer but also to a change of habits through technical support provided at every stage of production, to producers' willingness to cultivate forage crops, and to the development of forage crop culture with mixtures created with species and varieties suitable for the ecology of the region. As a result, the Roughage Production Project has made a significant contribution to raising regional producers' awareness of producing their own quality roughage and establishing forage crop culture.

1. Introduction

Feed expenses constitute approximately 70% of operating expenses in livestock enterprises (Alçiçek, 2002; Budağ & Keçeci, 2013). Roughage, which is called dry, green, or silage feed, which is rich in cellulose and has low digestibility and energy value, can be included in rations at a rate of 25-80%, depending on the type of animal feeding (Kutlu & Çelik, 2010; Özkan & Şahin Demirbağ, 2016). The most important source of quality roughage used in animal feeding is forage crop farming and natural pastures (Yavuz et al., 2020). Unfortunately, in Türkiye, due to uncontrolled grazing that has been going on for a long time, both plant coverage of pastures and their ability to produce high quality forage have decreased significantly, and they have become unable to meet the needs of the livestock in Türkiye (Sürmen et al., 2008; Yildiz & Özyazici, 2017).

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According to the calculation made based on the forage crop production and livestock data of TÜİK (2024), Türkiye can meet approximately 41% of the quality roughage needs of 16.7 million animal units. The fact that the desired level has yet to be reached in forage crop agriculture for a very long time has resulted in using intensive cereal straw to close the quality roughage gap. This negative situation regarding quality roughage production in Türkiye manifests itself in Kırşehir under more severe conditions. When the total roughage requirement and production of Kırşehir province were calculated according to TÜİK (2024) data, only 9.5% of the need for 252 thousand animal units was met. Undoubtedly, this is due to the forage crop agriculture carried out on only 6 thousand hectares of arable land in the city and cereal straw is used much more intensively than the country average. Kir et al. (2018) emphasized that the most suitable species for quality forage crop production in Kırşehir ecology were Hungarian vetch (*Vicia pannonica* Crantz.) and triticale (X *Triticosecale* Wittm.), and the most suitable mixture to be created with these species was a mixture of 70% Hungarian vetch and 30% triticale.

The inadequacy of forage crop production in Kırşehir, or the roughage deficit, is related to producer habits rather than negative environmental factors. The aim of the Roughage Production Project, supported by the Strategy and Budget Directorate of the Presidency of the Republic of Türkiye and carried out within the Pilot Agriculture and Geothermal Coordinatorship of Kırşehir Ahi Evran University, is to show producers engaged in plant and animal production in the region how to produce cheap and high-quality roughage, and to introduce and extend the culture and agriculture of forage crops. The Roughage Production Project applications were distinguished from other forage crop support project applied by extension services of Turkish Ministry of Agriculture and Forestry through the technical support provided at every stage of production and the seed and fertilizer support given to the producer. This difference increases the study's originality. In this presented study, the effects of the Roughage Production project on forage crop agriculture in Kırşehir were examined.

2. Materials and Methods

The Roughage Production Project farmer practices were carried out in 25 different locations, including twelve villages in the Central district, one village in the Akçakent district, two villages in the Boztepe district, four villages in the Mucur district, and six villages in the Kaman district (Figure 1).

Figure 1. Locations of the application area of Roughage Production Project

Within the scope of the project, to introduce and extend forage crop agriculture, a mixture of 70% Hungarian vetch and 30% triticale was grown in the selected farmer lands, especially among livestock producers, between the 2018-19 and the 2023-24 production seasons under contract with 67 producers. In the mixture prepared with the classical method, 14 kg of certified seed per decare was used, including 8 kg Hungarian vetch and 6 kg triticale. Along with sowing, 12-15 kg of diammonium phosphate was applied according to the soil analysis results, and 3 kg of pure nitrogen ammonium sulfate fertilizer was applied as topdressing.

Since forage crop agriculture is not sufficiently developed in the region, the producers were given one-on-one training in the field in all processes from sowing to harvest, such as soil preparation, seeder adjustment, and determination of top fertilization time or cutting time, in the productions carried out within the project's scope. The project budget covered all seed and fertilizer expenses used in the project, and an average of 20% of the obtained roughage was taken as the project management share.

The Forage Production Project's impact on farmers' production habits was evaluated through the feedback of the project participants. In a survey conducted in October 2023, the participants were specifically asked three questions: Have you ever sown a Hungarian vetch-triticale mixture before? Did the project contribute to your knowledge of forage crop farming? Are you satisfied with the project results? In addition, to determine the contribution of the Roughage Production Project to the forage crop production areas in Kırşehir, the production area and production amount of Hungarian vetch and triticale from TÜİK data between 2016 and 2023 were compared.

3. Results and Discussion

In the roughage production project, between the 2017-2018 season, when the first project application was made, and the 2020-2021 production seasons, 63 tons of quality roughage from the mixture of Hungarian vetch and triticale was obtained in an area of 355 decares in the university application areas. Within the project's scope, 67 farmers produced 1300 tons of quality roughage in an area of 5915 decares with the cooperation of the project personnel in the 2018-2019 and 2023-2024 growing seasons (Table 1).

Table 1. Growing areas and production amounts of quality roughage within the scope of the Roughage Production Project

Production Season	Number of Farmers	Area (da)	Production (tons)
2018-2019	5	238	62
2019-2020	7	714	160
2020-2021	11	1567	154
2021-2022	30	2748	678
2022-2023	5	259	129
2023-2024	9	389	117
2017-2021	UAA*	355	63
Total	**67**	**6270**	**1363**

*UAA: University Application Areas

Before the start of the Roughage Production Project in Kırşehir, the growing areas of triticale and Hungarian vetch in the year of 2016 were 625 decares and 150 decares, respectively. In the year of 2023, these growing areas increase to 5464 decares for triticale and 3630 decares for Hungarian vetch (Table 2). Support program of the Ministry of Agriculture and Forestry for forage crops production since the year of 2000 has played a vital role in the increase of forage growing areas in Türkiye (Anonymous, 2024; Can et al., 2024; Merdan, 2024; Turan et al., 2015; Yavuz et al., 2020). However, it is impossible to say that all of this development in forage crop production between 2016 and 2023 in Kırşehir is due to the Ministry's support.

Table 2. Changes in Hungarian Vetch and Triticale Production in Kırşehir Between 2016-23*

Years	Triticale		Hungarian Vetch	
	Area (da)	Production (tons)	Area (da)	Production (tons)
2016	625	140	150	135
2017	1700	535	7330	6027
2018	2124	781	5437	4513
2019	1766	610	4603	3846
2020	833	262	4994	4702
2021	4304	874	22743	17869
2022	4377	1259	11253	9834
2023	5434	1367	3630	2446

*(TÜİK, 2024)

The support program of the Ministry of Agriculture and Forestry has significantly contributed to sustainability by increasing production, it's clear that forage crop production has not yet reached the desired level, indicating a need for further improvement. Although increasing agricultural support increases producers' incomes, the success of the support is related to the producers' expectations and satisfaction (Çetin & Olhan, 2024).

According to the results of the survey conducted with project participant farmers in October 2023 to determine the effects of the Forage Production Project, 97.1% of the farmers stated that the project contributed to their knowledge of forage crop farming and said that they were satisfied with the project results, and 94.3% of them continued to grow the mixture of Hungarian vetch and Triticale after leaving the project. The high satisfaction and sustainability rates in the survey results are not only because the project covers the sowing costs but also due to the practical training provided in the field at every stage. These practices have significantly contributed to farmers' recognition of the forage crop culture and their knowledge of the subject. Therefore, it can be said that the Roughage Production Project was at least as effective as the support provided by the Ministry of Agriculture and Forestry in increasing the production of forage crops in Kırşehir province. Therefore, it is possible to say that the Roughage Production Project is more effective than the support provided by the Ministry of Agriculture and Forestry in the increase in the production of forage crops in Kırşehir.

4. Conclusion

According to the results of farmer applications in the Roughage Production Project, in addition to the financial support provided through the project to the producer, the technical support provided at every stage of production has made a significant contribution to the establishment and development of the forage crop culture by changing producer habits, making producers willing to do forage crop farming, and creating awareness of producing the quality roughage they need.

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Research

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Effects of Zinc on the Yield and Quality of Forage Pea [*Pisum sativum* ssp. *arvense* (L.) Poir.]

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A B S T R A C T

Zinc (Zn) is one of the most important micronutrients that can increase the growth, yield attributes, yield, quality and nutritional value of plants. This study aimed to evaluate the effects of zinc sulphate ($ZnSO_4 \cdot 7H_2O$) application at different concentrations (0, 5, 10, 15, and 20 kg ha^{-1}) on forage yield and quality and mineral content of the plant in forage pea [*Pisum sativum* ssp. *arvense* (L.) Poir.] (cv. Özkaynak) under semi-arid climate conditions. The response variables included stem diameter, plant height, green forage yield, hay yield, crude protein (CP), acid detergent fiber, neutral detergent fiber, total phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg). As a result of the research, it was determined that the Zn doses applied from the soil had meaningful effects on the green forage yield ($p<0.05$) and CP ($p<0.01$), total P ($p<0.05$) and Ca ($p<0.01$) contents of forage pea. The highest green forage yield of 43.60 t ha^{-1} was obtained at Zn dose of 10 kg ha^{-1}. Although it did not show statistically significant changes, improvements were also achieved in hay yield compared to the control at the same dose. In the study, Zn fertilization increased forage CP ratio significantly. In addition, soil Zn application also provided sufficient macronutrient accumulation in forage pea hay for ruminants. According to the research results, it was concluded that in the presence of low level extractable Zn in the soil, 10 kg Zn ha^{-1} application to forage pea would provide prominent increases in forage production, forage quality and nutritional value.

1. Introduction

Pea is one of the oldest crops grown for human food and animal feed, and in some places for green manure purposes (Delchev and Delchev, 2019; Özyazıcı and Açıkbaş, 2021). Pea which is the second most important legume crop in the world (Pawar et al., 2017) has a subspecies that has significant value as a forage and grain feed, *Pisum sativum* ssp. *arvense*, known as forage pea (Açıkgöz, 2001; Manga et al., 2003). With the help of its high biological nitrogen (N) fixation capacity, forage pea [*Pisum sativum* ssp. *arvense* (L.) Poir.] (Özyazıcı and Açıkbaş, 2021), which is an important component of sustainable agricultural systems that include both short-term crop rotation systems and green fertilization, is one of the most important roughage source of livestock enterprises with its high protein content and forage yield (Fraser et al., 2001).

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In recent years, focus has been placed on increasing the production of forage crops in field agriculture to meet the demand for roughage in animal production. This focus has led to an increase in the use of high-yielding forage crop varieties, intensive cultivation methods and micronutrient-free fertilization practices. This contributes to the reduction of essential micronutrients such as iron, copper, zinc (Zn) and manganese in agricultural soils. In this sense, Zn deficiency in agricultural soils has been particularly emphasized (Özyazıcı et al., 2015). Studies have shown that there is a geographical overlap between soils that is characterized by Zn deficient and plant populations experiencing Zn deficiency (Tarakçıoğlu et al., 2003; Özkutlu et al., 2015; Söylemez et al., 2017; Oya and Çimrin, 2023). Deficiency of micronutrients in soil can lead to nutrient imbalances in many plants, reducing the quality of crops and potentially affecting human and animal health (Barrett and Bevis, 2015).

Zinc has important functions in protein and carbohydrate metabolism and the activation of many enzymes (Sharma et al., 2010; Hassan et al., 2020). For this reason, Zn is one of the most important micronutrient elements required for optimum crop growth and development (Hanifuzzaman et al., 2022; Rion et al., 2022). Additionally, Zn directly affects yield and quality due to its remarkable effects on root and shoot growth throughout the growing season (Rengel, 2001; Priyanka et al., 2019).

Many of the minerals necessary for animals are easily met by plants due to their abundance in the soil (Reynolds-Marzal et al., 2021). However, some minerals, such as Zn, are generally found in soil in relatively low concentrations relative to other soil fractions (Reynolds-Marzal et al., 2021). This situation affects the uptake of some other nutrients, especially Zn and some other nutrients that interact with Zn, by plants and plays a role in the different concentrations of mineral contents of forages. When considering Zn application in agriculture, both soil and foliar applications play important roles in increasing plant growth and nutrient uptake (Özyazıcı, 2023; García-Latorre et al., 2024; Özyazıcı and Özyazıcı, 2024). Studies have shown that soil application can lead to increased Zn levels in the soil, which can positively affect plant growth and development (Toğay and Anlarsal, 2008; Özyazıcı, 2020; Boaretto et al., 2024; Devi et al., 2024).

This research aimed to evaluate the effects of soil Zn application at different concentrations on forage yield, quality and nutrient accumulation in forage pea (*P. sativum* ssp. *arvense*).

2. Materials and Methods

2.1. Study site

The study was carried out in the 2019-2020 growing season in the Siirt province (37°58'13.20" N - 41°50'43.80" E, 887 m) in the southeastern Türkiye (Figure 1), in the under rainfed semi-arid climate zone. Some physical and chemical properties of the research soil are given in Table 1. The soil of the research area is clayey in texture and slightly alkaline in character. Soil without salinity problems have a calcareous, low organic matter, high available phosphorus (P) and excess available potassium (K) content. The soil has a good level of available calcium (Ca) and magnesium (Mg) content, while the extractable Zn content is low (Table 1).

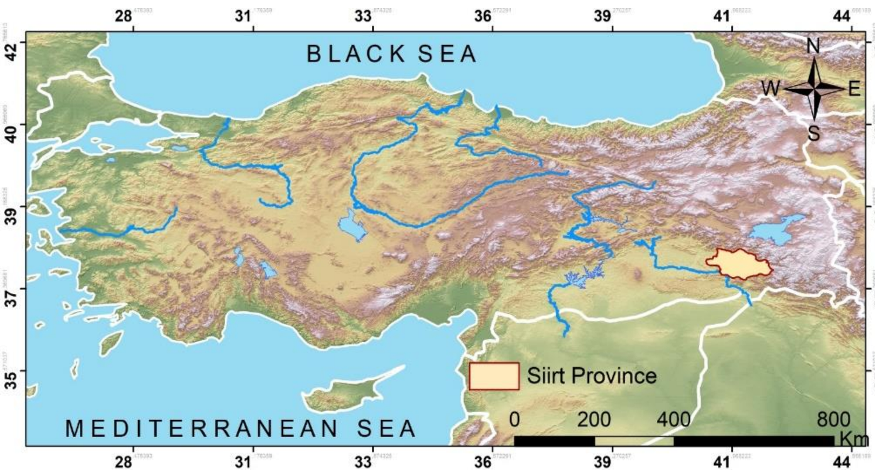

Figure 1. Research area location map

Table 1. Some physical and chemical properties of the soils where forage pea is grown (0-20 cm)*

Parameters	Unit	Value
Clay	%	38.9
Sand	%	43.1
Silt	%	18.0
pH		7.70
Electrical conductivity	dS m^{-1}	0.18
Calcareous ($CaCO_3$)	%	2.8
Organic matter	%	1.64
Available P	kg ha^{-1}	112
Available K	kg ha^{-1}	1882
Available Ca	kg ha^{-1}	11958
Available Mg	kg ha^{-1}	1114
Extractable Zn (DTPA)	ppm	0.79

*: The analyzes were carried out in the laboratory of Science and Technology Application and Research Center-Siirt University, $CaCO_3$: Calcium carbonate, DTPA: Diethylene triamine penta acetic acid

Monthly temperature averages during the period when the forage pea was grown were generally above the long-term average values. During the 7-month vegetation period, the total rainfall amount was 756.8 mm, while the long-term average of total rainfall in the same period was recorded as 652.0 mm. Especially in March and April very high amounts of precipitation has occurred which has supported crop development (Figure 2).

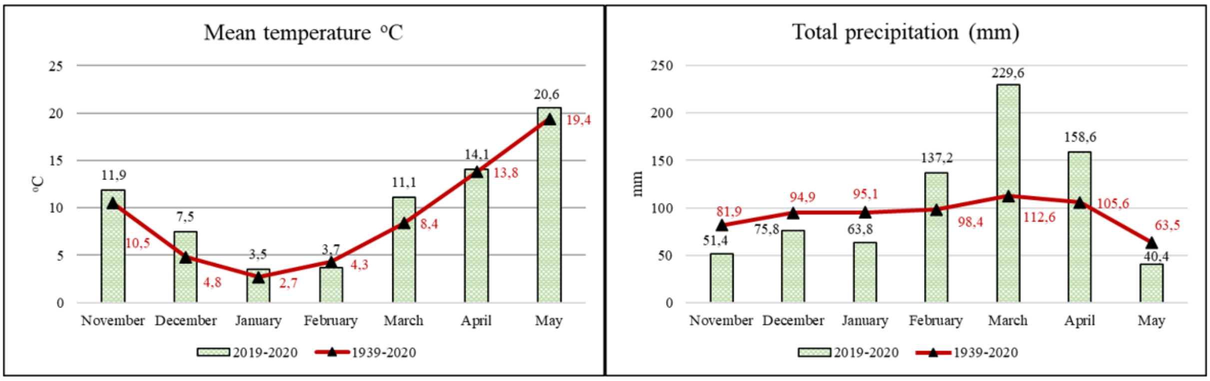

Figure 2. The long-term (1939-2020) and research year (2019-2020) average temperature and total precipitation data for the province of Siirt

2.2. Experimental design and crop management

Özkaynak forage pea (*P. sativum* ssp. *arvense*) variety was used as plant material in the research and 5 different doses of zinc (0, 5, 10, 15, and 20 kg ha^{-1}) were applied as zinc sulphate ($ZnSO_4 \cdot 7H_2O$). The field experiment was set up according to randomized complete block design with 3 replications. Plants were sown in each plot in 4 rows with 30 cm row spacing, and the plot size was 3.6 m^2 (1.2 m × 3 m).

The preliminary crop of the trial area was wheat, and after the wheat harvest, the field was deeply ploughed with a plough, and then the field was made ready for sowing by using a disc harrow, harrow and roller in the autumn of 2019. Before sowing, nitrogenous fertilizer (ammonium sulphate, 21% N) was applied homogeneously to all plots with the calculation of 40 kg ha^{-1} pure N, according to the soil analysis results (Table 1). Since the available P and K contents of the soil were sufficient, fertilization was not done for these elements. Zinc sulphate applied to the plots in different doses and mixed into the soil before sowing. The sowing process was carried out on 07 November 2019 with 100 live seeds per m^2 (Anonymous, 2019). The cutting process for the forage was carried out on May 13, 2020, when the plants were in full bloom in the research. During the cutting process, one row from the parcel edges and 50 cm sections from the parcel heads were removed to avoid edge effects.

2.3. Plant measurements and forage analyses

In the research, stem diameter, plant height, green forage yield, hay yield, crude protein (CP) ratio, acid detergent fiber (ADF) ratio, neutral detergent fiber (NDF) ratio, total P, K, Ca and Mg parameters were examined. Stem diameter and plant height measurements were made on 10 randomly selected plants before cutting in each plot and mean values were calculated for each plot. In each plot, the remaining part was harvested and weighed after removing the edge effects, and green forage yields were determined by taking the harvest area into account. 500 g fresh sample of herbage was taken from each plot for hay yield, forage quality and mineral analyses. These samples, which were withered for a while in laboratory conditions, were then dried in a drying cabinet at 65 $^{\circ}$C for 48 hours. Dried samples were weighed on a precision scale and the hay ratios (%) of the samples were established by calculation. Hay yields were determined by multiplying the hay ratios with the green forage yields of the plots. Dried samples were ground separately and prepared for analysis. Crude protein, ADF, NDF, P, K, Ca and Mg ratios of the ground samples were determined using the #IC-0904FE calibration set (Anonymous, 2020) with a NIRS (Near Infrared Reflectance Spectroscopy) device (Brogna et al., 2009).

2.4. Statistical analysis

The data were subjected to variance analysis according to the randomized complete blocks design, and according to the F test results, the differences between the groups were determined with the LSD (Least Significant Difference) multiple comparison test (Yurtsever, 1984).

3. Results and Discussion

3.1. Effect of zinc doses on forage yield and some yield components

Data on forage yield obtained from forage pea at different levels of Zn applications, and some parameters affecting the yield are presented in Table 2.

Stem diameter and plant height are one of the important morphological growth parameters that affect yield in forage crops and are affected by cultural practices such as fertilization applications. As a result of the research, the effect of Zn doses on stem diameter and plant height of forage pea was found to be statistically insignificant. Depending on the zinc doses, stem diameter varied between 4.80-5.67 mm and plant height varied between 73.00-78.33 cm (Table 2). Öncan Sümer and Yaraşır (2022) stated that the effect of Zn doses on stem diamater in *P. sativum* was insignificant. This result is consistent with the current research findings in terms of stem diamater that is reported by the researchers. Similar to the current research findings, the effect of different doses of Zn applications on plant height was found to be statistically insignificant in some studies conducted on different plant species such as rapeseed (*Brassica napus* ssp. *oleifera* L.) (Aytaç et al., 2016) and oat (*Avena sativa* L.) (Yılmaz and Sonkaya, 2020), as well as legume species such as chickpea (Kurt and Önder, 2024), pea (*Pisum sativum* L.) (Öncan Sümer and Yaraşır, 2022) and alfalfa (*Medicago sativa* L.) (Ersöz Çelik, 2022). On the other hand, in their studies conducted by Yashona et al. (2018) on pigeon pea (*Cajanus cajan*), Erdoğan (2022) on forage cowpea (*Vigna unguiculata* L.), Roy et al. (2022) on faba bean (*Vicia faba* L.), and Sayed et al. (2024) on grass pea (*Lathyrus sativus* L.), reported that the effects of Zn doses were significant and that Zn fertilization increased plant height. Differences between current research results and literature in terms of herbage yield components in forage pea can be explained by genotypic and ecological differences and different reactions of plant species depending on these differences.

In forage pea, 10 kg ha^{-1} Zn fertilization applied from soil significantly increased green forage yield compared to the control; in the following doses, a statistically insignificant decrease in green forage yield was observed at the 15 kg ha^{-1} dose and a significant decrease was observed at the 20 kg ha^{-1} Zn dose. According to this, the highest green forage yield was determined as 43.60 t ha^{-1} at 10 kg ha^{-1} Zn dose. In the study, the lowest green forage yields were determined at 0 (37.36 t ha^{-1}), 5 (37.45 t ha^{-1}) and 20 (37.50 t ha^{-1}) kg ha^{-1} Zn doses, which were statistically in the same group. This difference between Zn doses in terms of green forage yield was found to be statistically significant at $p<0.05$ level. A similar trend was observed in the hay yield in parallel with the increase in zinc doses, however, the variation in Zn doses in terms of hay yield was statistically insignificant (Table 2).

Table 2. Average values of forage yield and some yield components in forage pea applied with different Zn doses*

Zn doses (kg ha^{-1})	Stem diameter (mm)	Plant height (cm)	Green forage yield (t ha^{-1})	Hay yield (t ha^{-1})
0	5.15	73.00	37.36 b	6.09
5	4.80	73.67	37.45 b	6.26
10	5.64	78.33	43.60 a	7.12
15	5.67	75.33	40.37 ab	6.53
20	5.57	75.00	37.50 b	6.30
Mean	5.36	75.07	39.26	6.46
P	0.1426	0.0976	0.0117	0.2424

*: The difference between the means indicated by the same letter in the same group is not statistically significant, P: Significance level

The remarkable effects of zinc on forage pea yield, especially on green forage yield, may be due to the availability of more Zn in the soil during the growth phase and its uptake by plants, depending on the applied Zn levels. The beneficial effects on forage pea biomass can be attributed to soil-applied zinc when considering the roles of zinc in plant chlorophyll formation, biosynthesis of plant growth regulator (Indole-3-acetic acid, IAA), carbohydrate and N metabolism leading to high yield and yield components (Taliee and Sayadian, 2000; Sharma et al., 2010), as well as increasing N-fixation through photosynthesis (Hegazy et al., 1990) and nodule formation (Hegazy et al., 1990; Patel et al., 2011). Padma et al. (1989) and Deotale et al. (1998) stated that zinc also plays a role in the hormone synthesis and contributes to additional growth in the plant compared to the control due to these positive roles in plant metabolism. Moreover, Zn application is thought to be effective in increasing the availability of other nutrients and accelerating the translocation of photo assimilates (Guhey, 1999), which in turn increases the yield of forage in the plant. Enrichment of plant nutrition increases photosynthesis efficiency, assimilation and production (Ali, 2004).

In a study conducted with forage pea (*P. sativum* L.) in Mediterranean conditions in soil with low DTPA extractable Zn content, soil Zn application affected forage yield significantly ($p<0.01$); forage yield increased by 30% when 50 kg ha^{-1} zinc sulphate was applied to the soil before sowing (Reynolds-Marzal et al., 2021). It was established that Zn application in field pea (*P. sativum*) grown in calcareous soils significantly increased seed yield, stover yield and number of nodules per plant compared to the control (Quddus et al., 2018). It was reported that foliar Zn fertilization improves grain and straw yield and quality parameters (Dhaliwal et al., 2022), and Zn element should be applied together with the recommended fertilizer dose to maximize production and net profit (Reddy et al., 2023), in some other studies conducted with field pea (*P. sativum*).

In studies conducted with different species of forage legumes, results consistent with the current research findings were obtained. In fodder cowpea [*Vigna unguiculata* (L.) Walp.], application of 20 kg Zn ha^{-1} in the form of $ZnSO_4$ markedly ($p<0.05$) increased the green fodder yield (Rathore et al., 2015; Kumar et al., 2016). Grain and straw yields of *C. cajan*, a perennial legume forage plant from the Fabaceae family, were found to be 7-25% and 6-18% higher, respectively, under different mode of Zinc application, including Zn doses, compared to the control (no zinc application) (Yashona et al., 2018). In studies conducted with alfalfa (M. sativa) plant, Zn ($ZnSO_4.7H_2O$) application at a dose of 80 kg ha^{-1} applied from soil (Ceylan et al., 2009) and 120 mg L^{-1} applied from leaves (Ersöz Çelik, 2022) increased green forage and hay yield significantly compared to the control. It has been determined that grain yield and yield-effective parameters in grass pea (*L. sativus*) show a positive correlation with Zn application, in this sense the optimum Zn dose was determined as 1.95 kg ha^{-1} and there were decreases in all yield parameters in applications above this recommended Zn dose (Sayed et al., 2024). Similarly, Zn application has been reported to increase the yield of legumes such as chickpea (Valenciano et al., 2010), soybean (*Glycine max* L.) (Chauhan et al., 2013; Choudhary et al., 2014), mungbean (*Vigna radiate* L.) (Islam et al., 2017), garden pea (*Pisum sativum* L.) (Alam et al., 2020; Öncan Sümer and Yaraşır, 2022), faba bean (*Vicia faba* L.) (Roy et al., 2022).

On the other hand, micronutrient deficiencies, especially Zn, have been reported by many researchers as the main reason for the suboptimal yield of crops, including legume forage crop species (Das and Parida, 2020; Islam et al., 2021; Ghosh et al., 2021; Sridhar et al., 2021). For this reason, it is important to fertilize Zn in addition to other nutrients when its deficiency is observed in order to achieve optimum yield in forage peas.

3.2. Effect of zinc doses on forage quality

3.2.1. Crude protein, acid detergent fiber and neutral detergent fiber

Improving productivity in livestock is possible by providing farmers with sufficient amounts of better-quality feed. Forage quality varies depending on cultural practices such as fertilization applied in forage crop cultivation. In this sense, Zn deficiency in soil may be the main reason for the poor quality of legume forages. CP, ADF and NDF ratios of hay are important quality parameters in forage crops.

In the research, the CP content of forage pea hay was significantly affected by Zn doses ($p<0.01$). The CP ratio increased in parallel with the increase in Zn doses, but this increase was insignificant after the 5 kg ha^{-1} Zn dose. Therefore, this statistically significant difference at $p<0.01$ level occurred between the control and other Zn doses. According to this, while the CP rate was 20.17% in the control process, this rate varied between 21.60-25.97% depending on the Zn doses (Table 3). Soil Zn fertilization improved the CP content of forage compared to the control (0 kg ha^{-1} Zn) application in the study. This can be explained by the fact that Zn, which has an important function in nodule formation and N fixation, increases N fixation in the soil and therefore available nitrogen, and also increases N uptake by the plant. Furthermore, Zn plays an important role in various enzyme systems and protein metabolism, which is an important factor in improving the protein content in dry matter of forage pea. In the studies conducted with peas in the literature, results compatible with the current research findings were obtained; it was reported that Zn fertilization increased the CP rate in the plant. For example, in the study conducted with forage pea (*P. sativum*) under Mediterranean conditions, it was emphasized that foliar Zn application significantly affected the CP content of the feed ($p\leq0.01$), the CP level increased by approximately 8% compared to the control, and Zn application was the most effective application to increase the CP rate (Reynolds-Marzal et al., 2021). It was reported that the protein content in grains was significantly affected by Zn application in studies conducted with field pea (*P. sativum*) (Quddus et al., 2018; Dhaliwal et al., 2022). Besides, previous studies with different forage crop species reported that Zn application is an effective method to improve the CP ratio of forage in various crops such as forage sorghum (Sutaria et al., 2013), fodder cowpea (*Vigna unguiculata*) (Rathore et al., 2015), fodder maize (Kumar and Ram, 2021), triticale (x*Triticosecale*) (García-Latorre et al., 2024).

In the research, although the ADF and NDF ratios of forage pea increased according to Zn doses, this increase was found to be statistically insignificant. Accordingly, the ADF ratio of forage pea varied between 33.24-36.85% and the NDF ratio between 38.60-46.93% (Table 3). The effect of Zn applications on ADF and NDF rates was found to be statistically insignificant, in line with the current research findings in the study conducted by Reynolds-Marzal et al. (2021) on forage pea. It has also been reported in the research results conducted on fodder cowpea (*Vigna unguiculata*) (Rathore et al., 2015) and fodder maize (*Zea mays* L.) (Kumar et al., 2017) plants that zinc fertilization does not affect the ADF and NDF rates of feeds.

Table 3. Average values of forage quality parameters in forage pea applied with different Zn doses*

Zn doses (kg ha^{-1})	CP (%)	ADF (%)	NDF (%)
0	20.17 b	33.24	38.60
5	21.60 ab	34.35	42.21
10	25.70 a	34.52	42.24
15	25.74 a	35.52	43.38
20	25.97 a	36.85	46.93
Mean	23.84	34.89	42.67
P	0.0080	0.4616	0.2028

*: The difference between the means indicated by the same letter in the same group is not statistically significant, P: Significance level

3.2.2. Mineral element contents

When considering the forage quality of a forage plant, its nutritional value comes to mind. In determining the nutritional value of forage plants, chemical compositions such as CP, crude fiber, vitamins and minerals are one of the main criteria.

In forage pea, pre-sowing soil Zn fertilizer application significantly affected P ($p<0.05$) and Ca ($p<0.01$), which are mineral components of the hay. The P content in forage pea showed a descending trend with the increase in Zn application over control which gave the highest value of 0.52%. Whereas the Ca content exhibited ascending trend with the application of Zn and the highest value was observed for treatment 20 kg ha^{-1} Zn (1.32%) (Figure 3). The decreasing trend observed for total P content can be attributed to the antagonistic relationship between Zn and P. When elemental soil zinc is high in the soil, the availability of elemental soil phosphorus is in general always low (Ladumor et al., 2019). Due to this interaction between Zn and P, Zn applied to the soil reduced P uptake in the plant. The decrease in the amount of inorganic P with Zn application was also reported by Ghoneim (2016); similar to the current research findings, it has been documented in many studies that the total P amounts in the plant decrease in parallel with the increase in Zn dose (Li et al., 2003; Petković et al., 2019; Dhaliwal et al., 2022). It has also been reported in some studies that the amount of Ca in the plant improves compared to the control depending on the applied Zn fertilization (Petković et al., 2019; Reynolds-Marzal et al., 2021; García-Latorre et al., 2024).

Zn fertilization did not significantly affect the content of K and Mg elements in forage pea. In the study, the total K content of the hay varied between 3.32-3.47% and the total Mg content varied between 0.24-0.27%. At the same time, it was observed that the amount of K increased in parallel with the increase in Zn doses (Figure 3). It can be said that in soils with zinc deficiency, zinc fertilization may contribute to the improvement of the K content of the forage. Petković et al. (2019) reported that plant K and Mg ratios did not show significant changes according to Zn doses in alfalfa, Reynolds-Marzal et al. (2021) reported that soil Zn application did not affect the Mg content of the plant in forage pea, whereas foliar Zn application increased the Mg content significantly ($p<0.01$). It can be indicated that these results in the literature are generally compatible with the current research findings. Contrary to current research findings, García-Latorre et al. (2024) observed that total Mg content in the plant was affected by Zn fertilization, and that Zn application from soil in triticale plants gave better results than other application methods.

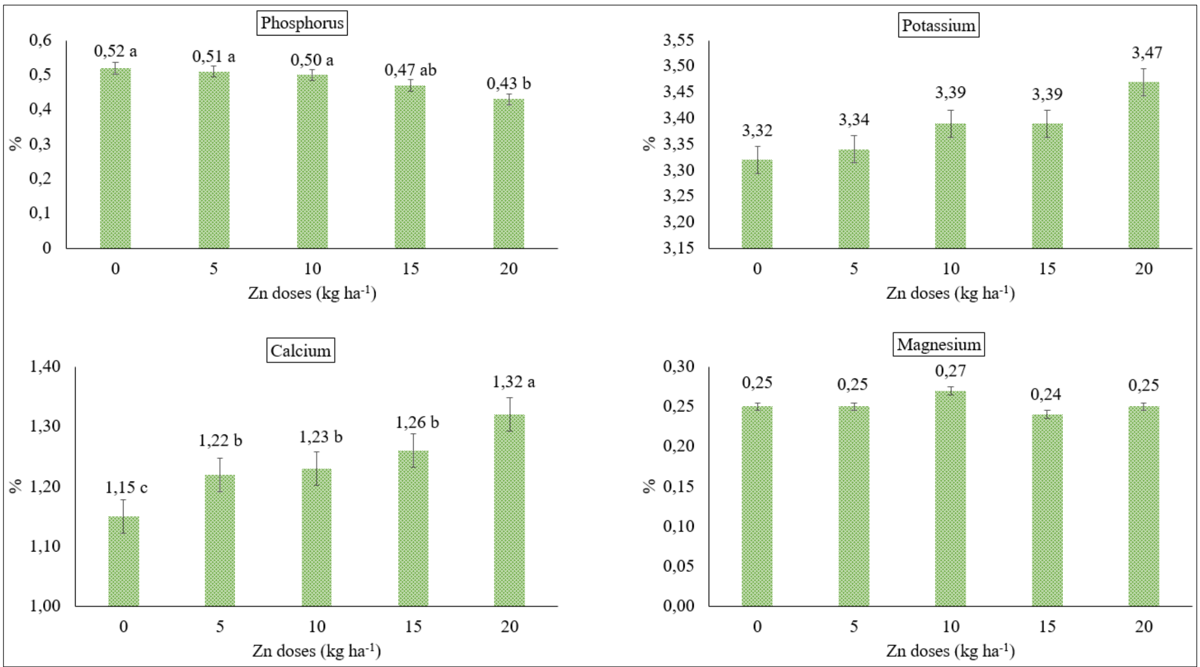

Figure 3. Average values (%) of some macro elements determined in forage pea hay applied with different Zn doses* *: The difference between the means indicated by the same letter in the same group is not statistically significant, Significance level: P_P= 0.0143, P_K= 0.6387, P_{Ca}= 0.0001, P_{Mg}= 0.5944

It is important to compare these macronutrients, which vary according to zinc doses, in terms of the amounts required in rations for animal nutrition. It is reported that in order to meet the macro element needs of animals at a minimum level in feed rations, feed should contain 0.40% P, 1.00% K, 0.90% Ca according to Muller (2009) and 0.25% Mg according to Anonymous (2001). According to these criteria in the literature, it was understood that the total P, K, Ca and Mg values determined in forage pea hay at different Zn doses were at a level to meet the needs of ruminants.

The research result showed that Zn fertilization of forage pea through soil can not only increase the fodder efficiency and quality, but also improve the nutrient uptake of the plant.

4. Conclusion

Research results showed that zinc application may be beneficial in increasing the growth, forage yield and quality of forage legumes such as forage pea in Zn deficient soils in the Southeastern Anatolia Region of Türkiye. In the research, it was concluded that 10 kg Zn ha^{-1} application to forage pea in the presence of low-level extractable Zn in the soil would provide significant increases in forage production. The same Zn dose provided significant improvements in both forage quality and mineral content of the forage.

Consequently, optimum application of micronutrients such as Zn is required for the forage yield and quality of legume forage crops. When considering the complex relationship between soil properties, Zn application and plant uptake, longer-term and more research on the effects of micronutrients is needed.

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Determination of Nutritional Values of Cattail (*Typha latifolia* L.) and Common Reed (*Phragmites australis* Cav.) Silages

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A B S T R A C T

Cattail (*Typha latifolia* L.) and common reed (*Phragmites australis* Cav.) are species that grow spontaneously in wetlands and produce high biomass. This research was carried out to reveal the potential of these species to be used as an alternative silage plant. These plants were harvested in two different growing stage (boot stage and flowering stage) in 2021 and 2022, and they were ensiled by adding rolled barley at four different rates (0, 5, 10 and 15%). In this study, the dry matter, crude protein, ndf and adf ratios of cattail plant at different harvesting times (boot stage and flowering), which were examined by adding rolled barley at certain ratios, varied between 30.5%-30.3%, 13.35%-12.14%, 57.07%-59.11% and 35.34%-34.21%, respectively, while in common reed plant, the parameters examined varied between 44.8%-48.3%, 17.51%-16.45%, 53.99%-56-28%.

1. Introduction

Global climate change and drought have led agricultural scientists and producers to search for alternative production models. Increasing energy, fertilizer and irrigation costs make agricultural production difficult and increase production costs. Especially the decrease in water resources has made it necessary to use water in agriculture more carefully. Climate change and drought also affect livestock activities, especially causing an increase in the cost of feed, which is the largest input. As direct food production has gained priority in irrigated areas, it has become difficult for forage crops to find a place in crop rotation systems. This situation has further increased the importance of alternative feed sources.

Feeding animals with silage is a technique that is very common and successfully implemented today. The most used plant for silage production around the world is corn. In recent years, less costly alternative silage materials have begun to be emphasized instead of plants with high water consumption, such as corn. In this context, cattail (*Typha latifolia* L.) and common reed (*Phragmites australis* Cav.) are among the plants that attract the most attention, and research on these plants has been concentrated. Cattail and common reed grow on the edges of streams, lakes and wetlands without the need for agricultural practices such as irrigation

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and fertilization. Both species are perennial rhizomatous grasses found in freshwater wetlands. Their use as a bioenergy plant and to reduce environmental pollution in water also attracts attention (Hayta and Erkan, 2019). These plant associations, which cover very large areas in some regions, can produce high amounts of biomass per unit area. Researchers such as Büyükkılıç Beyzi and Sırakaya (2019) and Baran et al. (2002) reported that these species can be used as forage plants. Cattle and horses graze this grass during winter as a protein source, but common reed and cattle is unpalatable after maturity. Due to their coarse structure, these species seem more suitable to be used as silage in animal nutrition (Musa et al., 2019). In order for these plant silages to ferment successfully, harvest periods must be determined correctly.

Büyükkılıç Beyzi and Sırakaya (2019) determined that Phragmites austrialis reached its highest feeding value in mid-June. To increase the chances of success of cattail and common reed silages, it may be necessary to apply some additives containing carbohydrates (Asano et al., 2018). WingChing-Jones and Leal-Rivera (2017) determined that 3% molasses addition was successful in Typha domingensis silage. Cattail and common reed form very large associations in wetlands and stream banks in Erzurum and surrounding provinces. They grow naturally in large areas in many regions of Türkiye. The aim of this research is to determine the silage quality parameters of cattail (*Typha latifolia* L.) and common reed (*Phragmites australis* Cav.) silages that are harvested at different periods and ensiled with barley crushed at different rates.

2. Materials and Methods

2.1. Study Site

The research was conducted in 2021 and 2022 at Atatürk University, Faculty of Agriculture, Department of Field Crops, Erzurum. Erzurum is located in Eastern Anatolia Region of Türkiye. The city is situated 1860 meters above sea level, and It's latitute and longitude coordinates are 39°54'31"N, 41°16'36.98"E. Erzurum is an important center for animal husbandry and is a region with a high need for forage due to the long winter period. Research material; it was obtained from cattail (*Typha latifolia* L.) and common reed (*Phragmites australis* Cav.) associations, which grow naturally in large areas in the Erzurum Plain and on the banks of the Karasu River.

2.2. Sampling and Experimental design

Sampling was done from the areas covered with cattail and common reed, located on the edge of the Karasu River in Erzurum, during the boot stage and the flowering stage of the plants. In this sampling carried out in July and August, an area of 1 m2 was mowed from 3 different points representing plant associations, leaving a stubble height of 10-15 cm (in the wet habitats associated). The samples taken from the field were chopped into 1-2 cm sized pieces in a laboratory type silage machine and silage was made on the same day. Silages were made by compressing and sealing the material in 2 kg glass jars in an airtight manner. In both plants, samples taken at 2 different stages (booting and flowering) were mixed with 4 different ratios of crushed barley grain (0, 5, 10 and 15%) on a weight basis. The research was arranged in completely randomized experimental design with 3 replications for each species in 2021 and 2022.

2.3. Chemical and statistical analysis

Silages were opened after 60 days and dry matter, crude protein, ADF and NDF ratios and silage pH were determined. Crude protein ratios were determined by the Micro Kjeldahl method (Kacar and İnal 2008). Silage NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) ratios were calculated by Van Soest et al. (1991) with the help of ANKOM Fiber Analyzer. The methods adopted by Kılıç (2010) were used to determine silage pH.

In the research, each species was evaluated on its own and no comparison was made with another species. Two-year data were subjected to analysis of variance for a complete randomized experimental design. When the ANOVA was significant, means were separated using Duncan's multiple range test at the level of $p \leq 0.05$.

3. Results

3.1. Dry Matter Ratio

While the effect of year, cutting stage and additive rate in cattail on the dry matter ratios of silages was found to be insignificant, the interaction of cutting stage time x additive rate was found to be significant ($p < 0.05$, Table 1). This is due to the different effects of additives depending on the harvest stage. In common reed, the effect of years was found to be insignificant, but the effects of cutting time and additive, and the interaction of cutting time × additive were found to be significant (Table 1). As the harvesting stage was delayed in common reed silages, the dry matter ratio increased from 44.8% to 48.3%. Addition rates also increased the dry matter ratios, and the dry matter ratio, which was 42.2% in non-additive silages, increased to 49.2% in 15% additive application. Additive rates also had a significant impact depending on the cutting time. While silage dry matter ratios fluctuated according to the increasing additives during the booting stage, a continuous increase occurred during the flowering stage (Table 1).

Table 1. Dry matter content of cattail and common reed silages (%)[1]

Harvest stage	Additive Ratio (%)	Cattail			Common Reed		
		2021	2022	Mean	2021	2022	Mean
Boot stage	0	29.4	28.7	29.1	43.3	42.2	42.8
	5	32.7	30.9	31.9	44.6	45.7	45.2
	10	30.9	30.9	30.9	44.3	45.2	44.8
	15	30.1	30.1	30.1	47.1	46.0	46.6
Mean		**30.8**	**30.2**	**30.5**	**44.8 B**	**44.7 B**	**44.8 B**
Flowering	0	29.0	28.4	28.7	42.1	41.1	41.6
	5	28.5	29.5	29.0	48.7	49.1	48.9
	10	30.6	30.7	30.7	50.4	51.4	50.9
	15	32.8	32.8	32.8	51.0	52.3	51.7
Mean		**30.2**	**30.4**	**30.3**	**48.1 A**	**48.5 A**	**48.3 A**
	0	29.2	28.6	28.9	42.7	41.7	42.2 B
	5	30.7	30.2	30.5	46.7	44.7	47.1 A
	10	30.8	30.8	30.8	47.4	48.3	47.9 A
	15	31.5	31.5	31.5	49.1	49.2	49.2 A
Mean		**30.5**	**30.3**	**30.4**	**46.5**	**46.6**	**46.6**
Additive ratio × Harvest stage			**			*	

[1]Means marked with different letters in the same column are different from each other.
*: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$

3.2. Crude Protein Ratio

In the research, crude protein ratios of cattail and common reed silages showed significant changes according to years, additives ratio and cutting times (Table 2). According to the two-year average results, the average crude protein rate is 12.75% in cattail silages and 16.98% in common reed silages. According to the two-year average results, advancing cutting stage significantly reduced the silage crude protein ratio in both species. The effect of additives was insignificant. While there was no difference in common reed, the crude protein content of the first-year silages of cattail was higher (Table 2).

Table 2. Crude protein content of cattail and common reed silages (%)[1]

Harvest stage	Additive Ratio (%)	Cattail			Common Reed		
		2021	2022	Mean	2021	2022	Mean
Boot stage	0	13.88	11.61	12.75	14.90	18.11	16.50
	5	14.39	11.91	13.15	17.00	1786	17.43
	10	14.50	13.48	13.99	17.20	18.57	17.89
	15	14.21	12.83	13.52	17.52	18.90	18.21
Mean		**14.25 A**	**12.45**	**13.35 A**	**16.66 A**	**18.36 a**	**17.51 A**
Flowering	0	12.19	12.31	12.25	14.43	16.69	15.56
	5	12.27	11.58	11.93	14.87	17.50	16.19
	10	11.55	12.38	11.97	15.96	17.86	16.91
	15	12.25	12.59	12.42	15.42	18.84	17.13
Mean		**12.06 B**	**12.22**	**12.14 B**	**15.17 B**	**17.72 b**	**16.45 B**
	0	13.04	11.96bc	12.50	14.67 B	17.40	16.03

	5	13.33	11.74 c	12.54	15.94 A	17.68	16.81
	10	13.02	12.93 a	12.98	16.58 A	18.21	17.40
	15	13.23	12.71ab	12.97	16.47 A	18.87	17.67
Mean		**13.16 A**	**12.34 B**	**12.75**	**15.92**	**18.04**	**16.98**
Additive ratio x Harvest stage			*			ns	

[1]Means marked with different letters in the same column are different from each other.
*: significant at $p \leq 0.05$, ns: non-significant.

3.3. NDF (Neutral Detergent Fiber) Ratio

NDF contents of silages did not change significantly over the years, but varied depending on the additive rate (Table 4). Although delaying in harvest stage generally increased the NDF rate, this increase was found to be significant in the two-year average in common reed. The interaction of additive rate x harvest stage was found to be significant in the two-year average results of both plants. The most significant effect on the NDF content of silages was the additive rates. As the additive rate increased, NDF contents decreased from 64.05 to 53.76% in cattail and from 58.81 to 50.96% in common reed.

Table 4. Neutral detergent fiber (NDF) content of cattail and common reed silages (%)[1]

Harvest stage	Additive Ratio (%)	Cattail			Common Reed		
		2021	2022	Mean	2021	2022	Mean
Boot stage	0	52.75	68.19	60.47	58.96	58.35	58.66
	5	50.79	60.82	60.31	58.08	55.29	56.69
	10	54.28	52.98	53.63	53.09	55.26	54.17
	15	55.27	52.51	53.89	46.84	46.06	46.45
Mean		**55.52 B**	**58.63**	**57.07**	**54.24**	**53.74**	**53.99 b**
Flowering	0	68.44	66.82	67.63	59.49	58.41	58.95
	5	62.66	54.35	58.51	57.87	57.62	57.74
	10	60.02	53.31	56.67	54.43	51.46	52.95
	15	55.72	51.54	53.63	55.06	55.88	55.47
Mean		**61.71 A**	**56.51**	**59.11**	**56.71**	**55.84**	**56.28 a**
	0	60.59 a	67.51A	64.05 A	59.22 A	58.38 a	58.81A
	5	61.23 a	57.59B	59.41 B	57.98AB	56.46 b	57.22A
	10	57.15ab	53.14BC	55.15 C	53.76BC	53.36ab	53.56B
	15	55.49 b	52.02C	53.76 C	50.95C	50.97 b	50.96B
Mean		**58.62**	**57.57**	**58.10**	**55.48**	**54.79**	**55.14**
Additive ratio × Harvest stage			*			*	

[1]Means marked with different letters in the same column are different from each other.
*: significant at $p \leq 0.05$.

3.4. ADF (Acid Detergent Fiber) Ratio

ADF contents of silages were affected by the additive rates in both cattail and common reed (Table 3). As the additive rate increased, the ADF rate decreased from 39.79 to 31.55% in cattail and from 31.84 to 27.81% in common reed. Harvesting stage did not have a statistically significant effect on the ADF ratio of the silages (except for the second year in common reed). ADF rates were found to be higher in the second year in both plants, and this difference was statistically significant in cattail.

Table 3. Acid detergent fiber (ADF) content of cattail and common reed silages (%)[1]

Harvest stage	Additive Ratio (%)	Cattail			Common Reed		
		2021	2022	Mean	2021	2022	Mean
Boot stage	0	39.23	42.56	40.90	33.31	34.92	34.12
	5	33.34	37.80	35.82	30.73	30.78	30.76

	10	27.94	37.41	32.68	27.96	28.21	28.08
	15	31.74	32.18	31.96	25.24	26.95	27.00
Mean		**33.19**	**37.49 A**	**35.34**	**29.31**	**30.22**	**29.76**
Flowering	0	37.46	39.91	38.69	28.09	31.05	29.57
	5	31.92	34.88	33.40	26.08	27.48	26.78
	10	31.23	31.34	31.29	32.09	25.91	29.00
	15	28.48	33.81	31.15	27.51	31.53	29.52
Mean		**32.27**	**34.99 B**	**34.21**	**28.44**	**28.99**	**28.72**
	0	35.85 A	41.23 A	39.79 A	30.70a	32.98 a	31.84 A
	5	32.88 B	36.34B	34.61 B	28.40ab	29.13 b	28.77 B
	10	29.58 C	34.38BC	31.98 C	30.02a	27.06 b	28.54 B
	15	30.11 C	33.00C	31.55 C	26.38b	29.24 b	27.81 B
Mean		**32.73 B**	**36.25 A**	**34.78**	**28.88**	**29.61**	**29.24**
Additive ratio × Harvest stage			ns			**	

[1]Means marked with different letters in the same column are different from each other.

**: significant at $p \leq 0.01$, ns: non-significant.

3.5. Silage pH

The pH values of the silages were statistically affected by both harvest stage and additive rate ($p \leq 0.05$). In general, mowing of the plants at advanced growing stage and integrating additives increased the fermentation of silages and reduced the silage pH (Table 5). The additive ratio of 10% and 15% resulted in lower pH values. In general, common reed silages appear to have higher pH than cattail silages.

Table 5. pH values of cattail and common reed silages (%)[1]

Harvest stage	Additive Ratio (%)	Cattail			Common Reed		
		2021	2022	Mean	2021	2022	Mean
Boot stage	0	4.98	5.70	5.34	5.81	6.23	6.02
	5	4.93	5.46	5.20	5.58	5.56	5.57
	10	4.45	5.21	4.83	5.19	5.35	5.27
	15	4.44	5.08	4.76	5.17	5.42	5.30
Mean		**4.70 B**	**5.36**	**5.03 B**	**5.44 A**	**5.64**	**5.54 a**
Flowering	0	5.77	5.63	5.70	5.17	6.40	5.79
	5	5.38	5.22	5.30	5.18	5.76	5.47
	10	4.76	5.05	4.91	5.34	5.22	5.28
	15	4.92	4.91	4.92	5.32	5.25	5.29
Mean		**5.21 A**	**5.20**	**5.21 A**	**5.25 B**	**5.66**	**5.46 b**
	0	5.38 a	5.67 a	5.52 a	5.49 a	6.32 a	5.91 a
	5	5.16 a	5.34 a	5.25 a	5.38 ab	5.66 b	5.52 b
	10	4.61 b	5.13 b	4.87 b	5.27 b	5.29 c	5.28 c
	15	4.68 b	5.00 b	4.84 b	5.25 b	5.34 c	5.30 c
Mean		**4.96 c**	**5.29b**	**5.12bc**	**5.35 b**	**5.65a**	**5.50**
Additive ratio × Harvest stage			*			*	

[1]Means marked with different letters in the same column are different from each other. *: significant at $p \leq 0.05$.

4. Discussion

As a result of the study, it was determined that different harvesting times and rolled barley added at different rates significantly affected some quality characteristics of cattail and common reed plants. However, this situation showed some differences depending on years and species. While the effect of harvest time and additive ratio on the silage dry matter ratio was found to be insignificant in cattail silages, delaying in the harvesting stage and adding rolled barley in common reed silages increased the silage dry matter ratio. Because the accumulation of structural substances in plants will increase with the progression of plant growth, it is expected that there will be an increase in the dry matter ratio

(Buxton and Mertens, 1995). It is thought that rolled barley increases the silage dry matter content because it contains husk. Dumlu and Tan (2009) and Dumlu Gül et al. (2015) pointed out similar results. Crude protein content was found to be higher in both plants in the early development stage. As the growth stage of forage plants progresses, the crude protein rate decreases as the structural substances in the plant increase (Bakoglu et al., 1999). ADF and NDF ratios, which express the crude protein ratio and the fibrous fraction, change inversely proportional to each other (Tan et al., 2019; Güllap et al., 2021). In this study, additive rates did not have a significant effect on crude protein ratio. Dumlu and Tan (2009) stated that the increasing effect of rolled barley on crude protein ratio occurs in material silages with low crude protein content.

The ADF ratio decreased with the advancement of the development stage in both plants, while this change was found to be statistically significant in cattail, it was insignificant in common reed. In general, the ADF ratio, which represents the fibrous fraction in plants, is expected to increase as the growing stage advanced (Tan et al., 2012). The increases in the generative parts during the flowering stage in cattail may have reduced the ADF rate in the silage. The addition of rolled barley and the increase in the additive ratio reduced the ADF content in both plants. It is estimated that this situation is due to the lower ADF content in rolled barley. Similarly, increasing additive rates resulted in significant decreases in NDF content. As a matter of fact, the results obtained by adding barley to silage in Dumlu Gül et al. (2015) support our study.

In this research, different results of harvest stage on silage pH were revealed depending on the species. As the cutting stage progressed, silage pH increased in cattail and decreased in common reed. It was determined that additives reduced pH in both species. Additives like rolled barley make ease fermentation and decrease the pH value of silages, because they have high soluble carbohydrate content (Umana et al., 1991). It is desirable that the silage pH be low, which is an indicator of successful fermentation. In this study, it is generally seen that gun pH is high in both plants. Harvesting stage and the addition of rolled barley did not cause decreases in the pH sufficiently. Similarly, Musa et al. (2020) determined the pH value of cattail silages as 5.39 and stated that the addition of urea and molasses was not sufficient to reduce the pH. For this reason, although it is considered more appropriate to harvest cattail and common reed in the boot stage for silage making, different additive applications should be tried to reduce the silage pH.

4. Conclusion

In the silage study in which the effects of harvesting at different periods and addition of rolled barley at different rates on different plant species were examined, we can suggest that the addition of rolled barley at a rate of 10-15% in addition to harvesting at early growth periods will positively affect the quality of silage.

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