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EFFECT OF VARIOUS FERTILIZER APPLICATIONS ON GROWTH, NUTRITIVE VALUE, AND NUTRITIONAL QUALITY OF BARLEY SPROUTS

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ABSTRACT

Effect of various fertilizing techniques on the growth, nutritive value, and nutritional quality of barley sprouts produced in a hydroponic chamber were examined in this study. Four experimental groups were designed as control (without fertilizer application); S (fertilizer application to seeds before planting); S1 (fertilizer application to sprouts fertilizer after 4 days of planting), and S2 (fertilizer application to seeds before planting and to sprouts after 4 days of planting). The highest shoot height, root length, and fresh and dry forage weights were seen in S2 group. Different fertilizer applications increased the dry matter content and decreased the dry matter loss of sprouts compared to the control group. Crude protein, ether extract, ash, in vitro dry matter digestibility, crude fiber, and acid detergent fiber contents of sprouts were greater in all the treatment groups compared to the control group. After harvesting, lower neutral and acid detergent fibers, nitrogen-free extract, and hemicellulose were detected in the fertilized groups than control group. The relative feed value and relative feed quality in S2 group were the highest among all groups. In conclusion, the application of fertilizer to both seed and sprout may improve the growth, nutrient content, and quality values of barley sprouts.

Keywords: Green sprout yield, hydroponic system, Nutritional composition, Hydroponic system.

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INTRODUCTION

Greenhouse industry, that protects the crops against external harsh weather conditions, has ecological benefits as it lowers the use of chemicals (Straten *et al*., 2011). However, soil- or air- borne diseases have become more prominent in greenhouse cultivation (Fiers *et al*., 2012) that can be avoided in soilless culture systems. Crop production in soilless conditions ensures disease control (Maucieri *et al*., 2019), therefore, this system is used in most European countries. Over the past decade, the environmental impact of climate change has increased resulting in unusual drought and water scarcity in the world (Fatima *et al*., 2020; Abbas *et al*., 2023). Considering the unsustainable use of water in agriculture along with climate change and drought, hydroponic system is an important substitute for conventional agriculture (Mukherjee *et al*., 2018). Soilless culture can be categorized into three classes based on its growing system; 1) solution culture or liquid hydroponics (circulating methods, nutrient film technique, and deep flow technique), 2) solid media culture (hanging bag system, grow bag system), and 3) aeroponics (Hussain *et al*., 2014). The liquid hydroponic system, used in this study, is a closed system that produces 750-800 kg fresh fodder daily. Many studies have reported a 5 to 9 kg of sprout production per kg seed and a 15-20 cm of sprout shoot height (Fazaeli *et al*., 2011; Fazaeli *et al*., 2012; Gumus and Bayir, 2020). The nutrient changes in sprouts caused by biochemical changes (such as big complex lipids and protein are broken down by enzymes into simple compounds) occur during soaking as well as physical modification (Sneath and Mclntosh, 2003). As fiber content of sprouts increases, energy levels and organic matter content decrease linearly after 7 days of growth. (Fazaeli *et al*., 2012; Karasahin, 2014, Gebremedhin *et al*., 2015). Dry matter (DM) and starch levels decrease whereas, fiber and cellulose levels increase since starch is converted to soluble sugars during sprouting (Shit, 2019). The low DM content of sprouts might be a problem due to fungal and mold proliferation (Hussain *et al*., 2014). In addition, low DM content can be a limitation factor for the use of sprouts in animal nutrition (Gumus and Bayir, 2020). Chemical changes in sprouts can affect feed quality by increasing the fiber content and decreasing DM content of sprout

(Gebremedhin *et al*., 2015). Therefore, it is worth mentioning that the relative feed value (RFV) relative to NDF and ADF contents is the best way to evaluate the feed quality as well as physical appearance and chemical composition of forage crops (Moore and Undersander, 2002). Some studies have reported the effects of fertilizer (Rivero *et al.*, 2016), water salinity (Natsheh, 2019), different crops (Jolad *et al.*, 2020), and seed rates (Assefa *et al*., 2020, Afzalinia and Karimi, 2020) on hydroponic fodders. However, there is no literature describing the effect of fertilizer application on growth and nutritional composition of sprouts. Hence, the present study was carried out to evaluate the effects of different fertilizer application on the yield, nutritive value, and nutritional quality of barley sprouts produced in a hydroponic system.

MATERIALS AND METHODS

Experimental details: The intensive hydroponic system was constructed by using a steel stand, size 2.80 m × 9 m × 7 m (H × L × W). An irrigation time and frequency of 90 sec/120 min, a temperature of 18-19 °C, relative humidity of 60%, 12 h lighting time of yellow light were set to grow the sprouts. Before planting, the trays, irrigation system, necessary tools, and production chamber were sterilized with formaldehyde to prevent mold contamination. About 50 mL of sodium hypochlorite was added to the irrigation water every day. Tap water was used as a source of irrigation. Barley seeds were planted on August 19, 2021, in a steel hydroponic chamber at the Center for Agriculture, Livestock and Food Research, Burdur Mehmet Akif Ersoy University (Southern Türkiye, 30° 53' E, 36° 53' N and 950 m above sea level). Hydroponic chamber had 196 polyethylene trays sized 70 × 30 × 5 cm (0.21 m^2). After the barley reached the green stage on August 29, 2021, it was harvested to assess yield, nutritive value, and digestibility of sprouts. The experiment was conducted in a completely randomized design using four experimental groups as control (without fertilizer application); S (fertilizer application to seeds before planting); S1 (fertilizer application to sprouts fertilizer after 4 days of planting), and S2 (fertilizer application to seeds before planting and to sprouts after 4 days of planting). Each group had 5 trays as replicates. Before planting, the fertilizer (boron 0.6 w/w, molybdenum 0.2 w/w and zinc 1.2 w/w) was placed into the plastic bucket containing 1000 g seed and 1400 mL water to allow mixing. Then, 2.5 mL of fertilizer was reconstituted in 1 L of tap water and sprayed onto the fresh sprout. Seeds were pre-soaked in water separately for 12 hours to eliminate the low-density material (wastes, straw, and others) and to accelerate the germination. Amount of water used for soaking was based on our previous studies (Gumus and Bayir, 2020; Akman *et al*., 2021) since no other studies have reported the amount of water sufficient for soaking before planting. According to previous studies, 1.4 mL water/g of seed is required to entirely submerge the seed in water. Since seeds can absorb water at a rate of 65% of their weight, 1 mL water was deemed sufficient for 1 g seed. Seed rate applied was 1,000 g/tray. Trays were placed on the shelves of hydroponic chamber.

Data recording: Shoot height, root length, and fresh and dry weight of sprouts were determined after 8, 9 and 10 days of planting. Shoot height and root length were measured using a ruler with a 1 mm scale; measurements were conducted in fifteen replicates for each shoot and nineteen replicates for each root. Fresh weight of sprouts was measured with a digital bench scale (scale graduations, 0.005 lb.). Dry weight of sprouts was calculated by multiplying fresh weight with DM content of the sprouts. Sprouts were dried at 65°C for 72 h in an oven to ascertain the DM content. Obtained dry sprouts (kg) were divided by dry seed weight to calculate the sprouts produced (kg) per kg dry seed (df/ds). Dried sprouts were ground to pass through a 1-mm screen using a mill. Nitrogen (N) was determined by Kjeldahl method (Vapodest 50s, Germany) and crude protein (CP) content was calculated by multiplying N by 6.25. Ether extract (EE, method 920.39) and ash (method 942.05) contents were analyzed according to Association of Official Analytical Chemists methods (AOAC, 2019). Non-fibrous carbohydrate [NFC = 100 – (NDF% + CP% + EE% + Ash%)] and nitrogen free extract [NFE= 100 – (CF% + EE% + CP% + Ash%)] contents were calculated according to the standards of the National Research Council (NRC, 2001). Structural carbohydrates were analyzed for: (i) crude fiber (CF) by the methods of Crampton and Maynard (1938); (ii) neutral detergent fiber (aNDFom) by the methods of Goering and Van Soest (1970) with both heat-stable amylase and sodium sulfite included within the NDF solution using the Ankom fiber analyzer (A2000, Ankom Technology, Method 13; Macedon, NY); and (iii) acid detergent fiber (ADF) and acid detergent lignin (ADL) by the method of Goering and Van Soest (1970) without preliminary digestion in neutral detergent using Ankom fiber analyzer (A2000, Ankom Technology, Method 11 and 8; Macedon, NY). Digestible dry matter (DDM) was determined by using ADF content of sprout [DDM% = 88.9 – (0.779 × ADF%)]. Then dry matter intake (DMI) was measured by using NDF content of sprout [DMI% =120 ÷ NDF%)]. Relative feed value was calculated by using (Rohweder *et al.*, 1978) DDM and DMI [RFV = DDM% × DMI% × 0.775]. Relative feed quality was calculated according to Zhou *et al*. (2022) by using TDN and DMI [RFQ = TDN × (DMI ÷ 1.23)]. Observations of shoot height, root length, fresh weight, and dry weight were analyzed as repeated measures.

Statistical analysis: The experiment was steered as a completely randomized design with three harvesting days and four treatments. Data were subjected to two-way analysis of variance with the fixed effects of treatments, harvesting day, and treatment by harvesting day interaction using the GLM procedures of computer-aided software package SPSS (version 22.0; IBM Corp., NY, US). One-way ANOVA was applied to evaluate the effects of different fertilizer applications on the nutritive value, RFV, and RFQ of barley sprouts. Significant differences between means were reported using the Tukey's test at $\alpha \leq 0.05$.

RESULTS

As shown in Table 1, shoot height was affected by harvest day and treatments ($P<0.001$), while harvest day × treatment interaction remained unaffected. Application of fertilizer increased the shoot height of barley sprouts having a sustained higher shoot height than control group throughout the harvesting period. Root length was affected by harvesting day and increased linearly by delaying harvest day ($P<0.001$). Effects of harvesting day, treatments, and their interaction on the fresh weight (kg/tray) were significant ($P<0.001$). Regardless of harvesting days (Figure 1), the dry yield (ton ha^{-1}) was greater in S, S1 and S2 groups than control group ($P<0.001$).

Nutritive value of sprouts has been presented in Table 2. Dry matter tendency (P 007) The dry sprout per unit of dry seed (df/ds) was the lowest (0.56 kg) in control group. There was a significant improvement in the CP content of sprouts treated with fertilizer ($P<0.05$). The fertilizer-applied sprouts had significantly greater ivDMD ($P<0.05$) than that of control group. Application of fertilizer decreased the NFE content ($P< 0.05$) of sprouts compared to control group. As seen in Figure 2, after d 10 of harvesting, the DM content of S2-group was higher in comparison with other groups that resulted in low DM loss ($P<0.05$). As shown in Table 3, S2-sprouts had lower NDF (34.73%), and hemicellulose (7.72%) contents compared to control group ($P<0.05$). The CF content was significantly greater in treated sprouts than those of control group ($P<0.05$). Cellulose content of sprouts tended to increase with the application of fertilizer regardless of the application method ($P = 0.09$). As indicated in Figure 3, the RFV and RFQ in S2-sprouts was greater than other groups ($P<0.05$).

Table 1: Effects of different fertilizer application on growth of barley sprouts.

Items	Days of Harvesting	Treatment				SEM	Significance				
		CON	S	S1	S2		D	T	D × T	L	Q
Shoot height (cm)	8	5.48	6.59	5.89	6.60	0.15	**	**	NS	**	*
	9	7.62	8.84	7.41	8.85						
	10	9.87	11.39	10.87	11.41						
Root length (cm)	8	1.66	1.56	1.57	1.43	0.13	**	NS	NS	**	NS
	9	1.91	2.25	2.18	2.04						
	10	2.21	2.39	2.26	2.48						
Fresh weight (kg/tray)	8	4.74	5.44	5.33	6.85	0.21	**	**	*	**	*
	9	5.30	5.79	5.80	7.34						
	10	5.82	6.34	6.26	8.12						
Dry weight (kg/tray)	8	0.35	0.43	0.46	0.62	0.36	**	**	*	**	*
	9	0.42	0.50	0.52	0.70						
	10	0.50	0.57	0.61	0.83						

CON (control), with no fertilizer; S, fertilizer applied to the seeds before planting; S1, fertilizer applied to the sprouts after 4 d of planting but not seeds; S2, fertilizer applied to both, the seeds before planting and sprouts after 4 d of planting. D: effect of harvesting day, T: effect of treatment, D × T: Interaction between harvesting day and treatment, L: Linear effect, Q: Quadratic effect

Table 2: Effects of different fertilizer application on nutritive value of barley sprouts

Treatment[1]	DM	df/ds	CP	EE	Ash	NFC	NFE	ivDMD
CON	8.61	0.56[a]	15.36[a]	3.62	3.15	35.11	59.98[b]	80.62[a]
S	8.95	0.64[ab]	16.64[ab]	3.81	3.20	37.02	57.16[b]	83.70[b]
S1	9.79	0.71[b]	18.01[ab]	3.86	3.32	35.34	52.73[a]	83.58[b]
S2	10.11	0.91[c]	18.60[b]	3.95	4.16	38.55	51.73[a]	83.92[b]
P	0.07	0.001	0.04	0.93	0.99	0.52	0.01	0.04

[1]CON (control), with no fertilizer; S, fertilizer applied to the seeds before planting; S1, fertilizer applied to the sprouts after 4 d of planting but not seeds; S2, fertilizer applied to both, the seeds before planting and sprouts after 4 d of planting. DM: Dry matter, df/ds: dry fodder per dry seed, CP: Crude protein, EE: Ether extract, NFC: Non-fiber carbohydrate, NFE: Nitrogen free extract, ivDMD: In vitro dry matter digestibility.

Table 3: Effects of different fertilizer application on structural carbohydrates of barley fodders.

Treatment[1]	CF	NDF	ADF	ADL	HEM	CEL
CON	17.89[a]	42.76[b]	21.06	11.73	21.69[b]	9.32
S	19.19[a]	39.32[ab]	24.71	10.41	14.60[ab]	14.30
S1	22.06[b]	39.45[ab]	25.02	10.66	14.43[ab]	14.35
S2	21.54[a]	34.73[a]	26.99	10.38	7.72[a]	16.61
P	0.03	0.02	0.24	0.30	0.01	0.09

[1]CON (control), with no fertilizer; S, fertilizer applied to the seeds before planting; S1, fertilizer applied to the sprouts after 4 d of planting but not seeds; S2, fertilizer applied to both, the seeds before planting and sprouts after 4 d of planting. CF: Crude fiber, NDF: Nötral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, HEM: Hemicellulose, CEL: Cellulose

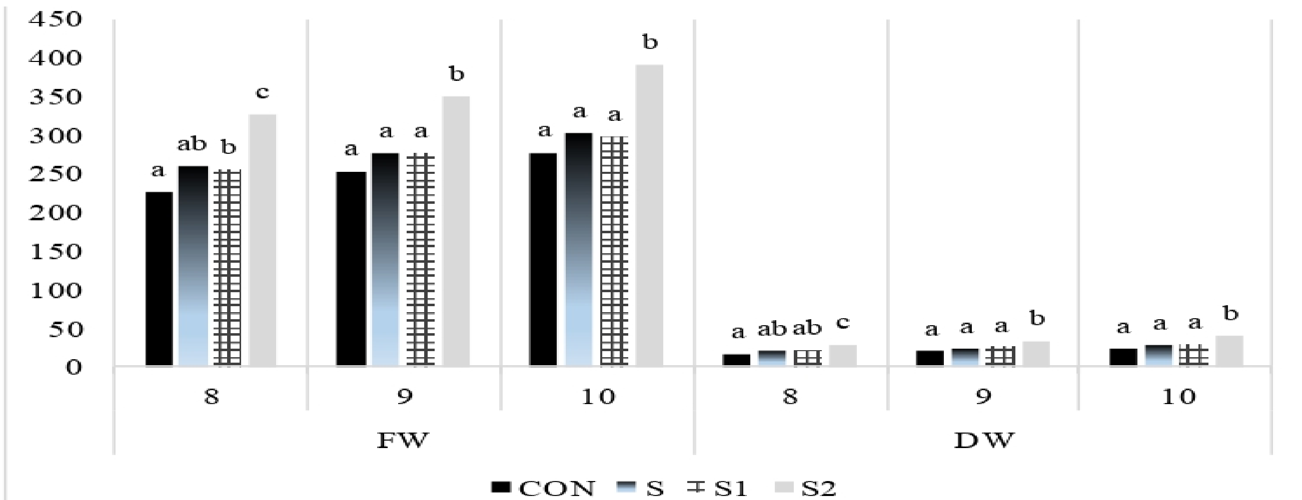

Figure 1: Effect of fertilizer application on fresh weight and dry weight of barley sprouts. FW: fresh weight; DW: dry weight; CON: control, without fertilizer; S: fertilizer applied to the seeds before planting; S1: fertilizer applied to the sprouts after 4 d of planting; S2: fertilizer applied to both, seeds before planting and sprouts after 4 d of planting. Bars bearing different superscripts on the same day represent significant differences among the groups (P < 0.05).

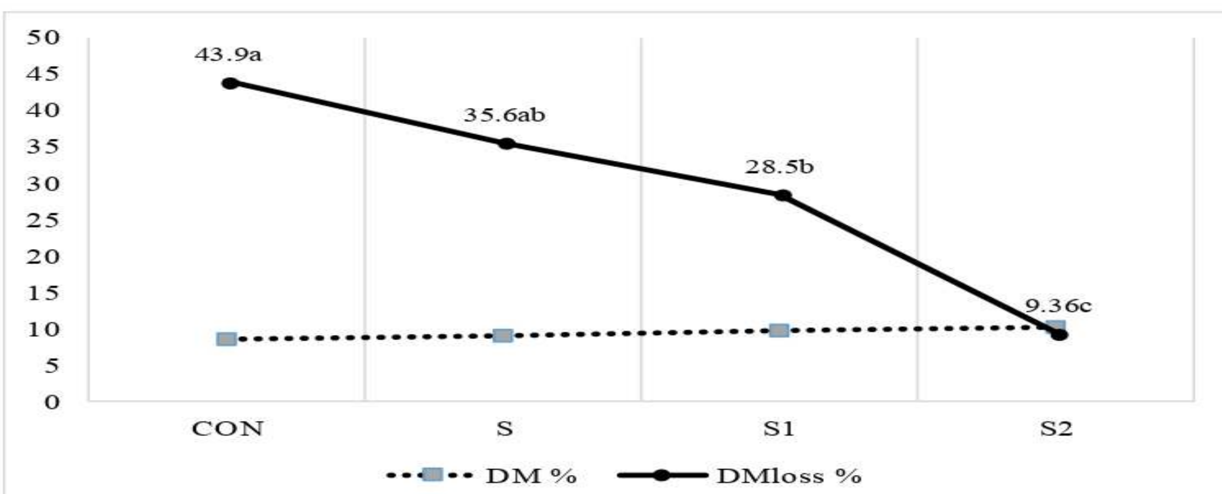

Figure 2: Effect of fertilizer application on dry matter (DM %) and dry matter loss (DMloss %) of barley sprouts. CON: control, without fertilizer; S: fertilizer applied to the seeds before planting; S1: fertilizer applied to the sprouts after 4 d of planting; S2: fertilizer applied to both, seeds before planting and sprouts after 4 d of planting. Different superscripts on the same line represent significant differences among the groups (P < 0.05).

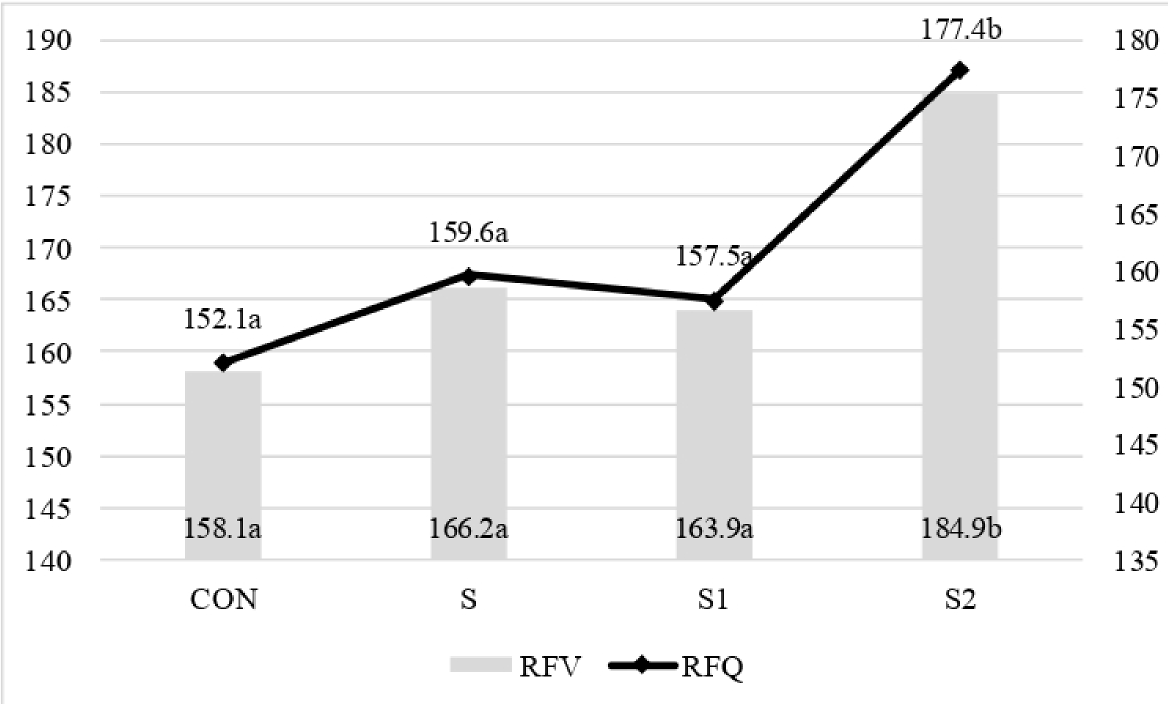

Figure 3: Effect of fertilizer application on relative feed value (RFV) and relative feed quality (RFQ) of barley sprouts. CON: control, without fertilizer; S: fertilizer applied to the seeds before planting; S1: fertilizer applied to the sprouts after 4 d of planting; S2: fertilizer applied to both, seeds before planting and sprouts after 4 d of planting. Different superscripts on the same line (RFQ) and inside bars (RFV) represent significant differences among the groups (P < 0.05).

DISCUSSION

After 10 days of planting shoot height ranged from 9.87 to 11.39 cm and root length ranged from 2.21 to 2.39 cm in the present study. In agreement with Emam (2016), shoot height of barley *(Giza 127 variety)* sprout reached to 10.10 cm. Shoot height and root length in S treated sprouts were quite closer to those in S2 treatment than S1 and control after 10 days of planting (Table 1). It is believed that the discrepancies in plant height occurring in hydroponic system could be attributed to many factors such as seeds type, harvesting time, and fertilization. For instance, Karasahin (2016) reported that the height of corn sprouts was increased by approximately 38% with the use of fertilizers. Akman *et al*. (2021) found that the shoot height and root length values in triticale and vetch sprouts were significantly increased by the addition of liquid fertilizer. As already discussed, the ability to reach to higher plant height likely explains the germination power of seeds depending on seeds type and harvesting days. Kusan *et al*. (2019) stated that the heights of triticale, barley, wheat, oat, and rye sprouts were determined as 16.08 cm, 17.47 cm, 14.21 cm, 13.90, and 11.67 cm, respectively, on the 8th day in a hydroponic chamber system. In the current study, shoot height of S2-sprouts reached to 11.41 cm, which was highest among the treatments. Importantly, positive effect on plant height (18 cm after 15 d after planting) was stated with addition of synthetic nutritious solution to ryegrass (Rivero *et al*., 2016). Considering the growth performance, therefore, the harvesting time and nutrient solution play a major role in the successful outcome of plant height. This outcome was in line with the opinion of Yurtseven *et al*. (2020), who stated that one week was not sufficient for effective germination of ryegrass possibly due to smaller seeds. The fresh weight in S2-sprouts were the highest among the treatments regardless of the days. The fresh weight ranged from 5.82 to 8.12 kg/tray (Table 1). The outcome of the present study is in agreement with Al-Karaki and Al-Momani (2011), who reported that the fresh weight of barley sprouts ranged from 4.74 and 6.0 kg kg^{-1} of barley seeds. Among treatments, control group had the highest (2.38 kg) and S2 group had the lowest (3.95 kg) dry weight per m^2. Our study was comparable to that of Assefa *et al*. (2020), who obtained 4.58 to 6.63 kg of dry sprouts per m^2. Mean value of dry barley sprouts (23.61-to-39.25-ton ha^{-1}) in the current study (Figure 1) were in line with that reported by Afzalinia and Karimi (2020) in which the values ranged from 23.96 to 42.51-ton ha^{-1}. Sometimes, it could be difficult to find the high-quality forage in the winter term lasting generally for 4 to 5 months in Turkiye depending on the topography (Bozyurt and Özdemir, 2021). Current hydroponic chamber (41.16 m2 of total trays) could approximately produce (5 months about 15 cycles) 35 to 40 ton of fresh green sprouts in such cases. At this point, sprouts should be carefully added to animal diets due to quite low DM content of sprouts leading to increase moisture ratio of ration (Akman *et al*., 2021). However, it is worth mentioning that the high moisture forage such as silage, haylage, green sprouts, and other have been important components of the rations (Coblentz and Akins, 2018) that increase the moisture of diet resulting in an increased DMI. The researchers have recommended that optimal DM content of ration should be more than 45% and fewer than 60% (Schingoethe, 2017; Dohme *et al*., 2008). This can be explained by too high DM content in the ration that reduces the DMI (Bargo *et al.,* 2002).

The DM contents of S, S1, and S2 sprouts were not different compared to control group. Similar or lower results were reported in previous studies on barley or sorghum sprouts. (Emam, 2016; Sriagtula *et al*., 2021). We believe that higher DM content of barley sprouts was partly associated with the application of fertilizer (Dung, 2010). This notion is supported by the plant height and wet weight of S2-sprouts in the present study that were 11.41 cm and 8.12 kg/tray, respectively. The number of dry sprouts harvested in hydroponic chamber per dry seed amount planted on the trays play an important role in hydroponic production. In the current study, fertilizer application increased the DM content and decreased the DM loss of sprouts. The df/ds ratio was found as 0.56, 0.64, 0.71 and 0.91 in control, S, S1 and S2 groups, respectively. The CP was highest in S2 group and lowest in control group. Our findings were in line with the results of Saidi and Omar (2015) who showed CP content reached up to 19.8% in barley sprouts produced in a hydroponic system. On the contrary, Fazaeli *et al*. (2012) described that the CP values of barley sprouts ranged from 13.69 to 14.47%. It is worth stating that these findings were inconsistent probably due to differences in research condition, seed type, seed quality, variety, addition of liquid fertilizer, and harvesting time. A study stated that (Akbag *et al*., 2014) the CP values were not different among the harvesting day 7 (17.11%), 10 (18.25), and 13 (17.59%). One possible reason for higher protein content in the sprouts could be because of the greater photosynthesis capability of young sprouts and its higher DM losses (Sneath and McIntosh, 2003). This idea is further supported by the fact that increased total protein content is associated with the positive effect of germination on enzyme activity that leads to change in amino acid profile (El-Morsy *et al*., 2013). Therefore, fertilizer application especially of nitrate and ammonium, can improve the CP content of the green sprouts (Girma and Gebremariam, 2018).

The highest mean EE value was recorded in S2 group, followed by S1, S, and control groups. Gumus and Bayir (2020) found that the EE values of barley and oat sprouts were 5.23 and 3.22% of DM, respectively. The observed higher value of EE content in fertilizer-treated was partly related to increase in the amount of chlorophyll and structural lipids during germination (Girma and Gebremariam, 2018). Regarding the ash content of barley sprout, it increased in all groups compared to their seeds on DM basis. Similar results were reported by Saidi and Omar (2015) and Emam (2016). The former stated that the ash content of sprouts produced in hydroponic chamber improved, while the latter stated that the ash content ranged between 2.27 and 3.43%. The reason for the increased ash content might be due to the mineral uptake (Morgan *et al*., 1992). Fazaeli *et al.* (2012) demonstrated that the calcium, iron and zinc content of sprouts were significantly higher than those of the seeds. The reducing effect of fertilizer on NFE was detected in the current study, particularly with a 13% decrease in comparison with control group, possibility due to the increase in CP and CF contents. Either seeds or sprouts of fertilizer treatments significantly affected the ivDMD compared to control group. Emam *et al*. (2018) indicated that ivDMD of barley sprouts was higher than that of seeds.

The highest CF value was found as 21.54% in S2-sprout and the lowest was 17.89% in control group. Emam *et al*. (2018) found that CF values of barley sprouts ranged from 8.13 to 12.43%. These results are in agreement with those of Girma and Gebremariam (2018) stating that structural carbohydrates such as CF, ADF, and NDF content was increased simultaneously with the increase in CP, EE, OM as well as NFE contents. The fertilizer application decreased NDF and hemicellulose of S2-sprout. These observations were in accordance with the findings of Adeyemi *et al.* (2020) who showed that organic and inorganic solutions decrease the NDF content and increase the ADF content of maize sprouts. Saidi and Omar (2015) reported that the NDF value was 3.5 times higher in barley sprouts compared with seeds. Fazaeli *et al.* (2012) reported that structural carbohydrates increase due to late harvesting.

The RFV and RFQ values play an important role as "Significant" for all forage according to the Quality Classifying Standard assigned by the Hay Market Task Force of American Forage and Grassland Council. The RFV is widely used by the United States to measure the feed value of alfalfa (Yang *et al.,* 2022). At the end of the experiment, the RFV and RFQ values were highest in S2-sprout, whereas it was the lowest in the CON. Our findings were in line with the results of Akman *et al.* (2021) who showed that the liquid fertilizer increased the RFV of triticale sprouts. Our previous study showed that RFV of barley sprout ranged from 180 to 200 (Gumus and Bayir, 2020). In a comparison of a hydroponic system and a soil system conducted by Sarıcicek *et al.*

(2018) it was identified that the hydroponic system yields the highest RFV.

Conclusion: In conclusion, compared with control, all fertilizer applications improved the shoot height, root length, and fresh weight. Improvements in an all the nutritive values, especially DM, CP, and ivDMD, were seen in the fertilizer treated groups. The RFV and RFQ were higher in the fertilizer treatments compared to control. The study showed that application of fertilizer to the seeds as well as sprouts can enhance the yield of green sprouts. These results suggest that the fertilizer may be applied to the seeds before planting.

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Author's contribution: Experimental design was created by HG, EK and UA. Samples were collected by DMK, EZO, and ET. Original manuscript was written by HG, EK and UA. All the authors contributed to the revision and final proofreading of the manuscript.

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