

1 **Barley (*Hordeum vulgare* L.) Cultivars Dry Forage Yield and Whole-Crop**
2 **Silage Quality as Affected by Interaction of Drought and Nitrogen**
3 **Fertigation**

4
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7 **Abstract**

8 Barley silage is a cornerstone of sustainable livestock feed, yet its production faces mounting
9 pressure from water scarcity and variable growing conditions. The objective of this study was
10 to explore how soil moisture conditions (50% and 85% soil-water depletion) and nitrogen (N)
11 fertigation (0 and 62.5 kg N/ha as urea, 46-0-0) shape the dry forage yield (DFY) and silage
12 quality of 10 barley cultivars across two distinct planting environments (E1: 22 October 2017;
13 E2: 4 March 2018). Conducted in field trials in 2017 and 2018, our results reveal a major
14 finding of a striking duality: N fertigation boosted dry matter yield (DMY) by 1474 kg/ha under
15 well-watered conditions, yet reduced it by 399 kg/ha under drought stress. This interaction also
16 influenced silage quality, with drought stress elevating silage dry matter (SDM), neutral
17 detergent fiber (NDF), and propionic acid while diminishing digestibility (DIG), relative feed
18 value (RFV), and lactic acid content, whereas N fertigation lowered NDF and, under adequate
19 moisture, enhanced DIG and RFV. The applied implication is that these insights enable
20 optimized N management strategies to enhance barley silage yield and quality in water-limited
21 regions.

22 **Keywords:** Barley cultivars, Digestibility, Fatty acids, Nitrogen, Soil moisture condition.

23 **Abbreviations:** ADF, acid detergent fiber; DDM, digestible dry matter, DMY, dry matter
24 yield; DIG14, digestibility in 14 hours; DIG30, digestibility in 30 hours; NDF, neutral
25 detergent fiber; NE_L, net energy for lactation; RFV, relative feed value; SDM, silage dry matter
26 content; TSC, total soluble carbohydrates; RDP, rumen degradable protein; MAD, maximum
27 allowable depletion; SAW, soil available water.

28
29 **1- Introduction**

30 Water scarcity, driven by dwindling rainfall, urbanization, industrialization, and climate
31 change, poses a growing threat to irrigation-dependent agriculture in arid and semi-arid regions
32 (Mancosu et al., 2015; Carraro and Di Iorio, 2022). This challenge has severely constrained

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33 forage production for livestock, with high-water-demand crops like corn silage and alfalfa hay
34 losing viability due to their warm-season growth patterns (Weller et al., 1992). Barley
35 (*Hordeum vulgare* L.), however, emerges as a resilient alternative, thriving in adverse
36 conditions such as drought, salinity, and heat stress (Feiziasl et al., 2022; Fischbeck, 2002). Its
37 adaptability to autumn and early spring planting allows it to capitalize on sparse rainfall,
38 making it a staple in water-limited environments (Bardehji et al., 2021). Globally, over 70% of
39 barley production supports livestock feed (Tricase et al., 2018), serving diverse species and
40 enhancing animal performance through high-quality whole-crop silage (Borowiec et al., 1998;
41 Wallsten and Hatfield, 2016). As an energy and protein source for milking herds, barley silage
42 holds untapped potential to boost farm profitability by improving yield and quality (Anderson
43 and Schroeder, 1999; Sun et al., 2021; Flaten et al., 2019).

44 Despite its drought tolerance, barley is not immune to water deficit, with drought stress
45 specifically causing 15–45% reductions in both grain and forage yield percentages under
46 varying stress intensities (Albrizio et al., 2010; Bardehji et al., 2021). These losses stem from
47 disrupted water and nutrient uptake (Mahlooji et al., 2018), with impacts varying across species
48 and cultivars. The effects on silage quality remain contentious: some report that drought stress
49 lowers leaf-to-stem ratios and digestibility while increasing crude protein (CP), acid detergent
50 fiber (ADF), and neutral detergent fiber (NDF) (Farahani and Chaichi, 2013; Uzun et al., 2017),
51 whereas others find ADF unaffected or fiber degradability unchanged (Islam et al., 2012;
52 Ferreira et al., 2021). Nitrogen (N) fertigation offers another lever to enhance forage outcomes.
53 Research demonstrates linear yield gains with N rates up to 132 kg/ha (Collen, 2006), alongside
54 increases in crude protein, digestibility, and leaf area, and reductions in fibrous components
55 (Nematpour et al., 2021; Vos et al., 2005). Yet, N effects on silage traits like water-soluble
56 carbohydrates, buffering capacity, and crude fiber remain inconsistent (Tremblay et al., 2005;
57 Brezink et al., 2002; Carr et al., 2004).

58 These conflicting findings, coupled with recent studies (2021–2025) on barley under deficit
59 irrigation and nitrogen management (e.g., Sabra et al., 2023; Al-Menaie et al., 2024; Bardehji,
60 et al., 2025), highlight a critical knowledge gap: while these works advance understanding of
61 individual effects on grain yield, the interactive impacts of soil moisture and N fertigation on
62 whole-crop silage yield and quality—across diverse genotypes in water-limited regions—
63 remain underexplored.

64 This study aims to assess the yield response of different barley genotypes to varying soil
65 moisture conditions and urea fertigation and evaluating the whole-crop silage quality,
66 providing insights to optimize production in drought-prone regions.

67

68 2- Materials and Methods

69 2.1 Experimental Design and Plant Material

70 This study was conducted at the Isfahan University of Technology research farm (Lavark) in
71 Najaf Abad, Iran (32°32' N, 51°23' E, 1630 m elevation) under two planting dates that
72 established distinct environmental conditions due to seasonal differences in climate and
73 photoperiod: E1 (fall planting on 22 October 2017) and E2 (spring planting on 4 March 2018).
74 A split-split plot design within a randomized complete block framework, replicated thrice, was
75 employed in each planting date. Main plots consisted of two soil moisture levels—control (50%
76 depletion) and water deficit (85% depletion)—while subplots featured two nitrogen fertigation
77 levels (0 and 62.5 kg N/ha as urea, 46-0-0). This N rate was selected based on preliminary soil
78 tests (Table 1), regional fertilizer recommendations for barley in semi-arid Iran, and previous
79 studies indicating significant yield responses at moderate rates (e.g., 50–80 kg N/ha) under
80 water-limited conditions. Sub-sub plots included 10 barley cultivars representing a diverse
81 range of drought tolerance levels (high, medium, and low) and spike row numbers (two- and
82 six-rowed types) (Table S1). Each sub-sub plot comprised 13 rows, 2 m long, spaced 15.4 cm
83 apart, with a planting density of 360–370 plants/m², averaging 1461 plants per plot and 112 per
84 row, sown by hand.

85

86 2.2 Soil and Weather Conditions

87 Soil physical and chemical properties are detailed in Table 1. Monthly weather data during
88 the growing seasons were sourced from the Najaf Abad Synoptic Weather Station (Table 2).

89

90 2.3 Soil Moisture and Nitrogen Treatments

91 Soil moisture treatments were based on the maximum allowable depletion (MAD) of soil
92 available water (SAW), ranging from 0.03 to 1.5 MPa. Control (55% ± 5% depletion) and water
93 deficit (85% ± 5% depletion) levels were set, monitored by measuring soil moisture at root
94 depth throughout growth.

95 Soil moisture was determined gravimetrically by periodic sampling (immediately before each
96 scheduled irrigation) from the 5–30 cm depth after removing the dry surface layer (0–5 cm) to
97 avoid measurement error. At least three subsamples were taken from both the control and

98 water-deficit regimes in each plot, placed immediately in sealed nylon bags to prevent moisture
 99 loss, and transported to the laboratory. In the laboratory, 100 g of fresh soil from each
 100 subsample was weighed and dried in a microwave oven for 25 min until constant weight was
 101 reached. Soil moisture content was then calculated on a gravimetric basis. These measured
 102 values were compared with the pre-determined field capacity ($\theta_{FC} = 25\%$) and permanent
 103 wilting point ($\theta_{PWP} = 16\%$) obtained from the soil laboratory of the Department of Soil Science,
 104 College of Agriculture, Isfahan University of Technology. The MAD thresholds were
 105 calculated as:

$$106 \quad \theta_{irrig} = \theta_{FC} - (\theta_{FC} - \theta_{PWP}) \times MAD$$

107 , where θ_{irrig} is the soil-water content at irrigation (m^3/m^3), θ_{FC} is field capacity (%), θ_{PWP} is
 108 wilting point (%), and MAD reflects depletion levels (Allen et al., 1998). Irrigation depth was
 109 determined by:

$$110 \quad D_{irrig} = (\theta_{FC} - \theta_{avg}) \times D_{rz}$$

111 with D_{irrig} as depth (cm), D_{rz} as root zone depth (cm), and θ_{avg} as pre-irrigation soil-water
 112 content (m^3/m^3) (Nematpour et al., 2020).

113 Irrigation occurred via drip tapes (16 mm diameter, 15 cm emitter spacing, 1.3 L/h flow),
 114 with tapes 30 cm apart, servicing two rows each. Water deficit began at Zadoks growth stages
 115 13 (3 leaves), after uniform irrigation at 55% MAD. Urea was applied in three equal doses (0
 116 or 20.83 kg N/ha) at Zadoks codes 13, 30 (stem elongation), and 50 (heading) via fertigation
 117 (Zadoks et al., 1974).

118 2.4 Yield Measurement and Silage Preparation

120 At the soft-dough stage (Zadoks code 85), plants from central rows were harvested and
 121 weighed. Subsamples (100 g) were oven-dried at 65°C for 48 h to determine dry matter
 122 percentage. Remaining material was chopped (~2 cm) using a two-row maize chopper
 123 (Barchinkar Industrial Co., Yazd, Iran) and packed into 240 three-liter PVC mini-silos at a
 124 packing density of 550 kg fresh matter m^{-3} (2 environments \times 2 moisture levels \times 2 nitrogen
 125 levels \times 10 cultivars \times 3 replicates). Silos were sealed after air removal with CO₂ and stored at
 126 room temperature for 90 days.

127 2.5 Silage Analysis

129 After 90 days, mini-silos were opened, and silage traits—pH, dry matter, ash, ADF, NDF,
 130 DDM, NEL, RFV, crude protein, total soluble carbohydrates (TSC), digestibility, and fatty
 131 acids—were assessed as follows:

132 **2.5.1 pH**

133 Fresh silage (30 g) was blended with 270 mL distilled water, filtered through double-layered
134 cheesecloth, and pH measured with a manual pH meter (Model 8601 AZ, China) (Nematpour
135 et al., 2020).

136
137 **2.5.2 Dry Matter and Ash**

138 Silage (200 g) was dried at 65°C for 48 h to calculate dry matter. Ash was determined by
139 incinerating dried samples at 550°C for 12 h, calculated as: %Ash = (ash weight/sample weight)
140 × 100.

141 **2.5.3 ADF, NDF, DDM, NEL, and RFV**

142 ADF and NDF were analyzed using an ANKOM 220 Fiber Analyzer (USA, Patent
143 5,370,007) per Van Soest et al. (1991). DDM, NEL, and RFV were derived from Yolcu et al.
144 (2008):

145 $DDM (\%DM) = 88.9 - (0.779 \times ADF);$

146 $DMI (\%BW) = 120/NDF;$

147 $RFV = (DMI \times DDM)/1.29;$

148 $NEL (Mcal\ kg^{-1}) = (1.044 - 0.0119 \times ADF) \times 2.205.$

149
150 **2.5.4 Crude Protein and TSC**

151 Crude protein was calculated as $6.25 \times$ total nitrogen, measured via Biancarosa et al. (1998).
152 TSC was quantified by Johnson et al. (1966), extracting 0.5 g silage with ethanol, reacting with
153 anthrone, and reading absorbance at 625 nm.

154
155 **2.5.5 Digestibility**

156 Dried, milled silage (0.5 g) was incubated in rumen bags within a fistulated cow for 14 and
157 30 h, then dried at 65°C for 48 h. Digestibility was calculated as: ((initial weight - final
158 weight)/initial weight) × 100 (Vanzant et al., 1998).

159
160 **2.5.6 Fatty Acids**

161 Fatty acids (acetic, lactic, propionic, butyric) were analyzed in four cultivars selected to
162 represent a range of genetic diversity (including local Iranian, improved, and international
163 varieties) and chosen based on their contrasting responses in preliminary yield and quality
164 assessments (Behrokh, M-93-16, Goharan, RGT-Planet) under normal nitrogen using HPLC
165 (HP 1090 Series, USA) per Arnetol et al. (2008).

166

167 2.6 Statistical Analysis

168 For all traits except fatty acids, a combined analysis of variance was performed on split-split
169 plot data (2 moisture levels × 2 nitrogen levels × 10 cultivars × 3 replicates) using SAS 9.4
170 (SAS Institute, Cary, NC, USA). For fatty acids, analysis used split plots (2 moisture levels ×
171 4 cultivars × 3 replicates). Means were compared with the LSD test ($P \leq 0.05$), and histograms
172 were drawn in Microsoft Excel 2016.

173

174 3- Results

175 3-1- Main results

176 **Soil moisture** conditions (50% and 85% depletion), nitrogen levels (0 and 62.5 kg N/ha as
177 urea), and cultivars affected nearly all traits (Table S2, Table 3). Exceptions included NEL and
178 acetic acid, which showed no cultivar variation. Overall, soil moisture emerged as the dominant
179 limiting factor, overriding nitrogen and cultivar effects for most yield- and quality-related
180 traits. Water deficit stress (85% depletion) reduced DMY, crude protein, DIG14, DIG30,
181 DDM, RFV, NEL, acetic acid, lactic acid, and butyric acid, but elevated SDM, ash, and
182 propionic acid relative to control conditions (50% depletion) (Tables 3 and 9). This shift
183 indicates a clear trade-off between biomass production and forage nutritive value under drought
184 stress, accompanied by altered fermentation profiles.

185 Nitrogen application boosted most traits—DMY, crude protein, DIG14, DIG30, DDM, RFV
186 and NEL —while reducing SDM, TSC, ADF, NDF, and pH (Table 3). These results confirm
187 the compensatory role of nitrogen in improving forage quality, particularly under favorable soil
188 moisture conditions.

189 Cultivar differences were notable, with Goharan showing the highest TSC and CB-84-10
190 exhibiting the lowest ADF and highest DIG14 (Table 3). These contrasting responses highlight
191 cultivar-specific strategies, where Goharan favors carbohydrate accumulation, while CB-84-
192 10 exhibits superior fiber digestibility, making it more suitable for high-quality forage
193 production.

194

195 3-2 Soil Moisture × Nitrogen Interaction

196 Soil moisture and nitrogen interacted significantly on DMY ($P \leq 0.01$; Table S2). Nitrogen
197 boosted DMY by 1474 kg/ha under control conditions but reduced it by 399 kg/ha under water
198 deficit stress. The highest DMY (11,580 kg/ha) occurred with control soil moisture and
199 nitrogen, while the lowest (6995 kg/ha) was under water deficit without nitrogen (Fig. 1). This

200 crossover interaction demonstrates that nitrogen efficiency is strongly moisture-dependent and
201 may become counterproductive under severe drought.

202

203 **3-3 Soil Moisture × Cultivar Interaction**

204 Under control soil moisture, cultivars varied significantly in DMY, but under water deficit,
205 only RGT-Planet differed (Table 4). This indicates a convergence of cultivar performance
206 under stress, except for RGT-Planet, which maintained partial yield stability. Water deficit hit
207 CB-84-10 hardest, slashing DMY by 5036 kg/ha, and raised ash by 18 g/kg in M-92-9 and 9
208 g/kg in RGT-Planet, with no effect on others (Table 4). Despite its superior digestibility, CB-
209 84-10 appears highly drought-sensitive, limiting its suitability for water-limited environments.

210

211 **3-4 Nitrogen × Cultivar Interaction**

212 Nitrogen increased RFV across all cultivars, with Fajr30 showing the largest gain (24 units)
213 and CB-84-10 the smallest (10 units) (Fig. S1). This suggests that cultivars differ in their
214 responsiveness to nitrogen fertilization, with Fajr30 being particularly efficient in converting
215 added nitrogen into forage quality improvements.

216

217 **3-5 Environment × Soil Moisture Interaction**

218 Water deficit stress effects varied by environment. In E1, it slashed DMY by 4730 kg/ha,
219 crude protein by 4 g/kg, and lactic acid by 10.3 g/kg DM, but raised propionic acid by 0.21
220 g/kg DM (Tables 5 and 10). In E2, DMY dropped by 2567 kg/ha, DIG30 by 0.02 g/kg, and
221 lactic acid by 12 g/kg DM, with propionic acid increasing by 0.41 g/kg DM; crude protein
222 remained unaffected (Tables 5 and 10). Water deficit stress consistently reduced lactic
223 acid but increased propionic acid in both environments (Table 10). These consistent shifts
224 indicate a stress-induced alteration in fermentation pathways, potentially affecting silage
225 stability and palatability. Environment 2 (March 2018) showed stronger stress-induced
226 changes for both acids compared to Environment 1 (October 2017) (Table 10). This
227 emphasizes the amplifying role of warmer late-season conditions on drought impacts.

228

229 **3-6 Environment × Nitrogen Interaction**

230 Nitrogen effects differed across environments (Table S2 and Table 6). In E1, it increased
231 DMY by 1298 kg/ha, DDM by 0.02, DIG30 by 0.04, and NEL by 0.13 MJ/kg, while cutting
232 ADF by 29 g/kg. In E2, DMY rose by 574 kg/ha, DDM by 0.02, and DIG30 by 0.04, with ADF
233 decreasing by 24 g/kg, but NEL showed no change (Table 6). This variability underscores the

234 need for environment-specific nitrogen management strategies rather than uniform
235 recommendations.

236

237 **3-7 Environment × Cultivar Interaction**

238 Environmental shifts altered ash in most cultivars except Behrokh, Fajr30, and RGT-Planet,
239 crude protein in Nik and M-94-15, ADF in all but M-92-9 and M-93-16, DIG30 in Reyhan03,
240 CB-84-10, M-93-16, and RGT-Planet, and DDM in all except M-92-9 and M-93-16 (Table
241 S3). M-93-16 consistently showed the highest ash. This stability suggests strong mineral uptake
242 capacity across environments.

243 Reyhan03 had peak ADF in E1, Behrokh in E2, while Reyhan03 led DIG30 in E2, and M-
244 93-16, M-94-15, and RGT-Planet topped E1. DDM peaked in Behrokh (E1) and M-94-15 (E2).
245 Lactic acid dropped by 5.6 and 10 g/kg DM in M-93-16 and RGT-Planet, respectively, from
246 E1 to E2, but remained stable in Behrokh and Goharan (Fig. 2). Such rank changes reveal
247 substantial genotype × environment interactions, reinforcing the importance of multi-
248 environment testing for cultivar selection.

249

250 **3-8 Environment × Nitrogen × Cultivar Interaction**

251 Nitrogen reduced NDF in all cultivars across environments, with Fajr30 (66 g/kg in E1) and
252 M-92-9 (69 g/kg in E2) showing the greatest reductions (Table 7).

253

254 **3-9 Environment × Soil Moisture × Nitrogen Interaction**

255 Water deficit stress raised SDM and NDF while lowering DIG and RFV in both
256 environments. Nitrogen reversed these trends under control soil moisture—reducing SDM and
257 NDF, and increasing DIG and RFV—but amplified them under water deficit (Table 8).

258

259 **3-10 Environment × Soil Moisture × Cultivar Interaction**

260 This triple interaction significantly affected SDM, TSC, NDF, pH, DIG14, and RFV (Table
261 S4). Peak changes occurred in Fajr30 (SDM: 92 g/kg; DIG14: 0.15), M-94-15 (TSC: 5.37 mg/g
262 DW), Behrokh (NDF: 94 g/kg; pH: 0.71; RFV: 51), while Goharan (SDM: 36 g/kg), CB-84-
263 10 (TSC: 1.92 mg/g DW), RGT-Planet (NDF: 27 g/kg; DIG14: 0.05), Nik (pH: 0.1), and M-
264 94-15 (RFV: 12) showed the least. Water deficit maximized SDM (except in Goharan and
265 RGT-Planet), and low-NDF treatments yielded the highest RFV across cultivars (Table S4),
266 under E2 conditions. These higher-order interactions collectively indicate that optimal forage

267 quality is achievable only when moisture availability, nitrogen supply, and cultivar choice are
268 jointly optimized.

269
270 **4- Discussion**

271 The interaction between soil moisture and nitrogen fertigation was the primary factor
272 determining barley silage yield and quality, with opposite effects under adequate versus deficit
273 irrigation. As expected, nitrogen application significantly increased DMY, DDM, DIG14,
274 DIG30, and NEL at 50% depletion (Table 3; Table 4), but these improvements were
275 consistently reduced or reversed at 85% depletion (Table 8). Mechanistically, under adequate
276 moisture, maintained root uptake and chlorophyll stability supported coordinated protein
277 synthesis. However, drought impaired stomatal conductance and photosynthetic nitrogen use
278 efficiency (Bista et al., 2018; Bardehji et al., 2021). Moreover, the negative yield response to
279 nitrogen under drought likely reflected osmotic stress, impaired nitrate assimilation, and a shift
280 of carbon toward nitrogen metabolism instead of biomass (An et al., 2025; Wang,
281 2021). Consequently, this carbon-nitrogen imbalance reduced forage quality by decreasing
282 protein and increasing fiber (Izadi et al., 2025; Liu et al., 2023). These findings confirm that
283 nitrogen application should be matched with water availability to avoid detrimental effects.

284 Environmental conditions strongly modified these responses. Compared to E2, the cooler and
285 longer growing period in E1 (autumn sowing, ~60 days longer) allowed nitrogen to improve
286 DMY, DDM, DIG30, NEL, and crude protein while reducing ADF and NDF (Table 6), leading
287 to superior digestibility. In contrast, the warmer E2 environment accelerated phenology and
288 increased respiration, weakening nitrogen assimilation and yield potential. Thus, higher
289 evaporative demand and a shorter grain-filling period in E2 likely explain the reduced nitrogen
290 effects on quality traits. This indicates that autumn sowing can enhance nitrogen use efficiency
291 in barley silage production.

292 The soil moisture \times nitrogen interaction also significantly affected silage chemical
293 composition and fermentation (Table 8; Table 9). Under control moisture, nitrogen reduced
294 SDM, NDF, and pH while increasing crude protein, DDM, DIG14, DIG30, RFV, and NEL.
295 Under water deficit, however, nitrogen increased SDM and NDF but reduced DIG and
296 RFV. This reversal is attributable to drought-induced lignin deposition and reduced cell wall
297 extensibility, which decreased substrate accessibility for microbial degradation (Choi et al.,
298 2023). Therefore, nitrogen application under drought not only fails to improve digestibility but
299 may actually impair it.

300 Across environments, water deficit consistently decreased lactic acid and increased propionic
301 acid (Table 10), indicating a shift in fermentation metabolism. This shift likely resulted from
302 drought-reduced soluble carbohydrates, which limited lactic acid bacteria activity, and from
303 selection for osmotolerant propionate-producing microbes under low pH and high osmotic
304 stress. Hence, reduced carbohydrate availability under drought constrains lactic fermentation
305 and alters organic acid profiles, affecting silage stability (Zi et al., 2022). This highlights that
306 moisture management is critical not only for yield but also for fermentation quality.

307 Cultivar-specific responses further modulated these interactions. Under control moisture,
308 Goharan and CB-84-10 excelled in TSC and DIG14, respectively (Table 4). Under water
309 deficit, performance differences narrowed except for RGT-Planet, which maintained partial
310 yield stability (Table 5). This variation likely reflects differences in root hydraulic
311 conductance, osmolyte accumulation, and nitrogen reduction capacity among cultivars
312 (Decouard et al., 2022; Kreszies et al., 2020). The nitrogen \times cultivar interaction showed
313 that Fajr30 had the greatest nitrogen-induced RFV increase and NDF reduction, while
314 CB-84-10 showed the smallest response (Fig. S1; Table 7). Thus, genotypic adaptation
315 determines both nitrogen efficiency and drought resilience.

316 Finally, the significant triple interactions (environment \times soil moisture \times nitrogen/cultivar)
317 (Tables S2, S4, S5) indicate that maximizing DMY, RFV, DIG30, and NEL requires joint
318 optimization of irrigation, nitrogen supply, and cultivar choice. Nitrogen enhanced yield and
319 nutritive traits only under well-watered conditions; under drought, its effectiveness declined or
320 became detrimental (Figure 1 and Table 8). Accordingly, integrated irrigation management,
321 adjusted nitrogen rates, and drought-tolerant cultivars are essential to maintain barley silage
322 productivity and quality across environments. These results provide practical guidance for
323 barley silage production in regions with contrasting water availability.

324

325 Conclusion

326 This study demonstrates the critical, context-dependent role of urea fertigation in barley
327 silage production, revealing a pronounced crossover interaction with soil moisture across two
328 distinct planting environments (fall vs. spring sowing). Water deficit stress (85% depletion)
329 raised silage dry matter, NDF, and propionic acid but lowered digestibility, RFV, and lactic
330 acid. Nitrogen fertigation ($62.5 \text{ kg N ha}^{-1}$) consistently reduced NDF; under adequate moisture
331 (50% depletion) it increased dry matter yield by 1474 kg ha^{-1} and enhanced digestibility and
332 RFV, whereas under drought it reduced yield by 399 kg ha^{-1} . By quantifying the three-way

333 interactions (environment × soil moisture × nitrogen) on key silage traits in a multi-
334 environment field trial with 10 genetically diverse barley cultivars, this work provides novel,
335 actionable insights into genotype × environment × management responses for whole-crop
336 silage in water-limited regions. Nitrogen's contrasting effects—beneficial with water, harmful
337 without—emphasize the need for moisture-based decision making. For farmers in semi-arid
338 regions, we recommend applying 62.5 kg N ha⁻¹ as urea fertigation when soil moisture
339 depletion is maintained at ≤50% to maximize dry forage yield and silage quality; however,
340 under anticipated drought conditions (≥85% depletion), nitrogen application should be reduced
341 or withheld to prevent yield penalties and preserve fermentation profiles. These quantitative,
342 site-specific recommendations will optimize sustainable barley silage production under
343 variable climate scenarios.

344

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348

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495 **Table 1.** Physico-chemical properties of soil at the experimental site.

Depth (cm)	Soil texture	TN (%)	K (mg/kg)	P (mg/kg)	OM (%)	pH	EC (dS/m)	Clay (%)	Silt (%)	Sand (%)
0-30	CL	0.063	350	38.03	0.534	7.91	1.18	30.0	31.0	39.0
30-60	CL	0.054	359	40.03	0.904	7.75	1.22	33.3	30.0	36.7

496 TN, total nitrogen; K, potassium; P, phosphorus; OM, organic matter; PH, potential hydrogen; EC, electrical
 497 conductivity.

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499 **Table 2.** Monthly mean minimum and maximum air temperatures, total rainfall, and
 500 evaporation during the growing period.

Environment	Month ⁱ	T min (°C)	T max (°C)	Rainfall (mm)	Evaporation (mm)
Environment1	October	9.9	26.9	0.000	166
	November	3.5	17.9	0.000	157
	December	-0.1	14.6	0.039	161
Environment2	January	-2.7	11.8	0.265	183
	February	4.0	16.4	0.429	209
	March	8.1	23.5	0.010	236
	April	9.5	21.6	1.149	260
	May	13.6	27.0	0.855	343
	June	20.0	35.6	0.097	351
	July	21.8	37.5	0.000	330

501 ⁱ1th Environments (22 Oct. 2017) and 2nd Environments (4 March, 2018).

502 **Table 3.** The main effects of environments, soil moisture condition, nitrogen levels and cultivars on forage yield and silage quality traits of barley
503 cultivars.

Experimental Factors	Levels	At the mid-dough stage (Z85)					In silage							
		DMY (kg/ha)	SDM (g/kg)	ash (g/kg)	Crude protein (g/kg DM)	TSC (mg/gDW)	ADF (g/kg)	NDF (g/kg)	DIG14 (g/kg)	DIG30 (g/kg)	pH	DDM (g/kg)	RFV	NEL (Mcal kg ⁻¹)
Environment	Environment 1	9899± 246.0 ^a	288± 2.2 ^b	113± 1.2 ^b	130.0± 0.75 ^a	6.7± 0.17 ^a	270± 2.8 ^a	434± 4.0 ^a	661± 4.2 ^a	692± 3.0 ^a	4.12± 0.037 ^a	683± 2.5 ^a	147± 1.7 ^a	6.45± 0.021 ^a
	Environment 2	8138± 151.0 ^b	314± 2.9 ^a	125± 1.3 ^a	127.0± 0.70 ^b	6.3± 0.17 ^b	275± 2.2 ^a	432± 3.1 ^a	663± 4.1 ^a	692± 4.1 ^a	4.13± 0.026 ^a	673± 2.2 ^a	146± 1.3 ^a	6.41± 0.021 ^b
Soil moisture condition ⁱⁱ	Control	10843± 182.0 ^a	286± 2.5 ^b	117± 1.3 ^b	129.0± 0.63 ^a	5.4± 0.14 ^b	265± 2.6 ^b	420± 3.6 ^b	683± 3.4 ^a	703± 3.0 ^a	4.07± 0.035 ^b	680± 2.2 ^a	152± 1.5 ^a	6.62± 0.017 ^a
	Stress	7194± 88.4 ^b	317± 2.2 ^a	121± 1.4 ^a	128.0± 0.82 ^b	7.6± 0.14 ^a	281± 2.2 ^a	446± 3.2 ^a	643± 3.0 ^b	675± 3.5 ^b	4.18± 0.028 ^a	673± 2.0 ^b	141± 1.4 ^b	6.33± 0.017 ^b
Nitrogen ⁱⁱⁱ	N0	8550± 188.0 ^b	307± 2.7 ^a	112± 1.2 ^b	123.0± 0.66 ^b	7.1± 0.17 ^a	286± 2.4 ^a	452± 3.4 ^a	642± 3.4 ^b	671± 3.4 ^b	4.18± 0.032 ^a	676± 2.4 ^b	138± 1.4 ^b	6.41± 0.021 ^b
	N1	9487± 239.0 ^a	295± 2.8 ^b	126± 1.2 ^a	134.0± 0.44 ^a	5.9± 0.16 ^b	259± 2.1 ^b	414± 2.8 ^b	673± 4.0 ^a	712± 3.0 ^a	4.06± 0.031 ^b	695± 2.0 ^a	155± 1.3 ^a	6.49± 0.025 ^a
cultivars	Goharan	9494± 508.0 ^{ab}	309± 7.6 ^{bc}	116± 3.8 ^{de}	130± 1.2 ^a	8.3± 0.39 ^a	276± 4.8 ^{bc}	430± 5.2 ^c	642± 6.2 ^{ef}	712± 6.2 ^b	4.29± 0.060 ^b	672± 4.2 ^{cd}	146± 2.5 ^c	6.45± 0.046 ^a
	Behrokh	8720± 419.0 ^c	300± 3.9 ^{de}	117± 2.0 ^{ce}	129± 1.8 ^{ab}	5.7± 0.35 ^c	267± 9.2 ^{cd}	417± 9.5 ^d	673± 8.0 ^c	670± 6.4 ^d	3.94± 0.058 ^{de}	685± 7.1 ^{bc}	154± 4.7 ^b	6.43± 0.063 ^a
	Nik	8997± 539.0 ^{bc}	304± 6.9 ^{cd}	117± 2.7 ^{bc}	130± 1.9 ^a	6.1± 0.29 ^{ce}	269± 4.2 ^{cd}	444± 7.8 ^b	660± 7.2 ^d	683± 5.3 ^c	3.91± 0.062 ^c	674± 3.4 ^d	142± 2.9 ^d	6.41± 0.050 ^a
	Fajr30	9575± 484.0 ^a	312± 5.5 ^b	122± 1.9 ^{bc}	128± 1.9 ^{ab}	5.8± 0.34 ^c	270± 5.3 ^{cd}	457± 7.3 ^a	683± 12.0 ^b	701± 5.3 ^b	4.12± 0.052 ^c	684± 4.0 ^{bc}	139± 2.9 ^d	6.45± 0.050 ^a
	Reyhan03	8743± 302.0 ^c	299± 5.9 ^{d-f}	115± 2.7 ^{de}	130± 1.4 ^{ab}	6.4± 0.30 ^{cd}	295± 5.5 ^a	464± 6.8 ^a	673± 7.2 ^c	705± 9.3 ^b	4.20± 0.067 ^{bc}	660± 4.1 ^c	133± 2.7 ^c	6.37± 0.050 ^a
	CB-84-10	9664± 597.0 ^a	294± 4.2 ^{c-g}	122± 2.4 ^b	126± 1.5 ^b	5.7± 0.23 ^c	257± 4.1 ^f	409± 7.3 ^d	634± 7.1 ^a	66.3± 8.0 ^d	3.82± 0.076 ^c	692± 3.3 ^a	158± 3.2 ^a	6.49± 0.046 ^a
	M-92-9	9446± 465.0 ^{ab}	281± 7.4 ^b	119± 3.7 ^{bd}	127± 1.6 ^{ab}	6.5± 0.34 ^c	289± 3.5 ^a	460± 7.3 ^a	655± 7.0 ^{de}	681± 5.1 ^c	4.49± 0.047 ^a	663± 3.0 ^c	135± 2.6 ^c	6.37± 0.050 ^a
	M-93-16	8529± 394.0 ^{cd}	331± 5.1 ^a	136± 2.6 ^a	129± 1.8 ^{ab}	6.0± 0.39 ^{de}	289± 2.8 ^a	412± 5.4 ^d	642± 6.1 ^f	683± 10.1 ^c	4.10± 0.075 ^c	693± 2.4 ^a	156± 2.4 ^a	6.54± 0.046 ^a
	M-94-15	8994± 460.0 ^{bc}	289± 4.6 ^g	115± 2.5 ^{de}	128± 1.6 ^{ab}	7.1± 0.44 ^b	265± 4.5 ^{de}	427± 4.4 ^c	653± 8.4 ^{de}	724± 5.4 ^a	4.23± 0.050 ^{bc}	680± 3.2 ^b	149± 2.1 ^c	6.49± 0.050 ^a
	RGT-Planet	8064± 472.0 ^d	292± 5.0 ^{fg}	112± 2.6 ^c	129± 1.5 ^{ab}	7.6± 0.32 ^b	269± 4.5 ^{cd}	409± 5.8 ^d	631± 6.5 ^f	711± 6.3 ^{ab}	4.10± 0.049 ^{cd}	682± 4.1 ^{bc}	155± 2.7 ^{ab}	6.45± 0.046 ^a

504 DMY, dry matter yield; SDM, silage dry mater content; TSC, total soluble carbohydrate; ADF, acid detergent fiber; NDF, neutral detergent fiber; DIG14, digestibility after 14
505 hours; DIG30, digestibility after 30 hours; DDM, digestible dry matter; RFV, relative feed value; NEL, net energy for lactation. ⁱEnvironment 1 (22 Oct. 2017) and Environment
506 2 (4 March, 2018), ⁱⁱIrrigation (control, 55% MAD and stress, 85% MAD), ⁱⁱⁱNitrogen (N0, 0 kg/ha and N1, 62.5 kg/ha). In each column and within each factor, numbers
507 followed by the same letter are not significantly different ($P<0.05$).

508 **Table 4.** The interaction effects of soil moisture conditions and cultivars on DMY and ash in barley.

Barley cultivars	At the mid-dough stage (Z85)		In silage	
	DMY (kg/ha)		Ash (g/kg)	
	Control ⁱ	Stress	Control	Stress
Goharan	11684±456.0 ^{ab}	7304±216.0 ^g	117±5.1 ^{ef}	114±6.0 ^{e-g}
Behrokh	10348±483.0 ^{d-f}	7091±227.0 ^g	113±2.9 ^{e-g}	120±2.6 ^{de}
Nik	10863±737.0 ^{cd}	7132±304.0 ^g	128±3.3 ^{de}	115±4.6 ^{ef}
Fajr30	11589±476.0 ^{ab}	7561±242.0 ^g	119±2.9 ^{de}	125±2.6 ^{cd}
Reyhan03	10117±572.0 ^{ef}	7288±356.0 ^g	115±3.6 ^{e-g}	116±4.3 ^{ef}
CB-84-10	12182±351.0 ^a	7146±313.0 ^g	118±3.6 ^{de}	125±3.2 ^{cd}
M-92-9	11226±538.0 ^{bc}	7666±281.0 ^g	110±4.2 ^{fg}	128±5.2 ^{bc}
M-93-16	9863±518.0 ^f	7196±295.0 ^g	135±3.6 ^{ab}	136±4.0 ^a
M-94-15	10706±586.0 ^{c-e}	7281±214.0 ^g	115±3.6 ^{e-g}	115±3.8 ^{ef}
RGT-Planet	9851±580.0 ^f	6278±233.0 ^h	107±3.3 ^g	116±4.0 ^{ef}

509 DMY, dry matter yield; ⁱ Soil moisture conditions (control, 55% MAD and stress, 85% MAD). In each trait,
 510 numbers followed by the same letter are not significantly different ($P<0.05$).
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512 **Table 5.** The interaction effects of environments and soil moisture conditions on DMY, CP and
 513 DIG30 in barley cultivars.

Traits		Environment 1 ⁱ		Environment 2	
		Control ⁱⁱ	Stress	Control	Stress
DMY (kg/ha)	At the mid-dough stage (Z85)	12264±194.0 ^a	7534±140.0 ^c	9422±169.0 ^b	6855±90.0 ^d
Crude protein (g/kg DM)	In silage	132.0±0.94 ^a	128±1.1 ^b	128.0±0.73 ^b	127±1.2 ^b
DIG30 (g/kg)	In silage	712±4.2 ^a	673±4.0 ^b	70.4±5.1 ^a	684±5.1 ^b

514 DMY, dry matter yield; DIG30, digestibility after 30 hours; ⁱ Environment 1 (22 Oct. 2017) and Environment 2 (4
 515 March, 2018). ⁱⁱ Soil moisture conditions (control, 55% MAD and stress, 85% MAD). In each trait, numbers followed
 516 by the same letter are not significantly different ($P<0.05$).
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518 **Table 6.** The interaction effects of environments and nitrogen levels on DMY, DIG30, DDM and
 519 NE_L of silage in barley cultivars.

Traits		Environment 1 ⁱ		Environment 2	
		N0 ⁱⁱ	N1	N0	N1
DMY (kg/ha)	At the mid-dough stage (Z85)	9250±309.0 ^b	10548±370.0 ^a	7851±179.0 ^d	8425±240.0 ^c
ADF (g/kg)	In silage	285±3.8 ^a	256±3.2 ^c	287±2.9 ^a	263±2.6 ^b
DDM (g/kg)	In silage	671±3.21 ^c	692±2.11 ^a	660±2.14 ^c	683±2.33 ^b
DIG30 (g/kg)	In silage	682±4.12 ^b	724±4.25 ^a	672±4.32 ^b	715±4.11 ^a
NE _L (Mcal kg ⁻¹)	In silage	6.41±0.029 ^b	6.54±0.034 ^a	6.41±0.029 ^b	6.49±0.034 ^{ab}

520 DMY, dry matter yield; ADF, acid detergent fiber; DDM, digestible dry matter; DIG30, digestibility after 30
 521 hours; NE_L, net energy for lactation. ⁱ Environment 1 (22 Oct. 2017) and Environment 2 (4 March, 2018), ⁱⁱ
 522 Nitrogen fertilizer (N0, 0 kg/ha and N1, 62.5 kg/ha). In each trait, numbers followed by the same letter are not
 523 significantly different ($P<0.05$).
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531**Table 7.** The interaction effects of environments, nitrogen levels and cultivars on NDF of barley silage.

cultivars	Environment 1 ⁱ		Environment 2	
	N0 ⁱⁱ	N1	N0	N1
Goharan	452±6.6 ^{d-f}	429±5.8 ^{h-k}	437±11.6 ^{f-h}	402±7.2 ^{m-o}
Behrokh	393±2.7 ^{o-q}	373±7.1 ^r	484±13.4 ^b	416±9.2 ^{j-m}
Nik	489±5.8 ^{ab}	437±2.5 ^{f-i}	449±11.1 ^{d-g}	402±5.8 ^{m-o}
Fajr30	504±4.9 ^a	438±9.4 ^{e-h}	464±7.4 ^{cd}	421±9.7 ^{i-l}
Reyhan03	493±6.8 ^{ab}	478±7.9 ^{bc}	462±9.5 ^d	424±12.5 ^{h-i}
CB-84-10	378±4.8 ^{qr}	379±7.4 ^{qr}	454±7.0 ^{dc}	424±8.2 ^{h-l}
M-92-9	492±8.7 ^{ab}	448±10.2 ^{d-g}	485±6.8 ^b	416±6.4 ^{k-m}
M-93-16	432±5.1 ^{h-j}	397±6.9 ^{u-p}	427±13.6 ^{h-l}	391±7.1 ^{o-q}
M-94-15	433±6.6 ^{g-i}	417±7.5 ^{j-m}	448±73.2 ^{d-g}	412±7.4 ^{l-n}
RGT-Planet	426±8.4 ^{h-l}	387±6.8 ^{o-r}	438±7.4 ^{f-h}	385±5.1 ^{p-r}

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ⁱEnvironment 1 (22 Oct. 2017) and Environment 2 (4 March, 2018), ⁱⁱNitrogen fertilizer (N0, 0 kg/ha and N1, 62.5 kg/ha). Numbers followed by the same letter are not significantly different ($P<0.05$).

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536**Table 8.** The interaction effects of environments, irrigation regimes and nitrogen levels on SDM, NDF, DIG14 and RFV in barley silage.

Environment	Soil moisture condition ⁱⁱ	Nitrogen ⁱⁱⁱ	SDM (g/kg)	NDF (g/kg)	DIG14 (g/kg)	RFV
Environment 1	Control	N0	278±3.0e	441±4.3c	663±7.21b	144±1.4c
		N1	268±2.6f	404±3.9f	701±5.11a	161±1.6a
	Stress	N0	291±3.3d	432±4.0d	662±4.22b	149±2.0b
		N1	314±3.9b	458±5.1b	624±9.15d	136±1.2d
Environment 2	Control	N0	302±6.0c	438±6.0cd	664±8.41b	143±2.3c
		N1	294±3.2d	396±4.4g	703±10.3a	162±2.8a
	Stress	N0	328±4.3a	422±3.8e	643±11.4c	150±1.3b
		N1	334±4.1a	472±5.0a	624±4.22d	131±2.2e

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SDM, silage dry matter content; NDF, neutral detergent fiber; DIG14, digestibility after 14 hours; RFV, relative feed value. ⁱEnvironment 1 (22 Oct. 2017) and Environment 2 (4 March, 2018), ⁱⁱSoil moisture condition (control, 55% MAD and stress, 85% MAD); ⁱⁱⁱNitrogen (N0, 0 kg/ha and N1, 62.5 kg/ha). In each column and within each factor, numbers followed by the same letter are not significantly different ($P<0.05$).

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544**Table 9.** The main effects of environments, irrigation regimes and cultivars on acetic, lactic, propionic and butyric acid content of barley silage.

Experimental Factors	Levels	In silage			
		Acetic (g/kg DM)	Lactic	Propionic	Butyric
Environments	Environment 1	14.5±0.41 ^a	40±1.4 ^a	1.77±0.045 ^b	0.11±0.006 ^a
	Environment 2	11.9±0.32 ^b	36±1.5 ^b	1.96±0.051 ^a	0.10±0.006 ^b
Soil moisture condition ⁱⁱ	Control	14.3±0.43 ^a	43.5±0.85 ^a	1.72±0.039 ^b	0.13±0.003 ^a
	Stress	12.0±0.36 ^b	33±1.1 ^b	2.01±0.045 ^a	0.08±0.004 ^b
Cultivar	Behrokh	13.4±0.53 ^{ab}	38±1.6 ^b	1.78±0.058 ^{bc}	0.10±0.007 ^b
	Goharan	13.1±0.74 ^{ab}	41±1.4 ^a	1.89±0.068 ^b	0.12±0.008 ^a
	M-93-16	12.2±0.55 ^b	33±2.3 ^c	1.71±0.063 ^c	0.09±0.008 ^c
	RGT-Planet	13.9±0.65 ^a	40±2.3 ^{ab}	2.09±0.048 ^a	0.11±0.009 ^a

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ⁱEnvironment 1 (22 Oct. 2017) and Environment 2 (4 March, 2018), ⁱⁱSoil moisture condition (control, 55% MAD and stress, 85% MAD). In each trait, numbers followed by the same letter are not significantly different ($P<0.05$).

549 **Table 10.** The interaction effects of environments and soil moisture condition on lactic and
 550 propionic acid content in barley silage.

Traits	Environment 1 ⁱ		Environment 2	
	Control ⁱⁱ	Stress	Control	Stress
Lactic (g/kg DM)	45.4±1.3 ^a	35.1±1.4 ^c	42.0±0.94 ^b	30±1.5 ^d
Propionic (g/kg DM)	1.67±0.054 ^c	1.88±0.055 ^b	1.73±0.051 ^{bc}	2.14±0.048 ^a

551 ⁱ Environment 1 (22 Oct. 2017) and Environment 2 (4 March, 2018), ⁱⁱ Soil moisture condition (control, 55%
 552 MAD and stress, 85% MAD). In each trait, numbers followed by the same letter are not significantly different
 553 (P< 0.05).
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555 **عملکرد علوفه خشک و کیفیت سیلاژ کامل جو (*Hordeum vulgare* L.) ارقام تحت تأثیر برهمکنش**
 556 **خشکی و کودآبیاری نیتروژن**

557 سیاوش برده جی، حمیدرضا عشقی زاده، مرتضی زاهدی، و مهرداد محلوجی

560 **چکیده**

561 سیلاژ جو سنگ بنای خوراک دام پایدار است، با این حال تولید آن با فشار فزاینده‌ای از کمبود آب و شرایط رشد متغیر مواجه
 562 است. هدف از این مطالعه بررسی چگونگی تأثیر شرایط رطوبت خاک (50٪ و 85٪ تخلیه آب خاک) و کوددهی نیتروژن (N
 563 0 و 62.5 کیلوگرم نیتروژن در هکتار به صورت اوره، 0-0-46) بر عملکرد علوفه خشک (DFY) و کیفیت سیلاژ 10 رقم
 564 جو در دو محیط کاشت مجزا (E1: 22 اکتبر 2017؛ E2: 4 مارس 2018) بود. نتایج ما که در آزمایش‌های میدانی در سال‌های
 565 2017 و 2018 انجام شد، یافته اصلی یک دوگانگی قابل توجه را نشان می‌دهد: کوددهی نیتروژن، عملکرد ماده خشک (DMY)
 566 را در شرایط آبیاری مناسب 1474 کیلوگرم در هکتار افزایش داد، اما در شرایط تنش خشکی آن را 399 کیلوگرم در هکتار
 567 کاهش داد. این تعامل همچنین بر کیفیت سیلو تأثیر گذاشت، به طوری که تنش خشکی باعث افزایش ماده خشک سیلو (SDM)،
 568 فیبر شوینده خنثی (NDF) و اسید پروبیونیک در حالی که قابلیت هضم (DIG)، ارزش نسبی خوراک (RFV) و محتوای اسید
 569 لاکتیک را کاهش داد، در حالی که کودآبیاری با نیتروژن، NDF را کاهش داد و در شرایط رطوبت کافی، DIG و RFV را
 570 افزایش داد. مفهوم کاربردی این است که این بینش‌ها، استراتژی‌های مدیریت بهینه نیتروژن را برای افزایش عملکرد و کیفیت
 571 سیلوی جو در مناطق کم‌آب امکان‌پذیر می‌سازد.
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