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Research article

Water regimes in selected fodder radish (*Raphanus sativus*) genotypes: Effects on nutritional value and *in vitro* ruminal dry matter degradability

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ABSTRACT

Fodder radish is widely used as a livestock supplement, however, the nutritional value of fodder radish under different water conditions remains insufficiently understood. This study aimed to assess the chemical components and *in vitro,* ruminal dry matter degradability of two fodder radish genotypes (Endurance and Line 2) subjected to three irrigation regimes: well-watered (W1), moderate water stress (W2), and severe water stress (W3). The analysis revealed statistically significant effects of the main factors on the chemical composition and estimates of fodder radish leaves and tubers, particularly in terms of Crude Protein (CP) and Ether Extract (EE) across genotypes. Both Endurance and Line 2 leaves exhibited interaction effects on N, P, Ca, Mg, K, Na, Fe, Zn, Cu, Mn and Al. Meanwhile only Na, K, Zn, and Cu were affected in tubers. Endurance tubers, specifically, displayed significantly higher ($p < 0.05$) CP content, with Line 2 tubers showing the highest CP content under W1. Furthermore, Endurance leaves had higher levels of Neutral Detergent Fibre, EE, and Non-Structural Carbohydrate (NSC) compared to Line 2 leaves under W1. Notable differences in tuber fibres were found, specifically in Acid Detergent Fibre for Endurance, with W3 exhibiting a higher concentration level. Both genotypes displayed higher NSC under W3. Significant variations in macro and mmicro minerals were observed between water levels in both genotypes. In terms of *in vitro* degradability during the 24 h and 48 h incubation periods, all treatments met the acceptable level of 60–80 %. Regardless of water regimes, both Endurance and Line 2 showed nutrient concentrations meeting the minimum

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requirements for optimal animal production. Though, Line 2 exhibits significantly higher nutritional value and *in vitro* ruminal dry matter degradability than Endurance, evident in both leaves and tubers. Notably, moderate water stress conditions yielded better nutritional quality and *in vitro* ruminal dry matter degradability compared to both well-watered and severe water stress treatments. This suggests that applying 180–220 mm of water per season can also yield better nutritive value of these genotypes.

1. Introduction

To ensure the food security of an increasing human population, it is crucial to increase crop production capacity both under normal conditions and during times of extreme moisture stress. The necessity to assess existing crops for drought tolerance is inevitable due to the increase of water deficit in the world caused by the competing water demands among livestock, humans, and industrial sectors [1]. Furthermore, in most rural parts of South Africa, drought has led to high deaths of cattle and sheep because of the unavailability of forages especially during dry and dormant seasons [2].

In rural households, grazing livestock contribute to social, cultural, and economic functions as they improve the well-being and income of the farming family. Livestock farming promotes family and community employment, social status, family nutrition, ritual purposes, food supply, family income, agricultural diversification, transport, soil productivity, asset savings, agricultural traction, and sustainable agriculture [3]. The production of livestock is integrated with crop production. Livestock and its by-products used as manure, play an important role in crop production.

Apart from livestock, plants are also regarded as the primary elements of agricultural systems as well as major sources of revenue [4]. One of the difficulties in grazing livestock mainly during the dry season, is the unavailability of fodder banks particularly among smallholder livestock producers. Around the world, a variety of forages and fodder crops have been used for animal diets [5]. Lablab, cowpea, soya bean, sorghum, fodder beet, kale, and fodder radish are just a few examples. Although at a very small scale, *Raphanus sativus* var *olefemis* Pers also known as Japanese radish is used as a feed resource for livestock in parts of South Africa [6]. The genotypes that were evaluated by the current study, namely Endurance and Line 2 were bred specifically for late flowering and thus provide high-quality forage yield for grazing livestock in late winter (August).

When compared to other fodder crops in the *Brassicaceae* family, species like the fodder radish (*Raphanus sativus* var *olefemis* Pers) are annual or biannual crops with a short cycle [7,8]. Ammann et al. [7] indicated that this plant has a five to seven-month growth cycle, and it has been employed as single graze fodder in several locations [9], including South Africa [6,10,11]. Agriculture heavily relies on climate and is negatively impacted by climate extremes primarily due to increasing climate variability and anthropogenic climate change. Furthermore, agricultural production risks are likely to become a problem in various areas globally as heatwaves and droughts may increase the occurrence of crop failure. Drought has a substantial effect on the economy, society, environment, and agriculture as it is one of the crucial natural hazards. Notably, agricultural drought focuses on the amount of soil water available for crop and forage growth and has no direct correlation between precipitation and infiltration into the soil [12]. This may warrant the need to introduce plant species that can adapt to climate changes and soil properties to address forage shortage during dry seasons.

Lack of water slows down plant development, and various morphological, anatomical, and biochemical changes may appear, lowering the crop's capacity for production [13]. Additionally, the lack of soil moisture might restrict the absorption of nutrients by roots, which can directly affect the translocation owing to the plant or crop's low transpiration level. Plants often exhibit flexibility in their acquisition and partitioning of resource availability when responding to environmental situations [14]. According to Iqbal et al. [15] and Seleiman et al. [16], the accumulation of solutes that permit cell expansion, maintain stomatal openness, and carbon dioxide absorption, as well as the development of xeromorphic characteristics, may boost a plant's tolerance to drought. These adaptable characteristics can aid plants in coping with intense stress. To survive during periods of water stress, plants have also been found to give additional nutrients for the growth of the roots [17,18]. However, Norman et al. [19], reported better nutritional value for radish under well-watered conditions. Omokanye et al. [20] assessed chemical composition of Daikon radish and Tillage radish under field experiment. Their findings showed that Daikon radish cultivar had crude protein (CP) 15.9 %, Acid Detergent Fibre (ADF) 24.6 %, Neutral Detergent Fibre (NDF) 32.9 %, phosphorus 0.37 % dry matter (DM) and potassium 2.31 % DM, while Tillage radish had CP 13.4 %, ADF 34.5 %, NDF 51.6 % phosphorus 0.2 % DM and potassium 2.09 % DM. Dairy cows showed an improvement in milk yield and feed conversion efficiency due to the consumption of fodder radish diet [21]. A nutritious diet or feed in grazing livestock promotes good health which results in optimal productivity. Information on the nutritional value of fodder radish under various water levels is poorly understood. Hence, the objective of this study was to evaluate chemical components and *in vitro* ruminal dry matter degradability in newly developed genotypes of fodder radish under different water regimes. We assumed that well-watered treatments would increase the CP, *in vitro* degradability, and mineral concentrations, and reduce fibre content. We further hypothesised that applying high water in forages either under rainfed or irrigation enhances the quality of forages. Literature has shown the quality parameters such as CP and *in vitro* degradability to increase while Acid Detergent Lignin (ADL), NDF, and ADF decrease with an increase in water applied to forages, [22–24]. Based on this background, we predicted that Endurance and Line 2 genotypes under a well-water regime (W1) would have the highest nutrient concentrations, and severe stress (W3) would have the lowest. We tested our prediction by assessing chemical components and *in vitro* ruminal dry matter degradability of two fodder radish genotypes under controlled environment field conditions.

2. Material and methods

2.1. Site description and soil quality attributes

The experiment was conducted in Roodeplaat, Agricultural Research Council (ARC), Pretoria, Gauteng Province (25° 60′ S; 28° 35′ E; 1168 m.a.s.l.) South Africa. It took place under normal field conditions and rainfall was excluded because the rain shelter was designed to close when rainfall starts [25] [Fig. 1(a, b)]. Nyathi et al. [1] presented long-term climate data from 1990 to 2015 which shows that the site receives 650 mm of summer rainfall annually and most of this takes place between (October–March). The maximum average temperature of 30 °C is experienced during the month of January. The soil texture class in the rain shelter was classified according to (USDA taxonomic system) as sandy clay loam [1] with a field capacity of 291 mm m^{-1} and the permanent wilting point was 20 mm m^{-1}. There are grazing livestock farmers around the area who are also facing a shortage of forage availability during dry seasons. The chemical properties of topsoil at the layer of 0.3 m are shown in Table 1.

2.2. Plant material

Seeds used for this experiment were sourced from ARC API Cedara seedbank. Fodder radish genotypes used in this study have some common characteristics of annual cool season crops: hairless soft leaves, shoot system (broad-leafy plants), and root system (large tube but differed in size), however, they differ in flowering behavior and flower color. In South Africa, fodder radish is commonly used as feed for livestock when there is a shortage of feed. This study evaluated two genotypes developed by the Agriculture Research Council of South Africa. These genotypes namely Endurance and Line 2 were bred to provide high-quality forage for grazing livestock in late winter (August). These genotypes were derived from a cross between a very late flowering fodder radish line "PG 1" from Pyne Gould Wrightson Seeds, New Zealand, and Agricultural Research Council- Range and forage unit, Cedara, South Africa, fodder radish cultivars Geisha and Sterling. Endurance and Line 2 were selected for late flowering and high yielding.

2.3. Trial layout and design

The experiment was conducted under a rain shelter at the Agricultural Research Council, Vegetables and Ornamental Plants, for the 2021/22 winter season. The experimental design was a 3 × 2 factorial design replicated thrice: factors were three irrigation water regimes {well-watered 30 % (W1), moderate stress 50 % (W2) and severe stress 80 % (W3)} and two fodder radish genotypes Endurance and Line 2. The treatments are outlined as follows: The plants were irrigated back to fill capacity when 30 % (W1) was lost, and the same was done when 50 % (W2) and 80 % (W3) of plant available water was lost.

The individual plot size was 4.6 m^2 with inter-row and intra row spacing of 0.3 m × 0.3 m making it a total of 111,111 plants ha^{-1}. Table 2 presents the meteorological conditions [maximum and minimum temperatures (°C), total solar radiation (MJ m^{-2}), cumulative reference evapotranspiration (mm), U2 Average Wind Speed (ms), CU Total Cold Units (unitless), HU Total Heat Units (unitless) and vapour pressure deficit (kPa)] during 2021/2022 growing season while the total rainfall (mm) was excluded because rain shelter excludes rain [25]. Prior to planting, aluminium access tubes were installed in the middle of each plot to a depth of 1 m. A neutron water meter (CPN, 503 DR Hydroprobe, USA) calibrated for the site with measurements from a wet and dry profile was used to measure soil water content. Compensating non-leaking (CNL) Urinam dripper lines, with a discharge dripper rate of 2.3 l h^{-1} were used for irrigation. Irrigation scheduling was based on irrigation regimes (W1, W2, and W3).

2.4. Irrigation management

An irrigation system that was used in the rain shelters is drip irrigation. Pump, filters, solenoid valves, water meter, control box, online drippers, 200-L water tank, main line, sub-main line, and laterals are all components of the irrigation system that were used in this study. The 200 kPa is the maximum operating pressure that can only be allowed by the system with an average discharge of 2 L per hour. Dripper line spacing was based on plant spacing (0.3 m × 0.3 m). The water seepage and lateral movement of water prevention

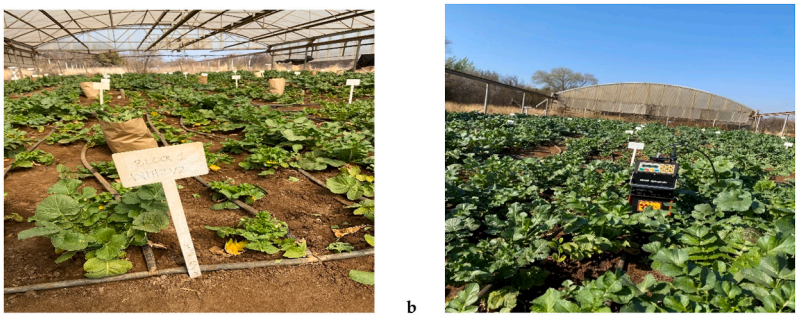

Fig. 1. a: Experimental design with the rain shelter closed due to rainfall; b: Rain shelter open due to normal weather conditions.

Table 1
Chemical properties ($mgkg^{-1}$, unless otherwise stated) of the topsoil layer (0.3 m) for the experimental site.

Nutrients	Range per depth	Fertility status
P	98.8	High
K	122	Low
Ca	1730	Moderate
Mg	2.89	High
Na	91.7	Fair
Clay (%)	18	
pH	7.8	Slightly alkaline

Table 2
Monthly meteorological data for the 2021/22 winter season.

Month	Tx	Tn	Rs	U2	ET_0	HU	CU	VPD
May	27.9	3.78	15.09	0.7	2.95	4.01	2.6	0.97
June	22.89	1.88	13.13	0.79	2.46	1.31	5.13	0.8
July	23.64	1.42	16.24	0.53	2.8	0.77	5.5	0.82
August	30.5	4.9	20.79	1.08	4.52	6.15	−1	1.09
September	29.63	8.87	19.54	1.13	1.56	4.14	9.09	1.61

The reported values are monthly means of daily climatic data during the 2021/2022 winter season; from planting day to end of the harvest; Tx = Daily Maximum Temperature °C, Tn = Daily Minimum Temperature °C, Rs = Total Radiation MJ/m2, U2 = Average Wind Speed ms, ET0 = Total Relative Evapotranspiration mm, HU = Total Heat Units Unitless and CU = Total Cold Units Unitless.

between plots were prevented by trenching a 200 μm thick polyethylene sheet at a depth of 1 m between plots. For plant establishment, treatments were given the same amount of water for three weeks, and then the treatments were imposed. Irrigation was applied three times every week. Plants were irrigated during the morning at the same time to ensure water availability during peak periods of demand in the day. The total amount of irrigation water applied was recorded for all irrigation levels including water that was applied prior to the introduction of treatments. The soil water status during the growing period was monitored using a neutron probe reading. The total water applied to the treatments for both genotypes was: W1 = 305 mm; W2 = 221 mm and W3 = 180 mm.

2.5. Agronomic practice and data collection

Prior to land preparation, soil samples from each plot were collected for chemical analysis by using a 30 cm augur. The land was mechanically ploughed, and a seedbed was prepared before planting. The weeds were removed by hand before planting and during the experiment. Fertilizer was applied as per the chemical analysis results (Table 1). In all rows, planting was done by hand at 1 cm depth opened by hand. Selected fodder radish genotypes were harvested after four months of planting, and only plants between rows were used for data collection to avoid the border effect. The harvesting was done by separating above and below fresh matter and oven-drying them at 70 °C until constant weight was reached. Then above and below plant material were ground to pass through a 1 mm sieve prior to chemical analysis. Milled samples were transferred into airtight sample bottles.

2.6. Chemical analysis

Fodder radish leaves and tubers plant material were ground to pass through a 1 mm sieve prior chemical analysis. Ground samples of fodder radish genotypes were analysed for the chemical composition in the North-West University Animal Science laboratory at the Molelwane University farm. Approximately 1 g of each sample was placed into pre-weighed crucibles and placed in an oven set at 105 °C for 12 h to estimate the dry matter (DM). The loss in weight was measured as moisture content and DM was calculated as the difference between initial sample weight and moisture weight. Organic matter (OM) concentration was determined by ashing the dried samples in a muffle furnace set at 600 °C for 6 h, and the loss in weight was measured as organic matter (OM) content. Total nitrogen content was determined following the standard macro Kjeldahl method [26] and was converted to crude protein (CP) by multiplying the percentage of the N content by a factor of 6.25 and expressed in g/kg DM. The NDF and acid detergent fibre (ADF) were determined using ANKOM2000 Fibre Analyser (M/s ANKOM Technology, New York), according to Van Soest et al. [27]. A heat stable bacterial α amylase was used for the NDF analysis. The acid detergent lignin (ADL) was determined by treating ADF residue in ADF residue in ANKOM F57 bags with 72 % sulphuric acid and estimated after drying (105 °C) the ADF residue for 12 h.

2.7. Mineral procedure

The mineral elements assessed were nitrogen (N), phosphorus (P), calcium (Ca), sodium (Na), magnesium (Mg), potassium (K), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and aluminium (Al) [28]. Fodder radish leaves and tuber samples underwent drying at 75 °C and were subsequently milled to pass through a 0.84-mm sieve. Subsamples weighing 0.5 g were then subjected to dry ashing at

450 °C overnight and dissolved in 25 ml of 1 M HCl. The resulting solutions were diluted fourfold with deionized water before analyzing P, K, Ca, Mg, Na, Cu, Zn, Fe, Mn, and Al using ICP-OES (inductively coupled plasma optical emission spectroscopy). Total nitrogen, carbon, and sulfur were determined through an automated Dumas dry combustion method using a LECO TruMac CNS (LECO Corporation, Michigan, USA; Matejovic) [29]. This method involved weighing 0.125-g samples into a ceramic boat and adding a combustion catalyst (COM-CAT), thoroughly mixed with the sample. The boat was then introduced into a horizontal furnace, where the sample underwent combustion in a stream of oxygen at 1350 °C. Nitrogen was determined (as N2) in a thermal conductivity cell.

2.8. Prediction of chemical constituents (total digestible nutrients, relative feeding value,/ metabolizable energy, and digestible energy

To predict the total digestible nutrient (TDN) the following formula was used 82.38 – (0.7515 × ADF) as described by Bath and Marble [29]. The formula for dry matter digestibility was DMDigest% = 88.9 – 2(0.770 × % ADF). Relative feed value (RFV) was calculated from the estimates of dry matterdigestibility (DMDigest) and dry matter intake (DMI). The relative feed value (RFV) = (% DMDigest × %DMI)/1.29 [30]. The dry matter digestibility values were used to estimate the digestible energy (DE, kcal/kg) using the regression equation reported by Fonnesbeck et al. [31], DE (Mcal/kg) = 0.27 + 0.0428 (DMDigest%). The digestible energy values were converted to ME using the formula reported by Khalil et al. [32], ME (Mcal/kg) = 0.821 × DE (Mcal/kg).

2.10 The determination of *in vitro* dry matter digestibility (DMD) of fodder radish leaves and tubers was done using the ANKOM DaisyII incubator, following ANKOM technology method number three for *in vitro* true digestibility. Rumen fluid was collected from a cannulated Bonsmara donor cow, weighing approximately 550 kg, in the morning prior to feeding. The Bonsmara breed was bred in South Africa by combining traits from *Bos indicus* and *Bos taurus* cattle and was chosen for its adaptability to harsh African conditions. The cannulated Bonsmara received a diet of lucerne and Buffel grass before the day of rumen fluid collection. The animal's care adhered to the guidelines of the institutional Federation of Animal Science Societies for animals involved in research and teaching, and ethical approval was obtained from the Agricultural Research Council of South Africa animal ethics committee (**approval number: APAEC [2019/27]).** Rumen fluid was collected, blended and strained through two layers of warm muslin cloth. The strained rumen fluid was maintained at 39 °C under a stream of carbon dioxide gas. Inoculation of fodder radish samples, sealed in ANKOM F57 filter bags, into four DaisyII jars containing 1600 ml of ANKOM buffer each was performed by adding 400 ml of rumen fluid to each digestion jar. The jars underwent regular purging with CO_2, were covered, and placed in the incubation chamber. The ANKOM F57 filter bags were withdrawn at 8, 16, 24, and 48 h after incubation. Withdrawn bags underwent a 15-min wash with cold water using the ANKOM Fibre Analyser. Time 0-h samples, which were not incubated, underwent the same washing procedure as the incubated samples. Subsequently, all samples were oven-dried at 105 °C for 12 h to determine *in vitro* ruminal DMD. Determination of *in vitro* ruminal DM degradability was by the following formular:

$$\text{IVDMD (DM basis)} = 100 - \frac{(W3 - (W1\ x\ C1))}{W2\ XDM} x\ 100$$

where W1 = bag tare weight, W2 = sample weight, W3 = final bag weight after *in vitro* treatment, and C1 = blank bag correction factor (final oven-dried weight ÷ original blank bag weight).

2.9. Total non-structural carbohydrate (NSC)

Standard wet chemistry was used as described by Marias et al. [33] to analyse the total non-structural carbohydrate. Concentrated sulphuric acid (H2SO4) (2.8 ml) was added to distilled H_2O (500 ml) and further diluted to 1l through slowing to make 0.05 M sulphuric acid reagent solution. Concentrated H2SO4 (0.56) was added to the distilled water (H_2O) (50 ml) and further diluted to 100 ml through slowing to make 0.1 M Sulphuric acid solution reagent. To prepare copper (Cu) I reagent solution sodium carbonate (Na2CO3) (30 g), NaHCO3 (20 g), (potassium sodium tartarate) KNaC4H4O6, 4H_2O (15 g) and sodium sulfate (Na2SO4) (180 g) were dissolved in distilled H2O (250 ml) four volumes of the solution was instantly mixed, one volume of solution. To prepare copper II dissolve Na2SO4 (45) and make a copper (II) sulfate5-hydrate CuSO4.5H2SO4 (2 g) in distilled water (250 ml). To prepare the Arsenomolbdate reagent solution (NH4) 6Mo7O24. 4H20 (25 g) was dissolved on distilled $H_2$0 (400 ml) and concentrated H2SO4 (21 ml) was added cautiously. Distilled H2O (400 ml) was used to dissolve sodium arsente7-hydrate (Na2HASO4-7H_2O) and was further added to the acidic ammonium molyddate solution and made up to 500 ml. A glass stoppered brown bottle was used to store the solution and it was incubated for 48 h at 37 °C to prepare 0.1 M sugar standard distilled H_2O (200 ml) was used to dissolve glucose.

***Procedure*:** The fodder radish plant material (0.3 g) samples were weighed into a test tube, and 0.05 M H2SO4 (10 ml) was added to each test tube and mixed. A blank solution which contains 10 ml 0.05 M H2SO4 standard solution of 0.1 M H2SO4 (5 ml) and 5 ml sugar standard. Solutions were heated in a water bath for 30 min and then cooled the samples to room temperature and transferred to a 250 ml volumetric flask. Filter the solution, take 1 ml of filtered solution to test tubes, and add 2 ml of H2SO4. 3 ml aliquot of blank and sugar standard other test tubes respectively. A copper mixed solution of 3 ml was added to each test tube and mixed well, heated in a boiling water bath for 20 min, and cooled to room temperature. The Arsenomolybdate reagent of 3 ml was added and mixed well until the formation of the bubble ceased then allowed colour to develop for 90 min and transferred the solution to a 200 ml volumetric flask. The absorbance was measured from visible spectrometer (UV) at 750 nm.

2.10. Statistical analysis

Two-way analysis of variance (ANOVA) that uses general linear model (GLM) procedures of SAS 9.3 [34] was used to analyse plant chemical composition and chemical estimates. Fixed factors were three water levels and two genotypes. Leaves and tubers were analysed separately.

The following general linear model was used:

$$Yijk = u + Wi + Gj + (W \times G)\, ij + \varepsilon ijk$$

where Yijk is the dependent variable, μ is the overall mean, Wi is the effect of water, Cj is the effect of two fodder radish genotypes, (W × G) ij is the interaction effect between three water level and two genotypes, and εijk is the random error term associated with observation ijk and assumed to be normally and independently distributed. Means were separated and compared using the Least Significant Difference (LSD) and a significant difference was declared at the $p < 0.05$.

3. Results

3.1. Plant chemical composition and chemical estimates

Table 3 shows the effect of the main factors on chemical composition and chemical estimates of radish leaves. Differences were observed in CP and EE across genotypes, water level, and their interaction. Significant differences in genotypes and water level were only found on NSC. The statistically significant difference was also observed on ADF, ADL, DMI, TDN, RFV, DE, and ME across genotypes. Endurance leaves under W2 and W3 had higher ($p < 0.05$) CP content, while W1 had higher ($p < 0.05$) EE and NSC. Within each genotype, Line 2 genotype leaves under W1 and W2 had higher ($p < 0.05$) CP content, whereas W2 and W3 had higher ($p < 0.05$) NDF content. Comparing across the genotypes, W1 and W2 of Line 2 had the highest ($p < 0.05$) CP content when compared to the same water levels on the Endurance genotype. Endurance leaves under W1 had higher ADF, EE, and NSC when compared to the same water level in the Line 2 genotype. Line 2 leaves under W2 had higher NDF and TDN when compared to the same water level in Endurance. In Endurance and Line 2 genotypes, there was no significant difference ($p > 0.05$) in DMDigest, RFV, and DMI. Line 2 under W1 had higher ($p < 0.05$) DE values.

The results on statistical significance of the effect of main factors on chemical composition and chemical estimates of fodder radish tubers are presented in Table 6. Difference was observed in CP and EE across genotypes, water level, and their interaction. There was no interaction effect observed in DMI, TDN, RFV, DE, ME, and DMDigest. The statistically significant difference was also observed on NSC across genotypes and water levels. Comparing across the genotypes, all water regimes of Line 2 had the lowest ($p < 0.05$) NDF content when compared to the same water levels on Endurance.

genotype. Endurance tubers under W1 and W2 had the highest ($p < 0.05$) CP content and ADL under W1. The highest ($p < 0.05$) NSC concentration level was also observed under W3 on both genotypes. The Line 2 tubers under W1 and W2 had the highest ($p < 0.05$) CP and ADL content. The W3 level had higher ($p < 0.05$) EE and NSC.

Table 3
Effect of water regime and genotype on chemical composition (g/kg DM, unless otherwise stated) and chemical estimates (%, unless otherwise stated) of fodder radish leaves.

	Endurance			Line 2						
Parameters	W1	W2	W3	W1	W2	W3	SE	G	W	G*W
ADF	188.50^{aA}	182.60^{aA}	182.95^{aA}	169.60^{aB}	174.97^{aA}	171.54^{aB}	3.42	**0.0007**	0.8571	0.2905
ADL	99.53^{aA}	134.89^{aA}	103.52^{aA}	78.93^{aA}	88.39^{aB}	103.49^{aA}	10.15	**0.0194**	0.1240	0.1129
NDF	237.75^{bB}	283.13^{aA}	274.06^{aA}	264.40^{aA}	242.189^{aB}	245.52^{aB}	8.04	**0.0503**	0.3560	**0.0028**
CP	172.50^{bB}	185.29^{aB}	175.15^{aA}	243.70^{aA}	245.70^{aA}	160.39^{bB}	1.16	< **0.001**	< **0.001**	< **0.001**
EE	58.25^{aA}	41.15^{cB}	47.37^{bB}	36.84^{cB}	46.33^{bA}	52.66^{aA}	1.42	**0.008**	**0.003**	< **0.001**
NSC (%)	3.75^{aA}	2.29^{cA}	3.02^{bA}	2.29^{aB}	1.58^{bB}	2.14^{aB}	0.19	< **0.001**	**0.004**	0.177
DMI (kg)	6.86^{aA}	7.23^{aA}	6.88^{aA}	7.37^{aA}	7.34^{aA}	7.05^{aA}	0.19	0.1192	0.2954	0.5436
TDN (% DM)	68.21^{aB}	68.65^{aA}	68.63^{bA}	69.63^{aA}	69.244^{aA}	69.48^{aA}	0.27	**0.0001**	0.8571	0.2905
RFV (g/kg DM)	372.51^{aA}	396.36^{aA}	376.99^{aA}	411.49^{aB}	406.71^{aA}	392.60^{aA}	10.83	**0.0307**	0.3344	0.4002
DE (Mcal/kg)	3.27^{aA}	3.29^{aA}	3.29^{aA}	3.35^{aA}	3.33^{bA}	3.34^{bB}	0.01	**0.007**	0.0871	0.290
ME (Mcal/kg)	2.68^{aB}	2.70^{aA}	2.70^{aB}	2.75^{aA}	2.73^{aA}	2.74^{aA}	0.01	**0.0007**	0.857	0.291
DM digest (%)	70.04^{aA}	70.63^{aA}	70.60^{aA}	71.93^{aA}	71.42^{aA}	71.74^{aA}	2.84	0.34	0.8571	0.290

^{abc} different lower-case superscripts within each genotype water levels symbolised significant differences ($p < 0.05$). ^{AB} different upper-case superscripts in each water level between genotypes symbolised significant differences ($p < 0.05$). W1: well-watered, W2: moderate stress, W3: severe stress, G; genotype effect, W; water level effect, G*W; interaction between genotype and water level effect, CP: crude protein, EE: Crude fat, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin and EE: ether extract, NSC; total non-structural carbohydrates, DMI: dry matter intake, TDN: total digestible nutrients, DE: Degradable energy, ME: metabolizable energy and NSC; total non-structural carbohydrates, DM digest: dry matter digestibility, SE: Standard error.

Table 4
Effect of water regime and genotype on minerals (DM) of fodder radish leaves.

	Endurance			Line 2						
	W1	W2	W3	W1	W2	W3	SE	G	W	G*W
N (%)	3.16bB	3.36 aB	3.19bA	4.29 aA	4.27 aA	3.01bB	0.033	< **0.001**	< **0.001**	< **0.001**
P (%)	0.48bB	0.54 aB	0.49bA	0.63 aA	0.64 aA	0.50bA	0.004	< **0.001**	< **0.001**	< **0.001**
Ca (%)	4.31 cA	5.40 aA	4.76bB	4.33bA	4.36bB	5.42 aA	0.049	**0.011**	< **0.001**	< **0.001**
Mg (%)	0.57bB	0.76 aA	0.58bB	0.66bA	0.71 aB	0.72 aA	0.004	< **0.001**	< **0.001**	< **0.001**
K (%)	3.26bB	3.91 aA	3.23bB	3.92 aA	3.64bB	3.38 cA	0.032	< **0.001**	< **0.001**	< **0.001**
Na (mg/kg)	860.50 aA	698.37bA	749.10bB	595.50bB	500.69 cB	1065.12 aA	30.70	0.075	< **0.001**	< **0.001**
Fe (mg/kg)	7151.77 aA	6350.33bB	4260.88 cB	6718.04bB	7220.0 aA	5146.79 cA	74.29	<**0.001**	< **0.001**	< **0.001**
Zn (mg/kg)	35.36bB	40.57 aB	40.27 aA	45.21 aA	46.68 aA	36.25bB	0.491	< **0.001**	< **0.001**	< **0.001**
Cu (mg/kg)	4.55 aB	4.69 aB	3.86bB	5.39bA	6.43 aA	4.67 cA	0.168	< **0.001**	< **0.001**	**0.026**
Mn (mg/kg)	94.30 cA	105.02bA	124.04 aA	95.18 cA	103.84bA	106.33 aB	0.994	< **0.001**	< **0.001**	< **0.001**
Al (mg/kg)	668.77 aA	568.00 cA	596.05bA	438.48bB	383.95 cB	565.38 aB	19.01	< **0.001**	**0.004**	**0.005**

abc Means in the same column with different lower-case superscripts are significantly different ($p < 0.05$). AB different upper-case superscripts in each water level between genotypes symbolised significant differences ($p < 0.05$). W1: well-watered, W2: moderate stress, W3: severe stress, G; genotype effect, W; water level effect and G*W; interaction between genotype and water level effect N: Nitrogen, P: Phosphorus; Ca: calcium; Na: sodium; Mg: magnesium; K: Potassium, Fe: iron, Zn: zinc, Cu: copper, Mn: manganese, Al: aluminium, SE: Standard error.

Table 5
Effect of water regime and genotype on *in vitro* dry matter degradability (IVDMD) (g/kg) of fodder radish leaves.

	Endurance			Line 2						
Incubation P.	W1	W2	W3	W1	W2	W3	SE	G	W	G*W
8 h	512.50^{bB}	570.58^{aA}	534.45^{bB}	632.41^{aA}	570.58^{cA}	616.30^{bA}	16.01	0.002	0.955	0.008
16 h	686.51^{aA}	721.06^{aA}	604.01^{bA}	731.89^{aA}	721.06^{aA}	654.20^{aA}	21.85	0.099	0.002	0.470
24 h	739.48^{aA}	734.19^{aA}	727.44^{aA}	837.28^{aA}	734.19^{aA}	809.39^{aA}	33.55	0.049	0.299	0.328
48 h	870.89^{aA}	867.92^{aA}	840.66^{aA}	890.53^{aA}	867.92^{aA}	823.04^{bB}	10.55	0.939	0.001	0.249

abc Means in the same column with different lower-case superscripts are significantly different ($p < 0.05$). AB different upper-case superscripts in each water level between genotypes symbolised significant differences ($p < 0.05$). Incubation P: Incubation periods; W1: well-watered, W2: moderate stress, W3: severe stress, G; genotype effect, W; water level effect and G*W; interaction between genotype and water level effect, SE: Standard error.

Table 6
Effect of water regime and genotype on chemical composition (g/kg DM, unless otherwise stated) and chemical estimates (%, unless otherwise stated) of fodder radish tubers.

	Endurance			Line 2						
Parameters	W1	W2	W3	W1	W2	W3	SE	G	W	G*W
ADF	345.70^{aA}	326.56^{aA}	314.16^{aA}	285.77^{aA}	308.60^{aA}	333.93^{aA}	28.84	0.420	0.960	0.410
ADL	148.02^{aA}	149.27^{aB}	130.90^{aB}	176.25^{aA}	194.19^{aA}	176.19^{aA}	9.640	**0.003**	0.210	0.610
NDF	506.37^{aA}	519.17^{aA}	543.64^{aA}	399.11^{aB}	437.35^{aB}	420.26^{aB}	26.31	**0.004**	0.500	0.730
CP	71.04^{bB}	92.35^{aA}	66.17^{cB}	88.57^{aA}	79.99^{bB}	72.80^{cA}	1.053	**0.006**	< **0.001**	< **0.001**
EE	58.26^{aA}	51.15^{cB}	57.37^{bB}	56.84^{cB}	56.33^{bA}	62.66^{aA}	1.427	**0.008**	**0.003**	< **0.001**
NSC (%)	3.11^{bB}	2.99^{bB}	5.55^{aA}	3.86^{bA}	4.01^{bA}	6.12^{aA}	0.232	**0.001**	< **0.001**	0.619
DMI (kg)	6.25^{aA}	6.44^{aA}	6.29^{aA}	7.03^{aA}	6.73^{aA}	6.42^{aA}	0.240	0.060	0.470	0.390
TDN (% DM)	56.40^{aA}	57.83^{aA}	58.77^{aA}	60.90^{aA}	59.19^{aA}	57.28^{aA}	2.170	0.420	0.950	0.410
RFV (g/kg DM)	263.51^{aB}	281.24^{aA}	280.13^{aA}	329.71^{aA}	302.00^{aA}	276.24^{aA}	18.78	0.090	0.610	0.210
DE (Mcal/kg)	2.59^{aA}	2.68^{aA}	2.73^{aA}	2.85^{aA}	2.75^{aA}	2.64^{aA}	0.120	0.430	0.940	0.410
ME (Mcal/kg)	2.13^{aA}	2.20^{aA}	2.24^{aA}	2.34^{aA}	2.26^{aA}	2.17^{aA}	0.100	0.440	0.960	0.400
DM digest (%)	54.32^{aA}	56.24^{aA}	57.48^{aA}	60.32^{aA}	58.04^{aA}	55.51^{aA}	2.880	0.420	0.960	0.410

abc Means in columns with different lower-case superscripts are significantly different ($p < 0.05$). AB different upper-case superscripts in each water level between genotypes symbolised significant differences ($p < 0.05$). W1: well-watered, W2: moderate stress, W3: severe stress, G; genotype, W; water level effect, G*W; interaction between genotype and water level effect, CP: crude protein, EE: ether extract, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin and EE: ether extract, NSC; total non-structural carbohydrates, DMI: dry matter intake, TDN: total digestible nutrients, DE: Degradable energy, ME: metabolizable energy and DM digest: dry matter digestibility SE: Standard error.

3.2. Macro and micro minerals accumulation of radish leaves and tubers

The results on the statistical significance of the effect of main factors on macro and micro mineral content of radish leaves and tubers are presented in Tables 4 and 7 respectively. In leaves, the difference was observed in all the parameters across genotypes, water level, and their interaction on leaves (Table 4). In tubers, difference was observed on tubers across genotypes, water level, and their interaction with Sodium, Potassium, Zinc, and Copper. Iron, Manganese, and Aluminium did not affect genotype, but the effect of

Table 7
Effect of water regime and genotype on minerals (DM) of fodder radish tubers.

	Endurance			Line 2						
Parameters	W1	W2	W3	W1	W2	W3	SE	G	W	G*W
N (%)	1.65^{aB}	1.76^{aB}	1.60^{aA}	1.97^{aA}	1.96^{aA}	1.66^{bA}	0.058	**0.0020**	**0.006**	0.132
P (%)	0.96^{bB}	1.01^{aA}	1.08^{aB}	1.07^{bA}	1.10^{bA}	1.20^{aA}	0.031	**0.0012**	**0.006**	0.797
Ca (%)	0.89^{bB}	1.00^{aB}	1.07^{aB}	1.11^{cA}	1.15^{aA}	1.13^{bA}	0.036	**0.005**	**0.039**	0.130
Mg (%)	0.42^{bB}	0.46^{bB}	0.56^{aB}	0.53^{bA}	0.54^{bA}	0.60^{aA}	0.017	**0.002**	< **0.001**	0.159
K (%)	7.75^{bB}	8.04^{aB}	6.61^{cB}	8.44^{aA}	8.46^{aA}	7.75^{bA}	0.082	< **0.001**	< **0.001**	**0.004**
Fe (mg/kg)	696.19^{bA}	670.92^{bA}	1241.24^{aA}	718.47^{bA}	757.89^{bA}	1038.12^{aB}	63.33	0.556	< **0.001**	0.094
Na (mg/kg)	10410.46 cA	14370.16 aA	12578.57bA	14353.30 aA	14350.21bB	11616.55 cB	142.69	<0.001	< **0.001**	<0.001
Zn (mg/kg)	41.10^{cB}	47.83^{bB}	58.92^{aB}	60.99^{cA}	62.66^{bA}	69.79^{aA}	0.491	< **0.001**	< **0.001**	**0.001**
Cu (mg/kg)	1.37^{bA}	1.76^{bB}	3.15^{aB}	2.06^{bA}	1.95^{bA}	7.38^{aA}	0.350	< **0.001**	< **0.001**	**0.002**
Mn (mg/kg)	29.82^{bA}	31.89^{bA}	41.15^{aA}	34.60^{bA}	34.98^{bA}	40.23^{aA}	1.760	0.132	**0.008**	0.286
Al (mg/kg)	651.07^{bA}	654.89^{bA}	990.03^{aA}	765.50^{bA}	775.96^{bA}	853.87^{aB}	50.67	0.439	**0.002**	**0.043**

abc Means in the same column with different lower-case superscripts are significantly different ($p < 0.05$). AB different upper-case superscripts in each water level between genotypes symbolised significant differences ($p < 0.05$). W1: well-watered, W2: moderate stress, W3: severe stress, G; genotype effect, W; water level effect, G*W; interaction between genotype and water level effect, N: Nitrogen, P: Phosphorus; Ca: calcium; Na: sodium; Mg: magnesium; K: Potassium, Fe: iron, Zn: zinc, Cu: copper, Mn: manganese, Al: aluminium, SE: Standard error.

water level was found in all the parameters. On macro minerals Endurance leaves under W2 had the higher Phosphorus, Calcium, Magnesium, and Potassium concentration when compared to W1 and W3 of the same genotype which were not significantly different from each other except Calcium. Line 2 leaves under W1 and W2 had the highest Nitrogen and Potassium compared to W3 of the same genotype. The W2 and W3 of Line 2 had higher Na concentrations when compared to the same water levels in Endurance. When it comes to micro minerals Endurance leaves under W1 had the highest Iron and Aluminium when compared to W2 and W3 of the same genotype. Line 2 leaves under W2 had the highest Zinc and Copper when compared to W1 and W3 while W3 had higher Iron, Manganese, and Aluminium concentration when compared to W1 and W2 of the same genotypes. In all water levels, Endurance had a higher Cu concentration when compared to the same water levels in Line 2. In all water levels, Line 2 had a lower Al concentration when compared to the same water levels in Endurance.

The results of the effect of water regimes and genotype on macro and micro minerals of fodder radish tubers are presented in (Table 7). Endurance tubers under W2 had higher Sodium, and Potassium concentrations when compared to W1 and W3 of the same genotype. Line 2 tubers under W1 and W2 had the highest Nitrogen when compared to W3 of the same genotypes. Line 2 tubers under W2 had the highest Na and K when compared to W1 and W3 of the same genotypes. Line 2 had higher N at W1 and W2 when compared to the same water levels. For micro mineral concentration, within each genotype, both Endurance and Line 2 tubers under W3 had higher Iron, Zinc, Copper, Manganese, and Aluminium when compared to W1 and W2 water levels. Within each water level, both Endurance and Line 2 tubers under W3 had higher Iron, Zinc, Copper, Manganese, and Aluminium when compared to W1 and W2 water levels. Zinc in Line 2 in all water levels was higher when compared to the Endurance genotype in the same water levels.

3.3. In vitro ruminal dry matter degradability (IVDMD) of fodder radish leaves and tubers

The results on the statistical significance of the effect of main factors on *in vitro* ruminal dry matter degradability (IVDMD) of radish leaves and tubers are presented in Tables 5 and 8. There was a significant effect of genotype on 8 h and 24 h incubation periods for leaves and 16 h incubation periods for tubers. While a statistically significant effect of water level was observed on 16 h and 48 h incubation periods for leaves and 8 h and 16 h incubation periods for tubers. Then the interaction between genotype and water level was observed on 8 h incubation periods for both measured plant parts. In 24 h and 48 h incubation periods, the degradability in all treatments was at an acceptable level above 60–80 %.

3.4 Regarding the influence of genotype on nutritive value, Line 2 showed the highest crude protein (CP) levels in both leaves and tubers (Table 9). In terms of the impact of water regime on nutritive value, W2 displayed the highest CP levels in both leaves and tubers, surpassing W1 and W3, which also exhibited significant differences from each other (Table 10).

Table 8
Effect of water regime and genotype on *in vitro* dry matter degradability (IVDMD) (g/kg) of fodder radish tubers.

		Endurance			Line 2					
Incubation P.	W1	W2	W3	W1	W2	W3	SE	G	W	G*W
8 h	502.97^{bA}	608.66^{aA}	484.07^{cB}	529.11^{bA}	574.98^{aB}	557.23^{bA}	18.63	0.176	0.002	0.043
16 h	499.27^{cA}	576.75^{aB}	557.53^{bB}	544.62^{cA}	616.51^{aA}	583.29^{bA}	17.03	0.021	0.002	0.841
24 h	653.00^{aA}	676.17^{aA}	623.39^{aB}	679.62^{bA}	664.27^{bA}	719.23^{aA}	21.59	0.058	0.971	0.077
48 h	771.12^{aA}	828.35^{aA}	817.27^{aA}	786.10^{aA}	784.27^{aA}	820.12^{aA}	34.67	0.763	0.515	0.676

abc Means in columns with different lower-case superscripts are significantly different ($p < 0.05$). AB different upper-case superscripts in each water level between genotypes symbolised significant differences ($p < 0.05$). Incubation P: Incubation periods; W1: well-watered, W2: moderate stress, W3: severe stress, G; genotype effect, W; water level effect, G*W; interaction between genotype and water level effect, SE: Standard error.

Table 9
Effect of genotype on chemical constituents of selected fodder radish.

Plant P.	G	ADF	ADL	NDF	CP	EE	NSC	DMI	TDN	RFV	DE	ME	DM digest
Leaves	Endura.	184.69[a]	112.65[a]	264.98[a]	177.65[b]	48.93[a]	3.02[a]	6.99[a]	68.50[a]	381.96[b]	3.28[b]	2.69[b]	70.43[b]
	Line 2	171.98[b]	90.28[b]	250.71[a]	216.59[a]	45.28[b]	2.00[b]	7.26[a]	69.46[b]	403.60[a]	3.34[a]	2.74[a]	71.70[a]
	SEM	1.97	5.86	4.64	0.67	0.82	0.11	0.11	0.15	6.25	0.008	0.007	0.19
	P-value	0.0007	0.019	0.0503	<0.0001	0.0087	<0.0001	0.1192	0.0007	0.0307	0.0007	<0.0001	0.0007
Tubers	Endura.	328.82[a]	142.73[b]	523.06[a]	76.52[b]	52.33[b]	3.88[b]	6.33[a]	57.67[a]	274.96[a]	2.67[a]	2.19[a]	56.02[a]
	Line 2	309.43[a]	183.31[a]	418.91[b]	80.45[a]	55.43[a]	4.66[a]	6.73[a]	59.13[a]	302.65[a]	2.75[a]	2.26[a]	57.97[a]
	SE	16.66	5.57	15.19	0.60	1.34	0.133	0.14	1.25	10.84	0.072	0.06	1.67
	p-value	0.4266	0.0003	0.0004	0.0006	<0.0001	0.0014	0.0647	0.4264	0.0961	0.4384	0.4423	0.4264

[ab] Means in columns with different lower-case superscripts are significantly different ($p < 0.05$),Endura; Endurance, G: genotype, CP: crude protein, EE: ether extract, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin and EE: ether extract, NSC; total non-structural carbohydrates, DMI: dry matter intake, TDN: total digestible nutrients, DE: Degradable energy, ME: metabolizable energy and DM digest: dry matter digestibility SE: Standard error.

Table 10
Effect of water regimes on chemical constituents of selected fodder radish.

Plant P.	Water R.	ADL	NDF	ADF	CP	EE	NSC	DMI	TDN	RFV	DE	ME	DM digest
Leaves	W1	89.23	251.08	179.05	208.12^{b}	47.55^{a}	3.018^{a}	7.12	68.92	392.01	3.30	2.716	70.99
	W2	111.64	262.66	178.69	215.50^{a}	43.74^{b}	1.935^{c}	7.29	68.95	401.54	3.31	2.718	71.03
	W3	103.51	259.79	177.25	167.77^{c}	50.02^{a}	2.578^{b}	6.97	69.06	384.79	3.32	2.723	71.18
	SEM	7.18	5.68	2.42	1.008	1.01	0.137	0.14	0.18	7.66	0.01	0.008	0.24
	p-value	0.124	0.356	0.8571	<0.0001	0.003	0.0004	0.295	0.857	0.334	0.857	0.857	0.8571
Tubers	W1	162.14	452.74	315.73	79.80^{b}	45.49^{a}	3.49^{b}	6.64	40.56	296.61	1.69	1.39	57.33
	W2	171.73	478.26	317.60	86.17^{a}	43.78^{a}	3.50^{b}	6.59	40.39	291.62	1.68	1.38	57.14
	W3	153.69	481.95	324.05	69.49^{c}	52.38^{a}	5.84^{a}	6.36	41.26	278.19	1.73	1.42	56.49
	SEM	6.82	18.60	20.39	0.744	1.65	0.16	0.17	1.74	13.28	0.099	0.08	2.04
	p-value	0.215	0.501	0.955	<0.0001	0.004	<0.0001	0.470	0.955	0.610	0.948	0.956	0.955

abc Means in columns with different lower-case superscripts are significantly different ($p < 0.05$). W1: well-watered, W2: moderate stress, W3: severe stress, WCP: crude protein, EE: ether extract, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin and EE: ether extract, NSC; total non-structural carbohydrates, DMI: dry matter intake, TDN: total digestible nutrients, DE: Degradable energy, ME: metabolizable energy and DM digest: dry matter digestibility SE: Standard error.

Table 11
Effect of genotype on mineral elements of selected fodder radish.

Plant P	G	N	P	Ca	Mg	K	Na	Fe	Zn	Cu	Mn	Al
Leaves	Endura.	3.24[b]	0.51[b]	4.83[a]	0.64[b]	3.47[b]	5921.01[b]	769.33[a]	38.74[b]	4.37[b]	107.79[a]	610.95[a]
	Line 2	3.86[a]	0.59[a]	4.71[b]	0.70[a]	3.65[a]	6361.61[a]	720.44[b]	42.71[a]	5.50[a]	101.78[b]	462.61[b]
	SE	0.019	0.003	0.027	0.002	0.019	42.890	17.727	0.097	0.971	0.574	10.973
	p-value	<0.0001	<0.0001	0.0119	<0.0001	<0.0001	<0.0001	0.0749	<0.0001	<0.0001	<0.0001	<0.0001
Tubers	Endura.	1.68[b]	1.02[b]	0.99[b]	0.48[b]	7.27[b]	13440.00[b]	869.45[a]	49.2862[b]	2.09[b]	34.29[a]	765.33[a]
	Line 2	1.86[a]	1.13[a]	1.13[a]	0.56[a]	8.22[a]	12453.10[a]	838.17[a]	64.4845[a]	3.80[a]	36.61[a]	798.45[a]
	SE	0.020	0.018	0.020	0.009	0.047	0.034	36.561	0.0525	0.205	1.014	29.260
	p-value	0.002	0.001	0.005	0.0002	<0.0001	<0.0001	0.0556	<0.0001	<0.0001	0.132	0.439

[ab] Means in the same column with different lower-case superscripts are significantly different ($p < 0.05$); Plant P.; Plant parts, Endura; Endurance, G: genotype; N: Nitrogen, P: Phosphorus; Ca: calcium; Na: sodium; Mg: magnesium; K: Potassium, Fe: iron, Zn: zinc, Cu: copper, Mn: manganese, Al: aluminium, SE: Standard error.

Table 12
Effect of water regime on mineral elements of selected fodder radish.

Plant P.	Water R.	N	P	Ca	Mg	K	Na	Fe	Zn	Cu	Mn	Al
Leaves	W1	3.73^{b}	0.56^{b}	4.32^{c}	0.62^{c}	3.59^{b}	6934.91^{a}	728.01^{b}	40.28^{b}	4.97^{b}	94.75^{c}	553.63^{a}
	W2	3.81^{a}	0.59^{a}	4.88^{b}	0.74^{a}	3.77^{a}	6785.17^{a}	599.53^{c}	43.63^{a}	5.57^{a}	104.44^{b}	475.98^{b}
	W3	3.10^{c}	0.49^{c}	5.09^{a}	0.65^{b}	3.31^{c}	4703.84^{b}	907.12^{a}	38.27^{c}	4.27^{c}	115.19^{a}	580.72^{a}
	SE	0.023	0.0033	0.035	0.003	0.228	52.529	21.710	0.351	0.119	0.703	13.439
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.0001	<0.0001	<0.0001	<0.001	<0.0001	0.004
Tubers	W1	1.81^{a}	1.01^{b}	1.00^{b}	0.47^{b}	7.81^{b}	$12381.^{b}$	707.33^{b}	51.05^{c}	1.71^{b}	32.21^{b}	708.29^{b}
	W2	1.86^{a}	1.06^{b}	1.07^{a}	0.50^{b}	8.24^{a}	$14360.^{a}$	714.40^{b}	55.24^{b}	1.85^{b}	33.44^{b}	715.42^{b}
	W3	1.63^{b}	1.14^{a}	1.10^{a}	0.58^{a}	7.18^{c}	$12097.^{b}$	1139.68^{a}	64.35^{a}	5.26^{a}	40.69^{a}	921.95^{a}
	SE	0.041	0.022	0.025	0.01	0.05	100.896	44.778	0.643	0.250	1.241	35.836
	p-value	0.0056	0.0057	0.038	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0008	0.0016

abc Means in the same column with different superscripts are significantly different ($p < 0.05$); Water R; water regimes, Plant P.; Plant parts, W1: well-watered, W2: moderate stress, W3: severe stress; N: Nitrogen, P: Phosphorus; Ca: calcium; Na: sodium; Mg: magnesium; K: Potassium, Fe: iron, Zn: zinc, Cu: copper, Mn: manganese, Al: aluminium, SE: Standard error.

When considering the impact of genotype on mineral elements, Line 2 demonstrated the highest levels of P, N, Ca, Mg, K, Na, and Cu in both leaves and tubers (Table 11). Regarding the effect of water regime on mineral elements, W2 showcased the highest levels of N, P, K, Zn, and Cu in leaves, compared to W1 and W3, which also exhibited significant differences from each other (Table 12).

Concerning the influence of genotype on IVDMD, Line 2 displayed the highest IVDMD value at the 16-h withdrawal period in tubers (Table 13). Regarding the impact of water regime on IVDMD, both W1 and W2 demonstrated the highest values at the 16-h withdrawal period, compared to W3, in leaves (Table 14).

4. Discussion

4.1. Effect of water regimes on chemical composition and chemical estimates

Abiotic factors such as the amount of water received by plants influence the crop metabolism and nutrient concentration of most fodder crops. Limited research has been conducted on the impact of varying irrigation water levels on the nutritional composition and digestibility potential of fodder radish. The chemical composition of the leaves of Endurance and Line 2 ADL showed little variation between treatments except for CP but EE, NSC, NDF, and ADF. The variation was mostly observed for NDF when genotypes on each water level were compared. The study hypothesised that Endurance and Line 2 genotypes under higher water levels will have better nutrient concentration than moderate stress and severe stress. The EE and NDF were the only parameters that supported the study hypothesis in Endurance leaves, while in Line 2 the ADF and EE supported it. As opposed to our study results for Line 2 which we found no variation. A study by Delfani et al. [35] found that under well-watered treatment (irrigation as a supplement) neutral detergent fibre (NDF) increased when compared to rainfed crops. Furthermore, Mahfouz et al. [36] also found a decrease in the fibre of some forage crops under water stressed conditions. Unlike our study results in which both genotypes had mixed reactions toward water regimes, Ashrafi and Razmjoo [37] found that plants under water stress treatment tend to decrease crude fat. While some studies found an increase in EE in some forage crops under water stressed treatment [35,38,39]. Sheaffer et al. [40] suggest that the reduction of fibre and increase in CP in forages under water stressed conditions, may be due to activities of enzymes responsible for the manufacturing of NDF, ADF, and ADL and this might be the case for our study results. Kara [41], reported crude protein (CP) and ash contents of yellow sweet clover herbage were higher during the vegetative and early flowering stages compared to the full flowering stage. This might also be the case for the current study results because when plants are stressed tend to mature or flower early to avoid drought stress. During the data collection for the current study, it was observed that severe water stressed plots flowered first and reached full flowering than well-watered and moderate-stressed plots. Line 2 had less CP under water and severe stress treatment. This finding is opposite to the study results by Kaplan et al. [42] who found that well-watered treatments increased both ADF and NDF but decreased CP and digestibility. Contrary to this study, the increase in CP under drought-stressed conditions was also observed in canola which also belongs to the brassica family [43]. Balazadeh et al. [44] also found water stress reduced the digestible dry matter (DDM) yield and content, CP yield, dry matter intake (DMI), and relative feed value (RFV) of some forages, while CP, water-soluble carbohydrates (WSC), neutral detergent fibre (NDF), and acid detergent fibre (ADF) increased.

In tubers, for Endurance, the hypothesis was only supported by EE, while in Line 2 it was observed in CP. In both genotypes, ADF, NDF, and ADL were not influenced by the water regimes. One possible reason for the current study results could be that since tubers are composed of 80 % water, which was adequate to get a similar chemical composition in all the water regimes. The reduced CP content for both genotypes Endurance and Line 2 concur with some studies that found a reduction of protein content in tubers under limited water conditions [45,46]. The insignificance of water treatments in fibre concentration for the current study results agrees with Schlering et al. [47] who found that water stress did not reduce the nutrient concentration of red radish. While severe stress treatment resulted in high NSC. One reasonable explanation for NSC to be high in tubers for the severe water stressed treatments could be that, since tubers are storage organs of fodder radish, implying that carbohydrates are stored in tubers for regrowth or growth. Tubers and storage roots as subterranean storage organs of plants are growing all their life span in soil, and they develop thick outer periderms as interfaces towards the soil environment [48]. Root systems allow for the efficient uptake of water and dissolved nutrients from soil [49]. Difference was observed in CP and EE across genotypes, water level, and their interaction. The statistically significant difference was also observed on NSC across genotypes and water levels. While in Line 2 tubers, some contradictory studies found that protein content in tubers was reduced by limited water [45,46]. On chemical estimates of fodder radish tubers, both Endurance and Line 2, the

Table 13
Effect of genotype on *in vitro* dry matter degradability of selected fodder radish.

		Incubation periods			
Plant parts	Genotypes	8 h	16 h	24 h	48
Leaves	Endurance	539.18[b]	670.5[a]	733.7[b]	859.8[a]
	Line 2	606.43[a]	702.4[a]	793.62[a]	860.5[a]
	SE	9.24	12.6	19.37	6.09
	p-value	0.0002	0.099	0.049	0.939
Tubers	Endurance	531.9[a]	544.5[b]	650.9[a]	805.6[a]
	Line 2	553.8[a]	581.5[a]	687.7[a]	796.8[a]
	SE	10.75	9.82	12.46	20.01
	p-value	0.176	0.020	0.058	0.762

[ab] Means in columns with different lower-case superscripts are significantly different ($p < 0.05$); SE: Standard error.

Table 14
Effect of water regime on *in vitro* dry matter degradability of selected fodder radish.

	Incubation periods			
Water regimes	8 h	16 h	24 h	48 h
W1	572.46[a]	709.201[a]	788.38[a]	880.71[a]
W2	570.58[a]	721.067[a]	734.19[a]	867.93[a]
W3	575.38[a]	629.107[b]	768.42[a]	831.86[b]
SE	11.321	15.450	23.719	7.459
p-value	0.955	0.023	0.299	0.0016
W1	516.04[b]	521.90[b]	666.31[a]	778.60[a]
W2	591.82[a]	596.60[a]	670.22[a]	806.30[a]
W3	520.65[b]	570.40[a]	671.31[a]	818.70[a]
SE	13.171	12.040	15.260	24.510
p-value	0.0024	0.0029	0.970	0.515

[ab] Means in columns with different lower-case superscripts are significantly different ($p < 0.05$); W1: well-watered; W2: moderate stress; W3: severe stress, SE: Standard error.

water levels did not affect DMI, TDN, RFV, DE, ME, and DM digest. Keogh et al. [50] have similar values of DMI and ME with our study results while differing in TDN, RFV DE, ME, and DM digest. Therefore, our study results contribute valuable information for optimizing the cultivation and utilization of fodder radish leaves and tubers as fodder. The identified genotype-specific responses and the impact of water levels provide a basis for informed decision-making in agricultural practices, emphasizing the importance of considering both genetic factors and water management strategies for enhanced nutritional quality in radish crops.

4.2. Effect of water regimes on macro and micro minerals of leaves and tubers of two fodder radish genotypes

The hypothesis of the study was not strongly supported by the study results. There was a trend of reduction of sodium in the Endurance genotype and potassium in Line 2 as influenced by water level. Moderate-stress treatment resulted in higher Nitrogen and phosphorus for both genotypes, and Calcium, Magnesium, and Potassium concentrations for the Endurance genotype. In Line 2 leaves, only Potassium supported the hypothesis and most of the macro minerals improved under moderate stress treatment.

In tubers for both genotypes the same trend was found, none of the mineral elements supported our hypothesis, while in Line 2 Nitrogen under well-watered was the same as moderately stressed treatment. Moderate stressed treatment resulted in higher Nitrogen, Sodium, and Potassium for both genotypes. Our study findings on minerals disagree with Delfani et al. [35] who found that well-watered plants resulted in high mineral concentrations of ash. In micronutrients, only iron was improved under well-watered Endurance leaves, the other micronutrients such as zinc, and copper were high in water-stressed treatments; and in Line 2, iron, manganese, and aluminium were high in stressed treatments. Across genotypes under well-watered treatment, Line 2 had relatively high values of N, P, K, and Mg than Endurance, this might be due to the genetic makeup because Line 2 was bred out of Endurance to stabilise colour and Line 2 has longer tuber length compared to Endurance which might help in nutrient absorption. Additionally, traits that contribute to genetic variation could potentially help forages respond to both abiotic and biotic factors. In general, both genotypes well-watered had lower macro mineral concentration levels. Our study results concur with the study results of Wang et al. [51] who found a decrease in total minerals in tubers under well-watered. One possible reason for this could be that more water applied to crops resulted in more soil moisture content which favors the nutrient uptake by the crop [52]. In general, micro-elements of tubers for both genotypes were high under water stressed treatments. Our results are consistent with Schlering et al. [47] who found an increase of Zinc, copper, and potassium under water stressed treatment on both leaves and tubers of radish. However, they also found a decrease in manganese and phosphorus under water stressed treatment on radish. Our study results revealed high iron, manganese, and aluminium concentrations on Line 2 under water-stressed treatments, and on both genotypes, tuber aluminium was found high under water-stressed treatments. These results concur with [53,54] who suggest that a high concentration of aluminium in plants is due to drought stress. While the study by Krizek & Foy [55] found an Aluminium increase in well-watered treatment which concurs with our result on the Endurance genotype that had high aluminium concentration. Our study results on macro and micro mineral content in fodder radish leaves and tubers provide valuable insights into the factors influencing nutrient composition. These findings have practical applications in grazing livestock nutrition, crop management, and the selection of fodder radish genotypes for specific agricultural goals. Those minerals are important elements that are needed by grazing livestock, particularly when the quality and quantity of grass decline due to drought or during the dormant season. These results also imply that even under limited water these two genotypes can possess high mineral accumulation.

4.3. Effect of water regimes on in vitro ruminal dry matter degradability of leaves and tubers of two fodder radish genotypes

The absorption of forage nutrients for ruminants depends on the rate of DM fermentation in the rumen [56]. While Kara and Özta [57], suggest that the total gas production and the concentration of end-products in the fermentation liquid are indicators of the fermentation level of feedstuffs or rations in the rumen. The leaves of Endurance and Line 2 genotypes had similar trends in different

water regimes and incubation periods. Endurance genotype statistically differed at 8 h and 16 h and no significant difference in the 24 h and 48 h incubation periods, while Line 2 statistical difference was observed in the 8 h and 48 h incubation periods. Across genotypes with the same water regime, the same trend was observed in the 8 h incubation period. This was not strange due to low ADL and high CP in all water regimes on both genotypes, which are the prerequisite for optimum microbial protein synthesis to get optimum animal performance. The study results partially supported the current study hypothesis, that Endurance and Line 2 under higher water levels will have better nutrient concentration than moderate and severe stressed treatments. On the contrary, Kara [58] reported that there were variations in CP contents between silages and herbage of crown vetch and there was no noticeable difference in ruminal ammonia-N concentrations during *in vitro* ruminal fermentation. He, further suggested that the non-significant difference was attributed to the rise in Non-Fibre Carbohydrate (NFC) contents resulting from the inclusion of barley and ammonia in the ruminal environment, potentially influencing microbial protein production. This could be the case with the current study results, water stress increased the level of non-structural carbohydrates and, possibly impacting the microbial protein production as water stressed treatments had higher *in vitro* DM values than well-watered. In all the water regimes irrespective of genotype the substrate degradation increased as incubation periods increased. Some studies also witnessed the same trend [5,59–61]. Detmann et al. [62] found that high nitrogen improves the growth of rumen fibrolytic bacteria which results in high-fibre ruminal digestion and agrees with our study results. Keim et al. [60] also found a high degradation rate on two fodder brassica species. While some studies found no effect of nitrogen on the degradability [63,64].

In both genotypes, the tubers did not support the hypothesis. High degradability was found on moderate and severe stress treatment. On the 8 h and 16 h incubation periods Endurance and Line 2 under moderate stress and only 48 h withdrawal period did not show significant difference. The high concentration of readily fermentable carbohydrates and digestibility is known for forage brassica [52], this could be the same trend as our study results for tuber high degradation rate due to high total non-structural carbohydrates possessed by water-stressed treatments. However, Line 2 had high CP under well-watered but low ADL value and high NSC under water-stressed treatments. Brito et al. [65] and Berthiaume et al. [66] suggest that NSC enhances the microbial protein synthesis in the rumen, and this could be the explanation for Line 2 where well-watered had high CP but less degradation than water stressed treatments. High lignin is the organic compound that lowers the nutritive value of forages and reduces the ability of microorganisms to break down the plant Ravhuhali et al. [67], and this might be the reasonable explanation for high degradability in both genotype-tubers since they had low lignin content. The non-significant difference at the 48 h period may be explained by Kara [41], who found similar values of *in vitro* digestibility of yellow sweet clover herbages at different phenological stages. These IVDMD from current study results provide a critical link between plant characteristics and their potential as ruminant feed. The observed effects of genotype and water level, along with the acceptable degradability levels, have practical implications for optimising the use of fodder radish as a forage crop in livestock production systems.

5. Conclusions

Across all water regimes, the CP content in both leaves and tubers exceeded the minimum requirements for difference livestock classes across different production levels. Furthermore, the degradability observed in all treatments surpassed acceptable levels, ranging above 60–80 %. These findings suggest that both fodder radish genotypes can serve as viable components in ruminant feeding systems during periods of low forage availability. However, Line 2 in both plant parts leaves and tuber demonstrates notably higher nutritional value and *in vitro* ruminal dry matter degradability in comparison to Endurance. Notably, moderate water stress conditions yield superior nutritional value and *in vitro* ruminal dry matter degradability compared to both well-watered and severe water stress treatments, regardless of genotype. This underscores the potential of irrigating Line 2 and Endurance with 180–220 mm per season to realize optimal production outcomes across all genotypes, even under water stress conditions. Further evaluation of these genotypes in natural environments, particularly in arid regions receiving 180 mm or less rainfall, is warranted to better understand their performance and adaptability.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

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Declaration of competing interest

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