agriculture

MDPI

Article

Influence of Land Configuration on the Proximate Composition, Amino Acid Profile, and Antinutritional Factors of Alfalfa (*Medicago sativa* L.): Implications for Ruminant Nutrition

Hosea Istifanus Finangwai [1], Shedrach Benjamin Pewan [2,*] and Luka Yangka Guluwa [1]

[1] Department of Animal Science, Faculty of Agriculture, Plateau State University, Bokkos 932112, Nigeria; hoseafinangwai@plasu.edu.ng (H.I.F.); guluwaluka@plasu.edu.ng (L.Y.G.)
[2] Extension Division, National Veterinary Research Institute, Vom 930001, Nigeria
* Correspondence: shedrach.pewan@my.jcu.edu.au

Abstract

This study at Plateau State University, Bokkos, examined how land configuration and crop establishment methods affect alfalfa (*Medicago sativa* L.) forage's composition, amino acid profile, and antinutritional factors, with implications for ruminant nutrition. Using a 2 × 2 factorial design, the study tested two land configurations (flat and ridge) and two methods (broadcast and drilled), resulting in four treatment combinations (flat × broadcast, flat × drilled, ridge × broadcast, and ridge × drilled), with four replicates. Data were analyzed via factorial ANOVA at $p \leq 0.05$. Land configuration significantly influenced crude protein (CP), crude fibre (CF), and ether extract (EE), with flat land yielding higher CP (23.08%) and CF (12.85%), while ridge land produced higher EE (7.88%). Establishment method notably affected EE, with broadcasting resulting in 7.55% compared with 7.15% under drilling. The interaction between land configuration and establishment method significantly impacted CF. Land configuration also affected lysine and histidine levels, while establishment method influenced leucine, lysine, isoleucine, valine, threonine, and histidine. Significant interactions were found for tryptophan and histidine. For non-essential amino acids, establishment method significantly impacted tyrosine, and land configuration × establishment interactions affected alanine, aspartic acid, glutamic acid, glycine, serine, tyrosine, proline, and arginine. Land configuration also significantly affected saponin, tannin, cyanide, and total phenol, while establishment method impacted saponin, alkaloids, and total phenol. Interactions were significant for saponin, alkaloids, flavonoids, tannins, cyanide, and total phenol. The findings reveal that both individual factors and their interactions influence forage quality, especially amino acids and secondary metabolites. Combining suitable land configurations and establishment methods could enhance alfalfa forage for ruminant feeding.

Keywords: flat cultivation; ridge cultivation; proximate analysis; saponins; alkaloids; phenolics; forage quality

Check for updates

Academic Editors: Cristiana Maduro Dias and Helder Nunes

Received: 8 September 2026
Revised: 11 October 2026
Accepted: 14 October 2026
Published: 19 October 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

As global demand for high-quality ruminant-derived animal protein rises, livestock production systems must adopt sustainable practices that improve feed efficiency and meat quality [1]. Unfortunately, the livestock industry faces persistent challenges, including feed shortages, rising costs of imported concentrates, and declining pasture productivity driven by land degradation and climate change [2]. Feed alone accounts for approximately 60–70%

of total livestock production costs [3]. As a result, changes in feed prices directly lead to notable declines in milk and meat production, adverse effects on animal health, reduced livestock productivity, and diminished farm profitability and sustainability [4,5].

In Nigeria, livestock feeding commonly relies on natural grazing, crop residues, browse, and cut-and-carry forages, yet seasonal feed deficits remain a major constraint [6,7]. Agronomic approaches that improve the nutritional value of adapted forage crops can reduce reliance on purchased feeds while supporting more sustainable crop–livestock systems.

Alfalfa (*Medicago sativa* L.) is a protein-rich perennial forage legume valued for its digestibility and nutrient density [8–13]. Crude protein, fibre, and other proximate components influence its feeding value, while amino acid composition provides further insight into protein quality [14,15]. Alfalfa also contains secondary metabolites, including saponins, tannins, flavonoids, and phenolic compounds, whose concentrations can vary with genotype, environment, maturity, and management [16–18]. Environmental conditions, genotype, soil nutrient availability, maturity, and agronomic practices influence forage quality [19–22]. Land configuration can modify soil moisture distribution, aeration, nutrient availability, and the near-surface microenvironment, while establishment method may alter plant density and spatial competition [23–25]. These factors may therefore influence forage biochemical composition. The expected annual yields on flat land are 26 tonnes of fresh herbage and 6.1 tonnes of dry matter per hectare, while ridge land produces 24 and 4.8 tonnes per hectare, respectively, for the same period.

Limited information exists on how land configuration impacts the proximate composition, amino acid profiles, and antinutritional factors of alfalfa (*Medicago sativa* L.) forage. Variations in land setup can change soil water availability and nutrient dynamics, which may affect forage quality and the buildup of antinutritional compounds. Crop establishment methods might also influence these effects, either on their own or through interactions with land configuration. Understanding these relationships can help develop agronomic practices to enhance alfalfa's nutritional value for ruminants. This study examined how land configuration and crop establishment methods affect the proximate composition, amino acid profiles (essential and non-essential), and antinutritional factors of alfalfa forage. It specifically assessed the individual impacts of each agronomic factor and their interaction on these compositional attributes. The study also evaluated how these variations could affect alfalfa's nutritional value in ruminant diets. The study tested three null hypotheses: that land configuration has no significant effect on forage composition; that crop establishment method has no significant effect; and that these factors do not significantly interact to affect proximate composition, amino acid profiles, or antinutritional factors.

2. Materials and Methods

2.1. Experimental Site and Climatic Conditions

The field experiment was conducted during the 2025 cropping season at the Faculty of Agriculture research farm, Plateau State University, Bokkos, Nigeria (9.3736° N, 8.9569° E; 1375–1400 m above sea level), about 70 km from Jos. The site lies in a near-temperate highland zone, with annual rainfall of approximately 1400–1460 mm falling from April to September and average daily temperatures of 18–22 °C. The soil is predominantly loamy to sandy-loam, with a reported pH of 6.5–7.5.

2.2. Experimental Design and Land Preparation

The experiment employed a 2 × 2 factorial design within a Randomised Complete Block Design (RCBD), with four replicates. It examined two factors, land configuration (flat and ridge) and establishment method (broadcasting and drilling), yielding 4 treatment

combinations across 16 units. Treatments were randomly allocated within each block. The field was prepared through ploughing and harrowing to ensure a uniform seedbed. Flat plots were levelled, while ridges were manually formed with 50–75 cm spacing. Alfalfa was planted at 30–40 cm row spacing where applicable, with intra-row spacing of 10–15 cm.

2.3. Planting and Crop Establishment

The experiment used a 2 × 2 factorial RCBD with two land configurations (flat land and ridge) and two establishment methods (broadcasting and drilling), yielding four treatment combinations. Treatments were randomly assigned to plots within each block. Alfalfa (*Medicago sativa* L.) seeds were either broadcast uniformly or drilled in rows, according to the assigned treatment. Certified Simlaw alfalfa (*Medicago sativa* L.) seeds were sown in May 2025 at the recommended rate of 15–20 kg ha^{-1}. In the flat land treatment, seeds were evenly broadcast across each plot to ensure uniform plant distribution. For the ridge treatment, seeds were drilled along the ridge crests at the same seeding rate to ensure comparable plant density across treatments.

Uniform agronomic practices were applied across all plots to evaluate the main and interaction effects of land configuration and establishment method on crop performance. Weed control involved hand weeding, hoeing, and the targeted use of the pre-emergence herbicide pendimethalin. Plots were routinely monitored to ensure consistent crop growth and to identify any biotic or abiotic stresses. Aside from variations in land configuration, management strategies remained unchanged. A basal application of P_2O_5 and K_2O at 40–80 kg/ha was applied 2–3 weeks before the final harrowing, with excessive nitrogen avoided after crop establishment. The study was conducted under rainfed conditions to mirror typical farmers' practices, providing practical insights into effective land configuration and establishment methods for successful alfalfa cultivation using natural rainfall, especially in areas with limited or expensive irrigation.

2.4. Sample Collection and Processing

Harvest was conducted once during the early-bloom stage, about 60 days after planting, to ensure consistent physiological maturity across the plots, which affects proximate and amino acid content as well as antinutritional factors. From each plot, we randomly selected 20 interior plants, avoiding border rows, and combined them into a single composite sample. Both leaves and stems were included. The top 10–15 cm of shoots were cut with clean stainless steel scissors to prevent contamination. Each replicate was kept separate, labelled, and transported to the lab in clean polyethylene bags. Samples were air-dried at room temperature for 48 h, then oven-dried at 60–65 °C until reaching a constant weight. After drying, the material was ground in a laboratory mill through a 1 mm sieve and stored in airtight containers for chemical analysis.

2.5. Proximate Composition Analysis

Proximate composition was measured using standard methods from the Association of Official Analytical Chemists [26]. Moisture content was assessed by oven drying at 105 °C until a constant weight was reached. Crude protein (CP) was analyzed using the Kjeldahl method, with nitrogen content multiplied by 6.25. Crude fat was extracted using Soxhlet extraction with petroleum ether as the solvent. Crude fibre was determined by sequentially digesting defatted samples with acid and alkali. Ash content was measured by incineration in a muffle furnace (CWF 11/5 laboratory chamber furnace, Derbyshire, UK) at 550 °C. Nitrogen-free extract (NFE) was calculated by subtracting the sum of the other components from the total.

$$\text{NFE (\%)} = 100 - (\text{Moisture} + \text{Ash} + \text{Crude Protein} + \text{Crude Fat} + \text{Crude Fiber}).$$

All values were expressed on a dry matter basis.

2.6. Amino Acid Analysis

Amino acid composition was analyzed following the method described by Benitez [27]. Dried samples were defatted using AOAC [28] procedures, then hydrolyzed with 6 N HCl under controlled conditions. After hydrolysis, the samples were evaporated with a rotary evaporator and reconstituted for analysis. Amino acids were subsequently measured with an Applied Biosystems PTH Amino Acid Analyser (Applied Biosystems, Foster City, CA, USA).

The profile included both essential amino acids (lysine, histidine, arginine, valine, methionine, isoleucine, leucine, and phenylalanine) and non-essential amino acids (alanine, aspartic acid, cystine, glutamic acid, glycine, proline, serine, and tyrosine). Tryptophan was measured separately after alkaline hydrolysis, following Yust et al. [29], because it is sensitive to acid degradation. Amino acid levels were expressed as mg per 100 g of dry matter.

2.7. Determination of Antinutritional Factors

Antinutritional compounds were measured in dried, ground forage samples from each treatment replicate. The analysis covered oxalates, saponins, alkaloids, flavonoids, tannins, cyanides, phytates, and total phenols. Quantification followed standard analytical procedures outlined by AOAC [26,30], with modifications tailored to forage samples. In brief, appropriate solvent extraction methods were used for each compound, and spectrophotometry was employed against calibration standards where applicable. Results are reported on a dry matter basis as mg per 100 g.

2.8. Statistical Analyses

The data underwent a 2 × 2 factorial ANOVA to evaluate the effects of land configuration, crop establishment method, and their interaction. Significant findings from the ANOVA were further analyzed through mean separation using Fisher's protected LSD test at $p < 0.05$. Treatment means were labelled with superscript letters in descending order, with 'a' indicating the highest mean. Means sharing the same letter were not significantly different at the 5% level.

The statistical model used was:

$$Y_{ijk} = \mu + B_k + L_i + E_j + (LE)_{ij} + E_{ijk}$$

where Y_{ijk} represents the observed response for the ith land configuration and jth establishment method in the kth block; μ is the overall mean, B_k denotes the effect of the kth block; L_i indicates the effect of the ith land configuration (flat or ridge); E_j is the effect of the jth establishment method (broadcast or drilled); $(LE)_{ij}$ is the interaction effect between land configuration and establishment method; and E_{ijk} is the random experimental error, assumed to be independently and normally distributed with mean zero and constant variance. This model analyzes the effects of land configuration, establishment method, and their interaction, while also accounting for variation among blocks.

3. Results

A 2 × 2 factorial analysis investigated how land configuration (flat or ridge) and establishment method (broadcast or drilled) affected eight antinutritional factors in alfalfa forage. Table 1 presents the factorial analysis of variance for antinutritional factors in alfalfa forage. Land configuration notably influenced saponin, tannins, cyanide, and total phenol, while the establishment method mainly impacted saponin, alkaloids, and total phenol

($p < 0.05$). Neither factor significantly affected oxalate, flavonoids, or phytate. Significant interactions were found for saponin, alkaloids, flavonoids, tannins, and cyanide ($p < 0.001$), with total phenol also showing a significant interaction ($p = 0.015$). Oxalate and phytate interactions were not significant, suggesting that the influence of establishment method on several antinutritional factors varies depending on land configuration.

Table 1. Factorial ANOVA for antinutritional factors of alfalfa.

Source of Variation	Oxalate	Saponin	Alkaloids	Flavonoids	Tannins	Cyanide	Phytate	Total Phenol
Land configuration (L), F	0.026	7.788 *	0.390	0.027	8.244 *	10.920 **	3.537	6.648 *
p-Value	0.876	0.021	0.548	0.873	0.018	0.009	0.093	0.030
Establishment method (E), F	4.950	5.298 *	5.325 *	0.940	0.542	4.945	2.602	6.299 *
p-Value	0.053	0.047	0.046	0.358	0.481	0.053	0.141	0.033
L × E, F	0.037	32.786 ***	51.216 ***	44.015 ***	26.347 ***	24.723 ***	2.498	8.947 *
p-Value	0.852	<0.001	<0.001	<0.001	<0.001	<0.001	0.148	0.015

Note: * = significant at $p <= 0.05$; ** = significant at $p <= 0.01$; *** = significant at $p <= 0.001$; L = land configuration; E = establishment method.

Table 2 compares the antinutritional factors of alfalfa grown on flat versus ridge land. Results show that flat land yields higher levels of saponin (1428.32 vs. 1346.01), tannin (13.68 vs. 12.55), cyanide (1.79 vs. 1.52), and total phenol (1065.60 vs. 1012.69 mg/100 g). In contrast, differences in oxalate (573.07 vs. 571.80), alkaloids (7300.98 vs. 7185.60), flavonoids (8001.85 vs. 8022.35), and phytate (43.46 vs. 42.16 mg/100 g) were not statistically significant. Overall, land configuration notably affected saponin, tannins, cyanide, and total phenol levels, with alfalfa grown on ridges showing lower concentrations of these compounds.

Table 2. Main effect of land configuration on antinutritional factors of alfalfa forage.

Land Configuration	Oxalate	Saponin	Alkaloids	Flavonoids	Tannins	Cyanide	Phytate	Total Phenol
Flat land	573.07 ^{a}	1428.32 ^{a}	7300.98 ^{a}	8001.85 ^{a}	13.68 ^{a}	1.79 ^{a}	43.46 ^{a}	1065.60 ^{a}
Ridge land	571.80 ^{a}	1346.01 ^{b}	7185.60 ^{a}	8022.35 ^{a}	12.55 ^{b}	1.52 ^{b}	42.16 ^{a}	1012.69 ^{b}
Significance	NS	*	NS	NS	*	**	NS	*

Note: Values are expressed in mg/100 g. Means within a column bearing the same superscript letter are not significantly different at $p > 0.05$. The highest mean is assigned superscript ^{a}, * = significant at $p <= 0.05$; ** = significant at $p <= 0.01$.

Table 3 indicates that the establishment method influenced certain antinutritional compounds in alfalfa. Drilling resulted in significantly higher saponin levels (1421.11 vs. 1353.22 mg/100 g), as well as higher alkaloids (7456.45 vs. 7030.12 mg/100 g) and total phenols (1064.90 vs. 1013.40 mg/100 g). Although oxalate and flavonoid levels were higher with drilling, the differences were not statistically significant at $p \leq 0.05$. Conversely, broadcasting produced marginally higher amounts of tannins, cyanide, and phytates, but these differences were not statistically significant between the two methods.

Table 4 indicates that land configuration and establishment method significantly interacted to influence several antinutritional compounds in alfalfa. The Flat × Drilled combination resulted in the highest saponin content (1546.71 mg/100 g), whereas Ridge × Drilled had the lowest (1295.51 mg/100 g). Alkaloid and flavonoid levels were highest in the Flat × Drilled treatment, averaging 8175.20 and 8475.20 mg/100 g, respectively. These were statistically similar to Ridge × Broadcast but exceeded other treatments. Tannin levels

varied notably, peaking under Flat × Broadcast (14.84 mg/100 g) and being lowest in Ridge × Broadcast (11.68 mg/100 g). Flat × Drilled also yielded significantly more total phenols (1122.05 mg/100 g) than the other methods, which did not differ significantly from each other. Nonetheless, the interaction effects on oxalate, cyanide, and phytate levels were not statistically significant, despite some numerical differences.

Table 3. Main effect of crop establishment method on antinutritional factors of alfalfa.

Crop Establishment Method	Oxalate	Saponin	Alkaloids	Flavonoids	Tannins	Cyanide	Phytate	Total Phenol
Broadcast	563.71 ^{a}	1353.22 ^{b}	7030.12 ^{b}	7951.75 ^{a}	13.26 ^{a}	1.75 ^{a}	43.37 ^{a}	1013.40 ^{b}
Drilled	581.16 ^{a}	1421.11 ^{a}	7456.45 ^{a}	8072.45 ^{a}	12.97 ^{a}	1.57 ^{a}	42.25 ^{a}	1064.90 ^{a}
Significance	NS	*	*	NS	NS	NS	NS	*

Note: Values are expressed in mg/100 g. Means within a column bearing the same superscript letter are not significantly different at $p > 0.05$. The highest mean is assigned superscript ^{a}, Note: * = significant at $p <= 0.05$; NS = not significant at $p > 0.05$.

Table 4. Interaction effect of land configuration x crop establishment method on antinutritional factors of alfalfa.

Land Configuration × Establishment Method	Oxalate	Saponin	Alkaloids	Flavonoids	Tannins	Cyanide	Phytate	Total Phenol
Flat × Broadcast	563.58 ^{a}	1309.93 ^{bc}	6426.75 ^{b}	7528.50 ^{b}	14.84 ^{a}	2.08 ^{a}	44.56 ^{a}	1009.16 ^{b}
Flat × Drilled	582.55 ^{a}	1546.71 ^{a}	8175.20 ^{a}	8475.20 ^{a}	12.52 ^{bc}	1.50 ^{b}	42.36 ^{ab}	1122.05 ^{a}
Ridge × Broadcast	563.83 ^{a}	1396.50 ^{b}	7633.50 ^{a}	8375.00 ^{a}	11.68 ^{c}	1.41 ^{b}	42.17 ^{b}	1017.63 ^{b}
Ridge × Drilled	579.78 ^{a}	1295.51 ^{c}	6737.70 ^{b}	7669.70 ^{b}	13.42 ^{b}	1.64 ^{b}	42.15 ^{b}	1007.75 ^{b}
L × E significance	NS	***	***	***	***	***	NS	*

Note: Values are expressed in mg/100 g. Means within a column bearing at least one common superscript letter are not significantly different at $p > 0.05$. The highest mean is assigned superscript ^{a}, * = significant at $p <= 0.05$; *** = significant at $p <= 0.001$; NS = not significant at $p > 0.05$.

Table 5 shows the factorial analysis of variance for alfalfa's proximate composition. Land configuration significantly impacted crude protein (CP), crude fibre (CF), and ether extract (EE) ($p \leq 0.05$), but did not significantly influence moisture content (MC), ash, or nitrogen-free extract (NFE) ($p > 0.05$). The crop establishment method significantly affected EE ($p = 0.0383$) but not MC, CP, CF, ash, or NFE. Additionally, the interaction between land configuration and crop establishment method was significant for crude fibre ($p = 0.0201$) but not for other proximate components.

Table 5. Factorial ANOVA for proximate composition of alfalfa forage.

Parameter	Land Configuration F (p)	Establishment Method F (p)	Land × Method F (p)
Moisture content	2.173 (0.175)	0.554 (0.476)	2.802 (0.128)
Crude protein	**12.155 (0.0069 **)**	0.261 (0.622)	0.155 (0.703)
Crude fibre	**18.929 (0.0018 **)**	0.170 (0.690)	**7.941 (0.0201 *)**
Ether extract	**41.189 (0.0001 ***)**	**5.883 (0.0383 *)**	0.614 (0.454)
Ash	0.491 (0.501)	0.627 (0.449)	0.024 (0.881)
Nitrogen-free extract	3.041 (0.115)	1.011 (0.341)	1.011 (0.341)

Note: Bold values indicate significance at $p <= 0.05$. Note: * = significant at $p <= 0.05$; ** = significant at $p <= 0.01$; *** = significant at $p <= 0.001$.

Table 6 illustrates how land configuration influences the proximate composition of alfalfa. Flat land significantly increases CP (23.08%) and CF (12.85%), compared with ridge

land, which shows 22.19% and 11.70%, respectively. Conversely, ridge land has a notably higher EE content (7.88%) than flat land (6.81%). Moisture content varies slightly, from 9.03% on flat land to 9.40% on ridge land. Ash content ranges from 9.24% to 9.55%, and NFE from 38.70% to 39.60%. However, these variations are not statistically significant.

Table 6. Main effect of land configuration on proximate composition (%) of alfalfa forage.

Land Configuration	Moisture Content	Crude Protein	Crude Fibre	Etter Extract	Ash	Nitrogen-Free Extract
Flat land	9.03 ^{a}	23.08 ^{a}	12.85 ^{a}	6.81 ^{b}	9.55 ^{a}	38.70 ^{a}
Ridge land	9.40 ^{a}	22.19 ^{b}	11.70 ^{b}	7.88 ^{a}	9.24 ^{a}	39.60 ^{a}

Note: Within each column, means bearing different superscript letters differ significantly at $p <= 0.05$.

Table 7 shows how different crop establishment methods affect alfalfa's proximate composition. The broadcast method produced a notably higher EE content (7.55%) than the drilled method (7.15%). Nonetheless, the establishment method does not significantly influence MC, CP, CF, ash, or NFE levels. Moisture content is slightly lower with broadcasting (9.12%) than with drilling (9.31%). CP levels are 22.57% for broadcasting and 22.70% for drilling. CF is 12.33% for broadcasting and 12.22% for drilling. Ash content stands at 9.57% versus 9.22%, and NFE is 38.89% compared to 39.41%.

Table 7. Main effect of crop establishment method on proximate composition (%) of alfalfa forage.

Establishment Method	MC	CP	CF	EE	Ash	NFE
Broadcast	9.12 ^{a}	22.57 ^{a}	12.33 ^{a}	7.55 ^{a}	9.57 ^{a}	38.89 ^{a}
Drilled	9.31 ^{a}	22.70 ^{a}	12.22 ^{a}	7.15 ^{b}	9.22 ^{a}	39.41 ^{a}

Note: Within each column, means bearing different superscript letters differ significantly at $p <= 0.05$. MC = moisture content; CP = crude protein; CF = crude fibre; EE = ether extract; NFE = nitrogen-free extract.

Table 8 reveals a significant interaction between land configuration and establishment method solely for CF. The Flat × Drilled treatment had the highest CF (13.17%), which was statistically similar to both broadcast treatments but notably higher than Ridge × Drilled (11.27%). Ridge × Broadcast was intermediate and did not differ significantly from either extreme. Interactions for MC, CP, EE, ash, and NFE were not significant ($p > 0.05$). Numerically, Ridge × Drilled recorded the highest MC (9.70%) and NFE (40.12%), Flat × Drilled the highest CP (23.20%), Ridge × Broadcast the highest EE (8.15%), and Flat × Broadcast the highest ash (9.76%). However, these numerical differences do not reflect significant treatment effects.

Table 8. Land configuration x crop establishment method interaction on proximate composition (%) of alfalfa.

Land Configuration	Establishment Method	MC	CP	CF	EE	Ash	NFE
Flat	Broadcast	9.15 ^{a}	22.97 ^{a}	12.53 ^{a}	6.95 ^{a}	9.76 ^{a}	38.70 ^{a}
Flat	Drilled	8.91 ^{a}	23.20 ^{a}	13.17 ^{a}	6.68 ^{a}	9.34 ^{a}	38.70 ^{a}
Ridge	Broadcast	9.10 ^{a}	22.17 ^{a}	12.13 ^{ab}	8.15 ^{a}	9.38 ^{a}	39.08 ^{a}
Ridge	Drilled	9.70 ^{a}	22.20 ^{a}	11.27 ^{b}	7.61 ^{a}	9.10 ^{a}	40.12 ^{a}

Note: Within each column, means bearing different superscript letters differ significantly at $p <= 0.05$. MC = moisture content; CP = crude protein; CF = crude fibre; EE = ether extract; NFE = nitrogen-free extract.

Table 9 shows the variance analysis of the essential amino acids in alfalfa. Land configuration significantly affected lysine and histidine ($p < 0.05$) but did not significantly impact leucine, isoleucine, phenylalanine, tryptophan, valine, methionine, and threonine. The method of crop establishment significantly influenced leucine, lysine, isoleucine, valine, threonine, and histidine, but not phenylalanine, tryptophan, or methionine ($p > 0.05$). Land configuration and crop establishment interacted significantly for tryptophan and histidine, but not with the other essential amino acids.

Table 9. Factorial ANOVA for essential amino acid profile of alfalfa forage.

Essential Amino Acid	Land F-Value	*p*-Value	Establishment F-Value	*p*-Value	Land × Establishment F-value	*p*-Value
Leucine	0.015	0.905 NS	14.419	0.004	0.029	0.869 NS
Lysine	8.068	0.019	36.676	<0.001	0.054	0.822 NS
Isoleucine	0.022	0.886 NS	30.258	<0.001	0.293	0.602 NS
Phenylalanine	0.776	0.401 NS	2.482	0.150 NS	2.316	0.162 NS
Tryptophan	1.771	0.216 NS	0.609	0.455 NS	10.618	0.010
Valine	0.001	0.982 NS	14.682	0.004	0.014	0.910 NS
Methionine	0.063	0.808 NS	0.104	0.754 NS	0.372	0.557 NS
Threonine	2.725	0.133 NS	94.349	<0.001	1.612	0.236 NS
Histidine	14.018	0.005	30.420	<0.001	31.314	<0.001

Note: NS = not significant ($p > 0.05$).

Table 10 illustrates the impact of land configuration on the essential amino acid profile of alfalfa. Ridge land showed significantly higher levels of lysine (3.761) and histidine (2.304) compared with flat land (3.562 and 2.188, respectively; $p < 0.05$). In contrast, land configuration did not significantly affect leucine, isoleucine, phenylalanine, tryptophan, valine, methionine, or threonine. The values for these amino acids ranged narrowly: leucine (7.067–7.088), isoleucine (3.659–3.677), phenylalanine (4.265–4.348), tryptophan (0.758–0.794), valine (3.979–3.980), methionine (1.275–1.284), and threonine (3.434–3.499).

Table 10. Main effect of land configuration on essential amino acid profile of alfalfa forage.

Treatment	Leucine	Lysine	Isoleucine	Phenylalanine	Tryptophan	Valine	Methionine	Threonine	Histidine
Flat land	7.088 ^{a}	3.562 ^{b}	3.659 ^{a}	4.265 ^{a}	0.794 ^{a}	3.980 ^{a}	1.275 ^{a}	3.499 ^{a}	2.188 ^{b}
Ridge land	7.067 ^{a}	3.761 ^{a}	3.677 ^{a}	4.348 ^{a}	0.758 ^{a}	3.979 ^{a}	1.284 ^{a}	3.434 ^{a}	2.304 ^{a}

Means within the same column bearing different superscripts differ significantly ($p < 0.05$). The highest separated mean is assigned superscript ^{a}.

Table 11 illustrates how crop establishment methods influence alfalfa's essential amino acid profile. The broadcast method significantly increased leucine (7.386), lysine (3.874), isoleucine (4.018), threonine (3.658), and histidine (2.331) compared with the drilled method (6.769, 3.450, 3.319, 3.275, and 2.160, respectively) ($p < 0.05$). Conversely, the drilled method resulted in a higher valine content (4.082) than the broadcast method (3.876). No significant differences were observed between the two methods for phenylalanine, tryptophan, and methionine.

Table 11. Main effect of crop establishment method on essential amino acid profile of alfalfa forage.

Treatment	Leucine	Lysine	Isoleucine	Phenylalanine	Tryptophan	Valine	Methionine	Threonine	Histidine
Broadcast	7.386 ^{a}	3.874 ^{a}	4.018 ^{a}	4.380 ^{a}	0.765 ^{a}	3.876 ^{b}	1.274 ^{a}	3.658 ^{a}	2.331 ^{a}
Drilled	6.769 ^{b}	3.450 ^{b}	3.319 ^{b}	4.232 ^{a}	0.786 ^{a}	4.082 ^{a}	1.285 ^{a}	3.275 ^{b}	2.160 ^{b}

Means within the same column bearing different superscripts differ significantly ($p < 0.05$). The highest separated mean is assigned superscript ^{a}.

Table 12 shows the interaction effect of land configuration and crop establishment method on the essential amino acid profile of alfalfa. A significant ($p < 0.05$) interaction was found for tryptophan and histidine. For tryptophan, the highest value was observed in Flat × Broadcast (0.828), followed by Flat × Drilled (0.760), Ridge × Drilled (0.812), and Ridge × Broadcast (0.703). Flat × Broadcast had the highest concentration, while Ridge × Broadcast had the lowest. For histidine, Flat × Broadcast had the highest value (2.360), while Flat × Drilled was the lowest (2.015). The responses for leucine, lysine, isoleucine, phenylalanine, valine, methionine, and threonine were not significantly affected by the interaction ($p > 0.05$), indicating these amino acids responded similarly across different land configurations.

Table 12. Land configuration × crop establishment method interaction on essential amino acid profile of alfalfa forage.

Treatment	Leucine	Lysine	Isoleucine	Phenylalanine	Tryptophan	Valine	Methionine	Threonine	Histidine
Flat × Broadcast	7.383 ^{a}	3.783 ^{a}	4.043 ^{a}	4.410 ^{a}	0.828 ^{a}	3.880 ^{b}	1.280 ^{a}	3.665 ^{a}	2.360 ^{a}
Flat × Drilled	6.793 ^{b}	3.342 ^{b}	3.275 ^{b}	4.120 ^{a}	0.760 ^{a}	4.080 ^{a}	1.270 ^{a}	3.333 ^{b}	2.015 ^{b}
Ridge × Broadcast	7.390 ^{a}	3.965 ^{a}	3.992 ^{a}	4.350 ^{a}	0.703 ^{b}	3.873 ^{b}	1.268 ^{a}	3.650 ^{a}	2.303 ^{a}
Ridge × Drilled	6.745 ^{b}	3.558 ^{b}	3.362 ^{b}	4.345 ^{a}	0.812 ^{a}	4.085 ^{a}	1.300 ^{a}	3.217 ^{b}	2.305 ^{a}

Means within the same column bearing different superscripts differ significantly ($p < 0.05$). The highest separated mean is assigned superscript ^{a}.

Table 13 shows the factorial ANOVA results for the non-essential amino acid profile of alfalfa, examining the effects of land configuration, crop establishment method, and their interaction. Land configuration did not significantly affect any of the non-essential amino acids studied ($p> 0.05$). Similarly, the crop establishment method did not significantly influence alanine, aspartic acid, glutamic acid, glycine, serine, proline, cystine, and arginine, but it did significantly affect tyrosine ($p < 0.05$). The interaction between land configuration and crop establishment method significantly affected alanine, aspartic acid, glutamic acid, glycine, serine, tyrosine, proline, and arginine. However, the interaction had no significant effect on cystine ($p > 0.05$).

Table 13. Factorial ANOVA for non-essential amino acid profile of alfalfa forage.

Source of Variation	Alanine	Aspartic Acid	Glutamic Acid	Glycine	Serine	Tyrosine	Proline	Cystine	Arginine
Land configuration	0.220 NS	0.038 NS	0.869 NS	1.131 NS	0.704 NS	0.018 NS	1.937 NS	0.479 NS	0.257 NS
Crop establishment	1.467 NS	0.021 NS	1.788 NS	1.166 NS	1.455 NS	8.104 *	2.244 NS	0.139 NS	0.225 NS
Land × Establishment	33.222 **	26.595 **	30.819 **	24.711 **	25.885 **	16.308 **	7.822 *	0.918 NS	16.042 **

NS = not significant ($p > 0.05$); * = significant at $p < 0.05$; ** = significant at $p < 0.01$. Values are F-statistics; amino acid unit = g/100 protein.

Table 14 illustrates how land configuration influences the non-essential amino acid profile in alfalfa. No significant effect ($p > 0.05$) was observed for any amino acid studied. Ridge land showed slightly higher levels of alanine (3.93), aspartic acid (7.82), glutamic acid (9.95), and tyrosine (4.10) compared with flat land. Conversely, flat land had marginally higher levels of glycine (4.92), serine (3.99), proline (3.43), cystine (0.82), and arginine (5.03). Despite these numerical differences, none were statistically significant.

Table 14. Main effect of land configuration on non-essential amino acid profile of alfalfa forage.

Land Configuration × Establishment Method	Alanine	Aspartic Acid	Glutamic Acid	Glycine	Serine	Tyrosine	Proline	Cystine	Arginine
Flat land + Broadcast	4.16 ^{a}	8.19 ^{a}	10.09 ^{a}	5.21 ^{a}	4.18 ^{a}	4.35 ^{a}	3.63 ^{a}	0.81 ^{a}	5.38 ^{a}
Flat land + Drilled	3.58 ^{b}	7.40 ^{b}	9.57 ^{b}	4.62 ^{b}	3.81 ^{b}	3.83 ^{c}	3.23 ^{b}	0.84 ^{a}	4.69 ^{b}
Ridge land + Broadcast	3.48 ^{b}	7.45 ^{b}	9.52 ^{b}	4.30 ^{b}	3.61 ^{b}	4.05 ^{bc}	3.24 ^{b}	0.82 ^{a}	4.69 ^{b}
Ridge land + Drilled	4.38 ^{a}	8.19 ^{a}	10.37 ^{a}	5.21 ^{a}	4.21 ^{a}	4.14 ^{ab}	3.36 ^{ab}	0.76 ^{a}	5.23 ^{a}
SEM	0.13	0.17	0.13	0.16	0.10	0.10	0.10	0.04	0.14

Means within the same column bearing different superscripts differ significantly ($p < 0.05$). The highest separated mean is assigned superscript ^{a}, amino acid unit = g/100 protein.

Table 15 shows how crop establishment method affects the non-essential amino acid profile of alfalfa. Crop establishment method did not significantly affect alanine, aspartic acid, glutamic acid, glycine, serine, proline, cystine, or arginine ($p > 0.05$). Nonetheless, broadcasting significantly increased tyrosine levels (4.20 ^{a}) compared with drilling (3.98 ^{b}) ($p < 0.05$). Although not statistically significant, drilling tended to have slightly higher levels of alanine (3.98), glutamic acid (9.97), glycine (4.92), and serine (4.01), while broadcasting showed higher approximate levels of aspartic acid (7.82), proline (3.43), cystine (0.81), and arginine (5.03).

Table 15. Main effect of crop establishment method on non-essential amino acid profile of alfalfa.

Crop Establishment Method	Alanine	Aspartic Acid	Glutamic Acid	Glycine	Serine	Tyrosine	Proline	Cystine	Arginine
Broadcast	3.82 ^{a}	7.82 ^{a}	9.81 ^{a}	4.75 ^{a}	3.89 ^{a}	4.20 ^{a}	3.43 ^{a}	0.81 ^{a}	5.03 ^{a}
Drilled	3.98 ^{a}	7.80 ^{a}	9.97 ^{a}	4.92 ^{a}	4.01 ^{a}	3.98 ^{b}	3.29 ^{a}	0.80 ^{a}	4.96 ^{a}
SEM	0.09	0.12	0.09	0.11	0.07	0.07	0.07	0.03	0.10
p-Value	0.249	0.888	0.206	0.301	0.251	0.015	0.160	0.716	0.644

Means within the same column bearing different superscripts differ significantly ($p < 0.05$). The highest separated mean is assigned superscript ^{a}, amino acid unit = g/100 protein.

Table 16 indicates that land configuration and establishment method significantly interacted ($p < 0.05$) for all evaluated non-essential amino acids except cystine. Ridge × Drilled recorded the highest alanine (4.38), glutamic acid (10.37), and serine (4.21), while sharing the highest aspartic acid (8.19) and glycine (5.21) values with Flat × Broadcast. Flat × Broadcast also produced the highest tyrosine (4.35), proline (3.63), and arginine (5.38). Tyrosine under Ridge × Drilled was statistically comparable to the maximum. Cystine ranged from 0.76 to 0.84, with no significant differences. Overall, establishment method effects on most amino acids depended on land configuration.

Table 16. Interaction effect of land configuration × crop establishment method on non-essential amino acid profile of alfalfa.

Land Configuration × Establishment Method	Alanine	Aspartic Acid	Glutamic Acid	Glycine	Serine	Tyrosine	Proline	Cystine	Arginine
Flat land + Broadcast	4.16 ^{a}	8.19 ^{a}	10.09 ^{a}	5.21 ^{a}	4.18 ^{a}	4.35 ^{a}	3.63 ^{a}	0.81 ^{a}	5.38 ^{a}
Flat land + Drilled	3.58 ^{b}	7.40 ^{b}	9.57 ^{b}	4.62 ^{b}	3.81 ^{b}	3.83 ^{c}	3.23 ^{b}	0.84 ^{a}	4.69 ^{b}
Ridge land + Broadcast	3.48 ^{b}	7.45 ^{b}	9.52 ^{b}	4.30 ^{b}	3.61 ^{b}	4.05 ^{bc}	3.24 ^{b}	0.82 ^{a}	4.69 ^{b}
Ridge land + Drilled	4.38 ^{a}	8.19 ^{a}	10.37 ^{a}	5.21 ^{a}	4.21 ^{a}	4.14 ^{ab}	3.36 ^{ab}	0.76 ^{a}	5.23 ^{a}
p-Value	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.016	0.357	0.002

Means within the same column bearing different superscripts differ significantly ($p < 0.05$). The highest separated mean is assigned superscript ^{a}. S = significant, NS = not significant, amino acid unit = g/100 protein.

4. Discussion

Factorial analysis revealed that land configuration, crop establishment method, and their interaction affect alfalfa's antinutritional and secondary metabolite profiles. Alfalfa naturally contains high levels of secondary metabolites such as saponins, flavonoids, and

phenolic compounds, and genotype, developmental stage, and environmental factors influence their concentrations [31,32]. Recent reviews highlight that flavonoids, phenolics, and triterpenoid saponins are key bioactive classes in *Medicago sativa* [33].

4.1. Effect of Land Configuration on Antinutritional Factors of Alfalfa Forage

Table 2 shows significantly higher concentrations of saponins, tannins, cyanides, and total phenols on flat land than on ridge land, while oxalate, alkaloids, flavonoids, and phytate showed no significant differences. These differences suggest that land configuration altered the growing environment sufficiently to modify some secondary metabolite accumulation pathways without producing a uniform response across all compounds [34]. This explanation is biologically plausible because secondary metabolites help plants adapt to their environment. For instance, saponins are key secondary compounds in alfalfa, and their levels fluctuate significantly depending on field conditions and plant samples [35]. Additionally, seasonal field studies have documented variations in alfalfa saponin levels across harvest times [36], suggesting that the plant's environment and developmental stage influence secondary metabolite accumulation.

Significant differences in total phenol levels among land configurations may also reflect variations in the plant microenvironment associated with the two land management practices. Phenolic compounds contribute to antioxidant defence and plant responses to environmental stresses. Research into alfalfa indicates that environmental stress can impact phenolic metabolism; for example, Páramo et al. [37] observed changes in total phenolic content under stress conditions. These results suggest that land configuration may affect conditions around the roots and shoots, influencing secondary metabolic processes. Nevertheless, without measurements of soil moisture, temperature, or nutrient levels alongside antinutrients, this experiment cannot determine a specific physiological mechanism.

4.2. Effect of Crop Establishment Method on Antinutritional Factors of Alfalfa Forage

The crop establishment method significantly influenced saponin, alkaloid, and total phenol levels (see Table 3). Drilling produced higher concentrations of saponins (1421.11 mg/100 g), alkaloids (7456.45 mg/100 g), and total phenols (1064.90 mg/100 g) than broadcasting, which recorded 1353.22, 7030.12, and 1013.40 mg/100 g, respectively. However, the establishment method did not significantly affect oxalate, flavonoids, tannins, cyanide, or phytate levels. These findings suggest that crop establishment techniques may influence alfalfa's secondary metabolic processes [38]. Drilling and broadcasting produce different spatial arrangements of plants, which may influence plant competition and environmental conditions. The varied response of certain metabolites, but not others, indicates that different classes of secondary metabolites in alfalfa respond differently to environmental factors [39]. Metabolomic studies show that environmental stress does not affect all metabolites equally; instead, specific pathways respond uniquely. For instance, Guo et al. [40] found that alkaline stress increased flavonoid synthesis and altered various metabolic pathways in alfalfa.

The absence of notable effects on phytate and oxalate is also important. This suggests these components remained fairly consistent across establishment methods in this study. Phytate is a key nutritional factor because it chelates minerals, reducing their absorption, notably of iron and zinc [41]. Therefore, each antinutrient's response to management should be assessed individually, rather than presuming that altering one secondary compound will influence all antinutritional factors.

4.3. Land Configuration × Crop Establishment Method Interaction

The significant interactions suggest that establishment method influences antinutritional composition differently across land configurations. Flat × Drilled favoured saponin,

alkaloid, flavonoid and total phenol accumulation, whereas Flat × Broadcast favoured tannin and cyanide. This variation indicates that treatment effects differ among compounds, with no combination uniformly maximizing their concentrations.

The observed strong interaction between flavonoids and total phenol aligns with the known environmental responsiveness of secondary metabolism. Alfalfa utilizes flavonoids and other non-enzymatic antioxidants as part of its response to environmental stress [42]. Recent metabolomic studies also reveal increased flavonoid biosynthesis under alkaline stress, highlighting alfalfa's ability to adapt its secondary metabolite profile to its environment [40]. Additionally, Singer et al. [42] reported physiological, biochemical, and transcriptional changes in alfalfa under drought conditions, further emphasizing the sensitivity of its metabolism to environmental factors.

The observed saponin interaction is also significant, given that saponins are key secondary metabolites in *Medicago*. Kiełbasa et al. [43] detailed the structural diversity of alfalfa saponins, including derivatives of medicagenic acid, hederagenin, bayogenin, and soyasapogenol B. Further field studies revealed differences in total saponin levels across alfalfa cultivars and sampling times [35]. Therefore, the notable Flat × Drilled response in this experiment might indicate a treatment-specific change in plant secondary metabolism.

From a nutritional perspective, these compounds should not be viewed solely as undesirable. Their biological effects depend on concentration, chemical structure, and dietary context. Saponins, flavonoids, and phenolic compounds have several potentially beneficial biological properties, although excessive concentrations of certain antinutritional compounds may interfere with nutrient utilization [44,45]. Recent systematic evidence on alfalfa phytochemistry emphasizes this diverse biological role, particularly for saponins, flavonoids, and other phenolic constituents [33].

Overall, the results show that, for most measured secondary metabolites, the combined influence of land configuration and crop establishment method outweighs the effects of each factor alone. Significant interactions in six of eight parameters suggest that agronomic management can alter alfalfa's phytochemical profile [46]. The lack of significant interactions for oxalate and phytate indicates that these compounds are relatively stable across the tested treatment combinations. Consequently, when significant interactions are present, interpretation should focus mainly on treatment combination means rather than the main effects considered separately.

4.4. Factorial ANOVA for Proximate Composition of Alfalfa

The factorial ANOVA in Table 5 showed that land configuration significantly ($p \leq 0.05$) affects crude protein (CP), crude fibre (CF), and ether extract (EE). By contrast, moisture content (MC), ash, and nitrogen-free extract (NFE) showed no significant effects. This suggests that flat and ridge land configurations yield different outcomes for alfalfa's key nutritional components, likely due to variations in soil–water interactions and nutrient supply. Hu et al. [47] also found that alfalfa forage quality, particularly CP and fibre traits, responds to changes in soil water conditions and environmental stresses.

The crop establishment method notably affected EE but did not significantly affect MC, CP, CF, ash, or NFE. Additionally, the interaction between land configuration and crop establishment method was significant only for CF. The overall lack of significant interactions suggests that broadcast and drilled methods produced similar effects on most components across different land configurations. Past studies show that sowing or establishment techniques can affect alfalfa performance, although their influence varies by the specific crop characteristic assessed [48].

4.5. Main Effect of Land Configuration on Proximate Composition

Table 6 shows that flat land had a notably higher crude protein (CP) content of 23.08%, compared with ridge land at 22.19%. As CP is a key measure of forage nutritive value, the relatively high levels in both land types highlight the nutritional potential of the alfalfa produced in this study. Hu et al. [47] noted that CP and fibre are primary forage quality traits and showed that their levels can vary with soil water availability and growth conditions.

The notably higher CP on flat land likely reflects differences in the growing environment between the two setups, although direct measurements of soil moisture and nutrient uptake are needed to confirm the underlying cause. Mak-Mensah et al. [49] similarly found that ridge-furrow rainwater-harvesting management influenced alfalfa's nutritional quality, including CP and fibre fractions. Their study also included biochar and various ridge-furrow configurations, supporting the idea that alfalfa quality is generally sensitive to field layout, but it did not directly compare with the current treatments.

Higher crude fibre on flat land (12.85%) than on ridge land (11.70%) suggests that land configuration affects alfalfa's structural composition. As structural carbohydrates influence forage digestibility and feeding value, this difference may have implications for the nutritional quality of alfalfa herbage. This finding aligns with Greveniotis et al. [50], who showed that alfalfa fibre characteristics vary with environmental conditions and growth stages. Ridge land resulted in notably higher EE (7.88%) than flat land (6.81%). Therefore, the land setup linked to higher CP and CF did not correspond to the highest EE. The responses of individual proximate components should be considered separately. MC, ash, and NFE showed no significant differences between flat and ridge land, indicating these components remained relatively stable across the two land types.

4.6. Main Effect of Crop Establishment Method on Proximate Composition

Broadcasting produced significantly higher EE than drilling (7.55% versus 7.15%), suggesting an effect on lipid content, while MC, CP, CF, ash, and NFE showed little variation. This implies that the choice of crop establishment method has a relatively minor influence on alfalfa's proximate composition [51], especially compared with land configuration effects. Gondal et al. [48] examined broadcast sowing and various row spacings in alfalfa and found that the sowing method strongly affects forage productivity. Although previous findings linked broadcast sowing to higher forage yield, our results suggest similar nutritional composition across establishment methods. Broadcast and drilled treatments showed similar CP (22.57% versus 22.70%) and CF (12.33% versus 12.22%), indicating limited effects on these traits.

4.7. Land Configuration × Crop Establishment Method Interaction on Proximate Composition

The notable interaction for CF shows that establishment effects vary by land configuration. Drilling raised CF by 13.17% on flat land but decreased it by 11.27% on ridges. Broadcasting resulted in intermediate changes, with increases of 12.53% and 12.13%, respectively. These opposing responses highlight the need to assess both factors together, as examining only main effects could mask differences in alfalfa fibre composition across different treatment combinations. Environmental and management factors affect alfalfa fibre properties [52], and field configuration also influences alfalfa fibre fractions and other nutritional features [49]. Land configuration affected CP, CF, and EE, while the establishment method affected only EE. Their interaction influenced only CF. The nonsignificant interactions for MC, CP, EE, ash, and NFE suggest that numerical differences do not define treatment effects. These findings suggest that the proximate composition of alfalfa varies selectively in response to specific agronomic practices, rather than all nutritional components responding equally [53].

4.8. Factorial ANOVA on Essential Amino Acid Profile of Alfalfa Forages

The ANOVA indicates different responses among essential amino acids: land configuration impacted lysine and histidine, while the establishment method affected leucine, lysine, isoleucine, valine, threonine, and histidine. Notably, significant interactions were observed only for tryptophan and histidine, suggesting both factors influence their responses. These results suggest that individual amino acids do not respond uniformly to agronomic practices, which is biologically plausible given alfalfa's diverse essential amino acids [54]. Their concentrations can vary based on crop management, growth stage, and forage handling. Recent analyses confirm that alfalfa contains amino acids such as threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, and histidine [55].

4.9. Impact of Land Configuration on Alfalfa's Essential Amino Acids

Ridge cultivation notably increased lysine and histidine levels compared with flat land, while other essential amino acids remained similar. These variations suggest that land configuration has a limited impact on the overall amino acid profile, consistent with previous findings that alfalfa's amino acid composition remains fairly stable across different conditions. For example, Du et al. [56] found that although amino acid levels decreased with alfalfa maturity and crude protein declined, the proportional composition of amino acids remained fairly constant across various growth stages. The distinct responses of lysine and histidine to land configuration suggest that some amino acids are more sensitive to environmental changes in crop cultivation than others. Agronomic practices can affect plant growth, nutrient absorption, and biomass composition, influencing forage quality. Liu et al. [57] found that forage amino acid profiles change with different cropping management, notably affecting key amino acids like histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine across various alfalfa treatments. Therefore, differences in lysine and histidine between ridge and flat planting methods reflect a broader trend: specific agronomic techniques can deliberately alter forage protein quality.

4.10. Effect of Crop Establishment Method on Essential Amino Acid Profile of Alfalfa Forages

The establishment method had a more significant impact on essential amino acids than land configuration. Broadcasting increased leucine, lysine, isoleucine, threonine, and histidine, while drilling specifically increased valine. However, phenylalanine, tryptophan, and methionine levels did not change significantly. Neither approach consistently improved the amino acid profile. These variations may reflect establishment and competition dynamics, although the study did not examine physiological mechanisms, which cannot be inferred from amino acid levels alone. Overall, the results align with previous studies showing that management influences alfalfa's essential amino acid content. For instance, Brito et al. [58] identified numerical differences in histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine concentrations when alfalfa was harvested at different times of day, showing that management practices can influence forage composition at the amino acid level. Additionally, Cheng et al. [59] reported that cropping treatment and harvest stage significantly affected several essential amino acids in alfalfa-based forage systems.

4.11. Effect of Land Configuration × Crop Establishment Interaction on the Essential Amino Acid Profile of Alfalfa Forages

Significant interactions involving tryptophan and histidine suggest that the responses to establishment vary with land configuration. Flat × Broadcast had the highest levels of both amino acids, while Ridge × Broadcast had the lowest tryptophan and Flat × Drilled had the lowest histidine. Histidine levels were similar across ridge methods but varied significantly on flat land, highlighting the importance of interaction effects. In contrast, the lack of significant interactions for leucine, lysine, isoleucine, phenylalanine, valine,

methionine, and threonine indicates that their main effects are more straightforward. For tryptophan and histidine, examining specific treatment combinations provides clearer insights than averaging main effects, which might hide land-specific responses. These variable responses align with evidence that alfalfa forage amino acid composition can vary due to management factors without all amino acids changing uniformly [58,60]. These results imply that agronomic practices mainly affect specific components of alfalfa's essential amino acid profile rather than causing uniform changes [57]. This is nutritionally important because alfalfa is a protein-rich forage containing the essential amino acids studied [55].

4.12. Factorial ANOVA on Non-Essential Amino Acid Profile of Alfalfa Forages

The stronger interaction effect on non-essential amino acid composition indicates that land configuration and establishment method should be evaluated together when assessing alfalfa protein quality, rather than separately. Composition studies confirm that alfalfa contains significant amounts of alanine, aspartic acid, glutamic acid, glycine, serine, proline, tyrosine, cysteine/cystine, and arginine, although levels vary by plant material and analytical method [61]. Recent protein characterization of alfalfa also revealed a broad range of amino acids, emphasizing its importance as a protein-rich forage and potential protein source [62].

4.13. Effect of Land Configuration on Non-Essential Amino Acids Profile of Alfalfa Forages

The lack of notable differences in all nine non-essential amino acids indicates that land configuration has little impact on alfalfa composition under the tested conditions, despite small variations between treatments. Therefore, neither landform consistently improved the amino acid profile across the different crop establishment methods, supporting Zhang [63].

The relatively stable main effect of land configuration suggests that the crop sustained protein and amino acid synthesis across both setups. This supports the broader understanding that amino acid levels in forage plants depend on multiple factors, including plant development, environmental conditions, tissue composition, and management practices [64], rather than a single field management factor. Evidence from alfalfa-based forage systems indicates that management and maturity can alter individual amino acid concentrations independently, rather than causing a uniform change across the entire amino acid profile [65]. In this experiment, glutamic and aspartic acids were among the most abundant amino acids, with average levels nearing 10 and 8, respectively. This pattern aligns well with biological expectations for alfalfa. Referenced compositional data indicate that aspartic and glutamic acids are key amino acids in alfalfa, and experimental analysis of *Medicago sativa* further confirms their significant presence in the amino acid profile [66].

4.14. Effect of Crop Establishment Method on Non-Essential Amino Acids Profile of Alfalfa Forage

The establishment method mainly affected non-essential amino acids, with only tyrosine showing a significant increase under broadcasting (4.20) compared with drilling (3.98). This response indicates that different amino acids vary in sensitivity to agronomic practices, consistent with reports of variation in alfalfa amino acid profiles across harvesting methods and plant parts. For example, Zhang et al. [38] found that separating the leaf-rich portion of alfalfa significantly changed crude protein and amino acid levels, including tyrosine and cysteine. Likewise, Liebhardt et al. [67] reported differences in both individual and total amino acid concentrations in alfalfa depending on cutting timing. These results suggest that management can selectively influence amino acid composition, rather than all amino acids responding uniformly.

4.15. Land Configuration × Crop Establishment Interaction on Non-Essential Amino Acids Profile of Alfalfa Forage

Interactions involving amino acids such as alanine, aspartic acid, glutamic acid, glycine, serine, tyrosine, proline, and arginine show that land configuration affected their levels differently. Cystine was an exception, indicating that non-essential amino acids respond differently. This suggests that whether broadcasting or drilling is more effective depends on whether alfalfa is grown on flat or ridge land [68]. These interactions reveal responses hidden by individual factors: Ridge × Drilled favours alanine, glutamic acid, and serine, while Flat × Broadcast favours tyrosine, proline, and arginine, along with higher levels of other amino acids, reflecting different treatment benefits. Overall, the two contrasting combinations (flat land with broadcast and ridge land with drilled) generally produced the highest amino acid concentrations [69]. This crossover response clarifies why there were few significant main effects despite notable interaction effects. For instance, as Liu et al. [70] reported, broadcasting was advantageous on flat land for several amino acids, while drilling generally yielded better results on ridge land. When averaging treatment combinations to determine main effects, these opposing responses often cancel each other out. Such interaction effects are crucial agronomically because they show that an establishment method's impact depends on the specific production environment. Forage research has also documented variations in amino acid profiles in response to crop management conditions. Poe et al. [65] found significant differences in amino acid profiles among alfalfa cropping systems and at different harvesting stages. Similarly, studies comparing harvesting methods in alfalfa showed that increases in protein-rich leaf material were linked to changes in amino acid levels [71,72]. These findings support the idea that agronomic practices that influence crop growth, development, or the leaf-to-stem ratio can ultimately affect forage protein characteristics.

Cystine stood out from other amino acids since neither its land configuration, establishment method, nor their interaction had a significant impact. Its limited variation across treatment combinations indicates relatively higher stability under the management practices studied. Additionally, our findings agree with Camli-Saunders and Villouta [73] that alfalfa contains cystine/cysteine at lower concentrations than several other amino acids, as shown in compositional datasets and experimental protein analyses.

Overall, land configuration and establishment method interacted to shape alfalfa's non-essential amino acid profile. Flat-land broadcasting and ridge-land drilling tended to increase several amino acids, while cystine levels stayed fairly constant. These notable interactions highlight the need to consider land configuration and crop establishment together as a combined management approach when assessing alfalfa forage quality, rather than analyzing each factor separately.

This study has some limitations. Variables such as changes in soil moisture, mineral nitrogen levels, nodulation strength, and biological nitrogen fixation were not directly measured, thereby hindering definitive mechanistic inferences about the observed amino acid responses. Additionally, the study did not assess physiological metrics such as photosynthesis rates and plant water status, limiting understanding of carbon–nitrogen interactions. Results on animal digestibility and performance were beyond the scope of this work, leaving implications for feeding value tentative. Future research incorporating soil nutrient analysis, rhizosphere microbial activity, nitrogen flux assessments, and livestock response experiments could better explain how land configuration affects forage biochemical makeup and its functional advantages in forage–livestock systems.

4.16. Implications for Ruminant Feeding

Variations in alfalfa composition have practical implications for its use across different ruminant species. Its relatively high crude protein content (22.19–23.08%) highlights its potential as a protein-rich feed for dairy cattle, growing beef cattle, sheep, and goats. For lactating animals, adequate protein and essential amino acids are vital for supporting milk production and milk protein synthesis. Differences in lysine, histidine, leucine, isoleucine, and threonine suggest that factors such as land setup and planting method may influence the protein quality of alfalfa fed to high-producing animals. These compositional differences can inform forage selection and ration formulation to meet specific animal needs and production stages. However, crude protein levels and amino acid profiles alone do not determine the feeding value, since amino acid availability depends on ruminal protein breakdown and intestinal digestibility. Consequently, these findings should be integrated with data on forage intake, digestibility, and overall diet composition when assessing their suitability for different ruminant groups.

5. Conclusions

Overall, land configuration and crop establishment techniques selectively impacted the composition, amino acid profile, and antinutritional traits of alfalfa forage. Flat land yielded higher crude protein and crude fibre, while ridge land had increased ether extract and elevated levels of lysine and histidine. The establishment method influenced ether extract and numerous essential amino acids, with significant interaction effects noted for certain antinutritional compounds, crude fibre, tryptophan, histidine, and most non-essential amino acids.

These findings suggest viewing agronomic practices as part of integrated management strategies, rather than relying on a single land configuration or establishment method to improve all forage quality traits at once. Since the results vary depending on site and season, the observed relationships should not be mistaken for physiological causality or guaranteed improvements in animal performance.

Under the studied conditions, certain treatment combinations that promote a desirable forage composition can be identified. However, additional multi-season and multi-location research, including assessments of soil and plant physiological variables as well as animal feeding responses, is necessary before general production guidelines can be established.

Author Contributions: Conceptualization, H.I.F., S.B.P. and L.Y.G.; methodology, H.I.F., S.B.P. and L.Y.G.; software, H.I.F. and L.Y.G.; validation, H.I.F., S.B.P. and L.Y.G.; formal analysis, H.I.F., S.B.P. and L.Y.G.; investigation, H.I.F., S.B.P. and L.Y.G.; resources, H.I.F., S.B.P. and L.Y.G.; data curation, L.Y.G.; writing—original draft preparation, H.I.F., S.B.P. and L.Y.G.; writing—review and editing, H.I.F., S.B.P. and L.Y.G.; visualization, H.I.F., S.B.P. and L.Y.G.; supervision, H.I.F.; project administration, H.I.F.; funding acquisition, H.I.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received financial support from Alternative Bank Limited, Abuja, Nigeria, with supplementary funding provided through the authors' personal contributions.

Institutional Review Board Statement: Not applicable for studies not involving humans or animals.

Data Availability Statement: The data and materials in this study are available from the corresponding author upon reasonable request.

Acknowledgments: We thank the management and staff of Plateau State University, Bokkos (PLASU) for the support they provided throughout this study into the adaptability of alfalfa (*Medicago sativa* L.) cultivation. We also thank Alternative Bank Ltd. for their financial support.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

EAAs	Essential amino acids
NEAAs	Non-essential amino acids
RCBD	Randomized Complete Block Design
AOAC	Association of Official Analytical Chemists
MC	Moisture content
EE	Ether extract
CP	Crude protein
NFE	Nitrogen-free extract

References

1. Esen, S. Optimizing ruminant nutrition: Insights from a comprehensive analysis of silage composition and in vitro gas production dynamics using nonlinear models. *Biosystems* **2023**, *234*, 105062. [CrossRef] [PubMed]
2. Finangwai, H.I.; Barje, P.P.; Danbauchi, S.E.; Guluwa, L.Y.; Pewan, S.B.; Gunya, D.Y. Socio-Economic and Institutional Predictors of Climate Change Adaptation Among Smallholder Dairy Farmers in Plateau State, Nigeria. *Agriculture* **2026**, *16*, 1671. [CrossRef]
3. Khanal, P.; Dhakal, R.; Khanal, T.; Pandey, D.; Devkota, N.R.; Nielsen, M.O. Sustainable Livestock Production in Nepal: A Focus on Animal Nutrition Strategies. *Agriculture* **2022**, *12*, 679. [CrossRef]
4. Alemayehu, S.; Olago, D.; Alfred, O.; Dejene, S.W. Climate Change Perceptions, Impacts, and Adaptation Strategies in Mixed Crop–Livestock Systems of Ethiopia's Lowlands. *Sustainability* **2025**, *17*, 10428. [CrossRef]
5. Pewan, S.B.; Otto, J.R.; Kinobe, R.T.; Adegboye, O.A.; Malau-Aduli, A.E.O. Fortification of diets with omega-3 long-chain polyunsaturated fatty acids enhances feedlot performance, intramuscular fat content, fat melting point, and carcass characteristics of Tattykeel Australian White MARGRA lambs. *Front. Vet. Sci.* **2022**, *9*, 933038. [CrossRef] [PubMed]
6. Amole, T.; Augustine, A.; Balehegn, M.; Adesogoan, A.T. Livestock feed resources in the West African Sahel. *Agron. J.* **2021**, *114*, 26–45. [CrossRef] [PubMed]
7. Nepali, S. Sustainable Feed Innovation for Mountain Livestock: Reducing the Need for Imported Feed with Local Resources First. *Front. Sustain. Food Syst.* **2026**, *9*, 1741602. [CrossRef]
8. Pauliukaitytė, I.; Žygytė, M.; Šalaševičienė, A.; Almonaitytė, K. Uncovering the Protein Conversion Potential of Alfalfa (*Medicago sativa* L.) and Duckweed (*Lemna minor* L.) Through Enzymatic Hydrolysis and Digestibility Assessment. *Foods* **2026**, *15*, 885. [CrossRef] [PubMed]
9. Suwignyo, B.; Mustika, A.; Kustantinah Yusiati, L.M.; Suhartanto, B. Effect of drying method on physical-chemical characteristics and amino acid content of tropical alfalfa (*Medicago sativa* L.) hay for poultry feed. *Am. J. Anim. Vet. Sci.* **2020**, *15*, 118–122. [CrossRef]
10. Hadidi, M.; Palacios, J.C.O.; McClements, D.J.; Mahfouzi, M.; Moreno, A. Alfalfa as a sustainable source of plant-based food proteins. *Trends Food Sci. Technol.* **2023**, *135*, 202–214. [CrossRef]
11. Shaikh, F.; Mohammed, R.; Uzgare, A.S. Efficient Protein Purification: From Basics to Advanced Analytical Techniques—A Review. *Anal. Bioanal. Chem. Res.* **2026**, *13*, 133–146.
12. Joolaei, A.P.; Nodeh, H.R.; Esfandiari, Z.; Ferrentino, G. Bioactive Phytochemical Compounds in *Medicago sativa* L. (Alfalfa): A Systematic Review of Biological Properties and Therapeutic Relevance in Nutraceutical and Functional Food Implications. *Food Front.* **2025**, *7*, e70189. [CrossRef]
13. Wang, S.-Y.; Yang, G.-L.; Jing, Y.-Y.; He, Z.-N.; Liu, B.; Sun, L.; Zhang, Y.; Gao, F. Alfalfa quality improvement and loss reduction technology advances. *Front. Anim. Sci.* **2025**, *6*, 1550492. [CrossRef]
14. Zhu, X.; Chen, W.; Li, M.; Liu, B.; Zhao, S.; Hu, M.; Li, J.; Li, D.; Shi, Y.; Sun, H.; et al. Comprehensive evaluation of the nutritional value and contaminants of alfalfa (*Medicago sativa* L.) in China. *Front. Nutr.* **2025**, *12*, 1539462. [CrossRef] [PubMed]
15. Bhandari, K.B.; Rusch, H.L.; Heuschele, D.J. Alfalfa Stem Cell Wall Digestibility: Current Knowledge and Future Research Directions. *Agronomy* **2023**, *13*, 2875. [CrossRef]
16. Domokos-Szabolcsy, É.; Yavuz, S.R.; Picoli, E.; Fári, M.G.; Kovács, Z.; Tóth, C.; Kaszás, L.; Alshaal, T.; Elhawat, N. Green Biomass-Based Protein for Sustainable Feed and Food Supply: An Overview of Current and Future Prospective. *Life* **2023**, *13*, 307. [CrossRef] [PubMed]
17. Adimas, M.A.; Delele, M.A.; Abera, B.D.; Worku, A.F.; Abera, M.K.; Kanellou, A.; Xanthakis, E. Addressing Anti-Nutritional Factors of Barley and Exploring Different Processing Conditions to Enhance Nutritional Value and Digestibility: A Review. *Appl. Food Res.* **2025**, *6*, 101605. [CrossRef]

18. Salim, R.; Nehvi, I.B.; Mir, R.A.; Tyagi, A.; Ali, S.; Bhat, O.M. A review on anti-nutritional factors: Unravelling the natural gateways to human health. *Front. Nutr.* **2023**, *10*, 1215873. [CrossRef] [PubMed]
19. Li, S.; Liu, Z. Fertilisation and environmental factors affect the yield and quality of alfalfa in China. *Plant Soil Environ.* **2024**, *70*, 276–286. [CrossRef]
20. Baral, R.; Jagadish, S.K.; Hein, N.; Lollato, R.P.; Shanoyan, A.; Giri, A.K.; Kim, J.; Kim, M.; Min, D. Exploring the impact of soil water variability and varietal diversity on alfalfa yield, nutritional quality, and farm profitability. *Grassl. Res.* **2023**, *2*, 266–278. [CrossRef]
21. Feng, Y.; Shi, Y.; Zhao, M.; Shen, H.; Xu, L.; Luo, Y.; Liu, Y.; Xing, A.; Kang, J.; Jing, H. Yield and quality properties of alfalfa (*Medicago sativa* L.) and their influencing factors in China. *Eur. J. Agron.* **2022**, *141*, 126637. [CrossRef]
22. Horvat, D.; Viljevac Vuletić, M.; Andrić, L.; Baličević, R.; Kovačević Babić, M.; Tucak, M. Characterization of Forage Quality, Phenolic Profiles, and Antioxidant Activity in Alfalfa (*Medicago sativa* L.). *Plants* **2022**, *11*, 2735. [CrossRef] [PubMed]
23. Arshad, M.A.; Abbas, R.N.; Baloch, R.; Ahmad, A.; Zulfiqar, U.U.; Haider, F.U.; El-Beltagi, H.S.; Alqahtani, M.D.; Prasad, P.V.V. Enhancing soybean (*Glycine max* L.) yield and quality through optimised weed-free periods and sowing techniques. *Front. Plant Sci.* **2026**, *17*, 1700878. [CrossRef] [PubMed]
24. Balliu, A.; Zheng, Y.; Sallaku, G.; Fernández, J.A.; Gruda, N.S.; Tuzel, Y. Environmental and Cultivation Factors Affect the Morphology, Architecture and Performance of Root Systems in Soilless Grown Plants. *Horticulturae* **2021**, *7*, 243. [CrossRef]
25. Xin, X.; Sun, Z.; Xiao, J.; Bai, W.; Zhang, Z.; Yan, H. Efficient Utilization Mechanism of Soil Moisture and Nutrients with Ridge Film Furrow Seeding Technology of Sloping Farmlands in Semi-Arid and Rain-Fed Areas. *Agriculture* **2023**, *13*, 1940. [CrossRef]
26. AOAC (Association of Official Analytical Chemists). *Official Methods of Analysis*, 19th ed.; AOAC International: Singapore, 2012.
27. Benitez, L.V. Amino acid and fatty acid profiles in aquaculture nutrition studies. In *Fish Nutrition Research in Asia*; Asian Fisheries Society: Manila, Philippines, 1989; pp. 23–35.
28. AOAC (Association of Official Analytical Chemists). *Official Method of Analysis of the AOAC 2026*, 18th ed.; Horwitz, W., Ed.; AOAC: Washington, DC, USA, 2011.
29. Yust, M.M.; Pedroche, J.; Girón-Calle, J.; Vioque, J.; Millán, F.; Alaiz, M. Determination of tryptophan by high-performance liquid chromatography of alkaline hydrolysates with spectrophotometric detection. *Food Chem.* **2004**, *85*, 317–320. [CrossRef]
30. Harborne, I.B. *Phytochemical Screening Methods: A Guide to Modern Techniques of Plant Analysis*; Chapman and Hall: London, UK, 1998; pp. 182–190.
31. Khal, L.H.; Rasul, K.S.; Lateef, D.D.; Halshoy, H. Comparative analysis of phytochemical composition, antioxidant activity, and chemotaxonomic relationships of Medicago species from the Kurdistan Region of Iraq. *Genet. Resour. Crop Evol.* **2026**, *73*, 284. [CrossRef]
32. Zheng, B.; Zhou, X.; Tao, X.; Qi, Y.; Wei, Y.; Xu, Z.; Wang, G. Study on differences in flavonoid synthesis in *Xanthoceras sorbifolia* leaves based on transcriptome analysis. *Front. Plant Sci.* **2026**, *17*, 1822700. [CrossRef] [PubMed]
33. Xu, L.; Duan, H.; Cui, G.; Zhang, Q.; Long, R.; Zhu, X.; Wu, F.; Wang, Y.; Li, Y.; Shen, J.; et al. Comprehensive transcriptome and metabolome analysis revealing the molecular and metabolic basis of methyl jasmonate response in alfalfa. *BMC Plant Biol.* **2026**. [CrossRef]
34. Safari, N.; Samuolienė, G.; Viršilė, A. Environmental Control and Controlled Elicitation Strategies to Modulate Phytochemical Quality of Medicinal and Aromatic Plants in Controlled Environment Agriculture. *Agronomy* **2026**, *16*, 1652. [CrossRef]
35. Nagy, B.; Makleit, P. Total saponin content (TSC) of different alfalfa (*Medicago sativa* L.) cultivars cultivated in field experiment. *Rev. Agric. Rural Dev.* **2022**, *10*, 176–180. [CrossRef]
36. Kovács, Z.; Soós, Á.; Kovács, B.; Kaszás, L.; Elhawat, N.; Razem, M.; Veres, S.; Fári, M.G.; Koroknai, J.; Alshaal, T.; et al. Nutrichemical alterations in different fractions of multiple-harvest alfalfa (*Medicago sativa* L.) green biomass fortified with various selenium forms. *Plant Soil* **2023**, *487*, 173–195. [CrossRef]
37. Páramo, L.; Feregrino-Pérez, A.A.; Vega-González, M.; Escobar-Alarcón, L.; Esquivel, K. *Medicago sativa* L. plant response against possible eustressors (Fe, Ag, Cu)-TiO_2: Evaluation of physiological parameters, total phenol content, and flavonoid quantification. *Plants* **2023**, *12*, 659. [CrossRef] [PubMed]
38. Zhang, S.; Zhu, H.; Cen, H.; Qian, W.; Wang, Y.; Ren, M.; Cheng, Y. Effects of various forms of selenium biofortification on photosynthesis, secondary metabolites, quality, and lignin deposition in alfalfa (*Medicago sativa* L.). *Field Crops Res.* **2023**, *292*, 108801. [CrossRef]
39. Wei, X.; Wang, W.; Zhao, Y.; Pu, X.; Lu, G.; Xu, C. Adaptive Mechanisms of White-Flowered Alfalfa (*Medicago sativa* L.) in High-Altitude Cold and Saline–Alkali Environments. *Biology* **2026**, *15*, 414. [CrossRef] [PubMed]
40. Guo, R.; Zhou, Z.; Cai, R.; Liu, L.; Wang, R.; Sun, Y.; Wang, D.; Yan, Z.; Guo, C. Metabolomic and physiological analysis of alfalfa (*Medicago sativa* L.) in response to saline and alkaline stress. *Plant Physiol. Biochem.* **2024**, *207*, 108338. [CrossRef] [PubMed]
41. Gibson, R.S.; Raboy, V.; King, J.C. Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. *Nutr. Rev.* **2018**, *76*, 793–804. [CrossRef] [PubMed]

42. Singer, S.D.; Subedi, U.; Lehmann, M.; Burton Hughes, K.; Feyissa, B.A.; Hannoufa, A.; Shan, B.; Chen, G.; Kader, K.; Ortega Polo, R.; et al. Identification of differential drought response mechanisms in *Medicago sativa* subsp. sativa and falcata through comparative assessments at the physiological, biochemical, and transcriptional levels. *Plants* **2021**, *10*, 2107. [CrossRef] [PubMed]
43. Kiełbasa, A.; Krakowska, A.; Rafińska, K.; Buszewski, B. Isolation and determination of saponin hydrolysis products from *Medicago sativa* using supercritical fluid extraction, solid-phase extraction and liquid chromatography with evaporative light scattering detection. *J. Sep. Sci.* **2019**, *42*, 465–474. [CrossRef] [PubMed]
44. Edo, G.I.; Ndudi, W.; Makia, R.S.; Jikah, A.N.; Yousif, E.; Gaaz, T.S.; Akpoghelie, P.O.; Opiti, R.A.; Isoje, E.F.; Igbuku, U.A.; et al. Nutritional immunological effects and mechanisms of chemical constituents from the homology of medicine and food. *Phytochem. Rev.* **2025**, *24*, 4183–4217. [CrossRef]
45. Agregán, R.; Guzel, N.; Guzel, M.; Bangar, S.P.; Zengin, G.; Kumar, M.; Lorenzo, J.M. The effects of processing technologies on nutritional and anti-nutritional properties of pseudocereals and minor cereal. *Food Bioprocess Technol.* **2023**, *16*, 961–986. [CrossRef]
46. Khairy, A.; Hammoda, H.M.; Metwally, A.M.; Zaatout, H.H.; Ibrahim, R.S. Therapeutic potential of *Medicago sativa* (Alfalfa): A comprehensive review of its bioactive compounds and pharmacological properties. *J. Adv. Pharm. Sci.* **2025**, *2*, 1–27. [CrossRef]
47. Hu, Y.; Kang, S.; Ding, R.; Zhao, Q. A crude protein and fiber model of alfalfa incorporating growth age under water and salt stress. *Agric. Water Manag.* **2021**, *255*, 107037. [CrossRef]
48. Gondal, M.R.; Arshad, M.; Ali, N.; Rizvi, S.A.; Jahangeer, A.; Ijaz, S.; Ashraf, M.S.; Khan, M.N.; Iqbal, N.; Idrees, M. Appropriate sowing technique to enhance productivity and maximize the net return of alfalfa crop. *Int. J. Agric. Technol.* **2022**, *18*, 1477–1486.
49. Mak-Mensah, E.; Zhao, W.; Zhou, X.; Zhang, D.; Zhao, X.; Wang, Q.; Obour, P.B. Influence of tied-ridge with maize straw biochar on alfalfa fodder yield, nutritional quality, and production economics. *J. Soil Sci. Plant Nutr.* **2023**, *23*, 339–350. [CrossRef]
50. Greveniotis, V.; Bouloumpasi, E.; Skendi, A.; Korkovelos, A.; Kantas, D.; Zotis, S.; Ipsilandis, C.G. Modeling Stability of Alfalfa Yield and Main Quality Traits. *Agriculture* **2024**, *14*, 542. [CrossRef]
51. Du, N.; Zhang, H.; Pu, X.; Xu, C.; Zhao, Y. Effects of Companion Crops on the Production Performance and Nutritional Quality of Alfalfa in Desertified Saline–Alkali Soils of the Qaidam Basin. *Agronomy* **2026**, *16*, 468. [CrossRef]
52. Zhang, L.; Jiang, Y.; Yin, M.; Ling, Y.; Li, H.; Gan, Y.; Yue, C.; Ma, Y.; Kang, Y.; Qi, G.; et al. Optimizing Phosphorus Fertilization Management Is Conducive to Improving Alfalfa Yield and Quality: A Meta-Analysis. *Agriculture* **2025**, *15*, 797. [CrossRef]
53. Samfira, I.; Horablaga, N.M.; Hulea, A.; Floares, D.; Imbrea, F.; Onișan, E.; Sărățeanu, V.; Stanciu, A.-Ș.; Hulea, C.; Imbrea, I.; et al. Integrated Characterisation of Elite Wild Lolium perenne Germplasm Reveals Valuable Parental Material for Sustainable Forage Breeding. *Appl. Sci.* **2026**, *16*, 9561. [CrossRef]
54. Bilea, F.; Garcia-Vaquero, M.; Magureanu, M.; Mihaila, I.; Mildažienė, V.; Mozetič, M.; Pawłat, J.; Primc, G.; Puač, N.; Robert, E.; et al. Non-thermal plasma as environmentally-friendly technology for agriculture: A review and roadmap. *Crit. Rev. Plant Sci.* **2024**, *43*, 428–486. [CrossRef]
55. Gurev, A.; Bulgaru, V.; Ciugureanu, I.; Netreba, N.; Dragancea, V.; Dianu, I.; Sandu, I.; Mazur, M.; Mitina, T.; Bandarenco, N.; et al. Effect of Conventional and Ultrasound-Assisted Extraction Conditions on the Physicochemical Properties, Phytochemical Content, Antioxidant Activity and Functional Properties of Alfalfa Protein Concentrates. *Foods* **2025**, *14*, 4309. [CrossRef] [PubMed]
56. Du, H.; Zhang, Y.; Ma, Y.; Jiao, W.; Lei, T.; Su, H. Rapid Determination of Crude Protein Content in Alfalfa Based on Fourier Transform Infrared Spectroscopy. *Foods* **2024**, *13*, 2187. [CrossRef] [PubMed]
57. Liu, L.; Hou, M.; Liu, Y.; Sa, D.; Lu, Q.; Qi, B. Salt-induced amino acid metabolism enhances nutritional quality of alfalfa: Evidence from metabolomic profiling. *Plant Soil* **2025**, *517*, 1981–1998. [CrossRef]
58. Brito, A.F.; Tremblay, G.F.; Bertrand, A.; Castonguay, Y.; Bélanger, G.; Michaud, R.; Lapierre, H.; Benchaar, C.; Petit, H.V.; Ouellet, D.R.; et al. Alfalfa cut at sundown and harvested as baleage improves milk yield of late-lactation dairy cows. *J. Dairy Sci.* **2008**, *91*, 3968–3982. [CrossRef] [PubMed]
59. Cheng, G.; Huang, Z.; Zhang, J.; Zhao, X.; Zhang, Y. Forage-based cropping systems enhance protein water productivity, economic performance, and environmental outcomes in the North China Plain. *Agric. Water Manag.* **2026**, *333*, 110571. [CrossRef]
60. Bo, P.T.; Dong, Y.; Zhang, R.; Soe Htet, M.N.; Hai, J. Optimization of alfalfa-based mixed cropping with winter wheat and ryegrass in terms of forage yield and quality traits. *Plants* **2022**, *11*, 1752. [CrossRef] [PubMed]
61. Sahni, P.; Sharma, S.; Surasani, V.K.R. Influence of processing and pH on amino acid profile, morphology, electrophoretic pattern, bioactive potential and functional characteristics of alfalfa protein isolates. *Food Chem.* **2020**, *333*, 127503. [CrossRef] [PubMed]
62. Getahun, A.; Kechero, Y.; Yemane, N.; Dessie, T.; Esatu, W. Fruit and Vegetable Peels as Alternative Feed for Sustainable Poultry Production: A Solution for Small-Scale Farms in Central and Southern Ethiopia. *Vet. Med. Sci.* **2026**, *12*, e70791. [CrossRef] [PubMed]
63. Zhang, G. Terrace-Mediated Nutrient Redistribution Enhances Nitrogen Assimilation and Amino Acid Accumulation in Tea Plantations Across Hillslope Positions. *Horticulturae* **2026**, *12*, 843. [CrossRef]
64. Abebe, H.; Tu, Y. Impact of Salt and Alkali Stress on Forage Biomass Yield, Nutritive Value, and Animal Growth Performance: A Comprehensive Review. *Grasses* **2024**, *3*, 355–368. [CrossRef]

65. Poe, T.B.; Bai, Y.; Dong, Y.; Shi, H.; Htet, M.N.S.; Samoon, H.A.; Zhang, R.; Tanveer, S.K.; Hai, J. Influence of Different Harvesting Stages and Cereals–Legume Mixture on Forage Biomass Yield, Nutritional Compositions, and Quality under Loess Plateau Region. *Plants* **2022**, *11*, 2801. [CrossRef] [PubMed]
66. Shen, S.; Pan, L.; Li, J.; Wang, J.; Ahmad, I.; Liu, H.; Bai, Y.; Kang, B.; Yin, J.; Gao, Y.; et al. The Involvement of Amino Acid Metabolism in the Mechanisms of Salt Tolerance Adaptation in Medicago sativa and *Medicago truncatula*. *Plants* **2025**, *14*, 929. [CrossRef] [PubMed]
67. Liebhardt, P.; Maxa, J.; Bernhardt, H.; Aulrich, K.; Thurner, S. Comparison of a Conventional Harvesting Technique in Alfalfa and Red Clover with a Leaf Stripping Technique Regarding Dry Matter Yield, Total Leaf Mass, Leaf Portion, Crude Protein and Amino Acid Contents. *Agronomy* **2022**, *12*, 1408. [CrossRef]
68. Liu, L.; Bi, Y.; Fu, Z.; Liu, Z.; Zheng, Z.; Zhao, H.; Huang, Y. Mechanism of hardpan remolding furrowing for alfalfa based on a shear-resistant reversible bonding model. *Soil Tillage Res.* **2026**, *262*, 107206. [CrossRef]
69. Choudhary, M.; Jat, H.S.; Prajapat, K.; Mukhopadhyay, R.; Krupnik, T.J.; Gathala, M.K. Soil biological quality and microbial functional diversity under diversified conservation agriculture systems in the Indo-Gangetic Plain. *J. Agric. Food Res.* **2026**, *27*, 102833. [CrossRef]
70. Liu, B.; Zhang, J.; Wang, D.; Zhang, X.; Gao, P. Exploration and Practice of Geological Drilling Technology in the Field of Oil and Gas. *Chem. Technol. Fuels Oils* **2025**, *60*, 1468–1474. [CrossRef]
71. Yavuz, S.R.; Alshaal, T.; Suhartini, W.; Bákonyi, N.; Kovács, Z.; Kaszás, L.; Veres, S.; Fári, M.G.; Elhawat, N.; Domokos-Szabolcsy, É. Leaf protein concentrates as a nutrient-dense protein source from vetch and triticale mixture: A comparative analysis of fermentation techniques. *Food Chem. X* **2025**, *27*, 103153. [CrossRef] [PubMed]
72. Nissen, S.H.; Lübeck, M.; Møller, A.H.; Dalsgaard, T.K. Protein recovery and quality of alfalfa extracts obtained by acid precipitation and fermentation. *Bioresour. Technol. Rep.* **2022**, *19*, 101190. [CrossRef]
73. Camli-Saunders, D.; Villouta, C. Root exudates in controlled environment agriculture: Composition, function, and future directions. *Front. Plant Sci.* **2025**, *16*, 1567707. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.