BMC Plant Biology

RESEARCH Open Access

Assessment of nutritional value, aerobic stability and measurement of in vitro fermentation parameters of silage prepared from several leguminous plants

ShiJia Yang[1,2], Kai Zhang[1,2], RuiXuan Ji[1,2], XueWen Chen[1,2], Jiao Wang[1,2], Imtiaz Hussain Raja[3], AnShanShan[4] and SuJiang Zhang[1,2*]

Abstract

The livestock sector plays a pivotal role in rural socio-economic development, particularly in arid regions. Making silage is one method for storing feed during the dry season, while leguminous species serve as indispensable resources in livestock production systems due to their nutritional and ecological benefits. This study aimed to select suitable forage resources by evaluating several available leguminous species (*Arachis hypogaea* (peanut vines), *Sophora alopecuroides*, *Glycyrrhiza glabra* (liquorice), and *Medicago sativa* (Alfalfa)) in Alar, Xinjiang, China, and comprehensive analyses of fermentation quality, aerobic stability, and in vitro rumen fermentation were conducted to assess their nutritional value and relieve arid regions feed shortages. The fermentation analysis indicated that *Sophora alopecuroides* exhibited the optimal quality, showing significantly higher lactic acid and lower ammonia nitrogen (NH_3-N) concentrations than the other species ($P < 0.05$). Following a 12-day aerobic exposure, all the legume silages exhibited increased dry matter (DM), pH, and NH_3-N concentrations alongside a decreased water-soluble carbohydrates (WSC) content. Alfalfa and *Sophora alopecuroides* silages maintained significantly higher lactic acid bacteria populations than other varieties ($P < 0.05$). In vitro rumen fermentation revealed alfalfa silage achieved peak biogas production at 72 h, while liquorice silage showed significantly lower NH_3-N content than other groups ($P < 0.05$). Alfalfa and *Sophora alopecuroides* silages demonstrated superior gas production and DM degradation rates, indicating their enhanced fermentative characteristics. In summary, alfalfa and *sophora alopecuroides* could be of the suitable plants for making high-quality silage. However, additional research is needed to study the effect of the silages on animal growth performance.

Keywords Leguminous silage, Nutritional analysis, Fermentation quality, Number of microorganisms, *In vitro* degradation of DM

*Correspondence:
SuJiang Zhang
zsjdky@126.com
[1]Key Laboratory of Tarim Animal Husbandry Science and Technology, College of Animal Science and Technology, Tarim University, Alar, Xinjiang 843300, PR China
[2]Key Laboratory of Livestock and Grass Resources Utilization around Tarim, Ministry of Agriculture and Rural Areas (Co-construction by Ministries and Provinces), Tarim University, Alar, Xinjiang 843300, PR China
[3]Department of Animal Nutrition, (FAP&T), Cholistan University of Veterinary and Animal Sciences, Bahawalpur 63100, Pakistan
[4]Institute of Animal Nutrition, Northeast Agricultural University, Heilongjiang Harbin 150030, PR China

Introduction

The sustainable advancement of ruminant livestock systems in developing countries is significantly constrained by the rapid expansion of animal husbandry and the limited availability of high-quality forage, particularly in arid regions [1–2]. To address these challenges, the strategic selection and utilization of forage resources have become essential for enhancing feed production efficiency and economic viability. Notably, leguminous plants have garnered significant attention as promising forage resources for livestock production in arid regions, particularly due to their adaptability and nutritional value [3].

Within the leguminous family, *Arachis hypogaea* is an important cash crop in arid and semiarid regions, characterized by its high nutritional value, high crude protein content, high fiber quality, and good palatability [4]. However, most of the peanut vines are typically disposed of through field incineration or abandonment, which causes environmental pollution [5–6]. *Sophora alopecuroides* has emerged as a promising forage candidate due to its high crude protein content (18–22%), rapid growth rate, and exceptional tolerance to barren soils, wind erosion, and drought [7–9]. Recent studies have demonstrated its potential as a supplemental feed, with in vitro trials showing improved digestibility and favorable rumen fermentation characteristics [10], though further research is needed to optimize its inclusion levels in livestock diets. Liquorice (*Glycyrrhiza spp.*), a perennial legume species, demonstrates exceptional drought tolerance and cold resistance, while containing substantial concentrations of amino acids, essential minerals, and crude protein (12–16%) [11]. While historically employed in pharmaceutical and flavoring applications, liquorice has recently emerged as a dual-purpose crop, offering high-quality forage and bioactive compounds with potential antioxidant and immunomodulatory effects in livestock nutrition [12–14]. *Medicago sativa* (alfalfa), a globally distributed perennial legume, is characterized by high biomass yield, superior nutritional value, and ecological adaptability, establishing it as a premier forage crop with significant soil amelioration properties [15–16].

Forage preservation for livestock feeding is usually done by drying the plant material and storing it as hay or ensiling it into silage [17]. In contrast, ensiling effectively preserves the nutritional quality through anaerobic fermentation, while extending the storage stability [18–20]. Among the leguminous species, *Medicago sativa* (alfalfa) dominates global cultivation as a primary forage crop [21–22]. However, limited scientific attention has been given to the ensiling potential and nutritional evaluation of alternative leguminous resources, including peanut vines, *Sophora alopecuroides*, and the aerial parts of liquorice. The current study was designed to address the critical shortage of roughage resources in arid and semiarid regions through a systematic evaluation of underutilized leguminous species. Using the peanut vines, *Sophora alopecuroides*, and the aerial parts of liquorice as test materials, with alfalfa as a reference, the study aims to assess the nutritional composition, evaluate the fermentation characteristics, and determine the digestibility parameters of these alternative forages. The findings will help in the development of selection of suitable feed resources that will ultimately help to alleviate feed shortage in arid areas.

Materials and methods

Forage harvesting and silage making

The four leguminous species - *Arachis hypogaea* (Yuhua-37), the aerial parts of liquorice (*Glycyrrhiza uralensis*), *Sophora alopecuroides* (wild variety), and alfalfa (*Medicago sativa*) - were cultivated in Alar, Xinjiang (40°30′N, 81°15′E) and harvested at the full flowering stage to ensure an optimal nutritional composition for ensiling. The fresh biomass was mechanically chopped into 2.5 cm particles using a multi-function chopper [2]. Silage preparation was performed using an industrial ensiling machine (RH-IBJ-400, Qufu Tianliang Trading Co., Ltd.), with each polyethylene-wrapped bale standardized to 60 kg. The ensiled materials were stored for 60 days under controlled room temperature conditions to facilitate proper anaerobic fermentation [23].

Quality analysis of silage

The 500 g sample of each silage (prepared) and fresh forage were taken for quality analysis. The samples were dried at 65 ℃ in an oven and then ground through a 1 mm sieve using a mill (Christy and Norris Co. Ltd., Suffolk, UK), and analysed in triplicate for dry matter (DM), crude protein (CP), crude ash (Ash) and ether extract (EE) contents [24]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methods of Van Soest et al. [25]. Water-soluble carbohydrates (WSC) were determined by a spectrophotometer following the methods of Koehler [26]. Alkaloids (TAs) were determined using an alkaloid kit (Suzhou Keming Biotechnology Co., Ltd.) [23].

After 60 days of ensiling, the wrapped silages were opened, and a 500 g fresh weight sample was collected from each polyethylene bag for analysis. A 15 g fresh weight subsample was homogenized with 135 mL of distilled water and filtered through two layers of cheesecloth. The pH of the filtrate was measured using a calibrated pH meter (Hanna Instruments Italia Srl, Villafranca Padovana, Italy). Two 15 mL aliquots of the filtrate were centrifuged at 2500 rpm for 10 min at 4℃, and the supernatant was subjected to acid extraction for subsequent ammonia (NH_3) and organic acids analysis

[27]. The NH_3 concentration was determined using a Pentra 400 analyzer (Horiba Ltd., Kyoto, Japan) following the method of Rhine et al. [28], while lactic, acetic, and butyric acids were quantified by gas chromatography (GC, Shimadzu Ltd., Kyoto, Japan) as described by Fussell and McCalley [29].

Aerobic stability analysis

The aerobic stability, defined as the time (hours) until the silage temperature exceeds the ambient temperature by 2℃, was evaluated following the methodology of Nishino and Touno [30]. The silage samples (150 g) were aerobically exposed in 500 mL sterile bottles covered with sterile gauze. The temperature was continuously monitored using data loggers, while subsamples (20 g) collected at 0, 3, 6, 9, and 12 days were homogenized with 180 mL of Ringer's solution, filtered, and analyzed. The pH of the filtrate was measured was using a calibrated pH meter (Hanna Instruments), and the samples were assessed for dry matter (DM), water-soluble carbohydrates (WSC), and ammonia nitrogen (NH_3-N) content to evaluate the stability dynamics.

Microbial colony determination

During the aerobic exposure (0, 3, 6, 9, 12 days), 10 g silage samples were homogenized with 90 mL of sterile saline (0.85% NaCl) for microbial analysis. Serial dilutions were plated on selective media: Plate Count Agar for the aerobic bacteria (30 °C, 48 h), MRS agar for the lactic acid bacteria (37 °C, 72 h, anaerobic), and Potato Dextrose Agar for the yeast/mold quantification (28℃, 72 h). Microbial enumeration was performed using a colony counter (Scan 1200, Interscience) [31], enabling the precise monitoring of the microbial population dynamics throughout the aerobic exposure.

Table 1 Nutrient composition of fresh forage (before ensiling) of the four legumes (dry matter basis) %

Items	Peanut vines	*Sophora alopecuroides*	Liquorice	Alfalfa
DM	42.91 ± 0.11^{a}	41.19 ± 0.01^{ab}	32.10 ± 0.01^{b}	42.64 ± 0.02^{a}
CP	10.23 ± 0.05^{c}	18.07 ± 0.35^{a}	15.20 ± 0.69^{b}	17.33 ± 0.44^{a}
NDF	35.00 ± 0.25^{d}	51.03 ± 1.30^{c}	54.25 ± 0.95^{b}	56.14 ± 0.85^{a}
ADF	23.36 ± 0.27^{d}	30.34 ± 1.06^{c}	32.32 ± 0.49^{b}	35.39 ± 0.22^{a}
EE	1.85 ± 0.57^{d}	4.69 ± 0.28^{a}	2.37 ± 0.26^{c}	3.93 ± 0.56^{b}
Ash	15.69 ± 0.20^{a}	5.71 ± 0.14^{d}	6.90 ± 0.05^{c}	9.28 ± 0.25^{b}
WSC	5.78 ± 0.02^{c}	6.06 ± 0.07^{b}	7.51 ± 0.16^{a}	4.67 ± 0.14^{d}
TA	0.79 ± 0.73	1.64 ± 0.77	1.18 ± 0.14	1.57 ± 0.19

Note DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, EE: ether extract, Ash: crude ash, WSC: water soluble-carbohydrates, TA: total alkaloids. In the same row, values with no letter or the same letter superscripts are not significantly different ($P > 0.05$), whereas those with different small letter superscripts are significantly different ($P < 0.05$)

Measurement of in vitro fermentation parameters

A sample of 0.22 g was weighed into a 100 mL glass syringe, and rumen buffer was prepared according to the method of Menke et al. [32]. Before morning feeding, the ruminal fluid obtained from three sheep fitted with a fistula was immediately taken to the laboratory, filtered through four layers of gauze, kept at 39 °C in a water bath and blended with buffer solution (1:2, v/v) under a continuous flush of CO_2, and several drops of 0.1% resazurin solution were added. The samples were added into each glass syringe with 30 mL of mixed solution. After the mixture was added, it was placed in an artificial rumen incubator in a 39 °C water bath. The amount of gas generated after 0, 1, 2, 3, 4, 6, 8,10, 12, 24, 36, 48 and 72 h was recorded, and the gas production (GPt) was calculated as $Pt = (Pt - Pt_0)/W$.

GPt: samples in t time of gas production ($mL \cdot g^{-1}$ DM); Pt: Volume read in time period t (mL); Pt_0: Volume of blank sample read in time period t (mL); W: weight of sample used in fermentation.

After 72 h of In Vitro fermentation, the pH of the fermentation broth was measured with a pH meter and placed in ice water to terminate the fermentation. The ammoniacal nitrogen (NH_3-N) concentration determined by method of Chaney et al. [33]. The In Vitro dry matter degradation (IVDMD) was calculated by the difference of the DM content before and after fermentation [22].

Statistical analysis

Statistical analysis was conducted using one-way analysis of variance (ANOVA) with SPSS software (version 26.0). The resultant data were shown as means ± standard errors ($n = 3$), and Duncan's multiple test was applied to detect differences between means($P < 0.05$). Nonlinear regression in SPSS software was adopted for the calculation of degradation parameters a, b and c.

Results

Nutritional characteristics of fresh and silage

The chemical compositions of the different legumes are presented in Table 1. The dry matter (DM) content of the peanut vines and alfalfa was significantly higher ($P < 0.05$) than that of the aerial parts of liquorice. The crude protein (CP) contents of the peanut vines were significantly lower ($P < 0.05$) than that of the other legumes. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of alfalfa were significantly higher ($P < 0.05$) than those of the other samples. The ether extract (EE) contents of the peanut vines were significantly lower ($P < 0.05$) than that of the others, while its ash content was significantly higher ($P < 0.05$). The water-soluble carbohydrate (WSC) content of the aerial parts of liquorice was significantly higher ($P < 0.05$) than that of the other

legumes, whereas no significant differences ($P > 0.05$) were observed in the total ash (TA) content among the four leguminous species.

The nutrient contents of the different legume silages are shown in Table 2. The DM content of the liquorice silage was significantly lower ($P < 0.05$) than that of the other silages. The CP content of the *Sophora alopecuroides* silage was significantly higher ($P < 0.05$) than that of the other samples, with no significant difference ($P > 0.05$) observed between the aerial parts of liquorice silage and the alfalfa silage. The NDF and ADF contents of the alfalfa silage were significantly higher ($P < 0.05$) than those of the other silages. The EE content of the *Sophora alopecuroides* silage was significantly higher ($P < 0.05$) than that of the others. Compared to the other silages, the ash and WSC contents of the peanut vines silage were significantly higher ($P < 0.05$), while its TA content was significantly lower ($P < 0.05$). These results demonstrate the distinct chemical and nutritional profiles among the legumes and their silages.

Table 2 Nutritional components of silage of four leguminous plants (DM) %

Items	Peanut vines	*Sophora alopecuroides*	Liquorice	Alfalfa
DM	38.95 ± 0.02^{a}	35.28 ± 0.03^{ab}	31.08 ± 0.01^{c}	37.92 ± 0.01^{ab}
CP	10.48 ± 0.05^{c}	17.94 ± 0.52^{a}	16.94 ± 0.03^{b}	16.89 ± 0.24^{b}
NDF	26.46 ± 1.78^{c}	43.37 ± 1.05^{b}	41.32 ± 0.75^{b}	49.59 ± 0.64^{a}
ADF	14.25 ± 0.92^{c}	26.24 ± 0.91^{b}	24.62 ± 0.94^{b}	33.63 ± 0.92^{a}
EE	1.27 ± 0.04^{d}	4.08 ± 0.54^{a}	2.18 ± 0.20^{c}	3.71 ± 0.31^{b}
Ash	14.92 ± 0.79^{a}	6.61 ± 0.04^{d}	7.77 ± 0.03^{c}	11.58 ± 0.04^{b}
WSC	3.90 ± 0.02^{a}	2.68 ± 0.30^{c}	3.41 ± 0.12^{b}	3.39 ± 0.27^{b}
TA	0.34 ± 0.03^{b}	1.06 ± 0.15^{a}	1.08 ± 0.51^{a}	0.94 ± 0.15^{a}

Note DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, EE: ether extract, Ash: crude ash, WSC: water soluble-carbohydrates, TA: total alkaloids. In the same row, values with no letter or the same letter superscripts are not significantly different ($P > 0.05$), whereas those with different small letter superscripts are significantly different ($P < 0.05$)

Table 3 Silage fermentation prepared from four legumes (DM) %

Items	Peanut vines	*Sophora alopecuroides*	Liquorice	Alfalfa
pH	4.66 ± 0.06^{c}	4.69 ± 0.08^{c}	5.30 ± 0.06^{a}	4.92 ± 0.02^{b}
LA	1.16 ± 0.02^{c}	3.81 ± 0.04^{a}	1.02 ± 0.09^{d}	2.85 ± 0.07^{b}
AA	2.19 ± 0.11^{a}	0.71 ± 0.06^{c}	2.24 ± 0.08^{a}	1.56 ± 0.09^{b}
BA	0.04 ± 0.01^{a}	ND	ND	ND
NH_3-N / TN	16.52 ± 1.23^{a}	8.56 ± 0.27^{d}	13.66 ± 0.96^{b}	10.87 ± 0.45^{c}

Note LA: lactic acid, AA: acetic acid, BA: butyric acid, NH_3-N: ammonia nitrogen. ND indicates that no data are detected. In the same row, values with no letter or the same letter superscripts are not significantly different ($P > 0.05$), whereas those with different small letter superscripts are significantly different ($P < 0.05$)

Fermentation quality of the legume silages

The fermentation qualities of the different legume silages are presented in Table 3. The pH of the peanut vines silage was significantly lower than that of the aerial parts of liquorice silage and the alfalfa silage ($P < 0.05$). The lactic acid (LA) content of the *Sophora alopecuroides* silage was significantly higher than that of the other silages ($P < 0.05$), while the acetic acid (AA) content of the aerial parts of liquorice silage was significantly greater than that of the *Sophora alopecuroides* silage and the alfalfa silage ($P < 0.05$). Additionally, the NH_3-N content of the *Sophora alopecuroides* silage was significantly lower than that of the other three legume silages ($P < 0.05$). These results highlight the distinct fermentation characteristics among the silages.

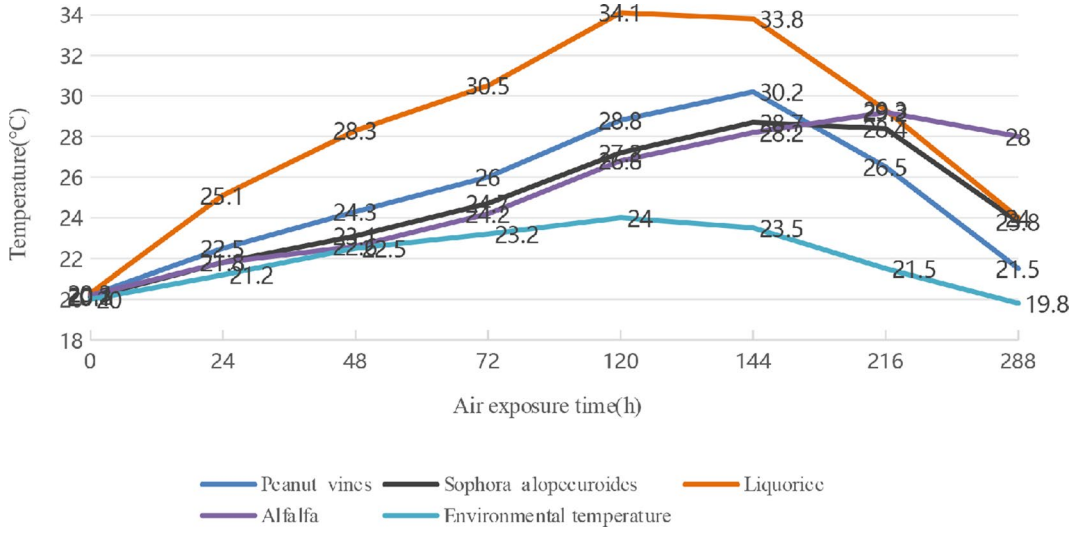

Fig. 1 Changes in Temperature during 12-day aerobic exposure(°C)

Table 4 Changes in DM, pH, WSC, and NH_3-N during 12-day aerobic exposure

Time(d)	Peanut vines	*Sophora alopecuroides*	Liquorice	Alfalfa
DM(%)				
0	38.95 ± 0.02^{a}	35.28 ± 0.03^{ab}	31.08 ± 0.01^{c}	37.92 ± 0.01^{ab}
3	45.93 ± 0.01^{a}	44.47 ± 0.02^{a}	35.80 ± 0.03^{c}	40.53 ± 0.02^{b}
6	46.99 ± 0.03^{a}	44.64 ± 0.03^{ab}	38.82 ± 0.03^{b}	42.60 ± 0.03^{ab}
9	50.40 ± 0.03^{a}	44.74 ± 0.04^{ab}	37.91 ± 0.02^{c}	42.42 ± 0.02^{bc}
12	50.74 ± 0.02^{a}	46.00 ± 0.05^{ab}	39.82 ± 0.05^{b}	44.14 ± 0.02^{ab}
pH				
0	4.66 ± 0.06^{c}	4.69 ± 0.08^{c}	5.30 ± 0.06^{a}	4.92 ± 0.02^{b}
3	4.86 ± 0.01^{d}	4.89 ± 0.01^{c}	5.39 ± 0.01^{a}	5.36 ± 0.01^{b}
6	4.88 ± 0.02^{d}	4.93 ± 0.02^{c}	5.88 ± 0.01^{a}	5.42 ± 0.04^{b}
9	4.84 ± 0.01^{d}	4.96 ± 0.01^{c}	6.07 ± 0.01^{a}	5.40 ± 0.05^{b}
12	4.88 ± 0.01^{c}	5.39 ± 0.07^{b}	6.20 ± 0.02^{a}	5.41 ± 0.04^{b}
WSC(%)				
0	3.90 ± 0.02^{a}	2.68 ± 0.30^{c}	3.14 ± 0.12^{b}	3.39 ± 0.27^{b}
3	3.69 ± 0.09^{a}	2.49 ± 0.02^{b}	2.78 ± 0.68^{b}	2.64 ± 0.28^{b}
6	3.49 ± 0.11^{a}	2.32 ± 0.58^{b}	2.69 ± 0.09^{b}	2.64 ± 0.08^{b}
9	3.37 ± 0.10^{a}	2.15 ± 0.15^{c}	2.73 ± 0.06^{b}	2.33 ± 0.06^{c}
12	3.22 ± 0.14^{a}	2.05 ± 0.06^{c}	2.39 ± 0.20^{b}	2.25 ± 0.04^{bc}
NH_3-N(%)				
0	3.15 ± 0.13^{b}	2.65 ± 0.06^{c}	3.80 ± 0.16^{a}	2.77 ± 0.17^{c}
3	3.36 ± 0.07^{b}	2.70 ± 0.03^{c}	3.83 ± 0.19^{a}	3.45 ± 0.01^{b}
6	3.39 ± 0.12^{a}	2.75 ± 0.06^{b}	3.91 ± 0.30^{a}	3.56 ± 0.47^{a}
9	3.63 ± 0.02^{b}	2.82 ± 0.21^{c}	4.32 ± 0.06^{a}	3.65 ± 0.24^{b}
12	4.29 ± 0.07^{ab}	2.91 ± 0.09^{c}	4.71 ± 0.82^{a}	3.80 ± 0.06^{b}

Note DM: dry matter, WSC: water-soluble carbohydrate, NH_3-N: ammonia nitrogen. In the same row, values with no letter or the same letter superscripts are not significantly different ($P > 0.05$), whereas those with different small letter superscripts are significantly different ($P < 0.05$)

Table 5 Changes in the number of microorganisms during aerobic exposure(lg /g FM)

Time(d)	peanut vines	*Sophora alopecuroides*	Liquorice	Alfalfa
Lactic acid bacteria				
0	5.03 ± 0.04^{b}	5.56 ± 0.05^{a}	4.73 ± 0.01^{c}	5.50 ± 0.01^{a}
3	4.21 ± 0.20^{d}	5.02 ± 0.04^{b}	4.50 ± 0.01^{c}	5.44 ± 0.01^{a}
6	3.98 ± 0.08^{c}	4.36 ± 0.35^{b}	4.08 ± 0.07^{bc}	5.33 ± 0.07^{a}
9	3.63 ± 0.20^{c}	4.26 ± 0.19^{b}	3.91 ± 0.11^{c}	5.00 ± 0.02^{a}
12	3.60 ± 0.07^{b}	4.06 ± 0.11^{ab}	3.70 ± 0.06^{b}	4.50 ± 0.59^{a}
Yeast				
0	3.04 ± 0.12	2.87 ± 0.25	3.08 ± 0.07	3.09 ± 0.03
3	3.16 ± 0.17^{b}	3.00 ± 0.03^{b}	3.43 ± 0.07^{a}	3.44 ± 0.07^{a}
6	3.21 ± 0.09^{d}	3.51 ± 0.05^{c}	3.96 ± 0.09^{a}	3.67 ± 0.05^{b}
9	3.44 ± 0.07^{c}	3.64 ± 0.05^{b}	4.26 ± 0.02^{a}	3.71 ± 0.15^{b}
12	3.88 ± 0.19^{b}	3.75 ± 0.17^{b}	4.42 ± 0.04^{a}	4.47 ± 0.05^{a}
Mold				
0	<2	<2	<2	<2
3	<2	<2	2.77 ± 0.11^{a}	<2
6	2.91 ± 0.07^{b}	2.14 ± 0.04^{c}	3.45 ± 0.03^{a}	2.20 ± 0.11^{c}
9	2.96 ± 0.03^{b}	2.35 ± 0.05^{d}	3.79 ± 0.05^{a}	2.66 ± 0.30^{c}
12	3.63 ± 0.08^{a}	2.63 ± 0.40^{c}	3.93 ± 0.07^{a}	3.21 ± 0.13^{b}
Aerobic bacteria				
0	2.86 ± 0.11^{c}	2.98 ± 0.18^{bc}	3.11 ± 0.08^{ab}	3.26 ± 0.08^{a}
3	3.05 ± 0.47^{b}	3.24 ± 0.01^{ab}	3.71 ± 0.08^{a}	3.37 ± 0.02^{ab}
6	3.37 ± 0.08^{c}	3.67 ± 0.04^{b}	3.87 ± 0.07^{a}	3.61 ± 0.10^{b}
9	3.41 ± 0.11^{c}	3.77 ± 0.15^{b}	4.65 ± 0.15^{a}	3.94 ± 0.06^{b}
12	3.83 ± 0.01^{c}	4.29 ± 0.03^{b}	4.84 ± 0.05^{a}	4.59 ± 0.34^{ab}

Note In the same row, values with no letter or the same letter superscripts are not significantly different ($P > 0.05$), whereas those with different small letter superscripts are significantly different ($P < 0.05$)

Aerobic stability of the legume silages

The changes in temperature, dry matter (DM), pH, water-soluble carbohydrates (WSC), and ammonia nitrogen (NH_3-N) during the aerobic exposure of the different legume silages are presented in Fig. 1; Table 4. In terms of temperature changes, the temperature of the silages was basically the same at 0 d. However, under aerobic environment, the temperature of silage continued to increase during 24 h and 72 h. The temperatures of peanut vines silage and liquorice silage were significantly greater than those of the others ($P < 0.05$), the onset of spoilage was observed at 72 h for liquorice and 120 h for peanut vines, liquorice silage underwent complete aerobic deterioration within 96 h, while *Sophora alopecuroides* and Alfalfa exhibited significantly delayed thermal peaks (144 h and 216 h), respectively, compared to liquorice, and Alfalfa maintained the longest stability (> 216 h). At 9 and 12 d, silage temperature decreased in all groups. By day 12, all silages maintained elevated center temperatures basically exceeding environmental temperatures ($P < 0.05$). The pH values of all four legume silages increased significantly with prolonged aerobic exposure time. At 0 d, the pH values, ranked from low to high, were as follows: the peanut vines silage, the *Sophora alopecuroides* silage, the alfalfa silage, and the aerial parts of liquorice silage. The pH of the peanut vines silage was significantly lower than that of the aerial parts of liquorice silage and the alfalfa silage ($P < 0.05$). By 12 d, the pH of the liquorice silage was significantly higher than that of the other three legume silages ($P < 0.05$).

The WSC content of the four legume silages exhibited a negative correlation with the duration of aerobic exposure. Throughout the exposure period, the WSC content of the peanut vines silage was significantly higher than that of the other three silages at all time points ($P < 0.05$). At 0 d, the NH_3-N content of the aerial parts of liquorice silage was significantly higher than that of the other silages ($P < 0.05$). Subsequently, the NH_3-N content of all four legume silages showed an increasing trend. By 12 d, the NH_3-N content reached its highest value, with the *Sophora alopecuroides* silage recording significantly lower NH_3-N content compared to the other silages ($P < 0.05$).

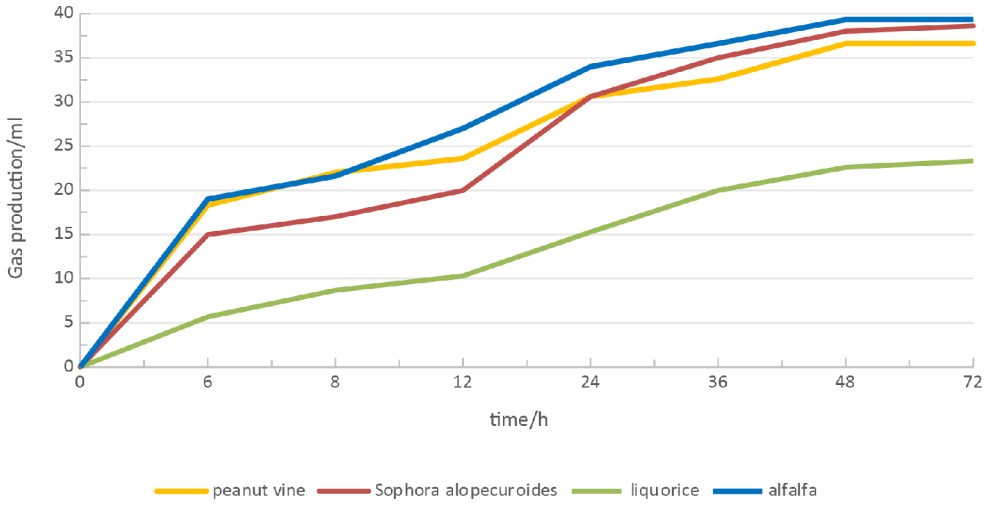

Fig. 2 In vitro gas production (mL·g^{-1} DM) of silages prepared from four legumes for 72 h

Table 6 Fermentation parameters and gas production parameters of four legume silages

Items	Peanut vines	*Sophora alopecuroides*	Liquorice	Alfalfa
a(mL)	4.75 ± 0.06^{a}	4.37 ± 0.24^{ab}	1.30 ± 0.64^{c}	3.71 ± 0.77^{b}
b(mL)	30.55 ± 1.26^{b}	35.83 ± 1.49^{a}	23.85 ± 3.45^{c}	34.88 ± 1.98^{a}
a + b(mL)	35.30 ± 1.27^{b}	40.20 ± 1.26^{a}	25.14 ± 2.82^{c}	38.59 ± 2.73^{ab}
c(mL/h)	0.10 ± 0.01^{a}	0.06 ± 0.01^{b}	0.04 ± 0.01^{b}	0.10 ± 0.02^{a}
pH	6.78 ± 0.08	6.82 ± 0.07	6.91 ± 0.01	6.89 ± 0.10
NH_3-N(mg/dL)	52.00 ± 1.25^{a}	54.55 ± 0.38^{a}	47.85 ± 2.07^{b}	53.24 ± 3.12^{a}

Note a: the fast gas production fraction, b: slow gas production section, a + b: potential gas production, c: the gas production rate constant, NH_3-N: ammonia nitrogen. In the same row, values with no letter or the same letter superscripts are not significantly different ($P > 0.05$), whereas those with different small letter superscripts are significantly different ($P < 0.05$)

Microbial population of the legume silages

As shown in Table 5, the lactic acid bacteria (LAB) counts in the alfalfa silage were significantly higher than those in the other silages at 12 d ($P < 0.05$). No molds were detected in any of the legume silages at 0 d, and the yeast counts showed no significant differences at this time point ($P > 0.05$). At 3 d, the yeast counts in the alfalfa silage were significantly higher than those in the peanut vines silage and the *Sophora alopecuroides* silage ($P < 0.05$), while the yeast counts in the aerial parts of liquorice silage were also significantly higher than those in the peanut vines silage and the *Sophora alopecuroides* silage ($P < 0.05$). Between 6 and 9 d, the yeast and mold counts in the aerial parts of liquorice silage were significantly higher than those in the other silages ($P < 0.05$). By 12 d, the yeast and mold counts in the *Sophora alopecuroides* silage were significantly lower than those in the liquorice silage and the alfalfa silage ($P < 0.05$). The aerobic bacterial counts increased progressively over time in all silages; however, at 12 d, the aerobic bacterial counts in the peanut vines silage were significantly lower than those in the other silages ($P < 0.05$). These results demonstrate the distinct microbial dynamics among the legume silages during the aerobic exposure.

In vitro gas production and kinetic parameters of the legume silages

As shown in Fig. 2, the gas production in all groups increased with the fermentation time. A rapid increase was observed from 0 to 24 h in all silages except the liquorice silage. After 24 h, the gas production curve gradually plateaued, with the majority of gas production occurring within 0–36 h for all four legume silages. Notably, the gas production of the aerial parts of liquorice silage was consistently lower than that of the other three legume silages at all time points, indicating the distinct fermentation characteristics.

As shown in Table 6, the fast gas production fraction (a) was significantly higher ($P < 0.05$) for the peanut vines silage compared to the liquorice silage and the alfalfa silage. The slow gas production fraction (b) and the potential gas production (a + b) were significantly higher ($P < 0.05$) for the *Sophora alopecuroides* silage than for the peanut vines silage and the liquorice silage, with no significant difference ($P > 0.05$) observed compared to the alfalfa silage. The gas production rate constants (c) were significantly higher ($P < 0.05$) for the peanut vines silage and the alfalfa silage than for the *Sophora alopecuroides* silage and the liquorice silage. After 72 h of in vitro fermentation, the pH values of the artificial rumen cultures for all four legume silages exceeded 7, with no significant differences ($P > 0.05$) among them. However, the NH_3-N content of the liquorice silage was significantly lower ($P < 0.05$) than that of the other silages, reflecting the distinct fermentation kinetics and end-product profiles.

Table 7 DM degradation rates of silage prepared from four legumes

Time(d)	Peanut vines	*Sophora alopecuroides*	Liquorice	Alfalfa
4	21.44 ± 0.52^{b}	18.86 ± 0.88^{c}	15.82 ± 1.59^{d}	25.79 ± 0.81^{a}
8	22.31 ± 2.38^{b}	19.57 ± 1.22^{bc}	18.28 ± 1.42^{c}	28.27 ± 1.14^{a}
12	23.99 ± 0.83^{b}	20.70 ± 2.21^{c}	19.52 ± 1.78^{c}	32.12 ± 1.67^{a}
24	32.17 ± 0.82^{a}	24.04 ± 1.53^{b}	20.59 ± 1.92^{c}	35.15 ± 1.91^{a}
36	33.46 ± 1.92^{b}	29.71 ± 0.67^{c}	23.12 ± 0.85^{d}	40.94 ± 1.28^{a}
48	37.39 ± 1.54^{b}	37.00 ± 2.64^{b}	26.51 ± 2.91^{c}	43.47 ± 1.29^{a}
72	38.32 ± 1.76^{b}	37.74 ± 0.92^{b}	28.67 ± 1.54^{c}	44.80 ± 0.73^{a}

Note In the same row, values with no letter or the same letter superscripts are not significantly different ($P > 0.05$), whereas those with different small letter superscripts are significantly different ($P < 0.05$)

As shown in Table 7, the ruminal degradation rates of DM for the different legume silages increased over time. From 4 to 12 h, the DM degradation rate of the alfalfa silage was consistently significantly higher than that of the other silages ($P < 0.05$). By 24 h, the DM degradation rates of the peanut vines silage and the alfalfa silage reached comparable levels ($P > 0.05$) and were significantly higher than those of the other silages ($P < 0.05$). At 36 h, the DM degradation rate of the liquorice silage was significantly lower than that of the other silages ($P < 0.05$). However, from 48 to 72 h, the DM degradation rate of the alfalfa silage was again significantly higher than that of the other silages ($P < 0.05$), highlighting its superior degradability over extended fermentation periods.

Discussion

Nutritional characterization and fermentation quality analysis of silage feeding

The dry matter (DM) content of the studied legumes ranged from 32 to 43% pre-ensiling, satisfying the optimal silage conditions, with post-ensiling values maintaining 31–39% DM content. This minimal DM loss (≈ 3–4%) during the ensiling process aligns with previous findings by Zhang [34], suggesting the effective preservation of the original forage material. The crude protein (CP) content remained stable throughout the ensiling process, with values exceeding 15% for most species, except the peanut vines (≈ 10%). This stability in CP content, consistent with Li's observations [35], underscores the potential of these legumes as high-quality protein sources in ruminant diets.

The reduction in the NDF and ADF contents post-ensiling ($P < 0.05$) suggests enhanced digestibility, likely resulting from the degradation of plant cell walls by organic acids during fermentation. This finding is particularly significant as it indicates improved feed efficiency, given the established negative correlation between ADF content and ruminant digestibility [36–37]. The observed decrease in the ether extract (EE) content, consistent with Fabrício et al.'s findings [38], may be attributed to the microbial conversion of lipids into volatile fatty acids during fermentation.

The scientific community has established that the optimal pH range for high-quality silage preservation falls between 3.7 and 4.2 [39], which promotes proper fermentation and ensures maximal nutrient preservation. The pH values of silage from the four leguminous species increased after ensiling, primarily due to their high protein and mineral content, as well as low water-soluble carbohydrate (WSC) levels. These characteristics endow leguminous plants with strong buffering capacity, thereby resulting in relatively higher pH values in their silage [40]. Fermentation quality indicators revealed distinct patterns among the silages. While only the *Sophora alopecuroides* silage achieved the optimal NH_3-N/TN threshold (< 10%), all silages demonstrated acceptable fermentation characteristics. The elevated CP content post-ensiling, despite higher NH_3-N/TN ratios in some species, aligns with previous research [39], suggesting that while protein degradation occurs, net protein preservation remains favorable. The higher lactic and acetic acid content in the peanut vines silage may reflect its unique chemical composition and microbial dynamics during fermentation.

Aerobic stability and microbial dynamics

When silage is exposed to air during the opening of a silo, or after its removal, fermentation acids and other substrates undergo oxidation due to the activity of yeasts, aerobic bacteria and molds [30, 41]. Therefore, aerobic stability is crucial in silage. Aerobic stability, a crucial parameter governing silage conservation efficiency and agricultural economics, was defined as the number of hours until silo temperature increased 2 °C above the baseline silo temperature [30]. Under aerobic conditions, silage temperatures continued to increase over the 24 h and 72 h period, the onset of spoilage was observed at 72 h for liquorice and 120 h for Peanut vines. Liquorice silage underwent complete aerobic deterioration within 96 h, characterized by sustained temperature elevation and pH exceeding 6.0, consistent with accelerated microbial proliferation under aerobic conditions [42], while *Sophora alopecuroides* and Alfalfa exhibited significantly delayed thermal peaks (144 h and 216 h), respectively, compared to liquorice, indicating liquorice silage exhibited the poorest aerobic stability with its elevated WSC concentration identified as the predisposing factor for accelerated oxidative deterioration through microbial respiratory dominance [46]. The initial rapid increase in the DM content, attributed to moisture evaporation [43], was followed by characteristic changes in microbial populations and chemical composition. In this study, the DM content increased initially during aerobic exposure due to moisture evaporation [44]. The pH elevation and the WSC depletion during aerobic exposure, consistent

with the established literature [45–46], reflect the metabolic activity of aerobic microorganisms. Notably, the *Sophora alopecuroides* silage exhibited slower pH elevation, potentially due to its lower residual sugar content and distinct microbial community structure. The decline in the lactic acid bacteria (LAB) populations, concurrent with the proliferation of aerobic microorganisms [44], reflects the competitive microbial ecology of exposed silage. The slower deterioration rate observed in the *Sophora alopecuroides* silage may be attributed to its lower WSC content, which limited the growth of spoilage microorganisms. This finding aligns with previous research identifying yeasts as the primary agents of aerobic deterioration in silage [47].

Gas production and gas production parameters in vitro rumen fermentation

The in vitro gas production reflects the nutritional value of feed and the rumen microbial metabolism [48–50]. In this study, the gas production increased over time, with the alfalfa silage exhibiting the highest cumulative gas production, indicating efficient utilization of fermentable carbohydrates [51–53]. The gas production parameters, including the fast and slow gas production fractions, provide insights into the feed fermentation kinetics [54]. The positive fast gas production fraction in all silages indicated no lag phase, while the higher slow gas production fraction in the *Sophora alopecuroides* and alfalfa silages suggested effective utilization of insoluble components like cellulose and hemicellulose [55–56].

The rumen fluid pH, ranging from 5.5 to 7.5, remained stable across all treatments, indicating no adverse effects on the rumen homeostasis [57]. The NH_3-N, a byproduct of protein degradation, ranged from 47 to 54 mg/dL, higher than the optimal range of 3.3–8.0 mg/dL for microbial growth [58–60]. This discrepancy may result from the high crude protein content of legumes, leading to NH_3-N accumulation [61]. The DM degradation rate, reflecting the feed digestibility, increased with the fermentation time, with the alfalfa silage exhibiting the highest rate (44.80%) at 72 h [62]. Combined with its high cumulative gas production, the alfalfa silage demonstrated superior nutritional value and digestibility for ruminants [63].

Conclusions

The results of this study demonstrated significant variation in silage quality among the tested leguminous species. *Sophora alopecuroides* silage exhibited optimal fermentation characteristics, while *Glycyrrhiza glabra* (liquorice) silage showed comparatively inferior fermentation quality. During aerobic exposure, *Arachis hypogaea* (peanut vines) and *Sophora alopecuroides* silages maintained better stability, as evidenced by minimal pH elevation and restricted proliferation of yeasts, molds, and aerobic bacteria. In vitro rumen fermentation analysis revealed superior gas production and dry matter degradation rates in *Medicago sativa* (alfalfa) and *Sophora alopecuroides* silages. Based on comprehensive evaluation of fermentation quality, aerobic stability, and rumen degradability, *Medicago sativa* and *Sophora alopecuroides* were identified as the most suitable species for high-quality silage production under the experimental conditions. Further research is warranted to investigate the effects of these silages on animal growth performance and production efficiency.

Acknowledgements
The authors are grateful to Tarim University for the necessary facilities and assistance to carry out this research work.

Author contributions
ShiJia Yang: Conceptualization, Methodology, Data curation, Formal analysis, Writing- original draft, Investigation, Validation. Kai Zhang: Writing- original draft, Investigation, Validation, Visualization. RuiXuan Ji: Data curation, Investigation, Validation. XueWen Chen and Jiao Wang: Formal analysis, Investigation, Validation, Visualization. AnShan and Imtiaz Hussain Raja: Writing- review & editing, Supervision. SuJiang Zhang: Writing- review & editing, Conceptualization, Methodology, Funding acquisition. All authors read and approved the final manuscript.

Funding
Not applicable.

Data availability
All relevant data can be found in the manuscript, and that all figures and illustrations are original and were created by the authors based on the research data. Therefore, no additional permissions or credits are needed for images.

Declarations

Ethics approval and consent to participate
Not applicable. Our study does not involve any experiments on live vertebrates or higher invertebrates. Therefore, ethical approval or consistency statements related to animal experiments are not applicable. Our research does not involve human participants, so informed consent statements are not required.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 6 February 2025 / Accepted: 2 May 2025
Published online: 15 May 2025

References

1. Cremilleux M, Coppa M, Bouchon M, Delaby L, Beaure G, Constant I, Natalello A, Martin B, Michaud A. Effects of forage quantity and access-time restriction on feeding behaviour, feed efficiency, nutritional status, and dairy performance of dairy cows fed indoors. Animal. 2022;16(9):100608–100608.
2. Wang J, Cheng L, Chaudhry AS, Khanaki H, Abbasi IHR, Ma Y, Abbasi F, Guo X, Zhang S. Silage mixtures of alfalfa with sweet Sorghum alter blood and rumen physiological status and rumen microbiota of Karakul lambs. Animals. 2022;12(19):2591.
3. Hartley M, Fyfe CL, Wareham NJ, Khaw KT, Johnstone AM, Myint PK. Association between legume consumption and risk of hypertension in the European

prospective investigation into Cancer and nutrition (EPIC)-Norfolk cohort. Nutrients. 2022;14(16):3363–3363.
4. Li Y, Zhang TL, Wang XX, Hua K, Zhao L, Han ZM. The composition of root exudates from two different resistant peanut cultivars and their effects on the growth of Soil-Borne pathogen. Int J Biol Sci. 2013;9(2):164–73.
5. Massarioli AP, Sartori AGO, Juliano FF, Santos RCD, Ramos JPC, Lima LM, Alencar SM. Optimizing procedures for antioxidant phenolics extraction from skin and kernel of peanuts with contrasting levels of drought tolerance. Foods. 2022;11(3):449.
6. Li L, Cheng X, Kong X, Jia P, Wang X, Zhang L, Zhang X, Zhang Y, Zhang Z, Zhang B. Comparative transcriptomic analysis reveals the negative response mechanism of peanut root morphology and nitrate assimilation to nitrogen deficiency. Plants. 2023;12(4):732.
7. Wei J, Yang H, Cao H, Tan T. Using polyaspartic acid hydro-gel as water retaining agent and its effect on plants under drought stress. Saudi J Biol Sci. 2016;23(5):654–9.
8. Zhu Y, Wang Q, Gao Z, Wang Y, Liu Y, Ma Z, Chen Y, Zhang Y, Yan F, Li J. Analysis of phytohormone signal transduction in Sophora alopecuroides under salt stress. Int J Mol Sci. 2021;22(14):7313–7313.
9. Rong ZJ, HuG S, Lin S, Yan T, Li N, Zhao Y, Jia JM, Wang AH. Constituents from the seeds of Sophora alopecuroides L. Molecules. 2020;25(2):411–411.
10. An YH, Wang H, Gao A, Li SZ, Yang J, Li B, Lü H. Effects of Sophora alopecuroides in a High-Concentrate diet on the liver immunity and antioxidant function of lambs according to transcriptome analysis. Animals. 2024;14(2):182–182.
11. Nargish Marjhan. Review on medicinal properties of Glycyrrhiza glabra Linn. (Liquorice). Int J Unani Integr Med. 2024;8(2):17–23.
12. Singh OP, Gupta P, Rawat A. A review on Pharmacological activities and traditional uses of Glycyrrhiza glabra (licorice). J Pharmacognosy Phytochemistry. 2024;13(2):772–5.
13. Varada Arun NV, Abhiram A, Anil, Baby Shajini Y, Deepika Krishnan, Bhadra S, Dev PR, Akhil KG, Athulya KG, Anil S, Ganesh P, Singh K, Ravikumar. Sandip Chakraborty, Hitesh Chopra, Shopnil Akash, Ruhul Amin, abhijit Dey, anil Kumar Sharma, Mahmoud Alagawany, Kuldeep Dhama, Deepak Chandran. Potential benefits of Glycyrrhiza glabra (Liquorice) herb, its chemical make-up and significance in safeguarding poultry health: current scientific knowledge. J Experimental Biology Agricultural Sci. 2023;11(3):462–78.
14. Dang H, Zhang T, Wang Z, Li G, Zhao W, Lv X, Zhuang L. Succession of endophytic fungi and arbuscular mycorrhizal fungi associated with the growth of plant and their correlation with secondary metabolites in the roots of plants. BMC Plant Biol. 2021;21(1):165.
15. Li JS, Wang RF, Zhang MJ, Wang Q, Yu YZ, Sun XB, Xu DH, et al. A method for estimating alfalfa (Medicago sativa L.) forage yield based on remote sensing data. Agronomy. 2023;13(10):2597–2597.
16. Wang FY, Wei YG, Fan JT, Jin M, Wei XB, Wang QJ. Effects of subsequent rainfall events with different intensities on runoff and erosion in a coarse soil. CATENA. 2018;170:100–7.
17. Dalibor Tomić V, Stevović, Dragan Đurović, Miloš MARJANOVIĆ, Milomirka Madić N, Pavlović Đ, Lazarevic. Mirjana Petrović, Mirjana Radovanović. Perennial forage legumes as an element of sustainable systems. Notulae Botanicae Horti Agrobotanici Cluj-napoca. 2023;51(3):13240–13240.
18. Salim Barbhuiya P, Basheer MW, Clark GIB, Rankin. Penny Frost. An investigation into the behaviour of concrete containing seawater-neutralised bauxite refinery residues in silage effluent. Biosyst Eng. 2010;106(4):433–9.
19. Cai Y, Benno Y, Ogawa M, Ohmomo S, Kumai S. Influence of Lactobacillus spp. From an inoculant and of Weissella and Leuconostoc spp. From forage crops on silage fermentation. Appl Environ Microbiol. 1998;64(8):2982–7.
20. Lynn M, Wendt BD, Wahlen C, Li G, Kachurin KL, Ogden J, Austin, Murphy. Evaluation of a high-moisture stabilization strategy for harvested microalgae blended with herbaceous biomass: part I—Storage performance. Algal Res. 2017;25:567–75.
21. Roberta Rossi,Alessio Pollice,Giovanni Bitella,Rosanna Labella,Rocco Bochicchio,Mariana Amato. Modelling the non-linear relationship between soil resistivity and alfalfa NDVI: A basis for management zone delineation. J Appl Geophys. 2018;159:146–56.
22. Appiah EA, Kovács AB, Ocwa A. József Csajbók, Erika Kutasy. Enhancing alfalfa (Medicago sativa L.) productivity: exploring the significance of potassium nutrition. Agronomy. 2024;14(8):1806–1806.
23. Zhang SJ, Chaudhry AS, Osman A, Shi CQ, Edwards GR, Dewhurst RJ, Cheng L. Associative effects of ensiling mixtures of sweet sorghum and alfalfa on nutritive value, fermentation and methane characteristics. Anim Feed Sci Technol. 2015;206(0):29–38.
24. AOAC. Official Methods of Analysis of AOAC International, 18th Edn. Gaithersburg, MD: AOAC International. (1995).
25. Van Soest PJ, Robertson JB. Lewis. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991;74(10):3583–97.
26. Leonore H. Koehler. Differentiation of carbohydrates by anthrone reaction rate and color intensity. Anal Chem. 1952;24(10):1576–9.
27. Chaudhry AS, Khan M. Impacts of different spices on in vitro rumen dry matter disappearance, fermentation and methane of wheat or ryegrass hay based substrates. Livest Sci. 2012;146(1):84–90.
28. Rhine ED, Mulvaney RL, Pratt E. Sims. Improving the Berthelot reaction for determining ammonium in soil extracts and water. Soil Sci Soc Am J. 1998;62(2):473–80.
29. Richard J, Fussell DV, McCalley. Determination of volatile fatty acids (C2—C5) and lactic acid in silage by gas chromatography. Analyst. 1987;112(9):1213–6.
30. Nishino N. Ensiling characteristics and aerobic stability of direct-cut and wilted grass silages inoculated with Lactobacillus casei or Lactobacillus buchneri. J Sci Food Agric. 2005;85(11):1882–8.
31. Shi YW, Zhang WG, Zhou GH. Effects of different Moisture-Permeable packaging on the quality of aging beef compared with wet aging and dry aging. Foods. 2020;9(5):649–649.
32. Menke KH, Raab L, Salewski A, Steingaß H, Fritz D, Schneider W. The Estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. J Agricultural Sci. 1979;93(1):217–22.
33. Chaney AL, Marbach EP. Modified reagents for determination of Urea and ammonia. Clin Chem. 1962;8:130–2.
34. Zhang Y, Tao X, Liu Q, Zhang YJ, Xu J, Zhang W, Wang J, Zhang D, Li B, Wang L, Cheng J, Zhang YQ. Succession changes of fermentation parameters, nutrient components and bacterial community of sorghum stalk silage. Front Microbiol. 2022;13:982489.
35. Li X, Xu W, Yang J, Zhao H, Pan C, Ding X, Zhang Y. Effects of applying lactic acid bacteria to the fermentation on a mixture of corn steep liquor and air-dried rice straw. Anim Nutr. 2016;2(3):229–33.
36. Wang Z, Wang W, Xu S, Ding J, Zeng X, Liu H, Wang F. Diets enriched with finely ground wheat Bran alter digesta passage rate and composition of the gut Microbiome in sows. Anim Nutr. 2023;12:32–41.
37. Fan W, Ge G, Liu Y, Wang W, Liu L, Jia Y. Proteomics integrated with metabolomics: analysis of the internal causes of nutrient changes in alfalfa at different growth stages. BMC Plant Biol. 2018;18(1):78.
38. Fabrício A, Magalhães. Sebastião de Campos Valadares Filho, Gustavo Chamon de Castro Menezes, Marcelo grossi Machado, Diego Zanetti, Douglas Dos Santos Pina, Odilon gomes Pereira, Mário Fonseca Paulino. Chemical composition and fermentative losses of sugar cane ensilage with different Brix degrees, with or without calcium oxide. Revista Brasileira De Zootecnia. 2012;41(2):256–63.
39. Wang C, He L, Xing Y, Zhou W, Yang F, Chen X, Zhang Q. Effects of mixing Neolamarckia cadamba leaves on fermentation quality, microbial community of high moisture alfalfa and Stylo silage. Microb Biotechnol. 2019;12(5):869–78.
40. Luciana Maria da Silva. Kátia aparecida de Pinho Costa, Laís Guerra Prado, Alexandre Garcia Rezende, Eduardo Da Costa Severiano, João victor Campos Pinho Costa, João Antônio Gonçalves e Silva, Adriano Carvalho Costa, Patrick bezerra Fernandes, Rosane Cláudia Rodrigues, Bruno de Souza Marques, Breno furquim de Morais, Fabrício Á. Rodrigues. Maize intercropped with Panicum maximum cultivars and pigeon pea improves silage yield and quality. Front Sustainable Food Syst. 2024;8:1416717.
41. Ma J, Xue F, Ma Z, Huang XB, Tang MS, Yin F, Zhao ZJ, Gan SQ. Silage additives improve fermentation quality, aerobic stability and rumen degradation in mixed silage composed of Amaranth and corn straw. Front Plant Sci. 2023;14:1189747.
42. Tahir M, Wang T, Zhang J et al. Compound lactic acid bacteria enhance the aerobic stability of Sesbania cannabina and corn mixed silage. BMC Microbiol. 2025, 68.
43. Wang Y, Zhou W, Wang C, Yang F, Chen X, Zhang Q. Effect on the ensilage performance and microbial community of adding Neolamarckia cadamba leaves to corn stalks. Microb Biotechnol. 2020;13(5):1502–14.
44. Le Cocq K, Brown B, Hodgson CJ, McFadzean J, Horrocks CA, Lee MRF, Davies DR. Application of monoclonal antibodies in quantifying fungal growth dynamics during aerobic spoilage of silage. Microb Biotechnol. 2020;13(4):1054–65.

45. Wang T, Zhang J, Shi W, Sun J, Xia T, Huang F, Liu Y, Li H, Teng K, Zhong J. Dynamic changes in fermentation quality and structure and function of the Microbiome during mixed silage of Sesbania cannabina and sweet Sorghum grown on Saline-Alkaline land. Microbiol Spectr. 2022;10(5):e0248322.
46. Li X, Xu W, Yang J, Zhao H, Xin H, Zhang Y. Effect of different levels of corn steep liquor addition on fermentation characteristics and aerobic stability of fresh rice straw silage. Anim Nutr. 2016;2(4):345–50.
47. Zhang M, Wang L, Wu G, et al. Effects of Lactobacillus plantarum on the fermentation profile and Microbiological composition of wheat fermented silage under the freezing and thawing low temperatures. Front Microbiol. 2021;12:671287.
48. Ma Y, Chen X, Khan MZ, Xiao J, Cao Z. A combination of novel microecological agents and molasses role in digestibility and fermentation of rice straw by facilitating the ruminal microbial colonization. Front Microbiol. 2022;13:948049.
49. Wang Y, Nan XM, Zhao YG, Jiang LS, Wang H, Hua DK, Zhang F, Wang YP, Li J, Yao JH, Xiong BH. Dietary supplementation with inulin improves lactation performance and serum lipids by regulating the rumen Microbiome and metabolome in dairy cows. Anim Nutr. 2021;7(4):1189–204.
50. Song HT, Yang YM, Liu DK, Xu XQ, Xiao WJ, Liu ZL, Xia WC, Wang CY, Yu X, Jiang ZB. Construction of Recombinant Yarrowia lipolytica and its application in bio-transformation of lignocellulose. Bioengineered. 2017;8(5):624–9.
51. Shen Y, Abeynayake R, Sun X, Ran T, Li J, Chen L, Yang W. Feed nutritional value of brewers' spent grain residue resulting from protease aided protein removal. J Anim Sci Biotechnol. 2019;10:78.
52. Blu¨mmel M, Ørskov ER. Comparison of in vitro gas production and nylon bag degradability of roughages in predicting feed intake in cattle. Anim Feed Sci Technol. 1993;40(2–3):109–19.
53. You L, Bao W, Yao C, Zhao F, Jin H, Huang W, Li B, Kwok LY, Liu W. Changes in chemical composition, structural and functional Microbiome during alfalfa (Medicago sativa) ensilage with Lactobacillus plantarum PS-8. Anim Nutr. 2022;9:100–9.
54. Tan H, Yan W, Ren ZY, Wang QJ, Mohamed A. Distributionally robust operation for integrated rural energy systems with broiler houses. Energy. 2022;254(0):124398–124398.
55. López Secundino, Dhanoa MS, Dijkstra J, Bannink A, Kebreab E. Some methodological and analytical considerations regarding application of the gas production technique. Anim Feed Sci Technol. 2007;135(1–2):139–56.
56. Zhu Y, Xiong H, Wen Z, Tian H, Chen Y, Wu L, Guo Y, Sun B. Effects of different concentrations of Lactobacillus plantarum and Bacillus licheniformis on silage quality, In vitro fermentation and microbial community of hybrid Pennisetum. Animals. 2022;12(14):1752.
57. An X, Zhang L, Luo J, Zhao S, Jiao T. Effects of oat hay content in diets on nutrient metabolism and the rumen microflora in sheep. Animals. 2020;10(12):2341–2341.
58. Mahdi Mahmoudiabyane D, Alipour H, Moghimi. Effects of different sources of nitrogen on performance, relative population of rumen microorganisms, ruminal fermentation and blood parameters in male feedlotting lambs. Animal. 2020;14(7):1438–46.
59. Cui J, Lu Z, Wang T, Chen G, Mostafa S, Ren H, Liu S, Fu C, Wang L, Zhu Y, Lu J, Chen X, Wei Z, Jin B. The genome of Medicago polymorpha provides insights into its edibility and nutritional value as a vegetable and forage legume. Hortic Res. 2021;8(1):47.
60. H. Hoover W. Chemical factors involved in ruminal Fiber digestion. J Dairy Sci. 1986;69(10):2755–66.
61. Bollati C, Xu R, Boschin G,,Bartolomei M, Rivardo F, Li J, Arnoldi A, Lammi C. Integrated evaluation of the multifunctional DPP-IV and ACE inhibitory effect of soybean and pea protein hydrolysates. Nutrients. 2022;14(12):2379–2379.
62. Luo C, Wang D, Lu N, Li H, Liu G, Cao Z, Yang H, Li S, Yu X, Shao W, Wang W. Analysis of chemical composition, amino acid content, and rumen degradation characteristics of six organic feeds. Animals. 2022;12(6):682–682.
63. Wang C, He LW, Xing YQ, Zhou W, Fang Y, Chen XY, Zhang Q. Effects of mixing Neolamarckia cadamba leaves on fermentation quality, microbial community of high moisture alfalfa and Stylo silage. Microb Biotechnol. 2019;12(5):869–78.

Publisher's note