2024 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 14, Issue 3: 171-184; May 25, 2024

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2024.21

NUTRITIONAL AND CHEMICAL COMPOSITION OF SAINFOIN (*Onobrychis viciifolia*) ACCESSIONS IN MID-ALTITUDE OF SODDO AND ABESHGIE WOREDAS OF ETHIOPIA

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Supporting Information

RESEARCH ARTICLE
PII: S222877012400021-14
Received: November 07, 2023
Revised: April 27, 2024
Accepted: May 10, 2024

ABSTRACT: Sainfoin (*Onobrychis viciifolia*) is a perennial herbaceous leguminous forage crop with a high content of crude protein, palatability, nutritive value and non-bloating. It can be offered in the form of green forage, grazing pasture, hay, or silage. To mitigate the dry season feed shortage, the agronomic performance and chemical composition of sainfoin (*Onobrychis viciifolia*) accessions were studied in the mid-altitude of Soddo and Abeshgie Woredas of Garage Zone of Ethiopia. For the screening, five International Livestock Research Institute (ILRI) accessions (No 5708, 16006, 6582, 10558, and 10556) and four wild sainfoin accessions were collected from Worabe, Albazer, Meskan Dubo Tuto and Gibie river basin with morphological variation. They were subjected to initial screening followed by field trials for a period of fifteen months per session. Among the twenty-nine agronomic parameters (for screening purpose), based on the dendrogram results, fifteen data points were used for location wise (Buee and Tatesa) evaluation purposes. The results indicated that, with the exception of leaf length, leaf width, and annual seed yield, all parameters were affected by the accession by location interaction. The highest (P<0.001) cumulative dry matter, seed yield, and crude protein (CP%) contents were recorded for Worabe sainfoin, ILRI 5708 and ILRI 16006, respectively. Lower (P<0.05) condensed tannin (CT) was recorded in ILRI 5708, Worabe's and Albazer's sainfoin without any significant difference. During the first 24-hour incubation period, the highest and least (P<0.001) gases were produced from ILRI 16006 and Albazer, and ILRI 5708, respectively. The highest and least (P<0.001) methane gas was recorded from ILRI 16006 and ILRI 5708 respectively. The results indicated that Worabe sainfoin was superior to the result in all evaluated parameters. So, Worabe sainfoin hay mixture with crop residues can be used to enhance the nutritional value of crop residue-based poor feed resources.

Keywords: Agronomic performance, Alternative feedstuffs, Crude protein, Dry matter yield, Sainfoin accessions.

INTRODUCTION

In the crop-livestock mixed farming system of Ethiopia, livestock are subjected to crop residue over a long period of the year due to seasonal variation and diminished grazing land. Crop residues are poor in feed value to support production and reproduction processes (Mekuria and Mekonnen, 2018; Mekonnen et al., 2022). During the dry period, multipurpose perennial legume species enhance long term forage production and provide positive environmental effects such as improvement of soil fertility, reduction of erosion and control of nitrate leaching (McCallum et al., 2004). In the past, the introduction of improved forage plants comprised the Ethiopian forage development strategies. However, this option did not bring the anticipated change because of the exotic forage species failed under the local environment and inappropriate technical practices (Mekoya et al., 2008).

In Ethiopia, some indigenous legumes and browse species proved their availability and nutritive value at a time when feed is scarce or is of low quality (Salem et al., 2006). Unfortunately, most of the preferred and high quality indigenous legume species are under pressure (Chettri et al., 2009). Sainfoin is one of such indigenous legume species in the mid-altitudes of Ethiopia, specifically, around Soddo Woreda. Sainfoin is a perennial forage and fodder legume, tolerant to varying climatic conditions and poor soil fertility, with high nitrogen fixing capacity.

It represents a valuable pollen and nectar source for honey production. Although sainfoin is considered an important forage legume with high hay and pasture production potential (Bhattarai et al., 2018), information on agro-morphological and nutritional traits was lacking for Ethiopia. Hayot et al. (2011) summarized the sainfoin taxonomy, biology, and agronomy mainly from European perspective but not inclusive in Equatorial as well as Sub Saharan region. Sainfoin is distributed in northern temperate regions of the world and more specifically in the eastern Mediterranean and Western Asia with an emphasis on Iran and Turkey (Yildiz et al., 1999; Irani et al., 2016) but did not indicate the Ethiopian potential for sainfoin. Therefore, the objective of this research was to evaluate sainfoin accessions using adaptability,

Citation: Abebe DG, Cherkos SD, Ejeta TT, Dejene M, Shignato TK and Geletu AS (2024). Nutritional and chemical composition of sainfoin (*Onobrychis viciifolia*) accessions in mid-altitude of Soddo and Abeshgie Woredas of Ethiopia. Online *J. Anim. Feed Res*., 14(3): 171-184. DOI: https://dx.doi.org/10.51227/ojafr.2024.21

biomass yield, seed production and nutrient composition in the mid-altitude of Soddo and Abeshgie Woredas of Garage Zone.

MATERIALS AND METHODS

Description of the study area

The initial establishment of sainfoin accessions was undertaken in Soddo Woreda of Guragie Zone while the agronomic performance of selected accessions was carried out both in Soddo and Abeshgie Woredas of Guragie Zone. The Soddo experimental location is found within the geographical location of 8.31⁰ 98' to 8.31⁰ 99 'N and 37.95⁰ 31' to 37.95⁰ 62 'E while Abeshgie Woreda is found within 8.31⁰ 59 '-8.31⁰ 62 'N and 37.95⁰ 31 '-37.95⁰ 62 'E. Soddo has a mean annual temperature ranging from 10 to 25°C and receives between 801-1200mm rain falls. Soddo Woreda is located at 103km south of Addis Ababa, while Abeshgie is 150km from Addis Ababa in south-west direction. Abeshgie Woreda has an altitude range from 1500-2900m above sea level, with an annual mean temperature in the range of 13°C-25°C and a mean annual rainfall in the range of 1000–1500 mm (Gugissa et al., 2022).

Screening sainfoin accessions

Five accessions from ILRI (International Livestock Research Institute) and four indigenous wild accessions with morphological variations were used as seed sources. Land size of 25m × 25m from black soil color was demarcated at Buee Construction and Industrial Collage compound. The land was ploughed and loosened manually. Each of the 5 ILRI accessions without replication and each of the 4 wild accessions on five plots (1 plot per block) was sawn on prepared 1m² (0.5m×2m) plot of land with a border side of 12. 5 cm (8.5m × 14m net demarcated plot size). During the wet season, on each plot, two parallel lines with a distance of 25cm were drawn for seed drilling. The space between blocks and plots was 1m and 0.5m respectively, as Alemayehu and Aklilu (2007). Seeds were dehulled and drilled at a depth of 2cm (Liu, 2006). The scholars' recommendations (Hume and Withers 1985; Shah et al., 1991 and Tufenkci et al., 2006) were applied for the conversion of available fertilizer into P_2O_5, N and K. To get the recommended types, 198.9kg ha^{-1} NPSB and 121 kg ha^{-1} KCl for initial and 212kg ha^{-1} NPSB and 161kg ha^{-1} KCl for regrowth were used. Plots were arranged using an Augmented Design.

Data collection for screening

Survival count was done on the 4th, 8th and 12th weeks after sowing, plant vigor, disease conditions, soil cover data, initial flowering date, date of 50% flowering, and plant height were recorded. Tiller number data until the first harvest, number of leaves per plant total stem, stem length, leaflet number, leaflet width, leaflet length, and the ratio of leaflet length to leaflet width data were collected. The leaf area was calculated using the formula used by Bianco et al. (2011).

$$LA = 0.691156 \times LWct + 0.3652754 \times LWlt \quad (1)$$

Leaf area = The central leaflet length x width (LWct) + 0.3652754 x [(left lateral leaflets length + right lateral leaflets length) x width].

Before reaching the 75% flowering stage, the method of Tarawali (1995) was applied for partitioning ILRI accessions in drilled lines. When the plants were at 75% flowering stage as Goplen et al. (1991), all plants from randomly assigned partitions were cut at a height of 10-15 cm and taken as standard cut (Tarawali, 1995). According to the Tarawali (1995) method, mowed partitions were again divided into two equal portions for minimum precipitation season yield evaluation. Before harvest, at 7th and 12th weeks of the regrowth during minimum precipitation season evaluation, soil cover, % of green leaf, pest incidence severity status data were recorded again. On randomly half of the above partitions, at 7th weeks, regrowth harvest was carried out as Bhattarai et al. (2018). Then after the 7th week's harvest, at 5th week, both the regrowth harvest and reserved halve were harvested independently, and these two were taken as the minimum precipitation season yield evaluation. The samples in every harvest season and week were weighted and sun - dried. Until reaching maximum precipitation season, data on diseases and coverage %, and vigor were repeatedly recorded. For maximum precipitation season evaluation, all randomly partitioned portions for biomass estimation including the border side were independently harvested just before maximum precipitation commenced. After border side demarcation (excluding the seed source part), all partitioned lines of the biomass estimation were divided equally into four for ILRI accessions and 2 parts for wild accessions. All four and the two partitions for ILRI and wild accessions, respectively from a single line were randomly assigned for 3rd and 15th, 6th and 15th, 9th and 15th and 12th and 15th week's herbage regrowth harvest. All the seasonal cuts were measured, sun-dried bulked, and analyzed for DM yield. Based on the suggestion of (Carleton et al., 1968), within every 4 days gap, the seed was collected. The biomass and seed yields from partitioned lines were extrapolated in to plot size and then in to hectares. As Tarawali (1995) suggestion, on the basis of the first year result, four accessions that set seeds with better biomass yield were transferred to agronomic field experimentation.

Land preparation and sowing methods for experimental establishment

Based on herbage mass and seed yield results, ILRI 5708, ILRI 16006, and wild sainfoin collected from Worabe and Albazer were selected for experimental field trial. Soil type, seed type, rate of fertilizer applications, sowing depth, and spacing between plots and blocks were similar to that of the initial establishment work. Demarcated experimental land was ploughed repeatedly at both sites. An individual plot size of $6m^2$ (2m×3m) with a border side of 25 cm was used for seeding with seed rate (Cupina and Erid, 1999). Plots were prepared in 9 rows. On prepared rows, dehulled seeds were drilled and fertilizer was applied in the screening phase. Sowing season was carried out based on available soil moisture season (Ethiopian moisture availability condition June-July). Each accession was randomly replicated on 6 plots. The 4 accessions were assigned to the plots in RCBD design with 6 replications.

Experimental establishment data collection

Biomass data was collected from randomly selected either left or right sides of each plot on 3 near sides' central rows of each plot. The first herbage harvest was carried out on the 12^{th} week after sowing, when the average flowering stage reached 50-75%. This harvest was taken as Standard Cut. After the Standard Cut, the two - times regrowth herbage harvest was undertaken at 50-75% flowering stage. These herbage harvests were taken as the minimum precipitation season evaluation. Before entering the maximum precipitation season, Standard Cut was carried out on those 3 rows and then the three - times regrowth harvest at 50-75% flowering stage. Ripe seeds were collected from the left side of each plot on 3 near side rows. These 3 rows either for biomass and /or seed samples were extrapolated into hectares.

Sample preparation

Representative wet samples were taken during each cut period and season. Fresh samples were weighted, sun-dried, pooled and subsampled. The samples were sent to Hawassa College of Agriculture, Jimma University College of Agriculture and Veterinary Medicine and Holeta Agricultural Research Centre for the determination of chemical composition, digestibility, Phenolic, *in vitro* gas and methane production.

Chemical analysis

Coarsely chopped sward samples were dried in an oven for about 16 hours at 105^oC. Oven-dried samples were ground to pass through a sieve, a size of 1mm. Dry matter, total ash, ether extract, and crude proteins were determined as AOAC (2005). Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were determined using the method of Van Soest et al. (1991). The N content was determined by the Kjeldahl method and the CP content was calculated as N*6.25. The *in vitro* digestibility was determined by Tilley and Terry (1963) and applied as the modification of Van Soest and Robertson (1985).

Phenolic and tannin determination

The sample was dried at $50\text{-}55^0C$ for 48 hours. Determinations of total phenols, total tannins (TT) and condensed tannins (CT) were done as Makkar (2003). Butanol–HCl–iron, as Porter et al. (1985) was used for tannin determination. Hydrolysable tannins (HT) were estimated as the difference between TT and CT. Total phenols and tannins were expressed as tannic acid equivalent. The CT was expressed as leucocyanidin equivalent as Makkar (2003) and Porter et al. (1985)

CT = (Absorbance at 550 nm × 78.26 × Dilution factor)/(%DM) (2)

This formula assumed the effective $E^{\%(\text{ leucocyanidin equivalent}),\ 1\text{ cm},\ 550\text{ nm}}$ of leucocyanidin at 460 (Porter et al., 1985). Phenolic and tannin determination was carried out in JUCAVM Post Harvest laboratory.

Methane production

Methane production was measured separately from duplicate bottles incubated for each sample at 24 hours. The procedure of Santoso et al. (2007) was applied for the conversion of CH_4 gas volume to energy and mass values by using the conversion factors of 9.45 kcal/l and 0.716 g/l respectively. The methane gas production analysis was carried out in Hawassa College of Agriculture Animal Nutrition Laboratory.

Relative feed value (RFV) of sainfoin

The Relative Feed Value (RFV) combines the estimates for forage digestibility and intake into a single number, and it was calculated from the estimation of ADF and NDF (Ward and Ondarza, 2008). The RFV index was estimated using the digestible dry matter (DDM) of the samples from ADF values and was calculated the dry matter intake (DMI) potential (as a percent of body weight, BW) from NDF values. The index was then calculated as Digestible Dry Matter (DDM) multiplied by Dry Matter Intake (DMI) as a % of body weight(BW) divided by 1.29 (Jeranyama and Garcia, 2004).

Digestible Dry matter (DDM) = 88.9 - (0.779x% ADF) (3)

DMI (% of BW) = 120/ (% NDF) (4)

Relative feeding value (RFV) = (DDM x DMI)/1.29 (5)

Statistical analysis

Analysis of variances were used to test the statistical significance of the treatments. Using the general linear model procedure of the SAS program version 9.3 (SAS, 2010) with a 5% probability with experimental model:

Yijk = μ + Li + αj+ βk +Lβik + eijk,

Where: Yijk= An observation; μ = was the overall mean Li= is the ith treatment locations (the 2 locations), i = 1Buee, 2 Tatesa; αj is the jth block effect (the 6 blocks), j= 1…6; βk = is the kth treatments(selected sainfoin accessions) , k= 1…..4 ; Lβik = The Lth site (i=2locations) and the βth treatment (k= 4 accessions)interaction; eijk = was the random error.

RESULTS AND DISCUSSION

Classical separation of agronomic parameters

Among the 29 parameters, the 15 parameters (cover percent, infection level, plant height, mean tiller number, leaf length, leaf width, leaf area, regrowth score, fresh biomass weight, leaf and stem percent, air dried weight, air dried %, annual dry matter yield and annual seed yield) were taken for Dendrogram distance separation. The separation was conducted using principal component Analysis (SAS, 2010). Those first fifteen dependent components were found near 1.0 Eigenvalues, which encompassed 62%. Using these eighteen parameters, the four accessions were evaluated.

From the report of Thomson (1951), the seed height and width of the studied sainfoin' are in the range of 2.5–4.5 and 2.0-3.5 mm respectively. With the exception of 5708's, the values of the seed height for three accessions nearly agreed with their record. Minor variation is attributed to weather conditions and the seed collection period. *In* the study of Thomson (1951) 15.3g of milled and 21.4g of un milled sainfoin were recorded for 1000 seed weights.

Accessions' leaf and seed morphology

Accession 5708's leaflets were arranged separately, but those of 16006 were arranged laterally in 9 pairs, with a single pinnate leaflet at the tip (Figure 1). Similarly, Worabe and Albazer accessions had similar lateral arrangements in 13 paired leaflets with a single pinnate leaflet at the tip point. The entire accessions bear creamy to grey seed pods. The seed pod of 5708, Worabe's, and Albazer's were pinnate (spiny at endpoints) but that of 16006 was rough and resembled tortilis Keratein crust. Pod of 5708's hold pair seed per pod but that of 16006's was single seeded indehiscent. Seed pods of Worabe and Albazer were different from those of 5708's and 16006's. Both bear two-strand pods which stuck together at mid-rib until dry and bear seeds independently. The single lateral pod holds 3–5 seeds; and in a total, 6–10 seeds per pair pod. Seed pod length for 5708, 16006, and Worabe and Albazer was ranged from 33-34, 5-6, and 25-27mm respectively. Seed lengths of 5708, 16006, Worabe and Albazer were 6-7, 3-3.1, 2-2.3, and 2.9-3 mm respectively. Similarly, the seed width of 5708, 16006 and Worabe and Albazer were 5-5.7, 1.9–2 and 2-2.1 mm respectively. A Thousand seed weights for 5708, 16006, Worabe, and Albazer were 81, 16.9, 1.7, and 1.9 g respectively. Attacking weevils (Coleoptera: Curculionidae) fed sainfoin foliage and pods were assessed as pod fluid sucker. In the present study, seed weight of, 16006 is similar to the report of Thomson (1951) and Hayot et al. (2011) for milled giant and common types. Dis agreement with the other accessions were attributed to population, accession, harvesting stage and season and soil nutrient variability. The nature of sainfoin in seed setting is as single-seeded indehiscent with brown colored pods with olive to brown or black color observation of Goplen et al. (1991) is agreed with present study result for 16006 accession pod and seed color.

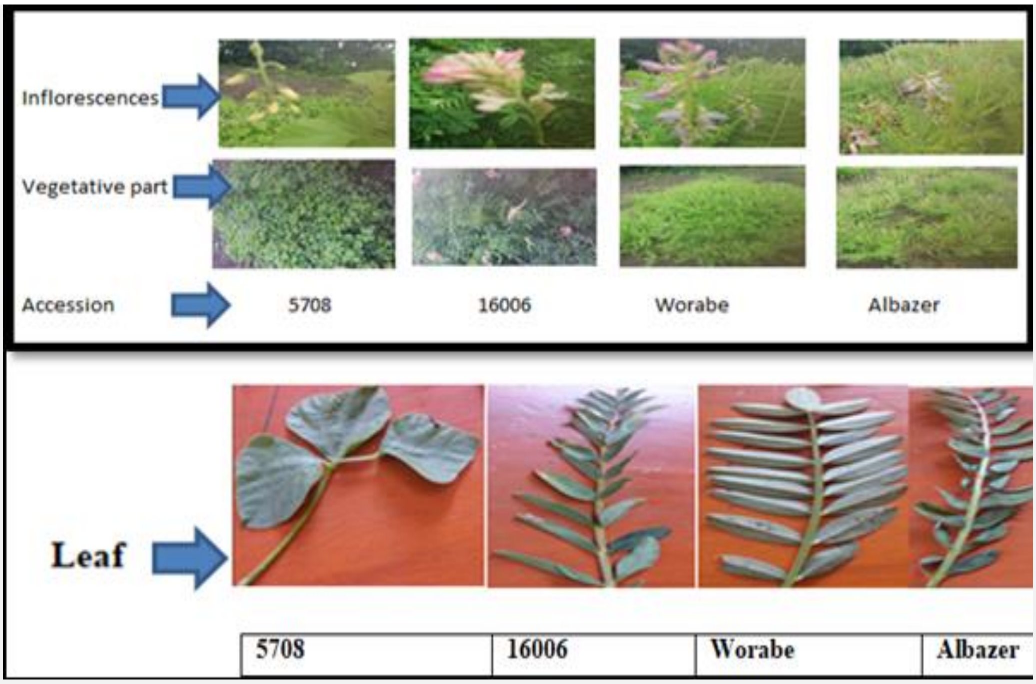

Figure 1 - Above ground biological part of selected accessions

Agronomic efficiency during screening phase

Measured plant phenology and yields during screening are presented in Tables 1 and 2. Germination failure was encountered for Meskan Dubo Tuto (MDDT), Gibie, ILRI 10558 and ILRI 10556 accessions. There were significant differences (P<0.001) in mean Phenological parts of the accessions. The mean disease tolerant score was higher for 16006, Worabe and Albazer without significant difference (P>0.05) but lower for 5708 accessions. Worabe was the tallest and 6582 was the shortest. Accession 16006 recorded the highest tiller-forming ability. Except for the accession 6582, a similar regrowth score was recorded (P>0.05) for the 4 accessions, with Worabe being the best compared to the others. As indicated in Table 2, significantly (P<0.0001) higher mean fresh herbage yield per square meter was recorded for Worabe followed by that of Albazer accession. The highest leaf percent was recorded for 6582, but the least was for Worabe and Albazer without showing a significant difference (P>0.05). The highest stem percent was recorded for Albazer but the least was for 16006. Air-dried weight per square meter was lower for Albazer and 6582 while all the others scored higher and/or comparable dried weight. Significantly (P<0.0001) higher annual DM (t ha^{-1}) yield was recorded for Worabe followed by 16006 accession. Relatively higher annual seed yield (kg ha^{-1}) was recorded for ILRI 5708 accession followed by Worabe, while seed setting ability failed in 6582 accession. Accession by block interaction was not significant (P>0.05) in all observed parameters. The effect of geographic location and moisture effect can cause differences among populations. The percentage of flowering plants in the sowing year and the speed of regrowth after the spring cut can help for differentiation of the two sainfoin types (giant/common) (Delgado et al., 2008). The results of the current study indicated that five accessions were able to flower in the sowing year, and the four accessions showed regrowth potential after successive harvest in dry and wet seasons. These characteristics led them to be giant type.

Agronomic efficiency of selected sainfoin accessions at two sites

Mean recorded accessions values are presented in Table 3 and Table 4. Tiller forming potential and leaf area records were found similar. Significantly higher (P<0.0001) mean fresh herbage mass per square meter was recorded for Worabe while the least was reported from ILRI accession 5708 (Table 4). Lower leaf and stem proportion was recorded for ILRI accession 16006. All the others found comparable in mean leaf stem percentage. The highest annual DM yield (t $h^{-1}y^{-1}$) and annual seed yield (kg $h^{-1}y^{-1}$) were obtained from Worabe whereas; all the other accessions were comparable in mean annual DM yield. The lowest mean annual seed yield was collected from ILRI accession 16006. In location wise, except for air-dried stem percentage, the Buee site was superior to all the others.

Table 1 - Agronomic performance of Sainfoin accessions during the screening phase.

Accession	Mean accession values							
	X1	X2	X3	X4	X5	X6	X7	
5708	40.74^{bc}	7.5^{b}	20.4^{c}	11.2^{e}	1.3^{a}	2.36^{a}	9.5^{a}	7.4^{b}
16006	56.7^{a}	9.0^{a}	15.2^{d}	40.2^{a}	1.3^{d}	0.8^{b}	1.93^{b}	8.06^{ab}
Worabe	41.7^{b}	9.0^{a}	38.7^{a}	15.4^{c}	1.6^{b}	0.48^{c}	1.06^{c}	8.1^{a}
Albazer	39.3^{cd}	9.0^{a}	34.2^{b}	12.7^{d}	1.5^{c}	0.42^{c}	0.98^{c}	7.6^{ab}
6582	38.9^{d}	8.0^{b}	13.7^{e}	32.1^{b}	1.3^{a}	0.74^{b}	1.97^{b}	7.3
MDDT	0	0	0	0	0	0	0	0
Gibie	0	0	0	0	0	0	0	0
10558	0	0	0	0	0	0	0	0
10556	0	0	0	0	0	0	0	0
SE	3.34	0.31	5.1	5.83	0.20	0.34	1.62	0.17
P	***	*	***	***	***	*	***	ns
Block	**Mean block value**							
1st	40.0^{b}	9.0^{ab}	36.3^{a}	14.4^{c}	1.32^{b}	0.46^{c}	1.06^{c}	7.75
2nd	41.0^{b}	9.0^{ab}	36.9^{c}	14.2^{c}	1.27^{a}	0.44^{c}	0.97^{c}	7.93
3rd	41.0^{b}	8.5^{b}	31.6^{b}	13.2^{d}	1.57^{bc}	1.1^{a}	3.9^{a}	7.47
4th	40.2^{b}	8.7^{ab}	29.1^{c}	20.3^{b}	1.54^{bc}	0.55^{b}	1.36^{c}	7.89
5th	45.3^{a}	9.0^{a}	28.4^{d}	22.2^{a}	1.27^{a}	0.56^{b}	1.30^{b}	8.02
SE	0.97	0.11	1.78	1.83	0.07	0.12	0.54	0.10
P	*	ns	*	*	*	*	***	ns
Accession by location interaction								
SE	1..31	0.13	2.54	2.41	0.08	0.15	0.65	0.11
P	ns	ns	ns	ns	ns	ns	ns	ns

a,b,c,d,e; Means within the column with different superscripts in accessions and blocks are differ significantly at P<0.05. 5708:Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; 6582: Accession collected from ILRI; MDDT: Accession collected from Meskan Dubotuto; Gibie: Accession collected from Gibie river basin; 10558: Accession collected from ILRI; 10556: Accession collected from ILRI; X1: Cover %; X2: Infection tolerance; X3: Plant height(cm); X4: Mean tiller number; X5: Leaf length(cm); X6:Leaf width(cm); X:Leaf area(cm^2); X8- Regrowth score. *: P<0.05; ***: P<0.0001; NS: not significant.

Table 2 - Yield efficiency of Sainfoin accessions during screening phase contd…

Accession	Mean accession values						
	9	10	11	12	13	14	15
5708	4.42^{d}	66.7^{c}	29.0^{c}	1.31^{a}	48.7^{a}	10.71	2163^{a}
16006	4.55^{c}	67.9^{b}	25.7^{e}	1.30^{a}	38.3^{b}	11.05^{b}	88.3^{d}
Worabe	5.24^{a}	52.2^{d}	32.9^{b}	1.33^{a}	31.6^{b}	12.49^{a}	927^{b}
Albazer	5.57^{b}	52.1^{d}	35.6^{a}	0.98^{b}	40.7^{c}	10.96^{c}	561^{c}
6582	4.90^{d}	70.1^{a}	26.6^{d}	1.03^{b}	46.8^{b}	0	0
MDDT	0	0	0	0	0	0	0
Gibie	0	0	0	0	0	0	0
10558	0	0	0	0	0	0	0
10556	0	0	0	0	0	0	0
SE	0.24	3.97	1.89	0.08	2.47	0.69	443.87
P	***	***	***	***	*	***	***
Block	**Mean block value**						
1st	5.49^{a}	51.97^{c}	34.5^{a}	1.20	35.6	11.75^{a}	767^{b}
2nd	5.45^{a}	52.3^{c}	34.3^{a}	1.19	36.4	11.74^{a}	691^{c}
3rd	5.16^{c}	57.07^{b}	32.4^{b}	1.45	40.39	11.39^{c}	1181^{a}
4th	5.17^{c}	58.21^{a}	31.7^{c}	1.14	39.7	11.73^{c}	750^{b}
5th	5.33^{b}	57.26^{a}	31.3^{d}	1.17	36.95	11.46^{b}	577^{d}
SE	0.07	1.33	0.66	0.01	0.82	0.22	102.53
P	*	*	*	ns	ns	ns	***
Accession by location interaction							
SE	0.12	1.97	0.97	0.05	0.89	0.33	144.6
P	ns	ns	ns	ns	ns	*	ns

a,b,c,d,e; Means within the column with different superscripts in accessions and blocks are differ significantly at P<0.05. 5708: Accession collected from ILRI, 16006- Accession collected from ILRI, Worabe- Accession collected from Worabe, Albazer- Accession collected from Albazer 6582- Accession collected from ILRI, MDDT- Accession collected from Meskan Dubotuto, Gibie- Accession collected from Gibie river basin, 10558-Accession collected from ILRI, 10556- Accession collected from ILRI. X9:Fresh biomass weight(kg) per meter square; X10:leaf%; X11:Stem %; X12:Air dried weight (kg); X13: Air dried %; X14: Annual dry matter yield(t); X15: Annual seed yield(kg). *: P<0.05; ***: P<0.0001; NS: not significant.

Table 3 - Agronomic efficiency of yield factors during experimental establishment

Accession	Mean accession values							
	1	2	3	4	5	6	7	8
5708	33.2^{c}	8.0^{b}	7.5^{d}	6.2^{d}	2.82^{a}	2.32^{a}	9.37^{a}	7.8^{c}
16006	41.6^{a}	7.8^{c}	19.1^{c}	33.3^{a}	2.08^{b}	0.76^{b}	1.81^{b}	4.5^{d}
Worabe	37.1^{b}	9.0^{a}	32.1^{a}	13.1^{b}	1.49^{d}	0.49^{c}	1.08^{c}	8.9^{a}
Albazer	30.6^{d}	9.0^{a}	26.1^{b}	12.0^{c}	1.59^{c}	0.40^{d}	1.07^{c}	8.3^{b}
SE	3.34	0.31	5.1	5.83	0.20	0.34	1.62	0.17
P	***	*	***	***	***	*	***	ns
Location	**Mean location value**							
Buee	41.1^{a}	8.5	29.1^{a}	25.6^{a}	2.06^{a}	1.01^{a}	2.79	8.5^{a}
Tatesa	30.1^{b}	8.4	13.4^{b}	6.6^{b}	1.94^{b}	0.97^{b}	2.73	6.4^{b}
SE	5.48	0.06	7.84	9.50	0.06	0.02	0.02	1.10
P	***	*	***	***	*	*	ns	***
Mean block								
SE	0.37	0.03	0.06	0.05	0.002	0.002	0002	0.04
P	ns	ns	ns	ns	ns	ns	ns	ns
Accession by location interaction								
SE	2.64	0.22	5.05	6.67	0.20	3.0	1.32	1.07
P	ns	ns	***	***	ns	ns	***	***

a,b,c,d; Means within the column with different superscripts in accessions and blocks are differ significantly at P<0.05. 5708: Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; X1: Cover %; X2: Infection tolerance; X3: Plant height(cm); X4: Mean tiller number; X5: Leaf length(cm); X6:Leaf width(cm); X:Leaf area(cm^2); X8- Regrowth score;.*:P<0.05; ***:P<0.0001; NS: not significant.

Table 4 - Yield efficiencies during experimental establishment contd…							
Accession \ Mean accession values	9	10	11	12	13	14	15
5708	1.24^{d}	54.9^{a}	22.7^{a}	42.3^{a}	0.85^{a}	7.49^{b}	573.8^{b}
16006	1.08^{b}	35.7^{b}	7.4^{b}	21.4^{d}	0.42^{c}	3.69^{b}	90.91^{d}
Worabe	2.53^{a}	54.0^{a}	21.3^{a}	36.6^{b}	1.1^{d}	9.69^{a}	701.57^{a}
Albazer	1.87^{c}	55.6^{a}	22.6^{a}	36.5^{c}	0.89^{b}	7.85^{b}	389.5^{c}
SE	0.33	4.80	3.70	4.47	0.14	1.26	142.60
P	***	***	***	***	***	***	***
Location	Mean location value						
Buee	2.3^{a}	69.8^{a}	22.13^{b}	46.2^{a}	1.04^{b}	9.12^{a}	499.18^{a}
Tatesa	1.04^{b}	30.3^{b}	14.7^{a}	22.2^{b}	0.6^{a}	5.23^{b}	355.96^{b}
SE	0.63	19.79	3.63	11.99	0.22	1.94	71.61
P	***	***	*	***	**	***	***
Block mean							
SE	0.001	0.05	0.06	0.08	0.004	0.039	0.244
P	ns	ns	ns	ns	ns	ns	ns
Accession by location interaction							
SE	0.34	8.87	2.93	5.79	0.14	1.19	103.23
P	***	***	***	***	***	***	ns

a,b,c,d; Means within the column with different superscripts in accessions and blocks are differ significantly at P<0.05. 5708: Accession collected from ILRI, 16006- Accession collected from ILRI, Worabe- Accession collected from Worabe, Albazer- Accession collected from Albazer; X9:Fresh biomass weight(kg) per meter square; X10:leaf%; X11:Stem %; X12:Air dried weight(kg); X13: Air dried %; X14:Annual dry matter yield(t); X15: Annual seed yield(kg). *:P<0.05; **:P<0.01; ***:P<0.0001; NS: not significant.

Agronomic performance similarity and variations in two experimental sessions

During the screening phase, plant vigor record was highest for Worabe's than that of Albazer's. During the experimental session, the cumulative value of the accessions at both agro ecologies was found to be comparable. The total leaf count during the screening phase for ILRI 16006 accession was more vigorous than all the others. However, the cumulative value of the two agro ecologies showed Worabe's observed more vigorous. During the screening session, regrowth scores for Worabe, Albazer and ILRI 16006 were found similar. However, during the experimental session, Worabe became superior, followed by Albazer. During screening sessions, fresh biomass weight per square meter was higher for Worabe Sainfoin than Albazer sainfoin, but during experimental sessions ILRI 16006 replaced Albazer sainfoin. Cumulative seed yield for Worabe accession was highest during the experimental period, but during the screening period, the highest record was found in ILRI 5708. Variability could be attributed to agroecology variation.

The conclusion of Hanna (1993) indicates that the longest with the superior in leaf percent can score the highest DM yield, could contradict the present study in that the lowest leaf percent with highest scored the highest DM yield. The cumulative DM yield of sainfoin is within the range of 7–15 t ha^{-1} based on growing conditions (Tarawali, 1995). At seed filling and the beginning of flowering stages, 5.1 t ha^{-1} and 6.5 t ha^{-1} DM yields are reported by Turk et al. (2011) and Goplen et al. (1991) respectively. The results of the current study are in agreement with the ranges of monoculture or mixture stands, except for pick production time records. This result shows that the Ethiopian agroecology is suitable for either indigenous or exotic accessions. Several years of drought are the threat of Sainfoin on DM yield (Biligetu et al., 2014). Despite the determination of suitability of the Brown, Dark Brown and Black soils types for sainfoin, the report of Goplen et al. (1991) has limitation in determining the crack effect of Black Vertis that causes plant death as observed at the Tatesa site.

Chemical composition of sainfoin accessions

The chemical composition of the four sainfoin accessions is presented in Table 5. Higher crude protein contents were recorded for 5708 compared to that of 16006 and Albazer. Significantly (P<0.05) higher NDF and ADF were recorded for 16006 and 5708 compared with that of 5708 and Worabe respectively. The phenol content was 21.9 and 22.9 for 5708 and 16006 respectively. Higher total tannin was recorded from 5708 compared with that of 16006 and Albazer accessions. The crude protein content of sainfoin depends on the growth stage and population. The CP content of accessions in the present study was within the range of 16.36 -18.77 during the filling stage. This result was in agreement with that of the earlier reports presented by Turk et al. (2011) and Azuhnwi et al. (2012). Crude protein content in the range of 11.4–17.7% was also reported for the flowering stage (Kaplan, 2011). Differences in CP content with other reports could be attributed to the stage of maturity, soil type, and available chemical composition in the soil. The NDF content of sainfoin at flowering was reported to be in the range of 37.2-45% (Parker and Moss, 1981). In contradict to this, the lower NDF values of the regrowth stage reported by Bal et al. (2006) was agreed with the present result. On the other hand, the minimum content of ADF composition for the vegetative stage and flowering stages are agreed with the reports of Bal et al. (2006) and McCallum et al. (2004) respectively with the present result, but disagrees neither with the maximum content nor the growing stages. Variation within fiber contents attributed to growth stage, population differences (Bhattarai et al., 2018), climatic conditions, and soil type, and soil chemical composition. Factors affecting the types, structures, and concentration levels of secondary metabolites in plants are many and complex. The two tannin types in plant parts and the CT distribution at the upper epidermis of leaf tissue varies from plant to plant (Jean-Blain, 1998). The tannin in sainfoin leaf, has a molecular weight of 17000–28,000 Da has lower efficiency in blood protein precipitation than the lower molecular weight (6000-7100 Da) present in Lotus species (Jones et al., 1976). The major causes of higher tannin concentration in plants are early growth stage (Bate-Smith, 1973), Phenological (leaf) (Mole et al., 1988), increased temperature (Lees et al., 1994), and poor soil fertility (Kelman and Tanner, 1990). On top of that, there is concentration variation in species and cultivars (Kelman and Tanner, 1990). Except for phenolic compounds, the present study indicates the existence of variations in secondary metabolite contents among accessions is in line with the reports of Kelman and Tanner (1990) and Roberts et al. (1993).

Roberts et al. (1993). The secondary metabolites in sainfoin is within the range of 5-8% /kg DM, and taken as a moderate level that has lower effects on digestion than the Lotus as reported by Waghorn et al. (1999). Waghorn et al. (1999) that has in agreement with Wang et al. (2015) report. This above range contradicts the report of Salem et al. (2006), who recorded 20–50 g/kg. DM and took it as a moderate level. Differences in accessions are attributed to leaf-to-stem ratio and growth stage variation. In the present study, the leaf percent ratio recorder holds the highest CT.

Table 5 - Chemical composition of experimental Sainfoin accessions

Accessions \ % DM	DM%	Ash	OM	CP	NDF	ADF	ADL	TP (mg/GAE/g)	CT (mg/g)
5708	90.29[a]	11.43[d]	90.5[ab]	18.77[a]	46.3[d]	31.09[a]	17.7[a]	21.90	7.64[a]
16006	90.33[a]	11.84[b]	88.5[a]	16.45[c]	39.25[a]	29.88[c]	9.4[bc]	22.14	7.47[b]
Worabe	90.55[a]	12.71[c]	91.1[b]	17.56[b]	37.38[c]	26.07[d]	11.0[b]	22.90	6.81[a]
Albazer	89.17[b]	13.53[a]	91.4[a]	16.36[c]	37.78[b]	30.03[b]	9.1[c]	22.11	7.12[ab]
SE	0.31	0.03	0.55	0.05	0.26	0.03	0.50	0.50	0.15
p	*	***	***	***	***	***	***	ns	*

a,b,c,d; Means in the same column with different superscript letters are significantly different at P<0.05. 5708: Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; DM: Dry matter; CP: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin; TP: Total phenol; GAE: Gallic acid equivalent; CT: Condensed tannin. *: P<0.05; **:P<0.01; ***:P<0.0001; NS: not significant

***In vitro* gas production potential of sainfoin accessions**

In vitro gas production of experimental sainfoin accessions is presented in Table 6 with its trend (Figure 2). There was a significant difference (P<0.05) in gas production and the characteristics of accessions in each incubation period. Accession 5708 was the lowest in gas production within the first 3 hours of the incubation period but 16006 and Albazer were found to be higher in gas production without showing a significant difference (P>0.05) between each other. Within 24 hour of incubation period, 16006 and Albazer sainfoin accessions significantly (P<0.0001) produced the highest gas, and the least was observed in 5708 accessions compared to others.

In vitro gas production is used for the estimation of the potential digestibility of the feed in the rumen (Getachew et al., 2000). However, there exist differences in gas production characteristics among laboratories that partially result from differences in chemical constituents (CP, NDF, ADL, TP, and CT), leaf-to-stem ratio, and cell wall composition, as indicated by Larbi et al. (2011) and Getachew et al. (2002). These causes for variations agree with the present study of gas production differences in the same species. Higher CP content is positively correlated with gas production extent, while higher fiber content reduces initial gas production and is negatively correlated with gas volume (Gasmi-Boubaker et al., 2005). The current study result contradicted the reports of Khazaal et al. (1994) and Tolera et al. (1997) because lower gas production was recorded from the highest CP content holder. The cause of low gas production vs higher CP could be attributed to the effect of higher affinities formed by CT and proteins than polysaccharides (Patra and Saxena, 2010). This affinity resulted the formation of insoluble CT-fibre and CT-protein complexes and implies fermentation reduction (Huyen et al., 2016).

The initial incubation period gas is related from the fermentation of the soluble and fast fermentable fraction of the substrate (i.e., soluble carbohydrates) and microbial protein synthesis, whereas the last portion of the incubation period is the result of fermentation of the insoluble but potentially degradable components (NDF) fraction (Groot et al., 1996). The NDF content in fermentation leads to proportionally greater amounts of gas production in the latter incubation periods (Lagrange and Villalba, 2016). Sainfoin is characterized by a very low fiber digestible legume at early incubation times (Niderkorn et al., 2011) due to the negative effect of its CT (Theodoridou et al., 2011). Accordingly, it can be concluded that along with other factors, a relatively higher CT in 5708 accessions may contribute to the reductions of the rate of gas production at initial as well as the potential gas production.

Sainfoin holds 12.6±0.6 g/kg CT with 5.5, 74.7, and 80.2 of the mean degree of polymerization (mDP), prodelphinidins percentage (%PD) and *cis* flavan-3-ols (%*cis*) respectively (Huyen et al., 2016). The percentage of prodelphinidins within CT had the largest effect on fermentation characteristics followed by average polymer size and percentage of *cis* flavan- 3-ols (Huyen et al., 2016). The prodelphinidins (PD%), rather than procyanidins (PC) has the potential of inhibition of growth of proteolytic bacteria (Valentin et al., 1999). These listed cases for gas production variation are in lined with the present study gas production results of experimental accessions. In support of this, in *vitro* fermentation of sainfoin caused low VFA concentration and gas production than that of alfalfa (Niderkorn et al., 2011). Other than the CP content effect, methodology (Valentin et al., 1999), substrates and types of experimental animals, number of measurements and mathematical model; and also personnel error, laboratory chemical constituents, consistent working period and personnel execution rate could also be taken as the cause for gas record variations.

Table 6 - Chemical composition of experimental Sainfoin accessions.

Accessions	Incubation times, hour								Gas characteristics			
	3	6	9	12	18	24	48	72	a	b	c	a+b (%)
5708	6.7[c]	9.6[c]	12.4[d]	15.0[c]	19.6[c]	23.6[c]	35.1[c]	41.6[c]	3.49[c]	46.7[c]	0.0236[c]	50.2[b]
16006	16.6[a]	25.3[a]	32.1[a]	37.4[a]	44.7[a]	49.2[a]	55.4[ab]	56.3[a]	6.41[a]	51.0[ab]	0.082[a]	57.4[a]
Worabe	11.7[b]	17.2[b]	21.9[b]	26.2[b]	33.2[b]	38.7[b]	50.9[b]	55.6[ab]	5.44[b]	53.3[a]	0.0416[b]	58.7[a]
Albazer	15.3[a]	23.6[a]	30.1[c]	35.1[a]	41.9[a]	46.0[a]	51.4[ab]	52.1[b]	5.49[b]	47.8[bc]	0.0861[a]	53.3[b]
SE	0.29	0.45	0.56	0.63	0.70	0.72	0.65	0.56	0.04	0.60	0.003	0.57
p	***	***	***	***	***	***	***	***	***	***	***	*

a,b,c,d; Means within the column with different superscripts are differ significantly at P<0.05. 5708: Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; a: Gas production from immediately soluble component (ml); b: Gas production from insoluble but potential degradable portion (ml); a+b(%): Potential gas production (ml); c: The rate constant of gas production (fraction/h). *: P<0.05; **: P<0.01; ***: P<0.0001.

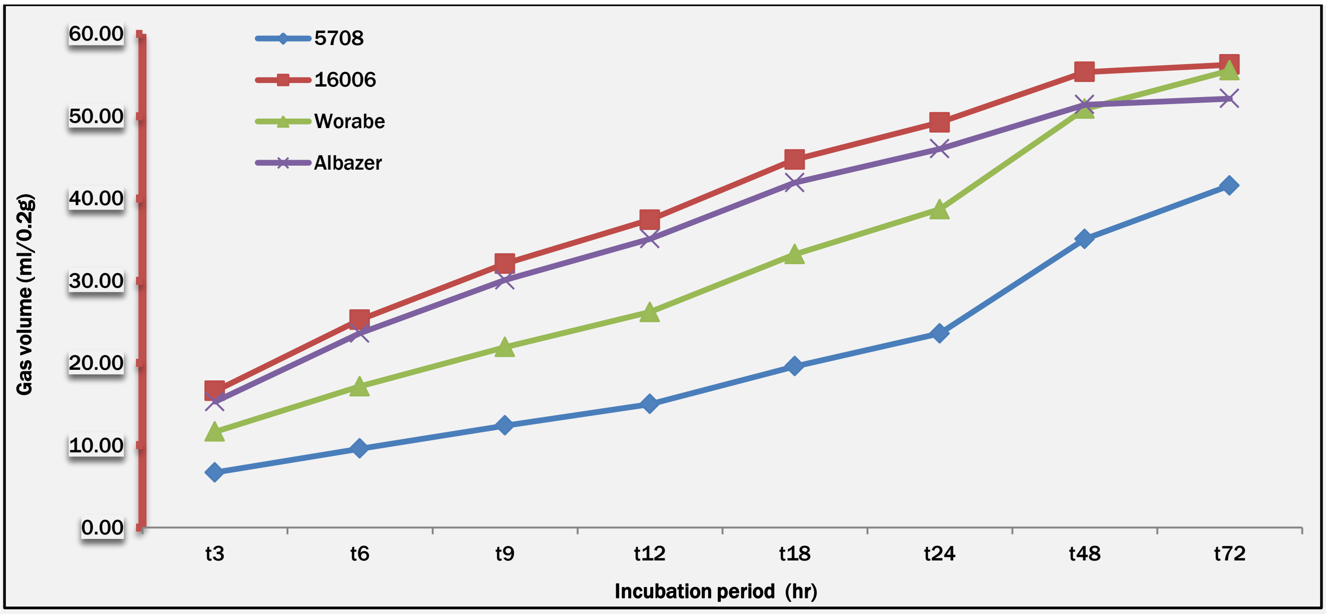

Figure 2 - *In vitro* gas production of experimental Sainfoin accessions

Nutritive value and methane emission potential of sainfoin accessions

As presented in Table 7, there were significant (P<0.05) variations between the experimental sainfoin accessions in chemical composition, estimated digestibility, energy value, and methane production potential. Except for 5708 accessions, all the others hold higher carbohydrate content. Significantly (P<0.001) lower *in vitro* dry matter digestibility (IVDMD) was recorded for Albazer followed by 16006 accessions. Organic matter digestibility (OMD) and metabolizable Energy (ME) were lower for 5708. Significantly (P<0.001) the highest methane gas (CH4), calculated energy, and its mass was recorded in Albazer while all the rest hold lower and similar value records (P>0.05).

The *in vitro* dry matter digestibility of sainfoin accession within the range of 55.6–73.3% is in agreement with the ranges reported by Yordanka and Viliana (2015). There exists higher OM degradability and rate of gas production for alfalfa than that of sainfoin (Lagrange and Villalba, 2016). The lower OM degradability and rate of gas production records for sainfoin are attributable to the greater contents of the cell wall and relatively more CT together with the advanced stage of maturity (Guglielmelli et al., 2011).

In the current study, metabolizable energy content in the range of 11.55-12.49 MJ/kg DM is higher than the report of Scharenberg et al. (2007). *Acacia tortilis* in mid-rift-valley grassland of Ethiopia holds lower ME as reported by Bezabih et al. (2014) than the present study result. Such variation could be attributed to differences in morphological composition (Larbi et al., 2011), harvesting season (Zafu et al., 2020), stage of maturity (Guglielmelli et al., 2011), and differences in CT that causes fermentation and end-product variation (Huyen et al., 2016).

Methane production from 5.6-9.9ml/200mg for sainfoin accessions is reported by Aderao et al. (2018). There is a positive correlation between methane and gas volume production (Huyen et al., 2016). In contrast to this, there are reports presented by Bueno et al. (2020) indicating the potential effect of CT on methane production reduction through reducing methanogens, shifting metabolic pathways, and reducing the availability of protein for microbial activity. In support of this, Huyen et al. (2016) reported the ability of all CT sources on CH4 production reduction. As a result, the liberation of 2[H]-ions from the fermentation of organic matter to acetate and butyrate that uses for CH4 production is suppressed. In contrast, increased propionate production has a role as [H]-ion sinker (Tavendale et al., 2005). Moreover, condensed tannins have higher affinities for proteins than polysaccharides (Patra and Saxena, 2010). From the current study, accession with the highest CHO and least IVDMD produced higher methane than those hold higher CP, since higher CP can suppress acetate production.

Table 7 - Digestibility, nutritive value and methane emission potential of experimental Sainfoin accessions

Sample	Chemical composition and energy			Digestibility (%)				Methane characteristics in 24 hrs		
	SCFA (mmol/200 mg $^{-1}$ DM) at 24 hr	CHO	ME (at 24 hr)	*IVDMD*	TDN	DCHO	OMD at 48hr	Volume (ml/200 mg^{-1} DM)	Energy (k Cal)$^{-1}$	Mass (mg ml^{-1})
5708	0.63^{b}	47.5^{b}	6.51^{d}	73.3^{a}	73.8^{c}	52^{c}	62.0^{c}	5.60^{bc}	52.9^{bc}	4.03^{bc}
16006	1.24^{a}	59.3^{a}	9.86^{a}	62.7^{c}	88^{a}	70.6^{a}	79.8^{a}	7.0^{b}	66.2^{b}	5.0^{b}
Worabe	0.99^{ab}	56.9^{a}	8.49^{c}	71.1^{b}	85.5^{ab}	66.5^{b}	75.7^{b}	7.0^{b}	66.2^{b}	5.0^{b}
Albazer	1.16^{a}	58.9^{a}	9.42^{b}	55.6^{d}	84.2^{b}	66.9^{b}	76.7^{b}	9.9^{a}	93.6^{a}	7.1^{a}
SE	0.11	0.86	0.13	0.38	1.03	0.38	0.70	0.65	6.13	0.46
p	ns	***	***	***	***	***	***	***	***	***

a,b,c,d; Means within the column with different superscripts are differ significantly at P<0.05. 5708: Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; SCFA: Short chain fatty acid; DM: Dry matter; CHO: Carbohydrate; ME: Metabolizable energy; IVDMD: *in vitro* dry matter digestibility; TDN: Total digestible nutrient; DCHO: Digestible carbohydrate; OMD: Organic matter digestibility. ***: P<0.0001; NS: not significant.

Relative feeding value

The Relative Feeding Value (RFV) of the four accessions is presented in Table 8. There was significant variation in relative feeding value among the accessions (P<0.05). The highest (P<0.001) relative feeding value was recorded for Worabe accessions. Significantly (P<0.001) the least RFV was observed for 5708.

Determination of RFV for legumes compared with that of grass mixture and grass forages bears limitations (Ward and Ondarza, 2008). Many scholars stated that, intake is a function of the NDF content. Alfalfa forage has greater than 100 RFV and is taken as a greater intake of digestible dry matter by the cow compared to more NDF holders (Ward and Ondarza, 2008). The result of the current study is in agreement with the above conclusions in that intake is inversely proportional to the NDF content but positively correlated with ADF content. The mean relative feeding value of alfalfa at the early and late cutting stages is within a range of 119-203 and 125-254 respectively, Hackmann et al. (2008). Having RFV of more than 100 in the present study shows the importance of sainfoin as feed source, and this result is in agreement with the report of Ward and Ondarza (2008). It can be concluded that the RFV of studied sainfoin accessions in the present study and studied alfalfa in another report are comparable with the existence of variability in secondary metabolites and their effect on animal production.

Table 8 - Relative feeding value of experimental Sainfoin accessions

Sample / Chemical composition and energy	DDM (%)	DMI (%BW)	RFV
5708	64.7^{c}	2.59^{c}	130.0^{c}
16006	65.6^{b}	3.06^{b}	155.5^{b}
Worabe	68.6^{a}	3.21^{a}	170.7^{a}
Albazer	65.5^{bc}	3.18^{ab}	161.3^{b}
SE	0.02	0.02	0.77
p	***	***	***

a,b,c,d; Means within the column with different superscripts are differ significantly at P<0.05. 5708: Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; DDM: Digestible dry matter; DMI(%BW): Dry matter intake in % of body weight; RFV: Relative feeding value. ***: P<0.0001; NS: not significant.

CONCLUSION

Testing locations caused inconsistency result in agronomic performances collected during the screening phase at one testing site and the evaluation session at two testing sites. Location that boosts manifestation for some potential of some accession could suppress the others potential. The highest CP, together with the higher secondary metabolite (Phenol) content in 5708 accession, suppressed *in vitro* gas fermentation. The lowest ADL content, together with higher CHO composition in Albazer sainfoin caused a higher fermentability and led to higher methane production. In this study, the lowest NDF content indicated a level of digestibility but couldn't indicate the RFV. Compared with other accessions, the highest in disease tolerance ability, regrowth potential, annual herbage biomass and seed yield, and better in CHO and RFV, but lower in NDF content scores assisted Worabe sainfoin to be the primary accession. In areas where crop residues become the dominant feed resources and in moisture scarce areas of Ethiopia, the use of Worabe sainfoin to enhance the nutritive value of poor feed resources seems appealing.

DECLARATIONS

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Authors' contribution

D. Getu: Designed the experiment, set methodology, conducts the experiment, collect, analyses and interpret and prepare the manuscript. S. Demeke, T. Tolemariam and M. Dejene: Reviewed, edited and supervised the experimental design, performed methodology, data quality, data analysis, data interpretation and manuscript writing.

Acknowledgements

The authors would like to thank the South Agricultural Research Institute, Worabe Agricultural Research Centre, and Ethiopian Institute of Agricultural Research for funding; Jimma University College of Agriculture and Veterinary Medicine Department of Animal Science and Postharvest, Hawassa University College of Agriculture, Department of Animal and Range Sciences and Holeta Agricultural Research Centre for facilitating the laboratory execution; Soddo Woreda, Buee Municipality, Buee Secondary School, Buee Construction College and Wolkite Research Centre administrative heads and officers for facilitating experimental sites and the International Livestock Research Institute (ILRI), Ethiopian branch for availing initial Sainfoin Seed.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

We, the authors, have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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