SHORT COMMUNICATION

Nutritive value and energy content of the straw of selected *Vicia* L. taxa from Tunisia

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Abstract

The chemical composition and energy value of straws of *Vicia sativa* L. (varieties Languedoc and Mghila, and subspecies *amphicarpa*) and *Vicia villosa* Roth. (variety Sejenane and accession 2565) were investigated. The plants were grown in a Mediterranean environment, under identical environmental conditions. Total digestible nutrients (TDN) and energy values (digestible energy, metabolisable energy, and net energy for lactation) were estimated according to the equations developed by the 2001 Dairy National Research Council. Both ether extract (EE) or total fatty acids (FA) amounts, and digestible neutral detergent fibre (dNDF) calculated from chemical analysis or measured using a 48-hour rumen *in vitro* assay were used for calculations. Significant differences were observed in the chemical composition and energy value of the straws of the considered *V. sativa* and *V. villosa* varieties. Within the same variety, the TDN was similar using either EE or FA values for calculation. The energy resulted largely dependent on the dNDF values. Energy was higher when *in vitro* dNDF was used for calculation in low-NDF straw samples, while the opposite occurred for high-NDF samples.

Introduction

Common vetch (*Vicia sativa* L.) is a multipurpose, cool season, annual and erect growth habit legume grown for livestock feed and soil fertility improvement in Mediterranean environments, where average annual rainfall ranges from 250 to 350 mm. The forage can either be grazed, or cut for hay or straw production (Larbi *et al*., 2011a).

Hairy vetch (*Vicia villosa* Roth) is a cool season and creeping growth habit legume grown for pasture, hay, silage, and grain production for livestock feed, as well as green manure and cover crop for weed control and soil productivity improvement. Hairy vetch tolerates cold better than common vetch and it is also more suitable for grazing because of its long vegetative growth, high forage production, and low harvest index (Larbi *et al.*, 2011b).

Despite the importance of common and hairy vetches as feed resources in dryland mixed farming systems, little information exists on intra- and inter-species variations in quality determinants of straw. Moreover, very few energy values of individual *V. sativa* and *V. villosa* varieties are currently available.

This study is part of a research aimed to evaluate the nutritive value of straw and seeds of different species and varieties of the genus *Vicia* growing in Mediterranean areas. In this paper, straw samples of five varieties, subspecies or accessions of *V. villosa* and *V. sativa,* grown in identical climate and soil conditions in North Tunisia, were analysed for their chemical characteristics. Total digestible nutrients (TDN) and energy values were determined according to the National Research Council (2001) using two different approaches [chemical and biological (from an *in vitro* assay)] for the assessment of neutral detergent fibre (NDF) digestibility. Fatty acids (FA) were also measured as an alternative approach to the National Research Council equation for the estimation of TDN and energy of feeds with less than 1% of ether extract (EE). The differences among results were used to suggest the most accurate and precise predictive approach for high fibre-low fat feedstuffs such as vetch straws.

Materials and methods

Biological material

The biological material consisted of straw samples of two species of the genus *Vicia* L.: i) *V. sativa* L., represented by two Tunisian varieties (Languedoc and Mghila) and one subspecies [*amphicarpa* (Dorthes) Asch.]; ii) *V. villosa* Roth, represented by a Tunisian variety (Sejenane) and one accession (2565) introduced from and provided by the International Center for Agricultural Research in the Dry Areas (ICARDA) in the frame of a germoplasm exchange.

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Each variety/subspecies/accession was sown on ten 2×2 m plots at the experimental station of the National Institute of Agronomy of Tunisia (INRAT), Tunis, Tunisia (latitude: 36°50'37" N; longitude: 10°11'28" E) on 1 November 2011. The seeding rate was 100 viable seeds/m^2; the plots were not fertilised and the soil texture was clay-loam. Straw harvesting occurred 230 days after sowing; total precipitation during the period was 516 mm (data recorded at the meteorological station of INRAT). The straw was harvested cutting the plants manually; after separation of the seeds by threshing, the stalks were mixed and sampled for chemical analysis.

Chemical analysis and calculations

Straws samples were ground in a 1-mm sieve Pulverisette 15 (Fritsch GmbH, Idar-Oberstein, Germany) and analysed in duplicate.

AOAC (2000) procedures were used to determine dry matter (DM) (method no. 930.15), ash (method no. 942.05), and crude protein (CP) (nitrogen ×6.25; method no. 984.13). Ether extract was determined following method no. 920.39 of AOAC (2003). Soluble protein (SolP) and rumen undegradable protein (RUP) were measured according to Licitra *et al*. (1998).

The Ankom 200 Fibre Analyzer (Ankom Technology, Macedon, NY, USA) was used to determine NDF, acid detergent fibre (ADF) and acid detergent lignin (ADL), following the procedure of Mertens (2002) for NDF and Van Soest *et al.* (1991) for ADF and ADL. For NDF, the detergent solution contained sodium sulfite and a heat-stable bacterial α-amylase (activity=17,400 Liquefon units/mL; Ankom Technology). The NDF, ADF and ADL were corrected for residual ash content. The neutral detergent insoluble protein (NDIP) and the acid detergent insoluble protein (ADIP) were determined as residual nitrogen (N×6.25) in Ankom fibre bags after extraction with neutral detergent or acid detergent solution.

The non-fibrous carbohydrates (NFCc) content was corrected for NDIP and calculated as follows: NFCc=100−[CP+ash+EE+(NDF − NDIP)].

Estimated TDN of straws was calculated with the equation 2-5 of the National Research Council (2001) as sum of digestible non-fibrous carbohydrates (dNFCc: equation 2-4a), digestible protein (dCP: equation 2-4b), digestible fatty acids (dFA: equation 2-4d) and digestible neutral detergent fibre (dNDF: equation 2-4e).

Total FA were determined using a combined direct transesterification and solid-phase extraction method (Alves *et al*., 2008), and quantified by gas chromatography as described by Renna *et al*. (2014).

The dNDF was either calculated from the lignin content of NDF (dNDFc: chemical approach), or measured from a 48-hour *in vitro* assay (dNDFm: biological approach) by the Ankom DaisyII incubator (Ankom Technology) following Robinson *et al*. (1999). For each sample, 2 bags (Ankom F57) were filled with ground material (1-mm sieve, 250 mg) and sealed. Filtered rumen fluid was collected at a slaughterhouse from beef cattle fed mixed grass hay (*ad libitum*) and a concentrate (400 g d^{-1}) containing ground corn (62%), soybean meal (10%), barley (20%), sunflower meal (5%), minerals and vitamins (3%). After 48 h of incubation, the bags were removed from jars, rinsed thoroughly with cold tap water and immediately analysed for NDF content using the Ankom200 Fibre Analyzer. After incineration and correction for the ash, the residual NDF was used to calculate NDF digestibility as percentage of DM. The TDN of straw samples were estimated using dFA and dNDFc (TDNc) or dFA and dNDFm (TDNm) in equation 2-5 of National Research Council (2001) for the chemical and biological approach, respectively.

As proposed by the National Research Council (2001) for the calculation of the digestible energy (DE) of straw samples, equation 2-8a instead of equation 2-1 was used. Metabolisable energy (ME) and net energy for lactation (NE_L) were calculated using equations 2-2 and 2-11 (National Research Council, 2001). All energy values were calculated using both the dNDFc (DEc, MEc and NE_Lc) and the dNDFm (DEm, MEm and NE_Lm) values in the equations.

Statistical analysis

The statistical analysis of data was performed using IBM SPSS Statistics v.20 for Windows (SPSS Inc., Chicago, IL, USA). Differences in the chemical composition, total FA concentration, dNDFc, dNDFm, TDN and energy values (DE, ME, NE_L) of straws were subjected to one-way analysis of variance according to the following model:

$$X_{ij}=\mu+\alpha_i+\ _{ij}$$

where: X_{ij} is observation; μ is overall mean; α_i is effect of variety/subspecies/accession; ε_{ij} is residual error. Pairwise multiple comparisons (Tukey's test) were performed to test the difference between each pair of means. A paired-samples Student's *t*-test was used to compare the energy values calculated using dNDFc or dNDFm. Significance was declared at P≤0.05.

Results

Chemical composition of the analysed vetch straws

The chemical composition of *V. sativa* and *V.*

Table 1. Chemical composition of *Vicia sativa* and *Vicia villosa* straw.

	V. sativa L.			*V. villosa* Roth.		SEM	P
	Variety Languedoc	Variety Mghila	Subspecies *amphicarpa*	Variety Sejenane	Accession 2565		
DM, %	91.3[b]	91.7[a]	91.9[a]	91.7[ab]	91.5[ab]	6.67	*
Ash, g 100g^{-1} DM	11.6[b]	7.3[d]	14.8[a]	7.5[cd]	7.9[c]	0.91	***
CP, g 100g^{-1} DM	13.4[a]	7.5[c]	9.3[b]	7.4[c]	7.0[c]	0.78	***
SolP, %CP	38.3[b]	37.4[b]	42.4[a]	29.9[d]	36.3[c]	1.35	***
RUP, %CP	40.7[d]	43.1[c]	40.0[d]	45.2[a]	44.2[b]	0.67	***
EE, g 100g^{-1} DM	0.7[a]	0.7[a]	0.5[c]	0.5[c]	0.6[b]	0.03	***
FA, g 100g^{-1} DM	0.4	0.3	0.3	0.3	0.3	0.01	ns
NDF, g 100g^{-1} DM	55.5[d]	77.4[a]	55.5[d]	72.4[c]	75.9[b]	3.25	***
ADF, g 100g^{-1} DM	43.4[b]	57.1[a]	39.6[c]	56.3[a]	56.6[a]	2.51	***
ADL, g 100g^{-1} DM	9.2[c]	12.3[a]	7.7[d]	11.4[b]	11.5[b]	0.57	***
NDIP, g 100g^{-1} DM	4.0[a]	4.0[a]	4.2[a]	2.7[c]	3.1[b]	0.20	***
ADIP, g 100g^{-1} DM	2.2[a]	2.1[ab]	2.0[ab]	1.8[b]	1.8[b]	0.05	**
NFCc, g 100g^{-1} DM	22.7[b]	11.2[d]	24.8[a]	14.9[c]	11.5[d]	1.90	***
dNDFc, g 100g^{-1} DM	21.7[d]	31.9[ab]	23.5[c]	30.7[b]	32.5[a]	1.51	***
dNDFm, g 100g^{-1} DM	25.0	25.0	26.4	27.3	28.5	5.17	ns
TDNc, g 100g^{-1} DM	48.8[a]	41.9[d]	48.6[a]	44.6[b]	42.9[c]	0.96	***
TDNm, g 100g^{-1} DM	52.1[a]	35.3[c]	51.5[a]	41.2[b]	38.9[bc]	2.28	***

DM, dry matter; CP, crude protein; SolP, soluble protein; RUP, rumen undegradable protein; EE, ether extract; FA, total fatty acids; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; NDIP, neutral detergent insoluble protein; ADIP, acid detergent insoluble protein; NFCc, non-fibre carbohydrate (corrected); dNDFc, digestible neutral detergent fibre (calculated); dNDFm, digestible neutral detergent fibre (Ankom measured); TDNc, total digestible nutrients (calculated); TDNm, total digestible nutrients (Ankom measured). [a-d]Means within a row with different letters differ significantly. *P≤0.05; **P≤0.01; ***P≤0.001; ns, not significant (P>0.05).

Table 2. Estimated energy content of *Vicia sativa* and *Vicia villosa* straw.

	V. sativa L.			*V. villosa* Roth.		SEM	P
	Variety Languedoc	Variety Mghila	Subspecies *amphicarpa*	Variety Sejenane	Accession 2565		
DEc	2.20[a]	1.83[e]	2.13[b]	1.94[c]	1.87[d]	0.049	***
DEm	2.34[a]	1.55[c]	2.26[a]	1.80[b]	1.70[bc]	0.104	***
MEc	1.77[a]	1.40[e]	1.71[b]	1.51[c]	1.44[d]	0.049	***
MEm	1.91[a]	1.12[c]	1.83[a]	1.37[b]	1.27[bc]	0.105	***
NE_Lc	1.05[a]	0.79[e]	1.01[b]	0.87[c]	0.82[d]	0.035	***
NE_Lm	1.15[a]	0.60[c]	1.10[a]	0.77[b]	0.70[bc]	0.074	***

DEc, digestible energy (calculated using dNDFc); dNDFc, digestible neutral detergent fibre (calculated); DEm, digestible energy (calculated using dNDFm); dNDFm, digestible neutral detergent fibre (Ankom measured); MEc, metabolisable energy (calculated using dNDFc); MEm, metabolisable energy (calculated using dNDFm); NE_Lc, net energy for lactation (calculated using dNDFc); NE_Lm, net energy for lactation (calculated using dNDFm). Values are expressed as Mcal kg^{-1} DM. [a-e]Means within a row with different letters differ significantly. ***P≤0.001; ns, not significant (P>0.05).

villosa straws is presented in Table 1. In general terms, the differences observed among varieties of *V. sativa* were more consistent than those observed for varieties of *V. villosa*.

In the current study, CP values ranged from 7.0 (*V. villosa* acc. 2565) to 13.4% of DM (*V. sativa* var. Languedoc). The CP and ash values of *V. villosa* samples were significantly lower than those of *V. sativa* var. Languedoc and subsp. *amphicarpa* (P≤0.001), but similar to those of *V. sativa* var. Mghila. The soluble protein (SolP) and the rumen degradable fraction (RDP) of CP were always higher in *V. sativa* than *V. villosa*, the latter being characterized by a significantly higher amount of rumen undegradable protein (RUP) (P≤0.001).

Significant differences between varieties were observed for their EE content (P≤0.001), which was lower than 1% of DM in all samples. Total FA of straws resulted similar in both species and all analysed varieties (P>0.05) and approximately equal to 50% of EE.

Regarding NDF, the values for *V. sativa* ranged from 55.5 (var. Languedoc and subsp. *amphicarpa*) to 77.4% of DM (var. Mghila), while those for *V. villosa* ranged from 72.4 to 75.9%. The samples of *V. sativa* var. Mghila showed the highest amounts of NDF and ADL within and between species (P≤0.001). The ADF of *V. sativa* var. Mghila did not differ significantly from the ADF of *V. villosa* varieties (56.3 to 57.1% of DM), but was significantly higher if compared to that of *V. sativa* var. Languedoc and subsp. *amphicarpa* (43.4 and 39.6% of DM, respectively; P≤0.001).

Significant differences were observed among species and varieties in their NFCc (P≤0.001). *V. sativa* var. Mghila and *V. villosa* acc. 2565 showed the lowest values of NFCc (about 11% of DM) due to their high amount of NDF, as previously described.

The digestible NDF (dNDFc) calculated from the chemical analysis values of NDF and ADL (equation 2-4e of National Research Council) showed differences among varieties of both

Table 3. Comparison of the energy values of vetch straws estimated using digestible neutral detergent fibre calculated with National Research Council equations or measured from Ankom *in vitro* assay.

	C	AM	SEM	P
DE	1.99	1.93	0.056	ns
ME	1.56	1.50	0.057	ns
NE_L	0.91	0.86	0.040	ns

C, energy values calculated using dNDFc; dNDFc, digestible neutral detergent fibre (calculated); AM, energy values calculated using dNDFm; dNDFm, dNDFm, digestible neutral detergent fibre (Ankom measured); DE, digestible energy; ME, metabolisable energy; NEL, net energy for lactation. ns, not significant. Values are expressed as Mcal kg^{-1} DM.

species of *Vicia* (P≤0.001); the highest values were observed, as expected, in *V. sativa* var. Mghila and *V. villosa* acc. 2565 (31.9 and 32.5% of DM, respectively). The digestible NDF from the 48-h *in vitro* assay (dNDFm) did not show significant differences within and between species. The dNDFm values of *V. villosa* were always lower than dNDFc; in *V. sativa*, only dNDFm of var. Mghila showed the same trend as observed for *V. villosa*.

Total digestible nutrients (calculated) and TDNm of *V. sativa* var. Mghila were always lower and significantly different from var. Languedoc and subsp. *amphicarpa* (P≤0.001). For *V. villosa*, only TDNc values were significantly different between varieties.

With the exception of var. Mghila, *V. sativa* samples always showed higher TDNc and TDNm values than *V. villosa* samples (P≤0.001). Var. Mghila resulted the least digestible variety among the 5 studied due to the high amounts of NDF and lignin.

Energy content of the analysed vetch straws

The energy values (DE, ME, NE_L) of straw samples are shown in Table 2. The energy values calculated from dNDFc (DEc, MEc and NE_Lc) were always significantly different within and between species (P≤0.001). In accordance with the chemical composition of straws, DEc, MEc and NE_Lc values ranked in the following order: *V. sativa* var. Languedoc>*V. sativa* subsp. *amphicarpa*>*V. villosa* var. Sejenane>*V. villosa* acc. 2565>*V. sativa* var. Mghila. In *V. sativa*, NE_Lc ranged from 0.79 Mcal kg^{-1} DM of. var. Mghila to 1.05 Mcal kg^{-1} DM of var. Languedoc; NE_Lc of *V. villosa* straws was comprised between 0.82 and 0.87 Mcal kg^{-1} DM (in acc. 2565 and var. Sejenane, respectively).

Using measured dNDF values (dNDFm), the estimated DEm, MEm and NE_Lm resulted lower than DEc, MEc and NE_Lc only in straw samples characterised by high NDF and ADF amounts (*i.e.*, *V. sativa* var. Mghila and *V. villosa* varieties); less fibrous samples (*V. sativa* var. Languedoc and subsp. *amphicarpa*) showed higher energy amounts.

No statistical difference was observed when the energy values were calculated using dNDFm or dNDFc in the National Research Council equations (Table 3).

Discussion

The chemical composition of vetch straws may significantly differ according to species and varieties, as previously observed by Larbi *et al*. (2011a, 2011b). These authors revealed significant intra-species variations in 45 accessions of *V. sativa* and 25 accessions of *V. villosa* ssp. *dasycarpa* in days to flowering, pod maturity and harvest index, as well as yields and quality determinants of hay, grains and straw. More recently, Kebede *et al.* (2014) evaluated the forage nutritive values of 20 accessions of different vetch species; their nutritional value varied across testing sites and harvesting stage. Intermediate maturing and erect growth habit vetch species had better ash and CP, and lower NDF, than early maturing and creeping growth habit species. Such results seem to be in accordance with those obtained in the current trial, with the exception of *V. sativa* var. Mghila.

In our study, the straw of *V. sativa* var. Languedoc and subsp. *amphicarpa* showed higher amounts of CP than the average range values (7.0 to 8.9% of DM) reported for common vetch by other authors (Hadjipanayiotou *et al*., 1985; Bruno-Soares *et al*., 2000; Haddad and Husein, 2000). Bruno-Soares *et al*. (2000) also reported – for *V. villosa* cv. Amoreiras – higher CP values (10.9% of DM) than those observed for hairy vetch in our trial.

According to some authors (Hadjipanayiotou *et al*., 1985; Bruno-Soares *et al*., 2000; Haddad and Husein, 2000; López *et al*., 2005), the NDF values of *Vicia* spp. straws may vary between 40.2 and 64.7% of DM. Neutral detergent fibre is highly variable among the same species and varieties of the genus *Vicia* according to different soil types and climate conditions (Kebede *et al.*, 2014). In the current trial, the NDF values of *V. villosa* varieties and of *V. sativa* var. Mghila were higher than the above mentioned maximum value, but the NDF values of *V. sativa* var. Languedoc and subsp. *amphicarpa* fell within the range and were similar to those observed by López *et al.* (2005).

According to the National Research Council (2001), if EE<1, then FA=0, and digestible FA (dFA)=0. In this study the total amount of FA and dFA were determined for a more accurate TDN calculation. Total digestible nutrients were therefore calculated using dFA and two different approaches (chemical and biological) for dNDF values, according to the equations developed by the National Research Council (2001). Both approaches require estimates of a similar group of several chemical components; they differ only in that the first approach utilizes the lignin content of the feed to estimate the digestibility of NDF (dNDFc), whereas the second specifies that a 48 h *in vitro* estimate of NDF digestion (dNDFm) can be substituted for the lignin-based estimate. Neutral detergent fibre digestibility is not simply a feed characteristic; therefore dNDFc and dNDFm are generally different (Yu *et al*., 2004), as confirmed by the results obtained in the current trial. The magnitude of this difference may vary within and among laboratories according to many factors (Hall and Mertens, 2012). Our results confirm that the quantity and digestibility of NDF greatly influence the accuracy of the estimate of the TDN and the energy content of feeds. Using the biological approach (dNDFm), the estimated DEm, MEm and NE_{L}m of straw samples characterized by high NDF amounts (*i.e.*, *V. sativa* var. Mghila and *V. villosa* varieties) resulted lower than the DEc, MEc and NE_{L}c calculated with the chemical approach. On the contrary, with less fibrous samples (*V. sativa* var. Languedoc and subsp. *amphicarpa*), the chemical approach resulted in higher energy amounts than the biological one. These results are partially in agreement with previous studies on other feeds such as corn, wheat, and corn or wheat distillers grain with solubles (Yu *et al*., 2004; Nuez-Ortín and Yu, 2011), in which the highest energy values were found when dNDF was measured with a biological approach (ruminal *in situ* assay).

Conclusions

This study showed that the chemical composition of straws of the considered varieties of *Vicia sativa* and *Vicia villosa* can be significantly different even if plants have grown in identical environmental conditions. The chemical composition of the two species showed differences for all the analysed parameters with the only exception of FA. Within the same species, the two varieties of *V. villosa* showed a more uniform chemical composition than the three varieties of *V. sativa*.

For feedstuffs characterized by very low amounts of EE (<1) such as straws, the National Research Council suggests that dFA=0 for the TDN calculation. The FA determination allows a more precise estimation of TDN, but the cost for the analysis may not be justified for this type of samples and the differences seem negligible.

The different approach in estimating the digestibility of NDF (chemical or biological) led to variable energy values. However, the results of the paired-samples Student's *t*-test indicated that the differences are negligible. Therefore, dNDFc (easier to be obtained and less expensive) can be used for predicting the energy value of low-EE and high-NDF samples such as straws.

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