

# Carbohydrate and Protein Fractions of Fresh and Dried Common Vetch at Three Maturity Stages

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## ABSTRACT

Mixed cereal and sheep (*Ovis aries*) production systems in the Mediterranean area and the Middle East region rely on annual forage legumes as a source of complementary forage. Fractionation of carbohydrates (CHO) and crude protein (CP) into chemical entities of refined biological significance may improve the forage utilization of these feed resources. Fresh and dried (field-cured) common vetch (*Vicia sativa* L.) samples were collected during two growing seasons (1996–1997 and 1997–1998) at La Poveda Field Station in central Spain with the objective of determining chemical CHO and CP fractions required for application of new feed models (Cornell System). Fresh and dried samples were harvested at three maturity stages—flowering (>50% of plants with flowers), seed filling phase 1 (280 g DM kg<sup>-1</sup> seed), and seed filling phase 2 (380 g DM kg<sup>-1</sup> seed)—and the treatments were arranged in a completely randomized design. Nonfiber carbohydrates (NFC) and neutral-detergent fiber (NDF), corrected for neutral-detergent insoluble protein (NDIP), were evenly distributed in fresh and dried samples, and were not affected by maturity. The B<sub>1</sub> and B<sub>2</sub> CHO fractions were the most abundant with mean values of 342 and 303 g kg<sup>-1</sup> of total carbohydrates (TC), respectively, across maturities, harvest forms, and years. Fraction CP B<sub>2</sub> was the most abundant CP fraction with mean value of 408 g kg<sup>-1</sup> of total CP. Fraction CP B<sub>3</sub> was <100 g kg<sup>-1</sup> of total CP and increased with maturity. Harvesting common vetch within the seed filling phase would increase ruminal escape protein in vetch.

THE CEREAL–SHEEP SYSTEM is the main agropastoral enterprise in most of the Mediterranean area, the Middle East, and even Central Asia regions. Crop residues and natural feed resources such as cereal stubble, fallows, shrub-steppe vegetation, and pastures are seasonal and generally of low-quality. However, integration of cereal and sheep requires year-long coordinated feeding programs. Information on forage quality of main complementary forage legumes, allowing mixed cereal and sheep production systems, is of paramount importance. Common vetch used as grazing, silage, and mostly as field-cured forage is the main complementary annual forage legume in these areas. Vetch field-cured forage can meet the forage deficit produced by seasonally distributed and low-quality natural feed resources and crop residues (Caballero, 1993).

The application of the Cornell Net Carbohydrate and Protein System (CNCPS) for prediction of metabolizable energy and protein requires accurate estimates of feed composition carbohydrate and protein fractions

and additional inputs. The metabolizable energy and metabolizable protein derived from forages are highly sensitive to variations in forage content of NDF, lignin, CP, and protein solubility (Fox et al., 1995).

The CNCPS has recently received considerable interest by the scientific community, but only a few temperate grasses and legumes such as tall fescue (*Festuca arundinacea* Schreb), bromegrass (*Bromus biebersteinii* Roem and Schult.), and alfalfa (*Medicago sativa* L.) have been tested with this nutrition model, and mostly for CP fractions (Agbossamey et al., 1998; Elizalde et al., 1999). Reports of both the carbohydrate and protein fractions are less common (Masoero et al., 1999), and analytical parameters based on the CNCPS for annual forage legumes have not been reported.

Unlike alfalfa, some authors (Hintz and Albrecht, 1994; Caballero et al., 1996) recommend harvesting annual legumes, such as soybean [*Glycine max* (L.) Merr.] and common vetch, well beyond bloom when the seed fraction accounts for a higher proportion of the harvested biomass. A maturity factor was thus included to test the CNCPS in comparing fresh and field-cured common vetch forage. The objective of this research was to assess the impact of maturity and field curing on vetch carbohydrate and protein fractions used in the CNCPS.

## MATERIALS AND METHODS

### Cropping and Sample Characteristics

Common vetch cv. Vereda was planted on 27 Nov. 1996 and 25 Nov. 1997 at La Poveda Field Station (30 km Southeast of Madrid, Spain) at seeding rate of 80 kg ha<sup>-1</sup> in rows 0.17 m wide. Plants were grown under rainfall continental Mediterranean climatic conditions of the Castilian Plain (elev. = 610 m; 30-yr mean rainfall = 425 mm; mean days with frost = 50), and were harvested as fresh or field-cured forage (harvest forms) between May and June of the following year. The soil is an alluvial sandy-loam Typic Xerofluvic with the following mean properties: pH = 7.7, C/N ratio = 7.9, organic matter = 17 g kg<sup>-1</sup>, N = 1.3 g kg<sup>-1</sup>, CaCO<sub>3</sub> = 42 g kg<sup>-1</sup>, available P = 90 mg kg<sup>-1</sup>, and available K = 120 mg kg<sup>-1</sup>.

Within harvest forms, vetch plants were harvested at three stages: flowering (more than 50% of plants with flowers), and two seed filling stages defined by progressively higher dry matter concentration in the seed (mean of 280 and 380 g DM kg<sup>-1</sup> for seed-filling phase 1 and 2, respectively). More

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**Abbreviations:** ADC, acid-detergent cellulose; ADF, acid-detergent fiber; ADIP, acid-detergent insoluble protein; ADL, acid-detergent lignin; CHO, carbohydrates; CNCPS, Cornell Net Carbohydrate and Protein System; CP, crude protein; DM, dry matter; EAA, essential amino acids; NDF, neutral-detergent fiber; NDIP, neutral-detergent insoluble protein; NEAA, nonessential amino acids; NFC, nonfiber carbohydrates; SC, structural carbohydrates; SEAA, semiessential amino acids; TC, total carbohydrates.

advanced stages of growth were discarded as vetch reached full pod growth before 500 g DM kg<sup>-1</sup> seed, and losses of quality and biomass of vegetative plant parts occurred thereafter (Caballero et al., 1996). Maturity and harvest form treatments were allocated to single plots measuring 40 m by 20 m in a completely randomized design with three replications.

Plots assigned to the flowering stage were harvested with a rotary mower on 28 May 1997 and 8 May 1998, respectively. Twenty individual plants were selected randomly within single plots and separated into leaves and stems. Mean leaf/stem ratios at this stage, on a DM basis, were 1.64 and 1.23 in 1997 and 1998, respectively. Dry matter content of fresh forage (yearly mean of 173 g DM kg<sup>-1</sup>) and plant parts were determined by oven-drying at 60°C for 22 h and subsequently at 80°C for 2 h.

Plots assigned to seed filling phase 1 were harvested on 12 June 1997 and 20 May 1998 and plots assigned to seed filling phase 2 were harvested on 21 June 1997 and 4 June 1998, respectively. In these cases, 30 randomly selected plants were separated into leaves, stems, and full pods. Whole fresh forage and plant parts were oven-dried in a similar way. For seed filling phase 1 and seed filling phase 2, mean yearly DM contents of fresh forage were 267 and 298 g DM kg<sup>-1</sup>, respectively. Leaf, stem, and pod proportions (on a DM basis) were, respectively, 430, 420, and 150 g kg<sup>-1</sup> in 1997 and 310, 350, and 340 g kg<sup>-1</sup> in 1998 at seed filling phase 1, and 310, 380, and 310 g kg<sup>-1</sup> in 1997 and 210, 190, and 600 g kg<sup>-1</sup> in 1998 at seed filling phase 2.

Samples of field-cured vetch were obtained from the swathes and just before pressing. A tined-circle rake was used on field-cured plots. The drying periods lasted <5 d. Yearly mean maximum air temperature during the three field curing periods (flowering, seed filling 1, and seed filling 2) were 24.5, 28.6, and 30.1°C, respectively. Corresponding mean minimum air temperatures were 10.2, 13.7, and 12.8°C, respectively. No rain fell during field curing. Morphological composition of field-cured samples was similar to that of fresh samples as field-cured forage was collected from the swathes, which avoided pod-shattering that can be produced by baling. Dry matter content of field-cured samples at harvest was between 790 and 820 g kg<sup>-1</sup>.

Approximately 3 kg of fresh and field-cured vetch were randomly collected from each plot for chemical analysis. Fresh forage samples were put into freeze-bags, frozen at -20°C, and then freeze dried. Samples of field-cured vetch were oven dried at 60°C for 22 h and subsequently at 80°C for 2 h. Both type of samples were milled to pass a 1-mm screen.

### Fractionation of Carbohydrates and Chemical Analyses

Fractionation of CHO was performed according to the CNCPS (Sniffen et al., 1992). Total carbohydrates (TC) were calculated by subtracting from 1000 the CP, crude fat, and ash contents, and expressed as g kg<sup>-1</sup> DM. Structural carbohydrates (SC) and nonfiber carbohydrates (NFC) represented, respectively, the carbohydrates that are insoluble and soluble in neutral detergent. Carbohydrate fraction A includes soluble sugars and represents the CHO fraction that is degraded rapidly in the rumen. Carbohydrate fraction B<sub>1</sub> has an intermediate rate of degradation and represents mainly starch and non-starch polysaccharides soluble in neutral-detergent (pectic substances, beta-glucans, galactans, gums) and has an intermediate rate of degradation. Carbohydrate fraction B<sub>2</sub> is available cell wall and its degradation rate is slow. Carbohydrate fraction C represents unavailable cell wall and is undegradable and

undigestible. These CHO fractions were expressed as g kg<sup>-1</sup> of TC and calculated as follows:

$$\text{SC} = \text{neutral detergent fiber (NDF)} - \text{neutral detergent insoluble protein (NDIP)}$$

$$\text{NFC} = \text{TC} - \text{SC}$$

$$\text{CHO fraction A} = \text{Soluble sugars}$$

$$\text{CHO fraction B}_1 = \text{NFC} - \text{sugars}$$

$$\text{CHO fraction B}_2 = \text{NDF (corrected for protein)} - \text{fraction C}$$

$$\text{CHO fraction C} = \text{Lignin} \times 2.4$$

Crude protein, crude fat, ash, and DM were determined by standard methods (AOAC Int., 1995). Conventional fractionation of structural carbohydrates and lignin was carried out by the detergent system. Neutral-detergent fiber was analyzed following Van Soest et al. (1991) using a heat-stable  $\alpha$ -amylase (Termamyl 120, Novo, Denmark), and acid-detergent fiber (ADF), acid-detergent cellulose (ADC), and acid-detergent lignin (ADL) were determined by the methods of Goering and Van Soest (1970) as modified by Robertson and Van Soest (1981). Soluble sugars were obtained by extraction with aqueous ethanol (80%, v/v) and measured colorimetrically by the anthrone method (Yemm and Willis, 1954). Starch was quantified from the amount of glucose released after its gelatinization and enzymatic hydrolysis by amyloglucosidase (EC 3.2.1.3.; Boehringer no. 102857) as described by Longstaff and McNab (1991).

### Fractionation of Crude Protein and Chemical Analyses

Fractionation of CP was carried out by the Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992). According to this system, CP is partitioned into three fractions: CP fraction A is nonprotein nitrogen (NPN)  $\times$  6.25; CP fraction B is true protein and CP fraction C is unavailable protein. Crude protein fraction B is further divided into three subfractions B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> of rapid, intermediate, and slow rates of ruminal degradation, respectively. Crude protein fractions A and B<sub>1</sub> are soluble in borate-phosphate buffer, CP fraction B<sub>2</sub> is insoluble in the buffer but soluble in neutral detergent solution, and CP fraction B<sub>3</sub> is insoluble in the buffer and in neutral detergent, but soluble in acid detergent. Crude protein fraction C is the protein that is insoluble in acid detergent (acid detergent insoluble protein, ADIP); it contains protein associated with lignin, tannin-protein complexes, and Maillard products that are highly resistant to microbial and mammalian enzymes.

Precipitated true protein, buffer insoluble protein, neutral-detergent insoluble protein (NDIP), and ADIP were analyzed as described by Licita et al. (1996). Crude protein fraction A was calculated as the difference between the total crude protein and precipitated true protein. True protein was determined by Kjeldahl analysis of the residue resulting after precipitation with tungstic acid followed by filtration. Crude protein fraction B<sub>1</sub> was estimated as true protein minus buffer insoluble protein, CP fraction B<sub>2</sub> as buffer insoluble protein minus NDIP, and CP fraction B<sub>3</sub> by subtracting the ADIP (fraction C) from the NDIP.

### Amino Acids

Amino acid analysis was done on the 1996 to 1997 samples by OPA (o-phthalaldehyde) precolumn derivatization following hydrolysis in 6 M HCl. Amino acids were separated on a HPLC system (Hewlett-Packard 5890, Waldronn, Germany)

according to the method of Jones et al. (1981). Cystine was determined as cysteic acid (Moore, 1963).

### Statistical Analysis

Variables determined were the cell-wall components (NDF, ADF, ADC, and ADL); starch; soluble sugars; crude fat; ash; TC, SC, and NFC; the concentrations of the four CHO fractions (on TC basis); CP concentration; and the concentrations of the five CP fractions (on total CP basis). A two-factorial model of fixed effects including interaction was used for statistical analysis:

$$Y_{ij} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{ij}$$

where  $Y$  = the  $i$ th maturity observation on the  $j$  harvest form;  $\mu$  = a common effect for the whole experiment;  $\varepsilon$  = the random error present in the  $i$ th on the  $j$ th;  $i = 1,2,3$ ; and  $j = 1,2$ . The main effects were maturity ( $A$ ) and harvest form ( $B$ ). The mean squares of the main effects and those of the interaction were compared with that of the residual. Analyses of variance were done by year as differences between years were found, as tested by year  $\times$  treatment interactions and heterogeneity of error. The 2V program of the BMDP statistical package was used (BMDP, 1992).

## RESULTS AND DISCUSSION

### Carbohydrate Fractions

The conventional partitioning of structural carbohydrates and lignin by the detergent system plus analyses of CP, crude fat, ash, starch, and soluble sugars are shown in Table 1. The results showed that common vetch fiber components varied with maturity. In the first growing season, most fiber components, except ADC, increased, but in the second growing season, increased from flowering to seed filling 1 and decreased at seed

filling phase 2. The different trend was the result of higher seed proportion in DM in the second than in the first season at seed filling phase 2 that may have compensated for the increasing content of fiber components in the vegetative plant parts. This offsetting effect was not apparent for CP that showed the higher content at flowering in both growing seasons.

In the first growing season, differences of most CHO components, except soluble sugars, between fresh and field-cured forage were not significant. In the second growing season, however, differences were significant, but it seems more the result of DM reallocation of different components than to respiration loss. The only fraction with consistently lower content in field-cured forage than in fresh forage was starch, while soluble sugars showed a contrasting trend between the first and the second growing season. Alzueta et al. (1995) have reported that while other soluble sugars may decrease from fresh to field-cured common vetch forage, sucrose may increase during drying.

The results in Table 2 illustrate that SC including lignin, and NFC are evenly distributed in common vetch DM, structural carbohydrate being greater than NFC in the first season and lower in the second. This can be explained by the higher proportion of grain in the second growing season with corresponding higher starch content in the DM. The TC increased significantly ( $P < 0.001$ ) from flowering to the seed filling 2 across seasons and harvest forms, but TC remained unchanged in the first growing season and decreased significantly from fresh to field-cured forage in the second.

When TC were fractionated (Table 2), the SC remained unchanged as maturity progressed in the first

**Table 1. Chemical composition of common vetch fresh and field-cured forage at three maturity stages.**

Item	Maturity stage†						SE	Significance‡		
	Flowering		Seed filling (phase 1)		Seed filling (phase 2)			MS	HF	MS $\times$ HF
	FF§	FCF	FF	FCF	FF	FCF				
g kg <sup>-1</sup> DM										
1996–1997										
Crude protein	221.0	221.1	200.8	209.3	189.2	184.2	6.60	**	NS	NS
Crude fat	25.6	17.1	25.7	19.4	13.2	13.4	0.45	***	***	***
Ash	131.1	130.3	101.5	119.6	102.6	102.6	4.72	***	NS	NS
NDF¶	344.3	373.9	358.4	391.6	427.6	390.3	5.73	***	NS	**
ADF	264.1	249.8	260.8	285.3	301.5	268.6	4.91	**	NS	**
ADC	212.7	193.5	205.2	224.1	232.6	209.9	4.70	NS	NS	*
ADL	46.9	53.5	55.9	63.2	69.7	59.8	2.29	***	NS	**
Starch	14.5	16.2	40.3	14.0	61.1	51.6	5.40	***	NS	NS
Sugars	88.1	117.3	117.0	133.1	67.9	85.0	6.08	***	*	NS
1997–1998										
Crude protein	198.2	219.4	173.5	184.8	169.1	190.6	2.36	***	***	NS
Crude fat	23.4	18.9	11.9	16.5	16.2	13.1	0.69	***	NS	***
Ash	86.7	103.6	74.4	87.5	72.3	77.3	2.73	***	***	NS
NDF¶	345.7	351.7	338.4	405.9	324.3	352.1	7.58	*	**	*
ADF	250.6	248.6	236.3	287.8	221.5	237.5	6.17	*	*	*
ADC	199.7	192.2	188.6	224.6	173.9	183.1	4.37	**	*	*
ADL	54.3	48.8	55.0	71.6	50.0	57.1	1.95	**	*	**
Starch	39.9	11.4	128.6	35.4	167.0	135.0	5.74	***	***	**
Sugars	139.6	137.2	104.4	117.4	94.6	52.2	3.81	***	*	***

\* Significant at the 0.05 level.

\*\* Significant at the 0.01 level.

\*\*\* Significant at the 0.001 level.

† Seed filling (phase 1) = 280 g DM kg<sup>-1</sup> seed; seed filling (phase 2) = 380 g DM kg<sup>-1</sup> seed.

‡ MS = maturity stage effect; HF = harvest form effect; MS  $\times$  HF = interaction effect; SE = standard error of a mean ( $n = 3$ ); NS = not significant.

§ FF = fresh forage; FCF = field-cured forage.

¶ NDF = neutral-detergent fiber; ADF = acid-detergent fiber; ADC = acid-detergent cellulose; ADL = acid-detergent lignin.

growing season and decreased significantly ( $P < 0.01$ ) at seed filling phase 2 in the second season. Correspondingly, the NFC fraction remained unchanged in the first growing season and increased significantly ( $P < 0.01$ ) at seed filling phase 2 during the second.

These results illustrate the combined effects of morphological changes and photosynthetic activity during seed filling. The general trend toward higher SC as maturity progressed was partially compensated for by higher proportion of seed in DM and starch accumulation on the seed fraction (Caballero et al., 1998). At seed filling phase 2, this compensatory effect was more apparent in the second (full pod proportion of 600 g kg<sup>-1</sup> DM) than in the first (full pod proportion of 310 g kg<sup>-1</sup> DM) growing season.

The biological significance of the conventional TC fractionation is not clear. Although the NFC fraction may represent the digestible fraction of TC, the rate of ruminal degradation of two of its main components (starch and total soluble sugars) is very different. Simple soluble sugars are rapidly fermented within the rumen but starch must first be degraded to simple sugars before it can be fermented (Nocek and Tamminga, 1991). In a protein-rich forage legume such as common vetch, the proportion of rapidly fermented soluble sugars relative to the CP content can affect the efficiency of microbial protein synthesis and forage CP utilization, as legumes presented a much lower soluble sugars/CP ratio than grasses (Givens, 1993).

The large difference found between NFC and sugars plus starch suggests a large *soluble fiber fraction* (non-starch polysaccharides soluble in neutral detergent) in common vetch (Tables 1 and 2). With our fractionation system, organic acids were not discounted, but legume forage tend to be high in pectic substances (Van Soest,

1994). Our results showed that common vetch as fresh or field-cured forage contained a soluble fiber fraction higher than the starch or soluble sugars and unrelated to the maturity stage (means of 178 and 172 g kg<sup>-1</sup> DM for fresh and field-cured samples across maturity and years, respectively). Pectic substances are rapidly and completely degraded within the rumen (White et al., 1993), but the rate of fermentation and utilization by rumen microorganisms of their degradation products cannot compare with soluble sugars (Dehority, 1993).

The CNCPS system stresses the availability of NFC fractions as estimated by the rate of fermentation. In forages, none of the soluble sugars escapes ruminal degradation (Sniffen et al., 1992). The rate of fermentation of CHO fraction B<sub>1</sub> may reach 25 to 40% h<sup>-1</sup>, but two of its components (starch and pectic substances) may differ in functionality. The rate of digestion of legumes starch is lower than that of cereal grains and, while pectic substances are completely degraded in the rumen, some starch may escape ruminal fermentation (Nocek and Tamminga, 1991). Although pectic substances can make a high contribution to CHO fraction B<sub>1</sub>, it is questionable whether these components can act as substitutes of soluble sugars in their ability to provide a readily available source of energy to the rumen microorganisms. Many cellulolytic bacteria have pectinolytic activities but cannot utilize the resulting oligogalacturonides and galacturonic acid to support their own growth (Dehority, 1993). Our results showed that at seed filling phase 2, in the second growing season, starch concentration reached a mean of 151 g DM kg<sup>-1</sup> across harvest forms (Table 1), but the concentration of CHO fraction B<sub>1</sub> was 340 g DM kg<sup>-1</sup> (Table 2). These results showed that even at the higher starch concentration, the common vetch plant may contain a substantial concentration

**Table 2. Carbohydrate fractions (Cornell System) of common vetch fresh and field-cured forage at three maturity stages.**

Item	Maturity stage†						SE	Significance‡		
	Flowering		Seed filling (phase 1)		Seed filling (phase 2)			MS	HF	MS × HF
	FF§	FCF	FF	FCF	FF	FCF				
<b>1996–1997</b>										
Total carbohydrates, g kg <sup>-1</sup> DM	622.4	630.5	672.0	651.7	695.0	700.0	10.55	***	NS	NS
SC¶, g kg <sup>-1</sup> TC	532.8	532.3	505.5	569.6	573.5	521.4	13.59	NS	NS	**
NFC, g kg <sup>-1</sup> TC	467.2	467.7	494.5	430.4	426.5	478.6	13.59	NS	NS	**
Carbohydrate fractions, g kg <sup>-1</sup> TC										
A#	141.6	185.2	174.7	204.1	97.7	121.7	12.41	***	**	NS
B <sub>1</sub>	325.6	282.5	319.8	226.3	328.8	356.8	16.93	**	*	*
B <sub>2</sub>	352.2	328.1	305.3	337.0	332.6	316.2	9.96	NS	NS	*
C	180.6	204.2	200.2	232.6	240.9	205.2	8.42	**	NS	**
<b>1997–1998</b>										
Total carbohydrates, g kg <sup>-1</sup> DM	691.7	658.1	740.2	711.2	742.4	719.0	4.52	***	***	NS
SC, g kg <sup>-1</sup> TC	476.3	503.6	434.2	530.7	412.7	456.0	15.84	**	**	NS
NFC, g kg <sup>-1</sup> TC	523.7	496.4	565.8	469.3	587.3	544.0	15.84	**	**	NS
Carbohydrate fractions, g kg <sup>-1</sup> TC										
A	201.9	208.5	141.0	165.0	127.4	72.5	7.22	***	NS	***
B <sub>1</sub>	321.8	287.9	424.9	304.3	459.9	471.6	14.59	***	**	**
B <sub>2</sub>	288.1	325.5	255.6	289.1	250.9	265.2	8.66	***	**	NS
C	188.3	178.1	178.5	241.7	161.8	190.8	9.90	*	**	*

\* Significant at the 0.05 level.

\*\* Significant at the 0.01 level.

\*\*\* Significant at the 0.001 level.

† Seed filling (phase 1) = 280 g DM kg<sup>-1</sup> seed; seed filling (phase 2) = 380 g DM kg<sup>-1</sup> seed.

‡ MS = maturity stage effect; HF = harvest form effect; MS × HF = interaction effect; SE = standard error of a mean ( $n = 3$ ); NS = not significant.

§ FF = fresh forage; FCF = field-cured forage.

¶ SC = structural carbohydrates including lignin; NFC = nonfiber carbohydrates, including starch and pectins.

# A = carbohydrate compounds instantaneously degraded in the rumen; B<sub>1</sub>, B<sub>2</sub> = intermediately and slowly degraded, respectively; C = unavailable fiber.

of pectic substances. The diversity, amounts, and differences in functionality of the CHO fraction B<sub>1</sub> stress the requirement of further investigation in vetch.

The CHO fraction A decreased ( $P < 0.001$ ) with advancing maturity stages during both seasons. From flowering to the seed filling phase 2, CHO fraction A decreased by 31% in fresh forage and by 34% in field-cured forage in the first growing season. Corresponding values in the second growing season were 37 and 65%, respectively (Table 2). Caballero et al. (1998) reported decreasing values of soluble sugars as maturity advanced, within the seed filling phase, that were mostly attributable to the decreasing contents of the seed and stem plant parts.

The relative proportion of CHO fractions A and B<sub>1</sub> in fresh and field-cured vetch were related, as CHO fraction B<sub>1</sub> is derived from NFC by difference of CHO fraction A. However, this relation varied with maturity. At flowering and at seed filling phase 1, the proportion of CHO fraction A was higher for field-cured forage compared with fresh forage while the proportion of CHO fraction B<sub>1</sub> decreased accordingly. At seed filling phase 2, however, the relative proportions of CHO fractions A and B<sub>1</sub> may depend on the proportion of starch-rich seed on DM. The CHO fraction B<sub>1</sub> was higher in the second than in the first growing season due to the effect of higher seed proportion in the former (Table 2). Higher starch concentration did not relate with proportional decrease of soluble sugars (CHO fraction A), because most starch is derived from photosynthesis and not redistributed from soluble sugars (Caballero et al., 1998).

The proportion of CHO fraction B<sub>2</sub> on TC was not affected by drying in the first growing season, but it had a significant effect in the second ( $P < 0.01$ ). As the proportion of plant parts did not vary between fresh

and field-cured samples, this is probably a redistribution effect of other TC components, mainly starch. At flowering and at seed filling phase 1, CHO fraction B<sub>1</sub> decreased significantly from fresh to field-cured forage and, accordingly, CHO fraction B<sub>2</sub> increased. At seed filling phase 2, the differences between fresh and field-cured samples were not significant, (Table 2). During this latter phase, differences in SC content are more attributable to redistribution of components and less to accumulation (Caballero et al., 1998).

The undegraded and undigestible fraction of TC (CHO fraction C) represented 211 and 190 g kg<sup>-1</sup> of TC in the first and second growing season, respectively, across maturity and harvest form (Table 2). The CNCPS model used this value for predicting the indigestible NDF using a linear equation. Traxler et al. (1998) have reported that this equation under-predicted daily gain in cattle (*Bos taurus*) because it overestimated the indigestible NDF fraction. Validation tests in sheep are much needed (Cannas, 2000).

### Crude Protein Fractions

The CP concentration of fresh and field cured common vetch forage did not vary in the first growing season and differed ( $P < 0.001$ ) in the second, being higher in the field-cured forage (Table 1). This can be the result of lower NFC in the field-cured samples of the second growing season and correspondingly higher relative proportion of other chemical components (CP and SC). In both seasons, the maturity effect was significant ( $P < 0.01$ ) and the interaction effect (harvesting stage × harvest form) was not significant. Across maturities and seasons, the CP concentration of fresh and field-cured common vetch forage showed an average value of 200 g kg<sup>-1</sup> of DM.

The harvest form did not affect the relative propor-

**Table 3. Crude protein fractions (Cornell System) of common vetch fresh and field-cured forage at three maturity stages.**

Item	Maturity stage†						SE	Significance‡		
	Flowering		Seed filling (phase 1)		Seed filling (phase 2)			MS	HF	MS × HF
	FF§	FCF	FF	FCF	FF	FCF				
<b>1996–1997</b>										
Crude protein, g kg <sup>-1</sup> DM	221.0	222.1	200.8	209.3	189.2	184.2	6.60	**	NS	NS
Protein fractions, g kg <sup>-1</sup> CP										
A¶	211.3	268.3	329.5	370.2	256.2	294.2	22.31	*	NS	NS
B <sub>1</sub>	215.0	145.0	185.6	137.3	236.1	234.5	16.15	**	NS	NS
B <sub>2</sub>	516.3	466.0	389.4	394.8	370.0	334.0	12.90	***	NS	NS
B <sub>3</sub>	19.0	69.1	49.0	42.9	75.0	78.3	4.92	**	*	**
C	38.4	51.6	46.6	54.9	62.4	59.1	2.89	**	NS	NS
<b>1997–1998</b>										
Crude protein, g kg <sup>-1</sup> DM	198.2	219.4	173.5	184.8	169.1	190.6	2.36	***	***	NS
Protein fractions, g kg <sup>-1</sup> CP										
A	181.3	293.4	262.9	286.3	173.8	181.8	14.18	**	*	*
B <sub>1</sub>	308.1	158.5	238.7	176.4	304.1	342.3	16.17	***	**	**
B <sub>2</sub>	428.9	455.1	399.7	383.2	416.4	347.6	13.61	**	NS	*
B <sub>3</sub>	38.7	50.9	56.0	85.5	56.0	68.7	2.33	***	***	*
C	43.1	42.1	42.7	68.5	49.7	59.6	3.58	NS	*	NS

\* Significant at the 0.05 level.

\*\* Significant at the 0.01 level.

\*\*\* Significant at the 0.001 level.

† Seed filling (phase 1) = 280 g DM kg<sup>-1</sup> seed; seed filling (phase 2) = 380 g DM kg<sup>-1</sup> seed.

‡ MS = maturity stage effect; HF = harvest form effect; MS × HF = interaction effect; SE = standard error of a mean ( $n = 3$ ); NS = not significant.

§ FF = fresh forage; FCF = field-cured forage.

¶ A = nonprotein nitrogen compounds instantaneously degraded in the rumen; B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> = rapidly, intermediately and slowly degraded protein, respectively; C = bound protein undegraded in the rumen and indigestible in the intestine.

tion of CP fraction A within maturity stages (Table 3). The field-cured forage always contained a higher concentration of CP fraction A than the fresh forage. This general trend was found across maturities and growing seasons. Harvesting common vetch at the recommended seed filling 2 maturity stage resulted in a lower proportion of CP fraction A in the second (higher seed proportion on DM), but not in the first growing season. Conversely, the CP fraction B<sub>1</sub> showed a decreasing trend from fresh to field-cured forage and the effect of maturity was significant ( $P < 0.01$ ). The relative proportion of CP fraction B<sub>1</sub> was higher at seed filling 2 than at the two previous harvesting stages. As a whole, CP fraction A increased from fresh to field-cured forage and CP fraction B<sub>1</sub> increased with maturity and decreased from fresh to dried. Both soluble fractions, the instantaneously fermented (CP fraction A) and the rapidly fermented (CP fraction B<sub>1</sub>), represented together between 410 and 530 g kg<sup>-1</sup> of total CP.

The CP fraction B<sub>2</sub> represented the greatest proportion of total CP with values ranging from 334 g kg<sup>-1</sup> in dry at seed filling 1 in the first growing season to 516 g kg<sup>-1</sup> of total CP in fresh forage at flowering in the first growing season (Table 3). Fraction B<sub>2</sub> decreased with maturity and was not affected by harvest form. Conversely, CP fraction B<sub>3</sub> increased at seed filling phase 2 and always constituted <100 g kg<sup>-1</sup> of total CP. The undegraded and indigestible CP fraction C made up some 50 g kg<sup>-1</sup> of total CP. These results illustrate that common vetch CP is likely to be largely degraded in the rumen. The available CP fraction should include the majority of CP fraction B<sub>3</sub> and a small proportion of CP fraction B<sub>2</sub>. The CP fraction B<sub>3</sub> presented a higher proportion of total insoluble CP (fractions B<sub>2</sub>, B<sub>3</sub>, and

C) at seed filling phase 2, showing the advantage of delaying harvesting.

As CP fraction B<sub>3</sub> represents CP insoluble in NDF but soluble in ADF, the proportion of CP fraction B<sub>3</sub> is linked to NDF (Elizalde et al., 1999). For the same reason, values of CP fraction B<sub>3</sub> are usually greater in grasses than in forage legumes at comparative growth stages and, within forage species, values of CP fraction B<sub>3</sub> are usually lower in fresh forage than in the corresponding field-cured forage (Sniffen et al., 1992; Agbosamey et al., 1998). Our results with common vetch showed a similar pattern in the two growing seasons (Table 3). The compensatory effect of the grain proportion on CP fraction B<sub>3</sub> was more apparent in the second growing season than in the first season. The CP fraction B<sub>3</sub> increased at seed filling phase 2 but to a lesser extent in the second than in the first growing season. In the second growing season, the unavailable and indigestible CP fraction C remained unchanged with maturity. As a whole, a compensatory effect of the grain proportion was not found for this latter fraction (Table 3). Andrés (1990) indicated an estimated CP ruminal degradation from grain samples of common vetch of 75% to which would correspond to an effective CP degradability of common vetch grain of 77.7% (Rodríguez et al., 1999). These results may suggest that grain-rich samples had little influence on CP degradation. Higher CP fraction B<sub>3</sub> at seed filling phase 2 in our samples seems to be the result of higher NDF in the vegetative components.

### Amino Acid Pattern of Vetch Protein

Information on essential amino acid (EAA), semies-  
sential amino acid (SEAA), and nonessential amino

Table 4. Amino acid composition of common vetch fresh and field-cured forage at three maturity stages (1996–1997).

	Maturity stage†						SE	Significance‡		
	Flowering		Seed filling (phase 1)		Seed filling (phase 2)			MS	HF	MS × HF
	FF§	FCF	FF	FCF	FF	FCF				
	g kg <sup>-1</sup> CP									
<b>Essential</b>										
Arginine	60.2	72.1	55.8	59.2	68.7	61.9	2.97	*	NS	*
Histidine	18.6	18.5	16.9	15.8	19.6	18.5	0.83	*	NS	NS
Isoleucine	41.2	39.7	32.4	31.1	33.8	32.6	1.65	***	NS	NS
Leucine	71.5	64.0	54.3	50.6	56.0	55.4	2.73	***	NS	NS
Lysine	48.0	42.9	38.8	35.4	40.7	40.7	1.90	**	NS	NS
Methionine	9.5	14.0	9.0	10.5	10.6	13.6	0.57	**	***	NS
Phenylalanine	46.6	46.5	36.9	34.9	37.0	37.5	1.91	***	NS	NS
Threonine	48.4	43.8	39.3	38.2	40.2	37.5	1.88	**	NS	NS
Valine	49.8	50.1	40.3	41.1	41.2	44.5	2.12	**	NS	NS
<b>Semiessential</b>										
Cystine	33.5	33.6	29.4	22.5	30.7	30.4	1.46	***	NS	NS
Tyrosine	37.6	37.7	28.9	27.2	30.7	30.4	1.54	***	NS	NS
<b>Nonessential</b>										
Alanine	51.6	51.7	42.3	41.1	43.9	43.4	2.16	**	NS	NS
Aspartic acid	145.7	145.9	150.4	142.9	151.2	136.3	6.56	NS	NS	NS
Glutamic acid	110.9	111.1	98.1	92.7	121.0	127.6	5.19	**	NS	NS
Glycine	43.4	43.5	35.4	33.4	37.5	36.4	1.81	***	NS	NS
Serine	45.7	45.8	39.3	37.7	42.3	42.3	1.98	*	NS	NS

\* Significant at the 0.05 level.

\*\* Significant at the 0.01 level.

\*\*\* Significant at the 0.001 level.

† Seed filling (phase 1) = 280 g DM kg<sup>-1</sup> seed; seed filling (phase 2) = 380 g DM kg<sup>-1</sup> seed.

‡ MS = maturity stage effect; HF = harvest form effect; MS × HF = interaction effect; SE = standard error of a mean ( $n = 3$ ); NS = not significant.

§ FF = fresh forage; FCF = field-cured forage.

acid (NEAA) composition of vetch CP can be relevant for comparison with other studies on CP fractionation. Also, until EAA needs of sheep have been quantified, information about EAA requirement and optimality of EAA pattern of vetch CP must be inferred from comparison with the EAA profile of sheep products (milk, tissue, and wool) or from comparison with the amino acid profile of extensively cultivated forage such as alfalfa (NRC, 1982).

The vetch CP amino acid pattern is presented in Table 4. The mean vetch CP amino acid profile across maturity and harvest forms showed some differences with the alfalfa profile (Broderick, 1994). Within the EAA group, arginine was higher in vetch than in alfalfa (63 vs. 46 g kg<sup>-1</sup> of CP) while the opposite occurred with lysine (41 vs. 51 g kg<sup>-1</sup> of CP). Within the SEAA group, the cystine concentration of vetch CP was twofold (30 vs. 15 g kg<sup>-1</sup> of CP). This fact can be of importance as cystine may spare the low content of both forage species in methionine (11 and 13 g kg<sup>-1</sup> of CP, respectively). Within the NEAA group, the highest concentrations of individual amino acid in vetch CP were found for aspartic acid and glutamic acid (145 and 110 g kg<sup>-1</sup> of CP, respectively).

Maturity had a significant effect on the concentration of individual amino acids and, in general, this concentration was significantly higher at the flowering stage as occurred with CP (Table 4). On the contrary, there were no significant differences between the two harvest forms for individual amino acid concentrations, except for methionine which showed higher concentration in field cured than in fresh samples.

### IMPLICATION SUMMARY

Our research shows that common vetch forage should be harvested near seed filling. The total carbohydrate and crude protein fractions of common vetch were influenced more by maturity than by the process of field curing.

The ratio of structural to nonfiber carbohydrates (SC/NFC) changed little with maturity in the first growing season (1.14 at flowering to 1.21 at seed filling phase 2). However, higher starch-rich seed proportion in the second growing season, determined a decrease of the SC/NFC ratio with maturity from 0.97 at flowering to 0.77 at seed filling phase 2.

The CP fraction B<sub>2</sub> was the largest single CP fraction in fresh and field-cured vetch forage and the available CP fraction B<sub>3</sub> escaping ruminal fermentation accounted for <100 g kg<sup>-1</sup> of total CP. The CP fraction B<sub>3</sub> increased with maturity, reinforcing the advantage of delaying harvesting if reduced ruminal protein degradation is an objective.

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### REFERENCES

- Agbassamey, Y.R., P. Savoie, and J.R. Seoane. 1998. Effect of maceration on nitrogen fractions in field cured forage and silage. *Can. J. Anim. Sci.* 78:399–405.
- Alzueta, C., A. Rebolé, C. Barro, J. Treviño, and R. Caballero. 1995. Changes in nitrogen and carbohydrate fractions associated with the field drying of vetch (*Vicia sativa* L.). *Anim. Feed Sci. Technol.* 52:249–255.
- Andrés, S.G. 1990. Efecto de las características físico-químicas de los concentrados proteicos sobre la degradabilidad ruminal de sus materias nitrogenadas. Effect of physico-chemical properties of some protein-rich feeds on the ruminal degradability of its nitrogen components. Tesis doctoral. Universidad Politécnica de Madrid, Spain.
- Association of Official Analytical Chemists (AOAC International). 1995. Official methods of analysis. In P. Kunniff (ed.) AOAC Int., Arlington, VA.
- BMDP. 1992. Statistical Software Inc. Univ. of California Press, Los Angeles, CA.
- Broderick, G.A. 1994. Quantifying forage protein quality. p. 200–228. In G.C. Fahey, Jr. (ed.) Forage quality, evaluation, and utilization. ASA, CSSA, and SSSA, Madison, WI.
- Caballero, R. 1993. An experts' survey on the role of legumes in arable cropping systems of the Mediterranean area. *J. Sust. Agric.* 3:133–154.
- Caballero, R., C. Barro, A. Rebolé, M. Arauzo, and P.J. Hernaiz. 1996. Yield components and forage quality of common vetch during pod filling. *Agron. J.* 88:797–800.
- Caballero, R., A. Rebolé, C. Barro, C. Alzueta, and L.T. Ortiz. 1998. Aboveground carbohydrate and nitrogen partitioning in common vetch during seed filling. *Agron. J.* 90:97–102.
- Cannas, A. 2000. Sheep and cattle nutrient requirement systems, ruminal turnover, and adaptation of the Cornell Net Carbohydrate and Protein System to sheep. Ph.D. diss. Cornell Univ., Ithaca, NY (Diss. Abstr. AAT 99-78206).
- Dehority, B.A. 1993. Microbial ecology of cell wall fermentation. p. 425–453. In H.G. Jung et al. (ed.) Forage cell wall structure and digestibility. ASA, CSSA, and SSSA, Madison, WI.
- Elizalde, J.C., N.R. Merchen, and D.B. Faulkner. 1999. Fractionation of fiber and crude protein content in fresh forages during spring growth. *J. Anim. Sci.* 77:476–484.
- Fox, D.G., M.C. Barry, R.E. Pitt, D.K. Roseler, and W.C. Stone. 1995. Application of the Cornell Net Carbohydrate and Protein Model for cattle consuming forage. *J. Anim. Sci.* 73:267–277.
- Givens, D.J. 1993. Evaluating energy and protein in grass and grass silage. *Grass Farmer* 45:26–28.
- Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analysis. USDA Handb. 379. U.S. Gov. Print. Office, Washington, DC.
- Hintz, R.W., and K.A. Albrecht. 1994. Dry matter partitioning and forage nutritive value of soybean plant components. *Agron. J.* 84: 59–62.
- Jones, B.N., S. Pääbo, and S. Stein. 1981. Amino acid analysis and enzymatic sequence determination of peptides by an improved o-phthalaldehyde precolumn labeling procedure. *J. Liq. Chromatogr.* 4:565–586.
- Licitra, G., M.T. Hernández, and P.J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.* 57:347–358.
- Longstaff, M.A., and J.M. McNab. 1991. The effect of concentration of tannin-rich bean hulls (*Vicia faba* L.) on activities of lipase (EC 3.1.1.3) and  $\alpha$ -amylase (EC 3.2.1.1) in digesta and pancreas and on the digestion of lipid and starch by young chicks. *Br. J. Nutr.* 66:139–147.
- Masoero, F., M. Moschini, F. Rossi, and G. Piva. 1999. Estimate of feed rumen degradable proteins by using the CNCPS carbohydrate and protein fractions (abstract). *J. Anim. Sci.* 77(supp. 1):250.
- Moore, S. 1963. On determination of cystine as cysteic acid. *J. Biol. Chem.* 238:235–237.
- Nocek, J.E., and S. Tamminga. 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. *J. Dairy Sci.* 74:3598–3629.
- National Research Council. 1982. United States–Canadian tables of

- feed composition. 3rd rev. National Academy Press, Washington, DC.
- Robertson, J.B., and P.J. Van Soest. 1981. The detergent system of analysis and its application to human food. p. 123–158. In W.P.T. James and O. Theander (ed.) The analysis of dietary fiber in foods. Marcel Dekker, New York.
- Rodríguez, C.A., J. González, M.R. Alvir, and C. Cajarville. 1999. Underestimation of in situ effective degradability of N due to microbial contamination. p. 67. In G.E. Lobley et al. (ed.) 8th Int. Symp. on Prot. Met. and Nutr., Aberdeen. 1–4 Sept. 1999. Wageningen Pers., the Netherlands.
- Sniffen, C.J., J.D. O'Connor, P.J. Van Soest, D.G. Fox, and J.B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II Carbohydrate and protein availability. J. Anim. Sci. 70:3562–3577.
- Traxler, M.J., D.G. Fox, P.J. Van Soest, A.N. Pell, C.E. Lascano, D.P.D. Lanna, J.E. Moore, R.P. Lana, M. Vélez, and A. Flores. 1998. Predicting forage indigestible NDF from lignin concentration. J. Anim. Sci. 76:1469–1480.
- Van Soest, P.J. 1994. Nutritional ecology of the ruminant. 2nd ed. Cornell Univ. Press, Ithaca, NY.
- Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.
- White, B.A., R.J. Mackie, and K.C. Doerner. 1993. Enzymatic hydrolysis of forage cell walls. p. 465–484. In H.G. Jung et al. (ed.) Forage cell wall structure and digestibility. ASA, CSSA, and SSSA, Madison, WI.
- Yemm, E.W., and A.U. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57:508–514.

## Performance of 15 *Miscanthus* Genotypes at Five Sites in Europe

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### ABSTRACT

*Miscanthus* is a genus of high-yielding perennial rhizomatous grasses with C<sub>4</sub> photosynthesis. Extensive field trials of *Miscanthus* spp. biomass production in Europe during the past decade have shown several limitations of the most widely planted clone, *M. × giganteus* Greef et Deu. A 3-yr study was conducted at five sites in Europe (Sweden, Denmark, England, Germany, and Portugal) to evaluate adaptation and biomass production potential of four acquisitions of *M. × giganteus* (No. 1–4) and 11 other genotypes, including *M. sacchariflorus* (Maxim.) Benth. (No. 5), *M. sinensis* Andersson (No. 11–15), and hybrids (No. 6–10). At each site, three randomized blocks containing a 5- by 5-m plot of each genotype were established (except in Portugal where there were two blocks) with micropropagated plants at 2 plants m<sup>-2</sup>. In Sweden and Denmark, only *M. sinensis* and its hybrids satisfactorily survived the first winter following planting. Mean annual yields across all sites for all surviving genotypes increased each year from 2 t ha<sup>-1</sup> dry matter following the first year of growth to 9 and 18 t ha<sup>-1</sup> following the second and third year, respectively. Highest autumn yields at sites in Sweden, Denmark, England, and Germany were 24.7 (*M. sinensis* hybrid no. 8), 18.2 (*M. sinensis* hybrid no. 10), 18.7 (*M. × giganteus* no. 3), and 29.1 t ha<sup>-1</sup> (*M. × giganteus* no. 4), respectively. In Portugal, where irrigation was used, the top-yielding genotype produced 40.9 t ha<sup>-1</sup> dry matter (*M. sinensis* hybrid no. 7). Highest-yielding genotypes in Sweden and Denmark were among the lowest yielding in Portugal and Germany, demonstrating strong genotype × environment interactions.

**M**ISCANTHUS × *giganteus* was introduced to Europe in the 1930s by Aksel Olsen and was observed

to have exceptionally vigorous growth (Linde-Laursen, 1993). In the late 1980s, interest in C<sub>4</sub> perennial rhizomatous grasses, such as *Miscanthus* spp. (Nielsen, 1987), switchgrass (*Panicum virgatum* L.) (Christian, 1994), *Cyperus* spp., and *Spartina* spp. (Potter et al., 1995), for biofuel production increased due to their high yield potential and rising energy prices. Since 1983, extensive field trials of *M. × giganteus* have been carried out in northern Europe, showing the capacity of this genotype for yields >20 t dry matter ha<sup>-1</sup> year<sup>-1</sup> (Nielsen, 1987; Schwarz et al., 1994).

There are several reasons why European-wide biomass production from a single genotype within the *Miscanthus* genus is inadequate. First, in northern Europe, a number of sites established with *M. × giganteus* failed to survive during the first winter (Jones and Walsh, 2001), principally due to insufficient freeze tolerance of the overwintering rhizome. Second, it is unlikely that one single clone is sufficient to fulfil all of the quality requirements of different uses (combustion and fiber). Third, *M. × giganteus*, being a sterile triploid (Greef and Deuter, 1993), must be propagated vegetatively, either with rhizome cuttings or by micropropagation, making establishment expensive compared with crops established from seed. Fourth, growing large areas of a single clone increases disease risk. A broad genetic base and the provision of different *Miscanthus* genotypes are required to overcome these limitations.

As part of the European *Miscanthus* Improvement Project, a *Miscanthus* gene pool was created by combining collections directly from Asia and material already made available in Europe by German, Danish, and Swedish breeders. *M. sinensis* is characterized by a tuft-forming rhizome with high shoot densities while *M. sacchariflorus* is characterized by a broad, creeping rhizome with thick tall stems. *M. × giganteus* shows an intermediate type of rhizome between *M. sinensis* and *M. sacchariflorus* and is most probably a natural hybrid of the two (Greef and Deuter, 1993; Hodkinson et al., 1997).

In this paper, we report on field trials planted with 15 *Miscanthus* genotypes, which can be broadly divided

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