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# Crude protein fractions in common vetch (*Vicia sativa* L.) fresh forage during pod filling<sup>1</sup>

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**ABSTRACT:** Crude protein (CP) of forages can be separated into fractions of differentiated abilities to provide available amino acids in the lower gut of ruminants. This knowledge is critical to develop feeding systems and to predict animal responses. We have measured during two growing seasons (1996 to 1997 and 1997 to 1998) the CP fractions of common vetch fresh forage with the objective being to assess the influence of maturity on concentration of CP fractions (as a percentage of total CP) and fraction yields. Fraction B<sub>2</sub>, which represents true protein of intermediate ruminal degradation rate, was the largest single fraction in com-

mon vetch forage (about 40% of CP across seasons and maturity stages). Soluble fractions (A plus B<sub>1</sub>) were less than 50% of total CP while the unavailable fraction C ranged from 4 to 8% of total CP. As a result, the remaining fraction B<sub>3</sub> (true protein of very low degradation rate) only represented 2 to 9% of total CP. Concentration and yield of fraction B<sub>3</sub> increased ( $P < 0.05$ ) from flowering to pod-filling. Results showed that undegraded dietary protein represented a small proportion of total CP in common vetch forage. Moving the harvesting stage from flowering to the pod filling phase allowed for greater yield of undegraded dietary protein.

Key Words: Forage, Protein, Vetch, *Vicia sativa*

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## Introduction

Common vetch (*Vicia sativa* L.) is a leguminous, annual, and commonly used forage in the Mediterranean basin and West Asia in rotation with winter cereal small grains, under rainfed conditions. This rotation facilitates the integration of small ruminants into agricultural systems as it contributes to meeting the structural forage deficit that is linked to the seasonality of other feed sources (natural pastures, cereal stubbles, or shrub-steppe vegetation). Feed legumes provide grazing in spring and early summer or hay, straw, and seed for the periods of forage deficit, such as winter (Thomson and Bahhady, 1992).

Our ultimate goal was to establish quality standards that would facilitate commercial pricing and complementary use of ingredients for specific animal requirements. A standard based on CP and detergent fiber

concentration has been developed (Caballero et al., 1996b), but this standard should be refined by an evaluation of CP fractions (Sniffen et al., 1992; Broderick, 1995). Quantification of various CP fractions has not been extensively conducted on fresh forage, and specific data on common vetch forage are not reported.

Seeds of annual legumes, used as forage, contribute to an essential proportion of the harvesting biomass (Hintz et al., 1992; Caballero et al., 1996a). It is important, however, to assess the balance between increasing the proportion of seed as pod filling progresses and decreasing the quality of vegetative plant parts.

The objective of this research was to assess whether CP concentration and the relative proportion of CP fractions in common vetch forage were affected by the stage of growth (i.e., from flowering to the pod-filling phase).

## Material and Methods

### *Research Site, Forage Practices, and Sampling*

Field studies were conducted under rain-fed conditions at La Poveda Field Station (30 km southeast of Madrid, Spain) on the Castilian Plain (continental Mediterranean climate, elevation = 610 m, 30-yr mean precipitation = 425 mm). The soil is an alluvial sandy-loam

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Typic Xerofluvent with means pH = 7.7, C/N ratio = 7.9, and OM = 17 g/kg.

Common vetch (*Vicia sativa* L. var. Vereda) was sown November 27, 1996, and November 25, 1997, at the rate of 80 kg of seed per hectare in rows with a 0.17-m spacing. Before planting, the experimental field was fertilized with 400 kg/ha of a compound fertilizer (N, 0; P, 6.1; K, 5.8), and linuron [N'-(3,4-dichlorophenyl)-N-methoxy-N-methylurea] was applied before emergence at the rate of 0.875 kg ai/ha for weed control. Common vetch forage was harvested between May and June of the respective following year at three successive growth stages: flowering (more than 50% of plants with flowers) and two stages within the early pod-filling phase. These latter stages were defined by DM concentration in the seed (mean of 28 g DM/100 g and 38 g DM/100 g for pod-filling Phases 1 and 2, respectively). More advanced stages of growth were discarded. Common vetch seed reached full growth before 50 g DM/100 g, and losses of biomass and quality of vegetative parts (leaves and stems) occurred thereafter (Caballero et al., 1996a).

Harvesting stage treatments were distributed in a single-factor completely randomized design with three replications and single plots measuring 4 × 20 m. Forage yields were determined by harvesting a 1.5 × 10-m area out of the center of the plots with a rotary disk forage harvester. Forage was cut approximately 0.05 m above the soil level. Samples of the forage were oven-dried for 22 h at 60°C and, subsequently, for 2 h at 80°C to determine DM content. Plant development was evaluated at the three harvesting stages to determine plant parts of forage samples. Individual plants (20 at flowering, 30 at pod-filling Phase 1, and 20 at pod-filling Phase 2) were selected at random and hand-separated into plant parts. At pod-filling Phase 1, more plants were sampled because of lower pod weight. At flowering, stem and leaf (leaflets plus petioles) were separated. At pod filling, stem, leaf, pod shell, and seed were separated, weighed, and oven-dried for 22 h at 60°C and, subsequently, for 2 h at 80°C to determine DM content. The proportions of plant parts were expressed on a DM basis. Seed moisture content and seed DM weight were conducted twice to evaluate stage of growth before each pod-filling harvesting stage.

Approximately 3 kg of fresh forage was collected at each sampling by cutting at the same height above the soil as the forage harvester. In an aliquot of the samples at the two pod-filling stages, full pods and vegetative parts (stems plus leaves) were hand-separated. Samples were immediately put into plastic bags and frozen at -20°C. The samples were freeze-dried and milled to pass a 1-mm screen before analyses.

#### Fractionation of CP and Chemical Analyses

Fractionation of CP in common vetch forage was conducted according to the Cornell net carbohydrate and protein system (Sniffen et al., 1992). According to this

system, CP is partitioned into three fractions: fraction A is nonprotein nitrogen (NPN × 6.25); fraction B is true protein, and fraction C is unavailable protein. Fraction B is further divided into three subfractions (B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>) that are believed to have different rates of ruminal degradation. Fractions A and B<sub>1</sub> are soluble in phosphate-borate buffer and are rapidly degraded in the rumen. Fraction B<sub>2</sub> is insoluble in the buffer but is soluble in neutral detergent solution and has an intermediate rate of ruminal degradation; some B<sub>2</sub> fraction escapes to the lower gut. Fraction B<sub>3</sub> is insoluble in the buffer and in neutral detergent but is soluble in acid detergent solution. This fraction is more slowly degraded in the rumen than are fractions B<sub>1</sub> and B<sub>2</sub> because it is associated with the cell wall. A high proportion of fraction B<sub>3</sub> escapes ruminal degradation. 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. All these CP fractions, when altered in diets, will have an impact on animal responses (Chalupa and Sniffen, 1994).

Crude protein was determined as Kjeldahl N × 6.25 (AOAC, 1995). Precipitated true protein, buffer-insoluble protein, neutral detergent-insoluble protein (**NDIP**), and acid detergent-insoluble protein (**ADIP**) were analyzed as described by Licitra et al. (1996). Fraction A was calculated as the difference between the total CP and precipitated true protein.

True protein was determined by Kjeldahl analysis of the residue resulting after precipitation with tungstic acid followed by filtration. Licitra et al. (1996) recommended tungstic acid as precipitant because it recovers peptides in the protein precipitate, and, in ruminants, peptides are metabolized similarly to soluble protein. Fraction B<sub>1</sub> was estimated as true protein minus buffer-insoluble protein, fraction B<sub>2</sub> as buffer-insoluble protein minus **NDIP**, and fraction B<sub>3</sub> by subtracting the **ADIP** (fraction C) from the **NDIP**.

#### Statistical Analysis

The variables analyzed were concentrations of total CP and the five CP fractions (on a total CP basis). Yields of each fraction were estimated by the product of concentrations (on a DM basis) by forage yield. Concentrations and yields were analyzed in the whole forage and only concentrations in the full pods and vegetative plant parts (stems plus leaves). The nine experimental plots were randomly assigned to the three harvesting stage treatments in a completely randomized design with three replications. Concentrations and yields were arranged as a single-factor experiment at three fixed levels because we were interested in comparing flowering and two pod-filling stages, just before seed reached full weight (about 50 g DM/100 g of seed). Statistical significance was determined using one-way analysis of variance followed by means comparison at

**Table 1.** Characterization of common vetch forage harvested at three stages of maturity during 2 yr<sup>a</sup>

Item	Flowering		Pod-filling Phase 1		Pod-filling Phase 2	
	1997	1998	1997	1998	1997	1998
Date of harvest	5/28	5/8	6/12	5/20	6/21	6/4
Forage DM content, g/100 g	14.0	20.7	29.6	25.0	33.4	31.9
Seed DM content, g/100 g			30.4	25.6	35.9	40.0
Plant parts proportion, g/100 g, on a DM basis						
Leaf	62.1	55.2	43.0	31.5	31.5	21.4
Stem	37.9	44.8	42.1	34.5	37.6	19.2
Full pods			14.9	34.0	30.9	59.4
Seed weight (mg/seed) on a DM basis <sup>b</sup>			21.7	20.5	33.8	49.3

<sup>a</sup>Each mean based on three replications, except seed weight that was based on 15 groups of 10 seeds selected at random.

<sup>b</sup>Ten-year mean weight of seed at maturity is 55 mg (Caballero et al., 1996a).

a value for *P* of less than 0.05. Analyses of variance were conducted by year as differences between years were found, based on year × treatment interaction and heterogeneity of error. The BMDP statistical package (Program 1V) was used (Statistical Software, Inc., Los Angeles, CA). Data are reported as means, pooled SEM values from the indicated number of plots on each harvesting treatment, and actual *P*-values.

## Results and Discussion

### Characterization of Common Vetch Forage

Morphological characteristics of common vetch forage samples showed differences between 1996 to 1997 and 1997 to 1998 (Table 1). Rainfall during the 2 mo before flowering was the principal determinant of annual legume forage and grain yields in Mediterranean continental environment (Caballero, 1986). During the first growing season (1996 to 1997), rainfall during the late pod-filling phase did not alleviate water stress and poorer filling conditions. As a result, the proportion of full pods in the total forage was lower in the first (1996 to 1997) than in the second (1997 to 1998) growing season. Higher temperatures in the second growing season caused the plants to advance to the flowering stage at a faster rate.

### Crude Protein Concentration and Concentration of CP Fractions

The CP concentration of common vetch forage decreased ( $P < 0.05$ ) during the two seasons as the stage of growth advanced beyond flowering (Table 2). The higher proportion of full pods in pod-filling Phase 2 did not compensate for lower concentration of CP in vegetative plant parts (Table 3) because the concentration of CP in the full pods remained almost unchanged between the two reproductive stages considered (Table

4). This fact has been previously reported (Caballero et al., 1998).

Across growing stages, the soluble fractions (A plus B<sub>1</sub>, as % of total CP) in whole forage averaged 50% for the two growing seasons. Sniffen et al. (1992) reported that soluble fractions averaged 46% of total CP for spring growth in alfalfa. Maturity of common vetch had contrasting effects on fraction A and fraction B<sub>1</sub>. Fraction A decreased ( $P < 0.05$ ) and fraction B<sub>1</sub> increased ( $P < 0.05$ ) from pod-filling Phase 1 to pod-filling Phase 2 in the whole forage of the second season (Table 2), in the vegetative plant parts (Table 3), and in the full pods (Table 4). As a result, the soluble CP changed little between the two reproductive stages. This contrasting pattern between the two soluble CP fractions can be explained by the process of accumulation and distribution of CP during the period of rapid seed growth from pod-filling Phase 1 to pod-filling Phase 2. During this period, most CP accumulated in the seed and CP was redistributed from vegetative structures (Caballero et al., 1998). Fraction A is NPN, whereas fraction B<sub>1</sub> is soluble true protein. As seed makes a larger fraction of total forage, these results suggest that a higher proportion of soluble true protein is accumulated in the seed as compared with NPN or that some NPN of vegetative plant parts is redistributed as true protein to the seed (Petronici and Lotti, 1969).

Across seasons and harvesting stages, results showed that the intermediately degraded fraction B<sub>2</sub> accounted for 40% of the total CP of common vetch forage. During the first growing season (1996 to 1997), fraction B<sub>2</sub> decreased ( $P < 0.001$ ) from flowering to the pod-filling phase. From pod-filling Phase 1 to pod-filling Phase 2, fraction B<sub>2</sub> remained almost unchanged in the whole forage (Table 2) and vegetative plant parts (Table 3), but increased ( $P < 0.01$ ) in the full pods (Table 4). During the second growing season (1997 to 1998), proportions of fraction B<sub>2</sub> at flowering and at pod-filling Phase 2 changed little. A higher proportion of seeds in the second growing season can be the cause of this difference

**Table 2.** Crude protein fractions of common vetch forage at three harvesting stages during 2 yr

Item	Harvesting stage <sup>a</sup>			SEM <sup>b</sup>	P-value
	Flowering	Pod-filling Phase 1	Pod-filling Phase 2		
1996–1997					
Crude protein, g/100 g DM	22.1 <sup>x</sup>	20.1 <sup>xy</sup>	18.9 <sup>y</sup>	0.68	0.0431
Protein fractions, g/100 g CP					
A <sup>c</sup>	21.1 <sup>x</sup>	32.9 <sup>y</sup>	25.6 <sup>xy</sup>	2.91	0.0719
B <sub>1</sub>	21.5 <sup>x</sup>	18.6 <sup>x</sup>	23.6 <sup>x</sup>	2.47	0.4068
B <sub>2</sub>	51.6 <sup>x</sup>	38.9 <sup>y</sup>	37.0 <sup>y</sup>	1.16	0.0002
B <sub>3</sub>	1.9 <sup>x</sup>	4.9 <sup>y</sup>	7.5 <sup>z</sup>	0.69	0.0036
C	3.8 <sup>x</sup>	4.7 <sup>x</sup>	6.2 <sup>y</sup>	0.31	0.0046
1997–1998					
Crude protein, g/100 g DM	19.8 <sup>x</sup>	17.4 <sup>y</sup>	16.9 <sup>y</sup>	0.32	0.0013
Protein fractions, g/100 g CP					
A <sup>c</sup>	18.1 <sup>x</sup>	26.3 <sup>y</sup>	17.4 <sup>x</sup>	2.17	0.0491
B <sub>1</sub>	30.8 <sup>x</sup>	23.9 <sup>y</sup>	30.4 <sup>x</sup>	1.79	0.0581
B <sub>2</sub>	42.9 <sup>x</sup>	40.0 <sup>x</sup>	41.6 <sup>x</sup>	1.22	0.3084
B <sub>3</sub>	3.9 <sup>x</sup>	5.6 <sup>y</sup>	5.6 <sup>y</sup>	0.40	0.0380
C	4.3 <sup>x</sup>	4.3 <sup>x</sup>	5.0 <sup>x</sup>	0.33	0.3108

<sup>a</sup>Pod-filling Phase 1 = 28 g DM/100 g seed; pod-filling Phase 2 = 38 g DM/100 g seed (seasons means, Table 1).

<sup>b</sup>SEM = Pooled standard error of the mean for each treatment (n = 3).

<sup>c</sup>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.

<sup>d</sup>Within a row, means without a common superscript letter differ (P < 0.05).

between growing seasons. As indicated in Table 4, fraction B<sub>2</sub> of full pods increased significantly in the 2 yr when seed accounted for a greater proportion of total forage. These results illustrate the fact that although the CP concentration was higher in the seed than in

the vegetative plant parts, the proportion of the main CP fraction B<sub>2</sub> changed little. Maturity had a greater effect on CP concentration than on the proportion of the main CP fraction among plant parts, similar to results of Elizalde et al. (1999) with alfalfa.

**Table 3.** Crude protein fractions of common vetch vegetative plant parts (stems plus leaves) at two harvesting stages during 2 yr

Item	Harvesting stage <sup>a</sup>		SEM <sup>b</sup>	P-value
	Pod-filling Phase 1	Pod-filling Phase 2		
1996–1997				
Crude protein, g/100 g DM	17.1	15.1	0.46	0.0405
Protein fractions, g/100 g CP				
A <sup>c</sup>	38.0	23.3	1.38	0.0017
B <sub>1</sub>	11.8	19.4	1.62	0.0291
B <sub>2</sub>	37.3	40.7	1.51	0.1849
B <sub>3</sub>	6.2	9.7	0.53	0.0097
C	6.7	6.9	0.28	0.6905
1997–1998				
Crude protein, g/100 g DM	15.0	12.9	0.26	0.0046
Protein fractions, g/100 g CP				
A <sup>c</sup>	17.0	12.5	1.82	0.1582
B <sub>1</sub>	29.8	29.3	1.50	0.8235
B <sub>2</sub>	40.9	31.8	0.90	0.5467
B <sub>3</sub>	5.0	7.7	0.46	0.0153
C	7.2	8.7	0.39	0.0575

<sup>a</sup>Pod-filling Phase 1 = 28 g DM/100 g seed; pod-filling Phase 2 = 38 g DM/100 g seed (seasons means, Table 1).

<sup>b</sup>SEM = pooled standard error of the mean of each treatment (n = 3).

<sup>c</sup>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.

**Table 4.** Crude protein fractions of the full pods of common vetch at two harvesting stages during 2 yr

Item	Harvesting stage <sup>a</sup>		SEM <sup>b</sup>	P-value
	Pod-filling Phase 1	Pod-filling Phase 2		
	1996–1997			
Crude protein, g/100 g DM	28.2	28.0	1.62	0.8201
Protein fractions, g/100 g CP				
A <sup>c</sup>	39.3	27.5	1.36	0.0035
B <sub>1</sub>	21.1	26.4	1.01	0.0204
B <sub>2</sub>	33.7	41.1	0.53	0.0006
B <sub>3</sub>	3.6	3.5	0.26	0.6576
C	2.1	1.5	0.05	0.0011
	1997–1998			
Crude protein, g/100 g DM	22.2	21.1	0.24	0.0314
Protein fractions, g/100 g CP				
A <sup>c</sup>	31.7	18.4	0.66	0.0001
B <sub>1</sub>	26.2	33.7	0.93	0.0046
B <sub>2</sub>	35.9	43.3	0.62	0.0011
B <sub>3</sub>	4.5	2.8	0.19	0.0031
C	1.7	1.9	0.05	0.0792

<sup>a</sup>Pod-filling Phase 1 = 28 g DM/100 g seed; pod-filling Phase 2 = 38 g DM/100 g seed (seasons means, Table 1).

<sup>b</sup>SEM = pooled standard error of the mean of each treatment (n = 3).

<sup>c</sup>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.

Fraction B<sub>2</sub> was also the largest single CP fraction in studies with alfalfa (*Medicago sativa* L.). Sniffen et al. (1992) reported that fraction B<sub>2</sub> accounted for 41% of total CP in alfalfa forage, and Elizalde et al. (1999) reported a mean value of 51.6%. Because alfalfa forage is not harvested after the flowering stage, only comparisons with common vetch at flowering are relevant. In our study, the value of fraction B<sub>2</sub> at flowering in the first growing season (51.6% of total CP) was the same as that of alfalfa value reported by Elizalde et al. (1999). During the second growing season, our values (42.9% of total CP) were similar to those of Sniffen et al. (1992). The difference in B<sub>2</sub> values at flowering between the two growing seasons is largely the result of a higher proportion of soluble fractions (A plus B<sub>1</sub>, as a percentage of CP) in the second growing season. As a whole, our results suggest that the proportion of fraction B<sub>2</sub> of common vetch at the flowering stage was similar to that reported for alfalfa.

The neutral detergent-insoluble fractions (B<sub>3</sub> plus C) contributed little to total CP of common vetch forage. Across maturity, each protein fraction contributed less than 10% to total CP, and the two added fractions contributed less than 20%. Results were similar in both growing seasons. Plant maturity had a significant ( $P < 0.01$ ) effect on proportions of fraction B<sub>3</sub> and fraction C in the first season. The proportion of fraction B<sub>3</sub> in the whole forage was lower at flowering than during the period of rapid seed development. Within the pod-filling phases, the proportions of fraction B<sub>3</sub> and fraction C were greater in the vegetative plant parts than in the whole forage as both fractions are linked to the cell

wall structures. The proportions of fraction B<sub>3</sub> and C in the full pods (Table 4) were lower than those of the whole forage and vegetative plant parts at similar pod filling stages.

Fraction B<sub>3</sub> includes CP that is insoluble in NDF but soluble in ADF. Changes in NDF concentration of plant parts with maturity may largely explain the differences in proportions of fraction B<sub>3</sub> (as percentages of total CP). The lower NDF concentration in seeds compared with those reported for vegetative plant parts at similar stages during pod-filling (Caballero et al., 1998) may largely explain the differences in fraction B<sub>3</sub> proportions among common vetch plant parts. The relationship between NDF concentration and proportion of fraction B<sub>3</sub> has also been reported by Elizalde et al., (1999) in fresh alfalfa forage. For the same reason, values of fraction B<sub>3</sub> are usually greater in grasses than in forage legumes at comparative growth stages, and values of fraction B<sub>3</sub> within forage species are usually lower in fresh forage than in the corresponding forage hay (Sniffen et al., 1992; Agbossamey et al., 1998). Values of fraction B<sub>3</sub> reported by these authors were about 10% of CP for alfalfa forage. Abdalla et al. (1988) reported values of 13% for fraction B<sub>3</sub> in a mixture of birdsfoot trefoil (*Lotus corniculatus* L.) and brome grass (*Bromus biebersteinii* Roem and Schult.). Our results showed that fraction B<sub>3</sub> in common vetch never reached 10% of total CP and that common vetch forage had higher values of fraction B<sub>3</sub> at pod filling than at the flowering stage.

Unavailable fraction C (ADIP) represents bound protein that is not degraded in the rumen and is not di-

gested in the small intestine. In the first growing season, maturity affected ( $P < 0.01$ ) the proportion of fraction C. This fraction increased in whole forage from flowering to pod-filling Phase 2 (Table 2), and the vegetative plant parts (Table 3) always had higher values than full pods (Table 4). Mean values of whole forage across harvesting stages were 4.9% in the first growing season and 4.5% in the second. These values were similar to those reported by Elizalde et al. (1999) for fresh alfalfa (4.8% of total CP) but higher than those reported for alfalfa (i.e., 2.2% of CP or ranging from 1.8 to 4.6% of CP) by Sniffen et al. (1992) and Cherney et al. (1997), respectively. Rubio (1994) reported ruminal effective CP degradability of 87% for common vetch hay. As the unavailable fraction C in our results only accounts for less than 5% of total CP, these results suggest that the majority of fraction B<sub>3</sub> (less than 10% of total CP) may escape ruminal degradation whereas much of fraction B<sub>2</sub> is likely to be degraded in the rumen.

The proportion of the insoluble CP fractions (B<sub>2</sub>, B<sub>3</sub>, and C) as percentages of the total insoluble CP for the three maturity stages is presented in Figure 1 for the two growing seasons. Fraction B<sub>3</sub> ranged from 3.3% to 7.6% of total insoluble CP at flowering and from 14.8% to 10.8% at seed filling Phase 2 in the first and second growing season, respectively.

#### Yields of CP and of CP Fractions on the Whole Forage

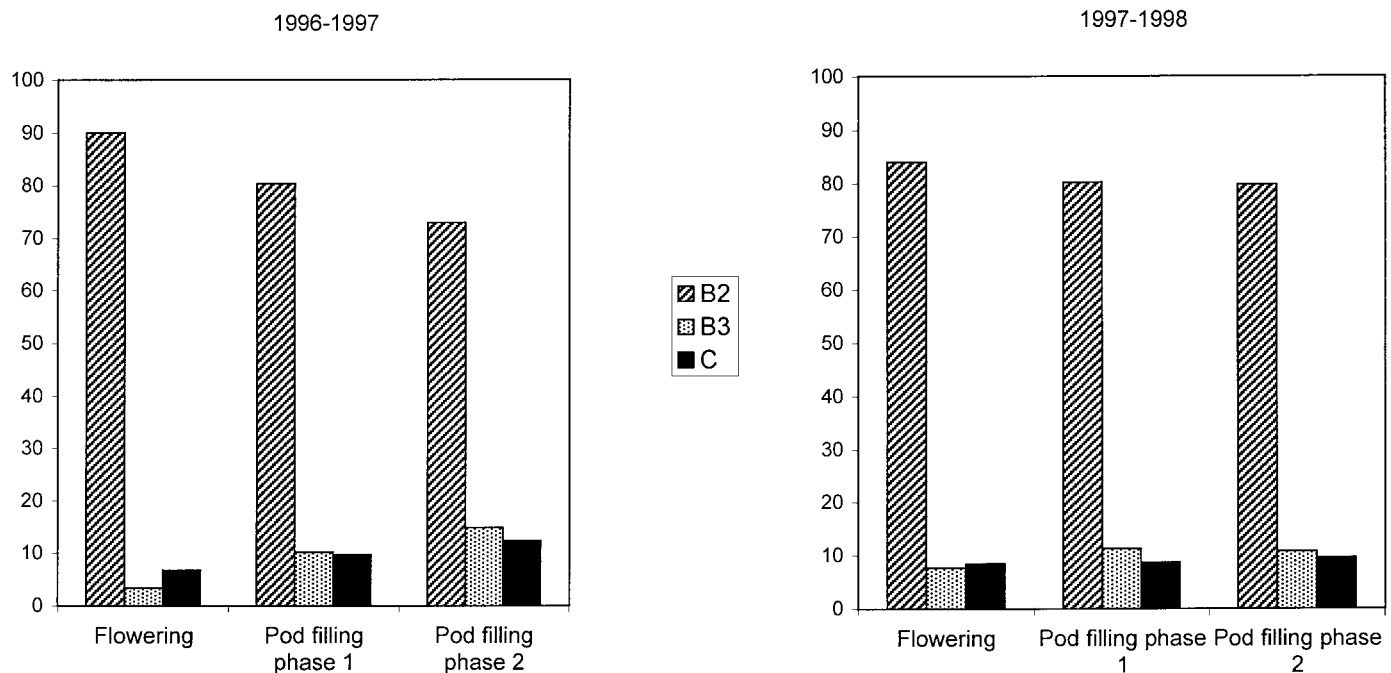
Yields of CP for common vetch forage may increase significantly as maturity advanced from early (20 g DM/100 g seed) to full (50 g DM/100 g seed) pod-filling

phases as relative seed weight may increase up to four times with maturity (Caballero et al., 1996a). In this experiment, the yield of CP varied little between flowering and pod filling because seed weight was not reached (Table 5).

Yields of CP fractions were more affected by season than by plant maturity. Cumulative yields of the two soluble fractions (A plus B<sub>1</sub>) remained almost unchanged as maturity progressed. Yields were higher in the second growing season as a consequence of higher DM yields (means of 8,400 ± 800 kg DM/ha and 5,590 ± 130 kg DM/ha, in the second and first seasons, respectively).

The yield fraction B<sub>2</sub> in the whole forage decreased at rates of 10 and 1.6 kg/ha<sup>-1</sup>·d<sup>-1</sup> between flowering and pod-filling Phase 1, and at rates of 8 and 4 kg/ha<sup>-1</sup>·d<sup>-1</sup> from the latter to the pod-filling Phase 2, in the first and second growing seasons, respectively. Across maturity, yields of fraction B<sub>2</sub> in the second growing season were higher than those of the first one, largely due to greater DM yields in the second season.

The slowly degraded fraction B<sub>3</sub> was the only one that showed yield increments as maturity progressed. Fraction B<sub>3</sub> increased ( $P < 0.01$ ) from flowering to pod filling in the first growing season. During the second growing season, fraction B<sub>3</sub> increased ( $P < 0.05$ ) from flowering to pod-filling Phase 1. Higher values at flowering in the second growing season were related to both higher proportion (percentages of total CP) and higher DM yields as compared with the first growing season. Yields of fraction C were not affected by maturity (Table 5).



**Figure 1.** The proportion of the insoluble CP fractions (B<sub>2</sub>, B<sub>3</sub>, and C) as percentages of total insoluble CP in common vetch forage at three harvesting stages during two years (B<sub>2</sub>, B<sub>3</sub> = intermediately and slowly degraded protein, respectively; C = bound protein undegraded in the rumen and indigestible in the intestine).

**Table 5.** Yields of CP fractions of common vetch forage at three harvesting stages during 2 yr

Item	Harvesting stage <sup>a</sup>			SEM <sup>b</sup>	P-value
	Flowering	Pod-filling Phase 1	Pod-filling Phase 2		
1996–1997					
Crude protein, kg/ha	1,205 <sup>x</sup>	1,213 <sup>x</sup>	1,079 <sup>x</sup>	74.2	0.4152
Protein fractions, kg/ha					
A <sup>c</sup>	256 <sup>x</sup>	403 <sup>x</sup>	277 <sup>x</sup>	47.5	0.1372
B <sub>1</sub>	259 <sup>x</sup>	222 <sup>x</sup>	255 <sup>x</sup>	29.6	0.6395
B <sub>2</sub>	622 <sup>x</sup>	472 <sup>y</sup>	399 <sup>y</sup>	31.3	0.0061
B <sub>3</sub>	22 <sup>x</sup>	59 <sup>y</sup>	81 <sup>y</sup>	8.6	0.0086
C	46 <sup>x</sup>	57 <sup>xy</sup>	67 <sup>y</sup>	5.2	0.0687
1997–1998					
Crude protein, kg/ha	1,556 <sup>x</sup>	1,622 <sup>x</sup>	1,404 <sup>x</sup>	87.6	0.2717
Protein fractions, kg/ha					
A <sup>c</sup>	276 <sup>x</sup>	424 <sup>y</sup>	247 <sup>x</sup>	30.2	0.0127
B <sub>1</sub>	483 <sup>x</sup>	389 <sup>x</sup>	424 <sup>x</sup>	40.6	0.3272
B <sub>2</sub>	668 <sup>x</sup>	649 <sup>x</sup>	584 <sup>x</sup>	40.5	0.3743
B <sub>3</sub>	61 <sup>x</sup>	91 <sup>y</sup>	79 <sup>xy</sup>	7.7	0.0846
C	68 <sup>x</sup>	69 <sup>x</sup>	70 <sup>x</sup>	8.1	0.9642

<sup>a</sup>Pod-filling Phase 1 = 28 g DM/100 g seed; pod-filling Phase 2 = 38 g DM/100 g seed (seasons means, Table 1).

<sup>b</sup>SEM = pooled standard error of the mean for each treatment (n = 3).

<sup>c</sup>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.

<sup>d</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

## Implications

Crude protein yields of common vetch were affected by growing season but not by plant maturity. Fraction B<sub>3</sub>, however, makes a higher contribution to total crude protein at pod filling than at the flowering stage. Thus, moving the harvesting stage of common vetch forage from flowering to the pod filling stage contributed to increased yields of dietary protein escaping ruminal degradation. Farmers should be advised on the productivity gains that can be achieved by delaying harvesting to near the pod-filling stage.

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