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Nutritive quality and fatty acid profile of Ravizzone (*Brassica campestris* L. var. *Oleifera*) seeds and plant during growth

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Abstract

Forage *Brassica* spp. have high quality and digestibility. It is important to define the nutritive quality and fatty acid (FA) profile of this type of crop during growth. The quality of *Brassica* forage changes according to its growth stage and an understanding of its evolution would enable cutting times to be planned, in order to optimise forage utilisation and to identify new sources of FAs for herbivores. Our aim was to study changes in the chemical composition, *in vitro* dry matter digestibility (IVDMD) and neutral detergent fibre digestibility (IVNDFD), gross energy, and FA profile of Ravizzone (RAV) (*Brassica campestris* L. var. *Oleifera*) seeds and plant samples collected from the vegetative through to the ripening stage.

The evolution of RAV quality during growth is characterised by a progressive decrease in crude protein and IVDMD and an increase in dry matter and fibrous fractions, while the lowest value of IVNDFD was reached at the bud stage. The main FAs of the plant during growth were: α -linolenic acid (ALA, C_{18:3n-3}), linoleic acid (LA, C_{18:2n-6}), cis-10 heptadecenoic acid (C_{17:1}), and palmitic acid (PA, C_{16:0}). The most abundant FAs in the seed were oleic acid (OA, C_{18:1n-9}), LA, ALA and PA.

The chemical composition and FA profile of RAV is closely connected with the development of the plant. A forage with good nutritive value is still obtained even when harvesting RAV at the flower stage.

Key words: *Brassicaceae, crude protein, digestibility, fibrous fractions, gross energy, lipid, stage*

Introduction

Brassica campestris L. var. *Oleifera* (RAV) is a plant of uncertain origin, cultivated in the countries of Central and Northern Europe. The plant is a member of the *Brassicaceae* tribe of the *Brassicaceae*, whose taxonomy is very difficult (Lysak et al 2005). In the Po Plain (NW Italy) it is known as "Ravizzone" and is cultivated as an annual forage crop. In Europe, *Brassicaceae* are cultivated as oil crops for the production of seed meal with a very high lipid and medium crude protein (CP) content, useful for animal feed. Moreover, the protein-rich meal, obtained as a by-product of oil pressing and that competes with soybean meal, is a leading option for Europeans to avoid importing products made from genetically modified organisms.

Brassica crops are highly productive and digestible, fast-growing crops, that are particularly suitable for grazing by livestock. They have the potential to provide additional or supplemental forage with high fresh yields (Altinok and Karakaya 2003) and can be grazed 80 to 150 days after seeding, depending on the species (Albayrak and Çamaş 2006). Most brassicas are relatively low in

dry matter (DM) content, but their total DM yield is high relative to most cereals and forage grasses (Rao and Horn 1986). A DM yield of 4 to 8 t/ha has been reported for *Brassica* spp (Rao and Horn 1986; Jung et al 1983; Jung et al 1986; Albayrak et al 2004; Albayrak and Çamaş 2005). In addition, CP levels are high, varying from 150 to 250 g/kg DM in the leaf and 80 to 150 g/kg DM in the roots, depending on the level of nitrogen fertilization and weather conditions (Albayrak and Çamaş 2006). The metabolisable energy content ranges from 11-14 MJ/kg DM. In general, environment had a significant influence on the fibre content of rape and kale, but less for swedes, turnip and turnip hybrids (Rao and Horn 1986; Guillard and Allison 1988). Fibre content and *in vitro* dry matter digestibility (IVDMD) values are reported to be influenced by the interactions of environment, season, and harvest date (Guillard and Allison 1988; Kunelius et al 1989).

The fatty acid (FA) composition of the oil of *Brassica* species is characterized by high erucic acid (EA, C_{22:1n-9}) content. Since EA is nutritionally undesirable, its removal has been one of the most important breeding objectives both for *Brassica campestris* L (Downey 1964) and for other *Brassica* crops (Chen and Heneen 1989).

The quality of *Brassica* forages, which are important sources of essential FAs, changes according to their morphological stage, making it important to define the nutritive characteristics that would increase the use of this crop as a dietary component and as a possible source of polyunsaturated fatty acid (PUFA) for ruminants.

Therefore, the objective of this experiment was to study changes in the chemical composition, IVDMD and *in vitro* neutral detergent fibre digestibility (IVNDFD), gross energy (GE) and FA profile of RAV at four stages of development.

Materials and methods

Plant material and environmental conditions

The study was conducted in the Western Po Valley near Cuneo, Italy (44° 41' N, 7° 11' E). The climate is characterised by high precipitation in the spring and autumn (96-110 mm/month) with little rainfall in the summer and winter. The mean daily temperatures increase from 0.5°C in January to 22°C by the end of July. A variety of RAV containing no EA was obtained from the Ornitalia Product Service s.a.s. (Colleredo di Monte Albano (UD), Italy). The stands were seeded on 8 May 2010 and no irrigation or fertilisers were applied after sowing. The herbage samples were collected with edging shears (0.1 m cutting width) from 1 m² subplots randomly located in 3 x 4 m² plots with three replicates at four progressive morphological stages from late vegetative to ripening stage from June to July 2010. Plants were cut to a 1-2 cm stubble height and sampling was performed in the morning after the disappearance of dew and was not carried out on rainy days.

The morphological stage was evaluated on a sample of about 50 stems clipped to ground level and classified according to the 6-stage classification system developed by Harper and Berkenkamp (1975).

Chemical analysis

The herbage samples were immediately dried in a forced-draft air oven to a constant weight of 65°C, brought to air temperature, weighed, ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass through a 1 mm screen and stored for qualitative analysis.

Dried seed and herbage samples were analysed to determine the total N content (AOAC 1990) and ash by ignition to 550°C. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined with the Ankom²⁰⁰ Fibre Analyser (Ankom Technology Corp., Fairport, NY, USA), following the procedure of Van Soest et al (1991) and corrected for residual ash content. The NDF was analyzed without sodium sulfite and α -amylase, as described by Van Soest et al (1991).

The herbage samples were also analysed to determine IVDMD and IVNDFD using the Daisy^{II} Incubator (Ankom, Tech. Co., Fairport, NY, USA), according to Robinson et al (1999). The *in vitro* rumen incubations were performed in two consecutive fermentative runs. Ground samples (size of grind, 250 mg) were inserted into filter bags (Ankom F57 bags) which were then sealed. Digestion jars were filled with pre-warmed (39°C) buffer solutions (266 ml of solution A: KH₂PO₄ 10 g/l, MgSO₄·7H₂O 0.5 g/l, NaCl 0.5 g/l, CaCl₂·2H₂O 0.1 g/l, Urea 0.5 g/l; 1330 ml of solution B: Na₂CO₃ 15.0 g/l, Na₂S·9H₂O 1.0 g/l) and placed into the Daisy^{II} Incubator. Rumen liquor was collected from rumen contents obtained at a slaughter house and 400 ml of liquor filtered through two layers of cheesecloth was introduced into each jar together with the filter bags. After 48 h incubation, the bags were removed, rinsed thoroughly with cold tap water and immediately analysed for NDF content with the Ankom²⁰⁰ Fibre Analyzer and incinerated to correct the residual NDF for the residual ash.

IVDMD was calculated as follows:

$$\text{IVDMD} = 100 - (W_3 - (W_1 \times C_1)) \times 100 / (W_2 \times \text{DM} / 100)$$

where W_1 is the filter bag weight, W_2 is sample weight, W_3 is the final weight (filter bag+residue) after *in vitro* digestion, C_1 is the comparative weight of the filter bag after and before digestion treatment and DM is the DM content (%) of the samples.

IVNDFD was calculated using the following equation:

$$\text{IVNDFD} = 100 - (W_3 - (W_1 \times C_1)) \times 100 / (W_2 \times \text{NDF} / 100)$$

where W_1 is the filter bag weight, W_2 is the sample weight, W_3 is the final weight (filter bag+residue) after *in vitro* and sequential treatment with NDF solution, C_1 is the comparative weight of the filter bag after and before digestion treatment and NDF is neutral detergent fibre content (%) of the sample.

Fatty acid analysis

Fresh samples (200 g) of the herbage were refrigerated, freeze-dried and ground to pass through a 1 mm screen. Lipid extraction was performed on freeze-dried samples according to Hara and Radin (1978), while transesterification of the FAs was performed according to Christie (1982), with the modifications described by Chouinard et al (1999). The FA methyl esters were then determined by gas chromatography according to Peiretti and Meineri (2008).

Statistical analysis

The variability in the FA and herbage quality characteristics harvested at four different stages of maturity was analysed for statistical significance via analysis of variance (ANOVA) using the Statistical Package for Social Science (SPSS Inc 2002) to test the effect of the growth stage. When

the values of F were significant (i.e., $P < 0.05$), the Duncan range test (Duncan, 1955) was used to detect differences among means.

Results and discussion

Crop quality

The evolution of RAV plant quality at the four different stages of development is reported in Table 1.

Table 1. Chemical composition (g/kg DM), gross energy (GE), *in vitro* neutral detergent fibre digestibility (IVNDFD), and *in vitro* dry matter digestibility (IVDMD) of *Brassica campestris* at four morphological stages and of the seed

Stage and Days after sowing	Vegetative	Bud	Flower	Ripening	SEM	Prob.	Seed
	49	56	63	70			
DM, g/kg FM	142 ^a	150 ^b	177 ^c	183 ^d	5.29	<0.001	933
OM	900 ^{ab}	912 ^{bc}	898 ^a	921 ^c	3.19	0.008	963
Crude protein	121 ^c	107 ^b	94.9 ^a	90.3 ^a	3.13	<0.001	227
Lipid	24.5 ^{ab}	28.1 ^b	18.0 ^a	17.8 ^a	1.63	0.028	434
Ash	99.7 ^{bc}	88.3 ^{ab}	101.8 ^c	79.0 ^a	3.19	0.008	37.1
NDFom	395 ^a	491 ^b	519 ^c	551 ^d	1.94	<0.001	418
ADFom	303 ^a	387 ^b	397 ^b	419 ^c	1.34	<0.001	297
Lignin	65.1 ^a	73.3 ^{ab}	86.3 ^{bc}	83.8 ^c	0.33	0.003	162
GE, MJ/kg DM	17.3 ^b	17.5 ^c	16.8 ^a	17.2 ^b	0.08	<0.001	29.0
IVNDFD, % NDF	62.6 ^b	46.4 ^a	52.5 ^a	48.7 ^a	2.04	0.002	85.5
IVDMD, % DM	84.4 ^b	73.5 ^{ab}	73.6 ^{ab}	70.4 ^a	1.64	<0.001	93.9

abcd Within a row, values with different letters differ ($P < 0.05$)

Growth of RAV was characterised by a DM accumulation in the herbage during development and a progressive increase in NDF and ADF contents, while lignin was higher at the flower stage than at the other stages. The CP and lipid content decreased with advancing maturity. Ash was highest and GE was lowest at the flower stage.

Seeds were higher in DM, OM, CP, lipid content and GE than the plant during the growth cycle. Ash content was very low in the seeds, while lignin content was twofold higher in the seed than in the plant during the growth cycle.

Pelletier et al (1976) and Jung et al (1984) reported that increasing nitrogen fertilization increased DM yield and CP content in forage turnip. Of more importance to the producer is the fact that quality of forage *Brassica* herbage is more comparable to a concentrate than traditional forage because of its relatively low fibre and high CP content (Wiedenhoeft and Barton 1994). Albayrak and Çamaş (2006) found that nitrogen fertilizer increased CP content in four forage turnip *cultivars*. The CP content of forage turnip resulted higher in leaves than in roots, but roots accumulated more NO₃ than leaves (Pelletier et al 1976).

Rao and Horn (1995) indicated that the CP content of all rape parts declined with maturity and that this decline was more rapid for the stem component. These authors attributed this rapid decline of CP to increasing DM accumulation rate and N dilution during this growth period. Other researchers concluded that the CP concentrations in the leaves of kale (*B. oleracea* L.) and turnips also declined with time (Pelletier et al 1976; Reid et al 1994).

Jung et al (1988) reported lower CP content for rape plants with delayed dates, most likely due to the greater stem production and leaf senescence shown by older plants.

Sincik et al (2007) found that the CP content of the whole plant and vegetative parts of the *Brassica* genotypes decreased as plant maturity progressed and reported that the full flowering stage with high leaf percentage and high CP content must be the preferred harvest stage of *Brassica* forage production.

Wiedenhoef and Barton (1994) determined the nutritive quality of initial and regrowth herbage from three *Brassica* spp. (rape, turnip and turnip hybrid) as influenced by planting and harvest date over a 3-year period. Nutritive levels declined with warmer temperatures and low soil moisture levels, particularly during July and August. NDF and ADF levels were higher, while CP levels were lower in herbage from the earliest planting date compared with the later planting dates, regardless of species and year.

The high energy content of forage brassicas is confirmed by Cassida et al (2007). Barry and Manley (1985) found that the energy content of forage brassicas is due in part to a high concentration of pectin.

In vitro digestibility

IVDMD and IVNDFD are presented in Table 1. Estimated digestibility at 48 hours of incubation showed significant decreases with increasing plant maturity. Generally, RAV is less digestible at the ripening stage. IVDMD ranged from 84.4%, at the vegetative stage, to 70.4% at the ripening stage. Similarly, IVNDFD ranged from 62.6% to 48.7% NDF. This suggests that the effect of lignification on the digestibility of NDF was not severe with the aging of the plant. The seeds are highly digestible.

Cassida et al (2007) confirmed that forage brassicas are readily digestible for cattle, sheep and goats.

Lambert et al (1987) reported that the disappearance of NDF residue was rapid during *in vitro* fermentation of forage rape samples. Wiedenhoef and Barton (1994) stated that with an increased fibre content, IVDMD and digestible energy content of *Brassica* forage declines.

Kunelius and Sanderson (1990) reported that forage rape, stubble turnip, and forage radish produced adequate yields with good quality with significant differences in DM yield, IVDMD and fibre content among cultivars.

Forage kale (*Brassica oleracea* L. 'Maris Kestrel') provided highly digestible feed from mid-September to early December and IVDMD ranged from 91.6 to 95.2% according to harvest date (Kunelius et al 1989)

Cassida et al (1994) determined the apparent digestibility of hay-supplemented brassica diets for lambs and found that the apparent digestibilities of DM, OM, NDF, ADF, cellulose, and CP were greater for tyfon than for hay. These authors reported that apparent digestibility coefficients for DM, NDF, and ADF of tyfon were slightly greater than values reported by Lambert et al (1987) for lambs fed rape.

Fatty acid profile

The FA profile in the plant during growth differs from the oil in the seed and shows quantitative differences for the various plant stages (Table 2).

Table 2. Fatty acid composition (g/kg of total FA) of *Brassica campestris* at four morphological stages and of the seed

Stage and Days after sowing	Vegetative	Bud	Flower	Ripening	SEM	Prob.	Seed
	49	56	63	70			
C _{8:0}	ND [#]	10.7	5.47	ND [#]	1.36	<0.001	ND [#]
C _{10:0}	ND [#]	21.3	11.2	ND [#]	2.74	<0.001	ND [#]
C _{12:0}	ND [#]	12.0	6.50	ND [#]	1.54	<0.001	ND [#]
C _{14:0}	ND [#]	5.55 ^b	6.04 ^b	2.23 ^a	0.758	<0.001	ND [#]
C _{16:0}	98.3	103	93.4	92.2	1.74	0.11	46.5
C _{16:1}	ND [#]	ND [#]	7.66	4.95	1.00	<0.001	2.63
C _{17:0}	3.81	5.10	6.56	5.81	1.42	0.94	ND [#]
C _{17:1}	132 ^c	111 ^b	104 ^b	61.8 ^a	7.90	<0.001	ND [#]
C _{18:0}	26.3 ^a	30.0 ^a	39.4 ^b	43.6 ^b	2.19	<0.001	25.7
C _{18:1n-9}	23.7 ^a	17.4 ^a	23.1 ^a	166.9 ^b	19.8	<0.001	571
C _{18:1n-7}	15.4 ^a	15.5 ^a	14.3 ^a	48.0 ^b	4.35	<0.001	34.7
C _{18:2n-6}	124 ^a	122 ^a	126 ^a	178 ^b	7.11	<0.001	190
C _{18:3n-3}	491 ^c	462 ^{bc}	435 ^b	309 ^a	21.2	<0.001	99.5
C _{20:0}	50.5 ^a	48.9 ^a	61.5 ^b	43.1 ^a	2.27	0.006	5.29
C _{20:2}	10.1 ^b	12.5 ^b	12.8 ^b	4.95 ^a	1.13	0.016	ND [#]
Others	24.7	23.8	47.7	39.7	4.89	0.20	24.5

abc Within a row, values with different letters differ ($P < 0.05$)

[#] Not detected

The main FAs of the plant during growth were α -linolenic acid (ALA, C_{18:3n-3}) and linoleic acid (LA, C_{18:2n-6}). ALA decreased from the vegetative stage to the ripening stage, while the highest content of LA was found at the ripening stage. The most abundant FAs in the seed were oleic acid (OA, C_{18:1n-9}), LA, ALA and palmitic acid (PA, C_{16:0}), but there was a lack of EA in the seed and plant due to the use of a commercial variety free of this FA.

In winter oilseed rape and other *Brassica* crops, there is a natural variability in the content of particular FAs, while the main FAs in the oil of currently grown oilseed rape cultivars are OA (59-68%), LA (17-21%) and ALA (7.8-10%) (Koprna et al 2006).

A great variability in the FA composition of seed oil in a germplasm collection of the genus *Brassica* was reported by Velasco et al (1998). Genotypic variations in FA composition have also been reported by Chen and Heneen (1989).

Ahmad and Abdin (2000) described the changes in the five fatty acids (PA, OA, LA, ALA and EA), that comprised over 90% of the total lipid in developing seeds of rapeseed. They showed that major changes occur in the OA and EA content during seed development and stated that the pattern of change in EA content may be consistent with the chain elongation of OA to give EA. Bano et al (2009) found that growth regulator treatments in the form of foliar spray could change the FA composition of *Brassica campestris* L. subsp. *oleifera* seed oil.

Conclusions

- The chemical composition and FA profile of RAV is closely connected with the development of the plant.
- The evolution of RAV quality during growth is characterised by a progressive decrease in CP and IVDMD and an increase in DM and fibrous fractions, while the lowest value of IVNDFD was reached at the bud stage.
- A forage with good nutritive value can still be obtained even when harvesting RAV at the flower stage. Further studies are necessary to determine the DM yield of this crop during growth.

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