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## Nutritive value and fatty acid content of soybean plant [*Glycine max* (L.) Merr.] during its growth cycle

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### ABSTRACT

Soybean [*Glycine max* (L.) Merr.] is mostly cultivated for grain in Asia, South and North America and it may be grown to be used as high-protein forage for grazing, haying or ensiling. Field trials with the soybean cultivar Eiko were conducted in North-West Italy to determine its chemical composition, gross energy, *in vitro* true digestibility (IVTD), neutral detergent fibre digestibility (NDFD) and fatty acid (FA) profile during growth. Herbage samples of cultivar Eiko were collected at seven progressive morphological stages, from the early vegetative to the seed-pod stage, during the 2014 growing season. The effect of plant growth was analysed by polynomial contrasts. Crude protein and ash decreased with increasing stage, whereas neutral detergent fibre, acid detergent fibre and lignin increased with progressive growth stage. No differences in lipid content during growth cycle were observed. IVTD decreased, whereas NDFD did not change with advancing growth stage. The most abundant FA during growth was  $\alpha$ -linolenic (C<sub>18:3n-3</sub>), which accounted for 464–538 g/kg of total FA. It decreased with advancing growth until the late vegetative stage when it increased. Significant differences were also found for  $\gamma$ -linolenic acid (C<sub>18:3n-6</sub>) and stearidonic acid (C<sub>18:4n-3</sub>), while no differences in the content of minor and unknown FAs were noted during growth.

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### Introduction

Soybean (*Glycine max* L. Merr.), a member of the *Fabaceae* family, is a protein and oil-seed crop mostly cultivated for grain in Asia, South and North America, where it may also be grown to be used as forage for grazing, haying or ensiling, either alone or in mixtures because of its high protein content (Chang et al. 2012; Touno et al. 2014; Spanghero et al. 2015). Soybean has extremely great importance in animal nutrition because of its high content of protein in the grain in the overhead biomass (Popović et al. 2014, 2015, 2016; Živanović and Popović 2016).

Researches had led to the discovery of new high-yielding soybean varieties developed specifically for forage production (Darmosarkoro et al. 2001; Koivisto et al. 2003; Bilgili et al. 2005; Rao et al. 2005) or to modify fatty acid (FA) composition in soybean oil (Hou et al. 2006; Oliva et al. 2006). No studies to date have examined the effect of growth stage on the FA profile of the soybean plant.

Sheaffer et al. (2001) suggested that grain varieties have lower dry matter (DM) yields than the forage varieties when the soybean is harvested at a similar stage of maturity. The small effect on forage quality and the large increase in forage DM yield associated with soybean varieties of later-than-normal maturity suggest that the later maturity varieties are often the better choice for soybean forage production than locally adapted grain varieties (Hintz et al. 1992). Furthermore, since soybean should ideally provide a forage for dairy and livestock production with qualities similar to alfalfa, it may be considered a viable alternative forage when crop damage limits grain yield (Sheaffer et al. 2001), other forage legumes are unavailable or clover or alfalfa are in short supply due to drought or winter-killing conditions (Mihailović et al. 2013).

Location and maturity stage at harvest are known to affect the soybean forage yields of grain-type cultivars (Hintz et al. 1992; Altinok et al. 2004), cultivar and

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**Figure 1.** Vegetative and reproductive stage of development of soybean.

management practices to affect the nutritive value of soybean (Nielsen 2011), and genotype and environmental interactions to influence the FA profile of soybean oil (Lee et al. 2007). We therefore thought it useful to determine the effect of growth cycle of an oilseed soybean cultivar on the chemical composition, gross energy (GE), *in vitro* true digestibility (IVTD), neutral detergent fibre digestibility (NDFD) and FA profile of the whole plant.

## Materials and methods

### Plant materials

Soybean seeds of the cultivar Eiko, with a high content of crude protein (CP) (436 g/kg), were purchased from Sipcam Italia S.p.A. (Pero, Milan, Italy). The study was carried out at the Department of Agriculture, Forestry, and Food Sciences of the University of Turin. Field trials were carried out in Grugliasco, Piedmont, Italy (45°03'57.9"N 7°35'36.9"E, 293 m a.s.l.) in sandy soil, low in organic matter with moderately alkaline pH and taxonomically classified as Entisol according to the USDA soil classification system (USDA 1999).

The climate of the study site is temperate sub-continental, characterised by two main rainy periods in spring and autumn. During the growing season, the total precipitation varies from 76.2 mm/month (May) to 139.0 mm/month (July), and the mean relative humidity and mean temperature are 68.6% and 20.3 °C, respectively.

The soybean stands were seeded in the spring (15 May 2014) in an experimental field (4 m wide and 14 m long). No fertilisers or irrigation was applied after sowing. The herbage samples were collected (from 18 June to 1 August 2014) with edging shears (0.1 m cutting width) at seven progressive stages of development (Figure 1) classified as V5, V6, R1, R2, R3, R4 and R5, respectively (according to Fehr et al. 1971). Two sample replicates for each stage were cut to a 1–2 cm stubble height from two subplots measuring 4 m<sup>2</sup> each.

Sampling was done in the morning after dew had evaporated and was never carried out on rainy days.

### Chemical analysis

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

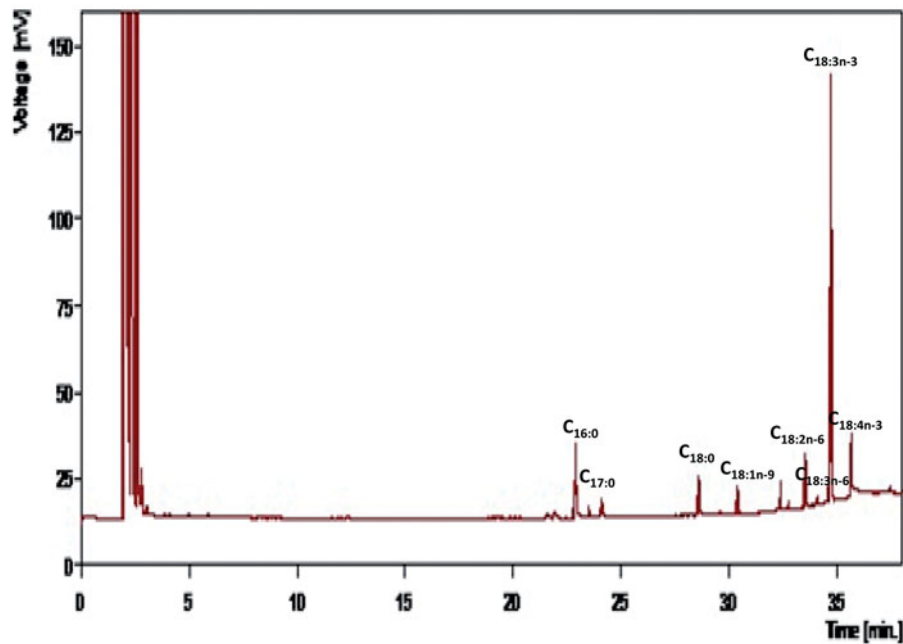
Dried herbage samples were analysed using the official methods of analysis of the Association of Official Analytical Chemists (AOAC 1995) for DM (#925.40), N (#984.13) and ash (#923.03). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined with an Ankom<sup>200</sup> Fibre Analyzer (Ankom Technology Corp., Macedon, NY), following the Ankom Technology Method and corrected for residual ash. Gross energy was determined using an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany).

Fresh herbage samples (200 g) were refrigerated, freeze-dried and ground to pass a 1-mm screen. Lipid content was quantified on freeze-dried samples and the FAs were determined as their methyl esters according to Peiretti et al. (2013) (Figure 2).

### In vitro digestibility

The *in vitro* digestion was conducted as follows. Approximately, 0.25 g of the freeze-dried herbage was weighed into filter bags (F57, Ankom Technology, Macedon, NY) in duplicate samples, sealed and incubated in a jar of Daisy<sup>II</sup> Incubator (Ankom Technology, Macedon, NY) containing pre-warmed (39 °C) buffer solution and rumen liquor collected at a slaughterhouse (Spanghero et al. 2010).

After incubation for 48 hours, the residuals in the filter bags were analysed for NDF content with an



**Figure 2.** GC chromatogram of fatty acid methyl ester derivatives of soybean plant.

Ankom<sup>200</sup> system. The IVTD and NDFD were calculated according to the following equations:

$$\begin{aligned} \text{IVTD g/kg of DM} &= 1000 \\ &\times [(DM_{\text{feed}} - NDF_{\text{residue}}) / DM_{\text{feed}}] \\ \text{NDFD g/kg of NDF} &= 1000 \\ &\times [(NDF_{\text{feed}} - NDF_{\text{residue}}) / NDF_{\text{feed}}] \end{aligned}$$

where  $DM_{\text{feed}}$  and  $NDF_{\text{feed}}$  are the amount of DM and NDF incubated;  $NDF_{\text{residue}}$  is the residual NDF after incubation.

### Statistical analysis

The variability in the FA and herbage quality characteristics harvested at seven different stages of maturity were analysed for their statistical significance by analysis of variance (ANOVA) using the Statistical Package for Social Science (SPSS Inc., Chicago, IL) (SPSS 2002) to determine the effect of growth stage. In addition, single degree-of-freedom polynomial contrasts were used to test for linear, quadratic and cubic effects of morphological stage (Steel and Torrie 1980).

## Results and discussion

### Chemical composition and nutritional quality

Table 1 presents the chemical composition and digestibility characteristics of soybean. The CP values decreased significantly (linear  $p < .01$ ; cubic  $p < .05$ ) from 301 g/kg (V5) to 154 g/kg (R5). Similar decreasing trend was found for ash (148 and 96 g/kg of DM,

respectively), whereas the amount of fibre fractions (NDF, ADF), and lignin increased with advancing maturity (linear  $p < .01$ ). No significant change in lipid content was noted. GE content varied with plant maturation and values ranged from 17.5 MJ/kg to 18.3 MJ/kg of DM (linear  $p < .01$ ; quadratic  $p < .05$ ). The results indicate that soybean was highly digestible, particularly the NDF fraction. A significant decrease in IVTD from 881 to 776 g/kg was observed (linear  $p < .01$ ), whereas no substantial changes in NDFD (from 605 to 757 g/kg of DM) with advancing growth stage were recorded.

Our results indicate that the quality of the soybean cultivar Eiko is excellent. Though the nutritional composition changed with advancing maturity, the quality remained high throughout the entire growth cycle.

The chemical values we obtained were in good agreement with those Mustafa et al. (2007) reported for other cultivars wilted before ensiling. As compared with other forage-type cultivars, we noted that Eiko was more comparable to the Kodiak cultivar, which Mustafa et al. (2007) found to be more nutritive for ruminants than the Mammoth cultivar. Heitholt et al. (2004) evaluated the quality of several soybean cultivars for forage (Tyrone, DP5110S, DP4344RR, AG4702RR) by developmental stage and found that the samples harvested at the full seed stage (R6) had an optimal quality with an average *in vitro* DM digestibility between 71.9 and 75.7% after 89 days of sowing.

Our observation that advancing maturity significantly alters digestibility of soybean forage is shared

**Table 1.** Chemical composition (g/kg DM basis), gross energy (GE), *in vitro* true digestibility (IVTD) and *in vitro* neutral detergent fibre digestibility (NDFD) of soybean plant at seven morphological stages.

Stage of development	V5	V6	R1	R2	R3	R4	R5	SEM	Contrasts		
									L	Q	C
Days after sowing	34	41	55	62	69	74	78				
DM, g/kg	190.5	185.1	197.7	184.2	181.9	204.4	199.8	2.45	*	ns	ns
Ash	147.7	142.6	96.5	101.5	92.5	97.6	95.8	6.18	**	**	ns
Crude protein	301.1	257.7	228.5	200.9	205.7	192.2	153.9	12.61	**	ns	*
Lipid <sup>a</sup>	15.8	12.9	12.8	15.3	10.6	13.1	15.2	0.68	ns	ns	ns
NDF	508.0	453.2	454.2	567.4	592.2	567.0	662.7	21.52	**	ns	ns
ADF	327.7	356.5	371.8	376.5	413.6	385.2	425.4	9.09	**	ns	ns
Lignin	58.0	64.7	69.5	72.6	76.3	71.8	81.2	2.18	**	ns	ns
GE, MJ/kg DM	17.5	18.1	18.5	18.0	18.2	18.5	18.3	0.10	**	*	ns
IVTD, g/kg DM	876.5	880.7	824.8	842.1	816.2	791.7	775.8	11.83	**	ns	ns
NDFD, g/kg NDF	756.9	736.6	605.0	721.7	689.8	634.0	661.8	18.06	ns	ns	ns

C: cubic contrast; L: linear contrast; Q: quadratic contrast; V5, V6, R1, R2, R3, R4, R5: vegetative and reproductive stage of development of soybean. Significance: ns: not significant.

<sup>a</sup>Lipid content was according to Peiretti et al. (2013).

\* $p < .05$ .

\*\* $p < .01$ .

**Table 2.** Fatty acid (FA) composition (g/kg of total FA) of soybean plant at seven morphological stages.

Stage of development	V5	V6	R1	R2	R3	R4	R5	SEM	Contrasts		
									L	Q	C
Days after sowing	34	41	55	62	69	74	78				
C <sub>16:0</sub>	55.1	49.2	63.7	57.1	57.5	59.5	55.4	2.29	ns	ns	ns
C <sub>17:0</sub>	32.3	38.9	28.4	32.6	30.2	26.0	28.9	2.47	ns	ns	ns
C <sub>18:0</sub>	33.7	31.1	36.3	35.4	38.5	35.8	32.0	1.12	ns	ns	ns
C <sub>18:1n-9</sub>	8.86	20.5	8.52	7.73	8.04	8.24	7.97	1.88	ns	ns	ns
C <sub>18:2n-6</sub>	59.2	69.7	82.4	65.3	70.2	68.1	59.5	2.82	ns	ns	ns
C <sub>18:3n-6</sub>	13.1	9.03	8.28	9.89	9.63	10.2	4.79	0.72	*	ns	**
C <sub>18:3n-3</sub>	537.8	507.9	464.5	493.0	471.6	494.0	514.6	8.27	ns	*	ns
C <sub>18:4n-3</sub>	84.3	64.7	66.8	81.8	79.6	77.8	70.4	2.48	ns	ns	*
Others	175.6	209.0	241.1	217.1	234.7	220.4	226.4	8.93	ns	ns	ns

C: cubic contrast; L: linear contrast; Q: quadratic contrast; V5, V6, R1, R2, R3, R4, R5: vegetative and reproductive stage of development of soybean. Significance: ns: not significant.

\* $p < .05$ .

\*\* $p < .01$ .

by Açıkgöz et al. (2007) who reported a decrease in *in vitro* DM digestibility from 75% (V5) to 58.3% (R6). We noted that while the IVTD gradually decreased from V5 to R5 (876 ÷ 776 g/kg of DM), the digestibility of NDF was not affected by maturing stage. It could be increased with ensiling (Spanghero et al. 2015), however. The reason for this, as given in previous studies (Sheaffer et al. 2001; Burke et al. 2007), is that, differently from a forage soybean, the starch content increases during maturity in a grain soybean, which compensates for the increase in NDF and ADF values without substantially changing the fibre content of the total crop.

Açıkgöz et al. (2007) demonstrated that CP, a degradable protein, and *in vitro* DM digestibility of soybeans managed for forage in a Mediterranean-type environment were not affected by row spacing and seeding rate. They also reported a mean content of 13.3% CP, 8.2% degradable protein and 60.6% *in vitro* DM digestibility. Seiter et al. (2004) found that soybean had a moderate content of ADF and NDF and a higher CP content when harvested at R6 and R7 stages, than at R3 and R4 stages (full bloom). Asekova et al. (2014) recommended forage varieties with late maturity

harvested at growth stages from R5 to R7 for producing high quality soybean forage.

### Fatty acid composition

Table 2 presents the FA composition of forage soybean. The most abundant FA was  $\alpha$ -linolenic (C<sub>18:3n-3</sub>), which accounted for 464–538 g/kg of total FA content. It decreased with progressive growth until the late vegetative stage when it increased (quadratic  $p < .05$ ). Significant differences were also found for  $\gamma$ -linolenic acid (C<sub>18:3n-6</sub>; linear  $p < .01$ ; cubic  $p < .05$ ) and stearidonic acid (C<sub>18:4n-3</sub>; cubic  $p < .01$ ). No differences in the content of palmitic acid (C<sub>16:0</sub>), linoleic acid (C<sub>18:2n-6</sub>) and other minor FAs were found. The  $\alpha$ -linolenic/linoleic acid ratio decreased from 9.08 (V5) to 5.64 (R1) and then increased till 8.65 (R5).

The FA profile of soybean is similar to those found for other legumes, such as birdsfoot trefoil (*Lotus corniculatus*) and white clover (*Trifolium repens*) (Peiretti et al. 2016). Another legume such as *Galega officinalis*, harvested at three morphological stages and at regrowth, was characterised by three dominant FA,



being:  $\alpha$ -linolenic, palmitic and linoleic acid (Peiretti and Gai 2006).

There is little published information on the FA composition profile of the soybean plant during growth. The FA profile of the seed oil is generally different from that of the corresponding plant during growth (Peiretti and Meineri 2008). High amounts of polyunsaturated fatty acids (PUFAs) in soybean seed were found by Sukhija and Palmquist (1988) and Grela and Günter (1995). Ezeagu et al. (1998) reported the presence of  $\gamma$ -linolenic acid in selected tropical soybean seed oils. Dornbos and Mullen (1992) reported that, while drought had little effect on the FA composition of the oil, high air temperature reduced the proportion of PUFAs.

## Conclusions

Our results indicate that the soybean cultivar Eiko can be employed as forage of high quality. The best combination of nutritive quality and FA content are obtained from soybean harvested for forage when the seeds fill the pods at the R5 stage, when IVTD is still good, and the  $\alpha$ -linolenic/linoleic acid ratio is more favourable. This is the stage when all the leaves on the plant are still green and the NDFD has not yet begun to decrease.

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## Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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