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Chemical Composition of Soybean Harvested in Different Stages of Maturity and Its Suitability for Forage Production

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Abstract. Climatic conditions for soybean cultivation in Latvia are challenging because of their variability. In addition, the sum of sunlight hours and the ratio of temperature and precipitation required to produce a quality crop are unpredictable. There is a risk that even early soybean varieties will not ripen due to weather conditions in a region. Studies show that soybean plants, including the green part of the crop, are well suited for animal nutrition prepared as hay or silage. The aim of this study was to evaluate the chemical composition of different soybean varieties harvested before ripening and assess them as hay or silage raw material. Weight, protein, fat, ash, fibre, acid detergent fibre (ADF) and neutral detergent fibre (NDF) of soybean varieties 'Erica', 'Bolgar' and 'Viola' green mass were determined at different stages of maturity. The results of the study showed a significant increase in protein (from 9.18% to 12.06%) and fat (from 1.18% to 4.40%) content of the dry matter variety 'Bolgar' from September to October. The protein content of the dry soya green mass was not affected by variety at the same developing stage but significantly changed among different stages of maturity. As the plant develops, the sucrose content in the green mass increases, the same as the total sugar content.
Key words: protein crop, *Glycine max*, feed, green mass.

Introduction

The soybean, without denying the possibility of using it as an oil source, is one of the most prevalently grown and used protein plants. Uses range from human foods to animal feeds, industrial products, ingredients, and precursor materials (Gaonkar, 2019). Soybeans have a long history as a nutritious hay and silage crop (Asekova Shannon, & Lee, 2014). Soybean cultivation areas have been an increasing trend in the last 20 years in Europe. The obtained results, published in the journal Nature Food, show that Europe could supply itself with 50–100% soybean products if 4%–11% of European agricultural lands were cultivated soybeans. The projected increase would be of considerable economic benefit and would protect the environment by reducing the use of nitrogen fertilisers (Guilpart, Iizumi, & Makowski, 2022). Very little is known about the environmental potential of soybeans in higher latitudes with relatively cool conditions. (Karges *et al.,* 2022). The estimated results show that the European croplands are more suitable for soybean growing in comparison with the currently used ones. Estimates show that a potential yield of 2 t ha^{-1} could be harvested if the climatic conditions do not change. This is possible even without fertiliser, and yields could increase in the predicted ones by +0.4 to +0.6 t ha^{-1} until 2050. This report shows a change in the most productive areas of the European continent from the south to the north and east as a result of climate change (Guilpart, Iizumi, & Makowski, 2022).

Soybeans are a new species for growing as a protein crop in Latvia promoted by climate change, increased demand for local resources and biodiversity. The length of soybean vegetation varies from 142 to 161 days at latitude 55° N (Toleikiene, 2021). Soybean is a photoperiod-sensitive crop, which means that soybean biomass is influenced by sunlight hours, including flowering and seed production. A longer photoperiod provides more vegetative biomass matter produced by soybean cultivar, greater internode elongation and leaf expansion (Seo *et al.*, 2019).

Unfortunately, there is a high risk that due to environmental conditions, soybean seeds are not able to ripen each year. Alternative uses for unripe soybean

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crops to reduce the risk of losing investment could be required. Studies show that soybean forage is as great as fodder. The feed value of properly harvested soybean forage can be approximately equivalent to alfalfa (Staton *et al.,* 2019). Soybean stems and leaves can be grazed and dried to make hay same as used for silage preparation. The green mass of soya is evaluated as very palatable to cattle, which has good digestibility and a high nutritional value (Koivisto *et al.,* 2003). The stubble that is left after harvesting on the field can be used for feeding dairy cows or heifers (Felix & Adebowale, 1997). Soybean straw left over from threshing the beans can be successfully fed as a roughage source for cattle (Sruamsiri, 2007; Rigueira *et al*., 2015). Soybean forage is palatable to sheep and goats (Bacchu *et al*., 2005; Luginbuhl, 2006) and deer in wildlife management.

Soybean varieties bred for forage production are characterised as taller than grain varieties, produce more biomass and have delayed maturity which is preferable for good-quality fodder (Sheaffer *et al*., 2001). Soybean forage is very valuable if it is harvested between full bloom to maturity, when the leaves lose their green colour, but before they start to fall (Blount *et al*., 2013; Luginbuhl, 2006). Soybean silage is a valuable animal feed which can replace, for example, soybean meal in ruminant diets (Rigueira *et al*., 2015).

Because soybeans have only grown in Latvia since relatively recently, the varieties most suitable for the local conditions are tested. At the same time, there is a lack of research into the use of green matter in cases when the crop must be harvested unripe. The aim of this study was to evaluate the chemical composition of different soybean varieties harvested before ripening and evaluate them as a material for hay or silage preparation.

Materials and Methods

This research was done at the Stende Research Centre, Institute of Agricultural Resources and Economics. The material used in the green mass evaluation is three varieties of soya – 'Bolgar', 'Erica' and 'Viola' – grown at the experimental fields of the research centre (lat. 57.1412° N, long. 22.5367° E) in the year 2020.

Experimental design and growing conditions

The field experiment was carried out using a block design with four replicates and the plot size was 10 m^2. The conventional plots were fertilised as follows – before sowing nitrogen 15 kg ha^{-1}, phosphorus and potassium 39 kg ha^{-1} (using standard fertiliser NPK 10-26-26 in amount 150 kg ha^{-1}), additional top fertiliser nitrogen 20 kg ha^{-1} and sulphur (using NS 26-14 in amount 77 kg ha^{-1}) were used. Agrochemical indicators of soil: organic matter – 32 g ha^{-1} of soil (by the Tyrin method), soil exchange reaction pH KCl 7.3, available for plants P_2O_5 contents – 378 mg kg^{-1}, available for plants K_2O contents 204 mg kg^{-1} of soil (by the Egner-Reihm method).

Soil type sod – gleyic soil, precrop was oat, sowing date for all varieties was 9.05.2020, sowing rate 50 seeds per 1 m^2. The sum of monthly precipitation and temperature during the growing season is shown in Figure 1.

The harvesting of samples was performed three times per season in different stages of development. The harvesting data and stage of development in

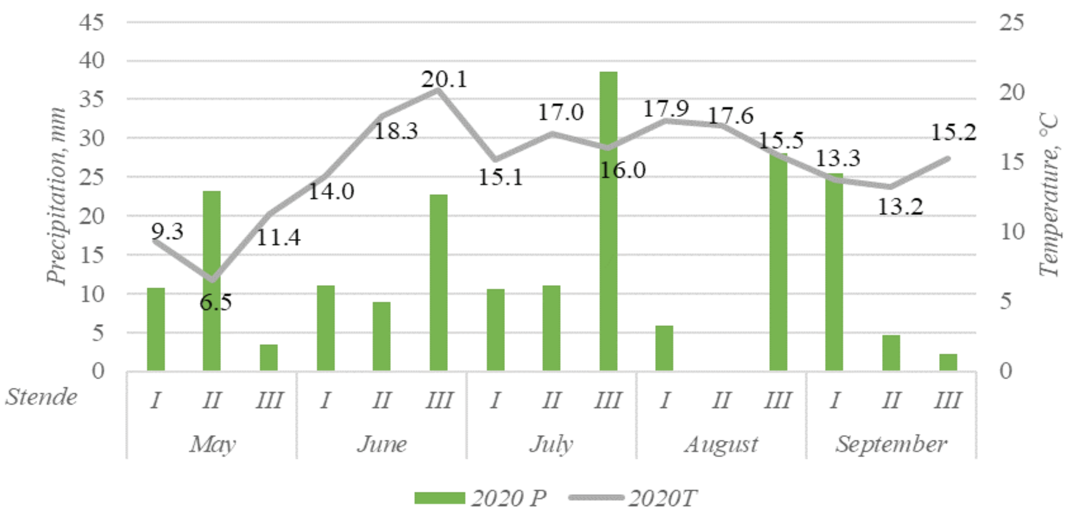

Figure 1. Mean air temperature (T) and precipitation (P) in monthly decades.

Source: SLLC "Latvian Environment, Geology and Meteorology Centre"

Table 1

The stage of development of soybean at the moment of harvesting

Harvesting data	Development stage by BBCH (development stage by PO)		
	'Bolgar'	'Viola'	'Erica'
06.09.2020	62–64 (R1)	72–74 (R3)	80–82 (R7)
20.09.2020	72–74 (R3)	80–82 (R7)	-
07.10.2020	80–82 (R7)	-	-

agreement with BBCH classification (Meier & Kuhn, 2018) and Plant Ontology (PO) classification (Jaiswal *et al.*, 2005) for each variety are determined and specified in Table 1.

Ten plants randomly selected formed one sample from each replication. Samples were weighed, chopped and sent for testing. Weight, protein, fat, ash, fibre, acid detergent fibre (ADF) and neutral detergent fibre (NDF) tests were made in duplicate.

Chemical analyses

The chemical analyses were carried out at the Laboratory of Cereal Technology and Agricultural Chemistry of the Institute of Agricultural Resources and Economics. Collected green mass samples of all four replicates were dried at 60 °C to constant weight, ground in a mill using a 1.0 mm sieve and collected for further analysis. Protein content was determined by the Kjeldahl method, the conversion factor 6.25 was used to convert total nitrogen to crude protein. Fat was extracted with petroleum ether (boiling range of 40–60 °C) by the Soxhlet extraction method and determined gravimetrically. Ash content was determined by ISO 5984:2002/Cor 1:2005. Fibre content, NDF and ADF were determined according to ISO 5498:1981; LVS EN ISO 16472:2006; and LVS EN ISO 13906:2008, respectively. The sugar profile was tested in the laboratory J.S. Hamilton Baltic according to internal standards using the internal method PB79/HPLC edV18.05.2017 for liquid chromatography.

Statistical analysis

The obtained results were statistically processed with MS Excel 2016 using methods of descriptive statistics – sample mean and standard deviation. Two-sample T-test with equal variance was used to compare means. Statistical significance was declared at $p < 0.05$.

Results and Discussion

Soybean development is influenced by sunlight because it is photoperiod photoperiod-sensitive crop. All of the soya varieties had different, genetically determined ripening times, therefore within the time

Figure 2. Comparison of examined soya varieties before harvesting on 20.09.2020 – first from the right 'Bolgar', next 'Erica' and 'Viola'.

Table 2

Chemical composition of examined soybean forage at different development stages

BBCH stage	Amount of plants per 1m^2			Amount of green mass, t ha^{-1}			Dry matter, %			Dry matter yield, t ha^{-1}		
	'Bolgar'	'Viola'	'Erica'	'Bolgar'	'Viola'	'Erica'	'Bolgar'	'Viola'	'Erica'	'Bolgar'	'Viola'	'Erica'
62–64	44.8	-	-	27.8	-	-	25.8	-	-	7.2	-	-
72–74	48.0	17.0	-	34.7	16.8	-	32.0	28.6	-	11.2	5.6	-
80–82	48.0	21.8	29.3	31.1	19.9	13.5	29.6	36.1	28.7	9.2	7.2	3.9

specified in the study, they had achieved different heights and amounts of green mass. A comparison of the tested varieties' visual assessment is shown in Figure 2.

Field germination differed by variety – 'Bolgar': 93.2%; 'Viola': 38.8%; 'Erica': 58.6% – and it affected the soybeans' biomass production. The biomass yield depends on the length of the growing season of the variety. Late varieties produce more green mass and mature later as we observed in the case of the 'Bolgar' variety. The highest green mass yield for the 'Bolgar' variety (34.7 t ha^{-1}) was obtained at the development stage 72–74, and later it decreased. At the same time for the variety Viola, the obtained green mass was 16.8 and 19.9 t ha^{-1} in both stages and dry matter was increased from 28.6% (72–74) to 36.1% (80–82). Evaluation of dry matter yield at stages 80–82 showed that the 'Bolgar' variety has the highest result – 9.2 t ha^{-1}**.**

Biochemical composition of soya green mass was determined and a comparison of protein, fat, starch and fibre after drying is shown in Table 3.

Research by Japanese scientists shows that late-ripening soybean varieties produced a higher amount of dry matter than those of early-ripening soybean varieties. The content of ADF and NDF was decreased along with growth stages for late-ripening varieties. In contrast, crude protein content increased along with growth stages for late-maturing cultivars resulting in higher crude protein weight (Prasojo, 2021).

The results of our study showed a significant increase ($p<0.05$) in protein and fat content from September to October. The protein content of the dry soya green mass was not affected by variety at the same developing stage but significantly changed among different growth stages. The protein content of the 'Bolgar' variety varied from 9.18% at stage 62–64 to 12.06% at stage 80–82. The results regarding protein content in this study were significantly lower than the results of Bohner *et al.* (2016), where protein content at different harvest stages varied from 18.1% to 20.1%. Bacchu *et al.* (2005) reported that soybean straw contained 10.4% crude protein, 1.6% fat content and 39.3% crude fibre. Evaluation of soybean varieties specially selected for forage production grown in the UK in 2001 showed similar results – crude protein of the 'Denegal' and 'Derry' varieties on 5 October were 125 g kg^{-1} (12.5%) and 132 g kg^{-1} (13.2%), but on 1 November, 133 g kg^{-1} (13.3%) and 155 g kg^{-1} (15.5%), respectively (Koivisto *et al.*, 2003). Koivisto *et al.* (2003) also reported an increase in crude protein content between the first and second harvest dates.

An increase in fat content with the developing stage was observed. The fat content determined in this study varied from 1.18% to 6.67% in dry matter.

Table 3

Biochemical composition of examined soybean forage at different development stages

Variety	BBCH stage	Crude protein	Ash	Fat	Crude fibre	ADF	NDF
		% (values in dry matter) ± standard deviation					
'Bolgar'	62–64	9.18^{a}±1.30	8.94±0.55	1.18^{a}±0.11	28.31±2.28	33.85±3.53	45.58±2.23
'Bolgar'	72–74	10.20^{b}±1.60	8.91±2.04	1.90^{b}±0.14	26.21±1.98	31.51±1.55	43.95±3.47
'Bolgar'	80–82	12.06^{c}±1.30	8.54±0.17	4.40^{c}±0.98	25.90±1.33	32.65±2.52	40.25±2.68
'Viola'	72–74	9.94^{b}±1.33	8.37±0.16	3.73^{c}±0.58	27.70±0.66	32.90±2.10	44.62±1.34
'Viola'	80–82	12.27^{c}±2.59	8.06±0.27	6.67^{d}±0.87	28.58±1.99	34.00±2.00	43.70±2.49
'Erica'	80–82	12.06^{c}±1.35	9.03±0.66	5.85^{e}±0.34	28.54±1.55	31.24±1.84	41.97±1.38

Table 4

The content and composition of sugars in soybean forage samples

Variety	BBCH stage	Total sugars	Glucose	Fructose	Sucrose	Maltose
		g 100 g^{-1}(values in dry matter) ± standard deviation)				
‘Bolgar’	62–64	4.9±0.5	2.2±0.2	2.4±0.2	0.2±0.1	0.3±0.1
‘Bolgar’	72–74	4.6±0.5	2.0±0.2	2.2±0.2	0.2±0.1	0.4±0.1
‘Bolgar’	80–82	5.2±0.5	2.0±0.2	2.2±0.2	0.7±0.1	0.3±0.1
‘Viola’	72–74	3.1±0.3	1.4±0.2	1.4±0.2	0.3±0.1	0.2±0.1
‘Viola’	80–82	3.8±0.4	1.3±0.2	1.1±0.2	1.2±0.1	0.2±0.1

A significant increase of fat content ($p<0.05$) was observed between stage 72–74 and stage 80–82 in samples of the ‘Bolgar’ variety, as well as in samples of the ‘Viola’ variety. Ash and crude fibre content did not differ by varieties; their contents were 8.06 – 9.03% and 25.9 – 28.58%, respectively. Acid detergent fibre of soybean forage was determined from 31.2% to 34.0%, but neutral detergent fibre from 40.3% to 45.6%. The results of the study showed that the content of NDF decreased as the growth stage increased. These results were in line with other studies, where ADF of green soya plants was reported from 28% to 34% and NDF from 38.6% to 45.7%. In addition, the highest value was determined at stage R5 and the lowest at stage R7 (Bohner, 2016). Evaluation of the composition of soybean cultivars and experimental lines at Cirencester, UK, in 2001 showed that ADF varied from 279 g kg^{-1} (27.9 %) to 342 g kg^{-1} (34.2%) and NDF varied from 384 g kg^{-1} (38.4%) to 455 g kg^{-1} (45.5%) (Koivisto *et al.*, 2003). Most studies show that soybean forage harvested from seed development (growth stage R5) to the beginning of maturity (growth stage R7) has been considered appropriate for animal feed because it has a good combination of high protein, low fibre content and greater digestible energy (Asekova *et al.*, 2014).

The total content of sugars and their profile was determined in samples of soybean varieties ‘Bolgar’ and ‘Viola’. The results are assumed in Table 4.

Evaluation of total sugar amount and composition significantly differed between the varieties ‘Viola and ‘Bolgar’ ($p<0.05$). The composition of sugars listed in Table 4 shows that total sugars composed of glucose and fructose, the sum of sugars in the green mass of variety ‘Bolgar’ determined 81–94% at different stages. The content of sucrose between stages 72–74 and 80–82 increased from 0.2 to 0.7 g 100 g^{-1} in samples of the ‘Bolgar’ variety and from 0.3 to 1.2 g 100 g^{-1} in samples of the ‘Viola’ variety. An increase in total sugar content with the developing stage was observed. To ensure good silage fermentation, the target sugar content is 3% or higher (Harrington 2020), because in the silage preparation, the aim is to convert sugars to lactic acid to drop the pH of the silage to around 4. This is where the silage is stable. From this point of view, the ‘Bolgar’ variety is more favourable for silage preparation at all tested stages of maturity.

The results of this study confirm that harvesting soy green mass at stage 80–82 provides more value than harvesting it at stage 72–74. The results of the study show that soybean could be used as a raw material for silage preparation if it is unable to ripen due to weather conditions. Other commonly used animal feeds in Latvia are cereal grass, meadow grass, red clover, corn silage and barley meal. The protein content of these feeds is respectively 10.5%; 5.8–8%; 14–15%; 7–8.4%; 10–12%, and crude fibre – 36.8%; 34–39%; 28.5%; 19.6–21.2%; 5.2-9%, respectively (Degola, Trupa, & Aplocina, 2016). Soybean shows useful potential for dry matter, crude protein and sugar content.

Conclusions

The study underlines the importance of the soybean growth stage and its effect on soybean forage green mass and chemical composition that can guide farmers in choosing the best harvesting time for using it as forage.

The results of the study showed a significant increase in protein and fat content from stage 72–74 to stage 80–82 where the protein content of the dry soya green mass at the last one was determined 12.06% to 12.27% not affected by variety at the same developing stage.

The content of NDF decreased with the maturity stage increasing, and at stage 80–82 varied from 40.25– 43.70%.

The composition of determined sugars shows that total sugars consist of glucose and fructose and they significantly differed between soybean varieties.

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