

Effect of cultivar and the number of harvests on the feed value, protein fractions and nutrient digestibility of *Festulolium braunii* forage in young cattle

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ABSTRACT. The effect of three *Festulolium braunii* cultivars (Felopa, Perun, and Lofa) and five successive cuts on the nutritional value of herbage and silage was evaluated. The chemical composition, carbohydrate and protein fractions, ensilability, fermentation parameters were determined. The nutrient digestibility of experimental diets containing silages made from different *F. braunii* cultivars was determined by the simple balance methods in 45 Holstein-Friesian bulls (three groups of 15 animals) at the end of fattening. The experiment lasted 90 days, the animals were fed a total mixed ration. *Festulolium braunii* cv. Lofa and third-cut and fourth-cut herbage were characterized by the most desirable chemical composition. *Festulolium braunii* cv. Felopa had the highest concentration of water-soluble carbohydrates ($P = 0.046$) and the highest buffering capacity ($P = 0.005$). First-cut silage had the highest content of lactic acid ($P = 0.018$). The protein fraction composition of silage varied across cuts, and significant differences between cultivars were found only for fraction B1 ($P = 0.046$), whose proportion was highest in cv. Perun. Fourth-cut silage had the lowest proportion of fraction A ($P \leq 0.01$) and the highest proportion of fraction B1 ($P = 0.037$). The nutrient digestibility coefficients of experimental diets were influenced by the number of cuts ($P \leq 0.05$) and the interaction of both factors ($P \leq 0.01$). The study confirmed the high feed value of *F. braunii*, which provides high-quality forage for ruminants. The significant differences in the nutritional value of *F. braunii* herbage and silage observed among cultivars and cuts indicate that various cultivars cut multiple times at different harvest dates should be tested in future studies.

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Introduction

High-quality grass silage is the basal diet in many dairy and beef production systems around the

world, especially in temperate regions (Huuskonen and Pesonen, 2017). Due to global climate change, recent years have witnessed an increased interest in the cultivation of hybrid grasses, mainly intergeneric

hybrids (Kitczak et al., 2021). This group of grasses includes *Festulolium braunii*, which combines the traits of both parent genera: the rapid growth, high yield, and high feed value of *Lolium*, and the persistence in the sward and resistance to adverse environmental conditions, including abiotic stresses such as drought and frost, of *Festuca* (Cernoch and Groenbaek, 2015).

The morphology, yield potential, and herbage quality of *F. braunii* are comparable to those of Italian ryegrass (*Lolium multiflorum*), but the former is characterized by greater winter hardiness and drought tolerance. *Festulolium braunii* is resistant to variable and extreme weather conditions (sub-zero temperatures during snowless winters, spring frosts, floods, droughts), provides stable and high herbage yields, and high carbohydrate and crude protein (CP) content of forage (Kitczak et al., 2021; Purwin et al., 2024). This species is suitable for ensiling when grown in pure stands and mixed with other grasses, alfalfa or red clover (Cernoch and Groenbaek, 2015).

Festulolium braunii maintains high growth and development potential in successive production years and is characterized by a high leaf-to-shoot ratio (Frankow-Lindberg and Olsson, 2008). The species can also be used to restore permanent grasslands (Kitczak et al., 2021). Previous research has shown that *F. braunii* can contribute to reducing milk and beef production costs due to its high yield potential and high nutritional value (Humphreys and Zwierzykowski, 2020).

The relationship between green fodder production vs. the frequency and intensity of cutting has been extensively investigated in terms of dry matter (DM) yield and quality of fodder crops, since these agronomic factors influence the growth and quality of perennial fodder grasses (Rojas-García et al., 2018). Bazzaz et al. (1987) analysed the response of fodder crops to cutting, where growth contributes to maintaining a dynamic equilibrium between plants and their environment to optimize the use of all resources allocated to growth and reproduction. Cutting disrupts the supply of carbohydrates needed for plant regrowth by reducing photosynthetic tissue. Cutting frequency should be adjusted to maintain high forage quality, cutting intervals should be long enough for plant regrowth and regeneration, and cutting practices must be consistent with seasonal and climatic conditions (Geren et al., 2014). It has been found that the DM yield, the proportion of stalks, and crude fibre (CF) concentration increase, whereas digestibility, CP content, and the proportion

of green leaves decrease with longer cutting intervals (Campos-de Quiroz, 2002).

The research hypothesis postulates that cultivar and successive cuts, or their interaction, affect the chemical parameters and digestibility of *F. braunii* herbage and silage. The aim of the present study was to identify the optimal combination of the experimental factors to produce *F. braunii* silage without additives.

Material and methods

Treatments and experimental design

The experiment was established in central Poland (52.738014, 20.881330) on soil of good rye complex according to the Polish soil quality classification system. Plot size was 2.5 ha. Seeds of three *F. braunii* cultivars were sown: Felopa (*F. pratensis* x *L. multiflorum*) – FF, Perun (*L. multiflorum* x *F. pratensis*) – FP, and Lofa (*L. multiflorum* (2x) x *F. arundinacea* cv. *Genuina* (6x)) – FL. The seeding rate was 30 kg seeds · ha⁻¹ in each cultivar. Cattle slurry at 30 m³ · ha⁻¹ and 70 kg · ha⁻¹ of nitrogen from urea were applied before sowing. In the first productive year, 316 kg of nitrogen (112 kg at the beginning of the growing season, 68 kg after each of three consecutive harvests), 60 kg of potassium after each cutting, and 150 kg of phosphorus as a single dose at the beginning of the growing season were applied. In the first year, five cuttings were made on the following dates: 1st cut – 18 May, 2nd cut – 14 June (27 days of regrowth), 3rd cut – 14 July (30 days of regrowth), 4th cut – 13 September (61 days of regrowth), 5th cut – 26 October (43 days of regrowth). The first cutting was made in the early heading stage.

Silage preparation

Herbage was cut at a height of 5 cm with a John Deere 328A disc mower (Deere & Company, Moline, Illinois, USA) around noon (10:00–14:00). The forage was left in the field and allowed to wilt for 24 h, during which time it was tedded with a Kuhn GF 5001 MH Digidrive tedder (Kuhn S.A., Saverne, France). The next day, the wilted grass was raked with a Fella 425 rake (Fella-Werke, Feucht, Germany) and then baled and wrapped with a Kuhn FBP 3135 baler-wrapper (Kuhn S.A., Saverne, France). The bales were wrapped with six layers of plastic film (thickness – 30 µm, width – 750 mm). The bales from each harvest were tagged for identification and stored in a vertical position.

Mowing, tedding, raking, and baling-wrapping were performed alternately to ensure uniform wilting time in all plots. Representative herbage samples were collected immediately after mowing and stored at -25°C , silage samples were collected each time during feed ration preparation and stored at -25°C . After thawing, the samples were partially dried at 55°C in Binder FED dryers with forced air circulation (Binder GmbH, Tuttlingen, Germany) and ground to a particle size of 1 mm in a mill for fibrous materials, Retsch ZM 200 (Retsch, Haan, Germany).

Animals and management

The Act of January 15, 2015, on the Protection of Animals Used for Scientific or Educational Purposes was followed in the care of the animals used in this experiment (Journal of Laws 2015, item 266). The animal handling and sampling procedures used in this study not require approval from the Local Ethics Committee for Animal Experiments.

The experiment was performed on 45 Holstein-Friesian Black-and-White bulls with average initial body weight of 618 kg, at the final stage of the fattening period. All animals originated from the same herd and were housed in a tie-stall barn with free access to water. During the four months prior to the trial, they were fed roughage and concentrates with grass silage as the main component.

The bulls were divided into three equal groups, of 15 animals each ($n = 15$). During the 90-day trial, they were fed *ad libitum* a total mixed ration (TMR) composed of experimental feeds and ground barley (70:30 ratio on a DM basis). Groups FF, FP, and FL received diets containing silages made from *F. braunii* cvs. Felopa, Perun, and Lofa, respectively. The feed ration was supplemented with the mineral-vitamin premix Blattin M92 ADE (Blattin Sp. z o. o., Poland) in the amount of $100\text{ g}\cdot\text{animal}^{-1}$ per day. The premix contains (per kg): calcium – 21%, phosphorus – 3.0%, sodium – 8.0%, vitamin A – 500 000 IU, vitamin D₃ – 80 000 IU, vitamin E – 300 mg, vitamin K₃ – 20 mg, vitamin B₁ – 20 mg, vitamin B₂ – 30 mg, vitamin B₆ – 25 mg, vitamin B₁₂ – 250 mcg, niacin – 160 mg, D-calcium pantothenate – 70 mg, folic acid – 4 mg, zinc – 7 000 mg, manganese – 3 000 mg, copper – 1 500 mg, cobalt – 20 mg, iodine – 100 mg, selenium – 40 mg. The TMR for each group was prepared every other day using a Seko Samurai 5 450/110 mixing wagon (SEKO Quality and Technology, Rieti, Italy). Feed was administered twice a day, at 8.00 and 16.00. The daily feed ration was calculated so that uneaten feed did not exceed 5%.

Digestibility

The nutrient digestibility of diets was determined by the simple balance method. The experiment does not need the Approval of the Relevant Ethics Committee of the procedures used in experiments on animals. The amount of feed offered to the animals, leftovers and feces was weighed and recorded between 30 and 35 days of controlled feeding. Digestibility within the tested variety was examined on 15 animals in each experimental group ($n = 15$). In order to test digestibility within the next cut in each experimental group, five subgroups of 3 animals each were separated ($n = 3$). During the collection period, uneaten feed was weighed daily at 7:00 and 5% samples were taken. Faeces were collected daily for 24 h, a bulk sample was weighed, then an average analytical sample of approximately 200 g was prepared for each animal and each day. All samples were frozen at -25°C . After thawing, average analytical samples were prepared. Faeces samples collected from all animals were pooled and homogenized, and an analytical sample was taken to determine CP content. The other part of the faecal sample was analysed for the content of DM, crude ash, and neutral detergent fibre (NDF). After thawing, feed, leftover, and faeces samples were partially dried at 55°C in Binder FED dryers with forced air circulation (Binder GmbH, Tuttlingen, Germany), and feed and leftover samples were ground to a particle size of 1 mm in a mill for fibrous materials, Retsch ZM 200 (Retsch, Haan, Germany), whereas faeces samples were ground in a laboratory mill (WŻ-1, Sadkiewicz Institute, Bydgoszcz, Poland).

Analytical methods

The samples were analysed for the content of: DM, crude ash, CP, ether extract (EE), and CF – by standard AOAC methods (AOAC International, 2016), structural carbohydrate fractions, including NDF – assayed with the use of heat-stable amylase and expressed exclusive of residual ash (aNDFom), acid detergent fibre (ADF) – expressed exclusive of residual ash (ADFom), acid detergent lignin (ADL) – as described by Van Soest et al. (1991) using the ANKOM 220 fibre analyser (ANKOM Technology Corp., Macedon, NY). The content of water-soluble carbohydrates (WSC) was determined by the anthrone method (Thomas, 1977) using the EPoll 20 BIO spectrophotometer (Poll Limited, Warsaw, Poland). The buffering capacity (BC) of herbage was determined according to the method proposed by Playne and McDonald (1966).

The pH of silage was measured with the CP-411 pH-meter (Elmetron sp. j., Zabrze, Grzybowice, Poland). The concentrations of short-chain fatty acids (SCFAs) and lactic acid (LA) were determined as described by Kostulak-Zielińska and Potkański (2001), and Gąsior (2002). The concentrations of acetic acid (AA), propionic acid, isobutyric acid, butyric acid (BA), isovaleric acid, and valeric acid were determined by gas chromatography using the Varian 450-GC gas chromatograph with the Varian CP-8410 autosampler (Varian Inc., Palo Alto, CA) and a flame-ionization detector (FID). The samples (1.0 µl) were placed on the CP-FFAP capillary column (length – 25 m, inner diameter – 0.53 mm, film thickness – 1.0 µm). Carrier gas was helium (flow rate – 5.0 ml·min⁻¹). Detector temperature was 260 °C, injector temperature was 200 °C, and column temperature was 90 °C→200 °C. Lactic acid content was determined by high-performance liquid chromatography (HPLC) using a SHIMADZU chromatograph (SCHIMADZU, Kyoto, Japan). Separation was carried out using METACARB 67H columns (Varian Inc, Palo Alto, CA) and a mobile phase – 0.002 M solution of sulphuric acid in de-ionized water, with a flow rate of 1 cm³·min⁻¹. The chromatograph was equipped with a UV detector.

The ammonia nitrogen (NH₃-N) content of silage was determined by direct distillation using the 2100 Kjeltex Distillation Unit (Foss Analytical A/S, Hillerød, Denmark) after the pH of the samples had been increased by adding MgO. Silage samples were also analyzed to determine the proportions of the following nitrogen fractions: protein nitrogen, neutral-detergent insoluble nitrogen (NDIN), and acid-detergent insoluble nitrogen (ADIN) – as described by Licitra et al. (1996), buffer-soluble nitrogen (BSN) - using McDougall's buffer, with separation into buffer-soluble protein nitrogen (BSPN) and non-protein buffer-soluble nitrogen (NPBSN), as described by Hedqvist and Uden (2006).

Calculations and statistical analyses

Organic matter (OM) content was calculated as the difference between DM content and crude ash content. The fermentability coefficient (FC) of herbage was calculated using the following equation: $FC = SM(\%) + 8 \cdot WSC/BC$ (Weissbach and Auerbach, 2013). Based on the analysis of the nitrogen fractions of silage, CP was divided into fractions according to the Cornell Net Carbohydrate and Protein System (CNCPS). Fraction A, which consists of non-protein nitrogen (NPN), was calculated as the difference between total nitrogen (N_{total}) and

protein nitrogen (N_{protein}). Fraction B, which consists of protein nitrogen, was divided into three subfractions (B1, B2, B3) based on solubility and ruminal degradability. Subfraction B1 is BSPN, subfraction B2 is neutral-detergent soluble protein calculated as the difference between buffer-insoluble protein (IP) and NDIN, subfraction B3 is acid-detergent soluble protein calculated as the difference between NDIN and ADIN. Fraction C consists of ADIN (Licitra et al., 1996).

The nutritional value of *F. braunii* herbage and silage was determined based on their chemical composition, using PrevAlim 3.23 software 3.23 (INRA 3.3). Energy value was expressed as the feed unit for milk production (UFL) and the feed unit for meat production (UFV), and protein value was expressed as the content of protein digested in the small intestine when nitrogen is limiting (PDIN) and protein digested in the small intestine when energy is limiting (PDIE) per kg DM forage.

The apparent digestibility coefficients (ADC) of DM, OM, CP, and NDF were calculated using the following equation: $ADC (\%) = (\text{nutrient ingested, g} / \text{nutrient intake, g}) \times 100\%$. The amount of nutrient ingested was calculated as the difference between the amount of nutrient intake and the amount of nutrient excreted in the faeces. D-value was calculated as described by Jarrige (1989).

The results were processed statistically by analysis of variance (ANOVA). The significance of differences between treatment group means for the analysed traits and parameters was determined by Tukey's test, and the significance of the combined effect exerted by both experimental factors was estimated by Levene's test, at a significance level of $P = 0.05$. All statistical calculations were performed using STATISTICA software (Statsoft version 13.1, TIBCO Software Inc., Palo Alto, CA).

Results

Herbage

Cultivar had a significant effect on the content of CP, EE, CF, NDF, and ADL ($P \leq 0.05$), and successive cuts had a significant effect on all analysed chemical parameters of *F. braunii* herbage ($P \leq 0.05$) except DM content ($P = 0.726$) (Table 1). Among the studied *F. braunii* cultivars, cv. Lofa had the highest content of CP ($P = 0.024$), CF ($P \leq 0.01$), NDF ($P = 0.034$), and ADL ($P \leq 0.01$), and the lowest EE content ($P = 0.007$). Herbage cv. Perun obtained statistically the least CP ($P = 0.024$), and cv. Felopa

Table 1. Chemical composition of *Festulolium* herbage

Item n = 15	Cultivar			Harvest					SEM	P-value		
	FF	FP	FL	1 st	2 nd	3 rd	4 th	5 th		Cultivar (Cv)	Harvest (H)	Interaction (Cv x H)
DM, g·kg ⁻¹ FM	224	228	238	223	210	219	222	216	2.479	0.789	0.726	0.090
Chemical composition, g·kg ⁻¹ DM												
OM	913	923	917	926 ^a	927 ^a	905 ^{bd}	911 ^b	919 ^{ac}	2.435	0.106	0.042	≤0.01
CP	196 ^{ab}	175 ^b	206 ^a	177 ^b	178 ^b	214 ^a	203 ^{ab}	190 ^{ab}	7.225	0.024	0.049	≤0.01
EE	26.1 ^a	24.3 ^{ab}	21.9 ^b	25.0 ^a	20.0 ^b	28.2 ^{ac}	25.0 ^a	22.5 ^d	0.733	0.007	0.002	≤0.01
CF	237 ^b	233 ^b	276 ^a	221 ^b	275 ^a	242 ^{ab}	245 ^{ab}	260 ^a	5.868	≤0.01	≤0.01	≤0.01
NDF	527 ^b	538 ^{ab}	577 ^a	475 ^b	571 ^a	533 ^a	581 ^a	576 ^a	2.005	0.034	≤0.01	≤0.01
ADF	299	297	314	265 ^b	318 ^a	306 ^a	313 ^a	315 ^a	2.664	0.222	≤0.01	≤0.01
ADL	16.2 ^b	15.7 ^b	26.5 ^a	11.2 ^b	25.5 ^a	20.3 ^a	18.4 ^{ab}	22.0 ^a	1.679	≤0.01	≤0.01	0.02

FF – *Festulolium braunii* cv. Felopa, FP – *F. braunii* cv. Perun, FL – *F. braunii* cv. Lofa; DM – dry matter; FM – fresh matter; OM – organic matter; CP – crude protein; EE – ether extract; CF – crude fibre; NDF – neutral detergent fibre; ADF – acid detergent fiber; ADL – acid detergent lignin; SEM – standard error of the mean; values followed by the same superscript letters (a – d) are not significantly different at $P \leq 0.05$

had the lowest NDF ($P = 0.034$) content. Both cvs. Perun and Felopa had statistically confirmed lower CF ($P \leq 0.01$) and ADL ($P \leq 0.01$) contents. First-cut herbage had the lowest content of NDF, ADF, and ADL ($P \leq 0.01$), which resulted in the lowest concentration of easily digestible structural carbohydrates – hemicellulose and cellulose. The concentrations of NDF, ADF, and ADL were higher in subsequent cuts ($P \leq 0.01$). The CP content of herbage was also affected by the number of cuts ($P = 0.049$) and was lowest in first-cut and second-cut forage. The interaction effect of both experimental factors was found for all components ($P \leq 0.05$) except DM ($P = 0.090$).

Festulolium braunii cvs. Felopa and Perun had the highest WSC concentration ($P = 0.046$) and the highest BC ($P = 0.005$) (Table 2). First-cut herbage was most suitable for ensiling, as confirmed by the proportion of WSC and the value of FC ($P \leq 0.01$), despite the highest BC ($P \leq 0.01$). Third-cut herbage was characterized by intermediate levels of WSC, the

highest BC, and the lowest value of FC ($P \leq 0.01$). The above parameters were significantly influenced by both experimental factors ($P \leq 0.05$). Cultivar had a significant effect on PDIN ($P = 0.042$). Successive cuts differed significantly in UFL and UFV values ($P \leq 0.01$). Third-cut and fourth-cut herbage had the lowest energy value ($P \leq 0.01$). The protein value did not differ significantly between successive cuts. The interaction effect of both experimental factors was noted for the values of UFL, UFV, and PDIN ($P = 0.002$).

Silage

The chemical composition of silages from all varieties was similar, except for the concentrations of EE ($P = 0.020$), CF ($P \leq 0.01$), and WSC ($P \leq 0.01$) (Table 3). Silage made from cv. Felopa had the highest EE content, while cv. Perun obtained the lowest values ($P = 0.020$). Statistically, the highest CF content was confirmed for silage made from cvs. Felopa and Perun, relative cv. Lofa ($P \leq 0.01$).

Table 2. Ensilability and nutritional value of *Festulolium* herbage

Item n = 15	Cultivar			Harvest					SEM	P-value			
	FF	FP	FI	1 st	2 nd	3 rd	4 th	5 th		Cultivar (Cv)	Harvest (H)	Interaction (Cv x H)	
Ensilability													
WSC, g·kg ⁻¹ DM	100 ^a	116 ^a	71.3 ^b	194 ^a	99.9 ^{bc}	69.2 ^b	45.5 ^{bd}	72.7 ^b	4.905	0.046	≤0.01	0.004	
BC, g LA·kg ⁻¹ DM	44.6 ^a	43.4 ^a	35.6 ^b	48.3 ^a	42.3 ^{ac}	48.3 ^a	30.6 ^{bd}	36.4 ^b	1.499	0.005	≤0.01	0.001	
FC	39.7	43.5	39.7	63.9 ^a	39.8 ^{bc}	29.4 ^{bd}	34.1 ^b	37.6 ^b	0.866	0.681	≤0.01	0.001	
Nutritional value													
UFL	0.96	0.96	0.96	0.97 ^a	0.97 ^a	0.95 ^b	0.95 ^b	0.96 ^{ab}	0.002	0.669	≤0.01	0.002	
UFV	0.92	0.92	0.92	0.92 ^{ab}	0.93 ^a	0.91 ^b	0.90 ^b	0.92 ^{ab}	0.002	0.661	≤0.01	0.002	
PDIN, g·kg ⁻¹ DM	123 ^{ab}	110 ^b	127 ^a	111	112	135	124	118	1.700	0.042	0.062	0.002	
PDIE, g·kg ⁻¹ DM	102	98.6	103	99.0	100	104	102	101	1.000	0.068	0.347	0.105	

FF – *Festulolium braunii* cv. Felopa, FP – *F. braunii* cv. Perun, FL – *F. braunii* cv. Lofa; WSC – water-soluble carbohydrates; DM – dry matter; BC – buffering capacity; LA – lactic acid; FC – fermentability coefficient; UFL – feed unit for milk production; UFV – feed unit for meat production; PDIN – protein digested in the small intestine when nitrogen is limiting; PDIE – protein digested in the small intestine when energy is limiting; SEM – standard error of the mean; values followed by the same superscript letters (a – d) are not significantly different at $P \leq 0.05$

Table 3. Chemical composition and nutritional value of *Festulolium* silage

Item n = 15	Cultivar			Harvest					SEM	P-value		
	FF	FP	FL	1 st	2 nd	3 rd	4 th	5 th		Cultivar (Cv)	Harvest (H)	Interaction (Cv x H)
DM, g·kg ⁻¹ FM	379	381	370	319 ^{bd}	362 ^{bc}	381 ^a	353 ^b	332 ^b	18.19	0.153	0.003	0.047
Chemical composition, g·kg ⁻¹ DM												
OM	912	915	903	919 ^a	924 ^a	904 ^{ab}	893 ^b	909 ^{ab}	5.312	0.160	≤0.01	≤0.01
CP	201	182	185	185	175	188	207	191	5.215	0.093	0.058	0.059
EE	34.6 ^a	28.1 ^b	30.0 ^{ab}	32.9	26.5	32.9	32.6	29.6	1.766	0.020	0.380	0.013
CF	276 ^a	287 ^a	199 ^b	262	213	279	273	243	26.11	≤0.01	0.458	0.047
NDF	558	549	543	496	579	579	538	558	9.682	0.879	0.067	0.109
ADF	290	333	346	267	332	339	339	336	20.75	0.099	0.072	0.059
ADL	20.0	27.3	25.8	10.2 ^{bd}	26.6 ^{bc}	20.7 ^b	34.0 ^a	30.3 ^a	1.863	0.147	≤0.01	≤0.01
WSC	42.5 ^a	20.9 ^{bc}	13.7 ^{bd}	19.7	19.8	19.7	19.6	19.7	5.912	≤0.01	0.999	≤0.01
Nutritional value												
UFL, per kg DM	1.15 ^b	1.20 ^a	1.16 ^b	1.20 ^a	1.18 ^{ab}	1.18 ^{ab}	1.15 ^b	1.17 ^{ab}	0.011	≤0.01	0.041	≤0.01
UFV, per kg DM	1.07 ^b	1.13 ^a	1.09 ^b	1.12 ^a	1.10 ^{ab}	1.10 ^{ab}	1.08 ^b	1.09 ^{ab}	0.011	≤0.01	0.040	≤0.01
PDIN, g·kg ⁻¹ DM	120	119	137	121	110	129	134	116	1.001	0.508	0.514	0.067
PDIE, g·kg ⁻¹ DM	112	101	109	101	99.8	98.9	111	104	0.099	0.499	0.418	0.065

FF – *Festulolium braunii* cv. Felopa, FP – *F. braunii* cv. Perun, FL – *F. braunii* cv. Lofa; DM – dry matter; FM – fresh matter; OM – organic matter; CP – crude protein; EE – ether extract; CF – crude fibre; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin; WSC – water-soluble carbohydrates; UFL – feed unit for milk production; UFV – feed unit for meat production; PDIN – protein digested in the small intestine when nitrogen is limiting; PDIE – protein digested in the small intestine when energy is limiting; SEM – standard error of the mean; values followed by the same superscript letters (a – d) are not significantly different at $P \leq 0.05$

Silage made from cv. Felopa had the highest content of residual WSC, and silage made from cv. Lofa had the lowest WSC concentration ($P \leq 0.01$). Silages produced from successive harvests differed significantly in the content of DM ($P = 0.003$) and OM ($P \leq 0.01$). Silage made from the third-cut had the highest DM concentration, while silage produced from the first-cut received the least DM ($P = 0.003$). Silage produced from first-cut and second-cut had the highest OM content, and silage made from fourth-cut obtained the lowest values ($P \leq 0.01$). Silage produced from the other cuts was characterized by intermediate values. Fourth-cut and fifth-cut silages were highest in ADL ($P \leq 0.01$). A significant interaction effect of both experimental factors was noted for the content of DM, OM, EE, CF, ADL, and WSC in the analysed silages ($P \leq 0.05$).

The energy value of silages varied significantly depending on cultivar ($P \leq 0.01$), successive cuts ($P \leq 0.05$), and the interaction of both experimental factors ($P \leq 0.01$) (Table 3). Silage made from cv. Perun was characterized by the highest values of UFL and UFV ($P \leq 0.01$). Net energy content was highest in first-cut silage and lowest in fourth-cut silage ($P \leq 0.01$).

An analysis of selected parameters of fermentation (Table 4) revealed that only the concentration of SCFAs was not significantly affected by cultivar ($P = 0.713$). The fermentation pattern was significantly different in silage made from cv. Felopa vs. silages made from cv. Perun and cv. Lofa, as indicated by the highest LA content ($P = 0.021$). Silage made from cv. Felopa had the highest LA content, the highest pH ($P = 0.025$), and the lowest AA content ($P = 0.001$).

Table 4. Fermentation parameters of *Festulolium* silage

Item n = 15	Cultivar			Harvest					SEM	P-value		
	FF	FP	FL	1 st	2 nd	3 rd	4 th	5 th		Cultivar (Cv)	Harvest (H)	Interaction (Cv x H)
pH	4.63 ^a	4.40 ^b	4.50 ^{ab}	4.52 ^{ab}	4.53 ^{ab}	4.63 ^a	4.32 ^b	4.48 ^b	0.047	0.025	≤0.01	≤0.01
LA, g·kg ⁻¹ DM	207 ^a	113 ^b	105 ^b	207 ^a	132 ^{bc}	50.6 ^{bd}	101 ^{bc}	117 ^{bc}	19.03	0.021	0.018	≤0.01
AA, g·kg ⁻¹ DM	1.95 ^b	1.85 ^b	5.87 ^a	1.94 ^b	1.07 ^b	4.85 ^a	5.13 ^a	3.10 ^b	1.148	0.001	≤0.01	≤0.01
BA, g·kg ⁻¹ DM	1.62 ^a	1.06 ^{bc}	0.10 ^{bd}	0.29 ^b	0.02 ^b	4.23 ^a	0.06 ^b	0.04 ^b	0.004	0.032	≤0.01	≤0.01
SCFAs, g·kg ⁻¹ DM	2.74	2.02	1.29	0.43 ^b	0.17 ^b	9.17 ^a	0.15 ^b	0.16 ^b	0.023	0.713	≤0.01	≤0.01
NH ₃ -N, g·kg ⁻¹ N _{total}	28.9 ^{ab}	26.5 ^b	32.9 ^a	19.6 ^b	26.6 ^{ab}	22.4 ^{ab}	24.32 ^{ab}	34.9 ^a	2.080	≤0.01	0.016	0.039

FF – *Festulolium braunii* cv. Felopa, FP – *F. braunii* cv. Perun, FL – *F. braunii* cv. Lofa; DM – dry matter; LA – lactic acid; AA – acetic acid; BA – butyric acid; SCFAs – short-chain fatty acids (SCFAs are the sum of propionic acid, isobutyric acid, isovaleric acid, and valeric acid); NH₃-N – ammonia nitrogen; N_{total} – total nitrogen; SEM – standard error of the mean; values followed by the same superscript letters (a – d) are not significantly different at $P \leq 0.05$

Table 4. Fermentation parameters of *Festulolium* silage

Item n = 15	Cultivar			Harvest					SEM	P-value		
	FF	FP	FL	1 st	2 nd	3 rd	4 th	5 th		Cultivar (Cv)	Harvest (H)	Interaction (Cv x H)
pH	4.63 ^a	4.40 ^b	4.50 ^{ab}	4.52 ^{ab}	4.53 ^{ab}	4.63 ^a	4.32 ^b	4.48 ^b	0.047	0.025	≤0.01	≤0.01
LA, g·kg ⁻¹ DM	207 ^a	113 ^b	105 ^b	207 ^a	132 ^{bc}	50.6 ^{bd}	101 ^{bc}	117 ^{bc}	19.03	0.021	0.018	≤0.01
AA, g·kg ⁻¹ DM	1.95 ^b	1.85 ^b	5.87 ^a	1.94 ^b	1.07 ^b	4.85 ^a	5.13 ^a	3.10 ^b	1.148	0.001	≤0.01	≤0.01
BA, g·kg ⁻¹ DM	1.62 ^a	1.06 ^{bc}	0.10 ^{bd}	0.29 ^b	0.02 ^b	4.23 ^a	0.06 ^b	0.04 ^b	0.004	0.032	≤0.01	≤0.01
SCFAs, g·kg ⁻¹ DM	2.74	2.02	1.29	0.43 ^b	0.17 ^b	9.17 ^a	0.15 ^b	0.16 ^b	0.023	0.713	≤0.01	≤0.01
NH ₃ -N, g·kg ⁻¹ N _{total}	28.9 ^{ab}	26.5 ^b	32.9 ^a	19.6 ^b	26.6 ^{ab}	22.4 ^{ab}	24.32 ^{ab}	34.9 ^a	2.080	≤0.01	0.016	0.039

FF – *Festulolium braunii* cv. Felopa, FP – *F. braunii* cv. Perun, FL – *F. braunii* cv. Lofa; DM – dry matter; LA – lactic acid; AA – acetic acid; BA – butyric acid; SCFAs – short-chain fatty acids (SCFAs are the sum of propionic acid, isobutyric acid, isovaleric acid, and valeric acid); NH₃-N – ammonia nitrogen; N_{total} – total nitrogen; SEM – standard error of the mean. Values followed by the same superscript letters (a – d) are not significantly different at $P \leq 0.05$

The concentration of BA was also significantly highest in this treatment ($P = 0.032$).

Significant differences in the fermentation patterns were found between successive cuts (Table 4). The pH of silages ranged from 4.32 (fourth harvest) to 4.63 (third harvest). The concentration of LA was highest in first-cut silage ($P = 0.018$), which however was characterized by significantly lower levels of the remaining acids ($P \leq 0.01$). Third-cut silage had the lowest LA content, but high concentrations of AA ($P \leq 0.01$) and BA ($P \leq 0.01$), SCFA content was also the highest ($P \leq 0.01$). A significant difference in NH₃-N concentration was observed only between first-cut and fifth-cut silages ($P = 0.016$). The silage from the first-cut was characterized by the lowest NH₃-N content, while in the fifth-cut these values were the highest.

An analysis of the quality of CP revealed that only the proportion of fraction B1 was influenced by cultivar ($P = 0.046$) (Table 5), whereas the proportions of all analysed CP fractions were significantly affected by successive cuts. The highest concentration of the B1 fraction was characterized by silage made from the cv. Perun relative to the other two cvs. Felopa and Lofa, between which no significant differences were observed. First-cut silage had the significantly highest content of fraction A ($P \leq 0.01$). The concentration of fraction B1 was significantly lower in second-cut and third-cut silages ($P = 0.037$) than in the other silages, which were characterized by similar values of this parameter. Second-cut and third-cut silages had the highest proportion of fraction B2 ($P = 0.023$), and significant differences in this parameter were also found between fourth-cut

Table 5. Nitrogen fraction composition of *Festulolium* silage (g·kg⁻¹ N_{total}) according to the Cornell Net Protein and Carbohydrate System

Item n = 15	Cultivar			Harvest					SEM	P-value		
	FF	FP	FL	1 st	2 nd	3 rd	4 th	5 th		Cultivar (Cv)	Harvest (H)	Interaction (Cv x H)
Fraction A	613.0	584.8	585.7	675.0 ^a	564.5 ^b	501.6 ^b	556.1 ^b	589.8 ^b	20.10	0.879	≤0.01	0.254
Fraction B1	82.1 ^b	100.0 ^a	93.5 ^b	114.5 ^a	82.1 ^b	87.4 ^b	111.4 ^a	119.8 ^a	5.878	0.046	0.037	0.045
Fraction B2	177.8	176.7	179.4	123.1 ^{bd}	211.4 ^a	233.3 ^a	176.3 ^{bc}	155.7 ^{bc}	3.765	0.765	0.023	0.165
Fraction B3	89.1	97.7	96.3	55.4 ^{bd}	107.3 ^{bc}	121.3 ^a	114.9 ^{bc}	105.8 ^{bc}	2.765	0.819	≤0.01	0.044
Fraction C	38.0	40.8	45.1	32.0 ^b	34.7 ^b	56.4 ^a	41.3 ^{bc}	28.9 ^{bd}	2.571	0.689	≤0.01	0.051

FF – *Festulolium braunii* cv. Felopa, FP – *F. braunii* cv. Perun, FL – *F. braunii* cv. Lofa; A – non-protein nitrogen; B1 – buffer-soluble nitrogen; B2 – neutral detergent-soluble nitrogen; B3 – acid detergent-soluble nitrogen; C – acid detergent-insoluble nitrogen; N_{total} – total nitrogen; SEM – standard error of the mean; values followed by the same superscript letters (a – d) are not significantly different at $P \leq 0.05$

Table 6. Apparent nutrient digestibility coefficients of the experimental diets

Item n = 15	Cultivar			Harvest					SEM	P-value		
	FF	FP	FL	1 st	2 nd	3 rd	4 th	5 th		Cultivar (Cv)	Harvest (H)	Interaction (Cv x H)
Apparent digestibility coefficient, %												
Dry matter	81.4	85.7	83.1	85.7 ^a	80.7 ^{bc}	85.6 ^a	77.4 ^{bd}	78.2 ^{bd}	0.741	0.895	0.045	≤0.01
Organic matter	83.5	87.2	85.1	87.6 ^a	82.4 ^b	87.5 ^a	81.3 ^b	82.2 ^b	0.689	0.903	0.024	≤0.01
Crude protein	80.3	82.7	80.8	82.8	80.8	83.1	81.7	83.2	0.789	0.854	0.096	≤0.01
NDF	69.8	75.7	71.4	74.2 ^a	69.1 ^{bc}	75.2 ^a	66.1 ^b	64.3 ^{bd}	0.880	0.723	0.030	≤0.01
D-value, g·kg ⁻¹ DM	762	798	768	805 ^a	761 ^{bc}	791 ^a	756 ^b	747 ^{bd}	5.860	0.673	0.028	≤0.01

FF – *Festulolium braunii* cv. Felopa, FP – *F. braunii* cv. Perun, FL – *F. braunii* cv. Lofa; NDF – neutral detergent fiber; D-value – digestible organic matter; SEM – standard error of the mean; values followed by the same superscript letters (a – d) are not significantly different at $P \leq 0.05$

and fifth-cut silages vs. first-cut silage. A comparison of the CP composition of silages showed that the proportions of fractions B3 and C varied significantly across cuts ($P \leq 0.01$) and were highest in third-cut silage. The interaction effect of both experimental factors was found for the valuable fractions B1 ($P = 0.045$) and B3 ($P = 0.044$).

Digestibility

The nutrient digestibility of experimental diets is presented in Table 6. Cultivar had no significant effect on the digestibility coefficients of selected nutrients, whereas differences were observed between successive cuts. First-cut and third-cut silages were characterized by the highest apparent digestibility coefficients of DM ($P = 0.045$), OM ($P = 0.024$), NDF ($P = 0.030$), and digestible OM (D -value) ($P = 0.028$). The digestibility coefficients of nutrients were significantly lower in fourth-cut and fifth-cut silages. Only the apparent digestibility coefficient of CP was similar in all cultivars ($P = 0.854$) and cuts ($P = 0.096$). The interaction effect of both experimental factors was noted for the values of all parameters in Table 6.

Discussion

Herbage

The NDF content of *F. braunii* herbage, observed in this study, was similar to the values reported by Østrem et al. (2015) for cv. Felopa (452, 495, 510, and 564 g NDF·kg⁻¹ DM in successive cuts). In the current experiment, the high concentration of NDF in cvs. Perun and Lofa (mean of five harvests) was comparable with the cited values, most likely due to the low proportion of leaves in the tested cultivars, relative to other *F. braunii* cultivars. Similar observations were made by Frankow-Lindberg and Olsson (2008), who found that leaf NDF content at successive harvest dates (25 May, 2 June, 7 June) reached 563, 565 and 589 g·kg⁻¹ DM in cv. Hykor and was much lower in cvs. Paulita and Perun harvested on the same dates (450, 460, and 465 g·kg⁻¹ DM, and 450, 465, and 485 g·kg⁻¹ DM, respectively). According to the cited authors (Frankow-Lindberg and Olsson, 2008), the genetic origin of the hybrids played a key role in determining their concentration of structural carbohydrates. Cultivars Paulita and Perun, with *F. pratensis* as one of the parent species, had identical qualitative traits as cv. Hykor, with *F. arundinacea* as one of the parent species. Frankow-Lindberg and Olsson (2008)

also demonstrated that the above cultivars differed significantly in leafiness depending on their parent species. Therefore, different cultivars should be harvested at different times to improve the composition and content of structural carbohydrates (Ghesquière et al., 1996; Frankow-Lindberg and Olsson, 2008).

In the present study, the NDF content of *F. braunii* cv. Perun was considerably higher than that reported by Frankow-Lindberg and Olsson (2008), partly due to the fact that all tested cultivars were harvested at the same time.

The CP content of *F. braunii* herbage, noted in this study, was high relative to that reported by Staniak and Harasim (2018) for cv. Sulino (132, 149, and 128 g CP·kg⁻¹ DM in three consecutive years, respectively). The highest values were recorded in the fourth cut, 172 and 165 g CP·kg⁻¹ DM in two consecutive years, respectively (Staniak and Harasim, 2018). These results are similar to the lowest values noted in the current experiment. The CP content of *F. braunii* herbage, determined in this study, was comparable with that reported by Houdek and Jambor (2010) for cv. Perun at the first harvest (174 g·kg⁻¹ DM). However, CP concentration decreased in subsequent harvests (144 and 145 g kg⁻¹ DM) (Houdek and Jambor, 2010). In the present study, cv. Lofa was characterized by higher CP content than the values noted by Houdek and Jambor (2010) in the same cultivar during three consecutive harvests (171, 173, and 133 g·kg⁻¹ DM, respectively).

Babić et al. (2022) analyzed CP concentration in different *F. braunii* cultivars, including cv. Felopa (169.6 g·kg⁻¹ DM) and cvs. Perun and Lofa (116.2 and 128.0 g kg⁻¹ DM, respectively, first harvest). In second-cut forage, CP content was higher in cv. Perun (131.9 g·kg⁻¹ DM) and cv. Lofa (145.5 g·kg⁻¹ DM), and lower in cv. Felopa (142.1 g·kg⁻¹ DM), compared with first-cut forage (Babić et al., 2022).

The *F. braunii* cultivars analysed in this study had higher CP content, which could be influenced by various factors such as fertilization and environmental conditions. Staniak (2016) conducted a pot experiment where the following fertilizers were applied (g·pot⁻¹): 3.6 g nitrogen (N) in three rates, 1.0 g phosphorus (P), 1.5 g potassium (K), and 0.5 g magnesium (Mg). The study demonstrated that greater amounts of CP were accumulated by forage grasses in response to long-term drought stress than under optimal soil moisture conditions. In turn, in a pot experiment carried out by Staniak and Harasim (2018), drought stress did not induce a significant increase in CP concentration in *F. braunii* supplied

with the following fertilizer rates ($\text{g} \cdot \text{pot}^{-1}$): 0.5 g N, 1.0 g P, 1.5 g K, and 0.5 g Mg before sowing, and 0.5 g N after each harvest.

These inconclusive results may indicate that the CP content of plants is determined by a variety of factors such as soil type, soil fertility, fertilization, and temperature (Staniak, 2016). Differences in CP concentration within cultivars and cuts may also be due to differences in the distribution of N forms in vegetative plant parts (Lemaire et al., 2005). According to Geren et al. (2014), prolonged cutting intervals increase the growth rate of grasses while decreasing the leaf-to-stem ratio; in turn, frequent cutting increases the proportion of leaves, which explains the differences in CP concentration between cuts.

Previous research has revealed differences in the chemical composition of primary growth and regrowth grasses (Kuoppala et al., 2008; Huuskonen and Pesonen, 2017). Kuoppala et al. (2008) found that the regrowth grass differed from the primary growth grass in terms of tillering, chemical composition, digestibility, and palatability. The regrowth grass had a higher content of crude ash and CP, and lower NDF content than the primary growth grass, but great variation in chemical composition was noted within cultivars and between cuts, mostly due to differences in harvest time and environmental factors (Kuoppala et al., 2008). The differences in the crude ash and CP content of *F. braunii* herbage, observed in successive harvests in this study, are consistent with previous findings, but NDF content was higher in all subsequent cuts than in the first cut.

The traits of *F. braunii* cultivars vary considerably depending on their parental components (Ghesquière et al., 2010). The chemical composition of grasses undergoes profound changes during maturation, between the vegetative growth stage and anthesis, with more rapid changes occurring in stems than in leaves (Østrem et al., 2015).

It is generally accepted that the ensilability of plant material is determined by the supply of substrate for lactic acid bacteria. The required amount of WSC is linked to BC, and both these parameters can be combined into the FC. In the present study, the value of FC exceeded 45 only in first-cut herbage, confirming its very high suitability for ensiling. The values of FC below 35, noted in the third and fourth cuts, indicate that these herbages were difficult to ensile (Weissbach and Auerbach, 2013).

In general, WSC concentration in the tested *F. braunii* cultivars and successive cuts was lower

than the optimal value for *Lolium*-dominated grass swards ($220\text{--}160 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$). The obtained results were closer to those reported for fresh grass regrowth supplied with a high N rate ($100 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$) (Weissbach and Auerbach, 2013). In the present study, the BC of *F. braunii* herbage was considerably lower than the values noted by Weissbach and Auerbach (2013) in *Lolium*-dominated grass swards ($55 \text{ g LA} \cdot \text{kg}^{-1} \text{ DM}$). Weisbjerg et al. (2012) found that BC was considerably higher in the primary growth than in the regrowth, which was also observed in this experiment. The values of BC increased with increasing concentrations of CP and crude ash, and decreased with increasing WSC concentration in alfalfa herbage (Stepanova and Volovik, 2021). During primary growth and subsequent summer regrowth, the values of BC decrease by $20 \text{ meq} \cdot \text{kg}^{-1} \text{ DM}$ per week on average, starting at approximately $400\text{--}450 \text{ meq} \cdot \text{kg}^{-1} \text{ DM}$. In autumn regrowth, BC is relatively stable ($350\text{--}450 \text{ meq} \cdot \text{kg}^{-1} \text{ DM}$) (Muck et al., 1991). Similar relationships were observed in the present study.

The *F. braunii* cultivars analysed by Obraztsov et al. (2022) had high WSC concentration ($147\text{--}194 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$). Kryszak et al. (2001) demonstrated that all *F. braunii* cultivars accumulated more WSC than other grass species, and WSC concentration was highest in cv. Sulino ($180 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$). In the current experiment, high rates of N fertilizer could negatively affect WSC concentration (Ciepiela et al., 2003; Weissbach and Auerbach, 2013). The increase in WSC concentration induced by decreased N fertilization can be attributed to the reduced use of carbon chains for protein synthesis and the production of energy required for nitrate reduction prior to protein synthesis (Ciepiela et al., 2003).

In the present study, the energy value and protein value of *F. braunii* herbage were high, higher than the values reported for second-cut *L. multiflorum* after 4 and 5 weeks of regrowth: 0.82 and 0.82 UFL, 0.75 and 0.76 UFV, 105 and 102 g PDIN, and 89 and 88 g PDIE per kg DM, respectively (IZ PIB-INRA, 2016). The high energy value of the forage was due to a low proportion of lignin in the tested cultivars, which significantly influences cell wall digestibility (Huuskonen and Pesonen, 2017), and a high proportion of hemicellulose, the NDF fraction characterized by the highest digestibility (Dehurst et al., 2003).

Silage

Differences in the chemical composition and energy value of silages in successive cuts were reported by Huuskonen and Pesonen (2017) who compared first-, second-, and third-cut Timothy grass silages. The analysis revealed that third-cut silage had 22–26 percentage points higher CP concentration than first- and second-cut silages, and NDF concentration in first- and second-cut silages was 33 and 20 percentage points higher, respectively, than in third-cut silage (Huuskonen and Pesonen, 2017). In the current study, the greatest differences in CP content were observed between second-cut and fourth-cut silages, and first-cut and fourth-cut silages. Fourth-cut silage contained 18.3 and 11.9 percentage points CP more than second-cut and first-cut silages, respectively. In this experiment, unlike in the study by Huuskonen and Pesonen (2017), first-cut silage had the lowest NDF content.

The significantly lowest content of residual WSC in silage made from cv. Lofa could be due to the fact that more substrate was used during fermentation (McDonald et al., 1991; Purwin, 2007). In contrast to the relationships observed by Huuskonen and Pesonen (2017), the WSC content of silages was similar at all harvest dates. In the cited study, WSC concentration was considerably higher in second-cut and third-cut silages (115 and 148 g·kg⁻¹ DM, respectively) than in first-cut silage (65 g·kg⁻¹ DM).

According to Kung et al. (2018), the pH of grass silage (25–35% DM) should typically range from 4.3 to 4.7, which was observed in this study. In silage made from cv. Felopa, average LA concentration was twice higher than the values typical of grass silages (60–100 g·kg⁻¹ DM) (Kung et al., 2018). Such a high concentration of LA could result from the highest WSC concentration in silage and high microbial activity in this silage (McDonald et al., 1991). Lactic acid is 10 to 12 times more potent than the other major acids found in silages, and is largely responsible for the decrease in pH during fermentation (Kung et al., 2018). However, in the current experiment, silage made from cv. Felopa had the significantly highest pH. This type of fermentation pattern may have resulted from several factors, including atypically high BC induced by high concentrations of CP and crude ash (Kung et al., 2018).

The concentration of AA, which exerts antifungal effects and inhibits the growth of lactate-assimilating yeasts that initiate aerobic spoilage in grass silages, should be at a level of 10–30 g·kg⁻¹ DM (Kung et al., 2018). However, the values noted in

the present study were much lower. Similarly to LA concentration, AA concentration is usually inversely proportional to DM content, which was observed in fourth-cut silage (McDonald et al., 1991). In this study, BA concentration was less than 5 g·kg⁻¹ DM, but it was not associated with poor silage quality or excessive counts and activity of *Clostridium* spp. (Kung et al., 2018).

The concentrations of organic acids and pH values of silages, observed in the present study, point to a high rate of fermentation. Purwin (2007) reported lower concentrations of organic acids in baled silage made from wilted grass. The LA content of silage is the outcome of the available WSC pool as well as the BC of the preserved crop and chop length (Purwin, 2007). According to Slottnér (2002), the ensiling environment is completely different in bales of unchopped forage and in bales of precision-chopped forage. It should be noted that in this experiment bales were formed with a baling press equipped with cutting knives.

The very low proportion of NH₃-N in the analyzed silages indicates a low extent of amino acid degradation in the fermentation process (McDonald et al., 1991). According to Kung et al. (2018), high-quality silage should contain less than 100–150 g NH₃-N·kg⁻¹ N_{total}. The levels of BA and N-NH₃ suggest that the activation of *Clostridium* spp. was largely limited during ensiling. The high rates of N fertilizer applied to the experimental grasses could contribute to increasing nitrate concentrations in herbage and, consequently, reducing the fermentative activity of *Clostridium* spp. (Kaiser et al., 2002). It can be concluded that the parameters determined in this study are typical of one of the three types of fermentation described by Huhtanen et al. (2003), namely extensive fermentation with high LA concentration and relatively low levels of NH₃-N and SCFAs.

The proportion of fraction A in the analyzed silages is consistent with the values reported by Fijałkowska et al. (2015) for grass silage (486–688 g·kg⁻¹ TN) and alfalfa silage (521–705 g·kg⁻¹ TN). Fraction A is a direct indicator of protein hydrolysis and a predominant CP fraction in alfalfa silage (Li et al., 2018) and grass silage (Du et al., 2020), which was also observed in this study. Du et al. (2020) found that fraction A accounted for 380 g·kg⁻¹ TN in the CP of silages made from native Mongolian grasses, and this value is much lower than that determined in the present study. Fraction A is rapidly degraded in the rumen, and the estimated rate of its degradation is 200%·h⁻¹. Thus, a lower proportion of this fraction is

more desirable and associated with higher CP quality in ruminant diets (Van Amburgh et al., 2015).

The proportion of fraction B1, which represents true soluble protein, in the analyzed silages was considerably higher than the values reported for grass silage ($77.8 \text{ g} \cdot \text{kg}^{-1} \text{ TN}$) by Du et al. (2020) and for alfalfa silage ($53\text{--}79 \text{ g} \cdot \text{kg}^{-1} \text{ TN}$) by Grabber and Coblenz (2009) and Chrenkova et al. (2014). This fraction is rapidly degraded in the rumen, and its amount decreases during ensiling, which favours an increase in the content of fraction A (Fijałkowska et al., 2015).

Fraction B2 represents true protein with an *intermediate degradation* rate in the rumen, which is highly digestible in the small intestine, and its content of the analyzed silages was comparable with that reported for alfalfa silage ($198 \text{ g} \cdot \text{kg}^{-1} \text{ TN}$) by Grabber and Coblenz (2009), and considerably lower than that reported for grass silage ($403.7 \text{ g} \cdot \text{kg}^{-1} \text{ TN}$) by Du et al. (2020). Fraction B2 was the second largest CP fraction in alfalfa silage (Li et al., 2018) and in grass silage (Du et al., 2020), which was also observed in silages made from *F. braunii* grass in this study. The rate of fraction B2 degradation is determined by the relative rates of digestion and passage, which in turn are affected by glutelin protein found in small grains (Li et al., 2018; Du et al., 2020).

The proportion of fraction B3, which is the difference between NDIN and ADIN in silages, is an important consideration because this CP fraction is characterized by a slow *degradation* rate in the rumen and high intestinal bioavailability, which influences the passage of by-pass protein (Van Amburgh et al., 2015). In the current experiment, all silages except for first-cut silage had a higher proportion of fraction B3 than alfalfa silage ($32.6\text{--}69.0 \text{ g} \cdot \text{kg}^{-1} \text{ TN}$) (Grabber and Coblenz, 2009). In the study by Du et al. (2020), fraction B3 accounted for $105.1 \text{ g} \cdot \text{kg}^{-1} \text{ TN}$ in grass silage. The proportion of fraction B3 increases in silage due to a spontaneous increase in the temperature of the ensiled forage mass. Some of the proteins contained in this fraction undergoes denaturation under the influence of heat, which decreases solubility (Licitra et al., 1996).

In the present study, the content of fraction C in the analyzed silages was in-between the values reported in the literature for high-quality silage. The proportion of fraction C in silages made from native Mongolian grasses examined by Du et al. (2020) was $33.6 \text{ g} \cdot \text{kg}^{-1} \text{ TN}$. Other researchers reported completely different values for fraction C in alfalfa silage,

ranging from $12.0 \text{ g} \cdot \text{kg}^{-1} \text{ TN}$ (Hymes-Fecht et al., 2013) to even $105.0 \text{ g} \cdot \text{kg}^{-1} \text{ TN}$ (Schwab et al., 2003).

Fraction C is an indicator of the extent of proteolytic changes and heat damage, and its concentration is determined by ADIN content. This CP fraction is not degraded in the rumen and is not digested in the small intestine (Fijałkowska et al., 2015). In general, the proportion of fraction C in silages is related to the cascade of Maillard reactions, which are also induced by high temperature. These reactions lead to the formation of products that are unavailable to digestive enzymes and ruminal microorganisms, as a result of the condensation of reducing sugars with amino acids. The properties of the resulting polymers are similar to those of lignin (Coblenz et al., 2008).

The quality of CP in silages made from different *F. braunii* cultivars in this experiment can be considered good or very good, compared to literature data for other silages used as important components of ruminant diets, such as alfalfa silage. However, the protein fractions of silages produced from *F. braunii* or its parent species have not been described to date, making it difficult to compare the present results with previous research findings.

The highest proportion of fraction A in first-cut silage was associated with a greater extent of proteolysis (Coblenz et al., 2008), which could be due to the lowest DM content in the first cut. Different rates of water loss from plant cells after cutting, determined by temperature, moisture, and air flow, affect the activation of proteolytic processes prior to ensiling, which could be the case in the present study (Coblenz et al., 2008). Edmunds et al. (2014) demonstrated that a higher wilting rate resulted in a lower proportion of fraction A in grass silage. This could also be due to the fact that successive cuts are harvested under different weather conditions, which affects the wilting process. However, it should be noted that the composition of CP fractions in silages is largely determined by the quality of CP in herbage (Coblenz et al., 2008; Edmunds et al., 2014).

Digestibility

The nutrient digestibility of experimental diets was high, which corroborates the findings of Steen and Kilpatrick (2000) and Steen et al. (2002) who evaluated the apparent digestibility of grass silage-based diets with different proportions of concentrates. In the current study, the *D*-value of the analyzed silages ranged from 756 to $805 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$. According to Steen et al. (2002), this range is typical of the primary growth of perennial

ryegrass, and highly digestible silages have a *D*-value of 743 g·kg⁻¹ DM, which was confirmed in the present study. Steen and Kilpatrick (2000) found that the control grass silage without concentrates was characterized by lower digestibility coefficients for DM (77.4%), CP (67.8%), and OM (72.3%). The cited authors reported that increasing the proportion of concentrates (rolled barley) in the diet, from 0 g in the control group to 360 g·kg⁻¹ DM, did not significantly affect DM or CP digestibility. However, it considerably increased the proportion of digestible OM in DM, and decreased fibre digestibility (Steen and Kilpatrick, 2000). In a subsequent study by Steen et al. (2002), increasing the proportion of concentrates (ground barley) in grass-based diets had no effect on DM or OM digestibility, either. The NDF digestibility coefficient decreased from 79.3 to 51.8% when the proportion of concentrates in the total DM of diets was increased from 20 to 80% (Steen et al., 2002). The apparent digestibility coefficient of the diets analyzed by Steen et al. (2002) was similar to that determined for DM in the present experiment (81.3–76.7%).

In a study by Pozdisek et al. (2003), the digestibility coefficients of CP and OM in *F. braunii* cv. Hykor were 59.48 and 70.50%, respectively, and were much lower than the values determined in the current experiment, whereas the NDF digestibility coefficient in cv. Hykor (74.63%) was similar to the value noted in cv. Perun in this study (75.7%). However, Pozdisek et al. (2003) demonstrated that cv. Hykor was superior to *F. arundinacea* in terms of digestibility, whereas Ghesquière et al. (1996) found that *L. multiflorum* × *F. arundinacea* hybrids were similar in nutrient digestibility to their respective parent species. According to Frankow-Lindberg and Olsson (2008), the harvest date of *F. braunii* and cutting intervals play an important role in nutrient digestibility.

Both the quantity and quality of NDF are important criteria when evaluating feed digestibility, and NDF digestibility affects OM digestibility (Huhtanen et al., 2006). In a study by Østrem et al. (2015), the content of total NDF and indigestible NDF (iNDF) in *F. braunii* increased with advancing maturity during spring growth. A similar trend was observed in the current experiment: NDF digestibility decreased in successive cuts regardless of cultivar. The DM digestibility of cvs. Hykor and Felopa decreased at successive harvest dates in the study by Østrem et al. (2015) and in the present study. At successive cutting times, cvs. Hykor and Felopa had DM digestibility coefficients of 81.6, 80.0, 77.3, and

74.8, and 84.8, 80.1, 80.0, and 74.9%, respectively (Østrem et al., 2015).

During spring growth, DM digestibility was around 6% higher in leaves and around 9% higher in stems (Østrem et al., 2015). The chemical composition of grasses changes considerably as they mature, and the changes occur more rapidly in stems than in leaves, which is reflected in nutrient digestibility. Changes are also observed between successive cuts and in response to different cutting intervals (Huhtanen et al., 2006; Østrem et al., 2015). Therefore, the leaf-to-stem ratio in *F. braunii* plays an important role in nutrient digestibility.

Literature data show that different *F. braunii* cultivars have different traits inherited from their parents: the genera *Lolium* and *Festuca*, characterized by high genetic variation, and they differ in chemical composition, which translates into differences in nutrient digestibility. *Festulolium braunii* hybrids have a high potential for genetic exchange, and their cultivars are characterized by new combinations of desirable traits. Allotetraploid cultivars of *F. braunii* are usually characterized by high genetic variation because they are derived from several species, and therefore may be a richer source of these traits than *L. perenne* (Ghesquière et al., 2010; Østrem et al., 2015). Thus, a better understanding of the effect exerted by plant growth and development processes on forage quality is important not only for optimizing forage production and use, but also for breeding purposes.

Silage made from the primary growth of grass harvested at an early stage is highly digestible, whereas regrowth silage is usually less digestible (Kuoppala et al., 2008), which was also observed in this study. A harvesting strategy involving a higher number of cuts has been gaining popularity because it ensures optimal use of the entire growing season (Hyrkäs et al., 2015). Hyrkäs et al. (2015) demonstrated that the three-cut harvesting strategy contributed to higher average forage digestibility than the two-cut harvesting strategy throughout the growing season in Finland. Thorvaldsson et al. (2007) also observed that third-cut grass may be highly digestible due to its low content of structural carbohydrates. These observations are consistent with the results of this study where third-cut herbage was characterized by significantly highest nutrient digestibility, identical to that of first-cut herbage. The high nutrient digestibility recorded in third-cut grass may be due to the low mean temperatures in the late summer season. Therefore, the nutritional value of silage made from the autumn

regrowth should be high (Huuskonen and Pesonen, 2017), which was not confirmed in the present study. According to Sairanen et al. (2016), the lower nutritional value of autumn-harvested grasses could be a consequence of more variable weather conditions supporting the growth of epiphytic microflora and the spread of plant diseases, which could negatively affect the fermentation process, and the palatability and digestibility of silage.

Conclusions

Both *Festulolium braunii* cultivar and successive cuts had a significant effect on the chemical composition and feed value of herbage and silage. The differences in the content of the most important components such as structural carbohydrates and protein fractions indicate that each *F. braunii* cultivar should be treated individually in terms of both cultivation and harvest time. *Festulolium braunii* cv. Lofa had the most desirable chemical composition, and fermentation parameters were optimal in cvs. Perun and Lofa. However, cultivar had no effect on the digestibility of diets based on the analyzed silages. In turn, an analysis of successive harvests revealed that the first and third cuts were characterized by optimal chemical composition and digestibility, which is consistent with literature data. The significant differences in the nutritional value of *F. braunii* herbage and silage observed among cultivars and cuts indicate that various cultivars cut multiple times at different harvest dates should be tested in future studies to determine intraspecific differences.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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