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ORIGINAL PAPER

EFFECTS OF CULTIVAR AND HARVEST DATE ON THE COMPOSITION OF NITROGEN FRACTIONS IN *FESTULOLIUM* HERBAGE*

Marta Borsuk, Maja Fijałkowska

Department of Animal Nutrition and Feed Science
University of Warmia and Mazury in Olsztyn, Poland

Abstract

Grasses of ryegrass (*Lolium*) and fescue (*Festuca*) genera play an important role in the nutrition of high-yielding dairy cows, but fescue x ryegrass hybrids (*Festulolium braunii)* are also increasingly used. The growing popularity of *F. braunii* can be attributed to its supreme agronomic traits, combining the high yield potential of ryegrass: *Lolium multiflorum* and *Lolium perenne*, and the high resistance of fescue: *Festuca pratensis*, *F. arundinacea* and *F. arundinacea var. glaucescens*, to biotic and abiotic factors. The aim of this study was to analyze the composition of nitrogen fractions in the herbage of different *Festulolium braunii* cultivars harvested at different dates. Three *F. braunii* cultivars were studied: Felopa, Lofa and Perun. The chemical composition of herbage (dry matter – DM, crude protein – CP, water-soluble carbohydrates – WSC, neutral detergent fiber – NDF, acid detergent fiber – ADF) and nitrogen fractions (A, B_1, B_2, B_3, C) were determined in accordance with the Cornell Net Carbohydrate and Protein System (CNCPS). In the analyzed forages, highly significant differences ($P < 0.01$) were noted in the content of DM and WSC, which were affected by both experimental factors. Significant ($P < 0.05$) differences in the NDF content of herbage were found between cultivars. In the analyzed forages, significant differences ($P < 0.05$) were noted in CP, which was affected by both experimental factors. The cultivar Felopa had the highest CP content. The content of WSC decreased at successive harvests. The cultivar Lofa had the highest crude fiber content and a lower content of WSC than the remaining *Festulolium* hybrids. The harvest date had a significant effect on the composition of all nitrogen fractions, and the proportions of fractions B_2, B_3 and C were also highly significantly ($P < 0.01$) affected by a cultivar. The composition of fractions B_3 and C was influenced by both experimental factors at $P < 0.01$. Significant ($P < 0.05$) differences were observed for fractions A. In all analyzed forages, fraction B_2 had the highest share of the total nitrogen pool. The share of fraction B_3 increased, whereas the content of fractions B_1 and B_2 decreased in fourth-cut herbage. The share of acid detergent-insoluble nitrogen (ADIN) increased at successive harvests, which decreased the nutritional value of forage.

Keywords: CNCPS, crude protein, *Festulolium braunii*, nitrogen fractions.

dr inż. Maja Fijałkowska, Department of Animal Nutrition and Feed Science, University of Warmia and Mazury, Oczapowskiego 5, 10-718 Olsztyn, Poland, e-mail: maja.fijalkowska@uwm.edu.pl

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INTRODUCTION

Grasses of ryegrass (*Lolium*) and fescue (*Festuca*) genera play an important role in the nutrition of high-yielding dairy cows, but fescue x ryegrass hybrids (*Festulolium braunii)* are also increasingly used (Charmet, Balfourier 1994, Humphreys et al. 2014). The growing popularity of *F. braunii* can be attributed to its supreme agronomic traits, combining the high yield potential of ryegrass, *Lolium multiflorum* (2*n* = 14) and *Lolium perenne* (2*n* = 14), and the high resistance of fescue, *Festuca pratensis* (2*n* = 14), *Festuca arundinacea* (2*n* = 42) and *Festuca arundinacea var. glaucescens* (2*n* = 28), to biotic and abiotic factors (Kopecký et al. 2008, Ostrem et al. 2013). Ryegrass is characterized by high palatability and digestibility, but it is sensitive to environmental factors, mainly weather conditions. Fescue has an extensive root system and is highly tolerant to water deficit, and *F. pratensis* harbors genes that encode winter hardiness (Humphreys et al. 1998, Kopecký et al. 2006). *Festulolium* varieties are produced by crossing intergenerational hybrids with different ploidy levels (Humphreys et al. 2014). The cultivars Bečva and Lofa, produced by backcrossing the *L. multiflorum* (2*n*) x *F. arundinacea* (6*n*) hybrid with *L. multiflorum* (4*n*) (Fojtík 1994), are characterized by more desirable agronomic performance traits than the parental species. They are commonly used for silage but can also be grown for grazing. These grasses are characterized by high digestibility, palatability and high yield potential (Fojtík 1994, Kopecký et al. 2006). The cultivar Perun produced by crossing two tetraploids, *L. multiflorum* (4*n*) x *F. pratensis* (4*n*), has similar morphological parameters to ryegrass, but higher yield potential than fescue (Kopecký et al. 2005). The cultivar Felopa is a cross between *F. pratensis* (4*n*) and *L. multiflorum* (4*n*) (Zwierzykowski et al. 1998). Despite its high yield potential, cv. Felopa is less resistant and winter-hardy than cv. Perun (Terlikowski 2017). The cultivars Peron and Felopa are also characterized by high tiller density and high resistance to treading (Thomas, Humphreys 1991, Casler et al. 2001). The high nutritional value and high yield potential of *F. braunii* hybrids fed to ruminants contribute to a decrease in milk and beef production costs (Terlikowski 2017).

Grass forage and silage can be rich sources of crude protein (CP) in feed rations for high-yielding dairy cows (Wilkins, Jones 2000, Purwin et al. 2014). However, the efficiency of nitrogen utilization in dairy cows fed grass silage is low (Castillo et al. 2000, Fijałkowska et al. 2015), which could be attributed to improper ensiling practices and/or extensive fermentation. Preservation methods and plant selection (species and varieties) are important considerations in the production of roughage. The Cornell Net Carbohydrate and Protein System (CNCPS) is widely used to evaluate the nutritional value of feed proteins. In the CNCPS model, protein and carbohydrates are divided into fractions based on their solubility and fate in the animal's digestive system (Fox et al. 1995). The quality of CP fractions can be accu-

rately determined based on their ruminal degradation rates (SNIFFEN et al. 1992). In the CNCPS model, plant proteins are classified as true proteins (TP) and non-protein nitrogen (NPN) compounds. Non-protein nitrogen constitutes fraction A, which is degraded to ammonia in the rumen. True protein is divided into three subfractions (B_1, B_2, B_3) based on their solubility and ruminal degradability. Fraction B_1 is soluble in a buffer with ruminal pH (6.7 - 6.8), and it is rapidly degraded in the rumen and characterized by 100% intestinal digestibility. Fraction B_2 is soluble in neutral detergent, it has an intermediate degradation rate in the rumen and is characterized by 100% intestinal digestibility. Fraction B_3 is soluble in acid detergent, it is slowly degraded in the rumen and is characterized by 100% intestinal digestibility. Fraction B_3 represents neutral detergent-insoluble nitrogen (NDIN) minus fraction C (acid detergent-insoluble nitrogen). Acid detergent-insoluble nitrogen (ADIN) is unavailable protein, which is not degraded in the rumen and not digested in the intestines (KRISHNAMOORTHY et al. 1982, LICITRA et al. 1996).

The aim of this study was to evaluate the quality of feed protein based on the composition of nitrogen fractions in *F. braunii* herbage harvested at four different dates.

MATERIALS AND METHODS

The analyzed plant material was the herbage of *F. braunii* hybrids cv. Lofa (*L. multiflorum* (2*n*) x *F. arundinacea* (6*n*) x *L. multiflorum* (4*n*)), cv. Felopa (*F. pratensis* (4*n*) x *L. multiflorum* (4*n*)) and cv. Perun (*L. multiflorum* (4*n*) x *F. pratensis* (4*n*), collected from experimental plots in Skaszewo (Region of Mazowsze, Poland). The plot size was 2.5 ha, and the experiment was established on 21 September 2016 on soil of very good rye complex according to the Polish soil classification system.

Grasses were sown on 15 October 2016, and were fertilized at the beginning of the growing season, on 20 April 2017. Fertilizer composed of 40.0% K, 6.0% MgO, 12.0% SO_3 and 4.0% Na was applied at 120 kg ha^{-1}, and ammonium nitrate containing 24.0% N and 0.2% Mg was applied at 250 kg ha^{-1}. Within a week after the first, second and third harvest, cattle slurry containing on average 3.6 kg N, 1.9 kg P_2O_5, 4.1 kg K_2O, 2.1 kg CaO and 0.8 kg MgO m^{-3} was applied at 30 m^3 ha^{-1}. After the second harvest, urea (46.0% N) was additionally applied at 120 kg ha^{-1}. Fourteen days after the application of fertilizers, the third harvest was carried out, and the plots were again fertilized with urea at 150 kg ha^{-1}; the same procedure was repeated after the fourth harvest.

Grasses were cut at a height of 5 cm in the evening (7:00 - 9:00 p.m.) using two mowers: a side-mower with the implement's working width of 2.8 m and a front-mower with the working width of 3.2 m, equipped with

a conditioner. In 2017, harvest dates were as follows: first harvest – 18 May, second harvest – 14 June, third harvest – 14 July, fourth harvest – 24 August.

Five herbage samples were collected from different locations within each field, and were combined to obtain bulk samples which were dried at a temp. of 35°C using a Binder FED 115 drying oven, and were ground in a Retsch SK 100 cross beater mill to a particle size of 1 mm.

All samples were analyzed to determine the content of dry matter (DM) and crude protein (CP) – by standard methods (AOAC 2005), water-soluble carbohydrates (WSC) – by the anthrone method (Thomas 1977), neutral detergent fiber (NDF) – as described by Mertens (2002), and acid detergent fiber (ADF) – by the method proposed by Van Soest et al. (1991).

The experimental forages were assayed for the content of the following nitrogen fractions: protein nitrogen – with the use of trichloroacetic acid (TCA), as described by Licitra et al. (1996); buffer-soluble nitrogen (BSN) – using McDougall's buffer, with separation into protein and non-protein nitrogen, according to the procedure of Hedqvist and Uden (2006); NDIN and ADIN – as described by Licitra et al. (1996).

Crude protein was divided into nitrogen fractions in accordance with the CNCPS model (Shniffen et al. 1992): fraction A – NPN calculated as the difference between total nitrogen and true protein (TP) precipitated with 10% TCA; fraction C – ADIN; fraction B – TP divided into three subfractions (B_1, B_2, B_3) based on their solubility and ruminal degradability: B_1 – BSN, B_2 – nitrogen soluble in neutral detergent (B_2 = IP - NDIN) and B_3 – nitrogen soluble in acid detergent (B_3 = NDIN - ADIN).

The results were processed statistically by two-way (cultivar and harvest date) ANOVA. The significance of differences between means was estimated by the Duncan's test. Statistical analysis was performed using Statistica 13.1 software.

RESULTS AND DISCUSSION

An analysis of the chemical composition of the experimental forages (Table 1) revealed the effect of *Festulolium* cultivars on the content of DM ($P < 0.01$) and NDF ($P < 0.05$). The dry matter content (mean values of four harvests) was the lowest in *Festulolium* cv. Lofa (155 g kg^{-1}), and it was by 19.5% and 24.5% higher in cvs. Felopa and Perun, respectively. The CP content of herbage was influenced by a cultivar and harvest date, and it was determined at 200 g kg^{-1} DM in *Festulolium* cv. Felopa and 195 g kg^{-1} DM in cv. Lofa. In a study by Edmunds et al. (2011), *F. braunii* hybrids were characterized by higher CP content than *L. perenne* (129 g kg^{-1} DM) and meadow grasses (167 g kg^{-1} DM). The CP content of cvs. Felopa and Lofa was similar to that of alfalfa (188 g kg^{-1} DM). One of the parental species,

Table 1

Chemical composition of *Festulolium* herbage (g kg^{-1} DM)

Cultivar	Harvest	DM (g kg^{-1})	CP	WSC	NDF	ADF
Festulolium braunii cv. Felopa	I	174.2	170.6	233.5	433.1	247.8
	II	204.6	165.4	101.9	571.1	321.9
	III	156.3	283.0	61.7	459.8	275.7
	IV	205.6	184.0	37.6	589.1	325.2
Festulolium braunii cv. Lofa	I	158.3	198.7	119.0	554.4	298.0
	II	133.4	196.6	65.0	582.5	325.1
	III	174.8	175.4	65.0	607.5	351.4
	IV	152.0	211.4	49.8	565.2	288.4
Festulolium braunii cv. Perun	I	187.2	161.8	228.0	436.0	250.4
	II	193.8	170.9	132.7	559.8	306.4
	III	182.2	184.5	79.8	529.8	291.0
	IV	209.3	182.6	49.3	590.0	323.8
SEM		0.554	0.759	1.139	2.072	0.921
P-value						
Cultivar		0.01	0.236	0.001	0.020	0.514
Harvest date		0.562	0.202	<0.0001	0.146	0.202
Cultivar x harvest date		0.218	0.049	0.004	0.226	0.633

DM – dry matter, CP – crude protein, WSC – water-soluble carbohydrates, NDF – neutral detergent fiber, ADF – acid detergent fiber, SEM – standard error of the mean

F. arundinacea, was characterized by somewhat higher CP content (214 g kg^{-1} DM) than the hybrids, and the CP content in brome grass was even higher (258 g kg^{-1} DM) as determined by Elizalde et al. (1999). According to Domański (2004), *F. pratensis* and *L. perenne* were characterized by similar CP content, which was comparable to the average value noted in *F. braunii* herbage (190 g kg^{-1} DM) in our study.

Festulolium of cv. Lofa was characterized by a lower content of water-soluble carbohydrates (WSC, $P < 0.001$) than cvs. Felopa (by 31.0%) and Perun (by 39.0%), and higher NDF content (by 11.0% and 8.32%, respectively). The content of WSC decreased at successive harvests, which was also reported by Ciepiela et al. (2003). The progressive decrease in WSC content could be attributed to higher ambient temperature and a higher rate of respiration, a process that utilizes sugars (Koter 1973, Nowacki 1981). The WSC content of the analyzed forages was similar to that noted by PodkówkA et al. (2011) in the parental species *F. pratensis* (21.49 g kg^{-1} DM). The second parental species, *L. perenne,* was characterized by lower WSC content at 9.45% DM on average (Ciepiela et al. 2003).

The results of the analysis of CP fractions are presented in Table 2.

Table 2

Crude protein fractions in the analyzed *Festulolium* hybrids (g kg^{-1} crude protein)

Cultivar	Harvest	Crude protein fractions				
		A	B_1	B_2	B_3	C
Festulolium braunii cv. Felopa	I	318.3	194.6	328.2	105.5	53.3
	II	272.2	163.2	328.2	168.0	68.3
	III	270.3	131.8	424.0	135.3	38.5
	IV	138.3	125.0	328.2	286.9	121.7
Festulolium braunii cv. Lofa	I	288.9	155.5	249.6	240.6	65.4
	II	229.4	164.3	312.8	227.9	65.6
	III	238.9	141.4	278.8	254.8	86.1
	IV	192.7	139.6	237.4	338.0	92.4
Festulolium braunii cv. Perun	I	320.9	177.3	300.3	126.7	74.8
	II	269.3	156.8	342.2	170.8	60.8
	III	148.8	143.7	360.9	256.0	90.6
	IV	181.0	108.9	339.3	256.9	113.8
SEM		1.134	0.532	1.022	1.233	0.417
P – value						
Cultivar		0.452	0.820	<0.0001	<0.0001	0.006
Harvest date		<0.0001	0.002	0.034	<0.0001	<0.0001
Cultivar x harvest date		0.011	0.539	0.309	0.003	<0.0001

SEM – standard error of the mean

It was found that *Festulolium* cultivars affected the proportion of insoluble (B_2, B_3) and indigestible (C) protein fractions. The share of all nitrogen fractions in the CP of *Festulolium* herbage was determined by the harvest date ($P < 0.05$). Both experimental factors affected ($P < 0.01$) the composition of fractions B_3 and C. Significant differences ($P < 0.05$) were noted for fractions A. On average, NPN accounted for 25% of plant protein, which is typical of *L. perenne* (Boudon, Peyraud 2001). The share of fraction A decreased in the second-cut herbage (Figure 2). The analyzed forages were characterized by lower NPN content than the silages examined by Schwab et al. (2003). Proteolysis during ensilage leads to changes in protein quality. Therefore, true protein had a higher share of the total nitrogen pool in herbage. Concentrates were characterized by much lower NPN content than green forages (Chrenkova et al. 2014). True protein was composed of three subfractions, and B_2 was the predominant fraction. The content of fraction B_1 decreased, whereas the content of fractions B_2 and B_3 increased at successive harvests. Significant differences in the share of fraction B_1 were observed in first-cut herbage (Figure 1). In second-cut herbage (Figure 2), the value of the above parameter was similar in each treatment (16.0% CP on aver-

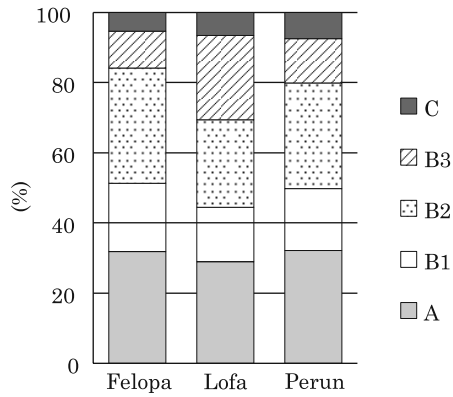

Fig. 1. Crude protein fractions in first-cut *Festulolium* herbage

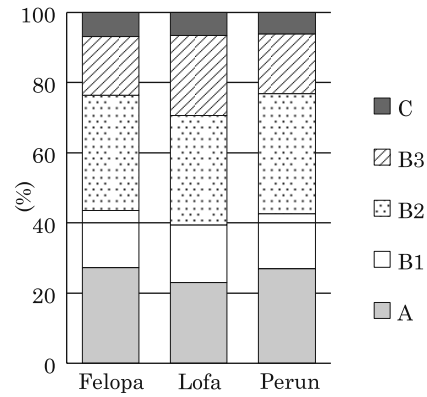

Fig. 2. Crude protein fractions in second-cut *Festulolium* herbage

age). The predominant fraction was B_2 (320 g kg^{-1} CP on average), which is characterized by 100% intestinal digestibility. The content of NDIN was higher in cv. Felopa than in the remaining hybrids, and it was the highest in third-cut herbage (424 g kg^{-1} CP) – Figure 3. The content of fraction B_2, characterized by an intermediate degradation rate in the rumen and 100% intestinal digestibility, is important to consider (KRISHNAMOORTHY et al. 1982, LICITRA et al. 1996). Protein soluble in acid detergent had an intermediate share of fraction B. The content of the above protein was the highest in cv. Lofa, in particular at the fourth harvest date. A comparison of the degradability of subfractions B in the forages analyzed in this study with a study on alfalfa carried out by GUO et al. (2008) revealed that the evaluated hybrids were characterized by lower BSN content and a higher content of protein soluble in neutral detergent. In our study, the average content of fraction B_3 in *Festulolium* herbage was similar to that reported in alfalfa forage by GUO et al. (2008). Fraction C, which is not degraded in the rumen, had the lowest share of the total nitrogen pool. The proportion of fraction C increased considerably at the fourth harvest date (Figure 4), which points to somewhat lower quality of fourth-cut herbage, which could result from the maturity stage of forage grasses. The highest average content of ADIN was noted in cv. Perun (85.0 g kg^{-1} CP). The proportion of ADIN was determined by both the cultivar and harvest date. The ADIN content of fourth-cut herbage was similar to that reported by SCHWAB et al. (2003) in alfalfa and red clover silages. In the cited study, the content of fraction C was very low only in bird's-foot trefoil silage. Rapeseed meal, soybean meal and sunflower meal were also characterized by similar ADIN values (CHRENKOVA et al. 2014). A low share of fraction C in the total nitrogen pool is highly desirable because fraction C represents unavailable protein. Fraction C is associated with lignin and Maillard reaction products; therefore, it is not a source of amino acids for animals (KRINAMOORTHY et al. 1982, WATERS et al. 1992). The analyzed *Festulolium* hybrids were characterized by a lower content

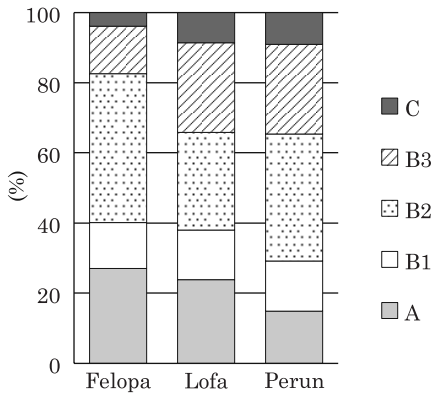

Fig. 3. Crude protein fractions in third-cut *Festulolium* herbage

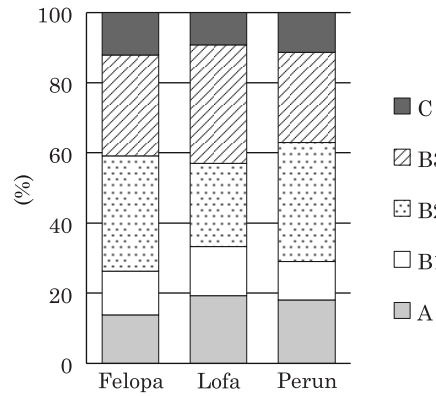

Fig. 4. Crude protein fractions in fourth-cut *Festulolium* herbage

of fraction B_2 and a higher content of fractions B_3 and C than alfalfa and brome grass studied by ELIZALDE et al. (1999). In *F. braunii* hybrids, the content of fractions A and B_1 was similar to that noted in fescue, the parental species. Fescue was characterized by a higher content of protein soluble in neutral detergent and a lower content of fractions B_3 and C. In a study by GRABBER (2009), the content of fraction B_2 was also higher (523 g kg^{-1} CP), whereas the content of fractions B_1 (96.0 g kg^{-1} CP) and B_3 (68.0 g kg^{-1} CP) was considerably lower in *Medicago sativa*. None of the analyzed *Festulolium* forages had nitrogen fractionation similar to that of the parental species *L. perenne* investigated by HOEKSTRA et al. (2008).

CONCLUSIONS

The results of this study, which focused on the composition of nitrogen fractions in *Festulolium* herbage with the use of the CNCPS model, indicate that the share of fractions B_2 and B_3 (most valuable for ruminants) and the content of indigestible fraction C in the total nitrogen pool are affected by a cultivar. Lofa is characterized by the most desirable composition of nitrogen fractions, therefore the herbage of this cultivar could partially replace expensive protein supplements in ruminant diets. Protein quality, determined according to the CNCPS model in the herbage of *Festulolium* hybrids, varies depending on a harvest date. The fourth-cut herbage is characterized by lower protein quality than the herbage harvested at the remaining dates.

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