Effects of grass species on ruminal degradability of silages and prediction of dry matter effective degradability

F. Jančík[1], V. Koukolová[1], P. Kubelková[1], B. Čermák[2]

[1]Institute of Animal Science, Prague-Uhříněves, Czech Republic
[2]Faculty of Agriculture, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic

ABSTRACT: Forty samples of grass silages, made from the five most widely used grass species in ruminant nutrition (*Dactylis glomerata* L., *Phleum pratense* L., *Lolium perenne* L., *Festuca arundinacea* L. and hybrid Felina) were tested in the present study. These grass species conserved by an ensiling process were compared among each other according to dry matter (DM) rumen degradability parameters (a = portion of DM solubilized at initiation of incubation, b = fraction of DM potentially degradable in the rumen, c = rate constant of disappearance of fraction b and ED_{DM} = effective degradability of DM, estimated for each ingredient assuming the rumen solid outflow rates of 0.02 (ED_{DM2}), 0.05 (ED_{DM5}) and 0.08 (ED_{DM8}) h^{-1}). Based on the chemical composition of grass silages the regression equations for prediction of ED_{DM} were evaluated. The influence of the ensiling process on dry matter degradability parameters was also assessed. The best values of ED_{DM} were determined for *Lolium perenne* (ED_{DM2} = 753.2, ED_{DM5} = 631.1 and ED_{DM8} = 567.7 g/kg DM). The best predictor was NDF (R^2-values of 0.757 (ED_{DM2}), 0.863 (ED_{DM5}) and 0.906 (ED_{DM8})). Using two predictors the accuracy level increased. The combination of CF and NDF gave R^2-values 0.892, 0.920 and 0.929 for ED_{DM2}, ED_{DM5} and ED_{DM8}, respectively. The regression equations based on the most important grass species harvested in different vegetation periods seem to be a useful tool for practical use. No significant ($P < 0.05$) effect of the ensiling process in relation to dry matter rumen degradability parameters was proved.

Keywords: grass silages; grass species; dry matter; *in sacco*; rumen degradability

Grass silage is the most important form of conserved forage for the nutrition of ruminants in many regions of Europe (Dawson et al., 2002). The nutritive value of silages or hay is highly influenced by species and cultivars (Pozdíšek et al., 2003). Ruminal degradability is influenced by many factors, mainly by the stage of maturity, forage species and preservation method (Hoffman et al., 1993; Komprda et al., 1996; Machačová et al., 1998; Elizalde et al., 1999).

Phleum pratense, *Dactylis glomerata*, *Festuca arundinacea* and *Lolium perenne* are the most important grass species not only in the Czech Republic but also in the whole of Europe, Canada and USA (Hoffman et al., 1993; Hetta et al., 2004; Jančík et al., 2008). Grass hybrids (hybridization

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of *Festuca* and *Lolium*) are useful and often used in Czech agriculture (Pozdíšek et al., 2003; Jančík et al., 2008).

Dry matter (DM) rumen degradability of roughages is a key value for the evaluation system of ruminant feeds. The rate and extent of rumen DM fermentation are very important determinants for the nutrients absorbed by the ruminants (Kamalak et al., 2005). *In sacco* (*in situ*) analyses are the most frequently used methods for determination of degradability parameters of DM, organic matter (OM), protein, fibre, minerals and other nutrients of feeds (Van Vuuren et al., 1991; Tománková and Kopečný, 1995; Čerešňáková et al., 2000, 2007; Homolka, 2000; Harazim et al., 2002; Třináctý et al., 2003; Homolka et al., 2002, 2007, 2008; Jančík et al., 2008).

However, these experiments are labour- and time-consuming and expensive for needs of fistulated animals. Therefore it is necessary to use/develop easier ways of determining degradability parameters. The simplest way of degradability determination is to use prediction equations based on a common chemical analysis of feeds (Nousiainen et al., 2003; Huhtanen et al., 2006; Jančík et al., 2008).

Relationships between degradability and chemical composition of forages were described in literature many times (Tamminga et al., 1991; Waters and Givens, 1992). For example according to Hoffman et al. (1993) and Von Keyserlingk et al. (1996) the increasing concentration of crude protein (CP) positively influences rumen degradability, on the contrary, the increasing amount of neutral-detergent fibre (NDF) has a negative influence. Based on information about the rumen degradability of feeds it is possible to predict the equations which are determined for forages growing in specific conditions. The relationships between chemical and even biological measurements to digestibility can be markedly different for the main grass species used for silage in different countries (Huhtanen et al., 2006).

The objectives of this experiment were to compare the most widely used grass species conserved by an ensiling process according to dry matter rumen degradability parameters and to evaluate the regression equations for prediction of effective dry matter rumen degradability (ED_{DM}) of grass silages based on the chemical composition of estimated samples. Following these results the effect of the ensiling process on dry matter degradability parameters was estimated.

MATERIAL AND METHODS

Samples

Forty samples of grass silages were evaluated in the present study. Five grass species (*Dactylis glomerata* L. – Dana variety, *Phleum pratense* L. – Sobol variety, *Lolium perenne* L. – Jaspis variety, *Festuca arundinacea* L. – Prolate variety and Felina hybrid), the most widely used in ruminant nutrition, were grown as a monoculture at the Větrov Breeding Station, Tábor region, Czech Republic (49° 31' 2.04'' N lat, 14° 28' 4.9'' E long; 620 m altitude). Individual grass species were ensiled from primary growth at two dates in 2004 and 2005. Grass forage was wilted on a table drier with cool air ventilation. After wilting up to 300–400 g/kg dry matter, grass was cut to 1–1.5 cm long pieces and ensiled without any additives into hermetic glass vessels (3 litre capacity). The vessels were stored in a dark and cold room for 10 and 20 weeks. After the opening of vessels, silage samples were oven-dried at 50°C for 48 h and milled to pass through a 1 mm screen. Grass forage was also dried, milled and analyzed as silage samples.

Chemical analysis

All samples were analyzed for DM, CP, ash, crude fat, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). DM was determined after drying at 105°C for 12 hours, and ash content was determined after combustion at 550°C for 4.5 hours (Regulation No. 497/2004, 2004). Crude fat was extracted for 6 h with petroleum ether, whereas the automated Kjeldahl method was used to determine nitrogen (N) (AOAC, 2005). CP was calculated as N × 6.25. NDF was determined according to the methods of Van Soest et al. (1991) and ADF and ADL were detected according to AOAC Official Method 973.18 (AOAC, 2005) using an ANKOM 220 Fibre Analyzer (ANKOM Technology Corporation, NY. USA). Fresh silage samples were analyzed for fermentation quality, i.e. pH, concentration of lactic, acetic and butyric acid (IONOSEP 3001 analyzer) according to Kvasnička (2000).

In sacco analysis

Ruminal DM disappearances were estimated for all silage samples and also for grass forage by an *in sacco* technique. Tested samples were incubated three times in the rumen of two Holstein steers fitted with rumen cannulas. Animals had *ad lib* access to meadow hay and were fed 1 kg of barley meal per day. Samples were weighed (1.5 g; 1 mm screen sieve) into nylon bags with pore size 42 µm (internal dimensions 50 × 120 mm) (Uhelon 130 T, Silk and Progress Moravská Chrastová). The bags with the samples were attached to a cylindrical carrier (Třináctý et al., 1996) and incubated in the rumen for 6, 12, 24, 48, 72 and 96 hours. Upon removal, bags were hand washed in cold water for 30 min. Zero time disappearances were obtained by washing unincubated bags. After that all nylon bags were dried in a forced-air oven at 50°C for 48 h.

Degradation of DM and effective degradability of dry matter (ED_{DM}) were calculated using the equations of Ørskov and McDonald (1979):

$$Deg_{(t)} = a + b \times (1 - \exp^{-ct})$$
$$ED_{DM} = a + b \times (c/(c + k))$$

where:

Deg = disappearance rate at time t

a = intercept representing the portion of DM solubilized at initiation of incubation (time 0)

b = fraction of DM potentially degradable in the rumen

c = rate constant of disappearance of fraction *b*

t = time of incubation

k = outflow rate of the rumen

Table 1. Chemical composition and fermentation quality of tested samples

	Mean	Minimum	Maximum	SD
Grass silages (*n* = 40)				
Ash (g/kg DM)	84.46	64.62	119.40	13.450
Fat (g/kg DM)	33.68	19.96	51.70	8.029
CP (g/kg DM)	146.10	93.71	214.10	33.990
CF (g/kg DM)	308.20	210.70	431.40	58.100
ADF (g/kg DM)	339.10	252.80	421.90	44.100
NDF (g/kg DM)	547.60	396.60	676.70	69.600
ADL (g/kg DM)	26.58	10.34	45.85	8.548
pH	4.87	4.04	5.68	0.470
Lactic acid (g/kg DM)	21.68	3.78	64.41	12.880
Acetic acid (g/kg DM)	7.52	3.00	18.30	4.110
Butyric acid (g/kg DM)	2.49	0.00	11.37	3.250
Proteolysis (%)	7.69	3.26	13.87	2.780
Grass forages (*n* = 20)				
Ash (g/kg DM)	76.83	54.35	105.30	13.880
Fat (g/kg DM)	23.87	13.73	36.08	6.201
CP (g/kg DM)	133.40	74.27	187.10	34.300
CF (g/kg DM)	277.40	194.00	327.40	36.770
ADF (g/kg DM)	299.00	214.70	365.00	42.270
NDF (g/kg DM)	539.90	417.30	610.10	58.600
ADL (g/kg DM)	22.22	10.93	41.04	8.130

SD = standard deviation; CP = crude protein; CF = crude fibre; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin

ED_{DM} was estimated for each ingredient assuming the rumen solid outflow rates of 0.02 (ED_{DM2}), 0.05 (ED_{DM5}) and 0.08 (ED_{DM8}) h^{-1}, which are representative of low, medium and high feeding amounts (Petit and Tremblay, 1992).

Statistical analysis

Data for *a*, *b* and *c* values and for ED_{DM} were evaluated by the general linear models procedure (SAS, 2002–2003) as a completely randomized design with steer as the replicate. Treatment sums of squares were partitioned to provide contrasts and compare grasses with each other. Prediction equations for ED_{DM} based on chemical composition were calculated using simple linear and multiple regressions (SAS, 2002–2003). The influence of ensiling on chemical composition and parameters of DM degradability was evaluated by one-way ANOVA.

RESULTS AND DISCUSSION

The chemical composition and fermentation quality of tested grass silages and grass forages are described in Table 1. Presented values of chemical composition are comparable with results of other authors (Huhtanen et al., 2002; Koukolová et al., 2004; Nousiainen et al., 2004). For example Blümmel et al. (1999) reported ADL contents ranging from 13 to 38 g/kg DM in silages made from *Lolium perenne*. Table 2 shows the values of parameters describing dry matter rumen degradability (*a*, *b*, *c*) and ED_{DM} calculated for various rumen solid outflow rates (0.02, 0.05 and 0.08 h^{-1}). The degradability parameters of grass silages tested by Petit and Tremblay (1992) varied from 180 to 530 g/kg DM (*a*), 350 to 670 g/kg DM (*b*), 0.022 to 0.056 h^{-1} (*c*), 580 to 710 g/kg DM (ED_{DM2}), 450 to 630 g/kg DM (ED_{DM5}) and 400 to 600 g/kg DM (ED_{DM8}). Coblentz et al. (1998) found similar ranges of degradability parameters, which ranged from 180 to 260, 460 to 540 g/kg DM and 0.031 to 0.056 h^{-1} for parameters *a*, *b* and *c*, respectively. Tables 1 and 2 show the high variability of chemical composition and degradability parameters of the observed grass silages. Elizalde et al. (1999) and Rymer and Givens (2002) reported the mean values of grass ED_{DM} (k = 0.026 and 0.06 h^{-1}) 470 and 560 g/kg DM, respectively. Averages of *a* (250 g/kg DM), *b* (550 g/kg DM) and *c* (0.053 h^{-1}) were published by Gosselink et al. (2004).

Table 2. Dry matter degradability of tested samples

	Mean	Minimum	Maximum	SD
Grass silages (*n* = 40)				
a (g/kg DM)	320.5	197.4	490.0	70.30
b (g/kg DM)	574.9	455.0	684.7	61.28
c (h^{-1})	0.040	0.030	0.056	0.008
ED_{DM2} (g/kg DM)	699.3	588.1	801.5	58.21
ED_{DM5} (g/kg DM)	572.3	463.4	693.1	64.76
ED_{DM8} (g/kg DM)	509.3	393.5	642.5	66.26
Grass forages (*n* = 20)				
a (g/kg DM)	299.2	241.0	416.3	56.42
b (g/kg DM)	569.6	486.2	666.6	51.02
c (h^{-1})	0.037	0.027	0.056	0.009
ED_{DM2} (g/kg DM)	665.4	583.3	810.6	72.51
ED_{DM5} (g/kg DM)	539.2	459.9	698.4	76.58
ED_{DM8} (g/kg DM)	478.1	406.0	636.0	74.89

SD = standard deviation; *a* = portion of DM solubilized at initiation of incubation (time 0); *b* = fraction of DM potentially degradable in the rumen; *c* = rate constant of disappearance of fraction *b*; ED_{DM2}; ED_{DM5} and ED_{DM8} = effective degradability of DM calculated for each ingredient assuming the rumen solid outflow rates of 0.02, 0.05 and 0.08 h^{-1}

Table 3. Comparison of species according to the parameters of rumen dry matter (DM) degradability of grass silages

Grass species	a^1	b^1	c^2	$ED_{DM2}{}^1$	$ED_{DM5}{}^1$	$ED_{DM8}{}^1$
Dactylis glomerata	316.2^{ab}	553.6^{a}	0.0416^{a}	687.4^{a}	565.8^{ab}	504.4^{ab}
Phleum pratense	245.9^{ac}	652.4^{abc}	0.0375^{b}	669.0^{b}	523.9^{ac}	453.1^{ac}
Lolium perenne	365.4^{ad}	561.5^{b}	0.0451^{bc}	753.2^{ab}	631.1^{acd}	567.7^{ad}
Festuca arundinacea	369.3^{bc}	524.7^{c}	0.0390^{c}	711.9^{bc}	596.0^{cd}	538.9^{bc}
Felina hybrid	290.0^{cd}	579.5^{c}	0.0344^{ac}	655.3^{ac}	525.5^{bd}	463.9^{bd}

^{1}g/kg DM; ^{2}h^{-1}

^{a,b,c,d}within a column means with the same superscript letters are different ($P < 0.05$)

a = portion of DM solubilized at initiation of incubation (time 0); *b* = fraction of DM potentially degradable in the rumen; *c* = rate constant of disappearance of fraction *b*; ED_{DM2}, ED_{DM5} and ED_{DM8} = effective degradability of DM calculated for each ingredient assuming the rumen solid outflow rates of 0.02, 0.05 and 0.08 h^{-1}

Averages of dry matter degradability parameters of grass silages determined for each grass species are presented in Table 3. The highest value of parameter *a* was determined for *Festuca arundinacea* and the lowest for *Phleum pratense*, parameter *b* was in opposite trend. Adding up the parameters *a* and *b*, *Lolium perenne* was the best (926.9 g/kg DM), followed by *Phleum pratense* (898.3 g/kg DM), *Festuca arrundinacea* (894 g/kg DM), *Dactylis glomerata* (869.8 g/kg DM) and Felina hybrid (869.5 g/kg DM). The rate of degradation (parameter *c*) was the highest for *Lolium perenne* and the lowest for Felina hybrid. Values of effective degradabilities were the highest for *Lolium perenne* and the lowest for hybrid Felina and *Phleum pratense*. These DM degradability results indicate *Lolium perenne* as the best grass species. Higher digestibility of *Lolium perenne* in comparison with *Festuca arrundinacea* was detected also by Wilman and Ahmad (1999). Pozdíšek et al. (2003) reported higher digestibility for *Festuca arrundinacea* in comparison with hybrid Hykor. Hoffman et al. (1993) compared ED_{DM} of different grass species, the highest values were found for *Lolium perenne*, lower for *Dactylis glom-*

Table 4. Prediction of effective dry matter degradability (ED_{DM}; g/kg DM) using multiple regression (n = 40)

	RMSE	R^2	P
Equation ED_{DM2}			
y = 1083 + 0.464 CF – 0.962 NDF	19.66	0.892	< 0.0001
y = 1073 – 1.861 ADL – 0.593 NDF	26.34	0.806	< 0.0001
Equation ED_{DM5}			
y = 1035 + 0.337 CF – 1.035 NDF	18.75	0.920	< 0.0001
y = 1028 – 1.329 ADL – 0.768 NDF	22.74	0.883	< 0.0001
Equation ED_{DM8}			
y = 998.3 + 0.220 CF – 1.017 NDF	18.13	0.929	0.0012
y = 992.0 – 0.832 NDF – 1.012 ADL	19.64	0.917	< 0.0001

RMSE = root mean square error; R^2 = determination coefficient; P = probability; ED_{DM2}, ED_{DM5} and ED_{DM8} = effective degradability of DM calculated for each ingredient assuming the rumen solid outflow rates of 0.02, 0.05 and 0.08 h^{-1}; CF = crude fibre; NDF = neutral detergent fibre; ADL = acid detergent lignin

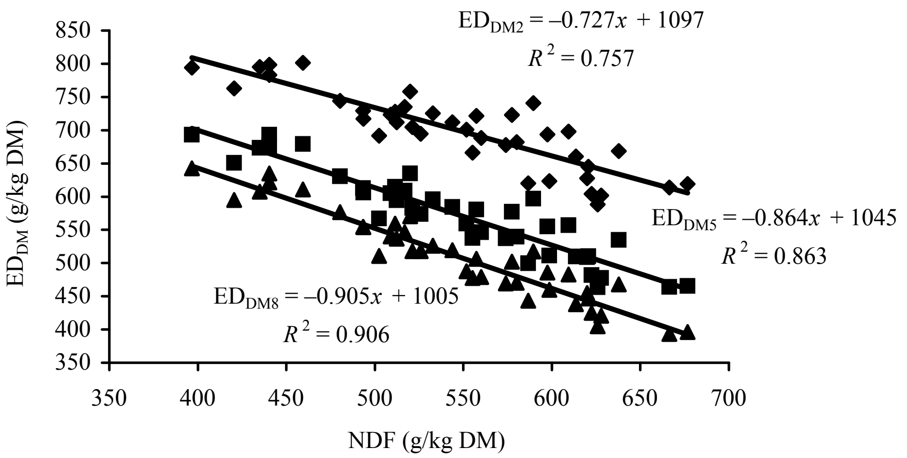

Figure 1. Prediction of DM effective degradabilities by NDF (ED_{DM2}, ED_{DM5} and ED_{DM8} = effective degradability of DM calculated for each ingredient assuming the rumen solid outflow rates of 0.02, 0.05 and 0.08 h^{-1})

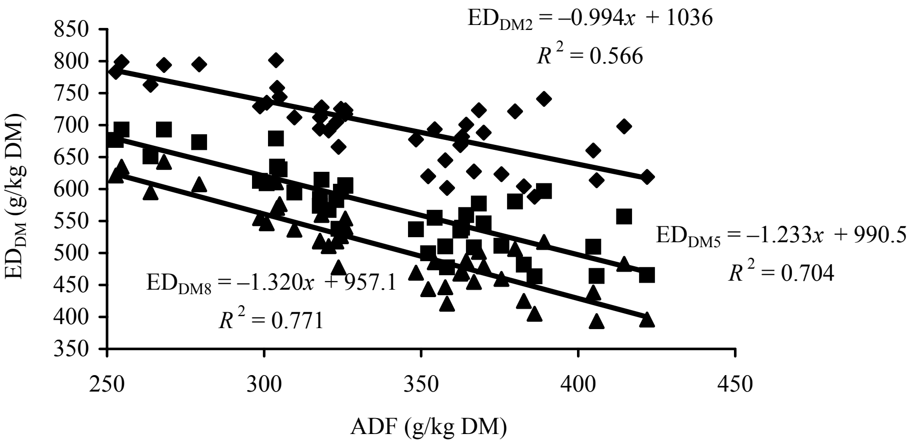

Figure 2. Prediction of DM effective degradabilities by ADF (ED_{DM2}, ED_{DM5} and ED_{DM8} = effective degradability of DM calculated for each ingredient assuming the rumen solid outflow rates of 0.02, 0.05 and 0.08 h^{-1})

erata and the lowest for *Phleum pratense.* Gosseling et al. (2004) found out a faster degradation rate for *Lolium perenne* compared to *Dactylis glomerata.*

In the present study simple linear (Figures 1 and 2) and multiple regressions (Table 4) were used for prediction of effective degradabilities of dry matter (ED_{DM2}, ED_{DM5}, ED_{DM8}). NDF was found to be the best single predictor of ED_{DM} (Figure 1), where R^2 (determination coefficient) was 0.757 for ED_{DM2}, 0.863 for ED_{DM5} and 0.906 for ED_{DM8}. Contrary to these findings, it was the worst predictor of CF content; R^2 was 0.06 for ED_{DM2}, 0.14 for ED_{DM5} and 0.21 for ED_{DM8}. Similar results were reported by Fonseca et al. (1998). Yan and Agnew (2004) predicted ED_{DM5} by NDF and ADF with 0.66 and 0.43 (R^2), respectively. Satisfactory regression equations based on ADF content only are in Figure 2. The ability to predict the equations (R^2) was higher for results of multiple regressions (Table 4) than for simple linear regressions (Figures 1 and 2), which is in agreement with Nousiainen et al. (2003). The multiple regression using two predictors showed the best ED_{DM} equation determination by the combination of CF and NDF. High determination of ED_{DM} equations was also found for NDF and ADL predictors.

There was not any difference ($P < 0.05$) between the ensiling process versus chemical composition and dry matter degradability parameters (*a*, *b*, ED_{DM2}, ED_{DM5}, ED_{DM8}). In general, higher values of CP and NDF values were found for ensiled samples compared to grass forage (Table 1). Figure 3 illustrates the relationship between the ensiling process and degradability parameters (*a*, *b*, ED_{DM2}, ED_{DM5}, ED_{DM8}). ED_{DM} was the highest for silages stored for 20 weeks, then for silages stored for 10 weeks and the lowest for grass forage. A similar trend was also detected for parameter *a*; parameters *b* and *c* showed just an opposite tendency. The values of parameter *c* were 0.037, 0.035 and 0.033 h^{-1} for grass forage, silages stored for 10 weeks and silages stored for 20 weeks, respectively (not tabulated values). The lower degradation rate of DM measured for ensiled grasses compared to grass forage is in

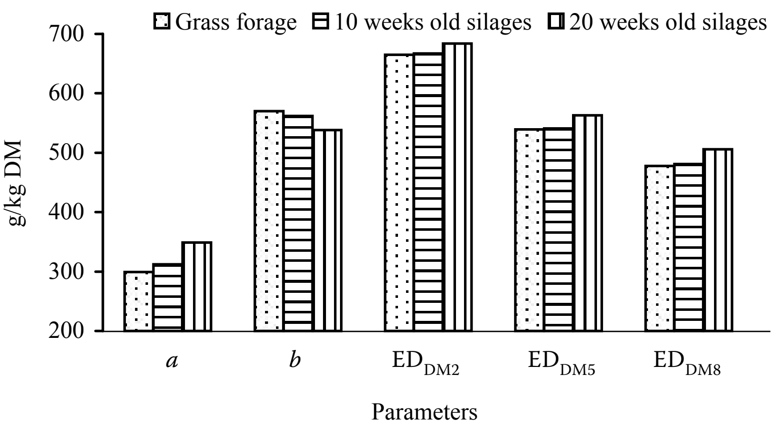

Figure 3. The influence of ensiling on degradability parameters (a = portion of DM solubilized at initiation of incubation (time 0); b = fraction of DM potentially degradable in the rumen; ED_{DM2}, ED_{DM5} and ED_{DM8} = effective degradability of DM calculated for each ingredient assuming the rumen solid outflow rates of 0.02, 0.05 and 0.08 h^{-1})

agreement with Gosseling et al. (2004). Yahaya et al. (2001) determined, also without statistical significance, higher digestibility of DM for ensilaged grass than for grass forage.

CONCLUSION

The best values of dry matter effective degradability (ED_{DM}) were determined for *Lolium perenne*. The prediction equations of ED_{DM} calculated on the basis of the chemical composition of grass silages suggested NDF as the best predictor. Using two predictors the accuracy level (R^2) of prediction increased. Regression equations based on the most important grass species harvested in different vegetation periods seem to be a useful tool for practical use. The ensiling process did not have a statistically significant influence on the values of dry matter rumen degradability parameters.

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Corresponding Author

Ing. Filip Jančík, Ph.D., Institute of Animal Science, Přátelství 815, Prague-Uhříněves, Czech Republic
Tel. +420 267 009 620, e-mail: jancik.filip@vuzv.cz