Fibre and ergosterol contents in forage of *Arrhenatherum elatius*, *Dactylis glomerata* and *Festulolium* at the end of the growing season

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ABSTRACT: The objective of this paper is to evaluate the contents of CF, NDF, ADF and ergosterol in the forage of *Festulolium, Dactylis glomerata* and *Arrhenatherum elatius* from stands harvested at the end of the growing season. The contents of CF, NDF and ADF were analysed using the ANKOM Fibre Analyzer instrument. The ergosterol content was analysed by the HPLC method. The lowest CF content was in the *Festulolium* forage matter (26.8%). Conversely, the highest content was in the *Arrhenatherum elatius* forage (30.2%). The CF content gradually increased during autumn from 28.0% to 29.4%. Likewise, the NDF and ADF contents were lowest in *Festulolium* (58.3% and 32.3%, respectively). The highest NDF content was in *Arrhenatherum elatius* (60.5%). The ADF contents in *Arrhenatherum elatius* and *Dactylis glomerata* were comparable (35.9% and 35.1%, respectively). The NDF content increased during autumn from 56.4% to 62.0% and ADF content from 32.8% to 36.4%. The ergosterol content suggests a lower infestation of the *Festulolium* forage by fungi. While this content in the *Festulolium* forage was 94.9 mg/kg of dry matter (DM), in the *Dactylis glomerata* forage it was 136.7 mg/kg DM and in the *Arrhenatherum elatius* forage 139.9 mg/kg DM. Forage samples taken in December contained ergosterol on a level of 248.6 mg/kg DM. The species under study and the time of use in autumn exhibited a statistically highly significant effect ($P < 0.01$) on the contents of CF, NDF, ADF and ergosterol in the forage matter.

Keywords: acid detergent fibre; neutral detergent fibre; ergosterol; winter grazing

In dependence on the course of weather, winter grazing is accompanied by the development of moulds and there is a risk of the occurrence of mycotoxins in the feed. The development of moulds occurs mainly in extremely overmature forage (Opitz von Boberfeld, 2001) or in forage growing in extreme conditions. Ergosterol ranks amongst the main sterols produced by lower and higher fungi. Its occurrence in other organisms is very limited, negligible concentrations of ergosterol in DM were detected only in some bacteria and yeasts. Due to this specific occurrence it is possible in practice to associate the occurrence of this sterol with the presence of moulds in the analysed sample (Marin et al., 2007). The issue of moulds is very urgent, in particular with forages from grass stands used at the end of the growing season. There are considerable differences between the species. Mould-resistant species include *Festuca arundinacea* and its hybrids (Opitz von Boberfeld and

Supported by the Czech Science Foundation of the Czech Republic (Grant No. PD 521/06/P253).

Banzhaf, 2006). The use of *Dactylis glomerata* at the end of the growing season was mentioned by Prigge et al. (1999). Jančovič et al. (2003) drew attention to the drop in the quality of *Dactylis glomerata* after exceeding the time limit for harvest in the first cut but they added that thanks to the perennial character, the time limit for the harvest in the next cut was wider. The deteriorated quality is due to the increased content of CF, NDF and ADF. The ADF content increases by approx. 0.2% per day. The highest increase is recorded in spring (0.4%). In autumn, it is around 0.1%. The ADF components have a relation to digestibility and/or to energy content (Opitz von Boberfeld, 1994). The objective of this paper is to evaluate changes in the content of CF, NDF and ADF at the end of the growing season in *Festulolium*, *Dactylis glomerata* and *Arrhenatherum elatius* forages and to evaluate the zoohygienic quality of the forage of these species in October, November and December via the ergosterol analysis.

MATERIAL AND METHODS

Site description

A small-plot experiment was established in 2004 in the Bohemian-Moravian Upland at an altitude of 560 m above sea level. In 1970–2000 mean annual precipitation was 617 mm and mean annual temperature amounted to 6.9°C. Two years which differed in weather were monitored. In 2005, the total annual precipitation amount was 660 mm and the average temperature reached 6.9°C. In the monitored months of October, November and December, the precipitation amounts reached 2.8 mm, 25.6 mm and 68.8 mm and the average daily temperature was 7.81°C, 0.72°C and –1.97°C, respectively. In 2006, the total annual precipitation amount was 727.5 mm and the average temperature reached 7.0°C. In the monitored months of October, November and December, the precipitation amounts were 25.7 mm, 76.6 mm and 22.2 mm and the average daily temperature was 7.75°C, 4.50°C and +2.57°C, respectively.

Experimental factors

The first experimental factor was the grass species (S). The monitored species were *Festulolium* (FS) cv. Felina, *Dactylis glomerata* (DGS) cv. Vega, *Arrhenatherum elatius* (AES) cv. Median. The second experimental factor was the intensity of use in summer (CS), when the sward was used as a single-cut stand (1CS) only in June or as a double-cut stand (2CS) in June and at the end of July. The third experimental factor was the time of harvest in autumn (A). The sward was harvested either in October (OA), November (NA) or December (DA). The time of the autumn harvest corresponded to the time of sampling for the chemical analyses. The experiment was repeated in two subsequent years (Y) 2005 (1Y) and 2006 (2Y).

Monitored characteristics

The monitored characteristics were dry matter yield, crude fibre (CF) content, neutral detergent fibre (NDF) content, acid detergent fibre (ADF) content and ergosterol content. Samples dried at 60°C and homogenised to a particle size of 1 mm were analysed. Ergosterol was determined by using the liquid chromatograph HP1100. CF, NDF and ADF contents were analysed using the ANKOM Fibre Analyzer. Based on the ADF content, the net energy of lactation (NEL) content was calculated according to the formula (Opitz von Boberfeld, 1994):

$$NEL\ (MJ/kg\ DM) = 9.23 - 0.105 \times ADF$$

where:

ADF = acid detergent fibre in %

Determination of CF

A total of 1 g of the sample was weighed into a filtration bag. Sealed bags were added petroleum ether (0.5 cm below the edge). After shaking, the bags were removed from the petroleum ether and inserted into the analyser after drying. They were lye-dipped in H_2SO_4 heated to a temperature of 100°C for a period of 45 min. After draining H_2SO_4, the samples were washed in water at a temperature of 100°C. Subsequently, NaOH heated to a temperature of 100°C was added for another period of 45 min. After draining NaOH, the samples were cooled with cold water. At the end, the analysed samples were dipped into acetone for a period of 2–3 min and then dried at a temperature of 105°C to a constant weight and burnt in a pre-annealed

and weighed cup at 550°C. The CF calculation was done according to the following formula:

$$CF\ (\%) = (W_3 - (W_1 \times C_1))/W_2 \times DM$$

where:

W_1 = the weight of an empty bag in g
W_2 = the weighted sample in g
W_3 = the weight of organic matter content in g (loss of cup weight and weight of the bag with the sample content after burning in g)
C_1 = the correction for the bag organic matter content (loss of the empty bag weight at burning)
DM = dry matter in %

Determination of NDF

A total of 0.5 g of the sample was weighed into the filtration bag, the bags being sealed 0.5 cm under the edge and put in the analyser. Subsequently, a solution of neutral detergent agent was added (a solution of chelaton III, sodium tetraborate decahydrate, sodium hydrogen phosphate, sodium laureth sulphate and ethylene glycol adjusted to pH 6.9 to 7.1), sodium sulphite and stable alpha-amylase. In the analyser, the samples were left for 75 min of continual mixing. After draining the solution, water heated to 85–90°C was added together with alpha-amylase for washing (3–5 min). The washing was repeated three times. After cooling, the samples were dipped in acetone for a period of 3 min and then dried at a temperature of 105°C (after complete evaporation of acetone). After weighing, the samples were burnt at a temperature of 525°C. The NDF content was calculated according to the following formula:

$$NDF\ (\%) = (W_3 - (W_1 \times C_1))100/W_2 \times DM$$

where:

W_1 = the weight of an empty bag in g
W_2 = the load in g
W_3 = the weight of organic matter in g (loss of cup weight and weight of the bag with the sample content after burning in g)
C_1 = the correction for the organic matter content in the bag (loss of the empty bag weight at burning)
DM = dry matter in %

Determination of ADF

A total of 0.5 g of sample was weighed into the filtration bag, the bags were sealed 0.5 cm below the edge and put in the analyser. Subsequently, an acid detergent solution was added (a solution of 1.0 N sulphuric acid and trimethylammonium). The samples were continually mixed in the analyser for 60 minutes. After draining the solution, water heated to 85–90°C was added for washing (5 min). The washing was repeated three times. After cooling, the samples were dipped in acetone for a period of 3 minutes and then dried at a temperature of 105°C (after complete evaporation of acetone). After weighing, the samples were burnt at a temperature of 525°C. The ADF content was calculated according to the following formula:

$$ADF\ (\%) = (W_3 - (W_1 \times C_1))100/W_2 \times DM$$

where:

W_1 = the weight of an empty bag in g
W_2 = the load in g
W_3 = the weight of organic matter in g (the cup and bag weight loss with the sample after burning in g)
C_1 = the correction of the bag organic matter content (loss of empty bag weight at burning)
DM = dry matter in %

Determination of ergosterol

A total of 250 mg of the sample and 2 ml of the 10%-solution of potassium hydroxide in methanol were weighed using analytical scales into 4 ml glass vials with a screw closure. The vials were then closed with lids with teflon antiseptic sealing. The content was intensively mixed for 30 s in a mixer (MS2 Minishaker IKA, USA) and the vial was kept in a thermostat (Evaterm, Labicom, CZ) for a period of 90 min at 80°C. 0.5 ml of distilled water, 1 ml of hexane was added after cooling up to the laboratory temperature and the vial content was mixed for 30 seconds. After a thorough separation of aqueous and organic phases, the content was centrifuged (Universal 32R, Hettich, Germany) at 4 000 r.m.p. for 5 minutes. The upper organic layer was poured into a 1.8 ml vial and evaporated under nitrogen flow. The remaining aqueous phase was added 1 ml of hexane and the whole extraction process was repeated twice more so as to achieve a quantitative ergosterol yield. The joint extracts were evaporated to the dry phase. The evaporation residue was finally dissolved in 400 µl mixture of methanol/toluene (75:25, v/v) and analysed with the use of liquid chromatography. The actual determination of ergosterol took place in a liquid chromatograph – reverse stage with using a Zorbax SB-C18 column sized 4.6 × 30 mm at a particle

Table 1. Effects of the species (S), intensity of use in summer (CS) and the time of harvest in autumn (A) on the content of CF, NDF, ADF and ergosterol at the end of the growing season

Factor	CF			NDF			ADF			Ergosterol		
	DF	MS	F	DF	MS	F	DF	MS	F	DF	MS	F
S	2	103.43	46.87**	2	101.3	12.91**	2	131.9	33.57**	2	22 729	10.71**
CS	1	692.27	313.72**	1	2 481.6	316.40**	1	1 034.3	263.34**	1	5 871	2.7700
A	2	16.62	7.53**	2	317.3	40.45**	2	118.8	30.24**	2	421 777	198.68**
S × CS	2	15.65	7.09**	2	12.7	1.62	2	13.0	3.31*	2	11 348	5.34**
S × A	4	4.26	1.93	4	6.0	0.76	4	0.3	0.08	4	18 111	8.53**
CS × A	2	3.81	1.72	2	2.4	0.31	2	1.6	0.40	2	16 922	7.97**
S × CS × A	4	2.37	1.07	4	7.9	1.00	4	5.4	1.38	4	2 507	1.1807
Error	36	2.21		36	7.8		36	3.9		36	2 123	

*$P < 0.05$; **$P < 0.01$; DF = degree of freedom; F = F-value; MS = mean square; S = species; CS = intensity of use in summer; A = harvest in autumn; S × CS = species × intensity of use interaction in summer; S × A = species × harvest interaction in autumn; CS × A = interaction of intensity of use in summer × harvest in autumn; S × CS × A = interaction of species × intensity of use in summer × harvest in autumn

size of 1.8 µm (Agilent Technologies, USA). The separation was carried out at a laboratory temperature using isocratic elution – mobile phase with the composition of methanol/water (97.5:2.5, v/v) at a volumetric velocity of 0.6 ml/min. Ergosterol was detected in the ultraviolet zone at 282 nm. The dosed extract volume was 2 µl. To measure the calibration curve, a method of standard addition was used. Various amounts of a standard ergosterol solution were poured into seven vials, corresponding after conversion to a range of 0.1 to 1.000 µg/g forage. The vial content was then evaporated by nitrogen flow. Each vial was inserted a weighed forage amount at 250 mg. The eighth vial was filled with forage without the added standard. The following procedure was the same as in the common sample. Each point of the calibration set had three replications.

Statistical evaluation

The obtained results were analysed by the multifactor analysis of variance and by subsequent verification based on Tukey's test (Meloun and Militký, 2006).

RESULTS

The content of CF in the analysed samples ranged from 18.4% to 36.6%. The significantly highest ($P < 0.05$) content was recorded in *Arrhenatherum elatius* (30.2%), Table 2. On the contrary, the significantly lowest ($P < 0.05$) content was observed in *Festulolium* (26.8%). In the *Dactylis glomerata* forage, the CF content was 28.9%. The CF content was gradually increasing in autumn. The CF content was significantly higher ($P < 0.05$) in December (29.4%) than in October (28.0%) or November (28.5%). The *Festulolium* forage had a significantly lower CF content in October and November than was the CF in the forage of *Dactylis glomerata* and *Arrhenatherum elatius* (Figure 2). The CF (Table 1) in forage was highly significantly affected ($P < 0.01$) not only by the species (S) and by the time of use in autumn (A) but also by the intensity of use in summer (CS). A significantly ($P < 0.05$) higher CF content was found in the forage from the grass stand of a higher physiological age (1CS). Swards used in summer only in June (1CS) had 31.2% of CF in autumn. On the contrary, swards used in

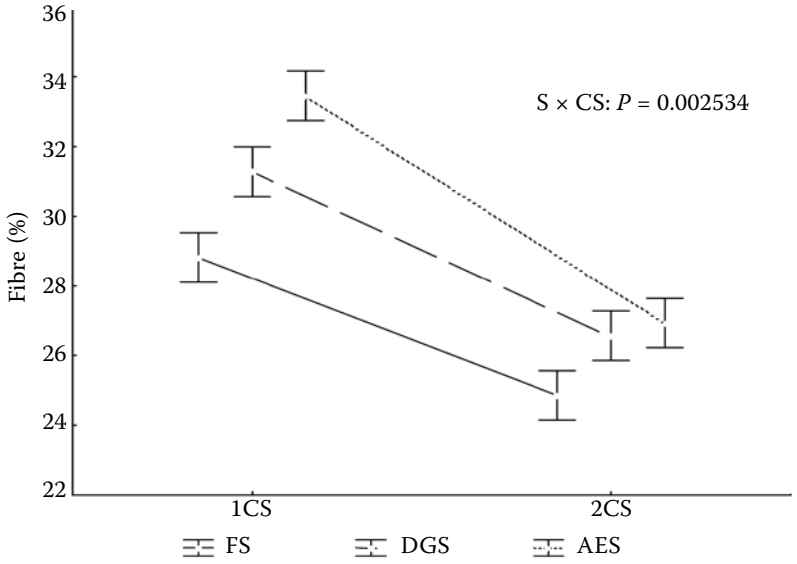

Figure 1. Fibre content in dependence on the species (S) and on the intensity of use in summer (CS)

June and towards the end of July (2CS) had 26.1% of CF in autumn. Figure 1 shows that in the case of 2CS there was no significant difference between CF contents in the *Arrhenatherum elatius* and *Dactylis glomerata* forages. On the contrary, in the case of 1 CS a significant difference existed between all monitored species. The interaction between S and CS was statistically highly significant ($P < 0.01$).

The NDF content in analysed samples ranged from 42.0% to 72.7% and the ADF content ranged from 24.3% to 45.8%. The demonstrably highest NDF content was identified in *Arrhenatherum elatius* (60.0%). Forage of this species also had a high ADF content (35.9%), which was however comparable with that of *Dactylis glomerata* (35.1%). The significantly lowest ($P < 0.05$) ADF content was found in the *Festulolium* forage, in which the value 34% (Figure 4) was not exceeded even in December. In October and November, the ADF content in *Festulolium* was significantly lower ($P < 0.05$) than in the forage of the other species (Figure 4). However, the statement does not apply to NDF (Figure 6). If we look at the average results of NDF and ADF at the end of the growing season (Table 2), it is obvious that the NDF content was comparable in October and November and a significant increase ($P < 0.05$) occurred only as in December. On the other hand, the ADF content was significantly higher already

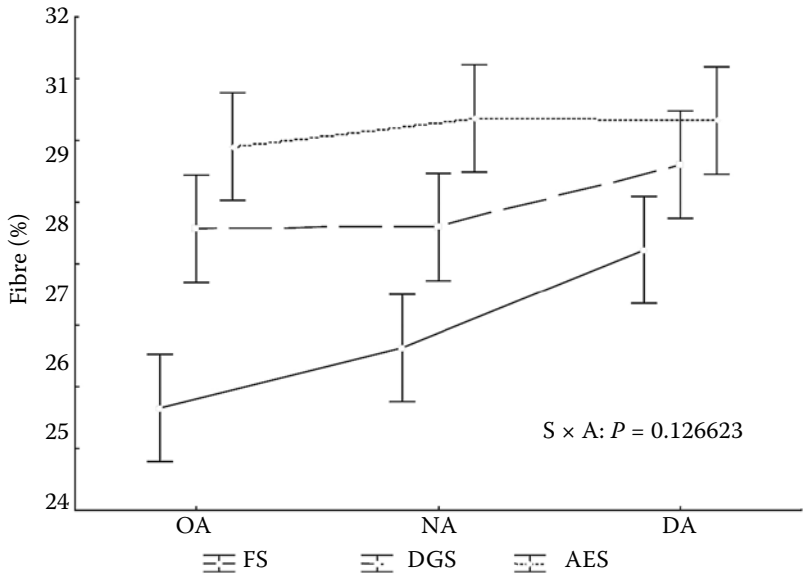

Figure 2. Fibre content in dependence on the species (S) and on the date of harvest in autumn (A)

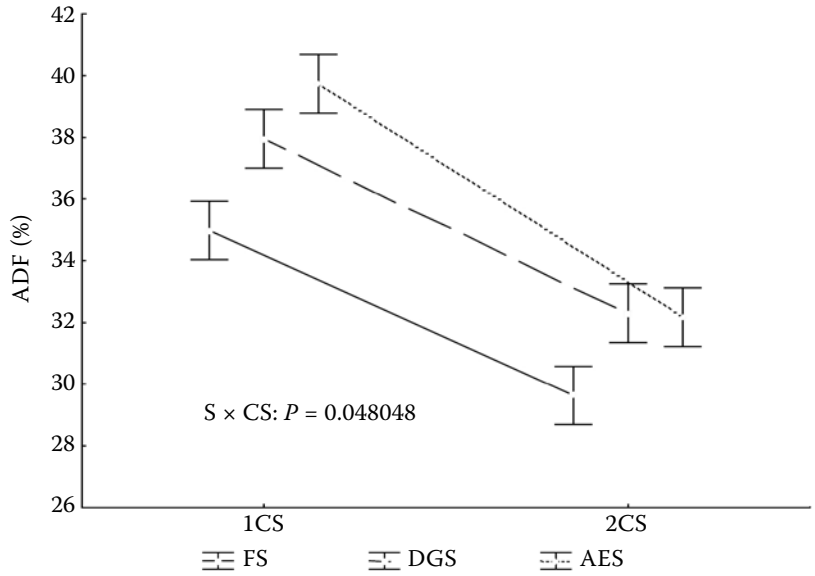

Figure 3. ADF content in dependence on the species (S) and on the intensity of use in summer (CS)

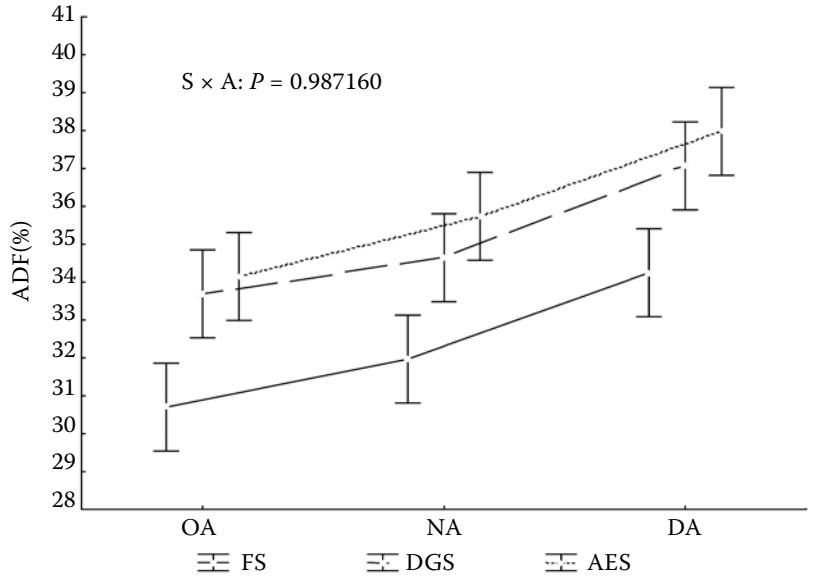

Figure 4. ADF content in dependence on the species (S) and on the date of harvest in autumn (A)

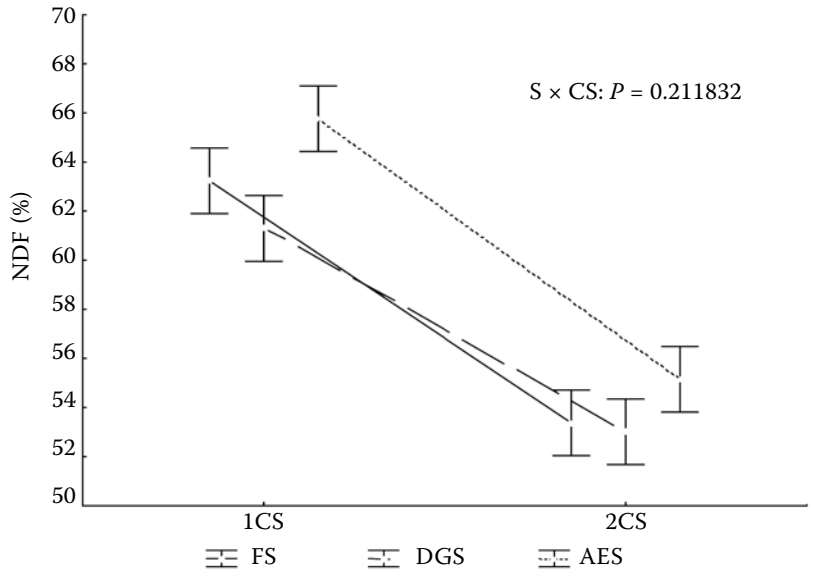

Figure 5. NDF content in dependence on the species (S) and on the intensity of use in summer (CS)

Table 2. Dry matter yields (t/ha), content of CF, ADF, NDF (%) and ergosterol (mg/kg) at the end of the growing season

Factor	*n*	DM	CF	ADF	NDF	Ergosterol
Species						
FS	36	1.71 ± 0.18 [a]	26.8 ± 0.53 [a]	32.3 ± 0.60 [a]	58.3 ± 1.16 [a]	94.9 ± 13.14 [a]
DGS	36	1.74 ± 0.21 [a]	28.9 ± 0.55 [b]	35.1 ± 0.60 [b]	57.1 ± 0.89 [a]	136.7 ± 19.90 [b]
AES	36	2.39 ± 0.22 [b]	30.2 ± 0.61 [c]	35.9 ± 0.82 [b]	60.5 ± 1.09 [b]	139.9 ± 20.58 [b]
Harvest in autumn						
OA	36	2.29 ± 0.25 [a]	28.0 ± 0.55 [a]	32.8 ± 0.64 [a]	56.4 ± 0.92 [a]	55.8 ± 8.33 [a]
NA	36	2.08 ± 0.20 [ab]	28.5 ± 0.50 [a]	34.1 ± 0.63 [b]	57.5 ± 0.87 [a]	67.0 ± 5.14 [a]
DA	36	1.47 ± 0.14 [b]	29.4 ± 0.74 [b]	36.4 ± 0.77 [c]	62.0 ± 1.17 [b]	248.6 ± 16.22 [b]
Intensity of use in summer						
1CS	54	2.83 ± 0.15 [a]	31.2 ± 0.36 [a]	37.6 ± 0.45 [a]	63.4 ± 0.54 [a]	131.2 ± 12.5 [a]
2CS	54	1.06 ± 0.07 [b]	26.1 ± 0.35 [b]	31.4 ± 0.37 [b]	53.8 ± 0.61 [b]	116.4 ± 17.18 [a]
Year of observation						
1Y	54	1.93 ± 0.17 [a]	27.7 ± 0.53 [a]	34.1 ± 0.65 [a]	58.0 ± 0.91 [a]	126.4 ± 17.67 [a]
2Y	54	1.96 ± 0.17 [a]	29.6 ± 0.42 [b]	34.8 ± 0.52 [a]	59.3 ± 0.82 [b]	121.3 ± 11.8 [a]

mean values in the same column with different superscripts ([a,b,c]) are significant at the $P < 0.05$ level; FS = *Festulolium;* DGS = *Dactylis glomerata;* AES = *Arrhenatherum elatius;* OA = harvest in October; NA = harvest in November; DA = harvest in December; 1CS = single-cut stand in summer; 2CS = double-cut stand in summer; 1Y = 1st year of observation; 2Y = 2nd year of observation

in November (Table 2) as compared to October ($P < 0.05$). Significantly ($P < 0.05$) higher NDF and ADF contents were also detected in autumn in the forage from the 1CS swards (63.4% and 37.6%, respectively) than in the forage from the 2CS swards (53.8% and 31.4%, respectively). The effect of S and CS on the NDF and ADF contents follows also from Figure 3 and Figure 5. The effect of S, CS and A on the NDF and ADF contents (Table 1), as well as on the CF content, was statistically highly significant ($P < 0.01$).

Ergosterol content in the analysed samples ranged from 6.6 to 473.2 mg/kg DM. The signifi-

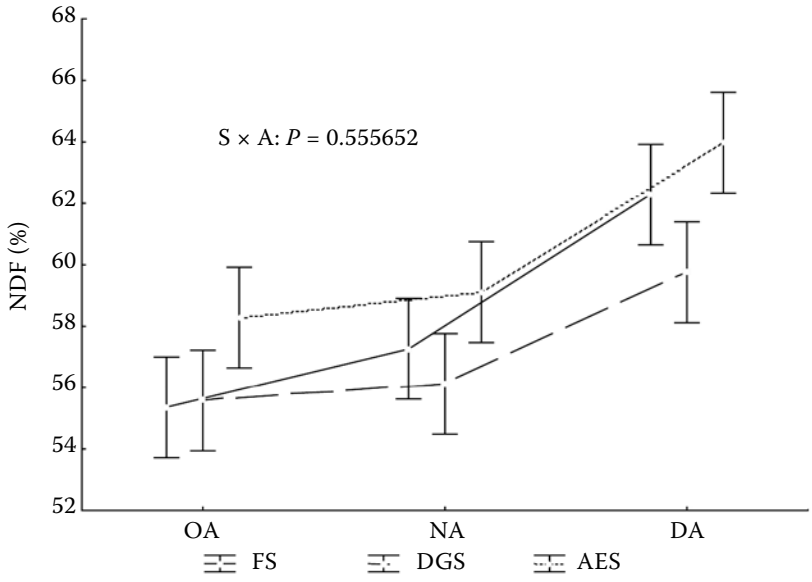

Figure 6. NDF content in dependence on the species (S) and on the date of harvest in autumn (A)

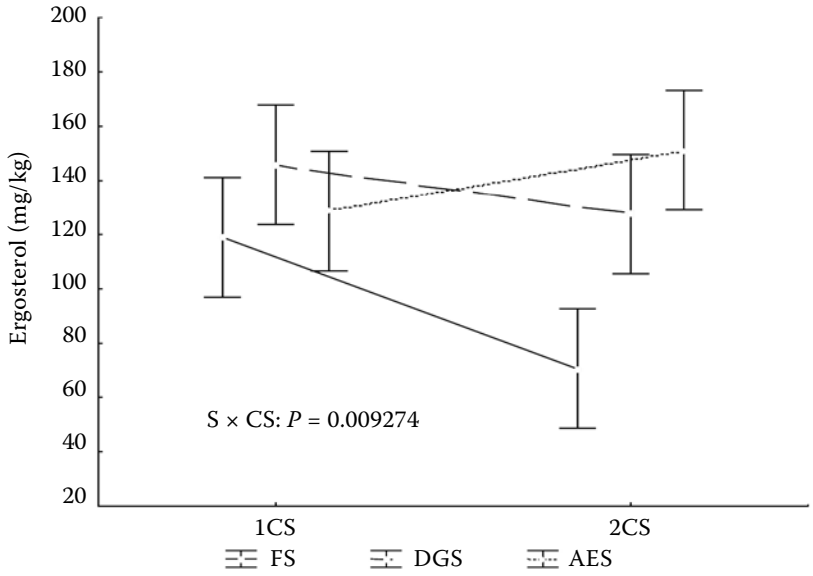

Figure 7. Ergosterol content in dependence on the species (S) and on the intensity of use in summer (CS)

cantly lowest ($P < 0.05$) ergosterol content (Table 1) was measured in *Festulolium* (94.9 mg/kg DM). The ergosterol content in *Dactylis glomerata* and *Arrhenatherum elatius* was comparable (136.7 mg per kg DM and 139.9 mg/kg DM, respectively). The ergosterol content (Table 1) was statistically highly significantly affected ($P < 0.01$) not only by S but also by A. The significantly highest ($P < 0.05$) ergosterol content was detected in December (248.6 mg per kg DM). The ergosterol content in October and November was stabilized (55.8 mg/kg DM and 67.0 mg/kg DM, respectively). Although CS did not have a significant effect on the ergosterol content at the end of the growing season, there was a highly significant ($P < 0.01$) interaction between S and CS (Figure 7). If the *Festulolium* and *Dactylis glomerata* stand was used in summer as a double-cut sward (2CS), the forage of these species had a lower ergosterol content in autumn. On the contrary, the stand used as a single-cut sward (1CS), and hence as a stand of physiologically higher age, had a higher ergosterol content in autumn. Opposite results were detected in *Arrhenatherum elatius*. Forage from the physiologically older stand had a lower ergosterol content in autumn than forage from the physiologically younger stand. A highly significant ($P < 0.01$) interaction was found between S and A (Figure 8). In October, the ergosterol content was slightly higher in *Festulolium* than in *Arrhenatherum elatius* and *Dactylis glomerata*. On

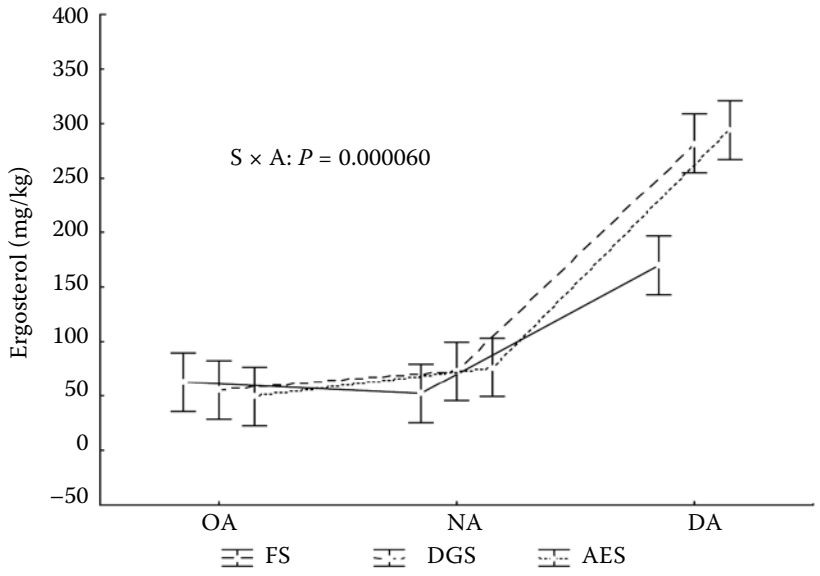

Figure 8. Ergosterol content in dependence on the species (S) and on the date of harvest in autumn (A)

Table 3. NEL content calculated on the basis of the ADF content

	ADF (%)	NEL (MJ/kg DM)
FS	32.3	5.8385
DGS	35.1	5.5445
AES	35.9	5.4605
OA	32.8	5.786
NA	34.1	5.6495
DA	36.4	5.408
1CS	37.6	5.282
2CS	31.4	5.933

FS = *Festulolium*; DGS = *Dactylis glomerata;* AES = *Arrhenatherum elatius;* OA = harvest in October; NA = harvest in November; DA = harvest in December; 1CS = single-cut stand in summer; 2CS = double-cut stand in summer

the contrary, the ergosterol content in *Festulolium* in winter was significantly ($P < 0.05$) lower than in *Dactylis glomerata* and *Arrhenatherum elatius*.

DISCUSSION

The detected CF content in *Arrhenatherum elatius* at the end of the growing season corresponds to the CF content in an average stand of *Arrhenatherum elatius*, which amounts to 30.5% in summer (Zeman, 1995). The CF content detected in *Festulolium* at the end of the growing season is comparable with *Festuca arundinacea*, with 24.9% a week prior to earing and 30.4% towards the end of earing (Zeman, 1995). By contrast, the value detected by us in the autumn increment of *Dactylis glomerata* forage was lower than that presented by Zeman (1995), according to whom its content in the older sward in the 1st cut amounts up to 34.2%.

Thanks to the NDF content, the individual species appear to be comparable in October and November but the ADF result indicates a better energy value of the *Festulolium* forage compared to the forage of *Dactylis glomerata* and *Arrhenatherum elatius* (Table 3). In contrast, Archer and Decker (1977) did not report any difference in the NDF or ADF content between *Festuca arundinacea* and *Dactylis glomerata*. Compared to this, our results show that the quality of the *Festuca arundinacea* × *Lolium multiflorum* hybrid is higher than that of *Festuca arundinacea*. The ADF content at the end of the growing season was studied by Opitz von Boberfeld and Banzhaf (2006), who determined the ADF content of up to 45% in December in *Festulolium* (variety Felina). The increasing ADF content between October and December indicates an overall deterioration in the energy value of the forage (Table 3) during autumn.

The results of the ergosterol content determined in the *Festulolium* forage correspond to the results of Opitz von Boberfeld and Banzhaf (2006), who studied the forage from swards in Central Germany at an altitude of 160 m. These authors also confirmed an increase in the ergosterol content during winter in *Festulolium*. The high ergosterol content in the winter months was also confirmed by Wolf and Opitz von Boberfeld (2003). It is remarkable that *Arrhenatherum elatius* forage taken in autumn from a physiologically older stand had a lower ergosterol content than forage sampled from a physiologically younger stand. Opitz von Boberfeld (1996) reported a higher ergosterol content in overmature oat grass meadows. *Arrhenatherum elatius* does not belong to the species that are acceptable for pasture lands. The occurrence in pasture matter is limited by the intensive use of pasture. It is possible to run a mixed system of grassland utilisation. For example to carry out the first cut that is followed by pasture use till the end of the growing season. However, in relation to a low occurrence of this species on intensively used pastures it is necessary to supply the herbage composition by additional sowing.

It can be concluded that CF content and NDF content in forage from grass stands is comparable in October and November. As to the increased CF content, a significant ($P < 0.05$) deterioration of forage quality occurs as late as in December. In contrast, the ADF content suggests a significant ($P < 0.05$) forage quality worsening as early as between October and November. The significantly lowest ($P < 0.05$) ADF content in autumn was detected in *Festulolium*. The ADF content at the end of the growing season indicates a better energy value contained in the *Festulolium* forage than in the *Dactylis glomerata* and *Arrhenatherum elatius* forages. The *Festulolium* forage also exhibited a significantly lower ($P < 0.05$) ergosterol content at the end of the growing season than the *Dactylis glomerata* and *Arrhenatherum elatius* forages. With respect to the zoohygienic aspect, the *Festulolium* forage is of a higher quality at the end of the growing season. On the other hand, the highest risk of

infestation by moulds was demonstrated to exist in *Arrhenatherum elatius* towards the end of the growing season.

Acknowledgement

The authors wish to thank Prof. Ing. Ladislav Zeman, CSc. and Prof. Ing. František Hrabě, CSc. for their valuable advice and comments provided during the elaboration of this paper.

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Received: 2007–09–18
Accepted after corrections: 2008–06–09

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