

CELL WALL COMPONENTS AND IN VITRO DRY MATTER DIGESTIBILITY OF ENSILED PEA: OAT MIXTURES

Jordan Marković¹, Snežana Anđelković¹, Vladimir Zornić¹, Ivica Kostić¹, Milomir Blagojević², Tanja Vasić³, Sanja Živković³

¹Institute for Forage Crops Kruševac, 37251 Globoder, Serbia

²University of Niš, Faculty of Agriculture kruševac, Kosančićeva 4, 37000 Kruševac, Serbia

³Agricultural Combine “Zlatibor”, 31315 Zlatibor, Serbia

Corresponding author: Jordan Marković, jordan.markovic@ikbks.com

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Abstract: Cell wall content generally is regarded as the most important factor affecting forage utilization because it comprises the major fraction of forage dry matter and is correlated with forage intake and digestibility. The experiment was carried out to evaluate the cell wall constituents of ensiled pea – oat mixtures sown at five different seeding rates. Three effects were studied: application of bacterial inoculant, seeding rate of pea and oat in the mixtures and stages of plant development. The NDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber), hemicellulose, lignin and DMD (Dry Matter Digestibility) were determined in silages. Pea and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac – Serbia, and were tested at five different mixture rates: pure pea and pure oat crop, 25% pea + 75% oat, 50% pea + 50% oat, 75% pea + 25% oat. Application of bacterial inoculant affected lower content of NDF and hemicellulose ($P < 0.05$). Content of cell wall constituents decreased with increasing pea content in the mixtures, but DMD increased. On the other hand, content of cell wall constituents increased with plant growth and development, but DMD decreased.

Key words: pea – oat silages, cell wall, digestibility

Introduction

Good quality silage and hay are important for the nutrition of ruminants, as well as for the quality and safety of dairy products. Silage quality depends on many factors. In terms of the nutritive value of the forage, the crop composition at harvest has a major impact on the ensiling process and quality of silage (*Buxton and O'Kiely, 2003*). The quality of grasses and legumes decreases with the growth

and plant development, primarily due to changes in the leaf to stem ratio, as well as due to a decrease in the content of nutrients in the stem. For that reason, it is necessary to cut these plants when the leaf to stem ratio is high. On the other hand, the quality of leguminous plants such as pea, cereals and combined crops of these legumes with cereals does not change in the same way with growth and development as those changes occur in grasses and other legumes, due to the influence of seed chemical composition and seed yield on quality (Salawu et al., 2001). Therefore, it is difficult to determine the time for using these plants in animal nutrition, especially ruminant nutrition. Several researches reported that annual legume – cereal mixtures resulted in high yields and high nutritional quality as compared with cereal alone (Yolcu et al., 2009; Balabanli et al., 2010; Kocer et al., 2012). One anticipated advantage of feeding bi-crop mixtures of cereal and legumes is an improvement in the efficiency of nutrient utilization due to the possible synchronous supply of readily fermentable energy and protein in the rumen (Blagojević et al., 2017). Pea forages generally have higher crude protein content and *in vitro* dry matter digestibility and lower NDF and ADF than oat (Marković et al., 2017).

The aim of the present study was to evaluate the cell wall components of pea-oat silages depending on application of inoculant, different seeding rate in the mixtures and stage of plant development.

Materials and Methods

Pea and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac-Serbia (21° 19' 35" E, 43° 34' 58" N). The experiment was designed with three replication according to a randomized complete block. The pea:oat mixtures were ensiled in the experimental containers holding 130 dm³, with three replications. After compaction, silomass was covered with plastic wrap, and covered with a layer of sand thickness of about 10 cm as the main load. Bacterial inoculant *BioStabil Plus* which contained homo-fermentative lactic acid bacteria (*Enterococcus faecium* and *Bacillus plantarum*) and hetero-fermentative lactic acid bacteria (*Bacillus brevis*) with a concentration of 5×10^{10} CFU per gram was added, and ensiled in containers for 45 days (a₁ – treatment with bacterial inoculant; a₂ – treatment without bacterial inoculant). The pea and oat were tested at five different mixture rates: b₁) 100% pea + 0% oat; b₂) 0% pea + 100% oat; b₃) 25% pea + 75% oat; b₄) 50% pea + 50% oat and b₅) 75% pea + 25% oat. Plant samples were taken at beginning of pea flowering and forming green seeds in 2/3 pea pods.

Neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicelluloses (HCL) and lignin content were determined according to Van Soest et al. (1991). Two stage pepsin-cellulase method was used for *in vitro* Dry Matter Digestibility - DMD (De Boever et al., 1986).

The experimental data were analyzed by a three-way analysis of variance for silage samples using a model that accounted for the main effects of addition of inoculant, pea:oat mixtures and stage of plant development. A computer program STASTICA -7.1 was used for data processing. Effects were considered significant at $P < 0.05$ level by Fisher test. The significance of differences between arithmetic means was tested by LSD test.

Results and Discussion

All three research factors had a significant impact on the NDF content in pea and oat silages (Table 1). The use of inoculants contributed to a significantly lower content of NDF ($588.8 \text{ g kg}^{-1} \text{ DM}$) compared to the treatment without the use of inoculants ($604.2 \text{ g kg}^{-1} \text{ DM}$). Pure oat silage contained the highest amount of NDF and compared to pure pea crop silage was richer in this cell wall constituent by 46.5%. The silage that contained the lowest proportion of pea in the mixture yielded silage with the highest NDF content ($646.9 \text{ g kg}^{-1} \text{ DM}$). With increasing pea proportion in the mixtures the NDF content decreased by 6.85% (pea proportion in the mixture was 50%), and 14.53% (pea proportion in the mixture was 75%). The NDF content in silage increased significantly from the pea flowering stage to the stage of forming green seeds in 2/3 of pea pods ($p < 0.05$).

Statistical analysis of the data showed that the application of bacterial inoculant had no effect on the ADF content. Contrary, the structure of the mixture had a significant effect on the ADF content. Due to the fact, that the highest ADF content was found in silage from pure oat crop, it is evident that with increasing seeding rate of pea in the mixtures content of ADF significantly decreased from 459.6 to $425.9 \text{ g kg}^{-1} \text{ DM}$. The results of investigation showed that higher content of ADF was found in silage when pea was in flowering stage of growth ($443.6 \text{ g kg}^{-1} \text{ DM}$), and with plant growth and development content of ADF decreased to $437.3 \text{ g kg}^{-1} \text{ DM}$, but differences between treatments were not significant.

The hemicellulose content in the silage with inoculant application was significantly lower ($146.0 \text{ g kg}^{-1} \text{ DM}$) compared to the silage without inoculant application ($167.7 \text{ g kg}^{-1} \text{ DM}$). The highest content of hemicellulose was found in silage contained pure pea crop. With increasing pea content in the mixtures the hemicellulose content decreased from 187.5 to $138.5 \text{ g kg}^{-1} \text{ DM}$. The silages prepared in the earlier stage of plant development had a significantly lower content of hemicellulose than silage prepared in the later stage of plant development.

The lignin content in pea and oat silage was also most influenced by the structure of the mixtures and stages of plant growth. The highest content of lignin was found in silage from pure oat crop ($94.5 \text{ g kg}^{-1} \text{ DM}$), whereas the lowest lignin content was found in silage from pure pea crop ($68.2 \text{ g kg}^{-1} \text{ DM}$). Increasing pea ratio in the mixtures caused a decrease in lignin content from 86.1 to 82.9 g kg^{-1}

DM. Utilization of plants in later stages of development caused an increase in lignin content (Table 2).

The DMD of pea and oat silages was significantly influenced by the structure of the mixtures and the stage of plant development. DMD is greatly influenced by the ADF and lignin content, so silages contained less lignin and ADF showed higher DMD compared to silages contained more lignin and ADF.

Table 1. Cell wall components of ensiled pea-oat mixtures

| Parameter | Average | b ₁ | b ₂ | b ₃ | b ₄ | b ₅ | Average AC | A | |
|--------------------------------------|----------------------------|--------------------|--------------------|---------------------|---------------------|--------------------|--------------------|---------------------|---------------------|
| NDF, g kg ⁻¹ DM | a ₁ | c ₁ | 476.5 ^g | 700.5 ^b | 628.5 ^d | 562.8 ^e | 535.5 ^f | 580.8 ^c | 588.8 ^b |
| | | c ₂ | 457.7 ^h | 650.8 ^c | 642.0 ^c | 642.1 ^c | 591.3 ^d | 596.8 ^b | |
| | AB | 467.1 ^e | 675.6 ^b | 635.2 ^{bc} | 602.4 ^c | 563.4 ^d | AC | | |
| | a ₂ | c ₁ | 491.1 ^g | 688.7 ^b | 636.9 ^c | 569.3 ^e | 564.6 ^e | 590.1 ^b | 604.2 ^a |
| | | c ₂ | 465.7 ^h | 730.9 ^a | 680.5 ^b | 647.3 ^c | 567.8 ^e | 618.4 ^a | |
| | AB | 478.4 ^e | 709.8 ^a | 658.7 ^b | 608.3 ^c | 566.2 ^d | | | |
| | BC | | 483.8 ^f | 694.6 ^a | 632.7 ^c | 566.1 ^d | 550.0 ^e | | |
| | | | 461.7 ^g | 690.8 ^a | 661.2 ^b | 644.7 ^c | 579.6 ^d | | 585.4 ^b |
| | B | | 472.8 ^e | 692.7 ^a | 646.9 ^b | 605.4 ^c | 564.8 ^d | | 607.6 ^a |
| | ADF, g kg ⁻¹ DM | a ₁ | c ₁ | 414.7 ^h | 525.1 ^a | 466.1 ^d | 427.9 ^g | 397.2 ⁱ | 446.2 ^a |
| c ₂ | | | 360.5 ^k | 483.0 ^c | 436.0 ^f | 479.0 ^e | 453.4 ^e | 442.4 ^a | |
| AB | | 387.6 ^f | 504.0 ^a | 451.0 ^d | 453.5 ^d | 425.3 ^e | AC | | |
| a ₂ | | c ₁ | 377.4 ^j | 484.7 ^c | 474.2 ^{cd} | 417.6 ^h | 451.3 ^e | 441.0 ^a | 436.6 ^{ns} |
| | | c ₂ | 352.2 ^k | 510.0 ^b | 462.0 ^d | 434.7 ^f | 401.9 ⁱ | 432.2 ^b | |
| AB | | 364.8 ^g | 497.4 ^b | 468.1 ^c | 426.2 ^e | 426.6 ^e | | | |
| BC | | | 396.0 ^e | 504.9 ^a | 470.1 ^b | 422.7 ^d | 424.3 ^d | | |
| | | | 356.3 ^f | 496.5 ^a | 449.0 ^c | 456.9 ^e | 427.6 ^d | | 443.6 ^{ns} |
| B | | 376.1 ^e | 500.7 ^a | 459.6 ^b | 439.8 ^e | 425.9 ^d | | 437.3 ^{ns} | |
| Hemicellulose, g kg ⁻¹ DM | a ₁ | c ₁ | 61.9 ^e | 175.3 ^c | 162.5 ^d | 135.0 ^f | 138.3 ^f | 134.6 ^c | 146.0 ^b |
| | | c ₂ | 112.8 ^g | 167.8 ^d | 206.0 ^b | 163.1 ^d | 138.2 ^f | 157.5 ^b | |
| | AB | 84.7 ^g | 171.5 ^d | 184.3 ^c | 149.1 ^e | 138.3 ^e | AC | | |
| | a ₂ | c ₁ | 113.7 ^g | 204.1 ^b | 162.7 ^d | 151.7 ^e | 113.2 ^g | 149.1 ^{bc} | 167.7 ^a |
| | | c ₂ | 113.6 ^g | 220.9 ^a | 218.5 ^a | 212.6 ^b | 165.8 ^d | 186.3 ^a | |
| | AB | 113.7 ^f | 212.5 ^a | 190.6 ^b | 182.2 ^c | 139.5 ^e | | | |
| | BC | | 87.8 ^g | 189.7 ^b | 162.6 ^c | 143.3 ^e | 125.7 ^f | | |
| | | | 113.2 ^f | 194.4 ^b | 212.3 ^a | 187.8 ^b | 152.0 ^d | | 141.8 ^b |
| B | | 100.5 ^d | 192.0 ^a | 187.5 ^a | 165.6 ^b | 138.9 ^c | | 171.9 ^a | |

a₁ - treatment with bacterial inoculant; a₂ - treatment without bacterial inoculant; b₁ - 100% pea + 0% oat; b₂ - 0% pea + 100% oat; b₃ - 25% pea + 75% oat; b₄ - 50% pea + 50% oat; b₅ - 75% pea + 25% oat; c₁ - 10% of pea flowering; c₂ - forming the green seeds in 2/3 pea pods; NDF - Neutral Detergent Fiber; ADF - Acid Detergent Fiber; Different letters denote significantly different means (P < 0.05)

Table 2. Lignin content and DMD of ensiled pea-oat mixtures

| Parameter | Average | | b ₁ | b ₂ | b ₃ | b ₄ | b ₅ | Average AC | A | |
|---|--------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|---------------------|---------------------|
| Lignin, g kg ⁻¹ DM | a ₁ | c ₁ | 70.9 ^e | 102.7 ^a | 81.3 ^d | 72.7 ^e | 67.7 ^e | 79.0 ^b | 84.3 ^{ns} | |
| | | c ₂ | 69.0 ^c | 93.9 ^c | 84.4 ^d | 98.1 ^b | 102.5 ^a | 89.6 ^a | | |
| | AB | | 69.9 ^c | 98.3 ^a | 82.8 ^b | 85.4 ^b | 85.1 ^b | AC | | |
| | a ₂ | c ₁ | 64.3 ^f | 81.7 ^d | 77.0 ^e | 68.3 ^e | 76.1 ^e | 73.5 ^b | | 81.5 ^{ns} |
| | | c ₂ | 68.4 ^c | 99.9 ^a | 102.0 ^a | 91.9 ^b | 85.1 ^d | 89.5 ^a | | |
| | AB | | 66.4 ^c | 90.8 ^a | 89.5 ^a | 80.1 ^b | 80.6 ^b | | | |
| | BC | | | 67.6 ^d | 92.2 ^b | 79.1 ^c | 70.5 ^d | 71.9 ^d | C | |
| | | | | 68.7 ^d | 96.8 ^a | 93.2 ^b | 95.0 ^a | 93.8 ^b | 76.3 ^b | |
| | B | | 68.2 ^d | 94.5 ^a | 86.1 ^b | 82.7 ^c | 82.9 ^c | | 89.5 ^a | |
| | Lignin, g kg ⁻¹ NDF | a ₁ | c ₁ | 148.8 ^b | 146.6 ^{bc} | 129.3 ^d | 129.1 ^d | 126.4 ^e | 130.6 ^b | 143.2 ^a |
| c ₂ | | | 150.7 ^b | 144.3 ^c | 131.5 ^d | 152.8 ^b | 173.3 ^a | 150.5 ^a | | |
| AB | | 149.8 ^a | 145.5 ^b | 130.4 ^d | 140.9 ^c | 149.9 ^a | AC | | | |
| a ₂ | | c ₁ | 130.9 ^d | 118.6 ^f | 120.9 ^{ef} | 119.9 ^f | 134.8 ^d | 125.0 ^b | 135.0 ^b | |
| | | c ₂ | 146.8 ^c | 136.7 ^{cd} | 149.9 ^b | 141.9 ^c | 149.8 ^b | 145.0 ^a | | |
| AB | | 138.9 ^{cd} | 127.7 ^f | 135.4 ^e | 130.9 ^d | 142.3 ^c | | | | |
| BC | | | | 139.9 ^c | 132.6 ^d | 125.1 ^e | 124.5 ^e | 130.6 ^d | | C |
| | | | | 148.8 ^b | 140.5 ^c | 140.7 ^c | 147.3 ^b | 161.5 ^a | | 130.5 ^b |
| B | | 144.3 ^a | 136.5 ^b | 132.9 ^c | 135.9 ^b | 146.0 ^a | | 147.8 ^a | | |
| Dry Matter Digestibility, g kg ⁻¹ DM | | a ₁ | c ₁ | 659.2 ^{bc} | 540.0 ^f | 544.7 ^{ef} | 600.2 ^d | 632.5 ^c | 595.3 ^a | 560.5 ^{ns} |
| | c ₂ | | 672.9 ^{ab} | 476.7 ⁱ | 472.6 ⁱ | 490.0 ^h | 516.4 ^g | 525.7 ^d | | |
| | AB | | 666.0 ^a | 508.4 ^{de} | 508.7 ^{de} | 545.1 ^c | 574.5 ^b | AC | | |
| | a ₂ | c ₁ | 681.6 ^a | 514.4 ^g | 554.9 ^e | 588.0 ^{de} | 555.0 ^e | 578.8 ^b | 560.1 ^{ns} | |
| | | c ₂ | 666.9 ^b | 478.0 ⁱ | 472.6 ⁱ | 491.0 ^h | 598.5 ^d | 541.4 ^c | | |
| | AB | | 674.3 ^a | 496.2 ^e | 513.8 ^d | 539.5 ^c | 576.7 ^b | | | |
| | BC | | | 670.4 ^a | 527.2 ^d | 549.8 ^c | 594.1 ^b | 593.7 ^b | | C |
| | | | | 669.9 ^a | 477.3 ^e | 472.6 ^e | 490.5 ^e | 557.4 ^c | | 587.0 ^a |
| | B | | 670.2 ^a | 502.2 ^d | 511.2 ^d | 542.3 ^c | 575.5 ^b | | | 533.5 ^b |

a₁- treatment with bacterial inoculant; a₂- treatment without bacterial inoculant; b₁- 100% pea + 0% oat; b₂- 0% pea + 100% oat; b₃- 25% pea + 75% oat; b₄- 50% pea + 50% oat; b₅- 75% pea + 25% oat; c₁- 10% of pea flowering; c₂- forming the green seeds in 2/3 pea pods; Different letters denote significantly different means (P < 0.05)

The highest DMD was found in silage from pure pea crop (670.2 g kg⁻¹ DM), whereas the lowest DMD was found in silage from pure oat crop (502.2 g kg⁻¹ DM). Silage with the highest pea ratio in the mixture was characterized by the highest DMD (575.5 g kg⁻¹ DM), whereas silage with the lowest pea ratio in the mixture showed the lowest DMD (511.2 g kg⁻¹ DM). Crop utilization in the later stages of development influenced lower DMD.

Similar results were obtained in the investigations of pea:oat mixtures quality depending on the stage of growth (Marković et al., 2018). Mustafa and Seguin (2004) found out that NDF and ADF content were not affected by harvest date in whole-crop pea-cereal mixture silages harvested 8 or 10 weeks after seeding. These authors indicated that lignin content was affected by harvest date. In pea plants, the greatest changes in chemical composition occur during the pouring of grains into pods (Åman and Graham, 1987). Nutrients, especially crude protein and carbohydrates are translocated from the vegetative parts of plants into the grain, while cellulose, hemicellulose and lignin are deposited in the leaves and especially in the stem. For this reason, the chemical composition of whole plants was approximately constant with plant growth and development, whereas soluble sugars are transformed to starch and the content of cell wall constituents increased (Åman and Graham, 1987). The content of dry matter, crude protein, starch, NDF, as well as organic matter digestibility in pure pea crop increases with plant growth and development, while the content of these components in the combined pea and oat crop depends on the seeding rate of individual crops in the mixtures and plant stage of development (Salawu et al., 2001).

Dry matter digestibility is higher in pure pea crop than in silage of combined pea and cereal crop (Salawu et al., 2002b). In addition, the intake of silage from the combined crops of legumes and cereals is higher than the intake of grass silage with a similar content of nutrients (Salawu et al., 2002a; Adesogan et al., 2004). The effect of pea silage or combined pea silage with cereals compared to cereal silage (Mustafa et al., 2002), or grass silage (Salawu et al., 2002a) on milk yield and milk composition is very different. According to investigations by Mustafa et al. (2002) feeding silage to animals has a similar effect on milk yield and milk composition as when animals are fed with barley or alfalfa silage.

Conclusions

A significant influence of proportion pea and oat in the mixtures and stage of plant development on cell wall components in silages was established in this investigations. On the other hand, the inoculant application did not significantly influenced content of cell wall components except on content of NDF and hemicellulose. In general, it can be concluded that silage of pure oat crop contained significantly higher content of cell wall components than silage of pure pea crop and their mixtures. The structure of mixtures significantly affected lignin content in

silages, and the highest value of lignin was obtained in silage of pure oat crop (94.5 g kg⁻¹ DM).

Komponente ćelijskog zida i *in vitro* svarljivost suve materije silaža graška i ovsu u smeši

Jordan Marković, Snežana Anđelković, Vladimir Zornić, Ivica Kostić, Milomir Blagojević, Tanja Vasić, Sanja Živković

Rezime

Sadržaj ćelijskih zidova se generalno smatra najvažnijim faktorom koji utiče na iskorišćavanje krme, jer predstavlja najvažniju komponentu suve materije i utiče na konzumiranje i svarljivost hrane. Eksperiment je sproveden kako bi se utvrdio sadržaj konstituenata ćelijskog zida u silažama smeša graška i ovsu u pet različitih odnosa ove dve biljne vrste. Ispitan je uticaj tri faktora: primena bakterijskih inokulanata, udeo graška i ovsu u smeši i faza razvića biljaka. Sadržaj NDF-a, ADF-a, hemiceluloze, lignina i svarljivost suve materije je utvrđen u ispitivanim silažama. Grašak i ovas su uzgajani na oglednom polju Instituta za krmno bilje Kruševac: čist usev graška, čist usev ovsu, 25% graška + 75% ovsu, 50% graška + 50% ovsu i 75% graška + 25% ovsu. Primena bakterijskih inokulanata je usloвила niži udeo NDF-a i hemiceluloze u ispitivanim silažama. Sadržaj konstituenata ćelijskih zidova se smanjivao sa povećanjem udela graška u smeši, ali se svarljivost suve materije povećavala. S druge strane, rastenje i razviće biljaka uslovio je povećanje sadržaja komponenti ćelijskog zida, dok se svarljivost suve materije smanjila.

Ključne reči: silaža graška i ovsu, ćelijski zid, svarljivost

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