Acta fytotechn zootechn, 23, 2020(1): 1–6
http://www.acta.fapz.uniag.sk

Original Paper

Natural variability of restriction profiles in non-coding part of *Prunus persica* (L.) Batsch. Pru p 3 gene

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Article Details: Received: 2019-08-31 | Accepted: 2019-12-29 | Available online: 2020-03-31

https://doi.org/10.15414/afz.2020.23.01.1-6

Peach is a popular sweet fruit with a very good climate adaptation and high production. It offers many health benefits determined by its biochemical composition. However, sensitive people can be sensitised by Pru p 3 non-specific lipid transfer protein family directly or through cross reaction as a member of Bet v 1 homologues. The majority of research is focused on the protein, less data can be found in genomics or transcriptomics. This study performed RFLP analysis by chosen restricted enzymes (*BfaI*, *MseI*, *NlaIII*) for non-coding region of Pru p 3 (NCBI: KC311811.1) as a tool to distinguish closely related isoforms of the allergen. *BfaI* cut amplicon into 5 fragments corresponding to the Yulu variety *in silico* cleavage and polymorphism was not detected. For *MseI* and *NlaIII* polymorphisms were found in the cleavage sites, two types of restriction profiles were created for both. None of the *NlaIII* profiles corresponds to the restriction profile of *in silico* cleavage. The study confirms the varietal differences in Pru p 3 gene and supports a hypothesis that allergenicity depends on both qualitative and quantitative factors that are different and specific to each variety.

Keywords: Pru p 3, nLTP, non-coding region, restriction variability, peach

1 Introduction

Prunus persica (L) Batsch is world-known popular fruit tree of the *Spiroideae* subfamily with a very good climate adaptation and high production in cultivation regions. It originated in China and for Chinese cultivars, higher diversity is reported in literature when compared to other peach germplasm collections (Zhang et al., 2006). Peach is a self-pollinated species with high degree of self-compatibility and homozygosity (Baird et al., 1996), but genetics and genomics analyses provided effective tools for its marker-assisted selection. Microsatellites and simple sequence repeat markers were reported to be suitable as DNA markers to evaluate genetic relationships between individuals, marker-assisted selections and for population genetic studies in different *Prunus* species (Aranzana et al., 2010; Wünsch et al., 2005; Cheng and Huang, 2009; Bouhadida et al., 2010; Xie et al., 2010). Actually, more than 500 simple sequence repeats have been mapped in the reference map, and many other microsatellites are available from the peach genome sequence data produced by the International Peach Genome Initiative (IPGI). Actual genomic knowledge of peach is collected in eight bioinformatic screened supercontigs that represents the sequential data of eight peach chromosomes with the numbering of appropriate tights. Proceeded genomic data cover near 99% of its genome with the relevance higher than 92% (Verde et al., 2013).

Peach fruit contains many of health benefits determined by its biochemical composition. The fruit is a rich source of elements as potassium, sodium, calcium, iron, silicon, zinc, phosphorus, manganese, cadmium, magnesium, copper, and vitamins as niacin, riboflavin, β-carotenes and vitamin C, and the content of these elements is affected by many factors, mostly by cultivar (Wills et al., 1983; Ashhammary and Al-Horayess, 2013; Mitic et al., 2019).

Beside the beneficiary characteristics, peach is a fruit that may be harmful for sensitive people suffering by allergy. Peach allergy was reported to have two different patterns. In Central Europe, it is connected mainly with oral allergy syndrome related to a primary sensitization to birch pollen Bet v 1 allergen and profilins. In Southern

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Europe, it is connected mainly with systemic symptoms, in many cases due to sensitization to lipid-transfer protein Pru p 3 (Gamboa et al., 2007). Pru p 3 is a non-specific lipid transfer protein of peach fruit and is proposed to be a model of true food allergens (Salcedo et al., 2008), because of its resistance to proteolytic digestion, oral sensitisation and severe clinical symptoms (Salcedo et al., 2007). It belongs to pan-allergens that are involved in IgE-mediated reactions to both plant food and pollens (Zuidmeer and Ree, 2007). Pru p 3 is well characterized on its biochemical and immunological level (García-Casado et al., 2003; Pasquato et al., 2006; Cubells-Baeza et al., 2017), but many unusual geographical profiles of LTP sensitization were reported across Europe in the clinical practice (Fernández-Rivaz et al., 2003, 2006; Reuter et al., 2006). These are explained by different dietary habits, specific exposure to pollens and natural differences in Pru p 3 content in peach varieties (Duffort et al., 2002; Ahrazem et al., 2007; Salcedo et al., 2008). Up to date, Pru p 3 gene and its expression is characterized only a few in the individual peach varieties (Carnés et al., 2002; Brenna et al., 2004) and no specific description of natural restriction variability of the gene exists. The aim of the study is to analyse the existing natural variability in restriction patterns of a non-coding part of the Pru p 3 gene of *Prunus persica* (L) Batsch.

2 Material and methods

2.1 Biological material and DNA extraction

Young healthy leaves of seven different undefined peach varieties, planted in gardens in south part of Slovakia, were collected in the region of Komárno, more specified Harčáš (figure 1). All of them were surface sterilized by ethanol, rinsed by distilled water and transported to laboratory immediately where were kept frozen until further processing. Total genomic DNA was extracted from 100 mg of frozen leaves following the manufacturer´s instruction for the Nucleospin Plant II kit. Quantity and quality of extracted DNA was checked by Nanophotometer P360 (Implen) and all the samples were diluted to 50 ng/µl.

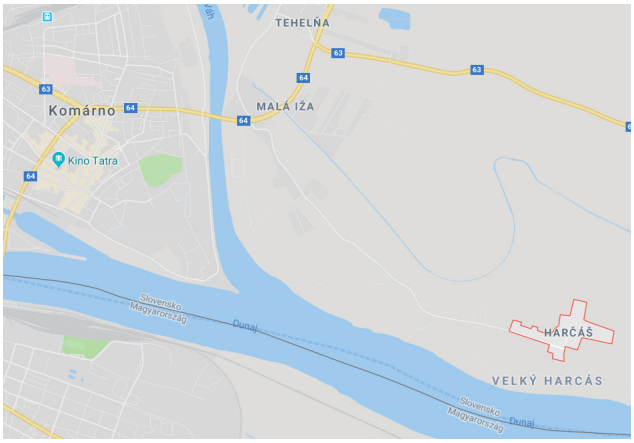

Figure 1 The location of accessions origin (GPS coordinates: lat. 47.747; long. 18.180)

2.2 PCR analysis and restriction cleavage

A non-coding region of Pru p 3 gene was subjected to the PCR amplification. Primers were designed to match a region of nucleotides 23 – 1050 of the NCBI accession number KC311811.1 corresponds to Pru p 3 sequence of peach variety Yulu (Figure 2). PCR thermal profile used in the analysis was as follows: 95 °C for 3 min followed by 35 cycles of 95 °C for 45 sec, 60 °C for 45 sec and 72 °C for 1 min, ended by last elongation step at 72 °C for 5 min and 55 sec. A specificity of obtained PCR amplicons was checked by agarose gel electrophoresis. Restriction endonucleases used in the restriction variability analysis were selected in a manner to meet the criteria – cleavage must be processed throughout the sequence, every endonuclease used must cleave at least three positions in the amplicon, different types of expected polymorphism – none, length polymorphism and presence of restriction site polymorphism. Based on the criteria, three different restriction endonucleases were used: *BfaI*, *MseI* and *NlaIII*. Restriction cleavage of PCR amplicons was performed as the manufacturer´s protocols recommended. The restriction fragments were separated in the 8% PAGE gels and stained by GelRed™.

1 tgtacttgta cgtgatgatg gctcttgcca tcttcctcag gcaatctata tcgttcgttt
61 attttccaca tgctagaaca atctttaatc tcgttcgttt cttttccatt tgcaagaaca
121 atcttaatta atctagttcg tttgtttaaa tatataattt tttttcctca tttgtttgaa
181 ggctcagtat cctcgaaggg atagggccca tatactataa tatcccaaag tcacacactc
241 atttgatatt ttgttaaatt tcatagagat ggaccatatt cattcatgca aaattttatt
301 gacaaagtaa aaaattatct gtggaagaat attatggggt ggatcccatc caaggctgta
361 tctaacggtc aagaaactta aataaaataa aatgaaatgt catgatcagt accaaaaata
421 aatttcttgt tttaaaagtt gaggtattca aagaagatat tctaacaaat tataaattgc
481 aatatttgaa taaagctttt tgtcacatcc tggatcggct ccgccgtagc atgatattat
541 ccgttttgag ccccctgcca tcaggttttg cttatgggaa ttcacgagca actttccagt
601 gggtcaccca ttctgggatt actctagccc aaacttactt aagttcggaa ttcctataat
661 tcaaaagcca gtaagcttcc aaaaggcctc gtgctagatg gaggtaagta tgtacatata
721 agacatatca ccccctctcc attgatcgat gtggaatgtt acacttttg ttgaagagag
781 tgggtgtatg aacaaatagc tggaaagctg aaaagtcggt cggatttacc atgaaagaga
841 tggttcttaa taatttgcct cgtttacaaa cataaaaaca atagattttg atgaccagaa
901 gttttttgt acctggtttt ggagcaaaac acgaatggca ggggagacac gggtctggtg
961 tactcgaacg tcaacaagaa acatttaagt gaaacaaatc aattatgcag tacgcaccat
1021 ttcacattaa tatcatggga ccccgacctt gattgtgcac ctaactaagt tgccagcaag

Figure 2 A fragment of non-coding sequence stored in NCBI under the accession KC311811.1

3 Results and discussion

Virtual cleavage profiles were designed for used endonucleases the non-coding segment of Pru p 3 gene. Those were compared further with the seven randomly chosen peach varieties to define the possible sequential polymorphism. Non-coding part of Pru p 3 gene in Yulu variety resulted in four cleavage sites in *BfaI* virtual restriction with the length of restriction fragment as 491 bp, 334 bp, 70 bp, 60 bp and 51 bp. All of the restriction sites were supposed to be without polymorphism, when based on *in silico* data of Pru p 3 genomic sequences stored in the public databases. All of these restriction sites were found in the analysed peach varieties with the correspondent restriction fragments and no changes of restriction profiles were observed. The only difference was presented in the three shortest fragments are of a low abundance and poor visibility (Figure 3).

Virtual restriction by *MseI* resulted eleven fragments in total with following length: 208 bp, 207 bp, 138 bp, 124 bp, 108 bp, 63 bp, 54 bp, 43 bp, 39 bp, 18 bp and 4 bp for peach variety Yulu, with two couples of non-separable fragments (208/207 bp and 43/39/18/4 bp) in the 8% PAGE gels, therefore a 15% PAGE gel was used. Here, polymorphism is described based on *in silico* data of Pru p 3 genomic sequence stored in the public databases. In the group of the shortest fragments, a deletion of 6 nucleotides exists among the stored genomic sequence of Pru p 3 for most of the compared peach varieties. Length polymorphism based on described deletion was obtained in the samples 1, 3 and 4 and confirmed the natural variability in the restriction profile of non-coding part of Pru p 3 gene. In the case of this restriction endonuclease, other type of polymorphism was found in the cleavage sites of analysed peach genotypes and two types of restriction profiles were obtained in the analysed samples. In three peach varieties, different restriction profiles exist as the virtual one and nine other restriction fragments were generated (Figure 4).

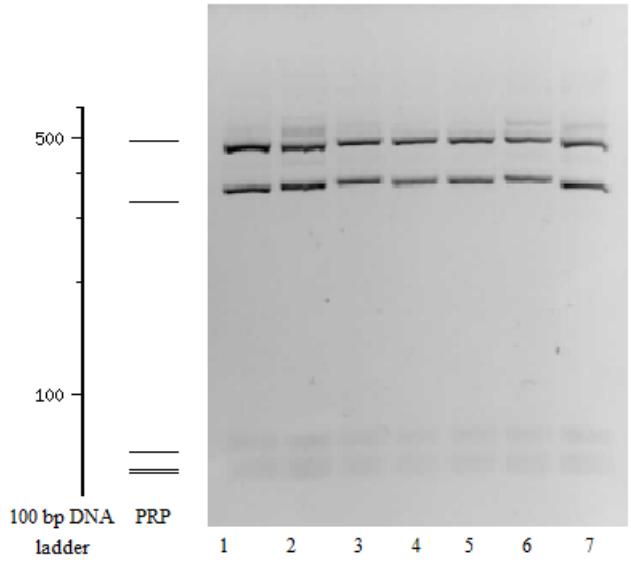

Figure 3 Obtained *BfaI* restriction profiles of analysed peach samples compared to the predicted restriction profile (PRP)

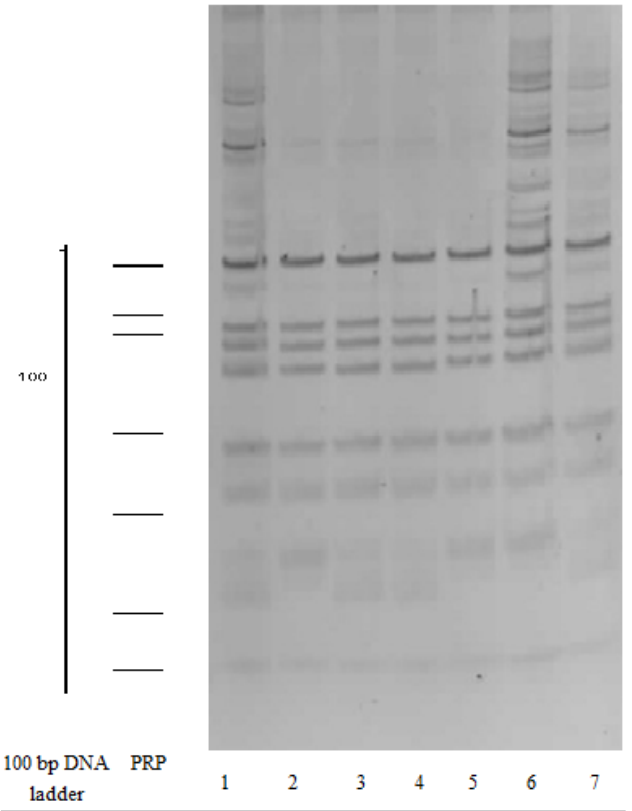

Figure 4 Obtained *MseI* restriction profiles of analysed peach samples compared to the predicted restriction profile (PRP)

Non-coding part of Pru p 3 gene resulted in six cleavage sites in *NlaIII* virtual restriction with the length of restriction fragment as 300 bp, 216 bp, 195 bp, 129 bp, 116 bp and 50 bp for peach variety Yulu. For this restriction endonuclease, a substitution-based polymorphism is described in the *in silico* data of Pru p 3 genomic sequences stored in the public databases. A substitution C/T exist in the cleavage site of *NlaIII* in the 69th nucleotide of analysed amplicon and the same substitution is in the 401st nucleotide of analysed amplicon. This results in the length polymorphism where two different restriction profiles can be obtained – 68 + 216 bp and 115 + 122 bp fragments or, if this restriction site absent, restriction fragments of 286 bp and 246 bp appear. Restriction profile of 115 + 122 bp fragments was obtained in samples 2,3 and 6. The restriction cleavage in the 69th and 286th nucleotide was obtained in none of the analysed samples. The same situation was observed for

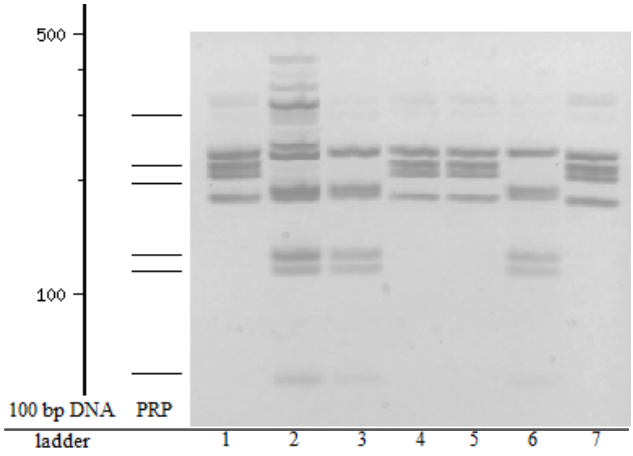

Figure 5 Obtained *NlaIII* restriction profiles of analysed peach samples compared to the predicted restriction profile (PRP)

the restriction fragment of 300 bp, which was obtained in none of the analysed samples, too (Figure 5). We suppose, that other type of nucleotide substitutions exists in this restriction cleavage sites in peach varieties. In all of the analysed samples, the shortest fragment is not present, too. In sample 2, restriction site of 286th nucleotide of analysed amplicon is missing and cytosines of both *NlaIII* sites are present in the 68th and 401st positions what resulted in the fragment with the length of 332 bp. In a summary, none of the analysed peach varieties correspond to the restriction profile of a virtual cleavage of Yulu peach variety. Samples 3 and 6 generated a completely different restriction profiles, where only a restriction fragment of 197 bp was identified from the prediction cleavage of the Yulu variety sequence and two other restriction fragments were generated as completely different. For *NlaIII*, the differences exist for the length of the generated restriction fragments, too.

Food allergy has an increasing prevalence, that is, why different useful tools are being developed for the strategy of selecting suitable varieties for breeding of hypoallergenic fruit (Hoffmann-Sommergruber et al., 2005). A similar approach was initiated in peach recently. It is assumed that genotypic variability of 4 allergen-coding gene families (Pru p 1–4) and different level of transcription could be responsible for different allergenic response (Zhong-Shan et al., 2016). The hypothesis is based on the assumption that allergenicity depends on both qualitative and quantitative factors that are different and specific to each variety (Chen et al., 2008). The hypothesis supports Gao et al. (2008) suggesting that the final structure of a protein and allele doses are linked to (hypo-) allergenicity. Protein structure is influenced by an origin gene sequence and as Ying-Tao et al. (2014) identified, in peach germplasm are three different allele sequences with variable frequency in cultivars what could be link to varying Pru p 3 content (Aranzana et al., 2019). Differences in gene sequences are confirmed by this article. However, the identification and description are impeded by the multiplicity of isoallergens of peach allergen families and are not sufficient by traditional immunological techniques. The real-time RT-PCR method, which is also a powerful tool for monitoring and quantifying gene expression, enables specificity and high sensitivity. The specificity given by the primers in RT-PCR is able to distinguish isoallergens at the transcribed mRNA level, allows to work with a single isoform at once and to provide information about isoforms related to peach allergy and shifts them to proteomics (Helsper et al., 2002; Monaci and Visconti, 2009). A preliminary study on peach allergen encoding genes (Botton et al., 2009) did not cover all members identified by genomic research.

Plant non-specific lipid transfer proteins are ubiquitous and encoded by multigene families. They are involved in many biological processes and their physiological functions are not clearly understood (Chae et al., 2009). Peach lipid transfer protein was reported previously as to be highly conserved in its coding sequences in *Prunus persica* (Ying-Tao et al., 2014). The allergen coding gene has three members encoding Pru p 3.01, Pru p 3.02 and Pru p 3.03 lipid transfer proteins (Chen et al., 2008), however LTP1 (Pru p 3.01), 9 kDa protein with an isoelectric point >9 (Pastorello et al., 1999) is expressed at high levels in peach fruit (Yang et al., 2011). Lipid transfer protein genomic variability was described for different *Prunus* species by Ying-Tao et al. (2014) with the results of following substitutions in exon1: G/A in 13th nucleotide, G/A in 104th nucleotide, G/C in 121st nucleotide, G/A in 154th nucleotide, C/T in 266th nucleotide, G/T in 280th nucleotide, A/C in 316th nucleotide, G/C in 325th nucleotide and C/A in 344th nucleotide. These nucleotide variability results in natural variants of signal peptides of LTP1 proteins.

Pru p 3 gene and its expression is characterized only a few in the individual peach varieties (Carnés et al., 2002; Brenna et al., 2004). Expression levels of LTP1 was measured in apples and significant differences were detected among varieties (Bolhaar et al., 2005; Borges et al., 2006; Sancho et al., 2008). Non-specific lipid transfer protein of peach was analysed for its expression previously and was characterized as two expressed isoforms – LTP1 and LTP2. LTP1 is expressed in pollinated flowers preferentially and LTP2 in ovary. In fruit, only LTP1 mRNA was detected (Botton et al., 2002, 2009). These analyses were performed for the peach varieties Springcrest, Royal Gem and Zorzi.

4 Conclusions

The Pru p 3 gene amplicons of the non-coding region were used in the RFLP analysis by three different restriction enzymes *BfaI*, *MseI* and *NlaIII*. The primers were designed for PCR according to the sequence of NCBI database with accession number KC311811.1 in a manner to be are capable of capturing multiple Pru p 3 allergen isoforms. The genomic variability was screened in a group of undefined peach varieties. *BfaI* restriction cleavage did not provide varietal specificity, unlike the next two restriction enzymes, where variability exists in restriction sites. For *NlaIII*, none of the analysed peach varieties correspond to the restriction profile of a virtual cleavage of Yulu peach variety. Obtained *in silico* and *in vitro* RFLP profiles do not match each other which points the necessity to obtain sequence records of each isoform, which would facilitate subsequent analysis.

For the future, construction of a phylogenetic dendrograms based on genomic sequences data could bring new insights into the development of allergen isoforms during the historic breeding of peach, find gene mutation sites, and ultimately successfully identify the specific area responsible for protein allergenicity.

Further research in the field could provide a simple and fast screening methodology for determining hypo-/hyper-allergenicity of the variety, which would benefit the final consumer.

Acknowledgments

Research was supported by European Community under project no 26220220180: Building research centre "AgroBioTech" and by The Danube strategy project DS-2016-0051 Genomics, transcriptomics, digestomics and a mouse allergy of sensitization to allergic lipid transfer proteins.

5 References

AHRAZEM, O. et al. (2007) Assessing allergen levels in peach and nectarine cultivars. *Annals of Allergy Asthma and Immunology*, 99(1), 42–47.

ALSHAMMARY, S.F. and AL-HORAYESS, O.S. (2013) Appraisal of mineral and heavy metal contents in peach and grape grown at some major agricultural companies in Saudi Arabia. *Oriental Journal of Chemistry*, 29(4), 1515.

ARANZANA, M.J. et al. (2019) *Prunus* genetics and applications after de novo genome sequencing: achievements and prospects. *Horticulture research*, 6 (58). https://doi.org/10.1038/s41438-019-0140-8

ARANZANA, M.J. et al. (2010) Genetic variation, population structure and linkage disequilibrium in peach commercial varieties. *BMC Genetics*, 11(1), 69.

BAIRD, W.V. et al. (1996) Progress in *Prunus* mapping and application of molecular markers to germplasm improvement. *Horticulture Science*, 31(7), 748–749.

BOLHAAR, S. et al. (2005) *In vivo* assessment with prick-to-prick testing and double-blind, placebo controlled food challenge of allergenicity of apple cultivars. *Journal of Allergy and Clinical Immunology*, 116(5), 1080–1086.

BORGES, J.P. et al. (2006) The lipid transfer proteins (LTP) essentially concentrate in the skin of *Rosaceae* fruits as cell surface exposed allergens. *Plant Physiology and Biochemistry*, 44(10), 535–542.

BOTTON A., et al. (2002) Differential expression of two lipid transfer protein genes in reproductive organs of peach (*Prunus persica* L. Batsch). *Plant Science*, 163, 993–1000.

BOTTON, A. et al. (2009) Peach (*Prunus persica* L. Batsch) allergen-encoding genes are developmentally regulated and affected by fruit load and light radiation. *Journal of Agricultural and Food Chemistry*, 57(2), 724–734.

BOUHADIDA, M. et al. (2010) Genetic variability of introduced and local Spanish peach cultivars determined by SSR markers. *Tree Genetics and Genomes*, 7(2), 257–270.

BRENNA, O.V. et al. (2004) Presence of Allergenic Proteins in Different Peach (*Prunus persica*) Cultivars and Dependence of Their Content on Fruit Ripening. *Journal of Agriculture and Food Chemistry*, 52(26), 7997–8000.

CARNÉS, J. et al. (2002) Pru p 3 (LTP) content in peach extracts. *Allergy*, 57(11), 1071–1075.

CHAE, K. et al. (2009) A gain of function mutation of Arabidopsis lipid transfer protein 5 disturbs pollen tube tip growth and fertilization. *Plant Cell*, 21(12), 3902–3914.

CHEN, L. et al. (2008) Genomic characterization of putative allergen genes in peach/almond and their synteny with apple. *BMC Genomics*, 9 (1), 543.

CHENG, Z. and HUANG, H. (2009) SSR fingerprinting Chinese peach cultivars and landraces (*Prunus persica*) and analysis of their genetic relationships. *Science Horticulture*, 120(2), 188–193.

CUBELLS-BAEZA, N. et al. (2017) Identification of the ligand of Pru p 3, a peach LTP. *Plant Molecular Biology*, 94(1–2), 33–44.

DUFFORT, O.A. et al. (2002) Immunoassay to quantify the major peach allergen Pru p 3 in foodstuffs. Differential allergen release and stability under physiological conditions. *Journal of Agriculture and Food Chemistry*, 50(26), 7738–7741.

FERNÁNDEZ-RIVAS, M. et al. (2003) Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. *Journal of Allergy and Clinical Immunology*, 112(4), 789–795.

FERNÁNDEZ-RIVAS, M. et al. (2006) Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. *Journal of Allergy and Clinical Immunology*, 118(2), 481–488.

GAMBOA, P.M. et al. (2007) Two different profiles of peach allergy in the north of Spain. *Allergy*, 62(4), 408–414.

GAO, Z.S. et al. (2008). Assessment of allelic diversity in intron-containing Mal d 1 genes and their association to apple allergenicity. *BMC Plant Biology*, 13(8), 116.

GARCÍA-CASADO, G. et al. (2003). Identification of IgE-binding epitopes of the major peach allergen Pru p 3. *Journal of Allergy and Clinical Immunology*, 112(3), 599–605.

HELSPER, J. et al. (2002). Quadrupole time-of-flight mass spectrometry: a method to study the actual expression of

allergen isoforms identified by PCR cloning. *Journal of Allergy and Clinical Immunology*, 110(1), 131–138.

HOFFMANN-SOMMERGRUBER, K. and SAFE consortium (2005). The SAFE project: 'plant food allergies: field to table strategies for reducing their incidence in Europe' an EC-funded study. *Allergy*, 60(4), 436–442.

MITIC, S. et al. (2019). Comparative Study on Minerals in Peel and Pulp of Peach (*Prunus persica* L.) Fruit. *Revista de Chimie Bucharest Original Edition*, 7(6), 2281–2285.

MONACI, L. and VISCONTI, A. (2009). Mass spectrometry-based proteomics methods for analysis of food allergens Tr. In TrAC Trends. *Analytical Chemistry*, 28(5), 581–591.

PASQUATO, N. et al. (2006). Crystal structure of peach Pru p 3, the prototypic member of the family of plant non-specific lipid transfer protein panallergens. *Journal of Molecular Biology*, 356(3), 684–694.

PASTORELLO, E.A, et al. (1999). The major allergen of peach (*Prunus persica*) is a lipid transfer protein. *Journal of Allergy and Clinical Immunology*, 103(3), 520–526.

REUTER, A. et al. (2006). A critical assessment of allergen component-based *in vitro* diagnosis in cherry allergy across Europe. *Clinical and Experimental Allergy*, 36(6), 815-823.

SALCEDO, G. et al. (2007). Plant non-specific lipid transfer proteins: an interface between plant defence and human allergy. *Biochimistry Biophysical Acta*, 1771(6), 781-791.

SALCEDO, G. et al. (2008). Review. Plant food allergens: peach non-specific lipid transfer protein Pru p 3 as a model. *Spanish Journal of Agricultural Research*, 6(Special issue), 30–37.

SANCHO, A.I. et al. (2008) Measurement of lipid transfer protein in 88 apple cultivars. *International Archives of Allergy and Immunology*, 146(1), 19–26.

VERDE, I. et al. (2013) The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. *Nature Genetics*, 45pr(5), 487–494.

WILLS, R. B. H. et al. (1983) Nutrient composition of stone fruit (*Prunus* spp.) cultivars: Apricot, cherry, nectarine, peach and plum. *Journal of the Science of Food and Agriculture*, 34(12), 1383–1389.

WÜNSCH, A. et al. (2005) Molecular characterization of local Spanish peach [*Prunus persica* (L.) Batsch] germplasm. *Genetic Resources and Crop Evolution*, 53(5), 925–932.

XIE, R. et al. (2010) Evaluation of the genetic diversity of Asian peach accessions using a selected set of SSR markers. *Science Horticulture*, 125(4), 622–629.

YANG, Z., et al. (2011) Differential transcript abundance and genotypic variation of four putative allergen-encoding gene families in melting peach. *Tree Genetics & Genomes*, 7(5), 903.

YING-TAO, M. et al. (2014) The PpLTP1 Primary Allergen Gene is Highly Conserved in Peach and Has Small Variations in Other *Prunus* Species. *Plant Molecular Biology Reporter*, 32(3), 652–663.

ZHANG, A.M. et al. (2006) Genetic diversity and ecogeographical phylogenetic relationships among peach and nectarine cultivars based on simple sequence repeat (SSR) markers. *Journal of American Society of Horticulture Science*, 131(4), 513–521.

ZHONG-SHAN, G. et al. (2016) Quantification of Peach Fruit Allergen Lipid Transfer Protein by a Double Monoclonal Antibody-based Sandwich ELISA. *Food Analytical Methods*, 9(4), 823–830.

ZUIDMEER, L. and van REE, R. (2007) Lipid transfer protein allergy: primary food allergy or pollen/food syndrome in same cases. *Current Opinion of Allergy and Clinical Immunology*, 7(3), 269–273.

Acta fytotechn zootechn, 23, 2020(1): 7–14
http://www.acta.fapz.uniag.sk

Original Paper

Assimilation capacity by non-destructive *in situ* measurements in longterm experiment of maize (*Zea mays* L.) under different plant density and nitrigen supply in Chernozem

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Article Details: Received: 2019-10-15 | Accepted: 2019-12-31 | Available online: 2020-03-31

https://doi.org/10.15414/afz.2020.23.01.7-14

We conducted a long term experiment (set up in 1983) to examine the effects of fertilization and plant density on SPAD and LAI values as well as yield in 2017 and 2018, in two maize hybrids (Sushi, Fornad) in chernozem soil. Hybrid yields varied between 10.2–16.7 t ha^{-1} in 2017 and between 9.3–15.9 t ha^{-1} in 2018, depending on fertilization doses and plant density. The hybrids had $SPAD_{max}$ values in early July (55.5–60.4 in 2017 and 54.9–63.9 in 2018), whereas they got LAI_{max} values in early July (3.0–5.3 m^2 m^{-2} in 2017) and early August (3.5–4.4 m^2 m^{-2} in 2018). Research data evaluated by Pearson correlation calculations proved that fertilization was the main factor that had a significant effect on SPAD and LAI values in the different maize phenophases (r = 0.6**–0.8** for SPAD, r = 0.5**–0.8** for LAI). Correlation among plant density, hybrid and SPAD and LAI values showed very weak correlations in both years (r = 0.1–0.3). The yield of the maize hybrids was most significantly affected by fertilizer in 2017 (r = 0.672**) and plant density in 2018 (r = 0.517**).

Keywords: long term experiment, LAI, SPAD, yield, Pearson correlation

1 Introduction

Maize has a significant role in both world (2nd largest production area) and Hungarian (largest production area of arable land) field crop production. It has very high genetic potential (it is a C4 photosynthetic type plant), but only 25–35% of which is currently utilised in Hungary (country average yield have been between 6.5–8.5 t ha^{-1} in last decade). In addition to its high yield potential, maize is largely affected by changes in ecological and agrotechnical conditions. From a crop formation perspective, changes in the dynamics of the assimilation capacity (leaf area, relative chlorophyll content) of the crop stand has a significant role in the vegetation period (Carter, 1994; Martinez and Guiamei, 2004; Hawkins et al., 2009). It is crucial to measure LAI and SPAD values in maize populations, as it makes it possible to gather data on photosynthetic activity via *in situ* non-destructive methods. LAI and SPAD values are affected by the year, the hybrid, fertilization and plant density as well. Fertilization makes huge changes in the SPAD (Yu et al., 2010; Széles et al., 2011) and LAI values of hybrids (Novoa and Loomis, 1981; Oikeh et al., 1998, Micskei et al., 2012). Some other references show that increasing plant density decreased the SPAD values (Su et al., 2012; Tajul et al., 2013) and increased the LAI values (Ahmad et al., 2010; Valadabadi and Farahani, 2010). Many researchers examined the relationship between maize SPAD and LAI values and yield. The relative chlorophyll concentration of maize (SPAD) had a positive correlation with nitrogen supply and maize yield (Széles, 2008; Bencze and Futó, 2017). Research results showed a strong positive correlation between LAI values at maize flowering and yield (Oikeh et al., 1998; Bavec and Bavec, 2002; Ma et al., 2005). However, research data by Esechie (1982), Remison and Lucas (1992) showed no correlation between leaf area index (LAI) and maize yield.

Several research shows that maize yield can be increased significantly by fertilization (Berzsenyi, 2010; Széll et

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al., 2010; Vári and Pepó, 2011). However, the effects of fertilizers were largely affected by the water supply of the year (Azeez, 2009; Ványiné et al., 2012) as well as the hybrid (Körshens, 2006; D'Haene et al., 2007). At the same time, there is a strong correlation between optimum plant density and yield in maize production. Optimal plant density was affected by the water supply of the vegetation period (Sárvári and Pepó, 2014; Nagy, 2010; Berzsenyi et al., 2011) as well the water reservoirs of soil (Fulton, 1970; Dóka, 2015). In addition to water supply and year, the plant density response of the hybrids also plays a significant role in yield formation. Up-to-date hybrids can utilise more yield potential at higher crop density (Carlone and Russel, 1987; Russel, 1991; Haegele et al., 2014). The experimental results of Pepó and Murányi (2014, 2015) show that the yield of crop hybrids is also affected by cropping area (row spacing).

In our long-term chernozem soil experiment we studied the responses of different maize hybrids to fertilization and plant density, as well as measure photosynthesis capacity values (LAI, SPAD) in the vegetation season of maize. We examined the relations between LAI and SPAD values and maize yield.

2 Material and methods

In 1983, a long-term trial on calcareous chernozem soils (code CH accordind to WRB classification) was set up in the Hajdúság (Eastern Hungary), 15 km from Debrecen (latitude 47° 33' N., and longitude 21° 27' E.). The chernozem soil of the long-term experiment contains 2.7–2.8% humus, and total depth of the humus enriched horizon was about 0.8 m (Table 1). When the trial was set up, the soil contained 130 mg kg^{-1} AL-soluble P_2O_5 and 240 mg kg^{-1} AL-soluble K_2O. The calcareous chernozem soil is characterized by a specific plasticity index (KA) of 40 and nearly neutral pH (pH_{KCl} = 6.46). The soil has favourable water management characteristics.

During the long-term trial we applied treatment with 6 nutrient doses. In addition to the control treatment, a basic dose of N =30 kg ha^{-1} + P_2O_5 = 22.5 kg ha^{-1} + K_2O = 26.5 kg ha^{-1} was applied in double, triple, quadruple and quintuple quantities. In the trial, among these doses, the following treatments were examined:

	N	P_2O_5	K_2O
kg ha^{-1}			
control	0	0	0
N_{90} + PK	90	67.5	79.5
N_{150} + PK	150	112.5	132.5

Nitrogen fertilizer was applied to the plots 50% in the autumn and 50% in the spring, before sowing. The full amount (100%) of the phosphorous and the potassium was applied in the autumn before ploughing.

Two plant densities (65 thousand ha^{-1} and 85 thousand ha^{-1}) were set up in our long-term experiment with two different genotype (Sushi (FAO 340), Fornad (FAO 420). The trial was arranged in a split-split-plot design. The gross and net plot areas were 9.12 m^2 and 7.60 m^2, respectively. The trial involved four repetitions. The forecrop was winter wheat. The optimal agricultural elements (tilling, sowing, crop protection, harvesting) were used, which matched to modern maize production.

Important weather information for trial years are shown in Tables 2 and 3.

The meteorical data of experimental years proved that the rainfall of pre-vegetation periods (from October to Mach) was slightly (+20.9 mm) and highly (+131.7 mm) higher, compared with the 30-years mean in 2017 and 2018, respectively. The amount and the distribution of rainfall in the vegetation period (April–September) were much favourable in 2017 year, compared with 2018 year. The monthly average temperatures of vegetation periods were over the mean average in both years which modified the absolute values and dynamics of SPAD and LAI readings.

Table 1 Most important traits of calcareous chernozem soil in the long-term experiment (Debrecen)

Soil layer (m)	pH (KCl)	Elasticity number	$CaCO_3$ (%)	Humus (%)	Total N (%)	$NO_3 + NO_2$ (mg kg^{-1})	AL soluble P_2O_5 (mg kg^{-1})	AL soluble K_2O (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	SO_4 (mg kg^{-1})
0–0.25	6.46	43.0	0	2.76	0.150	6.20	133.4	239.8	332.4	38.0	2.80	5.86	438	9.25
0.25–0.50	6.36	44.6	0	2.16	0.120	1.74	48.0	173.6	405.4	66.2	0.80	4.54	406	9.13
0.50–0.75	6.58	47.6	0	1.52	0.086	0.60	40.4	123.0	366.6	55.4	0.58	3.64	339	10.80
0.75–1.00	7.27	46.6	10.25	0.90	0.083	1.92	39.8	93.6	249.0	67.8	0.48	2.24	74	7.95
1.00–1.30	7.36	45.4	12.75	0.59	0.078	1.78	31.6	78.0	286.6	62.6	0.84	1.64	4	22.98

Table 2 Rainfall in pre-vegetation and vegetation period of maize (Debrecen)

Vegetation period	Pre-vegetation period (Oct–March) (mm)	Rainfall (mm)						
		Apr.	May.	June	July	Aug.	Sept.	Total
2017	235.0	50.4	31.9	62.3	71.6	47.5	91.7	355.4
2018	345.8	36.6	60.0	66.8	41.9	97.5	20.6	323.4
30-years mean	214.1	52.8	64.0	66.5	66.1	49.0	47.5	346.0

Table 3 Monthly average temperature in the vegetation period of maize (Debrecen)

Vegetation period	Monthly average temperature (°C)						Mean
	Apr.	May.	June	July	Aug.	Sept.	
2017	10.1	16.3	20.9	21.0	22.1	15.5	17.65
2018	15.5	19.0	20.1	21.7	23.2	17.1	19.43
30-years mean	11.1	16.6	19.4	21.3	20.7	15.8	17.48

In our long-term experiment the leaf area index (LAI) and relative chlorophyll content (SPAD) were measured 5 times in the vegetation period of maize. The LAI values were determined using a SunScan Canopy Analysis System (SSI) portable leaf area measuring instruments in four repetitions with five measurements per repetition. SPAD and LAI values were measured during the morning period between 9–11 a.m.

For the measurement of the SPAD values a portable Soil Plant Analysis Development (SPAD-502 Plus, Konica Minolta) instrument was used. The instrument measures the light absorption of leaves in the blue and red (R = 600–700 nm) spectrum range, which corresponds to the maximum light absorption of chlorophyll. The values are based on near infra-red band in addition to the visible light spectra. The SPAD values can be regarded equal to the leaf chlorophyll content, as there is a very close correlation between the SPAD value and the chlorophyll content in the different crops. The SPAD values were measured in four repetitions with fifteen measurements per repetitions.

The statistical evaluation of experimental data was performed using the programmes Microsoft Excel 2013 and SPSS for Windows 13.0. For the evaluation of the results analysis of variance and Pearson's correlation analysis were used. The average values were copmpared with post hoc statistical test.

3 Results and discussion

We measured the SPAD and LAI values of maize plants 5 times during the vegetation seasons in 2017 and 2018. (Tables 4 and 5). All assessments were carried out between late May/early June and early September. Despite the significant differences between the years, there were no significant differences in the SPAD dynamics and maximum values of the two vegetation seasons. The late May / early June SPAD values (49.7–54.1 in 2017, 43.7–52.0 in 2018) were continuously increasing and reached their $SPAD_{max}$ values in early July (04/07) in both years (55.5–60.4 in 2017 and 54.9–63.9 in 2018). After $SPAD_{max}$ the readings of SPAD had a moderate decreasement in 2017 (28.3–54.9 at the time of the 01/09 measurement) and a significant drop in 2018 (9.3–14.5 as measured on 07/09), which was due to the temperature differences of July and August between the two years. Fertilization had significant increasements in both hybrids at most measurement times, plant density made no differences on them. There was no significant difference in the relative chlorophyll content of the two genotypes either. The temporal changes in leaf area index (LAI) showed similar dynamics comparing with relative chlorophyll values (SPAD) (Table 4, 5). The hybrids gave their LAI_{max} values in early July in 2017 (3.0–5.3 m^2 m^{-2} on 04/07) and early August in 2018 (3.5–4.4 m^2 m^{-2} on 06/08). As opposed to SPAD values, the decreasement of LAI values followed a similar trend in both years (1.5–3.0 m^2 m^{-2}, on 01/09/2017 and 1.1–2.3 m^2 m^{-2} on 07/09/2018). The LAI_{max} values were higher in 2018 than in 2018. Increasing fertilization doses and plant density resulted the higher LAI values of hybrids at all times of measurements. As a result of fertilization, there were significant differences in the N_{150} + PK treatment in 2017, whereas differences were not significant in most cases in 2018 and LAI_{max} values were reached in the N_{90} + PK treatment.

The effects of excellent chernozem soil, favourable water supply and near-optimal agrotechnology could moderate the negative temperature conditions of both years, so we obtained high yields in our long-term

Table 4 Effect of crop year and agrotechnical elements on the SPAD and LAI of maize in the vegetation period (Debrecen, chernozem soil, 2017)

Hybrid	Fertilization	65,000 ha^{-1}					85,000 ha^{-1}				
		09. 06.	22. 06.	04. 07.	17. 08.	01. 09.	09. 06.	22. 06.	04. 07.	17. 08.	01. 09.
		SPAD									
Sushi	Ø	51.6^{ab}	54.5^{a}	55.5^{a}	48.0^{a}	31.8^{a}	50.7^{a}	53.9^{a}	54.0^{a}	45.3^{a}	28.3^{a}
	N_{90} + PK	54.1^{b}	57.8^{b}	59.2^{b}	56.9^{b}	47.8^{b}	53.6^{b}	55.0^{ab}	58.5^{b}	56.7^{c}	46.7^{c}
	N_{150} + PK	52.2^{ab}	56.5^{ab}	60.4^{b}	58.9^{bc}	46.5^{b}	51.7^{ab}	56.4^{b}	59.4^{b}	58.0^{c}	46.6^{c}
Fornad	Ø	49.7^{a}	56.8^{ab}	57.6^{ab}	52.2^{ab}	34.2^{a}	52.1^{ab}	54.8^{ab}	56.5^{ab}	50.9^{b}	35.7^{b}
	N_{90} + PK	52.4^{ab}	57.7^{b}	59.1^{b}	59.2^{c}	53.5^{c}	51.6^{ab}	56.4^{b}	58.8^{b}	58.3^{c}	51.3^{cd}
	N_{150} + PK	49.8^{a}	56.6^{ab}	60.3^{b}	58.8^{bc}	53.1^{c}	51.6^{ab}	57.7^{b}	60.6^{b}	59.1^{c}	54.9^{d}
		LAI (m^2 m^{-2})									
Sushi	Ø	1.0^{a}	2.0^{a}	3.8^{a}	2.1^{ab}	1.5^{a}	1.2^{a}	2.3^{ab}	3.9^{ab}	1.8^{a}	1.7^{a}
	N_{90} + PK	1.2^{a}	2.6^{b}	3.8^{a}	2.6^{b}	2.2^{c}	1.7^{ab}	2.9^{b}	4.9^{b}	2.6^{b}	2.2^{b}
	N_{150} + PK	1.8^{b}	2.4^{ab}	4.1^{a}	2.1^{ab}	2.2^{c}	2.0^{b}	3.0^{b}	5.3^{b}	2.2^{db}	2.2^{b}
Fornad	Ø	1.0^{a}	1.8^{a}	3.8^{a}	1.6^{a}	1.8^{b}	1.0^{a}	2.0^{a}	3.0^{a}	1.9^{a}	1.8^{a}
	N_{90} + PK	1.4^{ab}	2.2^{ab}	3.7^{a}	2.1^{ab}	2.0^{bc}	1.4^{ab}	2.9^{b}	4.1^{ab}	2.6^{b}	3.0^{c}
	N_{150} + PK	1.4^{ab}	2.5^{b}	3.8^{a}	1.9^{ab}	2.1^{c}	2.0^{b}	2.9^{b}	4.9^{b}	2.5^{b}	2.9^{c}

a, b, c, d letters are significantly different at $P \leq 0,05$ level

Table 5 Effect of crop year and agrotechnical elements on the SPAD and LAI of maize in the vegetation period (Debrecen, chernozem soil, 2018)

Hybrid	Fertilization	65,000 ha^{-1}					85,000 ha^{-1}				
		25. 05.	14. 06.	04. 07.	06. 08.	07. 09.	25. 05.	14. 06.	04. 07.	06. 08.	07. 09.
		SPAD									
Sushi	Ø	43.7^{a}	45.7^{a}	45.9^{a}	45.8^{a}	11.3^{a}	47.1^{a}	45.5^{a}	53.3^{a}	47.4^{a}	10.6^{ab}
	N_{90} + PK	51.0^{b}	55.9^{c}	62.5^{c}	54.7^{ab}	10.9^{a}	49.9^{b}	55.7^{b}	61.4^{b}	55.5^{b}	10.9^{ab}
	N_{150} + PK	50.7^{b}	59.7^{d}	62.4^{c}	54.2^{ab}	13.1^{b}	50.2^{b}	54.7^{b}	59.1^{b}	55.5^{b}	11.3^{ab}
Fornad	Ø	46.8^{ab}	49.1^{b}	58.4^{b}	49.8^{a}	11.3^{a}	49.7^{ab}	47.3^{ab}	58.3^{ab}	50.0^{ab}	9.3^{a}
	N_{90} + PK	49.8^{b}	59.6^{d}	63.9^{c}	59.9^{b}	10.7^{a}	52.0^{b}	57.5^{b}	60.5^{b}	54.7^{b}	12.1^{b}
	N_{150} + PK	50.0^{b}	58.7^{d}	61.8^{c}	56.4^{ab}	14.5^{b}	51.4^{b}	56.3^{b}	62.0^{b}	55.1^{b}	11.7^{b}
		LAI (m^2 m^{-2})									
Sushi	Ø	0.8^{a}	1.8^{a}	2.4^{ab}	3.5^{a}	1.1^{a}	0.8^{a}	2.0^{a}	2.5^{a}	4.1^{ab}	1.3^{a}
	N_{90} + PK	0.9^{ab}	2.3^{b}	2.6^{ab}	3.8^{b}	1.6^{ab}	0.8^{a}	2.3^{ab}	2.9^{ab}	4.4^{b}	2.0^{b}
	N_{150} + PK	1.0^{a}	2.4^{b}	2.1^{a}	3.6^{ab}	1.9^{b}	1.0^{b}	2.6^{b}	2.5^{a}	3.7^{a}	2.1^{b}
Fornad	Ø	0.8^{a}	1.9^{a}	2.1^{a}	3.9^{b}	1.5^{ab}	0.9^{ab}	2.2^{ab}	3.0^{ab}	3.6^{a}	1.3^{a}
	N_{90} + PK	0.9^{ab}	2.5^{b}	2.9^{b}	3.9^{b}	2.3^{b}	1.0^{b}	2.5^{b}	3.2^{b}	4.0^{ab}	2.2^{b}
	N_{150} + PK	1.1^{a}	2.6^{b}	2.3^{ab}	3.6^{ab}	2.0^{b}	1.2^{b}	2.7^{b}	3.0^{ab}	3.9^{ab}	2.1^{b}

a, b, c letters are significantly different at $P \leq 0,05$ level

experiments. The yields of hybrids varied between 10.2–16.7 t ha^{-1} in 2017 and between 9.3–15.9 t ha^{-1} in 2018, depending on fertilization and plant density (Table 6). In both years the fertilization had significant effects on the yield of maize hybrids. The yield surpluses of hybrids due to fertilization were 1.8–3.7 t ha^{-1} at 65 thousand ha^{-1} plant density, 2.1–5.9 t ha^{-1} at 85 thousand plant density, and 0.2–2.6 t ha^{-1} vs 1.1–2.3 t ha^{-1} in 2018, respectively. Increasing plant density lead to an increase in yield in both years, which was triggered by the significant amount of soil water supplies in spite of unfavourable temperature conditions in the vegetation season. The plant density of hybrids were different, i.e. in the Sushi hybrid increased plant density gave no significant yield increasement in the two years of our experiment (-0.2–+0.6 t ha^{-1} in 2017, vs 1.1–1.6 t ha^{-1} in 2018). In the Fornad hybrid, increased plant density realized a significant increase in yield (0.6–4.6 t ha^{-1} in 2017, 2.9–3.9 t ha^{-1} in 2018, respectively). The excellent nutrient and water supplies of the soil in the long-term experiment were proven by the high yield in the control treatment (10.2–11.1 t ha^{-1} in 2017, 9.3–13.6 t ha^{-1} in 2018, respectively). Sushi hybrid showed maximum yield in the N_{150} + PK (2017) and the N_{90} + PK (2018) treatment, whereas Fornad showed maximum yield in the $N_{90–150}$ + PK (2017) and N_{90} + PK (2018) treatment.

We applied the Pearson's correlation analysis to assess the correlations between the SPAD (Table 7) and LAI (Table 8) values with the hybrids, agrotechnological factors (fertilization, plant density) and yield at different measurement times. SPAD values showed relatively high correlations with nutrient supply in both years. In 2017, SPAD 3, SPAD 4 and SPAD 5 showed correlation coefficients of r = 0.630**, 0.773** and 0.795** with fertilization, respectively. In 2018, SPAD 2, SPAD 3 and SPAD 4 showed correlation coefficients of r = 0.799**, 0.530** and 0.593**, respectively. We found no correlations between the hybrids, plant density and SPAD values during the years of our experiments. According to our research data there were a very weak correlation between SPAD and yield (r = -0.018–0.424**), except at the time of the SPAD 4 (r = 0.619**) and SPAD 5 (r = 0.590**) measurements in 2017. Similarly, the Pearson's correlation analyses proved no significant correlations between LAI values measured at different times, hybrids and plant density. Fertilization

Table 6 Effect of genotype and agrotechnical elements on the yield of maize (Debrecen, chernozem soil, 2017–2018)

Hybrid	Fertilization	Yield (kg ha^{-1})			
		65,000 ha^{-1}		85,000 ha^{-1}	
		2017	2018	2017	2018
Sushi	Ø	11,123^{ab}	9,298^{a}	10,958^{a}	10,875^{a}
	N_{90} + PK	13,427^{b}	11,868^{b}	13,106^{b}	12,989^{b}
	N_{150} + PK	14,856^{b}	11,038^{ab}	15,439^{c}	12,322^{ab}
Fornad	Ø	10,246^{a}	10,534^{ab}	10,823^{a}	13,574^{b}
	N_{90} + PK	12,312^{ab}	12,990^{b}	15,433^{c}	15,915^{c}
	N_{150} + PK	12,072^{ab}	10,745^{ab}	16,682^{c}	14,647^{bc}

a, b, c letters are significantly different at $P \leq 0,05$ level

Table 7 Pearson correlation analysis among the agrotechnical elements (hybrid, plant density, fertilization) and yield and SPAD readings of maize (Debrecen, chernozem soil, 2017–2018)

Year		SPAD 1	SPAD 2	SPAD 3	SPAD 4	SPAD 5
2017	Hybrid	-0.254^{ns}	0.291*	0.174^{ns}	0.228^{ns}	0.303*
	Plant density	-0.062^{ns}	-0.231^{ns}	-0.129^{ns}	-0.089^{ns}	-0.025^{ns}
	Fertilization	0.074^{ns}	0.423**	0.630**	0.773**	0.795**
	Yield	0.018^{ns}	0.424**	0.275^{ns}	0.619**	0.590**
2018	Hybrid	0.190^{ns}	0.167^{ns}	0.222^{ns}	0.207^{ns}	0.065^{ns}
	Plant density	0.220^{ns}	-0.173^{ns}	-0.187^{ns}	-0.041^{ns}	-0.265^{ns}
	Fertilization	0.466**	0.799**	0.530**	0.593**	0.433**
	Yield	0.371**	0.297*	0.400**	0.339*	-0.224^{ns}

** – correlation at $LSD_{0.01}$ level, * – correlation at $LSD_{0.05}$ level, *ns* – non-significant

Table 8 Pearson correlation analysis among the agrotechnical elements (hybrid, plant density, fertilization) and yield and LAI (m^2 m^{-2}) readings of maize (Debrecen, chernozem soil, 2017–2018)

Year		LAI 1	LAI 2	LAI 3	LAI 4	LAI 5
2017	Hybrid	-0.111^{ns}	-0.137^{ns}	-0.164^{ns}	-0.089^{ns}	0.223^{ns}
	Plant density	0.239^{ns}	0.373**	0.230^{ns}	0.151^{ns}	0.361*
	Fertilization	0.593**	0.548**	0.315*	0.233^{ns}	0.752**
	Yield	0.486**	0.578**	0.496**	0.442**	0.659**
2018	Hybrid	0.188^{ns}	0.224^{ns}	0.200^{ns}	-0.036^{ns}	0.224^{ns}
	Plant density	0.093^{ns}	0.166^{ns}	0.391**	0.155^{ns}	0.106^{ns}
	Fertilization	0.470**	0.650**	0.012^{ns}	-0.023^{ns}	0.554**
	Yield	0.230^{ns}	0.349*	0.634**	0.306*	0.370**

** – correlation at $LSD_{0.01}$ level, * – correlation at $LSD_{0.05}$ level, *ns* – non-significant

Table 9 Pearson correlation analysis between the agrotechnical elements and the yield of maize (Debrecen, chernozem soil, 2017–2018)

	Hybrid	Plant density	Fertilization
2017	-0.450^{ns}	0.284^{ns}	0.672**
2018	0.374**	0.517**	0.247^{ns}

** – correlation at $LSD_{0.01}$ level, * – correlation at $LSD_{0.05}$ level, *ns* – non-significant

and LAI values showed moderate correlations at the vegetation periods of maize. In 2017 LAI 1, LAI 2, LAI 5 and fertilization treatments correlation values were r = 0.593**, 0.548** and 0.52**, whereas in 2018, LAI 1, LAI 2 and LAI 5 and fertilization showed correlations of r = 0.470**, 0.650** és 0.554**, respectively. Our results proved a moderate correlation between LAI values and yield in 2017 (r = 0.486**, 0.578**, 0.496**, 0.442, 0.659**), whereas in 2018 the correlation of the same factor was lower (r = 0.230, 0.349*, 0.634**, 0.306*, 0.370**), respectively.

Pearson's correlation analysis proved very weak correlations (r = -0.450–374**) between yields and hybrids in 2017 and 2018 (Table 9), but we had a relatively high correlation (r = 0.672**) between yields and fertilization in 2017. Similar correlation value (r = 0.517**) was between the plant density and the maize yield in 2018.

4 Conclusions

According to our 2017 and 2018 research results, the SPAD and LAI values of maize hybrids showed special dynamics in the vegetation season. SPAD and LAI values were growing from late May until early July (2017), and until early August (2018). Fertilization had a positive effect on SPAD values (Bencze és Futó, 2017, Yu et al., 2010) and the leaf area index (LAI) (Novoa and Loomis, 1981, Oikeh et al.; 1998; Micskey et al., 2012). As opposed to other researchers (Ahmad et al., 2010, Valadabadi and Farahani, 2010), in our experiments maize LAI values were not significantly increased by higher plant density.

Soil with excellent nutrient and water husbandry could significantly reduce the negative effects of the unfavourable weather conditions (high temperature values) of the vegetation season. The chernozem soil in our long-term experiment had excellent natural nutrient supplying capacity, which so we obtained high yields (10.2–11.1 t ha^{-1} in 2017, 9.3–13.6 t ha^{-1} in 2018, respectively) in the control plots. The maximum yield in 2017 was 15.4 t ha^{-1} in Sushi hybrid vs 16.7 t ha^{-1} in Fornad hybrid, whereas the maximum yield values in 2018 were 13.0 t ha^{-1} and 15.9 t ha^{-1}, respectively. Thus, our research results show that maize yield was affected by water supply (Azeez, 2009; Dóka, 2015; Ványiné et al., 2012), hybrid (2009; Körshens, 2006) and plant density (Sárvári and Pepó, 2014). Similarly to other research findings (Berzsenyi, 2010; Berzsenyi et al., 2011, Széll et al., 2010, Vári and Pepó, 2011), fertilization had the most significant effect on maize yield in our long-term experiment. As a result of fertilization, the yield surpluses compared to the control treatment were was 1.8–5.9 t ha^{-1} in 2017, and 0.2–2.6 t ha^{-1} in 2018, depending on hybrids and plant density. We have got a special interactive effect between fertilization and plant density in 2017. In case of no nutrient supply (control treatment), higher plant densities had minimal yield increasement (-0.2–+06 t ha^{-1}) as compared to the yield surpluses in the N_{150} + PK treatment (+0.6–+4.6 t ha^{-1}).

Using Pearson's correlation analysis we could state that fertilization was the main factor which significantly effects on SPAD and LAI values in the different maize phenophases. Correlations between fertilization and SPAD (r = 0.6**-0.8**) as well as LAI were relatively high (r = 0.5**-0.8**). According to our results we stated weak correlation (r = 0.1–0.3) among plant density, hybrid and SPAD and LAI values in both years. As opposed to research findings of Széles (2008), we found relatively weak correlations between SPAD and yield (r = -0.018–424**). We could prove moderate correlations (r = 0.4**–0.8**) between LAI values and yield at certain measurement times, as opposed to the research findings of Oikeh et al. (1998), Bavec and Bavec (2002) and Ma et al. (2005). The Pearson correlation analysis showed that hybrid (-0.450–0.374**) and plant density (0.284–0.517**) had very weak correlations with yield, whereas we could prove a moderate correlation between fertilization and yield (0.247–0.672**).

Acknowledgments

Supported by the GINOP-2.2.1-15-2016-00001 project

5 References

AHMAD, M. et al. (2010). Agro-physiological traits of three maize hybrids as influenced by varying plant density. *The Journal of Animal and Plant Sciences*, 20(1), 34–39

AZEEZ, J.O. (2009). Effect of nitrogen application and weed interference on performance of some tropical maize genotypes in Nigeria. *Pedosphere*,19(5), 654–662. https://doi.org/10.1016/S1002-0160(09)60160-0

BAVEC, F. and BAVEC, M. (2002). Effects of plant population on leaf area index, cob characteristics and grain yield of early maturing maize cultivars (FAO 100–400). *European Journal of Agronomy*, 16(2), 151–159. https://doi.org/10.1016/S1161-0301(01)00126-5

BENCZE, G. and FUTÓ, Z. (2017). Examination of the relationship between the relative chlorophyll content, leaf area index and yield of maize in a monoculture long term experiment. *Recent social and economic processes*, 12(3), 21–28.

BERZSENYI, Z. et al. (2011). Long-term effect of crop production factors on the yield and yield stability of maize (*Zea mays* L.) in different years. *Acta Agronomica Hungarica*, 59(3), 191–200 (2011). https://doi.org/10.1556/AAgr.59.2011.3.1

BERZSENYI, Z. (2010). Use of growth analysis to describe the N fertiliser responses of maize (*Zea mays* L.) hybrids. *Acta agronomica hungarica: a quarterly of the hungarian academy of sciences: an international multidisciplinary journal in agricultural science*, 58(1), 95–101.

CARLONE, M.R. and RUSSEL, W.A. (1987). Response to plant densities and N levels for four maize cultivars from different ears of breeding. *Crop Science*, 27(3), 465–470. https://doi.org/10.2135/cropsci1987.0011183X002700030008x

CARTER, G.A. (1994). Ratios of leaf reflectances in narrow wavebands as indicators of plant stress. *International Journal of Remote Sensing*, 15(3), 697–703. https://doi.org/10.1080/01431169408954109

DÓKA, L. F. (2015). The impact of different crop years on the water balance of the soil in mono- and biculture maize in different crop densities. *Crop production*, 64(2), 5–28.

D'HAENE, K. et al. (2007). Nitrogen and phosphorus balances of Hungarian farms. *European Journal of Agronomy*, 26(3), 224–234. https://doi.org/10.1016/j.eja.2006.10.005

ESECHIE, H. A. (1992). Effect of planting density on growth and yield of irrigated maize (*Zea mays*) in the Batinah Coast region of Oman. *The Journal of Agricultural Science*, 119(2), 165–169. https://doi.org/10.1017/S0021859600014076

FULTON, J.M. (1970) Relationship among soil moisture stress, plant populations, row spacing and yield of maize. *Canadian Journal of Plant Science*, 50(1), 31–38. https://doi.org/10.4141/cjps70-005

HAEGELE, J.W. et al. (2014). Row arrangement, phosphorous fertility, and hybrid contributions to managing increased plant density of maize. *Agronomy Journal*, 106(5), 1838–1846. https://doi.org/10.2134/cs2014-47-6-11

HAWKINS, T.S. et al. (2009). Modeling the Relationship between Extractable Chlorophyll and SPAD-502 Readings for Endangered Plant Species Research. *Journal for Nature Conservation*, 17(2), 123–127. https://doi.org/10.1016/j.jnc.2008.12.007

KÖRSCHENS, M. (2006). The importance of long-term experiments for soil science and environmental research – a review. *Plant Soil Environ*., 52(special issue), 1–8.

MA, B.L. et al. (2005). Comparison of Crop-Based Indicators with Soil Nitrate Test for Maize Nitrogen Requirement. *Agron. Journal*, 97(2), 462–471. https://doi.org/10.2134/agronj2005.0462

MARTINEZ, D.E. and GUIAMET, J.J. (2004) Distortion of the SPAD 502 chlorophyll meter readings by changes in irradiance and leaf water status. *Agronomie*, 24(1), 41–46. https://doi.org/10.1051/agro:2003060

MICSKEI, GY. et al. (2012). Relationships between maize yield and growth parameters in a long-term fertilization experiment. *Acta Agronomica Hungarica*, 60(3), 209–219. https://doi.org/10.1556/AAgr.60.2012.3.4

NAGY, J. (2010). The present and the future of cultivation of maize. *Crop Production*, 59(3), 85–111. https://doi.org/10.1556/Novenyterm.59.2010.3.6

NOVOA, R. and LOOMIS, R.S. (1981). Nitrogen and Plant Production. *Plant and Soil*, 58(1–3), 177–204. https://doi.org/10.1007/BF02180053

OIKEH, S.O. et al. (1998). Nitrogen Fertilizer Management Effects on Maize Grain Quality in the West African Moist Savanna. *Crop Science*, 38(4), 1056–1061. https://doi.org/10.2135/cropsci1998.0011183X003800040029x

PEPÓ, P. and MURÁNYI, E. (2014) Plant density impact on grain yield of maize (*Zea mays* L.) hybrids on chernozem soil of the Eastern Hungary. *Columella-Journal of Agricultural and Environmental Sciences*, 1(2), 95–100. https://doi.org/10.18380/SZIE.COLUM.2014.1.2.95

PEPÓ, P. and MURÁNYI, E. (2015). Examinations of cultivated area of different maize (*Zea mays* L.) hybrids. *Crop production*, 64(2), 1–17.

REMISON, S.U. and LUCAS, E.O. (1982). Effects of planting density on Leaf Area and Productivity of Two Maize Cultivars in Nigeria. *Experimental Agriculture*, 18(1) 93–100. https://doi.org/10.1017/S0014479700013478

RUSSEL, W.A. (1991). Genetic improvement of maize yields. *Adv. Agron*., 46(1), 245–298. https://doi.org/10.1016/S0065-2113(08)60582-9

SÁRVÁRI, M. and PEPÓ, P. (2014). Effect of Production Factors on Maize Yield and Yield Stability. *Cereal Research Communications*, 42(4), pp. 710–720. https://doi.org/10.1556/CRC.2014.0009

SU, Y.J. et al. (2012). Effects of planting density on growth and yield of summer maize Xundan 28. *Acta Agriculturae Jiangxi*, 24(6), 49–50, 53.

SZÉLES, A. (2008). The effect of crop year and fertilization on the interaction between the spad value and yield of maize (*Zea mays* l.) within non-irrigated conditions. *Cereal Research Communications*, 36(Supll. 5), 1367–1370.

SZÉLES, A. et al. (2011) Effect of N fertilisation on the chlorophyll content and grain yield of maize in different crop years. *Crop Production*, 60(Suppl.), 161–164.

SZÉLL, E. et al. (2010). Results of maize fertilization experiments on 4 different soil type. *Crop Production*, 59(4), 41–61.

TAJUL, M.I. et al. (2013). Influence of plant population and nitrogen-fertilizer at various levels on growth and growth efficiency of maize. *The Scientific World Journal*, 2013(1), 1–9. https://doi.org/10.1155/2013/193018

VALADABADI, S.A. and FARAHANI, H.A. (2010). Effects of planting density and pattern on physiological growth indices in maize (*Zea mays* L.) under nitrogenous fertilizer application. *Journal of Agricultural Extension and Rural Development*, 2(3), 40–47.

VÁNYINÉ, Sz. A. et al. (2012). Irrigation and nitrogen effects on the leaf chlorophyll content and grain yield of maize in different crop years. *Agricultural water management*, 107(1), 133–144.

VÁRI, E. and PEPÓ, P. (2011). Effect of agro-technical factors on the agronomy properties in long term experiment. *Crop Production*, 60(4), 115–130. https://doi.org/10.1556/Novenyterm.60.2011.4.6

YU, H. et al. (2010). Evaluation of SPAD and Dualex for In-Season Maize Nitrogen Status Estimation. *Acta Agronomica Sinica*, 36(5), 840–847. https://doi.org/10.3724/SP.J.1006.2010.00840T

Acta fytotechn zootechn, 23, 2020(1): 15–23
http://www.acta.fapz.uniag.sk

Original Paper

Morphological characteristics as a key attribute for a successful determination of selected *Cotoneaster* species

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Article Details: Received: 2019-12-17 | Accepted: 2019-01-24 | Available online: 2020-03-31

https://doi.org/10.15414/afz.2020.23.01.15-23

In this paper, morphological features, such as the number of pyrenes in pome and the number of pomes in infructescence, were used for determination of closely related tetraploid *Cotoneaster* species. Samples were collected from various localities in the Western Carpathians. The collection of samples, designed for counting of pyrenes in pome, included 2353 pomes of >130 individuals. Number of pyrenes in pome ranged from 1 to 5. Statistical analysis revealed a significant difference in pyrenes per pome mean values between *C. integerrimus* (3.01), *C. melanocarpus* agg. (2.46; including *C. matrensis*) and *C. tomentosus* (3.93). The collection of samples, designed for counting of pomes in infructescence, included 1019 infructescences of 141 individuals. Number of pomes in infructescence ranged from 1 to 5. Statistical analysis also revealed a significant difference in pomes per infructescence mean values between *C. integerrimus* (1.14) and *C. melanocarpus* agg. (1.54; including *C. matrensis*), and between *C. integerrimus* and *C. tomentosus* (1.50).

Keywords: pyrenes, pomes, infructescence, *Cotoneaster*, Western Carpathians

1 Introduction

According to the latest research, the *Cotoneaster* genus belongs to *Rosaceae* family, *Spiraeoideae* subfamily and tribe *Pyreae* (Potter et al., 2007). Overall, it includes approximately 300 species (Bartish et al., 2001). Many species, included in *Cotoneaster* genus, are cultivated as ornamental plants (Dickoré and Kasperek, 2010). Latest research also confirmed a presence of phenolic compounds and flavonoids in *Cotoneaster integerrimus*. Therefore, *C. integerrimus* has a great potential in health promotion. Novel food ingredients and medication could be developed from its twigs and fruit (Uysal et al., 2016; Kicel et al., 2019).

A total of 81 *Cotoneaster* species were recorded in Europe – 17 native and 64 alien species (Kurtto et al., 2013). The western part of Carpathian Mountains includes 3 native *Cotoneaster* species – *Cotoneaster integerrimus* Medik., *Cotoneaster melanocarpus* (Bunge) Fisch. et C. A. Mey and *Cotoneaster tomentosus* Lindl. (Marhold and Hindák 1998). Some authors distinguish *Cotoneaster matrensis* Domokos from *Cotoneaster melanocarpus* (Bunge) Fisch. et C. A. Mey. However, in this case, further research is needed (Baranec, 1992). The occurrence of a hybrid species *Cotoneaster alaunicus* × *integerrimus* in the area of the Low Tatras Mts., was also recorded (Baranec and Eliáš ml., 2004).

Cotoneaster tomentosus individuals usually grow in the community of *Prunion spinosae* and *Calamagrostion variae* from sub-montane to montane level. *Cotoneaster integerrimus* individuals grow in the community of *Seslerio-Festucion duriusculae* and *Prunion fruticosae* from colline to alpine level. *Cotoneaster melanocarpus* individuals typically grow in the community of *Seslerio-Festucion duriusculae* and *Prunion spinosae* from colline to montane level (Baranec, 1992).

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The classification of taxa included in *Cotoneaster* genus is relatively problematic. Morphological features, such as colour of fruit and bark or shape and size of leaves, are not always reliable attributes. A pome is a type of fruit specific to subfamily formerly known as *Maloideae*. In *Cotoneaster*, the ovary develops into the pyrene (Rohrer, 1991). The total amount of pyrenes in a single pome is variable. The number of pyrenes in pome and the number of pomes in infructescence are the most important morphological features for determination of previously mentioned *Cotoneaster* species. Other features include the presence of trichomes on leaves and pomes, and the colour of pomes (Baranec, 1992). Since we believe, that there is a lack of information and available data from contemporary articles, we decided to prove, whether these morphological characteristics are reliable for identification of selected *Cotoneaster* species. We also wanted to prove, whether it is possible to rely only on these selected characteristics. And last but not least, it should be mentioned, that such a large-scale study in the Western Carpathians has been lacking so far.

2 Material and methods

The majority of collected individuals grew in dry habitat with a permanent sunlight, mainly on grassy steppes or cliff edges. Some of the individuals grew in forest in the community of other shrubs and larger trees. Each population was relatively small and it included 1–5 individuals, rarely 6–10(–15). Pomes and infructescences were collected from various localities in four countries situated in the Western Carpathians – the Czech Republic, Hungary, Poland and Slovakia (Figure 1, 2). Infructescences were calculated in the field and from herbarium specimens stored in the herbarium NI (Herbarium Collection of Slovak University of Agriculture in Nitra). The list of localities selected for counting of pyrenes in pome and for counting of pomes in infructescence is listed in Appendix 1 and 2, respectively. Individuals were assigned to different species by the colour of pomes and the presence of trichomes according to determination key of Baranec (1992).

Samples were divided in two groups, one designed for counting of pyrenes in pome and the other for counting

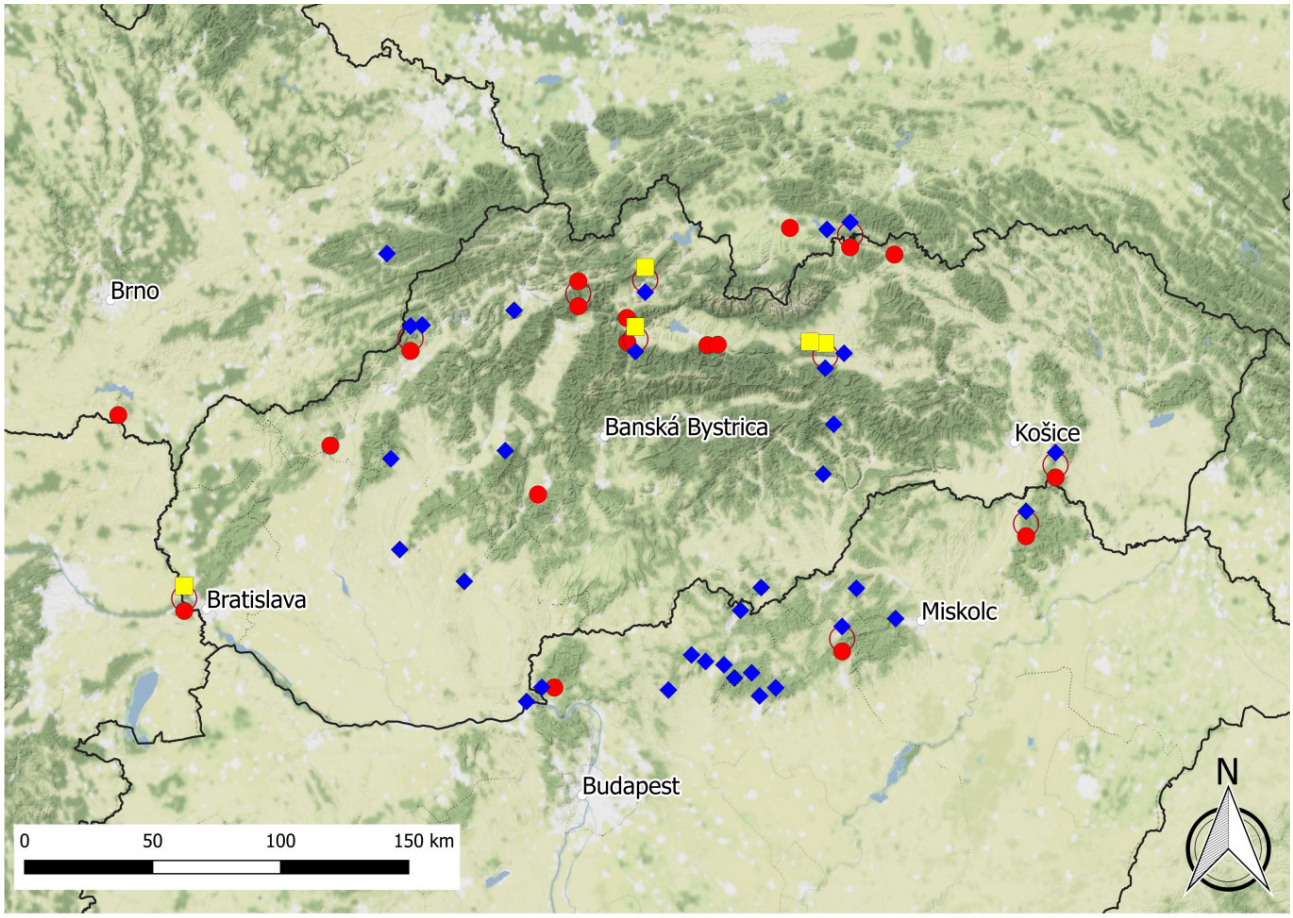

Figure 1 Localities within the Western Carpathians selected for counting of pyrenes in pome: red circles – *Cotoneaster integerrimus*, blue diamonds – *C. melanocarpus* agg., yellow squares – *C. tomentosus*. Background terrain layer is from Stamen Design with data by OpenStreetMap.

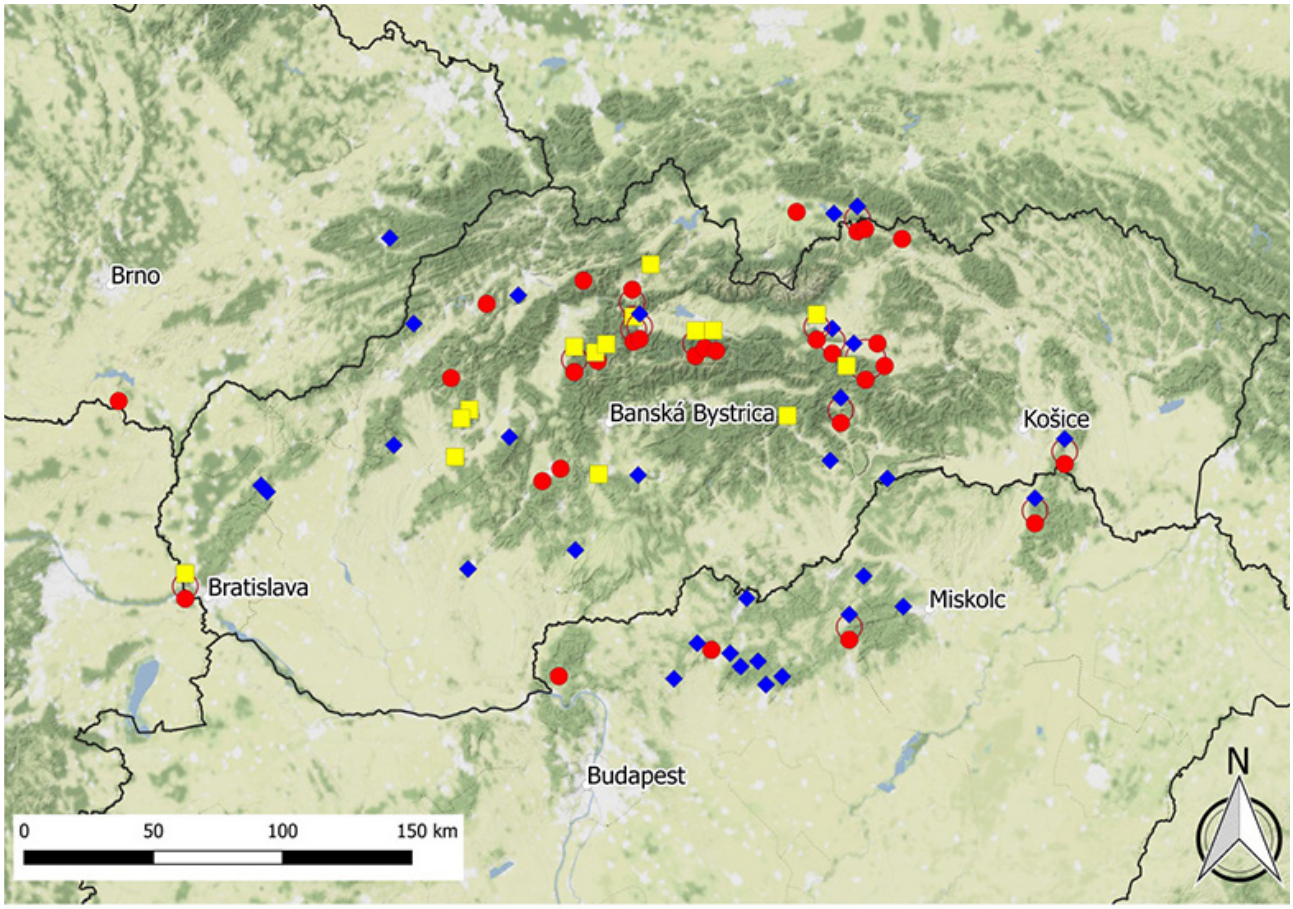

Figure 2 Localities within the Western Carpathians selected for counting of pomes in infructescence: red circles – *Cotoneaster integerrimus*, blue diamonds – *C. melanocarpus* agg., yellow squares – *C. tomentosus*. Background terrain layer is from Stamen Design with data by OpenStreetMap.

of pomes in infructescence. Overall, we collected 3–4 twigs per individual. Herbarium specimens from NI herbarium (Herbarium Collection of Slovak University of Agriculture in Nitra) were also used to increase the total amount of infructescences. The collection of samples, designed for counting of pyrenes in pome, included 2,353 pomes of >130 individuals. The collection of samples, designed for counting of pomes in infructescence, included 1019 infructescences of 141 individuals. The data were collected and further statistically analysed by Microsoft® Excel 2010 and STATISTICA, version 10 (StatSoft, Inc., 2011) data analysis software system (Tukey's HSD test). Values were calculated as a mean per species.

Herbarium abbreviations are according to Thiers (2019). Nomenclature of flowering plants follows Marhold and Hindák (1998). The map was created by the software QGIS, version 3.2 (QGIS Development Team, 2018) with QuickMapServices plug-in and terrain background layer from Stamen Design with data by OpenStreetMap.

3 Results and discussion

Statistical analysis (Tukey's HSD test; $\alpha = 0.01$) revealed a significant difference in pyrenes per pome mean values between *C. integerrimus* (3.01), *C. melanocarpus* agg. (2.46; including *C. matrensis*) and *C. tomentosus* (3.93). These results along with standard deviation are listed in Table 1.

Table 1 A difference in pyrenes per pome mean values between selected species

Species	Mean per species	Standard deviation
C. integerrimus	3.01	0.67
C. melanocarpus agg.	2.46	0.60
C. tomentosus	3.93	0.73

Table 2 Number of pyrenes in pome presented in this paper, compared with other published data

Species	This paper	Baranec (1992)	Kovanda (1992)	Bartha (2009)
C. integerrimus	(2–)3(–4)	3(–4)	(2–)3(–4)	2–3(–5)
C. melanocarpus agg.	2–3	2(–3)	2(–3)	2(–4)
C. tomentosus	3–5	3–5	–	3–5

Table 3 A difference in pomes per infructescence mean values between selected species

Species	Mean per species	Standard deviation
C. integerrimus	1.14	0.38
C. melanocarpus agg.	1.54	0.71
C. tomentosus	1.50	0.74

Table 4 Number of pomes in infructescence presented in this paper, compared with other published data

Species	This paper	Baranec (1992)	Kovanda (1992)	Bartha (2009)
C. integerrimus	1(–2–3)	1(–2)	–	–
C. melanocarpus agg.	1–2(–3–5)	(1–)2–5	–	–
C. tomentosus	1–2(–3–4)	3–7	–	–

Results, obtained by counting of pyrenes in pome of selected *Cotoneaster* species, compared with other published data, are listed in Table 2.

The majority of *C. integerrimus* pomes had 3 pyrenes (55.90 %), 2 pyrenes were present in 21.57 % of pomes and 4 pyrenes were present in 22.25 % of pomes. Both, 1 and 5 pyrenes in pomes represented only 0.14 % of the collection. Number of pyrenes in *C. melanocarpus* agg. pomes ranged mainly from 2 to 3. Pomes with 2 pyrenes represented 56.01 % of the collection and pomes with 3 pyrenes represented 38.39 % of the collection. Pomes with 1 pyrene (1.32 %), 4 pyrenes (4.22 %) and 5 pyrenes (0.06 %) were also present in the collection. Number of pyrenes in *C. tomentosus* pomes ranged from 3 to

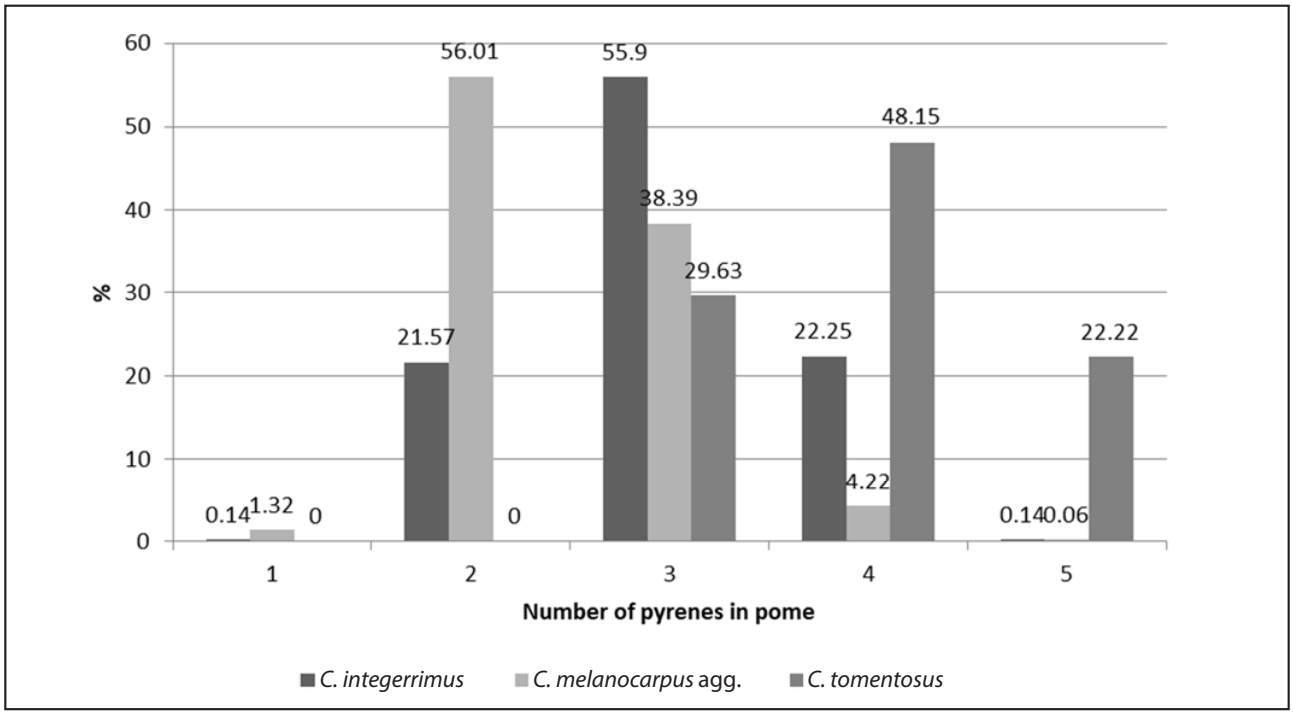

Figure 3 Number of pyrenes in pome of selected *Cotoneaster* species

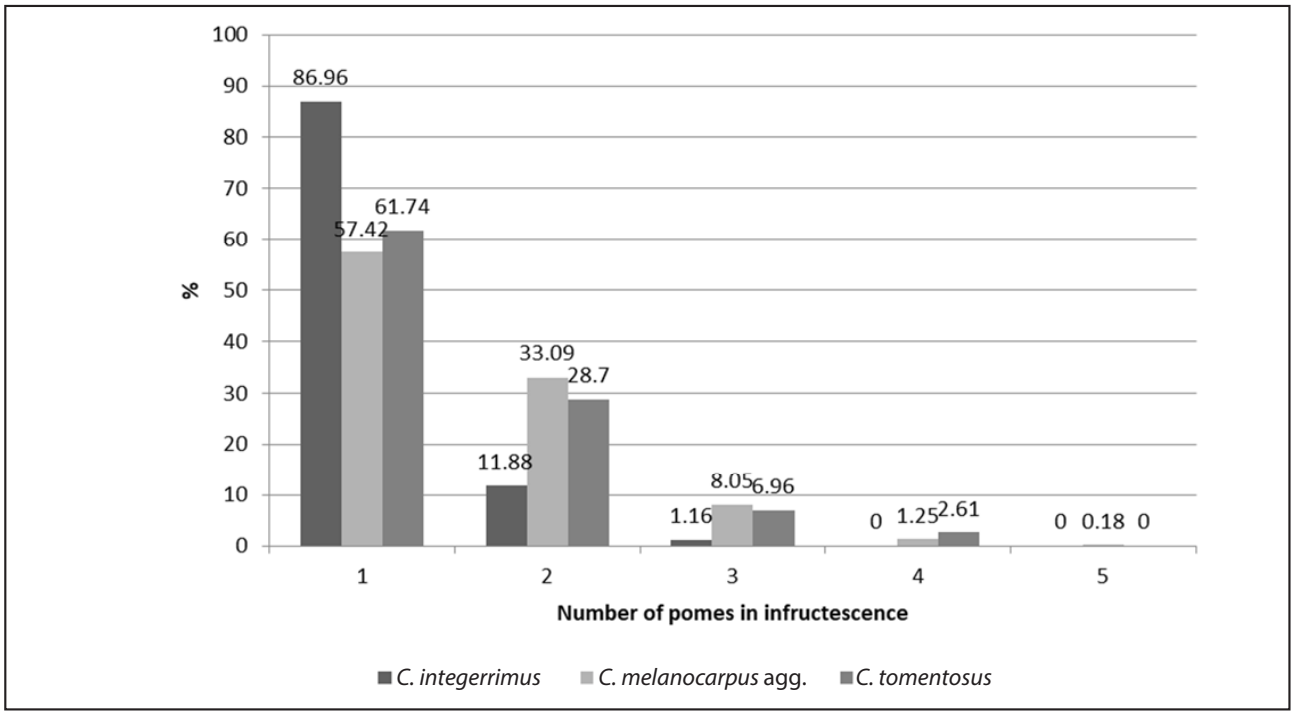

Figure 4 Number of pomes in infructescence of selected *Cotoneaster* species

5. Pomes with 3 pyrenes represented 29.63 % of the collection, 4 pyrenes were present in 48.15 % of pomes and 5 pyrenes were present in 22.22 % of pomes.

A significant difference (Tukey's HSD test; $\alpha = 0.01$) in pomes per infructescence mean values was also recorded between *C. integerrimus* (1.14) and *C. melanocarpus* agg. (1.54; including *C. matrensis*), and between *C. integerrimus* and *C. tomentosus* (1.50). There was no significant difference in pomes per infructescence mean values between *C. melanocarpus* agg. and *C. tomentosus*. However, the determination of these two species is not problematic. *C. tomentosus* individuals have hairy and red pomes while pomes of *C. melanocarpus* agg. individuals are glabrous and dark red-violet or black (Baranec, 1992; Bartha, 2009). These results along with standard deviation are listed in Table 3.

We also compared results from this paper, obtained by counting of pomes in infructescence, with other published data. These results are listed in Table 4.

The vast majority of *C. integerrimus* pomes grew one by one and did not form infructescences. Single pome represented 86.96 % of the collection. Infructescences with 2 pomes represented 11.88 % and infructescences with 3 pomes represented only 1.16 % of the collection. Number of pomes in infructescences of *C. melanocarpus* agg. ranged mainly from 1 to 2. Single pome represented 57.42 % of the collection and infructescences with 2 pomes represented 33.09 % of the collection. Infructescences with 3 pomes (8.05 %), 4 pomes (1.25 %) and 5 pomes (0.18 %) were also present. Number of pomes in infructescences of *C. tomentosus* ranged mainly from 1 to 2. Single pome represented 61.74 % of the collection. Infructescences with 2 pomes represented 28.70 % of the collection. Infructescences with 3 pomes (6.96 %) and 4 pomes (2.61 %) were also present.

Nowadays, modern methods, such as flow cytometry method, are very important in taxonomy of plants (Hajrudinović et al., 2015; Macková et al., 2017; Žabka et al., 2018), which is a big step forward in systematic botany. These methods in combination with classic morphological and population biology research (Rohrer et al., 1991; Ďurišová, et al., 2016; Ďurišová and Baranec, 2016) give us the opportunity to solve many different problems in complicated groups, like *Rosaceae*.

The purpose of these morphological analyses was to determine a difference between two closely related tetraploid species *C. integerrimus* and *C. melanocarpus* agg.) (Rothleutner et al., 2016), since *C. tomentosus* is considered to be pentaploid (Macková et al., 2018). Flow cytometry method, used in the last study, revealed no significant difference in the genome size of tetraploid *C. integerrimus* and *C. melanocarpus* agg., including *C. matrensis* (Kšiňan et al., 2019). Therefore, we decided to prove, if these morphological characteristics are reliable for identification of selected tetraploid *Cotoneaster* species.

4 Conclusion

Results from this paper proved, that even in the era of modern cytological and molecular methods, classic morphological methods are still relevant in the field of botany. However, we do not recommend relying only on morphological characteristics, because in some cases a determination of problematic closely related species can be more complicated. We suggest a combination of available methods, from classic morphological research to modern methods, or at least a combination of several morphological characteristics.

Acknowledgement

We thank to Gergely Király (Sopron), Zoltán Barina (Budapest) and András Schmotzer (Eger) for providing data of *Cotoneaster* localities in Hungary. We also thank Róbert Šuvada (Rožňava) for creating maps of sample collection. Work was supported by project VEGA No 1/0047/19 and AgroBioTech Research Centre built in accordance with the project Building "AgroBioTech" Research Centre ITMS 26220220180.

References

BARANEC, T. (1992). *Cotoneaster* Medicus. In Bertová, L. (Ed.) *Flora of Slovakia IV/3*. Bratislava: Veda (pp. 452–462). In Slovak.

BARANEC, T. and ELIÁŠ ml., P. (2004). Taxonomy and chorology of genus *Cotoneaster* Medicus in the territory of Nízke Tatry Mts. In Jasík, M. et al. (Eds.) *Nature of Low Tatras*. Banská Bystrica: State Nature Conservancy of the Slovak Republic, The Administration of the National Park Low Tatras (pp. 101–106). In Slovak.

BARTHA, D. (2009). *Cotoneaster* Medik. In Király, G. (Ed.) *New Hungarian Herbal. The Vascular Plants of Hungary. Identification key.* Jósvafő: Aggteleki Nemzeti Park Igazgatóság (pp. 229). In Hungarian.

BARTISH, I. V. et al. (2001). RAPD analysis of interspecific relationships in presumably apomictic *Cotoneaster* species. *Euphytica*, 120(2), 273–280.

DICKORÉ, W. B. and KASPEREK, G. (2010). Species of *Cotoneaster* (*Rosaceae*, *Maloideae*) indigenous to, naturalising or commonly cultivated in Central Europe. *Willdenowia*, 40, 13–45.

ĎURIŠOVÁ, Ľ. and BARANEC, T. (2016). The evidence of apomixis in *Cotoneaster matrensis*. In BAČKOR, M. and VALACHOVIČ, M. (Eds.) *Bulletin of Slovak Botanical Society 38, Supplement 1*. Bratislava: Slovak Botanical Society (pp. 25–33). In Slovak.

ĎURIŠOVÁ, Ľ. et al. (2016). Population and reproductive biology of *Cotoneaster matrensis* Domokos. In BRINDZA, J. and KLYMENKO, S. (Eds.) *Agrobiodiversity for improving nutrition, health and life quality, PART.* Nitra: Slovak University of Agriculture (pp. 134–138). In Slovak.

HAJRUDINOVIĆ, A. et al. (2015). When sexual meets apomict: genome size, ploidy level and reproductive mode variation of *Sorbus aria* s.l. and *S. austriaca* (*Rosaceae*) in Bosnia and Herzegovina. *Annals of Botany*, 116(2), 301–312.

KICEL, A. et al. (2019). Polyphenolic profile, antioxidant activity, and pro-inflammatory enzymes inhibition of leaves, flowers, bark and fruits of *Cotoneaster integerrimus*: A comparative study. *Phytochemistry Letters*, 30, 349–355.

KOVANDA, M. (1992). *Cotoneaster* Med. In HEJNÝ, S. et al. (Eds.) *Flora of the Czech Republic 3*. Praha: Academia (pp. 485–487). In Czech.

KŠIŇAN, S. et al. (2019). Identification of autochthonous West-Carpathian *Cotoneaster* species by using a flow cytometry method. In ELIÁŠ ml., P. (Ed.) *Book of Abstracts of 11th Congress of Slovak Botanical Society*. Nitra: Slovak Botanical Society & Slovak University of Agriculture. In Slovak.

KURTTO, A. et al. (2013). *Atlas Florae Europaeae. Distribution of Vascular Plants in Europe. 16. Rosaceae* (*Cydonia* to *Prunus*, excl. Sorbus). Helsinki: The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo.

MACKOVÁ, L. et al. (2017). Hybridization success is largely limited to homoploid *Prunus* hybrids: a multidisciplinary approach. *Plant Systematics and Evolution*, 303(4), 481–495.

MACKOVÁ, L. et al. (2018). Insight into the cytotype and reproductive puzzle of Western Carpathian *Cotoneaster integerrimus*. In ŠTĚPÁNKOVÁ, R. (Ed.) *Systematics, ecology and floristics in the light of flow cytometry*. Praha: Czech Botanical Society. In Czech.

MARHOLD, K. and HINDÁK, F. (1998). *Checklist of non-vascular and vascular plants of Slovakia*. Bratislava: Veda.

POTTER, D. et al. (2007). Phylogeny and classification of *Rosaceae*. *Plant Systematics and Evolution*, 266, 5–43.

QGIS Development Team (2018). *QGIS Geographic Information System.* Open Source Geospatial Foundation Project. Retrieved December 17, 2019 from https://www.qgis.org/en/site/

ROHRER, J. R. et al. (1991). Variation in Structure Among Fruits of *Maloideae* (*Rosaceae*). *American Journal of Botany*, 78(12), 1617–1635.

ROTHLEUTNER, J. J. et al. (2016). Ploidy Levels, Relative Genome Sizes, and Base Pair Composition in *Cotoneaster*. *Journal of the American Society for Horticultural Science*, 141(5), 457–466.

THIERS, B. (2019). *Index Herbariorum: A global directory of public herbaria and associated staff.* New York Botanical Garden's Virtual Herbarium, New York. Retrieved December 17, 2019 from http://sweetgum.nybg.org/ih/

UYSAL, A. et al. (2016). Chemical and biological insights on *Cotoneaster integerrimus*: A new (-)- epicatechin source for food and medicinal applications. *Phytomedicine*, 23(10), 979–988.

ŽABKA, M. et al. (2018). Genome size and ploidy level among wild and cultivated *Prunus* taxa in Slovakia. *Biologia*, 73(2), 121–128.

Appendix 1 List of localities selected for counting of pyrenes in pome (sorted by different species, by country and alphabetically. Sites are listed in order: geomorphological unit name, village, local site name, geographic coordinates)

Cotoneaster integerrimus:

Czech Republic (1 locality)

Pavlovské vrchy hills, Mikulov, Svatý kopeček hill, 48° 48' 23.2" N 16° 38' 48.3" E

Hungary (3 localities)
Börzsönyi Mts., Kóspallag, Só-hegy, 47° 52′ 28.0″ N 18° 53′ 29.4″ E
Bükk Mts., Bélapatfalva, Bél-kö hill, 48° 02′ 33.5″ N 20° 22′ 33.2″ E
Zemplényi-hegysék hills, Hejce, Sólyom-kő hill, 48° 26′ 12.7″ N 21° 19′ 09.5″ E
Poland (2 localities)
Pieniński Pas Skałkowy hills, Gronków, Skalka Cisowa hill, 49° 26′ 13.1″ N 20° 06′ 13.6″ E
Pieniny Mts., Trzy Korony, Okraglica 49° 24′50.4″N 20° 24′49.8″E
Slovakia (12 localities)
Biele Karpaty Mts., Vršatské Podhradie, ruins of the Vršatec castle, 49° 03′ 55.9″ N 18° 09′ 04.5″ E
Chočské vrchy Mts., Studničná, Sedem kostolov site, 49° 08′ 04.8″ N 19° 15′ 52.5″ E
Ľubovnianska vrchovina hills, Jarabina, rocks NW from the village, 49° 20′ 51.6″ N 20° 38′ 30.4″ E
Malá Fatra Mts., Terchová, Baraniarky hill, 49° 13′ 23.0″ N 19° 00′ 42.0″ E
Malá Fatra Mts., Terchová, Kraviarske hill, 49° 12′ 34.2″ N 19° 01′ 00.5″ E
Malé Karpaty Mts., Čachtice, Veľký Plešivec hill, 48° 42′ 11.1″ N 17° 44′ 17.8″ E
Malé Karpaty Mts., Devín, the foothills of the Devínska Kobyla hills, 48° 10′ 50.2″ N 16° 59′ 15.3″ E
Nízke Tatry Mts., Liptovský Hrádok, Skalka site, 49° 02′ 39.6″ N 19° 43′ 56.6″ E
Nízke Tatry Mts., Liptovský Ján, near the cemetery, 49° 02′ 35.6″ N 19° 40′ 45.0″ E
Slanské vrchy Mts., Slanec, slopes under the ruins of the Slanec castle, 48° 38′ 12.99″ N 21° 28′ 15.66″ E
Štiavnické vrchy Mts., Lehôtka pod Brehmi, Szabóova skala site, 48° 32′ 12.2″ N 18° 48′ 24.5″ E
Veľká Fatra Mts., Ružomberok, Malinô Brdo hill, 49° 03′ 09.1″ N 19° 16′ 02.9″ E

Cotoneaster melanocarpus agg.:
Czech Republic (1 locality)
Hostýnsko-vsetínská hornatina hills, Vsetín, Valova skála rock, 49° 21′ 02.9″ N 18° 01′ 47.0″ E
Hungary (14 localities)
Börzsöny Mts., Letkés, Nagy Galla hill, 47° 52′ 31.5″ N 18° 49′ 37.4″ E
Bükk Mts., Alsóhámor, Molnár-szikla hill, 48° 06′ 42.8″ N 20° 38′ 52.2″ E
Bükk Mts., Bélapatfalva, Bél-kö hill, 48° 02′ 28.7″ N 20° 22′ 07.5″ E
Bükk Mts., Uppony, SW slopes of Kalica-tetö, 48° 12′ 57.0″ N 20° 26′ 45.3″ E
Cserhát Mts., Bér, Nagy hegy hill, 47° 51′ 56.65″ N 19° 28′ 42.25″ E
Cserhát Mts., Mátraszőlős, Függőkő, 47° 57′ 51.63″ N 19° 40′ 10.66″ E
Cserhát Mts., Hollókő; Szár hegy hill, 47° 59′ 12.5″ N 19° 35′ 50.3″ E
Karancs-Medves hills, Salgóbánya, Kis Salgó (Boszorkánykő), 48° 8′ 24.40″ N 19° 50′ 57.86″ E
Mátra Mts., Mátraálmás, ruins of the Gyala-vár castle, 47° 55′ 31.40″ N 19° 54′ 24.65″ E
Mátra Mts., Mátraháza, Sas-kő hill, 47° 52′ 24.63″ N 20° 1′ 50.12″ E
Mátra Mts., Mátrakerestes, Bárány-kö hill, 47° 54′ 27.00″ N 19° 49′ 6.17″ E
Mátra Mts., Sástó, 47° 50′ 44.06″ N 19° 56′ 50.48″ E
Mátra Mts., Tar, Fehérkő-bánya stone pit, 47° 57′ 9.89″ N 19° 45′ 52.50″ E
Zemplényi-hegysék hills, Hejce, Sólyom-kő hill, 48° 26′ 12.7″ N 21° 19′ 09.5″ E
Poland (2 localities)
Pieniny Mts., Falsztyn, Zielone Skałki hills, 49° 25′ 55.5″ N 20° 17′ 40.5″ E
Pieniny Mts., Trzy Korony, Okraglica 49° 24′50.4″N 20° 24′49.8″E
Slovakia (16 localities)
Biele Karpaty Mts., Lednica, ruins of the Lednický hrad castle, 49° 06′ 38.0″ N 18° 12′ 40.8″ E
Biele Karpaty Mts., Vršatské Podhradie, ruins of the Vršatec castle, 49° 03′ 55.9″ N 18° 09′ 04.5″ E
Burda hills, Kamenica nad Hronom, Skaly site, 47° 49′ 34.00″ N 18° 44′ 53.68″ E
Cerová vrchovina hills, Hajnáčka, ruins of the Hajnáčka castle, 48° 13′ 06.5″ N 19° 57′ 20.4″ E
Muránska planina hills, Brdárka, Malý Radzim hill, 48° 46′ 31.1″ N 20° 19′ 44.4″ E
Nízke Tatry Mts., Hranovnica, Hranovnická dubina Nature Reserve, 49° 00′ 26.8″ N 20° 17′ 09.0″ E
Oravská vrchovina hills, Oravský Podzámok, Oravský hrad castle, 49° 15′ 46.9″ N 19° 21′ 32.9″ E
Podtatranská kotlina basin, Primovce, Primovské skalky Nature Reserve, 49° 00′ 56.2″ N 20° 22′ 57.5″ E
Podtatranská kotlina basin, Ružomberok, memorial of the Slovak National Uprising, 49° 03′ 47.6″ N 19° 18′ 30.6″ E
Podunajská nížina lowland, Čifáre, Podskalie site, 48° 14′ 22.5″ N 18° 25′ 40.9″ E
Považský Inovec Mts., Podhradie, ruins of the Topoľčiansky hrad castle, 48° 39′ 29.6″ N 18° 02′ 59.8″ E
Slanské vrchy Mts., Slanec, slopes under the ruins of the Slanec castle, 48° 38′ 12.99″ N 21° 28′ 15.66″ E
Slovenský kras karst, Gemerské Teplice, Stráň hill, 48° 36′ 23.3″ N 20° 16′ 29.2″ E
Strážovské vrchy Mts., Lietava, ruins of the Lietavský hrad castle, 49° 09′ 36.8″ N 18° 41′ 03.1″ E
Tribeč Mts., Nitra, Zoborská lesostep Nature Reserve, 48° 20′ 54.8″ N 18° 05′ 43.5″ E

Vtáčnik Mts., Podhradie, ruins of the Sivý Kameň castle, 48° 41' 08.8" N 18° 38' 19.8" E

Cotoneaster tomentosus:
Slovakia (5 localities)
Malé Karpaty Mts., Devín, Devínska Kobyla hills, 48° 10' 54.9" N 16° 59' 04.5" E
Nízke Tatry Mts., Hranovnica, Hranovnická dubina Nature Reserve, 49° 00' 26.8" N 20° 17' 09.0" E
Oravská vrchovina hills, Oravský Podzámok, Oravský hrad castle, 49° 15' 46.9" N 19° 21' 32.9" E
Podtatranská kotlina basin, Ružomberok, memorial of the Slovak National Uprising, 49° 03' 47.6" N 19° 18' 30.6" E
Nízke Tatry Mts., Svit, near the cemetery, 49° 03' 15.5" N 20° 12' 22.2" E

Appendix 2 List of localities selected for counting of pomes in infructescence (sorted by different species, by country and alphabetically. Sites are listed in order: geomorphological unit name, village, local site name, geographic coordinates, collector of herbarium specimen, year of collection)

Cotoneaster integerrimus:
Czech Republic (1 locality)
Pavlovské vrchy hills, Mikulov, Svatý kopeček hill, 48° 48' 23.2" N 16° 38' 48.3" E

Hungary (4 localities)
Börzsönyi Mts., Kóspallag, Só-hegy, 47° 52' 28.0" N 18° 53' 29.4" E
Bükk Mts., Bélapatfalva, Bél-kö hill, 48° 02' 33.5" N 20° 22' 33.2" E
Cserhát Mts., Mátraszőlős, Függőkő, 47° 57' 51.63" N 19° 40' 10.66" E
Zemplényi-hegysék, Hejce, Sólyom-kő, 48° 26' 12.7" N 21° 19' 09.5" E

Poland (2 localities)
Pieniński Pas Skałkowy hills, Gronków, Skalka Cisowa hill, 49° 26' 13.1" N 20° 06' 13.6" E
Pieniny Mts., Trzy Korony, Okraglica 49° 24'50.4"N 20° 24'49.8"E

Slovakia (24 localities)
Chočské vrchy Mts., Studničná, Sedem kostolov site, 49° 08' 04.8" N 19° 15' 52.5" E
Chočské vrchy Mts., Studničná, forest edge, 49° 08' 21.1" N 19° 15' 58.5" E, Eliáš jun. 2015 NI;
Ľubovnianska vrchovina hills, Jarabina, rocks NW from the village, 49° 20' 51.6" N 20° 38' 30.4" E
Malé Karpaty Mts., Devín, the foothills of the Devínska Kobyla hills, 48° 10' 50.2" N 16° 59' 15.3" E
Malá Fatra Mts., Terchová, Kraviarske hill, 49° 12' 34.2" N 19° 01' 00.5" E
Muránska planina hills, Brdárka, Malý Radzim hill, 48° 46' 31.1" N 20° 19' 44.4" E
Nízke Tatry Mts., Demänová, Siná hill, 48° 59' 58.9" N 19° 35' 15.7" E, Talapka 1996 NI
Nízke Tatry Mts., Hranovnica, Hranovnická dubina Nature Reserve, 49° 00' 26.8" N 20° 17' 09.0" E
Nízke Tatry Mts., Liptovský Ján, Krakova hoľa hill, 48° 59' 00.5" N 19° 38' 01.5" E, Talapka 1996 NI
Nízke Tatry Mts., Liptovský Ján, Ohnište hill, 48° 58' 28.7" N 19° 41' 34.7" E, Tóth 2004 NI
Nízke Tatry Mts., Svit, near the cemetery, 49° 03' 15.5" N 20° 12' 22.2" E
Pieniny Mts., Haligovce, Haligovské skaly site, 49° 22' 50.7" N 20° 27' 12.6" E, Eliáš jun. 2005 NI
Slanské vrchy Mts., Slanec, slopes under the ruins of the Slanec castle, 48° 38' 12.99" N 21° 28' 15.66" E
Slovenský raj region, Spišské Tomášovce, Tomášovský výhľad site, 48° 56' 42.3" N 20° 27' 34.3" E, Eliáš jun. 2017 NI
Slovenský raj region, Letanovce, Čertova sihoť hill, 48° 56' 42.9" N 20° 26' 25.2" E, Eliáš jun. 2017 NI
Slovenský raj region, Spišské Tomášovce, Tomášovský výhľad site, 48° 56' 42.3" N 20° 27' 34.3" E, Eliáš jun. 2017 NI
Strážovské vrchy Mts., Čierna Lehota, Sokolie skaly site, 48° 53' 01.4" N 18° 20' 31.1" E, Eliáš jun. 2003 NI
Strážovské vrchy Mts., Kostolec, Kavčia skala hill, 49° 07' 54.5" N 18° 31' 25.6" E, Palík 1997 NI
Štiavnické vrchy Mts., Lehôtka pod Brehmi, Szabóova skala site, 48° 32' 12.2" N 18° 48' 24.5" E
Štiavnické vchy Mts., ruins of the Šášovský hrad castle, 48° 34' 44.2" N 18° 53' 58.2" E, Eliáš jun. 2017 NI
Veľká Fatra Mts., Blatnica, Tlstá hill, 48° 56' 02.3" N 18° 58' 17.0" E, Talapka 1996 NI
Veľká Fatra Mts., Necpaly, Borišov, 48° 56' 28.0" N 19° 05' 23.4" E, Talapka 1996 NI
Veľká Fatra Mts., Ružomberok, Malinô Brdo hill, 49° 03' 09.1" N 19° 16' 02.9" E
Veľká Fatra Mts., Ružomberok, Biely Potok city part, 49° 02' 55.6" N 19° 17' 52.5" E, Eliáš jun. 2015 NI

Cotoneaster melanocarpus agg.:
Czech Republic (1 locality)
Hostýnsko-vsetínská hornatina hills, Vsetín, Valova skála rock, 49° 21' 02.9" N 18° 01' 47.0" E

Hungary (12 localities)
Bükk Mts., Alsóhámor, Molnár-szikla hill, 48° 06' 42.8" N 20° 38' 52.2" E
Bükk Mts., Bélapatfalva, Bél-kö hill, 48° 02' 28.7" N 20° 22' 07.5" E
Bükk Mts., Uppony, SW slopes of Kalica-tetö, 48° 12' 57.0" N 20° 26' 45.3" E
Cserhát Mts., Bér, Nagy hegy hill, 47° 51' 56.65" S 19° 28' 42.25" V

Cserhát Mts., Hollókő; Szár hegy hill, 47° 59' 12.5" N 19° 35' 50.3" E
Karancs-Medves hills, Salgóbánya, Kis Salgó (Boszorkánykő) 48° 8' 24.40" N 19° 50' 57.86" E
Mátra Mts., Mátraálmás, ruins of the Gyala-vár castle, 47° 55' 31.40" N 19° 54' 24.65" E
Mátra Mts., Mátraháza, Sas-kő hill, 47° 52' 24.63" N 20° 1' 50.12" E
Mátra Mts., Mátrakerestes, Bárány-kö hill, 47° 54' 27.00" N 19° 49' 6.17" E
Mátra Mts., Sástó, 47° 50' 44.06" N 19° 56' 50.48" E
Mátra Mts., Tar, Fehérkő-bánya stone pit, 47° 57' 9.89" N 19° 45' 52.50" E
Zemplényi-hegysék hills, Hejce, Sólyom-kő hill, 48° 26' 12.7" N 21° 19' 09.5" E

Poland (2 localities)
Pieniny Mts., Falsztyn, Zielone Skałki hills, 49° 25' 55.5" N 20° 17' 40.5" E
Pieniny Mts., Trzy Korony, Okraglica 49° 24'50.4"N 20° 24'49.8"E

Slovakia (16 localities)
Biele Karpaty Mts., Vršatské Podhradie, ruins of the Vršatec castle, 49° 03' 55.9" N 18° 09' 04.5" E
Muránska planina hills, Brdárka, Malý Radzim hill, 48° 46' 31.1" N 20° 19' 44.4" E
Malé Karpaty Mts., Driny hill, 48° 30' 03.3" N 17° 24' 14.3" E, Eliáš jun. 2014 NI
Malé Karpaty Mts., Buková, near ruins of the Ostrý Kameň castle, 48° 31' 20.0" N 17° 22' 24.2" E, Eliáš jun. 2010 NI
Podunajská nížina lowland, Čifáre, Podskalie site, 48° 14' 22.5" N 18° 25' 40.9" E
Podtatranská kotlina basin, Ružomberok, memorial of the Slovak National Uprising, 49° 03' 47.6" N 19° 18' 30.6" E
Považský Inovec Mts., Podhradie, ruins of the Topoľčiansky hrad castle, 48° 39' 29.6" N 18° 02' 59.8" E
Slanské vrchy Mts., Slanec, slopes under the ruins of the Slanec castle, 48° 38' 12.99" N 21° 28' 15.66" E
Slovenský kras karst, Gemerské Teplice, Stráň hill, 48° 36' 23.3" N 20° 16' 29.2" E
Slovenský kras karst, Silica, Sokolia skala site, 48° 32' 44.93" N 20° 33' 56.51" E
Strážovské vrchy Mts., Lietava, ruins of the Lietavský hrad castle, 49° 09' 36.8" N 18° 41' 03.1" E
Štiavnické vrchy Mts., Hontianske Nemce, Dianiš hill, 48° 18' 13.8" N 18° 58' 32.3" E
Nízke Tatry Mts., Hranovnica, Hranovnická dubina Nature Reserve, 49° 00' 26.8" N 20° 17' 09.0" E
Slovenský raj region, Spišské Tomášovce, Tomášovský výhľad site, 48° 56' 42.3" N 20° 27' 34.3" E, Eliáš jun. 2017 NI
Vtáčnik Mts., Podhradie, ruins of the Sivý Kameň castle, 48° 41' 08.8" N 18° 38' 19.8" E
Zvolenská kotlina basin, Vígľaš, elevation point 419 m, 48° 33' 26.5" N 19° 17' 45.4" E

Cotoneaster tomentosus:

Slovakia (15 localities)
Kremnické vrchy Mts., Zvolen, Poštárka hill, 48° 33' 37.7" N 19° 05' 40.6" E, Galvánek 2018 NI
Malé Karpaty Mts., Devín, Devínska Kobyla hills, 48° 10' 54.9" N 16° 59' 04.5" E
Muránska planina hills, Muráň, Cigánka hill, 48° 45' 27.5" N 20° 03' 27.2" E, Baranec and Eliáš jun. 1999 NI
Nízke Tatry Mts., Liptovský Ján, near the cemetery, 49° 02' 35.6" N 19° 40' 45.0" E
Nízke Tatry Mts., Demänová, Siná hill, 48° 59' 58.9" N 19° 35' 15.7" E, Talapka 1996 NI
Nízke Tatry Mts., Svit, near the cemetery, 49° 03' 15.5" N 20° 12' 22.2" E
Oravská vrchovina hills, Oravský Podzámok, Oravský hrad castle, 49° 15' 46.9" N 19° 21' 32.9" E
Strážovské vrchy Mts., Rokoš – Košútova skala, 48° 46' 39.3" N 18° 26' 06.5" E, Eliáš jun. 2003 NI
Strážovské vrchy Mts., Uhrovské Podhradie, Zrubiská site, 48° 44' 52.6" N 18° 23' 35.1" E, Eliáš jun. 2003 NI
Slovenský raj region, Spišské Tomášovce, Tomášovský výhľad site, 48° 56' 42.3" N 20° 27' 34.3" E, Eliáš jun. 2017 NI
Tribeč Mts., Partizánske, Podbralie site, 48° 37' 07.0" N 18° 21' 43.5" E, Eliáš jun. 2003 NI
Veľká Fatra Mts., Belianska dolina, near the cottage of Havranovo, 48° 58' 08.8" N 19° 04' 39.0" E, Širáň 2003 NI
Veľká Fatra Mts., Morávková hill, 48° 59' 48.9" N 19° 07' 59.8" E, Talapka 1995 NI
Veľká Fatra Mts., Folkušová, Pekárová hill, 48° 57' 28.4" N 18° 58' 07.9" E, Talapka and Jelšovský 1996 NI
Veľká Fatra Mts., Vlkolínec, 49° 02' 30.6" N 19° 16' 36.3" E, Baranec and Eliáš jun. 2003 NI

Acta fytotechn zootechn, 23, 2020(1): 24–28
http://www.acta.fapz.uniag.sk

Original Paper

Assessment of silage quality of phytogenic fortified feed samples in mini-silos for ruminants

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Article Details: Received: 2019-12-04 | Accepted: 2020-02-18 | Available online: 2020-03-31

https://doi.org/10.15414/afz.2020.23.01.24-28

This study was conducted to assess the silage quality of *Zingiber officinale* fortified samples in a completely randomized design. The samples consisted of four treatments as samples: Cassava peel (65%) + Moringa leaf (15%) + PKC (20%)+ Ginger (0 g), Cassava peel (65%) + Moringa leaf (15%) + PKC (20%) + ginger (200 g), Cassava peel (65%) + Moringa leaf (15%) + PKC (20%) + ginger (300 g), Cassava peel (65%) + Moringa leaf (15%) + PKC (20%) + ginger (400 g). The physical characteristics; colour, smell, texture, pH, temperature and mould status were observed. All samples retained their original colour, had pleasant alcoholic smell with a firm texture, the pH ranged from 4.2–4.4, temperature range of 25–26 °C with sample D having the highest temperature range of 26 °C while samples A and B had the same temperature of 25.5 °C. The mould status showed absence of mould. The chemical composition revealed that dry matter ranged from 40.86% (sample B) to 54.68% (sample D). Crude protein content ranged from 13.30% to 14.88%, crude fibre content of the samples was significantly (p <0.05) different and it ranged from 14.67% to 22.14%. The mineral concentrations of the samples were higher in *Zingiber officinale* samples except in sample A where potassium was higher (100.40 mg 100 g^{-1}) than in other samples. Volatile fatty acid composition showed that lactic acid (3.24–4.86%) had higher concentration than other acids. It can therefore be concluded that *Zingiber officinale* fortified sample showed better nutritional potential as ruminant feed.

Keywords: volatile fatty acid, silage, *Zingiber officinale*, ruminants

1 Introduction

Ruminant feed comprises forages,agro-industrial by-products and concentrates (Schroeder, 2004). However, the forages are inadequate and of low quality during the dry season. This results in stress, low productivity and even death of the animal causing great economic loss to the farmer (Ibhaze and Fajemisin, 2015), hence the need for consistent availability and supply of feeds is of paramount significance. Silage (a forage, crop residues or agro-industrial by-products preserved by fermentation) (Moran, 2005) production is one of the means to achieving this as it can be fed as basal ration as well as feed supplement especially during the dry season. In a bit to improve intake and digestion and consequent optimum utilization of feed by the animals, researchers adopt various measures such as addition of feed materials high in protein as well as intake enhancers (Phytogenics) of which ginger belongs to this category.

Many active ingredients from plants are regarded as phytogenic feed additives. Phytogenic feed additives are plant-derived products used in animal feeding to improve their performance (Mohammed and Yusuf, 2011). *Zingiber officinale* (ginger) is a perennial plant that may act as a pro-nutrient because of the vast active ingredients it has been reported to contain (Mohammed and Yusuf, 2011). It is a potential functional food/ingredient not only because of being known as good sources of antioxidants but also as a good source of phyto-medicine (Adediran et al., 2014). It is a herbal plant that is nutritionally adequate and locally available in Nigeria that can be harnessed as feed additives (Ademola et al., 2009). Volatile fatty acids (VFA's) such as lactic, acetic, butyric and propionic acids, as well as ammonia and ethanol are by-products of anaerobic fermentation of organic matter and these acids serve as source of energy to ruminants. The amount of the different acids produced has an unswerving effect

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on shelf- life and feeding quality. This study is therefore aimed at evaluating the silage quality made from *Moringa oleifera* leaves, cassava peel and palm kernel cake fortified with ginger.

2 Materials and Methods

2.1 Location of Experiment

The experiment was conducted at the small ruminants unit of the Teaching and Research (T & R) Farm while the laboratory analysis was carried out at the Nutrition Laboratory of Animal Production and Health, Federal University of Technology, Akure. Akure is located on longitude 4.944055 °E and 5.82864 °E, and latitude 7.491780 °N with annual rainfall ranging between 1,300 mm and 1,650 mm average maximum and minimum daily temperature of 38 °C and 27 °C respectively (Daniel, 2015).

2.2 Gross composition (g 100 kg^{-1}) of the experimental diet

Diet A: cassava peel (65 kg) + palm kernel cake (15 kg) + moringa leaves (20 kg) + ginger (0 g).

Diet B: cassava peel (65 kg) + palm kernel cake (15 kg) + moringa leaves (20 kg) + ginger (200 g).

Diet C: cassava peel (65 kg) + palm kernel cake (15 kg) + moringa leaves (20 kg) + ginger (300 g).

Diet D: cassava peel (65 kg) + palm kernel cake (15 kg) + moringa leaves (20 kg) + ginger (400 g).

2.3 Silage production

The cassava peel, palm kernel cake, moringa leaves and ginger were measured as stated in 2.2 and were mixed thoroughly (manually) on a cleaned floor and after mixing, each diet was put into different plastic silos of 4 litres and 120 litres. The materials were properly compressed, covered with nylon and weighted with sand bag to prevent entering of air and then covered with the plastic lids and kept to ferment for 21days.

2.4 Determination of physical characteristics

At 21 days of ensiling, the 4litres silo was used for the analysis. The physical characteristics of the ensiled diets in terms of smell, colour, texture, temperature and pH were determined. The colour was determined using the colour chart, the smell was perceived using the sense organ of smell while the texture was ascertained by taking a hand full of the sample and squeezing it to check for seepage of the juice. The pH value was determined by using a portable pH meter. A 100 gram sample of the silage was thoroughly mixed with 100 ml distilled water (*w*/*v*) and the mixture was allowed to stay for 10 mins, and stirred again. The liquid was poured into a beaker, the pH meter was inserted into the beaker and the value was noted. The temperature was determined by inserting a laboratory thermometer into the silage for 3 mins and the temperature was recorded. The mould status of the silage was determined by visual observation.

2.5 Analytical procedures

The proximate and mineral compositions were determined according to the methods of AOAC (1990) while the fibre fractions were done using the method of Van Soest and Robertson (1985). Metabolizable energy was calculated according to the method of Pauzenga (1985). The preparation of samples for determination of VFAs by gas chromatography method was based on Manni and Caron's procedure (Manni and Caron, 1995).

2.6 Experimental Design and Statistical Analysis

The experimental layout was a completely randomized design. Data obtained were subjected to Analysis of Variance ANOVA using SAS version 9.2 (SAS, 2012) and where significant difference between means exist, Duncan Multiple Test (DMRT) was used using the same statistical package.

3 Results and discussion

3.1 Physical Characteristics of experimental samples

The physical characteristic of ensiled experimental samples is presented in Table 1. The pH of ensiled materials is a measure of its acidity. The pH ranged from 4.2–4.4 indicating that all diets had adequate dry matter which did not restrict fermentation for the production of acids. The values are in tandem with the report of (Bilal, 2009; Nhan et al., 2009) for good silage (3.75–4.70) in the tropics. All samples had pleasant, alcoholic smell suggesting proper fermentation and they all retained their original colour. The samples were firm in texture, which may be due to high fibre content of cassava peel which helped in maintaining a firm structure within the silage. Also this could be attributed to the prior wilting of the materials which reduced the moisture content before ensiling. Temperature ranged from 25 °C to 26 °C with sample D having the highest temperature range of 26 °C while samples A and B had the same temperature of 25.5 °C. Good silage should be in the range of 25 °C to 30 °C as high temperature during ensiling reduces lactic acid concentration, aerobic stability, increase pH, DM losses (Weinberg et al., 2001; Ashbell et al., 2002) and maillard reaction in silage. The absence of mould observed in all the silages suggests that the low pH which indicates adequate concentration of lactic acid created an unfavorable condition for spoilage organisms like clostridia to thrive. Also, the air-tight laboratory

silos used in this study would have excluded possible microbial growth and oxidation from air contamination.

3.2 Chemical and Mineral compositions of experimental samples

As shown in Table 2, the dry matter ranged from 40.86% (sample B) to 54.68% (sample D). Although no significant (p >0.05) difference was observed in the crude protein content (13.30–14.88%) but diets containing ginger had higher values which may suggest that ginger could have the ability to release the proteins in feed materials when incorporated with ginger. However, these values are above the critical 8% crude protein requirement by ruminants for optimum microbial activities in the rumen (Norton, 2003). The crude fibre content of the samples which ranged from 14.67–22.14% suggesting that many of the soluble nutrients were not degraded and would be adequate to support rumination. The values were within the requirement of 8–33% crude fibre suggested by Castrillo (2001). Ether extract (EE) values of the diets were moderate as higher values indicates an increase in the energy density of the diets which may allow maximal fat intake but may alter rumen microbial metabolism (Jenkins and McGuire, 2006). Minerals are needed for the maintenance of body fluid and tissues, prevention of decreased appetite, weight loss, secretion of hormones, enzymes and "pica". The mineral composition of ensiled samples is presented in Table 3. Results showed that the mineral concentrations of the samples were higher in *Zingiber officinale* fortified samples except in sample A where potassium was higher (100.40 mg 100 g^{-1}) than in other samples. The mineral concentrations recorded in all the samples indicated that all the samples would be adequate in supplying the mineral requirements of the animals. Potassium requirements for ruminants are within 50–80 mg 100 g^{-1} (NRC, 1984).

3.3 Volatile fatty acids and ammonium concentrations of experimental samples

The volatile fatty acids and ammonium concentrations in the silage are presented in Table 4. The presence of volatile fatty acid denotes that the dietary treatments have potential to make energy available to animals (Aluwong et al., 2010). Amongst the acids produced during ensiling, lactic acid should be the primary acid in good silage and is usually responsible for most of the drop in silage pH (Kung and Shaver, 2001). The high concentrations of lactic acid than other acids produced would result in lowest DM losses and energy (Kung and Shaver, 2001). The lactic acid values ranged from 3.24– 4.86% indicating a range of 65.94–67.17% of the total acids produced. This further shows that the silage was a good one as (Kung and Shaver, 2001) reported a range of 65–70% lactic acid of the total silage acids in good silage. The butyric acid concentration range (0.08–0.16%) indicates that the silage did not

Table 1 Physical characteristics of ensiled experimental samples

Diets (%)	Colour	Smell	pH	Texture	Temperature (°C)	Mould Status
A (0 g)	slightly brown	pleasant alcoholic	4.4	Firm	25.5 °C	absent
B (200 g)	slightly brown	pleasant alcoholic	4.3	Firm	25.5 °C	absent
C (300 g)	slightly brown	pleasant alcoholic	4.4	Firm	25 °C	absent
D (400 g)	slightly brown	pleasant alcoholic	4.2	Firm	26 °C	absent

Table 2 Chemical composition of ensiled experimental samples

Parameters	A (0 g)	B (200 g)	C (300 g)	D (400 g)
Dry Matter (%)	47.35 ±0.00^{b}	40.86 ±0.00^{d}	44.70 ±0.00^{c}	54.68 ±0.00^{a}
Crude protein (%)	13.30 ±0.70	13.50 ±14.00	14.59 ±0.84	14.88 ±0.88
Crude fibre (%)	14.67 ±0.82^{c}	17.53 ±0.49^{cb}	22.14 ±1.91^{a}	19.10 ±0.25^{ab}
Ether extract (%)	17.17 ±0.48	19.02 ±2.55	19.08 ±0.49	16.42 ±3.32
Ash (%)	5.29 ±1.39^{b}	5.94 ±0.37^{b}	3.30 ±2.71^{c}	7.27 ±0.43^{a}
NFE (%)	49.57 ±1.03^{a}	40.01 ±11.56^{c}	40.89 ±0.43^{c}	42.32 ±4.02^{b}
NDF (%)	36.64 ±3.68^{b}	22.72 ±21.52^{cd}	23.82 ±21.52^{c}	41.39 ±4.57^{a}
ADF (%)	29.29 ±0.05 ^{ab}	23.77 ±4.45^{b}	31.82 ±1.41^{ab}	37.89 ±5.14^{a}
ME (kcal kg^{-1})	3,396.42	3,644.54	3,552.26	3,656.22

a, b, c, d – means along the same row with different superscripts are significantly (P <0.05) different; NFE – nitrogen free extract, NDF – neutral detergent fibre, ADF – acid detergent fibre, ME – metabolizable energy

Table 3 Mineral compositions (mg/100 g) of experimental samples

Parameters	A (0 g)	B (200 g)	C (300 g)	D (400 g)
Potassium	100.40 ±0.00^{b}	95.90 ±0.00^{c}	108.10 ±0.00^{a}	90.5 ±0.00^{d}
Calcium	61.90 ±0.00^{d}	68.30 ±0.00^{c}	75.00 ±0.00^{a}	72.5 ±0.00^{b}
Magnesium	4.53 ±0.00^{d}	4.86 ±0.00^{b}	4.71 ±0.00^{c}	5.22 ±0.00^{a}
Manganese	0.33 ±0.00^{d}	0.72 ±0.00^{c}	0.83 ±0.00^{a}	0.81 ±0.00^{b}
Copper	0.36 ±0.00^{d}	0.52 ±0.00^{b}	0.40 ±0.00^{c}	0.58 ±0.00^{a}
Iron	1.03 ±0.00^{b}	1.46 ±0.00^{a}	0.70 ±0.00^{c}	0.45 ±0.00^{d}
Zinc	0.47 ±0.00^{b}	0.57 ±0.00^{a}	0.35 ±0.00^{d}	0.41 ±0.00^{c}
Phosphorus	0.08 ±0.00^{a}	0.07 ±0.00^{b}	0.05 ±0.00^{c}	0.03 ±0.00^{d}

a, b, c, d – means on the same row but with different superscripts are statistically different (P <0.05)

Table 4 Volatile fatty acids and ammonium compositions (%) of experimental samples

Volatile fatty acids	A (0 g)	B (200 g)	C (300 g)	D (400 g)
Acetic	1.53^{d} ±0.02	1.85^{b} ±0.02	1.68^{c} ±0.02	2.07^{a} ±0.02
Lactic	3.24^{d} ±0.02	4.27^{b} ±0.02	3.97^{c} ±0.02	4.86^{a} ±0.02
Propionic	0.11^{c} ±0.02	0.21^{b} ±0.02	0.15^{c} ±0.02	0.28^{a} ±0.02
Butyric	0.08^{c} ±0.02	0.14^{b} ±0.02	0.11^{b} ±0.02	0.16^{a} ±0.02
Ammonium	6.87^{d} ±0.02	9.13^{b} ±0.02	8.79^{c} ±0.02	9.38^{a} ±0.02

a, b, c, d – means on the same row but with different superscripts are statistically different (P <0.05)

undergo clostridia fermentation. High butyric acid has sometimes induced ketosis in lactating cows due to poor energy value; intake and production can suffer (Kung and Shaver, 2001). The acetic acid produced would not depress intake when fed to ruminants. Values >4–6% depressed intake (Kung and Shaver, 2001). The ammonium values (6.87–9.38%) show that there was no excessive protein breakdown in the silo and the problem of ammonium toxicity would not occur when fed to ruminants. Most silages typically are low in propionic acid. The low concentration (0.11–0.28%) of propionic acid observed could be due to the dry matter of the silage as Kung and Shaver (2001) reported that very high values is associated with very wet (<25% DM) silage. The normal concentrations of these acids produced indicate that the silage was aerobically stable.

4 Conclusion

Ensiling *Moringa oleifera* leaves, cassava peels and palm kernel cake with *Zingiber officinale* resulted in stable silage with normal concentrations of the volatile fatty acids and ammonium that will not cause deleterious nutritional effect to ruminant animals when consumed. Hence, *Zingiber officinale* could be incorporated up to 400 g in ruminant diets.

5 References

ADEDIRAN, O. A, UWALAKA, E. C. and KOLAPO, T.U. (2014). Antihelminthic and Anticoccidial Effects of *Vernonia amygdalina* in Goats. *Journal of Veterinary Advances*, 4(7), 610–615.

ADEMOLA, S. G., FARINU, G. O. and BABATUNDE, G. M. (2009). Serum lipid growth and hematological parameters of broilers fed garlic, ginger and their mixtures. *World Journal of Agricultural Science*, 5(1), 9–104.

ALUWONG, T., KOBO, P. I and ABDULLAHI, A. (2010). Volatile fatty acids production in ruminants and the role of monocarboxylate transporters. *African Journal of Biotechnology*, 9(38), 6229–6232.

ASHBELL, G. Z.G. et al. (2002). The effects of temperature on the aerobic stability of wheat and corn silages. *J. Ind. Microbiol. Biotechnol*., 28, 261–263.

ASSOCIATION of Analytical Communities (AOAC) (1990). Official Methods of Analysis. 15th edn. *Association Official Analytical Chemists*, 805–845.

BILAL, M.Q (2009). Effect of molasses and corn as silage additives on the characteristics of mott dwarf elephant grass silage at different fermentation periods. *Pakistan Veterinary Journal*, 29, 19–23.

CASTRILLO C. (2001). The effect of crude fibre on apparent digestibility and digestible energy content of extruded dog foods. *Journal of Animal physiology and Animal nutrition*, 231–236.

DANIEL, O. A. (2015). Urban extreme weather: a challenge for a healthy Living environment in Akure, Ondo State, Nigeria. *Climate*, 3(4), 775–791.

IBHAZE, G.A and FAJEMISIN, A.N. (2015). Feed intake and nitrogen metabolism by West African Dwarf does fed naturally fermented maizecob based diets. *World Journal of Animal Science Research,* 3(2), 1 – 8.

JENKINS, T.C. and McGUIRE, M.A. (2006). Major Advances in Nutrition: Impact on milk composition. *Journal of Dairy Science*, 89(10),1302–1310.

KUNG, L. and Shaver, R. (2001). Interpretation and use of silage fermentation analyses reports. *Focus on Forage*, 3(13).

MANNI, G. and CARON, F. (1995). Calibration and determination of volatile fatty acids in waste leachates by gas chromatography. *J. Chromatogr. A*, 690, 237.

MOHAMMED, A. A and YUSUF, M. (2011). Evaluation of ginger (*Zingiber officinale*) as a feed additive in broiler diets. *Livestock Research for Rural Development*, 23(9).

MORAN, J. (2005). *Making quality silage. Tropical dairy farming. Feeding management for small holder dairy farmers in the tropics.* Landlinks Press.

N.R.C (1984) *Nutrient Requirements of sheep.* National Research Council- National Academy of Sciences, Washington DC.

NHAN, N.T.H., HON, N.V., and PRESTON, T.R. (2009). Ensiling with or without additives to preserve pineapple residue and reduce pollution of the environment. *Livestock Research for Rural Development*. 21, Article #96. Retrieved November 29, 2010, from http://www.lrrd.org/lrrd21/7/nhan21096.htm

NORTON, B.W (2003). *Studies of the nutritive of Austrialian goat* : thesis (D.Agric; Sc). University of Melbourne.

PAUZENGA, U. (1985). Feeding Parent Stock. *Zootecnica International*., 22–24.

SAS (2012). *Statistical Analysis System. SAS Version 9.2 user's guide.* Cary, NY: SAS institute.

SCHROEDER, J.W. (2004). *Corn Silage management.* Pub. AS-1253. NDSU Coop. Ext.Ser.pub. 1254.

VAN SOEST, P.J. and ROBERTSON, J.B. (1985). *Analysis of forages and fibrous foods. Lab Manual for Animal Science no. 613.* Department of Animal Science, Cornell University, Ithaca, 105–106.

WEINBERG, Z.G. et al. (2001). The effect of temperature on the ensiling process of corn and wheat. *J Appl. Microbial*., 90, 561–566.

Acta fytotechn zootechn, 23, 2020(1): 29–36
http://www.acta.fapz.uniag.sk

Original Paper

Slaughter performance, chemical composition and physical technological parameters of Holstein veal fed with total mixed ration (TMR) and alfalfa hay

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Article Details: Received: 2020-01-07 | Accepted: 2020-01-24 | Available online: 2020-03-31

https://doi.org/10.15414/afz.2020.23.01.29-36

This study was conducted to evaluate the selected fattening characteristics, carcass parameters and analysing of parameters associated with the meat quality of twenty Holstein male calves fed with different diets. Calves were after weaning divided into two groups with respect of feeding; control group fed with untreated total mixed ration (TMR) and experimental group, which received industrially dried alfalfa hay. Calves of both groups were after about 193 days slaughtered in the experimental abattoir. Subsequently, after chilling for 24 hours, detailed dissection of the right – carcass half was performed. For the nutritional characteristics, physical technological parameters and sensory properties, slices of loin (*M. longissimus thoracis*) and top round (*M. semimembranosus*) muscles were taken 24 hours *post mortem*. The average daily gains were higher in the experimental group of calves (P >0.05). Although calves of the control group had higher dressing percentage (50.23%; P >0.05), no significant differences were determined in the carcass weight (P >0.05). Proportions of some of intraabdominal fats were influenced by feeding concept; calves of alfalfa hay treated group had lower proportion of intestinal fat (P >0.05) and higher proportion of kidney fat (P >0.05). Differences in total amount of meat from right – half carcass were not significant (P >0.05); proportion of separable fat was higher in the experimental group of calves. In terms of individual valuable meat cuts, higher proportion of tenderloin was determined in the group fed with alfalfa hay (1.80%); however results were no significant. Statistical significant variety at the level P <0.05 were revealed in the moisture content, intramuscular fat content and energy value. The loin muscle from experimental group was lighter (CIE L^*; P >0.05) 24 hours *post mortem*.

Keywords: different diet, veal quality parameters, bull calves, meat colour, right – half carcass

1 Introduction

The definitions for the term ´veal´ vary according to the country of provenance. In many countries for calves raised for meat production are limited age of 7 months and a weight of 250 kg. However, in accordance with current European Union legislation, veal is defined as a meat from bovine animals aged less than 8 months at slaughter (category V). Animals aged between 8 and 12 months at slaughter are marketed as category Z (EC, 2008). In European market veal derived from dairy herds is an important part of the meat industry. The economic efficiency of this production is dependent on increasing growth rate of calves and efficient feed utilization, as well (Santos et al., 2013). In terms of the health and wellbeing of calves is significant to optimise the growth of calves for meat production, following weaning. Furthermore, for producers is important the smoothly transition of calves from liquid to solid feeding and effective utilising of solid feed, which relies mainly on pre-weaning and post-weaning management (Drake, 2017). Meat production is based on the animal growth rate, which depends on several environmental factors as well as management practices. Animals for the meat production, such as livestock differ in genetics, age, sex, nutritional and environmental effects (Irshad et al., 2013).

There is currently no consensus about precise explanation of the concept of meat quality, because is

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generally considered to be a combination of two main elements. On the one hand, the overall quality of meat and meat products includes measurable properties – microbiological state, tenderness, colour, juiciness, shelf life, pH value. On the other hand, meat quality includes personal consumers´ perception of the value of meat and meat products (Feiner, 2006). In addition to the quality of the carcass itself, the priority interest of consumers as well as meat producers is the inherent quality of the meat, hence pure muscle or fat tissue, respectively. This quality is expressed by chemical composition, physical technological properties and sensory properties (Foltys and Mojto, 2009). Nutrition contributes to the quality of meat directly or indirectly, in particular by increasing of intramuscular fat content (Brewer, 2010). Intramuscular fat content and also composition is influenced by the feeding method, sex of animals, slaughter weight and slaughter age, as well as the duration of suckling (Moreno, 2006). Nowadays, when dairy breeds predominate in cattle population whose milk is the main market commodity, males of these breeds will be the main source of beef (Lengyel et al., 2003). Meat from milking breeds shows the good quality in lower slaughter weights and therefore it is possible to cover the lack of veal mainly with meat from calves of dairy breeds.

The aim of this work was to bring knowledge about the possibilities of veal production and baby beef, respectively, which is in accordance with legislation, welfare rules and is economically acceptable to the farmer as well.

2 Material and methods

For this experiment twenty bulls of Holstein calves ($n = 20$) were studied. Calves were born in about the same period, within a week in a local milking farm. During first stage of the experiment calves were housed in individual outdoor crates. Calves were fed a milk replacer and a starter concentrate *ad libitum*. Calves had free access to fresh drinking water. After about two months, calves were moved to group igloos, ten bulls each. Subsequently, the period of habit for solid feeding began. The calves of both groups – control and experimental were fed with liquid milk replacer once per day with *ad libitum* access to the starter feed mixture. Gradually they received a small amount of solid feed; control group received a small amount of total mixed ration (TMR) and experimental group obtained a small amount of industrially dried alfalfa hay. The experimental fattening period started at about 70 days of age.

2.1 Housing and feeding of calves during experiment

The fattening period was carried out from weaning (about 70 days of age) to the final live weight of 180 kg. After weaning and addictive period (from 70 days) calves were divided at random into two groups with different diets, ten calves each. Calves of the control group was fed an untreated feed mixture TMR (total mixed ration) with 6.25% of hay, 43.25% alfalfa hay, 18.75% maize silage and 31.75% starter feed mixture HD-02. The net energy fattening of TMR diet was 6.80 MJ kg^{-1} of dry matter (DM); organic matter was 824 g kg^{-1} of DM. Calves of the experimental group received the diet with 31.75% of industrially dried alfalfa hay, 3.16% barley straw, 1.59% beet molasses, 31.75% water and 31.75% starter feed mixture HD-02. The net energy fattening of experimental diet was 6.1 MJ kg^{-1} of DM; organic matter was 853.9 g kg^{-1} of DM. Calves were housed under the same conditions with daily straw landings with *ad libitum* access to fresh drinking water. After reaching required weight about 192.58 days of treatment the calves were slaughtered in the Experimental abattoir which is a part of the Department of Animal Husbandry, Slovak University of Agriculture in Nitra.

2.2. End of experiment

Immediately after slaughter, carcasses were split into right – half carcass (RHC) and left – half carcass (LHC) and subsequently on the forequarter and hindquarter. The weight of the meat (carcass weight) includes headless half-carcasses, without the limbs separated in the elbow or heel joint, without the thoracic and abdominal organs (except the kidneys) and without the genital organs. After chilling for 24 hours on 2–5 °C, the detailed dissection of the right – half carcass was performed. Individual valuable meat cuts were weighted with and without bone. Moreover, individual tissues of right – half carcass – trimmed fat, muscles and bones (marrow, technical, pelvis and scapula) were weighted on scale. Physicochemical and sensory properties of the meat were observed from a sample of *Longissimus lumborum* et *thoracis* (MLT). The individual chemical and physical technological parameters were determined using laboratory techniques of SUA in Nitra from samples taken from the tenderloin (*M. longissimus thoracis*) and top round (*M. semimembranosus*) muscles. Samples were taken 24 hours after slaughter. The pH value and electrical conductivity values were measured 1 hour and 24 hours *post mortem* using pH meter Titan and a Biotech instrument. The free bound water was determined 24 hours after slaughter and after chilling at 4 °C as a percentage of drip loss from a 50 g sample of the loin muscle. The chemical composition of the veal was analyzed from 100 g of sample from MLT using a Spectrometer Nicolet 6700. The energy value (*EV*) of the meat was calculated from the protein and intramuscular fat content according to the equation, as follows: *EV*

(kJ 100 g^{-1}) = 16.75 * protein content (g 100 g^{-1}) + 37.68 * IMF content (g 100 g^{-1}).

2.3 Statistical evaluation

Basic variability and statistical characteristics of fattening, slaughter, physical, technological and sensory properties reported as means and standard deviations were analyzed using a two-factor analysis of variance. The significance of the differences between the individual treatment groups was tested using the t-test at the levels of significance P >0.05; P <0.05; P <0.01 and P <0.001. All statistical analyzes were calculated using statistical package (SAS) version 9.3 (TS1M2) Enterprise Guide 5.1. (SAS INSTITUTE Inc., 2011).

3 Results and discussion

The fattening and slaughter parameters of monitored groups of calves are shown in Table 1. Differences were found in the average daily gains from birth to the end of fattening, whereas control group fed with maize silage had lower gains at 197 days than experimental group at 185 days of fattening (687 vs. 748 g). Dias et al. (2018) found in Holstein calves fed with corn silage slaughtered at 179.8 kg average daily gain of 811 g. Noon et al. (1998) noted higher ADG (1.55) in calves fed with 50% corn and 50% barley. In general, dressing percentage of Holstein cattle is lower than from beef breeds (Schaefer, 2007). Higher, but statistically not significant differences were found in carcass weight with higher weight in the control group (84.19 kg). These differences were also reflected in the calculated dressing percentage; calves of the first group had higher dressing percentage than second group (P >0.05). Yim et al. (2015a) found lower carcass weight (83.4 kg) and higher dressing percentage (59.1%) at higher slaughter weight of Holstein calves (270 kg). According to Bartoň et al. (2003), the carcass value is significantly decreased with increasing utility and genotype representation in dairy herds. Compared to Czech Pied cattle, the Holstein breed have a lower proportion of muscle (79.03 vs. 76.61%) as well as a lower dressing percentage (57.29 vs. 54.88%). González et al. (2014) evaluated veal quality of Rubia Gallega calves fattening with oil supplement. Calves with linseed oil supplement had carcass weight 178.71 kg and dressing percentage 50.04%.

Feeding concept influenced proportion of kidney and intestinal fat (Figure 1); however results were not significant. Calves of control group had lower proportion of kidney fat (1.07%) and higher proportion of intestinal fat (0.68%) than second group (1.19% and 0.51%). Slightly higher proportion of kidney fat (1.61%)

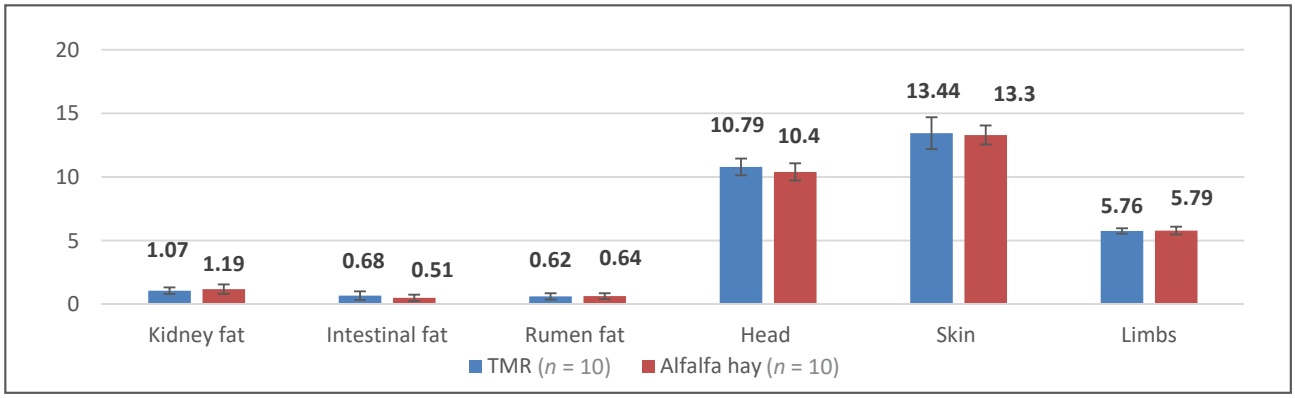


Figure 1 Proportions of by-products of veal carcasses from Holstein calves not included to the carcass weight (%)

Table 1 Fattening and slaughter parameters of veal from Holstein male calves fed TMR or alfalfa hay ($\bar{x} \pm s_x$)

	TMR (n = 10)	Alfalafa hay (n = 10)	*Sign.*
Birth weight (kg)	39.17 ±1.40	38.38 ±1.81	*ns*
Final live weight (kg)	174.33 ±12.97	175.25 ±9.59	*ns*
Starved live weight (kg)	167.67 ±10.68	169.60 ±11.26	*ns*
Number of feeding days	197.33 ±7.87	184.83 ±18.87	*ns*
Hot carcass weight (kg)	84.19 ±5.48	82.87 ±6.77	*ns*
Hot dressing percentage (%)	50.23 ±1.39	48.44 ±1.24	*ns*
ADG (g)	687 ±89	748 ±96	*ns*

ADG – average daily gain from birth to slaughter; *ns* – not significant; % – values were calculated from slaughter weight

Table 2 Proportion of tissues from right – half carcass from Holstein calves fed TMR or alfalfa hay ($\bar{x} \pm s_x$)

		TMR (n = 10)	Alfalfahay (n =10)	*Sign.*
Right – half carcass	(kg)	41.67 ±2.58	41.25 ±3.62	*ns*
RHC forequarter	(kg)	18.62 ±0.86	18.55 ±1.53	*ns*
RHC hindquarter	(kg)	22.75 ±2.02	22.41 ±1.81	*ns*
Meat from RHC	(kg)	27.17 ±2.24	26.67 ±2.63	*ns*
	(%)	65.17 ±2.29	64.62 ±2.05	*ns*
Bones	(kg)	11.29 ±0.53	11.13 ±0.90	*ns*
	(%)	27.14 ±0.68	27.04 ±1.71	*ns*
Separable fat	(kg)	2.89 ±0.55	3.16 ±0.58	*ns*
	(%)	6.95 ±1.19	7.70 ±1.54	*ns*

ns – not significant; % values were calculated from right – half carcass; RHC – right – half carcass

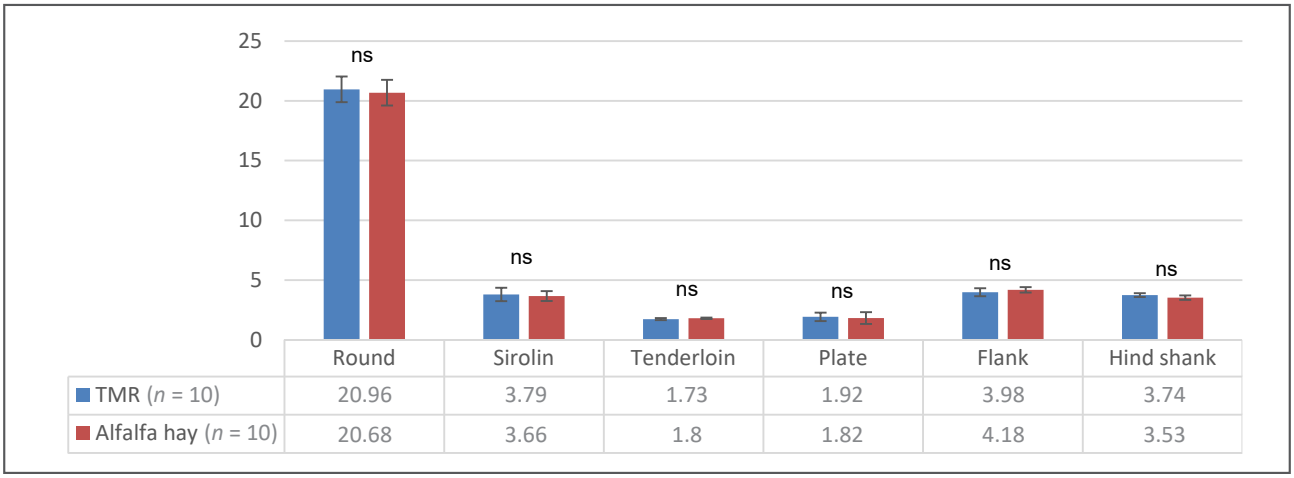
was noted for Buffalo calves in Holló et al. (2013) at slaughter weight of 196.06 kg. In Holstein calves with carcass weight of 187.8 kg found Titi et al. (2008) 1.7 kg of kidney fat. As noted in Schaefer (2007), Holstein cattle as a dairy type require 20% more maintenance energy. High milk production as ´lactability´ is associated with liver and intra-abdominal fat proportions. On the other hand, minimal differences in weight and proportion of rumen fat were found. Likewise, all other non-carcass component measurements were not different the two feeding groups (P >0.05).

Dressing data (%) of Holstein calves after manual dissection are presented in Table 2. No significant differences in the weight of right – half carcass between monitored groups were revealed. Our results are similar to those of Moran et al. (1992), who reported for milk – fed calves weight of forequarter 18.94 kg from side weight predicted at 40 kg. They found higher proportion of meat (69.3%) and lower proportion of bones (25.6%) and separable fat (6.0%). Furthermore, differences in weights and proportions of individual carcass quarters were not significant (P >0.05). When calculate the amount of the meat to percentage from weight of right halves of the carcasses, between two feeding groups were minimal differences (65.17%, resp. 64.62%; P >0.05). The nutrition contributes to the meat quality directly or indirectly, mainly by fat content increasing (Brewer, 2010). The percentage of bones in the carcasses (spiked, technical, scapula and pelvis) were similar between groups; whereas proportion of trimmed fat was higher in the experimental group (P >0.05). In comparison to Buffalo male calves slaughtered at higher weight (Holló et al., 2013), our Holstein calves had higher content of muscle tissue, similar proportion of bone and fat tissues in the carcass.

In table 3 and Figure 2 are presented amounts of most commercially valuable beef cuts of Holstein calves fed with different diets. We did not found significant differences between the two feeding groups. The average amount of whole round and tenderloin were similar (P >0.05). Holstein calves tend to have poorer conformation of the hindquarters and therefore carcasses are often of lower in conformation (Moran and Curie, 1992). In contrast Holló et al. (2013) reported that Buffalo calves compare to bovines have a higher percentage of forequarter in the carcass. Concerning our results, the proportion of meat from hindquarter was greater. Our values are different from those of Yim et al. (2015b); in Holstein male calves slaughtered at 159 kg they found proportion of tenderloin 2.67%, sirloin 2.67%, shortloin

Table 3 Proportion of boneless commercial meat cuts from fore quarter of right-half carcass from Holstein calves ($\bar{x} \pm s_x$)

	TMR (n = 10)	Alfalfahay (n = 10)	*Sign.*
Shoulder (%)	6.80 ±0.37	6.71 ±0.26	*ns*
Neck (%)	4.51 ±0.47	4.47 ±0.46	*ns*
Chuck (%)	4.95 ±0.29	4.69 ±0.33	*ns*
Short plate (%)	10.19 ±0.76	10.26 ±1.05	*ns*
Brisket (%)	5.29 ±0.69	5.09 ±1.03	*ns*
Fore shank (%)	4.64 ±0.31	4.93 ±0.27	*ns*



	Round	Sirolin	Tenderloin	Plate	Flank	Hind shank
TMR (*n* = 10)	20.96	3.79	1.73	1.92	3.98	3.74
Alfalfa hay (*n* = 10)	20.68	3.66	1.8	1.82	4.18	3.53

Figure 2 Proportion of boneless commercial meat cuts from hind quarter of right-half carcass from Holstein calves (%)

17.76%. Our calves had a lower proportion of tenderloin compare to Maciel et al. (2016).

Table 4 represents the moisture, intramuscular fat and protein contents as well as calculated energy value of 100 g of Holstein veal. The nutritional composition of the meat vary depending on the breed, the feeding concept, the season of slaughter and the meat cuts of carcass. In general, however, red meat has a low fat content, an adequate content of cholesterol and it is rich in contents of protein, vitamins and minerals (Williams, 2007). Energy value of loin samples increased with an increase in intramuscular fat content (P <0.05). The moisture content of loin muscle from the TMR fattened group (73.20%) was significantly higher than those from experimental group (68.91%; P <0.05). Moisture content of the muscles varies depending on the species, breed, age of animal, as well as its morphological-anatomical origin and nutrition of animal (Huff-Lonergan, 2010). Biel et al. (2019) found in *Musculus semitendinosus* muscle moisture content of 67.67% in calves weighing 95 kg. High levels of nutrition, especially during the final phase, may increase the intramuscular fat content to a greater or lesser extent depending on the species, breed, age of the animals and other factors. Moreover, the IMF content is influenced by type of muscle, as describe Gálvez et al. (2018); while higher IMF content is associated with higher physical activity of muscle and content of red oxidative muscle fibre. Authors reported values for individual muscles from Rubia Galega x Holstein cross calves slaughtered at 9 months of age – shoulder (1.29%), inside round (0.94%), eye of round (1.10%), bottom round (1.25%), heel of round (0.75%), knuckle (1.15%) and tenderloin (2.80%). Highly marbled meat is traditionally considered ideal because of the effect of fat on taste and tenderness (Brewer, 2010). Similar to our results, Holló et al. (2013) found in loin muscle from Buffalo calves protein content of 20.99%.

The mean values of selected physical technological parameters of *M. longissumus lumborum* et *thoracis* associated with the quality of veal are presented in Table 5. Numerical differences in drip loss values between monitored groups of calves were determined; however results were not significant (P >0.05). According to Ripoll et al. (2013) values of drip loss is a result of *post mortem* lateral contraction of myofibrils, causing the secretion of free water into the extracellular space of the muscles. Moreover, content of free water is associated with the content of dry matter in meat (Gariépy et al., 1998). Slightly higher results (1.38%) reported Skřivanová et al. (2007) in Holstein male calves fed with TMR. In contrast, Campbell et al. (2013) determined in grain-fed calves drip loss values 4.40% for *Longissimus* muscle and 3.56% for *M. semitendinosus*.

Table 4 Chemical composition of loin muscle from Holstein calves of different feeding group ($\bar{x} \pm s_x$)

	TMR (*n* = 10)	Alfalfahay (*n* = 10)	*Sign.*
Water (g 100 g^{-1})	73.20 ±2.59	68.91 ±2.55	*
Protein (g 100 g^{-1})	20.34 ±1.31	19.41 ±0.39	*ns*
IMT (g 100 g^{-1})	5.26 ±1.19	6.69 ±0.66	*
Energy value (kJ 100 g^{-1})	538.73 ±27.23	576.95 ±28.20	*

* P ≤0.05;** P ≤0.01; *** P ≤0.001; *ns* – not significant

The pH value measured 24 hours *post mortem* did not vary significantly; in case of *M. semitendinosus* (Table 6) there were no numerical differences between monitored feeding groups of calves. Different results reported Yim et al. (2015b), who found in Holstein calves slaughtered at 5 months of age pH values 5.77 in MLT muscle and 5.73 in *M. semimembranosus*. Ultimate pH is influenced by animal nutrition, as noted in Pateiro et al. (2013). On the other hand, the effect of pH is often referred to by other veal quality characteristics, especially color, and is generally measured as a consecutive factor; the rate of pH decline influences the meat color to a greater or lesser extent depending on the pigment content in each muscle (Ngapo and Gariépy, 2006). In addition, decline in muscle pH and temperature could influence meat color of veal carcasses when pigment concentrations do not significantly differ. Depending on muscle type, pigment content and rate of pH fall influence the meat color, i. e. pigment content in the loin muscle is more important than the rate of pH decline (Klont et al., 2000).

The differences in color parameters of the loin and top round muscles measured 24 hours and 7 days *post mortem* are presented in Tables 5 and 6. Significant differences (P <0.05) in the yellowness (b*) of the loin muscle 24 h after slaughter were observed. The most of differences in color parameters between meat cuts are associated with anatomical location, proportions of red fibres and haemoglobin content in blood (Gálvez et al., 2018; Cho et al., 2014).

Furthermore, anatomical location of muscles influences most of the color parameters, including pigment content, reflectivity, redness and rate of meat decolorization (Ngapo and Gariépy, 2006). The top round muscle

Table 5 Physical technological parameters of loin muscle associated with the veal quality of Holstein breed ($\bar{x} \pm s_x$)

		TMR (n = 10)	Alfalfahay (n = 10)	Sign.
pH_1		7.02 ±0.19	6.95 ±0.13	*ns*
pH_{24}		6.00 ±0.05	5.97 ±0.06	*ns*
Drip loss (%)		1.22 ±0.76	2.01 ±0.56	*ns*
Electrical conductivity – 1 (µS)		2.68 ±0.42	3.28 ±0.68	*ns*
Electrical conductivity – 2 (µS)		2.18 ±0.17	3.23 ±0.68	**
Meat color 24 h	CIE L*	46.3 ±3.29	44.41 ±4.29	*ns*
	CIE a*	7.38 ±1.71	7.06 ±2.56	*ns*
	CIE b*	10.36 ±0.62	9.22 ±0.70	*
Meat color 7 d	CIE L*	46.33 ±3.47	47.17 ±3.87	*ns*
	CIE a*	8.01 ±0.78	7.33 ±2.22	*ns*
	CIE b*	10.39 ±1.23	9.96 ±0.80	*ns*

* $P \leq 0.05$;** $P \leq 0.01$; *** $P \leq 0.001$; *ns* – not significant

Table 6 Physical technological parameters of top round muscle associated with the veal quality of Holstein breed ($\bar{x} \pm s_x$)

		TMR (n = 10)	Alfalfahay (n = 10)	*Sign.*
pH_1		6.98 ±0.10	6.88 ±0.11	*ns*
pH_{24}		5.91 ±0.08	5.91 ±0.11	*ns*
Electrical conductivity – 1 (µS)		4.02 ±0.23	4.10 ±0.60	*ns*
Electrical conductivity – 2 (µS)		3.17 ±0.30	3.32 ±0.43	*ns*
Meat color 24 h	CIE L*	48.51 ±3.33	49.79 ±3.39	*ns*
	CIE a*	10.16 ±3.39	9.87 ±2.49	*ns*
	CIE b*	12.13 ±0.84	12.42 ±1.43	*ns*
Meat color 7 d	CIE L*	47.81 ±3.83	48.30 ±3.19	*ns*
	CIE a*	9.73 ±3.21	9.79 ±2.68	*ns*
	CIE b*	11.98 ±1.52	11.70 ±0.98	*ns*

ns – not significant

had greater lightness (*L**) 24 hours *post mortem* than loin muscle (48.51 and 49.79 vs. 46.3 and 44.41). These results are consistent with those reported by Gálvez et al. (2018), in which *L** values were greater in the eye of round muscle. No significant and numerical differences were found in the color measurements 7 days after slaughter for both – loin and top round muscles ($P > 0.05$). For consumers is meat color very important attribute of satisfaction, while dark and pale color is associated with loss of freshness (Vieira et al., 2005). The lightest meat characterized 7 days *post mortem* with higher *L** values (49.79) were determined in samples from top round muscle of the experimental group of calves ($P > 0.05$). The loin muscle from experimental group was also pinkest (*a** – 7.33). Slightly higher values than our results for meat lightness reported Yim et al. (2015b) in MLD from 5-months old Holstein calves (50.44). Mojto et al. (2009) determined in cows to 4 years of age *L** value 29.20.

4 Conclusions

Analysing of growth, carcass characteristics, veal quality and mutual correlation between analysed characteristics of Holstein calves differentiated according feeding concept has become an object of concern. Bull calves of the control group had higher dressing percentage than those of experimental group ($P > 0.05$). Proportion of kidney and intestinal fat was influenced by feeding concept; control group of calves had lower proportion of kidney fat ($P > 0.05$) and higher proportion of intestinal fat ($P > 0.05$). Differences in weights and proportions of individual carcass quarters as well as in terms of individual retail meat cuts were not significant. Statistical significant variety of the moisture content, intramuscular fat content and energy value as well, were revealed ($P < 0.05$). Physical technological parameters of both the muscles (pH, drip loss, electrical conductivity) showed similarity among the two feeding groups. In colour spectrum of *M. longissimus thoracis* measured 7 days after slaughter we observed lighter (*L** 47.17; $P > 0.05$) and pinker (*a** 7.33; $P > 0.05$) meat in group fed with alfalfa hay. No significant differences in the fattening, carcass characteristics as well as in chemical and physical technological parameters of Holstein veal fed with these feeding concepts were revealed.

Acknowledgments

Work was supported by Scientific Grant KEGA No. 015SPU – 4/2019.

References

BARTOŇ, L. et al. (2003). Growth, feed efficiency and carcass characteristics of Czech Pied and Holstein bulls. *Czech Journal of Animal Science*, 48(11), pp. 459–465.

BIEL, W. et al. (2019). Offal Chemical Composition from Veal, Beef, and Lamb Maintained in Organic Production Systems. *Animals*, 9(8), pp. 489. https://doi.org/10.3390/ani9080489

BREWER, S. (2010). *Handbook of Meat Processing.* Ames: Blackwell Publishing, p. 584.

CAMPBELL, C. P. et al. (2013). Packing plant differences in meat quality for grain-fed veal. *Canadian Journal of Animal Science*, 93(2), 205–215. https://doi.org/10.4141/cjas2012-147

DIAS, A. M. O. et al. (2018) Performance and fatty acid profile of Holstein calves slaughtered at different weights. *R. Bras. Zootec.*, 47. https://doi.org/10.1590/rbz4720170208

DOMARADZKI, P. et al. (2017). Slaughter value and meat quality of suckler calves: A review. *Meat Science*, 134, 135–149. https://doi.org/10.1016/j.meatsci.2017.07.026

DRAKE, N. A. (2017). *The performance of veal calves fed concentrate, total mixed ration, or free choice in the post-weaning period:* Master of Sustainable Animal Nutrition and Feeding Thesis. Aarhaus: Aarhus University.

EC (2008). *Council Regulation (EC) No. 361/2008 of 14 April 2008 amending Regulation (EC) No. 1234/2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products.*

FEINER, G. (2006). *Meat products handbook: Practical science and technology.* London: Woodhead Publishing Limited and CRC Press.

FOLTYS, V. and MOJTO, J. (2009). *Produkty hovädzieho dobytka a ich kvalita*. [Online]. Retrieved 2019-01-15 from http://old.agroporadenstvo.sk/zv/hd/chovhd10.htm?start

GARIÉPY, C. et al. (1998). Effect of calf feeding regimes and diet EDTA on physico-chemical characteristics of veal stored under modified atmospheres. *Meat Science*, 49(1), 101–115. https://doi.org/10.1016/S0309-1740(97)00115-0

GÁLVEZ, F. et al. (2019). Nutritional and meat quality characteristics of seven primal cuts from 9-month-old female veal calves: a preliminary study. *J Sci Food Agric*, 99, 2947–2956. https://doi.org/10.1002/jsfa.9508

GONZÁLEZ, L. et al. (2014). Effect of supplementing different oils: Linseed, sunflower and soybean, on animal performance, carcass characteristics, meat quality and fatty acid profile of veal from "*Rubia gallega*" calves. *Meat Science*: *Part A*, 96(2), 829–836. https://doi.org/10.1016/j.meatsci.2013.09.027

HUFF-LONERGAN, E. (2010). *Chemistry and Biochemistry of Meat*. In TOLDRÁ, F. (2010) Handbook of Meat Processing. 1st ed., Blackwell Publishing. 2121 State Avenue, Ames, Iowa 50014-8300, USA, 2010, pp. 584. ISBN 978-0-8138-2182-5. DOI: 10.1002/9780813820897

HOLLÓ, G. et al. (2013). Characterisation of carcass composition and meat quality of male suckling buffalo calves kept on natural grassland. *Archives Animal Breeding*, 56(1), 1060–1065. https://doi.org/10.7482/0003-9438-56-107

CHO, S. et al. (2014). Physico-chemical Meat Qualities of Loin and Top Round Beef from Holstein Calves with Different Slaughtering Ages. *Korean Journal for Food Science of Animal Resources*, 34(5), 674–682. http://dx.doi.org/10.5851/kosfa.2014.34.5.674

IRSHAD, A. et al. (2013). Factors influencing carcass composition of livestock: a review. *Journal of Animal Production Advances*, 3(5), 177–186. https://doi.org/10.5455/japa.20130531093231

KLONT, R. E. et al. (2000). Effects of rate of pH fall, time of deboning, aging period, and their interaction on veal quality characteristics. *Journal of Animal Science*, 78(7), 1845–1851. https://doi.org/10.2527/2000.7871845x

LENGYEL, Z. et al. (2003). Fatty acid composition of intramuscular lipids in various muscles of Holstein-Friesian bulls slaughtered at different agens. *Meat Science*, 65(1), 593–598. https://doi.org/10.1016/S0309-1740(02)00252-8

MACIEL, R. P. (2016). Performance, rumen development, and carcass traits of male calves fed starter concentrate with crude glycerin. *R. Bras. Zootec.,* 45(6), 309–318. http://dx.doi.org/10.1590/S1806-92902016000600005

MOJTO, J. et al. (2009). Effect of age at slaughter on quality of carcass and meat in cows. *Slovak Journal of Animal Science*, 42(1), 34 – 37.

MORAN, J. B. et al. (1992). Growth, carcass and meat quality in veal calves fed diets based on wholemilk or milk replacers. *Proc. Aust. Soc. Anim. Prod.*, 17, 254–257.

MORAN, J. B. and Currie, J. R. (1992). The distribution of meat in pink veal carcasses as influenced by carcass weight, breed and diet. *Proc. Aust. Soc. Anim. Prod.*, 19, 65–67.

MORENO, T. et al. (2006). Nutritional characteristics of veal from weaned and unweaned calves: Discriminatory ability of the fat profile. *Meat Science*, 73(2), 209–217. https://doi.org/10.1016/j.meatsci.2005.11.016

NGAPO, T. M. and GARIÉPY, C. (2006). Factors affecting the meat quality of veal: Review. *Journal of the Science of Food and Agriculture*, 86, 1412–1431. https://doi.org/10.1002/jsfa.2507

NOON, C. D. et al. (1998). The use of corn and barley in diets for veal calves: Effects on performance, diet digestibility and carcass quality. *Canadian Journal of Animal Science*, 78(3), 351–358. https://doi.org/10.4141/A97-100

PATEIRO, M. et al. (2013). Meat quality of veal: Discriminatory ability of weaning status. *Spanish Journal of Agricultural Research*, 11(4), 1044–1056. http://dx.doi.org/10.5424/sjar/2013114-4363

RIPOLL, G. et al. (2013). Instrumental meat quality of veal calves reared under three management systems and color evolution of meat stored in three packaging systems. *Meat Science*, 93(2), 336–343. https://doi.org/10.1016/j.meatsci.2012.09.012

SANTOS, P.V. et al. (2013). Carcass physical composition and meat quality of Holstein calves, terminated in different finishing systems and slaughter weights. *Ciência e Agrotecnologia*, 37(5), 1413–7054. http://dx.doi.org/10.1590/S1413-70542013000500008

SAS INSTITUTE Inc. (2011). *Base SAS® 9.3 Procedures Guide.* Cary, NC: SAS Institute Inc., Carry.

SCHAEFER, D. M. (2007). Yield and Quality of Holstein Beef [Online]. Retrieved 2019-12-02 from https://pdfs.semanticscholar.org/90ea/1591cfbbaed6a756e6d9c483ff48cb3f6802.pdf

SKŘIVANOVÁ, E. et al. (2007). Influence of dietary selenium and vitamin E on quality of veal. *Meat Science*, 76(3), 495–500. https://doi.org/10.1016/j.meatsci.2007.01.003

TITI, H. H. et al. (2008). Growth and carcass characteristics of male dairy calves on a yeast culture-supplemented diet. *South African Journal of Animal Science*, 38(3), 174–183. http://dx.doi.org/10.4314/sajas.v38i3.4125

VIEIRA, C. et al. (2005). Effect of diet composition and slaughter weight on animal performance, carcass and meat quality, and fatty acid composition in veal calves. *Livestock Production Science*, 93(3), 263–275. https://doi.org/10.1016/j.livprodsci.2004.11.020

WILLIAMS, P. (2007). Nutritional composition of red meat. *Nutrition & Dietetics*, 64(4), S113–S119. https://doi.org/10.1111/j.1747-0080.2007.00197.x

WILLIAMS, J. L. (2008). *Genetic control of meat quality traits.* In TOLDRÁ, F. (2008) Meat Biotechnology. New York: Springer Science-Business-Media, 21–60. https://doi.org/10.1007/978-0-387-79382-5

YIM, D.G. et al. (2015a). Meat quality of Loin and Top Round Muscles from the Hanwoo and Holstein Veal Calves. *Korean Journal for Food Science of Animal Resources*, 35(6), 731–737. http://dx.doi.org/10.5851/kosfa.2015.35.6.731

YIM, D. G. et al. (2015b). Physicochemical traits of Holstein loin and top round veal from two slaughter age groups. *Journal of Animal Science and Technology*, 57(24), 1–5. https://doi.org/10.1186/s40781-015-0058-0

Acta fytotechn zootechn, 23, 2020(1): 37–41
http://www.acta.fapz.uniag.sk

Original Paper

The effect of different feeding system on fatty acids composition of cow's milk

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Article Details: Received: 2020-01-16 | Accepted: 2020-01-22 | Available online: 2020-03-31

https://doi.org/10.15414/afz.2020.23.01.37-41

The aim of the experiment was study the effect of different feeding system on fatty acids (FA) profile of cow's milk. The tank's samples from two farms were collected. On these farms breed: the Slovak Spotted cattle was reared. Feeding system was realized on the base pasture + supplementary feeding without silage – grazing feeding system (farm A) and silage feeding system (farm B). The FA profile in the milk samples with the apparatus (Agilent 6890A GC, Agilent technologies, USA) were analysed. Feeding system affects FA profile of cow's milk. Significantly higher proportion of FA in milk samples: C4:0, C17:0, C18:1 cis-n9, C18:2 cis-n9, C18:3-n3 and C20:0 in milk from grazing feeding system (farm A) was detected. The samples of milk only from this feeding system contained C20:5 n3. Significantly higher content of 18:2 cis n6 and presence of C13:0, C20:3 n6 and C20:4 n6 only in milk samples from silage feeding system were determined. Significantly lower proportion of saturated FA was typical for milk from farm A and significantly higher proportion of polyunsaturated FA was characteristic for the samples from farm B. The influence of the feeding system on the monounsaturated FA content was not confirmed. In milk samples from both feeding systems very different n6/n3 FA ratio was detected, with lower value for milk from grazing feeding system (1.36 vs. 9.12).

Keywords: dairy cattle, milk, fatty acids, feeding system

1 Introduction

Milk and dairy products are traditional foods in human nutrition (Haug et al., 2007; Kubicová and Habánová, 2012; Lorková et al., 2017). Milk contains water, lipids, FA, amino acids, proteins, minerals, and vitamins. It includes the available basic and essential nutrients needed for growth and development for the neonates in population of human's and animal's (Filipejová et al., 2010; Boro et al., 2016). Cow's milk has an average content of 3.5% proteins (80.0% caseins, 20.0% serum proteins), 3.0–4.0% lipids, 4.6% lactose, 1.0% ash (Ca, P, K, Mg, Na), vitamins (particularly thiamin, riboflavin, pyridoxine, tocopherol, retinol, carotenes) and 12.0% of dry matter (Muehlhoff et al., 2013). Slovak spotted breed, one from two national breeds in Slovakia, is combined meat-dairy utility type with medium to larger body frame, harmony structure of body and very good musculature (Kadlečík et al., 2013). According to performance control in 2017 and 2018, the average utility of 6 626 and 6 843 kg of milk with average fat content of 3.95 and 3.93% and 262 and 269 kg production of fat, with an average protein content of 3.39 and 3.40% in protein production 224 and 233 kg for lactation (BSSR 2017; 2018). Genetic and non-genetic factors play a significant effect on the variations of milk yield and their components (Boro et al., 2016; Miluchová et al., 2014; Bujko et al., 2018). In terms of nutrition, not only is the fat content of milk but also its qualitative parameters forcefully on the content and ratio of FA is crucial. Cow's milk is an important source of saturated FA (Bagnicka et al., 2010; Gálik et al., 2011; Szwajkowska et al., 2011). The fat of bovine milk was often associated with cardiovascular disease because of saturated FA (Kajaba et al., 2009; Kalač and Samková, 2010). Changing the composition of FA is a long-term nutrition strategy.

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The forages, although are low in fat content, are the main and cheapest source of unsaturated FA in ruminant nutrition. Current research is focussed on effects of different forages (fresh, silage, haylage) on fat content and FA profile in milk. Intake of pasture herbage rich on grassland and legume species positively affects ratio of FA (unsaturated/saturated) and increases content of conjugated linoleic acid (CLA) and vaccenic acid in cow's milk in comparison with milk from cows fed with conserved forages (hay or silage) (Kalač and Samková, 2010). Milk from cows grazed by pasture with wider biological diversity of plants contains lower portion of saturated FA, higher portion of polyunsaturated FA and it's characterized by lower n6/n3 FA ratio contrast to the milk from housed cows (Martin et al., 2002; Martin et al., 2004; D'urso et al., 2008).

2 Material and methods

2.1 Farm A: grazing feeding system

Farm A operates in Central Slovakia and cultivates 397 hectares of agricultural land in the foothills. The altitude is in the range from 400 to 700 m. above see level. Annual average precipitation in the year 2016 was 750 mm and has a decreasing trend. The average annual temperature reached 8 °C. At the time of the realization of the experiment, 64 cows of Slovak spotted cattle were bred. Daily milk production was about 150 kg and for the entire herd 55,000 kg of milk per year. Milking was provided by Alfa Laval Agri no. 906601-82 series 415 MA, with frequency 2× daily.

2.2 Farm B: silage feeding system

The agricultural grange is located in the south of western Slovakia, a maize production area with an altitude of 150 m above see level. The company managed 1,820 ha of agricultural land in 2016. Average annual precipitation reached 568 mm in 2016 and an average temperature of 9.6 °C. At the time of the research, on the farm were 280 pieces of dairy cattle – Slovak spotted breed with an average daily production of 5,000 kg and an annual production of 1,800,000 kg per year. Milking was realized twice a day in herringbone parlour Fullwood 2 × 10. Type of stabling – free.

2.3 Feeding

2.3.1 Farm A: grazing feeding system

The dairy cows were grazed daily between 6:30 and 15:30. Pasture grassland was from a botanical point of view consisted of 53% of grasses dominated by *Trisetum flavescens*, 4% of legumes with predominant *Trifolium repens*, 40% of other meadow and pasture herbs and 3% of blank places. The botanical evaluation of grassland was done by the method of reduced projective dominance (*D* in %) according to Regal (1956).

At the time off grazing, dairy cows were supplementary fed with a feed ration of 15 kg of meadow hay, 25 kg of fresh clover, 2 kg of feed mixture (50% wheat and 50% barley), and 0.5 kg of molasses. The intake of pasture, water and mineral block was *ad libitum*.

2.3.2 Farm B: silage feeding system

Feed ration (on the base total mixed ration – TMR): maize silage 17 kg, protein-energy feed (residue of corn grain) 6 kg, 8 kg of sugar beet pulps, 8 kg of feed mixture, 2 kg of extracted rapeseed meal, 1 kg of straw and 1 kg of meadow hay was fed by dairy cows. Water intake and mineral block were *ad libitum*.

2.4 Sampling and laboratory analysis

Collection of tank's milk samples (200 ml) from both farms ($n = 12$) was carried out on the 16th of June 2016 (summer feeding season). The content of crude fat and FA profile were analysed in the milk samples. Analyses in Laboratory of Quality and Nutritive Value of Feeds (Department of Animal Nutrition, FAFR, SUA in Nitra) were realized. The crude fat content after acid hydrolysis was determined by extraction according to the Soxhlet principle. For determination of FAME´s (FA methyl esters) was used GC system Agilent 6890A (Agilent technologies). The GC system was equipped with split injection autosampler, DB 23 analytical column (lenght 60 m, diam. 0.250 mm, film 0.15 µm, Agilent technologies) and flame ionisation detector (FID).

2.5 Statistical analysis

Statistical evaluation of results was realized using one-way ANOVA by IBM SPSS v. 20.0. For evaluation of statistical significance between variables (FA and fat content) Tukey test was used.

3 Results and Discussion

Average content of fat in analysed milk samples was 3.38% from farm with grazing feeding system and 3.46% from farm with silage feeding system (Table 1.). Mendoza et al. (2016) also confirmed tend to decrease fat yields with increasing pasture in the diet of dairy cows. Significant ($P < 0.05$) differences between milk samples in the content of butyric acid (C4:0) were found. The higher percentage (2.30%) was found in milk from grazed cows. In the myristic acid (C14:0) percentage, the higher ($P < 0.05$) value was determined in the milk from silage system (12.01%) in comparison with the milk from grazing system (9.04%). Palmitic acid (C16:0) was detected as a major acid in the analysed milk samples from both feeding systems. Differences in palmitic acid

content were not statistically significant. Significance (P <0.05) was determined in heptadecanoic acid (C17:0) with higher concentration in samples from grazing system. Cow's milk is the richest source of oleic acid (24%) (Markiewicz-Keszycka et al., 2013). In C18:1 cis n9 (oleic acid) concentration, the higher (P <0.05) value was found in the milk from farm with grazing feeding system. Morales-Almaráz et al. (2017) confirmed that the concentration of 18:1 cis n9 increase in cow's milk with increasing grazing time. In linoleidic acid (C18:2 cis n6), significant (P <0.05) differences were detected, with higher content in samples from silage feeding system. The TMR feeding system resulted in milk with increased concentrations of C18:2 cis n6 in experiment of O'Callaghan et al. (2016) too. Guler et al. (2010) reported the average total CLA (conjugated linoleic acid) content in milk samples from Turkey 1.02% and Blaško et al. (2010) reported the average CLA content in summer cow's milk from 6 farms in Slovakia 0.08%. In our experiment, the concentration of CLA (C18:2 cis n9) had values 0.35 and 1.19%. Higher (P <0.05) CLA value in samples from grazing feeding system was determined. This is in agreement with the results of Alothman et al. (2019) and Rolinec et al. (2018). The predominant n-3 FA in milk fat of the majority of mammals is α-linolenic acid (Markiewicz-Keszycka et al., 2013). Significantly

Table 1 Fatty acid profile in cow's milk from different feeding system (g 100 g^{-1} FA)

Trait	Grazing feeding system		Silage feeding system	
	mean	S.D.	mean	S.D.
C4:0	2.30*	0.05	1.83*	0.07
C6:0	1.55	0.01	1.78	0.16
C8:0	0.91	0.01	1.31	0.19
C10:0	1.93	0.01	3.37	0.39
C12:0	2.22	0.01	4.14	0.42
C13:0	n.d.	/	0.15	0.01
C14:0	9.04*	0.01	12.01*	0.20
C14:1	0.64	0.01	0.84	0.10
C15:0	1.43	0.01	1.37	0.13
C16:0	27.52	0.04	31.00	1.08
C16:1	1.62	0.01	1.44	0.35
C17:0	0.97*	0.01	0.62*	0.06
C18:0	11.09	0.04	9.05	1.10
C18:1 cis n9	24.60*	0.11	19.76*	0.92
C18:2 cis n6	1.94*	0.01	3.45*	0.03
C18:2 cis n9	1.19*	0.01	0.35*	0.02
C18:3 n3	1.29*	0.01	0.42*	0.01
C20:0	0.22*	0.02	0.14*	0.02
C20:3 n6	n.d.	/	0.13	0.02
C20:4 n6	n.d.	/	0.21	0.02
C20:5 n3	0.13	0.01	n.d.	/
PUFA	3.37*	0.01	4.20*	0.01
MUFA	26.85	0.11	22.04	1.36
SFA	59.17*	0.17	66.78*	1.44
n3/n6	0.73*	0.01	0.11*	0.01
n6/n3	1.36*	0.01	9.12*	0.33
Fat %	3.38*	0.01	3.46*	0.01

FA – fatty acids, PUFA: polyunsaturated FA, MUFA: monounsaturated FA, SFA – saturated FA, S.D. – standard deviation, n.d.: not detected, * – the values with identical superscripts in rows are significantly different at P <0.05

(P <0.05) higher content of α-linolenic acid (C18:3 n3) in milk from grazing feeding system was found. Samples of milk collected from the farm with silage feeding system contained less arachidic acid (C20:0) compared to milk from the farm with different feeding system (P <0.05). Eicosapentaenoic acid (C20:5 n3) was detected only in milk from pasture fed cows. Presence of tridecanoic acid (C13:0), eicosatrienoic acid (C20:3 n6) and arachidonic acid (C20:4 n6) was determined only in milk samples from farm with silage feeding system. The polyunsaturated FA (PUFA) in cow milk represents small proportion, less than 3% of all FA (Lindmark-Månsson, 2008). Differently in the experiment, a PUFA portion of more than 3% in the milk from both types of feeding was found. The effect of different feeding system on PUFA was significant (P <0.05). Higher proportion of PUFA was determined in the milk of cows fed on the base of silage. Similar as in this study, Markiewicz-Keszycka et al. (2013) reported more than 4% of PUFA in cow milk. In MUFA content, non-significant (P >0.05) differences were determined. In saturated FA (SFA) content, a significant (P <0.05) lower content was found in milk samples from farm with grazing feeding system, consistent with Elgersma (2015). According to Hudečková et al. (2011) recommended n6/n3 FA ratio in human and animal nutrition is 5 : 1. Ratio of n6/n3 in samples from farm with grazing feeding system was under this recommendation and from different feeding system was above the recommend ratio (1.36 farm A and 9.12 farm B). The data were consistent with the results of Barca et al. (2018) where n6/n3 ratio was greater in milk of cows fed with TMR than in milk of grazing cows.

4 Conclusions

Milk is an important source of nutrients in animal and human nutrition. Feeding system affects FA profile of cow's milk. Significantly higher proportion of butyric acid, heptadecanoic acid, oleic acid, α-linolenic acid, arachidic acid in milk samples from farm with grazing feeding system (farm A) was detected. Conjugated linoleic acid was more than three times higher in milk from grazing system. Milk samples only from this feeding system contained the eisosapentaenoic acid. The results confirmed that in milk from farm with silage feeding system (farm B) significantly higher content of linoleic acid and presence of tridecanoic acid, eicosatrienoic acid and arachidonic acid was determined only in these samples. Significantly lower proportion of saturated FA was typical for the examined samples from grazing feeding system and significantly higher proportion of polyunsaturated FA was characteristic for the samples from silage feeding system. The influence of the feeding system on the monounsaturated FA content was not confirmed. In milk samples from both feeding systems very different n6/n3 FA ratio was detected (1.36 vs. 9.12, milk from farm A vs. farm B).

Acknowledgement

Thanks to the project "The application of additives in animal nutrition for nutrients transformation improve with the accent on quality, safety and sustainability of animal production" no. 1/0474/19.

References

ALOTHMAN, M. et al. (2019). The "grass-fed" milk story: understanding the impact of pasture feeding on the composition and quality of bovine milk. *Foods*, 8(8), 350.

BAGNICKA, E. et al. (2010). Expression and polymorphism of defensins in farm animals. *Acta Biochimica Polonica*, 57(4), 487–497.

BARCA, J. et al. (2018). Milk fatty acid profile from cows fed with mixed rations and different access time to pastureland during early lactation. *Journal of Animal Physiology and Animal Nutrition*, 102(3), 620–629.

BLAŠKO, J. et al. (2010). Fatty acid composition of summer and winter cows' milk and butter. *Journal of Food and Nutrition Research*, 49(4), 169–177.

BORO, P. et al. (2016). Genetic and non-genetic factors affecting milk composition in dairy cows. *International Journal of Advanced Biological Research*, 6(2), 170–174.

BSSR. (2017). The breeding services of the Slovak republic, s.e. The results of dairy herd milk recording in Slovak republic year 2017. Retrieved January 7, 2020 from http://test.plis.sk/volne/RocenkaMagazin/Rocenka.aspx?id=mlhd2017/

BSSR. (2018). Breeding services of the Slovak Republic, s.e. The results of dairy herd milk recording in Slovak Republic for year 2018. Retrieved January 7, 2020 from http://test.plis.sk/volne/rocenkamagazin/rocenka.aspx?id=mlhd2018/

BUJKO, J. et al. (2018). The impact of genetic and non-genetic factors on somatic cell count as a monitor of udder health in Slovak Simmental dairy cows. *Acta fytotechnica et zootechnica*, 21(4), 166–168. https://doi.org/10.15414/afz.2018.21.04.166-168

D'URSO, S. et al. (2008). Influence of pasture on fatty acid profile of goat milk. *Journal of Animal Physiology and Animal Nutrition*, 92(3), 405–410.

ELGERSMA, A. (2015). Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: A review of the contributing factors, challenges and future perspectives. *European Journal of Lipid Science and Technology*, 117(9), 1345–1369.

FILIPEJOVÁ, T. et al. (2010). Evaluation of selected biochemical milk parameters of dairy cows and their correlations. *Potravinárstvo*, 4, 12–15.

GÁLIK, B. et al. (2011). *Biotechnology and animal food quality*. Nitra: Slovak University of Agriculture in Nitra.

GULER, G. O. et al. (2010). Fatty acid composition and conjugated linoleic acid (CLA) content of some commercial milk in Turkey. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 16*(Supplement-A), 37–40.

HAUG, A., HØSTMARK, A. T. and HARSTAD, O. M. (2007). Bovine milk in human nutrition – a review. *Lipids in health and disease*, 6(25), 1–16.

HUDEČKOVÁ P. et al. (2011). Plants oil in the diet for laying hens. In STRAKOVÁ, E. and SUCHÝ, P. (eds.): *9th Kabrt's dietetic days.* Brno: Tribun EU, 144–147. In Czech.

KADLEČÍK, O. et al. (2013). Diversity of cattle breeds in Slovakia. *Slovak Journal of Animal Science*, 46(4), 145–150.

KAJABA, I. et al. (2009). Role of milk and dairy products in prevention of cardiovascular distribution. In FATRCOVÁ ŠRAMKOVÁ, K. (eds.): *Zobor day and West Slovakia days about osteoporosis 2009*. Nitra: SUA in Nitra, 70–77. In Slovak.

KALAČ, P. and SAMKOVÁ, E. (2010). The effects of feeding various forages on fatty acid composition of bovine milk fat: A review. *Czech Journal of Animal Science*, 55(12), 521–537.

KUBICOVÁ, Ľ. and HABÁNOVÁ, M. (2012). Development of milk consumption and marketing analysis of its demand. *Potravinarstvo Slovak Journal of Food Sciences*, 6(4), 66–72.

LORKOVÁ, M. et al. (2017). Consumption of milk and dairy products in patients with cardiovascular disease. In GÁLIK, B. and ZELINKOVÁ, G. (eds.): *Proceedings of Slovak community for agricultural, forestry, food and veterinary sciences*. Nitra: SUA in Nitra, 98–107. In Slovak.

LINDMARK-MÅNSSON, H. (2008). Fatty acids in bovine milk fat. *Food & Nutrition Research,* 2008, 52.

MARKIEWICZ-KĘSZYCKA, M. et al. (2013). Fatty acid profile of milk-a review. *Bulletin of the Veterinary Institute in Pulawy*, 57(2), 135–139.

MARTIN, B. et al. (2002). Variabilité de la teneur des laits en constituants d'intérêt nutritionnel selon la nature des fourrages consommés par les vaches laitières. *Rencontres Recherche Ruminants*, 9, 347–350.

MARTIN, B. et al. (2004). Effects of grass-based diets on the content of micronutrients and fatty acids in bovine and caprine dairy products. In LÚSCHER, A. et al. (eds.): *Land use systems in grassland dominated regions. Proceedings of the 20th general meeting of the European grassland federation, Zurich: Hochschulverlag* AG an der ETH, 876–886.

MENDOZA, A., CAJARVILLE, C. and REPETTO, J. L. (2016). Intake, milk production, and milk fatty acid profile of dairy cows fed diets combining fresh forage with a total mixed ration. *Journal of Dairy Science*, 99(3), 1938–1944.

MILUCHOVÁ, M., GÁBOR, M. and TRAKOVICKÁ, A. (2014). Analysis of genetic structure in Slovak Pinzgau cattle using five candidate genes related to milk production traits. *Genetika*, 46(3), 863–875.

MORALES-ALMARÁZ, E. et al. (2017). Parity and grazing-time effects on milk fatty acid profile in dairy cows. *Animal Production Science*, 58(7), 1233–1238.

MUEHLHOFF, E., BENNETT, A. and McMAHON, D. (2013). *Milk and dairy products in human nutrition.* Rome: Food and Agriculture Organization of the United Nations (FAO).

O'CALLAGHAN, T. F. et al. (2016). Effect of pasture versus indoor feeding systems on raw milk composition and quality over an entire lactation. *Journal of Dairy Science*, 99(12), 9424–9440.

REGAL, V. (1956). Microscopic method for evaluating feed quality. In *Proceedings ČSAZV, Plant production,* 6, 31 – 40. (in Czech).

ROLINEC, M. et al. (2018). Change of feeding affects fatty acids profile of goat's milk. *Journal of Central European Agriculture*, 19(4), 883–889.

SZWAJKOWSKA, M. et al. (2011). Bovine milk proteins as the source of bioactive peptides influencing the consumers' immune system–a review. *Animal Science Papers and Reports*, 29(4), 269–280.

