

Dragonflies (*Odonata*) of Botanical Garden's Pond of SUA in Nitra

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The faunistic research of dragonflies was realized during 2016 and 2017. The research was carried out under the conditions of Botanical garden's pond of Slovak University of Agriculture (SUA) in Nitra. 229 dragonfly individuals (105♂, 124♀) were trapped during the monitored period. Trapped individuals represented 10 species and 3 families of dragonflies. The aim of the research was to determine the species composition of dragonflies of the selected locality. Based on the representation of individual species for the monitored locality, its dominance was also calculated.

Keywords: dragonflies, *Odonata*, bioindicator, habitat, pond, dominance, climate change

1 Introduction

The dragonflies (*Odonata*) are definitely one of the most obvious and various group of the insects. The biology of nature protection calls the dragonflies as an umbrella species. So, the protection of dragonfly habitats helps to protect the wide spectrum of other aquatic animals with similar requirements on the environment (Noss, 1990; Lambeck, 1997; Hreško et al., 2006). Adult dragonflies are excellent predators and flyers, but larval stages live in the aquatic environment (Holuša, 2013). Dragonfly larvae represent an important intermediate stage of trophic relationships. Larvae are hunted by larger invertebrates and vertebrates and are themselves predators of many aquatic animals (Corbet, 1999). The occurrence of dragonflies can give us a lot of information about the environment and its current state. Some species occur only in an undisturbed environment with original ecosystems (Šácha et al., 2007; Šácha, 2010). The knowledge of dragonfly occurrence can be used to assess the changes in its species spectrum, or to assess the importance and regime of environment where it lives (Holuša, 2013). Hreška et al. (2006) states that results of dragonfly research can be used for revitalization, conservation and legislative measures. The presence of dragonflies helps to indicate the overall status of both aquatic and terrestrial habitats. Various environmental

changes reflect changes in the structure of dragonfly communities. Dragonflies are currently also being used to assess the impact of climate change. For example, the so-called Loosers include *Calopteryx splendens*, whose population density will decrease due to global warming because it is sensitive to oxygen deficiency in water (Beracko et al., 2017). Due to the increasing use of dragonflies for the indication of global climate changes, the aim of the research was to determine the impact of environment on species composition of dragonflies.

2 Material and methods

Faunistic research of dragonflies was carried out under the conditions of the park pond situated in the Botanical Garden of the Slovak University of Agriculture (SUA) in Nitra during 2016 and 2017 from May to August. The Botanical Garden of SUA is a scientific and pedagogical workplace founded on 1st January 1982. Botanical Garden covers an area of 21.2 ha. The collection of the Botanical Garden contains 3,765 taxa and about 1,000 cultivars. In addition to tropical and subtropical species, plants of temperate zone, domestic flora, ornamental plants and crops are represented. The area of the Botanical Garden is of irregular shape. The pond is situated in the middle of Botanical Garden. Shores of pond are slightly inclined and reinforced by concrete blocks. The pond is

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filled by groundwater and rainwater. *Iris pseudacorus*, *Sparganium erectum*, *Phragmites australis*, *Nuphar lutea*, representatives of Typhaceae and Nymphaeaceae plant species there are extended around the pond. The aquatic ecosystem of pond is inhabited by fish, frogs and ducks.

The method of trapping of dragonfly adult individuals by entomological net (Ø 0.40 m, handle length of 1.5 m) was used for the research. The trapping was carried out above the water surface and close to vegetation under the ideal meteorological conditions (clear to cloudy, warm, complete windless or just a light breeze). The biological material was preserved with 96% alcohol. The following keys were used to determine the dragonflies: Askew 1988, Hanel and Zelený (2000), Dijkstra and Lewington (2006), Heidemann and Seidenbusch (1993), Kohl (1998) and Šácha et al. (2008). The classification and nomenclature of dragonflies by Wasscher and Bos (2000) were performed. Based on the representation of individual species of the monitored locality, dominance by Losos (1992) was calculated:

$$D = n_i / N \times 100 \quad (1)$$

where:

- n_i – number of individuals of species i
- N – total number of individuals

According to Tischler (1949) the individual species were included into the dominance classes: 1 – subrecedent (<1%), 2 – recedent (1–2%), 3 – subdominant (2–5%), 4 – dominant (5–10%) and 5 – eudominant (>10%) (Holuša and Vaněk, 2008).

3 Results and discussion

229 (105♂, 124♀) individuals of dragonflies (10 species and 3 families) were trapped and classified during 2016 and 2017. 5 species of dragonflies were classified as Zygoptera suborder and 5 as Anisoptera. The number of identified species represents 14.49% of the total number of species of Slovakia (David, 2013). Eudominant ($D > 10\%$) species were: *Ischnura elegans*, *Coenagrion puella*, *Nallagma cyathigerum* and *Sympetrum sanguineum*. Dominant ($D > 5 < 10\%$) were: *Orthetrum cancellatum*, *Sympetrum vulgatum*, *Lestes barbarus* and *Lestes sponsa*. Subdominant ($D > 2 < 5\%$) species were: *Libellula depressa* and *Sympetrum striolatum*. Recedent and subrecedent ($D < 2$) dragonfly species were not recorded in this experimental locality during the research (Table 1).

Suborder: Zygoptera

Family: Coenagrionidae

Coenagrion puella (Linnaeus, 1758)

Year 2016: 16th May: 2♂, 1♀; 21st June: 5♂, 2♀; 12th July: 3♂, 1♀; 16th August: 3♂, 2♀.

Year 2017: 24th June: 5♂, 2♀; 30th June: 4♂, 1♀; 17th July: 7♂, 5♀; 4th August: 4♂, 3♀; 26th August: 2♂.

Enallagma cyathigerum (Charpentier, 1840)

Year 2016: 16th May: 2♂; 21st June: 3♂, 2♀; 12th July: 2♂, 2♀; 16th August: 2♂.

Year 2017: 24th June: 3♂, 2♀; 30th June: 2♂, 3♀; 17th July: 2♂, 2♀; 4th August: 2♂; 26th August: 1♂, 1♀.

Ischnura elegans (Vander Linden, 1820)

Year 2016: 16th May: 3♂, 1♀; 21st June: 5♂, 3♀; 12th July: 4♂, 2♀; 16th August: 3♂, 3♀.

Year 2017: 24th June: 4♂, 3♀; 30th June: 5♂, 3♀; 17th July: 3♂, 2♀; 4th August: 2♂, 1♀; 26th August: 1♂, 1♀.

Table 1 Representation of dragonfly species and its dominance in 2016 and 2017

Species	2016	D (%)	2017	D (%)	Σ
<i>Coenagrion puella</i> (Linnaeus, 1758)	19	17.75	33	27.05	52
<i>Enallagma cyathigerum</i> (Charpentier, 1840)	13	12.15	18	14.75	31
<i>Ischnura elegans</i> (Vander Linden, 1820)	24	22.43	25	20.49	49
<i>Lestes barbarus</i> (Fabricius, 1798)	7	6.54	5	4.09	12
<i>Lestes sponsa</i> (Hansemann, 1823)	6	5.61	8	6.56	14
<i>Libellula depressa</i> (Linnaeus, 1758)	5	4.67	3	2.46	8
<i>Orthetrum cancellatum</i> (Linnaeus, 1758)	8	7.47	7	5.74	15
<i>Sympetrum sanguineum</i> (Müller, 1764)	13	12.16	10	8.19	23
<i>Sympetrum striolatum</i> (Charpentier, 1840)	4	3.73	7	5.74	11
<i>Sympetrum vulgatum</i> (Linnaeus, 1758)	87.49	6	4.93	14	
Total	107	100.00	122	100.00	229

Family: Lestidae

Lestes barbarus (Fabricius, 1798)

Year 2016: 16th May: 1♂; 21st June: 2♂, 1♀; 12th July: 2♂; 16th August: 1♂.

Year 2017: 24th June: 1♂, 1♀; 30th June: 1♂; 4th August: 1♂; 26th August: 1♂.

Lestes sponsa (Hansemann, 1823)

Year 2016: 21st June: 1♂; 12th July: 2♂, 1♀; 16th August: 1♂, 1♀.

Year 2017: 24th June: 2♂; 30th June: 2♂, 1♀; 17th July: 2♂; 26th August: 1♂.

Suborder: Anisoptera

Family: Libellulidae

Libellula depressa (Linnaeus, 1758)

Year 2016: 21st June: 1♀; 12th July: 2♂, 1♀; 16th August: 1♂.

Year 2017: 17th July: 3♂.

Orthetrum cancellatum (Linnaeus, 1758)

Year 2016: 21st June: 2♂, 1♀; 12th July: 2♂; 16th August: 2♂, 1♀.

Year 2017: 30th June: 2♂; 17th July: 1♂, 1♀; 4th August: 2♂; 26th August: 1♀.

Sympetrum sanguineum (Müller, 1764)

Year 2016: 16th May: 2♂; 21st June: 3♂, 1♀; 12th July: 2♂, 2♀; 16th August: 2♂, 1♀.

Year 2017: 24th June: 2♂, 1♀; 30th June: 2♂; 17th July: 1♂; 4th August: 2♂, 1♀; 26th August: 1♂.

Sympetrum striolatum (Charpentier, 1840)

Year 2016: 12th July: 2♂; 16th August: 1♂, 1♀.

Year 2017: 30th June: 1♂, 1♀; 17th July: 2♂; 4th August: 2♂, 1♀.

Sympetrum vulgatum (Linnaeus, 1758)

Year 2016: 21st June: 3♂, 1♀; 12th July: 2♂, 1♀; 16th August: 1♂.

Year 2017: 24th June: 1♂, 1♀; 17th July: 2♂, 1♀; 4th August: 1♂.

Most of the classified dragonfly species of the monitored locality were typically stagnicolous. Stagnicolous species inhabit various types of aquatic habitats of calm waters (David and Ábelová, 2015). *Ischnura elegans*, *Libellula depressa* and *Orthetrum cancellatum* were classified as eurytopic dragonfly species. Eurytopic species inhabit different habitats and can survive under the different environmental conditions. Monitored locality is not

a habitat with running water, species typical for such localities were not identified.

The vegetation species composition and vegetation cover of aquatic habitats is an important environmental factor influencing dragonflies (Hreško et al., 2006). Submerge and natant water vegetation presence is important for dragonfly egg laying and its development. The dragonfly species (also called as thermophilic species) of calm eutrophic water habitats of lowlands to uplands prefer overheated water, such as small water reservoirs, flooded sand pits, gravel pits, etc.

The presence of dragonflies can be considered as an expression of the favourable state and stability of the natural ecosystem. The largest number of dragonfly species live in habitats that consist of a wide range of micro habitats with different environmental characteristics. Olberg et al. (2000) states that Anisoptera responds much more strongly to ecosystem pollution than Zygoptera. It was found that environmental interventions and changes of environmental quality caused changes in the species spectrum of odonatocenoses in practice (Sahlén and Ekestubbe, 2001; Wildermuth, 2001; Foote and Hornung, 2005; Butler and Demaynadier, 2008; Simaika and Samways, 2008; Harabiš and Dolný, 2010). On the basis of the above it can be stated that common species of dragonflies typical for calm waters were recorded in the monitored locality.

The Palearctic species (*Coenagrion puella*, *Lestes sponsa*, *Libellula depressa*, *Orthetrum cancellatum*, *Sympetrum striolatum*, *Sympetrum vulgatum*), Circumboreal species (*Enallagma cyathigerum*) and Eurosiberian species (*Ischnura elegans*, *Lestes barbarus*, *Sympetrum sanguineum*) there are widely spread on the Earth (Šácha et al., 2008).

4 Conclusions

During the years 2016 and 2017 was carried faunistic research of dragonflies. Were trapped 229 individuals of dragonflies and 10 species were classified. Most of the classified dragonfly species were typically stagnicolous. Common dragonfly species of calm waters were recorded in the monitored locality. Eudominant species were: *Ischnura elegans*, *Coenagrion puella*, *Enallagma cyathigerum* and *Sympetrum sanguineum*. Typical species of running water habitats were not identified during the monitored period. Research of dragonflies is important in terms of its species diversity, which confirms the favourable status of habitats. The results showed that none of the identified dragonfly species indicates climate change.

5 References

- ASKEW, R. R. (1988) *The dragonflies of Europe*. Colchester: Harley Books, 291 p.
- BERACKO, P. et al. (2017) *Bentic invertebrates and its habitats*. Bratislava: Faculty of Natural Sciences of Comenius university, 291 p. (in Slovak).
- BUTLER, R. G. and DE MAYNADIER, P. G. (2008) The significance of littoral and shoreline habitat integrity to the conservation of lacustrine damselflies (*Odonata*). In *Journal of Insect Conservation*, vol. 12, pp. 23–36.
- CORBET, P. S. (1999) *Dragonflies: behavior and ecology of Odonata*. New York: Cornell University Press, 829 p.
- DALECKÝ, V. (2011) Influence of landscape structure on bionomics of forest species of reophilic dragonflies. Bachelor thesis. Brno: Mendel University, 63 p. (in Czech).
- DAVID, S. (2013) Annotated Checklist of dragonflies (*Odonata*), Slovakia. In BRYJA, J. (eds.): *Zoological days. Brno 2013: Abstracts from the conference*. Brno: Mendel University, pp. 1–52.
- DAVID, S. and ÁBELOVÁ, M. (2015) Dragonflies (*Odonata*) of the Protected Area Mlyňany Arboretum. In *Folia faunistica Slovaca*, vol. 20, no. 2, pp. 135–139.
- DIJKSTRA, K. D. B. and LEWINGTON, R. (2006) *Field guide to the dragonflies of Britain and Europe including western Turkey and north-western Africa*. London: British Wildlife Publishing, 320 p.
- FAŠKO, P. and ŠTASTNÝ, P. (2002) Average annual rainfall. In Zaňko, M. (eds.) *Initial landscape structure. Atlas of the Slovak Republic*. Banská Bystrica: Ministry of the Environment of Slovak Republic, Slovak Agency of Environment, 344 p. (in Slovak).
- FOOTE, A. L. and HORNUNG, C. L. R. (2005) Odonates as biological indicators of grazing effects on Canadian prairie wetlands. In *Ecological Entomology*, vol. 30, pp. 273–283.
- HANEL, L. and ZELENÝ, J. (2000) Dragonflies (*Odonata*): research and protection. Vlašim: Czech Union for Nature Conservation, 240 p. (in Czech).
- HARABIŠ, F. and DOLNÝ, A. (2010) Ecological factors determining the density-distribution of Central European dragonflies (*Odonata*). In *European Journal of Entomology*, vol. 107, pp. 571–577.
- HEIDEMANN, H. and SEIDENBUSCH, R. (1993) *Die Libellenlarven Deutschlands und Frankreichs*. Handbuch für Exuviansammler. Keltern: Verlag Erna Bauer Keltern, 391 p.
- HOLUŠA, O. and VANĚK, J. (2008) Fauna of Dragonflies (*Odonata*) Krkonoš. In *Opera Corcontica*, vol. 45, pp. 81–98 (in Czech).
- HOLUŠA, O. (2013) Taxonomy, ecology and zoogeography of Cordulegaster dragonflies (*Odonata*: Corgulegastridae) in Central Europe. Dissertation thesis. Bratislava: Comenius University, 179 p. (in Slovak).
- HREŠKO, J. et al. (2006) Nitra and its surroundings – Initial phase of research. Scientific Monograph. Nitra: Constantine the Philosopher University, 182 p. (in Slovak).
- KOHL, S. (1998) *Odonata. Anisoptera – Exuvien (Grosslibellen-Larvenhäute) Europas. Bestimmungsschlüssel*. Berlin: Kohl, 27 p.
- LAMBECK, R. J. (1997) Focal species: A multispecies umbrella for nature conservation. In *Conservation Biology*, vol. 11, no. 4, pp. 849–856.
- LOSOS, B. (1992) *Exercise of animal ecology*. Brno: Masaryk University, 229 p.
- NOSS, R.F. (1990) Indicators of monitoring biodiversity: A hierarchical approach. In *Conservation Biology*, vol. 4, pp. 355–364.
- OLBERG, R.M. et al. (2000) Prey Pursuit and Inception in Dragonflies. In *Journal of Comparative Physiology A: Sensory Neural and Behavioral physiology*, vol. 186, pp. 155–162.
- SAHLÉN, G. and EKESTUBBE, K. (2001) Identification of dragonflies (*Odonata*) as indicators of general species richness in boreal forest lakes. In *Biodiversity and Conservation*, vol. 10, pp. 673–690.
- ŠÁCHA, D. et al. (2007) *Dragonflies of Slovak Republic*. [Online]. Retrieved 2019-03-20 from <http://www.vazky.sk>, 10/2008 (in Slovak).
- ŠÁCHA, D. et al. (2008) *The key to identifying our species of dragonflies*. [Online]. Retrieved 2019-05-12 from <http://www.vazky.sk> (in Slovak).
- ŠÁCHA, D. 2010. Dragonflies (*Odonata*) detected during „Monitoring of species of European importance“ in southern Slovakia. In *Folia faunistica Slovaca*, vol. 15, no. 6, pp. 43–46 (in Slovak).
- SIMAİKA, P. and SAMWAYS, M. J. (2008) *Valuing dragonflies as service providers*. In Córdoba-Aguilar A. (eds.): *Dragonflies: Model Organisms for Ecological and Evolutionary Research*. Oxford: Oxford University Press, pp. 23–55.
- TISCHLER, W. (1949) *Basic features of terrestrial animal ecology*. Wiesbaden: Springer trade media, 220 p. (in German).
- WASSCHER, M. T. and BOS, F. G. (2000) The European dragonflies: notes on the checklist and on species diversity. In *Odonatologica*, vol. 29, pp. 31–43.
- WILDERMUTH, H. (2001) The rotation model for the care of small bog waters. In *Conservation and Landscape Planning*, vol. 33, pp. 269–273 (in German).

Performance of maize (*Zea mays* L.) cultivars and community structure of Arbuscular Mycorrhizal Fungi in response to tillage practices and soil amendments in a derived Savanna

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Arbuscular mycorrhizal fungi (AMF) are often negatively affected in agro-systems. This investigation tested the hypothesis that community structure of AMF would vary in soil sown with maize (*Zea mays* L.) and amended with inorganic NPK fertilizer and tillage practice; that varietal variation of maize would have implication on their performance in a derived savanna. Field experiments were conducted at the Teaching and Research Farm, Federal University of Agriculture, Abeokuta in the early and late cropping seasons of 2013. Three tillage practices [conventional (CT), minimum (MT) and zero (NT)] were imposed on maize cultivars (Oba super 2 and Swan 1) in soil amended with NPK fertilizer (120 kg N/ha + 60 kg P₂O₅/ha + 60 kg K₂O/ha) and no fertilization. The treatments were in split-split arrangement fitted into randomized complete block design, replicated thrice. The main plot consisted of tillage practises, the sub plot consisted of maize varieties, while the sub-sub plot was made of soil amendments. *Glomus* was identified in the soil in the order NT > MT > CT (early and late seasons of 2013). Similar pattern was observed on specie richness (late season), specie evenness and diversity (both seasons). Spore count, percentage AMF colonization, specie richness, evenness and diversity were significantly higher in non-amended soil than amended. Significantly higher spore count was observed in the rhizosphere of Oba super 2 than Swan 1. Conversely more *Acaulospora* was observed in Swan 1 than Oba super 2. These evidences suggested that NT supported enriched community structure of AMF with a predominance of *Glomus*. Conversely, amending soil with NPK in this agroecology reversed this pattern, except for *Glomus*. Improved performance of maize in amended soil could have implied complimentary role of *Glomus* apart from nutritional. Cultivar differences of maize and seasons could have explained variation in species diversity of AMF in a derived savanna.

Keywords: *Acaulospora*, *Glomus*, NPK, spore count, Shannon-diversity index

1 Introduction

A change in demographic profile of the populace is going to compromise the food and nutritional security of most people, especially in the sub-Sahara region of Africa (SSA). Maize is one of the most cultivated crop in the continent that is used by man, animal and industry to meet their needs. Considering the abovementioned problem there is the need to cultivate maize intensively to increase its productivity per unit area. Tillage and application of synthetic soil amendments form a very important component in this quest for intensive cultivation of maize in the continent. Despite its agronomic advantages, in

the long run tillage practices might not be sustainable. Comparatively, it had been reported that under no-till there is an improvement in the community structure of Arbuscular mycorrhizal fungi (AMF) (Melero et al., 2009), reduced hyphae breakdown and increased fungi biota in agroecosystem (Drijber et al., 2000). The presence of AMF in soils have been reported to be associated with improved nutrition (P, Zn, and Cu uptake) (Evans and Miller, 1988), and other beneficial non-nutritional effects (improved drought tolerance, enhances resistance to pest and diseases and better soil structure) (Gosling et al. 2006). The effect of tillage on the colonization of the root

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however is dependent on the soil type (Kabir et al., 1999; Mulligan et al., 1985). Few studies have been conducted in the derived Savanna on the implication of tillage on AMF community structure and infectivity of roots in soils cultivated under hybrid maize. Furthermore there have been inconsistent reports on the role of AMF in most agroecosystems (Ryan and Graham, 2002). Modern maize hybrid have been posited to be less dependent on AMF but responded better to application of inorganic fertilizer than the presence of AMF (Hetrick et al., 1993). When examined in the context of the presence of time of AMF infection, early infestation of most arable crops by AMF have been linked to reduced performance (Ryan and Graham, 2002), despite their dependency on AMF (Daniell et al., 2001). The need to resolve these conflicting reported justified the implementation of this investigation.

Application of inorganic fertilizer remains one of the sources of nutrients to maize. Other sources though desirable in the long run due to their sustainability, in the short term might not be economically attractive to farmers. There have been studies of their effects on AMF in the literature. Jensen and Jakobsen (1980) reported that application of inorganic fertilizer would reduce reliance of cultivated crops on AM symbiosis. Kahiluoto et al. (2001) indicated that under fertiliser application there would be a reduction in propagules density that would decrease AM colonization of the arable crops like maize. Furthermore and Johnson (1993) showed that increased inorganic fertilizer application would reduce AM sporulation and reduce AM community structure. Given the above evidences Wang et al. (2011) varied the combination of NPK together with other nutrient sources in their investigation to ascertain their efficacy on AM spore density and their species composition. Their investigation concluded that balanced NPK could be desirable for good yield and AM specie composition. Empirical evidence is lacking to validate this fact in other agroecological systems with different test crops, such as maize. More so, Davison et al. (2011) reported contrasting results of host preferences of AMF. Extending this logic further on the effect of cultivar differences on host preferences in a derived Savanna could allow us to further gain insight on the host-AMF symbiosis in maize in a derived Savanna.

This investigation tested the hypothesis that there would be a significant varietal variation on AMF community structure and the performance of maize cultivars in a derived Savanna. It hypothesized that tillage practices and inorganic N application would have significant effect on the AMF community structure and performance of maize cultivars in a derived Savanna.

2 Material and methods

2.1 Characterisation of site and location

Two field experiments were conducted during the early and late wet seasons of 2013 at the Teaching and Research Farms, Federal University of Agriculture, Abeokuta, Nigeria. The location lies in the South-Western Nigeria (Latitude 7° 15' N, Longitude 3° 28' E, 75 m a.s.l.). The highest rainfall was observed in May and June (128.2 mm) in early and August (202.6 mm) in the late season. The maximum temperature during the cropping season was observed in March (29.8 °C), while the minimum was observed in August (24.3 °C). The textural class (USDA textural triangle) of the soil was sandy with a pH (McLean, 1982) that was strongly acidic (5.34). It has total nitrogen (Jackson, 1962) of 2.16 mg/kg and available phosphorus (Bray and Kurtz, 1945) of 4.0 mg/kg with soil organic carbon (Allison, 1965) of 1.76%.

2.2 Experimental treatments and design

The field experiments consisted of maize varieties [Oba Super 2 (hybrid) and Suwan-1 (open pollinated)] established under different tillage practises [conventional tillage (CT), minimum tillage (MT) and no-tillage (NT)] and amended with inorganic NPK fertilizer (120 kg N/ha + 60 kg P₂O₅/ha + 60 kg K₂O/ha) and without. The treatments were in split-split plot arrangement fitted into randomised complete block design replicated three times. Tillage methods were in the main plot. The sub plot consisted of the maize varieties with sub-sub plot made of soil amendments. The seeds were sourced from the Ogun State Ministry of Agriculture, Nigeria. Oba Super 2 is a single cross maize hybrid, with a maturity of 110 days on the average. Suwan-1 is an open pollinated variety (OPV) developed in Thailand.

2.3 Cultural operations

Conventional tillage was achieved through maximum soil disturbance. Ploughing and harrowing was conducted twice and once respectively. Conversely, minimum tillage was achieved through relatively minimal soil disturbance with hand hoe. The soil was left undisturbed throughout the duration of the experiments under no-tillage. The gross plot size was 5 × 5 m (25 m²) and net plot size of 3 × 3 m (9 m²). Sowing of maize seeds was conducted on May 5 and August 18, 2013 for early and late season experiments respectively. Planting was done at a spacing of 0.75 × 0.25 m at one plant per stand to give a plant population of 53,333 plants per hectare and 140 plants per plot. Conventionally and minimally tilled plots were weeded with hoes at 3 and 6 weeks after planting in both seasons while zero tilled plots were kept weed free with the application Atrazine at 3.5 kg a.i/ha as pre-emergence herbicide at planting, while Glyphosate

applied before planting. Fertiliser was applied at 120 kg N/ha+ 60 kg P₂O₅/ha + 60 kg K₂O/ha) using inorganic NPK fertilizer (NPK 20 : 10 : 10) at 600 kg/ha at two weeks after planting (2WAP). Harvesting was done on the August 10 and November 30, 2013 for early and late season trial respectively.

2.4 Sampling and data collection

2.4.1 Soil sampling

A composite soil sample was collected randomly from the depth of 0–20 cm. It was later air dried and sieved before the evaluation of soil physical and chemical properties.

2.5 Preparation of soil and root samples

Soil samples were collected the depth of 0–20 cm from each plots. Collected root samples were rinsed in water and preserved in 50% ethanol solution. Root samples were prepared using the methods modified by Phillips and Hayman (1970). Preserved roots were washed free of ethanol, bleached in 10% KOH at 90 °C for 30 minutes. The bleached roots were rinsed to remove excess KOH and stained in acidic glycerol containing methyl blue lacto-glycerol (1 : 1 : 1 : 0.5 g) at 90 °C for 2 minutes. Prepared root samples were rinsed off staining solution with clean tap water, cut into 1 cm pieces. They were preserved with 40% glycerol solution for further viewing under compound microscope to determine percentage root colonisation.

2.6 Isolation of spores from soil and identification of AMF

Extraction of spores from soil was done using the wet sieving method described by Gerdemann and Nicolson (1963). Twenty grams of soil samples were collected from each plot and air-dried. The samples were wet sieved in order of decreasing fineness. Screenings from the sieves were washed into 50 ml centrifuge tubes using a small stream of distilled water. Tubes were centrifuged at 4000 rpm for 2 minutes. The supernatant was decanted and replaced with 50% sucrose solution, then centrifuged at earlier mentioned conditions. Spores were extracted from the supernatant by washing off sucrose solution and pouring them over a clean 0.045 mm sieve. AMF spores were collected in clean sample bottles and spores were counted under a dissecting microscope at ×80 magnification. Each spore type was mounted in polyvinyl-lactic acid-glycerine (PVLG) (Koske and Tessier, 1983) and the PVLG mixed with Melzer's reagent (Brundrett and Abbott, 1994). The spores were identified at the genus level on the basis of size, spore-wall structure, Melzer's reaction, colour and presence or absence of subtending hyphae and compared with descriptions of fungal genera according to taxonomic criteria (Pérez and Schenck, 1990).

The assignment of AMF morphotypes to families followed the consensus classification of Redecker et al. (2013).

2.7 Data collection

Spore count and percentage AMF colonisation were sampled at 2, 4, 6, 8 and 12 WAP in both seasons. The total number of spore per sample was evaluated as the presence of spores from all the species in a sample. Relative species abundance was determined as the ratio of the number of spores from a particular species to the total number of spores recovered from the soil sample. Community structure of AMF was evaluated as the product of species diversity, richness and evenness. The Shannon Wiener's diversity index (H) (Shannon and Weaver, 1949) was used to determine the diversity of AMF species in each sample. Species richness was represented as total number of species recovered in each site. Species evenness was computed by dividing the Shannon diversity by the log of species richness (Abdelhalim, Finckh, Babiker and Oehl, 2014). Percentage AMF colonisation was determined according to (Giovannetti and Mosse, 1980).

2.8 Grain yield

Harvesting was conducted at harvest maturity. Grain yield was evaluated from the net plot in the middle rows with an area of 3 × 3 m and extrapolated to grain yield kg/ha.

3 Results and discussion

Tillage had significant effect on the spore count at 6 WAP in the late season (Table 1). Tillage effect on the spore count was in the order zero > minimum > conventional tillage. Similar pattern was observed on the percentage AMF colonisation at 2 WAP (early and late seasons) and 6 WAP (late season) (Table 2). Conversely it was observed that *Glomus* was the most AMF specie (early and late season) in the order Conventional > minimum > zero tillage (Table 3). In the late season specie richness was in the order zero > minimum > conventional tillage (Table 4). Similar pattern was observed on specie evenness (both seasons) (Table 5) and species diversity (both seasons) (Table 6).

Inorganic fertiliser had a significant effect on spore count at all period of investigation except at 2 WAP (Table 1). Significantly higher spore count was observed in soil without soil amendments than plots with the application of NPK fertiliser. Similar pattern was observed on percentage AMF colonisation at both seasons in the 4 WAP, 6 WAP (early season), 8 WAP (late season) and 10 WAP (both seasons) (Table 2). At both seasons *Glomus* was significantly higher in soil amended with NPK than those without (Table 3). Conversely, in both seasons significantly higher *Gigaspora* species was observed in soils without soil amendments than those amended

Table 1 Effect of tillage, inorganic fertiliser application rate on the spore count of AMF in soils sown with maize variety (early and late planting season, Abeokuta)

Treatment	2 WAP		4 WAP		6 WAP		8 WAP		10 WAP	
	early	late	early	late	early	late	early	late	early	late
Tillage (T)										
Conventional	6.50	7.42	33.83	30.00	64.92	77.7	122.6	98.3	137.8	99.8
Minimum	12.67	16.67	33.75	33.67	74.83	113.9	124.5	130.9	140.3	127.2
Zero	9.42	9.42	28.92	28.42	67.50	116.5	105.5	131.9	124.9	131.3
LSD	4.412	4.254	NS	NS	NS	23.76	NS	0.2441	NS	15.99
Fertilizer (F)										
without	9.39	9.72	34.67	33.67	74.50	116.1	126.2	133.6	143.2	132.9
NPK fertilizer	9.67	9.094	29.67	27.72	63.67	89.3	108.8	107.1	125.4	105.9
LSD	NS	NS	4.903	5.331	6.981	9.46	14.4	9.66	16.06	7.64
Variety										
Oba super 2	9.22	9.61	32.39	31.33	68.56	104.2	121.6	122.8	141.3	121.3
Suwan 1	9.83	10.06	31.94	30.06	69.61	101.2	113.5	117.9	127.3	117.5
LSD	NS	NS	NS	NS	NS	NS	7.17	5.88	5.18	4.96
T × F	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T × V	NS	NS	*	NS	NS	NS	NS	NS	NS	NS
F × V	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T × F × V	NS	NS	*	NS	NS	NS	NS	NS	NS	NS

NS – not significant, LSD – least significant difference, WAP – weeks after planting, * – significant at 5% probability level, NPK fertilizer (120 kg N/ha + 60 kg P₂O₅/ha + 60 kg K₂O/ha)

with NPK. Similar pattern was observed on *Acaulospora* species in the early season (Table 3). In both seasons unidentified AMF was observed to be significantly higher in unamended soil than amended with NPK. The order of specie richness and evenness at both seasons was unamended soil >NPK (Tables 4 and 5). Similar pattern was observed on species diversity at both seasons (Table 6). Conversely, grain yield at both seasons was in the order NPK> unamended soil (Table 7).

Significant varietal variation was observed on spore count at both seasons in 8 and 10 WAP (Table 1). Significantly higher spore count was observed in soils that had hybrid maize sown than those sown with OPV. Conversely, in the early season significantly more *Acaulospora* was observed in soils where OPV was sown than those with hybrid maize (Table 2).

Tillage and application of synthetic input form an integral component of intensification of agroecosystem. Under zero tillage the significantly increased spore count of AMF observed at 6 WAP (late season) could have been associated with the significantly higher AMF colonization than other tillage practices in the same period. Under conventional tillage the reduced root colonization could

also be linked with soil inversion, burying of propagules (Kabir, 2005) and reduced volume of soil exploited by AMF (Evans and Miller 1988). Under zero tillage the increased spore count observed could also have been linked with the species diversity. While observed increased specie evenness and richness under zero tillage could have suggested increased species diversity under this tillage practice. Changes in the community structure under zero tillage could also be associated with increased labile carbon (Melero et al., 2009), reduced fluctuation in temperature (Zhang et al., 2014) and increased enzymic activities together with nutrient turnover (Frey, Elliott, and Paustian, 1999). Specie richness was observed in this study to be more in the late season than in the wet season. Similar observation was made by Tchabi et al. (2008). In their study they observed that specie richness decreased with increasing wet seasons in the tropics. Their observed specie richness was in the order Sudan > Northern guinea > Southern guinea. This could be explained by the availability of water and nutrients in the soil. Dry season is characterized by reduced precipitation and nutrient availability compared to the wet season. AMF species survive mostly in marginal soils with low fertility

Table 2 Effect of tillage, fertilizer application and variety on mycorrhizal colonization (%) of maize in 2013 early and late planting

Treatment	2 WAP		4 WAP		6 WAP		8 WAP		10 WAP	
	early	late	early	late	early	late	early	late	early	late
Tillage (T)										
Conventional	0.00	0.00	9.90	10.27	54.10	54.1	66.6	67.1	75.51	72.2
Minimum	1.11	1.66a	10.01	10.01	46.90	46.9	72.3	70.8	81.62	79.7
Zero	1.66	2.50a	8.88	8.33	47.70	38.0	62.00	53.6	73.86	68.3
LSD	0.227	1.090	NS	NS	NS	7.00	NS	NS	NS	NS
Fertilizer (F)										
without	1.29	1.66	11.66	11.29	54.0	49.4	71.2	65.7	83.11	76.8
NPK fertilizer	0.55	1.11	7.59	7.77	45.1	43.3	62.8	61.9	70.89	70.0
LSD	NS	NS	1.808	2.169	8.12	NS	NS	15.85	3.52	5.07
Variety										
Oba super 2	0.55	1.29	9.27	9.08	52.2	48.3	68.4	65.7	77.2	72.5
Suwan 1	1.29	1.48	9.99	9.99	46.9	44.3	65.6	61.9	76.8	74.2
LSD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T × F	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T × V	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
F × V	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T × F × V	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS – not significant, LSD – least significant difference, WAP – weeks after planting,* – significant at 5% probability level, NPK fertilizer (120 kg N/ ha + 60 kg P₂O₅/ha + 60 kg K₂O/ha)

status (Ratnayake, Leonard and Menge, 1978). The most predominant specie of AMF under conventional tillage was *Glomus*. Increasing land intensification resulted in changes in specie composition in the order *Glomus* > *Aculospora* > *Gigaspora* in the study conducted by Tchabi et al. (2008). The persistence of *Glomus* specie under land intensification was reported to be linked with the speed and intensity of their spore formation and their rapidity in colonizing the root (Oehl et al., 2003). Other reasons provided for the survival of *Glomus* species under conventional tillage was linked with anastomosis, the fusion of their hyphae under soil disturbance. Increased anastomosis of *Glomus* species under conventional tillage had been associated with reduced disruption of hyphae network (Sbrana et al., 2011).

The percentage of AMF colonization in the root of maize with no fertilizer application observed in the early and late seasons of the year under investigation could have been explained by the spore count during those periods. Similar observation was made by Jensen and Jakobsen (1980), where they reported that under soil amendments there was reduced reliance on AMF symbiosis in crops as indicated in the reduced percentage of AMF colonization

and reduced propagules density (Kahiluoto et al., 2001; Treseder and Allen, 2002), which resulted in reduced sporulation (Johnson, 1993). These earlier reported response pattern of AMF under inorganic fertilizer application have been reported not to be general in all cases as reported by Jumpponen et al. (2005). This could be explained by the dependency of the crop on AMF symbiosis (Evans and Miller, 1988), soil type and its nutrient availability (Titus and Lepš, 2000), the combination of nutrients involved and the length of soil amendment from inorganic sources. Wang et al. (2011); Wang et al. (2008) had reported that increase in spore density under long term fertilization of the field in China was in the order NP > PK > NPK. It could be speculated that such pattern of response of spore density of AMF could also have been displayed under short term soil amendment from inorganic sources in this study. There is need to provide empirical validation for the effect of different combination of macronutrients under short term studies in this agroecology. Hepper (1983) posited that in long term study of the effect of inorganic soil amendments on AMF spore density the presence of N and K could have offset the negative effect of high P in

Table 3 Effects of tillage, fertilizer and variety on AMF spore abundance (%) of maize in 2013 early and late planting season

Treatment	Glomus		Gigaspora		Acaulospora		Scutellospora		Unidentified	
	early	late	early	late	early	late	early	late	early	late
Tillage (T)										
Conventional	76.8	87.6	6.75	5.59	8.77	7.68	7.24	3.98	0.77	0.85
Minimum	67.4	73.5	7.48	7.80	12.94	13.41	9.91	9.40	2.06	1.74
Zero	52.8	56.6	10.58	10.25	16.66	15.01	13.09	10.16	3.38	3.30
LSD	8.24	13.46	NS	NS	NS	NS	NS	NS	NS	NS
Fertilizer (F)										
without	59.1	63.9	10.70	9.36	14.47	12.45	11.0	8.71	2.85	2.64
NPK fertilizer	72.2	81.2	5.72	6.40	11.10	11.61	9.05	6.98	1.29	1.29
LSD	4.52	13.96	3.87	2.53	3.45	NS	NS	NS	0.3226	0.602
Variety										
Oba super 2	66.5	72.3	7.96	8.37	11.85	11.56	10.48	9.09	2.495	2.10
Suwan 1	64.8	72.8	8.46	7.39	13.59	12.50	9.67	6.61	2.446	1.82
LSD	7.40	NS	NS	NS	1.96	NS	NS	NS	NS	NS
T × F	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T × V	NS	NS	NS	NS	**	NS	NS	NS	NS	NS
F × V	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T × F × V	NS	NS	NS	NS	*	NS	NS	NS	NS	NS

NS – not significant, LSD – least significant difference, WAP – weeks after planting, * – significant at 5% probability level, ** – significant at 1% probability level, NPK fertilizer (120 kg N/ha+ 60 kg P₂O₅/ha + 60 kg K₂O/ha)

the soil. Contrary to the short term effect of inorganic fertilizer on AMF spore density, the observed increase of this variable was explained to be due to the effect of inorganic fertilizer on growth of the host crop. Improved growth under this condition would have increased the supply of carbon to AMF symbionts, consequently the observed increase in AMF spore density under long term effect. Probably the presence of nutrient combination in this study could not offset the negative effect of the presence of P in the nutrient combination. Alternative explanation could be the acidic nature of the soil could have affected spore count in both seasons with the application of inorganic NPK. This observation was corroborated by the findings of Mohammad et al. (2003) in their studies. The observed changes in the community structure under NPK amendment could be associated with the specie richness and evenness compared to that of soil that was not amended in this study. Wang et al. (2011) reported that application of inorganic soil amendments in China resulted in a significant decrease in specie richness and diversity of AMF. Liu et al. (2009) further indicated that changes in community structure of AMF in their study were dependent on host phenology, edaphic factors and the habitat of host. The soil samples

taken in this experiment were at the vegetative growth stage. It had earlier been reported by Schalamuk et al. (2006) that AMF species diversity increases with the growth stage. This factor could have confounded the effect of inorganic NPK amendment on the community structure in this experiment. The pH of the soil and its particle distribution are some of the edaphic factors that could have combined with NPK application in changing the community structure of AMF compared with unamended soil. Thompson (1991) reported that the activities of AMF are most pronounced in alkaline, heavy clay soil. The soil under this investigation was acidic with sandy textural class. These two factors could predispose the soil to reduced AMF species diversity compared to unamended soil in this agroecology. In this study *Acaulospora* specie of AMF was observed to be predominant in unamended soil in the early season. Tchabi et al. (2007) had earlier reported that duration of the dry season significantly changed the species diversity in favor of *Gigasporaceae* while that of the *Acaulospora* and *Glomus* were reduced. This could have explained the observed pattern of species in our study with respect to the seasons. *Acaulospora* was significantly higher than other species of AMF under no soil amendments than

Table 4 Effects of tillage, fertilizer and variety on AMF species richness, evenness, Shannon Weiner diversity index and grain yield in 2013 early and late planting season

Treatment	Species richness (S)		Species evenness (E)		Shannon Weiner diversity index (H)		Grain Yield (kg/ha)	
	season		season		season		season	
	early	late	early	late	early	late	early	late
Tillage (T)								
Conventional	4.33	4.08	0.53	0.41	0.77	0.58	2251	1775
Minimum	4.50	4.50	0.65	0.62	0.98	0.92	1874	1270
Zero	4.58	4.67	0.78	0.73	1.18	1.11	1649	1724
LSD	NS	0.23	0.14	0.07	0.19	0.14	NS	NS
Fertilizer (F)								
without	4.72	4.83	0.72	0.64	1.12	1.01	1272	1333
NPK fertilizer	4.22	4.00	0.58	0.52	0.84	0.73	2577	1846
LSD	0.41	0.65	0.06	0.09	0.11	0.14	447.3	365.7
Variety								
Oba super 2	4.39	4.39	0.65	0.60	0.97	0.89	1920	1698
Suwan 1	4.56	4.44	0.65	0.51	0.99	0.84	1929	1481
LSD	NS	NS	NS	NS	NS	NS	NS	NS
T × F								
T × V	NS	NS	NS	NS	NS	NS	NS	NS
F × V	NS	NS	NS	NS	NS	NS	NS	NS
T × F × V	NS	NS	NS	NS	NS	NS	NS	NS

NS – not significant, LSD – least significant difference, WAP – weeks after planting, * – significant at 5% probability level, ** – significant at 1% probability level, NPK fertilizer (120 kg N/ha+ 60 kg P₂O₅/ha + 60 kg K₂O/ha)

those soils amended with inorganic NPK. Oehl et al., 2004 observed that the predominance of *Acaulospora* was linked to organically managed soil. This could not be substantiated in this experiment, since post-planting soil analysis was not conducted. It could be inferred that the presence of organic residue from litters could have altered this C content of the soil. This is however subject to further empirical validation. AMF specie *Glomus* was observed in this study to be more under inorganic NPK amendment. This is consistent with earlier studies that indicated that AMF specie composition under intensive land use was in the order *Glomus* > *Acaulospora* > *Gigaspora* (Tchabi et al., 2007). This observed pattern could also have been linked with the fact that *Acaulospora* sporulate later than *Glomus* (Oehl et al., 2004). It had been reported that N or any of the water soluble fertilizers adversely affects *Gigasporaceae* population than earlier enumerated AMF species (Egerton-Warburton and Allen, 2000). The presence of other unidentified AMF species in this agroecology under the use of inorganic fertilizer is consistent with studies earlier conducted by Tchabi et al. (2007). In their study they were able to identify high specie richness in West Africa in varied agroecological

zones under different land use. Despite the observed improved community structure under zero application of fertilizer as occasioned by their specie richness and evenness grain yield of plots treated with inorganic fertilizer was observed to be higher than those without any soil amendment, this could have suggested that changes in community structure had effect on the performance of maize under this treatment in this agroecology. Available evidence in this study could have allow us to infer that *Glomus* specie of AMF could have played a predominant role among other species in conferring a positive effect on the performance of maize since they were the dominant specie of AMF when the soil was amended with NPK fertilizer. The underlying role of *Glomus* specie in this regard could not be explicated. This position conforms to the position of Jansa et al. (2003) where he posited that effect of changes in the community structure of AMF on maize performance could not be explicated. However, Wang et al. (2011) opined that a balanced NPK is good if crop yield and together with species diversity is considered. However this was not confirmed in our own study, since it was only the performance of maize that was observed without

a corresponding improvement in species diversity. The observed pattern here could also be explained by the fact that modern hybrid of maize are bred to respond to high fertilizer application with low AMF dependency (Hetrick et al., 1993). Other factors that could have explained the observed community structure changes under soil amendments and the performance of maize in this agroecology could have been the phenology of the host, nutrient availability in the soil and plant (Liu et al., 2009). The time of sampling the soil for AMF spore count was conducted at the vegetative growth stage. Increasing growth stage irrespective of the treatment imposed it was observed resulted in significant increase AMF species diversity (Schalamuk, Velazquez, Chidichimo and Cabello, 2006). This could have explained the observed pattern of AMF species diversity in this study considering the tolerance of *Glomus* specie in all circumstance in this agroecology. The pre-planting acidic nature of the soil of the experimental site could have affected AMF community structure (Thompson, 1991). Other line of evidence was provided by Oliveira et al. (2009), where he posited that AMF colonization in the tropics was associated more with the P efficiency in maize than soil available P. All these evidences corroborated the fact that the performance of maize under this soil was related to nutrient availability to them than changes in the community structure of AMF. The performance of maize could be explained by the fact that probably in the early season there was a reduced infestation of maize by AMF; hence the Carbon cost incurred by the host would be reduced considering reduced AMF species diversity (Graham, 2000). All these hypotheses still needed further empirical validation.

Spore count had been reported to be related to the AMF colonization in cultivated crops (Khalil, Loynachan and McNabb, 1992). The observed spore count in hybrid at the later vegetative growth stage in both cropping season could have suggested that the Carbon cost to AMF would be reduced compared to the OPV. This would have implication on the yield, though this was not established in this experiment, a pattern of higher yield in the hybrid than OPV though not significant was established in the late cropping season. This pattern was established despite the fact that modern maize hybrid were established to benefit more from fertilizer application than their dependency on AMF (Hetrick et al., 1993). However, the possibility of maize encountering low nutrient availability in the late season is high especially under low precipitation observed from August till the end of the cropping season in this investigation. Hence this is where their P use efficiency would be more germane, especially for the hybrids in the tropics. Though we could not establish the P use efficiency of the maize

cultivars used we hypothesize that a high P use efficiency in hybrid maize could favor increased root exudation with increased AMF colonization (Ratnayake et al., 1978). This could have serve as a proximate mechanism for the high spore count observed under hybrid maize. This is subject to further testing. The underlying mechanism response for the significantly higher number of *Acaulospora* in OPV compared to hybrid could not be explicated in this experiment.

5 Conclusion

AMF colonization of the maize cultivars under tillage practices was in the order zero tillage > minimum > conventional tillage in both seasons. Similar pattern was observed in the spore count. It could be inferred that AMF colonization is associated with the spore count in the soil. AMF species diversity under different tillage practices also followed similar pattern. This could be linked with the observed species richness and evenness under different tillage practices. It was observed that the most dominant AMF specie was *Glomus* under conventional tillage in both seasons. In both seasons (4 and 8 WAP) percentage AMF was significantly higher in unamended soil than those treated with inorganic N. This pattern was also observed in the spore count. AMF species diversity was observed (both seasons) to be higher in unamended soil than those with inorganic N. In unamended soil *Gigaspora* sp was significantly more than other AMF species in both seasons. However, *Acaulospora* sp was more in the early season in unamended soil. Conversely in soil amended with inorganic N *Glomus* species was observed to be more than other. This observation is consistent with earlier reports in the literature, where it was observed that with increasing duration of the dry season *Acaulospora* and *Glomus* are lesser in the soil than. It was suggested that this specie of AMF is more resilient under intensive cropping system. In both seasons the observed performance of maize cultivar with the application of inorganic N could have suggested that modern maize are bred to be more responsive to fertilizer application than their dependency on AMF. The observed pattern of spore count at the later stage (8 and 10 WAP) in both seasons for both maize cultivars used could have suggested the growth stage dependency of AMF infestation. However, this was more pronounced in the hybrid than the OPV. The assertion that modern hybrid maize are more dependent on nutrient application might need a second look, considering the low assimilate remobilization efficiency of maize. In conditions of increasing nutrient deficiency especially at the late growth stage maize might benefit from association with AMF. This would be most pronounced if there were differences in P utilization efficiencies between hybrid and OPV. Variation in this factor would elicit differences in the release of exudates

and efficacy of maize-AMF association in a derived savanna. This hypothesis is subject to further empirical validation.

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References

- ABDELHALIM, T. S., FINCKH, M. R., BABIKER, A. G. and OEHL, F. (2014). Species composition and diversity of arbuscular mycorrhizal fungi in White Nile state, Central Sudan. In *Archives of Agronomy and Soil Science*, vol. 60, no. 3, pp. 377–391.
- ALLISON, L. E. (1965). Organic carbon. In C.A Black (Ed.), *Methods of soil analysis. Part 2* (pp. 1307–1378). Madison: American Society of Agronomy.
- AVIO, L., CASTALDINI, M., FABIANI, A., BEDINI, S., SBRANA, C., TURRINI, A. and GIOVANNETTI, M. (2013). Impact of nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a Mediterranean agroecosystem. In *Soil Biology and Biochemistry*, vol. 67, pp. 285–294.
- BRAY, R. H. and KURTZ, L. T. (1945). Determination of total, organic and available forms of phosphorus in soil. In *Soil Science*, vol. 59, pp. 39–45.
- BRUNDRETT, M. C. and ABBOTT, L. K. (1994). Mycorrhizal fungus propagules in the jarrah forest. In *New Phytologist*, vol. 127, no. 3, pp. 539–546.
- DANIELL, T. J., HUSBAND, R., FITTER, A. H. and YOUNG, J. P. W. (2001). Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. In *FEMS Microbiology Ecology*, vol. 36, no. 2–3, pp. 203–209.
- DAVISON, J., ÖPIK, M., DANIELL, T. J., MOORA, M. and ZOBEL, M. (2011). Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. In *FEMS Microbiology Ecology*, vol. 78, no. 1, pp. 103–115.
- DRIJBER, R. A., DORAN, J. W., PARKHURST, A. M. and LYON, D. J. (2000). Changes in soil microbial community structure with tillage under long-term wheat-fallow management. In *Soil Biology and Biochemistry*, vol. 32, no. 10, pp. 1419–1430.
- EVANS, D. G. and MILLER, M. H. (1988). Vesicular-arbuscular mycorrhizas and the soil-disturbance-induced reduction of nutrient absorption in maize: I. Causal relations. In *New Phytologist*, vol. 110, no. 1, pp. 67–74.
- FREY, S. D., ELLIOTT, E. T. and PAUSTIAN, K. (1999). Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. In *Soil Biology and Biochemistry*, vol. 31, no. 4, pp. 573–585.
- GERDEMANN, J. W. and NICOLSON, T. H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. In *Transactions of the British Mycological Society*, vol. 46, no. 2, pp. 235–244.
- GIOVANNETTI, M. and MOSSE, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. In *New Phytologist*, vol. 84, no. 3, pp. 489–500.
- GOSLING, P., HODGE, A., GOODLASS, G. and BENDING, G. D. (2006). Arbuscular mycorrhizal fungi and organic farming. In *Agriculture, Ecosystems & Environment*, vol. 113, no. 1–4, pp. 17–35.
- HEPPER, C. M. (1983). The effect of nitrate and phosphate on the vesicular-arbuscular mycorrhizal infection of lettuce. In *New Phytologist*, vol. 93, no. 3, pp. 389–399.
- HETRICK, B. A. D., WILSON, G. W. T. and COX, T. S. (1993). Mycorrhizal dependence of modern wheat cultivars and ancestors: A synthesis. In *Canadian Journal of Botany*, vol. 71, no. 3, pp. 512–518.
- JACKSON, M. L. (1962). *Soil chemical analysis*. New Delhi: Prentice Hall of India Pvt, Ltd.
- JASPER, D. A., ABBOTT, L. K. and ROBSON, A. D. (1989). Soil disturbance reduces the infectivity of external hyphae of vesicular-arbuscular mycorrhizal fungi. In *New Phytologist*, vol. 112, no. 1, pp. 93–99.
- JENSEN, A. and JAKOBSEN, I. (1980). The occurrence of vesicular-arbuscular mycorrhiza in barley and wheat grown in some Danish soils with different fertilizer treatments. In *Plant and Soil*, vol. 55, no. 3, pp. 403–414.
- JOHNSON, N. C. (1993). Can fertilization of soil select less mutualistic mycorrhizae? In *Ecological Applications*, vol. 3, no. 4, pp. 749–757.
- JUMPPONEN, A., TROWBRIDGE, J., MANDYAM, K. and JOHNSON, L. (2005). Nitrogen enrichment causes minimal changes in arbuscular mycorrhizal colonization but shifts community composition—evidence from rDNA data. In *Biology and Fertility of Soils*, vol. 41, no. 4, pp. 217–224.
- KABIR, Z., O'HALLORAN, I. P. and HAMEL, C. (1999). Combined effects of soil disturbance and fallowing on plant and fungal components of mycorrhizal corn (*Zea mays* L.). In *Soil Biology and Biochemistry*, vol. 31, no. 2, pp. 307–314.
- KABIR, Z. (2005). Tillage or no-tillage: Impact on mycorrhizae. In *Canadian Journal of Plant Science*, vol. 85, no. 1, pp. 23–29.
- KAHILUOTO, H., KETOJA, E., VESTBERG, M. and SAARELA, I. (2001). Promotion of AM utilization through reduced P fertilization 2. Field studies. In *Plant and Soil*, vol. 231, no. 1, pp. 65–79.
- KHALIL, S., LOYNACHAN, T. E. and McNABB, H. S. (1992). Colonization of soybean by mycorrhizal fungi and spore populations in Iowa soils. In *Agronomy Journal*, vol. 84, no. 5, pp. 832–836.
- KOSKE, R. E. and TESSIER, B. (1983). A convenient, permanent slide mounting medium. In *Mycological Society of America Newsletter*, vol. 34, p. 39.
- LIU, Y., HE, L., AN, L., HELGASON, T. and FENG, H. (2009). Arbuscular mycorrhizal dynamics in a chronosequence of *Caragana korshinskii* plantations. In *FEMS Microbiology Ecology*, vol. 67, no. 1, pp. 81–92.
- MCCLEAN, E. O. (1982). Soil pH and lime requirement. *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties (methodsofsoilan2)*, pp. 199–224.
- MELERO, S., LÓPEZ-GARRIDO, R., MURILLO, J. M. and MORENO, F. (2009). Conservation tillage: Short- and long-term effects on soil carbon fractions and enzymatic activities under Mediterranean conditions. In *Soil and Tillage Research*, vol. 104, no. 2, pp. 292–298.

- MOHAMMAD, M. J., HAMAD, S. R. and MALKAWI, H. I. (2003). Population of arbuscular mycorrhizal fungi in semi-arid environment of Jordan as influenced by biotic and abiotic factors. In *Journal of Arid Environments*, vol. 53, no. 3, pp. 409–417.
- MULLIGAN, M. F., SMUCKER, A. J. M. and SAFIR, G. F. (1985). Tillage Modifications of Dry Edible Bean Root Colonization by VAM Fungi 1. In *Agronomy Journal*, vol. 77, no. 1, pp. 140–144.
- OEHL, F., SIEVERDING, E., INEICHEN, K., MÄDER, P., BOLLER, T. and WIEMKEN, A. (2003). Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. In *Applied and Environmental Microbiology*, vol. 69, no. 5, pp. 2816–2824.
- OLIVEIRA, C. A., SA, N. M., GOMES, E. A., MARRIEL, I. E., SCOTTI, M. R., GUIMARAES, C. T., ... ALVES, V. M. (2009). Assessment of the mycorrhizal community in the rhizosphere of maize (*Zea mays* L.) genotypes contrasting for phosphorus efficiency in the acid savannas of Brazil using denaturing gradient gel electrophoresis (DGGE). In *Applied Soil Ecology*, vol. 41, no. 3, pp. 249–258.
- PÉREZ, Y. and SCHENCK, N. C. (1990). A unique code for each species of VA mycorrhizal fungi. In *Mycologia*, vol. 82, no. 2, pp. 256–260.
- PHILLIPS, J. M. and HAYMAN, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. In *Transactions of the British Mycological Society*, vol. 55, no. 1, pp. 158–181.
- RATNAYAKE, M., LEONARD, R. T. and MENGE, J. A. (1978). Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. In *New Phytologist*, vol. 81, no. 3, pp. 543–552.
- REDECKER, D., SCHÜSSLER, A., STOCKINGER, H., STÜRMER, S. L., MORTON, J. B. and WALKER, C. (2013). An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). In *Mycorrhiza*, vol. 23, no. 7, pp. 515–531.
- RYAN, M. H. and GRAHAM, J. H. (2002). Is there a role for arbuscular mycorrhizal fungi in production agriculture? In *Plant and Soil*, vol. 244, no. 1–2, pp. 263–271.
- SBRANA, C., FORTUNA, P. and GIOVANNETTI, M. (2011). Plugging into the network: Belowground connections between germlings and extraradical mycelium of arbuscular mycorrhizal fungi. In *Mycologia*, vol. 103, no. 2, pp. 307–316.
- SCHALAMUK, S., VELAZQUEZ, S., CHIDICHIMO, H. and CABELLO, M. (2006). Fungal spore diversity of arbuscular mycorrhizal fungi associated with spring wheat: Effects of tillage. In *Mycologia*, vol. 98, no. 1, pp. 16–22.
- SHANNON, C. E. and WEAVER, W. (1949). *The mathematical theory of communication* (Urbana), IL. University of Illinois Press IL.
- TCHABI, A., COYNE, D., HOUNTONDJI, F., LAWOUI, L., WIEMKEN, A. and OEHL, F. (2008). Arbuscular mycorrhizal fungal communities in sub-Saharan Savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone. In *Mycorrhiza*, vol. 18, no. 4, pp. 181–195.
- THOMPSON, J. P. (1991). Improving the mycorrhizal condition of the soil through cultural practices and effects on growth and phosphorus uptake by plants. *Phosphorus Nutrition of Grain Legumes in the Semi-Arid Tropics*: (Eds C Johansen, KK Lee, KL Sahrawat), pp. 117–137.
- TITUS, J. H. and LEPŠ, J. (2000). The response of arbuscular mycorrhizae to fertilization, mowing, and removal of dominant species in a diverse oligotrophic wet meadow. In *American Journal of Botany*, vol. 87, no. 3, pp. 392–401.
- TRESEDER, K. K. and ALLEN, M. F. (2002). Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: A model and field test. In *New Phytologist*, vol. 155, no. 3, pp. 507–515.
- WANG, F. Y., HU, J. L., LIN, X. G., QIN, S. W. and WANG, J. H. (2011). Arbuscular mycorrhizal fungal community structure and diversity in response to long-term fertilization: A field case from China. In *World Journal of Microbiology and Biotechnology*, vol. 27, no. 1, pp. 67–74.
- WANG, Y. Y., VESTBERG, M., WALKER, C., HURME, T., ZHANG, X. and LINDSTRÖM, K. (2008). Diversity and infectivity of arbuscular mycorrhizal fungi in agricultural soils of the Sichuan Province of mainland China. In *Mycorrhiza*, vol. 18, no. 2, pp. 59–68.
- ZHANG, B., LI, Y., REN, T., TIAN, Z., WANG, G., HE, X. and TIAN, C. (2014). Short-term effect of tillage and crop rotation on microbial community structure and enzyme activities of a clay loam soil. In *Biology and Fertility of Soils*, vol. 50, no. 7, pp. 1077–1085.

The technological meat quality of the White Mangalitsa breed

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The aim of the experiment was to evaluate the technological meat quality of the breed White Mangalitsa through the pH, electric conductivity, drip loss and meat color parameters. Totally, 20 pigs of the breed White Mangalitsa (10 barrows and 10 gilts) were evaluated. Pigs were bred under the intensive breeding conditions. The animals were fed *ad libitum* using a complete feed compound with the added silage. Pigs were slaughtered upon reaching 110 kg of live weight. The muscles of MLD (*Musculus longissimus dorsi*) and MSM (*Musculus semimembranosus*) were evaluated. The meat quality analysis showed that pH₁ was similar between the muscles. The evidently lower pH₂ value was in MLD ($P < 0.01$). The EC₁ value ($P < 0.01$) was significantly higher in the MSM muscle. The EC₂ values in MLD and MSM were similar. Between the muscles, an evidentiary difference was observed in water drip loss ($P < 0.01$), higher losses were recorded in MLD. In the SCI a^* and SCI b^* values, which express the redness and yellowness of the meat, the values in MSM were higher. The lightness of the meat (SCI L^*) was the same in both muscles. The differences between the sexes in the observed qualitative parameters were not detected.

Keywords: mangalitsa, *Musculus longissimus dorsi*, *Musculus semimembranosus*, pork, quality

1 Introduction

According to Honikel (1998a), a technological quality of meat is particularly important for its processing and preparation. At present, we follow a range of criteria for assessing the technological quality of pig meat, such as pH, colour of meat, conductivity, dripping of meat juice, fat content, collagen content, etc. (Honikel, 1992).

The indigenous breeds, such as Iberian and Mangalitsa, are known to have desirable quality properties of meat that could be of interest to many farms giving them the possibility to produce unique high-quality meat products (Straadt et al., 2013). Mangalitsa is one of the most popular rustic pig breeds in Europe (Pârvu et al., 2012). It is a typical representative of a fatty pig breed. This means that of the total body weight, fat tissue accounts for 65–70% and proportion of meat represents 30–35% (Egerszegi et al., 2003). Fresh meat of this breed is darker, more juicy and softer than the meat of other pig breeds. Its smell is more specific. Fragility is also much higher compared to other pig breeds (Flegler, 2015). Meat has

got excellent properties, such as taste, marbling and a low content of cholesterol (Pârvu et al., 2012). The meat is of very good quality, but it has a very high content of fat at a bad lifestyle (Steffen et al., 2008).

Within the context of the practical control of the meat quality, the most important qualitative criteria are, in particular, the pH value, electrical conductivity, dripping of meat juice (Honikel, 2007). The meat colour plays a key role for a consumer as he/she combines the red colour of the meat with freshness, palatability and softness, although there does not have to be any connection between these qualitative parameters (Tikk et al., 2008; Mancini and Hunt, 2005). Productive parameters of pigs and meat quality traits may be influenced by multiple interacting factors before and after slaughter. These include breed, sex, genotype, feeding, production systems, pre-slaughter handling, stunning method, slaughter procedure, chilling and storage conditions (Rosenvold and Andersen, 2000; Olsson and Pickova, 2005; Nevrkla et al., 2016).

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The aim of this study was to evaluate the meat quality of the breed White Mangalitsa in the *Musculus longissimus dorsi* and *Musculus semimembranosus* under the intensive breeding conditions with regard to sex.

2 Material and methods

2.1 Biological material

The experiment was implemented at the Experimental Centre of Animals at the Slovak University of Agriculture (SUA) in Nitra. Totally, 20 pigs of the breed White Mangalitsa (10 barrows and 10 gilts) were evaluated.

2.2 Feeding and rearing conditions

Pigs were housed in the group pens on a full concrete floor with litter. Automatic feeders for dry fodder and two pin feeders were part of the pen. The temperature in the building was maintained at 18–20 °C. The air exchange in the building worked on the principle of vacuum ventilation. The air is drawn into the building through the ventilation self-regulating flaps, which were located in the suction channels and supply the fresh air from the outside. The livestock manure removal was carried out manually by means of a rotary swathe. The housing pen was manually cleaned every day and manure was temporarily stored in a container.

The pigs were fed by using a conventional compound feed with the addition of voluminous feed (clover-grass silage and maize silage in a ratio 1 : 1). Animals were fed and watered *ad libitum*. The basic nutrient composition is to be seen in Table 1.

Table 1 Basic Content of Nutrients in Feed

In % in the original mass	Feed compound	Silage mix
Dry matter	90.20	34.89
Crude protein	14.85	5.76
Fat	3.27	0.90
Fibre	4.41	9.16
Ash	3.58	3.50
Nitrogen free extract	64.09	15.57
Metabolisable energy (MJ/kg)	13.10	2.88

2.3 Technological parameters of the meat quality

Pigs were slaughtered upon reaching 110 kg of live weight. Firstly, the animals were electrically stunned by electric forceps during 4 s with the voltage 250 V and the amperage 1.3 A. The slaughtering was realized according to Government Regulation (SR) no. 432/2012 of the coll. of the Slovak Republic establishing the protection of animals during the slaughter. The meat quality parameters were evaluated in the longest MLD back

muscle (*Musculus longissimus dorsi*) at the level of the last thoracic vertebra and in the MSM muscle (*Musculus semimembranosus*).

The values pH 45 minutes (pH₁) a 24 hours (pH₂) *post mortem* were measured by the pH meter Hanna HI99161 in units-log₁₀[H⁺]. The electric conductivity was determined 45 minutes (EC₁) and 24 hours (EC₂) *post mortem* by using an instrument Tecpro in unit mS/cm. Drip losses in MLD and MSM were measured 24 h *post mortem* by the method according to Honikel (1998b). The meat colour was measured in MLD and MSM 24 hours *post mortem* by using the spectrophotometer CM-2600d with CIE Lab space and illuminate D65 (Konica Minolta, Japan). The following colour coordinates were determined: L* (lightness, white ± black), a* (redness, red ± green) and b* (yellowness, yellow ± blue). The values were recorded from the average of the three random readings across each muscle surface.

2.3 Statistical analysis

The data were analysed using the SAS statistical program, Version 9.1. The following was calculated within the descriptive statistics: number (*n*), average, minimum (min), maximum (max), standard deviation (*S_x*) and variation coefficient (*V_x*). Within a detailed statistical analysis, normality was tested in individual groups and indicators using the Shapiro-Wilk test. The statistically evidentiary differences between the compared groups were tested in the case of the normal distribution using the General Linear Model method. If the file did not have a normal distribution, a non-parametric Mann-Whitney *U*-test was used.

3 Results and discussion

Table 2 shows the results of the pH values compared according to muscles and sex. According to Kim et al. (2016), the initial pH and the final *post mortem* pH belong to the essential factors in determining the quality of pork. The differences in the pH₁ values between the muscles and sex were not statistically significant. We have recorded a lower pH₁ in the MSM muscle, where the minimum values pointed to the occurrence of the PSE qualitative variation (pale, soft, exudative), since according to Honikel (2007), meat is considered PSE when the pH₁ value is lower than 5.80. The variability of the measured values was relatively low and ranged from 2.60 to 5.36%. 24 hours after slaughter, we have found a statistically significant difference in pH₂ between the MLD and MSM muscles (*P* < 0.001). We have recorded a greater decrease of pH₂ in the MLD muscle (MLD 5.74 and MSM 5.84). The variation coefficient was relatively low and ranged from 0.78 to 1.04%. Unlike us, a more significant decrease in pH₂ was recorded in the mangalitsa breed

in MLD (5.69 ± 0.07) (Lípová et al., 2019). Parunovic et al. (2013) found the pH_2 values at the level of 5.77 ± 0.05 in the breed White Mangalitsa, which are comparable with our results. We have not found any gender differences between the groups. Similarly, Kasprzyk et al. (2015) have not find any gender differences in pH_1 and pH_2 values when comparing different pig genotypes.

Thanks to the *post mortem* changes in the muscle, detection of electrical conductivity enables to determine quality deviations. According to Honikel (2007), the conductivity in the intact and lively muscle is very low, since the cell membrane prevents the flow of ions. The death start leads to a partial destruction of the cell membrane, which becomes ion-permeable and

electrical conductivity increases. Table 3 demonstrates the values of electrical conductivity by muscle and sex 45 minutes (EC_1) and 24 hours (EC_2) after slaughter. We have found statistically evidentiary higher conductivity of EC_1 ($P < 0.001$) in the MSM muscle (6.15 mS/cm), while the maximum value of the electrical conductivity in MSM was 17.10 mS/cm. The EC_1 value in the MLD was at the level of 3.46 mS/cm. We have not found any statistically evidentiary differences between the sexes in the EC_1 values. The variability was considered relatively high and ranged from 44.12 to 78.02%. We have not found any statistically evidentiary differences in EC_2 between the sexes, nor between MLD and MSM. The average EC_2 values ranged from 10.81 to 11.34 mS/cm. The relatively steady values have been also confirmed by the coefficient

Table 2 Comparison of the pH Values by Muscle and Sex

Parameter	Group	<i>n</i>	Mean	S_x	Min	Max	V_x (%)	Significance
pH_1	MLD	20	6.25	0.18	5.90	6.49	2.96	$p > 0.05$
	MSM	20	6.05	0.28	5.55	6.38	4.61	
pH_1 MLD	Barrows	10	6.26	0.16	6.04	6.43	2.60	$p > 0.05$
	Gilts	10	6.24	0.22	5.90	6.49	3.59	
pH_1 MSM	Barrows	10	5.99	0.24	5.61	6.23	4.05	$p > 0.05$
	Gilts	10	6.11	0.33	5.55	6.38	5.36	
pH_2	MLD	20	5.74	0.05	5.66	5.83	0.81	$p < 0.01$
	MSM	20	5.84	0.05	5.77	5.94	0.93	
pH_2 MLD	Barrows	10	5.74	0.04	5.66	5.76	0.78	$p > 0.05^*$
	Gilts	10	5.74	0.05	5.69	5.83	0.93	
pH_2 MSM	Barrows	10	5.85	0.05	5.77	5.89	0.89	$p > 0.05$
	Gilts	10	5.83	0.06	5.79	5.94	1.04	

*Mann-Whitney *U*-test

Table 3 Comparison of the EC Values by Muscle and Sex

Parameter	Group	<i>n</i>	Mean	S_x	Min	Max	V_x (%)	Significance
EC_1	MLD	20	3.46	0.41	3.10	4.40	11.89	$p < 0.01$
	MSM	20	6.15	4.15	3.80	17.10	67.40	
EC_1 MLD	Barrows	10	3.48	0.54	3.10	4.40	15.39	$p > 0.05^*$
	Gilts	10	3.44	0.30	3.10	3.80	8.87	
EC_1 MSM	Barrows	10	7.14	5.57	4.50	17.10	78.02	$p > 0.05^*$
	Gilts	10	5.16	2.28	3.80	9.20	44.12	
EC_2	MLD	20	11.34	1.28	9.20	13.60	11.29	$p > 0.05^*$
	MSM	20	10.81	1.53	6.90	12.50	14.13	
EC_2 MLD	Barrows	10	11.82	1.48	9.50	13.60	12.54	$p > 0.05^*$
	Gilts	10	10.86	0.96	9.20	11.50	8.80	
EC_2 MSM	Barrows	10	10.66	2.20	6.90	12.50	20.64	$p > 0.05^*$
	Gilts	10	10.96	0.59	10.20	11.50	5.38	

*Mann-Whitney *U*-test

of variation, which was ranging from 8.80 to 20.64%. According to Mörlein et al. (2007), the PSE meat has got the value 24 hours *post mortem* higher than 9–7 mS/cm. It follows that some animal individuals might have had deteriorated meat quality. The lower EC₂ values in MLD (9.31 ±1.91 mS/cm) were found at the evaluation of the Mangalitsa meat quality by Lípová et al. (2019).

Water loss caused by dripping is not only considered an aspect of the meat quality, it is also an important economic factor due to the weight loss of the carcass. A good water binding characterizes a high grade of the pork quality (Otto et al., 2005). Between the MLD (5.95%) and MSM (1.99%) muscles, we have found statistically evidentiary differences ($P < 0.001$) in water

loss by dripping (Table 4). Intersexual differences were not found. Similarly, Kasprzyk et al. (2015) have not found any statistical differences between the sexes, when comparing different breeds of pigs. A good quality meat should keep the value of the water, lost through dripping, up to 7–9% (Mörlein et al., 2007). We can state for the reasons given that the meat of the tested animals has shown good quality. A higher drip loss in Mangalitsa in MLD (7.15 ±2.99%) was found by Lípová et al. (2019). In organic farming, Millet et al. (2005) have found the water loss by dripping at the level of 7.3% and Hansen et al. (2001) from 6.25 to 6.53%.

Results of the meat colour are shown in the Table 5. The SCI L* values reflect the lightness of the meat. The higher the

Table 4 Comparison of the Free Water Losses by Dripping according to Muscle and Sex

Parameter	Group	n	Mean	S _x	Min	Max	V _x (%)	Significance
Drip loss	MLD	20	5.95	1.65	3.62	7.80	27.81	$p < 0.01^*$
	MSM	20	1.99	2.31	0.31	6.81	116.11	
Drip loss MLD	Barrows	10	6.21	1.47	4.45	7.80	23.61	$p > 0.05$
	Gilts	10	5.69	1.96	3.62	7.79	34.45	
Drip loss MSM	Barrows	10	0.63	0.28	0.36	1.02	43.98	$p > 0.05$
	Gilts	10	3.34	2.70	0.31	6.81	80.88	

*Mann-Whitney U-test

Table 5 Comparison of the Meat Colour Values by Muscle and Sex

Parameter	Group	n	Mean	S _x	Min	Max	V _x (%)	Significance
SCI L*	MLD	20	54.03	4.41	48.87	63.59	8.16	$p > 0.05^*$
	MSM	20	54.30	10.52	40.41	65.09	19.37	
SCI L* MLD	Barrows	10	52.32	3.53	48.87	56.98	6.74	$p > 0.05$
	Gilts	10	55.73	4.91	50.69	63.59	8.80	
SCI L* MSM	Barrows	10	53.18	11.29	40.41	65.09	21.24	$p > 0.05$
	Gilts	10	55.42	10.87	40.92	64.75	19.62	
SCI a*	MLD	20	4.00	1.45	2.41	7.21	36.38	$p < 0.01$
	MSM	20	9.03	4.23	3.95	14.56	46.85	
SCI a* MLD	Barrows	10	4.45	1.89	2.42	7.21	42.39	$p > 0.05$
	Gilts	10	3.55	0.83	2.41	4.45	23.42	
SCI a* MSM	Barrows	10	9.36	4.34	3.95	14.56	46.34	$p > 0.05$
	Gilts	10	8.70	4.61	4.43	13.99	52.92	
SCI b*	MLD	20	11.39	1.80	8.82	14.51	15.82	$p < 0.01$
	MSM	20	13.92	1.76	11.45	15.84	12.62	
SCI b* MLD	Barrows	10	11.02	1.92	8.82	13.82	17.40	$p > 0.05$
	Gilts	10	11.76	1.81	10.26	14.51	15.41	
SCI b* MSM	Barrows	10	13.74	1.96	11.45	15.77	14.29	$p > 0.05$
	Gilts	10	14.10	1.73	12.09	15.84	12.30	

*Mann-Whitney U-test

value, the lighter the meat. The average SCI L^* in MLD was 54.03 and in MSM 54.30, with no evidentiary differences between the muscles. The values ranged from 52.32 to 55.73 between the sexes in the MLD muscle. They ranged from 53.18 to 55.42 in MSM. The gilt meat was lighter, but the differences were not statistically significant. Unlike us, Tomovic et al. (2014) found evidentiary differences in the meat lightness of the breed Swallow-Belly Mangalitsa between the MLD and MSM (46.29 ± 2.00 against 40.86 ± 5.83). Lípová et al. (2019) have found that the Mangalitsa breed has evidently darker meat than the crossbreed Mangalitsa \times Slovak Large White (53.06 ± 4.34 vs. 58.12 ± 4.93). Similarly, Ender et al. (2002) have found that mangalitsa has evidently darker meat than other breeds of pigs: Mangalitsa 38.80, German Saddle Pig 47.40 and German Landrace 48.50. A significantly lower SCI L^* value in Mangalitsa found by Ender et al. (2012) could have been caused by the fact, that the mentioned authors slaughtered the Mangalitsa at an average live weight of 155 kg, that is, at a higher age.

In the SCI a^* values, which reflect the redness of the meat, we have found statistically significant differences ($P < 0.001$) between MLD and MSM. The redder meat was found in the MSM muscle (9.03) versus the MLD (4.00). In accordance with our results, Tomovic et al. (2014) found out that the MSM muscle was evidently redder compared to MLD (16.59 ± 0.52 versus 12.79 ± 1.20). The barrows were redder than gilts in both MLD and MSM, but the differences were not statistically significant. Contrary to our findings, Kasprzyk et al. (2015) found, when comparing the breeds Pulawska and Polish Landrace, that the gilts had evidently redder meat than barrows. In the SCI b^* color scale describing the blue-yellow spectrum, we have found out a statistically evidentiary difference between the muscles ($P < 0.001$), whereas the yellower meat was found in the MSM muscle (13.92) compared to MLD (11.39). Comparable values in MLD for the breed Mangalitsa (10.41 ± 1.53) and the crossbreeds Mangalitsa \times Slovak Large White (11.89 ± 1.45) were found by Lípová et al. (2019). Bednářová et al. (2014) found the average values of SCI b^* in the range of 9.53–10.14 in the muscle MSM. Significantly lower levels of the meat yellowness were found in the muscle of the Swallow-Belly Mangalitsa Tomovic et al. (2014), which was at the level of 6.47 ± 1.08 in MSM and 5.21 ± 0.81 in MLD.

4 Conclusions

This study provides the data on the technological parameters of the fresh meat of the breed White Mangalitsa bred under the intensive farming conditions. Comparing the technological parameters of the MLD and MSM quality, we can conclude that regarding the pH and EC indicators, the MSM meat showed a kind of worse results

because some individuals had the pH₁ values below 5.8 and the EC₁ were provably higher ($P < 0.001$) compared to MLD. However, from the point of view of the water loss through dripping, the MSM has achieved evidently lower losses than MLD ($P < 0.001$). Similarly, for the colour assessment, the MSM muscle was evidently redder (SCI a^*) and yellower (SCI b^*) compared to MLD ($P < 0.001$). The lightness of the meat (SCI L^*) was the same in both muscles. We have not recorded any differences between the sexes in the observed qualitative parameters. Based on the complex assessment of the average values of all the observed technological indicators we can state, that the White Mangalitsa breed is suitable for production of the quality pork and production of traditional durable meat products.

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5 References

- BEDNÁŘOVÁ, M. et al. (2014) Monitoring of color and pH in muscles of pork leg (*M. adductor* and *M. semimembranosus*). In *Potravinárstvo Slovak Journal of Food Sciences*, vol. 8, no. 11, pp. 48–53. doi: <https://doi.org/10.5219/337>
- EGERSZEGI, I. et al. (2003) Mangalica – an indigenous swine breed from Hungary: Review. In *Archives Animal Breeding*, vol. 46, no. 3, pp. 245–256. doi: <https://doi.org/10.5194/aab-46-245-2003>
- ENDER, K. et al. (2002) Fleisch und Fett von Mangalitzaschweinen im Labor. In *Fleischwirtschaft*, vol. 82, no. 6, pp. 125–128.
- FLEGLER, J. (2015) Das Wollschwein. In *Das Wollschwein auf wollschwein-register.de*. Witzenhausen, DE: Gesellschaft zur Erhaltung alter und gefährdeter Haustierrassen e.V. (GEH). [Online]. Retrieved 2015-12-11 from <https://wollschwein-register.de/das-wollschwein/die-historie/index.html>
- HANSEN, L. L., MAGNUSSEN C. C. and ANDERSEN, H. J. (2001) Meat and eating quality of organically produced pigs. In *Økologisk og udendørs svineproduktion*: Internal report no. 145. Tjele, DK: Danish Institute of Agricultural Sciences, pp. 39–40.
- HONIKEL, K. O. (1992) Qualitätsprodukte erfordern geeignete Messmethoden. In *Qualitätssicherung im Fleischbereich: Konferenzband*. Kulmbach, DE: Bundesanstalt für Fleischforschung, Kulmbacher Reihe, Band 11, pp 1–19.
- HONIKEL, K. O. (1998a) Qualität ökologisch erzeugter Lebensmittel tierischer Herkunft. In *Deutsche tierärztliche Wochenschrift*, vol. 105, no. 8, pp. 327–329.
- HONIKEL, K. O. (1998b) Reference methods for the assessment of physical characteristics of meat. In *Meat Science*, vol. 49, no. 4, pp. 447–457. doi: [https://doi.org/10.1016/S0309-1740\(98\)00034-5](https://doi.org/10.1016/S0309-1740(98)00034-5)
- HONIKEL, K. O. (2007) Physikalische Messmethoden zur Erfassung der Fleischqualität. In *Qualität von Fleisch und Fleischwaren*. 2. Auflage. Frankfurt am Main, DE: Verlagsgruppe Deutscher Fachverlag. Band 2, pp. 855–881.

- KASPRZYK, A., TYRA, M. and BABICZ, M. (2015) Fatty acid profile of pork from a local and a commercial breed. In *Archives Animal Breeding*, vol. 58, no. 2, pp. 379–385. doi: <https://doi.org/10.5194/aab-58-379-2015>
- KIM, T. W. et al. (2016) Pork Quality Traits According to postmortem pH and Temperature in Berkshire. In *Food Science of Animal Resources*, vol. 36, no. 1, pp. 29–36. doi: <https://doi.org/10.5851/kosfa.2016.36.1.29>
- LÍPOVÁ, P. et al. (2019) Effect of intramuscular fat content on physical-chemical parameters of pork from mangalitsa and their crossbreeds. In *Potravinárstvo Slovak Journal of Food Sciences*, vol.13, no. 1, pp. 422–428. doi: <https://doi.org/10.5219/1095>
- MANCINI, R.A. and Hunt, M.C. (2005) Current research in meat color. In *Meat Science*, vol. 71, no. 1, pp. 100–121. doi: <https://doi.org/10.1016/j.meatsci.2005.03.003>
- MILLET, S. et al. (2005) Performance and meat quality of organically versus conventionally fed and housed pigs from weaning till slaughtering. In *Meat Science*, vol. 69, no. 2, pp. 335–341. doi: <https://doi.org/10.1016/j.meatsci.2004.08.003>
- MÖRLEIN, D. et al. (2007) Suitability of three commercially produced pig breeds in Germany for a meat quality program with emphasis on drip loss and eating quality. In *Meat Science*, vol. 77, no. 4, pp. 504–511. [Online]. Available at: <https://doi.org/10.1016/j.meatsci.2007.04.030>
- NEVRKLA, P. et al. (2016) Effect of Farm on reproductive and productive performance in sows of Prestice Black-pied pig. In *Acta Universitatis Agriculturae Mendelianae Brunensis*, vol. 64, no. 4, pp 1233–1237. doi: <https://dx.doi.org/10.11118/actaun201664041233>
- OLSSON V. and Pickova, J. (2005) The influence of production systems on meat quality, with emphasis on pork. In *Ambio*, vol. 34, no. 4–5, pp. 338–343.
- OTTO, G. et al. (2005) Genmarker: Jetzt im Paket gegen Tropfsaftverluste vorgehen? In *SUS – Schweinezucht und Schweinemast*, vol. 2005, no 2, p. 42.
- PARUNOVIĆ, N. et al. (2013) Carcass properties, chemical content and fatty acid composition of the musculus longissimus of different pig genotypes. In *South African Journal of Animal Science*, vol. 43, no. 2, pp. 123–136. doi: <http://dx.doi.org/10.4314/sajas.v43i2.2>
- PÂRVU, M. et al. (2012) Influence of Cold Stress on the Chemical Composition of Carcass to Mangalica pigs. In *Scientific Papers: Animal Science and Biotechnologies*, vol. 45, no. 2, pp. 394–396.
- ROSENVOLD, K., and Andersen, H.J. (2003) Factors of significance for pork quality – A review. In *Meat Science*, vol. 64, no. 3, pp. 219–237. doi: [https://doi.org/10.1016/S0309-1740\(02\)00186-9](https://doi.org/10.1016/S0309-1740(02)00186-9)
- SLOVENSKO (2012) Government regulation (SR) no. 432/2012 of the coll. of Slovak Republic of 12 December 2012 establishing the protection of animals during the slaughter. In *Collection of Slovak republic laws*, Edition 105/212. [Online]. Retrieved from <http://www.epi.sk/zz/2012-432>
- STEFFEN, P., SCHARDAX, K. and KÜRZL, G. (2008) *Schweineglück – Die Bibel der Schweine*. Hart b. Graz, AT: Agentur am Kunsthaus.
- STRAADT, I. K., AASLYNG, M. D. and BERTRAM, H. CH. (2013) Sensory and consumer evaluation of pork loins from crossbreeds between Danish Landrace, Yorkshire, Duroc, Iberian and Mangalitza. In *Meat Science*, vol. 95, no. 1, pp. 27–35. doi: <https://doi.org/10.1016/j.meatsci.2013.04.026>
- TIK, K. et al. (2008) The significance of diet, slaughter weight and aging time on pork colour and colour stability. In *Meat Science*, vol. 79, no. 4, pp. 806–816. doi: <https://doi.org/10.1016/j.meatsci.2007.11.015>
- TOMOVIC, V.M. et al. (2014) Sensory, physical and chemical characteristic of meat from free-range reared Swallow-Belly Mangulica pigs. In *The Journal of Animal & Plant Sciences*, vol. 24, no. 3, pp. 704–713.

Cytotoxic effect of aluminium ions on unicellular eukaryotic organism

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Aluminium is abundant in nature, food, or water and thus its exposition is part of everyday life. However, overexposure can result in cellular malfunctions. Therefore, the aim of this study was to investigate the effects of aluminium on eukaryotes, with the use of *Schizosaccharomyces pombe* as model organism. Spectrophotometry at OD₆₀₀, inductively-coupled plasma optical emission spectroscopy (ICP-OES) and microscopy techniques were used to analyse aluminium responses on the living system. Our results revealed that exposition of increasing aluminium concentrations lead to cell growth inhibition in a concentration dependent manner. Furthermore, aluminium incorporation by the cell from media markedly increased with rising Al concentration. Our results indicate that the yeast self-protection system in the presence of lower Al(OH)₃ concentration in the environment avoids to large extent dramatic uptake of aluminium by the cell while cells surrounded by higher aluminium concentrations lose this ability. Supplementation of the growth media with 100 µM Al(OH)₃ doubled the amount of Al in the cell compared to untreated control (232 mg/kg vs. 459 mg/kg), whereas addition of 1 mM Al(OH)₃ caused more than hundred fold increase of intracellular Al content (27,781 mg/kg). Here we also show that high concentrations of aluminium have an impact on cell morphology leading to cell integrity disruption. Findings presented in this study have the ambition to bring more light in an issue of how aluminium mediates impairments of the living organism.

Keywords: aluminium, *Schizosaccharomyces pombe*, toxicity, absorption, cell cycle

1 Introduction

Aluminium is widely used in daily life, and that is the reason of easy exposure to organisms. Uptake of aluminium in humans is possible from food, drinking water, cooking dishes or aluminium foils. Aluminium also occurs in agricultural soils, and through the crops can enter animal or human organism. In neutral pH, aluminium is bound in various minerals (most frequent is bauxite). In acidic environment (pH lower than 5) aluminium is released as aluminium ions Al³⁺ which are toxic to many organisms.

Chronic exposure of aluminium causes excessive accumulation in tissues thus having adverse effect on health. In the past, osteomalacia, microcytic anaemia and dialysis encephalopathy was observed in patients with kidney diseases due to presence of aluminium in dialysis fluid (Short et al., 1980; Li et al., 2012; Chen et

al., 2018). Additionally, aluminium exposure can cause damages on cellular level as it increases the level of oxidative stress in cells. Elevated oxidative stress leads in mammals to apoptosis of brain cells contributing to development of neurodegenerative diseases (Maya et al., 2016; Colomina and Peris-Sampedro, 2017). Oxidative stress caused by aluminium enhances lipid peroxidation in brain and decreases activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (Kaizer et al., 2005; Nehru and Anand, 2005). Moreover, formation of 8hydroxydeoxyguanosine, a common biomarker of oxidative DNA damage, is elevated in mitochondrial DNA after aluminium exposure. Expression of p53 protein has been increased as a result of oxidative DNA damage caused by Al³⁺ ions. Elevated expression of regulatory protein cyclin D1 due to Al³⁺ exposure can lead to cell cycle arrest in G1/S phase (Kumar

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et al., 2009). Investigations of effects of aluminium on the cell cycle revealed that its ability to interfere with cell components, disrupting the organization of microtubules and microfilaments results in alterations of cell cycle regulation. Defects after aluminium exposure were observed in microtubule cytoskeleton organization of mitotic cells of *Triticum turgidum* root tips. Stability of microtubules is affected by aluminium, tubulin forms atypical tubulin bundles, or ringlike aggregates causing incorrect spindle organization. Overdoses of aluminium impair kinetochore alignment during metaphase leading to abnormal arrangement of chromosome arms (Frantzios et al., 2000). Aluminium ions are responsible for slight depolymerization of cytoskeleton in alga *Spirogyra decimina* (Přibyl et al., 2008). Aluminium excess causes nucleolus disassembly which persists in the cell during mitosis (Qin et al., 2010; Zhang et al., 2014).

Studies analysing the possible mechanisms of aluminium toxicity for eukaryotic organism performed on yeasts *S. cerevisiae*, one of the most frequent model organisms (MacDiarmid and Gardner, 1996), showed that the yeast can undergo programmed cell death induced by aluminium exposure (Zheng et al., 2007). Analysis of the yeast genome has identified aluminium tolerant genes. Genes related to vesicle transport, genes responsible for signal transduction, and genes associated with protein mannosylation are aluminium tolerant, and can be responsible for aluminium metabolism and maintaining of the aluminium compromised cell integrity (Kakimoto et al., 2005). Intracellular aluminium ion accumulation can also generate abnormalities on protein level. In combination with diamide induces disulphide stress through generation of disulphide bonds between cysteines within, or between proteins leading to production of misfolded proteins that can be a cause of a disease (Wu et al., 2012; Tun et al., 2013). Another research has shown that even aluminium and acid tolerant yeast strain *Cryptococcus humicola* can be damaged by high concentration of aluminium. Incubation with concentration of aluminium above the tolerance level causes oxidative damage of membrane lipids resulting in size reduction and cell death (Nian et al., 2012).

It has been previously shown that aluminium ions are capable of interaction with many cellular components which has negative impact on homeostasis of microorganisms, plants and animals. Because of the toxicity of aluminium, for studies on its effect on living systems, the use of model organisms is required. *Schizosaccharomyces pombe*, a single-celled eukaryote, also known as fission yeast is a widely used model organism, as its genome comprises of many genes which are orthologous to genes reported to be responsible for

certain diseases. In addition, this cell divides by mitosis similar to mitosis of multicellular organisms (Wood et al., 2002). Influence of aluminium exposition on cell cycle progression is to date only poorly examined. We used *S. pombe* to investigate the effect of aluminium on cell morphology, cell growth and proliferation, as this model organism exhibits similar responses as multicellular organisms and can thus help to elucidate toxic effects of the metal on cellular level.

2 Material and methods

2.1 Model organism and the growth conditions

The prototroph wild type (h⁺) strain JG 15458 of the fission yeast *Schizosaccharomyces pombe* kindly provided from Doc. Dr. Juraj Gregan was used to analyse the sensitivity to aluminium ions. The standard rich YE+5S medium (YES), where indicated supplemented with defined Al(OH)₃ (Centralchem, Bratislava, Slovakia) concentrations was used for yeast growth. For media preparation the protocol from Sabatinos and Forsburg (2010) was used. Accordingly, for solid media, 20 g/L agar was added. Incubation temperature for the organism growth was 30 °C in both solid or liquid media. Moreover, aeration for yeast growth in liquid media was ensured by shaking at 130 rpm.

2.2 IC₅₀ value determination

The half maximal inhibitory concentration (IC₅₀) represents the concentration of Al that causes the growth inhibition of the cells by 50%. Model organisms from the over-night culture were incubated for 3 hours at 30 °C with decreasing aluminium concentrations in a log 2 serial dilution from 1 mM to 1.5652 mM. The culture sample without aluminium addition was used as a control. Spectrophotometric analysis performed on Cary 60 UV-VIS spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) was used to determine the effect of tested metals on the cell growth. The differences in light absorbance at 600 nm before and after 3 hours of aluminium exposure denoted changes in the cell density revealing alterations in growth ability with the increasing metal concentrations. Cell growth ratio was calculated and IC₅₀ value was determined using an IC₅₀ calculator <http://www.ic50.tk/>.

2.3 Growth rate and cell morphology

To determine growth ability of the model organism under Al environment, cells from the over-night culture, diluted to OD₆₀₀ 0.3, exposed to decreasing Al concentrations (1,000, 500, 250, 125, 60, 30 mM and 0 as a control) were incubated at 30 °C in water bath, and after 3 hours OD₆₀₀ was measured on Cary 60 UV-VIS spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Increase of

the cell density compared to time point 0 h defines the ability of cells to divide. The effect of the increasing Al concentrations to cell division is expressed as impairment of the cell density increase over time (Sabatinos and Forsburg, 2010). Cell morphology (length and width) was determined by the use of bright-field microscopy with 40× magnification on inverted microscope (Leica DMI 6000, Leica microsystems, Wetzlar, Germany). The length and width in [mm] was measured and compared in 100 cells exposed to 500, 150, 100 and 0 mM Al(OH)₃ by ImageJ software.

2.4 Spot test analyses

Spot test analysis was used in previous research (Pozgajova et al., 2013). Three aluminium concentrations 100, 150 and 500 µM were added to YES media containing agar, after sterilization by autoclaving. Cells from the over-night culture containing approximately 1×10^7 cells/mL were counted, serially diluted, and 10 µl of diluted cultures containing 1×10^4 , 1×10^3 , 1×10^2 and 1×10^1 cells/spot were plated on YES plates. Afterwards, plates were incubated at 30 °C for 2–3 days until growing cells formed colonies (spots). Individual spots formed on plates enriched by different Al concentrations were compared among each other and to the control. The number, size, and density of spots were evaluated.

2.5 Pre-analytical sample preparation of yeast

Cells were cultured overnight in 50 mL YES medium by shaking at 130 rpm and 30 °C. After culture growth, aluminium was added to sample in final concentrations: 100, 150, 500 and 1,000 µM. Control sample was left untreated. Cells were exposed to Al(OH)₃ for 3 hours followed by thorough washing at least three times with deionized water. Cells were pelleted by centrifugation, supernatant was removed, and samples were incubated at 55 °C for 12 hours. Dried yeast pellets were placed into PTFE digestion tubes and weighted. Five mL of ultra-pure TraceSELECT™ HNO₃ (Honeywell Fluka™, Seelze, Germany) is used for mineralization of the sample by pressure microwave digestion system ETHOS-One (Milestone, Srl., Italy). Mineralized samples were subsequently filtered with the use of quantitative Munktell filter paper No. 390 (Munktell & Filtrak, Bärenstein, Germany) into 50 mL volumetric flasks and filled with deionized H₂O (Kovacik et al., 2019). Three replicates of each sample were prepared to ensure statistical scoring.

2.6 Determination of aluminium content in yeast

Content of aluminium in mineralized yeast solutions was determined by optical emission spectroscopy with inductively coupled plasma ICP-OES (ICP-OES 720, Agilent Technologies Australia (M) Pty Ltd) (Kovacik et al.,

2019). The detection limit for Al of the sample dry matter is 0,2 µg/kg.

2.7 Statistical analysis

Statistical significance of detected differences was evaluated by Student's *T*-test using STATISTICA v.10 software (StatSoft Inc. Tulsa, OK, USA). To detect normality and homogeneity of variances the Cochran-Hartley-Barlett and Levene's tests were used. The limit of statistical significance was set up at $P < 0.05$ *, 0.01 **, 0.001 *** for all statistically analysed samples.

3 Results and discussion

3.1 Effect of aluminium ions on the growth rate of cell culture in liquid medium

To investigate the influence of aluminium exposure on *S. pombe* growth, the spectrophotometry light absorbance measured at 600 nm was used (Figure 1). Optical density of the cell culture was determined after 3 hours of incubation with various concentrations of aluminium in liquid YES medium. As expected, increasing concentration of Al(OH)₃ led to cell culture growth inhibition. Similarly, growth rate inhibition of *S. pombe* was reported also after addition of the other toxic cations e.g. Cd (Clemens, 2003), As (Salgado, 2012) or Ni (Pozgajova et al. 2019). Significant drop in the cell growth was observed at concentration 250 µM. It demonstrates that exposure to high concentration of aluminium can reduce mitotic division, and thus disrupt normal growth of the cell culture compared to the control sample without an addition of aluminium. The half of the maximal inhibitory concentration IC₅₀ was calculated and set on 234.53 µM Al(OH)₃.

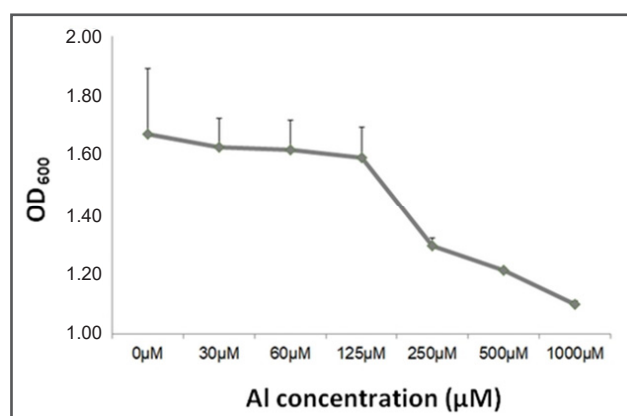


Figure 1 Dynamics of the growth of *S. pombe* cell culture after aluminium exposure in liquid YES medium. Change in optical density with increasing concentration of Al(OH)₃: 30, 60, 125, 250, 500 and 1,000 µM. Control sample was untreated with Al (0 µM). OD at 600 nm was measured immediately after Al(OH)₃ addition and after 3 hours of incubation

3.2 Spot test of cells on the solid medium with addition of aluminium

Spot test analysis confirmed the sensitivity of *S. pombe* to aluminium (Figure 2). In this test, the solid YES medium was enriched by 0, 100, 150 and 500 μM of aluminium. After serial dilution of the yeast culture 10, 100, 1,000, and 10,000 cells were spotted on plates, the growth ability was determined three days after incubation at 30 °C. Results of spot test corresponded to the results of the growth rate test, showing that lower $\text{Al}(\text{OH})_3$ concentrations did not alter cell proliferation, whereas higher concentration, such as 500 μM $\text{Al}(\text{OH})_3$, markedly reduced growth of cells.

Although small variations in growth rate of cell culture in these two methods could be observed due to different growth condition (liquid vs. solid medium, distribution of the cells on/in the medium, shaking, aeration), the trend of the cell culture growth under aluminium stress remained similar, and it was evident that growth rate decreased with rising $\text{Al}(\text{OH})_3$ concentrations. Our results confirm that mitotic division of *S. pombe* cells was inhibited by high $\text{Al}(\text{OH})_3$ concentrations, and thus we can conclude, that *S. pombe* yeast is sensitive to increasing aluminium exposure in the environment. Sensitivity of cells to aluminium exposure resulting in the growth inhibition was also confirmed on other model systems such as budding yeast or plants. Mitotic activity of root cells decreased with increasing concentration of aluminium ions (Huang et al., 2014; Jaskowiak et al., 2018). Wild type budding yeasts exposed to different aluminium concentrations showed growth rate inhibition in a dose dependent manner (Wu et al., 2012). However, in our hands, cell growth of *S. pombe* was inhibited by lower concentration of aluminium compared to experiments presented by Wu et al. (2012) with wild type *S. cerevisiae*.

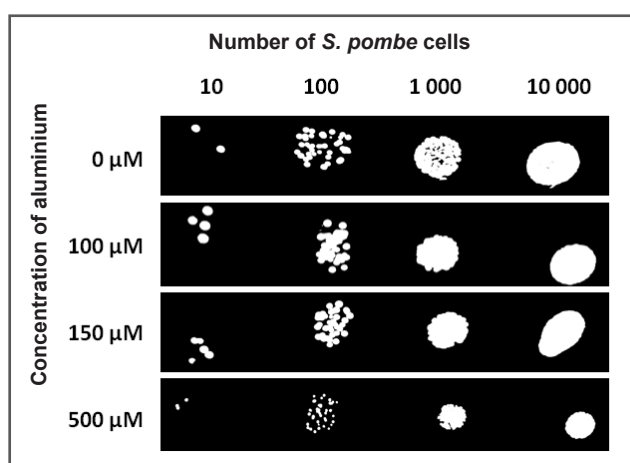


Figure 2 Spot test with 10, 100, 1,000 and 10,000 cells of *S. pombe* on agar plates in solid YES medium incubated for 3 days with concentrations of $\text{Al}(\text{OH})_3$ 0, 100, 150 and 500 μM

We assume, that the resistance of *S. cerevisiae* cells to higher Al concentrations might be due to different experimental conditions and is caused by the use of AlCl_3 , which shows lower toxicity to the cell compared to $\text{Al}(\text{OH})_3$ (Zheng et al., 2007; Li et al., 2011).

Inhibition of the cell culture growth can be a result of aluminium interactions with intracellular structures responsible for proper mitosis. Experiments with plants revealed that accumulation of aluminium ions induces severe changes in the microtubular cytoskeleton and mitotic spindle, leading to significant destruction of mitotic cells. Furthermore, chromosome fragmentation, and chromosome stickiness was observed. This type of chromosome aberration is irreversible and leads to programmed cell death (Zhang et al., 2014, Vardar et al., 2016). Presence of aluminium in acid soil alters root growth by reducing the mitotic activity of the root tip cells as its exposure inhibits DNA replication resulting in the delay of the cell cycle (Jaskowiak et al., 2018). Thus, we suggest that the possible mechanism of the *S. pombe* cell culture growth inhibition is connected to aluminium interactions with intracellular components related to regulation of the cell cycle. Additionally, our results clearly demonstrate that aluminium effect on the cell division is largely concentration dependent.

3.3 ICP measurement of absorbed aluminium by the cells

In order to determine the amount of aluminium absorbed by the *S. pombe* cells, samples were analysed by ICP-OES. Concentration of incorporated $\text{Al}(\text{OH})_3$ was measured after 3 hours of incubation. Obtained results have shown that concentration of absorbed aluminium rose with increasing concentration of aluminium present

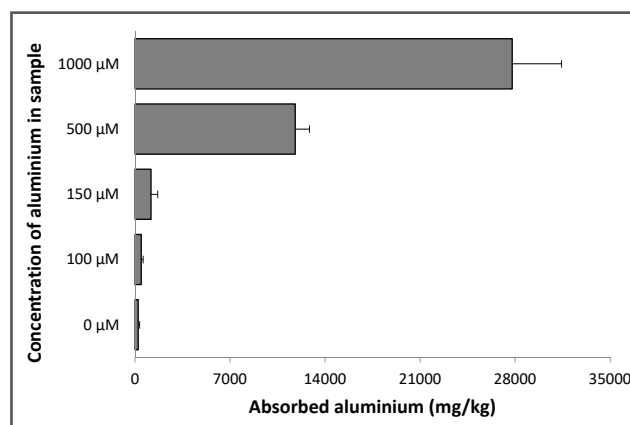


Figure 3 ICP-OES measurement of Al absorption. Atomic content of absorbed aluminium from $\text{Al}(\text{OH})_3$ after 3 hours of incubation. Comparison of aluminium absorption into *S. pombe* cells in dependence on the increasing concentration of $\text{Al}(\text{OH})_3$ in cell culture

Table 1 Added vs. absorbed Al³⁺, measured by ICP-OES after 3 hours of incubation

Added Al(OH) ₃ (μM)	Added Al ³⁺ (μg)	Absorbed Al ³⁺ (μg)
0	0	1.21 +/-0.67
100	54	2.63 +/-0.53
150	81	4.03 +/-0.69
500	270	48.10 +/-7.39
1,000	540	112.41 +/-18.83

in the *S. pombe* cell culture growth media. In the control sample, without aluminium addition, cells contained 232 mg/kg of aluminium. After addition of 100 μM of Al(OH)₃ to the growth medium content of aluminium in yeast cells doubled (459 mg/kg). Medium enrichment by 1 mM Al(OH)₃ caused more than hundredfold increase of aluminium content (27,781 mg/kg) in the cell (Figure 3). Uptake of aluminium by the cell exponentially rises with increasing aluminium concentration in the culture medium. Addition of 100 or 150 μM Al(OH)₃ led to incorporation of only one twentieth of Al³⁺ whereas under presence of 500 or 1,000 μM Al(OH)₃ in the medium cells were able to integrate one fifth of Al³⁺ ions (Table 1). We suggest that cells are capable to release aluminium up to certain concentration, while higher aluminium concentrations in the media cause aluminium persistence in the cell. Process of aluminium absorption and excretion by the cell is still not fully understood, however experiments with *S. cerevisiae* revealed some possible mechanisms through aluminium tolerant genes that are associated with secretory pathways (Kakimoto et al., 2005).

For instance, level of aluminium tolerance in plants is associated with citrate, oxalate and malate chelation of Al³⁺ to form a non-toxic complex. Release of aluminium from the cell is afterwards triggered by elution of aluminium complexes formed in the presence of elevated concentration of Al³⁺ in the environment (Jones and Ryan, 2003; Brunner and Sperisen, 2013). Accordingly, increased production of citrate was observed in aluminium tolerant yeast *Rhodotorula taiwanensis* RS1 after addition of aluminium above the tolerance level, which could be connected with possible detoxifying mechanism (Wang et al., 2013). Similarly, another research shows that accumulation of citrate in cellular and extracellular pools can mediate aluminium tolerance in *S. cerevisiae* strains (Anoop et al., 2003). Citrate and malate are present in all organisms, thus this mechanism of aluminium detoxification could be possibly used also by fission yeast. However, as these molecules are important for basic biochemical cycles (e.g. citric acid cycle), the capacity of binding Al³⁺ ions is probably limited, and therefore the mechanism of Al³⁺ detoxification

cannot be conducted at high aluminium concentrations. Although, to date known molecular mechanism of toxic metal/metalloids, such as antimony, mercury, arsenic or cadmium, metabolisms in *S. cerevisiae* were described by Wysocki and Tamas (2010), and detoxification mechanism of cadmium and arsenic in *S. pombe* was suggested by Guo et al. (2016), the exact mechanism of how *S. pombe* deals with aluminium ions remains to be elucidated. Uptake of aluminium by *S. pombe* cells is still poorly understood and accordingly, many other aspects of metal biology remain largely elusive.

3.4 Cell morphology

Cells respond to nutritional stress e.g. starvation or presence of toxic elements, causing interruption of energy production, by immobilization of cellular compartments in a size-dependent manner (Heimlicher et al., 2019). Different types of cells exhibit different pattern of cell destruction and apoptosis caused by aluminium exposure (Zheng et al., 2007; Wu et al., 2012; Huang et al., 2014). Deformation of cell morphology was observed on *S. cerevisiae* cells upon aluminium exposure, size of the yeast cells was modified with typical apoptotic signs as cell shrinkage, nuclear fragmentation, vacuolization, and chromatin marginalization (Zheng et al., 2007). Cells of normal newly generated *S. pombe* divided by mitosis should be oval, 8 μm long, and 3.5 μm wide (Hoffman et al., 2015). Differences of the cell shape caused by aluminium ions analysed under microscope using 40× magnification, were detected after 3 hours of incubation (Figure 4A). Although, some cells were longer as they started a new cell cycle, it was obvious that increasing concentration of Al(OH)₃ caused conformational deformation of the cell. At high concentration of 500 μM, the morphology of cells was altered and irregular, and cells were markedly destructed. Attributes of 100 cells from each treated and untreated sample were measured and compared (Figure 4B). The results suggest that addition of aluminium causes modifications of *S. pombe* cells morphology in a dose dependent manner. Average length of the cells was higher, though average width was lower (although high concentration of aluminium led to complete destruction of some cells). When cells are in mitosis, the

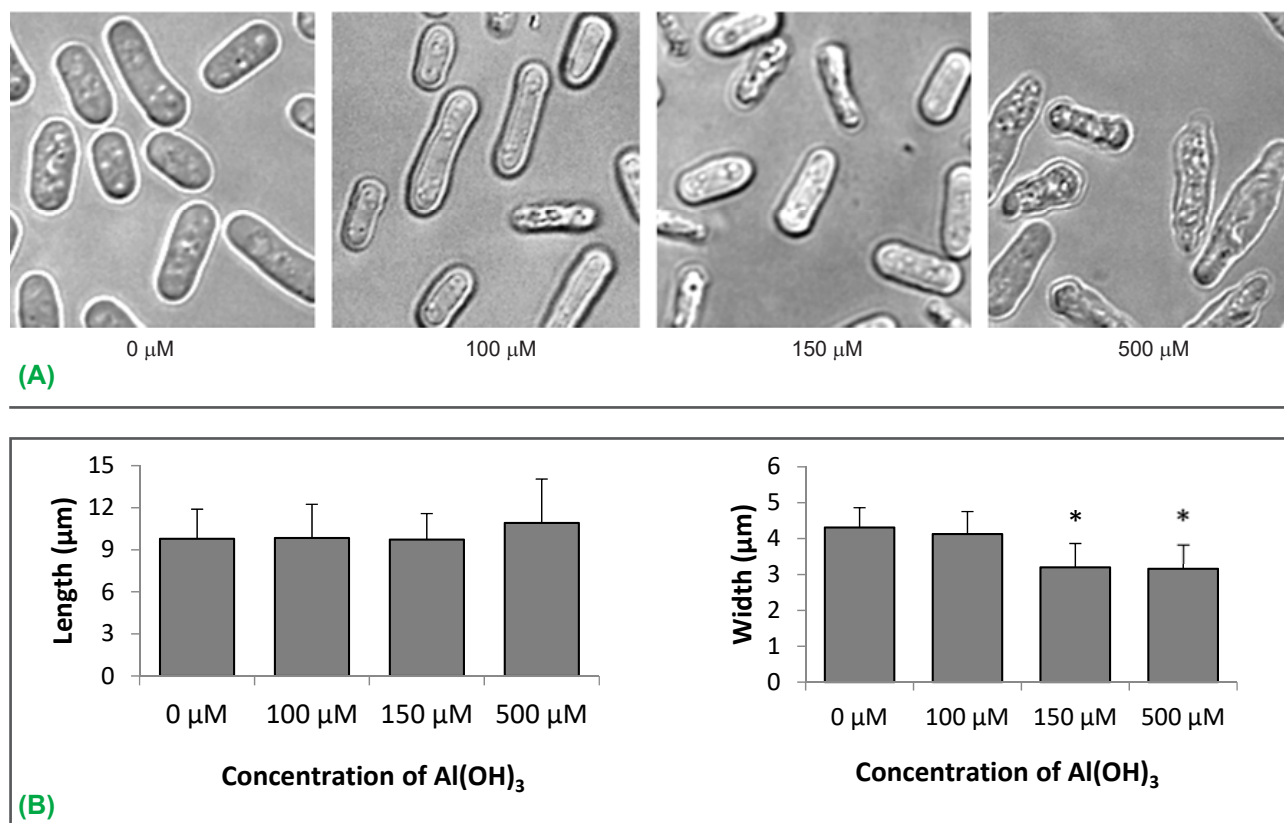


Figure 4 Morphology of the yeast cell after 3 hours of incubation with various concentrations of Al(OH)₃. (A) Representative pictures taken under the light microscope, magnification 40x. (B) Average length and width of the *S. pombe* in µm. Graph was created on the base of the pictures, length and width of 50 cells was measured by programme ImageJ. Statistical significance of obtained differences was determined by Student’s *T*-test, limit for significance was set up at *P* < 0.05*, 0.01**, 0.001 ***

length increases, but the width should stay the same. Our results show that according to the measurement, addition of high aluminium doses cause that cells seem to be “stretched” in their length and “shrunked” in their width. Accordingly, similar cell destructions after aluminium exposure were visible on *S. cerevisiae* under the electron microscope (Zheng, 2007).

4 Conclusions

Exposition of aluminium to the yeast *S. pombe* inhibits cell culture growth as it negatively affects the mitotic activity, together with cytoskeletal components responsible for cell division in a dose dependent manner. Additionally, higher aluminium concentrations cause irregular shape formation of the cells resulting in the overall cellular structure deformation. Intriguingly, our results show that uptake of aluminium by the cells is concentration dependent, as the amount of intracellularly absorbed aluminium is much greater in cells cultured under high aluminium concentration compared to conditions with lower Al content. This suggests that cells are able to eliminate certain proportion of aluminium from growth

medium when they grow under conditions enriched by low although, compared to standard, still elevated aluminium concentrations. Mechanisms of aluminium excretion are not fully understood so far, thus further investigation is required. The effect of aluminium exposure on the cell, cell cycle and aluminium metabolism in model organism could broaden the understanding of fate of the aluminium compounds after exposure in multicellular organisms.

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5 References

ANOOP, V.M. et al. (2003) Modulation of citrate metabolism alters aluminum tolerance in yeast and transgenic canola overexpressing a mitochondrial citrate synthase. In *Plant Physiology*, vol. 132, no. 4, pp. 2205–2217. DOI: <https://doi.org/10.1104/pp.103.023903>

- BRUNNER, I. and SPERISEN, C. (2013) Aluminum exclusion and aluminum tolerance in woody plants. In *Frontiers in Plant Science*, vol. 4, 172. DOI: <https://doi.org/10.3389/fpls.2013.00172>
- CHEN, Y. et al. (2018) Advances in dialysis encephalopathy research: a review. In *Neurological Sciences*, vol. 39, no. 7, pp. 1151–1159. DOI: <https://doi.org/10.1007/s10072-018-3426-y>
- CLEMENS, S. and SIMM, C. (2003) *Schizosaccharomyces pombe* as a model for metal homeostasis in plant cells: the phytochelatin-dependent pathway is the main cadmium detoxification mechanism. In *New Phytologist*, vol. 159, no. 2, pp. 323–330. DOI: <https://doi.org/10.1046/j.1469-8137.2003.00811.x>
- COLOMINA M.T. and PERIS-SAMPEDRO F. (2017) Aluminum and Alzheimer's Disease. In Aschner M., Costa L. (eds.) *Neurotoxicity of Metals. Advances in Neurobiology*, vol. 18 Springer, Cham, pp. 183–197. DOI: https://doi.org/10.1007/978-3-319-60189-2_9
- FRANTZIOS, G. et al. (2000) Aluminium effects on microtubule organization in dividing root-tip cells of *Triticum turgidum*. I. Mitotic cells. In *New Phytologist*, vol. 145, no. 2, pp. 211–224. DOI: <https://doi.org/10.1046/j.1469-8137.2000.00580.x>
- GUO, L. et al. (2016) Global fitness profiling identifies arsenic and cadmium tolerance mechanisms in fission yeast. In *G3: Genes, Genomes, Genetics*, vol. 6, no. 10, pp. 3317–3333. DOI: <https://doi.org/10.1534/g3.116.033829>
- HEIMLICH, M.B. et al. (2019) Reversible solidification of fission yeast cytoplasm after prolonged nutrient starvation. In *Journal of Cell Science*, vol. 132, no. 21, pp. 1–17. DOI: <https://doi.org/10.1242/jcs.231688>
- HOFFMAN, C.S. et al. (2015) An ancient yeast for young geneticists: a primer on the *Schizosaccharomyces pombe* model system. In *Genetics*, vol. 201, no. 2, pp. 403–423. DOI: <https://doi.org/10.1534/genetics.115.181503>
- HUANG, W.-J. et al. (2014) Aluminum induces rapidly mitochondria-dependent programmed cell death in Al-sensitive peanut root tips. In *Botanical Studies*, vol. 55, 67. DOI: <https://doi.org/10.1186/s40529-014-0067-1>
- JASKOWIAK, J. et al. (2018) Analysis of aluminum toxicity in *Hordeum vulgare* roots with an emphasis on DNA integrity and cell cycle. In *PLoS ONE*, vol. 13, 2: e0193156. DOI: <https://doi.org/10.1371/journal.pone.0193156>
- JONES, D. and RYAN, P. (2003) Aluminum toxicity. In: Thomas, B. et al. (eds.) *Encyclopedia of Applied Plant Science*. London: Elsevier Academic Press, pp. 656–664.
- KAIZER, R.R. et al. (2005) Acetylcholinesterase activation and enhanced lipid peroxidation after long-term exposure to low levels of aluminum on different mouse brain regions. In *Journal of Inorganic Biochemistry*, vol. 99, no. 9, pp. 1865–1870. DOI: <https://doi.org/10.1016/j.jinorgbio.2005.06.015>
- KAKIMOTO, M. et al. (2005) Genome-wide screening of aluminum tolerance in *Saccharomyces cerevisiae*. In *Biometals: An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine*, vol. 18, no. 5, pp. 467–474. DOI: <https://doi.org/10.1007/s10534-005-4663-0>
- KOVACIK, A. et al. (2019) Trace metals in the freshwater fish *Cyprinus carpio*: Effect to Serum Biochemistry and Oxidative Status Markers. In *Biological Trace Element Research*, vol. 188, no. 2, pp. 494–507. DOI: <https://doi.org/10.1007/s12011-018-1415-x>
- KUMAR, V. et al. (2009) Aluminium-induced oxidative DNA damage recognition and cell cycle disruption in different regions of rat brain. In *Toxicology*, vol. 264, no. 3, pp. 137–144. DOI: <https://doi.org/10.1016/j.tox.2009.05.011>
- LI, X. et al. (2011) Regulating cytoplasmic calcium homeostasis can reduce aluminum toxicity in yeast. In *PLoS ONE*, vol. 6, 6: e21148. DOI: <https://doi.org/10.1371/journal.pone.0021148>
- LI, X. et al. (2012) Aluminum induces osteoblast apoptosis through the oxidative stress-mediated JNK signaling pathway. In *Biological Trace Element Research*, vol. 150, no. 1, pp. 502–508. DOI: <https://doi.org/10.1007/s12011-012-9523-5>
- MACDIARMID, C.W. and Gardner, R.C. (1996) Al toxicity in yeast (A role for Mg?). In *Plant Physiology*, vol. 112, no. 3, pp. 1101–1109. DOI: <https://doi.org/10.1104/pp.112.3.1101>
- MAYA, S. et al. (2016) Multifaceted effects of aluminium in neurodegenerative diseases: a review. In *Biomedicine & Pharmacotherapy*, vol. 83, pp. 746–754. DOI: <https://doi.org/10.1016/j.biopha.2016.07.035>
- NEHRU, B. and ANAND, P. (2005) Oxidative damage following chronic aluminium exposure in adult and pup rat brains. In *Journal of Trace Elements in Medicine and Biology*, vol. 19, no. 2, pp. 203–208. DOI: <https://doi.org/10.1016/j.jtemb.2005.09.004>
- NIAN, H. et al. (2012) Physiological and transcriptional analysis of the effects of aluminum stress on *Cryptococcus humicola*. In *World Journal of Microbiology and Biotechnology*, vol. 28, no. 6, pp. 2319–2329. DOI: <https://doi.org/10.1007/s11274-012-1039-9>
- POZGAJOVA, M. et al. (2013) Prp4 kinase is required for proper segregation of chromosomes during meiosis in *Schizosaccharomyces pombe*. In *Acta Biochimica Polonica*, vol. 60, no. 4, pp. 871–873. DOI: https://doi.org/10.18388/abp.2013_2075
- POZGAJOVA, M. et al. (2019) Impact of cadmium and nickel on ion homeostasis in the yeast *Schizosaccharomyces pombe*. In *Journal of Environmental Science and Health, Part B*, pp. 1–8. DOI: <https://doi.org/10.1080/03601234.2019.1673613>
- PŘIBYL, P. et al. (2008) Cytoskeletal alterations in interphase cells of the green alga *Spirogyra decimina* in response to heavy metals exposure: II. The effect of aluminium, nickel and copper. In *Toxicology in Vitro*, vol. 22, no. 5, pp. 1160–1168. DOI: <https://doi.org/10.1016/j.tiv.2008.03.005>
- QIN, R. et al. (2010) Effects of aluminum on nucleoli in root tip cells and selected physiological and biochemical characters in *Allium cepa* var. *agrogarum* L. In *BMC Plant Biology*, vol. 10, p. 225. DOI: <https://doi.org/10.1186/1471-2229-10-225>
- SABATINOS, S.A. and FORSBURG, S.L. (2010) Molecular genetics of *Schizosaccharomyces pombe*. In *Methods in Enzymology*, vol. 470, pp. 759–795. DOI: [https://doi.org/10.1016/S0076-6879\(10\)70032-X](https://doi.org/10.1016/S0076-6879(10)70032-X)
- SALGADO, A. et al. (2012) Response to arsenate treatment in *Schizosaccharomyces pombe* and the role of its arsenate reductase activity. In *PLoS one*, vol. 7, no. 8, pp. e43208. DOI: <https://doi.org/10.1371/journal.pone.0043208>

SHORT, A.I. et al. (1980) Reversible microcytic hypochromic anaemia in dialysis patients due to aluminium intoxication. In *Proceedings of the European Dialysis and Transplant Association*. In *European Dialysis and Transplant Association*, vol. 17, pp. 226–233.

TUN, N.M. et al. (2013) Disulfide stress-induced aluminium toxicity: molecular insights through genome-wide screening of *Saccharomyces cerevisiae*. In *Metallomics*, vol. 5 no. 8, pp. 1068–1075. DOI: <https://doi.org/10.1039/C3MT00083D>

VARDAR, F. et al. (2016) Determination of aluminum induced programmed cell death characterized by DNA fragmentation in Gramineae species. In *Caryologia*, vol. 69, no. 2, pp. 111–115. DOI: <https://doi.org/10.1080/00087114.2015.1109954>

WANG, C. et al. (2013) Proteomic analysis of a high aluminum tolerant yeast *Rhodotorula taiwanensis* RS1 in response to aluminum stress. In *Biochimica et Biophysica Acta (BBA) – Proteins and Proteomics*, vol. 1834, no. 10, pp. 1969–1975. DOI: <https://doi.org/10.1016/j.bbapap.2013.06.014>

WOOD, V. et al. (2002) The genome sequence of *Schizosaccharomyces pombe*. In *Nature*, vol. 415, no. 6874, pp. 871–880. DOI: <https://doi.org/10.1038/nature724>

WU, M.J. et al. (2012) Delineation of the molecular mechanism for disulfide stress-induced aluminium toxicity.

In *Biometals: An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine*, vol. 25, no. 3, pp. 553–561. DOI: <https://doi.org/10.1007/s10534-012-9534-x>

WYSOCKI, R., and TAMÁS, M. J. (2010) How *Saccharomyces cerevisiae* copes with toxic metals and metalloids. In *FEMS microbiology reviews*, vol. 34, no. 6, pp. 925–951. DOI: <https://doi.org/10.1111/j.1574-6976.2010.00217.x>

ZHANG, H. et al. (2014) Accumulation and cellular toxicity of aluminum in seedling of *Pinus massoniana*. In *BMC Plant Biology*, vol. 14, 264. DOI: <https://doi.org/10.1186/s12870-014-0264-9>

ZHENG, K. et al. (2007) Programmed cell death-involved aluminum toxicity in yeast alleviated by antiapoptotic members with decreased calcium signals. In *Plant Physiology*, vol. 143, no. 1, pp. 38–49. DOI: <https://doi.org/10.1104/pp.106.082495>

The comparison of the selected morphometric traits in three medium-sized rabbit breeds

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The aim of the study was to determine the growth characteristics of three rabbit breeds (Czech Solver, CSo, $n = 11$; Czech Spotted, CS, $n = 28$; Blanc de Hotot, BH, $n = 24$) under small-scaled stock conditions as a basis for their potential meat performance. The body morphometric characteristics live weight (LW), head length (HL), body length (BL), ear length, thoracic circumference (TC) and body compact index TC/BL were recorded in growing rabbits from 21st to 91st day of their age. At the end of the trial, the highest LW value was recorded in the BH breed (2,700.0 g) as compared to the CSo and CS breeds, respectively (1,887.5 and 1,545.4 g). The values of the HL were significantly affected by a rabbit breed up to the 63rd day of their age ($P < 0.01$). The highest values of BL and TC were found in the BH breed as compared to the CSo breed and also the CS breed ($P < 0.01$). The BH breed showed also the longest ears ($P < 0.01$) while the different dynamics of the ear growth among the evaluated breeds was found. Concerning the body compact index TC/BL, the growing rabbits of the BH breed showed wider body given by their musculature proportion as compared to the representatives of the CSo and CS breeds ($P < 0.01$). Our findings suggest that the Blanc de Hotot breed possesses suitable growth and morphometric characteristics for intended meat production.

Keywords: rabbit, breeds, growth analysis, body measurement, morphometric parameters

1 Introduction

According to the EU overview report, a substantial proportion (34%) of produced rabbit meat came from backyard small-scaled stocks that is different to other livestock species (EU, 2017). Under conditions of the Czech Republic, this proportion is underlined by local historical background of husbandry of domestic rabbit and its main role as a productive animal (Mach et al., 2010). Approximately 95% of fattened rabbits reared in the Czech Republic originated from small-scaled stocks. Although, annual consumption of the rabbit meat per capita shows a decreasing tendency in the Czech Republic currently, local meat consumption ranks still among European countries with the highest stated consumption of this meat (Josrová, 2018). Number of studies dealing with productive and reproductive traits of rabbit breeds included in the Czech genetic resources

programme revealed that some selected breeds show a good potential for meat production (Zita et al., 2010; Tůmová et al., 2011; Tůmová et al., 2014). Similarly, a Nitra rabbit breed, Slovak national breed, showed suitable promise for meat production in small-scaled stocks (Fik et al., 2018). Generally, morphometric measurement of animals is served for recording of their phenotypic characteristics (Khan et al., 2017), what aids to selection in animal genetic improvement (Hassan et al., 2012). The measurement of the specific parts of the rabbit carcass belongs to the important meat traits finding after rabbit slaughtering (Blasco and Ouhayoun, 1993; Hernández et al., 1996). Whereas, chosen specific morphometric parameters were used successfully for assessment of the rabbit body composition and can serve as indirect indicators of adipose tissue mass in rabbits (Sweet et al., 2013).

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The aim of the study was to evaluate selected morphometric parameters among three rabbit breeds normally reared in the Czech Republic. An integral part of the work was to determine the growth characteristics of these rabbit breeds under small-scaled stock conditions as a basis for their potential meat performance.

2 Material and methods

2.1 Animals and husbandry conditions

The study was performed on a total of 63 young rabbits belonging to the medium-sized breeds, specifically the Czech Solver (CSo, $n = 11$), the Czech Spotted (CS, $n = 28$) and the Blanc de Hotot (BH, $n = 24$). The adult rabbits of the CSo breed weigh usually around 3.50–4.25 kg and possess a unique genotype for coat colour which seems a yellowish river sand. The adults of the CS breed (3.30–4.00 kg) show a typical coloured marking where coloured patterns are situated on the pure white base colour. Adult rabbits of the BH breed (4.00–5.00 kg) possess a maximally reduced colour marking, just in form of the gentle black eye bands, while the rest of the body is of pure white colour (Zadina, 2003; Whitman, 2004). The rabbits included in the present study came from the common hobby stocks which perform exhibition activities under guidelines of the Czech Association of Breeders. Parents of these rabbits belonged to the typical representatives of the respective breeds. The rabbits were housed in the outdoor hutches with pens of a recommended size. Rabbit kits were housed with their does up to 8th week of their age. Subsequently, after weaning, the growing rabbits were housed in small groups (2–3 rabbits per group) up to the end of the monitored period (91st day of age). The young growing rabbits received a complete pelleted diets with similar nutrient composition. The rabbits were fed once a day. The animals had unlimited access to drinking water and meadow hay.

2.2 Morphometric parameters and data collection

The body morphometric measurement was performed according to Dalle Zotte et al. (2013) with certain technical modifications. The characteristics such as the live weight (LW), head length (HL), body length (BL), ear length (EL), thoracic circumference (TC) and body compact index TC/BL were recorded in growing rabbits. Orientation using specific anatomical points at the rabbit body was used within the measurements. The measuring tape was used for recording the BL (measured from atlas to the first coccygeal vertebra), the HL (measured from the tip of the nostrils to atlas) and the TC (measured behind a shoulder blade). Subsequently, the TC/BL index was calculated. The special ear measuring ruler was used for the EL recording. The base of this instrument was placed

between the ears at the ears' base and both ears were put on the measuring scale, then the value at the top of the ear was recorded.

All monitored morphometric parameters of individual rabbits were recorded for the first time at the age of 21 days and subsequently in two-week period up to the age of 91 days. All data collection was performed at the same day period and prior to feeding. All measurements were performed by the same person with the aim to eliminate measurement errors.

2.3 Statistical analysis

The obtained data were statistically analysed using a software STATISTICA CZ, version 10 (StatSoft Inc., 2011). One-way variance analysis (ANOVA) was used to determine differences in the evaluated morphometric traits. When ANOVA showed significant differences among the evaluated breeds a post-hoc HSD test was used. The differences were considered significant at $P < 0.05$.

3 Results and discussion

The breed effect on the evaluated morphometric parameters in pre-weaning rabbit kits is presented in Table 1. The effect of rabbit breeds on the evaluated morphometric parameters in the post-weaning period is presented in Table 2.

Generally, it follows from the results that all monitored morphometric parameters were significantly affected by a particular rabbit breed.

In meat-type rabbits, their live weight (more specifically slaughter weight) is essential trait in relation to the economy of rabbit meat production (Szendrő et al., 2012). In our trial, the rabbits of the CS breed showed the lowest LW values among monitored breeds within an entire monitored period ($P < 0.01$). These findings were expectable due to foregoing selective breeding of the CS breed. Martinec et al. (2017) state that the CS breed is a typical hobby breed historically selected mainly for an exhibition purpose, while its exterior traits that have been included in the selection effort in the past are not directly related to meat performance of rabbits. This fact was repeatedly verified also in studies dealing with evaluation of meat performance of rabbit breeds included in the Czech genetic resources of animals (Tůmová et al., 2011; Tůmová et al., 2014). On the other hand, the highest LW values were found in the BH breed. At the age of 91 days, the BH rabbit showed significantly higher LW ($P < 0.01$) as compared to the CS rabbit (+1,545.4 g) and also the CSo rabbit (+812.5 g). It can be highlighted that the final LW of the BH purebred rabbits is similar to that of common slaughter weights in meat-type rabbit hybrids (Szendrő et al., 2012, Josrová, 2018). The very good growth potential

Table 1 Selected morphometric parameters in the monitored rabbit breeds in pre-weaning period.

Parameter	Breed						P
	Czech Solver		Czech Spotted		Blanc de Hotot		
	x	SEM	x	SEM	x	SEM	
Age 21 days							
LW (g)	211.8 ^B	10.86	178.9 ^B	9.40	392.5 ^A	15.41	**
HL (mm)	82.2 ^{A, B}	0.18	77.1 ^B	1.29	87.1 ^A	0.79	**
BL (mm)	141.3 ^B	3.58	121.7 ^C	3.44	178.5 ^A	1.96	**
EL (mm)	47.0 ^B	0.69	42.8 ^B	0.92	60.9 ^A	1.05	**
TC (mm)	94.7 ^B	4.05	96.1 ^B	2.50	148.0 ^A	4.11	**
Index TC/BL	0.67 ^B	0.03	0.78 ^A	0.01	0.83 ^A	0.02	**
Age 35 days							
LW (g)	460.9 ^B	26.81	371.1 ^B	25.26	823.8 ^A	26.08	**
HL (mm)	89.1 ^{A, B, b}	1.9	86.8 ^{B, b}	1.9	108.0 ^{A, a}	0.95	**
BL (mm)	202.4 ^B	7.21	191.1 ^B	4.76	239.3 ^A	2.38	**
EL (mm)	71.5 ^B	0.65	61.9 ^C	1.33	89.2 ^A	1.64	**
TC (mm)	120.5 ^B	5.71	115.2 ^B	4.08	186.4 ^A	5.44	**
Index TC/BL	0.60 ^B	0.02	0.60 ^B	0.01	0.78 ^A	0.02	**
Age 49 days							
LW (g)	812.2 ^B	23.73	614.8 ^B	30.64	1353.7 ^A	46.28	**
HL (mm)	106.3 ^B	1.8	99.0 ^B	1.57	120.4 ^A	1.48	**
BL (mm)	255.4 ^{A, B, b}	6.4	225.7 ^{B, b}	4.31	280.7 ^{A, a}	2.41	**
EL (mm)	87.1 ^{B, a}	0.96	78.5 ^{B, b}	1.38	104.7 ^A	1.79	**
TC (mm)	148.4 ^B	4.61	141.8 ^B	5.06	216.1 ^A	5.59	**
Index TC/BL	0.58 ^B	0.01	0.62 ^B	0.01	0.77 ^A	0.02	**

x – arithmetic mean; SEM – standard error of the mean; LW – live weight; HL – head length; BL – body length; EL – ear length; TC – thoracic circumference

a, b – means within a row with different superscript letters differ at ($P < 0.05$); **, A, B, C – means within a row with different superscript letters differ at ($P < 0.01$)

of the BH breed was not described in recent literature yet. At the present time, the BH breed is considered as a typical hobby rabbit breed reared mainly for exhibition purposes. However, its original breeding goal was focused to create a multi-purpose breed using for meat and fur productions and also for exhibition activities. The BH rabbits showing the large body frame, white coloured coat and black eyes (Whitman, 2004), while their standard markings are underlined by the combination of English spot gene and Dutch gene (Hinkle, 2011); this gene combination is rare among the rabbit breeds. The present form of the BH rabbits was obtained from a breeding programme that based on crossing of the Giant Papillon, the White Flemish Giant, the Vienna White and other breeds (Whitman, 2004). The crossbreeding leads to increase of level of heterosis, whereas the breed complementarity and correct selection process must be taken into consideration to achieve favourable traits for

meat performance (Ouyed et al., 2011; McNitt et al., 2013). An initial increase of the rabbit genetic diversity normally occurs because of initial crossbreeding (Queney et al., 2002). It can be assumed that the favourable growth rate of the BH breed in the present study could be influenced by its various genotypes passed from the initial crossing of included breeds due to a heterosis effect.

Besides that, when compared to ideal standard LW values at the age of 3 months (Zadina, 2003), LWs found in the CS breed and also in the CSo breed are closed to the ideal standard LW values (1,500 and 1,800 g, resp.) according to Zadina (2003). Concerning the BH rabbits, they showed the obviously higher LW as compared to the published standard LW value (1,500 g). It should be pointed out that the exterior traits of each breed are developing with ongoing time and therefore the breed standards are regularly updated. Besides that, a recent study of Tůmová et al. (2011) showed slightly different

Table 2 Selected morphometric parameters in the monitored rabbit breeds in post-weaning period.

Parameter	Breed						P
	Czech Solver		Czech Spotted		Blanc de Hotot		
	x	SEM	x	SEM	x	SEM	
Age 63 days							
LW (g)	1,205.0 ^B	27.45	901.2 ^C	41.50	1,853.3 ^A	34.37	**
HL (mm)	123.6 ^B	15.707	112.9 ^C	1.50	138.9 ^A	1.37	**
BL (mm)	260.6 ^B	25.64	256.8 ^B	4.03	323.2 ^A	3.83	**
EL (mm)	98.1 ^B	0.93	92.7 ^B	1.15	115.2 ^A	1.28	**
TC (mm)	165.4 ^B	1.69	160.6 ^B	4.24	232.1 ^A	3.25	**
Index TC/BL	0.77	0.19	0.62	0.01	0.72	0.01	ns
Age 77 days							
LW (g)	1,606.3 ^B	34.48	1,188.8 ^C	44.63	2,267.6 ^A	58.19	**
HL (mm)	136.6	2.48	128.4	1.65	148.4	1.64	ns
BL (mm)	321.5 ^A	6.14	282.2 ^B	5.22	346.8 ^A	3.57	**
EL (mm)	106.1 ^B	1.08	104.0 ^B	1.09	120.2 ^A	1.60	**
TC (mm)	192.0 ^B	4.3	174.7 ^B	4.75	244.0 ^A	4.44	**
Index TC/BL	0.60 ^B	0.02	0.62 ^B	0.01	0.70 ^A	0.01	**
Age 91 days							
LW (g)	1,887.5 ^{B, a}	72.23	1,545.4 ^{B, b}	50.25	2,700.0 ^A	54.46	**
HL (mm)	140.9	2.72	137.7	1.45	157.7	1.85	ns
BL (mm)	355.8 ^A	3.19	322.5 ^B	5.24	369.2 ^A	3.71	**
EL (mm)	111.1 ^B	1.32	112.0 ^B	0.98	121.2 ^A	2.02	**
TC (mm)	204.6 ^B	3.13	190.8 ^B	4.07	264.4 ^A	6.54	**
Index TC/BL	0.58 ^B	0.01	0.59 ^B	0.01	0.72 ^A	0.02	**

x – arithmetic mean; SEM – standard error of the mean; LW – live weight; HL – head length; BL – body length; EL – ear length; TC – thoracic circumference; ns – not significant

a, b – means within a row with different superscript letters differ at ($P < 0.05$); **, A, B, C – means within a row with different superscript letters differ at ($P < 0.01$)

LW of the 91-day-old CSo rabbits (2,453 g) and CS rabbits (2,240 g). In accordance with this finding, the CS breed showed a lower LW as compared to the CSo breed in the present study.

The rabbit head is a part of the carcass when a hot carcass weight is determined. The proportion of the head play a role when the carcass dressing percentage is calculated (Blasco and Ouhayoun, 1993). Wang et al. (2016) found that the proportion of the rabbit head is significantly affected by a rabbit genotype, while the commercial meat-type hybrid rabbits show a lower proportion of the head as compared to the purebred rabbits. Concerning findings in the present study, HL was significantly affected by a rabbit breed up to the 63rd day of their age ($P < 0.01$). Thereafter, HL didn't differ significantly among monitored breeds ($P > 0.05$).

The dorsal linear length belongs to the important meat traits measured after rabbit slaughtering. This trait is associated with length of the *m. longissimus lumborum*, one of the main part of the rabbit carcass (Blasco and Ouhayoun, 1993). In the present study, the breed had a significant effect on values of BL in course of an entire monitored period. At the end of the monitored period, the highest values of BL were found in the BH breed ($P < 0.01$) as compared to the CSo breed (+13.4 mm) and also the CS breed (+46.7 mm).

In present study, the highest values of EL were recorded in the BH breed within the entire monitored period. At the age of 91 days, the BH rabbits showed significantly ($P < 0.01$) longer ears as compared to the CSo rabbits (+10.1 mm) and also the CS rabbits (+9.2 mm). The EL belongs to the important exterior traits stated in a breed standard. The found ELs of 91-day-old rabbits of all

the evaluated breeds were in accordance with values (CSo, 110–120 mm; CS, 105–110 mm; BH, 120–125 mm) published in the current breed standards (Zadina, 2003). It can be noted that values for EL at 21 day-old rabbit represented only 38.2% from its value at the age of 91 days in the CS breed, while the BH rabbits only doubled (+49.8%) their EL within the monitored period. Besides that, Lukefahr and Ruiz-Feria (2003) found in rabbits the moderate to high positive correlation between the EL and growth traits, while they point out that growth rate of rabbits may be influenced also by their fur clipping.

The body compact index TC/BL determines relationship between the length and width proportions of a rabbit body. When the TC/BL increases, the rabbit shows wider body given by musculature proportion whereas the decreasing TC/BL index results in poor muscling of their body. Except for the age of 63 days in the present study, it can be highlighted that the growing rabbits of the BH breed showed the highest values of TC/BL index among the monitored breeds ($P < 0.01$). At the end of the monitored period, the BH rabbits showed a higher value ($P < 0.01$) for TC/BL index as compared to the CSo rabbits (+0.14) and also the CS rabbits (+0.13). Parameters of the *m. longissimus lumborum* are essential for meat quality of rabbits (Blasco and Ouhayoun, 1993; Gondret et al., 1998, Tůmová et al., 2014). Generally, based on findings of the present study, it can be assumed that the growing rabbits of the BH breed displayed promising preconditions for the meat purpose.

4 Conclusions

Based on the obtained results, it can be concluded that both live weight and all monitored morphometric parameters of rabbits were significantly affected by a rabbit breed.

As for meat production potential, the least favourable values for observed morphometric measures were found in the Czech Spotted breed. Thus, the Czech Spotted breed can be considered still as a breed suitable mainly for exhibition purpose, while its meat production potential remains low. On the other hand, the highest values for the live weight and morphometric parameters were found in the non-traditional Blanc de Hotot rabbit breed. Especially, the values of the live weight and body length in the 91-day-old growing rabbits were quite high, what are desirable traits of this breed. These preliminary findings suggest that the Blanc de Hotot breed possesses suitable growth and morphometric characteristics for intended meat production. However, further studies are needed to deepen our knowledge about productive and reproductive traits of the Blanc de Hotot rabbits reared also under intensive farming conditions.

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5 References

- BLASCO, A. and OUHAYOUN, J. (1993) Harmonization of criteria and terminology in rabbit meat research. Revised proposal. In *World Rabbit Science*, vol. 4, no. 2, pp. 93–99. DOI: <https://doi.org/10.4995/wrs.1996.278>
- DALLE ZOTTE, A., RICCI, R., SARTORI, A., LUKEFAHR, S. and PACI, G. (2013) Body morphometric development during growth and maturity of colored dwarf rabbits available in the Italian market. In *World Rabbit Science*, vol. 21, no. 4, pp. 227–233. DOI: <https://doi.org/10.4995/wrs.2013.1386>
- EUROPEAN UNION (2017) *Overview Report: Commercial Rabbit Farming in the European Union*. Luxembourg: Publications Office of the European Union. 16 s. DOI: 10.2772/62174.
- FIK, M., ANDREJI, J., HRNČÁR, C., ARPÁŠOVÁ, H. and NEIRUREROVÁ, P. (2018) Reproduction performances, growth and slaughter traits analysis of rabbit of Nitra breed. In *Acta fytotechnica et zootechnica*, vol. 21, no. 4, pp. 162–165. DOI: <https://doi.org/10.15414/afz.2018.21.04.162-165>
- GONDRET, F., JUIN, H., MOUROT, J. and BONNEAU, M. (1998) Effect of age at slaughter on chemical traits and sensory quality of *Longissimus lumborum* muscle in the rabbit. In *Meat Science*, vol. 48, no. 1–2, pp. 181–187. DOI: [https://doi.org/10.1016/S0309-1740\(97\)00088-0](https://doi.org/10.1016/S0309-1740(97)00088-0)
- HASSAN H.E., ELAMIN K.M., YOUSIF I.A., MUSA, A.M. and ELKHAIREY, M.A. (2012) Evaluation of body weight and some morphometric traits at various ages in local rabbits of Sudan. In *Journal of Animal Science Advances*, vol. 2, no. 4, pp. 407–415.
- HERNÁNDEZ, P., PLA, M. and BLASCO, A. (1996) Prediction of carcass composition in the rabbit. In *Meat Science*, vol. 44, 1–2, pp. 75–83. DOI: [https://doi.org/10.1016/S0309-1740\(96\)00078-2](https://doi.org/10.1016/S0309-1740(96)00078-2)
- HINKLE, A. (2011) Color genetics of the Dwarf Hotot. In HINKLE, A. *American Dwarf Hotot Specialty Club Guidebook*, pp. 112–116.
- JOSROVÁ, L. (2018) *Situation and Prospect Report: Rabbits*. Praha: Ministry of Agriculture of the Czech Republic. 20 pp.
- KHAN, S., KHAN, M.H., MUHAMMAD, S., KALEEM, K., SHAH, P., KHAN, A. SHAKIR, M.I.M. and KHAN, N. (2017) Phenotypic and morphometric characteristics of Angora rabbits in rabbit model farm JabbaMansehra Khyber Pakhtunkhwa-Pakistan. In *Journal of Biology, Agriculture and Healthcare*, vol. 7, no. 5, pp. 68–71.
- LUKEFAHR, S.D. and RUIZ-FERIA C.A. (2003) Rabbit growth performance in a subtropical and semi-arid environment: effects of clipping, ear length, and body temperature. In *Livestock Research for Rural Development*, vol. 15, no. 2.
- MACH K., MARTINEC, M., VOSTRÝ, L., ADREJSOVÁ, L. and MAJZLÍK, I. (2010) History and development of rabbit breeding in the Czech Republic. In *Acta fytotechnica et zootechnica – Mimoriadne číslo*, pp. 111–114 (In Czech).
- MARTINEC, M., ŠIMEK, V. and JAHODA, J. (2017) Development of rearing of national rabbit breeds included into genetic resources preserve programme in the Czech Republic.

- In *New trends in intensive and hobby rabbit breeding*. Praha 29.11.2017. Praha: Institute of Animal Science, pp. 54–56 (In Czech).
- McNITT, J.J., LUKEFAHR, S.D., CHEEKE, P.R. and PATTON, N.M. (2013) *Rabbit production*. 9th ed. Wallingford: CABI, 300 pp.
- OUYED, A., RIVEST, J. and BRUN, J.M. (2011) Heterosis, direct and maternal additive effects on rabbit growth and carcass traits from a Canadian experiment. In *World Rabbit Science*, vol. 19, no. 1, pp. 31–41. DOI doi: <https://doi.org/10.4995/wrs.2011.783a>
- QUENEY, G., VACHOT, A.-M., BRUN, J.-M., DENNEBOUY, N., MULSANT, P. and MONNEROT, M. (2002) Different levels of human intervention in domestic rabbits: Effects on genetic diversity. In *Journal of Heredity*, vol. 93, no. 3, pp. 205–209. DOI <https://doi.org/10.1093/jhered/93.3.205>
- TŮMOVÁ, E., MARTINEC, M. and CHODOVÁ, D. (2011) Analysis of Czech rabbit genetic resources. In *Scientia Agriculturae bohemica*, vol. 42, no. 3, pp. 113–118.
- TŮMOVÁ, E., BÍZKOVÁ, Z., SKŘIVANOVÁ, V., CHODOVÁ, D., MARTINEC, M. and VOLEK, Z. (2014) Comparison of carcass and meat quality among rabbit breeds of different size and hybrid rabbits. In *Livestock Science*, vol. 165, pp. 8.14. DOI: <http://dx.doi.org/10.1016/j.livsci.2014.04.019>
- STATSOFT Inc. (2011) STATISTICA Data analysis software system, version 10. StatSoft Inc., Tulsa.
- SWEET, H., PEARSON, A.J., WATSON, P.J. and GERMAN, A.J. (2013) A novel zoometric index for assessing body composition in adult rabbits. In *Veterinary Record*, vol. 173, no. 15, pp. 369. DOI: <https://doi.org/10.1136/vr.101771>
- SZENDRŐ, K., METZGER S., OEDRMATT, M., RADNAI, I., GARAI, É., HORN, P. and SZENDRŐ, Zs. (2012) Effect of age and weight of rabbits at slaughter on carcass value. In *Acta agriculturae Slovenica*, suppl. 3, pp. 333–337.
- WANG, J., SU, Y., ELZO, M.A., JIA, X., CHEN, S. and LAI, S. (2016) Comparison of carcass and meat quality traits among three rabbit breeds. In *Korean Journal of Food Science of Animal Resources*, vol. 36, no. 1, pp. 84–89. DOI: <http://dx.doi.org/10.5851/kosfa.2016.36.1.84>
- WHITMAN, B.D. (2004) *Domestic rabbits and their histories: Breeds of the World*. Leawood: Leathers Publishing, 456 pp.
- ZITA, L., TŮMOVÁ, E. and BÍZKOVÁ, Z. (2010) Genetic resources of rabbits in the Czech Republic. In *Acta fytotechnica et zootechnica – mimoriadne číslo*, pp. 34–36 (In Czech).
- ZADINA, J. (2003) *Rabbit breed standard*. Praha: Czech Association of Breeders. 371 pp. (in Czech)