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EFFECT OF SEWAGE SLUDGE COMPOST TREATMENT ON CROP YIELD

Attila TOMÓCSIK[1]*, Marianna MAKÁDI[1], Viktória OROSZ[1], Tibor ARANYOS[1], Ibolya DEMETER[1], József MÉSZÁROS[2], György FÜLEKY[3]

[1]Research Institute of Nyíregyháza, University of Debrecen, Nyíregyháza, Hungary
[2]Nyírségvíz Closed Shareholder Group, Nyíregyháza, Hungary
[3]Department of Soil Science and Agricultural Chemistry, Szent István University, Gödöllő, Hungary
*Corresponding author: tomcsik@freemail.hu

ABSTRACT

Due to the increasing number of sewage cleaning plants, the amount of sewage sludge also increases. We have to solve the environmentally sound disposal of the sludge. Results of many experiments show that sewage sludge and sewage sludge compost can be recycled as nutrient suppling material in agriculture. Municipal sewage sludge compost could cause the occurrence and accumulation of toxic elements in the soil. A small-plot experiment with sewage sludge compost was established in the spring of 2003. The applied compost contains 40% sewage sludge, 25% straw, 30% rhyolite, 5% betonite. The small-plot experiment was re-treated in the autumn of 2006, 2009, 2012and 2015. There are 4 treatments in five blocks, where the sewage sludge compost was applied at a rate of 0, 9, 18 and 27 tha^{-1} and then ploughed into the soil. Triticale as autumn cereal, maize and green pea as spring crops were sown in crop rotation every year. Plant samples were collected before harvesting. In this paper the results of crop yield between 2010-2012 are presented. Crops of triticale and maize were higher in the treated plots than in control one in 2010 and 2011. Treatment effect was not observed on green pea yield.The results show that the effect of applied compost doses depends on plant species and time. Our aim is to maintain this unique long-term experiment for studying the composted sewage sludge as a nutrient and organic matter source, applying it similarly to the farmyard manure.

Keywords: *sewage sludge, long-term experiment, crop yield, compost treatment.*

INTRODUCTION

The uses of sewage sludge include industrial utilization, landfill, combustion and composting for yield utilization (Sánchez et al. 2004). Composting is one of the most efficient waste treatment technologies to handling waste and enables to recycling of organic matter, reduce the amount of biodegradable organic matters

(Epstein, 1997; Stamatelatou et al., 2011; Amir et al., 2005; Ramirez et al., 2008; Marttinen et al., 2004; Oleszczuk, 2007; Poulsen and Berster 2010). Sandy soils, that are poor in mineral and organic colloids, have low fertility as determined by low water retention and a shortage of macro-and micronutrients. Good agricultural practices involve frequent applications of organic fertilizers, both conventional such as manure or plant residues, as well as non-conventional, such as peat, brown coal, sewage sludge or different kinds of composts (Chandra etal., 2009). Several years of sludge usage experiments demonstrate the effect of sewage sludge fertilizer. The plants responded favourable to the sludge treatment. Beneficial effects of sludge on soil are the increase of organic matter content, improvement of soil fertility, increase of nutrient content and microbiological activity and its ability for working as a complex fertilizer. The negative effects of the continuous application of composted sewage sludge can be the appearance of toxic elements, and in case of long-term usage the accumulation of the toxic elements in the soil and in the food chain (Vermes, 1998). Many countries introduce compost standards for regulating product quality including maximum-minimum trace elements or pollutant elements concentrations (Madrid, 2010).

Our more than 10 years old experiment is suitable to study the soil-plant system and to decide whether these elements are accumulated or not in this experimental condition which are similar to a general field application. The main objective was to observe the effect of composted sewage sludge on test plants. We analysed the relationship between the crop yield of test plants and some soil chemical properties.

MATERIALS AND METHODS

The experimental design is randomized block in five repetitions. Soil type is acidic sand(sand: 86.01%, silt: 4.08%, clay: 9.64%) at the Research Institute of Nyíregyháza,University of Debrecen, in the NE part of Hungary (GPS coordinates: 47º96' North, 21º72' East). Plot size is 12 x 18 m. Test plants are maize (*Zea mays* L.'MV NK 333'), triticale (*x Triticosecale Wittmack* 'Titán') and green pea (*Pisum sativum* L. 'Zita'). Plant samples were collected before harvesting. Green pea and triticale samples were collected from 4x1 square metres while maize samples were collected from 4x1 m rows. Crop yields were calculated from these samples.

The composted sewage sludge contained 40 m/m% dry matter(DM) of sewage sludge, 25 m/m% DM of rye straw, 30 m/m% DM of rhyolite and 5 m/m% DM of bentonite and was applied to the 0-30 cm soil layer in every 3rd year at the rate of 0, 9, 18 and 27 t ha^{-1} after harvesting in 2003, 2006, 2009, 2012 and 2015. Table 1 contains the important parameters of the applied compost which has more than 25 m/m% DM organic matter content therefore it is good for improving the organic matter content of soil. The addition of composted sewage sludge improves some physical and chemical properties of sandy soil. The pH(H_2O) of compost was near 7, thereby we can increase the pH of acid soil with application of compost. The nitrogen (N), phosphorus (P) and potassium (K) content of composted sewage

sludge can enhance the available nutrients content of soil. The toxic elements content of compost was under the Hungarian limit values.

Table 1. Main characteristics of the applied compost

Parameter	Average	
pH (H_2O 1:10)		6.5-8.5
Dry matter content [m/m% row matter]	at least	50
Organic matter content [m/m % dry matter]	at least	25
Water soluble total salt [m/m% dry matter]	at least	4
Total N-content [m/m% dry matter]	at least	1
Total P_2O_5-content [m/m% dry matter]	at least	0,5
Total K_2O-content [m/m% dry matter]	at least	0,5
As (mg kg^{-1})	maximum	10
Cd (mg kg^{-1})	maximum	2
Co (mg kg^{-1})	maximum	50
Cr (mg kg^{-1})	maximum	100
Cu (mg kg^{-1})	maximum	100
Hg (mg kg^{-1})	maximum	1
Ni (mg kg^{-1})]	maximum	50
Pb (mg kg^{-1})	maximum	100
Se (mg kg^{-1})	maximum	5

Statistical analysis was done by SPSS 22.0 statistical program. Treatment effect was evaluated for each year separately by one-way ANOVA followed by Tukey's test ($p<0.05$). Pearson correlations were applied to determine the relationships between crop yield of test plant and soil chemical properties.

RESULTS AND DISCUSSION

We observed the stronger green colour and healthier crops of triticale in the treated fields. The yields of treated plants were higher than the control ones (Table 2.) in three years (2010-2012). These data show that the favourable beginning developmental conditions couldresult in the increase of the crop yield. We found significant differences among the treatments. The highest crop yield was caused by the 27 t ha^{-1} dose of compost in 2010. The combination with fertilizers application, which is used insewage sludge compost, had the most effect on crop yield of cereal crops (Jalilian, 2015).

Table 2.Crop yield of triticale in the sewage sludge compost experiment from 2010 to 2012

Dose of compost t ha^{-1}	Crop yield of triticale t ha^{-1}		
	2010	2011	2012
Control	1.3 a	1.9 a	1.8 a
9	1.6 ab	2.4 ab	2.3 ab
18	1.6 ab	2.8 b	2.7 b
27	1.8 b	3.0 b	2.9 b

There were significant differences between treatments, according to the Tukey's test (p<0.05). Therefore we signed the "a, b" index in the table.

In 2011 and 2012 the 18 and 27 t ha^{-1} treatments resulted in the highest plant products of triticale (in 2011: 2.8 and 3.0 t ha^{-1}, in 2012: 2.7 and 2.9 t ha^{-1}). The use of complex fertilizers made from artificial fertilizers and organic manure can improve soil biological activity and chemical, physical properties of soil (Berecz et al., 2005). The improved soil condition increases the yield of cultivated plants. The changes of maize yield after treatments were similar to crop yield of triticale (Table 3.). In 2010 9.0 and 9.5 t ha^{-1}crop yields were measured in 18 and 27 t ha^{-1} treated plots and similar results were obtained in 2011. These results indicated the favourable effects of sewage sludge compost on maize as a nutrient source. Delgado et al. (2002) also treated this plant with sewage sludge compost without observing any toxic effects. Moreover, lower zinc (Zn) and nickel (Ni) concentrations were measured in treated than in non-treated plants in their experiment.

Table 3.Crop yield of maize in the sewage sludge compost experiment from 2010 to 2012

Dose of compost t ha^{-1}	Crop yield of maize t ha^{-1}		
	2010	2011	2012
Control	6.3 a	5.5 a	4.8 a
9	7.4 ab	7.1 ab	4.6 a
18	9.0 b	8.2 b	5.1 a
27	9.5 b	8.4 b	4.9 a

There were significant differences between treatments, according to the Tukey's test (p<0.05). Therefore we signed the "a, b" index in the table.

In case of green pea the treatments effects were favourable in 2010 (Table 4.). In the other years the treatment was not caused any increase of the crop yield, moreover,in 2012 It was a higher crop yield washarvested in all treatments.

Table 4.Crop yield of green pea in the sewage sludge experiment in from 2010 to 2012

Dose of compost t ha^{-1}	Crop yield of green pea t ha^{-1}		
	2010	2011	2012
Control	1.3 a	1.0 a	2.9 a
9	1.7 b	1.1 a	3.2 a
18	1.8 b	1.0 a	3.3 a
27	1.6 b	0.8 a	3.1 a

There were significant differences between treatments, according to the Tukey's test ($p<0.05$). Therefore we signed the "a, b" index in the table.

The measured data did not show typical results of crop production of green pea because weather conditions were unfavourable in 2010-2011. The highest recorded mean value (3.3 t ha^{-1}) was resulted by the treatment 18 t ha^{-1} in 2012.Farmers have been recognized that adopting N-efficient management strategies can significantly affect the growth, development and crop yield of many plant species (Binder et al., 2000; Hirel et al., 2007). The lowest recorded mean value (0.8 t ha^{-1}) was measured in the treatment of 27 t ha^{-1} in 2011.

Table 5. Correlation between crop yield of test plants and soil chemical properties in 2010

	Treatments		pH (KCl)	pH (H_2O)	P_2O_5 mg kg^{-1}	K_2O mg kg^{-1}	Na mg kg^{-1}	Mg mg kg^{-1}
Crop yield of triticale t /ha^{-1}	Control	Pearson Correlation	n.s.	n.s.	n.s	n.s.	-0.551*	n.s.
	9 t ha^{-1}		n.s.	n.s.	n.s	n.s.	n.s.	n.s.
	18 t ha^{-1}		n.s.	n.s.	0.607*	n.s.	n.s.	n.s.
	27 t ha^{-1}		n.s.	n.s.	n.s	n.s.	n.s.	n.s.
Crop yield of maize t /ha^{-1}	Control	Pearson Correlation	n.s.	n.s.	n.s	n.s.	n.s.	n.s.
	9 t ha^{-1}		-0.649**	-0.647**	-0.586*	n.s.	n.s.	0.584*
	18 t ha^{-1}		-0.729**	-0.730**	n.s	0.618*	0.702**	0.603*
	27 t ha^{-1}		-0.641**	-0.659**	n.s	n.s.	0.601*	0.718*
Crop yield of green pea t /ha^{-1}	Control	Pearson Correlation	n.s.	n.s.	n.s	n.s.	n.s.	n.s.
	9 t ha^{-1}		n.s.	n.s.	n.s	n.s.	n.s.	n.s.
	18 t ha^{-1}		-0.557*	-0.547*	n.s	n.s.	n.s.	n.s.
	27 t ha^{-1}		n.s.	n.s.	n.s	n.s.	n.s.	n.s.

n.s. non-significant differences

**Correlation is significant at the 0.05 level (2-tailed).*

***Correlation is significant at the 0.01 level (2-tailed).*

In this year we observed negative correlation in all treatments among the soil pH and the yield of maize. Although, the maize grows on a wide range of soil types in the pH range of 5 – 8, but best growth is achieved in the range of pH 5.6 - 7.5 (Bocz, 1992). In the experiment the pH was between 4.5 - 6.8. We got strong positive correlations for Mg content in the crop yield of maize. As well as we studied the correlation between crop yield of test plants and soil chemical properties in 2012 (Table 6.). In the 3rd year (2012) after compost application (2009) we did not observe correlation between crop yield of triticale and soil chemical properties.

Table 6.Correlation between crop yield of test plants and soil chemical properties in 2012

	Treatments		pH (KCl)	pH (H_2O)	P_2O_5 mg kg^{-1}	K_2O mg kg^{-1}	Na mg kg^{-1}	Mg mg kg^{-1}
Crop yield of triticale t /ha^{-1}	Control	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	9 t ha^{-1}		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	18 t ha^{-1}		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	27 t ha^{-1}		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Crop yield of maize t /ha^{-1}	Control	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	9 t ha^{-1}		n.s.	n.s.	-0.684**	n.s.	n.s.	n.s.
	18 t ha^{-1}		-0.710**	-0.653**	n.s.	n.s.	n.s.	n.s.
	27 t ha^{-1}		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Crop yield of green pea t /ha^{-1}	Control	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	9 t ha^{-1}		n.s.	n.s.	0.593*	-0.528*	-0.781**	-0.570*
	18 t ha^{-1}		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	27 t ha^{-1}		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. non-significant differences
**Correlation is significant at the 0.05 level (2-tailed).*
***Correlation is significant at the 0.01 level (2-tailed).*

Similar to the results of 2010, we observed negative correlation among the pH(KCl) and pH(H_2O), and the yield of maize but only in 18 t ha^{-1} compost treated plot. The pH has strong effect on the availability of P for plants (Bakker et al., 2005) which is proved by our results, too. Moreover, the water content of soil has strong effect on the nutrient availability. The year of 2010 was extra humid in Hungary, the yearly precipitation was 995 mm (the average is 550-600 mm in our region), while the year of 2012 was dry with 382 mm precipitation. Dry soil conditions decrease the nutrient availability (Binkley and Vitousek, 1989).

CONCLUSION

This study confirmed that the composted sewage sludge can be successfully used in the production of triticale (*x Triticosecale Wittmack*'Titán') and maize (*Zea mays* L.'MV NK 333') because the increase in the average value of crop yield of these test plants were proved. However, any treatment effects on green pea yield were not found.The results show that the effect of applied compost doses depends on plant species, the elapsed time after compost treatment and the actual climate of the vegetation period.Crops of triticale and maize were higher in the treated plots than

in control one in 2010 and 2011.In our experiments we have found, that the composted sewage sludge could be used efficiently in crop production but more studies needed to screening the reactions of other plant species by planning more effective crop rotations based on this plant nutrient supplying method.

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REFERENCES

Amir S., Hafidi M., Merlina G., Hamdi H., Jouraiphy A., El Gharous M., Revel J.C. (2005). Fate of phthalic acid esters during composting of both lagooning and activated sludge.Process Biochem. 40. 2148-2190.

Bakker C., Rodenburg J., and Bodegon van P. M. (2005). Effect of Ca- and Fe-rich seepage on P availability and plant performance in calcareous dune soils.Plant and Soil. 275. 111-122.

Berecz K., Kismanyoky T. and Debreczeni K. (2005).Effect of organic matter recycling in long-term fertilization trials and model pot experiments.Communications in Soil Science and Plant Analysis. 36 (1-3). 191-202.

Binder D. I., Sander D. H., Walters D. T. (2000). Maize response to time of nitrogen application as affected by level of nitrogen deficiency.Agron. J. 92. 1228-1236.

Binkley D. and Vitousek P. (1989).Soil nutrient availability. Plant Physiological Ecology. 75-96.

Bocz E. (Ed.) (1992).Field crop production (In Hungarian). Mezőgazda Kiadó, Budapest.

Chandra R., Bharagava R.N., Yadav S., Mohan D. (2009). Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. Journal of Hazardous Materials 162.1514–1521.

Delgado A., Porcel C., Miralles De I. H., Beltrán R., Beringola B., Martín S. (2002).Sewage sludge compost fertilizer effect on maize yield and soil heavy metal concentration. Rev. Int. Contam. Ambient. 18 (3) 147-150.

Hirel B., Le Gouis J., Ney B., Gallais A. (2007). The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J. Exp. Bot. 58. 2369-2387.

Jalilian J. (2015). The effect of farm slope and application of different fertilizer sources on wheat on wheat yield. Sixth International Scientific Agricultural Symposium "Agrosym 2015". Bosnia and Herzegovina, Jahorina, 15-18 October 2015. Book of proceedings 209-213.10.7251/AGSY1505209J.

Epstein E. (1997). The science of composting.CRC Press Pensilvania USA.

Madrid L. (2010). “Heavy metals”: reminding a long-standing and sometimes forgotten controversy. Geoderma 155.128-129.

Marttinen S., Hanninen K., Rintala J. 2004. Removal of DEHP in composting and aeration of sewage sludge.Chemosphere 54.262-272.

Oleszczuk P. (2010). Toxicity of light soil fertilized by sewage sludge or compost in relation to PAH content.Water Air Soil Pollut. 210. 347-356.

Poulsen T.G., Berster K. (2010). Organic micro pollutant degradation in sewage sludge during composting under thermophilic conditions. Environ. Sci. Technol. 44. 5086-5091.

Ramírez W.A., Domene X., Ortíz O., Alcaniz J.M. (2008). Toxic effects of digested, composted and thermally-dried sewage sludge on three plants. Bioresource Technol. 99. 7168-7175.

Sánchez Monedero M.A., Mondini C.D., De Nobili M., Leita L., Roig A. (2004). Land application of biosolids: soil response to different stabilization degree or treated organic matter. Waste Manage. 24. 325-332.

Stamatelatou K., Pakou C., Lyberator G. (2011). Occurrence, toxicity, and biodegradation of selected emerging priority pollutants in municipal sewage sludge. In: Murray, M.-Y.(Ed.), Comprehensive Biotechnology, 473-484.

Várallyay Gy. (2002). Basics in soil science of agricultural water management (In Hungarian).Egyetemi jegyzet, Szent István Egyetem, Mezőgazdaság- és Környezettudományi Kara, Gödöllő. 128.

Vermes L. (1998).Waste management and waste utilization (In Hungarian). Mezőgazda Kiadó, Budapest.

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GLOBAL FOOD MARKET – NEW FACTORS INFLUENCING DEVELOPMENT

Grzegorz DYBOWSKI*, Anna BUGAŁA

Institute of Agricultural and Food Economics - National Research Institute, Warsaw, Poland
*Corresponding author: dybowski@ierigz.waw.pl

ABSTRACT

During the long period before 2002 the global index of real food prices showed downward tendency. This resulted from the dynamic economic growth, technological progress and supporting of agriculture in many OECD countries. The supply factors mainly determined global food price levels. Meeting food needs in developing countries was highly dependent onimports. This increased the role of food exporting countries which drove towards liberalization of international trade.
In the years of sharp price increase followed by financial crisis, the new market forces appeared such as: support granted to biofuels in US and EU, competition for land of food and non-food agricultural products, links between food and fuel markets, increase in demand for food in emerging economies. In effect demand factors determining food market development prevailed over the supply ones.
However, the duality existing in the global economic system has been also spreading to the global food sector. The developing countries could hardly benefit from high prices on international agricultural markets because they had no potential to start additional production in a short time. Price transmission from the global food market to local agricultural sectors was insufficient as well. This has brought about the loss of reliabilities of free market as a source of food for states with scaresnational food supply. The policy response of many developing countries has resulted in tendencies to increase self-sufficiency in food production.

Key words: *global food market, factors, development, food price crisis.*

INTRODUCTION

Population growth, economic development and persistence of high food prices in the years 2007-2011 have increased concerns for food access and food security in development strategies in many countries. Agriculture becomes more and more capital, water and energy intensive. Other economic sectors compete with agriculture not only for energy and water, but also for land. Demographic pressure and unsustainable agricultural practices have increased the risk of production capacity breakdown and threaten biodiversity. International trade was for a long time an essential component of most countries` agricultural development strategies.

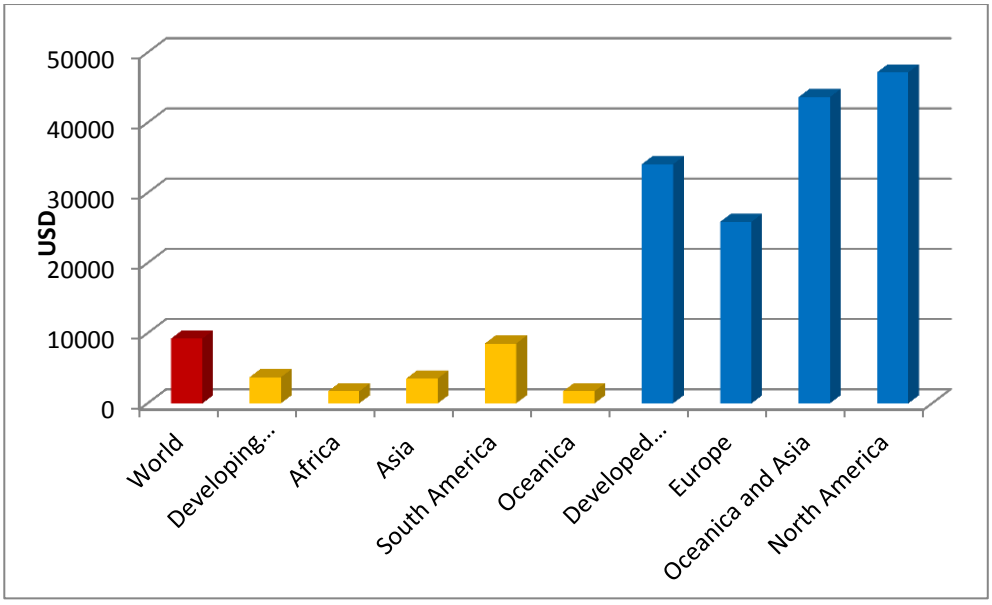
Food price crisis of 2007-2011 has brought about important changes in the global, regional and national food systems.

MATERIALS AND METHODS

The aim of the paper is to draw the attention on the outer factors determining the development opportunities of some less developed countries, which are independent on their strategies and undertaken policy measures. Regarding the character of analysed phenomena the indirect observation method has been used as a base for deductive thinking as well as for revealing causal relationships. The analysis and conclusion presented in the paper have been based mainly on the Food and Agricultural Organization (FAO) of the UN data, especially on materials of theCommittee on Commodity Problems and Committee on World Food Security.

RESULTS AND DISCUSSION

During the long period before 2002 the global index of real food prices showed downward tendency. This resulted from the dynamic economic growth measured by GDP increase per capita, technological progress as well as supporting of agricultural sectors granted mainlyin the OECD countries. However, despitethis growth the diversification in economic development levels around the World remained enormous.

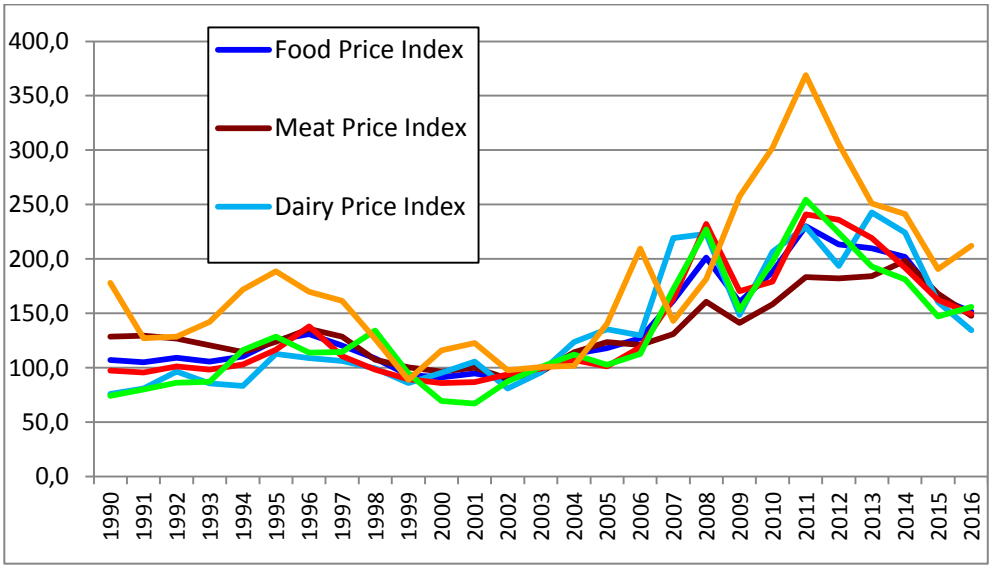


**Source: based on the FAO data*

Figure 1. GDP in USD per capita in regions (2010)

The long period of relatively low food prices had both positive and negative effects. Countries with agriculture as a dominating economic sector obtained low

incomes. In the same time meeting the food needs in many less developed countries was highly dependent on relatively cheap imports. This in turn increased the role of food exporting states which formed rather limited group located mainly in the temperate zone. Theyhad food surpluses so they drove towards liberalization of international trade in food products. Such liberalization was to some extend achieved due to Uruguay Round Agreement on Agriculture monitoring by the World Trade Organization (WTO). Most of the developing countries have benefited little from this Agreement and felt disappointed. That is why they put on the table quite different proposal for further trade liberalization than did the developed countries during another round (Doha Round) of international negotiation under the WTO. The differences of interests are such that the Doha round can hardly been expected to terminate successfully. However, despite the negotiations standstill,WTO proved to be very useful during the food crisis on the global market.



**Source: FAO*

Figure 2. Annual food price indices (2002-2004=100)

The food price crisis was caused by two sets of market factors. First, the traditional ones, such as: reduction of basic food products supply, low level of global stocks – the lowest for 30 years, increasing production costs caused by higher prices for basic agricultural inputs, increasing demand for meat and meat products resulted from higher incomes in some developing countries. Second, the new market forces which appeared in the years of sharp food price increase followed by economic and financial crisis started in 2009.Among them: very high level of world oil prices, support granted to biofuels in the US and the EU, competition for land between food and non-food agricultural raw materials, links of food and fuel markets,

increasing demand for food in emerging economies, inflow of speculative capital from the real estate market and higher turnover on agricultural future markets, seem to be the most important. In effect the demand factors started to prevail over the supply ones in determining food prices development.

Financial crisis contributed a lot to restraining further upward trend in food prices, but the second pick of their increase took place in 2011. Domestic prices in many countries were then even higher than in 2007. After 2011 world food prices decreased constantly. However, they were still staying well above their pre-2007 levels.

Rapid and high growth of food prices in a very short time evoked price shock in many food deficit countries. The dynamic of price increase for crops was faster than that for animal products while the consumption development structure was divers. This brought about increase in agricultural production, but its pace waslower relative to the initiating incentive.All these events exerted impact on the global food market. The main effects were as follows:

- the transmission of prices from global food market to local agricultural markets was insufficient to enable local farmers to benefit from high international and retail prices,
- many food import-depending countries have lost confidence in the global food market as a source of filling in the gaps in domestic food supply,
- up till the food price crisis food import was increasing faster than food production. In the years 2008-2011 the pace of imports growth was slower relative to production growth.

Table 1. Effects of food price crisis on global food market

Years	In USD per capita			World population	Food prices 2002-04=100
	food production	food imports	% of imports		
2000	216	49	22,7	6,1	91,1
2008	429	112	26,1	6,8	201,4
2011	529	130	24,6	7,0	229,9
2013	542	136	25,1	7,2	209,8
2013/2008 %	126,3	121,4	X	105,9	X
2008/2000 %	198,6	228,6	X	111,5	X

**Source: calculated basing on the FAO data*

While analysing the basic reasons for food crisis of 2007-2011it becomes obvious that this is not population increase to be responsible for this, but sudden and sharp rise in world and retail food price levels which was accompanied by insufficient procurement power of consumers in many regions. This in turn brought about

lowering of imports share in total food supply.Slowing down in the dynamic of food imports increase versus food production growth also took place in the years 2008-2013.

The reasons for this should be looked for mainly among the ways in which the import-dependent countries respondedto the sudden price shock. First of all the most of undertaken measures were of an immediate character and were aimed at meeting short term goals such as protecting the consumers or assuring access to food for the majority of population. The main measures included: the reduction of import duties, subsidies to production inputs, preferential rates of working credits, price control, a ban or higher taxes on food exports. The measures concerned first of allproduction and trade spheres and the response to the incentive was delayed. The introduced measures often violated the international rules that had been previously achieved - often with difficulty. Most of these political decisions led to reversal of liberalization trends indeveloping countries which again focused on self-sufficiency in food production.Food importing countries changed their development strategies giving priority to support for domestic production.

Table 2. Measures undertaken in developing countries in response to food price crisis

Measure	Central Africa	Near East and North Africa	Latin America	Asia
Food aid	xxx	-	xxx	xx
Food for labour	xx	-	-	xx
Giving extra food	xx	-	xx	x
Food price control	xxx	-	xx	x
Subsidies to food prices	x	xxx	-	x
Tax reduction	xxx	x	xxx	xxx
Subsidies to inputs	xx	-	-	-
Subsidies to production	x	x	-	x
Ban to food exports	xxx	x	xx	xxx
Reduction of import duties	xxx	xxx	xxx	-

**Source: based on the FAO documents*

The duality prevailing in the global economic system has been also spreading to the global food sector. Technological progress in agriculture contributed to the acceleration of food production dynamic, but the globalisation of the agri-food market resulted in continuing drastic inequalities in access to food in many less

developed countries. The developing countries could hardly benefit from high prices on international agricultural markets because they had no potential to start additional production. Agricultural inputs were expensive and price transmission from the global food market to local agricultural sectors was delayed and insufficient. Economic surplus was being captured by middlemen and over-national corporations.

In the vast majority of countries, either the policy or the level of agricultural income excludes the switch to sustainable growth of food sector, which would be a highly desirable development path at the moment. The competition between the production goals and the environmental aims is still too strong. This is also the main obstacle to sustainable development of agriculture at the global level.

The increasing demand for food due to both supply-related (decrease in agricultural land reserves, climate change) and demand-related factors (growing population, increasing income in some regions, changing mode of consumption) makes the issue of securing access to food for the entire world population increasingly serious. In the short-term perspective, the shortage of food in relation to the constantly growing demand is to a large extent an economic phenomenon. In the global perspective, there is spatial diversity of the development of agricultural sectors, and particularly, the condition of their surroundings. However, economic growth in many countries, which results in increasing demand for food, calls for the increase in global food production.

Taking account of the fact that the opportunities for further territorial expansion of agriculture are limited, the increasing demand for food requires the increase in agricultural production from the current agricultural area. From the long-term perspective, this should be sustainable development, i.e. development that would not be a burden to future generations' consumption. Hence, what is needed is an increase in the efficiency of agricultural production factors on the global scale. Land, which is a specific factor for agriculture, becomes the place where goods that compete with food and traditional agricultural raw materials for the processing industry are produced. At the same time, it is the factor that is mostprone to risks related to civilisation, such as climate change, water shortage, pollution.

Demographic, social and technological factors are becoming more and more important while creating development strategies at the both local and regional levels. Shifting from natural agriculture based on land use to the capital, water and energy intensive agriculture has resulted in weakness of the position of traditional farmer who is not able any more to assure capital necessary to finance modern technology. This leads to diminishing of the number of small farms and increases the dependence on financial institutions.

CONCLUSION

Adequate supply of food does not guarantee food security at the household level. Access to food depends first of all on income, food prices and ability to obtain social aid. In other words, it depends on social factors like gender, location in

society and an individual household hierarchy, social infrastructure as well as political and economic stability.

As far as global scale is concerned, rising incomes and the urbanisation process of lifestyles, drive changes in diets. Food consumption patterns are shifting from being cereals-based to being more diversified and protein-rich. More and more meals are also consumed away from home. These changes are expected to continue.

Developing countries will remain the engine of growth for global production and consumption of food products. The changing structure of demand should promote stronger increase in livestock production compared to the crop sector. Human consumption will continue to evolve in favour of proteins, fat and sugar although cereals will remain at the core of it.

The food price crisis has typically been viewed as a general phenomenon and interpreted from the global standpoint. However, its effects have variedacross commodities, regions and countries. Because of impact of policies, infrastructure and exchange rates domestic food prices often behave differently and were less volatile than world food prices. It is also worth noting that their increases were not solely the effect of growing prices on the world food market. Social, demographic, institutional and policy factors play a big role as well.

REFERENCES

Biofuel Support Policies: an economic assessment (2009). FAO working papers for the 67th CCP Session, Rome.

Human Development Report (2013). United Nations Development Program, Oxford University Pres.

McCullough E.B., Pingali P.L., Stamonlis K.G. (2008). Small farms and the transformation of food systems (in) The transformation of agri-food systems: globalization, supply chains and small holder farms. FAO papers, Rome.

OECD-FAO Agricultural Outlook 2008-2017 (2008). OECD Publishing.

Policy responses to higher food prices (2009).FAO working papers for the 67th CCP Session, Rome.

Schusky E. (1989). Culture and agriculture: an economic introduction to traditional and modern farming system. New York, Bergin and Garvey.

The environmental food crisis (2009).United Nations Environment Programme.

The Millennium Development Goals Report (2013). United Nations, New York.

The State of Agricultural Commodity Markets (2009). High Food Prices and the Food Crisis – Experiences and lessons learned. Electronic publishing Policy and Support Branch Knowledge and Communication Department.

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APPLICATION OF FARMYARD MANURE IN GRASSLAND PRODUCTION

Aleksandar SIMIĆ[1*], Bojan STOJANOVIĆ[1], Savo VUČKOVIĆ[1], Jordan MARKOVIĆ[2], Aleksa BOŽIČKOVIĆ[1], Zorica BIJELIĆ[3], Violeta MANDIĆ[3]

[1]Faculty of Agriculture, University of Belgrade, Belgrade, Serbia
[2]Institute for forage crops, Globoder-Kruševac, Serbia
[3]Institute for Animal Husbandry, Autoput 16, Zemun, Belgrade, Serbia
*Corresponding author: alsimic@agrif.bg.ac.rs

ABSTRACT

This experiment aimed at determining the effect of farmyard manure (FYM) applicationon a natural pasturein Western Serbia, with a 30 t ha^{-1}treatment in comparison to control (no fertilizer added) during two years (2012-2014).The FYM was applied in the autumn of 2012 and the trial plots were harvested twice a year. Dry matter (DM) yield and forage quality - content of crude protein (CP), nonprotein N (%CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and net energy for lactation(NE_L), were estimated for each production year. Treatment with manure gave a higher DM yield compared to control plots in both experimental years (5.91 t ha^{-1} vs. 3.01 t ha^{-1} in 2013, and 2.76 vs. 2.03 t ha^{-1} in 2014). As expected,the yield in the second cuts of both years was much lower than in the first cuts. The FYM application did not affect chemical composition and net energy concentration of forages, whereas significant effects of different cuts were found, but were inconsistent between the first and second experimental year. In general, it can be concluded that application of FYM did not have a significant effect on forage quality ina permanent grassland, whereas chemical composition was significantly affected by different cuts and experimental years. Based on the results, a grassland may have a good DM yield response if FYM is used as a fertilizer, while the effect on forage qualitymay be much weaker.

Keywords: *manure, pasture, quality, yield.*

INTRODUCTION

One of possible applications of farmyard manure in food production is natural grasslands fertilization (Bukvić *et al*., 2013), in a case when mineral nitrogen (N) use for forage production is prohibited (European Union Council Regulation No 834/2007).Natural grasslands occupy large areas in hilly-mountainous regions of Serbia. Herbage grasses respond favorably to high fertilization by abundant production of vegetative biomass (Vučković *et al*., 2010).The investigation carried out on a natural grassland (Vučković *et al*., 2005) showed that maximum yield is

obtained through high fertilization rate. However, the high fertilization rates are not economically justified, since lower rates produce much higher ratio of biomass per kg of nutrient applied (Vučković *et al*., 2004). Application of mineral fertilizers increases production costs and awareness of economic and environmental consequences of N loss.

In general the permanent grasslands in Serbia are situated on soils with low natural fertility, are of low productivity and have sub-optimal botanical composition. The main means for improving quality of these grasslands include adjusting soil fertility, changing the dominance in the vegetation canopy and a good management. Increased productive potential of grasslands can be achieved through fertilization at different rates and with different types of organic and mineral fertilizers. The investigations carried out to date have shown positive effects of manure application on grasslands (Bouwman *et al*., 2002; Bittman *et al*., 2005).

Animal production is largely dependent upon two factors: energy intake and absorbed protein. These factors are highly dependent upon forage quality as well as the interaction of forage with the rumen microbial population, animal factors and other dietary ingredients (Allen, 1996). Content of crude protein (CP) and nonprotein N, fiber (neutral detergent fiber-NDF and acid detergent fiber-ADF), available energy concentration are important indicators of nutritive value for grazing forages. Pasture is characterized with the higher concentration of soluble and rumen degradable protein than the needs of the high-producing ruminants and rumen bacteria (Stojanović *et al*., 2015). Addition of nitrogen fertilizer increases CP concentration of grasses as well as yield (Buxton, 1996). The effect of N fertilization on NDF and ADF is variable (Coleman *et al*. 2004). Year-to-year and seasonal variation in environment alter forage quality, even when forages are harvested at similar maturity stages (Buxton and Casler, 1993).

In the present study, cattle manure was applied as a sustainable organic fertilizer. The objective of this study was to investigate and launch a sustainable manure-based nutrition for pastures in Serbia.It was therefore expected for the application of manure to increase the forage yield and quality.

MATERIAL AND METHODS

The field trial was established in vicinity of Šabac (Varna, 44°40′40″N 19°39′05″E, 123 asl.), Serbia by the method of RCB design of plots in 4 replications. The experiment carried out in the field included treatments:

a) control (without fertilization);
b) manure fertilization (30 t ha^{-1});

Prior to application, fresh cattle manure was homogenously mixed and fermented during 3 months.The manure was applied in autumn 2012. The decomposition of fermented manure and its contribution tothe nutrients pool was as expected (first year - 50% of manure decomposition, second year - 30% of manure decomposition).

The plots were harvested in May and July of both vegetation seasons; dry matter (DM) of the harvests was measured.All samples were air-dried before chemical analyses. Parameters of proximate analysis were determined.Analytical DM content of air-dried samples were determined by oven-drying at 105^{o}C for 5 h. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (ADL) were determined according to procedure by Van Soest (1991); protein fractions (true protein and NPN) were determined as described by Licitra *et al*. (1996). The net energy concentration of forage was determined according to the Nutrient Requirements of Dairy Cattle Seventh Revised Edition (NRC, 2001). The main characteristics of the soils were determined (tab. 1) and meteorological data (tab. 2)for experimental field was collected from the Sremska Mitrovica Weather Station, located near the experimental site. Total DM yield and pasture DM quality in each of the two cuts were analyzed through analysis of variance (ANOVA) and LSD test, in order to recognize significant effects of fertilization treatments.

Table 1. Chemical properties of soil

Depth	pH		OM %	AL-P_2O_5 mg/100g	AL-K_2O mg/100g	Total C %	Total N %
	$CaCl_2$	H_2O					
0-20 cm	5.07	5.73	4.31	1.98	11.51	1.37	0.16

Soil from experimental field had a low P content and moderately acidic pH.

Table 2. Average monthly temperature, ^{0}C and monthly precipitation sum, mm (2012-2014)

Production year	VIII	IX	X	XI	XII	I	II	III	IV	V	VI	VII	Total
Temperatures - ^{o}C													
First	23.8	19.3	12.8	9.3	1.1	3.2	3.9	6.3	13.1	17.2	19.9	21.2	12.6
Second	15.9	13.7	8.4	1.7	4.2	6.8	9.5	13.3	16.1	19.8	21.9	21.2	12.7
Precipitation sum - mm													
First	1.0	17.6	36.2	24.0	57.5	56.2	47.8	65.3	32.0	119	62.0	44.5	563
Second	61	71.6	34.1	5.8	51	17	47	76	188	38	75	56	720

According to the meteorological data, the total precipitation was 563 mm in the first vegetation season, and 720 mm in the second vegetation season. The maximum and minimum temperatures were registered in August and December of the first vegetation season, and in July and November of the second vegetation season.

RESULTS AND DISCUSSION

Forage yield

Fertilizer treatment with manure affected yield in 2013, especially in the case of the first cut: the yield was more than doubled by fertilization, in comparison to control (Table 3). Manure also showed an extended effect in the second cut (1.38 vs 1.02 t ha^{-1}), but without a statistical significance. In total, both cuts and fertilization treatment showed significant effect on the DM yield in 2013.

Table 3. Forage yield (t ha^{-1}) and quality from permanent grassland in 2013 year, Western Serbia (% DM)

treatments	DM yield	CP	NPN	EE	Ash	NDF	ADF	Lignin	NE_L
I cut									
Control	1.99	9.71	26.37	2.66	7.67	62.8	38.4	5.70	4.53
Manure	4.53	10.1	35.21	2.83	7.99	63.9	38.7	6.17	4.40
LSD 0,05	*****	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
II cut									
Control	1.02	9.04	15.77	4.25	9.13	69.5	37.1	6.07	4.23
Manure	1.38	9.72	26.07	4.13	9.35	66.7	38.8	6.73	4.15
LSD 0,05	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Cut									
I cut	3.26	9.89	30.79	2.74	7.83	63.4	38.6	5.94	4.47
II cut	1.20	9.38	20.92	4.19	9.24	68.1	38.0	6.40	4.19
LSD 0,05	*****	**NS**	**NS**	*****	*****	*****	**NS**	**NS**	**NS**
Fertilizer									
Control	1.51	9.37	21.07	3.45	8.40	66.2	37.8	5.89	4.38
Manure	2.95	9.89	30.64	3.48	8.67	65.3	38.7	6.45	4.28
	*****	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

DM – dry matter (t ha^{-1}), CP – crude proteins (% DM), NPN-non protein nitrogen (% of crude proteins), EE–Ether extract (% DM), NE_L – Net energy lactation (MJ/kg DM)

In the second production year, the effect of applied manure on pasture yield was diminished, but again with significant effect on total DM yield of two cuts (Table 4). Generally, it seems that manure application was a good way to increase yield on a permanent grassland during two years. The positive effects of manure applied on grasslandsagree with earlier studies performed byBouwman *et al*., 2002 and Bittman *et al*., 2005.

Table 4. Forage yield (t ha^{-1}) and quality from permanent grassland in 2014 year, Western Serbia (% DM)

treatments	DM yield	CP	NPN	EE	Ash	NDF	ADF	Lignin	NE_L
I cut									
Control	1.24	8.95	33.3	3.15	7.50	59.7	35.0	5.46	4.89
Manure	2.09	9.18	31.9	3.16	7.84	60.2	35.3	5.44	4.85
LSD 0,05	*****	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
II cut									
Control	0.79	11.8	26.0	2.72	9.84	55.9	36.8	7.54	4.36
Manure	0.67	10.5	27.5	2.55	9.71	58.8	39.5	7.48	4.26
LSD 0,05	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Cut									
I cut	1.67	9.07	32.6	3.16	7.67	59.9	35.2	5.45	4.87
II cut	0.73	11.15	26.8	2.63	9.77	57.3	38.2	7.51	4.31
LSD 0,05	*****	*****	**NS**	**NS**	*****	**NS**	*****	*****	*****
Fertilizer									
Control	1.02	10.39	29.7	2.93	8.67	57.8	35.9	6.50	4.62
Manure	1.38	9.82	29.7	2.86	8.77	59.5	37.4	6.46	4.55
	*****	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

DM – dry matter (t ha^{-1}), CP – crude proteins (% DM), NPN-non protein nitrogen (% of crude proteins), EE–Ether extract (% DM), NE_L – Net energy lactation (MJ/kg DM)

Forage quality

This research showed no significant effect of manure fertilization on forage quality, neither in the first nor the second year of the experiment.

According to an earlier study (Puoli *et al*., 1991), proper N fertilization of grasses generally increases CP. But the effect of N fertilization on NDF and ADF is variable. Higher N rates tended to increase the NDF concentrations of the plants in wetter years resulting from change in the leaf:stem ratio in favour of less digestible stems (Buxton and Fales, 1994). In contrast, Coleman *et al*. (2004) reported that higher N rates provided a delayed plant maturity for later harvests and in turn increased total plant digestibility. A better understanding of the nutritive values of grasses as affected by N fertilization can be helpful in making grassland management decisions.

A significant effect of different cuts on chemical composition and quality of herbage was found. In the first experimental year the determined values were significantly higher in the second than in the first cutfor NDF (7.41%) and ether extract content (52.92%). In the second experimental year, significantly higher values for CP (22.93%), ADF (8.50%) and lignin content (37.80%) and lower value for net energy concentration (11.50%) were recorded in the second compared to the first cut.

These findings agree with earlier studies (Mandaluniz *et al*. 2015, Müller and Jänicke, 2015), with the exception of CP concentration in the second experimental year. Mandaluniz *et al*. (2015) reported that the CP content decreased (21.4%), while NDF and ADF content increased (10.4% and 18.1%) for grazing herbage mass during the spring grazing period (April-June), when the grazing management regime is characterized by 20-25 days of grazing and resting period, in total. The highest crude protein concentrations of grass pasture are found in spring and autumn (Van Vuuren and Van Den Pol 2006). The increase of CP content within the second cut of second production year could likely be a result of other environmental conditions and possibly changes in botanical composition of herbage mass.

There were no significant differences between treatments (without and with manure fertilization) and between different cuts, for NPN (%CP) concentration, however, results indicate higher values for the first cut in both experimental years, also for the herbage with manure application in the first production year.

Results obtained for herbage NE_L concentration indicate no significant effect of fertilization. Considering different cuts, results of this study agree to an extent with findings of Van Vuuren and Van Den Pol (2006) who reported that the energy value (NEL) of grass pasture is the highest in April, but remains rather stable throughout the year.

The differences in forage chemical composition between production years could be explained by different environmental factors. The change in forage quality in grasses investigated at the same day of different years indicating their maturation had the greatest effect on whole-plant nutritive values (Waramit *et al*., 2012). The most important environmental factors are temperature, water deficit, solar irradiation, and soil nutrient availability (Buxton and Fales, 1994). Among these environmental factors, temperature usually has the greatest influence over forage quality.

CONCLUSION

In general, it can be concluded that applying farmyard manure did not have a significant effect on forage quality ina permanent grassland, whereas chemical composition was significantly affected by different cuts and experimental years. Based on the results, a grassland may have a good DM yield response if farmyard manure is used as a fertilizer, while the effect on forage quality could be much weaker . Also, manure can be recommended for agricultural purposes in terms of sustainable fertilizing and improving the system cattle farm – manure - organic fertilizer for forage crops. Future studies should focus on including additional sites with different soil types in areas with contrasting climate.

REFERENCES

Allen M.S. (1996). Relationship between forage quality and dairy cattle production. Anim. Feed Sci. Technol. 59, 51-60.

Bittman S., Forge T. A., Kowalenko C. G. (2005). Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer. Soil Biology and Biochemistry, 37(4), 613-623.

Bouwman A. F., Boumans L. J. M., Batjes N. H. (2002). Estimation of global NH_3 volatilization loss from synthetic fertilizers and animal manure applied to arable lands and grasslands. Global Biogeochemical Cycles, 16(2), 8-14.

Buxton D.R. (1996). Quality-related characteristics of forages asinfluenced by plant environment and agronomicfactors. Anim. Feed Sci. Technol. 59, 37-49.

Buxton D.R., Fales S.L. (1994). Plant environment and quality. In: G.C. Fahey, Jr. *et al*. (Editors), Foragequality, evaluation, and utilization. American Society of Agronomy, Madison, WI, pp. 155-199.

Buxton D.R., Casler M.D. (1993). Environmental and genetic effects on cell wall composition anddigestibility. In: H.G. Jung *et al*. (Editors), Forage cell wall structure and digestibility. American Society ofAgronomy, Madison, WI, pp. 685-714.

Bukvić G., Gantner R., Steiner Z., Karalić K. (2013). Crop rotation and association design for N budgeting in organic dairy farms.In: Green Design, Materials, and Manufacturing Processes. Bartolo, H. M. *et al*. (editors). Leiden, Netherlands: CRC Press/Balkema (Taylor & Francis Group), 179-184.

Coleman S.W., Moore J.E., Wilson J.W. (2004). Quality and utilization. In: Moser, L.E., *et al*. (Eds.), Warm Season Grasses. ASA, CSSA, SSSA, Madison, WI, pp. 267–308.

Licitra G., Hernandez T.M., Van Soest P.J. (1996). Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Sci. Feed Technol., 57, 347-358.

Mandaluniz N., Arranz J., Ruiz R. (2015). A comparison of two grazing regimes during lactation forimproving the sustainability of Latxa dairy sheep system. In: Grassland and forages in high output dairy farming systems, Grassland Science in Europe, Vol. 20., p. 253-255.

Müller J., Jänicke H. (2015). Forage quality screening of *Lolium multiflorum* Lam. cultivarsappropriate for high carbohydrate rations. In: Grassland and forages in high output dairy farming systems, Grassland Science in Europe, Vol. 20., p. 271-273.

National Research Council (2001). Nutrient Requirements of Dairy Cattle 7th Revised Edition. National Academy Press. Washington, D.C.

Puoli J.R., Jung G.A., Reid R.L. (1991). Effects of nitrogen and sulfur on digestion and nutritive quality of warm-season grass hays for cattle and sheep. J.Anim. Sci. 69, 843–852.

Stojanović B., Grubić G., Đorđević N., Božičković A., Ivetić A. (2015).Paša u ishrani muznih krava. Zbornik naučnih radova Instituta PKB Agroekonomik, vol.21, br. 3-4: 55-64, 2015.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Van Vuuren A.M., Van Den Pol V.D. (2006). Grazing systems and feed supplementation. In: Fresh Herbage for Dairy Cattle: The Key to a Sustainable Food Chain (Wageningen UR Frontis Series), ed. Elgersma, A., Dijkstra, J., Tamminga, S. Springer, London.

Vučković S., Ćupina B. Simić A., Prodanović S. Živanović T. (2005). Effect of nitrogen fertilization and undersowing on yield and quality of *Cynosuretum cristati*-type meadows in hilly-mountainous grasslands in Serbia. Journal of Central European Agriculture, 6 (4):515-520.

Vučković S., Simić A., Ćupina B., Stojanović I., Stanisavljević R., Vojin S., Dubljević R. (2004). The effect of nitrogen fertilizer on the productivity of *Cynosuretum cristati* type meadows on the Sjenica-Pešter plateau. *Acta agriculturae Serbica*, 9(17):123-127.

Vučković S., Simić A., Ćupina B., Krstić Đ., Duronić G. (2010). Effect of mineral fertilization on yield of *Agrostidetum vulgaris* – type meadows in mountainous grasslands in Serbia. XII International Symposium on Forage Crops of Republic of Serbia, Biotechnology in Animal Husbandry 26 (spec. issue), 389-394.

Waramit N., Moore K.J., Fales S.L. (2012). Forage quality of native warm-season grasses in response to nitrogenfertilization and harvest date. Anim. Feed Sci. Technol. 174, 46– 59.

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THE USE OF CRUDE PALM OIL IN FINISHING PIGS' DIET: EFFECTS ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY

Wandee TARTRAKOON*, Parichat KHANCHIT, Nipont PHOMSAK, Sonram THONGDEE

Department of Agricultural Science, Faculty of Agriculture Natural Resource and Environment, Naresuan University, Phitsanulok, Thailand
*Corresponding author: wandeeta@nu.ac.th

ABSTRACT

The goal of this study was to evaluate the effect of including crude palm oil (CPO) in the diet of finishing pigs in terms of growth performance and nutrient digestibility. In the first experiment, 40 barrows and 40 gilts (Duroc×LargeWhite×Landrace) were divided into five groups using a randomized complete block design. CPO, soybean oil (SBO) and poultry fat (PF) were mixed and divided into CPOmix11 (CPO 50% + PF 50%), CPOmix13 (CPO 50% + SBO 50%), CPOmix21 (CPO 75% + PF 25%) and CPOmix23 (CPO 83% + PF 17%). Each group of pigs was randomly fed two diets at 50-80 and 80-100 kgBW. Group 1 comprised the pigs fed diet10 (without oil inclusion) and diet20 (without oil inclusion). Group 2 comprised the pigs fed diet11 (1% CPOmix11) and diet21 (1% CPOmix21). Group 3 comprised the pigs fed diet13 (3% CPOmix13) and diet23 (3% CPOmix23). Group 4 comprised the pigs fed diet10 and diet 21. Group 5 comprised the pigs fed diet10 and diet23. The results showed that the pigs fed diet23 (Group 5) at 80-100 kgBW tended to have the greatest performance and lowest feed cost. In the second experiment, nutrient digestibility was examined in six barrows (initial 50 kgBW) using a 3×3 double Latin square design. Each set of two pigs was randomly fed diet10, diet11 or diet13. The highest ($P<0.01$) digestibility of dry matter, protein, crude fiber and ash and the greatest ($P<0.01$) digestible energy and metabolizable energy were found in diet13. The inclusion of 3% CPOmix23 in the diet at80-100 kgBW might improve finishing pig performance, and the 3% of CPOmix13 in the diet improved nutrient digestibility.

Keywords: *crude palm oil, finishing pigs, growth performance, nutrient digestibility.*

INTRODUCTION

Fats and oils have the highest average energy density of all macronutrients. Besides having high caloric value, some fats and oils like crude palm oil(CPO) can be a primary source of essential fatty acids, which cannot be synthesized by pigs, as well as fat-soluble vitamins and antioxidants such as phytosterols, tocopherols and carotenoids that help preserve and stabilize fats.

These micronutrients are essential for animal health, growth and carcass quality. Moreover, the use of particular types of fats and oils in pig rations affect the metabolizable energy of the total rations beyond the calculated energy of the diet. Finally, fats and oils can play a significant role in creating pelleted products and controlling mill and barn dust. CPO is extracted from the mesocarp of the fruit of the oil palm tree, Elaeis guineensis. Palm oil and its refinery products are now consumed worldwide as cooking oil and in a wide variety of foodstuffs (Pantzaris, 1995).

CPO has a dark orange-red color due to its high carotenoid content. It is also a rich source of vitamin E, namely tocopherols and tocotrienols (Nesaretnam and Muhammad, 1993). Both β-carotene and vitamin E are well-known nutritional antioxidants. The over-expanding production of palm oil in Thailand and other tropical countries offers the possibility of an increased and constant availability of CPO-based feedstuff for pig feed formulations. The chemical and energy demands of the palm oil refining process could be reduced. CPO could be used as energy feedstuffs directly.

The objective of this study was to evaluate the effect including CPO in the diet had on growth performance and nutrient digestibility in finishing pigs.

MATERIALS AND METHODS

This experiment was conducted in 40 barrows and 40 gilts (Duroc×LargeWhite×Landrace), which were dividedintofivegroups using a randomized complete block design. CPO, soybean oil (SBO) and poultry fat (PF) were mixed and divided into CPOmix11 (CPO 50% + PF 50%), CPOmix13 (CPO 50% + SBO 50%), CPOmix21 (CPO 75% + PF 25%) and CPOmix23 (CPO 83% + PF 17%). Each group of pigs was randomly fed two diets at50-80 and 80-100 kgBW.

Group 1 included the pigs fed diet10 (0% oil) and diet20 (0% oil).
Group 2 included the pigs fed diet11 (1% CPOmix11) and diet21 (1% CPOmix21).
Group 3 included the pigs fed diet13 (3% CPOmix13) and diet23 (3% CPOmix23).
Group 4 included the pigs fed diet10 and diet21.
Group 5 included the pigs fed diet10 and diet23.

The composition and proximate analysis of the diets are shown in Table 1. The nutrient compositions of all of the diets followed NRC (1998) recommendations. The pigs were housed 8 pigs to a pen, whichgavethe*ad libitum* access to feed and water.The pigs'body weights and feed consumption were recorded and measured

from the beginning of the trial to a final average live weight of 100±5 kg to calculate theiraverage daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (Feed: Gain; FCR).

In experiment 2, thetotal tract digestibility (%) of DM, crude protein (CP), crude fiber (CF), EE and ash were determined, and the dietary energy (DE) and metabolizable energy (ME) were calculatedin six barrows (initial 50 kgBW) using a3×3 double Latin square design. Titanium dioxide (TiO_2) was added to the feed at a dose of 0.5% for the duration of sevendays as an indigestible marker for digestibility determination.Each set of two pigs were randomly fed diet10, diet11 or diet13 for 7dwith a 4d adjustment period. They were then maintained for a 3d collection period. Fresh fecal samples were mixed, pooled, and stored in a freezer at -20ºC until they were analyzed. Before chemical analysis, each fecal sample was thawed and dried at 60ºC for 72 h, after which it was finely ground to 1-mm sized grains.

All feed and fecal sampleswere then analyzed for DM, CF, EE and N according tothe procedures outlined by the AOAC (1995). TiO_2 was analyzed using UV absorption spectrophotometry according tothe method described by Short et al. (1996), Kavanagh et al. (2001) and Glindemann et al. (2009).The proper care and use of the animals in theseresearch procedures were approved by the Naresuan University Animal Care and Use Committee (NUACUC No.57 04 0031).

All data were subjected to statistical analysis by one-way analysis of variance (ANOVA) using the SPSS statistical software (Ver. 15 for Windows, SPSS Inc., Chicago, IL, USA). Differences among treatments were examined using Duncan's multiple range tests, which were considered significant at $P<0.05$. The means and standard errors of the means are presented.

Table 1**.** Dietary treatment composition for finishing pigs 1 (50-80 kgBW) and finishing pigs 2 (80-100 kgBW).

Items	**Finishing pig diets 1**			**Finishing pig diets 2**		
	10	**11**	**13**	**20**	**21**	**23**
Ingredients (g/kg as fed basis)						
Corn	330	500	50	704	364	200
Broken rice	330	135	520	-	305	404
Soybean meal	200	205	180	155	150	145
Defatted rice bran	120	130	200	120	150	200
Crude palm oil	-	5	15	-	7.5	25
Soybean oil	-	-	15	-	-	-
Poultry fat	-	5	-	-	2.5	5
Lysine	10	10	10	1	1	1
Di-calcium phosphate (P18)	5	5	5	4	4	3
CaCo3	2.5	2.5	2.5	11	11	12
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin and mineral premix[b]	330	500	50	2.5	2.5	2.5
Calculated composition (g/kg DM basis)						
Metabolizable energy (Kcal/kg)	3187	3237	3255	3217	3218	3260
Protein	155	155	155	132.1	138.8	141.3
Fiber	29.3	31.0	28.3	43.8	41.9	45.0
Ether extract	7.6	15.3	38.8	31.6	30.4	44.2
Calcium	5.3	5.3	5.3	6.0	6.0	6.2
Total phosphorus	6.7	7.0	7.4	5.7	5.8	6.2
Lysine	8.1	8.2	7.8	7.8	7.8	7.8
Methionine+ cystine	5.4	5.6	5.1	5.2	5.0	4.9
Tryptophan	3.7	4.5	2.1	5.3	3.6	2.7
Threonine	5.9	6.0	5.6	5.3	5.2	5.1
Fatty acid Composition						
Total unsaturated fatty acid	9.0	16.9	19.0	16.2	12.8	14.3
Total saturated fatty acid	1.6	5.1	7.0	2.8	4.5	9.4
[a]U:S ratio	6.02	4.04	3.91	6.2	3.90	3.02
[c] CPOmix cost (Baht/kg)	-	25.00	37.50	-	25.00	25.00
[c] *Feed cost (Baht/kg.)*	13.11	13.80	12.96	12.77	12.50	12.61

[a] U:S ratio = Unsaturated fatty acid: Saturated fatty acid

[b] Vitamin and mineral premix provided per kilogram of diet: 450 mg Fe; 400 mg Cu; 250 mg Zn; 150 mg Mn; 0.5 mg I; 0.25 mg Se; 8,000 IU vitamin A; 2,000 vitamin D_3; 37.5 mg vitamin E; 0.925 mg vitamin K-3; 8.43 mg vitamin B_2; 0.04 mg vitamin $B_{12;}$ 34.5 mg nicotinic acid; 26 mg pantothenic acid

[c] Theprice of feed ingredients calculated during July-September 2015

RESULTS AND DISCUSSION

As the experiment only had two replications per treatment that were housed in groups (8 pigs per group), statistical analysis could not support it. The results shown in Table 2 only demonstrate the tendency of the pigs in groups 2 to 4 to have smaller experimental periods, FCRs and feed costs. In agreement with previous research, the addition of fat and oil in this research has been reported to improve feed efficiency (Goodband et al., 1989; DeRouchey et al., 2007; and Shannon, no date). Feed efficiency did not significantly differ by type of fat source (Park et al., 2012).The pigs in group 5, which were fed with diet10 (50-80kgBW) and diet23 (80-100 kgBW), tended to have the lowest FCR and feed cost. According to DeRouchey et al. (2007) and Shannon (no date), for each 1% of added fat in grower-finishing pigs, feed efficiency is usually improved 1.8%. Meanwhile, the ADG is reported to increase approximately 2% in grower diets and 1% in late finisher diets for each 1% of added fat. Additionally, previous research found that the inclusion of 4% of fat from soybean oil, coconut oil or choice white grease showed an increase in feed intake and feed efficiency compared with the non-fat control (Goodband et al., 1989). The current study did not find any effects of gender on the pigs' FCR. However, it was found that barrows tended to have better ADG than gilts. This differs from previously published research (Latorre et al., 2003; Serrano et al., 2013; Tartrakoon et al., 2016). The different results of the current study might be related to the type of oil or fat supplementation.

Table 2**.** Effect of dietary treatments on growth performance of finishing pigs (50-100 kgBW)

Items	Group[a]					Gender[b]	
	1	2	3	4	5	**B**	**G**
Initial weight, kg	50.08	50.20	50.03	49.86	49.95	50.01	50.03
Final weight, kg	100.01	99.95	100.45	100.16	100.65	100.24	100.24
Weight gain, kg	49.93	49.75	50.42	50.30	50.70	50.23	50.21
Period of trial, d	84.00	72.00	71.00	69.50	70.00	67.60	79.00
Average daily feed intake, kg/d	2.26	2.23	1.80	1.95	1.68	2.12	1.84
Average daily gain, kg/d	0.60	0.71	0.72	0.73	0.73	0.75	0.64
Feed conversion ratio (FCR)	3.32	2.91	2.54	2.73	2.31	2.72	2.79
Feed cost, Baht/kgWG	42.46	37.92	32.47	34.36	29.37	34.86	35.71

[a]Group1, the pigs fed diet without oil inclusion in diet10 and diet20; Group2, the pigs fed diet11 and diet21; Group3, the pigs fed diet13 and diet23; Group4, the pigs fed diet10 and diet21; Group5, the pigs fed diet10 and diet23 at50-80 and 80-100 kgBW, respectively.

[b]B = Barrows; G = Gilts

Improving the FCR or feed efficiency of the pigs' fed diets supplemented with fat and oil could also be supported by the 2nd experiment in this research. The highest ($P<0.05$) digestibility of dry matter, protein, crude fiber, ash and the greatest

(P<0.05) digestible energy and metabolizable energy were found in the pigs fed diet13, which was 3% CPOmix13. It may be due to the fact that the oil in CPOmix13 was plant oil and that the higher nutrient digestible was found indiet11, which contained CPO11 (50%CPO+50%PF). These two groups were different not only because of the type of oil or fat but also because of the inclusion level of diet13, which was 3%CPOmix13. The explanation could be supported by the results of Albin et al., (2001) who found that adding high levels of soybean and palm oil to a semi-purified swine diet increased the apparent ileal digestibility of some amino acids. Also, the fat sources affected some apparent ileal amino acid digestibility (Albin et al., 2001), digestible energy and ether extract digestibility (Mitchaothai et al., 2008; Yongbo et al., 2015).

When fat is oxidized, ADG and ADFI in nursery pigs will decrease; however, free fatty acid (FFA) concentrations of at least 53% in choice, white grease fat does not adversely affect its utilization in nursery pigs (DeRouchey et al., 2004). The FFA analysis results of fat and oil source and experimental diet in Table 4 showed the highest FFA in CPO and the lowest FFA in SBO. After the oil was mixed into CPOmix11, which contained 50% CPO+50% PF in diet11 at a 1% inclusion level, a significantly higher FFA concentration was found in diet11 than the control, diet10 (2.03% vs. 1.67%). However, there was no significant difference in the FFA concentrations of diet11 and diet 13 (CPOmix13, 50%CPO+50%SBO)

Table 3**.** Total tract nutrient digestibility (%) of finishing pigs fed experimental diets

Treatments	**Total tract digestibility (%)[e]**						**Energy[f]**	
	DM	**CP**	**CF**	**EE**	**Ash**	**Energy**	**DE**	**ME**
Diet10	91.75^{c}	92.45^{c}	74.59^{c}	97.52^{a}	78.82^{c}	91.32^{c}	3,441^{b}	3,376^{b}
Diet11	92.94^{b}	93.63^{b}	78.89^{b}	97.15^{ab}	82.41^{b}	92.83^{b}	3,567^{a}	3,501^{a}
Diet13	94.98^{a}	95.61^{a}	84.70^{a}	96.61^{b}	87.25^{a}	94.36^{a}	3,646^{a}	3,577^{a}
[d]SEM	0.37	0.38	1.18	0.15	0.98	0.37	25.97	25.17

[a,b,c]Means within rows with different superscripts differ (*P*<0.05)

[d]SEM = Standard error of the mean

[e]DM = Dry matter; CP = Crude protein; CF = Crude fiber

[f] DE = Digestible energy (kcal/kg); ME= Metabolizable energy (kcal/kg)

Table 4**.** Free fatty acids content (%) in oil, fat and experimental diet.

Oil and Fat[d]			Experimental Diet [c]			SEM [e]	p-value [f]
CPO	PF	SBO	Diet10	Diet11	Diet13		
8.71	4.31	0.25	1.67[b]	2.03[a]	1.80[ab]	0.069	*

[a,b]Means within rows with different superscripts differ ($P<0.05$)

[c] Diet10, the pigs fed diet without oil inclusion; Diet 11, diet comprised CPOmix11 (50% CPO+50%PF); Diet13, diet comprised CPOmix13 (50%CPO+50%SBO).

[d] CPO = Crude palm oil; PF = Poultry fat; SBO = Soybean oil

[e] SEM = standard error of the mean; [f]*= $P<0.05$

CONCLUSION

The inclusion of CPOmix23 (83%CPO+17%PF)as 3% ofthe diet at80-100 kgBW of the pigs might improve finishing pig performance, andCPOmix13 (50%CPO+50%SBO)as 3% of the diet improves nutrient digestibility.

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REFERENCES

AOAC. (1995). Official methods of analysis. 16th ed. Arlington, VA: Association of Official Analytical Chemists.

Albin, D. M., Smiricky, M. R., Wubben, J. E., Gabert, V. M. (2001). The effect of dietary level of soybean oil and palm oil on apparent ileal amino acid digestibility and postprandial flow patterns of chromic oxide and amino acids in pigs. Can J Anim Sci, 81, 495-503.

DeRouchey, J. M., Hancock, J.D., Hines, R.H., Maloney, C.A., Lee, D.J., Cao, H., Dean, D.W., Park, J.S. (2004). Effects of rancidity and free fatty acids in choice white grease on growth performance and nutrient digestibility in weanling pigs. J Anim Si,82, 2937-2944.

DeRouchey, J.M., Dritz, S.S., Goodband, R.D., Nelssen, J.L., Tokach. M.D. (2007). General Nutrition Principles for Swine. Kansas State University Agricultural Experiment Station and Cooperative Extension Service. MF-2298, 2-44.

Glindemann, T., Tas, B.M., Wang, C.,Alvers, S., Susenbeth, A. (2009).Evaluation of titanium dioxide as an inert marker for estimating faecal excretion in grazing sheep. Anim Feed Sci Tech,152, 186-197.

Goodband, R.D., Hines, R.H., Neissen, J.L., Nichols, D.A. (1989). Effects of Various Fat Sources on Growth Performance of Finishing Pigs. Swine Day, Manhattan Kansas, 154-156.

Kavanagh, S., Lynch, P.B., Mara, F.O., Caffrey, P.J. (2001). A comparison of total collection and marker technique for measurement of apparent digestibility of diets for growing pig. Anim Feed Sci Tech,89, 49-58.

Latorre, M.A., Lázaro, R., Gracia, M.I., Nieto, M., Mateos, G.G. (2003). Effect of sex and terminal sire genotype on performance, carcass characteristics, and meat quality of pigs slaughtered at 117 kg body weight. Meat Sci, 65, 1369–1377.

Mitchaothai, J., Yuangklang, C., Wittayakun, S., Vasupen, K., Wongsuthavas, S., Srenanul, P. (2008). Effect of Dietary Fat Type on Digestibility and Deposition of Energy in Growing-finishing Pigs. Proceedings, The 15th Congress of FAVA 27-30 October FAVA - OIE Joint Symposium on Emerging Diseases Bangkok, Thailand, 211-214.

NRC. 1998. Nutrient requirements of swine. 10th Ed. National Academy Press, Washington, DC.

Nesaretnam, K., Muhammad, B. (1993).Nutritional properties of palm oil. Selected Readings on Palm Oil and its Uses. Kuala Lumpur: PalmOil Research Institute of Malaysia. p 57–67.

Pantzaris, T.P. (1995).Pocketbook of Palm Oil Uses, 3rd edn. Kuala Lumpur: Palm Oil Research Institute of Malaysia.

Park, J.C., Kim, S.C., Lee, S.D., Jang,H.C., Kim,N.K., Lee, S.H., Jung, H.J.I., Kim, C., Seong, H.H., Choi, B. H. (2012). Effects of Dietary Fat Types on Growth Performance, Pork Quality, and Gene Expression in Growing-finishing Pigs. Asian-Australas J Anim Sci, 25, 1759–1767.

Serrano, M.P., Cámara, L., Morales, J.I., Berrocoso, J.D., López Bote, C.J., Mateos, G.G. (2013).Effect of gender, housing density and the interaction on growth performance and carcass and meat quality of pigs slaughtered at 110 kg body weight. Spanish J Agr Res, 11, 89-99.

Shannon, M., No date. Which Fat Source Should I Feed my Pigs? University of Missouri Extension, 1-3.

Short, F.J., Gorton, P., Wiseman, J., Boorman, K.N. (1996). Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim Feed Sci Tech,15, 215-221.

Tartrakoon, W., Tartrakoon, T., Kitsupee, N. (2016). Effects of the ratio of unsaturated fatty acid to saturated fatty acid on the growth performance, carcass and meat quality of finishing pigs. J Anim Nutr, 2, 79-85

Yongbo, S., Xiaohua, B., Huang, Q., Liu, L., Piao, X., Li, D. (2015).The effect of inclusion level and basal diet on the determination of the digestible and metabolisable energy content of soybean oil and its digestibility when fed to growing pigs. Anim Prod Sci, 56, 1167-1173.

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THE CONCENTRATION ANALYSIS OF AGRICULTURAL SECTOR IN TURKEY: ARE THERE ANY CHANGES IN THE PERIOD 2010 - 2015?

Funda GENCLER[1*], Berna TURKEKUL[2]

[1]School of Tobacco Expertise, Celal Bayar University, Manisa, Turkey
[2]Faculty of Agriculture,Ege University, Izmir, Turkey
*Corresponding author: funda.gencler@cbu.edu.tr

ABSTRACT

Despite the regional policies taken into agenda in all development plans in Turkey, inter-regional imbalances could not be resolved. Dynamics of the region must be examined in detail to resolve the inter-regional imbalance problems and in the light of these data differing policies should be developed in each specific region. Thus, detailed studies are needed on basis of regions and provinces. It is critically important to establish the priorities in agro-food production for utilizing the resources effectively and for competing against others like in Turkey whose agro-food sector is relatively dominant both in terms of population and employment. Therefore, it is aimed to determine the sectorial priorities in agriculture for using geographic concentration coefficient analysis in agricultural sub-sectors in Turkey.Inthiscontext, considering the Nomenclature of Territorial Units for Statistics (NUTS) - Level 2 (26 regions), the weight of employment structure of rural areas in Turkey will be analyzed for each region. Location coefficients will be calculated for each sector by comparing the population that is living in rural areas and working in agricultural, industrial and service sectors,with sector values of Turkey's rural areas. Later,agricultural sector will be analyzed on the basis of sub-sector for Level 2 regions. These analyses will be done for both 2010 and 2015 to compare the differences during this period. The reason of these diversities between mentioned time periods will be clarified by utilizing the agricultural policy applications in Turkey.

Keywords: *regional development, geographic concentration coefficient, location coefficient.*

INTRODUCTION

Inter-regional imbalances experienced today not only cause economic issues in the country but also affect global competitive strength negatively.In this context, countries worldwide have endeavored to eliminate inter-regional imbalances. However, this issue is considerably difficult for developing countries where financing needed for investments is limited. Regional development policies should

be produced based on regional dynamics so as to make undeveloped countries reach level of developed regions.

Although regional development concept has been in agenda since past, it has been intensively talked over during last twenty years. One reason for it are the changes occurred in regional development concept at first glance. While regional development has a predominant state view in past, recently it has changed to a market centered view. The solution based on provision of direct government income and from public source stated before for reduction of development deficit has now been replaced with necessity to use the resources owned by the regions at market conditions (Ercan, 2006).

What is expected from the government here is the adoption of legal regulations/legislations needed for such transformation and allocating incentives based on productivity principle rather than equality principle. The resources that are difficult to return in case of use in inefficient regions should be used in another region of the country efficiently, which may contribute to economy and growth of the country. This is a pre-condition for enhancing regional competitiveness and regional development (Aydemir, 2002),

Upon change addressing regional development, local economies and local actors have started to gain importance and SMEs with flexible and adaptive structures and new industries have started to emerge in parallel to increasing competition (Elvan et al., 2005). In this context, it is likely to say that in particular the efforts of international capital to shift production process to regions having relatively cheap workforce and raw material or increasing competitiveness by means of agreements with small and medium size enterprises performing activities in such regions have been effective. It is claimed that the changes in production process will serve for strengthening local economies and thus regional development (Ercan, 2006).

Agriculture sector, one of fundamental sectors, has not remained out of such developments. Particularly, upon high technology and bio-technology, agriculture sector not seen as high profit areas by developed countries in past has now been within interest of capital intensively today.

In countries like Turkey where rural regions and agriculture have an essential place in respect to population and employment, it is important to identify priorities for agriculture and food products in respect to effective use of resources as well as competitiveness.This study aims to analyze concentration of agriculture sector on basis of sub-sectors and establish sectoral priorities.

MATERIAL AND METHODS

This study analyses location coefficient of sectoral and geographical concentrations of regions, geographic concentration coefficient, concentration coefficient and concentration rate. In this context, statistical regional classification Level 2 regions (26 regions) were studied. Population living in rural areas and engaged in agricultural, industrial, trade and service sectors in the said regions were compared to the sectoral values of other rural areas in Turkey and location coefficients were

calculated for all sectors. Location coefficient has been calculated by use of following formula:

$$\text{Location coefficient} = (E_{ij} / E_i) / (E_j / E) \quad (1)$$

E_{ij}: Employment in region i of sector j E_i: Sum of employment in region i
E_j: Employment of sector j in Turkey E: Sum of employment in Turkey

Then geographic concentrations were analyzed based on vegetal, animal production and stocks values. Taking into account the geographical concentration coefficient and areas of regions, the concentration structures were analyzed for sectors. Geographical concentration coefficient has been calculated by use of following formula.

$$\text{Geographical concentration coefficient}: (D_{ij} / D_j) / (Y_i / Y) \quad (2)$$

D_{ij}: Total value in region i of sector j D_j: Total value of sector j in Turkey
Y_i: Area of region i Y: Area of Turkey

Location and geographical concentration coefficients were calculated for 2010 and 2015. Related data were obtained from the Turkish Statistical Institute(TUİK)-Agricultural Structure and Workforce Statistics.

RESULTS AND DISCUSSION

Location coefficients have been calculated for four major sectors taking into account the employment of population in Level 2 Regions (Figure 1). Thus regional potentials have been identified based on sectoral concentration. For a sector the region where location coefficient is above 1 is defined as intensively concentrated for such sector.

When agricultural sector is considered, 19 regions with location coefficient above 1 have been identified. The highest location coefficient is in TRA2 region. It is followed respectively by TRA1, TR90, TR82, TR83, TRB1, TR81, TR33, TR22, TR71, TRB2, TR63, TR52, TR61, TR32, TR62, TR72, TRC3 and TRC2. The region where agriculture concentration is the least is TR10 region. Black Sea and Eastern Anatolia Regions are the ones having high concentration of agriculture sector (location coefficient above 2)(Figure 1).

When industrial sector is considered, 7 regions with location coefficient above 1 have been identified. TR41 is the one having the highest location coefficient for industrial sector. It is followed by TR10, TR21, TR42, TRC1, TR31, TR72 regions. The region where industrial concentration is the least is TRA1 region.

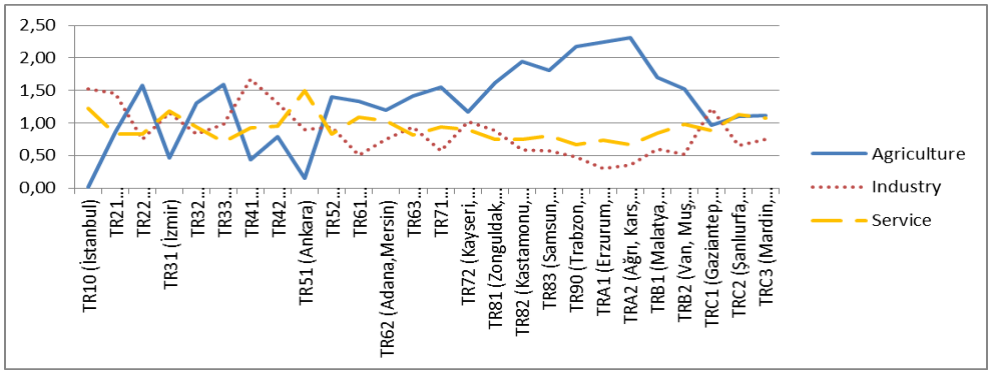

Figure 1. Location coefficients based on employment in rural residential regions (2010)

When service sector is studied, TR51 region is the one having the highest concentration. It is followed by regions TR10, TR31, TRC2, TR61, TRC3, and TR62. TRA2 region is the one having the least concentration for service sector. When concentrations between 2010 and 2015 are considered for agriculture sector, it is noticed that some regions show important differences. When location coefficients difference for 2010 and 2015 are considered, the biggest change is seen in TRB2 region (Figure 2).

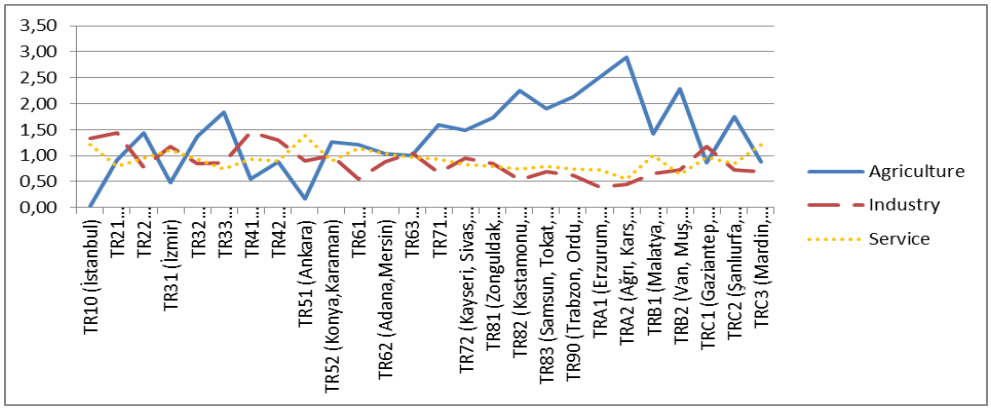

Figure 2. Location coefficients based on employment in rural residential regions (2015)

This part analyzes geographical concentration in respect to three structures. They are vegetal production values, animal productions and livestock values. Taking into account the geographical concentration coefficient method and areas of regions, the concentration structures in the regions were analyzed for sectors. The geographical concentration coefficient of Turkey is 1. Accordingly,it is seen that the production level is low where geographical concentration coefficient is under 0.5, and production level is close to Turkey's average in the regions where geographical

concentration coefficient is between 0.5 and 1.5 and production is gradually concentrated wheregeographical concentration coefficient is 1.5-3 and 3+(Elvan et al., 2005).

Vegetal production has concentrated in Mediterranean and Aegean coastline regions and TR22 region in 2010 (Table 1). Vegetal production has been at low level in Eastern and South-Eastern Anatolia Regions (TRB1, TRB2, TRA2, TRA1), TR72 in Central Anatolia Region and TR81 and TR82 in West and Central Black Sea region. Vegetal production in other regions has occurred at average of Turkey. In 2015, vegetal production has concentrated in TRC1 and TR90 regions. What is noticed here is that geographical concentration coefficients of regions TR31, TR61, TR62 and TR22 in Aegean Region have decreased when compared to those of 2010. Despite higher geographical concentration coefficients for years, it can be said that these two regions have got away from vegetal production.

Table 1. Vegetal and animal production and livestock concentrations

2010	**Vegetal Production**	**Livestock**	**Animal Production**	**2015**	**Vegetal Production**	**Livestock**	**Animal Production**
TR10	0.48	1.01	1.04	TR10	0.41	0.71	0.70
TR21	1.38	1.40	1.17	TR21	1.34	1.35	0.69
TR22	1.50	2.05	2.53	TR22	1.12	1.88	0.86
TR31	2.76	2.55	3.50	TR31	2.34	3.18	1.39
TR32	1.59	1.30	1.12	TR32	1.36	1.12	0.70
TR33	1.06	1.20	1.46	TR33	0.96	1.24	0.42
TR41	1.15	0.80	0.87	TR41	1.15	0.83	0.34
TR42	1.17	1.56	3.87	TR42	1.39	1.37	0.51
TR51	0.92	0.57	0.58	TR51	0.79	0.77	0.27
TR52	0.72	0.84	0.70	TR52	1.13	0.98	0.45
TR61	2.48	0.73	0.61	TR61	1.79	0.86	0.42
TR62	2.37	0.87	1.02	TR62	2.14	0.79	0.41
TR63	1.83	0.81	0.67	TR63	1.60	0.84	0.39
TR71	1.27	1.01	0.97	TR71	1.45	1.49	0.66
TR72	0.38	0.75	0.67	TR72	0.44	0.68	0.36
TR81	0.47	1.08	0.79	TR81	0.52	0.72	0.28
TR82	0.38	0.77	0.74	TR82	0.33	0.65	0.39
TR83	1.29	1.09	1.17	TR83	1.35	1.02	0.43
TR90	0.90	0.59	0.83	TR90	1.57	0.52	0.57
TRA1	0.18	0.76	0.68	TRA1	0.17	0.81	0.43
TRA2	0.13	1.81	1.31	TRA2	0.20	1.66	0.76
TRB1	0.33	0.67	0.53	TRB1	0.41	0.72	0.29
TRB2	0.18	1.34	0.78	TRB2	0.20	1.10	0.45
TRC1	1.33	0.78	0.54	TRC1	1.67	1.05	0.42
TRC2	1.32	0.91	0.64	TRC2	1.29	0.97	0.41
TRC3	0.68	0.85	0.59	TRC3	0.69	0.79	0.37

When geographical concentration coefficients are studied in respect to animal production in 2010, it is seen that animal production has concentrated in regions TR31, TR22, TRA2 and TR42 (Table 1). Other Level 2 regions have production

levels close to Turkey average. In 2015 only region TR31 concentrated in animal productions while all other regions were close to Turkey average.
In 2010, regarding livestock, regions TR42, TR31 and TR22 concentrated above Turkey average and comparative superiority of these regions are seen in the sector (Table 1). Other regions have livestock at Turkey average. In 2015, concentration coefficients of regions TR42, TR31 and TR22 went below 1.5.

CONCLUSION

Studies and practices for elimination of regional differences are considerably complex and difficult as each region has different structure and dynamics. For that reason, the primary condition to achieve the target is to identify the structural features of each region and make a planning accordingly.
In this study Level 2 regions have been studied for agricultural sector. In this frame, location coefficients have been found out in order to identify the regions where agriculture has priority. Location coefficients calculated separately for 2010 and 2015 have also allowed commenting on differences in this process. According to the results, Black Sea and Eastern Anatolia Regions are the ones where agriculture concentration has occurred. When concentrations in 2010 and 2015 are considered, the biggest change in terms of agriculture sector has been in region TRB2. Then the geographical concentrations in agriculture were studied in respect to vegetal production, animal production and livestock production. The concentrations were calculated for 2010 and 2015 separately. The results indicate that the most concentration in vegetal production is in Aegean and Mediterranean Sea coasts in 2010. It is likely to say that vegetal production level in Eastern and south-Eastern Anatolia (TR72, TR81 and TR82) is low. When comparison is made in respect to 2010 and 2015, it is seen that vegetal production has decreased in Aydın, Denizli, Muğla (TR32) located in Aegean Region while vegetal production in TR61 and TR42 has increased. Animal production has concentrated in TR42 consisting of Kocaeli, Sakarya, Düzce, Bolu, Yalova and Izmir, Balıkesir, Çanakkale regions in 2010. Regarding livestock, concentration in TR31 (Izmir), TR22 (Balıkesir, Çanakkale) TRA2 (Ağrı, Kars, Iğdır, Ardahan) above Turkey average for each period suggests comparative superiority of the regions for the sector.When the study is evaluated in general, agriculture sector in employment terms concentrate in Eastern Anatolia and Black Sea Regions but concentration remains at low level in terms of production. Indicating low concentration in terms of agriculture sector Izmir (TR31), Adana, Mersin (TR62), Antalya, Isparta, Burdur (TR61), Tekirdağ, Edirne, Kırklareli (TR21), Aydın, Denizli, Muğla (TR32), the concentration in other sectors in terms of employment has an important place. Particularly, it is likely to say that Izmir is the most important city forming inter-sectoral integration. Low agricultural production level of regions having agricultural employment concentration is the most important finding to be considered. Particularly, contrary to Erzurum, Erzincan, Bayburt (TRA1), Ağrı, Kars, Iğdır, Ardahan (TRA2) known as important regions for animal growing is considerably under Turkey average contrary to what's expected. Although priority

will be given to regions where resources are utilized more efficiently as per policies in effect and potential incentives, some social factors should not be ignored as seen in Eastern Anatolia region. Concentration of employment in agriculture sector in such regions is a factor decreasing competitiveness strength.In this framework, in addition to works for enhancement of efficiency in the sector, projects providing transfer of work force concentrated in agriculture to different sectors should be achieved. The studies to be made in order to ensure regional development and achievement of development targets should be performed in the light of consideration of some fundamental findings exemplified above. Studies to analyze all sectors and sub-sectors should be conducted for discovery of sectoral integration level and identifying the issues since regional development is targeted today. It is also essential that the studies to be conducted cover the EU harmonization process.The most important duty of the regional development agencies in this respect is to discover internal dynamics of each region and distribution of available financial resources in the most effective way. For instance, study of projects conducted for sectoral integration and development for Izmir displaying an essential level in terms of development, will constitute a good and important example for other regions. At this point, it should not be ignored that each region has different structure.

REFERENCES

Aydemir, Z. C. (2002). Resource utilization efficiency by the regional competitiveness from the city scope: data envelopment analysis application. General Directorate of Economic Sectors and Coordination, State Planning Organization, Proficiency Thesis, Item No: SPO: 2664, Ankara, pp. 53.

Elvan, L., Sorguç, N., Kazancık, L.B., Öztürk, A. (2005).Regional development policies in Turkey; sector-region agglomeration. Turkish Industry and Business Association, Item No: TÜSİAD-T/2005-09/408, Istanbul, pp.134.

Ercan, F. (2006). Changes in regional development: regional development of the state-centric markets to regional deposition. Regional Development Policies and New Dynamics, Derin Publications, Istanbul, pp. 45-116.

http://rega.basbakanlık.gov.tr 08.02.2006:26074, 5449 with the number of legislation.

Küçük, O. (2007). EU adaptation process development agency and EU grant project investigation of contribution to regional development: Samsun, Kastamonu and Erzurum nuts 2 regions case study. Journal of Graduate School of Social Science, 10/2: 488-500.

Ünsaldı, M., (2006). State incitements and their effects on local development differences. Journal of Research in Eastern Anatolia Region, 4/2: 122-125.

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LOTUS spp: BIOTECHNOLOGICAL STRATEGIES TO IMPROVE THE BIOECONOMY OF LOWLANDS IN THE SALADO RIVER BASIN (ARGENTINA)

Cristian J. ANTONELLI[1], Pablo I. CALZADILLA[1], Francisco J. ESCARAY[1,3], María F. BABUIN[1], María P. CAMPESTRE[1], Rubén ROCCO[1], César D. BORDENAVE[1], Ana PEREA GARCÍA[1.3], Amira S. NIEVA[1], María E. LLAMES[1], Vanina MAGUIRE[1], Gustavo MELANI[2], Daniel SARENA[2], Matías BAILLERES[2], Pedro CARRASCO[3], Francesco PAOLOCCI[4], Andrés GARRIZ[1], Ana MENÉNDEZ[5], Oscar A. RUIZ[1]*

[1]Institute of Biotechnological Research-Technological Institute of Chascomús (IIB-INTECH), Argentina
[2]INTA-MAA Integrated Experimental Farm (CEICh), Argentina
[3]University of Valencia, Spain
[4]IBBR-CNR, Division of Perugia, Italy
[5]Faculty of Natural Sciences, Buenos Aires University, Argentina
*Corresponding author: ruiz@intech.gov.ar; oaruiz@conicet.gov.ar

ABSTRACT

The Salado River Basin region is the most important livestock breeding area in Argentina, wherethe *Lotus* species has been traditionally cultivated as forages. Nearly 60% of their land surface is dominated by salt-affected soils with severe constraints for crop cultivation. In order to cope with that limitation, farmers have utilized species such as non-native *L. tenuis* (ex- *Lotus glaber*), which shows a very good adaptation. As a result, inter-seeding of *L. tenuis* has been proposed as a strategy of choice for improving forage production in marginal areas. The increase in soil quality by these means is achieved by an increment of the organic matter content, improvement of soil fertility as well as microbial biodiversity. Thus, the introduction of *L. tenuis* and/or other *Lotus* genotypes could have enormous benefits for similar constrained lands around the world. We are developing an integrated analysis of the changes that occur in soils under legume production. We will not only analyze the microbial diversity associated, but also soil physical and chemical characteristics and the impact of different legume-microbes association on mitigation of GHG emissions. In addition, we are identifying the main genetic determinants associated with interesting agronomic traits such as plant tolerance against biotic and abiotic stresses and the content of condensed tannins. Our future and present research will build a solid base for the improvement of agronomically-important species and the development of better strategies for the management of constrained lands such as the lowlands in the Argentinean Pampas.

Keywords: *Lotus spp, flooding pampas, Salado River basin, condensed tannins, constrained soils.*

INTRODUCTION

The *Lotus* genus is included in Fabaceae, which is the third largest family of Angiosperms. This family has a high biological diversity, with approximately 720 genera and more than 18,000 species worldwide. The legume species take profit from root symbiosis with mycorrhizal fungi (Sannazzaro *et al*, 2004), characterized by their ability to establish symbiotic interactions with nitrogen-fixing bacteria (Estrella *et al*, 2009). These associations increase their competitiveness, turning them as "pioneer" plants in constrained soil environments. These legumes advantages became an important part of sustainable agricultural systems and others marginal soil area. In particular, the tribe Loteae DC is a monophyletic group composed by four genera. The name *Lotus* was introduced by Linnaeus in 1753 and since then, there were several changes in the species delimited by this name. Recently, developed molecular tools have significantly contributed to restrict the genus to 100–130 species. Most *Lotus* species are native to Europe, Asia, Africa, Australia and some to the Atlantic and Pacific Ocean Islands. Few species were described as native to the New World, but they were later segregated in non-Lotus genera. However, Lotus species have a worldwide distribution, except in very cold regions and certain tropical areas of Southeast Asia and Central America. This worldwide distribution is partially due to their introduction to non-native areas by human activities and its adaptability to different environmental stresses (Escaray *et al*, 2010).

Lotus genus crops species in South America

The genus *Lotus* has been historically used in the Southern Cone of South America forpasture improvement. Taking in mind this agronomical target, there are intends to contribute to the sustainability of them in the region through the generation of biotechnological tools to allow the development of better adapted genotypes of the forage species (*Lotuscorniculatus* L., *L. tenuis* Waldst. et Kit. (Syn. *L. glaber* Mill.), *L. uliginosus* Schkuhr (Syn. *L. pedunculatus* Cav.) and *L. subbiflorus* Lag.) in agricultural marginal soils.. All of these projects took advantage of the model species *Lotus japonicus* (Regel) to develop new strategies to accelerate breeding of cultivated species. In parallel, there are some research groups working in the identification, characterization and selection of microorganism that could guarantee the optimum N fixation in those soils improving legume nutrition, thereby increasing productivity, quality and sustainability of *Lotus* pastures in marginal soils (Escaray et al, 2012a).

The Salado River Basin and the importance of *Lotus spp.*

The Salado River Basin is located in the center of the Flooding Pampa, a vast area located in the Buenos Aires province, Argentina. It is a very flat region, and constantly affected by periods of flood/drought. About 40% of the soils in this area are composed of humus with a great water retention capacity and excellent quality

for growing many important crops. It is, therefore, considered one of the richest agricultural regions in Argentina. Most of the wheat, corn and soybean production comes from this area and it is, of course, essential for the economy of the country, where the agricultural activities are among the major economic resources. However, the economic development of the area has lagged because of the presence of thousands of hectares of poor quality salt-affected soils, representing about 60% of the total area. These soils are very conspicuous in Argentina, placing this country in third place among those with the broadest surface dominated by them. In fact, approximately 11.58 Mha (equivalent to 11% of the total area) of saline soils are estimated to occur under a sub humid-humid climate in this country. It has been largely demonstrated that saline soils hinder plant growth due to their high pH, low structural stability and low water and nutrient availability. In this sense, the distribution of natural grasslands in the Salado River Basin is determined by alkalinity levels and flooding frequency. All plant communities consist mainly of grasses and plants belonging to the family *Compositae*, whereas native legumes are largely absent. The grassland community growing on sodic soils is composed by low value foraging grasses, such as *Distichlis scoparia*, *D. spicata*, *Paspalumvaginatum*, *Sporobolus pyramidatus* and the alga *Nostoc*. Although they comprehend a considerable fraction of the forage available to livestock, their net primary productivities and palatability are clearly lower than those of communities growing in more fertile soils. In addition, these species are associated to low soil organic matter (SOM) contents, which may show wide variations among different Great Soil Groups. As will be described later, high SOM values are preferred since they are associated to reduce rates of gas emission to the atmosphere.

The economical practices of the Salado River Basin have suffered notable changes in the last decades. This is mainly due to the advance of agriculture at the expenses of grazing pastures, a phenomenon that has constrained livestock to marginal areas such as the case of native grasslands growing on sodic soils. Therefore, these soils are currently subjected to increasing use-pressures to improve forage offer. In this trend, many attempts to replace grassland communities by exotic species have been made with relative success. For instance, one of the species that has gained considerable importance is the naturally adapted legume *L. tenuis*. The tolerance of this species to alkalinity and long term flooding periods has allowed a wide and fast spread over the Salado River Basin. *L. tenuis* can also grow under low extractable phosphorus (P) concentrations, as it usually happens in most sodic soils, because its association with mycorrhiza enables the acquisition of P by plant roots (Sannazzaro *et al* 2004, Echeverría *et al* 2013). In addition, the introduction of *L. tenuis* represents a substantial benefit for the region since it is an important source of N for the soil (Castagno *et al* 2014). Even though *L. tenuis* has high nutritional value, similar to other forager legumes, it is unable to accumulate adequate levels of condensed tannins in the foliage and this deficiency is one of the principal aims for legume breeders in Argentina (Escaray *et al* 2014).

Abiotic stresses

Alkaline stress refers to the presence of alkaline salts (Na_2CO_3 or $NaHCO_3$) in the soil, whereas saline stress is related only to neutral salts such as NaCl or Na_2SO_4.Although saline stress has been addressed in numerous studies, alkalinity rather than salinity is the main constraint for cropping activity (Paz *et al* 2012). Studies on abiotic stress in *L. tenuis* have been focused principally on NaCl tolerance (Sánchez *et al* 2005; Sannazzaro *et al* 2006, 2007; Echeverria *et al* 2008; Uchiya *et al*, 2016), whereas less studies has addressed its tolerance level to alkaline and mixed salt–alkaline stresses. Alkaline salts have a more severe effect on plant growth than neutral salts. When soil salinity is high and/or the pH is 8.5 or above, a number of micronutrients such as P, Fe, and Zn become deficient,triggering differential gene expression (Paz *et al*, 2014b). Both saline and alkaline conditions frequently coexist in nature, with the proportion of neutral salt to alkaline salt varying in different soils. However, little is known about the effects of alkaline and salt–alkaline stresses on plants. In this sense, root architecture has been directly related to plant productivity because in no-till systems it can provide clues about resource cost, transport, and exploration efficiency, especially under limiting edaphic conditions. Salinity affects root developmental processes in different ways. Evident differences in the morphology of the root system as a function of salt stress treatment were found in *L. tenuis*(Echeverria *et al*, 2008). Neutral and alkaline salts produced a similar detrimental effect on *L. tenuis* growth, whereas the effect of their combination was synergistic (Paz et al 2012). The pattern of morphological changes in *L. tenuis* root architecture after the alkaline treatment (in the absence of NaCl) was similar to that found in the mixed salt–alkaline treatment and different from that observed in neutral salt. A unique root morphological response to the mixed salt-alkaline stress was the reduction in the ratio between xylem vessels and root cross-sectional areas (Paz *et al* 2014a).

Moreover, we characterized phenotipically the response to alkaline stress of the most widely used *L. japonicus* ecotypes, Gifu B-129 and MG-20, and analyzed global transcriptome of plants subjected to 10 mM $NaHCO_3$ during 21 days, by using the Affymetrix *Lotus japonicus*GeneChipH (Babuin *et al*, 2014). Plant growth assessment, gas exchange parameters, chlorophyll a fluorescence transient (OJIP) analysis and metal accumulation supported the notion that MG-20 plants displayed a higher tolerance level to alkaline stress than Gifu B-129. Overall, 407 and 459 probe sets were regulated in MG-20 and Gifu B-129, respectively. The number of probe sets differentially expressed in roots was higher than that of shoots, regardless the ecotype. Gifu B-129 and MG-20 also differed in their gene regulation related to Fe/Zn homeostatic cellular condition, synthesis of stress response compounds, protein-degradation, damage repair and root senescence, as well as in glycolysis, gluconeogenesis and TCA. In addition, there were differences between both ecotypes in the expression patterns of putative transcription factors that could determine distinct arrangements of flavonoid and isoflavonoid compounds (Babuin *et al*, 2014).

Likewise, we evaluated cold acclimation of the genus by studying *L. japonicus*(Gifu B-129 ecotype) over a stress period of 24 h. High-throughput RNA sequencing was used to identify and classify 1077 differentially expressed genes, of which 713 were up-regulated and 364 were down-regulated. Up-regulated genes were principally related to lipid, cell wall, phenylpropanoid, sugar, and proline regulation, while down-regulated genes affected the photosynthetic process and chloroplast development. Together, a total of 41 cold-inducible transcription factors were identified, including members of the AP2/ERF, NAC, MYB, and WRKY families; two of them were described as putative novel transcription factors. Finally, DREB1/CBFs were described with respect to their cold stress expression profiles. This is the first transcriptome profiling of the model legume *L. japonicus* under cold stress. Data obtained may be useful in identifying candidate genes for breeding modified species of forage legumes that more readily acclimate to low temperatures (Calzadilla *et al*, 2016a, 2016b).

Biotic stress

Legume production is also hampered in many areas by biotic stress originated by pathogenic microorganisms. This factor is the cause of considerable losses every year. Therefore, a deeper understanding of the defense responses deployed by legume plants against pathogens is a crucial step in the development of tolerant cultivars and the establishment of effective disease control strategies. However, there is a rather lack of information on the defense responses that control attacking pathogens in legumes. Even worse, the resistance mechanisms identified in plants belonging to other families cannot be fully extrapolated to legume species, probably due to differences in genomic organization. We examined the interaction between *L. japonicus* (Gifu B-129 and Miyakojima MG-20) and *P. syringae* pv. tomato DC3000, a strain that cause bacterial speck in tomato and *Arabidopsis*(Bordenave *et al* 2013). Our analysis demonstrated contrasting phenotypic differences in the two ecotypes during the response to the bacteria. On these grounds, we next performed a transcriptomic analysis aimed to identify the genes associated with such differential response. We were able to recognize a large number of transcripts differentially expressed, many of them showing high homology to well-known defense genes in other plant species. Importantly, some of the genes associated to virulence in this strain diverge from those described in other legume-parasitic races of this species, as pv. *glycinea*, pv. *phaseolicola* and pv. *pisi*. Thus, these partners could be developed into a useful model pathosystem to study the most general defense mechanisms deployed in this legume against non-pathogenic microorganisms (Bordenave *et al* 2013).

Microorganism symbiotic associations

Like most legumes, *L. tenuis* has the ability to establish mutualistic symbiotic relationships with soil N-fixing bacteria collectively known as rhizobia, thereby rendering this plant more competitive than non-legumes in soils with low N content. Taking into account the relatively recent naturalization of this legume in

Argentina (less than 80 years), it is not surprising that the selection of efficient strains and the development of high quality inoculants for *L. tenuis* is still incipient. However, in many cases *L. tenuis* seeds are inoculated before sowing, by using rhizobia formulations based on strains whose taxonomic identity has not been established. These strains were originally isolated from soils different to those of the Salado River Basin and selected for their ability to symbiotically fix N in environments not necessarily similar to those typical of this region. It is well known that, in the soil, the strains used for inoculation are subjected to the effects of numerous biotic and abiotic factors which could decrease the symbiotic efficiency. In the particular case of the Salado River Basin, the adaptation and survival of rhizobia, as well as their symbiotic efficiency can be affected by soil salinity. Thus, when efficient strains are introduced in environments different to those from which they were isolated, they can be outcompeted by well adapted native rhizobia. In this way, it is reasonable to hypothesize that rhizobia isolates from the Salado River Basin should be better adapted to the soil conditions of this region than introduced strains. In this sense, the use of inoculants based on efficient native strains could be an affordable and sustainable resource to improve the yield of *L. tenuis* in this region. As a consequence, we evaluatedinoculants isolated from soils of the region of Salado River Basin (Estrella *et al.* 2009; Sannazzaro *et al*, 2011).

Moreover, low P soils levels is also an important issue in the Salado River Basin, and symbiotic associations could diminished its detrimental effect. We isolated and characterized phosphate-solubilizing strains from a constrained environment in the region improve the implantation and persistence of *L. tenuis*. The most efficient isolate was identified as *Pantoea eucalypti*(*M91* strain), a novel species in terms of plant growth-promoting rhizobacteria (Castagno *et al*, 2011). Inoculation of *L. japonicus* with this strain, alters the root, resulting in a herringbone pattern of root branching. Additional features include improvement in Fe^{2+} transport to the shoots, acidification of the hydroponic solution of the plant cultures, and an accompanying increase in the efficiency of the PSII parameters. As a whole, *P. eucalypti M91*showed a beneficial effect on the Fe acquisition, suggesting its potential use as an inoculant for legume crops cultivated in alkaline soils (Campestre *et al*, 2016). Interestingly, in others studies developed in collaboration with our lab, Actinobacteria also showed promoting growth activity on *Mesorhizobium loti* symbiosis with*L. tenuis* (Solans *et al* 2015).

Condensed tannins

Proanthocyanidins (PAs) are secondary metabolites that strongly affect plant quality traits. The concentration and the structure of these metabolites influence the palatability and nutritional value of forage legumes. Hence, modulating PAs in the leaves of forage legumes is of paramount relevance for forage breeders worldwide (Escaray *et al*, 2012b). The lack of genetic variation in the leaf PA trait within the most important forage species and the difficulties in engineering this pathway via the ectopic expression of regulatory genes, prompted us to pursue alternative

strategies to enhance this trait in forage legumes of agronomic interest. The *Lotus* genus includes forage species which accumulate PAs in edible organs and can thus be used as potential donor parents in breeding programs. Taking in mind this, we recovered a wild, diploid and PA-rich population of *L. corniculatus* and crossed it with an argentinean naturalized germplasm of *L. tenuis*. The last species does not accumulate PAs in the herbage. The resulting interspecific hybrid displayed several traits of outstanding agronomic relevance such as rhizome production, PA levels in edible tissues sufficient to prevent ruminal bloating (around 5 mg of PAs/g DW) and adaptability to marginal lands. This material represents the first example of the introgression of the condensed tannins trait in forage legumes to levels known to provide nutritional and health benefits to ruminants (Escaray *et al*, 2014). Our studies suggest that the hybrids and their progeny are an invaluable tool to gain a leap forward in our understanding of the genetic control of PA biosynthesis and tolerance to stresses in legumes.

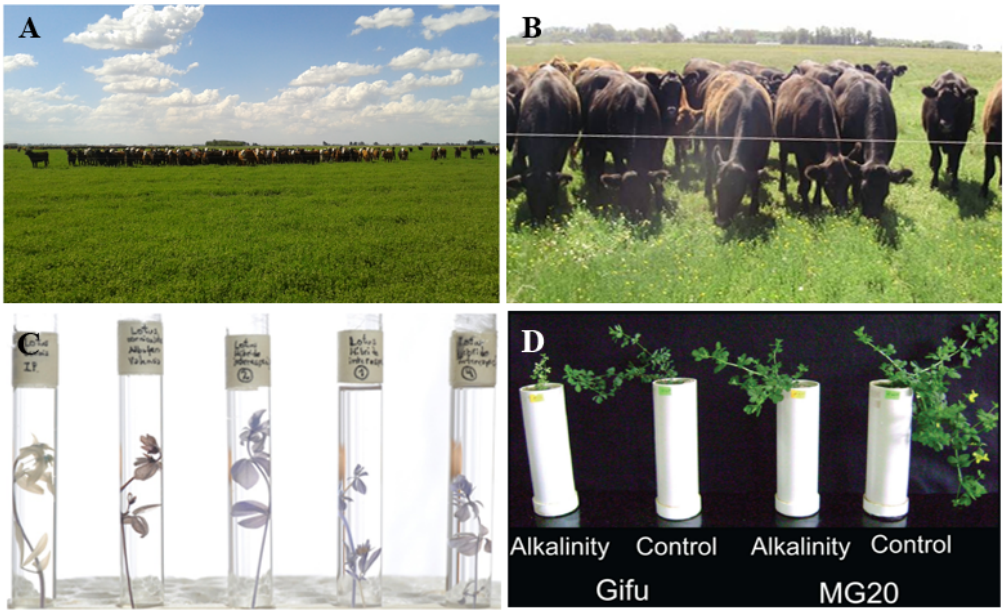

Figure 1.A and B. Cattle grazing paddock of *L. tenuis* in the Salado River Basin, Argentina.**C.** Condensed tannins content in different accessions of *Lotus* spp and hybrid *L. tenuis* x *L. corniculatus*. Adapted from Escaray *et al.* 2014.**D.** Alkalinity stress response of *L. japonicus* ecotypes after 21 d. Adapted from Babuin *et al.* 2014.

CONCLUSION AND PERSPECTIVES

Actually, our lab is increasing the knowledge on soil microbial diversity in the rhizosphere of *Lotus* species plants growing in the Salado River Basin (Nieva *et al*, 2016),and evaluating its relationship with soil C sequestration (Nieva *et al.*

Manuscript in preparation). Moreover, farm productivity of *L. tenuis* and the implementation of the different technological protocols designed is being also analyzed (Bailleres *et al.* Manuscript in preparation). Simultaneously we are evaluating the phenotype of different accessions of *L. japonicus* during the attack of the phytopatogenic fungi and bacteria and identifying the main traits associated to plant tolerance looking to improve it in forage crops of outstanding economical relevance for our region. Potential genes involved in the tolerance to biotic and abiotic stresses in *Lotus* model and crops species are under evaluation by High-throughput RNA sequencing and trans-genesis approaches (Espasandin *et al* 2010, 2014). Others lab aims include the better understanding of the molecular basis of condensed tannins biosynthesis in *Lotus* spp and the role of these secondary metabolites on plant stress responses and GHG mitigation. Moreover, we continue exploring the interspecific hybridization as a strategy to generate superior *Lotus* genotypes.

The evaluation under "cow farm conditions" of the incidence of *L. tenuis, L. corniculatus* and their interspecific hybrids on the generation of GHG by ruminants and the design and evaluation of a Spatial Distribution Model (SDM) for *L. tenuis* in the Flooding Pampa, and estimate potential GHG emission rates from sodic grasslands on a regional scale complete our actual aims and plan work targets[1].

Finally, we are interested in creating an international working group to coordinate the future transfer of the obtained knowledge to constrained lands around the globe, and the potential of the genus *Lotus sp* in the soil restoration and reclamation activities.

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REFERENCES

Babuin M.F.; Campestre M.P.; Rocco R.A.; Bordenave C.; Escaray F.J.; Antonelli C.; Calzadilla P.; Gárriz A.; Serna E.; Carrasco Sorlí P.; Ruiz O.A.; Menéndez A.B. (2014) Response to long-term $NaHCO_3$-derived alkalinity in model *Lotus*

[1] PICT 2014-3718 Project: Forage legumes in the Salado River Basin: integrated analysis of soil microflora on its productivity and GHG mitigation potential. (2015-2019).

japonicus ecotypes Gifu B-129 and Miyakojima MG-20: Transcriptomic profiling and physiological. PLOS ONE. vol.9 n°. p97106 - 97107. ISSN 1932-6203.

Bordenave C.D., Escaray F.J., Menendez A.B., Serna E., Carrasco Sorli P., Ruiz, O.A. and Gárriz A (2013). Defense responses in two ecotypes of *Lotus japonicus* against Non-Pathogenic *Pseudomonas syringae*.PLOS ONE. Volume 8. Issue 12. e83199.

Calzadilla P.I., Maiale S.J., Ruiz O.A. and Escaray F.J. (2016a).Transcriptome response mediated by cold Stress in *Lotus japonicus*. Frontiers in Plant Science.Vol:7. Article 374 doi: 10.3389/fpls.2016.00374.

Calzadilla P.I., Signorelli S., Escaray F.J., Menéndez A.B., Monza J., Ruiz .A. and Maiale S.J. (2016b).Photosynthetic responses mediate the adaptation of two *Lotusjaponicus* ecotypes to low temperature. Plant Science. 250: 59-68

Campestre M.P.; Castagno L.N.; Estrella M.J. and Ruiz O.A. (2016) *Lotus japonicus* plants of the Gifu B-129 ecotype subjected to alkaline stress improve their Fe^{2+}bio-availability through inoculation with *Pantoea eucalypti M91*. Journal of Plant Physiology 192: 47–55.

Castagno L.N., Estrella M.J., Sannazzaro A.I., Grassano A.I. and Ruiz O.A. (2011). Phosphate-solubilization mechanism and *in vitro* plant growth promotion activity mediated by *Pantoea eucalypti* isolated from *Lotus tenuis* rhizosphere in the Salado River Basin (Argentina). Journal of Applied Microbiology. 110 (5).1151-1165.

Castagno L.N.; García I.V.; Sannazzaro A.I.; Bailleres M. A.; Ruiz O.A.; Mendoza R.E.; Estrella M.J.. (2014). Growth, nutrient uptake and symbiosis with rhizobia and arbuscular mycorrhizal fungi in *Lotus tenuis* plants fertilized with different phosphate sources and inoculated with the phosphate-solubilizing bacterium. *Pantoea eucalypti M91*.Plant and Soil. vol.385 n°. p357 - 371.

Echeverría M., Scambato A.A.; Sannazzaro A.I., Maiale S.J. Ruiz O.A and Menéndez A.B. (2008). Phenotypic plasticity with respect to salt stress response by Lotus glaber: the role of its AM fungal and rhizobial symbionts. Mycorrhiza. 18: 317-329.

Echeverría M., Sannazzaro A.I., Ruiz O.A and Menéndez A.B. (2013) Modulatory effects of *Mesorhizobium tianshanense* and *Glomus intraradices* on plant proline and polyamine levels during early plant response of *Lotus tenuis* to salinity.Plant and Soil. 364: 69-79.

Escaray F.J., Collado Rosique F.J., Scambato A.A., Bilenca D, Carrasco Sorlí P.M., Matarredona A. V., Ruiz O.A. and Menéndez A.B.. (2010). Evaluation of a technical revegetation action performed on foredunes at the Valencian Devesa de la Albufera, Valencia, Spain. Land Degradation and Development. 21: (3). 239-247.

Escaray F.J., GárrizA., Estrella M.J., Pieckenstain F.L., Castagno L.N., Carrasco-Sorli P., Sanjuán Juan and Ruiz O.A (2012a)Ecological and agronomic importance of the plant genus *Lotus*. It's application in grassland sustainability

and the amelioration of constrained and contaminated soils. Plant Science. 182: 121-133.

Escaray F.J., Passeri V., Babuin F.M., Marco F., Carrasco-Sorli P., Damiani F., Pieckenstain F.L., Paolocci F. and Ruiz O.A. (2014) *Lotus tenuis x L. corniculatus* interspecific hybridization as a means to breed bloat-safe pastures and gain insight into the genetic control of proanthocyanidin biosynthesis in legumes. BMC Plant Biology 2014, 14:40. 1-18.

Escaray, F. J; Rosato M.; Pieckenstain F.L.; Menéndez A.B.; Roselló J.A.; Carrasco P. and Ruiz O. A (2012b). The proanthocyanidin content as a tool to differentiate between *Lotus tenuis* and *L. corniculatus* individuals. Phytochemistry Letters. 5: 37-40.

Espasandín F. D., Collavino M. M., Luna C. V., Paz R. C., Tarragó J. R., Ruiz O. A., Mroginski L. A. and Sansberro P. A..(2010). *Agrobacterium tumefaciens*-mediated transformation of *Lotus tenuis* and regeneration of transgenic lines. Plant Cell, Tissue and Organ Culture.102: 181-189.

Espasandín F.D, Maiale S.J., Calzadilla P., Ruiz O.A., Sansberro P.A. (2014). Transcriptional regulation of 9-cis-epoxycarotenoid deoxygenase (NCED) gene by putrescine accumulation positively modulates ABA synthesis and drought tolerance in *Lotus tenuis* plants. Plant Physiology and Biochemistry.76: 29-35.

Estrella M.J., Muñoz S., Soto M.J., Ruiz O.A. and Sanjuán J. (2009). Genetic diversity and host range of rhizobia nodulating *Lotus tenuis* in typical soils of the Salado River Basin (Argentina). Applied and Environmental Microbiology. 75 (4). 1088-1098.

Nieva A.S.; Bailleres M.A.; Corriale M.J.; Llames M.E.; Menéndez A.B. and Ruiz O.A (2016) Herbicide-mediated promotion of *Lotus tenuis* (Waldst. & Kit. ex Wild.) did not influence soil bacterial communities in soils of the Flooding Pampa, Argentina. Applied Soil Ecology (98) 83–91.

Paz, R. C.; Rocco, R.A.; Reinoso H.; Menéndez, A. B.; Pieckenstain, F. L. and Ruiz, O. A. (2012) Comparative study of alkaline, saline and mixed Saline-Alkaline Stresses with regard to their effects on growth, nutrient accumulation and root morphology of *Lotus tenuis*. Journal of Plant Growth Regulation. 31: 448-459.

Paz, R.C.; Reinoso, H.; Espasandín, F.D.; González Antivilio, F.A.; Sansberro, P.A.; Rocco, R.A.; Ruiz, O.A. and Menéndez, A. B. (2014a). Alkaline, saline and mixed saline-alkaline stresses induce physiological and morfo-anatomical changes in *Lotus tenuis* shoots. Plant Biology. 16: 6. 1042-1049.

Paz, R.C.; Rocco, R.A.; Jiménez Bremont, J.F.; Rodríguez Kessler, M.; Becerra Flora, A.; Menéndez, A. B.; Ruiz, O. A. (2014b). Identification of differentially expressed genes potentially involved in the tolerance of *Lotus tenuis* to long-term alkaline stress. Plant Physiology And Biochemistry . vol.82 n°. p279 - 288. ISSN 0981-9428.

Sánchez D.H.; Cuevas J.C., Chiesa M.A. and Ruiz O.A. (2005). Free spermidine and spermine content in *Lotus glaber* under long-term salt stress. Plant Science. 168: 241-245.

Sannazzaro A.I., Ruiz O.A., Albertó E. and Menéndez A.B. (2004). Presence of different arbuscular mycorrhizal infection patterns in roots of *Lotus glaber* plants growing in the Salado River basin. Mycorrhiza. 14: 139-142.

Sannazzaro A.I.; Ruiz O.A., Albertó E. and Menéndez A.B (2006) Alleviation of salt stress in *Lotus glaber* by *Glomus intraradices*. Plant and Soil. 285: 279-287.

Sannazzaro A.I.; Echeverría M., Albertó E., Ruiz O.A., and Menéndez A.B (2007) Alleviation of salt stress in *Lotus glaber* by *Glomus intraradices*. Plant Physiology and Biochemistry. 45. 39-46.

Sannazzaro A.I., Bergottini V.M., Paz R.C., Castagno L. N., Menéndez A.B., Ruiz O.A., Pieckenstain F.L. and Estrella M.J. (2011). Comparative symbiotic performance of native rhizobia of the Flooding Pampa and strains currently used for inoculating *Lotus tenuis* in this region. Antonie van Leeuwenhoek Journal of Microbiology. 99:371-379.

Solans M..; Ruiz, O.A.; Wall, L. G. (2015). Effect of actinobacteria on *Lotus tenuis* - *Mesorhizobium loti* symbiosis: preliminary study. Symbiosis. vol.65 n°1. p33 - 37.

Uchiya P.; Escaray F.J.; Bilenca D, Pieckenstain F.L.; Ruiz O.A. and Menéndez A.B.. (2016). Salt effects on functional traits in model and in economically important *Lotus* species. Plant Biology.Vol. 18. 4: DOI: 10.1111/plb.12455.

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FARMERS' SHARE OF MONEY SPENT ON FOOD IN AUSTRIA

Erika QUENDLER[1*], Franz SINABELL[2]

[1]Federal Institute of Agricultural Economics, Vienna, Austria
[2]Austrian Institute of Economic Research, Vienna, Austria
*Corresponding author: erika.quendler@awi.bmlfuw.gv.at

ABSTRACT

There has been renewed interest in the farmer's share of retail food sales recently, in the wake of sharp fluctuations in farm-gate or retail prices. Statistics on farmers' shares were already being developed decades ago and recent updates are a response to these fluctuations. We define the farm share as the average portion from each monetary unit spent on food by consumers that is received by farmers for their agricultural commodities. The same calculation gives the marketing margin, which is the remainder. Calculations show that the farmer's share from retail food sales has been on the decline for more than 60 years. Therefore, the gap between farmers' share and marketing margin is widening. This paper presents (i) the development of the farmers' share for Austria mainly for the period 1995 to 2013 and, where data is available, from 1971 onwards and(ii) compares the results with the developments in Germany and the United States as well as Switzerland. For Austria, two calculation methods are shown: the Agristat method (developed in Switzerland) and the method of the Austrian Institute of Economic Research (WIFO). Both calculations are based on official statistical data (Economic Accounts for Agriculture, National Accounts and trade data) but use different approaches. This paper uses the findings of these calculations to hypothesize on necessary areas for future research.

Key words: *farm-to-retail price spread, farmers' share, marketing margin, Austria.*

INTRODUCTION

The production of crops and livestock on farms is just a first step in the often long process of providing consumers with the goods and the food they require. On the way to the consumer agricultural commodities are transformed and value is added. From time to time price changes trigger discussions on the fairness of prices and the farmers' share of expenditure for food, ie. the question of what farmers get for their agricultural commodities relative to consumer expenditure for food consumption.

Consumer expenditure covers two main components besides the duties and taxes: remuneration for (i) agricultural commodities at the farm gate (equal to the

farmers' share) and (ii) the share for processing, trading and marketing of products on the way from the farm gate to the consumer (cf. Elitzak 1997; Orgen 1956; Sinabell 2010). In the remainder of the paper we call 'marketing margin' the part of consumer expenditure for food which does not cover the share farmers get.

The concept of marketing margin, or farm-to-retail price spread, was developed to show the difference between consumer expenditure for food and an associated farm value (Orgen 1956). Data has been collected and calculations have been done for decades by different institutions (El Benni, Hediger 2014; Giuliani 2015; Sinabell 2010; USDA 2015; Wendt, Peter 2014).

In light of the renewed recent interest and given the number of different calculation methods used to identify the respective farmers' share and marketing margin, the question poses itself on two levels: (i) is it possible to harmonize the approaches to achieve more transparent results by employing a standardized method and (ii) what has to be added to make the information useful and transferable to all relevant actors (e.g. policy-makers, farmers, consumers etc.).

With these questions in mind, this paper not only looks at(i) the development of the farmers' share for Austria mainly forthe period 1995 to 2013 using two slightly different methodologiesbut also (ii) compares the results with the developments in Germany, Switzerland and the United States. Finally, this paper outlines some prospective for further research.

MATERIAL AND METHODS

Comparing the total expenditure for food to the value of the agricultural commodities contained within it can be done using the sector-based (sometimes referred to as 'global') approach or by product specific calculations (e.g. Observatoire de la formation des prix et des marges des produits alimentaires[1]). This paper deals with twoglobal marketing margin approaches to calculating the Austrian farmers' share. The marketing margin can be understood as the total value, added to the agricultural commodities by downstream market stages, i.e. processing, marketing, trading, etc., which is included in the expenditure for food consumption of the population within a year (Sinabell 2010). On the other hand, the farmers' share is the average share of the expenditure for food consumption of the population that is received by farmers for their agricultural commodities. This can be expressed in equations as follows:

Farmers' share in % = (total production value of agricultural inputs for food in mn€ / domestic consumers' total expenditure in mn€) * 100
Marketing margin in % = 100 – farmers' share in %

One calculation of the Austrian farmers' share for the period from 1995 to 2013 is based on the Agristat method (Giuliani 2015), whereasfrom 1971 onwards the Austrian Institute of Economic Research (WIFO) (Sinabell 2016) has also provided such data but not over a continuous time series. Regardless of method, the

[1]see https://observatoire-prixmarges.franceagrimer.fr/Pages/default.aspx for most recent reports (accessed 25 Aug 2016)

development of the margin and the farmers' share can also be illustrated both in absolute terms and as an index.Both methods have a great deal in common, but there are still important differences. In both cases the calculations rely on primary data as shown in Table 1. The aim is to calculate the Austrian marketing margin or farmers' share for food without beverages, however, the WIFO method includes wine. One main difference, therefore, is in the definition of agrarian commodities for food. Neither calculation takes into account agricultural subsidies. The value added tax (VAT) is not considered in the Agristat method. The VAT producers or consumers have to pay is part of the marketing margin according to the WIFO method.Generally, the main difference is that the food consumption according to the WIFO method is based on the supply balances, whereas the production value of domestic agricultural products is the source forthe Agristat calculation ofthe commodities for food consumption adjusted by import and export values.
In light of these differences in the two methods of calculation applied, currently the usefulness of the information very much depends on perspective. One might surmise that the Agristat method be more useful to deal with issues from the farmers' side and the WIFO method be more relevant to the consumer side (see Table 1).

Table 1. Data comparison

Data	Method	
	Agristat	WIFO
Annual production value of domestic agricultural commodities for food production (Economic Accounts for Agriculture, Statistics Austria 2016a)	x	
Farm prices (Economic Accounts for Agriculture, Statistics Austria 2016a)		x
Annual imports and exports of food commodities and products (foreign trade database, Statistics Austria 2016b)	x	
Annual domestic expenditure made by end consumers for food (National accounts, Statistics Austria 2016c)	x	x
Expenditure for eating out (Input-Output-statistics, Statistics Austria 2016d)	x	x
Self sufficiency ratio, domestic food production (Supply balances, Statistics Austria 2016e)		x
VAT		x

**Source: own elaboration according to Giuliani 2015; Sinabell 2010*

The problem is therefore, again, the issue of how to package sensibly the information in a homogenous form for policy-makers and further relevant stakeholders in the sense of sustainable agricultural development. The marketing margin illustrates the value added to agricultural commodities on the way from the farm gate to the consumer. However, no information is provided on whether the actual incomes in the agricultural sector or the downstream processing, trading and marketing sectors have increased or decreased or whether the returns at each market stage are economically justified, adequate or fair.

Furthermore, these calculations give no details on the cost categories or the shares at the different stages in the value chain beyond the farm gate (Giuliani, 2015). When interpreting the results, one has to bear in mind that the calculations show developments and not their causes. Principally, the calculations merely highlight developments over time. This notwithstanding, they call for continuous updating, a possible expansion of the time series and a harmonisation of the calculation methods. For in-depth knowledge further analysis is required. In concrete terms, an analysis of the results must consider the respective frame conditions such as (i) the support from the government, (ii) the (average) degree of processing of the products, (iii) the consumers' habits and needs as well as (iv) the diversification of the range of products and so on.

RESULTS AND DISCUSSION

Farmers' share of food in Austria

The comparison of marketing margins over longer periods shows that the gap between the shares farmers get compared to others is widening. Figure 1 reveals a continuous growth of the marketing margins for the period 1971 to 2013. For the most part, this growth has been progressive.

On the one hand the WIFO method displays a continuous decline in the farmers' share from 41.7% in 1971 over 24.8% in 1995 to 27.1% in 2013. On the other hand the Agristat method shows a drop from 29.5% in 1995 to 22.2% in 2013. The discrepancies in the values given can be attributed to the fact that the value of food consumed, ie. value of food produced in Austria assessed using farm prices according to the WIFO method shows stronger annual fluctuations than the total production value of foodstuff of the Agristat method. This is due to the farm prices used. Overall, however, the current trend shows that with increased national prosperity the farmers' share declines and the marketing margin becomes larger.

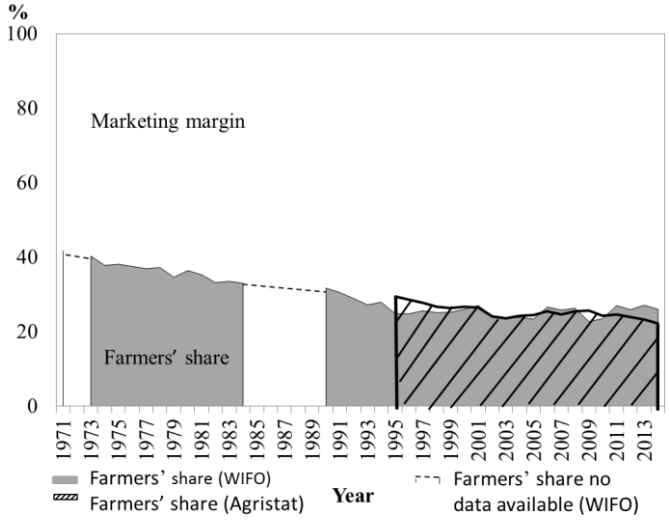

Figure 1. Long-term development of the farmers' share and marketing margin in Austria (in percent from 1971 to 2013) (own calculation; Sinabell 2016).

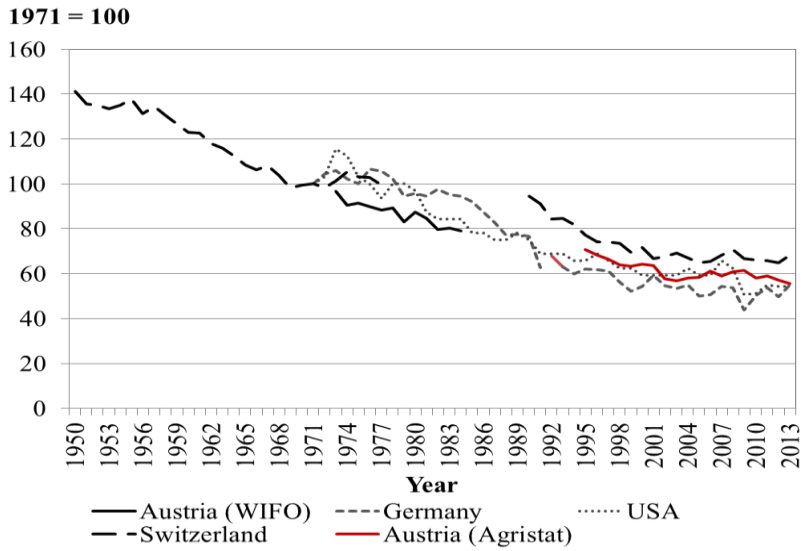

Figure 2. Development of the farmers' share in Austria, Switzerland, Germany and the United States (Index basis 1971 = 100 from 1950 to 2013) (own visualisation according to Elitzak 2016; Giuliani 2015; Senti in Schneider 1980; USDA 2015; Wendt, Peter 2014).

International developments

In Germany (Wendt and Peter, 2014), Switzerland (Giuliani 2015) as well as in the United States (Elitzak 2016; USDA 2015) the farmers' share in the food sector has been analysed for decades. While the method, the levels of margins and their development are different, the direction of change in the margins is similar and definitely shows a trend – as shown in Figure 4. Apart from the common trend a closer look reveals that the coverage of products, the treatment of taxes and subsidies and the sources of information are very different in the various countries. While other statistics, like the economic accounts of agriculture are based on internationally standardized procedures, the methods to calculate farmers' shares are vary from country to country.In these countries the marketing margin, as shown by available data, has grown relatively continuously and so the farmers' share of food expenditure has correspondingly decreased every year since 1971 and since 1950 in Switzerland, respectively. It would appear, however, that this trend is levelling off to a certain extent. The causes are well documented (Department for the Environment and Rural Affairs 2004; König, Senti 2001; McCorriston 2015, Schneider 1980). The international evidence is, of cause, very interesting for the purposes of forecasting.Currently, in-depth comparisons between these countries are hardly possible due mainly to the differences in the statistical data and the methods applied to obtain the results. Nevertheless, one can claim that the countries mentioned have a similar level of prosperity and consumption patterns as well as structure and development of consumer expenditure for food.

CONCLUSION

The developments of farmers' share frequently arouse public interest. The concept of farmers' share, or marketing margin, was developed to measure the difference between consumer expenditure for food and an associated farm value. Specifically, the marketing margin is calculated by subtracting the net farm value equivalent from food sold at retail price. These price spreads have been examined on many occasions, often in response to concern of the sharp movements in farm-gate or retail prices. Coupled with additional statistics, this information could give insight into trends in the actual formation of marketing margins and the farmers' share and thus contribute to more objectivity when discussing the agricultural and food price formation process. However, as calculations are based on estimates, additional information – such as production and marketing structure, support from the government, (average) degree of processing of the products, the consumers' habits and needs, diversification of the range of products – is needed to draw conclusions about the efficiency and performance of markets in the price formation process. The current results only reveal considerable growth in the marketing margins and a corresponding decline in the farmers' share. Since the nature and cause of these changes are not easy to identify, there is clearly room for additional empirical analysis. Important fields of research include in-depth trans-national comparisons and rigorous econometric or statistical analyses on the: (i) possible explanation behind both the extent of the price divergence between producer and consumer prices and its recent development; (ii) estimation of vertical price alterations from farm to retail level to test whether there is empirical evidence of the use of market power in the food chain, (iii) understanding of market drivers, and hence the economic well-being of producers operating in today's marketplace and (iv) development of cost elements or the profits of the various actors in the value chain. These all together, when properly applied could contribute to a holistic monitoring tool on trends, customs and drivers within the value chain based on agricultural produce. Furthermore, combining it with other instruments will potentially provide a viable tool for political decision making and stakeholder awareness.

REFERENCES

Department for the Environment and Rural Affairs (2004). Investigation of the determinants of farm-retail price spreads. London Economics. http://londoneconomics.co.uk/blog/publication/ investigation-of-the-determinants-of-farm-retail-price-spreads/[17 Jun 2016].

El Benni, N., Hediger, W. (2014). Wettbewerbsfähigkeit Landwirtschaft – Nachgelagerte Industrien. Untersuchung zuhanden des Bundesamtes für Landwirtschaft (BLW), Zentrum für Wirtschaftspolitische Forschung (ZWF), HTW Chur, Juli 2014 [Competitive agriculture–Downstream industries].

Elitzak, H. (2016). Re: Historical farm share data from the Marketing Bill series 1929 to 2008. *E*mail from 17 Feb 2016.

Elitzak, H. (1997). Food Cost Review, 1996, U.S. Department of Agriculture, Economic Research Service, AER-761.

Giuliani, S. (2015). Der Anteil der Landwirtschaft am Konsumentenfranken. Methode. Schweizer Bauernbund Agristat [Farmers' share from each Swiss franc spent on food].

McCorriston, S. (2015). Food Price Dynamics and Price Adjustment in the EU. Oxford University Press.

Orgen, K. (1956). The farmers' share: Three measurements. Agricultural Economic Research, vol. 8, pp. 43-50.

Schneider, M. (1980). Verbrauch von Nahrungsmitteln und Getränken und Anteil der Landwirtschaft an den Ernährungsausgaben. Studie im Auftrag des Bundesministeriums für Land- und Forstwirtschaft. Österreichisches Institut für Wirtschaftsforschung. Wien [Consumption of food and drinks as well as farmers' share on food expenditures]

Sinabell, F. (2016). Re: Marketing margin calculation by Austrian Institute of Economic Research. Email from 9 Feb 2016.

Sinabell, F. (2010). Marktspannen und Erzeugeranteil an den Ausgaben für Nahrungsmittel unter besonderer Berücksichtigung von Milchprodukten. Studie des Österreichischen Instituts für Wirtschaftsforschung im Auftrag des Bundesministeriums für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, Wien [Marketing margin and farmers' share of food expenditure with particular focus on dairy products].

Statistics Austria (2016a). Economic Accounts for Agriculture. http://www.statistik.at/web_de/statistiken/wirtschaft/land_und_forstwirtschaft/gesamtrechnung/index.html [21 Jun 2016].

Statistics Austria (2016b). Foreign trade. http://www.statistik.at/web_de/services/wirtschaftsatlas_oesterreich/aussenhandel/index.html [21 Jun 2016].

Statistics Austria (2016c). Expenditure made by end consumers for food (nominal private consumption). National account. http://www.statistik.at/web_de/statistiken/wirtschaft/volkswirtschaftliche_gesamtrechnungen/bruttoinlandsprodukt_und_hauptaggregate/jahresdaten/index.html [21 Jun 2016].

Statistics Austria (2016d). Input-Output-Statistics. http://www.statistik.at/web_de/statistiken/wirtschaft/volkswirtschaftliche_gesamtrechnungen/input-output-statistik/index.html [21 Jun 2016].

Statistics Austria (2016e). Supply balances. http://www.statistik.at/web_de/statistiken/wirtschaft/land_und_forstwirtschaft/preise_bilanzen/versorgungsbilanzen/index.html [7 Jul 2016].

USDA (2015). Food Dollar Series. http://www.ers.usda.gov/data-products/food-dollar-series.aspx[11 May 2016].

Wendt, H., Peter, G. (2014). Gestiegene Erzeugerpreise für tierische Erzeugnisse treiben den Erzeugeranteil nach oben. Anteilsberechnung 2013. Thünen-Institut für Marktanalyse. Braunschweig/Germany [Higher producer prices for livestock products drive up the farmers' share].

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URBAN AGRICULTURE AS A PLANNING TOOL FOR ACHIEVING SUSTAINABLE URBAN DEVELOPMENT

Maya MITEVA

Department of Urban Planning, Faculty of Architecture, University of Architecture, Civil Engineering and Geodesy, Sofia, Bulgaria
Corresponding author: mayamiteva@gmail.com

ABSTRACT

Diminishing population process causes new spatial changes in the urban environment. Local authorities of depopulated settlements are facing the challenge of managing the existing urban infrastructure with decreasing consumers and tax incomes. The question is raised about how to ensure high quality of life of the remaining residents. According to the United Nations (UN, 2015), Bulgaria will lose 27.9 % of its population until 2050, as a comparison Japanese population will decline by 15.1%. The projections lead towards specific changes in urban planning practices and shift the focus from city development to city shrinkage. Hence, the implementation of urban agriculture as a tool for creating viable cities and sustainable local communities could be expected to ensure food security, social inclusion and employment. The study aim is to propose responses to shrinking phenomena and to discuss benefits of urban agriculture development for binding local communities. In order to achieve this aim, three objectives are set: (i) to identify research and practical experience in the field of urban agriculture in Japan; (ii) to analyse the Japanese local authorities approach in facing declining and aging population consequences; and (iii) to discuss its transferability to Bulgarian planning practice. Started in 2010 in Kashiwa city, Japan, Kashiniwa program is innovative local governance system for tackling vacant lots in shrinking cities. The case study method was chosen for researching the planning approaches developed and implemented in Japan, such as urban agricultural planning method for establishing and maintaining green open spaces and building sustainable local community.

Keywords: *urban agriculture, shrinking city, spatial planning, sustainable urban development.*

INTRODUCTION

Shrinking cities are characterised by decreasing and aging population. Problems in the process of shrinkage appear due to low population density (Oswalt and Türetken, 2008). Local communities lose their old structure and need to be transformed into new ones. The observed decreasing municipal revenues and the

loss of community cause low efficiency of the technical, transport and social infrastructure; and environmental degradation. Furthermore, urban structure which is "perforated" by abandoned and unused urban places, left from past activity or due to unexecuted development, requires new spatial planning approach (Dimitrova and Scurrell, 2002). This paper sets out to outline the arguments for achieving sustainable urban development by a participatory planning approach for the implementation of urban agriculture in cities with diminishing population. Urban and peri-urban agricultural activities add values of "rebuilding productive urban landscapes and enhancing community cohesion" (Yokohari and Bolthouse, 2011).

Japan faced shrinking cities phenomena consequences after the total population peak in the country was reached in mid-2000s., Municipalities with declining population then developed local strategies to tackle vacant lots through "temporary utilization and acquisition" by citizens (Yamada et al., 2016). For instance, Kashiniwa program is a local governance method for tackling vacant lots in cities with declining and aging population. The initiative represents land-matching system between landowners and citizens to lease land for public activities and use the vacant lots as green open spaces. By changing land-use policy the municipality provides short term usage of vacant lots by local groups and ensures city greenery, food consciousness and community resilience.

MATERIALS AND METHODS

The article discussed the linkage of four aspects, namely urban agriculture, shrinking cities, spatial planning, and sustainable development, and their consequences: (1) tendency of growing city boundaries despite shrinking economy and social activities, causing environmental pressure (Dimitrova and Scurrell, 2002); (2) achieving environmental balance through the establishment of green open spaces on "perforated" urban areas; (3) reduction of induced local tax revenues by establishing spatial planning policy using local people resources for maintenance of public spaces and vacant lots; (4) such an approach is development of urban agriculture; and ensures (5) employment; (6) ecosystem services exploitation; (7) biodiversity conservation; (8) building resilient local communities. Japanese urban planning policy addressing depopulation and aging society implemented the strategies of compact city, smart city and low carbon city development. The Compact city concept focuses on dense and proximate development patterns, urban areas linked by public transport systems, and accessibility of local services and jobs (OECD, 2012). The concept of low carbon compact city structure relies on the compilation of "transport/urban structure", "energy" and "greenery" (MLITT, 2011). Particularly, greenery is implemented through the policy of conserving and creating green areas in collaboration with citizens, etc. The study focused on urban greening and more specifically on urban agriculture practices (community gardens).

The so called "Strategic choice approach" (Friend and Hickling, 2005) has been developed and applied for defining the linkage between different decisions made in

the planning field, in the process of integrated problem solving and achieving a balance of decisions to accomplish sustainable urban development. Therefore, the sustainability of choices is influence by selection between 1) scope defined through focused or synoptic actions; 2) complexity addressed through simplifying or elaborating actions; 3) conflict elaborated through reactive or interactive actions; 4) uncertainty elaborated through reducing or accommodating actions; 5) progress elaborated through exploratory or decisive actions.

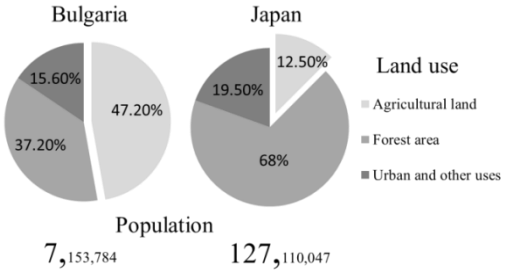

Figure 1. Land and human resources in Bulgaria and Japan

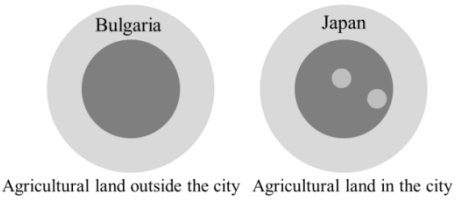

Figure 2. Location of farm land inBulgaria and Japan

Alongside the differentproportion of urban and rural land and the population number and density in Bulgaria and Japan (Figure 1), a strong border between urban and rural areas is observed in Bulgaria, in contrast with the mixed land use in Japan. Urban agriculture in Bulgaria is represented by individual houses' backyard gardens and developing "second homes" in the city periphery. Farm land can be found in the Japanese cities (Figure 2).

Kashiwa (Japan) is selected for a case study as an innovative municipal approach for tackling declining and aging population and the consequences of the shrinkage tendency. Kashiwa city subsidises citizen's group activities through Kashiniwa program (Kashiniwa in Japanese means "renting a garden"). The program provides an opportunity to create open green public spaces initiated by the local residents through the establishment of community gardens on vacant public and private lots or open gardens on citizens' backyards (UT, 2015). For the purposes of the current study the Japanese urban planning system and policy background, the program website content and the research documents related with the Kashiniwa system were considered. The study findings are based on exploring and analysing: 1) Kashiwa City strategic planning framework corresponding to the aging and shrinking society in the concept of low carbon city development; 2) semi-structured interviews with the creators of the program; 3) field visit observation and analyse vacant lots characteristics and urban agriculture activities in the area.

RESULTS AND DISCUSSION

Kashiniwa program has established a platform with database containing vacant lots information and rent conditions submitted by landowners and also citizen' groups request. The data is available on the municipal website and actors have opportunities to find all needed information about the procedure. The role of the government scheme is maintaining updated database and ensure secure negotiations and control in signing contract between actors. Kashiniwa scheme is structured by Kashiniwa Joho Bank (Rental Garden Databank) and Kashiniwa Kokai (Public Rental Garden). Kashiniwa Joho Bank (Figure 3) plays a role of intermediary between landowners and citizens' groups. Kashiniwa Kokai (Public Rental Gardens) regulates procedures for leasing land to be used for open green space.

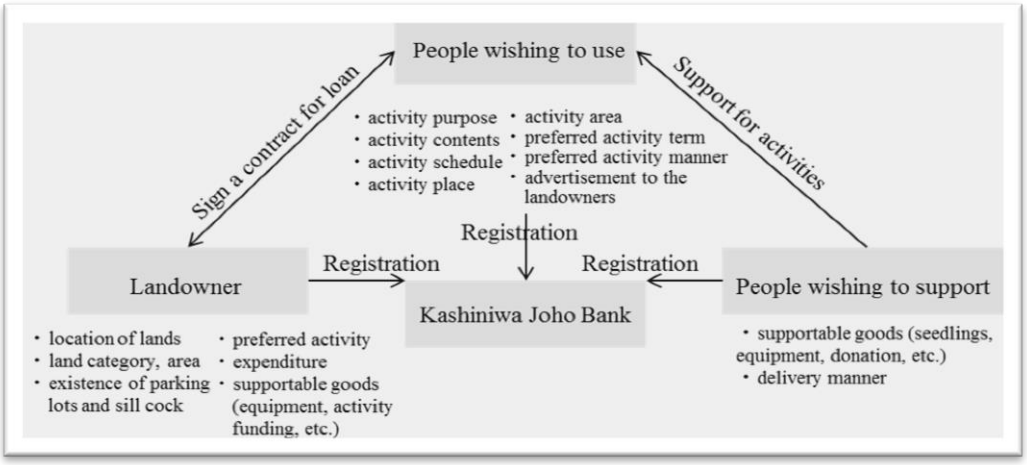

Figure 3. Kashiniwa Joho Bank – a conceptual model adopted MLITT, 2013and UT, 2015

Kashiniwa system (Table 1) could be regarded as a "win-win" situation - the owners win reduced taxes; community wins the opportunity to grow food and develop social capital; the local authority wins new function for unused and degraded areas.

The program was thenevaluated based on the concept of strategic choices to determine whether the decision is linked to other levels of decision making in integrated spatial planning process. Strategic choice approach (Table 2) is considered in five dimensions: scope, complexity, conflict, uncertainty, progress (Friend and Hickling, 2005).

Table 1. Advantages of Kashiniwa program in shrinking context

Consequences of shrinking	Benefits of urban agriculture	Inputs of the program
Lack of human resources	Using citizen' resources for maintaining land and participation activities	Lifelong learning and building social capital
High cost of city management	Exploitation of ecosystem services	Vacant land management
Low quality of life	Building sustainable local community	Social participation and community activities
Low environmental quality	Creating green infrastructure	Greening and using empty space for growing food

After preliminary literature review about shrinking consequences and urban agriculture benefits, including Kashiniwa program evaluation, the article established the program contribution to the sustainable use of human resources by "shift from government-led management to government-mediated management" (UT, 2015) through vacant lots management. Further improving the quality of life and environmental quality through greening and growing food on empty spaces is achieved by the collaboration of all local actors: local authority, landowners, citizens, civic groups, NGOs, etc.

Table 2. Strategic choice approach applied to Kashiniwa program

Strategic choice dimensions	Kashiniwa program
Scope	Ensured provisional open space on vacant lots (public or private ownership) for citizen's group activities (Terada et al., 2012)
Complexity	Local authority initiative for establishment of database insurance and mediation between stakeholders; land preparation for urban agriculture
Conflict	Insufficient level of consensus (land usage and invested effort) between stakeholders (Terada et al., 2012)
Uncertainty	Lost opportunities during the contract period (landowners); and future actions after the expiry of the contract period (citizens group)
Progress	Increasing number of gardens

Alternative choices for decision making process should investigate and evaluate potential benefits of developed strategy. According to Kashiwa case, preliminary steps for decision making are: investigation about vacant lots (number, location, ownership); intention of owners of vacant lots for future use of their land, as a function (parking, garden) and land ownership, and usage (MLITT, 2013).
For the study purpose, PETUS (Practical Evaluation Tools for Urban Sustainability) research project (Dimitrova, 2007) assessment method is adapted. Criteria defining efficiency of the evaluating management policies at local level are set out as major in theoretical terms - credibility, salience, and legitimacy (EEA, 2001). Further, credibility refers to scientific believability; salience ensures

intelligibility for all participants; and legitimacy is a measure for acceptability for all participants. Hence, concerning the main discussed aspects; urban agriculture, shrinking cities, spatial planning and sustainable development; the analysis framework of Kashiniwa program (Table 3) is outlined (Dimitrova, 2009).

Table 3. Local program assessment approach: Kashiniwa program, Kashiwa, Japan

Criteria / *Aspects*	**Credibility** (scientific believability)	**Salience** (understandability for all participants)	**Legitimacy** (acceptability for all participants)
Sustainable development	Low carbon society; Compact cities	Local authority as initiator and mediator of the program (contract negotiation)	Coherence with national policies for food security and production; Ensuring economic stability; social inclusiveness; and environmental protection
Urban agriculture	Community garden	Combining traditions and modern tendency; Establishing green open spaces; Improving quality of life	Designing plot rules; Regulation of participation
Spatial planning	Vacant lots	Investigation of ownership; and willingness: to lease the land, to use the land, to support	Land use management; Urban planning regulation: temporary land use, mobile facilities
Shrinking cities	Declining and aging population	Using citizen resources; Creating green infrastructure; Ensuring employment; Causing sense of significance in elderly	Building resilient local communities: tax reduction, food production, building social capital, maintaining vacant lots, etc.

Theimplementation of the investigated system in the current Bulgarian context would face several barriers and would require relevant changes: (1) establishment of national policy for promoting local food production and consumption, as well as building food consciousness through food education and generating farmers' market regulations; (2) creating sustainable development awareness on spatial measures for planning shrinking cities; (3) defining the function (urban planning zones), past activities (military reform; deindustrialization; unrealized urban

planning projects) and ownership of "perforated" urban fabric; (4) identifying citizens' awareness, information access and interest (individual plots, community gardens) for developing urban agriculture; (5) establishment of financial instrument for tax reduction of landowners and local groups' subsidy; (6) ensuring political and expert understanding for the significance and effect of initiated and developed urban agricultural program.

CONCLUSION

Cities'declining population trends require preliminary investigation for better problem understanding and linking the strategical choices to other taken local authorities planning actions. The transfer of Kashiniwa methodological approach to Bulgarian and other cases would provide a solution for complex issues - family income, vacant lots management, building sustainable local communities. That would however require a comprehensive consideration of cultural and spatial contexts. Urban agricultural development requires public policy focused on "developing sustainable food systems and promoting healthy diets" (Forster et al., 2015) and environmental protection awareness.Furthermore, achieving integrated urban planning, and utilization of local resources for urban planning reforms in shrinking cities implies sufficient scientific research and studies on local expert capacity.

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REFERENCES

Dimitrova, E., B. Scurrell (2002). "Shrinking" European Cities: Challenges to sustainable urban development. Jubilee Anniual, University of Architecture, Civil Engineering and Geodesy, Sofia, pp. 177-186 (In Bulgarian).

Dimitrova, E. (2007). Testing PETUS: Expectations and outcomes of the Theory-Practice Dialogue on Urban Sustainability. In Indoor and Built Environment. International Society of the Built Environment, Vol. 16, Iss. 3, pp. 216-225.

Dimitrova, E. (2009). Ecotourism: integrated approaches to spatial planning and management. Engineering Sciences, Vol.3, pp. 77-94 (In Bulgarian).

EEA (European Environment Agency) (2001). Designing effective assessments: The role of participation, science and governance, and focus. Environmental issue report No26, Website:http://www.unep.org/DEWA/water/MarineAssessment/reports/germany_report/EEA-report-issue_26.pdf. Accessed 28/06/2016.

Forster, Th., Egal, Fl., Renting, H., Dubbeling, M., and Getz Escudero, A (2015). Milan Urban Food Policy Pact. Selected Good Practices from Cities. Feltrinelli.

Friend, J., A. Hickling (2005). Planning under Pressure. The strategic Choice Approach. Third edition, Elsevier Butterworth-Heinemann.

MLITT (Ministry of Land, Infrastructure, Transport and Tourism) (2011). Low Carbon City Development Guidance. Ministry of Land, Infrastructure, Transport and Tourism, Website: http://www.mlit.go.jp/common/000996971.pdf. Accessed 28/06/2016.

MLITT (Ministry of Land, Infrastructure, Transport and Tourism) (2013). Trends Concerning Land in FY2012, Basic Measures in Relation to Land in FY2013. Website: http://tochi.mlit.go.jp/english/wp-content/uploads/2014/07/Trends-Concerning-Land-in-FY2012-Basic-Measures-in-Relation-to-Land-in-FY2013.pdf. Accessed 29/06/2016.

OECD (2012). Compact City Policies: A Comparative Assessment. OECD Green Growth Studies, OECD Publishing.

Oswalt, P., & Türetken, F. (2008). Shrinking Cities: Complete Works 3 Japan. Project Office Philipp Oswalt, Berlin.

Terada, T., Amemiya, M., Hosoe, M., Yokohari, M., & Asami, Y. (2012).A study of the maintenance and management scheme for provisional open spaces. Journal of The Japanese Institute of Landscape Architecture, Vol. 75(5), pp. 651–654 (In Japanese).

UN (United Nations) (2015). World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. Department of Economic and Social Affairs, Population Division. Working Paper No. ESA/P/WP.241.

UT (University of Tokyo) (2015). Kashiwa City, Mitsubishi Research Institute, Inc., 2015, Urban Reformation Program for Realization of a "Bright" Low-Carbon Society: Final report. Tokyo; Website: http://low-carbon.k.u-tokyo.ac.jp/news_15_05_26EN.html. Accessed 28/06/2016.

Yamada, C., Terada, T., Tanaka, T., & Yokohari, M. (2016). Directions for vacant lot management in the outer suburbs of the Tokyo Metropolitan Region. Urban and Regional Planning Review, Vol. 3, pp. 66–84.

Yokohari, M., & Bolthouse, J. (2011). Planning for the slow lane: the need to restore working greenspaces in maturing contexts. Landscape and Urban Planning, Vol. 100(4), pp.421-424.

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GROWTH OF ORGANIC FOOD INDUSTRY IN INDIA

Varghese MANALOOR[1]*, Divyanshu SRIVASTAVA[2], Shahidul ISLAM[3]

[1]University of Alberta, Canada
[2]IIT Kharagpur, India and UARE 2016 intern, University of Alberta, Canada
[3]Grant MacEwan University, Canada
*Corresponding author: manaloor@ualberta.ca

ABSTRACT

The organic food industry in India is in the early stages of growth. Higher disposable income and greater health awareness have resulted in an increased domestic demand for organic food. There is huge premium in selling organic products, not only to export markets but also to affluent, health conscious domestic consumers. India is endowed with an abundance of labour and has diverse agro-climatic region that is well suited to year round agriculture. It still has strong traditional agricultural practices. Can India make use of this comparative advantage to introduce sustainable agriculture practices and at the same time improve incomes of small and marginal farmers?On the supply side, small and marginal farmers realize that there is an opportunity to get higher net incomes even if yields are low in organic agriculture. This is because the price of pesticides and chemicals has increased significantly over the last few decades resulting in a significant increase in the cost of production. Organic farming cost could be 50% to 60% less when compared to inorganic farming practices.In addition to domestic demand side, globalized markets provide significant opportunities for Indian agriculture to capture a larger share of the global demand for organic food. This paper analyzes the growth of the organic food industry in relation to domestic and export demand. We also look at the supply side to determine if organic farming and sustainable agricultural practices could help improve farmers' income. Finally, this paper analyses existing policy framework towards organic agriculture and how small and marginal farmers could possibly benefit in this niche market.

Keywords: *food supply, organics, sustainable agriculture, India.*

INTRODUCTION

The organic food industry has experienced astonishing growth in the past few years. Still, the total percentage of agricultural land in the world that is certified organic still remains around 1% at 43.16 million hectares. This is almost four times the area covered under organic agriculture in 1999 recorded at 11 million hectares (Lernoud and Willer 2016). A large proportion (approximately 90%) of the total organic food and drink sales take place in the developed countries of North

America and the European Union (EU). There is a huge growth potential in developing countries to increase organic production owing to the largely prevalent traditional farming techniques which are in accord with organic agricultural practices. Strong institutional support is required to further push the organic industry from a small niche market into a mainstream agricultural industry.

India has a total agricultural land of 143 million hectares, out of which only 5.2 million hectares (3.64%) of land is under organic certification. The agricultural sector in India is characterized by a large proportion of households (85%) possessing less than 2ha of land (Agricultural Census Division 2014). Only 36% of India's land uses irrigation systems, while the rest is rain-fed (Directorate of Economics and Statistics 2012-13). The rain-fed area presents many opportunities to improve the socio-economic status of the farmers by adopting organic farming methods. The state of Sikkim has the highest percentage (54.66%) followed by Madhya Pradesh (16.80%) (Lok Sabha 2014). The government has made constant efforts towards improving the institutional support to growers by introducing several policies and programmes. The present paper aims to examine the possible supply side challenges and ways to overcome them in the context of Indian agriculture.

Looking at the demand side of organic food markets, there are several factors that affect the consumer choices like certification of the products, perceived health benefits and prices of organic food. However, as noticed earlier, demand for organic food and drink is heavily concentrated in the EU and North America. The market in India is very small, estimated at around USD 100 million as compared to the United States market, valued at around USD 36 billion (Lernoud and Willer 2016)(Technopak 2012). Due to such a large foreign market for organic produce, a significant proportion of organic goods are exported. However, there is a rising growth in demand of organic produce in India due to the increasing income of the population and growing health concerns. The paper tries to look at the various aspects of demand and trade of organic food in India.

Organic agriculture is often looked upon as a sustainable alternative to chemical farming. However, there is a debate between food security and environmental sustainability aspects. This paper also looks to analyze how the sustainability of small farms involved in organic agriculture.

MATERIALS AND METHODS

This paper is based on review of secondary data, official reports and previous studies relevant to the organic food industry. The majority of reviewed content is based on studies conducted in India while a few concerning Canada were chosen for comparisons. Most of the data collected is sourced from various Ministries of the Government of India and international organizations such as International Federation of Organic Agriculture Movements (IFOAM, 2016).

Estimates for costs of cultivation for paddy and income of paddy farmers in Andhra Pradesh have been taken from a previous study to conduct further analysis

(Sudheer 2013). Price data for several brands of organic and non-organic food had been taken from an NGO, Gurgaon Moms, for the aforementioned purpose.

RESULTS AND DISCUSSION

Supply Side

There exist several challenges in the supply dimension of the organic food industry. Firstly, the yield of the two types of systems depends upon the source of water; irrigation channels or rain. Previous studies have shown that organic agriculture has potential to increase crop yield in areas with traditional rain-fed agriculture and in drought conditions. In intensive farming systems, conventional crop yields outperform the organic yields (Ramesh, Singh and Rao 2005) (Stanhill 1990). Some studies point to a reduction in yield for a small period (around 4 years) for conventional systems that are in conversion to organic, but yields are comparable thereafter due to developed soil fertility (Neera, Katano and Hasegawa 1999). Other studies did not have such an effect observed (Stanhill 1990). Since a majority of India's arable land is rain-fed, adoption of organic agriculture may enhance the productivity of those soils, leading to a higher food supply in general, and specifically, a higher supply of organic food. The farmers' incomes will increase as a result of increased produce from their lands.

Secondly, comparing the costs of production between the two systems offer a few insights. Many of traditional farming practices, which have been developed over centuries, are concurrent with organic farming practices. Both these methods tend to employ as few chemicals as possible and make farming sustainable (Narayan 2005). Organic agriculture has lower variable costs, owing to reduced or non-use of industrially produced inputs like fertilizers and pesticides, in favour of farm-derived inputs like manure (Chandrashekar 2010). However, organic agriculture requires more labour than conventional systems, increasing the labour costs (Narayan 2005). Many studies have shown that costs in total reduce as a result of adoption of organic farming (Narayan 2005) (Sudheer 2013).

Finally, marketing plays a big role in the supply of organic food. The organic food market in India is still a very niche market with total organic production of 1.35 million MT as compared to total agricultural production of 253 million MT. Around 80% of the total organic consumption in India occurs in the cities classified as metros or mini-metros (Technopak 2012). But most of the production occurs in the rural areas far off from these cities. Marketing costs are high in these cases due to limited market access. One possible solution to this problem is introduction of contract farming. According to FAO, "contract farming involves an agreement between farmers and processing/marketing firms for production and supply of agricultural products under forward agreements, frequently at predetermined prices". The advantage of contract farming is that supply chains are more integrated and the products reach a larger segment of the market. The buyer firms are better at advertising and marketing than farmers, which benefits all involved parties. The consumers get access to larger amounts/varieties of products, while the buyer firm and the farmers face lower supply and price risks respectively.

A previous study conducted in the state of Andhra Pradesh in 2010-11 on 250 paddy farmers; 150 organic and 100 chemical; estimated the costs and incomes of the farmers (Sudheer 2013). Table 1 shows the estimates of costs of cultivation, revenues and gross incomes at different premiums for rice paddy. The study assumed comparable yields on both types of farms. For simplicity, this paper assumes the yield from the two types of farms are identical. Currently, the organic products fetch lower prices than their conventional counterparts. An interesting observation is that, even though per unit price of organic food to the farmers is lower, they are getting higher gross incomes, due to lesser costs of organic food. The study found that none of the organic farmers had formal certification, leading to lower prices of organic produce. Market price of different brands of organic and non-organic rice are shown in Table 2 (Gurgaon Moms 2012). Organic products yield at least 20% premiums but farmers do not gain much due to lack of certification.

Table 1. Cost and Revenues of Rice Paddy farmers

	Organic*	Conventional*
Cost of Cultivation per acre	21,549	23,989
Gross Income per acre	30,221	28,717
Revenue per acre	51,770	52,706
Current Premium	-1.78%	
Revenue at 20% premium	63,247.2	
Gross Income at 20% Premium	41,698.2	
Increase in Profits	38.0%	
Certification Costs	20,000 to 30,000	

Source: Sudheer, 2013
**All figures except percentages in INR*

If proper marketing and certification can be gained, the profits will go up by 38%. But certification has its own costs as well. Certification costs are between INR 20,000-INR 30,000 depending upon farm size. This is enough to lower the profits overall. Therefore, farmers will benefit from organic farming if there is a cheaper method of certification or with financial assistance schemes such as PKVY wherein the government assists the farmers in getting certification. A cheaper alternative in the form of Participatory Guarantee System exists, which is a decentralized way of inspection of farms leading to lower costs (IFOAM n.d.). India currently has 19,984 farmers in 954 farmer groups participating in PGS covering an area of about 23,482 ha which is less than 1% of the total organic area in India. More areas need to be covered by PGS to improve the economic status of organic farmers.

Table 2. Prices and premiums of different brands of organic vs non-organic rice

	Organic		Non-organic	Premium	
Market Price /kg	24 Letter Mantra	Navdanya		24 Letter Mantra	Navdanya
Basmati Premium Brown Rice	165	110	91	81.32%	20.88%
Basmati Premium White Rice	164	110	87	88.51%	26.44%

Source: Gurgaon Moms Website
**All figures except percentages in INR*

Demand side

In India, there has been a huge growth in the consumption of organic products in the past decade. In 2002-03, the total production was estimated around 14,000 MT with total organic exports of 11,925 MT (85%) (Garibay and Katke 2003). In 2015-16, the production was 1.35 million MT with exports of 0.26 million MT (19.26%)(APEDA n.d.). It suggests that the consumption of organic products in India has grown very rapidly. The compounded annual growth rate (CAGR) of production is 46.34% and that of exports is 29.44%. The domestic consumption has been growing faster than exports, expanding the domestic market. There are several reasons for this. First, most of India's land was already being farmed according to organic agricultural practices, but it was only after proper certification standards were adopted in early 2000s that certification took place. This lead to a huge rise in certified organic consumption over such a short period. The consumption is 2002-03 was estimated at around 2075 MT whereas it crossed the 1 million MT mark in 2015-16 (Garibay and Katke 2003)(APEDA n.d.).

Most of the studies show that factors like health and environmental concerns and lack of chemicals in the food are increasing the demand. Other factors like higher price and lack of availability are hindrances to demand (Hughner et. al. 2007).

A survey of households in major Indian Cities found that only 17% of the households (175) were consumers of organic food. Among the consumers, the major percentage pointed out health concerns (82%) and lack of pesticides and other chemicals (58%) as the major reasons for buying organic food. The study also noted that the major problems faced by buyers was that organic products were priced higher (64%) and limited availability of organic products in the market (60%). However, not many consumers were concerned with the impact organic farming has on the environment (21%). Of the remaining non-users, only 5% had good knowledge about organic food, with the rest having little to no knowledge. The major reasons for not buying among non-consumers was found to be limited

availability (72%) and high prices (52%). The place of purchase for organic food in most cases was either a supermarket or a speciality food stores as opposed to local markets and stores for regular food. Majority of purchasers preferred branded products (58%) with a high preference for Indian brands (78%) (Technopak 2012).

The limited availability of organic products coupled with the fact that majority of sales is concentrated in larger cities shows that the supply chains of organic food from the farms to the domestic consumers are not very well established. There is a lack of knowledge about organic products leading to a low penetration amongst potential consumers. Information dissemination campaigns can help increase the demand and lead to development of the domestic market.

Trade, Sustainability and Food Security

The organic food trade in India is currently limited to only exports. Most of them are targeted towards US, Canada and EU (84.6%) (Lok Sabha 2011). These are the areas with very high demand concentration. The developed countries import both organic and regular food from developing countries. Therefore, the share of "clean agricultural goods" consumption is higher in developed countries. As a result, countries where organic food is produced are not enjoying the ecological benefits of growing organic food (Lernoud and Willer 2016). This hinders the sustainability of organic agriculture in developing countries. Domestic organic food markets need to develop to consume more and more of organic products to realize the benefits of growing organic food.

Organic agriculture is generally believed to be more environment-friendly than conventional farming methods. This is because of the fact that organic farming involves practices which do not use chemicals and also have lesser energy requirements (Pimentel et. al. 2005). Currently, agriculture forestry and other land use (AFOLU) contributes around 25% of total greenhouse gas emissions (US-EPA n.d.)**.**There is a rising concern about the emission levels and organic farming can be a way to mitigate pollution.

There is a debate between sustainability and the food security aspects of organic farming. In intensively farmed areas, organic agriculture decreases yield but in irrigated lands, both methods have similar yields. For these cases, conversion to organic while being more sustainable, poses a threat to food security. Majority of the lands in North America and Europe are intensively farmed. This creates several problems for conversion. There is little economic incentive to convert these lands. But in traditional areas, organic agriculture increases yield. This could help improve food security issues in the developing countries like India where majority of agricultural land is traditional. The supply of food is higher, improving food security.

CONCLUSION

A new approach is required to realize the immense potential of organic food industry in India. There are several challenges at every stage of the market which needs to be addressed in order to develop the industry further. At the production level, farmers face problems regarding certification of their products, due to very high certification costs. There have been policies introduced to assist the farmers financially for certification and also organize them into Participatory Guarantee Systems (PGS) which is a low-cost certification method. While these are very helpful measures, they are not widespread. More farmers need to be included under these schemes and organized into farmer groups which will help them get greater access to markets where their products are demanded. Further, if organized into bigger groups, agricultural inputs might be available to them at a cheaper costs due to bulk purchasing. This will reduce the production costs and increase the profit margin.

Small and marginal farmers find it difficult to get access to markets where their products fetch premiums. Most of the consumption is concentrated in a few cities currently, but production happens far away in the rural areas. Retail firms can help improve the market access for the farmers. Most of the organic consumers in India prefer branded products. This is an incentive for retail firms to improve the rural-urban linkages by purchasing organic produce from farmers and selling them in urban markets where the demand for organic produce is higher. There are many retailers but awareness is pretty low among farmers and consumers both. Partnerships between the government and private retailers could prove beneficial for both parties involved as well as the farmers. More farmers will have access to better markets and get higher price premiums, which will enable them to increase their incomes. Retailers will have access to a larger quantity and variety of organic produce driving up their profits. Increase in farm incomes arising out of organic agriculture would help government policy makers to focus attention on other needs of the rural communities. Going organic will also lead to agriculture that is more sustainable.

REFERENCES

Agricultural Census Division (2014). Agricultural Census 2010-11. All India Report on Number and Area of Operational Holdings. Department of Agriculture and Co-operation, Ministry of Agriculture, Government of India.

APEDA. n.d. Organic Products. Accessed May 26, 2016. http://apeda.gov.in/apedawebsite/organic/Organic_Products.htm.

Chandrashekar, H. M. (2010). "Changing scenario of organic farming in India: An OVERVIEW." International NGO Journal 5 (1): 34-39.

Directorate of Economics and Statistics(2012-13). Land Use Statistics at a glance. Dept of Agriculture, Co-operation and Farmer Welfare. Ministry of Agriculture and Farmers Welfare, Govt. of India.

Garibay Slavador V., Katke, J. (2003). Market Opoortunities and Challenges for Indian Organic Products. Research Institue of Organic Agriculture FiBL, CH-Frick.

Gurgaon Moms. 2012. Price Comparison – Organic food vs conventional food. July 13. Accessed June 1, 2016. http://gurgaonmoms.com/2012/07/13/price-comparison-organic-food-vs-conventional-food/.

Shaw, H. R., McDonagh, P.,Prothero, A., Shultz II, C. J., Stanton, J.(2007). "Who are organic food consumers? A compilation and review of why people purchase organic food." Journal of Consumer Behaviour 6: 94-110.

IFOAM. n.d. PARTICIPATORY GUARANTEE SYSTEMS (PGS). Accessed May 17, 2016. http://www.ifoam.bio/en/organic-policy-guarantee/participatory-guarantee-systems-pgs.

Lernoud, J., Willer, H.(2016). The World of Organic Agriculture. Statistics and Emerging Trends 2016. Bonn: Research Institute of Organic Agriculture (FiBL), Frick, and IFOAM - Organics International.

Sabha, L.(2014). "Reply to Lok Sabha unstarred question no. 3130." Annexure 1A.

Sabha, L(2011). "Reply to unstarred question no. 5368."

Narayan, S. (2005). Organic Farming in India : Relevance, Problems and Constraints. Mumbai: National Bank for Agriculture and Rural Development.

Preecha, N., Katano, M., Hasegawa, T.(1999). "Comparison of Rice Yield after Various Years of Cultivation by Natural Farming." Plant Production Science 2 (1): 58-65.

Pimentel, D., Hepperly, P., Hanson, J., Douds, D., Seidel, R. (2005). "Environmental, Energetic, and Economic Comparisons of Organic and Conventional Farming Systems." BioScience 55 (7): 573-582.

Ramesh, P., Singh, M., A. Subba Rao. (2005). "Organic farming: Its relevance to the Indian Context." Curent Science 88 (4): 561-568.

Stanhill, G. (1990). "The Comparative Productivity of Organic Agriculture." Agriculture, Ecosystems and Environment (Elsevier Science Publishers B.V.) 30: 1-26.

Sudheer, P. Sri Krishna (2013). "Economics of organic versus chemical farming for three crops in Andhra Pradesh, India." Journal of Organic Systems 8 (2): 36-49.

Technopak. (2012). "Organic Food Market in India." http://www.organic.moc.go.th/sites/default/files/attachments/market_abroad/2012_india_ota-food-report.pdf.

US-EPA. n.d. Global Greenhouse Gas Emissions Data. Accessed May 27, 2016. https://www3.epa.gov/climatechange/ghgemissions/global.html.

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THE USE OF UREASE INHIBITOR FERTILIZERS (AGROTAIN) AND THEIR EFFECT ON CEREAL CROPS AND COTTON YIELD

Kyriakos D. GIANNOULIS, Dimitrios BARTZIALIS, Elpiniki SKOUFOGIANNI, Nicholaos G. DANALATOS

University of Thessaly, Department of Agriculture, Crop Production & Rural Environment, Volos, Greece
*Corresponding author:kyriakos.giannoulis@gmail.com

ABSTRACT

Many commercial compounds exist that promise the increasing efficiency of urea fertilizers by inhibiting urease activity in soils. Such a compound gaining in commercial importance in the last decades is N-(n-butyl) thiophosphoric triamide, broadly known with its registered trade name of "Agrotain". In this paper, the effect of nitrogen fertilizer dressings using Agrotain versus conventional (urea) fertilizers was studied under field conditions. In particular, the effect of three different nitrogen dressings using conventional N-fertilizers and Agrotain was investigated on the growth and final yield of (rainfed) durum wheat, and (irrigated) maize and cotton, grown on a fertile clay loamy soil in Velestino (Thessaly plain) area in central Greece in the year 2015. It was demonstrated that all three crops fertilized Agrotain obtained greater chlorophyll contents and reached significantly higher biomass and grain yields comparing to the crops receiving traditional nitrogen fertilization, obviously due to the more effective nitrogen release and uptake by the crops.Therefore, application of urease inhibitor fertilizers, such as Agrotain, might reduce nitrogen application dressings, reduce N-losses and nitrification, and their introduction to existing crop rotations is highly advisable.

Keywords: *urea inhibitors, yield, chlorophyll, wheat, cotton, maize*.

INTRODUCTION

Nitrogen is an essential plant nutrient and key to maintaining higher yield production and worldwide economic viability of agricultural systems. Farmers apply different N fertilizers such as urea, ammonium nitrate, ammonium sulphate and potassium nitrate to increase yields. However, this increase in N use, with N-response efficiency reported to be between 33 and 50%, is contributing to higher worldwide N losses via NH_3 volatilization and NO_3^-leaching that impact air and water quality (Raun and Johnson, 1999; Howarth et al., 2002; Nosengo, 2003).

Nitrogen is a major essential element for plant growth and is limited in agricultural soils (Kawakami et al., 2013). For its high N content (460 g N kg^{-1}), urea has been used as the primary solid N-fertilizer in agricultural crop production (Soares et al.,

2012; Glibert et al., 2006). However, the increase of pH and surface soil NH_4^+ concentrations resulting from urea hydrolysis can exacerbate NH_3 emission. This can cause low Nitrogen Use Efficiency, especially in alkaline soil or soil with low sorption capacity, which limits the use of urea fertilizer in Europe (Sommer et al., 2004). Nitrogen loss by NH_3 emission not only brings about economic loss to farmers, but also detrimental effects to ecosystems and human health (Bremner, 1995). There is a need to improve the efficiency of urea-based fertilizers through new technologies and management approaches. One of the most promising approaches is to apply urea in combination with the urease inhibitor (N-(n-butyl) thiophosphoric triamide, nBTPT or NBPT) at low concentrations ranging from 0.01 to 0.5% (NBPT, w/w) (Watson and Miller, 1996; Rawluk et al., 2001; Sanz-Cobena et al., 2008). Urease inhibitor (NBPT) is commercially available under the trade name of Agrotain. Agrotain refers to a liquid product containing 25% NBPT as the active ingredient. Granular urea applications with NBPT have been reported by a number of researchers to be effective in delaying urea hydrolysis as well as increasing productivity under a range of cropping and pasture systems (Chen et al., 2008; Martin et al., 2008). Urease inhibitor (NBPT) is commercially available under the trade name of Agrotain. Urease inhibitors inhibit the enzyme urease, decrease the urease activity and block the hydrolysis of urea to NH_3 (Varel, 1997). Urea can damage the seedlings after it hydrolyses by the enzyme urease, where the produced ammonia (NH_3) and ammonium (NH4+) can cause ammonia toxicity and osmotic damage (Bremner, 1995). Urea toxicity can be reduced by applying urease inhibitor to the fertilizer granule (Grant and Bailey, 1999; Malhi et al., 2003, Karamanos et al., 2004).

This study was conducted in the main agricultural plain (Thessaly) to evaluate the effect of new types of fertilizers containing urease inhibitor on the yield of the main arable crops (durum wheat, cotton and maize) in Greece.

MATERIALS AND METHODS

For the purposes of the study, field experiments were established in East Thessaly (Velestino, Volos). The selected crops were the same with previous year (2014), to assess the impact of a new fertilizer type in their performance: "durum wheat, corn and cotton", which are the most prevalent arable crops in Greece.

Soil characteristics

Velestino soil is characterized as Calcixerollic Xerochrept according to USDA (1975). It is a clay loam (sand 19-21%; clay 39-41%, silt 38-42%) calcareous (pH = 8.1-8.3) rich in organic matter (2.3-2.7% in soil profile of 40cm).

Cultivation practices

The experimental plots were demarcated by fixed points in previous year 2014, both on the outer perimeter and the sub-plots of each replication (block), as to remain stable the treatments for the study.

Variety and plant density were exactly the same as in 2014 for each crop. Specifically, sowing of durum wheat took place using a modern seeding machine applying 20 kg ha^{-1} variety "*Simeto*" at the end of November 2014. Maize and cotton was sown using a pneumatic precision seeder machine "Gaspardo 520". Maize sowing distances were 75cm between rows and 15cm on each line and took place at the begging of April 2105 using the Pioneer Hi-Bred hybrid "PR32P26". At least the "Flora" variety of Bayer Crop Science (cotton variety) was sown at distances of 95cm between rows and 4,5cm on each line during the first week of May 2105. There was performed pre- and post-emergence herbicide application, as well as manual control of weeds.

Basic fertilization took place one-two days before sowing using a dispenser and then the fertilizer was incorporates using a rotary cultivator. Finally, the irrigation dose for the emergence applied using a sprinkler system and then a drip irrigation system was established.

Experimental design

Durum Wheat

The experimental design of durum wheat was a completely randomized design with 25 fertilization treatments and three replications (blocks). During seeding period, there were applied three different N-levels (60, 120 and 180 kg ha^{-1}), using four different types of fertilizers (2 conventional simple: 20-10-0 and 16-20-0, and 2 with urease inhibitor: 30-15-0 and 20-24-0) as basic fertilization. The rest amount of the N-fertilization (top dressing) was applied using two simple fertilizers (calcium ammonium nitrate 26-0-0 and ammonium nitrate 34.5-0-0), and two with urease inhibitor (40-0-0 and 46 -0-0). Of course in each block there was a blank plot of zero fertilization.

Maize

The same experimental design was used for maize using different amounts and types of fertilizers. Therefore, three levels of N-fertilization was applied for basic fertilization (120, 240 and 360 kg ha^{-1}), using two simple (20-10-0 and 27-7-5M+0.5 Zn) and two with inhibitor urease (30-15-0 and 24-8-8M+0.5 Zn). The top dressing applied using two simple fertilizers: the ammonium nitrate (34.5-0-0) and urea (46-0-0), while in the case of urease inhibitor were used the 40-0-0 and 46-0-0. Of course in each block there was also a blank plot of zero fertilization.

Cotton

In cotton case was used a completely randomized experimental design with 37 treatments, in three replications (blocks). There were applied three levels of N-fertilization (70, 140 and 210 kg ha-1) using six types of fertilizers for basic fertilization. Three of them were simple (20-10-0, 16-20-0 and 15-15-15) and the other three with urease inhibitor (30-15-0 and 20-24-0 and 15-15-15). The top dressing applied on the plots with the simple basic fertilization using the simple fertilizers ammonium nitrate (34.5-0-0) and urea (46-0-0), while for the other plots

the fertilizers 40-0-0 and 46-0 -0 with urease inhibitor were applied. Also in cotton case, in each block there was a blank plot of zero fertilization.

Yield

There was used the same methodology with 2014. Specifically, in case of Durum wheat the yield was calculated by harvesting by hand $1m^2$ in each plot and after that a threshing of whole plot took place using an experimental harvester machine of the University of Thessaly on 17 June 2015. In case of maize and cotton, $3,75m^2$ and $3,8\ m^2$ were harvested, respectively.

Meteorological data

Meteorological data were recorded in Velestino from the established meteorological station of University of Thessaly.

RESULTS AND DISCUSSION

Meteorological data

In Figure 1 is illustrated the average temperature and precipitation during crop growth (durum wheat, maize, cotton).

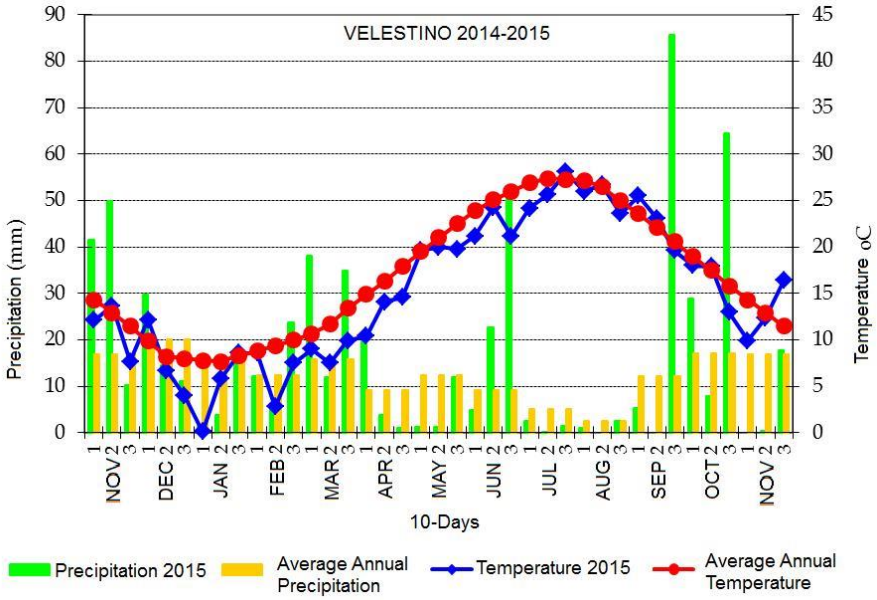

Figure 1.Average air temperature and precipitation at Velestino in 2015

Durum wheat

Normal and increased air temperatures by 2-3 oC for the season were prevailed during durum wheat sowing (late November), which combined with the rainfall in early December resulted in a satisfactory germination without problems in terms of the final population plants and the successful establishment of the crop.

Notable rainfall occurred from late February to early April (114 mm), which favored the growth of wheat in conjunction with the rise of temperature and application of top dressing. Both in April and May unsatisfactory rainfall occurred, so it was considered necessary to add irrigation water in early May, at the end of the crop flowering in both regions. Heavy rains on June 20 occurred had no effect on durum wheat cultivation, once the harvest had already taken place.

Maize

Couple a days after corn sowing took place; there was recorded satisfactory rainfall (≈ 20 mm) that contributed to a satisfactory germination. Thereafter there was required significant addition of irrigation for the satisfactory crop development, although low precipitation was recorded during summer period. Finally, the rainfall occurred after September 20 postponed crop harvest.

Cotton

Lack of precipitation during sowing time of cotton, necessitating the irrigation application to aid germination. Germination irrigation application resulted in successful establishment and desired plant population. There were not recorded extreme weather events that would cause problems to crop development. High precipitation during September 20 till early October (115 mm) hampered a uniform and satisfactory mature of cotton.

Yield and growth characteristics

Durum wheat

The results have shown differences between fertilization levels for all the examined characteristics, while hysteresis exhibited at low nitrogen level than the other two, which demonstrates once again the importance of fertilization on crop yield.

There was noticed statistically significant difference at the low N-level on chlorophyll and seed yield. On field it was clearly shown that plants fertilized with the lower N-level (60 kg ha^{-1}) were chlorotic with smaller ears.

Chlorophyll content measurements of the plants showed not only a superiority of fertilizers containing urease inhibitor but in some cases also statistically differences. In case of biomass yield, statistically differences were noticed for the different fertilizer combinations in pairs (Table 1). In the case of seed yield which is the main economic product of durum wheat, it appears that fertilizers with urease inhibitor gave higher yields. The amount of 120 and 180 kg ha^{-1} found to have statistically significant superiority compared to the low N-level of 60 kg ha^{-1}. Finally, the 8^{th} fertilizer combination with urease inhibitor reached the higher yield of 5060 kg ha^{-1}.

Table 1. Chlorophyll content, biomass and grain yield of durum wheat under different N-fertilization levels and fertilizer combinations.

Factor	Characteristic	Chlorophyll	Biomass (kg ha^{-1})	Grain (kg ha^{-1})
Nitrogen (kg ha^{-1})	0	16,5	7160	2830
	60	24,9	10940	4290
	120	26,9	11370	4900
	180	29,0	11840	4880
$LSD_{0.05}$		2,62	ns	354,0
Fertilizers	20-10-0 & 26-0-0	22,3	10360	4280
	30-15-0 & 40-0-0 Inhibitor	25,3	11110	4760
	16-20-0 & 26-0-0	26,6	11560	4730
	20-24-0 & 40-0-0 Inhibitor	30,0	12960	5050
	20-10-0 & 34,5-0-0	25,3	10550	4310
	30-15-0 & 46-0-0 Inhibitor	27,8	11440	4610
	16-20-0 & 34,5-0-0	27,7	10850	4730
	20-24-0 & 46-0-0 Inhibitor	30,4	12220	5060
$LSD_{0.05}$		4,28	1670,0	578,0
CV (%)		16,8	15,5	13,0

Therefore, it could be concluded that the N- level of 120 kg ha^{-1} was more profitable, while fertilizers with urease inhibitor produced higher biomass and higher seed yield. Moreover, chlorophyll measurements showed better plant nutrition those which fertilized with urease inhibitor fertilizers. The most efficient combination of fertilizers is the basic with 20-24-0 and the top dressing with 46-0-0.

Maize

The results concerning growth/development and seed yield of maize in 2015 is similar to the results of previous year 2014. Increasing nitrogen fertilization level showed a corresponding increase in chlorophyll content, biomass and seed yield. High hysteresis against all fertilization levels showed the blank plot with zero fertilization, which shows the importance of fertilization on crop yield. It thus becomes clear that the non-use of fertilizers is not an option for reducing the production cost.

The three nitrogen levels showed statistically differences in chlorophyll content with the highest giving darker green plants, which raises expectations for higher productivity and therefore better grain yields (Table 2).

Table 2. Chlorophyll content, biomass and grain yield of maize under different N-fertilization levels and fertilizer combinations.

Characteristic. / Factor		Chlorophyll	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})
Nitrogen (kg ha^{-1})	0	15,2	14560	6300
	120	31,6	26940	12660
	240	44,2	32230	15510
	360	49,1	35100	16460
$LSD_{0.05}$		3,10	2105	999
Fertilizer	20-10-0 & 34,5-0-0	38,0	29730	14260
	30-15-0 & 40-0-0 inhibitor	44,5	31350	15490
	26-7-5 & 34,5-0-0	39,3	30650	13980
	24-8-8 & 40-0-0 inhibitor	41,0	32710	15250
	20-10-0 & 46-0-0	42,5	30860	14480
	30-15-0 & 46-0-0 inhibitor	44,3	31650	14980
	26-7-5 & 46-0-0	40,1	31650	14960
	24-8-8 & 46-0-0 inhibitor	42,3	32770	15620
$LSD_{0.05}$		ns	ns	ns
CV (%)		12,8	11,5	11,6

Although fertilizers with urease inhibitor showed higher chlorophyll levels compared to conventional fertilizers, the differences were not statistically significant (Table 2). Total biomass production showed an upward trend from the lowest to the highest nitrogen level, with statistically significant differences from the lower level to the higher. Urease inhibitor fertilizers compared with common ones in all cases had a numerical superiority.

Finally in case of grain yield, which is the economic crop product, showed that all combinations of urease inhibitor fertilizers outweigh common fertilizers, but there was not found a statistically significant difference. Nevertheless, the mere fact of steadily higher performance of fertilizers with urease inhibitors in all cases, whether comparisons are made in pairs or individually, demonstrated the best provided nourishment versus common fertilizers.

In the case of the of the three N-fertilization levels it was found that the supply of 120 kg ha^{-1} to maize produced 12660 kg ha^{-1} seed, the level of 240 kg ha^{-1} increased the yield to 15510 kg ha^{-1} and final the level of 360 kg ha^{-1} reached the yield of 16460 kg ha^{-1}. Even if the seed yield is increasing by increasing the fertilization level it is cleary shown that the more is applied the smaller is the degree of performance.

Therefore, it could be concluded that the N-fertilization level of 240 kg ha^{-1} using fertilizers with urease inhibitor lead to the same yield with the fertilization of 360 kg ha^{-1} using simple fertilizers, which means probably less Nitrogen losses from leaching and evaporation, and less production costs.

Cotton

Cotton is a less demanding crop in nitrogen, therefore it is not expected any major differences in fertilization. Table 3 shows the chlorophyll content, biomass production and seed yield.

In chlorophyll cases there were found statistical significant differences only between the different N-fertilization levels while among the different combinations of the fertilizers no statistically differences were observed. The higher N-fertilization level the darker green plants were.

In case of biomass production the first N-fertilization level (70 kg ha^{-1}) lags considerably behind the top two levels. The fertilizers using urease inhibitors in all combinations figure slightly over conventional fertilizers.

Cotton seed yield was lower in the first N-level (70 kg ha^{-1}) than the top two N-levels (140, 210 kg ha^{-1}). The blank plot (zero N-fertilization) provides a yield of about 2600 kg ha^{-1}, similar to the results of previous year (2014). Statistical significant differences were found only in case of the different N-fertilization levels (Table 3). Supplying a dose of 70 kg ha^{-1} leads to the seed yield of 3210 kg ha^{-1}, while adding extra 70 kg ha^{-1} the final seed yield increases more or less 460 kg ha^{-1}. Finally at the top N-level the seed yield increased only 80 kg ha^{-1} (Table 3). Among the different fertilizer combinations, compared them in pairs of similar simple and including urease inhibitor fertizers, recorded an increase in yield to the urease inhibitor fertilizers by 6-12%.

Table 3. Chlorophyll content, biomass and seed cotton yield of cotton under different N-fertilization levels and fertilizer combinations at Velestino.

Factor	Characteristic	Chlorophyll	Biomass (kg ha^{-1})	Cotton seed yield (kg ha^{-1})
Nitrogen (kg ha^{-1})	0	35	6300	2600
	70	44,5	8030	3210
	140	48,2	8770	3670
	210	49,5	9200	3750
$LSD_{0.05}$		2,43	610	147
Fertilizer	20-10-0 & 34,5-0-0	46,7	8270	3360
	30-15-0 & 40-0-0 inhibitor	48,7	8820	3620
	16-20-0 & 34,5-0-0	46,2	8210	3450
	20-24-0 & 40-0-0 inhibitor	47,9	8690	3690
	15-15-15 & 34,5-0-0	46,2	8890	3510
	15-15-15 & 40-0-0 inhibitor	51,3	9510	3710
	20-10-0 & 46-0-0	48,0	8430	3360
	30-15-0 & 46-0-0 inhibitor	48,1	8450	3580
	16-20-0 & 46-0-0	44,8	8840	3340
	20-24-0 & 46-0-0 inhibitor	46,7	9060	3650
	15-15-15 & 46-0-0	46,6	8390	3400
	15-15-15 & 46-0-0 inhibitor	47,6	8420	3810
$LSD_{0.05}$		ns	ns	294
CV (%)		10,9	15,0	8,8

CONCLUSION

In the case of Durum Wheat higher values of chlorophyll showed better nutrition of the plants fertilized with a urease inhibitor fertilizer. Fertilizing with a slow-release nitrogen fertilizer gave greater biomass production and ultimately higher yield in durum wheat seed at 35-40 kg ha^{-1}.

In maize case the fertilized plots with fertilizers using urease inhibitor showed higher chlorophyll levels and therefore higher rates of photosynthesis confirming the trend recorded in the previous year (2014). The highest chlorophyll values may have led to higher rates of photosynthesis and thus to increased biomass production perhaps due to smoother and stable nitrogen nutrition. The use of fertilizers with

urease inhibitor resulted in increased yield in proportion of 3.5 to 9.1% with an average of 6.4%.

At least in cotton which is a hermit plant and covers a large part of its needs from the soil available nitrogen and therefore the addition of nitrogen fertilizer beyond a limit does not show spectacular results, fertilizers with urease inhibitor showed superiority over conventional in all the studied characteristics. These fertilizers did not confirm the greater superiority in the middle fertilization level also at the second year of experimentation. An average increased yield fluctuated from 6 to 12% with an average of 8% was observed for the fertilizers with urease inhibitor.

Finally as general conclusions it was found that fertilizers with urease inhibitor gave constant voltage supremacy against conventional fertilizers in almost all the studied characteristics for all crops, confirming the results and the trend attributed by experiments of the previous year, in all the studied characteristics. The second N-fertilization level using fertilizers with urease inhibitor gained greater than or equal odds with the high N-fertilization level with simple fertilizers, demonstrating the superiority of these types of fertilizers. The second N-fertilization level was the most effective. Due to the fact that the above results were found through two years of experimentation, safer conclusions expected to arise after the repetition of the experiments in the same place for a third year. In case that the results of the coming year will confirm the previous results then fertilizers with urease inhibitor should be proposed in future fertilization schemes.

REFERENCES

Bremner J.M., (1995). Recent research on problems in the use of urea as a nitrogen fertilizer. Fert. Res. 42, 321–329.

Chen D., Suter H., Islam A., Edis R., Freney J.R., Walker C.N., (2008). Prospects of improving efficiency of fertiliser nitrogen in Australian agriculture: a review of enhanced efficiency fertilisers. Aust. J. Soil Res. 46, 289–301.

Glibert,P.M., Harrison J., Heil C., Seitzinger S., (2006). Escalating worldwide use of urea – a global change contributing to coastal eutrophication. Biogeochemistry 77, 441–463.

Grant C.A., Bailey L.D., (1999). Effect of seed-placed urea fertilizer and N-(n-butyl) thiophosphoric triamide (NBPT) on emergence and grain yield of barley. *Can. J. Plant Sci.* 79, 491–496.

Howarth R., Marino R., (2006). Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades. Limnol. Oceanogr. 51, 364–376.

Karamanos R.E., Harapiak J.T., Flore N.A., Stonehouse T.B. (2004). Use of N-(n-butyl) thiophosphoric triamide (NBPT) to increase safety of seed-placed urea. Can. J. Plant Sci. 84, 105–116.

Kawakami E.M., Oosterhuis D.M., Snider J.L. (2013). Nitrogen assimilation and growth of cotton seedlings under NaCl salinity and in response to urea application with NBPT and DCD. J. Agron. Crop Sci. 199, 106–117.

Malhi S.S., Oliver E., Mayerle G., Kruger G., Gill K. S. (2003). Improving effectiveness of seedrow-placed urea with urease inhibitor and polymer coating for durum wheat and canola. *Commun. Soil Sci. Plant Anal.* 34, 1709–1727.

Martin R.J., Weerden V.D., Riddle M.U., Butler R.C. (2008). Comparison of Agrotaintreated and standard urea on an irrigated dairy pasture. Proc. N.Z. Grassl. Assoc. 70, 91–94.

Nosengo N., (2003). Fertilized to death. Nature 425, 894–895.

Raun W.R., Johnson G.V., (1999). Improving nitrogen use efficiency for cereal production. Agron. J. 91, 357–363.

Rawluk C.D.L., Grant C.A., Racz G.J. (2001). Ammonia volatilization from soils fertilized with urea and varying rates of urease inhibitor NBPT. Can. J. Soil Sci. 81, 239–246.

Sanz-Cobena A., Misselbrook T.H., Arce A., Mingot J.I., Diez J.A., Vallejo A., (2008). An inhibitor of urease activity effectively reduces ammonia emissions from soil treated with urea under Mediterranean conditions. Agric. Ecosyst. Environ. 126, 243–249.

Soares J.R., Cantarella H., Vargas V.P., Carmo J.B., Martins A.A., Sousa R.M., Andrade C.A., (2015). Enhanced-efficiency fertilizers in nitrous oxide emissions from urea applied to sugarcane. J. Environ. Qual. 44, 423–430.

Sommer S.G., Ersboll A.K., (1996). Effect of air flow rate, lime amendments, and chemical soil properties on the volatilization of ammonia from fertilizers applied to sandy soils. Biol. Fert. Soils 21, 53–60.

Varel V.H., (1997). Use of urease inhibitors to control nitrogen loss from livestock waste. *Bioresour. Tech.* 62, 11–17.

Watson C.J., Miller H., (1996). Short-term effects of urea amended with the urease inhibitor N-(n-butyl) thiophosphoric triamide on perennial ryegrass. Plant Soil 184, 33–45.

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IMPACT OF INULIN ON CALVES' GROWTH AND POSSIBLE REDUCTION OF GREENHOUSE GAS EMISSION

Aija ILGAZA*, Astra ARNE, Sintija GORODKO, Agris ILGAZS

Preclinical institute, Faculty of Veterinary medicine, Latvia University of Agriculture, Latvia
*Corresponding author:aija.ilgaza@llu.lv

ABSTRACT

To reduce greenhouse gas emission (GGE) researchers propose to shorten the period of breeding calves of dairy breeds (Mirzaei-Aghsaghali et al., 2015, Fao, 2010). However, producers try to prolong the time of rising animals in order to get more valuable production. The aim of this research was to determine the impact of different dosages of inulin concentrate (50%) produced in Latvia by using special technologies on calves' health, amount of obtained production and to evaluate possible reduction of GGE. Research has been supported by the National research programme AgroBioRes (2014-2017).Four week old clinically healthy *Holstein Friesian* calves which were kept in barn in individual cages were included in this research. Ten were in control group (CoG) and thirty were fed with additional flour supplement (groups: Pre6 (n=10), Pre12 (n=10), Pre24 (n=10)) until the groups' median weight was above 90kg. We found out that inulin supplement reduced the cases of diarrhoea especially in Pre12 (P=95%) less than in CoG. Also, the overall health condition in Pre12 was the most stable. The best rate of live weight showed calves from Pre12 and Pre24, the desired weight was exceeded on 42nd test day (median increase rate respectively 0.85 kg/day and 0.95 kg/day). Pre6 reached that goal on day 56 (0.76 kg/day), CoG only on 70 (0.55 kg/day). Conclusion: the optimal dose of that supplement for speeding up the growth rate is 12g which can stabilize the health and reduce breeding time. Besides shortening breeding time minimum to 3 weeks (i.e. 17%), GGE can be reduced too.

Keywords: *calf,inulin, greenhouse gas emission, live weight.*

INTRODUCTION

Climate changes come along with new challenges that are actual in agricultural production both in Latvia and the whole world. After regaining the independence Latvia takes active part in reducing negative climate changes in the world. However since year 2005 due to raise of economic activities in Latvia the GGE in agriculture sector is with progressive tendency furthermore agriculture sector is the second largest source of GGE, creating 21.5% of all GGE in the country. According to collected GGE data about the situation in Latvia in agriculture sector,

the main sources of GGE are: 1) from processing agricultural land and from soil nitric oxide (N_2O); 2) from ruminant digestive tract where methane (CH_4) is produced due to biologic food processing and fermentative processes; 3) methane (CH_4) and nitric oxide (N_2O) production from manure management. In addition, ruminants, mostly cattle in year 2013 were the main cause of all GGE in agriculture sector (35%). It is due to ruminant morphofunctional particularities and methanogenic bacteria in digestive tract. Studies reveal that for reducing GGE specific methane inhibitor 3NOP (3-nitrooxypropanol) can be used. Significantly, feed intake, fiber digestibility and milk production by cows that consumed the supplement did not decrease. Author also noticed the increase in milk protein and lactose. The emitted methane reduced by 30% (Hristovet al., 2015). In other studies researchers reached the methane reduction by 20%, at the same time the weight gain increased by additional 75g/day and milk yield increased by 1l/day in dairy cattle (McGinn,Beauchemin, 2009). It means that with significant reduction of CH_4 emission the animal productivity can improve but we need to find out the optimal feeding recipe which will be compatible with conditions and available food sources in Latvia, taking into account economic factors and obtained production. The other way to decrease GGE is to reduce to raisedairy cattle for meat purpose. Therefore it is advised to slaughter those claves which are not used for heard reproduction before they have become full ruminants respectively before the emission of the gases from the rumen (basically greenhouse gas emission) into surrounding environment has not significantly risen.However producers try to prolong the time of rising animals in order to get more valuable production from one animal. We consider that shorter animal rising time can be achieved by making corrections in animal feeding strategy and by using natural food supplements which can promote to use the animal interior body reserves.The aim of this research was to determine the impact of different dosages of prebiotics inulin concentrate on calves' health, amount of obtained production and to evaluate possible reduction of GGE.

MATERIALS AND METHODS

Four week old clinically healthy dairy breed (*Holstein Friesian*) calves which were kept in barn in individual cages were included in this research. All calves after birth within 30 minutes got 2 litres of colostrum, later on they were fed with up to 6 litres of whole milk (depending on age of calves'). At the beginning of this research each calf was clinically exanimated. Only clinically healthy, 23+/-5 days' old calves weighting 50kg +/- were included in this research.

Ten calves were in control group (CoG) (n=10), and thirty calves were fed with additional prebiotic - specially produced flour of Jerusalem artichoke, organized in groups Pre6 (n=10), Pre12 (n=10) and Pre24 (n=10) until the groups' median weight was above 90 kg. Calves' from group Pre6 were fed with additional 6g flour of Jerusalem artichoke (containing 3g of inulin), group Pre12 – 12g of this flour (containing 6g of inulin) and Pre 24 – 24g of prebiotic flour (containing 12g of inulin). Prebiotic was added to milk. The study included prebiotics inulin concentrate flour of Jerusalem artichoke (50%) produced in Latvia by using special

technologies on calves' health, amount of obtained production and to evaluate possible reduction of GGE. Usually flour of Jerusalem artichoke contains 10% of inulin, but it is possible to increase its amount up to 48.5%-50% by using special technologies. (Fleming et al., 1979; Valdovska et al., 2012)

All calves had free access to water and hay and starting from the second week of the research they also got fodder.

Each day we evaluated health status of all calves, paying more attention to faecal consistency. It was evaluated in scores 0-3, were 0 was solid faeces but 3 was watery faeces. (Larson et al., 1977).

During the research, every other week (respectively 4., 6., 8., 10. week old) we determined animal weight and performed additional clinical examination (respiratory rate, heart rate, body temperature). To analyse all data we used computer program MsExcel.

RESULTS AND DISCUSSION

Every other week additional clinical examination was performed. We did not find any statistically significant changes in body temperature, respiratory rate and heart rate; all results were in normal physiological range (Table 1).

Table 1. Heart rate, respiratory rate and temperature of all groups.

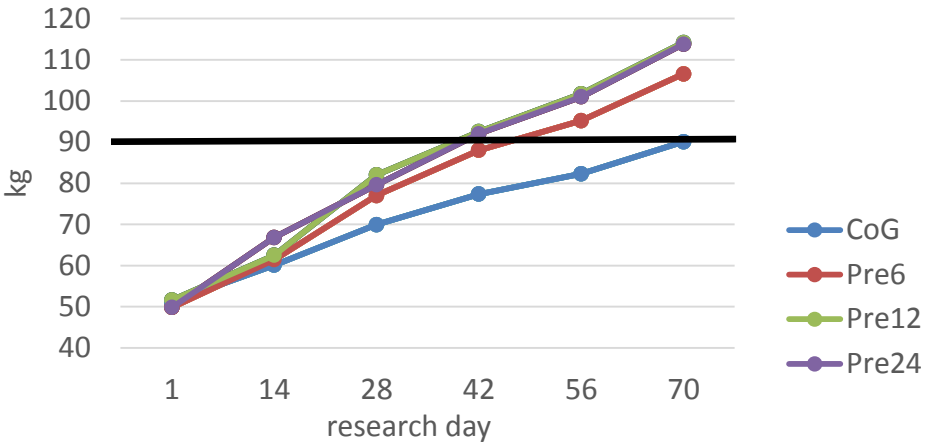
Groups	**Heart rate x/min ±SN**	**Respiratory rate x/min ± SN**	**T^0 ± SN**
CoG	124±16.76	31.3±2.19	39.20±0.87
Pre6	113±10.48	25.75±7	38.81±0.61
Pre12	108±12.00	31.2±3.35	39.02±0.62
Pre24	112.3±10.37	23.8±4.72	38.04±0.38
Normal values (Mohra et al., 2002)	86-125	15-40	38.0-39.5

We found out that inulin supplement reduced the cases of diarrhoea especially in group Pre12 (significantly (P=95%) less than in CoG). Also, the overall health condition (physiological parameters) in Pre12 was the most stable.

Every day the consistency of faeces was evaluated in scores 0-3 (Larson et al., 1977). It should be noted that watery faeces (score 3) we did not notice during this research. The median score during first two weeks of the research was 0.5, later it increased to 0.8 but faeces of seven weeks old calves' got more liquid-like (score 1.47). This could be due to adding fodder to feed.

Comparing CoG to groups Pre6 and Pre 12, faeces of CoG calves' were more watery that normal till week 10 but consistency of faeces of CoG calves' was scored 0.7 and for group Pre24 – scored 0.4. At week 12 consistency of faeces of all groups was close to score 0. This could be because at this age calves eat more

hay and during first 12 weeks of age bacteria grow very rapidly (Yutaka U. et al., 2015) and it provides the stabilisation of gastrointestinal activity.
As it was expected, digestive channel disorders had significant impact on animal live weight increase. Calves fed with inulin supplement showed higher live weight increase than animals from control group (Picture 1).



Picture 1. Live weight increase of calves during the research

On the first day of the research median weight of all calves was without relevant differences. It should be like this because we chose all animal groups with similar age and body weight. On 28th day of the research median weight of CoG calves was already significantly lower than median weight of groups Pre6 and Pre12 (P=95%) and Pre24 (P=99%) (respectively 69.9±7.33 and 77±1.1; 82.0±6.56; 79.6±1.14). At the end of the research on day 56 this difference between CoG and other groups became even greater (P=99%). This significant difference remained till the end of the research.
The best rate of live weight showed calves from groups Pre12 and Pre24, the desired weight of 90kg was exceeded on day 42nd (respectively 92.5± 3.87kg and 92±1.58 kg). Group Pre6 reached that goal on day 56 (95.2±1.92 kg), but group CoG only on day 70 of this research (90.1±4.54 kg).On the Table 2 we can see the average weight gain and standard deviation of each group at 1st and 70th day of research, live weight gain comparing 1st and 70th day of research and average daily body weight gains.

Table 2. Calf growth performance

Groups	Average weight (kg) 1st research day	Average weight (kg) 70th research day	Live weight gains (kg), 1st -70th research day	Average daily body weight gains (kg/d)	Results of T-test comparing Pre with CoG on 70th research day (p value)
CoG	51.1±3.08	90.1±4.54	39.0±7.13	0.55±0.15	-
Pre6	49.8±0.75	106.6±6.23	56.8±3.47	0.76±0.09	0.000225
Pre12	52.0±2.31	114.2±6.87	62.2±6.39	0.85±0.11	0.0001163
Pre24	49.8±0.75	113.8±5.71	64.0±4.29	0.95±0.09	0.0000714

Average daily body weight gain shows that in CoG it was the lowest – only 0.55±0.15 kg/d. Significantly higher it was noticed in groups fed with additional inulin supplement, respectively Pre6 0.76±0.09, Pre12 0.85±0.11 and the highest average daily body weight gain was in Pre24 - 0.95±0.09 kg/d (Table 2). But is it sufficient to be significantly higher? Results of T-test show that calves of Pre6 fed with 6g of prebiotics did not give significantly higher life weight gain comparing to Pre12 (12g of prebiotics) and Pre24 (24g of prebiotics). Calves from Pre24 showed the best average weight gain during 70 research days (64.0±4.09 kg), but this gain was not significantly higher than in Pre12 (62.2±6.39 kg). Our results show that double dose of prebiotics do not give significantly higher body weight gain.

There have been lot of researches about prebiotic impact on reducing GGE. The prebiotics or oligosaccharides are used in rumen manipulation along with nitrate, probiotics and yeast and had potential to reduce methane production. They are speculated to enhance the propionate production by stimulating *Selenomonas, Succinomonas* and *Megasphera* with simultaneous inhibition of acetate producers such as *Ruminococcus, Butyrivibrio* (Mwenyaet al., 2004). Administration of galacto-oligosaccharides (GOS) supplementation decreased nitrite accumulation in rumen and plasma and nitrate-induced methemoglobin, while retaining low methane production. 11% reduction in methane emission (liters/day) in GOS supplemented diet compared to control diet has been reported (Zhou et al., 2004).

Another research also indicates that GOS are efficacious in reducing methane production in dairy cows. Supplementation of dairy cows with GOS resulted in an 11% reduction in methane production (Charalampopoulos and Rastall, 2009). The use of probiotics or the stimulation of rumen microbial populations capable to decrease CH4 emissions remains a potentially interesting approach.

To reduce greenhouse gas emission (GGE) researchers propose to shorten the period of breeding calves of dairy breeds (Mirzaei-Aghsaghali et al., 2015, Fao, 2010).In our research by using inulin containing supplement we achieved significant decrease in breading time (minimum to 3 weeks (i.e. 17%). In groups Pre12 and Pre24 desired body weight, which was set 90kg, was exceeded on 42nd test day but in CoG just on day 70. Feed intake per day, including dry matter intake (DMI), in all groups was the same. Therefore according to the following predictive methane equations developed from measurements in respiration chambers: 1) Methane (MJ/d) = 0.92 × DMI (kg/d) + 5.93 (Mills et al.); 2) Methane (g/d) = 18.5 × DMI (kg/d) 9.5 0 (Grainger et al.) we can conclude that prognostically there is reduction in methane production and its distribution because calves' breeding time significantly decreased. However, more research is warranted in this area and our next step is to continue this research and prove our theory.

Currently, once again, we are studying the effect of inulin supplement on the live weight gain and possible reduction of greenhouse gas emission by direct methane measurements in rumen gases, liquid, as well as in faeces.

CONCLUSION

Calves from Pre24 showed the best average weight gain during 70 research days, but this gain was not significantly higher than in Pre12. Our results show that double dose of prebiotics do not give significantly higher body weight gain. We can conclude that the optimal dose of that special flour of artichoke (inulin 50%) for speeding up the growth rate of calves is 12 grams which can stabilize the health status and reduce fattening time. Average daily body weight gain in control group was significantly lower than in other groups fed with additional inulin supplement. There was no negative impact on calves' health status (heart rate, respiratory rate, body temperature). Consistency of faeces was more stable than in control group. Besides shortening breeding time minimum to 3 weeks (i.e. 17%), GGE can be reduced too.

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REFERENCES

Alexander N. Hristov, Joonpyo Oh, Fabio Giallongo, Tyler W. Frederick, Michael T. Harper, Holley L. Weeks, Antonio F. Branco, Peter J. Moate, Matthew H. Deighton, S. Richard O. Williams, Maik Kindermann, and Stephane Duvale. (2015). An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production, Proc Natl Acad Sci U S A. 2015 Aug 25; 112(34): 10663–10668. Published online 2015 Jul 30. doi: 10.1073/pnas.1504124112 Agricultural Sciences

Charalampopoulos, D., Rastall, R.A. (2009): Prebiotics and Probiotics. Science and

Technology, Springer, Vol. 1, pp. 1146-1148.

Grainger C., Clarke T., Mcginn S.M., Auldist M.J., Beauchemin K.A., Hannah M.C., Waghorn G.C., Clark H., Eckard R.J. (2007).Methane emissions from dairy cows measured using sulfur hexafluoride (SF6) and chamber techniques. J. Dairy Sci. 2007;90:2755–2766. doi: 10.3168/jds.2006-697

FAO (2010). Greenhouse Gas Emissions from the Dairy Sector: A LifeCycle Assessment. Food and Agriculture Organization of the United Nations, Rome, Italy

Fleming S., Groot Wassink J. (1979) Preparation of high-fructose syrup from the tubers of the Jerusalem artichoke (Helianthus tuberosus). CRC Critical reviews in Food Science and Nutrition, 12 (1), pp. 1-28.

Larson L., Owen F.G., Albright J.L., Appleman R.D., Lamb R.C., Muller L.D. (1977) Guidelines Toward More Uniformity in Measuring and Reporting Calf Experimental Data. Journal of Dairy Science, 60, pp. 989-991. DOI: http://dx.doi.org/10.3168/jds.S0022-0302(77)83975-1

Mills J.A.N., Kebreab E., Yates C.M., Crompton L.A., Cammell S.B., Dahnoa M.S., Agnew R.E. (2003) France J. Alternative approaches to predicting methane emissions from dairy cows. J. Anim. Sci. 2003;81:3141–3150

Mirzaei-Aghsaghali A., Maheri-Sis N., Alireza Siadati S., Jalilnejad N. (2015). Factors Affecting Mitigation of Methane Emission from Ruminants:Management Strategies, ECOLOGIA BALKANICA, Vol. 7, Issue 1, pp. 171-190

Mohr E., Langbein J., Nürnberg G. (2002). Heart rate variability: A noninvasive approach to measure stress in calves and cows. Physiology and Behavior, 75, pp. 251–259.

Mwenya B, Santoso B, Sar C, Gamo Y, Kobayashi T, Arai I amd Takahashi J. (2004). Effects of including β1-4 galacto-oligosaccharides lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep. Animal Feed Science and Technology, 115: 313-326.

Valdovska A., Jemeļjanovs A., Zītare I., Krastiņa V., Pilmane M., Proškina L. (2012). Impact of prebiotic on chicken digestive tract morphofunctional status. In: Conference on Current events in veterinary research and practice, LLU, Jelgava, pp. 63-67.

Yutaka U., Suguru S., Takeshi S. (2015). Effects of Probiotics/Prebiotics on Cattle Health and Productivity. Microbes and Enviroments, 30 (2), 126-132. doi.org/10.16/jsme2.ME14176

Zhou X, Sar C, Kobayashi T, Takahashi J, Santoso B, Gamo Y and Mwenya B. (2004). Effects of probioticvitacogen and ß1-4 galacto-oligosaccharides supplementation on methanogenesis and energy and nitrogen utilization in dairy cows.Asian-Australasian Journal of Animal Sciences, 17(3): 349-354.

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HIGH DENSITIES OF *CERVIDAE* EFFECT TO FOREST REGENERATION IN MIXED BROADLEAF FOREST IN SOUTH PART OF LITHUANIA

Gintare SABALINKIENE*, Kastytis SIMKEVICIUS, Kestutis PETELIS, Jolanta STANKEVICIUTE, Dovydas TALIJUNAS

Institute of Forest Biology and Silviculture, Aleksandas Stulginskis University, Lithuania
*Corresponding author: gintare.sabalinkiene@asu.lt

ABSTRACT

The abundance of *Cervidae* species is increasing in Lithuania as well as in the whole Baltic region. High densities of forest ruminants: Moose (*Alces alces*), Red deer (*Cervus elaphus*), Roe deer (*Capreolus capreolus*), Fallow deer (*Dama dama*) and European bison (*Bison bonasus*) cause a significant damage both to natural forest regeneration and afforestation. Strong negative impact on forest regeneration lead to increased costs of forest regeneration, formation and maintenance. Thus, the balance among intensive silviculture and big game management must be found for sustainable use of environment recourses. In our study, we observed the influence of cervids density increment to natural forest regeneration in mixed broadleaf forest, which is located near the Zuvintas strict reserve, and in landscape point of view connected with other forest arrays by natural migration corridors. We found that natural regenerated forest understory is hardly damaged as well as cultural forest plantations. Thus, hunting pressure in our research area is obviously too low and the current abundance of wild ruminants will not allow the economically effective intensive silviculture. After investigation we found a steady *Cervidae* abundance, not exceeding the highest optimal densities. Cervid winter pasture quality is relatively good – economically and browsing very intensively did not effect ecologically important tree species regeneration in forest understory. However, damage degree to forest regeneration in our observed stands was very high. Natural as well as artificial forest regeneration is rather difficult, thus cervid densities should be reduced of expensive protection tools should be implemented in forest regeneration stands.

Keywords: *cervids, afforestation, damage to forest regeneration, hunting pressure.*

INTRODUCTION

The intensive forest management has promoted rapid increase of population size of ungulates in Lithuania. Higher population densities cause major damage to forest regeneration. Population densities of cervid species, specially moose and red deer are spread not evenly. Moose herbivory plays an essential role in the dynamic of

natural forests (Risenhoover and Maass 1987). The feeding behavior of ruminants is regulated by the amount and quality of the food available (Heikila 1990).In the long term, high-density moose populations can damage forest habitats in the absence of predation or human control (McLaren et al 2004). Our research area is highly managed Bukta forest, represented by wet and fertile soils, preferred by moose and red deer in winter period regarding rich feed supply. Therefore, natural and artificial forest regeneration suffers in Bukta forest in winter period. Nature conservation preserves are often located in close proximity to managed forests that are occupied by moose (Heikkila and Tuominen 2009), Bukta forest is located near Zuvintas wetland strict reserve, thus moose population is naturally high in our research area. The main objective of our work was to determine the role of moose population size in browsing and bark stripping intensity and damage extent in rich mixed deciduous forest.

MATERIALS AND METHODS

The abundance of cervid population was determined by annual reports of hunters. As a control, we performed cervid census in April of 2016, using R. McCain pellet-counting method (McCain 1948) by line transects of 4 meters width. This method is used as one of the most accurate for cervid census. The aim of this method is to count pellets in a transect line. Particular shape pellets are left during one winter season of 130 days, when animals are using winter pastures – shoots and bark of young trees and bushes. During a winter season, in average one moose is leaving 2800 pellets, one red deer (Maral deer) is leaving 2085 pellets and one roe deer is leaving 2028 pellets (Balčiauskas 2004). To get the result as accurate as possible, transects must cover not less than 1.2 % of area. Total length of our transect was 26.1 km.

Total amount of pellets in all research area was counted using formula: S=P/s/p, were: S – total number of pellets in all the research area; P – total area; p – area of transects; s – pellets amount in transect. Amount of wintering animals by separate species were counted in area using formula: G=S/n; were: G – amount of wintering animals; S – pellets amount multiplied to all the research area; n – amount of pellets left by one individual during the winter.

Cervid winter pasture quality was evaluated according to S. Aldous method (Aldous, 1944), by regenerated forest understory (up to 4 meter height), the presence (S) and abundance (G) of understory species in cervid wintering places, the intensity (I) of understory use and each understory species portion in total winter feed balance (Q) were evaluated during the investigation. Winter pasture quality was evaluated together with pellet counting census. In each 200 meters of transect, 5,65 diameter sample plots were defined. In these sample plots (100 sq m diameter) all healthy and damaged (twig breaking, shoot browsing and bark scratching) understory trees and bushes were counted.

The presence (S) of particular understory species was counted using formula: S=(n/N) x100%, were: n – the number of sample plots, where each understory species was present; N – total number of sample plots. The abundance (G) of

particular understory species in winter pastures was counted by formula: G=(A/C) x 100%, were: A – total number of particular understory species in all sample plots; C – total number of all understory species in all sample plots. The intensity of understory use in winter pastures was counted by formula: I=(B/A) x 100%, were: B – the number of damaged understory by particular species in all sample plots; A – total number of particular understory species in all sample plots. The utilization factor (U) of particular understory species was counted by formula: U=G x I %, were: G - the abundance of particular understory species, %; I - the intensity of understory use, %. Each understory species portion in total winter feed balance (Q) was counted by formula: Q=(U/∑U) x 100%, were: U - the utilization factor of particular understory species, %; ∑U – the sum of utilization factor of all understory species.

Moose damage to forest regeneration was evaluated according to methodology approved by Ministry of Environment (2001 Feb. 28. No 120, Vilnius). For investigation of moose impact to forest regeneration we have chosen stands by criteria: no protection used in stands (fence or repellents), average height of understory trees and shrubs was 0.2 – 1.6 m for browsing and more then 4 m for bark stripping. In every stand included in our research we established 4 – 6 (depending on stand area) sample plots of 100 sq. m. area; in each sample plot we have estimated every tree or shrub. Trees were divided to four categories according to damage degree: (1) healthy and little damaged trees, (2) weakly damaged trees, (3) average damaged trees, (4) hardly damaged trees. Totally 6 forest stands were investigated. The criteria of stands are given in Table 1. The investigation was started in June 2010 y., four forest stands were selected for moose damage evaluation. In 2011 the investigation was repeated in the same 4 stands and two more stands were added. Investigation was repeated in exactly the same sample plots (the center of sample plot was marked by GPS and also by wood paints) in 2012, 2014 and 2016 y.

Table 1. Criteria of investigated forest stands

Stand No.	Taxonomic stand No.	Area, ha	No of sample plots in stand	Tree species composition
1.	23 quarter, 19 stand.	2.9	4	7 aspen 1 ash 1 oak 1 birch
2.	40 quarter, 1 stand.	1.7	4	9 aspen, 1 ash
3.	62 quarter, 11 stand.	17.9	6	5 aspen, 2 ash, 2 alder, 1 hornbeam
4.	65 quarter, 14 stand.	3.3	4	3 ash, 3 aspen, 2 oak, 1 spruce, 1 alder
5.	56 quarter, 5 stand.	1.2	5	6 aspen, 2 ash, 1 spruce, 1 birch
6.	72 quarter, 5 stand.	1.4	6	6 aspen, 2 ash, 1 spruce, 1 alder

RESULTS AND DISCUSSION

Cervid abundance dynamics in Bukta forest

The results of cervid census shows that cervid abundance in Bukta forest is relatively stable (Fig.1.). Though, wintering cervid abundance in Bukta forest has decreased in the last winter of 2015/2016 y. The decrease could be related to intensified timber harvesting in recent winter – increased noise on overnight labor could have a disturbing effect to cervid population size. Our results show optimal densities of cervid species in mixed broadleaved forest, not exceeding the highest allowable limits (Kibisa et al 2015).

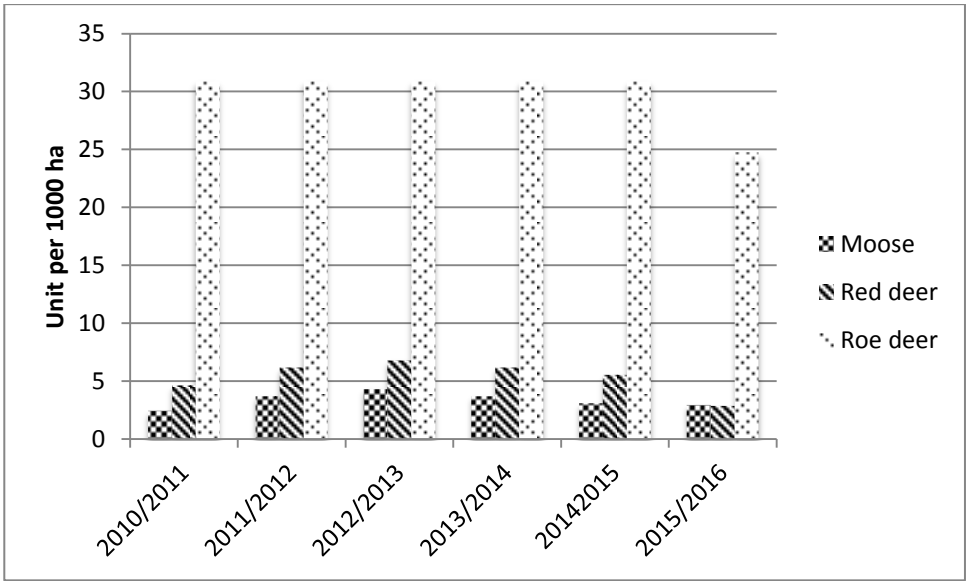

Figure 1. Wintering ervid density in Bukta forest from winter 2010/2011 to winter 2015/2016

Winter pasture quality

After investigation in Bukta forest in spring 2016 y. in cervid winter pastures we found 20 tree and shrub species (Fig. 2). The most abundant species (cover) was *Corylus avellana* (15.8%), *Lonicera xylosteum* (13,9%), *Carpinus betulus* (12,8%), *Fraxinus excelsior* (12.0%), *Betula pendula* (8.1%) and *Populus tremula* (7.8%). In cervid winter pastures most intensively was used *Salix caprea* (200%) and other *Salix* spp. (65%), *Acer platanoides* (52.2%), *Frangula alnus* (42.0%) and *Populus tremula* (33.5%), the use of other tree species did not reached 30% (Fig. 3).

Bukta forest is located in Southwest of the country, where soils are rich in fertility as well as water amount is sufficient. For this reason Bukta forest covered by mixed broadleaved species typical for rich soils as walnut, ash, hornbeam,

honeysuckle and aspen. However, economically important and abundant species are hornbeam, ash and aspen while other economically important species as oak, Norway spruce and Scots pine in Bukta forest are rare. From ecological point of view very important is the presence of ash as well as hornbeam in forest understory, as ash is very sensitive to Ash dieback disease (caused by fungus *Chalara fraxinea*) in Lithuania and hornbeam is presented in forestland only in southwest part of Lithuania.

The intensity of use of particular tree species in cervid winter pastures shows the species preference of animals. The most abundant species hazel was not used by cervids very intensively, while not so abundant Willow species were used most intensively. Economically most important tree species as Scots pine, Norway spruce and oak was used lightly, indicating that cervids do not cause significant damage to economically important tree species regeneration in Bukta forest. However, natural regeneration of ecologically important species - hornbeam tends to suffer, as the use intensity in winter pastures was nearly high (Fig 3). Natural regeneration of maple is also complicated, because the intensity of use reaches 52.2 %.

However, biggest portion in cervid winter feeding balance contains ash (18.9 %), aspen (14.1 %), Salix sp. (11 %), honeysuckle (11 %), buckthorn (10.3 %), hornbeam (9.3 %) and hazel (8.5 %), other species consist negligible portion.

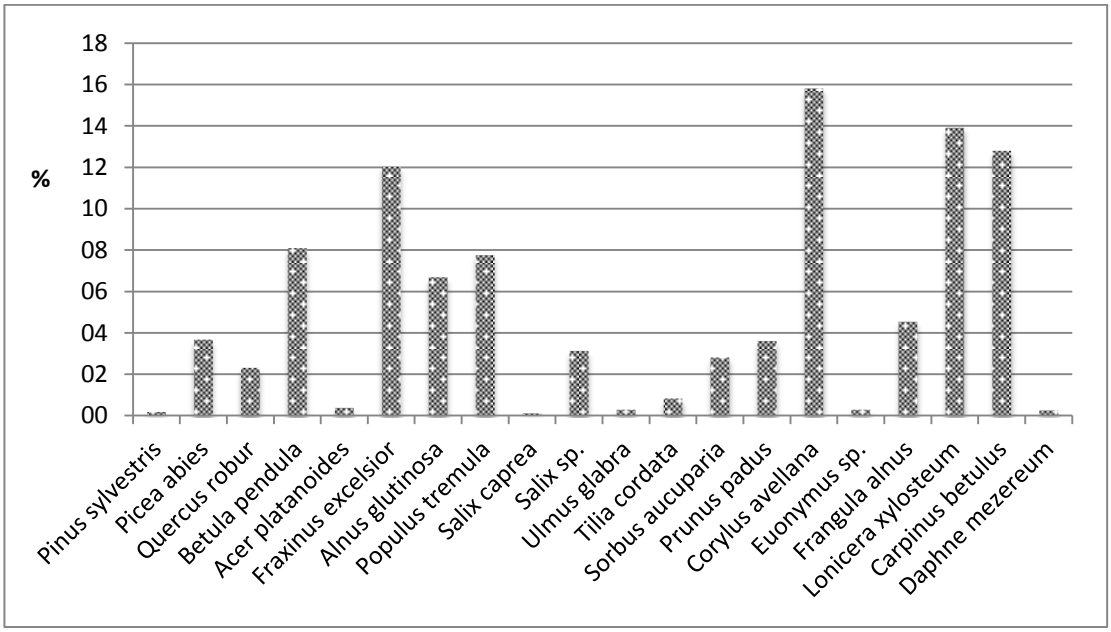

Figure 2.The abundance (G) of tree and shrub species in cervid winter pasture in understory of Bukta forest

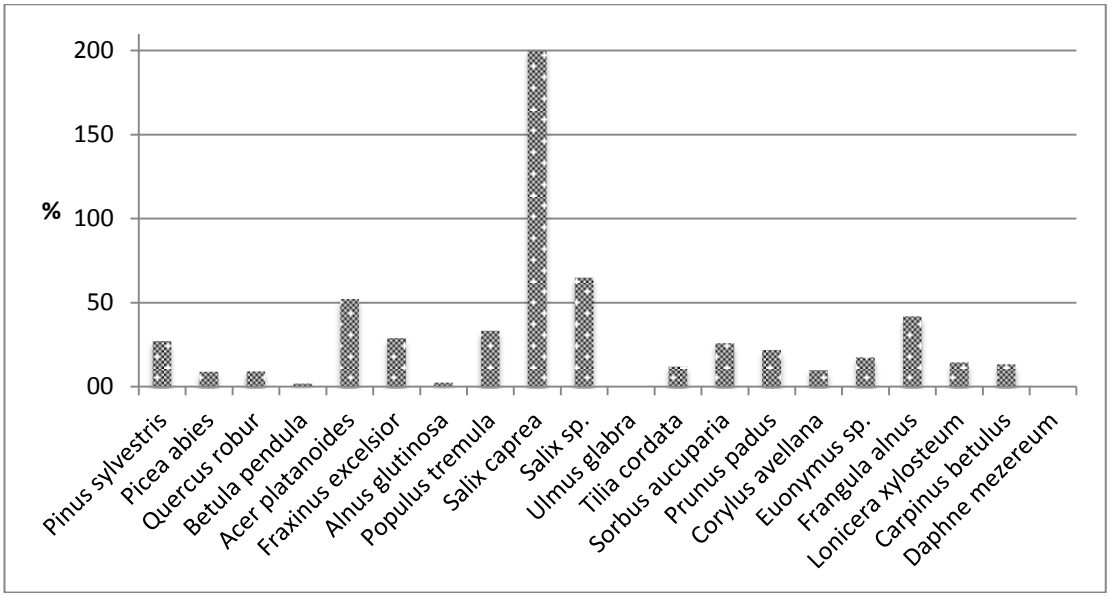

Figure 3.The intensity of use in cervid winter pastures

Moose density influence to forest regeneration on implemented stands

Our results of cervid abundance shows that moose and deer densities in Bukta forest are not exceeding the highest limit (see above), and winter pastures are in relatively good condition, surprisingly we have observed very high damage degree to forest regeneration indicating bad condition (Narauskaite et al 2011). However, damage degree was significantly decreasing every year in all investigated stands (Fig. 4) (ANOVA test: $p<0.0001$, $F=23,11$). The regeneration of economically and ecologically most important species: aspen, oak, ash and hornbeam mostly suffer of moose and deer browsing, indicating the need of cervid population reduction. The highest damage peak was observed in 2011, even 25 % of black alder was browsed by cervids. Black alder is least favorite species by cervids, thus browsing on black alder indicates the starvation period (Padaiga 2010). Smaller amount of wintering cervids in Bukta forest had a very important impact to reduction of damage degree. After winter 2015/2016 damage degree to forest regeneration dropped to 14,6%, providing a possibility to recover forest understory. Thus, our results show that current cervid population density is tolerable for successful forest regeneration in mixed broadleaved Bukta forest. Even if damage degree was reduced in recent year, earlier damage made by animals, specially bark stripping will have a negative impact to timber quality. Bark, thought to be only normally stripped from trees and eaten after all other forms of brows are depleted, is reportedly only eaten in late winter and generaly considered a "starvation food" (Renecker and Schwartz 1998). Trees, specially aspen, has a capacity to treat – regrow damaged bark, damaged bark is usually effected by fungus. Thus damaged steam will not grow a qualitative timber.

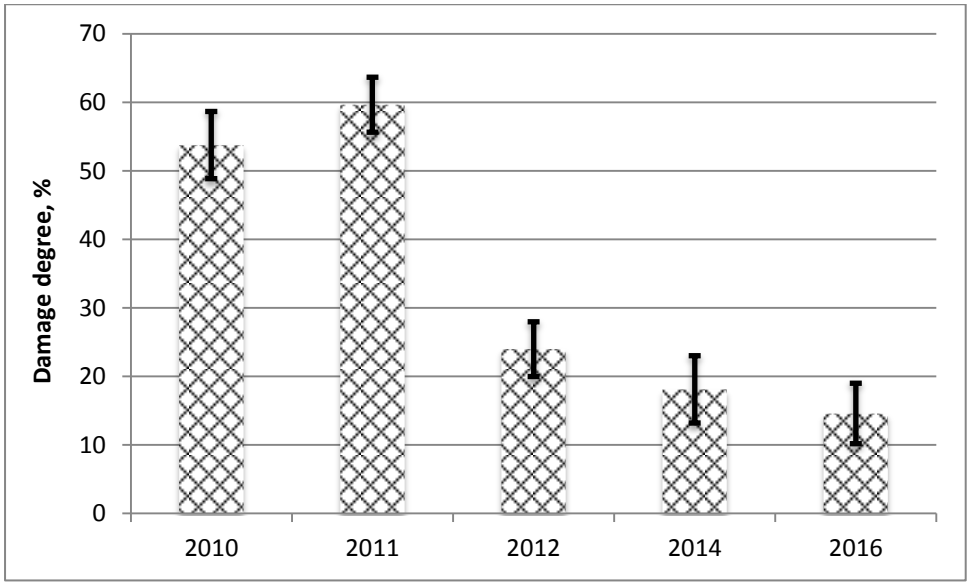

Figure 4.The change of damage degree to forest regeneration caused by *Cervidae* in Bukta forest

CONCLUSION

Cervid dencities was reduced till established optimal numbers in Bukta forest recently, due to higher hunting limits and increased bisturbance by intensive forest management (harvesting) labour. Reduced cervid dencities couse significantly smaller damage to forest regenration in comparison to last four winters. However, regeneration of economicaly most important tree species as ash, aspen and maple as well as one ecologicaly important species – hornbeam in Bukta forest still suffer from browsing and bark stripping. This results suggests that establisment of cervid optimal numbers must be re-evalueted and viewed. In order to protect unick forest of Bukta, which is located nearby Zuvintas strick nature reserve, cervid abundance must be reduced futher.

REFERENCES

Aldous S.E. 1944. A deer brows survey method. Journal of Mammology, 25(2): 130 – 136.

Balciauskas L. Research methods of terrestrial ecosystems. 1st part. Animal census. Vilnius. p.183.

Heikkila R. 1990. Effect of plantation characteristics on moose browsing on Scots pine. Silva Fennica, Vol. 24, 4:341 – 351.

Keikkila R., Tuominen M. 2009. The influence of moose on tree species composition in Liesjarvi national park in southern Finlamd. Alces 45: 59 – 58.

Kibisa A., Narauskaite G., Petelis K., Simkevicius K., Marozas V. 2015. Large herbivore abundance, distribution and winter pasture quality in two game farms

in North Kazakhstan. *Acta Biologica Universitatis Daugavpilensis* 15:105 – 112.

McCain R. 1948. A method for measuring deer range use. *Transactions of North America Wildlife conference*, 13:431-441.

McLaren B.E., Roberts B.A., Djan-Chekar N., Lewis P.K. 2004. Effects of overabundant moose on the Newfoundland landscape. Alces 40: 45-59.

Narauskaite G., Petelis K., Gackis M., Maksvytis M., Simkevicius K. 2011. Moose Damage to Forest Regeneration in Bukta Forets. Proceedings of Rural Development 2011 (2): 103 – 105.

Padaiga V. Miskas ir medziokle (*Forest and Hunting*). 2010 Kaunas, p. 271.

Risenhoover K.L., and Maas S.A. 1987. The influence of moose on the composition and structure of Isle Royal Forests. Canadian Journal of Forest Research 17: 357-364.

Renecker L.A., Schwartz C.C. 1998. Food habits and frrding behavior. Ecology and management of the North American Moose. Smithsonian Institution Press, Washington, DC. p. 403 – 439.

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IMPORTANCE OF THE BUFFER ZONE OF THE WATER INTAKE FOR THE MAINTENANCE OF AGROSYSTEM PHYTODIVERSITY

Renata GAMRAT[1*], Małgorzata GAŁCZYŃSKA[2], Monika MAJDOSZ[1], Andrzej ŁYSKO[1]

[1]Department of Ecology, Environmental Protection and Management, West Pomeranian University of Technology, Szczecin, Poland
[2]Department of General and Ecological Chemistry, West Pomeranian University of Technology, Szczecin, Poland
*Corresponding author:Renata.Gamrat@zut.edu.pl

ABSTRACT

A change of the agricultural policy in Poland after 1990 caused significant changes in the structures of the agricultural landscape functioning in the area of West Pomerania (Pomorze Zachodnie). The field fragments, which had been in a form of barren land in the protective zone of Miedwie Lake for 30 years, were chosen for the detailed phytosociological studies. The aim of the current study was the analysis of anthropogenic impacts on flora in the surveyed terrain. In the studied area, the values of the synanthropization indices of the flora were as follows: synanthropization - 2.58, apophytisation - 2.0, anthropophytisation - 0.56, archaeophytisation - 0.38, kenophytisation - 0.16, and fluctuation changes in the flora - 0.04. It is assumed that the larger the share of nonsynanthropic species, the larger the naturalness of the flora inthese biotopes. The large values for the apophytisation index showed degradation in natural habitats A large share of anthropophytes indicates dominance of the processes of a decline in native species in a given biotope, which means that there are disturbances of the ecological balance of a given ecosystem. The 30 year cessation of agricultural use around the lake resulted in the improvement of the chemical state of waters. A significant role in stopping water runoff played dense sodification of the surveyed area. The state of the flora found in this area was influenced by both natural and anthropogenic factors. This is proved by thedominance of synanthropic over nonsynanthropic, and spontaneophytes over anthropophytes species.

Keywords: *intake protection buffer, flora, biodiversity, agroecosystem, Poland.*

INTRODUCTION

Economic changes that occurred in Poland in the 1990s caused a significant transformation of the agrocenoses structure. In the area of West Pomerania, in particular, treated as feed area, the so-called polders, the area of meadows used has been dramatically reduced (Kochanowska, 1981). Many of these abandoned grasslands degraded, as a result of failure to perform mechanical treatments and fertilizing, - turning into worthless natural hairgrass or tall herb terrains

(Kochanowska and Rygielski, 1994; Kochanowska, 1997). The area of arable land has also changed. In order to protect drinking water intakes, as a result of the increasing pollution of surface waters, many field areas were excluded from use, ordering the compliance with numerous decrees and restrictions related to land and water use. A similar action was undertaken on the banks of Lake Miedwie - a reservoir of drinking water for 400 thousand residents of Szczecin. On the fertile soils around the lake, cereal crops, mainly wheat, were abandoned. Currently, the fallowed area in Poland accounts for 20% of arable land. Natural succession of plants takes place on the abandoned fields, which gradually, increasing the growth over the surface become a protective buffer – they capture any excess of nutrients and all types of poison getting into the habitat or already present in there. This vegetation is one of the components most strongly related to all elements of the natural environment, that is why it is so frequently used to study the structure and the functioning of the whole environment (Roo-Zielinska *et al.,* 2011). The phytodiversity of fallow land depends on many factors: soil type, degree of anthropopressure, the time of fallowing (Żarczyński *et al.,* 2008;Ziemińska-Smyk *et al.,* 2015). The species composition can serve to assess the degree of habitat synanthropization with the use of such anthropogenic indicators, as naturalness of flora, synanthropization, apophytization, archeophytization, and kenophytization. They allow to make an assessment of anthropogenic changes in time of the flora, and to set out the direction and rate in a given area being under the influence of a specific anthropogenic factor (Kutyna and Malinowska, 2011, Jírová *et al.,* 2012). In the last twenty years, it has become very important to block the process of synanthropization of plant communities, and to restore the ecological balance. These tasks were to prevent the degradation of agrocenoses diversity (Holzel *et al.,* 2012). The purpose of the research was to evaluate the transformation of the protection zone by analyzing indicators of flora synanthropization at a field fragment which had been fallow for 30 years.

MATERIAL AND METHODS

Between 2002-2003 and 2015 field flora research was conducted in the protection zone of a water intake on Lake Miedwie (West Pomeranian Region, Poland). Between 2002 and 2003 studied of the flora species which was verified in the year 2015.A part of the fields (7.1 hectares) set aside for 30 years (Fig. 1) was selected to assess the degree of transformation of the flora in the protection zone. The test area is located between the two towns Szczecin and Stargard. It covers an area of grasslands located around the lake Miedwie. In the neighborhood there is an area single-family housing. Floristic inventories were made in the area studied, which were used to calculate the indicators of flora synanthropization, ie.:naturalness, synanthropization and proper and potential apophytisation, and archeophytisation and kenophytisation (Jackowiak, 1990). The value of the synanthropization index of (total S_t/ permanent S_p) flora showed the extent of the impact of human activity on the studied flora. Apophytisation index od (total Ap_t/ permanent Ap_p) flora determines the ability to maintain and spread of native species from habitats that have arisen and remain through human activity. Large values of indicator sindicate that the synanthropization process wasassociated with the transformation of natural habitats.The values ofthe antropophytisation index (total An_t/ permanent An_p) the

presence of alien geographical species in the flora. Archeophytisation index of flora (total Ar_t/ permanent Ar_p) expresses the percentage of archeophytes (species that arrived in Europe before 1500 year) in the total flora.Kenophytisation index (total Kn_t/ permanent Kn_p) determines the degree of transformation occurring in plant communities. It determines the percentage of the "younger newcomers" in the flora of the total habitat. Index fluctuaction changes (F) in the flora antropophytes determines the percentage of diaphytes. Index defines the sharediaphytes throughout flora (Sp + A). Participation diaphytes within antropophytes testifies to the high lability floristic surveyed phytocoenoses.

$S_t = \frac{Ap+A}{Sp+A} \times 100\%$, $S_p = \frac{Ap+M}{Sp+M} \times 100\%$; $Ap_t = \frac{Ap}{Sp+A} \times 100\%$, $Ap_p = \frac{Ap}{Sp+M} \times 100\%$;

$An_t = \frac{A}{Sp+A} \times 100\%$, $An_p = \frac{M}{Sp+M} \times 100\%$; $Ar_t = \frac{Ar}{Sp+A} \times 100\%$, $Ar_p = \frac{Ar}{S+M} \times 100\%$;

$Kn_t = \frac{Kn}{Sp+A} \times 100\%$, $Kn_p = \frac{Kn}{Sp+M} \times 100\%$; $F = \frac{D}{Sp+A} \times 100\%$

Ap – apophytes = synanthropic spontaneophytes, Sp – nonsynanthropic spontaneophytes, A – antropophytes, Ar = archeophytes, Kn = kenophytes, D – diaphytes, M – metaphytes

The flora was classified according to the forms of space use, socio-ecological along with geographical and historical groups (Chmiel, 2006).

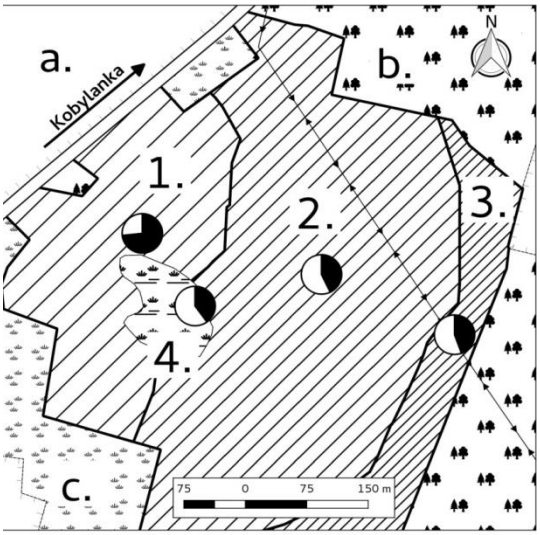

Figure 1. Location of the studied area
a. – buildings, b. – forests, c. – wet meadows; 1-4 habitats; ○ participation *Poaceae* species relative to the *Fabaceae*

RESULTS AND DISCUSSION

In consequence of political and economic transformations that occurred in the late 1980s and in the 1990s, a lot of fallow land appeared in the agricultural landscape in Poland, which is subject to spontaneous secondary succession, leading to the formation of numerous plant communities (Adamczyk and Kurzyp, 2014). In the studied site, the abandoned cultivation of cereals contributed to primary succession, or a sequential change in the combination of plants species in places previously unoccupied by vegetation. 157 species of vascular plants, altogether were found out here which belonged to 43 families. *Asteraceae* (16%), *Poaceae* and *Fabaceae* (12% each), as well as *Rosaceae* were most abundantly represented. Research after 15 years showed no significant differences in the floristic composition A similar share of species within the largest syntaxonomic groups was shown by Ziemińska-Smyk *et al.* (2015) in the segetal flora of rendzine soil near Zamość. These authors have noted 130 species, belonging to 30 families, of which the most represented were *Asteraceae, Fabaceae, Poaceae, Lamiaceae, Scrophulariaceae* and *Brassicaceae*.

The increase in the phytodiversity of fallow land is related to the duration of being set aside. Much more taxa occurred in areas with a long-term human failure to act than in case of several years (Marks *et al.,* 2000). The more diverse the phytocenoses were, the more intensive soil respiration took place. That is why, the highest values were proven on the fallowed soils - 1185 g C m^{-2}, then, in communities of single season grasses - 1020 g m^{-2} and crops of soybeans and corn - 750 g C m^{-2}. Areas strongly overgrown featured better soil breathing than crop fields, which indicates a greater soil biological activity in these habitats. By the same, these areas can act as a buffer in relation to stress factors (Tufekcioglu*et al.*, 2001).Numerous research studies are undertaken in relation to the forecast of the plant succession as a possible manner to restore degraded or heavily transformed land. Research carried out on the succession of communities by Jírová *et al.* (2012) after 30 years showed that a spontaneous natural succession of plants quickly occupies the areas previously characterized by low intensity farming. Only in such areas, is there a wealth of seeds of plant species appropriate for given soil habitats. Floristic composition of the studied area of fallow land proves its substantial phytodiversity, confirmed by a diversified pool of seeds of 72 species associated with meadows complex, 44 forest species, 24 segetal and 17 ruderal species. For 15 years the species of forest habitats have significantly increased occupied space Fallows sown over with perennial plants (perennials such as *Galega orientalis*) start more frequently the movement of nutrient elements and incorporate them into circulation than the natural vegetation of the fallow dominated by therophytes (Żarczyński *et al.,* 2008). However, attention must be drawn to maintaining an adequate variety, as the dominance of papilionaceous plants may cause a risk of nitrates (V) transmission into groundwater. This is prevented by the presence of grasses e.g. *Bromus inermis*. In the study on the fallow in the protection zone of lake Miedwie, perennial plants, including hemicryptophytes accounted for 53%,

phanerophyte 10%, chamephytes 3%, cryptogams 10%, and therophytes 24%. Such a share of the perennial plants prevents soil nutrients from being washed out and thus protects the water in the lake against any degradation of quality.

The environment can be evaluated on a landscape scale, with the use of landscape indicators (matrix) of assessing synanthropization (Solon, 2002). It is worth noting that even a partially depleted vegetation does not lose its indicator value. Basic indicators: synanthropization, apophytisation, antropophytisation, archeophytisation, kenophytisation, fluctuation changes in the flora were used in the studied area to assess the degree of changes in the landscape.

Flora synanthropization is defined as a whole of changes in vegetation caused by human activity (anthropopressure). It is featured by native elements being driven away by foreign, cosmopolitan ones. It consists of two processes taking place paralelly - apophytisation and antropophytisation. The apophytisation is an earlier process of changes in phytocenoses as it is associated with the original forms of anthropopressure. Its essence has been the transition of native species from natural habitats to anthropogenic ones. In the studied site of fallow land, the native species, i.e. apophytes and spontaneophytes dominated (21 and 95 respectively). The values of synanthropization indicators of flora ranged from 0.04 to 4.76 (Table 1).

Table 1. Synanthropization index.

Index	The value of the study area
Synanthropization index of total flora/ permanent	2.58/2.64
Apophytisation index of total flora/ permanent	2.00/2.08
Anthropophytisation index of total flora/ permanent	0.58/0.56
Archeophytisation index of total flora/ permanent	0.38/0.39
Kenophytisation index of total flora/ permanent	0.16/0.17
Indicator of fluctuactions changes	0.04

**Source: Own results*

It is assumed that the greater the share of non-synanthropic species, the greater the natural flora of a given biotope. Large values of apophytisation indicators signal the transformation of natural habitats, and a large share of antropophytes shows the prevalence of processes of native species regression in a given habitat, which is evidence of an ecological balance disturbance of the ecosystem.

Floristic research of Kutyna (Kutyna and Malinowska, 2011) on a dozen or so years old fallow of winter cereals in the south-western part of Szczecin Lowland and a fragment of Drawskie Lake Land showed 286 taxons (number of flora

species), at an indicator of naturalness of 4.5 and respectively 4.6, and archeophytisation 18.6 and 16.0 respectively, and kenophytisation 4.2 for both regions. The high values (generally exceeding 90%) of the sustainability rate of the total flora testify to relative stability of the floristic composition of communities of long-term fallows.

Following the rapid invasion on Zamość fallow rendzine soil, such segetal species as *Consolida regalis, Cichorium intybus, Sinapis arvensis,* and *Papaver rhoeas,* were defined as threatening to the biodiversity (Ziemińska-Smyk *et al.*, 2015). In the studied area, due to the occupied area, 49 species showed the most dynamic trends - as strongly expansive individuals.

The anthropogenic factors associated with various forms of space being used have an impact on the abundance and expansiveness of individual species. They can influence positively or negatively a given taxon (Table 2). 96 species having positive influence on the forms of use of space were shown to exist in the studied area, including 79 species with the only positive effect. These were, for example *Anthriscus sylvestris*, *Chelidonium majus*, *Urtica dioica*having an impact on most forms. There were 82 species negatively affecting the space, including 62 species with the only negative effect. These included among other things: *Agrostemma githago*, *Alium rotundom*, *Anagalis arvensis*, *Armeria elongata*, *Bromus mollis*, *Bupleurum rotundifolium*, *Cardamine pratensis*, *Chrysanthemum segetum*, *Ononis spinosa*, *Erigeron canadensis*, *Erysimum diffusum*, *Euphorbia helioscopia*, *Fallopia convonvulus*. Of all the forms of use of the space **(**agriculture; communication; forestry; recreation,urbanization), agriculture had the greatest impact (115 species („+"36 /„-"79 species), next forestry 56 (35/3) and communication 51 (51/0), and the leastinfluences positively urbanization (26) and recreation (19).

Too intensive farming led to the degradation of natural vegetation and the unification of species composition. The recreation and holiday makers did not have such a material adverse effect on vegetation. All forms of space use had a positive impact on the inventory of the species and stimulated the expansiveness of a large group of synanthropic species. These results come from the complexity of impacts. Several factors of space use affect one species. Furthermore, the efficiency of anthropogenic factors may have been strengthened or shielded by natural elements. Only owing to natural features of habitats can there gather numerous native species. However, all elements geographically foreign shaped by anthropogenic factors are not wanted (Krupa, 2010).

Table 2.The impact of the forms use the resources of the species.

Flora	The impact of use	
	„-"	„+"
Anthriscus sylvestris, Chelidonium majus, Trifolium repens, Urtica dioica	-	UAFCR
Lolium perenne, Senecio vulgaris, Stellaria media, Taraxacum officinale,	-	AUCR
Amaranthus retroflexus, Artemisia vulgaris, Capsella bursa-pastoris, Convonvulus arvensis, Dactylis glomerata, Melandrium album, Plantago major, Polygonum aviculare	-	AUC
Allaria officinalis, Galium aparine, Oxalis europaea, Rumex obtusifolius	-	AUF
Festuca rubra, Galium mollugo, Potentilla reptans, Viola arvensis	-	AFC
Calamagrostis epigejos, Convallaria majalis, Holcus mollis, Geranium robertianum,Lapsana communis,Lupinus polyphyllus, Stenactis annua,	-	FR
Achillea millefolium, Agropyron repens, Arrhenatherum elatius, Heracleum sphondylium, Leontodon autumnalis, Plantago lanceolata 6	-	CA
Berteroa incana, Melilotus album, M. officinalis	-	CR
Alopecurus pratensis, Acorus calamus,Lycopus arvensis, Potentilla anserina, Phalaris arundinacea, Phleum pratense,Trifolium pratense	-	A
Artemisia absinthium,Chenopodium album, Matricaria chamomilla,Poa trivialis, Solidago gigantea, Tripleurospermum inodorum	-	U
Bromus sterilis, Echium vulgare, Lotus corniculatus, Oenothera biennis, Sedum acre, Tanacetum vulgare, Jasione montana,	-	C
Acer platanoides c, Betula pendula a, c, Crataegus monogyna c, Fraxinus excelsior c, Helichrysum arenarium, Juncus effusus,Padus avium b, Pinus sylvestris b, c, Pirus communis c, Populus tremula a, c, Prunus cerasus c, Prunus spinosa b, Quercus robur a, c, Rosa canina b, Rubus caesius c, Salix cinerea b, Solidago virgaurea, Syringa vulgaris c, Ulmus minor	-	F
Anchusa officinalis, Carex hirta, Cerastium arvense, Coronilla varia, Daucus carota, Equisetum arvense, Euphorbia cyparisias, Galium verum, Hieracium pilosella, Hypericum perforatum, Lathyrus pratensis,Linaria vulgaris,Pimpinella saxifraga, Ranunculus acris, Trifolium arvense,Veronica chamaedrys, Vicia cracca	A	C
3 species negatively affects agriculture and forestry, 59 species negatively affects agriculture		

A –agriculture, C – communication, F –forestry, R – recreation, U – urbanization, a – trees, b- bushes, c – herbs (phase of growth of bush)

The species response to the forms of the space use can be interpreted in conjunction with other indicators, as the features of pressure on the environment (Roo-Zielińska *et al.*, 2011). In terms of compliance of the fallow studied with its buffer function, the fallow studied was divided into 4 habitats (Fig. 1). Of these, three (1 - dominated by *glaucae* species 2 – by meadow plants and 3 – by scrubs) demonstrated the appropriate ratio of grasses - fulfilling a protective role - to the number of legume plants which increase the fertility of soil (1 – 14/11, 2 – 12/9, 3-10/8).

Changes in the structure of Polish landscape caused by new political or economic view, were to counteract the degradation of biodiversity. The main objective of the conservation and ecological research is to explore and anticipate further successional vegetation and to develop methods to drive them towards the desired habitats and ecosystems (Holzel *et al.*, 2012). However, the natural succession of plants should be aided in the form of sowing or seeds in consequence of a significant transformation of soils, occurring in many agricultural regions (Jírová *et al.*, 2012).

CONCLUSION

The establishment of a protection zone around the lake waters Miedwie - which is a drinking water reservoir for Szczecin - hindered the process synanthropization plant communities. Low values of synanthropization and high apophytisation are evidence of slight invasion of synanthropic species. A significant share of grass on the fallow under study contributes to its role of buffer zone - lake water protection being fulfilledand thus the area of research is in the ecological balance.

REFERENCES

Adamczyk J., Kurzyp R. (2014). Using of fallows in agricultural lanscape in planning of ecological architecture. Journal of Civil Engineering, Environment And Architecture JCEEA, 31, 61 (3/II/14): 9-17.

Chmiel J. (2006). Spatial diversity of flora as the basis for nature conservation in the agricultural landscape. Prace Zakładu Taksonomii Roślin Uniwersytetu im. Adama Mickiewicza, Poznań. Wydawnictwo Naukowe Bogucki, 14: 250.

Holzel N., Buisson E., Dutoit T. (2012). Species introduction a major topic in vegetation restoration. Applied Vegetation Science, 15: 161–165.

Jackowiak B. (1990). Anthropogenic transformation of the flora of vascular plants of Poznan. Wydaw. Naukowe im. Adama Mickiewicza, Poznań., Ser. Biol. 42: 232.

Jírová A., Klaudisová A., Prach K. (2012). Spontaneous restoration of target vegetation in old fields in a central European landscape: a repeated analysis after three decades. Applied Vegetation Science, 15: 245–252.

Kochanowska R. (1981). The dynamics of the development and growth of some grass species in different habitat conditions. Rozprawy Naukowe, 115, AR w Poznaniu: 3-62.

Kochanowska R. (1997). Natural consequences of the regress of the economy meadow Western Pomerania.Przegląd Przyrodniczy, VIII-1/2: 73–76.

Kochanowska R., Rygielski T. (1994). Changes and threats meadow ecosystems as a resultof human pressure. Wiadomości Melioracyjne i Łąkarskie, 1: 40–42.

Krupa A. (2010). Landscape protection in agri-environmental program. Wydawnictwo Biblioteczka Programu Rolno-środowiskowego 2007-2013, Warszawa: 20.

Kutyna I., Malinowska K. (2011). Geographical and historical structure of the flora communities of winter crops cultivation and of a dozen year old fallow. Folia Pomeranae Universitatis Technologiae Stetinensis Agricultura, Alimentaria, Piscaria et Zootechnica, 283 (17): 31–40.

Marks M., Nowicki J., Szwejowski Z. (2000). Uncultivated land and fallow in Poland. Reasons for resting and accompanying phenomena. Fragmenta Agronomica1:6–19.

Roo-Zielińska E., Solon J., Degórski M. (2011). The use of environmental indicators to assess the state and changes in the geographical environment. Wydawnictwo Instytutu Geografii i Przestrzennego Zagospodarowania, PAN, Warszawa: 49–87.

Solon J. (2002). Evaluation of landscape diversity on the basis of the analysis of the spatial structure of vegetation. Wydawnictwo Prace Geograficzne, Warszawa: 185.

Tufekcioglu A., Raich, J.W., Isenhart, T.M., Schultz, R.C. (2001). Soil respiration within riparian buffers and adjacent crop fields. Plant and Soil 229(1): 117–124.

Ziemińska-Smyk M., Wyłupek G.T., Skwaryło-Bednarz B. (2015). Flora in abandoned fields and adjacent crop fields on rendzina soils in the Zamość region. Acta Agrobotanica, 68(3): 197–203.

Żarczyński P., Sienkiewicz St., Krzebietke S. (2008). Accumulation of macroelements in plants on newly established fallows. Journal of Elementology 13(3): 455–461.

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WAYS TO PRESERVE SOIL FERTILITY BASED ON AGROLANDSCAPE

Anna LINKINA*, Elena NEDICOVA

Department of Land Survey and Landscaping, Voronezh State Agrarian University named after Emperor Peter the Great, Russia
*Corresponding author anna_linkina@rambler.ru

ABSTRACT

As it is known, fertility is the most important property of soils to meet the needs of plants in nutrient elements, moisture or air and to provide conditions for their normal life. It is clear that yield of crops depends on soil quality. That is why the preservation of fertility is an important task in the implementation of agricultural production. Agrolandscape systems based on environmental based should be introduced in agriculture to solve the problem of preventing the degradation of soils, reducing water and wind erosion. Principles and methodology of landscape planning can be successfully adopted in farming systems that combine high environmental and economic indicators. During the years with unfavorable conditions such as drought or excessive moisture in farms with adaptive farming the yield is 30 % higher than in agricultural enterprises with the traditional farming system. The article discussed that the minimum value of the environment-stabilizing farm land agrolandscapes should not exceed 40% of the total land. The conducted experiments proved that the humus content in the households under consideration was increased by 0.24%. Average crop yields in the years with unfavorable weather conditions in the test farm (Kantemirovsky District, Voronezh region) are the following: grains 29 dt/ ha, sunflower 24 dt/ha, and for Kantemirovsky District, on average, 19.4 dt/ha and 17 dt/ha respectively. Stability of agrolandscapes depends on correlation between sustainable and destruct lands. Percentage ratio of agricultural lands for the sustainable agrolandscapes is determined.

Keywords: *agrolandscape, adaptive landscape system, soil fertility, Chernozem region.*

INTRODUCTION

As you know, soil fertility is the most important property of soils, which is to provide plant nutrients, moisture, soil and air for their normal functioning.

Crop yield depends on soil quality. Soil is one of the most valuable resources in the world and is by nature of a complex and dynamic ecosystem. Every year the demand for agricultural products increases, so the burden on agricultural land rises (Odum, 1953, reissued 1998).

Soil are degraded due to water and wind erosion, however the imperfect methods of land cultivation, violation of farming practices and agricultural technologies even worsen their condition. There are 196.44 million hectares of eroded land in the world that is 15% of the agricultural land («Food and Agriculture Organization of the United Nations» (FAO, 2014).

In Russia this figure is over 60 million ha of agricultural land that is more than 30%. According to «Food and Agriculture Organization of the United Nations» (FAO, 2014) Russia takes the 3rd place in the world on this indicator. In the Central Chernozem region eroded areas are more than 3 million hectares.

The aim of the study is the development of ways to increase the fertility of soils on the ecological and landscape base for sustainable land management.

The objective of the study is to determine the economic and environmental efficiency under the new introduced elements of the agricultural landscape.

MATERIALS AND METHODS

The agro landscapes in the agricultural enterprises of the Central Chernozem region of Russia are studied. Farms where agricultural landscapes elements increasing theirenvironmental sustainability are introduced, are located in Kantemirovsky district, Voronezh Region (49º 40 N, 39º 51E.), Chernyansky district, Belgorod Region (50°55' N, 37°48' E), Belgorod district of Belgorod Region (50°36' N, 36° 36' E), figure 1.

Figure 1. The Central Chernozem region of Russia

The research was carried out during 7 years, from 2008 to 2015. (A field experiment was conducted during seven years, 2008-2015).

The regions under study are characterized by temperate-continental climate, frost-free period is 140-165 days. The annual precipitation is 480-520 mm. Predominant

soil type is black soil, which are represented by subtypes of podzolized, leached, typical and ordinary chernozems. The relief on the territory of the studied farms is largely dissected by ravines, gullies and hollows. According to the degree of potential erosion hazards arable areas belong to different classes and have from a minor (2 t/ha) to critical truncation (more than 16 t/ha) (State standard 17.4.4.03-86, 1986).

The efficiency of the landscape specific agriculture was evaluated on environmental and economic indicators, among which are the percent forest cover of arable land, area under forest, ratio of environment-stabilizing and destabilizing land, plough disturbance of areas, the content of humus in the soil (WRB, 2014) , yield, cost of 1 center.

Graphical models were developed using spatial geoinformatical environment of Arcgis 10.3.All land was divided into two types: environment-stabilizing and destabilizing. The first ones increasing the environmental capacity of the agricultural landscape include forest belts, perennial plantings, shrub-stage, perennial grasses in crop rotations, ponds, ecotones, grasslands, and hayfields. The second group of lands which reduce the environmental capacity, include arable land without perennial grasses, roads, gullies, landslides. The ratio of wetlands was calculated by the formula:

$$K = \frac{A}{B}$$

Where A - environment-stabilizing areas,

B - environment-stabilizing.

RESULTS AND DISCUSSION

The following elements were put in the basis of adaptive system of farming that ensure sustainable ecosystem, high productivity, protection of soil erosion, stabilization and increase of soil fertility:

1. Differential land use. This means that arable land is cultivated by using different technologies, with different intensity. For example, in the farm under study in Kantemirovsky district four technologies, and three types of rotation such as beet, grain (without beets), soil conservation (more than 70% perennial herbs) are used. Marginal arable land is forced out from the rotation, grassing of ravine by means of perennial grasses and shrub vegetation is performed (Lopyrev, Linkina, 2012).

Figure 2.The consolidation of hollows with trees and shrubs. Belgorod district of Belgorod Region

2. The increase in the total forest covers of the territory. This is achieved through a range of activities, which includes the creation of protective forest strips on arable land, the creation of forest strips near hollows and gullets (Volkov, 2005), shrub scenes development, complete forestation of ravines.
In the studied farms blown and openwork forest strips were formed where the main species dominated is lombardy poplar (Pōpulus pyramidālis), among bushes golden currant (Ribes aureum) prevails.
3. The development of anti-erosion hydraulic engineering structures (revetments, bulkhead dams).
4. Contour mechanized cultivation. Cultivation is carried out in contour-straight-line and contour.
5. Increase in the mosaic of agricultural landscapes alternating areas of arable land, forest belts, perennial grasses, etc (Dokuchaev, 1883).

Figure 3. Contour tillage, Voronezh region

6.Development of micro wild reserves for wild fauna, ornithifauna, and entomological micro wild reserves. Example of entomological reserve is presented in figure 4.

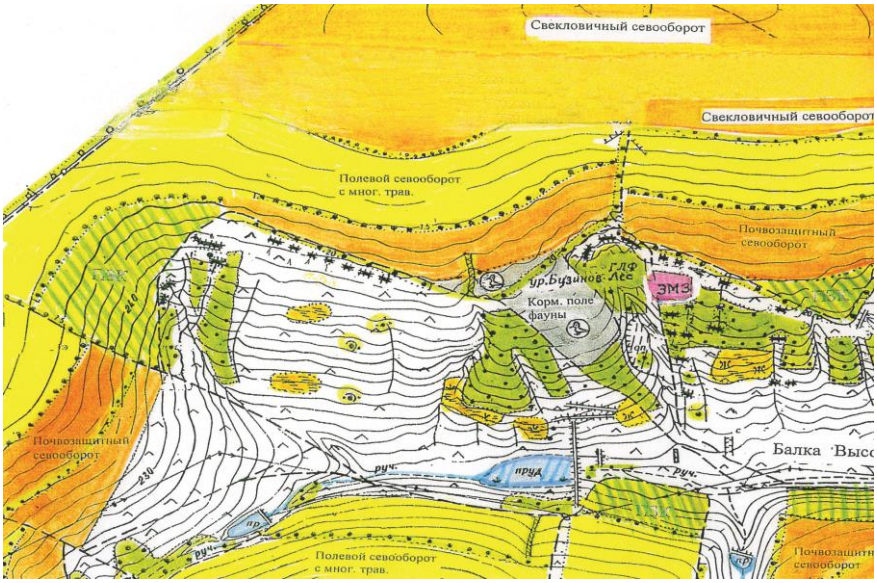

Figure 4. Example of entomological micro wild reserves, Kantemirovsky District. (Lopyrev at all, 2013)

Increasing the share of environment-stabilizing land after the implementation of the ecological-landscape system of agriculture is presented in figure 5.

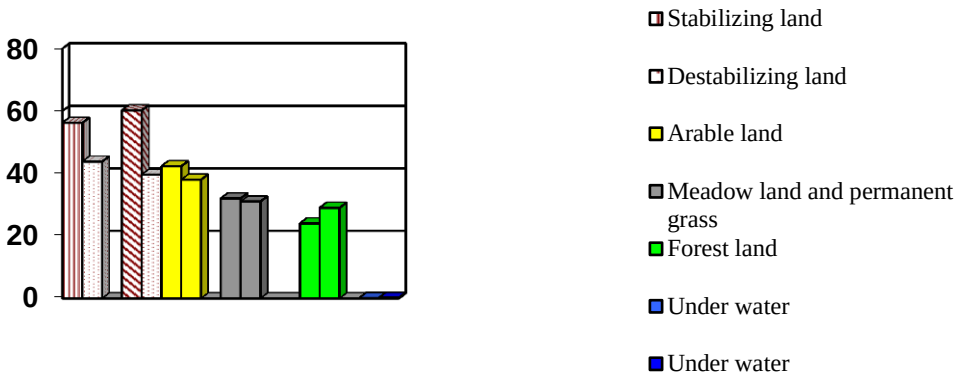

Figure 5. The ratio of wetlands in the agricultural landscape "Losovoy" before and after the introduction of agro landscape farming systems, Kantemirovsky district

Greater efficiency of the implemented activities shows in years with abnormal weather conditions (droughts or, conversely, excessive water logging). The damage from the drought is reduced, the productivity of agricultural crops in comparison with agricultural enterprises with traditional system of land management is increased (Cumani, Rojas, 2016).

The proportion of humus content is increased. Due to the soil studies carried out in 2005 by the staff of the Department of soil science under supervision of Professor K. E Stekolnikov it was proved that the humus content in the households under consideration was increased by 0.24%. Average crop yields in the years with unfavorable weather conditions in the test farm (Kantemirovsky District, Voronezh region) is following: on grains was 29 dt/ ha, sunflower 24 dt/ ha, and for Kantemirovsky District, on average, 19.4 dt / ha and 17 dt / ha respectively. Stability of agro landscapes depends on correlation between sustainable and destruct lands. Percentage ratio of agricultural lands for the sustainable agro landscapes is determined (Lopyrev at all, 2013).

CONCLUSION

As a result of activities on implementation of ecological-landscape farming systems, we can draw the following conclusions. These elements of agricultural landscapes contribute to the increase of environmental capacity, reduce erosion, and stabilize soil fertility. There is a tendency to increase the humus horizon,

improve water and thermal regimes, and to increase in the types of ornito - and entomofauna as well. The need to use pesticides is reduced. Crop yields are risen up to 30% on soils with the same score of bonitet around the area.

REFERENCES

Cumani, M.; Rojas, R. (2016). Characterization of the Agricultural Drought Prone Areas on a Global Scale, FAO, p. 42

Food and Agriculture Organization of the United Nations (FAO). Global plans of action endorsed to halt the escalating degradation of soils [Electronic Recourse]. – Access Regime:
http://www.fao.org/news/story/ru/item/239702/icode/. Accessed on 20/05/2016.

Dokuchaev V. (1883). Russian Chernozem, // Israel Program for Scientific Translations Ltd. (for USDA-NSF), S. Monson, Jerusalem, 1967. (Translated from Russian into English by N. Kaner).

Lopyrev M., Linkina A. (2012). Modernization of farming systems on the ecological landscape basis, Vestnik of the Voronezh State Agricultural University, Voronezh, p.49-56 (in Russian with English abstract).

Lopyrev M. at all (2013).Directory of agricultural landscapes and farming projects (soil conservation, territorial organization of farming systems, resistance to climate change), Voronezh, Voronezh State Agricultural University, 183

Odum E. (1953). Fundamentals of ecology, Philadelphia, Saunders, 384.

Odum E. (1998). Ecology and our endangered life-support systems, Sunderland, Sinauer Associates, 350.

Volkov S. (2005). Agroecological assessment of land, designing of adaptive - landscape systems of agriculture and agro-technologies, Rosinformagrotech, Moskow, p.784

World Reference Base (WRB), (2014). A framework for international classification, correlation and communication, World Reference Base for soil resources, food and agriculture organization of the UK, Rome. [Electronic Recourse]. – Access Regime:
http://www.fao.org/3/a-i3794e.pdf Accessed on 20/05/2016.

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EFFECT OF SOMATIC ANISAKIS SIMPLEX EXTRACT TO DEVELOPMENT CHICK EMBRYOS

Olga I. LAZAREVA, Tatiana N. SIVKOVA*

Department of infectious diseases, Perm State Agricultural Academy, Russia
*Corresponding author: tatiana-sivkova@yandex.ru

ABSTRACT

Helminthes have embryotoxic and teratogenic effects to host's cells and tissues. Investigations were carried out mainly on embryo and fetus in mammals. Blaszkowska, J. (1998); Kadlubowski R. (2000) conducted studies of in chick embryos. Dose-dependent effect is also studied and found that small doses cause embryotoxic effect, while higher doses - teratogenic. Anisakis simplex antigens have also embryotoxic effect has been found. The aim of our research was to examine the effect of A. simplex somatic antigen to the development of chick embryos in its experimental introduction in various ways and at different stages of embryogenesis. For the study chicken embryos were used at different stages of embryogenesis: 7.5, 10.5 and 12.5 days. Methods for introducing antigen chicken embryos: in the allantois cavity, on-chorion allantois membrane and yolk sac at 0.2 ml. The autopsy was performed on embryos 2 and 8 days. The eggs and embryos were weighed and evaluated the embryonic development. As a result of the first experience the greatest change observed by mass, and when administered in the development of antigen in both yolk sac on day 2 (1.68 ± 0.06, unlike control 2.25 ± 0.20), and at 8 days (9.97 ± 6.21 in the experiment versus 14.89 ± 0.78 in the control), respectively. In the second experiment eggs were used at 1 and 5 day incubation embryos. The antigen was administered in both cases also in the yolk sac at a dose of 0.2 ml. In the second experiment there was a delay in the development of both cases at autopsy after 2 days in non-incubated eggs missing the development of, unlike the control, the development of which 48 hours corresponded; including 5 daily observed decrease in weight 0.88 ± 0.22, against 1.05 ± 0.05 and 8 days in a similar way. As a result of experiments on the effect of the Anisakis simplex somatic antigen on the development of chick embryos embryotoxic action installed. Thus antigen has the greatest effect on development of early embryos and putting it into the yolk sac.

Keywords: *Anisakis simplex antigen, chicken embryos, embryotoxic, teratogenic.*

INTRODUCTION

Helminthes and their metabolites have embryotoxic and teratogenic effects to host's cells and tissues. *Anisakis simplex* antigens have also embryotoxic effect

(Sivkova and Berezhko, 2011)Scientists have conducted research mainly on embryos and fetuses in mammals (Bekish et al., 2010;Blaszkowska, 1998a; 2000; 2008;, Kadlubowski). Researchers conducting similar experiments on chick embryos are very few (Blaszkowska, 1998b, Kadlubowski and Blaszkowska, 2000).The researchers studied the dose-dependent effect and found that small doses cause embryotoxic effect, while higher doses - teratogenic. Embryotoxic effect is accompanied by a decrease in weight and / or death of the embryo.

Chicken embryos for the study of vertebrate embryogenesis are very comfortable model, because there is no harm to the mother's body. For research may be applied various methods: embryonic, histological and cytological and other (Trunova, 2008). The aim of the study was to investigate the influence of A.simplex somatic antigen to the development of chick embryos in the experimental introduction of a variety of ways and at different stages of embryogenesis.

MATERIALS AND METHODS

For the study chicken embryos were used at different stages of embryogenesis: 7.5, 10.5 and 12.5-days. Egg was one of the same parties, the breed Ross-308. Automatic Incubator Microprocessor-controlled RCom 50 Pro (South Korea) was used for the incubation of eggs. We used the following incubation conditions: temperature (37.4-37.8° C), humidity (60-70%), ventilation and turning the eggs every hour, was created for the development of chicken embryos.Ovoscope was used to control the experiment and assess the viability of the embryo.

The investigated *A.simplex*extract was injected to chicken embryos according to the standard method(Blieva, 2010). Before the introduction of the antigen eggs appeared through, checking the availability of contribution of blood vessels of the embryo and have noted pencil borders pneumatic bag, the location of the embryo and place of antigen injection. Injection of the antigen was performed in aseptic conditions using of a sterile instrument. Disinfection of eggs is performed by treatment with 70% ethyl alcohol solution and by flame. The *A.simplex*extract was injected with a sterile insulin syringe and a sterile needle. Place of injection were sealed sterile tissue adhesive plaster, and then embedded in paraffin. For the study chicken embryos were used at different stages of embryogenesis: 7.5, 10.5 and 12.5 days. Methods for antigen introducingto chicken embryos: in the allantois cavity, on-chorion allantois membrane and yolk sac at 0.2 ml. For each method of administration of the extract and control were taken at three chick embryo.

After injectionof the *A.simplex*extract embryos were placed to the incubator.The autopsy was performed on embryos 2 and 8 days,pre for 4-6 hours at 4°C embryos were placed into the cold. The eggs and embryos were weighed and evaluated the embryonic development. Egg's shell is treated with alcohol and removed gently, examined on-chorion allantois membrane, amniotic fluid and removed embryo.Chicken embryo was evaluated age and its degree development during incubation, by comparing the standard values(Dyadichkinaet al., 2010)Control remained intactbiocontrol conducted during the experiment to monitor the development of chicken embryos. For the second experiment we used 1-day and 5-day chick embryos. In both cases the antigen injected at a dose of 0.2 ml in the yolk sac.

RESULTS AND DISCUSSION

In the intact group changes are not were observed, the weight, the age of the chicken embryo and the degree of their development corresponded to established standards. In the experimental group were observed changes in weight, results are presented in Table 1.

Table 1. Development of 7-12-day-old chick embryos after influence of *A. simplex* somaticantigen extract

Age of chick embryos, day	2 days Incubation			
	Control		Experience	
	egg weight, g	embryo weight, g	egg weight, g	embryo weight, g
7,5	56,35±0,30	2,25±0,20	54,07±0,49*	1,68±0,06*
10,5	53,40±1,05	5,48±0,03	54,87±0,31	6,13±0,21*
12,5	56,08±4,23	14,00±0,85	55,87±0,79	13,83±0,82
8 days Incubation				
7,5	59,83±1,78	14,78±0,78	56,27±0,91	9,97±6,21
10,5	54,13±1,38	26,6±1,25	55,83±0,26	26,33±0,39
12,5	54,30±0,90	46,60±0,75	52,80±1,50	44,58±1,38

*P≥0,05

We have found that in the control after two days of incubation in 7.5 day embryos which antigen was injected into the yolk sac of development corresponds to 9 days. They were formed: a curved beak, egg tooth forever elliptical shape, and the beginnings of feathers. Chicken embryos lagged behind in development, were visually less in experience than in the controls.

Two days later the embryos dissected and evaluated their age. Embryos after antigen injection into the allantois cavity corresponded to 11 days incubation in the control. In the experiment the age and development of chicken embryos match to 12 days of incubation.

At chick embryos observed slight decrease in weight in contrast to the control. In embryos were clearly visible claws on his fingers, ¾ cornea was covered with the lower eyelid, the opening between the eyelids in the form of a narrow slit.

In the control group it was found 12.5-day embryos were which antigen injected in yolk sac, the development corresponded to 14-15 days after two days incubation. We were observed yolk sac has been reduced, the eyelids were closed. We found that in the experience, not all at in embryos were closed eyelids; their age corresponds to 13 days.

We have discovered that 7.5-day-old embryos in the control after eight days of their development corresponded to 15 days.The yolk sac has been reduced, the amount of allantois fluid was increased, the protein tunica was very small and the eye lids were closed. However, it was noted that in experiment in one embryo the development was absent, namely stopped for 6-7 days. It was observed that the

embryo's beak has been pushed forward, egg tooth is missing, the second finger of the wing was longer than the others, the toes are webbed, and the mass was less than one gram to contrast to other the rest embryos, mass was which 14.0 grams (Fig.1).

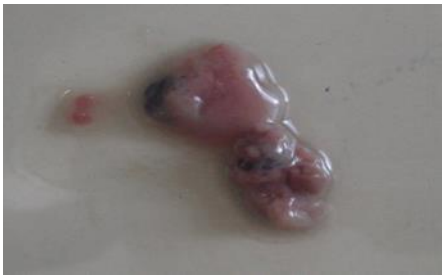

Figure 1. 7.5 day embryos, after *A.simplex* antigen introduction of in the yolk sac, the incubation of 8 days

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With the antigen injection into the allantois cavity of 10.5 daily, developing chick embryos controls correspond to 18 days. In the control group all the embryos head was under the right wing, and was sent to the air chamber. In the experiment we found that one embryo of the head was in the opposite direction that is downward, in other embryos head is also directed towards the air chamber, but are not under the right wing, a little lagged behind at development.

In control 12,5- days embryos conform by age 20 days. We found when compared control with experiment in which at two embryos was not hole at on-chorion allantois membrane, at one embryo was not retracted yolk sac, and was also allantois fluid, which in this should not be aged. In the second experiment, the following results were obtained Table 2.

Table 2. Development of 1-5-day-old chick embryos after *A. simplex* somatic antigen extracts influence

Age of chick embryos, day	2 days Incubation			
	Control		Experience	
	egg weight, g	embryo weight, g	egg weight, g	embryo weight, g
1	64,40±1,70	-	62,73±1,21	-
5	60,98±2,13	1,05±0,05	61,80±2,20	0,88±0,22
8days Incubation				
1	66,88±0,17	1,43±0,08	65,87±2,86	0,40±0,50
5	60,63±0,23	11,70±0,40	64,17±1,08*	9,10±3,27

*P≥0,05

In the control group was foundafter 48 hours where in the antigen injected into the yolk sac 1-day chicken embryos their development correspond to 48 hours of incubation, blastodisk was formed, were visible blood vessels, which is the norm. In the experiment it was found the lag in the development. The age of two embryos

corresponded to 24 hours (Fig. 2), and observed in an egg yolk uneven coloration and was a gas bubble.

After 2 days, we evaluated the age of 5-day-old embryos in the control group corresponded seven days of incubation; and in the experimental group at one embryo weight was 2-fold less than the other (Figure 3).

Figure 2. Experiment 1-day chicken embryos injection*A.simplex* antigen 2 days incubation, development corresponds to 24 hours

Figure 3. Experiment 5-day chicken embryos injection antigen A.simplex 2 days incubation, the arrow shows the embryo lagging by weight

The autopsy revealed that the age of 1-day embryos after 8 days of incubation in control age was 7-8 days. We did not observe any changes in the development of. In the experimental group, development stopped at two chicken embryos 72 hours and 48 hours; one embryo was observed lag by weight (Fig. 4).

After 8 days, we did not detect changes in development among the 5-day embryos in control; they were on the description 13 days.In the experience of the development has stopped with one embryo for 7-8 days. He had the characteristic shape of the head, beak, feathers beginnings, he was prone to maceration, and his cloths were dirty pink and flabby, due to hemolysis(Fig. 5).

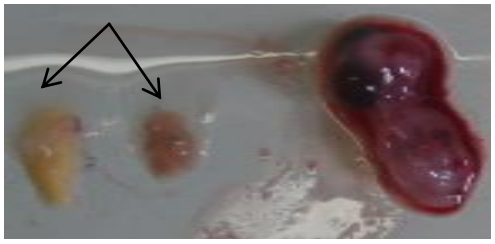

Figure 4. Experience 1-day chicken embryos under the *A.simplex* antigen action, incubation of 8 days, and the arrows indicate the embryos whose development stopped at 72 and 48 hours of incubation

Figure 5. Experience 5-day chicken embryos under the *A.simplex* antigen action, incubation of 8 days

CONCLUSION

As a result of these experiments is set:

1.*Anisakis simplex*somatic extract has embryotoxic effect on embryo chicken eggs;

2.Chicken embryos were more sensitive to *A.simplex*somatic extract 1-5 days of embryonic development.

3.*A.simplex*somatic extract has a greater impact on chicken embryos when injected into the yolk sac, than when it is introduced into the allantois cavity, on-chorion allantois membrane.

REFERENCES

Bekish, V., Gidranovich, V., Bekish, L. / Bekish, V., Gidranovich, V., Bekish, L., Konevalova, NA, Zorin, V., Pashinskaya, E. (2010). Effects of parasites on the body of mammals and human during pregnancy. Herald VSMU, Vol. 9(2), P.1-12.

Blaszkowska, J. (1998a).Embryotoxic and teratogenic action of trypsin inhibitor of Ascaris lumbricoides in mice. Acta Parasitological, Vol. 43(2), P. 103–108.

Blaszkowska, J. (1998b).The effect of Ascaris suum homogenate and its proteolysis inhibitors on chicken embryos. Helminthology, Vol. 35(1), P. 37–42.

Blaszkowska. J. (2000). Disturbance of mouse pregnancy after injection of Ascaris homogenate during early organogenesis. Wiad. Parazytol., Vol. 46(3), P.369-378.

Blaszkowska, J. (2008). Prenatal toxicity of Ascaris pepsin inhibitor in mice. Reprod. Toxicol., Vol.25(2), P.263-270.

Blieva, L. (2010). Methods cultivation, indication and identification of viruses. Methodical recommendations on the organization of a self-study course "General Virology" Kab.-Balk. Univ, Nalchik, P.48.

Dyadichkina, L., Pozdnyakov, N., Glavatskih, O., Melekhina, T., Tishenkov, A. et al. (2010).Manual for the biological control of the incubation of the eggs of poultry. Guidelines. State Scientific Institution. VNITIP RAAS, Sergiev Posad, P.84.

Kadlubowski, R. (1993). Teratogenic action of alpha-chymotrypsin inhibitor of Ascaris lumbricoides in mice. Med.Dosw.Mikrobiol.Vol.45(3),P.401-405.

Kadlubowski R., Blaszkowska J. (2000) The effect of Ascaris lumbricoides suis homogenate and its trypsin inhibitor in chick embryo. Wiad. Parazytol., Vol. 46(3), P. 369-378.

Sivkova, T., Berezhko, V. (2011). Products of metabolism of larvae *Anisakids* their action kariopatic and pathological effects: monograph, Perm State Agricultural Academy, Perm, P.132.

Trunova, A. (2008). Chicken embryo under the influence of ambrosial antigen and features of development immunegenesis: the dissertation ... candidate. biol. Sciences, Stavropol, P.181.

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SPATIAL STRUCTURE OF THE LIPIZZAN HORSE GENE POOL BASED ON MICROSATELLITE VARIATIONS ANALYSIS

Radovan KASARDA*, Nina MORAVČÍKOVÁ, Ondrej KADLEČÍK

Department of Animal Genetics and Breeding biology, Slovak University of Agriculture in Nitra, Nitra, Slovakia
*Corresponding author: radovan.kasarda@uniag.sk

ABSTRACT

The aim of this study was to determine the current state of genetic diversity and to assess the substructure and spatial structure at individual level based on analysis of microsatellite variations within Lipizzan horse population. The genomic DNA samples were obtained from totally 418 horses, originating from Slovenian (357) and Slovak (61) studs. A set of 13 microsatellite markers (AHT4, AHT5, ASB2, HMS1, HMS2, HMS3, HMS6, HMS7, HTG10, HTG4, HTG6, HTG7, and VHL20) have been used for analysis of genetic variability. Across all microsatellite loci the average number of alleles 6.65 and effective allele number at level 3.37 were found. The obtained Shannon's information index (I=1.37) indicated high degree within population genetic diversity. The prevalence of heterozygous genotype in sample confirmed also the average value of observed heterozygosity (Ho=0.67) and FIS index (-0.026). The most of the genetic variation in sample was conserved within individuals (95%) and the subdivision of horse populations explained only 4%. Similarly, the obtained pairwise values of FST index (0.02) and Nei's genetic identity (0.90) reflected mainly common ancestors used in breeding history of both population. But the principal coordinate analysis showed the division of individuals into the two separate clusters according to the studs where they come from. The membership probability resulted from spatial structure analysis suggested that the frequencies of alleles varied across the two regions that indicated the evidence of strong distinction in relation to the current breeding status of analysed populations and strategy of studs.

Keywords: *diversity, genetic markers, Lipizzan, population substructure.*

INTRODUCTION

In the last years the issue of conserving the genetic diversity as a component of the conservation of the environment has been raised at an international level. In this respect, one of main aspects of scientific research activities is conserving the biodiversity of local genetic resources, especially those of economic and cultural interest (Georgescu and Costache, 2012). Worldwide, the populations of numerous domestic animals, especially horses, are in steady decline, with some already

extinct, thereby affecting both inter and intra-breed diversities (Bömcke et al., 2011). Among many genetic issues involved in the conservation of such populations, a crucial question is how much gene flow should be maintained between populations (Mahrous et al., 2011).

Despite lower production and abilities to compete with high-productive breeds, local breeds are still important for countries as their heritage. Out of 27 horse breeds kept in the Slovak Republic 8 could be considered as endangered. Within them the population of Lipizzan horses belong based on the population size to critically endangered groups. Its effective management requires comprehensive knowledge of the population characteristics, including data on effective population size andstructure, geographical distribution, the production environment, and within and between-breed genetic diversity.Integration of these types of data will result in themost complete representation possible of biological diversityand will thus facilitate the effective preservation of breed (Groeneveld et al., 2010).

Generally, the Lipizzan as one of the oldest European horse breed deserve special attention because they represent an important gene pool, containing genetic material from several important historical breeds, some of which are almost extinct (Dovc et al., 2006; Barcaccia et al., 2013). The original herd in Lipica has been formed by stallions and mares imported from Spain. Later, imports of Andalusians, Barbs, and Italian horses at the beginning of 19th century, Arab horses contributed to the formation of the Lipizzan gene pool (Barcaccia et al., 2013). Currently, the base population of Lipizzan horses is divided into a number of rather large, mostly state-owned, studs with limited exchange of horses over the last few decades. The breeding goals of the studs are partly changing over time. Whereas the primary goal of the Austrian stud at Piber is still to provide horses for classic dressage at the Spanish Riding School Vienna, the Hungarian stud in Szilvásvárad has specialised in breeding of top horses for coach driving. The Slovenian, Slovakian and Croatian studs are breeding riding horses while the Romanian studs are providing stallions for improvement of the local farm horse population. Such differences in breeding may be reflected in the morphology of the horses and lead to a separation of the genetic pools of the breed (Zechner et al., 2001; Zechner et al., 2002).

The molecular genetic studies of diversity regarding the horse populations are largely based upon microsatellite markers which offer advantages that are particularly appropriate for conservation projects (Bordonaro et al., 2012). During the last years many studies of horse populations have described their usefulness for estimation of genetic relationship on both intra- and interbreed level as well as for determination of genetic diversity among and within populations (Achmann et al., 2004; Dovc et al., 2006; Bordonaro et al., 2012). Nowadays, microsatellites are being progressively replaced by SNP markers, but in small populations or breeds genotyping with high density SNP chips turns out to be very expensive, thereby limiting the availability of such data (Conant et al., 2011).

The aim of this study was to determine the current state of genetic diversity in the Slovak nucleus of the Lipizzan horse breed in comparison to the Lipizzan population originating from Slovenian studs based on microsatellite markers. The

analyses of substructure and spatial structure of populations have been prepared to provide information of potentially separation of both populations from each other that could result due to the differences of breeding strategy within each stud.

MATERIALS AND METHODS

The genotyping data have been collected from a total of 418 individuals with Slovak (61) and Slovenian (357) origin. The collected sample of 61 Slovak animals represents the nucleus of Lipizzan horses kept in Slovakia. Across and within populations, the variations of thirteen microsatellite markers primarily recommended to paternity testing (*AHT4*, *AHT5*, *ASB2*, *HMS1*, *HMS2*, *HMS3*, *HMS6*, *HMS7*, *HTG10*, *HTG4*, *HTG6*, *HTG7*, and *VHL20*) have been analysed to evaluate the Lipizzan horse gene pool.

The genetic diversity within and across both Lipizzan populations was measured as the mean number of alleles (MNA), observed heterozygosity (H_o), gene diversity expressed as expected heterozygosity (H_e), effective allele number (N_e) and Shannon's information index (I) using the Genalex version 6.1 (Peakall and Smouse, 2012). The significance of differences between observed and expected genotype frequencies that reflects the departure of HWE (Hardy-Weinberg equilibrium) has been tested by Chi-square test. The genetic differentiation (F_{ST}) and amount of inbreeding-like effect across (F_{IT}) and within (F_{IS}) Lipizzan populations were assessed based on Wright's F-statistic according to Weir and Cockerham (1984).

The molecular variance analysis (AMOVA) that estimates the genetic structure indices based on information about allelic content of haplotypes, as well as their frequencies stored enter as a matrix of Euclidean squared distances was performed based on 10,000 permutations using the Arlequin v3.5 (Excoffier et al., 2005). The genetic relationships among analysed individuals arising from the microsatellite variations were evaluated on individual level using Nei's distance and on population level based on Wright's F_{ST} index. Subsequently, the population genetic structure was tested based on the principal coordinate analysis (PCoA) using Genalex version 6.1 and discriminant analysis of principal components (DAPC) implemented in R package *adegenet* 1.3-0 (Jombart and Ahmed, 2011). The spatial Bayesian clustering algorithm adopted in Tess 2.3.1 (Chen et al., 2007) was then used to assign individuals into the clusters and evaluate their membership probability. In the analysis assuming admixture both the CAR and the BYM models was used to define the spatial prior for admixture proportions. Twenty runs were simulated from K=2 to K=10. The run with lowest DIC value was considered as the best and provided information about the hard clustering assignment of individuals and their neighbourhood system. The results from analyses were visualized by R software v 3.2.2 (R Core Team, 2013).

RESULTS AND DISCUSSION

Across thirteen analysed microsatellites totally 114 alleles were identified. The number of alleles per locus ranged from 5 (*AHT5*) to 12 (*HTG10*). The effective

allele numbers per locus was in average 3.27±0.18. The value of Shannon's information index of phenotypic diversity for molecular profiles at level of 1.35±0.06 was found. The average value of I index across all loci also reflected high degree of overall genetic variability across populations, because the Shannon's information index generally reflected the effectiveness of microsatellite markers to reveal the genetic variations. The value of observed heterozygosity ranged across microsatellites from 0.58 (*HMS1*) to 0.80 (*HMS7*), whereas the gene diversity varied from 0.55 (*HMS1*) to 0.77 (*HMS3*). Both of indices signalised sufficient proportion of genetic variability across evaluated individuals in relation to maintain of populations biodiversity. Moreover, the inbreeding-like F_{IS} index had an average negative value (-0.026), suggesting excess of heterozygous genotypes across animals. Using Chi-square test the departure from HWE ($P<0.05$) was identified up to 7 microsatellites (*VHL20*, *HTG4*, *HTG6*, *HMS6*, *ASB2*, *HTG10*, and *HMS3*).
Within the Slovak and Slovenian populations comparable state of diversity was found. A summary of analysed genetic diversity indices across all markers for both populations is given in table 1. The Wright's inbreeding coefficients F_{IS} and F_{IT} were considered as a measure of heterozygosity excess or deficiency. In accordance with identified level of heterozygosity the F_{IS}=0.03±0.02 (single animals compared to the subpopulation) and F_{IT}=0.05±0.03 (single animals compared to total population) also signalized only very low excess of homozygous loci across the Lipizzan populations. The F_{ST} index at level 0.02 indicated expected low degree of genetic differentiation between Slovak and Slovenian studs and reveals that only 2% of genetic variation was conserved between both populations.

Table 1. The descriptive statistic of analysed populations related to diversity evaluation

Population	Diversity indices					
	MNA	N_e	I	H_o	H_e	F_{IS}
Slovak Lipizzan	6.92±0.49	3.59±0.26	1.43±0.07	0.65±0.04	0.70±0.02	0.71±0.02
Slovenian Lipizzan	6.38±0.45	3.14±0.18	1.30±0.06	0.69±0.02	0.67±0.02	-0.03±0.01

To explain the proportion of differences influenced by the origin of evaluated Lipizzan horses the analysis of molecular variance was applied on genotyping data. The AMOVA showed similarly as F-statistic that most of the variance was explained by the differences conserved across individuals within separate populations (95%). The subdivision of Lipizzan horses into the subpopulation according to country of origin reflected only 4% of genetic variation and the rest of variability (1%) was divided within the individuals in whole population. In this regard the expected high level of genetic connectedness between both Lipizzan subpopulations confirmed also the value of Nei's genetic distance. Based on generally accepted criteria obtained D_A value at level 0.10 can be considered as low and the animals from Slovak and Slovenian studs only slightly genetically

differentiated. Figure 1B showed the minimum spanning network among analysed individuals constructed based on Nei's genetic distance matrix.

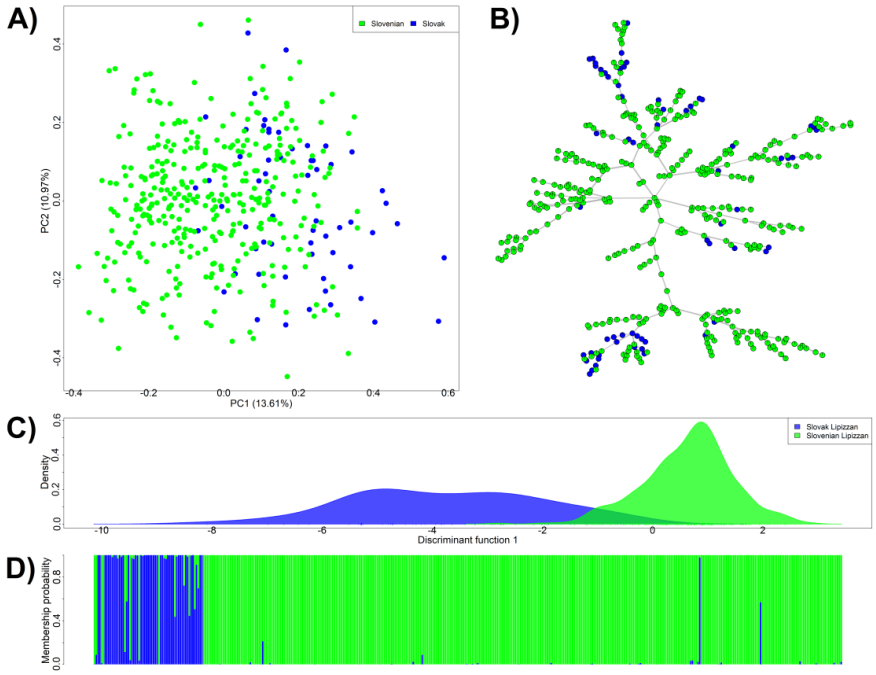

Figure 1. The structure of population based on PCoA analysis (A), the minimum spanning network representing genetic relationship between individuals based on Nei's distances (B), genetic clusters determined based on first discriminant function (C), and membership probability resulting from TESS analysis (D).

The principal coordinate analysis of population structure (Figure 1A) showed non-significant division of individuals in to the clusters according to its origin that can signalized relatively high degree of admixture between individuals originated from Slovak and Slovenian studs mainly due to the migration events during their breeding history. However, the discriminant analysis of principal components indicated the strong division among individuals into the clusters in relation to country of origin. The distinction of individuals into the groups produced by Bayesian Information Criteria (BIC) analysis showed that inferred cluster corresponded to the initial defined population membership. Based on the BIC analysis the K=2 was chosen as optimal. For sufficient reassignment of individuals 48 PCA axes were retained in DAPC that corresponded to more than 50% of variance conserved in analysed dataset. Based on the first discriminant function we were able to clearly detect two main genetic clusters in relation to the each of analysed population (Figure 1C). Similarly, the Bayesian assignment analysis

adopted in TESS that takes alongside genotyping data also the spatial distribution of samples into account showed lowest DIC value for K=2. The membership probability resulted from spatial structure analysis suggested that the frequencies of alleles varied across the two regions that indicated the evidence of strong distinction in relation to the current breeding status of analysed populations and strategy of studs (Figure 1D).

The genetic diversity of the Slovak Lipizzan horses was investigated as a part of studies included European countries representing a large fraction of the Lipizzan population (Achmann et al., 2004; Dovc et al., 2006). The analysis revealed comparable state of genetic diversity with our results within each of analysed population. Moreover, the authors similarly showed that the breeding history of the Lipizzan horses was transferred to the current genetic relationship among fragmented populations. Achmann et al. (2004) found that the Slovak Lipizzan population seems to be genetically closer to the Croatian and Hungarian than to subpopulations of Austria, Italy and Slovenia which represent the classical gene pool of Lipizzan horse breeding. One of the reason that can explain these results is mainly the fact that the Slovakian subpopulation, which was formed after the 1ST World War from the horses from Lipica, has been later due to breeding goal, geographical and socio-political barriers exchanged relatively more with horses from Croatia, Hungary and Austria than with other subpopulations.

Being among the oldest horse breeds in Europe and because of its historical connection to the Austro-Hungarian Empire, the Lipizzan horse is a living part of the European cultural heritage (Achmann et al., 2004). Within the context of horse population and breed conservation, genetic characterization is the first step in the development of proper management strategies, and molecular information is crucial for both preserving genetic diversity and preventing undesired loss of rare alleles in the Lipizzan breed (Barcaccia et al., 2013). In the connection to the pedigree data and morphological measures the genetic markers provide the basis to support the improvement of classical breeding strategies and allow the molecular genetic control of purebred status of the Lipizzan horse breed.

CONCLUSION

The present study was prepared in order to characterize current state of diversity within two Lipizzan subpopulations from Slovak and Slovenian studs based on microsatellite variability. The Slovak population represent the nucleus of Lipizzan breed that is due to the small effective population size considered in Slovakia as endangered. The results showed that both of the populations revealed sufficient level of genetic variability in the context of maintain the local genetic resources of each country. The information of the current state of molecular inbreeding and also genetic relationship between individuals within each population and among studs has important impact on maintaining of its gene pools on local level. Our study showed that the differences in breeding strategy has led to their separation from each other that indicated the evidence of strong distinction in relation to the current breeding status and strategy of studs.Due to the close genetic connection among the

Lipizzan studs resulting from the intensive animals exchange during their history the use of breeding line typical for each studs is currently prefer in order to preserve the local genetic resources of each country.

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REFERENCES

Achmann, R., Curik, I., Dovc, P., Kavar, T., Bodo, I., Habe, F., Marti, E., Sölkner, J., Brem, G. (2004). Microsatellite diversity, population subdivision and gene flow in the Lipizzan horse, Animal Genetics, 35, 285 - 292.

Barcaccia, G., Felicetti, M., Galla, G., Capomaccio, S., Cappelli, K., Albertini, E., Buttazzoni, L., Pieramati, C., Silvestrelli, M., Verini Supplizi, A. (2013). Molecular analysis of genetic diversity, population structure and inbreeding level of the Italian Lipizzan horse, Livestock Science, 151, 124 - 133.

Bömcke, E., Gengler, N., Cothran, E.G. (2011). Geneticvariability in the Skyrospony and its relationship with otherGreek and foreignhorsebreeds, Genetics and Molecular Biology, 34 (1), 68 - 76.

Bordonaro, S., Guastella, A. M., Criscione, A., Zuccaro, A., Marletta, D. (2012). Genetic diversity and variability in endangered Pantesco and two other Sicilian donkey breeds assessed by microsatellite markers, Scientific World Journal, 2012, 648427.

Chen, C., Durand, E., Forbes, F., François, O. (2007). Bayesian clustering algorithms ascertaining spatial population structure: A new computer program and a comparison study, Molecular Ecology Notes, 7, 747 - 756.

Conant, E. K., Juras, R., Cothran, E. G. (2011). A microsatellite analysis of five Colonial Spanish horse populations of the southeastern United States, Animal Genetics, 43 (1), 53 - 62.

Dovc, P., Kavar, T., Sölmner, H., Achmann R. (2006). Development of the Lipizzan Horse Breed, Reproduction in Domestic Animals, 41, 280 - 285.

Excoffier, L., Laval, G., Schneider, S. (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis, Evolutionary Bioinformatics Online, 1, 47 - 50.

Georgescu, S. E., Costache, M. (2012).Genetic Characterization of Romanian Local Breeds Using Microsatellite Markers.In M. Caliskan (eds.) Analysis of Genetic Variation in Animals (InTech), 27 - 42.

Groeneveld, L. F., Lenstra, J. A., Eding, H., Toro, M. A., Scherf, B., Pilling, D., et al. (2010). Genetic diversity in livestock breeds, Animal Genetics, 41 (Suppl. 1), 6 - 31.

Jombart, T., Ahmed, I. (2011). Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data, Bioinformatics, 1, 3070 - 3071.

Mahrous, K. F., Hassanane, M., Abdel Mordy, M., Shafey H. I., Hassan, N. (2011). Genetic variations in horse using microsatellite markers, Journal of Genetic Engineering and Biotechnology, 9, 103 - 109.

Peakall, R., Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update, Bioinformatics, 28, 2537 - 2539.

R Core Team. 2013. R: A Language and Environment for Statistical Computing (v3.2.2). R Foundation for Statistical Computing, Vienna, Austria.

Weir, B. S., Cockerham, C. C. (1984).Estimating F-statistics for the analysis of population structure, Evolution, 38, 1358 - 1370.

Zechner, P., Sölkner, J., Druml, T., Baumung, R., Achmann, R., Bodo, I., Marti, E., Habe, F., Brem, G. (2002). Analysis of diversity and population structure in the Lipizzan horse breed based on pedigree information, Livestock Production Science, 77, 137 - 46.

Zechner, P., Zohman, F., Sölkner, J., Bodo, I., Habe, F., Marti, E., Brem, G. (2001). Morphological description of the Lipizzan horse population, Livestock Production Science 69, 163 - 77.

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MORPHOLOGICAL AND CHEMICAL PROPERTIES OF MEDLAR (*MESPILUS GERMANICA* L.) FRUITS AND CHANGES IN QUALITY DURING RIPENING

Melekber SULUSOGLU DURUL[1,2]*, Hulya UNVER[3]

[1]Kocaeli University, Arslanbey Agricultural Vocational School, 41285, Kocaeli, Turkey
[2]Department of Horticulture, Graduate School of Natural and Applied Sciences, Kocaeli University, 41380 Kocaeli, Turkey
[3]Department of Horticulture, Agriculture and Natural Science Faculty, Duzce, Turkey
*Corresponding author: meleksl@kocaeli.edu.tr

ABSTRACT

Medlar has acquired increasing popularity in recent years for its edible fruits and some healing properties in modern medicine. Medlar fruits are often stored under non-cold conditions in straw. This study was conducted to determine morphological and biochemical characteristics of medlar fruits and changes in fruit quality occurring under ordinary storage conditions. For this purpose, eight types of medlar trees were evaluated. The trees were at a mature stage and productive. Morphological properties such as the fruit and seed weight (g), length, and width (mm) were measured after the harvest. Fruit soluble solid content (%) and pH values were determined at physiological maturity after harvesting, and the fruit soluble solid content was measured again at ripening (edible stage), after 25 days of storage under ordinary storage conditions (mean temperature of 10 °C and mean humidity of 65–70%). Leaf characteristics were also determined. The tree productivity was very different between the types, and it was determined to range from 5.9 and 17.8 kg. The fruit weight varied from 9.69 to 24.45 g, while the water content decreased nearly to the half of the harvest values in some genotypes. The soluble solid content changed depending on the water losses and increased during the ripening period. The seed numbers ranged from 1.7 to 4.7 among the types, and the seed weight varied from 0.12 to 0.45 g. The fruits were able to reach ripening maturity in three weeks under the ordinary storage conditions depending on the type. Thus, this method of storage is practical for the medlar producer, but the results clearly showed that the storage period was too short and that the fruit quality was negatively affected. Cold storage conditions are needed to ensure quality and a long selling time.

Keywords: *medlar, fruit characteristics, storage, ripening.*

INTRODUCTION

The medlar (*Mespilus germanica* L.) is a deciduous species belonging to the family Rosaceae and native to Southeastern Europe, Anatolia, and Caucasia. Medlar trees produce brown, sometimes reddish-tinged, pear- and apple-shaped fruits (Selcuk and Erkan, 2015). The fruits are astringent and hard at harvesting time but become sweet and reasonably pleasant when fully ripe, with a white flesh surrounding a few small stones inside. The fruits are eaten fresh, and rich in potassium (Glew *et al.*, 2003a) and amino acids (Glew *et al.*, 2003b). The fruits of medlar are used as a nutritional material by local customers and are consumed as marmalade and jellies (Baytop, 1999). It is a very healthy fruit, with phytochemicals including antioxidants (Ayaz *et al.*, 2008; Selcuk and Erkan, 2015). Medlar fruits are used as a treatment for constipation, as a diuretic, and for kidney and bladder stones (Baird and Thieret, 1989). The medlar shows better pest and climate resistance than most other fruit species of landscaping importance.

Recently, more attention has been paid to morphological and biochemical properties of different types of medlar. Some researchers have investigated the distribution of medlar (Yılmaz and Gerçekcioğlu, 2013), phenological stages (Atay, 2013), and pollen characteristics (Cavusoglu and Sulusoglu, 2013). The fruit is a typical climacteric fruit, which becomes brown, soft, sweet, and edible after and during storage (Dirr, 1990). The fruits are harvested during October and November, and ripening occurs later under storage conditions. The fruits are stored in a dry cool place until they are over-ripe. The green and hard flesh of the fruit softens and changes its color to light brown. This process is called overripening, and after that, the fruit becomes suitable for consumption. The pulp then has a distinctive, slightly sour flavor and can be consumed directly (Baird and Thieret, 1989).

Changes in sugars, organic acids, and fatty acids of consumed medlar have been reported during the post-harvest period (Glew *et al.*, 2003). In previous studies, mechanical properties and the chemical composition of medlar were described during maturation and ripening (Rodriguez *et al.*, 2000; Rop *et al.*, 2011; Veličković *et al.*, 2013). Changes in fruit quality are important for marketing of medlar fruits. Therefore, in this study, in addition to physical properties, mechanical and chemical properties of medlar fruits were investigated at physiological maturity and during the ripening period.

MATERIALS AND METHODS

This study was carried out in Kocaeli province (Turkey) for two growing seasons, between 2013 and 2014. The province of Kocaeli is located in the northwestern part of Turkey, at the latitude of 40°42′ N, longitude of 30°01′ E, and at 76 m above the sea level. The research area has a hybrid climate with a humid subtropical climate on the south Marmara Sea coast, an oceanic climate on the Black Sea coast, and a humid continental climate in the interior. Summers are warm to hot, humid, and moderately dry, whereas winters are cold, wet, and

sometimes snowy. The material for the study consisted of eight types of medlar trees cultivated in the research garden of the Kocaeli University Arslanbey campus. Each tree was considered a type. All trees were at the same age (10 years old) and productivity period and represented healthy mature plants.

Fruit characteristics: Fruit characteristics of the types, such as the fruit weight, width, and length, were determined for 10 fruit samples picked up randomly from each type. The weight of the fruit was determined using a 0.01-g sensitive balance. The measurements of the length and width (diameter) of the fruits, the length of the fruit stalk, and the width, and length of the calyx basin were carried out using a 0.01-mm sensitive digital compass caliper.

Leaf characteristics: The leaf width and length and the length of the leaf stalk were measured for 10 leaves with a 0.01-mm sensitive digital compass caliper.

Seed characteristics: The seed weight, length, width, and thickness were determined for 10 seeds. The weight of the seed was determined using a 0.01-g sensitive balance. The measurements of the length and width of the seeds were carried out using a 0.01-mm sensitive digital compass caliper.

Biochemical characteristics: Medlar fruits are picked at a hard and green stage, but they are not edible until they become half-rotten or 'overripe', i.e., turn brown and soft. Soluble solid content and pH values of the fruits were measured at the harvest time. The harvested medlars were stored in a cool, dark place until they were suitably overripe and developed an aromatic flavor. At this stage, the soluble solid content was determined again. The weight was recorded again for the same sample fruits that were selected at the green stage to determine the water losses during ripening.

Experimental design and statistical analysis: All measurements (fruit, stone, and leaf) were done for randomly selected samples, and three replicates were used ten samples each. Data were subjected to analysis of variance using the Minitab software (Minitab, Inc.). The data were transformed by arcsine square for percentage means. The means were separated by the Duncan's multiple range test ($P< 0.05$).

RESULTS AND DISCUSSION

Table 1. The characteristics of Fruit and Productivity of Medlar Types

Type	Tree Productivity (kg/tree)*	Fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	Fruit stalk length (mm)	Calyx leaf length (mm)	Calyx leaf width (mm)	Calyx basin length (mm)
1	5.9	6.6 c	22.2de	25.7c	4.9a	12.1ab	0.86	15.4
2	14.5	10.7 c	27.0c	28.3b	2.0c	14.9a	0.81	16.4
3	10.1	10.0cd	26.0c	27.6b	2.3c	12.4ab	0.96	15.9
4	17.8	20.1 a	33.3a	33.6a	3.3bc	11.4ab	0.86	17.6
5	10.6	7.6 de	23.8d	24.1d	2.4c	11.4ab	0.88	16.0
6	10.9	5.2e	21.2e	21.0e	4.4ab	9.61	0.92	13.2
7	11.5	15.1b	29.7b	28.9b	2.9c	13.0ab	1.00	14.7
8	12.5	12.4bc	27.8c	29.4b	3.3bc	11.7ab	0.84	16.7
Mean	11.7	11.0	26.4	27.3	3.2	12.1	0.89	15.7

Only one tree for each type was considered and no statistical analysis was conducted.

The tree productivity and fruit characteristics are given in Table 1. The tree productivity ranged between 5.9 and 17.8 kg. The most productive type in the orchard was type 4. Comparisons were made for all collected and weighed fruits (Table 1; Figures 1 and 2). The highest fruit weight was observed in type 4. Statistically significant differences were obtained for the means of the fruit weight, fruit length, fruit diameter, fruit stalk length, and calyx leaf length.

Figure 1. Medlar fruits at the harvest time.

Figure 2. Ripened medlar fruits.

The fruit diameter and length ranged among the types from 21.2 to 33.3 mm and from 21.0 to 33.6 mm, respectively. The calyx basin diameter and structure directly affected the quality of the fruit (Figure 1). A crack on the fruit lowers its marketable quality. Types 1 and 6 showed more cracks and abnormalities on the calyx side (Figure 3). In previous studies, the fruit weight, length, and diameter were found to range between 9.46 and 40.80 g, 26.53 and 48.73 mm, and 23.67 and 42.51 mm, respectively (Bostan and İslam, 2007), which is comparable with our results. The highest fruit pH value was found in type 6 (4.02), while pH values were between 2.89 and 3.22 in another study (Özkan *et al.*, 1997).

Figure 3. Deformations on the calyx basin side.

The fruit soluble solid contents were measured between 16.4 and 22.2% at the time of harvest. The soluble solid contents varied between 17.0 and 24.0% in earlier studies (Özkan *et al*., 1997; Yılmaz, 2015). The soluble solid content increased in the ripening period for all of the medlar types (Table 2). The fruits lost water during the ripening period (Figure 4), which caused a decrease in quality. The highest water loss occurred in Type 5. Some fresh fruits are susceptible to enzymatic browning during the ripening period, with flesh browning, which generally results in the loss of weight, as well as nutritional and organoleptic qualities (Altunkaya *et al*., 2009). The same results were obtained in this study.

Figure 4. Water losses and deformation of fruits.

Table 2.Changes in biochemical characteristics of medlar fruits during the ripening period

Type	pH at harvest time*	Soluble solid contents at harvest time (%)	Soluble solid contents after ripening (%)	Humidity loses during the ripening period (%)
1	3.68	18.4	22.9	17.16
2	3.97	16.4	22.2	16.75
3	3.75	17.8	23.9	15.45
4	3.88	21.5	27.5	13.57
5	3.78	21.8	26.2	14.41
6	4.02	18.6	22.6	15.52
7	3.80	22.2	25.8	16.44
8	3.85	21.8	25.5	15.28
Mean	3.84	19.8	24.6	15.23

The leaf and leaf stalk lengths showed small and unimportantdifferences among the types; however, there were statistically significant differences in all measured stone characteristics among the types. The average stone number varied from 1.7 to 4.8 per fruit (Table 3).

Table 3. The characteristics of leaf and stone of medlar types

Type	Leaf length (cm)	Leaf width (cm)	Leaf stalk length (mm)	Stone number per fruit	Stone weight (mm)	Stone width (mm)	Stone length (mm)	Seed thickness (mm)
1	7.4bc	3.2	0.57c	1.7b	0.16e	6.4d	10.4d	3.8b
2	7.8bc	3.0	0.57c	4.3a	0.24d	7.5bc	11.5abc	4.2b

3	8.3b	3.4	0.59bc	4.6a	0.23d	7.80bc	11.1bcd	4.1b
4	10.0a	3.5	0.81a	4.7a	0.45a	9.0a	12.5a	5.4a
5	7.9bc	2.9	0.62bc	4.7a	0.45a	7.6bc	11.2bcd	5.4a
6	6.5c	2.9	0.44d	4.7a	0.22d	7.1cd	10.5cd	4.0b
7	7.2bc	3.0	0.58bc	4.8a	0.34b	8.1b	12.5a	5.2a
8	8.1bc	2.9	0.69b	4.7a	0.29c	7.9bc	12.1ab	5.02a
Mean	7.9	3.1	0.61	4.3	0.30	7.7	11.5	4.63

CONCLUSION

After 25 days, darkening, softening, water loss, and flavor development were observed in the fruits. During the ripening period, some fruits lost their marketable value. Ripe medlar fruits are an important source of nutrition, and they become edible only after natural softening and browning. Studies must continue to prevent quality losses and improve quality during overripening.

REFERENCES

Altunkaya A., Becker E.M., Gokmen V., Skibsted L.H. (2009). Antioxidant activity of lettuce extract (Lactuca sativa) and synergism with phenolic antioxidants.Food. Chem. 115:163–168.

Altuntaş E., Gül E.N., Bayram M. (2013). The Physical, Chemical and Mechanical Properties of Medlar (*Mespilus germanica* L.)During Physiological Maturity and Ripening Period. Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi, 30 (1): 33–40.

Atay E. (2013). Phenological Stages of Medlar (*Mespilus germanica* L. 'İstanbul') according to the BBCH Scale J. Biol. Environ. Sci., 7(20), 103–107

Ayaz F.A., Demir O., Torun H., Kolcuoglu Y., Colak A. (2008). Characterization of polyphenoloxidase (PPO) and total phenolic contents in medlar (*Mespilus germanica* L.) fruit during ripening and over ripening. Food Chemistry Volume 106, Issue 1, p. 291–298

Baytop T. (1999). Therapy with medicinal plants in Turkey-past and present, 2nd, Nobel Tip Basimevi, p. 299, Çapa-Istanbul.

Baird JR., Thieret JW. (1989). The medlar (*Mespilus germanica*, Rosaceae) from antiquity to obscurity. Econ Bot 43:328–372

Bostan S.Z., İslam A. (2007). Doğu Karadeniz Bölgesi muşmulalarının (*Mespilus germanica* L.) seleksiyon yoluyla ıslahı üzerine bir araştırma.(An Investigation on breeding of East Black Sea Region medlar (*Mespilus germanica* L.) types). Türkiye V. Ulusal Bahçe Bitkileri Kongre Bildirisi: 494–501, 4–7 Eylül 2007, Erzurum

Cavusoglu A., Sulusoglu M. (2013). In vitro Pollen Viability and Pollen Germination in Medlar (*Mespilus germanica* L.) International Research Journal of Biological Sciences 2(5), 49–53, May (2013)

Glew R.H., Ayaz F.A., VanderJagt D.J., Millson M., Dris R., Niskanen R.(2003a). A research on mineral composition of medlar (*Mespilus germanica* L.) fruit at different stages of maturity, *J. Food Qual.* **26**,441–447.

Glew RH., Ayaz FA., Sanz C., VanderJagt DJ., Huang HS., Chuang LT., Strnad M.(2003b). Changes in sugars, organic acids and amino acids in medlar (Mespilus germanica L.) during fruit development, Food Chem. 83: 363–369.

Rodriguez A.R., Lozano J.S., Oderiz L.V., Hernandez J.L., Gonzalez M.J.(2000). Physical, physicochemical and chemical changes during maturation of medlars and persimmons, Dtsch. Lebensm-Rundsch. 96:142–145.

Rop O., Sochor J., Jurikova T., Zitka O., Skutkova H., Mlcek J., Salas P., Krska B., Babula P., Adam V., Kramarova D., Beklova M.A, Provaznik I., Kizek R.(2011). Effect of five different stages of ripening on chemical compounds in medlar (*Mespilus germanica* L.), Molecules 16:74–91.

Selcuk N., Erkan M. (2015). The effects of 1-MCP treatment on fruit quality of medlar fruit (Mespilusgermenica L. cv. Istanbul) during long term storage in the palliflex storage system. Postharvest Biology and Technology 100 ;81–90.

Yılmaz A., Gerçekçioğlu R. (2013). Tokat ekolojisi muşmula (*Mespilus germanica* L.) popülasyonu ve dağılımı. (Distribution of medlar (*Mespilus germanica* L.) population in Tokat Province ecology). Tarım Bilimleri Araştırma Dergisi 6 (2): 01–04.

Yılmaz P. (2015). Trabzon İli Tonya İlçesi'nde Doğal Olarak Yetişen Muşmula Tiplerinin (*M. germanica* L.) Seleksiyonu. (Selection of wild types medlars in Tonya District of Trabzon Province). MSc. Thesis, Ordu University, Ordu-Turkey.

Veličković MM., Radivojević DD., Oparnica CD., Nikićević NJ., Živković MB., Đorđević NO., Vajs VE., Tešević VV.)2013).Volatile compounds in Medlar fruit (*Mespilus germanica* L.) at two ripening stages 3 Hem. Ind. 67 (3) 437–441.

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DESIGNING OF A VARIABLE VOLUME COLD STORE

Serap AKDEMIR

Air-Conditioning and Refrigeration Programme, Technical Sciences Vocational School, Namik Kemal University, Tekirdağ, Turkey
Corresponding author: sakdemir@nku.edu.tr

ABSTRACT

Cold stores do not work for all time as full loaded during storage period. There is always empty space when cold store is not fully loaded. Unnecessary energy is consumed because this empty volume must be cooled even there isn't any stored agricultural product. In this study a system was developed to adjust cold store volume according to the amount of stored agricultural product. Therefore, effective volume of the cold store where agricultural product stored will only be cooled and energy saving will be established. In addition, better cold air distribution will be established in the cold store and the quality of product will also be better preserved. Developed system is a moving wall-door system located inside of the cold store. It can be moved automatically. Sliding-wall door system can be moved by an electrical engine on a rail located at side walls. Leakage of cold air is prevented by a silicone seal balloon. The silicone seal balloon is located all-around of the sliding wall-door. The sliding wall change volume of cold store due to amount of stored product. Sliding wall-door system is a new approach for manufacturing of cold storage. This system will not be used only for new cold stores, but also can be used for old cold stores. Energy saving is expected by using developed system in cold stores. Submission of Petit patent for this developed system was approved by Turkish Patent Institute.

Key words: *cold store, energy saving, variable volume, sliding wall-door.*

INTRODUCTION

The main criteria for successful product storage are temperature and humidity, air movement within store, packaging and stacking in storage, as well as pre-storage applications (Eris, 2001). Cold storages are special structures designed for storing various agricultural products. They are manufactured in various types. The most important types are the large cold storages with commercial purpose. Products in cold storages are unloaded depending on market conditions. The cooling system refrigerates the same storage volume regardless of whether the store is full at 80, 50 or 30% (Figure 1). It is unnecessary to cool the empty volume without products. Cooling of empty volumes leads to energy waste. As less agricultural products are

stored, the respiration heat spread by products will decrease depending on product amount.

During the establishment phase of the storage, pre-storage measures and in-store special applications enable proper calculation of cooling load and isolation, prevention of unnecessary energy consumption and reduction of product deterioration (Terzioglu, 1990). Certain tests were conducted by Patel and Patel (2012) to reduce energy consumption in cold storages, using different isolation materials, 3 different wall thicknesses and areas in consideration of compressor control parameters. Latest developments in cooling systems, electronic control systems are now used for energy saving through control of entire system instead of command on each individual device (Anonymous, 2009).

The objective of this study is to develop a sliding door system that enables reduction of cold storage volume for various loading conditions. In addition; study seeks developing a system that can be used in large commercial storages and containers.

MATERIALS AND METHODS

Cold storage with varying volume: The size of cold storage is 2.5 x 5.0 x 2.5 m (width x length x height) and volume will be 37.5 m^3.All walls and floors of cold store are made of prefabricated sandwich panels with polyurethane filling. Technical specifications of cooling system are given in Table 1.

Table 1. Technical specifications of cooling system

Evaporator/Condenser temperature :	-5/+40°C
C.O.P :	2,79
Cold Store temperature	+2°C
Capacity	4.876 Watt
Nominal Compressor Power	1,75 kW
Refrigeration	R 404A
Supply line	380 V - 50 Hz

Cooling system consists of an internal unit that transmits the cold air from rear wall to frontwards via evaporator, a condenser that condenses cold fluid circulating within the system, and an external unit that compresses the cooler to the system.

Moving door-wall system renders the cold store variable-volume for adjusting environmental conditions due to load amount. Various load rates of variable volume storage and the volumes of chests have been given in Figure 1.

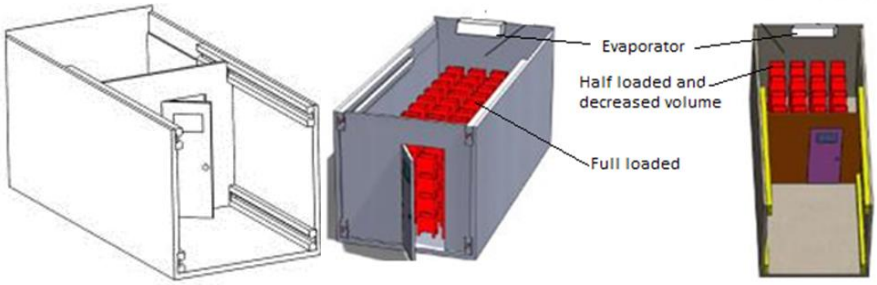


Figure 1. Moving door wall system and various load rates of variable volume storage

Size of the moving door-wall are 80 x 2500 x 2500 mm and made of the same material as cold room walls. The system is moved on the rail, placed behind the front door, by a mechanism to be operated by user, or via electric motor.
The door-wall mechanism reduced the cold storage volume depending on loading rate. The mechanism can be used in two different ways so as to ensure requested reduction.

Manual Controlled Moving Door Wall System:

Linear bearing (rail-slide) and Tube ball car :3500 mm long 2 linear bearings (rail-slides) TSX45E-G2-3500 mm and 4 tube ball cars INA RWU45EH-G2-V0 of -45 are used as support between bearings. Additionally, a metal profile is used in order to connect the bearings on the underside.
Support shaft and moving bearing set: In order to prevent overturn and twist of the 2.5 m high wall, 2 support shafts of 3500 mm length, and 4 moving bearing sets IN 40 KGBO40-PP-AS, which move on these shafts (IN 40 TSWWA40-3500 mm), are installed on the left and right side of the movable wall (Figure 2). Therefore the door-wall system can be moved back and forth in a parallel and smooth manner.

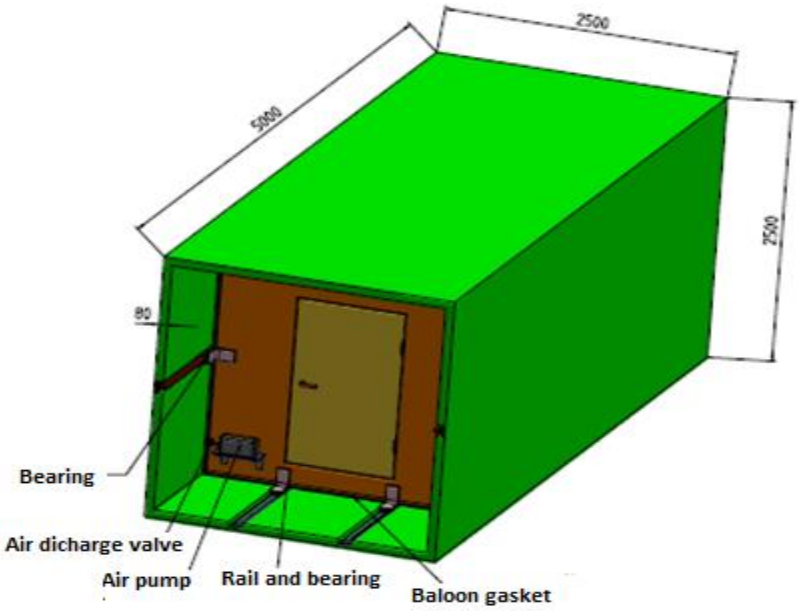
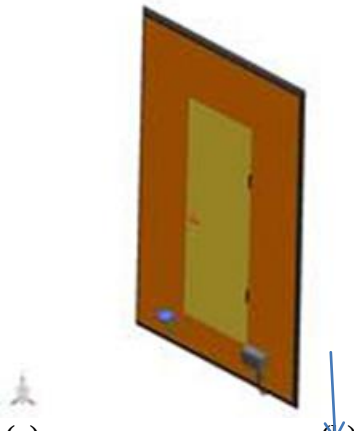


Figure 2. Moving wall elements of cold store with rail

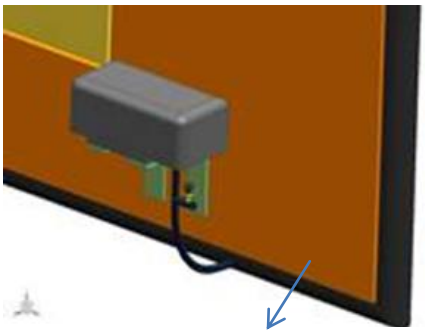
Air relief valve:Itensures that the air within balloon gasket is released for moving door-wall mechanism (Figure 3a).

Balloon gasket: The balloon gasket, essentially made of silicone, is encircling the entire moving wall for sealing (Figure 3b). Part of the gasket, which run into moving wall corners, are welded to establish perpendicular corner. The gasket will be formed as a rectangular closed hose. The gasket has a wall thickness of 3 mm, made of a transparent material with a density of 2.33 g/cm3; once inflated, it attains 80 mm in diameter. A sensitive sealing sponge of 3-5 mm is adhered to the gasket parts that run into surrounding walls in order to prevent possible slight leakages.



(a)

Figure 3a. Air relief valve



(b)

Figure 3b. Balloon gasket

Rubber gaskets: The thickness of rubber gaskets used in centring and bearing sections is 10-15 mm. (Figure 3-b). Hardness of rubber sealing elements will be chosen as 20-25 Shore. The rubber sealing elements will be connected to moving bearings, cars on bottom linear bearings and side centring bearings. These materials, which serve as special gasket, are connected on 4 tube ball cars and moving bearing sets.As is seen on Figure 3b, the gasket, placed in its specific housing on four sides of the wall, will be inflated by an air pressure of 10-15 bars. As the moving wall is drawn to the required spot, the gasket will be deflated; thus, the moving wall will be separated from exterior walls. Consequently, the wall can be easily pushed to desired point without significant friction or resistance. Once the wall reaches the prescribed point, electric air pump will inflate the balloon gasket via related valve; thus, the gasket will adhere to side walls and ensure both sealing and fixation of the wall.

Automatic Controlled Moving Door Wall System:

Electric air pump: Electric air pump is used to inflate the balloon gasket by an air pressure of 10-15 bars to enlarge its diameter up to 80 mm (Figure 4).

Electric Engine and Reductor:For automatic movement of moving wall via electric engine, a standard engine of 2.2 kW and 1400 rpm, as well as a reductor for revolution adjustment, are installed on the system. The reductor output speed will be 20 rpm .

Two wheel connected via Shaft:The 2 wheels connected via shaft will be attached by means of gear and chain system on the electric motor, which enables automatic positioning of the system. The wheels have diameter of Ø 230 mm, and are covered with polyurethane. The shaft will be supported with cast bearings.

Gear and chain system: The system will employ double-row chain. The shaft will be supported by cast bearings.

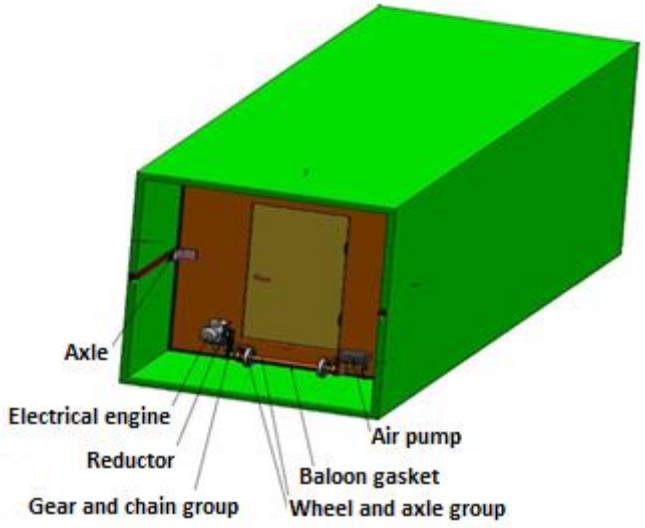


Figure 4. Automatic mowing wall-door elements

Automatic stopping and fixing at requested spot via electric motor: the moving wall operates with 2 wheels, connected to one another via a shaft, as well as with electric motor that revolves these wheels and shaft, in addition to the reducer, gear and chain system (Figure 4). Motor and reducer are mounted on moving wall. The torque from reducer is transmitted by means of a chain to the shafts and gears to which wheels are connected. Wheels and shaft will be supported with bearings on two ends, and the motor and reducer group will be placed on a small platform that will have the wall in between. Motor and reducer system will provide wheels with a speed of 4-6 m/min. Therefore, since the movement is very slow, the motor can be stopped any time by means of a button.

CONCLUSION

In current cold stores, the entire volume is cooled regardless of the loading rate (full, ¾ full or ¼ full). The cooling of vacant volume brings along redundant energy consumption depending on storage volume. Both domestic and imported cold storages are of constant volume. In other words, the cooled volume remains constant even if the amount of agricultural products decreases after delivery from cold store. Indeed, incoming orders may lead to whole or partial sale of products. In cases where the store is not completely full, any reduction in storage volume will abolish the necessity for refrigeration of the entire empty section. As a result, energy consumption, which constitutes the most significant part of operating costs in cold stores, will be reduced. Hereby study develops a moving wall-door system that can be adapted to both future and currently running cold stores. Moreover, the system can be applied in containers for situations where the products, carried to various countries in containers, decrease in the course of time. The system will reduce storage volume depending on the amount of products stored in cold room.

In the stores using this new system, the cooling will be carried out for only the required volume; therefore, notable saving will be obtained regarding energy consumption. Energy saving depends on storage volume and the power of cooling system to cool it. This system will decrease especially the operating costs of cold stores. Vegetable cultivation increased by 2.5% compared to previous year, and attained 28.5 million tonnes; while fruit production rose by 1.2% to 18.2 million tonnes. Apple enjoyed a production rise of 8.3% in 2013 and 8.3% in 2015, (TUIK, 2016). The production amounts of fruits and vegetables are sufficient to put forth the potential with respect to cold storage in Turkey. This fact underlines the importance of hereby study in regard to national economy. Energy saving is not the only advantage of the system. As storage volume decreases, the loss of quality and quantity in agricultural products will decrease. Consequently, agricultural products will be sold at more favourable prices. Moreover, the proper distribution of cold air in cold store is another influential factor on agricultural products. In a cold room with varying fullness level, the distribution of air within the room varies as well. Thus, agricultural products are cooled under varying storage conditions in cold rooms. The new system eliminates any cooled empty volume; therefore, agricultural products will be stored under better conditions. According to Bayboz *et*

al. , (2004), the maintenance of same temperature at every section of cold room air would influence reduction in frosting; indeed, simple cooling units or packet coolers may ensure temperature homogeneity within cold room air for small volume stores. Nevertheless, they indicate that in large volume cold stores, separate and specific air duct systems should be added to cooling units or packet coolers in order to ensure temperature homogeneity. As our present study provides cooling as per loading rate, no additional costs will be in question for large volume stores.

REFERENCES

Anonymous. (2009). Energy management optimization with computerized refrigeration control, http:// http://www.logix-controls.com/docs/energy_mgmt_opt_w_ref_controls.pdf (acess date: 23/06/2016)

Bayboz B., Yalçın E., Savaş S. (2004). Conventional methods in cold store, inapplicable trues and quality. (in Turkish: Soğuk depoculukta alışılagelen yöntemler,uygulanmayan doğrular ve kalite). Installation Engineering Journal (in Turkish: Tesisat Mühendisliği Dergisi), 81, p:1-9.

Eris A. (2001). Storage potential and targets od stone fruits in Turkey (in Turkish:Türkiye'de sert çekirdekli meyvelerin muhafaza potansiyeli ve hedefler). I. Sert Çekirdekli Meyveler Sempozyumu, 25-28 September, Yalova, 17-28.

Patel A.M., Patel R.I. (2012). Optimization of different parameter of cold storage for energy conservation, International Journal of Modern Engineering Research (IJMER), 2(3):1001-1005.

Terzioglu S. (1990). Capacites of cold store in İzmir region (processing and storage for fishery) (in Turkish:İzmir Bölgesinde Soğuk Hava Depolarının Kapasiteleri (Su ürünleri işleyen ve Muhafaza eden)) (MSc Thesis). Ege University, İzmir, Turkey, 52.

TUIK (2016). Pome Fruits 1083-2015 (in Turkish: Yumuşak çekirdekli meyveler, 1983-2015, http://www.tuik.gov.tr/PreTablo.do?alt_id=1001 (Access date:23/06/2016)

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DETERMINING THE NEED OF ALFALFA FOR WATER IN THE CONDITIONS OF SARAJEVO AREA (BOSNIA AND HERZEGOVINA)

Tanja JAKIŠIĆ*, Siniša BERJAN

Faculty of Agriculture,University of East Sarajevo,East Sarajevo, Republic of Srpska, Bosnia and Herzegovina
*Corresponding author: tanjaj26@yahoo.com

ABSTRACT

Alfalfa has a great need for water, because it creates a huge plant mass. For this reason, yield level, similar to the other species with large green mass, primarily depends on provided water during the growing season. Alfalfa uses water well from the pre-vegetation reserve in land, especially of rainfall during the growing season. In this paper, the need for water in alfalfa for average, the most rainy and dry hydrological year in the conditions of Sarajevo area was established. Evapotranspiration of alfalfa is 567 mm for the most dry, 569 mm for the most rainy and 540 mm for average hydrological year. Water deficit occurs in May, June, July and August in the amount of 323.9 mm for the most dry, 178.4 mm for the most rainy and 222.1 mm for average hydrological year. In the study area, alluvial soil is predominant (fluvisol), which is of a light mechanical composition (sandy loam) and favorable chemical characteristics for alfalfa growing.

Keywords: *alfalfa, evapotranspiration, water deficit, land.*

INTRODUCTION

Crop water demand can be defined as the amount of water required to compensate for water losses in crop occurring in evapotranspiration of crop which grows in terms of the lack of readily available water and nutrients and achieves the maximum development of green matter. Water needs of crops are expressed through evapotranspiration (Doorenbos and Pruitt, 1977) which includes plants transpiration and evaporation from land covered with vegetation cover.

Alfalfa requires and consumes large amounts of water; it has a very high transpiration coefficient and high production of green matter per hectare (Lukic and Katic, 1994). Alfalfa uses water well from the pre-vegetation reserve in land, especially of rainfall during the growing season (Balaceanu and Balaceanu, 1994). In the case of drought alfalfa vegetates thanks to its powerful roots. The depth of the roots of 5-6 or more meters (Miskovic, 1986), or 7-8 or even up to 10 m (Fazakas et al., 2006), allows alfalfa to use water from deeper layers. Alfalfa in favourable years, with favorable moisture, achieves high yields (Bosnjak et al., 1988). Although alfalfa is drought tolerant, it responds well to irrigation (Dragovic et al., 2000).

The needs of plants for water depend on several factors, primarily from the area, weather and soil conditions, the level of nutrition, applied agricultural technology, features genotype and so on. Knowing the need for water provides a rational watering, saving water (Neufeld and Davison, 1998). In addition to familiarity with the type of climate and water balance of a given area it is necessary to know some basic characteristics of soil types, because it is very important to know how much water sediment of rainfall reaches the land, and how much is retained in the soil, which primarily depends on the type of soil and its mechanical composition.
Taking into account Sarajevo area, the aim is to determine the real needs of alfalfa for water, and to determine the characteristics of one of the dominant soil types.

MATERIALS AND METHODS

The calculation of reference evapotranspiration (ET_0) was made by the FAO-56 Penman-Monteith method, which was proposed by FAO as a standard applicable in all weather conditions and in all time periods (Allen et al., 1998). Climate data for part of the Sarajevo area has been set for a twenty-year period (1995-2015), and potential evapotranspiration, effective precipitation, and water deficit have been determined for the most dry, the most rainy and average hydrological year.
Evapotranspiration of alfalfa has been calculated using the following formula:

$ET_k = ET_0 * k_c$
ET_k - crop evapotranspiration,
ET_0 - potential evapotranspiration,
k_c - crop coefficient,

USDA Soil Conservation Service method has been used for calculation of the effective rainfall:

Water deficit has been calculated using the following relationship:
$D_V = ET_0 - Pef$
D_V – water deficit,
ET_0 – potential evapotranspiration,
Pef – effective rainfall,

Mechanical composition of the soil has been determined by international B method, and textural classes of land have been determined by Fere triangle (Soil Survey Manual,1955).
Basic chemical properties have been determined by the following methods:

- pH value – by means of pHmeter in suspension with water and 1M KCl ;
- content of $CaCO_3$ volumetric;
- humus content by Kotzman;
- N content by Kjeldahl method;
- P content by Al method;
- K content by Al method.

RESULTS AND DISCUSSION

The most necessary amount of water for crop cultivation in the open field, in our climatic conditions during the growing season comes from precipitation. The difference between the total water needs of crops and the inflow of water precipitation (effective or useful for the plant) makes water deficit that must be compensated by irrigation.

Tables 1, 2 and 3 show the total rainfall, effective precipitation (precipitation beneficial for the plant), potential evapotranspiration, alfalfa evapotranspiration and water deficit at different stages of the alfalfa vegetation period of average hydrological year. Effective rainfall for average hydrological year amounted to 773.5 mm (Table 1), 927.6 mm for the most rainy hydrological year (Tab.2), and 588 mm for the most dry hydrological year (table 3). Evapotranspiration of alfalfa is 540 mm (average), 569 mm (wettest), 567 mm (driest) hydrological year (Tab.1, 2, 3). Water deficit for alfalfa occurs in May, June, July and August in a total amount of 222.1 mm, and the largest water deficit was recorded in July in an amount of 111.1 mm.

In the most rainy hydrological year (Table 2), deficit of water for alfalfa was lower and amounted to 178.4 mm, expressed also in May, June, July and August, with the largest deficit in June, in the amount of 56.8 mm.

In the rainiest hydrological year (Tab.3), deficit of water for alfalfa was amounted to 323.9 mm, expressed also in May, June, July and August, with the largest deficit in August in the amount of 104.5 mm.

Water requirements determine amount of irrigation (Schwankl and Prichard, 2003). Schewmakera et al. (2001) found that water surplus does not contribute to increase in yield. Therefore, rational irrigation is necessary by applying climate formulas (Zypries and Yevtushenko, 1980), where the coefficients should be adapted to stages of growth and development.

Table 1. Alfalfa needs for water (average hydrological year -2012)

Months	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**I**	**II**	**III**	**year**
P (mm)	45	63	148	26	32	95	48	113	100	105	70	62	1.144
P_{ef} (mm)	41.8	56.6	113	24.9	30.4	80.6	44.3	92.6	84	87.4	62.2	55.8	773.5
ET_0	45	78	117	124	116	70	30	26	0	0	0	20	626
ET_I	30	78	123	136	110	63	0	0	0	0	0	0	540
D_V	0	21.4	10	111.1	79.6	0	0	0	0	0	0	0	222.1

P- precipitation; P_{ef} – effective rainfall, ET_0 – potential evapotranspiration; ET_I- alfalfa evapotranspiration;

D_V-water deficit;

Table 2. Alfalfa needs for water (the most rainy hydrological year -1999)

Months	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II	III	year
P (mm)	106	72	74	100	84	106	56	142	298	49	59	57	1203
P_{ef} (mm)	88.0	63.7	65.2	84.0	72.7	88.0	51.0	109.7	154.8	45.2	53.4	51.8	927.6
ET_0	52	95	116	119	122	79	48	12	0	0	0	18	661
ET_l	34	95	122	131	116	71	0	0	0	0	0	0	569
D_V	0	31.3	56.8	47	43.3	0	0	0	0	0	0	0	178.4

P- precipitation; P_{ef} – effective rainfall, ET_0 – potential evapotranspiration; ET_l -alfaalfa evapotranspiration;
D_V -water deficit;

Table 3. Alfalfa needs for water (the most dry hydrological year -2000)

Month	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II	III	year
P (mm)	52	69	23	50	17	73	84	76	98	34	48	33	657
P_{ef} (mm)	47.7	61.4	22.2	46.0	16.5	64.5	72.7	66.8	82.6	32.2	44.3	31.3	588
ET_0	55	91	112	127	127	68	68	51	33	2	0	0	734
ET_l	36	91	118	140	121	61	0	0	0	0	0	0	567
D_V	0	29.6	95.8	94	104.5	0	0	0	0	0	0	0	323.9

P- precipitation; P_{ef} – effective rainfall, ET_0 – potential evapotranspiration; ET_l -alfaalfa evapotranspiration;
D_V -water deficit; S_V-water surplus;

According to the soil map of Bosnia and Herzegovina (Resulović et al., 2008) in the area of Sarajevo, one of the most common types of soil is alluvial soil (fluvisol).

Table 4. Mechanical composition of soil type alluvium (fluvisol)

Sampling depth (cm)	**Large sand 2-0,2 mm (%)**	**Small sand 0,2-0,02 mm (%)**	**Powder 0,02-0,002 mm (%)**	**Clay <0,002 mm (%)**
0-30	10.99	46.85	23.00	19.16
30-60	10.5	44.65	25.90	18.70

According to texture, soil type fluvisol belongs to a class of sandy loam (Table 4), which means that this soil has favorable physical properties due to the appropriate ratio of water and air in its micro and macro pores.

Table 5. The chemical composition of soil type alluvium (fluvisol)

Sampling depth (cm)	**pH/H_2O**	**pH/KCl**	**$CaCO_3$ (%)**	**Humus (%)**	**Total N**	**P_2O_5 (mg/100 g)**	**K_2O (mg/100 g)**
0-30	7.28	6.18	1.2	2.4	0.151	36.8	35.6
30-60	7.51	6.80	1.4	2.5	0.158	37.9	36.6

Chemical analysis of samples taken from soil type fluvisol shows that the land is slightly acidic to neutral, and from that standpoint, it is favorable for the cultivation of alfalfa. This soil is with lower carbonate content, medium secured humus, well secured accessible phosphorus and potassium (Tab.5).
According to Resulović et al., (2008) in soil type fluvisol, humus content is not evenly distributed regarding depth profile, which means that deeper layers of the profile can be more humous, which is proved by these soil samples.

CONCLUSION

Investigated area is perfect for growing alfalfa, due to favorable climatic and soil conditions. The deficit of water for alfalfa occurs mainly in the summer months, when there is not enough effective rainfall (rainfall useful for the plant) and then irrigation should be applied. The dominant soil type on the part of Sarajevo area is alluvial soil (fluvisol), which is suitable for growing alfalfa due to its physical and chemical properties.

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REFERENCES

Allen, R.G., Pereira L.S., Raes, D., Smith, M. (1998) Crop Evapotranspiration in FAO Irrigation and Drainage Paper NO.56, FAO, Rome, Italy. Guidelines for Computing Crop Water Requirements.

Balaceanu, C., Balaceanu Cornelia (1994): Perioada de aplicare a udarilor la unele culturi agricole in depresiunea Jijia-Bahlui Analele institutulni deceretare si inginerie tehnologica pentru irigatisi drenaje (ICITID), Bucuresti, VII(XVIII), pp. 151-156.

Doorenbos, J., Pruitt, W.O. (1977). Crop Water Requirements. FAO Irrigation and Drainage paper 24, FAO, Roma.

Fazakas, P., Chiš, E., Chiš, S., Riviš I.D (2006). Iencin, Amišoara: Irigarea culturilor. Editura EUROBIT, Timišoara, Romania.

Neufeld J. And Davison J. (2003). Alfaalfa irrigation Scheluding with an Automated Evaporation Pas System. University of Nevada, Reno-Cooperative Extension. Available at: www.unce/unr/publications.

Resulović, H., Čustović H., Čengić, I. (2008). Sistematika zemljišta. Poljoprivredno-prehrambeni fakultet, Sarajevo.

Schwankl L. and Prichard T. (2003). Improving irrigatio Water managment of Alfaalfa. In Proceedins California Alfaalfa an Forage Symposium, Monterey, CA, UC, Cooperative Extension, University of California, Davis, Available at_ http://alfaalfaucdavis.edu.

Soil Survey Mannual (1995). Washington D.S.

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APPLICATION OF MYCORRHIZAL FUNGI IN LANDSCAPE TURFGRASS ESTABLISHMENT UNDER ARID AND SEMIARID ENVIRONMENTS

Ghazi N. AL-KARAKI

Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan
*Corresponding author: gkaraki@just.edu.jo

ABSTRACT

Turf grasses are considered an integral part of landscape ecological systems worldwide which provide functional, recreational and aesthetic benefits to society and the environment. In arid and semiarid regions (e.g., Mediterranean region), turf grass is usually grown under harsh and unfavorable growing conditions with low rainfall and high rates of evapotranspiration as well as in soils with nutrient deficiencies. Hence, growing turf grass in these regions becomes dependent on application of high levels of fertilizers as well as on excessive use of irrigation water, resulting in an environmental pollution. Therefore, it is important that turf grass plantations are managed in a sustainable way to reduce the impact of turf grass cultivation on ecosystems while maintaining healthy and productive turf through using such practices as mycorrhizal fungi technology. The application of mycorrhizal fungi technology is an option that can benefit both agronomic plant health and ecosystems. Mycorrhizae confer numerous benefits to host plants including improved plant growth, mineral nutrition, water uptake, tolerance to diseases and stresses such as drought and salinity. The aims of this paper were to review how mycorrhizal fungi might play a role in enhancing landscape turf establishment and productivity in arid and semiarid regions and to evaluate the effectiveness of application of commercial mycorrhizal inoculum to enhance plant growth and survival under field conditions. Field experiment was conducted to study the effects of arbuscular mycorrhiza (AM) fungi inoculation on water use efficiency and establishment of a landscape turf. The results showed that turf grass inoculated with AM fungi used water more efficiently, established lawn more quickly and had more biomass than uninoculated turf grass. The conclusions of this paper indicated the potential of mycorrhiza inoculation in improving the fast establishment of turf landscape plants under arid and semiarid environments.

Keywords: *Arbuscular mycorrhizal fungi, sustainability, urban, irrigation, Jordan.*

INTRODUCTION

Turf grasses are consider an integral part of landscape ecological systems worldwide, which provide functional, recreational and aesthetic benefits to society and the environment. However, to maintain homogeneous and dense turf lawn, with an intense color, and free of diseases, it becomes dependent on application of high levels of chemical fertilizers and pesticides as well as on excessive use of irrigation water (Al-Karaki et al., 2007).

The present paper aimed at to provide an overview of how mycorrhizal fungi might play a role in enhancing landscape turf establishment and productivity in arid and semiarid regions and to evaluate the effectiveness of application of commercial mycorrhizal inoculum to enhance plant growth and survival under experimental field conditions.

Mycorrhizal fungi role in enhancing landscape turf establishment in arid and semiarid regions: Review

In arid and semiarid regions (e.g., Mediterranean region), turf grass is usually grown under harsh and unfavorable growing conditions with low rainfall and high rates of evapotranspiration as well as in soils with nutrient deficiencies (Al-Karaki, 2013). In these regions, water use for watering lawns is a major concern due to limited access to water resources, and application of high levels of fertilizers and plant protection chemicals which might not always offer the desired result and resulting in an environmental pollution (Law et al., 2004). Furthermore, the costs of maintaining a lawn are very high when considering the full cost of water and the fertilizer and plant protection products application. Therefore, it is important that turf grass plantations are managed in a sustainable way that use of appropriate water supply and reduce the impact of application of fertilizer and plant protection products on ecosystems while maintaining healthy and productive turf through using such biological agents as mycorrhizal fungi inoculants (Al-Karaki, 2013;Gemma, et al., 1997).

Arbuscular mycorrhizal (AM) fungi are beneficial symbiotic fungi that form an association on the roots of most of the world's plants (Marschner, 1995). The mycorrhizal fungi once established on the host plant root system radiate out from the roots to form a dense network of filaments. These filaments form an extensive system of hyphae that extends from root system, grow into the surrounding soil, and greatly improve access to water and nutrients far from roots (Marschner and Dell, 1994). AM fungi provide a variety of benefits for the host plants, including improved access to immobile nutrients especially P, Cu and Zn (Miyasaka and Habte 2001) as well as inorganic N (Govindarajulu et al. 2005), thus reducing fertilizer requirements (Gemma et al. 1997), improved water use (Naghashzadeh et al., 2015), improved tolerance to disease (Matsubara, *et al.* 2001), and tolerance to abiotic stresses such as drought (Rapparini and Peñuelas. 2014; Al-Karaki, *et al.* 2004), and salinity (Aroca and Ruiz-Lozano, 2012; Al-Karaki, 2006). AM fungi also enhance soil aggregation and water-holding capacity both by producing external hyphae and by exuding glomalin, a glycoprotein, from extraradical hyphae (Wright and Upadhyaya, 1998).

Although the majority of grass species form an AM symbiosis (Newman and Reddel, 1987), less research has been conducted until recent times on the

mycorrhization of turf, due to the believes that turf grass are less dependent on AM fungi because it is grown under almost perfect growth conditions. Recent scientific studies and practical applications of AM fungi inoculants demonstrated that many species of AM fungi are associated with highly maintained turf grasses (Miyasaka and Habte 2001; Govindarajulu et al. 2005). In arid and semi-arid climates, an efficient management of irrigation water as well as fertilizer and plant protection products application are of ecological and economic importance, which calls for mycorrhizae application as growth promoters, biofertilizers and bioprotectors. Research studies have shown that AM fungi can improve plant growth, fertilizer utilization, rooting depth, the speed of establishment, disease and drought and salinity resistance of turf (Al-Karaki et al., 2007; Gemma etal., 1997; Pelletier and Dionne, 2004; Aroca and Ruiz-Lozano, 2012). Warm-season grasses with coarse root systems such as bermuda grass are very dependent upon mycorrhiza for sustained growth (Hetrick et al 1988). Some other studies indicate that cool-season, finer rooted bentgrass species also form abundant mycorrhiza and benefit from the relationship, especially where the phosphorous levels are not too high (Gemma et al., 1997; Koske et al., 1997). It is also well documented that inoculation of grasses with mycorrhizal fungi in soil with low phosphorous concentrations can produce greater shoot and root biomass (Hetrick et al., 1991). Gemma et al. (1997) reported that Kentucky bluegrass produced more aboveground biomasses over time when inoculated with mycorrhizae compared with the uninoculated control. Besides positive impacts of AM fungi on plant nutrition and water uptake, mycorrhizal fungi protect annual grasses from root pathogenic fungi and nematodes in the field (Newsham et al. 1995; Little and Maun 1996), since these pathogens can be a severe problem in turf grass management.

Evaluation of application of mycorrhizal inoculum in enhancing plant growth and survival under experimental field conditions: Experimentation

MATERIALS AND METHODS

To confirm its practical application and to corroborate the mycorrhiza functionality, a field study was conducted during the summer of 2012 at the Experimental Farm of Faculty of Agriculture, Jordan University of Science & Technology, Irbid, Jordan. Experimental plots [(1m x 1 m x 0.35m); LxWxD] were constructed and filled with 90:10 (v: v) sand: peat rootzone. Plots were seeded with a standard commercial lawn seed mixture composed of 30% Kentucky bluegrass (*Poa pratensis* L. Nustar and Rugby II) and 70% perennial ryegrass (*Loliumm perenne* L. Goal Keeper) at 25 g m^{-2}. AM fungal treatments included inoculation (AM) or no inoculation (nonAM) with a commercial AM BioMyc inoculum. Both AM inoculum and seeds were uniformly sprinkled by hand over the surface plots and mixed into the top 1 cm of the soil. The turf plots were watered daily with 5 L m^{-2} for the first month after seeding, and 10 L m^{-2} thereafter. No rainfall is usually received during summer. Prior to seeding plots, inorganic fertilizer was applied at a rate of 40 g N, 3.3 g P and 27 g K per m^2 and all were incorporated into the upper 20 cm of plots.

Percentage of plot area covered by turf grass was evaluated by visually estimating the surface covered by turf grass4 and 8 weeks after seeding. To assess shoot and root growth and mycorrhizal colonization of roots, ten plants were collected randomly with

their intact roots from each plot 8 weeks after seeding. After that, plants were clipped to the height of about 5 cm from soil level, and clippings were collected to determine clipping yield. Five of sampled plants were used for determination of shoot and root growth parameters. Root samples from the other sampled five plants were cleared with 10% (w/v) KOH solution and stained with 0.05% trypan blue in lactophenol as described by Phillips and Hayman (1970), and microscopically examined for colonization using a gridline intercept method (Giovannetti and Mosse, 1980).

The experiment was arranged in a randomized complete block design with three replicates. Data were statistically analyzed using analyses of variance (ANOVA). Probabilities of significance among treatments were used to compare means between treatments.

RESULTS AND DISCUSSION

Turfgrass roots in AM plots after 8 weeks of seeding had been colonized extensively with AM fungi (~83%), while no root colonization with AM fungi has been noted in the control plots (Table 1). It has been reported that extensive system of hyphae that are growing out of colonized roots into the surrounding soil can provide a variety of benefits to the host plant (Pelletier and Dionne, 2004). The distributed network of hyphae beneath soil surface greatly increases the capacity of root system to absorb water and uptake of nutrients (especially low mobile nutrients in soil). The network of fungal filaments also helps in improving soil structure, porosity and aeration by binding soil particles (Pelletier and Dionne, 2004).

Highly significant differences in turf seedling size (shoot and root lengths an weights) andclipping yields were observed for the AMplots compared to the nonAM plots 8 weeks after seeding (Table 1). These results revealed that the mycorrhizal plants produced more biomass (high clipping rate) per unit of water applied through irrigation than control plants. Early establishment of turfgrass might reduces the need for high water use which help growers to increase irrigation intervals (less number of irrigation times) due to improved water holding capacity and extended root system by mycorrhiza, so help the plants to utilize water more efficiently (Pelletier and Dionne, 2004).

Table 1. Root colonization, shoot and root growth and clipping yield of mycorrhized (AM) and non-mycorrhized (NonAM) turf grass 8 weeks after seeding.

Treatment	**Root colonization**	**Clipping yield**	**Shoot dry weight**	**Root dry weight**	**Shoot length**	**Root length**
	%	g m-2	mg plant-1	mg plant-1	cm plant-1	cm plant-1
AM	0 b	32 b	18 b	5.1 b	7.3 b	6.8 b
Non-AM	83 a	182 a	38 a	13.5 a	18.1 a	10.7 a

Different letters denote significant differences between means of treatments.

Several studies have shown that grass species benefit greatly from mycorrhizal inoculation in terms of enhancement in the shoot and root growth (Gemma and Koske, 1989; Koske et al., 1995). Enhanced mycorrhizal inoculated turf was related to improved plant nutrition (Gemma and Koske, 1989), higher chlorophyll concentrations which enhance photosynthates production (Gemma et al., 1997), improved rooting depth, speed of establishment, disease and drought and salinity resistance of turf (Gemma etal., 1997; Koske et al., 1995).

After 4 weeks of seeding, turfgrass inoculated with AM fungi established more quickly than turfgrass not inoculated with AM fungi. This attained when percentage of plots area covered by turfgrass significantly higher in AM than nonAM inoculated plots (Fig. 1). After 8 weeks of seeding, no significant differences were observed between AM and nonAM plots (Fig 1.).

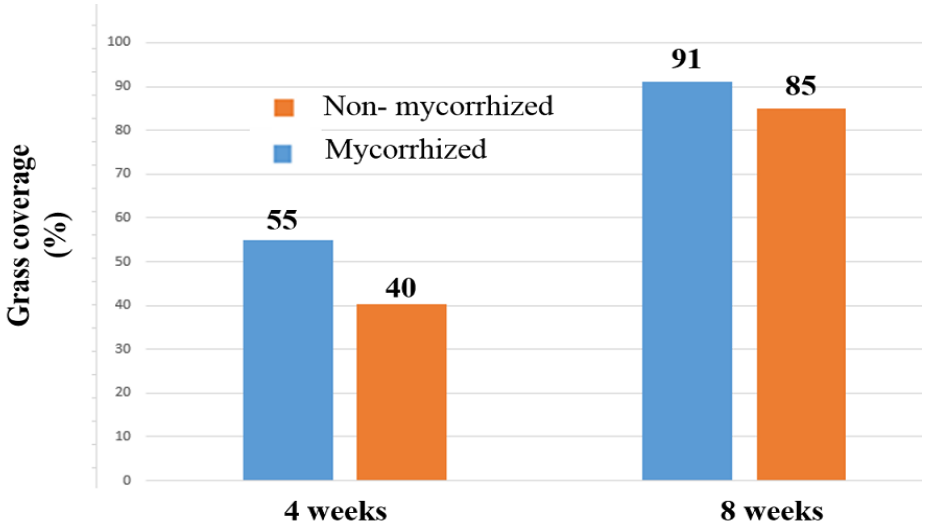

Fig. 1. Average grass coverage of mycorrhized and non-mycorrhized turf grass after 4 and 8 weeks of seeding.

However, shoot and root shapes were clearly different, AM plants having a greater size of shoots and longer and extensive roots (Fig. 2). These results suggest that inoculation with AM fungi is very efficient in increasing the speed of establishment of turfgrass seedlings in comparison to the controls at early time. The increase in establishment of turf grass is an important benefit to landscape owners as it improves the aesthetics of lawns, covering the surface and increasing the quality of grasses by reducing weed development. A dense cover may require less fertilizer due to high utilization of fertilizer directly into turf plants growth which reduces the fertilizer movement into ground water (Amaranthus, 2001).

Fig. 2. Shoot and root growth of mycorrhized (left) and nonmycorrhized (right) turf grass after 8 weeks of seeding.

CONCLUSIONS

It is apparent that mycorrhizal fungi are essential components of landscape ecological systems. Inoculation techniques for the management of turf grass plantations may be used, especially with improved inocula production methods development. The application of mycorrhizal fungi technology is an option that can benefit both agronomic plant health and ecosystems. Results of the field study indicated that inoculation of turfgrass with AM fungi is very efficient in increasing plant biomass and the speed of establishment of a standard turfgrass lawn seed mixture in comparison to uninoculated turf grown with low fertilizer inputs under arid and semiarid environments.

REFERENCES

Al-Karaki, G.N. 2006. Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Scient.Hortic. 109: 1-7 .

Al-Karaki, G.N. 2013. The role of mycorrhiza in the reclamation of degraded lands in arid environments. P. 823-836. In: Shabbir A.S., F.K.Taha,and M.A. Abdelfattah (eds)Developments in Soil Classification, Land Use Planning and Policy Implications. Springer Netherlands.

Al-Karaki GN, McMichael B, Zak J. 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263-269.

Al-Karaki, GNO; Othman, Y; Al-Ajmi, A. 2007. Effects of mycorrhizal fungi inoculation on landscape turfestablishment under Arabian Gulf region conditions. Arab Gulf J. Sci. Res. 25(3):147-152.

Amaranthus, M (2001) Mycorrhizae and Turfgrass:Biological Tool Improves Establishment, Growth,Disease and Drought Resistance of Golf TurfGrasses. Mycorrhizal applications Inc. Brochure,pp. 7.

Aroca, P. and J. Ruiz-Lozano. 2012. Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agronomy for Sustainable Development, 32 (1):181-200 .

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuringvesicular arbuscular mycorrhizal infection in roots. New Phytol. 84:489-500.

Gemma, JN, and Koske, RE (1989) FieldInoculation of America Beachgrass (*Ammophilaareviligulata*) with VA. Mycorrhizal Fungi. *J.Environ. Manag*. 29 (2):173-182.

Gemma, JN, Koske, RE, Roberts, EM, and Jackson, N (1997) Enhanced Establishment of Bentgrasses by Arbuscular Mycorrhizal Fungi. Journal of Turfgrass Science 73 (1): 9-14.

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, LammersPJ, Shachar-Hill Y (2005). Nitrogentransfer in the arbuscular mycorrhizal symbiosis. Nature 439: 819–823

Hetrick, B.A.D., Wilson, G.W.T. and Leslie, J.F. (1991): Root architecture of warm-and cool-season grasses: Relationship to mycorrhizal dependence. Can J. Bot. 69: 112-118.

Koske, RE, Gemma, JN and Jackson, N (1995).Mycorrhizal Fungi Benefit Putting Greens.USGA Green Section Record 33(6) (1): 12-14.

Law, N.L., LE. Band and J. M. Grove. 2004. Nitrogen Input from Residential Lawn Care Practices in Suburban Watersheds in Baltimore County, MD. J. Env. Plan. andManag. 47: 737–755.

Little L. R. and Maun M. A. (1996): The `Ammophila Problem' revisited: A role for mycorrhizal fungi. J. Ecol. 84(1): 1-7.

Marschner, H. and B.Dell. 1994. Nutrient uptake in mycorrhizal symbiosis. Plant Soil159:89-102.

Marschner, H, and Dell, B (1994) NutrientUptake in Mycorrhizal Symbiosis. Plant Soil159 (1): 89–102.

Matsubara, Y, Ohba, N, and Fukui, H (2001)Effect of Arbuscular Mycorrhizal FungusInfection on theIncidence of Fusarium RootRot in Asparagus Seedlings. J. Jpn. Soc. Hortic.Sci. 70 (2): 202-206.

Miyasaka SC, Habte M (2001) Plant mechanisms and mycorrhizal symbiosis to increase P uptake. Comm. Soil Sci Plant Anal 32: 1101-1147.

Naghashzadeh, M., K. Bour, and P. Pezeshkpour. 2015. Response of water use efficiency to mycorrhizalbiofertilizer in maize under water stress conditions. Bulletin of Environment, Pharmacology and Life Sciences, 4 [3]: 152-157 .

Newman, EI, and Reddel, P (1987) TheDistribution of Mycorrhizas among Families ofVascular Plants. New Phytol. 72 (1):127-136.

Newsham, K.K., Fitter, A.H. and Watkinson, A.R. (1995): Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. J. Ecol. 83, 991-1000.

Pelletier, S, and Dionne, J (2004) Inoculation Rate of Arbuscular Mycorrhizal Fungi *Glomus intraradices* and*Glomus etunicatum*affects establishment of

landscape turf with no irrigation or fertilizer inputs. Crop Science 44 (1): 335-338.

Phillips, J. and D. Hayman. 1970. Improved procedures for clearing roots and staininparasitic and vesiculararbuscular mycorrhizal fungi for rapid assessment of infection.Trans. Br. Mycol. Soc. 55: 158-161.

Rapparini, F. and J. Peñuelas. 2014. Mycorrhizal Fungi to Alleviate Drought Stress on Plant Growth .M. Miransari (ed.), Use of Microbes for the Alleviation of Soil Stresses, Volume 1, Springer Science+Business Media New York.

Wright, SF, Upadhyaya, A (1998) A Survey of soils for aggregate stability and glomalin, AGlycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil198 (1): 97-107

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- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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