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PRODUKTIVNE OSOBINE STRNIH ŽITA U ZAVISNOSTI OD PRIMENE MINERALNIH ĐUBRIVA SA POVEĆANIM SADRŽAJEM FOSFORA I KALIJUMA

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Sažetak: Cilj ovoga rada bio je da se na zemljištu tipa pseudoglej ispita uticaj primene mineralnih đubriva sa povećanim sadržajem fosfora i kalijuma na produktivne osobine nekih strnih žita. Istraživanja su obavljena u okolini Kraljeva tokom 2011/2013. godine. U ogledu su bili uključeni pšenica, ozimi ječam i tritikale i 3 varijante đubriva (K – kontrola; V1 – N_{90} P_{60} K_{60}; V2 – N_{90} P_{80} K_{80} i V3 – N_{90} P_{100} K_{100}). Pored prinosa zrna praćeni su apsolutna i hektolitarska masa zrna. Dobijeni rezultati su obrađeni primenom analize varijanse. Značajnih razlika u prinosu, apsolutnoj i hektolitarskoj masi zrna između strnih žita nije bilo. Primena mineralnih đubriva sa povećanim sadržajem fosfora i kalijuma dovela je do značajnog povećanja prinosa zrna, apsolutne i hektolitarske mase strnih žita u odnosu na kontrolu. Između varijanti V1 i V2, kao i varijanti V2 i V3 nisu postojale statistički značajne razlike u apsolutnoj i hektolitarskoj masi zrna. Prosečne vrednosti prinosa zrna za strna žita kretale su se od 1510 kg ha^{-1} na kontrolnoj varijanti do 3209 kg ha^{-1} na varijanti sa najvećom dozom fosfora i kalijuma. Značajne razlike u prinosu zrna zabeležene su između varijanti V1 i V2, kao i varijanti V2 i V3. Primenom samo mineralnih đubriva na kiselim zemljištima prinosi su relativno niski.

Ključne reči: strna žita, pseudoglej, mineralna đubriva, apsolutna masa, hektolitarska masa, prinos.

Uvod

Efikasnost upotrebe hraniva zavisi od nivoa plodnosti zemljišta, klimatskih uslova i plodoreda (Stewart et al., 2005).

Puni efekat NPK hraniva se može ostvariti samo ako su i drugi faktori koji modeliraju prinos dovedeni u optimum (Starčević et al., 2006). Posebno se to odnosi na azot zbog njegovog uticaja na prinos i kvalitet zrna, ali i zbog velike mobilnosti u zemljištu (Malešević et al., 2005). Kiselost, alkalnost, salinitet,

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monokultura, vetar, voda i erozija su glavni faktori degradacije poljoprivrednih zemljišta (Lal, 1990; Clarκ i Baligar, 2000).

Niska proizvodna sposobnost pseudogleja je rezultat loših fizičko-mehaničkih, toplotnih i vodno-vazdušnih osobina (Malešević et al., 2008; Jaćimović et al., 2012; Jelić et al., 2012). Otuda je proizvodnja strnih žita na ovom tipu zemljišta niska i nerentabilna. Vreme primene mineralnih đubriva potrebnih za formiranje visokog i kvalitetnog prinosa, kao i količina i vrste mineralnih đubriva, razlikuju se i u zavisnosti od plodnosti zemljišta (Popović, 2010; Jelić et al., 2004).

Od svih makroelemenata azot, fosfor i kalijum najviše utiču na normalan rast i razvoj pšenice. Međutim, uticaj azota na prinos zrna značajno opada bez drugih hranljivih elemenata, naročito fosfora.

Otuda se sve više potencira primena većih količina fosfornih đubriva, odnosno NPK-đubriva sa većim udelom fosfora, jer je uticaj ishrane fosforom naročito izražen na kiselim, kao i većini drugih degradiranih zemljišta (Jelić et al., 2005; Đekić et al., 2013).

Cilj ovoga rada bio je da se na zemljištu tipa pseudoglej ispita uticaj primene mineralnih đubriva sa povećanim sadržajem P i K na produktivne osobine nekih strnih žita.

Materijal i metode

Istraživanje je sprovedeno u okolini Kraljeva, na imanju Poljoprivredno-hemijske škole „Dr Đorđe Radić", tokom 2011/2013. godine. Eksperiment je uključivao pšenicu, ozimi ječam i tritikale i tri varijante đubriva (K – kontrola; V1 – N_{90} P_{60} K_{60}; V2 – N_{90} P_{80} K_{80} i V3 – N_{90} P_{100} K_{100}). Pored prinosa praćena je apsolutna i hektolitarska masa zrna. Od mineralnih đubriva korišćena su NPK đubriva. Ogled je postavljen po blok sistemu u tri ponavljanja. Pre osnovne obrade zemljišta, mineralna đubriva su rasturena na površini zemljišta, a zatim zaorana. Druga polovina azota korišćena je u prihranjivanju u obliku amonijačnog azota. Osnovna obrada zemljišta izvršena je na klasičan način, oranjem na 25 cm dubine. Setva je izvedena u oktobru. Žetva je sprovedena u fazi pune zrelosti, a prinos je obračunat sa 14% vlage. Rezultati su predstavljeni kao dvogodišnji prosek i obrađeni analizom varijanse uz upotrebu programa SPSS Statistics 20 (tabela 5).

Zemljišni i klimatski uslovi

U tabelama 1 i 2 su prikazane hemijske osobine zemljišta na kome je izveden ogled i meteorološki uslovi tokom izvođenja ogleda.

Ovo zemljište pripada tipu pseudoglejnih zemljišta, koja se odlikuju lošim fizičko-hemijskim osobinama sa izraženom kiselom reakcijom (pH u nKCl<4,5).

Sadržaj humusa je u granicama srednje obezbeđenosti, dok je sadržaj lakopristupačnog fosfora i kalijuma nizak (6,70–6,90 mg/100g zemljišta, odnosno 7,80–9,80 mg/100g zemljišta). S obzirom na loše fizičko-hemijske osobine može se očekivati da će primena đubriva pozitivno uticati na prinos gajenih biljaka.

Tabela 1. Hemijske osobine zemljišta.
Table 1.Chemical properties of the soil.

Dubina (cm) *Depth (cm)*	pH		Humus (%) *Humus* (%)	Dostupno (mg/100g zemlj.) *Available (mg/100g of soil)*	
	H_2O	nKCl		P_2O_5	K_2O
0-20	5,24	4,34	3,57	6,70	7,80
20-40	5,55	4,48	2,35	6,90	9,80

Tabela 2. Meteorološki uslovi tokom izvođenja ogleda (2011/2013).
Table 2. Meteorological conditions during the conduct of the experiment (2011/2013).

Meseci *Months*	Prosečne mesečne temperature vazduha (^{0}C) *Average monthly temperatures of air (^{0}C)*		Mesečne sume padavina (mm) *Monthly sums of precipitation (mm)*	
	2011/12	2012/13	2011/12	2012/13
X	10,4	13,7	30,4	56,7
XI	3,2	9,1	1,7	11,1
XII	3,3	0,4	63,7	97,6
I	-0,1	1,2	107,1	95,4
II	-4,2	1,9	54,9	48,7
III	8,8	8,4	24,5	56,7
IV	12,7	13,4	69,1	73,2
V	16,0	17,0	105,5	90,3
VI	23,1	24,4	17,8	13,8
Prosek/Suma *Average/Sum*	8,1	9,9	474,7	553,5

Prosečne mesečne temperature vazduha u 2011/2012. godini su bile nešto niže, posebno u januaru i februaru, u poređenju sa 2012/2013. godinom. Međutim, u obe godine,srednje mesečne temperature vazduha su se kretale u granicama optimalnih i nisu imale negativan uticaj na prinos. Više padavina sa boljim rasporedom bilo je u 2012/2013. godini, naročito u oktobru, novembru i decembru, što je doprinelo boljem rastvaranju mineralnih đubriva.

Rezultati i diskusija

U tabeli 3 dat je prikaz vrednosti prinosa, apsolutne i hektolitarske mase zrna strnih žita u zavisnosti od primene mineralnih đubriva sa povećanim sadržajem fosfora i kalijuma.

Tabela 3.Uticaj primene mineralnih đubriva na neke proizvodne osobine strnih žita.
Table. 3. The influence of the application of mineral fertilizers on some production properties of small grains.

Osobine *Properties*	B. Strna žita *Small grains*	A. Varijante đubrenja/*Variants of fertilizing*				Prosek B *Average B*
		K Kontrola *Control*	V1 N90 P60K60	V2 N90 P80 K80	V3 N90 P100 K100	
Apsolutna masa (g) *Absolute mass (g)*	Pšenica/*Wheat*	39,9	41,2	42,0	43,0	41,5
	Ječam/*Barley*	39,7	40,3	41,1	42,2	41,0
	Tritikale/*Triticale*	40,2	41,3	42,2	43,0	41,7
	Prosek A/*Average A*	39,9	40,9	41,8	42,7	41,3
Hektolitarska masa (kg) *Hectoliter mass (kg)*	Pšenica/*Wheat*	69,9	71,4	72,2	72,9	71,6
	Ječam/*Barley*	69,0	70,6	71,2	71,5	70,6
	Tritikale/*Triticale*	69,4	71,1	72,0	72,3	71,2
	Prosek A/*Average A*	69,4	71,0	71,8	72,2	71,1
Prinos (kg ha^{-1}) *Yield (kg ha^{-1})*	Pšenica/*Wheat*	1467	2867	3123	3327	2696
	Ječam/*Barley*	1587	2233	2705	3043	2392
	Tritikale/*Triticale*	1475	2445	3147	3256	2581
	Prosek A/*Average A*	1510	2515	2992	3209	2556

Prosečne vrednosti apsolutne mase zrna za strna žita kretale su se od 39,9 g na kontrolnoj varijanti do 42,7 g na varijanti sa najvećom dozom fosfora i kalijuma. Prosečne vrednosti apsolutne mase zrna za sve varijante đubriva, između strnih žita, bile su približno iste. Sve varijante đubrenja u odnosu na konrolu, kod svih strnih žita, dovele su do značajnog povećanja apsolutne mase zrna, dok između varijanti V1 i V2, kao i varijanti V2 i V3 nisu postojale statistički značajne razlike. Između varijanti V1 i V3, kod svih strnih žita, postojale su statistički značajne razlike u apsolutnoj masizrna. Apsolutna masa zrna je osobina sorte i otuda su između različitih genotipova veća variranja nego između varijanti mineralne ishrane (Jelić et al., 2002; Lalević et al., 2012).

Prosečne vrednosti hektolitarske mase zrna za strna žita kretale su se od 69,4 kg na kontrolnoj varijanti do 72,2 kg na varijanti sa najvećom dozom fosfora i kalijuma. Prosečne vrednosti hektolitarske mase zrna za sve varijante đubriva,

između strnih žita nisu se značajno razlikovale. Sve varijante đubrenja u odnosu na kontrolu, kod svih strnih žita, dovele su do značajnog povećanja hektolitarske mase zrna, dok između varijanti V1 i V2, kao i varijanti V2 i V3 nisu postojale statistički značajne razlike. Između varijanti V1 i V3, kod svih strnih žita, postojale su statistički značajne razlike u hektolitarskoj masi zrna. Naši rezultati su u saglasnosti sa rezultatima koje su naveli Jelić et al. (1998) i Lalević et al. (2012) i koji ističu da primena mineralnih đubriva dovodi do porasta hektolitarske mase, ali da su variranja između različitih varijanti đubrenja mala.

Pored genotipa, na prinos zrna u velikoj meri utiče đubrenje koje treba uskladiti sa uslovima zemljišta i klime, ali i zahtevima sorte.

Prosečne vrednosti prinosa zrna za strna žita kretale su se od 1510 kg ha^{-1} na kontrolnoj varijanti do 3209 kg ha^{-1} na varijanti sa najvećom dozom fosfora i kalijuma. Prosečne vrednosti prinosa zrna, za sve varijante đubriva, kretale su se od 2392 kg ha^{-1} kod ječma do 2696 kg ha^{-1} kod pšenice. Najveći prinos (3327 kg ha^{-1}) ostvarila je pšenica na varijanti V3. Sve varijante đubrenja u odnosu na kontrolu, kod svih strnih žita, dovele su do značajnog povećanja prinosa zrna. Takođe, značajne razlike u prinosu zrna zabeležene su između varijanti V1 i V2, kao i varijanti V2 i V3 (s izuzetkom kod tritikalea), gde je veća doza fosfora i kalijuma dovela do povećanja prinosa zrna pšenice i ječma. S obzirom na to da se radi o zemljištu koje je siromašno fosforom i kalijumom, primena mineralnih đubriva sa povećanim sadržajem fosfora i kalijuma dovela je do značajnog povećanja prinosa zrna strnih žita. Milivojević et al. (2012) preporučuju fosfatizaciju kao meru meliorativnog unošenja fosfornih đubriva u cilju povećanja produktivnosti. Prinosi strnih žita na kiselim zemljištima, ostvareni upotrebom samo mineralnih đubriva, relativno su niski. Da bi se prinosi strnih žita na kiselim zemljištima povećali, neophodno je, pored mineralnih đubriva, upotrebiti organska i krečna đubriva, s obzirom na loše fizičko-hemijske osobine kiselih zemljišta (Kovačević et al., 2006; Jelić et al., 2006; Biberdžić et al., 2013, 2014, 2015).

Veza između prinosa zrna i apsolutne mase kao i prinosa zrna i hektolitarske mase istražena je pomoću koeficijenta Pirsonove linearne korelacije (tabela 4). Izračunata je jaka pozitivna korelacija između prinosa zrna i hektolitarske razlike (r = 0,92), kao i između prinosa zrna i apsolutne mase (r = 0,88).

Tabela 4. Korelacija između prinosa, apsolutne i hektolitarske mase.
Table. 4. Correlation between yield, absolute mass and hectoliter mass.

		Prinos zrna *Grain yield*	Apsolutna masa *Absolute mass*	Hektolitarska masa *Hectoliter mass*
Prinos zrna *Grain yield*	Pearson correlation	1	,880**	,920**
	Sig. (2-tailed)		,000	,000
	N	36	36	36

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

Tabela 5. Analiza varijanse
Table 5. Analysis of variance

Strna žita *Small grains*	Mineralna đubriva *Mineral fertilizers*		Prinos *Grain yield*		Apsolutna masa *Absolute mass*		Hektolitarska masa *Hectoliter mass*	
			Mean difference	Sig.[b]	Mean difference	Sig.[b]	Mean difference	Sig.[b]
Pšenica *Wheat*	K	V1	-1399,333*	,000	-12,667*	,034	-14,667*	,005
		V2	-1655,667*	,000	-21,000*	,000	-23,333*	,000
		V3	-1859,333*	,000	-30,667*	,000	-30,000*	,000
	V1	K	1399,333*	,000	12,667*	,034	14,667*	,005
		V2	-256,333*	,004	-8,333	,298	-8,667	,180
		V3	-460,000*	,000	-18,000*	,001	-15,333*	,003
	V2	K	1655,667*	,000	21,000*	,000	23,333*	,000
		V1	256,333*	,004	8,333	,298	8,667	,180
		V3	-203,667*	,027	-9,667	,164	-6,667	,445
	V3	V1	1859,333*	,000	30,667*	,000	30,000*	,000
		V2	460,000*	,000	18,000*	,001	15,333*	,003
		V3	203,667*	,027	9,667	,164	6,667	,445
Ječam *Barley*	K	V1	-646,667*	,000	-5,667	,711	-16,000*	,002
		V2	-1118,667*	,000	-14,000*	,016	-21,333*	,000
		V3	-1456,667*	,000	-25,333*	,000	-25,000*	,000
	V1	K	646,667*	,000	5,667	,711	16,000*	,002
		V2	-472,000*	,000	-8,333	,298	-5,333	,685
		V3	-810,000*	,000	-19,667*	,001	-9,000	,151
	V2	K	1118,667*	,000	14,000*	,016	21,333*	,000
		V1	472,000*	,000	8,333	,298	5,333	,685
		V3	-338,000*	,000	-11,333	,070	-3,667	,922
	V3	K	1456,667*	,000	25,333*	,000	25,000*	,000
		V1	810,000*	,000	19,667*	,001	9,000	,151
		V2	338,000*	,000	11,333	,070	3,667	,922
Tritikale *Triticale*	K	V1	-969,333*	,000	-10,667	,100	-17,000*	,001
		V2	-1671,667*	,000	-19,667*	,001	-26,333*	,000
		V3	-1780,667*	,000	-28,333*	,000	-29,000*	,000
	V1	K	969,333*	,000	10,667	,100	17,000*	,001
		V2	-702,333*	,000	-9,000	,223	-9,333	,126
		V3	-811,333*	,000	-17,667*	,002	-12,000*	,026
	V2	K	1671,667*	,000	19,667*	,001	26,333*	,000
		V1	702,333*	,000	9,000	,223	9,333	,126
		V3	-109,000	,493	-8,667	,259	-2,667	,983
	V3	K	1780,667*	,000	28,333*	,000	29,000*	,000
		V1	811,333*	,000	17,667*	,002	12,000*	,026
		V2	109,000	,493	8,667	,259	2,667	,983

*The mean difference is significant at the 0.05 level. b. Adjustment for multiple comparisons: Sidak.

Zaključak

Na osnovu rezultata istraživanja može se zaključiti sledeće: Značajnih razlika u prinosu, apsolutnoj i hektolitarskoj masi zrna, između ispitivanih vrsta strnih žita, nije bilo. Primena mineralnih đubriva sa povećanim sadržajem fosfora i kalijumadovela je do značajnog povećanja prinosa zrna, apsolutne i hektolitarske mase strnih žita u odnosu na kontrolu. Izmeđuslabije i srednje doze fosfora i kalijuma kao i između srednje i najjače doze fosfora i kalijuma nisu postojale statistički značajne razlike u apsolutnoj i hektolitarskoj masi zrna. Značajne razlike u prinosu zrna zabeležene su između slabije i srednje doze fosfora i kalijuma, kao i između srednje i najjače doze fosfora i kalijuma (s izuzetkom kod tritikalea), gde je veća doza fosfora i kalijuma dovela do značajnog povećanja prinosa zrna pšenice i ječma. Između prinosa s jedne strane, i apsolutne i hektolitarske mase, s druge strane, postojala je jaka pozitivna korelacija. Prinosi strnih žita na kiselim zemljištima, ostvareni upotrebom samo mineralnih đubriva, relativno su niski.

Zahvalnica

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PRODUCTION CHARACTERISTICS OF SMALL GRAINS DEPENDING ON THE APPLICATION OF MINERAL FERTILIZERS WITH HIGH CONTENT OF PHOSPHORUS AND POTASSIUM

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A b s t r a c t

The aim of this study was to examine the impact of the application of mineral fertilizers with a high content of phosphorus and potassium on the productive characteristics of some small grains grown on pseudogley soils. The research was carried out in the vicinity of Kraljevo during the period 2011/2013. The experiment included wheat, winter barley and triticale and 3 variants of fertilizers (K – control; V1 – N_{80} P_{60} K_{60}; V2 – N_{80} P_{80} K_{80} and V3 – N_{80} P_{100} K_{100}). In addition to grain yield, absolute mass and hectoliter mass were observed. The obtained results were analyzed using the analysis of variance. There were no significant differences in yield, absolute mass and hectoliter mass of the grain between small grains. The use of fertilizers with an increased content of phosphorus and potassium led to a significant increase in grain yield, absolute mass and hectoliter mass of small grains compared to control. Among the variants V1 and V2, and V2 and V3 variants, there were no statistically significant differences in the absolute mass and hectoliter mass of the grain. The average values of grain yield of small grains ranged from 1510 kg ha^{-1} in the control variant up to 3209 kg ha^{-1} in the variant with the highest dose of phosphorus and potassium fertilizers. Significant differences in grain yield were observed between the variants V1 and V2, as well as between the variants V2 and V3. The application of only mineral fertilizers on acid soils leads to relatively low yields.

Key words: small grains, pseudogley, mineral fertilizers, absolute mass, hectoliter mass, yield.

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CLASSIFICATION OF GENETIC DIVERSITY FOR DROUGHT TOLERANCE IN MAIZE GENOTYPES THROUGH PRINCIPAL COMPONENT ANALYSIS

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Abstract: Water scarcity is a universal environmental constraint for agricultural sustainability and production. Two field experiments were accomplished during the 2012 and 2013 growing seasons in two sites: the experimental farm of Suez Canal University, Ismailia and Romana Province, North Sinai, Egypt to evaluate 21 genotypes of maize comprising six inbred lines and their 15 F_1 crosses for their drought tolerance. The experiments were arranged as a split-plot design with three replications, where moisture levels (100 and 50% of evapotranspiration) and maize genotypes were allocated to main plots and sub-plots, respectively. Results showed reduction in performance for most measured traits in response to water stress with varying degrees with yield plant^{-1} being the most affected. Inversely, proline and relative water content and anthesis-silking interval were increased. Correlation results confirmed the reduced grain yield with the increasing anthesis-silking interval, and suggested kernels row^{-1}, relative water content, peroxidase activity and rows ear^{-1} in Ismailia, and rows ear^{-1}, relative water content, peroxidase activity, kernel weight in Romana were indirect selection criteria for increasing yield in water scarcity environments. Principal component (PC) analysis showed that three PCs having Eigen value >1 explained 70.67 and 70.16%; 69.79 and 71.38% of the total variability among genotypes in control and stress conditions in Ismailia and Romana, respectively. The crosses P1×P3, P4×P6, P3×P5 and P1×P5 were classified as drought tolerant under Ismailia and Romana conditions. On the other hand, P1xP4, P3xP4, and P4 were considered as drought sensitive in Ismailia conditions. In addition, P5, P2×P4, P1×P4 and P5×P6 were the most affected by water deficiency under Romana conditions.

Key words: maize, drought, principle component analysis, yield, correlation.

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Introduction

Crop productivity is faced with environmental stresses such as drought, salinity, nutrient deficiency and heat. Drought is the most disturbing abiotic stress for stable crop production in developing countries. Maize is the third staple cereal crop worldwide after wheat and rice. Rapid changes in populations, society, and economies are expected to increase the demand for maize compared with wheat and rice by 2020 (Pingali, 2001). Different studies have been conducted to identify the most sensitive stages to drought stress in maize. While the flowering period was assigned as the most critical, the seedling stage was identified as influential on plant stand establishment and the resulting yield (Bänziger et al., 2000; Li et al., 2015). In the same context, Lauer (2003) stated that maize yield is most sensitive to water stress during flowering and pollination, followed by grain filling and finally vegetative growth stages. Since drought tolerance is a complex trait, maize researchers and breeders have found that using specific morpho-physiological traits at different growth stages would be extremely useful in the development of new cultivars (Bruce et al., 2002). Consequently, using identified secondary traits in parental lines that were inherited to good yielding hybrids would be ideal (Meeks et al., 2013).

The success of breeding depends generally on the genetic variability among the parental lines as a lack of this variability may limit breeding progress and the gain from selection (Singh et al., 2016). Genetic diversity among maize lines for root architecture under drought stress at the seedling stage was studied by Li et al. (2015). Others stated considerable genetic variation at flowering in modern commercial maize (Barker et al., 2005). In the same context, Bolaños and Edmeades (1996) highlighted the critical importance of the flowering stage in producing kernel number ear^{-1} and improving maize yield under stress. Correlation studies recorded by the same authors revealed the close dependence of yield on kernel number ear^{-1} (up to $r = 0.9$), and moderate to strong associations of both with anthesis-silking interval ($r = -0.4$ to -0.7). Araus et al. (2012) confirmed that ASI is the most contributing trait for yield determination under drought compared to any other secondary trait, therefore a significant proportion of variation observed in grain yield was predicted when ASI was measured at flowering. Due to the complexity of drought resistance, constructing an integrated evaluation assay to combine many factors based on investigations of multiple environments is a preliminary task.

Various studies have employed multivariate statistical analysis such as principal component analysis (PCA) to evaluate the magnitude of genetic diversity among the crop germplasm (Brown-Guedira, 2000; Wijewardana et al., 2016) and to reduce a large number of observed traits into a smaller set of

traits that have the maximum contribution in separating the genotypes. Bin Mustafa et al. (2015) found that the first four principal components explained 88.4 and 86.7% of the total variation among maize inbred lines in response to 100 and 40% moisture levels, respectively. Others recorded two PCs that contributed to 94.01% and 91.15% of total variation in root traits of 103 maize inbred lines evaluated in control and water-stressed conditions (Li et al., 2015).

Therefore, the objectives of the present study were to: i) assess the responses of maize genotypes to water stress; ii) classify and rank maize genotypes based on PCA to determine which traits are best suited for screening drought tolerance.

Materials and Methods

Genetic materials and selection of parental lines

Twenty maize inbred lines self-pollinated for eight generations, selected from the inbred line development program (Ali, 2004) belonging to local and exotic resources, were evaluated for their drought tolerance at germination (7 days) and seedling stages (20 days) in a pot experiment under greenhouse conditions. The tolerance response was quantified using polyethylene glycol (PEG, MW: 6000, The Panchi Chemicals, Hyderabad, India). Four PEG concentrations (0, 10, 20 and 30%) were applied to pots containing maize grains. Three doses of 300 ml PEG were given to each treatment at three-day intervals. Plants were assessed at germination and early seedling stages based on the following traits: germination percent, survival ability, shoot and root lengths, shoot and root fresh weights and shoot: root ratio. Based on trait means across PEG concentrations, three tolerant (P1, P3 and P5), two sensitive (P2 and P4) and one moderately tolerant (P6) lines were selected for further field experiments. The selected parents P3 and P5 were originated from the local DC-202; P1, P2 and P4 were originated from the cross between local Giza-2 and Ukraine line (AK135) and P6 was originated from the cross between DC-Youpiline (Ukraine) and local OP-Nabelgamal.

Experimental design and treatment application

Diallel crosses were made among the six maize inbreds to develop 15 F_1 crosses in the 2011 season. Field experiments were established in two locations during the 2012 and 2013 growing seasons; the experimental farm of Suez Canal University, Ismailia, and Romana Province, North Sinai, Egypt. Samples were taken from the 0–75 cm soil profile to determine physical properties and chemical compositions of the experimental sites (Table 1).

Table 1. Physical properties and chemical compositions of the experimental sites.

Properties	Ismailia	Romana
Particle size distribution (%)		
Sand	97.65	96.12
Silt	1.51	2.38
Clay	0.84	1.5
Textural class	Sand	Sand
Field capacity (%)	18.0	17.20
PH	7.88	8.19
ECe (dS m^{-1})	1.23	2.35
Soluble cations (meq l^{-1})		
$Ca2^{+}$	6.2	11.50
Mg^{+2}	4.0	7.50
Na^{+}	2.13	4.93
K^{+}	0.39	0.98
Organic C (g kg^{-1})	1.39	1.72
Available N (mg kg^{-1})	4.10	5.50
Available P (mg kg^{-1})	10.32	11.17

The experiments were laid out in a randomized complete block split-plot design at two drip irrigation rates: 100 (WW) and 50% (WS) of the estimated evapotranspiration and 21 maize genotypes were assigned as the main and split-plots, respectively. Each split-plot consisted of two polyethylene lateral drip lines with a length of 3 m and 0.70 m between lines and 0.3 m between drippers. The total area for each subplot was 4.2 m^2. The total amount of water (I) was determined from the calculated water requirement for maize (mm) as determined from the crop coefficient (kc) and the daily reference evapotranspiration (ETc) using the following equation:

$$I = ET_o k_c \qquad \text{Eq. (1)}$$

Evapotranspiration (ETo) was calculated by the Penman–Monteith equation (Allen et al., 2011) using daily data from a meteorological station located within 500 m from the experimental site. FAO computer programs (CROPWAT Version 7.0 and CLIMWAT) were used to calculate ETo. Accordingly, the total amounts of water applied for irrigation treatments were 6280 and 3140 m^3 ha^{-1}, for 100 and 50% irrigation levels, respectively.

Three seeds around each dripper were sown on May 20 and 25 at Ismailia and Romana in 2012 and 2013, respectively. One week after seeding, plants were thinned to one plant per dripper to obtain a final plant density of 47600 plants ha^{-1}. All agricultural practices were applied as recommended.

Measurements

The anthesis-silking interval (ASI) was calculated on a plot basis as differences between silking and anthesis dates. At harvest, plant height (PH) was measured on ten representative plants from each sub-plot, then ears were separated and the following traits were recorded: rows ear^{-1} (RE), kernels row^{-1} (KR), 100-kernel weight (KW) and yield plant^{-1} (YP). Relative water content (RWC%), peroxidase activity and proline content (μg g dw^{-1}) were determined using 0.50 g fresh leaves at 50% silking stage. Relative water content was determined using the method of Mata and Lamattina (2001) and calculated according to the formula: RWC (%) = (FW – DW)/(TW – DW) × 100, where: FW, DW and TW are fresh, dry and turgor weight of leaves. Proline content was estimated by the ninhydrin method of Bates et al. (1975). The chromophore formed was extracted with 4 ml of toluene and the absorbance of the resulting organic layer was measured at 520 nm and calculated using the formula:

$$\text{Proline content} = \frac{34.11 * OD520 * V}{2 * F} \quad \text{Eq. (2)}$$

where: OD is the optical density; F, leaf sample weight; V, extract volume. Calibrations were made with 1mM L-proline as a standard. Peroxidase activity was assayed according to Liu and Huang (2000), briefly 0.5 g of fresh leaves was homogenized in 4 ml of 50 mM potassium phosphate buffer, pH 7.0 and 1 mM Na_2EDTA. POD reaction solution (3 ml) was assayed at 470 nm every 30 seconds; one unit of peroxidase activity was defined as an absorbance change of mg fresh weight min^{-1}.

Statistical analysis

Data were averaged across years within sites and analysis of variance was performed to test the effects of irrigation levels, genotypes and their interaction on the measured traits using COSTAT software (version 6.311). The significance of differences was tested using least significant difference tests at $P = 0.05$. Principal component analysis (PCA), which is a tool to identify parameters that best describe the tolerance to response variables, was used to separate genotypes into tolerant groups. PCA was performed on the correlation matrix of 21 genotypes and nine response variables. Eigenvectors generated by PCA were used to identify parameters that best differentiated cultivars for drought tolerance. The first two PC scores, PC1 and PC2 that accounted for maximum variability of the parameters tested, were used to group the genotypes. XLSTAT software (2016.02.275370) was used to perform the analysis and biplot visualization. Eigenvectors generated by PCA were used to rank tested genotypes for their drought tolerance.

Results and Discussion

Responses of maize genotypes to irrigation treatments

Statistical analysis showed the significant differences among the genotypes, irrigation levels, and genotype × irrigation interval combinations for the measured traits in both locations (Tables 2 and 3). The significant variations among genotypes and genotypes × irrigation regimes under two different irrigation conditions indicate that adequate genetic variation existed among the tested maize genotypes (parents and F_1 crosses). Mean values of most traits were decreased after WS compared to WW, except ASI, POD and PC. ASIs of 5.15 and 5.75 d under WS regime in Romana and Ismailia were prolonged compared with 3.59 and 4.50 d of WW regime, respectively. While genotypes showed 16.22, 19.61, 20.62, 27.23, 31.73 and 44.84% reduction in RE, 100-GW, RWC, PH, KR and YP due to water stress; ASI, POD and PC increased by about 27.88, 59.88 and 151.15% compared to regular irrigation in Ismailia. In Romana, water stress reduced mean values for the same traits by 17.03, 19.93, 12.95, 26.53, 31.36 and 50.53%, in contrast, genotypes showed 43.47, 66.73 and 139.46% increases for ASI, POD and PC, respectively. While less reduction due to WS was recorded for ASI and YP in Ismailia, average reduction in RWC was higher in Romana. Morphological and other yield-related traits showed the negligible disparity between sites. Studies show that maize is susceptible to water scarcity during mid to late vegetative development and flowering significantly affects its growth and productivity (Chen et al., 2012; Svačina et al., 2014). Brar et al. (2016) recorded 35.9 and 40.9% increases in maize yield under 100% over 60% replacement of the cumulative pan evaporation in a two-year trial.

Comparing experimental sites, at WS treatments, mean values for measured traits were higher for crosses compared to ILs in Romana. In Ismailia location, inbred lines P1 and P3 recorded the tallest plants, heaviest kernel weight and maximum KR values. Generally, parents exhibited non-visible variation for ASI being P2, and P4 was the most prolonged in ASI. Generally, P4 was the most sensitive parent that showed lower mean values for the majority of traits. Two single crosses, P4×P6 and P3×P5, having the highest GY under WS were in the first order for KR, KW, POD activity and RWC. Two crosses, P1xP3 and P1xP5, showing moderate grain yield were characterized by moderate PH, high RE, KW and RWC. Conversely, crosses P2×P3 and P2×P5 possessed the lowest GY, low RE and KR compared with other crosses. In Romana site, only P1 revealed the highest yielding ability accompanied by the highest values for yield components, PC and RWC. In contrast, P4 was the most sensitive parent to water stress and recorded the lowest values for the majority of traits. While all lines showed similar ASI values, lines 2 and 4 were the latest.

Table 2. Mean values for nine variables measured on maize genotypes evaluated across two growing seasons in Ismailia. Regular and italic letters show values at control and 50% of evapotranspiration.

Genotypes	ASI	PH	RE	KR	KW	POD	PC	RWC	YP
P1	4.3	178.45	14.65	38.85	32.85	37.95	13.50	82.86	126.30
	5.6	142.50	13.65	29.35	27.15	61.50	32.40	69.20	86.55
P2	4.7	166.40	12.65	33.15	30.65	32.45	11.45	70.59	116.00
	6.2	141.05	11.50	26.35	23.9	45.65	21.40	60.63	55.80
P3	3.3	181.55	14.80	34.15	32.90	46.95	11.10	79.74	143.65
	5.5	136.00	11.20	30.35	26.75	69.10	24.50	67.89	77.50
P4	5.0	157.60	13.50	29.70	27.95	26.30	11.10	75.45	81.30
	7.0	120.60	11.00	18.50	28.00	54.35	21.25	60.09	61.55
P5	5.3	181.45	15.15	38.35	36.15	55.75	11.05	85.11	147.20
	5.6	122.05	13.00	24.50	25.50	91.15	24.70	67.90	75.75
P6	4.3	143.50	14.30	31.80	29.55	37.80	10.85	77.74	101.30
	5.3	129.40	11.25	25.50	23.75	65.60	23.60	60.02	68.20
P1xP2	4.0	181.30	15.15	29.00	34.40	38.65	13.20	79.99	98.45
	6.2	143.25	12.50	22.70	26.15	63.90	34.35	57.91	59.15
P1xP3	4.7	233.75	16.00	41.00	35.55	51.85	13.20	81.04	170.60
	6.0	137.95	14.00	26.30	27.70	73.80	43.40	64.93	73.90
P1xP4	4.7	186.35	16.00	32.85	31.35	25.45	12.90	77.92	110.90
	5.6	129.40	12.15	21.65	25.20	62.00	21.40	62.55	61.85
P1xP5	5.0	211.05	16.55	37.65	33.15	35.90	12.35	80.54	168.25
	4.6	144.55	13.35	23.20	27.75	69.20	20.85	63.89	74.90
P1xP6	4.0	175.40	14.35	37.85	35.85	35.15	12.85	80.30	140.60
	5.4	136.95	12.35	27.15	27.75	51.05	36.15	56.97	69.45
P2xP3	5.0	176.70	16.00	35.50	31.80	60.75	12.35	85.90	146.75
	6.2	134.05	14.65	22.00	30.10	77.20	40.95	62.22	55.25
P2xP4	5.7	179.65	15.60	36.50	27.75	48.35	13.25	82.26	128.05
	6.2	130.40	13.15	21.85	25.55	45.85	33.45	62.48	69.65
P2xP5	5.7	170.75	15.35	35.35	30.35	32.65	12.30	84.48	114.40
	5.3	136.35	12.00	20.50	25.60	50.65	29.25	47.77	49.75
P2xP6	3.7	180.45	15.80	37.00	33.75	27.30	12.60	74.38	114.10
	6.5	138.85	11.65	22.15	26.65	46.10	39.65	63.00	68.05
P3xP4	3.7	187.05	14.30	39.50	30.20	64.80	13.05	77.46	93.45
	5.7	141.00	12.50	22.65	20.75	79.85	22.70	61.16	73.80
P3xP5	4.0	214.40	15.35	39.80	35.45	59.20	11.85	86.16	146.00
	5.6	138.70	14.00	27.25	26.65	94.90	28.70	67.15	85.95
P3xP6	3.7	193.35	14.65	42.50	34.05	25.70	13.10	74.59	105.10
	5.4	137.45	13.15	29.30	24.10	40.75	38.45	71.07	72.30
P4xP5	5.3	199.80	15.15	29.30	31.85	42.05	13.00	75.94	95.50
	6.3	136.50	12.85	21.50	25.90	69.25	39.95	59.70	67.60
P4xP6	4.0	229.70	15.50	41.85	34.30	22.40	13.30	82.01	154.25
	4.8	140.70	13.00	27.30	29.70	73.30	40.25	66.85	86.20
P5xP6	4.3	186.30	16.15	36.85	31.55	34.65	10.10	76.95	139.10
	5.7	131.30	13.65	27.80	23.20	61.10	31.75	73.41	63.70
LSD									
≠IL	0.97	3.79	0.44	1.93	0.71	0.19	0.32	1.48	8.94
G	1.82	9.83	1.32	4.38	1.25	0.72	0.84	3.25	18.02
IL*G	3.18	13.30	2.67	5.75	2.42	2.29	1.26	1.42	6.75

≠IL: Irrigation level, G: Genotypes, IL×G: Irrigation level × Genotype, ASI = Anthesis-silking interval, PH = Plant height, RE = Rows ear^{-1}, KR = Kernels row^{-1}, KW = Kernel weight, POD = Peroxidase activity, PC = Proline content, RWC = Relative water content, YP = Yield plant^{-1}.

Table 3. Mean values for nine variables measured on maize genotypes evaluated across two growing seasons in Romana. Regular and italic letters represent values at control and 50% of evapotranspiration.

Genotypes	ASI	PH	RE	KR	KW	POD	PC	RWC	YP
P1	3.8	167.4	13.0	37.95	27.10	35.40	14.80	78.93	105.70
	4.5	126.5	11.6	23.45	24.05	58.85	35.00	68.39	61.30
P2	3.2	156.5	12.8	33.85	25.60	29.95	15.40	69.66	100.20
	6.2	124.8	10.3	25.55	21.75	42.75	27.15	61.88	50.90
P3	3.4	169.4	13.4	32.70	25.65	44.25	16.30	76.65	108.30
	4.8	120.9	10.4	23.80	22.15	66.40	27.95	67.38	52.70
P4	3.6	147.2	12.1	30.35	26.65	23.60	14.35	70.13	85.70
	6.5	113.9	10.3	20.35	18.80	51.50	22.85	56.38	44.50
P5	3.8	167.6	13.3	37.00	29.05	53.05	16.10	77.03	131.25
	4.5	108.8	11.1	21.30	20.40	88.60	27.80	70.76	52.80
P6	3.3	132.4	12.8	32.70	25.85	35.25	17.15	73.05	101.10
	4.5	112.1	10.4	24.15	19.25	63.10	27.30	62.07	51.05
P1xP2	4.2	159.3	12.8	36.45	27.30	49.55	12.35	73.49	110.30
	5.8	126.4	10.3	26.45	21.15	90.45	23.30	72.29	70.05
P1xP3	3.8	201.3	13.3	36.75	30.10	63.00	13.05	77.70	136.70
	4.5	121.0	11.2	30.10	23.70	86.90	42.65	73.66	60.90
P1xP4	3.3	164.8	13.7	34.15	26.55	45.50	12.75	73.79	112.45
	5.8	113.8	10.4	25.35	21.80	79.80	27.15	63.73	59.60
P1xP5	3.3	181.3	13.6	35.30	31.10	33.95	13.25	74.72	134.20
	4.2	129.4	11.7	26.90	26.85	85.95	29.35	69.88	78.35
P1xP6	3.8	151.5	12.4	32.85	27.90	54.60	12.90	73.47	126.45
	4.7	121.0	10.6	25.40	24.05	94.15	40.70	65.96	66.85
P2xP3	3.6	150.80	13.3	36.80	30.50	37.25	11.65	75.54	121.60
	5.7	117.50	11.4	23.35	22.65	75.50	40.00	63.65	70.60
P2xP4	3.5	149.20	11.6	36.50	26.65	31.20	12.40	73.41	111.55
	6.0	116.1	10.0	22.85	20.55	89.95	23.85	61.08	56.70
P2xP5	4.0	145.6	12.7	33.75	27.00	38.70	12.60	75.96	118.75
	5.5	119.55	10.4	23.35	23.40	61.10	34.70	64.80	62.85
P2xP6	3.8	155.85	13.4	32.45	27.55	53.20	12.75	69.55	114.25
	5.0	122.1	10.7	23.05	23.60	68.55	26.45	67.98	44.80
P3xP4	3.1	153.95	12.0	34.05	25.10	32.80	11.90	74.54	115.10
	5.0	124.30	9.4	21.50	20.85	53.25	33.20	50.03	54.90
P3xP5	3.5	180.9	14.4	39.55	31.55	45.15	11.60	76.99	158.95
	4.5	121.6	11.0	26.90	24.35	82.00	26.40	63.69	61.90
P3xP6	3.8	168.4	12.0	40.25	31.55	60.85	12.30	72.53	99.45
	5.8	121.9	10.4	26.30	24.15	76.75	38.95	64.05	48.85
P4xP5	3.8	169.9	11.3	36.55	28.30	45.50	13.50	69.50	104.35
	5.3	121.2	10.4	23.20	20.95	61.35	40.15	63.70	49.75
P4xP6	3.2	200.2	14.0	39.55	30.65	54.60	13.20	75.94	132.30
	4.5	125.3	11.4	26.75	24.65	87.15	39.75	59.50	62.15
P5xP6	3.3	161.8	12.0	33.75	26.00	64.35	12.00	74.14	148.65
	4.8	115.5	10.7	20.10	21.45	89.40	41.35	64.29	64.05
LSD									
≠IL	1.28	3.15	1.09	2.67	2.85	1.49	0.98	1.85	14.38
G	1.15	10.80	1.29	4.44	2.79	4.01	1.27	3.79	13.87
IL×G	3.17	9.68	2.74	5.56	1.09	2.21	1.32	2.48	14.61

≠IL: Irrigation level, G: Genotypes, IL×G: Irrigation level × Genotype, ASI = Anthesis-silking interval, PH = Plant height, RE = Rows ear^{-1}, KR = Kernels row^{-1}, KW = Kernel weight, POD = Peroxidase activity, PC = Proline content, RWC = Relative water content, YP = Yield plant^{-1}.

The cross P1×P5 exhibited the highest YP (78.35g) closely followed by crosses P2×P3 (70.60g) and P1xP2 (70.05g) with the lowest yield recorded for P1×P4 (59.60g). Similar trends were detected for ASI, PH, RE, KR, KW, POD and RWC, where the cross P1×P5 combined high values for these traits. The minimum values for RE, KR, 100-KW, RWC and POD were observed for the cross P3×P4. Maize is a commercial crop, and most experiments evaluating drought tolerance have been focused on hybrids rather than on inbred lines. In this study, inbred lines were considered sources for excellent genes for drought tolerance and high yield. Overall, it is suggested that promising hybrids combine at least one drought tolerant parent. In accordance with previous results, the inbred lines that make hybrids tolerant could be having favorable alleles for GY under drought (Makumbi et al., 2011). Moreover, Monneveux et al. (2008) stated that three-way hybrids involving two drought tolerant population lines yielded more than those involving one only, indicating the feasibility of gene pyramiding for drought tolerance.

Association of traits and classification of drought tolerance using PCA

PCA gives two important pictures of association among traits and classification of tested genotypes based on their tolerance/sensitivity to water stress. Since it is not sufficient to consider one of yield-correlated traits as indicative for stress resistance, we considered these traits all together as indicators for drought-resistant maize. The cosine of the angles between vectors shows the magnitude of correlation between traits, whereas the acute angles represent positive correlations, the wide obtuse angles show a negative correlation (Figures 1 and 2). The length of the vectors connecting traits to the origin shows the extent of variability. Water stress treatments at both sites showed that ASI was negatively correlated with all measured traits. A very strong association was recorded for KR, RWC, POD and RE with YP in Ismailia, while in Romana, YP correlated positively with RE, RWC, POD and KW. In Ismailia, RE correlated positively with POD and PC; RWC correlated with KR, on the other hand, KW showed positive correlations with PH and PC. In Romana, increasing GY was associated with increasing RE, KW and POD under WS conditions and KW recorded positive associations with PH, RE, KR and PC. Results by Ziyomo and Bernardo (2013) recorded negative correlations of ASI with YP and PH. The study by Monneveux et al. (2008) suggested that selection for bigger grains and smaller tassels may help to increase grain yield in water-limited environments in the near future.

PCA for nine traits and 21 genotypes at two irrigation treatments and two experimental sites was performed to identify the principal components of measured parameters that best described the response to irrigation treatments and, thus, to identify tolerant and sensitive genotypes (Figures 1 and 2). Results showed that the first three PCs contributed 70.67 and 70.16%; and 69.79 and 71.38% to the total

variation among genotypes at WW and WS in Ismailia and Romana, respectively (Table 4). Bin Mustafa et al. (2015) stated that 59.3 and 55% of the total variation were contributed by the first two PCs when evaluating maize genotypes at 100 and 40% moisture levels. PCA showed that the three factors had eigenvalue > 1, moreover 56.13 and 57.2; 56.22 and 58.94% of the total variability were explained by the first two PCs under the same conditions. Generally, the contributions of PC1 for the nine traits were more than two times that of PC2. The first PC was positively correlated with YP, PH, KR, RE and 100-KW, whereas, POD and RWC were the most important contributing traits to PC2 under WW conditions in Ismailia. At WS treatment, YP, KR, RWC and RE contributed with PC1, while KW and PC were the most important in PC2. In Romana, PC1 presented higher values for PH, KW, YP and KR, while RE and RWC were the most contributing traits for PC2 at WW. At WS, PC2 was related to diversity among maize genotypes due to PH with positive and high loadings and negative loadings of POD, while KW, RE, RWC and YP were the most positive contributors for variation in PC1 and ASI. Previous studies revealed the significant importance of high contributing variables for primary selection in the investigated germplasm and further breeding to generate more variability (Kumar et al., 2016).

Table 4. Eigenvectors (normal values) and factor loadings (italic values) for the principal components obtained for nine traits measured on maize genotypes.

Traits	Ismailia						Romana					
	100%			50%			100%			50%		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
ASI	0.06	-0.14	0.82	-0.43	0.16	-0.04	-0.11	-0.42	0.68	-0.31	0.17	0.56
	0.11	*-0.17*	*0.94*	*-0.76*	*0.21*	*-0.04*	*-0.21*	*-0.49*	*0.74*	*-0.61*	*0.20*	*0.59*
PH	0.43	-0.23	-0.20	0.22	0.37	-0.36	0.42	0.07	0.003	0.27	0.63	-0.25
	0.82	*-0.28*	*-0.23*	*0.40*	*0.49*	*-0.40*	*0.82*	*0.08*	*0.003*	*0.54*	*0.73*	*-0.27*
RE	0.37	-0.10	0.13	0.32	0.28	0.49	0.31	0.50	0.18	0.36	-0.10	0.42
	0.71	*-0.12*	*0.15*	*0.58*	*0.37*	*0.55*	*0.60*	*0.58*	*0.20*	*0.70*	*-0.12*	*0.447*
KR	0.38	-0.07	-0.31	0.40	-0.09	-0.49	0.39	-0.24	0.15	0.36	0.18	-0.14
	0.72	*-0.09*	*-0.36*	*0.72*	*-0.12*	*-0.54*	*0.75*	*-0.27*	*0.16*	*0.71*	*0.22*	*-0.15*
KW	0.37	-0.07	0.17	0.20	0.51	0.02	0.40	-0.25	0.15	0.43	0.31	0.10
	0.701	*-0.09*	*0.20*	*0.358*	*0.67*	*0.02*	*0.79*	*-0.28*	*0.16*	*0.84*	*0.37*	*0.11*
POD	0.18	0.60	0.06	0.30	-0.23	0.60	0.32	-0.05	-0.54	0.31	-0.51	0.08
	0.337	*0.73*	*0.07*	*0.542*	*-0.31*	*0.66*	*0.62*	*-0.06*	*-0.58*	*0.61*	*-0.60*	*0.09*
PC	0.23	-0.57	0.09	0.19	0.56	0.06	0.32	0.32	0.34	0.19	0.20	0.63
	0.445	*-0.68*	*0.10*	*0.354*	*0.737*	*0.06*	*0.62*	*0.77*	*0.37*	*0.38*	*0.23*	*0.67*
RWC	0.34	0.43	0.30	0.38	-0.25	-0.11	-0.20	0.59	0.23	0.33	-0.36	-0.01
	0.646	*0.51*	*0.34*	*0.691*	*-0.331*	*-0.12*	*-0.39*	*0.69*	*0.25*	*0.66*	*-0.42*	*-0.01*
YP	0.43	0.19	-0.21	0.45	-0.24	-0.11	0.40	-0.01	0.01	0.39	-0.11	-0.12
	0.826	*0.23*	*-0.24*	*0.824*	*-0.316*	*-0.13*	*0.78*	*-0.02*	*0.012*	*0.78*	*-0.13*	*-0.13*
Eigenvalue	3.61	1.44	1.31	3.33	1.73	1.22	3.79	1.34	1.18	3.94	1.37	1.12
Contribution%	40.06	16.07	14.54	37.02	19.20	13.57	42.11	14.91	13.14	43.74	15.20	12.44

ASI = Anthesis-silking interval, PH = Plant height, RE = Rows ear^{-1}, KR = Kernels row^{-1}, KW = Kernel weight, POD = Peroxidase activity, PC = Proline content, RWC = Relative water content, YP = Yield plant^{-1}.

Cultivars were classified into four groups based on biplots of PC1 vs. PC2 (Figures 1 and 2). Accordingly, under WS treatment, P1xP3, P4xP6 and P3xP6 were considered tolerant genotypes showing high scores for PC1 and PC2; P1, P3, P1×P5, P3×P5 and P5xP6 were categorized as moderately tolerant genotypes. The remaining genotypes were classified as moderately sensitive to sensitive under Ismailia conditions. In Romana, only P1 was categorized as the tolerant parent, both P4 and P5 were the most sensitive. Regarding crosses, eight crosses were distinguished as tolerant and moderately tolerant with varying degrees, with special regard to P4xP6, P3×P5, P1×P3 and P1×P5, in contrast, P2×P4, P1×P4 and P5×P6 showed the least tolerance to water shortage. According to previous studies (Wijewardana et al., 2015), the scores of PC1 and PC2 together revealed the importance of the variables studied in the cultivar separation for stress tolerance. Therefore, in Romana, genotypes with higher scores for PC1 (P1xP5) tended to have higher values for KR, KW, RE and YP and lower values for PH, PC and ASI. In Ismailia, the cross P4xP6 had higher values for RE, KR, POD, RWC and YP and low values for PH and PC.

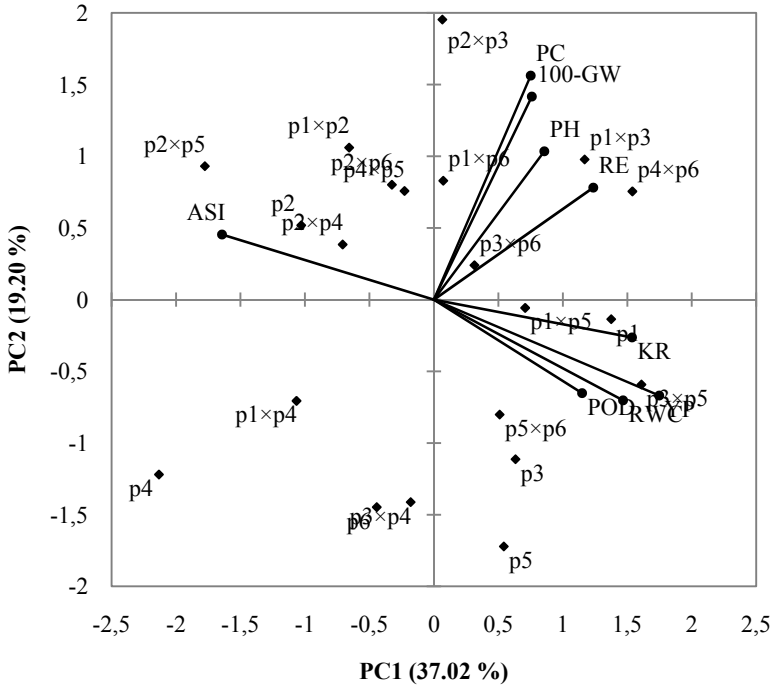

Figure 1. A biplot for the first two principal component (PC) scores, PC1 vs. PC2, related to the classification of maize genotypes for drought tolerance in Ismailia. Diamond and circle symbols refer to genotypes and variables, respectively.

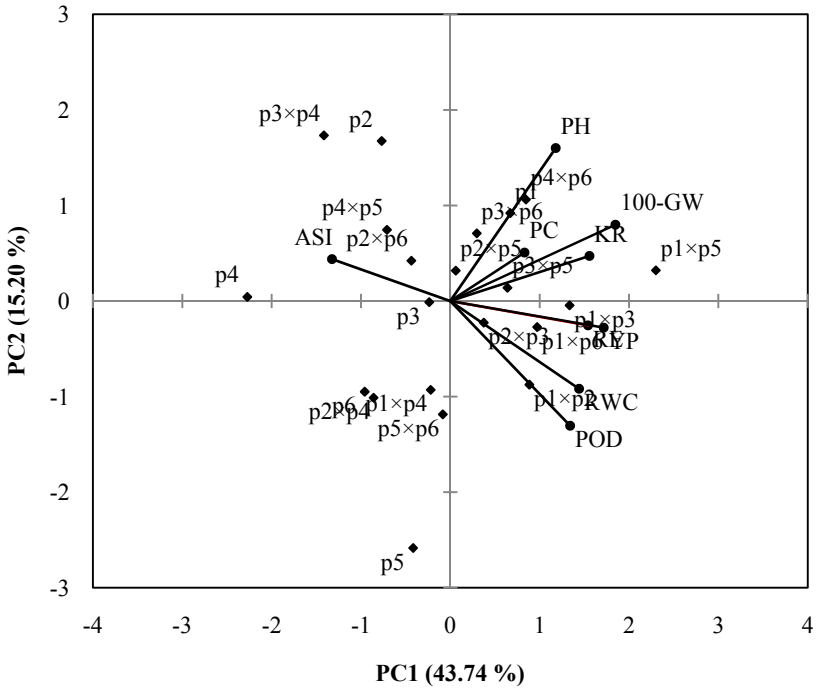

Figure 2. A principal component analysis (PCA) biplot for the first two principal component (PC) scores, PC1 vs. PC2, related to the classification of 21 maize genotypes for drought tolerance in Romana.

Conclusion

The tested maize genotypes exhibited substantial variability in their responses for all measured traits. PCA identified P1xP3, P4xP6, P3xP5 and P1xP5 as tolerant crosses for drought effects at both sites. Moreover, ASI, KR, RWC, POD and RE in Ismailia; and RE, RWC, POD, KW in Romana were ascribed as variables to best describe drought tolerance of investigated genotypes due to their strong correlations with YP. Experiments evaluating drought tolerance have been focused more on hybrids than on inbred lines. In this study, inbred lines were considered as sources for excellent genes for drought tolerance and high yield. Overall, it is suggested that promising hybrids combine at least one drought tolerant parent. In accordance with previous results, the inbred lines that make tolerant hybrids could be having favorable alleles for GY under drought. Moreover, analysis shows that three-way hybrids involving two drought tolerant population lines yielded more than those involving only one line, indicating the feasibility of gene pyramiding for drought tolerance.

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KLASIFIKACIJA GENETIČKE DIVERGENTNOSTI GENOTIPOVA KUKURUZA ZA TOLERANCIJU PREMA SUŠI PUTEM ANALIZE GLAVNIH KOMPONENTI

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R e z i m e

Nestašica vode predstavlja univerzalno ograničenje životne sredine za održivu poljoprivrednu proizvodnju. Sprovedena su dva poljska ogleda tokom 2012. i 2013. godine na dva lokaliteta: na eksperimentalnom gazdinstvu Univerziteta Suecki kanal, Ismailija i u pokrajini Romana, Severni Sinaj, Egipat. U ogledu je izvršeno procenjivanje 21 genotipa kukuruza koji obuhvataju šest inbred linija i njihovih 15 F_1 hibrida, radi ispitivanja njihove tolerantnosti prema suši. Ogledi su bili koncipirani kao sistem podeljenih parcela sa tri ponavljanja, gde su nivoi vlažnosti (100 i 50% evapotranspiracije) i genotipovi kukuruza dodeljeni glavnim parcelama odnosno potparcelama. Rezultati su pokazali smanjenje učinka za većinu merenih osobina kao odgovor na različite stepene vodnog stresa i sa prinosom po biljci kao najviše pogođenom osobinom. Nasuprot tome, prolin i relativni sadržaj vode i povećanje intervala metličanje-svilanje su bili povećani. Rezultatima korelacione analize su uvrđeni smanjeni prinos zrna sa povećanim intervalom metličenje-svilanje, i sugerisano je da su broj zrna u redu, relativni sadržaj vode, aktivnost peroksidaze i broj redova po klipu u Ismailiji, i broj redova po klipu, relativni sadržaj vode, aktivnost peroksidaze, masa zrna u Romani bili indirektno selekcioni kriterijumi za povećanje prinosa u sredinama sa nestašicom vode. Analiza glavnih komponenti (engl. *principal component* – PC) pokazala je da tri glavne komponente sa vrednošću >1 objašnjavaju 70,67 odnosno 70,16%; 69,79 odnosno 71,38% ukupne varijabilnosti među genotipovima u kontrolnim i uslovima stresa u Ismailiji odnosno Romani. Hibridi P1×P3, P4×P6, P3×P5 i P1×P5 klasifikovani su kao tolerantni prema suši u Ismailiji i Romani. S druge strane, P1×P4, P3×P4, i P4 smatrali su se osetljivim prema suši u Ismailiji. Pored toga, P5, P2×P4, P1×P4 i P5×P6 najviše su bili pogođeni nestašicom vode u Romani.

Ključne reči: kukuruz, suša, analiza glavnih komponenti, prinos, korelacija.

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FLORISTIČKI SASTAV KOROVA, MORFOLOŠKE I PRODUKTIVNE OSOBINE GENOTIPOVA RAZLIČITIH ALTERNATIVNIH VRSTA PŠENICE U ORGANSKOJ PROIZVODNJI

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Sažetak: Cilj rada jeste ocena florističkog sastava korova, proučavanje kvantitativnih osobina (visina stabla, dužina poslednje internodije, dužina klasa, masa hiljadu zrna, prinos zrna) različitih genotipova alternativnih vrsta pšenice i odnosa između ispitivanih osobina i mase korova u sistemu organske proizvodnje. Ispitivanje je realizovano prema planu slučajnog blok sistema u četiri ponavljanja, u dvogodišnjem periodu (2009/2010–2010/2011). Ispitivana je po jedna sorta tvrde pšenice *Triticum durum* (Dolap), kompaktum pšenice *Triticum compactum* (Bambi) i krupnika *Triticum spelta* (Nirvana). U okviru tretmana za održavanje i povećanje biološke plodnosti zemljišta ispitivane su dve varijante đubrenja: T_1 – đubrenje samo mikrobiološkim đubrivom u prihranjivanju (5,0 l ha^{-1}); T_2 – đubrenje biohumusom (3,0 t ha^{-1}) i mikrobiološkim đubrivom u prihranjivanju (5,0 l ha^{-1}); T_0 – kontrola – bez primene đubriva. Rezultati su pokazali da genotip i tretman, kao ispitivani faktori, veoma značajno utiču na suvu masu korova, visinu stabla, dužinu poslednje internodije i klasa, kao i na prinos zrna proučavanih genotipova, dok je uticaj njihove interakcije slabiji. U uslovima niskih ulaganja, između visine stabla i suve mase korova konstatovana je veoma značajna negativna korelacija (r = - 0,58), kao i značajna negativna korelacija između mase korova i visine prinosa zrna (r = - 0,44). Dobijeni rezultati ukazuju da pravilan odabir genotipa, uz primenu đubriva, može da ima značajan uticaj na zakorovljenost i uspostavljanje stabilne proizvodnje u organskoj poljoprivredi.

Ključne reči: organska poljoprivreda, alternativne vrste pšenice, kvantitativne osobine pšenice, korovi.

Uvod

Korovi predstavljaju veoma važan ograničavajući činilac u biljnoj proizvodnji jer mogu značajno da smanje prinos i kvalitet gajenih biljaka. Korovi akumuliraju

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veće količine azota, fosfora, kalijuma, kalcijuma i magnezijuma u odnosu na useve, čime iscrpljuju hranljive materije iz zemljišta (Barker et al., 2006). Osim za hraniva, korovi gajenim usevima predstavljaju aktivne konkurente za svetlost, vlagu i životni prostor, čime prouzrokuju velike gubitke u prinosu useva i povećavaju troškove proizvodnje.

Problem suzbijanja korova na proizvodnim površinama dobija na značaju naročito u sistemu organskog upravljanja, koji isključuje mogućnost upotrebe sintetičkih herbicida. Veliki broj istraživanja širom sveta ukazuje na povećanje diverziteta korovskih sinuzija, gustine populacija, proizvodnje biomase i banke semena korova pri konverziji sa konvencionalne na organsku proizvodnju (Hald, 1999; Van Elsen, 2000; Riemens et al., 2007). U sistemu organske proizvodnje, na zemljištu dobrih proizvodnih karakteristika, broj korovskih vrsta može biti i do deset puta veći u odnosu na konvencionalnu proizvodnju istog useva (Van Elsen, 2000). Osim većeg biodiverziteta, biomasa korova može biti petostruko veća (Hald, 1999), nadzemna biomasa useva žita i do 25% manja (Hald, 1999), a prinosi i preko 20% niži (Taylor et al., 2001) u uslovima organskog gajenja.

Konkurentski odnosi između gajene biljke i korova naročito su izraženi u agrofitocenozama jer je životni prostor ograničen, a činioci na koje utiče čovek prilagođeni gajenoj biljci. Razlike u jačini konkurentske sposobnosti postoje i među gajenim biljkama. Kada su u pitanju strna žita, najjačim konkurentom se smatra ječam, zatim slede raž, pšenica i ovas. Taylor et al. (2001) primetili su da je ječam jači konkurent korovima za resurse koji se nalaze u zemljištu, dok je kod ovsa i pšenice jače izražena konkurentnost za svetlost. Istovremeno, ovi autori ukazuju na značaj razvoja nadzemne biomase, odnosno robusnosti, kao i veće pokrovnosti useva u potiskivanju korova i sprečavanju njihovog rasta (Taylor et al., 2001).

Sposobnost gajenih biljaka da ispoljavaju konkurentnost za najvažnije životne činioce kontrolisana je kombinacijom nekoliko specifičnih morfoloških, fizioloških i bioloških osobina. To su pre svega osobine koje utiču na zasenjivanje i presretanje fotosintetski aktivnih zraka kao što su položaj lista, dobar početni rast, visina biljke, kapacitet bokorenja, veličina semena, nicanje i brzina rasta korena (Bertholdsson, 2005).

Brojna istraživanja pokazala su da visina biljke ima veliki doprinos u supresiji korova (Hulc, 1996; Cosser et al., 1997; Piliksere et al., 2013). Ispitujući razlike u konkurentskoj sposobnosti između četiri genotipa jare pšenice, Hucl (1998) je ustanovio da su sorte sa visokim stablom i jačim kapacitetom bokorenja ostvarile veće prinose u uslovima zakorovljenosti, u odnosu na sorte koje su bile niže i slabije bokorile. Konkurentnije sorte su ostvarile 7–9% veće prinose u uslovima zakorovljenosti (Hucl, 1998). Bolja konkurentnost sorti sa visokim stablom potvrđena je i u ispitivanjima drugih autora (Mason et al., 2007). Mason et al. (2007) ističu da bi, interakcijom visine sa drugim specifičnim osobinama

koje doprinose efikasnijem korišćenju životnih činilaca, bila stvorena bolja tolerantnost i veća sposobnost useva da guši korove. Cilj ispitivanja jeste proučavanje kvantitativnih osobina (visina stabla, dužina poslednje internodije, dužina klasa, masa hiljadu zrna, prinos zrna) različitih genotipova alternativnih vrsta pšenice, kao i odnosa između ispitivanih osobina i mase korova u sistemu organske proizvodnje.

Materijal i metode

Ispitivanje je obavljeno na „Radmilovcu", eksperimentalnom školskom dobru Poljoprivrednog fakulteta u Beogradu, u toku dve sezone (2009/2010–2010/2011). Korišćena je metoda slučajnog blok sistema u četiri ponavljanja, a površina elementarne parcele iznosila je 6 m^2. Ispitivanja su obavljena na zemljištu tipa izluženi černozem. Hemijska svojstva zemljišta su sledeća: pH (u H_2O) 8,04, ukupan sadržaj azota iznosio je 0,13%, P_2O_5 22,18 mg, K_2O 19,10 mg, sadržaj humusa u orničnom sloju kretao se od 2,09% do 2,82%.

Ispitivana je po jedna sorta tvrde pšenice *Triticum durum* (Dolap, G1), kompaktum pšenice *Triticum compactum* (Bambi, G2) i krupnika *Triticum spelta* (Nirvana, G3). Količina semena za setvu prilagođena je intenzitetu bokorenja, načinu i vremenu setve kako bi se obezbedio optimalan broj biljaka po jedinici površine za datu vrstu i sortu. Setva je obavljena ručno, krajem druge dekade oktobra meseca.

Za održavanje i povećanje biološke plodnosti zemljišta ispitivane su dve varijante đubrenja: T_1 – đubrenje samo mikrobiološkim đubrivom u prihranjivanju (5,0 l ha^{-1}); T_2 – đubrenje biohumusom (3,0 t ha^{-1}) i mikrobiološkim đubrivom u prihranjivanju (5,0 l ha^{-1}); T_0 – kontrola – bez primene đubriva. Organsko đubrivo, biohumus, zaoravano je u količini od 3,0 t ha^{-1} sa osnovnom obradom. Za prihranjivanje u proleće, u fenofazi vlatanja, korišćeno je mikrobiološko đubrivo u dozi od 5,0 l ha^{-1}.

Neposredno pred klasanje žita, određivan je floristički sastav korovske sinuzije determinacijom vrsta kao i broj individua po m^2 (Kojić et al., 1997). Na polju je određivan floristički sastav, broj vrsta, broj jedinki korova i merena je sveža biomasa, a nakon sušenja i vazdušno suva masa korova.

Prinos zrna je meren sa cele elementarne parcele, preračunat na 14% vlage i izražen u kg ha^{-1}.

Obrada podataka vršena je u statističkom paketu Statistica 5.5. Korišćena je metoda analize varijanse (F test) za dvofaktorijalne oglede, a značajnost razlika između tretmana testirana je LSD testom na nivou značajnosti od $p \leq 0,01$ i $p \leq 0,05$. Za ispitivanje zavisnosti između ispitivanih parametara, korišćena je metoda proste linearne korelacione i regresione analize.

Rezultati i diskusija

Posmatrano za dvogodišnji period, može se konstatovati da korovsku zajednicu, u ispitivanim usevima, gradi relativno veliki broj vrsta (22), što je jedna od karakteristika organske njivske proizvodnje. Jednogodišnji korovi čine 68,2%, a višegodišnji 31,8% od ukupnog broja evidentiranih vrsta. Prema Koocheki et al. (2009), u organskim i racionalnim sistemima gajenja useva, višegodišnji korovi čine 66% odnosno 56% ukupne populacije korova, dok su u uslovima visokih ulaganja značajno ređi. Alternativnim žitima u ovom ispitivanju u obe godine prethodila je okopavinska kultura, kukuruz, čime se može objasniti veća zastupljenost jednogodišnjih korova. Na osnovu analize brojnosti individua m^{-2} evidentiranih korovskih vrsta, najzastupljeniji jednogodišnji korovi su *Stellaria media* (L.) Vill., *Veronica persica* Poir., *Capsella bursa pastoris* L., a višegodišnji *Agropyrum repens* L., *Convolvulus arvensis* L. i *Sonchus arvensis* L. Ove vrste zakorovljavaju mnoge useve, a posebno strnine i okopavine.

Rezultati ispitivanja pokazuju da je zakorovljenost parcela na kontroli veća u poređenju sa varijantama na kojima su primenjena đubriva. Osim toga, sveža (55,9 g m^{-2}) i važušno suva masa korova (13,8 g m^{-2}) na kontroli, takođe je veća u poređenju sa varijantama na kojima su primenjena đubriva (tabela 1). Dobijeni rezultati ukazuju da se primenom đubriva u sistemu organske proizvodnje alternativnih vrsta pšenice može uticati na zakorovljenost. Ispitivanja drugih autora takođe ukazuju da plodnost zemljišta i dostupnost hranljivih materija mogu uticati na diverzitet i dinamiku korovskih vrsta. Analizirajući rezultate dugogodišnjeg ogleda, Ryan et al. (2009) konstatovali su da se povećanjem plodnosti zemljišta u organskim sistemima može uticati na konkurentnost korova, a samim tim i na produktivnost useva.

Rezultati analize varijanse i LSD testa ukazuju da genotip i varijanta đubrenja imaju značajan uticaj na vazdušno suvu masu korova u uslovima organske proizvodnje. Najniža vrednost suve mase korova zabeležena je kod krupnika (8,09 g m^{-2}), a najveća kod tvrde pšenice (15,78 g m^{-2}) (tabela 2). Na visoku konkurentsku sposobnost krupnika prema korovima u uslovima organske proizvodnje, ukazuju i ispitivanja koja su sproveli Zuk-Golaszewska et al. (2015). Razlike su ustanovljene i između varijanti đubrenja. Najmanja suva masa korova zabeležena je na varijanti sa samostalnom primenom mikrobiološkog đubriva (8,09 g), dok razlika dobijena između kontrole i varijante sa kombinovanom primenom đubriva nije imala statističku značajnost. Liebman i Davis (2000) ističu da je đubrenje faktor koji najviše utiče na masu korova u usevu žita. Zbog statistički značajne interakcije (tabela 3), suva masa korova se razlikovala između različitih varijanti đubrenja kod ispitivanih genotipova pšenice, pri čemu su razlike u poređenju sa kontrolom uglavnom bile značajne. Najveća masa korova u usevu

tvrde pšenice zabeležena je na kontroli, dok je kod kompaktum pšenice i krupnika masa korova na varijanti T_2 bila veća u poređenju sa kontrolom i varijantom T_1.

Tabela 1. Floristički sastav korovske sinuzije u usevu alternativnih žita u dvogodišnjem periodu (2009/2010–2010/2011).
Table 1. Floristic composition of weed community in crops of alternative small grains in a two-year period (2009/2010–2010/2011).

Korovske vrste/*Weed species*	Tretman/*Treatment*		
	T_0	T_1	T_2
Jednogodišnji korovi/*Annual weeds*			
Ambrosia artemisiifolia L.			1,0
Avena fatua L.	2,7	2,0	
Bilderdykia convolvulus (L.) Dum.	1,7		1,8
Capsella bursa pastoris (L.) Medic.	3,0	3,0	2,3
Chamomila recutita L.	1,5		
Chenopodium album L.			1,5
Consolida regalis S.F. Gray	2,0		
Erigeron canadensis L.	1,5	1,7	2,0
Papaver rhoeas L.		1,0	
Polygonum aviculare L.	2,5		2,0
Sinapis arvensis L.	2,3		1,5
Sonchus oleraceus L.	2,0	2,0	2,3
Stellaria media (L.) Vill.	3,0	3,3	4,8
Veronica persica Poir.	11,0	2,0	8,0
Višegodišnji korovi/*Perennial weeds*			
Agropyrum repens (L.) Beauv.	9,0	2,0	3,0
Convolvulus arvensis L.	3,0	3,0	2,5
Lepidium draba L.			3,7
Euphorbia cyparissias L.	1,0		
Sonchus arvensis L.	2,5	2,0	
Sorghum halepense (L.) Pers.	1,0		1,5
Taraxacum officinale Weber.	3,0		
Trifolium pratense L.	3,0	5,7	6,0
Ukupan broj jedinki korova (m^{-2}) *Total number of weeds (m^{-2})*	55,6	27,7	43,8
Ukupan broj vrsta korova *Total number of weed species*	18	11	15
Broj jedinki jednogodišnjih korova (m^{-2}) *Number of annual weeds (m^{-2})*	42,1	17,0	30,2
Broj jedinki višegodišnjih korova (m^{-2}) *Number of perennial weeds (m^{-2})*	13,5	10,7	13,7
Sveža nadzemna masa korova (g m^{-2}) *Fresh aboveground weed weight (g m^{-2})*	55,9	32,3	52,8
Vazdušno suva masa korova (g m^{-2}) *Air-dried weed weight(g m^{-2})*	13,8	8,1	13,4

Tabela 2. Prosečne vrednosti ispitivanih parametara u dvogodišnjem periodu (2009/2010–2010/2011).
Table 2. Average values of tested parameters in a two-year period (2009/2010–2010/2011).

Genotip/*Genotype*	Tretman/*Treatment*			Prosek/*Average*
	T_O	T_1	T_2	
	Suva masa korova (g m^{-2})/*Weed dry weight (g m^{-2})*			
G_1	21,15	8,33	17,86	15,78
G_2	11,71	8,15	13,55	11,14
G_3	7,65	7,75	8,88	8,09
Prosek/*Average*	13,50	8,08	13,43	11,67
Visina stabla pšenice (cm)/*Wheat stem height (cm)*				
G_1	60,12	61,35	62,83	61,43
G_2	64,91	69,50	73,95	69,45
G_3	78,98	81,77	85,70	82,15
Prosek/*Average*	68,00	70,87	74,16	71,01
Dužina poslednje internodije (cm)/*Last internode length (cm)*				
G_1	22,59	24,77	26,85	24,74
G_2	21,11	22,53	23,58	22,41
G_3	27,27	31,95	34,44	31,22
Prosek/*Average*	23,66	26,42	28,29	26,12
Dužina klasa (cm)/*Spike length (cm)*				
G_1	6,74	6,76	6,96	6,82
G_2	4,04	4,13	4,13	4,10
G_3	8,89	9,5	10,21	9,53
Prosek/*Average*	6,56	6,80	7,10	6,82
Masa hiljadu zrna (g)/*Thousand-grain weight (g)*				
G_1	37,67	41,13	42,20	40,33
G_2	31,37	31,12	29,95	30,81
G_3	40,75	48,04	44,22	44,34
Prosek/*Average*	36,60	40,10	38,79	38,50
Prinos zrna (kg ha^{-1})/*Grain yield (kg ha^{-1})*				
G_1	1.887,75	2.358,08	3241,02	2.495,62
G_2	2.551,50	2.826,25	3.560,08	2.979,28
G_3	3.494,75	4.194,68	5.150,24	4.279,89
Prosek/*Average*	2.644,67	3.126,34	3.983,78	3.251,60

Genotip: G_1 – Dolap, G_2 – Bambi, G_3 – Nirvana, Tretman: T_0 – kontrola, T_1 – mikrobiološko đubrivo, T_2 – organsko + mikrobiološko đubrivo.
Genotype: G_1 – Dolap, G_2 – Bambi, G_3 – Nirvana, Treatment: T_0 – control, T_1 – microbiological fertiliser, T_2 – organic + microbiological fertilisers.

Visina stabla, dužina poslednje internodije kao i dužina klasa ispitivanih genotipova alternativnih vrsta pšenice, gajenih u uslovima niskih ulaganja, značajno se razlikuju (tabela 3). Najveća visina stabla zabeležena je kod krupnika (82,15 cm), zatim kompaktum pšenice (69,45 cm), a najmanja kod tvrde pšenice

(61,43 cm) (tabela 2). Najveća dužina poslednje internodije kao i dužina klasa konstatovana je kod krupnika, zatim kod tvrde pšenice, a najmanja kod kompaktum pšenice. Duži klas ima veću sposobnost fotosinteze što se zajedno sa mineralnom ishranom direktno odražava na intenzitet stvaranja organske materije i veći broj fertilnih cvetova u klasiću, što određuje broj zrna u klasu (Miralles i Slafer, 2007).

Đubrenje, kao ispitivani faktor, ispoljilo je veoma značajan uticaj na visinu stabla i dužinu poslednje internodije, ali ne i na dužinu klasa. Na varijanti sa kombinovanom primenom organskog i mikrobiološkog đubriva visina stabla u proseku je veća za 9,1%, a na varijanti sa samostalnom primenom mikrobiološkog đubriva za 4,2% u poređenju sa kontrolom. Primena đubriva u ovom ispitivanju uticala je na povećanje visine stabla za 2,05%, 3,53% i 7,07% na varijanti T_1, dok je na varijanti T_2 to povećanje iznosilo 4,51%, 8,51% i 13,93% kod tvrde pšenice, krupnika odnosno kompaktum pšenice. Uzimajući u obzir prosečne vrednosti, dvostruko bolji efekti konstatovani su kod uticaja đubrenja na dužinu poslednje internodije. Do sličnih rezultata dolaze i Jablonskytė-Raščė et al. (2013), koji navode da kombinovana primena ekološkog đubriva i bio-aktivatora značajno utiče na povećanje dužine klasa krupnika.

Tabela 3. Analiza varijanse ispitivanih parametara.
Table 3. Analysis of variance of the tested parameters.

Ispitivani parametri *Tested parameters*		2009/2010–2010/2011			
		a nivo	G	T	G*T
Vazdušno suva masa korova *Air-dried weed weight*	F test		244,121**	157,742**	63,246**
	LSD	0,05	0,571	0,807	1,141
		0,01	0,783	1,107	1,566
Visina stabla *Stem height*	F test		143,761**	12,514**	1,139ns
	LSD	0,05	1,877	2,654	-
		0,01	2,574	3,640	-
Dužina poslednje internodije *Last internode length*	F test		224,622**	57,851**	5,518*
	LSD	0,05	0,707	0,999	1,413
		0,01	0,969	1,371	1,938
Dužina klasa *Spike length*	F test		215,128**	2,158ns	1,124ns
	LSD	0,05	0,417	-	-
		0,01	0,571	-	-
Masa hiljadu zrna *Thousand-grain weight*	F test		80,772**	5,236*	2,826ns
	LSD	0,05	1,809	2,559	-
		0,01	2,481	3,509	-
Prinos zrna *Grain yield*	F test		185,355**	100,146**	1,969ns
	LSD	0,05	148,869	210,533	-
		0,01	204,164	288,731	-

G – genotip, T – tretman, ns = P>0,05 * = P<0,05 ** = P<0,01.
*G – genotype, T – treatment, ns = P>0.05 * = P<0.05 ** = P<0.01.*

Na masu, kao i prinos zrna različitih genotipova alternativnih vrsta pšenice, veoma značajno utiče ne samo genotip, već i primena đubriva, dok interakcija ova dva faktora nije ostvarila značajne efekte (tabela 3). Najveća masa hiljadu zrna (44,34 g), kao i najveći prinos zabeleženi su kod sorte Nirvana (4.279,89 kg ha^{-1}). Značajno manji prinos zrna zabeležen je kod sorte Bambi (2.979,28 kg ha^{-1}), dok je najmanji prinos zrna konstatovan kod sorte Dolap (2.495,62 kg ha^{-1}) (tabela 2). Ranija ispitivanja su pokazala da se efikasnost korišćenja hranljivih materija i vode kod tvrde pšenice može povećati odabirom sorti koje su kompatibilne sa korisnim mikroorganizmima (Singh et al., 2012). Veoma značajne razlike u visini prinosa ustanovljene su i između varijanti. Uzimajući u obzir prosečne vrednosti, povećanje prinosa primenom đubriva kretalo se od 18% do 50%, na varijantama T_1 odnosno T_2. Slične rezultate saopštava i Roljević (2014) u vezi sa ispitivanjem produktivnosti alternativnih vrsta žita u uslovima organske zemljoradnje.

Rezultati korelacione analize (tabela 4) ukazuju da u uslovima niskih ulaganja između visine stabla i suve mase korova postoji veoma značajna negativna korelacija (r = - 0,58).

Tabela 4. Rezultati korelacione analize.
Table 4. Results of correlation analysis.

	Suva masa korova *Weed dry weight*	Visina stabla *Stem height*	Dužina poslednje internodije *Last internode length*	Dužina klasa *Spike length*	Masa hiljadu zrna *Thousand-grain weight*	Prinos zrna *Grain yield*
Suva masa korova *Weed dry weight*	1,00	-0,58**	-0,33^{ns}	-0,19^{ns}	-0,16^{ns}	-0,44*
Visina stabla *Stem height*	-0,58**	1,00	0,71**	0,50**	0,31^{ns}	0,87**
Dužina poslednje internodije *Last internode length*	-0,33^{ns}	0,71**	1,00	0,86**	0,80**	0,78**
Dužina klasa *Spike length*	-0,19^{ns}	0,50**	0,86**	1,00	0,86**	0,50**
Masa hiljadu zrna *Thousand-grain weight*	-0,16^{ns}	0,31^{ns}	0,80**	0,86**	1,00	0,40*
Prinos zrna *Grain yield*	-0,44*	0,87**	0,78**	0,50**	0,40*	1,00

LSD $\alpha_{0,05/2}$ = 1,96; $\alpha_{0,01/2}$ = 2,58, ns = P>0,05 * = P<0,05 ** = P<0,01.
*LSD $\alpha_{0.05/2}$ = 1.96; $\alpha_{0.01/2}$ = 2.58, ns = P>0.05 * = P<0.05 ** = P<0.01.*

Na osnovu jednačine proste linearne regresije ($\hat{y}i = 34,411 – 0,322*xi$) može se konstatovati da povećanje visine stabla za 1 cm utiče na smanjenje mase korova za 0,32 g m^{-2}. Suva masa korova u ovom ispitivanju nije imala značajan uticaj na masu hiljadu zrna (r = - 0,16), dok je između mase korova i

prinosa zrna ustanovljena značajna negativna korelacija (r = - 0,44). U usevu alternativnih vrsta pšenice povećanje mase korova za 1 g m^{-2} utiče na smanjenje prinosa od 72,4 kg ha^{-1} ($\hat{y}_i = 3951,81 – 72,388*x_i$). Rezultati ovih ispitivanja pokazali su da je kod genotipa sa najvećim stablom (krupnik) zabeležena i najmanja suva masa korova. Ispitivanja drugih autora takođe ukazuju na značaj visine kao jedne od važnih osobina koja doprinosi supresiji korova u usevu pšenice (Cosser et al., 1997; Piliksere et al., 2013; Roljević, 2014). Cosser et al. (1997) ističu da se prednosti sorti sa visokim stablom mogu koristiti kako bi se tolerisala zakorovljenost u uslovima organske proizvodnje, ali i ističu da se kontrola korova ne može osloniti samo na odabir sortimenta. Naime, u nekim slučajevima visoke sorte mogu da podstaknu infestaciju određenim vrstama korova stvaranjem povoljnog mikroklimata za njihov rast i razvoj.

Na osnovu korelacione analize ustanovljena je i negativna korelacija između suve mase korova i dužine poslednje internodije (r = - 0,33), kao i između suve mase korova i dužine klasa (r = - 0,19), ali zavisnost između ovih promenljivih nije imala statističku značajnost. U ispitivanjima koja su sproveli Murphy et al. (2007) visina genotipova pšenice takođe je bila jedini faktor koji je imao uticaja na masu korova.

Zaključak

Na osnovu dobijenih rezultata može se zaključiti da u uslovima organske proizvodnje ispitivani genotipovi alternativnih vrsta pšenice, uz primenu đubriva, mogu značajno da utiču na smanjenje mase korova. Osobine poput visine stabla, dužine poslednje internodije i klasa, mase hiljadu zrna i prinosa pod dominantnim su uticajem genotipa. Osim toga, zapažene su i značajne razlike kod ispitivanih osobina između različitih varijanti đubrenja. U uslovima niskih ulaganja prinos zrna ispitivanih genotipova zavisi od mase korova koja je u negativnoj korelaciji sa visinom stabla, što ukazuje na značaj ove osobine za konkurentsku sposobnost pšenice. Prema tome, pravilan odabir sorti, uz adekvatnu primenu đubriva, može doprineti kontroli, ali se borba protiv korova u organskoj proizvodnji ne može osloniti samo na ovu meru.

Zahvalnica

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FLORISTIC COMPOSITION OF WEEDS, MORPHOLOGICAL AND PRODUCTIVE CHARACTERISTICS OF DIFFERENT WHEAT GENOTYPES OF ALTERNATIVE SMALL GRAINS IN ORGANIC PRODUCTION

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A b s t r a c t

This study was aimed at assessing the floristic composition of weeds, studying properties of different wheat genotypes of alternative small grains (stem height, last internode length and spike length, thousand-grain weight, grain yield) and the relationship between the studied traits and dry weight of weeds in an organic production system. Testing was carried out in a randomized block design with four replications, in a two-year period (2009/2010–2010/2011). The study included one variety of durum wheat *Triticum durum* (Dolap, G1), the compact wheat *Triticum compactum* (Bambi, G2) and spelt *Triticum spelt* (Nirvana, G3). A treatment for maintaining the increase in the biological soil fertility included two different fertilisation variants: T_1 – fertilisation only with microbiological fertiliser as a top dressing (5.0 l ha^{-1}); T_2 – biohumus fertilisation (3.0 t ha^{-1}) and microbiological fertiliser as a top dressing (5.0 l ha^{-1}); T_0 – control – without the use of fertilisers. The results have shown that the genotype and treatment, as studied factors, very significantly affected the dry weight of weeds, stem height, last internode length and spike length, as well as the yield of different wheat genotypes of alternative small grains, while the effect of their interaction was lower. In terms of the low-input system, a highly significant negative correlation (r = - 0.58) was observed between the stem height and the dry weight of weeds. In addition, a significant negative correlation was noticed between the grain yield and the dry weight of weeds (r = - 0.44). These results showed that proper selection of the genotypes with the application of fertilisers could have a significant effect on the weeds and could lead to establishing a stable production in the organic production system.

Key words: organic production, wheat genotypes of alternative small grains, weeds.

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RODNOST SREDNJE KASNIH I KASNIH SORTI KAJSIJE U BEOGRADSKOM PODRUČJU

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Sažetak: Karakteristike rodnosti i rasta (prinos po stablu, površina poprečnog preseka debla, koeficijent rodnosti i masa ploda) proučavane su kod 30 sorti kajsije srednje kasnog i kasnog vremena zrenja u beogradskom području u periodu od šest godina (2009–2014). Kao kontrola za poređenje korišćena je sorta Mađarska najbolja. Period ispitivanja je podeljen na početnu rodnost (starost stabala tri i četiri godine) i punu rodnost (starost stabala od pet do osam godina). Utvrđene su značajne razlike u prinosu, kako između proučavanih sorti, tako i između godina ispitivanja. Prosečan prinos u periodu početne rodnosti se kretao od 0,6 do 6,4 kg po stablu, a u periodu pune rodnosti od 4,5 do 18,4 kg po stablu. U odnosu na kontrolnu sortu, statistički značajno viši prinos u punoj rodnosti je dobijen kod 11 sorti. Nepovoljne vremenske prilike uticale su na značajno smanjenje prinosa u dve od šest godina proučavanja. Kumulativni koeficijent rodnosti je varirao od 0,10 do 0,85 kg/cm^2 i kod 13 sorti je bio značajno veći u odnosu na kontrolu. Masa ploda je bila u rasponu od 26,6 do 81,1 g. U odnosu na kontrolu, devet sorti je imalo značajno veću, a deset sorti značajno manju masu ploda. Na osnovu visoke rodnosti i velike krupnoće ploda, za gajenje u beogradskom području se mogu preporučiti introdukovane sorte Roksana, Mari de Čenad i Silverkot, kao i domaće sorte Novosadska rodna, NS-4 i NS-6.

Ključne reči: *Prunus armeniaca*, prinos, površina poprečnog preseka debla, koeficijent rodnosti, masa ploda.

Uvod

Jedna od najvažnijih osobina pri izboru sorti voćaka za gajenje je njihova rodnost. Kajsija se odlikuje neredovnom rodnošću, tj. velikim variranjem prinosa po godinama. To je u prvom redu posledica izmrzavanja cvetova i zametnutih plodića usled pojave poznih prolećnih mrazeva (Bassi et al., 1995; Rodrigo et al., 2006; Milatović et al., 2013), a u manjoj meri i izmrzavanja cvetnih pupoljaka usled pojave jačih zimskih mrazeva (Miletić et al., 2006; Ozturk et al., 2006;

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Szalay et al., 2006; Szabó et al., 2010; Gorina i Korzin, 2016). S obzirom na veliku varijabilnost prinosa kajsije po godinama usled dejstva niskih temperatura, objektivni podaci o rodnosti sorti se mogu dobiti samo na osnovu višegodišnjeg proučavanja (Vachůn, 2002a).

Sortiment kajsije u Srbiji karakteriše se relativno malim brojem sorti i kratkim periodom sazrevanja. Glavna sezona potrošnje je u prvoj polovini jula, od početka zrenja sorte Mađarska najbolja, do desetak dana posle toga. Introdukcija novih sorti i njihovo proučavanje u našim agroekološkim uslovima omogućava proširenje izbora sorti za gajenje, a time i unapređenje proizvodnje kajsije (Milatović et al., 2012).

Vachůn (2002a) je proučavao rodnost kod 24 sorte kajsije u periodu od šest godina i utvrdio variranje prosečnog prinosa od 320 kg po stablu. Takođe, Vachůn (2002b) navodi da izražavanje rodnosti preko koeficijenta rodnosti (prinosa po jedinici površine poprečnog preseka debla) ima prednost u odnosu na prinos u kg po stablu, jer obezbeđuje lakšu uporedivost sa rezultatima drugih autora.

Milatović et al. (2006) su ispitivali rodnost kod 35 sorti kajsije na području Beograda u periodu od osam godina. Prosečan prinos se kretao od 2,0 do 32,6 kg po stablu.

Cilj ovog rada je bio da se prouči rodnost većeg broja introdukovanih sorti kajsije srednje kasnog i kasnog vremena zrenja u dužem vremenskom periodu. Dobijeni rezultati će biti korisni za izbor sorti za gajenje ili za njihovo korišćenje u daljem oplemenjivačkom radu.

Materijal i metode

Ispitivanja su obavljena u kolekcionom zasadu kajsije Poljoprivrednog fakulteta u Beogradu, na Oglednom dobru „Radmilovac", u periodu od šest godina (2009–2014). Zasad je podignut 2007. godine i u periodu ispitivanja stabla su bila starosti 3–8 godina. Ispitivanjem je obuhvaćeno 30 sorti kajsije srednje poznog i poznog vremena zrenja. Najveći broj ispitivanih sorti (šest) potiče iz Češke (Marlen, Kandela, Lebela, Betinka, LE VP 12/2 i Minaret). Pet sorti potiče iz Kanade (Harodžoj, Vikot, Harglo, Harostar i Hargrand), a po četiri sorte iz Bugarske (Gergana, Roksana, Silistrenska kompotna i Kasna Drjanovska) i Srbije (Čačanska pljosnata, NS-4, NS-6 i Novosadska rodna). Po dve sorte potiču iz Italije (Sabatani i Real d'Imola), Ukrajine (Vognik i Amos), Moldavije (Kospotenskij i Moldavska krupnoplodna), a po jedna sorta iz Južnoafričke Republike (Pika), Francuske (Silverkot), Rumunije (Mari de Čenad), Slovačke (Vestar) i Rusije (Poljus južnij). Sorta Mađarska najbolja je uzeta kao kontrola za poređenje. Sve sorte su okalemljene na sejancu džanarike (*Prunus cerasifera* Ehrh.) i zastupljene su u zasadu sa po pet stabala. Uzgojni oblik je piramidalna kruna, a razmak sadnje je 4,5 m × 3 m.

Prinos je određen merenjem mase ubranih plodova i izražen je u kg po stablu. Površina poprečnog preseka debla (PPPD) je izračunata na osnovu prečnika debla koji je izmeren na visini od 30 cm iznad spojnog mesta. Kumulativni koeficijent rodnosti (KKR) je izračunat kao odnos kumulativnog prinosa za šest godina (2009–2014) i PPPD u poslednjoj godini istraživanja (2014) i izražen je u kg po cm^2. Masa ploda je određena na uzorku od 25 plodova po sorti.

Podaci su analizirani statistički metodom analize varijanse dvofaktorijalnog ogleda. Značajnost razlika između srednjih vrednosti određena je LSD testom za verovatnoću 0,05.

Rezultati i diskusija

Prosečno vreme zrenja ispitivanih sorti je bilo od jedan dan pre do 12 dana posle kontrolne sorte – Mađarske najbolje (tabela 1). S obzirom na to da je prosečan datum zrenja sorte Mađarska najbolja bio 3. jul, ispitivane sorte su prosečno sazrevale u periodu od 2. do 15. jula.

Rodnost kajsije u ispitivanom periodu bila je neujednačena, kako po godinama, tako i po sortama (tabela 1). Razlike u prinosu između sorti i godina su bile statistički značajne.

Iako je prvi rod dobijen u trećoj godini nakon sadnje (2009), prvi ekonomski značajan prinos (prosečno 5,3 kg po stablu) dobijen je u četvrtoj godini (2010). Prosečan prinos po stablu u periodu početne rodnosti kretao se od 0,6 do 6,4 kg. Ranim ulaskom u period rodnosti i visokim početnim prinosima odlikovale su se sorte: Novosadska rodna, Real d'Imola, Harostar, LE VP 12/2, Vognik i Silverkot. Ove sorte su imale i statistički značajno viši prinos u periodu početne rodnosti u odnosu na kontrolu. S druge strane, kasni ulazak u period rodnosti i niski početni prinosi dobijeni su kod sorti: Amos, Moldavska krupnoplodna, Gergana i Kospotenskij.

Nepovoljne vremenske prilike uticale su na značajno smanjenje prinosa u dve od šest godina proučavanja. U 2012. godini zabeležen je zimski mraz 9. februara intenziteta -20,7°C, kao i pozni prolećni mraz 10. aprila intenziteta -3,0°C, nakon završetka cvetanja. Kod 33 sorte kajsije, prosečno izmrzavanje cvetnih pupoljaka od zimskog mraza iznosilo je 32,8%, a prosečno izmrzavanje plodića od poznog prolećnog mraza iznosilo je 61,4% (Milatović et al., 2013). U 2013. godini zabeležen je pozni prolećni mraz 25. marta od -3.4°C koji je prouzrokovao izmrzavanje cvetova kod sorti ranog vremena cvetanja koje su tada bile u fenofazi početka cvetanja. Međutim, glavni razlog slabije rodnosti u ovoj godini je bilo hladno vreme u periodu cvetanja koje je uticalo na slabiji let pčela i slabo zametanje plodova.

Tabela 1. Vreme zrenja i prinos (kg po stablu) sorti kajsije u periodu početne i pune rodnosti.
Table 1. Time of maturation and yield (kg per tree) of apricot cultivars in the periods of initial and full bearing.

Sorta *Cultivar*	VZ* Br. dana *No. of days*	Početna rodnost *Initial bearing*			Puna rodnost *Full bearing*				
		2009	2010	Mx	2011	2012	2013	2014	Mx
Vognik	-1	2,0	8,2	5,1	22,6	2,0	3,2	21,2	12,2
Marlen	-1	0,9	6,4	3,7	17,9	3,4	6,0	31,2	14,6
Kandela (LE-2927)	0	0,1	5,1	2,6	5,9	2,5	4,0	6,7	4,8
Pika	0	3,9	3,7	3,8	20,4	8,1	4,3	18,2	12,7
Harodžoj	1	0,8	6,9	3,9	22,7	4,8	11,9	22,7	15,5
Silverkot	1	1,1	8,2	4,7	23,5	0,5	1,6	16,2	10,4
Lebela	1	0,3	6,6	3,4	12,4	3,8	2,5	12,1	7,7
Betinka (LE-3276)	2	0,3	7,2	3,7	33,2	3,3	6,8	10,6	13,5
LE VP 12/2	2	2,9	8,4	5,7	16,4	5,2	7,6	17,6	11,7
Gergana	2	0,2	2,2	1,2	11,5	12,1	0,1	24,2	12,0
Amos	2	0,2	1,1	0,7	8,0	3,6	0,1	6,4	4,5
Vikot	2	0,2	7,7	3,9	22,7	11,2	0,3	5,2	9,8
Harglo	2	1,5	3,1	2,3	5,7	4,4	5,2	12,4	6,9
Harostar	2	1,1	10,8	5,9	17,9	5,1	3,8	21,6	12,1
Kospotenskij	2	0,1	2,4	1,3	14,2	3,6	10,6	10,9	9,8
Poljus južnij	3	0,2	3,8	2,0	7,6	1,4	4,8	25,9	9,9
Mold. krupnoplodna	3	0,2	2,1	1,2	20,5	2,4	0,7	22,2	11,5
Mari de Čenad	4	0,6	7,7	4,2	24,3	12,8	0,5	26,9	16,1
Vestar	4	0,5	6,4	3,4	24,5	7,0	8,7	15,8	14,0
NS-4	4	1,2	1,7	1,4	11,0	10,9	8,0	28,6	14,6
Sabatani	4	1,4	6,2	3,8	5,6	11,7	7,4	3,2	7,0
Roksana	5	0,8	6,8	3,8	16,7	11,4	1,3	36,3	16,4
Novosadska rodna	5	1,3	11,5	6,4	39,9	5,6	6,7	21,2	18,4
Čačanska pljosnata	6	0,5	3,1	1,8	5,9	10,0	2,2	10,9	7,3
NS-6	6	0,3	1,5	0,9	7,5	11,6	4,7	33,4	14,3
Hargrand	7	1,4	2,2	1,8	8,6	2,0	0,7	8,6	5,0
Real d'Imola	7	2,6	9,9	6,3	14,9	10,9	13,8	22,1	15,4
Minaret	8	3,5	4,0	3,8	14,7	0,3	14,1	7,8	9,2
Silistrenska kompotna	11	0,5	6,6	3,6	11.7	11.5	3.6	16.3	10,8
Kasna Drjanovska	12	0,1	1,0	0,6	1,8	14,5	3,1	14,0	8,4
M. najbolja (kontrola)	0	0,3	2,1	1,2	10,8	2,0	3,0	10,3	6,5
LSD 0,05				3,1					5,7

*VZ – Vreme zrenja: broj dana u odnosu na sortu Mađarska najbolja.
Time of maturation: No. of days compared to the cultivar Hungarian Best.

U 2014. godini cvetanje je bilo ekstremno rano (početak cvetanja od 23. februara do 17. marta). Mraz intenziteta -3,0°C zabeležen je 1. marta i on je naneo izvesnu štetu sortama ranog vremena cvetanja. Uprkos tome, u ovoj godini je

zabeležen najveći prosečan prinos (18,4 kg po stablu). Pored toga, visok prinos je zabeležen i u 2011. godini (prosečno 14,5 kg po stablu).

Prosečan prinos po stablu u periodu pune rodnosti je varirao od 4,5 kg kod sorte Amos do 18,4 kg kod sorte Novosadska rodna. U odnosu na kontrolnu sortu, statistički značajno viši prinos je dobijen kod 11 sorti. Dobijeni podaci za prinos sorti kajsije su u skladu sa prethodnim istraživanjima (Vachůn, 2002a; Milatović et al., 2006; Fajt et al., 2013; Polat i Çalışkan, 2014; Milošević et al., 2015).

Na osnovu prinosa po stablu Pejkić i Ninkovski (1987) su izvršili klasifikaciju sorti po rodnosti na četiri grupe: loša <10 kg, srednja 10–15 kg, dobra 15–20 kg i odlična >20 kg. Na osnovu ove podele, najveći broj ispitivanih sorti u periodu pune rodnosti je imao srednju rodnost (13 sorti) ili lošu rodnost (12 sorti, kao i kontrola). Visoku rodnost je imalo samo pet sorti i to: Novosadska rodna, Roksana, Mari de Čenad, Harodžoj i Real d'Imola.

Kontrolna sorta, Mađarska najbolja, imala je mali prinos u periodu pune rodnosti (6,5 kg po stablu) i može se svrstati u slabo rodne sorte. To je suprotno od rezultata koji su dobili Milatović et al. (2006) gde je ona svrstana u rodne sorte. Moguće je da su za ispitivanje korišćeni različiti klonovi, jer je u pitanju stara sorta sa većim brojem selekcionisanih klonova (Vachůn, 1988). U ovom ispitivanju je korišćen klon Mađarske najbolje C235. Interesantno je da je drugi, češki klon ove sorte (LE VP 12/2) u ovom periodu pokazao značajno veću rodnost (prosečno 12,7 kg po stablu).

Prosečan prinos po hektaru u periodu pune rodnosti za sve sorte bio je 7,4 t, sa variranjem od 3,3 do 13,6 t. Kod pojedinih sorti u mediteranskom području u uslovima visoke agrotehnike, uključujući i navodnjavanje, može se dobiti prinos od 40 do 50 t/ha (Egea et al., 1995). Ipak, u uslovima kontinentalne klime, prosečan prinos za višegodišnji period je znatno niži zbog pojave poznih prolećnih mrazeva, kao i sušenja stabala usled pojave apopleksije (Vachůn, 2001; Milatović et al., 2006).

Najmanju bujnost izraženu preko površine poprečnog preseka debla je imala sorta Hargrand – 66,2 cm^2, a najveću bujnost je imala sorta Amos – 191,2 cm^2 (tabela 2). Značajno veću bujnost u odnosu na kontrolnu sortu je imalo osam sorti.

Koeficijent rodnosti je značajan pokazatelj koji objedinjuje prinos i bujnost. Kumulativni koeficijent rodnosti (KKR) u ispitivanom šestogodišnjem periodu se kretao od 0,10 kg/cm^2 kod sorte Amos do 0,85 kg/cm^2 kod sorte Roksana. U odnosu na kontrolnu sortu, značajno veći KKR imalo je 13 sorti.

Naši rezultati o KKR u skladu su sa onim koje navode drugi autori: Ogašanović et al. (1991) 0,48–1,63 kg/cm^2; Vachůn (2002b) 0,22–1,36 kg/cm^2; Milatović et al. (2006) 0,07–0,96 kg/cm^2; Licznar-Malanczuk i Sosna (2013) 0,07–0,41 kg/cm^2; Milošević et al. (2013) 0,17–0,82 kg/cm^2.

Rodnost sorti nije ista ako se izvrši njihovo rangiranje na osnovu prinosa u kg po stablu ili na osnovu KKR u kg/cm^2 površine poprečnog preseka debla. Na

osnovu KKR neke slabo bujne sorte, npr. Vestar, Harglo i Lebela, znatno su bolje rangirane nego na osnovu prinosa u kg po stablu. Situacija je obrnuta kod bujnih sorti, kao što su npr. Poljus južnij i Vognik.

Tabela 2. Površina poprečnog preseka debla (PPPD) u 2014. godini, kumulativni koeficijent rodnosti (KKR) i masa ploda (prosek, 2009–2014. godine) sorti kajsije.
Table 2. Trunk cross-sectional area (TCSA) in 2014, cumulative yield efficiency (CYE) and fruit weight (average, 2009–2014) of apricot cultivars.

Sorta *Cultivar*	PPPD/*TCSA* (cm^2)	KKR/*CYE* (kg/cm^2)	Masa ploda *Fruit weight* (g)
Vognik	179,6	0,33	46,6
Marlen	135,5	0,49	48,9
Kandela (LE-2927)	116,8	0,21	68,4
Pika	109,7	0,53	39,3
Harodžoj	98,2	0,71	48,1
Silverkot	143,1	0,36	61,7
Lebela	86,7	0,43	53,7
Betinka (LE-3276)	118,8	0,52	65,7
LE VP 12/2	120,1	0,48	52,8
Gergana	115,0	0,44	70,8
Amos	191,2	0,10	47,3
Vikot	124,2	0,38	49,3
Harglo	71,7	0,45	41,0
Harostar	118,0	0,51	38,4
Kospotenskij	125,3	0,33	41,7
Poljus južnij	183,4	0,24	26,6
Mold. krupnoplodna	134,7	0,36	52,0
Mari de Čenad	97,0	0,75	77,4
Vestar	86,7	0,72	50,1
NS-4	127,4	0,48	72,2
Sabatani	187,3	0,19	47,1
Roksana	85,8	0,85	70,4
Novosadska rodna	134,6	0,64	81,1
Čačanska pljosnata	158,8	0,21	38,3
NS-6	109,0	0,54	71,4
Hargrand	66,2	0,35	43,7
Real d'Imola	154,1	0,48	40,6
Minaret	121,1	0,37	34,3
Silistrenska kompotna	147,2	0,32	46,3
Kasna Drjanovska	133,8	0,26	55,7
M. najbolja (kontrola)	101,5	0,28	53,3
LSD 0,05	41,1	0,18	8,1

Masa ploda je varirala od 26,6 g kod sorte Poljus južnij do 81,1 g kod sorte Novosadska rodna. U odnosu na kontrolnu sortu, devet sorti je imalo značajno

veću, a deset sorti značajno manju masu ploda. Naši rezultati o masi ploda su u skladu sa prethodnim navodima za pojedine sorte (Vachůn, 2003; Szani et al., 2006; Krška et al., 2013; Licznar-Malanczuk i Sosna, 2013; Szalay et al., 2013).

Zaključak

Rodnost sorti kajsije u beogradskom području u periodu ispitivanja (2009–2014) bila je neujednačena, kako po godinama, tako i po sortama. Nepovoljne vremenske prilike uticale su na značajno smanjenje prinosa u dve od šest godina proučavanja. Na osnovu visoke rodnosti i velike krupnoće ploda, za gajenje u beogradskom području mogu se preporučiti introdukovane sorte Roksana, Mari de Čenad i Silverkot, kao i domaće sorte Novosadska rodna, NS-4 i NS-6.

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PRODUCTIVITY OF APRICOT CULTIVARS OF MEDIUM LATE AND LATE MATURITY IN THE BELGRADE AREA

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A b s t r a c t

Characteristics of productivity and growth (yield per tree, trunk cross-sectional area, yield efficiency and fruit weight) were studied in 30 apricot cultivars of medium late and late maturity in the Belgrade area during the six-year period (2009–2014). The cultivar 'Hungarian Best' was used as a control for comparison. The period of study was divided into initial bearing (trees aged three to four years) and full bearing (trees aged five to eight years). Significant differences in yield between cultivars and years were found. An average yield in the period of initial bearing ranged from 0.6 to 6.4 kg per tree, and in the period of full bearing from 4.5 to 18.4 kg per tree. Compared to the control cultivar, significantly higher yield was obtained in 11 cultivars. Adverse weather conditions resulted in a significant reduction in yield in two out of six years of study. Cumulative yield efficiency varied from 0.10 to 0.85 kg/cm^2 and in 13 cultivars it was significantly higher compared to the control cultivar. Fruit weight ranged from 26.6 to 81.1 g. Compared to the control, fruit weight was significantly higher in nine cultivars, and significantly lower in ten cultivars. On the basis of high yield and large fruit size, introduced cultivars 'Roxana', 'Mary de Cenad' and 'Silvercot' as well as domestic cultivars 'Novosadska Rodna', 'NS-4' and 'NS-6' can be recommended for growing in the Belgrade area.

Key words: *Prunus armeniaca*, yield, trunk cross-sectional area, yield efficiency, fruit weight.

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INFLUENCE OF FOUR DIETARY OILS ON SELECTED BLOOD CONSTITUENTS IN EGG-TYPE CHICKENS

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Abstract: The enrichment of eggs with polyunsaturated fatty acids (PUFA), particularly with omega-3 fatty acids, has attracted the attention of both researchers and the food industry, because these fatty acids are essential for normal body development and play an important role in the prevention of heart diseases. This experiment was conducted to evaluate the effect of four dietary oils on selected blood metabolites in egg-type chickens. One hundred and five (105) Isa Brown laying hens at 34 weeks of age were used and the study lasted for 6 weeks in a completely randomised design. Hens were randomly allotted to seven dietary treatments namely: basal diet (T_1), basal diet + 1.5% palm oil (T_2), basal diet + 1.5% soybean oil (T_3), basal diet + 1.5% sesame seed oil (T_4), basal diet + 1.5% fish oil (T_5), basal diet + 0.75% soybean oil + 0.75% fish oil (T_6) and basal diet + 0.75% sesame seed oil + 0.75% fish oil (T_7). The treatments had 5 replicates of 3 hens each. Blood samples (5mls) were collected from the jugular vein of a bird per replicate for serum and haematological parameters. There were no significant differences observed in packed cell volume, haemoglobin, red blood cell, white blood cell, lymphocyte, heterophils and basophils of birds on experimental diets but diets had an influence ($P<0.05$) on the monocytes, eosinophils and platelets of birds. Monocytes of birds on the control diet were similar to those of birds on T_3 (basal diet + 1.5% soyabean oil) but significantly ($P<0.05$) higher than monocyte counts for birds on other diets. Eosinophils of birds on the experimental diets were similar except for those on T_4 (basal diet + 1.5% sesame seed oil) with significantly higher eosinophil values compared with others. A similar trend was observed in platelets of birds on the experimental diets. There were no significant differences observed in triglycerides, high density lipoproteins and cholesterol of birds on experimental diets. Low density lipoprotein (LDL) of birds on the control diet was similar to those recorded for birds on different dietary oils supplemented diets except for those on T_6 (basal diet + 0.75% soybean oil + 0.75% fish oil) with significantly ($P<0.05$) reduced LDL. It can be concluded that dietary oils combination of 0.75% soybean oil + 0.75% fish oil could be effective in reducing serum low density lipoprotein in laying birds.

Key words: palm oil, sesame seed oil, soybean oil, fish oil, laying hens, blood profile.

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Introduction

People with hypercholesterolemia have three times higher risk of heart attacks than those who have normal blood lipid profiles. It was reported that hypercholesterolemia contributed to 45% of heart attacks in Western Europe and 35% of heart attacks in Central and Eastern Europe from 1999 to 2003. In human nutrition, animal products such as meat, milk and egg play an important role. Consumers have changed their attitude towards egg consumption because of fear that eggs will raise their blood cholesterol levels. Eggs therefore have been singled out by diet-heart advocates as food to be avoided even though the egg contains the best and least expensive high quality protein of high biological value and balanced distribution of minerals and vitamins except vitamin C (Shrimpton, 1987; Connor, 2000). The many attempts to reduce cholesterol in egg content have had little practical application. An alternative way to reduce the cholesterol effects of eggs is by altering the yolk fatty acid composition. Egg yolk total fat content cannot be altered; however, fatty acid composition can be altered by using PUFA-rich dietary oils in hen's diet (Milinsk et al*.,* 2003). The enrichment of eggs with polyunsaturated fatty acids (PUFA), particularly with omega-3 fatty acids has attracted the attention of both researchers and the food industry, because these fatty acids are essential for normal body development and play an important role in the prevention of heart diseases, diabetes, arthritis, inflammatory and auto-immune conditions, and cancer (Simopoulos, 2000). The use of different dietary oils as energy sources in the diets of laying hens is one of the methods used nutritionally to change the lipid profile of eggs (Baucells et al., 2000; Grobas et al., 2001; Shafey et al., 2003; Cabrera et al., 2005). Some of the oil sources are rich in long chain polyunsaturated fatty acids that can change the proportion of the constituents of egg yolk (Hargis and Van Elswyk, 1993; Eseceli and Kahraman, 2004).

The nutritional and clinical status of an animal can be determined by myriad of metabolites and other constituents contained in the blood. Hence, World Health Organization recommends the use of blood and biochemical parameters in medical nutritional assessment. Several studies have been conducted to evaluate the effects of different omega-3 fatty acid sources on the cholesterol and fatty acid composition of egg yolk and meat (Caston and Leeson, 1990; Cherian and Sim, 1991; Coetzee and Hoffman, 2002; Komprda et al., 2003). However, its effect on blood constituents of laying birds is scanty. It was therefore, the objective of this study to determine the effect of four dietary oils on some selected blood constituents of egg-type chickens.

Materials and Methods

Experimental site

This experiment was carried out at the Poultry Experimental Unit of the Teaching and Research Farm, University of Ibadan, Oyo State, Nigeria. The University is located at latitude 7^{o} 10^{o} N and longitude 3^{o} 2^{o} E and lies in the south-western part of Nigeria with a prevailing tropical climate with a mean rainfall of about 1037mm per annum. The mean monthly ambient temperature ranges from 28^{o}C in December to 36^{o}C in February with an average yearly humidity of about 82%. The vegetation at the University represents an interphase between the tropical rain forest and the derived savannah. The project complied with the University of Ibadan ethics requirements for animal handling. The study was executed within the University of Ibadan Teaching and Research Farm; the project did not include any activity that contravened animal welfare and humane handling in animal husbandry.

Experimental diets and management of experimental birds

One hundred and five Isa brown laying hens at 34 weeks of age were used for this study. Hens were housed in a battery cage in a semi-controlled environment. The layers were randomly allotted into seven dietary treatments by body weight. Each dietary treatment had 5 replicates of 3 hens per replicate. The basal diet was a corn-soybean meal diet formulated to meet the nutrient requirements (NRC, 1994) for layers (Table 1). Treatment 1 (T_1) was a basal diet (no dietary oil); treatment 2 (T2), basal diet + 1.5% palm oil; treatment 3 (T_3), basal diet + 1.5% soybean oil; treatment 4 (T4), basal diet + 1.5% sesame seed oil; treatment 5 (T_5), basal diet + 1.5% fish oil; treatment 6 (T_6), basal diet + 0.75% soybean oil and 0.75% fish oil and treatment 7 (T7), basal diet + 0.75% sesame seed oil and 0.75% fish oil. Hens had free access to experimental diets and water during the study period that lasted for 6 weeks. Diet composition was analysed and compared with the calculated values (Table 2).

Experimental design

The experimental design was a completely randomised design.

Haematological parameters
Blood collection

At day 42, blood was collected from the jugular vein into two vaccutainer tubes for each hen, one containing Ethylene Diamine Tetra Acetic Acid (EDTA) for haematological study and the other sterile vaccutainer tubes without EDTA.

The second set of tubes was covered and centrifuged, serum separated out, decanted, deep-frozen for serum biochemical analyses.

Table 1. Gross compositions (g/100g DM) of experimental diets.

Treatments Ingredients	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Maize	58.05	53.55	53.55	53.55	53.55	53.55	53.55
Soya bean meal	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Wheat offal	9.20	12.20	12.20	12.20	12.20	12.20	12.20
Palm oil	0.00	1.50	0.00	0.00	0.00	0.00	0.00
Soya bean oil	0.00	0.00	1.50	0.00	0.00	0.75	0.00
Sesame oil	0.00	0.00	0.00	1.50	0.00	0.00	0.75
Fish oil	0.00	0.00	0.00	0.00	1.50	0.75	0.75
Limestone	8.50	8.50	8.50	8.50	8.50	8.50	8.50
Di-calcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Vit-min premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis							
Crude protein	17.11	17.16	17.16	17.16	17.16	17.16	17.16
ME, kcal/kg	2699.80	2718.61	2723.75	2723.02	2720.54	2722.15	2721.78
Fat	2.73	2.70	2.70	2.70	2.70	2.70	2.69
Crude fibre	3.22	3.46	3.46	3.46	3.46	3.46	3.46
Calcium	3.60	3.61	3.61	3.61	3.61	3.61	3.61
Total phosphorus	0.49	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Lysine	0.98	0.99	0.99	0.99	0.99	0.99	0.99
Sodium	0.22	0.23	0.23	0.23	0.23	0.23	0.23

DM – Dry matter; T_1 = Basal diet; T_2 = Basal diet + 1.5% palm oil; T_3 = Basal diet + 1.5% soya bean oil; T_4 = Basal diet + 1.5% sesame seed oil; T_5 = Basal diet + 1.5% fish oil; T_6 = Basal diet + 0.75% soya bean oil + 0.75% fish oil; T_7 = Basal diet + 0.75% sesame seed oil + 0.75% fish oil.
Vit-Min premix = Vitamin-mineral premix, ME = Metabolisable energy *2.5kg Premix supplied contain Vit. A = 10,000,000 IU; Vit. D_3 = 2,000,000 IU; Vit. E = 23,000mg; Vit. K_3 = 2,000mg; Vit .B_1 = 3000mg; Vit. B_2 = 6,000mg; Nicotinic acid = 50,000mg; Calcium pantothenate = 50,000mg; Vit. B_6 = 5,000mg; Vit. B_{12} = 25mg; Folic acid = 1000mg; Biotin = 50mg; Choline chloride = 400,000mg; Manganese = 120,000mg; Iron = 100,000mg; Zinc = 80,000mg; Copper = 8,500mg; Iodine = 1,500mg; Cobalt = 300mg; Selenium = 120mg and Anti-oxidant = 120,000mg.
Source: Agboola et al. (2016).

Packed cell volume estimation

The blood samples collected in bottles containing EDTA were gently mixed and drawn up in a micro-haematocrit capillary tube to ¾ of its length. One end of the tube was sealed with plasticine. The capillary tube was placed in a micro-

haematocrit centrifuge ensuring that the plasticine end is outward. After closing, these were then centrifuged at 12,000 rpm for 4 minutes. The tubes were then read in the haematocrit reader. The reading expressed the packed red blood cells as a percentage (%) of the total volume of blood (Mitruka and Rawnsley, 1981).

Haemoglobin

Haemoglobin concentration was determined by a cyanmethaemoglobin method using Drabkin's solution as diluents.

Red blood cell (RCB) and platelets counts

The properly mixed blood sample from the bottles containing EDTA was drawn up to 0.5 mark of a red blood cell pipette. The pipette was immersed into normal saline and carefully drawn up to exactly 101 marks after which the dilute blood was mixed by shaking for about half a minute. About a quarter of the content was expelled before filling the haematocytometer counting chamber and was allowed to stand for about a minute to settle after filling. All the red cells were counted using the x 40 objective lens and x 8 eye piece of microscope, with the aid of a counter.

RBC Total count =RBC counts x 10 x dilution factor;
=RCB counts x 10,000.

Platelets were determined by the phase microscopy method of Brecher and Cronkite (1950).

White blood cell (WBC) and differential leukocyte counts

The total leukocyte counts were determined using Neubauer haemocytometer after appropriate dilution, and differential leukocyte counts were performed using the oil immersion objective examination of blood films stained with the modified Romanowsky's Giemsa stain (Wittekind, 1979).

Serum Metabolites

Total serum cholesterol, triglycerides, and high density lipoprotein (HDL) were assayed by the method of Roschlan et al. (1974) while low density lipoproteins (LDL) were estimated using the Friedewald equation [LDL= Total cholesterol – (HDL-Tryglycerides/5)].

Chemical and statistical analyses

The proximate composition of diets was determined by the methods of Association of Official Analytical Chemists, AOAC (2000). Data were analysed

using descriptive statistics and analysis of variance, ANOVA ($P<0.05$) SAS (2008). Means differences were separated using Duncan's multiple range test (Duncan, 1955).

Results and Discussion

The result of the proximate composition of experimental layers' diets is as shown in Table 2. The crude protein ranged from 13.95 to 18.18%, crude fibre ranged from 3.20 to 3.40% while ether extract of the diets ranged from 7.23 to 10.10%. A high level of ether extract recorded across the dietary treatments could be due to dietary oil supplementation.

Table 2. Proximate composition (%) of experimental diets.

Parameter	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Dry matter	92.83	92.60	92.79	92.81	93.14	92.91	92.86
Crude protein	13.95	13.86	18.18	16.89	16.56	14.09	14.77
Crude fibre	3.20	3.00	3.22	3.30	3.40	3.30	3.30
Ether extract	7.48	10.10	7.39	7.23	7.30	7.40	7.30
Ash	17.00	15.07	13.00	13.00	13.00	17.00	13.00
Nitrogen free extract	58.37	57.97	58.21	59.58	59.74	58.21	61.63

T_1 = Basal diet; T_2 = Basal diet + 1.5% palm oil; T_3 = Basal diet + 1.5% soya bean oil; T_4- = Basal diet + 1.5% sesame seed oil; T_5- = Basal diet + 1.5% fish oil; T_6 = Basal diet + 0.75% soya bean oil + 0.75% fish oil; T_7 = Basal diet + 0.75% sesame seed oil + 0.75% fish oil.

The result of haematological indices of egg-type chickens on experimental diets is as shown in Table 3. There were no significant differences observed in packed cell volume, haemoglobin, red blood cell, white blood cell, lymphocyte, heterophils and basophils of birds on experimental diets, but diets had an influence ($P<0.05$) on the monocytes, eosinophils and platelets of birds. Monocytes of birds on the control diet were similar to those of birds on T3 (basal diet + 1.5% soya bean oil), but significantly ($P<0.05$) higher than the monocyte count for birds on other diets. Eosinophils of birds on the experimental diets were similar except for those on T4 (basal diet + 1.5% sesame seed oil) with significantly higher eosinophil values compared with others. A similar trend was observed in the platelets of birds on the experimental diets. The blood contains a myriad of metabolites and other constituents, which provide a valuable medium for the clinical investigation and nutritional status of the animals. Dietary components have measurable effects on blood components; hence, blood constituents are widely used in nutritional evaluation and survey of animals (Olorode et al., 1995).

Table 3. Haematological indices of experimental laying birds.

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM
PCV (%)	19.86	23.00	24.40	24.00	22.60	24.60	24.40	2.54
Hb (g/100mL)	7.84	7.40	7.84	7.94	6.90	8.02	7.62	0.65
RBC ($x10^6/\mu L$)	2.17	2.26	2.38	2.69	2.66	2.32	2.32	0.33
WBC ($x10^3/\mu L$)	24.02	24.22	23.94	25.27	24.64	22.27	22.49	1.86
Lymphocytes ($x10^3\mu L$)	57.40	63.00	55.20	55.60	64.20	55.00	53.60	3.45
Heterophils ($x10^3\mu L$)	35.40	29.40	38.40	36.80	30.40	39.20	40.20	3.34
Monocytes ($x10^3\mu L$)	4.00^a	1.60^b	4.20^a	2.60^b	2.60^b	2.80^b	3.00^b	0.63
Eosinophils ($x10^3\mu L$)	3.20^b	3.80^b	2.60^b	5.20^a	2.40^b	2.80^b	3.20^b	0.82
Basophils ($x10^3\mu L$)	0.20	0.20	0.00	0.20	0.20	0.20	0.00	0.17
Platelets ($x10^9/\mu L$)	244.70^b	282.20^{ab}	281.00^b	314.00^a	248.00^b	218.40^b	216.00^b	27.24

Means in the same row with different superscripts are significantly ($P<0.05$) different, SEM = Standard error of mean. Means in the same row with no superscripts do not differ significantly ($P>0.05$); PCV = Packed cell volume, RBC = Red blood cell, WBC = White blood cell, Hb = Haemoglobin; T_1 = Basal diet; T_2 = Basal diet + 1.5% palm oil; T_3 = Basal diet + 1.5% soya bean oil; T_4 = Basal diet + 1.5% sesame seed oil; T_5 = Basal diet + 1.5% fish oil; T_6 = Basal diet + 0.75% soya bean oil + 0.75% fish oil; T_7 = Basal diet + 0.75% sesame seed oil + 0.75% fish oil

The result of the present study agrees with the findings of Odunsi et al. (2007) who stated that packed cell volume, haemoglobin, white blood cell, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration values were not affected by 15 g kg^{-1} oil supplementation in broiler diets. This suggests a normal physiological functioning and better circulatory gaseous exchange since haematocrit and Hb measure the concentration of red blood cells and transportation of oxygen and carbon dioxide respectively. Eosinophils are mobilised at the site of antigens-antibody reactions, and this mobilisation is accompanied by an increase in the number of eosinophils in the blood stream (Deldar, 1994). A very low value of eosinophils recorded for birds on experimental diets could mean that the immune response of birds on this diet was enhanced. Circulatory monocytes are tissue macrophages, known as the mononuclear phagocyte system (MPS), playing an important role in phagocytising and destroying intra cellular organisms (fungi, protozoa and viruses) and transformed cells (Deldar, 1994) while eosinophils play a primary

role of detoxification (Coles, 1986). In this study, the monocyte and eosinophil counts recorded were within the normal physiological range reported by Nirmalan and Robinson (1971), who suggest that there is no presence of an intracellular organism to elicit the activities of MPS which could abnormally increase the monocyte count and parasitic infestation to raise eosinophil counts than normal. However, the high monocyte values observed in birds on the control and 1.5% soya bean oil supplemented diets probably suggest the presence of the condition known as monocytosis.

Serum metabolites of laying birds fed experimental diets

The results of serum metabolites of laying birds on experimental diets are shown in Table 4. There were no significant differences observed in triglycerides, high density lipoproteins and cholesterol of birds on experimental diets. Low density lipoprotein (LDL) of birds on the control diet was similar to those recorded for birds on different dietary oil supplemented diets except for those on T_6 (basal diet + 0.75% soyabean oil + 0.75% fish oil) with significantly ($P<0.05$) reduced LDL. Dietary lipids can alter the blood composition and serum lipoprotein level (Hermier and Dillon, 1992).

Table 4. Serum metabolites of laying birds on experimental diets.

Parameter (mg/dL)	T1	T2	T3	T4	T5	T6	T7	SEM
Triglycerides	361.50	469.50	344.00	560.00	414.20	332.80	437.20	77.67
HDL	11.33	10.31	11.93	10.51	12.40	10.57	10.72	1.10
LDL	33.72^{ab}	41.52^{a}	23.23^{ab}	44.78^{a}	40.67^{a}	14.45^{c}	44.58^{a}	7.53
Cholesterol	69.37	73.26	63.95	77.66	64.26	62.69	69.07	7.99

Means in the same row with different superscripts are significantly ($P<0.05$) different, SEM = Standard error of mean, Means in the same row with no superscripts do not differ significantly ($P>0.05$); HDL = High density lipoprotein, LDL = Low density lipoprotein T_1 = Basal diet; T_2 = Basal diet + 1.5% palm oil; T_3 = Basal diet + 1.5% soya bean oil; T_4 = Basal diet + 1.5% sesame seed oil; T_5 = Basal diet + 1.5% fish oil; T_6 = Basal diet + 0.75% soya bean oil + 0.75% fish oil; T_7 = Basal diet + 0.75% sesame seed oil + 0.75% fish oil.

Cholesterol is widely distributed in the body and plays an important role in the synthesis of steroid hormones, bile salts and vitamin D. Generally, saturated fatty acids increase plasma low density lipoproteins (LDL), which are very atherogenic, partly by reducing receptor-mediated up-take of cholesterol, whereas unsaturated fatty acids promote the production of plasma high density lipoproteins (HDL) which provide protection against atherosclerosis by transportation of cholesterol from tissue to liver for conversion to bile acids and

excretion (Grundy, 1989). Polyunsaturated fatty acid (PUFA) of dietary oils can decrease the plasma cholesterol concentrations in laying hens (Mori et al., 1999). The result of the present study showed that dietary oils did not influence the cholesterol, triglycerides and high density lipoprotein of the experimental diets. This is probably a normocholesterolemic condition which could be attributed to the n-3 and n-6 effect of the dietary oils and their ability to bind cholesterol in the small intestines. This agrees with the findings of Mighelenj et al. (2004), who reported no significant change in serum cholesterol levels of laying hens receiving canola seed and linseed at 2.5, 5 and 7.5% of the ration. Also, Ansari et al. (2006) reported that plasma cholesterol levels in laying hens receiving varying levels of linseed (0, 5, 10 and 15% of diet) were not significantly different across the diets. However, diets had an influence on the low density lipoprotein of birds in the present study and this is in consonance with the findings of Fébel et al. (2008), who reported that plasma total cholesterol and LDL-cholesterol decreased when birds' diets were fortified with 3% linseed oil, with no significant change in HDL-cholesterol. Similarly, Crespo and Esteve-Garcia (2002a) reported lower plasma very low density lipoprotein (VLDL) and total cholesterol levels in broilers fed diets supplemented with linseed oil. The authors averred that the decrease in cholesterol concentration may be explained by the suppression of hepatic cholesterol production. Celebi and Utlu (2006) were able to find a significant decrease in total cholesterol and VLDL-cholesterol in the blood serum of hens fed rations containing 4% linseed oil with an increase in serum HDL-cholesterol of hens. Similarly, Švedova et al. (2008) observed a decrease in serum total cholesterol and an increase in HDL cholesterol in laying hens fed 3% linseed oil in the ration. Many studies showed a decrease in serum triglyceride (TG) levels in the birds receiving diets rich in n-3 PUFA. An et al. (1997) found that n-6 and n-3 PUFA differ in their effect on TG concentration in the birds. According to the authors, n-3 PUFA reduced the serum TG level but n-6 PUFA did not. Celebi and Utlu (2006) observed a decrease in serum TG level of the laying hens receiving diets fortified with 4% linseed oil and added that dietary n-3 PUFA can decrease TG synthesis and secretion from intestinal cells and can suppress hepatic fatty acid synthesis as TG is produced. Schumann et al. (2000) reported a decrease of almost 25% in plasma TG of laying hens fed 4% linseed oil in their diet. According to Crespo and Esteve-Garcia (2002b), broilers fed n-3 PUFA rich diets showed lower concentrations of TG in blood serum. In contrast, Svedova´ et al. (2008) reported an increase in plasma TG level in the hens fed 3% linseed oil when compared to the control. In another study carried out by Fébel et al. (2008), n-3 PUFA supplementation (3% linseed oil in the diet) to the birds did not alter the plasma TG levels. It has been further reported that HDL-cholesterol concentrations were not affected by any of oil sources such as fish, canola and soya bean oils in laying hens (Murata et al., 2003).

Conclusion

Haematological parameters (PCV, Hb, RBC, WBC, platelets and white blood cell differential counts) observed in this study showed that addition of dietary oils to layers' diets did not have any adverse effects on blood profile. The serum biochemical indices (triglycerides, HDL and cholesterol) examined also showed that dietary oils had no effect on serum metabolites except low density lipoprotein (LDL) which was significantly reduced in birds on 0.75% soya bean oil + 0.75% fish oil supplemented diet compared to birds on other dietary treatments. It can therefore be recommended that dietary oils could be added to diets of laying birds especially the combination of 0.75% soya bean oil + 0.75% fish oil with an expected reduction in low density lipoprotein.

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UTICAJ ČETIRI DIJETETSKA ULJA NA ODABRANE SASTOJKE KRVI KOD KOKOŠI ZA PROIZVODNJU KONZUMNIH JAJA

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R e z i m e

Obogaćivanje jaja polinezasićenim masnim kiselinama (engl. *polyunsaturated fatty acids* – PUFA), posebno omega-3 masnim kiselinama, privlači pažnju kako istraživača tako i prehrambene industrije, zato što su ove masne kiseline neophodne za normalan razvoj tela i imaju važnu ulogu u prevenciji bolesti srca. Eksperiment je sproveden kako bi se procenio uticaj četiri dijetetska ulja na odabrane metabolite u krvi kod kokoši za prozvodnju jaja. Korišćeno je stotinu i pet (105) *Isa Brown* kokoši nosilja starosti 34 nedelje, a istraživanje je trajalo 6 nedelja u potpuno slučajnom dizajnu ogleda. Kokoši su nasumično raspoređene u sedam hranidbenih tretmana, odnosno: osnovni obrok (T_1), osnovni obrok + 1,5% palminog ulja (T_2), osnovni obrok + 1,5% sojinog ulja (T_3), osnovni obrok + 1,5% susamovog ulja (T_4), osnovni obrok + 1,5% ribljeg ulja (T_5), i osnovni obrok + 0,75% sojinog ulja + 0,75% ribljeg ulja (T_6) i osnovni obrok + 0,75% susamovog ulja + 0,75% ribljeg ulja (T_7). Tretmani su imali pet ponavljaja svaki sa po 3 kokoši. Uzorci krvi (5 ml) su uzimani iz jugularne vene svake ptice radi određivanja serumskih i hematoloških parametara. Nije bilo značajnih razlika u pogledu vrednosti hemokrita, hemoglobina, crvenih krvnih ćelija, belih krvnih ćelija, limfocita, heterofia i bazofia ptica na eksperimentalnoj ishrani, ali je sastav obroka imao uticaj ($P<0,05$) na monocite, eozinofile i trombocite ptica. Broj monocita ptica na kontrolnoj ishrani bio je sličan kao kod ptica na tretmanu T_3 (osnovni obrok + 1,5% sojinog ulja), ali značajno ($P<0,05$) viši nego broj monocita kod ptica koje su dobijale druge obroke. Eozinofili ptica na eksperimentalnim obrocima bili su slični osim kod ptica na tretmanu T_4 (osnovni obrok + 1,5% susamovog ulja) sa značajno većim vrednostima eozinofila nego kod ostalih tretmana. Sličan trend je zabeležen kod trombocita ptica u eksperimentalnim grupama. Nije bilo značajnih razlika u vrednostima triglicerida, lipoproteinima visoke gustine i holesterolu ptica u eksperimentalnim grupama. Lipoprotein niske gustine (engl. *low density lipoprotein* – LDL) ptica na kontrolnoj ishrani bio je sličan onima zabeleženim kod ptica hranjenih obrocima sa dodatkom različitih dijetetskih ulja osim za one na tretmanu T_6 (osnovni obrok + 0,75% sojinog ulja + 0,75% ribljeg ulja) sa značajno ($P<0,05$) sniženim LDL. Može se zaključiti da bi kombinacija 0,75% sojinog ulja + 0,75% ribljeg ulja u obroku mogla biti efikasna u smanjivanju količine lipoproteina niske gustine u serumu kod kokoši nosilja.

Ključne reči: palmino ulje, susamovo ulje, sojino ulje, riblje ulje, kokoši nosilje, profil krvi.

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PREBIOTIC POTENTIAL OF XYLANASE ENZYME SUPPLEMENTED WHEAT OFFAL IN BROILER CHICKENS

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Abstract: The recent development of antibiotics-resistant pathogens in poultry, which poses a threat to human health, has necessitated the search for an alternative to antibiotic growth promoters (AGPs) to improve the gut microflora in poultry diets. One of the alternatives to AGPs is probiotics which are beneficial organisms. The by-products of the digestion of polysaccharides for which poultry do not have enzymes to digest are called prebiotics. Prebiotics serve as food for probiotics. The application of enzymes makes this digestion possible. The prebiotic potentials of enzyme supplemented high fibre feedstuffs (HFFs) are not known. This study was conducted to assess the prebiotic potential of xylanase enzyme supplemented wheat offal on broiler chickens (*in-vivo*). The replacement of maize with wheat offal irrespective of levels supplemented with 100ppm xylanase enzyme caused a reduction in feed intake and an increase in weight gain and better feed conversion ratio. Birds fed diet with 20% wheat offal supplemented with a xylanase enzyme outperformed birds fed diets with 10 or 30% wheat offal supplemented with a xylanase enzyme and birds fed the control diet. The birds had normal weights of vital organs with good structural consistency. The identification of microbes (fungi and bacteria) showed that dietary levels of wheat offal (10, 20 or 30% inclusion) with supplementation of a xylanase enzyme enhanced the growth of beneficial microbes, which resulted in the inhibition or elimination of the opportunistic/pathogenic microbes. It was concluded that enzyme supplementation of high fibre feedstuffs could improve the growth performance, nutrient retention and increase concentration of beneficial microbes in the guts. The use of enzymes is therefore recommended when HFFs are required as a prebiotic source in the guts of broilers.

Key words: prebiotics, enzymes, intestinal microflora, broiler nutrition, digestibility.

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Introduction

Wheat offal is one of the high fibre feedstuffs that were screened and that met the first criteria for classification of any feedstuff as a potential prebiotic. Currently, there is a lot of interest in improving gut health by managing the colonic microbial population. This is traditionally done by the consumption of probiotics which are live microbial food supplements (Atteh, 2000). Probiotics will multiply faster in an environment that is rich in their food. Prebiotics are food for probiotics. Prebiotics are mostly oligosaccharides, some of which are by-products of fibre breakdown. They are mostly non-digestible by enzymes in the gut (Bailey et al., 1991). Fructans and arabinoxylans are naturally occurring non-digestible oligosaccharides in wheat that exhibit prebiotic properties. Wheat offal is made up of the coarse outer covering of wheat kernel, the seed coat, the major part of the germ, as separated from clean and scoured wheat in the process of wheat milling. Wheat bran is low in energy and high in fibre and does not allow for large inclusions in the poultry diet. This is because arabinoxylans (pentosans) being a major component of the non-starch polysaccharide fraction in cereals are not easily digested by monogastrics, and are abundantly present in wheat bran and wheat (Montagne et al., 2003). The anti-nutritive activity of arabinoxylans in wheat and wheat bran has been widely studied and found to depress the growth rate, feed digestibility and feed conversion efficiency when used in high quantity in broiler diets (Montagne et al., 2003).

Supplementation of wheat-based diets with xylanase enzymes such as arabinoxylanases and pentosanases has been reported to be capable of breaking down the arabinoxylans, as well as to reduce the adverse effects of some anti-nutritional compounds (Cowieson and Acamovic, 2003). Enzyme supplementation of wheat offal causes the breakdown of its cell wall with the release of oligosaccharides which can improve the probiotic population, thus improving digestibility and gut health. There is, however, little or no information on the prebiotic potentials of enzyme supplemented wheat offal in chickens. This study is therefore designed to assess the prebiotic potential of xylanase enzyme supplemented wheat offal on broiler chickens.

Materials and Methods

Sourcing and management of birds – A total of one thousand, nine hundred and twenty (1,920) day-old broiler chicks of the Arbor Acre strain purchased from Yammfy Farm hatchery at Ilemona, Kwara State were used for this experiment. The birds were housed in an electrically heated battery cage and fed the experimental diet shown in Table 1. A xylanase enzyme used is a bacteria xylanase feed enzyme (nutrase, a pure endo-1, 4-beta-xylanase) produced by *Bacillus subtilis* to break down the arabinoxylan fraction into shorter polysaccharides

(xylose monomers) with a decrease of viscosity, liberation of nutrients and improved zootechnical performances. It was supplied by Nutrex, Belgium.

Experimental design – Birds were fed a control diet (50% maize) or diets in which wheat offal was added at 10, 20, or 30% replacing maize in the control diet. Each of these diets was given with or without 100 PPM xylanase enzyme in a 4 x 2 factorial combination. Thus, there were 8 treatments each with 8 replicate cages of 30 birds. The experiment was conducted for a period of five (5) weeks. Live weight was recorded weekly while feed intake was recorded daily in grams and excreta samples were collected over a 72-hour period. The experimental diets and excreta samples were analyzed for their chemical constituents using the procedures outlined by AOAC (2008).

Table 1. Composition of experimental diet (%).

Ingredients	1	2	3	4	5	6	7	8
Maize (%)	50	50	40	40	30	30	20	20
Wheat offal (%)	0	0	10	10	20	20	30	30
Xylanase (PPM)	0	100	0	100	0	100	0	100
Basal ingredients (%)	50	50	50	50	50	50	50	50
Total (%)	100	100	100	100	100	100	100	100

Basal diets: Ground nut cake (GNC) – 26%, corn bran – 1%, soybean meal – 12%, fishmeal (72%) – 4%, palm oil – 2%, oyster shell – 2%, bone meal – 2%, salt – 0.25%, methionine – 0.25%, lysine – 0.25% and vitamin premix – 0.25% (*Vitamin/ mineral premix contained the following: (Univit. 15 Roche) 1500 I.U, Vit. A, 1500 I.U, Vit. D, 3000 I.U, Vit. E, 3.0g, Vit. K, Vit. B_2, 0.3g, Vit.B_6, 8.0mg, Vit.B_{12}, 8.0g, Nicotinic acid, 3.0g, Ca-pantothenate, 50mg, Fe, 10.00g, Al, 0.2g, Cu, 3.5mg, Zn, 0.15mg, I, 0.02g, CO_2, 0.01g, Se.

At the end of the experiment, 10 birds per replicate from each treatment were randomly selected and euthanized by bleeding through the carotid artery for collection of digesta from the gastro-intestinal tract (GIT) to determine the microbial profile. The carcasses were subsequently opened and the entire GIT removed using aseptic techniques. It was excised and the luminal contents of the crop, jejunum, duodenum, ileum and caecum were collected and pooled together according to the procedure described by Kalantar et al. (2014). This was done to determine the population and profile of microorganisms present in the broiler gut. Five grams of each sample were put into a sterile universal bottle containing 100ml of sterile water and shaken thoroughly. The mixture was allowed to settle and the supernatant was decanted into separate test tubes to be used as a stock solution. Serial dilutions of the stock were made and a 0.1ml aliquot was spread on potato dextrose agar (PDA) plate containing 1% streptomycin (to inhibit bacterial growth).The plates were incubated at room temperature (27–31^{0}C) for 48 hours (Bengmark, 2001). After incubation, representative fungal colonies were picked from each plate and purified on fresh PDA (for other fungi) and YPDA (yeast peptone dextrose agar) plates for yeast. The purified isolates were transferred to

PDA and YPDA slants incubated at room temperature for 48 hours and stored at 4^0C. The isolates were identified according to the scheme of Chio et al. (1994). In isolation of bacteria, identification was based on their Gram's reaction and biochemical tests with reference to Bergey's manual of determinative bacteriology. The direct culturing of the samples was carried out by pipetting 1ml of the dissolved sample in sterile distilled water aseptically on sterile Petri dishes. The pour plate technique was used with the NA (nutrient agar) plates incubated at 35^0C for 2–3 days. Bacteria were identified in their unique colonies. They were subcultured by streaking out on nutrient agar. Bacterial cells on NA were left for 2–3 days at 35^0C before their pure cultures were isolated and stored on slants containing nutrient agar at 4^0C. These slants served as the stock culture for bacteria. Other tests were carried out to identify the morphological and biochemical characteristics of the microbes such as Gram's staining reaction, spore staining, motility test, catalase test, Voges-Proskauer test, methyl-red test, starch hydrolysis, casein hydrolysis, gelatin hydrolysis, oxidase test, indole test, nitrate reduction, sugar fermentation test, growth at different temperatures, NaCl concentration, anaerobic growth, citrate utilization test, hydrogen sulphide production, urease test, acid production, sporulation test, growth in liquid medium at varying temperatures, NaCl solution, and deamination of amino acids.

Cost-benefit analysis – Cost-benefit analysis was carried out, taking into consideration the cost of maize, wheat offal and the enzyme (xylanase) as they are related to the performance of birds.

Statistical analysis – All data collected were subjected to two-way analysis of variance (ANOVA) using the PRO GLM (General Linear Model) of SAS (2008) at the 5% level of significance. All significantly different means were separated using the Duncan's multiple range test of the same software package.

Results and Discussion

Table 2 shows the effects of dietary levels of wheat offal with or without enzyme supplementation on the performance of broilers. Feed intakes by birds fed the control diet and those fed diet with 10% wheat offal were comparable and they were significantly lower than those of birds fed diets with 20% wheat offal ($p<0.05$). Feed intakes of birds fed 20% and 30% wheat offal were not significantly different. Birds fed diets with wheat offal gained more weight than those fed the control diet ($p<0.05$). Weight gains of birds fed diets with 20% or 30% wheat offal were comparable and significantly higher than those of birds fed diet with 10% wheat offal ($p<0.05$). Feed gain ratios of birds fed the control diet to those fed diets with 10% or 30% were comparable and significantly higher than those fed diet with 20% wheat offal ($p>0.05$). Thus, birds fed diet with 20% wheat offal had a better feed gain ratio compared to other levels of wheat offal ($p<0.05$). Enzyme supplementation increased feed intake and weight gain ($p<0.05$) but had a

numerical decrease effect on feed/gain ratio, though not significant ($p>0.05$). There was no significant effect of the interaction between dietary levels of wheat offal and enzyme supplementation on the performance parameters ($p>0.05$).

Table 2. Effects of dietary levels of wheat offal with or without xylanase supplementation on performance of broilers (0–5wks) and nutrient digestibility.

BDG (%)	Feed consumed (g/bird/day)	Weight gain (g/bird/day)	FCR	Crude protein (%)	Crude fibre (%)	Ether extract (%)
0	75.40^{b}	40.60^{c}	1.90^{a}	62.90^{a}	75.10^{a}	63.90^{a}
10	78.90^{b}	44.00^{b}	1.80^{a}	61.20^{ab}	71.10^{a}	61.50^{ab}
20	86.80^{a}	52.80^{a}	1.60^{b}	61.00^{ab}	65.30^{b}	60.90^{b}
30	88.40^{a}	49.70^{a}	1.70^{a}	58.00^{b}	59.50^{c}	57.80^{b}
SE	75.40^{b}	40.60^{c}	1.90^{a}	62.90^{a}	75.10^{a}	63.90^{a}
Enzyme supplementation (ES) (ppm)						
0	85.50^{a}	45.40^{b}	1.80	60.10	67.20	61.70^{b}
100	79.30^{b}	48.20^{a}	1.60	61.40	68.60	65.40^{a}
SEM±	1.59	1.10	0.24	1.59	1.62	1.09
WO x ES	NS	NS	NS	NS	NS	NS

Column means with different superscripts are significantly different ($p<0.05$), NS: Not significant, S: Significant; FCR – Feed conversion ratio (kg of feed intake/kg of weight gain).

Cost-benefit analysis for replacing maize with wheat offal with or without enzyme supplementation is shown in Table 3. Cost-benefit analysis showed that the 20% inclusion level of wheat offal supplemented with xylanase enzyme gave the best numerical result of a beneficiary reduction in the cost of production with the best improved broiler performance in terms of weight gain.

Table 3. Cost-benefit analysis for replacing maize with wheat offal with or without enzyme supplementation.

Sources of variation	Cost of producing/kg (N)	Percentage reduction in price of feed (%)	Cost of raising 1kg of broilers (N)	Percentage reduction in raising 1kg of broilers (%)
Dietary levels of WO without xylanase				
0	130.00	0.00	260.00	0.00
10	120.00	8.33	240.00	8.33
20	110.00	18.18	242.00	7.44
30	100.00	30.00	230.00	13.04
Dietary levels of WO with xylanase				
0	133.00	-2.26	239.00	8.79
10	123.00	5.69	209.00	24.40
20	113.00	15.04	180.80	43.81
30	103.00	26.21	185.40	40.24

The effects of dietary levels of wheat offal with or without enzyme supplementation on nutrient retention are shown in Table 4. Enzyme supplementation had no significant effect ($p>0.05$) on crude protein and ether extract retention. However, enzyme supplementation caused an increase in crude fibre retention ($p<0.05$). Crude protein retention in birds fed the control diet was significantly higher than in those fed diet with 30% wheat offal ($p<0.05$), but comparable with those of birds fed diet with 10% or 20% wheat offal ($p>0.05$). Protein retention in birds fed diets with wheat offal irrespective of levels was comparable ($p>0.05$). There was a decrease in the crude fat retention with an increase in the dietary level of wheat offal in birds fed diets with 20% or 30% wheat offal being significantly lower ($p<0.05$). The birds fed the control and those fed diet with 10% wheat offal were comparable ($p>0.05$) in crude fat retention. The crude fibre retention of birds fed the control diet was significantly higher than of those of birds fed diets with 20% or 30% wheat offal ($p<0.05$), but comparable with the crude fibre retention of birds fed diet with 10% wheat offal ($p>0.05$). Fibre retention of birds fed diets with wheat offal irrespective of levels was comparable ($p>0.05$). There was no significant effect of the interaction between the inclusion of enzymes and the dietary level of wheat offal on crude protein, ether extract and crude fibre ($P>0.05$).

Table 4. Effects of dietary levels of wheat offal with or without enzyme supplementation on nutrient retention of broiler chickens.

	Crude protein (%)	Ether extract (%)	Crude fibre (%)
Dietary levels of wheat offal (WO) (%)			
0	62.90^{a}	75.10^{a}	63.90^{a}
10	61.20^{ab}	71.10^{a}	61.50^{ab}
20	61.00^{ab}	65.30^{b}	60.90^{b}
30	58.00^{b}	59.50^{c}	57.80^{b}
Enzyme supplementation (ES) (ppm)			
0	60.10	67.20	61.70^{b}
100	61.40	68.60	65.40^{a}
WO x ES	NS	NS	NS
SEM±	1.59	1.62	1.09

Column means with different superscripts are significantly different; NS: Not significant, S: Significant.

Table 5a shows the effects of dietary levels of wheat offal with or without enzyme supplementation on organs and body part weights. An increase in dietary levels of wheat offal from zero to 30% had no significant effect on the heart, proventriculus, abdominal fat, liver, back, head, drumstick, shank and thigh weights ($p>0.05$). However, there were significant effects on the bursa of Fabricius, crop, full and empty gizzard, spleen, neck, wing and breast weights ($p<0.05$).

Table 5a. Effects of dietary levels of wheat offal with or without enzyme supplementation on organs and body parts expressed in percentage of live body weight of broilers.

	Heart	Bursa	Crop	Proven-triculus	Full gizzard	Empty gizzard	Abdominal fat	Liver	Spleen	Back	Neck	Head	Drumstick	Wings	Shank	Thigh	Breast
WO (%)																	
0	0.50	0.10^{b}	0.50^{b}	0.50	2.70^{b}	1.90^{b}	2.10	2.20	1.40^{a}	12.00	5.40^{a}	3.50	9.90	7.90^{b}	4.70	10.30	14.50^{b}
10	0.50	0.20^{b}	0.60^{b}	0.50	3.10^{ab}	1.90^{b}	1.50	1.90	0.20^{c}	15.80	4.80^{b}	3.40	10.60	9.00^{a}	4.00	9.60	15.30^{b}
20	0.50	0.40^{a}	0.90^{a}	0.60	3.90^{a}	2.50^{a}	2.00	1.90	0.90^{b}	14.00	5.00^{ab}	3.20	10.80	7.90^{b}	5.40	9.60	15.70^{b}
30	0.40	0.10^{b}	0.70^{b}	0.60	3.70^{a}	2.40^{a}	0.30	1.90	0.10^{c}	12.00	4.30^{c}	3.60	10.10	7.90^{b}	5.30	16.80	17.10^{a}
ES (ppm)																	
0	0.40	0.20^{a}	0.20	0.60	3.40	2.20	1.80	2.10	0.10	14.20	4.60^{b}	3.30	10.40	7.80^{b}	4.90	12.70	15.00^{b}
100	0.50	0.01^{b}	0.70	05.	3.20	2.10	1.10	1.90	0.10	13.00	5.20^{a}	3.50	10.60	8.50^{a}	4.80	10.50	16.30^{a}
WO x ES	NS	NS	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	S
SEM	0.22	0.32	0.45	0.22	0.17	0.75	0.41	0.11	0.12	0.69	0.13	0.86	0.25	0.18	0.24	1.72	0.39

The weights of the bursa of Fabricius of birds fed the control diet and of those fed diet with 30% wheat offal were comparable and significantly lower than those of birds fed diets with 10% and 20% wheat offal ($p<0.05$). Crop weights of birds fed the control diet and of those fed diets with 10% or 30% wheat offal were comparable and significantly lower than of those birds fed diet with 20% wheat offal ($p<0.05$). The weight of the crop of birds fed diet with 20% wheat offal was significantly higher than of those fed diets with 10% or 30% wheat offal. The weight of the full gizzard of birds fed the control diet was significantly lower than of those fed diets with 20% or 30% wheat offal ($p>0.05$), but comparable with the weight of the full gizzard of birds fed diet with 10% wheat offal ($p<0.05$). The full gizzard weights of birds fed diets with wheat offal irrespective of levels were comparable ($p<0.05$). However, the weight of the empty gizzard of birds fed the control diet and the weight of the empty gizzard of birds fed diet with 10% wheat offal were comparable ($p>0.05$), but were significantly lower than the weight of the empty gizzard of birds fed diets with 20% or 30% wheat offal ($p<0.05$). The empty gizzard weights of those birds fed diets with 20% and 30% wheat offal were not significantly different, but were significantly higher than the weight of the empty gizzard of birds fed diet with 10% wheat offal ($p>0.05$). The weight of the empty gizzard of birds fed the control diet were comparable ($p>0.05$). The spleen weight of birds fed the control diet was significantly higher than of those birds fed diets with wheat offal ($p<0.05$). The weights of spleen of birds fed diets with 10% and 30% wheat offal were comparable but significantly lower than of those fed diet with 20% wheat offal ($p>0.05$). The neck weight of birds fed the control diet was significantly higher than of those birds fed diets with 10% or 30% wheat offal ($p>0.05$), but it was comparable with the neck weight of birds fed diet with 20% wheat offal ($p>0.05$). The neck weights of birds fed diets with 10% and 20% wheat offal were comparable, but were significantly higher than those of birds fed diet with 30% wheat offal ($p<0.05$). The wing weights of birds fed the control diet and of those fed diets with 20% or 30% wheat offal were comparable ($p>0.05$), but were significantly lower than of those birds fed diet with 10% wheat offal ($p<0.05$). The wing weights of birds fed diet with 10% wheat offal were significantly higher than of those birds fed diets with 20% or 30% wheat offal ($p>0.05$). The breast weights of birds fed the control diet and of those fed diets with 10% or 20% wheat offal were comparable ($p<0.05$), and were significantly lower than of birds fed diet with 30% wheat offal. The breast weight of birds fed diet with 30% wheat offal was significantly higher than of those birds fed diets with 10% or 20% wheat offal ($p<0.05$). There was no significant effect of enzyme supplementation on the weight of the heart, crop, proventriculus, full and empty gizzard, abdominal fat, liver, spleen, back, head, drumstick, shank and thigh. However, there were

significantly increased effects on weights of neck, wings and breast but a significantly decreased effect on weight of the bursa of Fabricius. There was no significant ($p>0.05$) effect of the interaction between inclusion of enzymes and dietary levels of wheat offal on all the parameters except for the abdominal fat and breast meat ($p<0.05$), the details of which are shown in Table 5b. Thus, enzyme supplementation of the control diet and diet with 20% wheat offal increased the breast weight ($p<0.05$), while there was no significant effect of this parameter at 10% or 30% wheat offal level ($p>0.05$).

Table 5b. Effects of the interaction between dietary levels of wheat offal and enzyme supplementation on abdominal fat and breast weight ($p>0.05$).

Dietary wheat offal supplementation (%)					
Parameters	ES (100ppm)	0	10	20	30
Abdominal fat	0	2.40^{a}	1.20^{c}	0.60^{c}	0.30^{d}
	100	1.90^{b}	1.80^{b}	0.10^{d}	0.20^{d}
Breast weight	0	13.40^{c}	14.40^{bc}	15.40^{bc}	14.90^{bc}
	100	15.70^{b}	16.20^{b}	19.30^{a}	16.00^{b}

Table 6 shows the effects of dietary levels of wheat offal with or without enzyme supplementation on the microbial gut profile of broilers. An increase in dietary levels of wheat offal from 0% to 30% had a significant effect on the fungi colony count (FCC) and fecal count (FC) ($p<0.05$), but it had no significant effect on pH, total viable count (TVC), total colony count (TCC) and lactobacillus count (LBC) ($p>0.05$). The FCC for the birds fed the control diet was comparable with those of birds fed diets with wheat offal irrespective of the levels ($p<0.05$). Birds fed diet with 20% wheat offal had the significantly lower FCC compared to those of birds fed diets with 10% or 30% wheat offal ($p<0.05$). The FC for the birds fed the control diet was comparable with those of birds fed diets with wheat offal irrespective of the levels ($p<0.05$). Birds fed diet with 20% wheat offal had the significantly lower FC than birds fed diet with 30% wheat offal ($p<0.05$), but comparable with those of birds fed diet with 10% wheat offal ($p<0.05$). Enzyme supplementation had no significant effects on pH, TVC, TCC, FCC, LBC and FC ($p<0.05$), but it had a numerically increased effect on TVC, LBC and FC. There was no significant effect of the interaction between inclusion of enzymes and dietary levels of wheat offal on all the microbial gut profile parameters ($p>0.05$).

The FCC for the birds fed the control diet was comparable with those of birds fed diets with wheat offal irrespective of the levels ($p<0.05$). Birds fed

diet with 20% wheat offal had the significantly lower FCC compared to those of birds fed diets with 10% or 30% wheat offal ($p<0.05$). The FC for the birds fed the control diet was comparable with those of birds fed diets with wheat offal irrespective of the levels ($p<0.05$). Birds fed diet with 20% wheat offal had the significantly lower FC than birds fed diet with 30% wheat offal ($p<0.05$), but comparable with those of birds fed diet with 10% wheat offal ($p<0.05$). Enzyme supplementation had no significant effects on pH, TVC, TCC, FCC, LBC and FC ($p<0.05$), but it had a numerically increased effect on TVC, LBC and FC. There was no significant effect of the interaction between inclusion of enzymes and dietary levels of wheat offal on all the microbial gut profile parameters ($p>0.05$).

Table 6. Effects of dietary levels of wheat offal with or without enzyme supplementation on the microbial gut profile of broilers.

	pH	TVC 1 0^7cfu/ml)	TCC (10^7cfu/ml)	FCC (10^7cfu/ml)	LBC (10^7cfu/ml)	FC (10^5cfu/ml)
WO (%)						
0	6.10	3.00	2.50	0.90^{ab}	2.00	2.50^{ab}
10	6.20	3.90	1.80	1.20^{b}	3.50	2.60^{ab}
20	6.10	6.80	1.00	0.30^{a}	4.50	3.20^{a}
30	6.10	5.90	1.60	1.40^{b}	3.80	2.90^{b}
ES (ppm)						
0	6.10	5.10	1.90	1.20	3.30	1.40
100	6.10	5.90	1.00	0.70	6.00	2.90
WO x ES	NS	NS	NS	NS	NS	NS
SEM	0.53	0.28	0.24	0.17	0.96	0.11

a, b – Means within the column having different superscripts differ significantly; Acid-base balance (pH), total viable count (TVC), total coliform count (TCC), feacal coliform count (FCC), lactobacillus count (LBC) and fungi count (FC).

Tables 7 and 9 show colonal and morphological characteristics used in identifying and classifying the various species of fungi. The fungi identified were found to belong to five genera, namely: *Penicillium*, *Aspergillus*, *Fusarium*, *Saccharomyces* and *Rhizopus.*

Table 7. Colonal and morphological characteristics of fungi in the experimental broiler gut.

Isolates	Colonal characteristics	Morphological characteristics under microscope	identification
1	Loose white mycelia changing to yellowish black on sporulation under 72 hours of incubation	Conidiophore is a smooth wall, non–septate hyaline near the vesicle. It has a globose reside and conidia were in chains, rough with no spine. Phialide is uniform. Hyphae were septate and conidia in chains were more or less globose and rough.	*Aspergillus niger*
2	Loose white mycelia turning yellowish green with colourless reverse side on the Petri dish	Heavily walled conidiophore, uncoloured, nonseptate, coarsely roughened. A vesicle is globose at maturity and spinnate. A matured vesicle was globose and spinnate although some were elliptical.	*Aspergillus flavus*
3	Fluffy white mycelia becoming dirty brown later covering about 10cm width on PDA plate.	Sporangia are supported by a large apophysate columella atop a long stalk, the sporangiophore. Sporangiophores arise among distinctive, root-like rhizoids. Rhizoids and stolons were light brown and the collumelae were light brown. The sporangiophores were irregular, round, oval and elongated.	*Rhizopus stolonifer*
5	Colonies are flat, smooth, moist, glistening or dull, and cream in colour.	Cell buds were observed under the microscope. They are unicellular, globose, and ellipsoid to elongate in shape. Multilateral (multipolar) budding is typical. Pseudohyphae, if present, are rudimentary. Hyphae are absent. *Saccharomyces* produces ascospores which are globose and located in asci. Each ascus contains 1□4 ascospores. Ascospores are stained with Kinyoun stain and ascospore stain. When stained with Gram stain, ascospores appear Gram-negative, while vegetative cells appear Gram-positive.	*Saccharomyces cerevisae*
6	A pale green blue velvety colony which later turned darker green shades. The surface is covered by a thin overgrowth of hyaline, mycelium bearing some conidiophores appearing flocculent.	Numerous erect single conidiophores are seen under the microscope forming a colony with a radial pattern, margin and lobed. Conidiophores are smooth-walled while the metulae are cylindrical, smooth and walled bearing 3–6 phialides which are flask-shaped and thick-walled and the conidia are subglobose.	*Penicillium chrysogenum*

The results of biochemical tests on bacteria in the gut of experimental broiler chickens are shown in Table 8. It shows the various tests used in identifying and classifying the bacteria present in the broiler chicken gastrointestinal tract.

Table 8. Results of biochemical tests on bacteria in the gut of experimental broiler chickens.

Tests	Isolates								
	Salmonella	*Lactobacillus*	*Streptococci*	*Campylobacter*	*E.coli*	*Bifidobacterium*	*Bacillus*	*Clostridium*	*Staphylococcus*
Gram stain	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Spore	-ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve
Nitrate reduction	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Starch hydrolysis	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve
Methyl red	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Voges proskeauer	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve
Casein hydrolysis	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Gelatin hydrolysis	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
Indole test	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
H_2S production	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
Citrate reduction	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve
Urea hydrolysis	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	Variable
Deamination test	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
Oxidase test	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve
Motility	Motile	Non-motile	Non-motile	Non-motile	Motile	Non-motile	Non-motile	Non-motile	Non-motile
Shape	Rod	Rod	Cocci	Rod	Rods	Cocci	Rod	Rod	Cocci/ Rod
Capsule	-ve	-ve	+ve	=ve	+ve	+ve	-ve	-ve	+ve

+ = positive; - = negative.

Table 9. Results of biochemical tests on *Saccharomyces cerevisae*.

Test	Result
Acid production	-ve
Urease	+ve
Glucose	+ve
Raffmose	+ve
Fructose	-ve
Galactose	+ve
Sucrose	-ve
Mannitol	+ve
Lactose	+ve
Maltose	+ve
Inositol	+ve

Table 10 shows the effects of dietary levels of wheat offal with or without enzyme supplementation on the summary of the identified fungi and bacteria in the crop, ileum and caecum pooled together. An increase in dietary levels of wheat offal from 0 to 30% had no effect on both the number of beneficial fungi and the pathogenic/opportunistic fungi identified in the gut of the birds. *Penicilium chrysogenum* was the only beneficial fungus identified in the gut of birds fed the control diet and of those birds fed diets with dietary levels of WO. Four species of opportunistic/pathogenic fungi, namely: *Fusarium solani, Rhizopus stolonifer, Aspergillus niger* and *Aspergillus flavus* were identified in the gut of birds fed the control diet and of those birds fed diets with dietary levels of WO. Enzyme supplementation of the dietary levels of wheat offal had an increased effect on the the number of beneficial fungi identified and a decreased effect on the number of the opportunistic/pathogenic fungi identified in the gut of the birds. Two beneficial fungi species were identified in the gut of those birds fed diets with enzyme supplemented dietary levels of WO, namely: *Penicilium chrysogenum* and *Saccharomyces cerevisiae*, while birds fed the control diet with enzyme supplementation had only *Saccharomyces cerevisiae* identified in their gut. There were four species of pathogenic fungi identified in the gut of birds fed the control diet with enzyme supplementation as were observed with the control diet without enzyme supplementation, but the number of pathogenic fungi identified in the gut of birds fed diet with 10% WO with enzyme supplementation reduced to two species (*Fusarium solani* and *Rhizopus stolonifer*), while *Fusarium solani* was the only opportunuistic/pathogenic fungus identified in the gut of those birds fed diets with 20% or 30% wheat offal. In the gut of birds fed diets with dietary levels of wheat offal from 0 to 30% without xylanase enzyme supplementation, *Lactobacillus* sp was the only beneficial bacterium identified, while the pathogenic bacteria identified belonged to six genera, namely: *E. coli, Streptococcus, Staphylococcus, Clostridium, Campylobacter* and *Salmonella* species. Enzyme

supplementation of the dietary levels of wheat offal significantly increased the number of beneficial bacteria, while subduing the number of the pathogenic bacteria identified. The number of beneficial bacteria was constant (*Lactobacillus* species) with a reduction in the number of the pathogenic bacteria identified to five genera compared to 0% WO without xylanase supplementation. In the gut of those birds fed diets with 10% or 20% or 30% WO, the beneficial bacteria increased to three genera, namely: *Lactobacillus sp, Bacillus subtilis* and *Bifidobacterium* species while the number of pathogenic bacteria was reduced to four (*E. coli, Clostridium* species, *Streptococcus* species *and Staphylococcus* species), one (*Streptococcus* species) and two (*E. coli and Streptococcus* species), respectively.

Wheat offal is high in fibre and is known to affect feed intake and energy utilization of birds. The primary factor in the voluntary feed intake of chicks appears to be the need for energy (Atteh, 2000). Birds will ordinarily eat to satisfy their energy requirements. As fibre content of diets increases, the density of the diets decreases. The inclusion of fibre in feed dilutes energy concentration of diets (Sriver et al., 2003). Hence, for birds to keep a constant energy level, they have to change their feed intake as the energy density of the feed changes, hence the need for an increase in feed intake. Fibre has been included in experimental diets for monogastric animals for many years, primarily to promote constant passage of materials through the gut (Lee et al., 2003). Fibre reduces the digestibility time of feed in the gut not allowing intestinal secretions to act on the feed.

Observation from this trial showed that the feed became bulky as 30% of the maize in the control diet was replaced with wheat offal. An increase in feed intake as the dietary level of wheat offal increased without xylanase enzyme supplementation was observed. This may be a result of the birds trying to eat more to satisfy their energy requirements. This is in line with the report of Bailey et al. (1991) that birds are known to eat to satisfy their energy requirements. The reduction in feed intake in the presence of enzyme supplementation of the feed observed in this trial showed that the birds consumed less feed to meet their energy requirements. This finding is consistent with Annison and Choct (1992) and Montagne et al. (2003) who alluded that decreased feed intake in broilers fed enzyme supplemented diets could be due to the ability of the birds to fulfil their requirements with less feed. Similarly, Alander et al. (2001) reported reduced feed intake in birds fed diets supplemented with Roxaxyme G compared with the control. An increase in feed/gain ratio (FCR), when there was no enzyme in the feed, indicated that the feed was not efficiently utilized but when the feed was supplemented with xylanase, the FCR was improved. This observation was supported with published reports that the supplementation of non-starch polysaccharidases could improve the growth performance of birds fed on a wheat and/or rye diet (Annison and Choct, 1992; Bedford, 1996; Pettersson and Aman, 1989).

Table 10. Identification of fungi and bacteria.

Without enzyme	Beneficial fungi	Opportunistic/ pathogenic fungi	Beneficial bacteria	Pathogenic bacteria
Control	*Penicilium chrysogenum*	*Rhizopus stolonifer, Aspergillus niger*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp, Clostridium sp, Campylobacter sp, Salmonella sp*
10%	*Penicilium chrysogenum*	*Fusarium solani, Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp, Clostridium sp, Campylobacter sp, Salmonella sp*
20%	*Penicilium chrysogenum*	*Fusarium solani, Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp, Clostridium sp, Campylobacter sp, Salmonella sp*
30%	*Penicilium chrysogenum*	*Fusarium solani, Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp, Clostridium sp, Campylobacter sp, Salmonella sp*
With enzyme				
Control	*Penicilium chrysogenum*	*Fusarium solani, Rhizopus stolonifer, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp, Clostridium sp, Campylobacter sp*
10%	*Penicilium chrysogenum, Saccharomyces cerevisiae*	*Aspergillus flavus*	*Bacillus subtilis, Lactobacillus plantarum, Bifidobacterium*	*Campylobacter sp, Staphylococcus sp.*
20%	*Penicilium chrysogenum, Saccharomyces cerevisiae*	*Rhizopus stolonifer, Aspergillus flavus*	*Lactobacillus plantarum, Bacillus subtilis, Bifidobacterium*	*Campylobacter sp, Staphylococcus sp.*
30%	*Penicilium chrysogenum, Saccharomyces cerevisiae*	*Rhizopus stolonifer, Aspergillus flavus*	*Lactobacillus plantarum, Bacillus subtilis, Bifidobacterium*	*Campylobacter sp, Staphylococcus sp.*

Considerable improvement in performance rates especially feed conversion ratio as a result of enzyme supplementation has also been reported in poultry (Fuller, 1997; Petterson and Aman, 1989; Ohimain and Ofongo, 2013). Similarly, Malmen et al. (2002) showed that Roxaxyme^{R} G2 supplementation significantly improved weight gain and FCR of broilers. Thus, birds fed diet with 20% wheat offal supplemented with enzymes had a relatively better feed/gain ratio compared to those fed the control diet. There were no significant effects of the interaction between levels of wheat offal and xylanase enzyme supplementation on feed intake, weight gain and feed/gain ratio.

Reduction in nutrient digestibility with an increase in dietary levels of wheat offal observed in this study is associated with the fibre that has a high level of non-starch polysaccharides. This is in line with the work of Pylkas et al. (1998) who reported that increasing the levels of wheat offal without enzyme supplementation resulted in the difficulty in feed digestibility. Enzyme xylanase supplementations efficiently broke down NSP and enhanced digestibility. It was observed that digestibility of fibre, fat and protein were higher when supplemented with enzyme. This supports the report of Zhang et al. (2005) that xylanase supplementation resulted in improvements in the degradation of NSP in the gastro-intestinal tract of broilers fed wheat-based diet relative to the control group. However, the use of exogenous enzymes in poultry results in improvement of nutrient digestibility (Malimen et al., 2002). Enzyme allows improved performance or a more efficient use of cheap low-quality carbohydrate sources without adversely affecting animal performance (Santin et al., 2001).

An increase in the weight of the bursa of Fabricius, gizzard and spleen in the birds fed diet without enzyme supplementation may be as a result of increased activity of these organs (Montague et al., 2003). Increased viscosity of the intestinal contents decreases the rate of diffusion of substrates and digestive enzymes and hinders their effective interaction leading to significant modification of the structure and function of the digestive organs (Jorgensen et al., 1996). To adapt to these changes, the activities of the intestinal secreting mechanism may be enhanced possibly leading to hypertrophy of the digestive organs. The increased weight of the crop is thought to be due to a need to store more feed as a result of the bulky nature of wheat offal, while an increased gizzard size is due to the need for more grinding activities resulting in the increased musculature consequent on the increased fibre content of the diet (Chio et al., 1994). With enzyme supplementation, a greater proportion of non-starch polysaccharide (NSP) was digested, thereby attenuating the increased function of the responding organs.

E.coli, Streptococcus species, *Staphylococcus* species, *Clostridiu* species, Campylobacter species, Salmonella species were the pathogenic bacteria and *Lactobacillus* species, *Bacillus subtilis* and Bifidobacterium species were the beneficial bacteria identified in the GIT of the broiler chickens. The concentrations of these organisms were high in this trial. This observation is in line with the work of Skorupski et al. (1997) who concluded that environmental factors, including pH, temperature, osmolarity, and certain amino acids, could increase the activity of a transcriptional activator (ToxR) and result in more virulent bacteria.

A prebiotic is a selectively fermented food ingredient that stimulates specific changes in the composition of and (or) activity in the gastrointestinal microflora, which in turn confer benefits on the host well-being and health (Roberfroid, 2007). Prebiotics, such as fructooligosaccharides (FOS), inulin, galactooligosaccharides (GOS) and glucooligosaccharides, are dietary carbohydrates that escape digestion

in the upper gastrointestinal tract and alter the bacterial composition of the gut by changing the type of the substrate provided to the existing gut microbiota (Chio et al., 1994; Gibson and Roberfroid, 1995; Alander et al., 2001; Malinen et al., 2002). Prebiotics are fermented by probiotic bacteria, producing short-chain fatty acids (SCFAs) as the end products of fermentation. The major SCFAs produced are acetate, propionate, and butyrate (Pylkas et al., 2005), and the profile of SCFAs varies among the different prebiotic sources. SCFAs have beneficial effects on human health as energy sources and by inhibiting the growth of pathogenic bacteria (Bailey et al., 1991; Blottiere et al., 1999; Pylkas et al., 2005). SCFAs also decrease colonic pH, which demonstrates the fermentability of the non-digestible carbohydrates. Beneficial microbes such *as Lactobacillus* species, *Bifidobacterium* species and *Bacillus subtilis*, which were results of fermentation of fibres, were observed in this trial. *Lactobacillus* species are capable of controlling populations of *E. coli* in the crop, and their effects are both bacteriostatic (depressing the growth of other bacteria by their secretions) and bacteriocidal (killing other bacteria by secretions) (Collins and Gibson, 1999). *Bacillus subtilis* spores in broilers exert their beneficial effects by one or more of these mechanisms. It makes use of available oxygen, thereby creating a more favourable environment for the beneficial anaerobic species to proliferate. This action enhanced the proliferation of *Lactobacillus* that helps control the growth of pathogenic bacteria especially *E. coli*. It enhances enzyme production and immune responses (Kalantar et al., 2014).

The fungi identified were both beneficial and non-beneficial. *Aspergillus niger, Aspergillus flavus, Fusarium solani* and *Rhizopus stolonifer* were the pathogenic/opportunistic fungi identified. This may be a result of contamination from the feed/diet (Dalcero et al., 1997). Replacement of maize with wheat offal supplemented with xylanase has the effect on the growth of beneficial fungi: *Saccharomyces cerevisiae* and *Penicilium chrysogenum.* It was observed in this study that inclusion of wheat offal without enzyme supplementation increased the concentration of pathogenic/opportunistic microbes (fungi and bacteria) against the beneficial microbes while enzyme supplementation increased the concentration of beneficial microbes. This finding corroborates the report of Ohimian and Ofongo (2013) who reported that coliforms and *E. coli* were consistently higher ($P<0.05$) in the control (maize–soybean meal diet) than in feeds supplemented with wheat offal with or without enzyme supplementation in the gut of chickens and that the population of coliform and *E. coli* were the lowest in the diet containing wheat offal and Roxazyme G2.

The beneficial microbes (both fungi and bacteria) have been shown to improve bird performance and decrease mortality (Miles and Bootwalla, 1991; Santin et al., 2001). Enzyme supplementation has a beneficial effect on animal performance (Fasuyi, 2010).

Conclusion

The replacement of maize with wheat offal irrespective of levels supplemented with 100ppm xylanase enzyme caused a reduction in feed intake and an increase in weight gain and better FCR. The birds fed diet with 20% wheat offal supplemented with xylanase enzyme outperformed birds fed diets with 10% or 30% wheat offal supplemented with xylanase enzyme and birds fed the control diet. It can be deduced that enzyme supplementation of wheat offal helped in increasing and improving protein and fibre digestibilities. The weights of vital organs showed that the birds were in good health conditions. The result of identification of microbes (fungi and bacteria) showed that dietary levels of wheat offal (10, 20 or 30% inclusion) with supplementation of xylanase enzyme enhanced the growth of beneficial microbes which resulted in inhibition or elimination of the opportunistic/pathogenic microbes. Cost-benefit analysis showed that the 20% inclusion level of wheat offal supplemented with xylanase enzyme gave the best result of a beneficiary reduction in the cost of production with the best improved broiler performance.

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PREBIOTSKI POTENCIJAL PŠENIČNOG STOČNOG BRAŠNA SA DODATKOM ENZIMA KSILANAZE KOD BROJLERSKIH PILIĆA

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R e z i m e

Nedavni razvoj patogena otpornih prema antibioticima kod živine, što predstavlja pretnju za ljudsko zdravlje, zahteva traženje alternative za antiobiotske stimulatore porasta (engl. *antibiotic growth promoters* – AGPs) kako bi se poboljšala crevna mikroflora u ishrani živine. Jedna od alternativa za antiobiotske stimulatore porasta (AGPs) jesu probiotici, koji predstavljaju korisne organizme. Nusproizvodi varenja polisahirida za koje živina ne poseduje enzime za varenje nazivaju se prebiotici. Prebiotici služe kao hrana za probiotike, pri čemu primena enzima omogućava njihovo iskorišćavanje. Prebiotski potencijali hraniva sa visokim sadržajem vlakana (engl. *high fibre feedstuffs* – HFFs) sa dodatkom enzima nisu poznati. Ovo istraživanje je sprovedeno kako bi se ocenio prebiotski potencijal pšeničnog stočnog brašna sa dodatkom enzima ksilanaze na brojlerske piliće (*in vivo*). Zamena kukuruza, pšeničnim stočnim brašnom sa dodatkom enzima ksilanaze od 100ppm, nezavisno od učešća, dovela je do smanjenja konzumiranja hrane, povećanja prirasta i bolje konverzije hrane (engl. *feed conversion ratio* – FCR). Brojleri hranjeni smešom sa 20% pšeničnog stočnog brašna sa dodatkom enzima ksilanaze, odlikovali su se boljim performansama u odnosu na brojlere koji su konzumirali smešu sa 10 odnosno 30% pšeničnog stočnog brašna uz dodatak enzima ksilanaze, kao i u odnosu na kontrolnu grupu. Pilići su imali normalnu težinu vitalnih organa sa dobrom strukturalnom konzistencijom. Identifikacija mikroorganizama (gljivica i bakterija) pokazala je da sadržaj u obroku pšeničnog stočnog brašna (10, 20 ili 30%) sa dodatkom enzima ksilanaze povećava rast korisnih mikroba, što je uticalo na inhibiciju ili eliminaciju oportunističkih/patogenih mikroorganizama. Zaključeno je da dodavanje enzima hranivima sa visokim sadržajem vlakana može poboljšati performanse porasta, iskorišćavanje hranljivih materija i povećati koncentraciju korisnih mikroorganizama u digestivnom traktu. Upotreba enzima se stoga preporučuje kada su hraniva sa visokih sadržajem vlakana potrebna, kao izvor prebiotika u digestivnom traktu brojlera.

Ključne reči: prebiotici, enzimi, crevna mikroflora, ishrana brojlera, svarljivost.

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EXTRACTION OF RED GRAPE POMACE ANTIOXIDANTS WITH AQUEOUS ORGANIC ACID SOLUTIONS USING KINETIC MODELLING

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Abstract: Grape pomace is a food industry residual material which may contain a high load of polyphenolic antioxidants and a number of methods have been implemented for their effective recovery. Nevertheless, eco-friendly processes should embrace environmentally benign and non-toxic solvents. On this basis, this study investigated the extraction of antioxidants, by employing aqueous acetic acid and citric solutions and monitoring the reducing power (P_R) of the extracts. The scope was to evaluate the effect of acid concentration on the extraction yield using kinetics. The kinetic model established allowed for the credible comparison of the extraction efficiencies, achieved with acetic acid and citric acid solutions of variable concentration. The results suggested that citric acid solutions were more effective in recovering red grape pomace antioxidants. Using a 4% (w/v) citric acid solution, a maximum P_R of 229.8 μM ascorbic acid equivalents per g of dry pomace could be attained. This investigation demonstrated that aqueous media used for the extraction of antioxidant compounds from food industry wastes could be significantly influenced by the acidifying agent and its concentration.

Key words: antioxidants, extraction, grape pomace, kinetics, organic acids, reducing power.

Introduction

The agri-food sector is constantly under legislative pressure to reuse and/or recycle waste biomass, in an effort to minimise the associated environmental risks (Devesa-Rey et al., 2011). Industrial scale vinification produces inevitably the large volume of residues, composed of organic biomolecules (stems, seeds, skins, etc.). It is estimated that 14.5 million tonnes of grape processing residues are produced on annual basis from the viti-viniculture sector only in Europe (Pinelo et al., 2006). This waste has attracted significant attention, because of its important content in polyphenols, which may have beneficial biological properties (Yu and Ahmedna, 2013).

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The examinations on the effective polyphenol extraction from vinification wastes have focused mainly on red grape pomace (RGP), which is characterised by a significant content of polyphenols with high antioxidant potency (Makris et al., 2007). The antioxidant recovery of wine industry residues and their valorisation as food additives, cosmetic ingredients and dietary supplements are a high-value prospect (Galanakis, 2012). Numerous methodologies have been deployed to develop strategies for polyphenol extraction from RGP, with particular emphasis being given to the use of eco-friendly means, such as water/ethanol solutions (Boussetta et al., 2012; Bucić-Kojić et al., 2007; Carrera et al., 2012; Makris et al., 2008). In the examinations pertaining to extraction optimisation, the focus has been on the role of pH, a factor that may impact the extraction yield to a significant degree (Makris et al., 2008), but also it may act as a selectivity parameter, e.g. for flavanol extraction (Karvela et al., 2009a, b). The regulation of pH by the addition of acidifying agents in the extraction medium could have a profound effect on the overall efficiency of the extraction process.

Citric acid has been tested for the extraction of onion peel polyphenols (Kiassos et al., 2009; Makris, 2010) and olive leaf polyphenols (Mylonaki et al., 2008). Nonetheless, other natural organic acids have never been used for such a purpose. Both citric and acetic acids are natural organic acids occurring in foods and a more thorough study on the use of these acids in extraction processes could be of value to the cost-effective and sustainable exploitation of residues from the agri-food sector. On such grounds, this study was carried out for assessing the effect of citric and acetic acids on the efficiency with regard to the extraction of antioxidants from RGP. The extraction process was monitored using a ferric-reducing power test, which has been effectively used in previous studies on the recovery of antioxidants (Garcia-Perez et al., 2010), and the kinetic modelling was accomplished including extraction time and acid concentration as the critical variables.

Materials and Methods

Chemicals

Ascorbic acid (AA), 2,2′-dipyridyl and ferric chloride hexahydrate were obtained from Sigma Chemical Co (St. Louis, MO, U.S.A.). Trichloroacetic acid (TCA), citric acid and acetic acid were provided from Merck (Darmstad, Germany).

Waste material

RGP was obtained from the native Greek Agiorgitiko variety (*Vitis vinifera* spp.), provided by the Department of Food Science & Human Nutrition

(Agricultural University of Athens). RGP was dried in an oven at 65 °C for 48 h and then pulverized in a domestic blender (Bosch MMB 112R). The material was stored at – 20 °C until used.

Batch extraction process

The amount of 2.4 g of pulverised RGP was transferred in a 250-mL glass vial and mixed with 120 mL of acid (citric, acetic) solution (1, 2 or 4% w/v). Extractions were performed under stirring at 80 rpm in a thermostated chamber, at 20°C. Sampling was accomplished by acquiring the volume of 1 mL of extract at regular intervals, up to 320 min.

Determination of the reducing power (P_R)

Prior to analysis, all samples were centrifuged in an Eppendorf centrifugator at 10,000×g for 10 min. Measurement of the P_R was carried out using ferric chloride as the oxidant (Psarra et al., 2002). An aliquot of 0.05 mL of extract was mixed with 0.05 mL of ferric chloride (3 mM in 5 mM citric acid) and the mixture was incubated at 50 °C, in a water bath, for 30 min. Then 0.9 mL of 2,2′-dipyridyl solution (5 g L^{-1} in 1.2% TCA) was added and the absorbance was measured at 525 nm. Quantification was performed by means of a calibration curve, using ascorbic acid as standard. The P_R was expressed as μM AA equivalents (AAE) per g of dry pomace weight (dpw), as follows:

$$P_R\ (\mu M\ AAE\ g^{-1}\ dpw) = \frac{[0.932 \times A_{515} - 0.008] \times V}{m} \quad (1)$$

where V is the extraction medium volume (mL) and m is the dry weight of grape pomace (g).

Statistics

Determinations were performed in triplicate. Values reported are means ± standard deviation. Non-linear regression correlations were performed at least at the 95% significance level ($p < 0.05$). Statistical analyses were carried out using SigmaPlot™ 12.0 and Microsoft Excel™ 2010.

Results and Discussion

Kinetics

Monitoring of the P_R within a period up to 320 min gave a series of P_R/t points (Figure 1) and non-linear regression between P_R values and t gave the best fit of a hyperbola described by the equation:

$$y = \frac{ax}{1+bx} \quad (2)$$

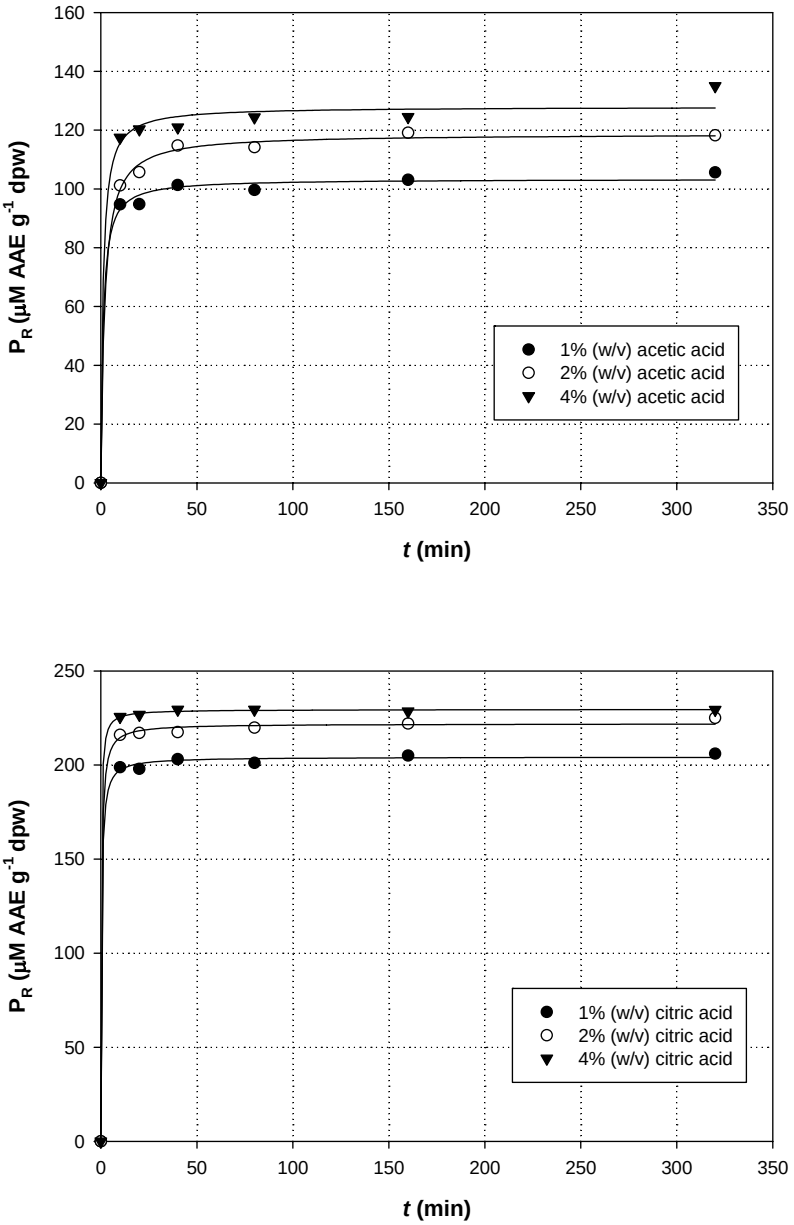

Figure 1. Time course of P_R during antioxidant extraction from RGP using acetic acid (upper plot) and citric acid (lower plot) solutions. Extractions were performed at 20 °C, 80 rpm and 1:50 solid-to-liquid ratio.

Model fitting was significantly high (Table 1), suggesting that prediction of the extraction yield as a function of *t* can be performed using the equation (2). This corresponds to the 2nd-order extraction model, in line with previous findings (Ho et al., 2005; Rakotondramasy-Rabesiaka et al., 2007), considering the boundary conditions $t = 0$ to t and $P_{R(t)} = 0$ to $P_{R(t)}$. The outcome indicated that there were two phases involved in the antioxidant extraction, an initial washing stage and a slow, rate-determining stage, governed by internal diffusion. These assumptions were made by admitting that (i) leaching of antioxidants occurred through diffusion, and (ii) at the equilibrium (saturation), the P_R remained constant. Thus, extraction kinetics could be described as follows:

$$P_{R(t)} = \frac{P_{R(s)}^2 kt}{1 + P_{R(s)} kt} \tag{3}$$

Table 1. Statistical parameters determined after correlating P_R and *t*, using non-linear regression.

	Statistical parameters	
C_{acid} (% w/v)	R^2	*p*
Acetic acid		
1	0.997	< 0.0001
2	0.998	< 0.0001
4	0.994	< 0.0001
Citric acid		
1	0.999	< 0.0001
2	0.999	< 0.0001
4	1.000	< 0.0001

$P_{R(s)}$ and *k* correspond to the P_R at saturation and the extraction rate constant. Modification of the equation (3) provides its linearized form:

$$\frac{t}{P_{R(t)}} = \frac{1}{kP_{R(s)}^2} + \frac{t}{P_{R(s)}} \tag{4}$$

When *t* approaches 0, the initial extraction rate, *h*, can be determined as:

$$h = kP_{R(s)}^2 \tag{5}$$

Plotting $t/P_{R(t)}$ as a function of *t* gives a straight line in the form of $y = ax + b$ is (Figure 2), where $a = 1/P_{R(s)}$ and $b = 1/h$. Thus, in every case, $P_{R(s)}$, *k* and *h* could be determined graphically. The correlation between $t/P_{R(t)}$ and *t* ($R^2 > 0.99$, $p < 0.0001$) enabled the estimation of the representative kinetic parameters (Table 2).

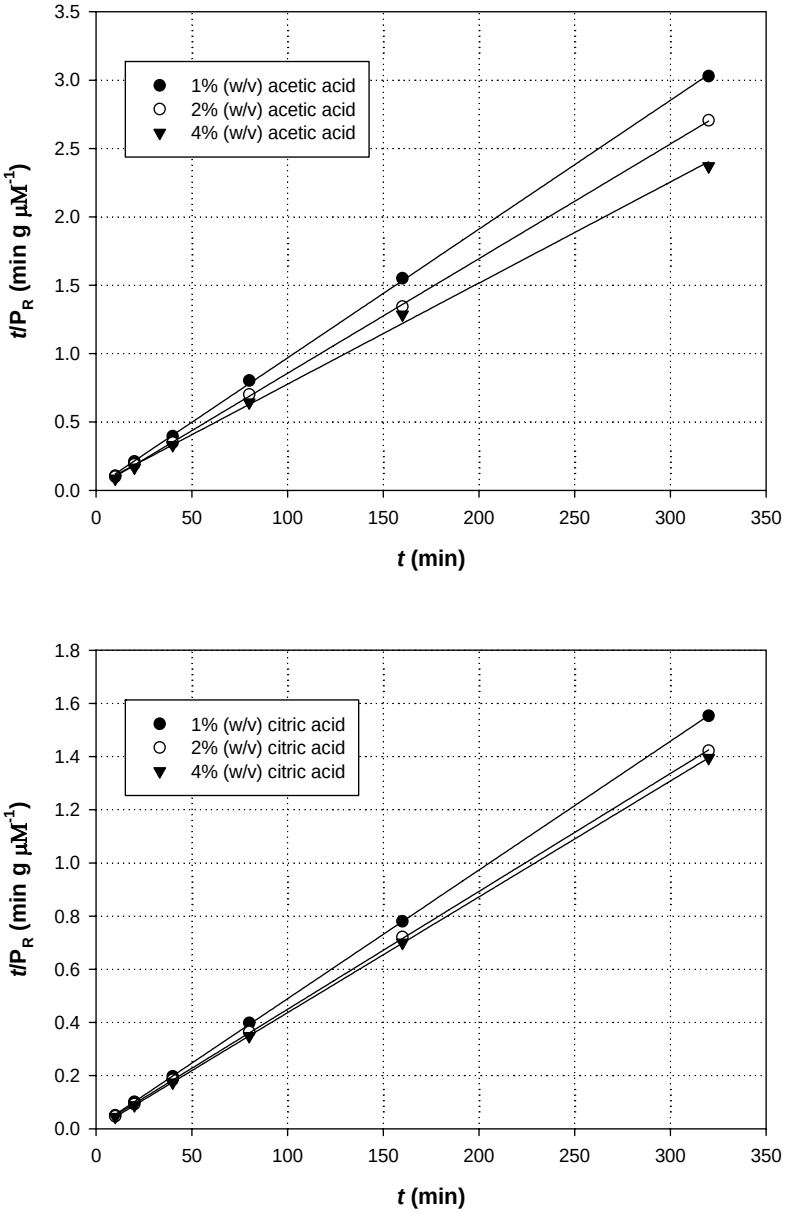

Figure 2. The second-order kinetics of antioxidant extraction from RGP with aqueous acetic acid (upper plot) and citric acid (lower plot) solutions. Extractions were performed at 20 °C, 80 rpm and 1:50 solid-to-liquid ratio.

The effect of acid concentration

The increased acetic acid concentration yielded decreased *k* values (Table 2), but $P_{R(s)}$ values exhibited an increasing tendency in response to C_{acid} and the highest $P_{R(s)}$ (128 μM AAE g^{-1} dpw) was achieved with 4% (w/v) acetic acid. Irrespective of the t/C_{acid} combinations used, *h* and $P_{R(s)}$ values were constantly higher in citric acid solutions, but *k* values were in all cases lower. The extract with the highest $P_{R(s)}$ value (229.8 μM AAE g^{-1} dpw) was obtained using 4% (w/v) citric acid.

Table 2. Parameters of the 2nd-order kinetics for the extractions performed with acetic acid and citric acid solutions.

	Kinetic parameters		
C_{acid} (% w/v)	*k* (g μM^{-1} min^{-1}) × 10^{-3}	*h* (μM g^{-1} min^{-1})	$P_{R(s)}$ (μM AAE g^{-1} dpw)
Acetic acid			
1	6.98	92.6	103.5
2	2.60	62.6	118.8
4	1.53	119.2	128.0
Citric acid			
1	1.90	579.0	204.3
2	2.28	647.5	222.0
4	3.06	1272.9	229.8

For the extractions carried out using acetic acid solutions, non-linear regression between $P_{R(s)}$ and C_{acid} obeyed a single rectangular hyperbola correlation, as follows:

$$P_{R(s)} = -3.57C_{acid}^2 + 26C_{acid} + 81.07 \qquad (R^2 = 1.000,\ p < 0.0001) \qquad (6)$$

Similarly, a correlation between *h* and C_{acid} obeyed a quadratic function:

$$h = 19.43C_{acid}^2 - 88.3C_{acid} + 161.47 \qquad (R^2 = 1.000,\ p < 0.0001) \qquad (7)$$

In a similar manner, the equations obtained for the extraction with citric acid solutions were:

$$P_{R(s)} = -4.6C_{acid}^2 + 31.5\ C_{acid} + 177.4 \qquad (R^2 = 1.000,\ p < 0.0001) \qquad (8)$$

$$h = 81.4C_{acid}^2 - 175.7C_{acid} + 673.3 \qquad (R^2 = 1.000,\ p < 0.0001) \qquad (9)$$

Rearrangement of the equation (3) would enable calculation of Y_{TF} at any time, *t*:

$$P_{R(t)} = \frac{t}{\frac{1}{h} + \frac{t}{P_{R(s)}}} \qquad (10)$$

For the extraction with acetic acid solutions, combination of equations (6), (7) and (10), would give:

$$P_R(t, C_{acid}) = \frac{t}{\frac{1}{19.42C_{acid}^2 - 88.26C_{acid} + 161.47} + \frac{t}{-2.87C_{acid}^2 + 26C_{acid} + 81.07}} \quad (11)$$

Likewise, the combination equations (8), (9) and (10) would give the corresponding function for citric acid solutions:

$$P_R(t, C_{acid}) = \frac{t}{\frac{1}{81.41C_{acid}^2 - 170.81C_{acid} + 178.2} + \frac{t}{-4.81C_{acid}^2 + 21.01C_{acid} + 71.74}} \quad (12)$$

These equations describe an empirical model for antioxidant extraction from RGP with aqueous acetic and citric acids and provide the values for P_R at any time *t* and any concentration C_{acid} within the limits set by the experimental design.

Model validation

Several combinations of C_{acid} and *t* were used to assure model validity (Table 3) and for this purpose linear regression was performed between the observed and the predicted values to ascertain the degree of correlation (Figure 3).

Table 3. Predicted and observed P_R values obtained using the extraction models.

Run	Time (min)	C_{acid} (% w/v)	P_R (μM AAE g^{-1} dpw)			
			Acetic acid		*Citric acid*	
			Observed	Predicted	Observed	Predicted
1	10	1	83.7	93.1	198.8	197.3
2	160	1	103.1	102.8	205.0	203.9
3	320	1	105.6	103.1	206.0	204.1
4	10	2	101.2	99.8	216.0	214.6
5	160	2	119.1	117.4	222.0	221.5
6	320	2	118.2	118.1	225.0	221.8
7	10	4	117.5	115.5	225.7	225.7
8	160	4	124.5	127.1	228.6	229.5
9	320	4	135.0	127.5	229.4	229.7

The values showed a high correlation, suggesting that, within the limits set by the experimental design, a credible prediction of P_R as a function of C_{acid} and *t* can be done using the equations (11) and (12). The trends in P_R recorded were given as 3D plots (Figure 4).

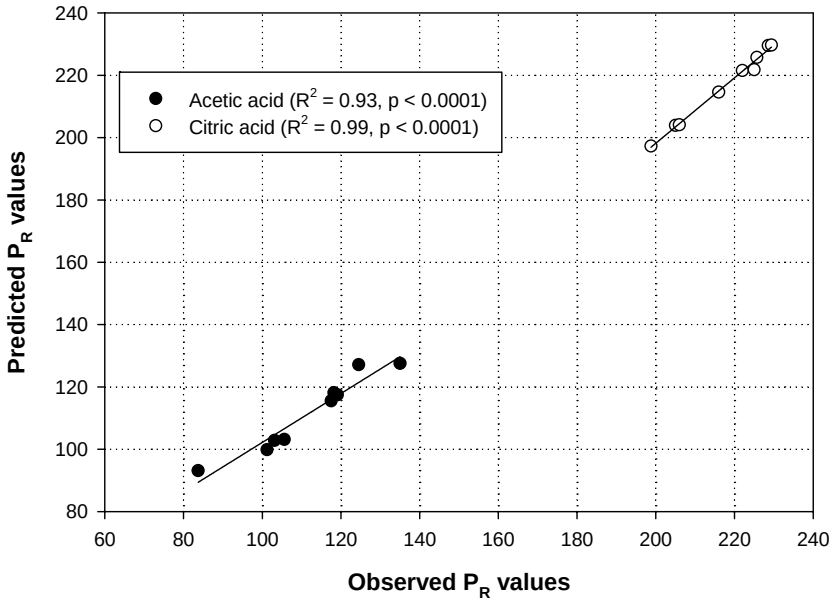

Figure 3. Linear regression between observed and predicted P_R values.

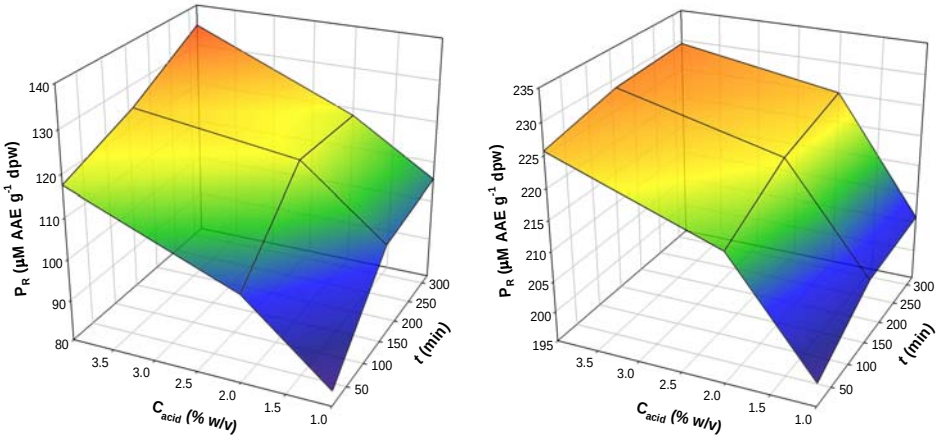

Figure 4. 3D graphs illustrating the effect of C_{acid} and *t* on P_R, using acetic acid solutions (upper plot) and citric acid solutions (lower plot).

Conclusion

Antioxidant extraction from RGP with acetic acid and citric acid solutions was shown to depend on both the acids and their concentration. Extractions were described by the determination of basic kinetic parameters, which permitted the establishment of a reliable model. This model enabled the reliable prediction of the P_R of the extracts as a function of both *t* and $C_{acid,}$ under the experimental conditions employed. This investigation illustrated that aqueous solvents used for antioxidant extraction from RGP can be profoundly affected by the acidifying agent. This is of particular importance for the development of the efficient extraction process. Similar examinations might be used for engineering extraction procedures involving eco-friendly and food compatible solvent systems.

Nomenclature: AA, ascorbic acid; AAE, ascorbic equivalents; Cacid, acid concentration (% w/v); dpm, dry pomace weight; *h*, initial extraction rate (μM g-1 min-1); *k*, extraction rate constant (min g μM-1); PR, reducing power (μM AAE g-1 dpw); *t*, time (min); PR(s), reducing power at saturation (μM AAE g-1 dpw); PR(Cacid,*t*), reducing power as a function of Cacid and *t;* TCA, trichloroacetic acid.

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EKSTRAKCIJA ANTIOKSIDANASA IZ KOMINE CRVENOG GROŽĐA VODENIM RASTVORIMA ORGANSKIH KISELINA KORIŠĆENJEM KINETIČKOG MODELOVANJA

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R e z i m e

Komina grožđa je rezidualni materijal prehrambene industrije koji može sadržati veliku količinu polifenolnih antioksidanasa i poznate su brojne metode za njihovo efikasno dobijanje iz navedenog materijala. Međutim, ekološki procesi bi trebalo da obuhvate i ekološki neškodljive i netokstične rastvarače. Na osnovu toga, u ovom istraživanju ispitana je ekstrakcija antioksidanasa, upotrebom vodenih rastvora sirćetne i limunske kiseline, kao i praćenje redukcione moći (engl. *reducing power* – PR) ekstrakta. Cilj je bio da se pomoću kinetike ispita uticaj koncentracije kiseline na prinos ekstrakcije. Uspostavljeni kinetički model omogućio je verodostojno poređenje efikasnosti ekstrakcije, postignute korišćenjem rastvora sirćetne i limunske kiseline različitih koncentracija. Rezultati ukazuju da su rastvori limunske kiseline efikasniji u dobijanju antioksidanasa iz komine crvenog grožđa. Korišćenjem 4% (w/v) rastvora limunske kiseline, mogla bi se postići maksimalna redukciona moć, PR, od 229,8 μM ekvivalenata askorbinske kiseline po g suve komine. Ovo istraživanje je pokazalo da sredstvo za zakišeljavanje i njegova koncentracija mogu značajno uticati na vodenu sredinu koja se koristi za ekstrakciju jedinjenja sa antioksidativnim osobinama iz otpada u prehrambenoj industriji.

Ključne reči: antioksidanti, ekstrakcija, komina grožđa, kinetika, organske kiseline, redukciona moć.

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QUANTITATIVE RELATIONSHIPS BETWEEN INVESTMENTS IN FARMS AND ENVIRONMENTAL EFFECTS IN SOME EUROPEAN COUNTRIES

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Abstract: Since the early 1970s, in many European Union member countries, there has been a significant improvement in their own agricultural economic growth due to the Common Agricultural Policy. The increasing levels of investment in land and in machinery have been one of the pivotal pillars in improvement levels of food self-sufficiency. However, a growth in investments has increased pollutant emission in terms of carbon dioxide, nitrogenous compounds and a deterioration of an environment. The purpose of this paper was to investigate the impact of investments on agricultural and arable land in improving agricultural production towards environmental quality. The quantitative methods have used a neural relation analysis through the Kohonen's maps or self-organizing maps (SOMs). Findings of Kohonen's maps have pointed out a good ability to classify the time series data and the spatial evolution of data over the time; furthermore, SOMs have been useful in describing the impact of investment on production specialization, levels of pollution and food self-sufficiency in the European countries. To sum up, an improvement of food self-sufficient levels has been tightly linked to an increase of pollution in terms of carbon dioxide emissions.

Key words: Kohonen's maps, Common Agricultural Policy, rural development, investments, productive specialization, multifunctionality.

Introduction

In the European Union, from 1955 to 1990, there was a significant implementation of economic conditions in the countryside due to a growth of levels of food self-sufficiency with positive consequences on the farmers' income through the Common Agricultural Policy (CAP) by a direct price support of ag-commodities and through a protection of the domestic market by customs barriers (Vieri, 1994). In fact, the first and foremost purpose of the CAP was to ensure a decent level of income in the countryside by an allocation of specific actions able to protect the domestic European market, which in the nineties was one of the

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predominant reasons of a radical structural change in the Common Agricultural Policy towards a post-productivist model of production (Shucksmith, 1993; Marsden 1995; Ilbery, 1998). It is impossible tofail to mention that the CAP has been one of the key pillars for getting better living conditions in the primary sector by ensuring an adequate socio-economic welfare in the countryside as well (Galluzzo, 2013a; Galluzzo 2013b) with a meaningful positive change in relationships among agriculture and environment throughout the multifunctionality (Van der Ploeg et al., 2002; Van der Ploeg, 2006, 2009).

Focusing the attention on the enlargement of the European Union in 2004 and in 2007 and on the changes imposed by the international agreements such as GATT and WTO, there has been a cut-off in direct payments to farmers, or rather there has been a radical political turning point in the first pillar of the Common Agricultural Policy. The direct consequence has been the development of a new agricultural common policy aimed at supporting diversified measures in order to promote a holistic rural development by multifunctionality and by a diversification of activities in the countryside through a surge of pluriactivity farms financed by the second pillar of the CAP (Vieri, 1994, 2001, 2012; Belletti, 2003; Henke, 2004; Cooper et al., 2009; Hassink et al., 2013; Dufour et al., 2007; Galluzzo, 2015).

The improvement in the levels of food self-sufficiency has implied the levels of technical efficiency in terms of investments in agrarian land, in zootechnic productions and in machinery used in agriculture which, however, have increased the emission of pollutants into the atmosphere such as carbon dioxide, nitrogenous compounds, etc. (Nguyen et al., 2010, Olesen and Bindi, 2002). Multifunctionality has been a direct consequence of a new vision of the European Union agriculture in protecting the environment by a production of positive externalities in particular in the upland rural areas at risk of socio-economic marginalization as a consequence of the liberalization processes (Dibden et al., 2009). Excessive specialization and intensification of agricultural production have had some effects on ecological, environmental, economic-financial management and governance in all European Union member states (Vieri, 2001; Cunha and Swinbank, 2011) in order to render the environment more sustainable in the countryside. All this has led many European citizens to a greater awareness towards the second pillar of the Common Agricultural Policy towards the rural development by awarding and recognition of the role of agriculture as a public good with effects on territorial specialization, landscape and ability to produce positive externalities (Belletti, 2003; Henke, 2004; Cooper et al., 2009). Agriculture is given a multifunctional role in terms of protecting the environment and promoting the development of rural cohesion and protection of rural territories through multifunctionality (Hassink et al., 2013; Dufour et al., 2007).

The purpose of this research was to evaluate, through the use of a quantitative method based on the self-organizing maps (SOMs), the impact of investments in land capital and machinery with the goal to improve agricultural production of commodities and the level of food self-sufficiency in the European Union countries from 1990 to 2012, and the environmental quality in terms of effects on agricultural and arable land. In order to assess these relationships, the FAO statistics database published on its website has been used.

Materials and Methods

The methodology has employed a quantitative approach as the self-organizing maps (SOMs) proposed by Kohonen (Kohonen, 1995), using the open source software Spice-Som version 2.1 for the estimation of the parameters. The main goal of the paper was aimed at assessing if there was a unique winner neuron (black hexagons in the SOMs) in terms in the European countries able to produce the highest level of carbon dioxide emission with a nexus to the highest levels of investments and agrarian production.

In general, self-organizing maps are particularly useful to estimate, in time series, the structure and the evolution of several variables, such as poverty, lifestyle, health situation, development and welfare features in different countries in order to obtain a unique parameter and a visualization of different clusters of states or rather groups of hexagons with lots of analogies to the principal component analysis (Kasky and Kohonen, 1996; Mehmood et al., 2011). The self-organizing map (SOM) is a particular quantitative model of an artificial neural network able to produce a low two-dimensional representation of inputs in some maps (Kohonen, 1995). Self-organizing maps are different from other artificial neural networks because they use a neighbourhood function in order to preserve the topological properties of the input space considered in the model of analysis (Meraviglia, 2001).

The self-organizing maps are useful to visualize low dimensional views of high dimensional data and they consist of components called nodes or neurons. Each node is a weight vector of the same dimension as the input data vectors and also as a specific position in the map space. The usual arrangement of nodes is a two-dimensional regular spacing made by a hexagonal grid or rectangular box. The self-organizing map describes a mapping from a higher dimensional input space to a lower dimensional map space. The procedure for placing a vector in the map is to find out a node located nearest to the winner neuron considering that each vector in the map is linked with every neuron in the used dataset (Kohonen, 1995). The network in the SOM is characterized by a pattern in two different layers, one layer is made up by input and the other layer, commonly called the Kohonen's layer, is constituted by output (Kohonen, 2001). The neurons of the two layers are

completely connected to each other, while neurons of the output layer are linked to different output neurons (Kohonen, 1984).

The purpose of the learning process in the SOM is to define different parts in the network made by the SOM able to match with patterns of different inputs. The weights of the neurons are initialized either to small random numbers or values sampled from a subspace crossed by the two larger eigenvalues able to increase the training because initial weights are a good approximation of weights in the SOM (Kohonen, 1995). When a training sample input is put in the network, its Euclidean distance is calculated from all vectors of weights. The neuron with the weight vector most similar to the input is called the best matching unit (BMU). Weights of the BMU and neurons close to it in the SOM lattice made by hexagons or component planes are closer to the input vector and the intensity of the approach decreases over time and in function of the distance of the neurons by the BMU (Kohonen, 1984).

The formula used for updating the weights of a neuron is Wv (Lucchini, 2007):

$$Wv(t+1) = Wv(t) + \Theta(v, t)\,\alpha(t)\,[D(t) - Wv(t)] \quad (1)$$

where $\alpha(t)$ is a monotone decreasing learning coefficient and D(t) is the input vector. The function that defines the neighborhood $\Theta(v, t)$ depends on the distance in the lattice between the BMU and the neuron v. In a simplified form of the competitive network, the value is equal to 1 for all neurons close to the BMU and 0 for others, even if the most common choice is similar to a Gaussian function; hence, the winner neuron is in a central position and the losers are distant. In general, regardless of the kind of used function, the neighborhood function decreases over time; initially, when the neighborhood is broad, the self-organizing map takes place on a global scale and when the neighborhood is reduced to only a few neurons, weights converge to a local estimate called a tuning phase. This process is repeated for each input vector and it is also reiterated for a variable large number of cycles.

Results and Discussion

Findings in some analyzed variables such as arable land and permanent crops over time of investigation have pointed out on average a significant and sharp growth of arable land and by contrast a steady development of permanent crops lower than 5 million of hectares (Figure 1). Considering different European nations, the rural population on average seems to fluctuate significantly in all countries even if the highest diffusion of rural population has affected Germany, Italy, the United Kingdom and France where scattered small villages marked rural territories (Figure 2).

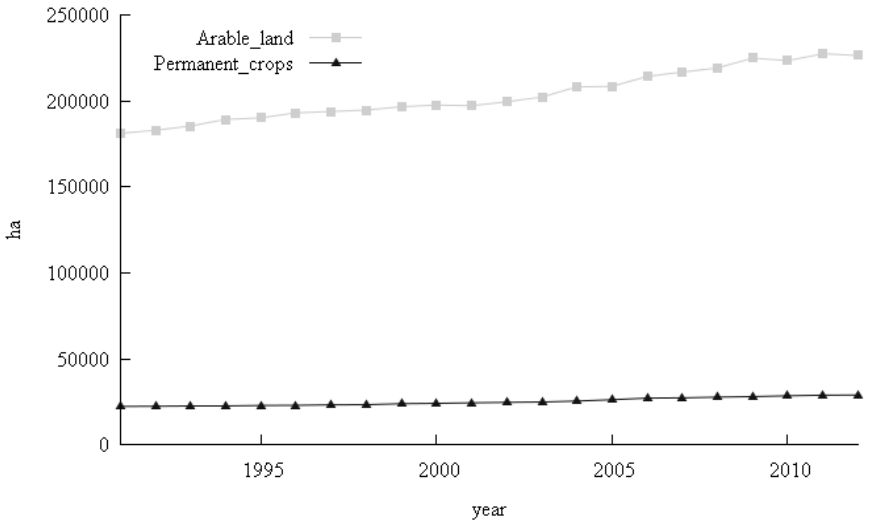


Figure 1. Evolution of arable land and permanent crops in thousands of hectares in all European countries.
Source: Author's elaboration on data http://faostat3.fao.org/faostat-gateway/go/to/home/E with the software GRETL 1.9.

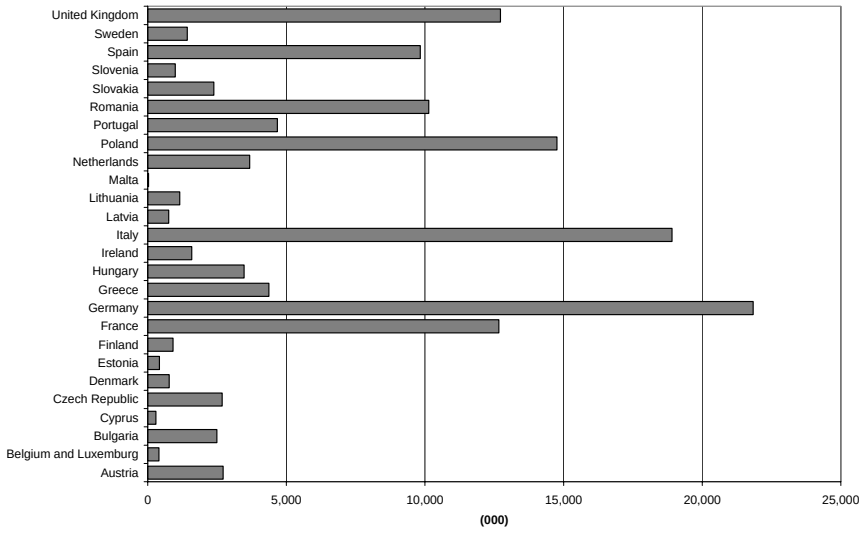


Figure 2. Average of rural population in investigated European countries from 1991 to 2012.
Source: Author's elaboration on data http://faostat3.fao.org/faostat-gateway/go/to/home/E.

In more than 20 years of investigation, the average value in the time series of protein and energy consumed every day by European citizens has highlighted a significant and consistent level of ingestion of food even if findings in some newcomer states of the European Union, such as Bulgaria, Latvia and Slovakia, have pointed out the lowest level of protein per day (Figure 3).

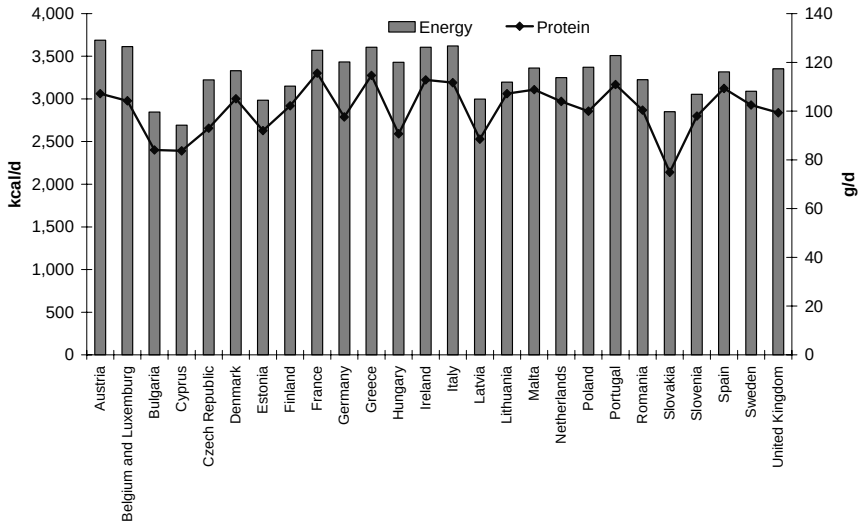


Figure 3. Daily average levels of protein and energy in European countries from 1991 to 2012.

Source: Author's elaboration on data http://faostat3.fao.org/faostat-gateway/go/to/home/E.

The rural population and also the urban population have been positively influenced by the level of intake protein (Table 1). In general, a growth of arable land has implemented the level of energy and protein consumed by the European citizens even if an increase of this latter has affected the use of fertilizers and CO_2 emissions. Investments in machinery and in land capital have acted directly to the rising level of protein per day and they did not act on the level of consumption of food in terms of energy.

Focusing the attention on all analyzed variables, such as protein and energy per day, arable land, agricultural areas, rural and urban population, investments in land capital and machinery, CO_2 emissions, the Kohonen's map has pointed out a meaningful level of horizontal variability with low level of interactions, as shown in white hexagons, in Italy and in some newcomer member states of the European Union such as Slovakia and Lithuania (Figure 4). Malta, Poland and Germany have been three countries characterized by the highest level of investigated variables such as arable land, level of investments, rural and urban population and food ingestions.

Table 1. Correlation among different investigated variables over time in all European countries.

	Arable land	CO_2 emission	Energy (kcal/day)	Forest	Land capital investments	Machinery investments	Meadows	Protein per day	Rural population	Urban population
Arable land	1.00	0.8820	0.3109*	0.4810	0.8092	0.8142	0.7810	0.2686*	0.8132	0.8040
CO_2 emission	0.8820	1.00	0.4810	0.3885	0.5291	0.8617	0.8096	0.3145*	0.8126	0.9275
Energy (kcal/day)	0.3109*	0.3919	1.00	0.0263*	0.2479*	0.3892	0.3772*	0.8004	0.3893	0.3848
Forest	0.4810	0.3885	0.0263*	1.00	0.3239*	0.4218	0.2934*	0.2257*	0.3008*	0.3535*
Land capital investments	0.8092	0.5291	0.2479*	0.3239*	1.00	0.4874	0.6542	0.2656*	0.6641	0.5156
Machinery investments	0.8142	0.8617	0.3892	0.4218	0.4874	1.00	0.5940	0.2663*	0.9182	0.8936
Meadows	0.7810	0.8096	0.3772*	0.2934*	0.6542	0.5940	1.00	0.3657*	0.6931	0.7965
Protein per day	0.2686*	0.3145*	0.8004	0.2257*	0.2656*	0.2663*	0.3657*	1.00	0.2339*	0.2588*
Rural population	0.8132	0.8126	0.3893	0.3008*	0.6641	0.9182	0.6931	0.2339*	1.00	0.9159
Urban population	0.8040	0.9275	0.3848	0.3535*	0.5156	0.8936	0.7965	0.2588*	0.9159	1.00

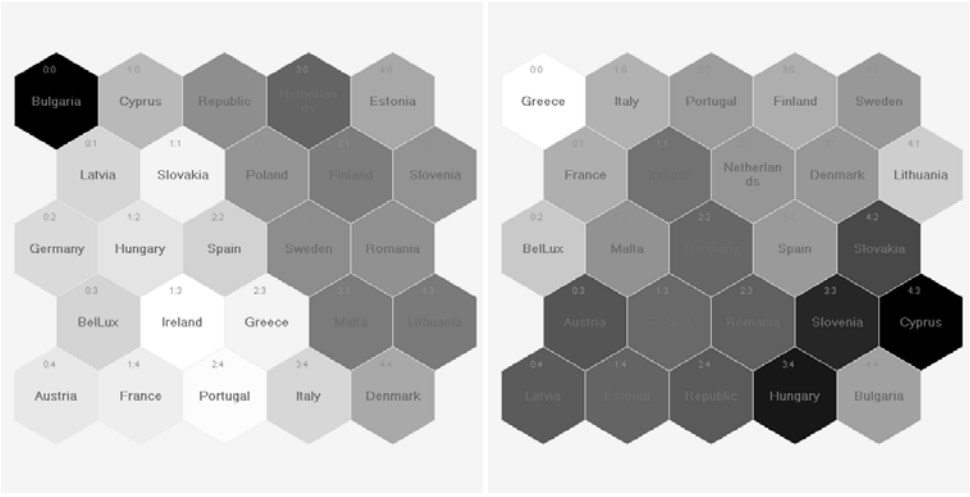
* denotes significance at 5%.

Source: Author's elaboration on data http://faostat3.fao.org/faostat-gateway/go/to/home/Ewith the software GRETL 1.9.

Figure 4. Component planes of all analysed variables.

Source: Author's elaboration on data http://faostat3.fao.org/faostat-gateway/go/to/home/E.

The Kohonen's maps comparing the level of intake protein and energy have pointed out that Bulgaria and newcomers of the European Union such as Cyprus, Hungary and Slovenia have been characterized by black hexagons, which have implied the highest levels of these variables (Figure 5). Component planes of nutrition levels, in terms of kilocalories and protein per day, which are a useful tool to understand over time the level of food self-sufficiency as a result of improving in the level of wellbeing and personal income, have showed a fairly homogeneous situation in all different European countries with the exception of some nations located in the European peripheral areas. In general, the level of intake protein per day has not underscored a great diversity among member countries of the EU. The heterogeneity in nodes, due to a greater allocation of agricultural areas, has concerned northern European countries such as France and Ireland, where there are extensive farming systems and large-sized farms.



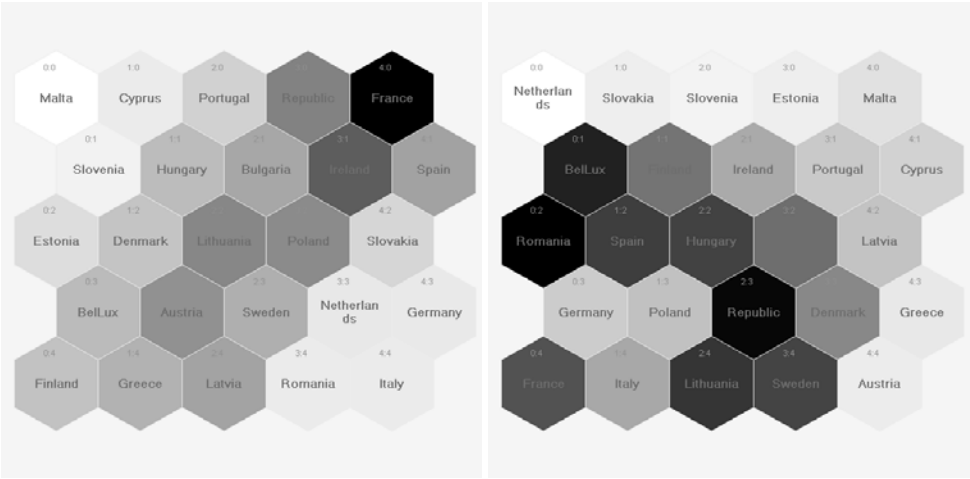
(A) (B)

Figure 5. Component planes of some inputs such as kilocaloriesper day (A) and level of protein per day (B).

Source: Author's elaboration on data http://faostat3.fao.org/faostat-gateway/go/to/home/E.

Findings in component planes of the variables such as agricultural areas and arable land have highlighted that there has been the highest level of this latter variable in France (black hexagon); newcomer states of the EU such as Romania, Lithuania, Belgium and Luxemburg have also pointed out the highest level of arable land. In contrast, Italy, Germany and Malta, on one hand, and the Netherlands, Austria, Greece and Slovenia (white and greyish scale), on the other hand, have shown the lowest levels of arable land (Figure 6). The analysis of the component planes for the arable land variable has pointed out a higher homogeneity in nodes among all EU countries, even if newcomer states have

demonstrated a greater endowment of arable land compared to small countries of the Mediterranean basin (Cyprus and Malta) or in other nations, where the orography does not allow cultivating of wide agrarian areas. Summing up, different component planes of self-organizing maps have highlighted a different intensity of connections. The analysis revealed a considerable horizontal variability in the agricultural areas, focusing the attention on the case of the arable land in all member states of the European Union.



(A) (B)

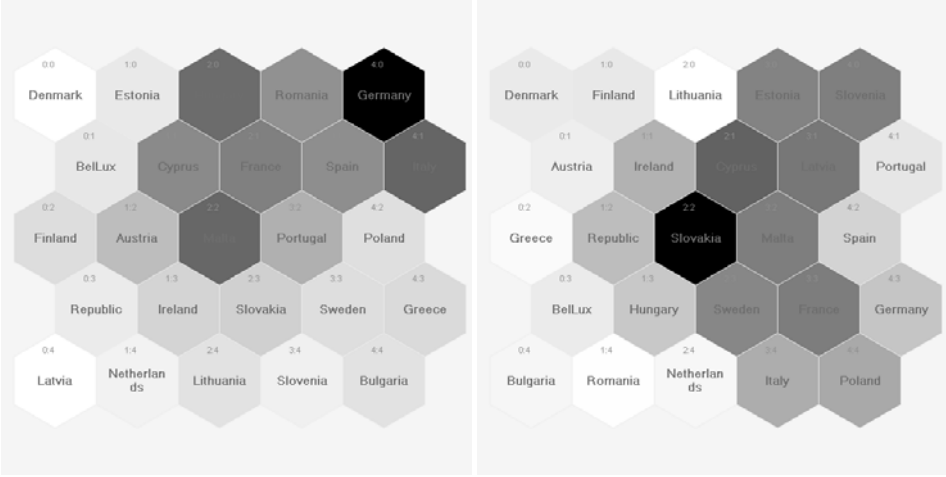
Figure 6. Component planes of some inputs such as agricultural areas in hectares (A) and arable land (B).

Source: Author's elaboration on data http://faostat3.fao.org/faostat-gateway/go/to/home/E.

It is interesting that the analysis of component planes refers to the variables such as the rural population and the urban population as well, concentrated predominately in Germany (Figure 7); the SOMs have showed that in countries, with higher agricultural areas with scattered rural villages, there is a high concentration of citizens unlike Slovakia and other countries, which have been members of the European Union since 2004, and where the population is predominantly concentrated in few urban areas.

Findings in SOMs investigating the level of investments in machinery and in land capital have pointed out that Latvia has invested more than Slovakia and Finland in machinery (Figure 8); in contrast, Denmark and France (black hexagons) have invested more in agrarian capital than newcomer states of the EU such as Bulgaria and Cyprus. Outcomes in European countries have pointed out that the higher is the rate of agricultural employment the higher is the level of agricultural population living in rural spaces. Addressing the research on the variable level of investments in countries that became members of the EU during the enlargement in

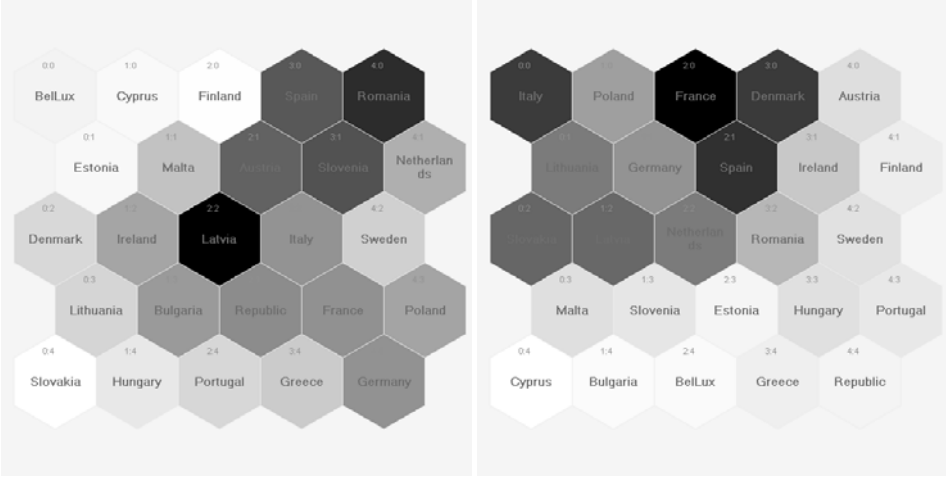
2004, findings have underlined that the most important financial resources have been used on investments in intensifying the endowment in agricultural machinery and equipment. By contrast, investments in increasing the endowment of land capital have involved predominately the old member states of the European Union, with the exception of Italy, and only few newcomer nations such as Poland.



(A) (B)

Figure 7. Component planes of some inputs such as the rural population (A) and the urban population (B).

Source: Author's elaboration on data http://faostat3.fao.org/faostat-gateway/go/to/home/E.

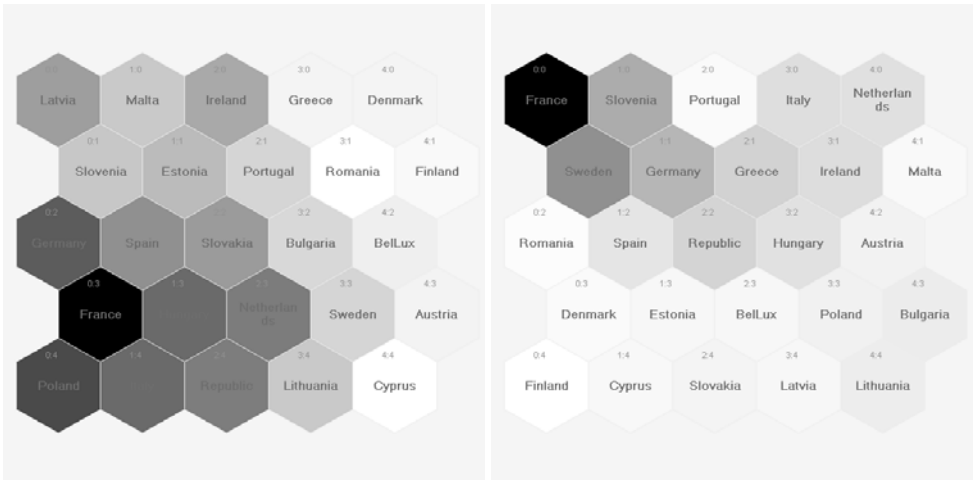


(A) (B)

Figure 8. Component planes of investments in machinery (A) and land capital (B) in different European countries.

Source: Author's elaboration on data http://faostat3.fao.org/faostat-gateway/go/to/home/E.

The Kohonen's maps assessing the endowment of agricultural areas in terms of meadows and pastures have pointed out a greater level of homogeneity among all European Union countries (Figure 9).



(A) (B)

Figure 9. Component planes of the carbon dioxide emission (A) and meadows and pastures (B) in all analysed European countries.

Source: Author's elaboration on data http://faostat3.fao.org/faostat-gateway/go/to/home/E.

The emission of carbon dioxide, as a consequence of a greater use of fertilizers and investment in the primary sector, has been homogeneous in all European countries (Figure 10).

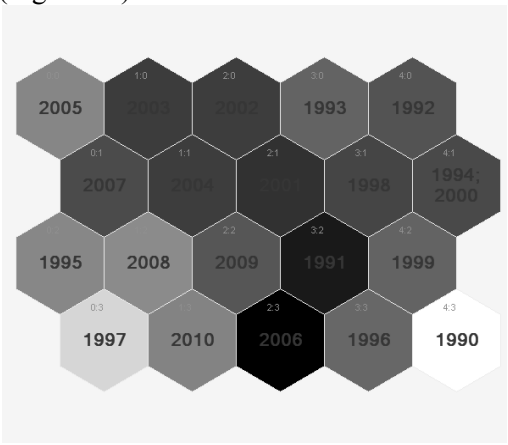


Figure 10. Self-organizing maps about the level of carbon dioxide emissions in all analysed countries.

Source: Author's elaboration on data http://faostat3.fao.org/faostat-gateway/go/to/home/E.

The level of emissions of carbon dioxide and other greenhouse gases, such as NO_2 from fertilizers and soil, has underlined that there has been a growth of the level of discharge due to an increase of the agricultural productions in all European countries over 20 years of study. Findings have indicated that since 2007 there has been a significant drop in CO_2 emissions as a consequence of several new actions carried out by the Common Agricultural Policy aimed at implementing the level of set-aside and not cultivated areas or afforested areas and at reducing the chemical negative impact on the environment through lots of actions correlated to the multifunctionality (Figure 10).

Conclusion

The Kohonen's maps have pointed out a good ability of this quantitative approach in order to investigate and classify time series data and its spatial evolution. Roughly speaking, this method has been useful in describing the impact of investment on production specialization, levels of pollution and food self-sufficiency.

The level of food self-sufficiency has increased since 2004, despite the accession of new member states of Eastern Europe in the EU, which came from the conditions of extreme structural productive weakness and economic disadvantage; hence, the role of pre-accession funds have been pivotal to stimulate farmers to improve the irfactors of production and land capital by investments able to improve both economic and also food self-sufficiency reducing, on the other hand, the ecological impact of emissions of pollutants such as carbon dioxide by new ecological strategies proposed by the Common Agricultural Policy focused on protecting the environment and on reducing chemical use of pesticides and fertilizers through financial subsidies towards organic farms.

Results have underscored that countries with wide arable land areas have produced a high level of emissions in terms of CO_2 from the fertilizers. In general, farmers with poorer areas than other European countries are able to put a lower level of carbon dioxide in the atmosphere, hence, this has implied that small farmers are able to produce a multifunctional effect compared to very large areas.

To sum up, the analysis has underlined that an improvement of the food self-sufficient level has been tightly linked to an increase of pollution in terms of carbon dioxide emissions; therefore, some proposals of the European Union Commission about the greening approach or as an alternative reforestation seem to be the first and foremost strategies in reducing this ecological problem. It is important that policy makers take into account specificities of the different typologies of European agricultures, with the aim to disburse financial subsidies by the CAP in favor of quality agrarian productions such as certified quality food and traditional food.

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KVANTITATIVNI ODNOSI IZMEĐU INVESTICIJA NA GAZDINSTVIMA I EFEKTI NA ŽIVOTNU SREDINU U NEKIM EVROPSKIM ZEMLJAMA

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R e z i m e

Od ranih sedamdesetih godina dvadesetog veka, u mnogim zemljama članicama Evropske unije, došlo je do značajnog poboljšanja njihovog poljoprivrednog ekonomskog rasta usled Zajedničke poljoprivredne politike (engl. *Common Agricultural Policy*). Sve veća ulaganja u poljoprivredno zemljište i mehanizaciju bila su ključni stubovi u nivoima poboljšanja prehrambene samodovoljnosti. Međutim, rast investicija povećao je emisiju zagađujućih supstanci u pogledu ugljen-dioksida, azotnih jedinjenja i pogoršanja životne sredine. Cilj ovog rada bio je da se ispita uticaj investicija na poljoprivredno i obradivo zemljište u unapređenju poljoprivredne proizvodnje prema kvalitetu životne sredine. Kvantitativne metode su obuhvatale analizu neuronskih odnosa putem Kohonenovih mapa ili samoorganizujućih mapa (engl. *Kohonen's maps/self-organizing maps* – SOMs). Rezultati Kohonenovih mapa ukazali su na dobru sposobnost da se klasifikuju podaci vremenskih serija i prostorna evaluacija podataka tokom vremena; pored toga, ove mape su se pokazale korisnim u opisivanju uticaja ulaganja na specijalizaciju proizvodnje, nivoe zagađenja i prehrambenu samodovoljnost u evropskim zemljama. Da rezimiramo, poboljšanje nivoa prehrambene samodovoljnosti blisko je povezano sa povećanjem zagađenja u pogledu emisija ugljen-dioksida.

Ključne reči: Kohonenove mape, Zajednička poljoprivredna politika, ruralni razvoj, investicije, produktivna specijalizacija, multifunkcionalnost.

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FACTORS INFLUENCING THE LEVEL OF USE OF CLIMATE-SMART AGRICULTURAL PRACTICES (CSAPs) IN SOKOTO STATE, NIGERIA

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Abstract: Climate-Smart Agriculture (CSA) is paramount to the success of farming activities today in the face of the menace of climate change. This study therefore investigated the frequency of usage of CSA and the factors influencing its level of usage in the Northern agricultural zone of Sokoto State. A well-structured questionnaire was used for data collection. The data used for the analysis were cross sectional data collected from 120 rural farming households in six (6) villages across two (2) local government areas. Descriptive statistics, Adaptation Strategy Use Index (ASUI) and ordered probit regression model were used for data analysis. Results indicated that the majority of the respondents were male (83.33%), married (83.33%), had Quranic education (73.33%), household size of 1−10 persons (58.33%), farming experience of 16–30 years (49.17%) and were between the ages of 46 and 60 (44.17%). Similarly, the practice of conservation agriculture was the most used CSAP in the study area, while the results of the ordered probit regression showed that years of education and membership of a social group were significant explanatory variables influencing the level of use of CSAP among the low user and high user categories at the 10% and 1% level of significance respectively, while access to credit significantly influenced only the low user category at the 10% level of significance. The study therefore concluded that CSAPs were being practiced at different levels in the study area with various factors influencing their usage, and it therefore recommended that the farming households be well enlightened by extension agents on the benefits of CSAPs.

Key words: Climate-Smart Agriculture, Adaptation Strategy Use Index, ordered probit and conservation agriculture.

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Introduction

Agricultural production is still the main source of livelihood for rural communities in sub-Saharan Africa, providing employment to more than 60 percent of the population and contributing about 30 percent of gross domestic product in this region (Nhemachena and Hassan, 2007). With likely long-term changes in rainfall patterns and shifting temperature zones, climate change is expected to significantly affect agricultural production, which could be detrimental to the region's food security and economic growth.

According to Intergovernmental Panel on Climate Change (IPCC) (2007), the relationship of agriculture to climate change is a topic of increasing interest. Worldwide agricultural production is expected to decrease under climate change projections, posing a threat to global food security. However, it is also important to note that agriculture contributes a significant amount of global emissions annually, which would increase with the intensification or expansion of production to meet higher demand. In addition, estimates attribute as much as 80% of global deforestation to agriculture (Kissinger et al., 2012 as cited by Elizabeth and Sophie, 2014). The IPCC 4th Assessment Report predicts that climate change could cause yields to decrease by as much as 50% in some highly vulnerable areas, including sub-Saharan Africa (Elizabeth and Sophie, 2014). According to this report "warming in Sub-Saharan Africa (SSA) is expected to be greater than the global average and rainfall will decline in certain areas. Also, cereal production growth for a range of crops in SSA is projected to decline by a net 3.2 percent in 2050 as a result of climate change". Ringler et al. (2010) have stated that under climate change, the largest negative yield impacts are projected for wheat followed by sweet potatoes. However, millet and sorghum yields are projected to be slightly higher under climate change, probably given their higher tolerance to higher temperatures and drought stress.

Steenwerth et al. (2014) opined that the term Climate-Smart Agriculture (CSA) was developed to represent a set of strategies that can help combat the above stated challenges of climate change by increasing resilience to weather extremes, adapting to climate change and decreasing agriculture's greenhouse gas (GHG) emissions that contribute to global warming. These strategies used by farmers are conceptualized in this paper as Climate-Smart Agricultural Practices (CSAPs). Therefore, "CSAP focuses on contributing to economic development, poverty reduction and food security; maintaining and enhancing the productivity and resilience of natural and agricultural ecosystem functions, thus building natural capital; and reducing trade-offs involved in meeting these goals". This stresses the need for farmers to adopt the use of CSAP, which will help in boosting agriculture to produce more on the same amount of land while adapting to a changing climate

and becoming more resilient to the risk derived from extreme weather conditions, such as droughts, floods, high temperature and low rainfall (World Bank, 2011).

Meybeck and Gitz (2013) state that Climate-Smart Agriculture has the potential to provide 'triple wins', which include: (a) increased resilience to climate change; (b) reduced GHG emissions; and (c) improved food security. The importance of these benefits is as follows:

a. Agriculture is projected to be negatively affected by climate change, so adaptation is necessary;

b. Agriculture is a major contributor to annual global emissions, requiring mitigation of emissions; and

c. Agriculture is also important to the issue of global food security, which could be threatened if productivity levels are affected by climate change.

Following the Second Global Conference on Agriculture, Food Security and Climate Change in Hanoi in 2012, "Climate-Smart Agriculture Sourcebook" was published to further advance the concept with the intention of benefiting primarily smallholder farmers and vulnerable people in developing countries (FAO, 2013). In the work of Fanen and Olalekan (2014) on "Assessing the role of Climate-Smart Agriculture in combating climate change, desertification and improving rural livelihood in Northern Nigeria", they have found out that many smallholder farmers have inadvertently practiced CSA as part of their traditional farming system in Northern Nigeria. This study therefore seeks to identify the extent of use of CSAPs and also to determine the factors influencing their level of usage in the study area.

Materials and Methods

Study area

This study was conducted in Sokoto State. The state is located between latitude 11^0 3' to 13^0 5' N and longitude 4^0 to $5^0$15'E. The climatic condition of the state is semi-arid with two distinct seasons; the raining season lasting for 3−4 months from mid- May to mid-September and the dry season from October to early May. The State has a mean annual temperature of 34.9^{o}C. Farming is the major occupation of the people in the State. The major crops grown include millet, cowpea, sorghum, maize, rice and other vegetables such as amaranthus and spinach. The major livestock reared are cattle, sheep and goats (Maikasuwa and Ala, 2013).

Sampling techniques and sample size

The sampling technique used for this study is a multi-stage simple random sampling technique. The first stage included the purposive selection of Northern

agricultural zone of Sokoto state. In the second stage, two (2) local government areas were randomly selected from the selected agricultural zone, while the third stage involved the random selection of six (6) villages from the two local government areas already selected. The last stage was the selection of a total of 120 respondents as used for this study.

Analytical techniques

Data collected for this research were analyzed using descriptive statistics, Adaptation Strategy Use Index (ASUI) for determining the frequency of use of CSAPs and the ordered probit regression model for determining the factors influencing the level of use of CSAPs. The ASUI will reflect the relative position (ranking) of each of the CSAPs identified in the study area in terms of their frequency of usage. The ASUI was adapted from Adesoji and Famuyiwa (2010) in Umunna et al. (2013). The frequency of use of the CSAPs was expressed using a four-point Likert scale, that is, 3, 2, 1, and 0 for frequently used, occasionally used, rarely used and not used respectively. The formula is as stated below:

$$ASUI = \frac{[(N_1 \times 3) + (N_2 \times 2) + (N_3 \times 1) + (N_4 \times 0)]}{M} \quad (1)$$

where:
N_1 = Number of farm households that frequently used a particular CSAP;
N_2 = Number of farm households that occasionally used a particular CSAP;
N_3 = Number of farm households that rarely used a particular CSAP;
N_4 = Number of farm households that did not use a particular CSAP;
$M = n \times 3$;
n = Total number of respondents.

Composite score

This was used to classify the farming households based on the level of use of CSAPs among the rural farming households in the study area. Respondents were made to respond to questions relating to the level of use of CSAPs. These practices included Conservation agriculture, Agro-forestry, Use of organic manure, Crop rotation, Crop diversification, Mulching, Use of wetland (Fadama), Planting of drought resistant crops, Planting of cover crops and Soil conservation techniques. A binary scale, that is, scoring 1 point for yes and 0 for no responses, regarding the use of any of these CSAPs, was used to rate their responses. If a respondent was asked 10 questions; such respondent would be scored a maximum of 10 points and a minimum of 0 points. The categorisation into high, medium and low users was achieved using a composite score as given below and as used by Salimonu, 2007 as cited by Adepoju et al., 2011).

High user = Between 10 points to (mean + S.D) points;
Medium user = Between upper and lower categories;
Low user = Between (mean – S.D) points to 0 point.

Ordered probit model

The ordered probit regression model was used to determine the factors influencing the level of use of CSAPs by the farming households in the study area. The various levels of CSAP usage (which is the dependent variable) were derived from the composite score. The ordered probit model is thus expressed:

$$Y_i^* = \chi'\beta+\varepsilon_i \qquad Y\mu i \qquad (2)$$

where Y_i^* is the unobserved discrete random variable, x_i is the vector of independent variables, β is the vector of parameters of the regression to be estimated and ε_i is the vector of error term (Greene, 2003). Thus, Y_i, which is the observed ordinal variable, takes on the following values:

$$\begin{aligned} &Y_i = 0 \text{ if } Y_i^* \leq 0 \\ &Y_i = 1 \text{ if } 0 < Y_i^* \leq \mu_1 \\ &Y_i = 2 \text{ if } \mu_1 < Y_i^* \leq \mu_2 \\ &Y_i = 2 \text{ if } \mu_1 < Y_i^* \leq \mu_2 \\ &Y_i = J \text{ if } \mu_{J-1} < Y_i^* \end{aligned} \qquad (3)$$

The dependent variable is Y_i = level of usage of Climate-Smart Agricultural Practices (2 = high user, 1 = medium user, 0 = low user).

The independent variables are:

X_1 = Age of household head (years);
X_2 = Gender of household head (D = 1 if male; 0 = otherwise);
X_3 = Marital status of household head (single = 1; married = 2; widowed = 3; divorced/separated = 4);
X_4 = Household size (number);
X_5 = Educational status of household head (years);
X_6 = Farming experience of household head (years);
X_7 = Farm size (hectares);
X_8 = Farm income (naira);
X_9 = Off-farm income (naira);
X_{10} = Membership of a social group (D = 1 if member; 0 = otherwise);
X_{11} = Access to agricultural credit (D = 1 if yes; 0 = otherwise);
X_{12} = Contact with extension agents (number);
X_{13} = Livestock ownership (D = 1 if owned; 0 = otherwise).

STATA version 13 was used for all the statistical analysis.

Results and Discussion

The results from Table 1 showed the socio-economic characteristics of the respondents. The majority of the respondents (44.17%) were between the ages of 46 to 60, which forms the active years of the farmers and therefore, they are strong enough to engage in agricultural practices. About 83.33% of the farmers are male, which is a common practice in North-western Nigeria, where women are only allowed to partake in activities like harvesting, winnowing, and processing of farm produce. About 73.33% of the respondents only had Quranic education followed by 12.50% with primary education. Quranic education is the prevalent form of education in Northern Nigeria, most especially among rural dwellers.

Table 1. Socio-economic characteristics of the respondents.

Variables	Frequency	Percentage (%)
Age		
16–30	13	10.83
31–45	50	41.67
46–60	53	44.17
61 and above	4	3.33
Gender		
Male	100	83.33
Female	20	16.67
Education		
No formal education	2	1.67
Quranic education	88	73.33
Primary education	15	12.50
Secondary education	11	9.17
Tertiary education	4	3.33
Marital Status		
Single	1	0.83
Married	100	83.33
Divorced/separated	2	1.67
Widowed	17	14.17
Household size		
1–10	70	58.33
11–20	47	39.17
21 and above	3	2.50
Farming experience		
1–15	29	24.17
16–30	59	49.17
31–45	29	24.17
46 and above	3	2.50
Total	120	100

Source: Field survey, 2016.

About 83.33% of the respondents were married, while about 58.33% had a household size of between 1 and 10, followed by 39.17% with a household size of 11 to 20 persons. A large household size is a source of family labour in rural Nigeria where farming is a major occupation. Results of the farming experience in Table 1 revealed that the majority of the rural farmers (49.17%) had farming experience of 16 to 30 years. Farming experience is very important in farming activities, as it helps the farmer in the area of proper farm management to maximize profit.

Table 2 reveals the results of the perception of farmers on the impact of climate change on their farming activities in the past five years. The ability of farmers to perceive climate change is a vital requirement for their choice of CSAPs. The results indicate that the majority of the respondents (95.8%) affirmed that there had been an increase in temperature in the study area for the past five years. This confirms the existence of global warming, which has brought about an increase in temperature with a negative effect on crop production, while only 4.2% affirmed otherwise. Also, 70.8% of the respondents perceived an increased rainfall pattern in the past five years.

Table 2. Perception of respondents on a climate change impact.

Features of climate change	Increasing	Decreasing	No change
Change in temperature	115 (95.8)	5 (4.2)	-
Change in rainfall pattern	85 (70.8)	34 (28.3)	1 (0.8)
Effect of climate change on crop yield	Positive	Negative	No change
	33 (27.5)	84 (70.0)	3 (2.5)
Effect of climate change on water supply	Improved	Worsened	No change
	98 (81.7)	22 (18.3)	-
Magnitude of drought	Mild	Moderate	Severe
	66 (55.0)	53 (44.2)	1 (0.8)

Source: Field survey (2016).
Note: Figures outside the brackets are frequencies, while the ones in the brackets are percentages (%).

Rainfall/precipitation is a vital requirement for agricultural activity, but increased rainfall which results in flooding or soil erosion is detrimental to crop production. This result is in line with Gbetibouo (2011), who opined that over the years, temperature had been on the increase. But this differs from the work of Gbetibouo (2011), who opined that there had been a decrease in the rainfall pattern due to a climate change impact. Regarding the effect of climate change on crop yield, 70.0% of the respondents asserted that climate change had a negative effect on their crop yield, while 27.5% perceived a positive effect of climate change on their crop yield. About 81.7% of the respondents perceived an improved water supply in their environment as a result of climate change, and this is in accordance with the perception of the majority of the respondents on increased rainfall due to the effect of climate change, while 18.3% perceived a worsened water supply as a

result of climate change. As for the magnitude of drought, 55.0% stated that the magnitude of drought in their farming environment was mild, while 44.2% perceived that the magnitude of drought was moderate. These findings affirmed that the farmers in the study area perceived the negative effect of climate change on their cropping activities and therefore the need to use CSAPs as a remedy to these problems.

Table 3 shows the results of the frequency of use of CSAPs. The results indicated which of the CSAPs was used most in ranking order in the study area. It may be noticed that the five (5) most used CSAPs in the study area included Conservation agriculture, Use of organic manure, Crop diversification, Use of wet land (Fadama) and Planting of drought and heat tolerant crops in descending order, while Agro-forestry is the least used CSAP in the study area. These results also showed that CSAP was being practiced in the study area, but at different levels of usage, which might be a result of some factors influencing their usage as shown in Table 4.

Table 3. Frequency of use of CSAPs by the respondents.

S/N	CSAPs	ASUI	Ranking
1	Conservation agriculture	0.9722	1st
2	Agro-forestry	0.1778	10th
3	Use of organic manure	0.9167	2nd
4	Crop rotation	0.2333	9th
5	Crop diversification	0.7139	3rd
6	Mulching	0.4111	6th
7	Use of wetland (Fadama)	0.4528	4th
8	Planting of drought and heat tolerant crops	0.4222	5th
9	Planting of cover crops	0.3444	8th
10	Soil conservation techniques	0.3694	7th

Conservation agriculture was the major CSAP in the study area. It involves minimum soil disturbance which reduces run-off and soil water loss. This is in line with the findings of Dumanski et al. (2006), who state that Conservation agriculture provides direct benefits to environmental issues of global importance. These include land degradation, air quality, climate change, bio-diversity and water quality. CSAPs should therefore be encouraged among farmers in order to lessen the effect/menace of climate change on crop production and also protect the eco-system.

The results of the ordered probit model which showed the factors influencing the level of usage of CSAPs in the study area are as shown in Table 4. The Log likelihood of -98.2285 with a p-value of 0.0000 revealed that the model as a whole was statistically significant. The estimated cut-off points (μ) showed that the categories were ranked in an ordered way of $\mu 2 > \mu 1 > \mu 0$. The dependent variables

were low user (Y = 0), medium user (Y = 1) and high user (Y = 2). The marginal effect estimates indicated that years of education of the respondents and membership of a social group were the significant explanatory variables that influenced the usage of CSAPs among the low user and high user categories in the study area at the 10% and 1% level of significance respectively. Access to credit also influenced the usage of CSAPs among the low user category. On the other hand, none of the explanatory variables significantly influenced the medium user category. Education is a vital tool for knowledge acquisition.

Table 4. Ordered probit regression model for the factors influencing the level of use of CSAPs.

		Low user				Medium user				High user			
Variable	Coefficient	SE	P-value	ME		SE	P-value	ME		SE	P-value	ME	
Age of household head	0.0003895	0.00599	0.983	-0.0001243		0.00047	0.983	-9.68e-06		.00645	0.983	0.000134	
Gender of household head	0.1501062	0.3662	0.893	-0.0494465		0.01829	0.969	-0.0007127		.34931	0.886	0.0501592	
Marital status	-0.3988126	0.17456	0.466	0.1272876		0.02282	0.664	0.0099127		.18755	0.464	-0.1372003	
Education	0.0553309	0.01056	0.095*	-0.0176598		0.00274	0.616	-0.0013753		.01141	0.095*	0.019035	
Household size	0.0498417	0.01141	0.163	-0.0159078		0.00252	0.623	-0.0012388		.01233	0.164	0.0171466	
Farm income	6.04e-07	0.0000	0.694	-1.93e-07		0.0000	0.753	-1.50e-08		0.0000	0.694	2.08e-07	
Off-farm income	1.02e-06	0.0000	0.824	-3.25e-07		0.0000	0.845	-2.53e-08		0.0000	0.825	3.50e-07	

Table 4. Continuation.

		Low user			Medium user			High user		
Variable	Coefficient	SE	P-value	ME	SE	P-value	ME	SE	P-value	ME
Farming experience	0.0072622	0.00645	0.719	-0.0023178	0.00062	0.769	-0.0001805	.00696	0.720	0.0024983
Membership of a social group	0.7728677	0.0835	0.003***	-0.2466735	0.03679	0.602	-0.01921	.08905	0.003***	0.2658835
Variable	Coefficient	SE	P-value	ME	SE	P-value	ME	SE	P-value	ME
Access to credit	0.5249819	0.08503	0.074*	-0.1520597	0.04492	0.396	-0.0381006	.12216	0.120	0.1901603
Contacts with extension officer	-0.1326864	0.04082	0.300	0.0423491	0.00682	0.629	0.003298	.0436	0.295	-0.045647
Livestock ownership	-0.5798643	0.11561	0.197	0.1493095	0.12282	0.567	0.0703809	.23446	0.349	-0.2196904
Total farm size	0.0158766	0.00441	0.251	-0.0050673	0.00079	0.617	-0.0003946	.00468	0.243	0.0054619
Cut 1	-0.5295117									
Cut 2	0.682739									

The more educated a farmer is, the easier he or she adopts modern technology/innovations. This is in line with the studies of Mamudu et al. (2012), who stated that educated members of the farming household would easily adopt modern agricultural production technologies and strategies. Therefore, the usage of CSAPs and their propagation among rural farmers depend on this factor.

Another important factor that influenced the use of CSAPs in the study area was being a member of a social group, for instance, farmers' cooperative society. Social groups play a very important role in the enlightenment of their members. Farmers who belong to such groups are easily enlightened and exposed to new farming technologies that will help boost agricultural production. Probably most of the farmers in the study area became aware of CSAPs through the social group they belong to.

Access to credit was also a vital tool that will enable a low user of CSAPs to rise up to being a high user. When farmers are given access to credit, it will enable them to acquire more technology which might be expensive to purchase. This agrees with the findings of Amao and Ayantoye (2015), who opined that access to credit in the form of loanable funds (soft loans) can be used to expand production through the purchase and use of modern improved inputs.

The marginal effects of all the variables influencing the three categories of 'low user', 'medium user', and 'high user' are as shown in Table 4. As it can be seen from Table 4, among the low user and medium user categories, age of household head, gender of household head, years of education of household head, household size, farm income, off-farm income, farming experience, membership of a social group, access to credit and total farm size were all negatively related. The negative sign implies that an increase in all these variables will cause the farmers who are low users and medium users of CSAPs to increase their usage of CSAPs. Regarding the high user category, on the other hand, all the above stated explanatory variables were positively related. This positive sign implies that as these variables increase, there will be a boost in the usage of CSAPs among the high user category in the study area.

Conclusion

The most used CSAPs in the study area in order of ranking were Conservation agriculture, Use of organic manure, Crop diversification, Use of wet land (Fadama) and Planting of drought and heat tolerant crops. A number of factors influenced the extent of use of these CSAPs among the three categories of users in the study area. The significant factors included years of education of the respondents, membership of a social group and access to credit, which all had a negative signed marginal effect on the low user category. This implies that if these factors are increased, the low user category will increase their usage of CSAPs. It is therefore recommended that farmers should be encouraged to join one or more farmer social groups, which would expose them to agricultural innovations. Also, the farmers should be well educated and enlightened by extension services on the benefits of CSAPs in agricultural production, which are the panacea for reducing the negative impact of climate change on their farming activities.

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FAKTORI KOJI UTIČU NA NIVO KORIŠĆENJA KLIMATSKI PAMETNIH POLJOPRIVREDNIH PRAKSI (CSAPs) U DRŽAVI SOKOTO, NIGERIJA

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R e z i m e

Klimatski pametna poljoprivreda (engl. *Climate-Smart Agriculture* – CSA) najvažnija je za uspeh poljoprivrednih aktivnosti danas, uprkos opasnosti od klimatskih promena. Ovo istraživanje je ispitivalo učestalost primene klimatski pametne poljoprivrede i faktore koji utiču na njen nivo upotrebe u Severnoj poljoprivrednoj zoni države Sokoto. Dobro strukturiran upitnik korišćen je za prikupljanje podataka. Podaci koji su korišćeni za analizu bili su podaci preseka stanja sakupljeni od 120 seoskih domaćinstava iz šest sela iz dve oblasti lokalne samouprave. Deskriptivna statistika, indeks korišćenja strategije adaptacije (engl. *Adaptation Strategy Use Index* – ASUI) i naručeni probit regresioni model korišćeni su za analizu podataka. Rezultati su pokazali da je većina ispitanika muškog pola (83,33%), u braku (83,33%), steklo versko obrazovanje (73,33%), veličine domaćinstva 1–10 članova (58,33%), poljoprivrednog iskustva 16–30 godina (49,17%) i starosti između 46 i 60 godina (44,17%). Pored toga, konzervacijska poljoprivreda je bila najviše korišćen metod u ispitivanoj oblasti, dok su rezultati regulisane probit regresije pokazali da su godine obrazovanja i članstvo u nekoj društvenoj grupi značajno važne promenljive, koje utiču na nivo upotrebe klimatski pametnih poljoprivrednih praksi među kategorijama „niskih" i „visokih korisnika" na nivoima značajnosti od 10% odnosno 1%. Pristup kreditu je značajno uticao samo na kategoriju „niskih korisnika" pri nivou značajnosti od 10%. Istraživanjem se zaključuje da su klimatski pametne poljoprivredne prakse korišćene pri različitim nivoima u ispitivanoj oblasti sa različitim faktorima koji utiču na njihovu upotrebu, pa se preporučuje da savetodavni agenti informišu poljoprivredna domaćinstva o koristima klimatski pametnih poljoprivrednih praksi.

Ključne reči: klimatski pametna poljoprivreda, indeks korišćenja strategije adaptacije, naručen probit i konverzacijska poljoprivreda.

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The References section should contain only papers cited in the main text. The paper cited in the text should contain the last (family) name and the year. If the citation is comprised of one author, it is stated as Jalikop (2010) or (Jalikop, 2010). When the citation is comprised of the two authors it is stated as Sadras and Soar (2009) or (Sadras and Soar, 2009). If more than two authors are cited, after the last (family) name of the first author, the abbreviation "et al." is given, and then the year. This citation is stated as Lehrer et al. (2008) or (Lehrer et al., 2008). If more than one paper are cited simultaneously for a certain problem, they should be listed chronologically. A large number of cited papers out of brackets should be separated by comma (,) and if in brackets, by semicolon (;). If two or more papers of the same author are cited, they must be listed chronologically (1997, 2002, 2006, etc.). If a certain author appears several times for the same year, the letters are added (2005a, b, c, etc.). The citations of personal communication and unpublished papers should be avoided, except that it is an absolute necessity. Such citations should appear in the text only as (Brown, personal communication), and not in the list of References.

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The examples of listing references are the following:

Periodicals

Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Books

Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Book chapter

Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R. M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Proceedings
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Thesis
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Report
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Web site
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

Summary
The summary in Serbian is given at the end of the paper and should comprise 100 to 250 words. Before the main text of the summary, as well as in English, the title of the paper, first name, middle initial(s) and last (family) name of all authors and the names and addresses of affiliations should be given.The title of the summary is centred and written separately. Below the title, the text of the summary should follow, without any indentation, and immediately after the text of the summary, the key words are given with the full stop at the end. The e-mail address of the corresponding author should be given at the bottom of the page.

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Tables numbered with Arabic numerals (1, 2, etc.), followed by the title should be placed in the text using 9 font size and a maximum width of 13 cm. They should be clear, simple and unambiguous. The vertical sections should be avoided, and the number of columns should be limited so that the table is not too wide. Also, an unnecessary usage of horizontal sections should be avoided. The title of the table, single spaced above the table, justified, and with the full stop at the end should be given. The detailed explanation of abbreviations, symbols and signs used in the table should be provided below the table. Each table must be mentioned in the text.

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All graphs, diagrams and photographs should be titled "Figure" (1, 2, etc.). They should be placed in the text. Graphs and diagrams should be computer drawn, using 9 font size and a maximum width of 13 cm, so that they can be legible and distinct after the size reduction. The overuse of colours and hues should be avoided for aesthetic reasons. The detailed legend without abbreviations for each graph and

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Only standardised abbreviations should be used in the paper. Measure units should be expressed using International System of Units (SI). The abbreviations can be used for other expressions provided these expressions are stated in the full form when appear for the first time with the abbreviated form in the brackets. Values from 1 to 9 can be written in letters, but others numerically.

Nomenclature

The complete nomenclature (chemical and biochemical, taxonomical, genetic etc.) must be adjusted to international codes and commissions, such as *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* etc.

Formulae

All formulae and equations in the paper should be worked out by means of the programme "WORD Equation". An ample space should be left around the formulae for the sake of visibility. Subscripts and superscripts should be clear. Greek letters and other non-Latin symbols should be explained when they are first used. The meaning of all symbols should be given immediately after the equation where these symbols are first used. Equations should be numbered by Arabic numerals, serially in brackets, at the right-hand side. Each equation must be mentioned in the text as Eq. (1), Eq. (2), etc.

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Ključne reči
Ključne reči su termini ili fraze koje najbolje opisuju sadržaj članka za potrebe indeksiranja i pretraživanja. Broj ključnih reči može biti od 3 do 10. Navode se ispod sažetka. Naslov „Ključne reči“ piše se boldovano i uvlači jednim tabulatorom. Nakon toga slede dve tačke, a zatim nabrajanje ključnih reči malim slovima, sa tačkom na kraju. Treba izbegavati korišćenje ključnih reči koje se nalaze u naslovu rada. Ključne reči se dostavljaju na srpskom i engleskom jeziku posle sažetaka na oba jezika.

Uvod
Uvod treba da sadrži informacije o dosadašnjim istraživanjima po navedenom pitanju i šta se datim istraživanjem želi postići. Prilikom osvrta na literaturu, navesti autora i godinu, a autora citirati u spisku literature. Naslov „Uvod“ piše se sa prvim velikim slovom, centrirano i boldovano, nakon čega sa jednim razmakom ispod naslova sledi tekst uvoda poravnat po levoj i desnoj margini. Svaki novi pasus uvlači se jednim tabulatorom. Ova pravila važe i za sva ostala poglavlja.

Materijal i metode
Materijal i metode treba izložiti jasno uz objašnjenje svih primenjenih postupaka u radu. Opšte poznate metode izložiti kratko, a detaljnije ih objasniti ukoliko se odstupa od ranije objavljenih postupaka. Za radove eksperimentalnog karaktera obavezno navesti način statističke obrade podataka. U ovom poglavlju, kao i u poglavlju „Rezultati i diskusija“, po potrebi se mogu dati i određena podpoglavlja.

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U poglavlju „Rezultati i diskusija“ interpretiraju se podaci dobijeni na osnovu zapažanja i izvršenih eksperimenata. U komentaru rezultata treba se pozivati na literaturu koja se navodi na kraju rada, čime se obezbeđuje poređenje dobijenih rezultata sa dosadašnjim saznanjima u toj oblasti.

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U zaključku treba ukratko navesti najznačajnije rezultate dobijene u radu. Izbegavati nabrajanje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija“. Zaključak ne sme da sadrži reference.

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Zahvalnica treba da sadrži naziv i broj projekta, odnosno naziv programa u okviru koga je rad nastao, kao i naziv institucije koja je finansirala projekat ili program.

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Primeri navođenja referenci su sledeći:

Periodičan časopis
Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Knjiga
Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Poglavlje u knjizi
Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R.M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Zbornik
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon.

Teza
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Izveštaj
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Veb sajt
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

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Tabele
Tabele obeležene arapskim brojevima (1, 2, itd.) praćene naslovom treba da se nalaze na odgovarajućem mestu u tekstu, u fontu 9. Maksimalna širina tabela treba da bude 13 cm. One treba da budu jasne, što jednostavnije i pregledne. Treba izbegavati vertikalne crte, a broj kolona ograničiti tako da tabela ne bi bila preširoka. Takođe, treba izbegavati nepotrebnu upotrebu horizontalnih crta. Naslov tabele, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom iznad tabele. Ispod tabele treba dati detaljno objašnjenje skraćenica, simbola i znakova korišćenih u samoj tabeli. Svaka tabela mora biti pomenuta u tekstu.

Ilustracije
Svi grafikoni, dijagrami i fotografije treba da se nazovu „Slika“ (1, 2, itd.). Prilažu se na odgovarajućem mestu u tekstu. Grafikone i dijagrame treba uraditi fontom 9, u crno-beloj tehnici i sa maksimalnom širinom od 13 cm. Voditi računa da oni budu čitki i jasni i nakon redukcije veličine. Za svaki grafikon i dijagram treba obezbediti detaljnu legendu bez skraćenica. Fotografije moraju biti visokog kvaliteta da bi se tehnički mogle dobro reprodukovati. Prilažu se u „TIF“ ili „JPG“ formatu, u crno-beloj tehnici. Naslov ilustracije, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom ispod ilustracije. Svaka ilustracija mora biti pomenuta u tekstu.

Skraćenice i jedinice
U radu treba koristiti samo standardne skraćenice. Merne jedinice treba izražavati u internacionalnom sistemu jedinica (SI). Kod navođenja jedinica posle broja treba da stoji razmak (osim za % i °C). Skraćenice se mogu koristiti i za druge izraze pod uslovom da se ti izrazi navedu u punom obliku prilikom prvog pominjanja, sa skraćenim oblikom u zagradi. Vrednosti od 1 do 9 mogu se izražavati slovima, a ostali brojevi isključivo numerički.

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Celokupna nomenklatura (hemijska i biohemijska, taksonomska, genetička itd.) mora biti usklađena sa međunarodnim kodeksima i komisijama, kao što su *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* itd.

Formule
Sve formule i jednačine u radu moraju biti urađene pomoću programa „Word Equation“. Pri pisanju formula, radi preglednosti, ostaviti dovoljno praznog prostora oko same formule. Subskripti i superskripti treba da budu jasni. Prilikom pisanja jednačina treba dati smisao svih simbola odmah posle jednačine u kojoj se simbol prvi put koristi. Jednačine treba da budu numerisane arapskim brojevima, serijski u zagradama, na desnoj strani linije. Svaka jednačina mora biti pomenuta u tekstu kao Eq. (1), Eq. (2), itd.

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