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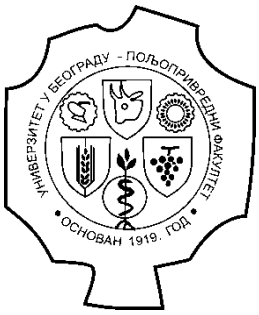

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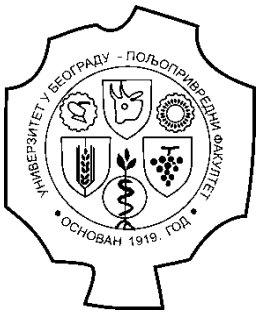

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THE EFFECT OF TOASTED *ADENANTHERA PAVONINA* SEED MEAL ON HAEMATOLOGY AND BLOOD CHEMISTRY OF FINISHER BROILER CHICKENS

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Abstract: The effect of graded levels of toasted *Adenanthera pavonina* seed meal (TAPSM) on haematological and biochemical indices of finisher broiler chickens was investigated in a five-week feeding trial with 84 (5-week old) broilers divided into four groups of 21 birds per group. Each group had three replicates of 7 birds. Four experimental diets were formulated to contain 0, 10, 20 and 30% TAPSM, represented as T_1, T_2, T_3 and T_4 respectively. Birds were allocated to the four diets in a completely randomized design and housed in a deep litter pen. Feed and water were offered to birds *ad libitum*. Haematological parameters showed a significant ($P<0.05$) decreasing trend in haemoglobin (10.20, 9.03, 8.60 and 7.63 g/dl), packed cell volume (28.33, 26.10, 25.60 and 25.20%), total red blood cell (4.23, 4.00, 3.99 and 3.99 $\times 10^6$/µl), mean corpuscular volume (66.97, 65.25, 64.16 and 63.15 fl), mean corpuscular haemoglobin (24.11, 22.57, 21.55 and 19.12pg) and mean corpuscular haemoglobin concentration (35.66, 34.59, 33.59 and 30.27%) with an increase in the level of TAPSM in the diets. Total white blood cells (71.76, 73.40, 75.07 and 76.17 $\times 10^3$/µl) increased significantly ($P<0.05$) as the TAPSM level increased. The other values were as follows: urea (4.00, 5.00, 6.67 and 10.00mg/dl), creatinine (0.33, 0.25, 0.43 and 0.46mg/dl), cholesterol (149.67, 135.67, 113.67 and 102.67 mg/dl), total protein (3.33, 3.43, 2.97 and 2.50mg/dl), glucose (146.14, 208.49, 179.66 and 135.33 mg/dl), alkaline phosphatase (105.65, 111.2, 132.67 and 145.00 iu/*l*) and aspartate transaminase (68.68, 70.00, 78.00 and 85.67 iu/*l*). In conclusion, 10% TAPSM in broiler diets most favourably influenced haematological and biochemical parameters.

Key words: *Adenanthera pavonina*, toasted, finisher broilers, haematology, blood chemistry.

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Introduction

There is a need for readily available, high quality alternative plant proteins that are inexpensive and capable of reducing production cost of meat and other animal products. Intensive poultry farming in Nigeria has been greatly affected by high cost of feeds and feed ingredients, especially the protein and energy sources like soybean, groundnut cake and maize (Ani and Adiegwu, 2005). This has been blamed on the competition between man, livestock and industries on conventional feedstuff (Akinmutimi, 2004). The importance of nutrients in intensive poultry production is evidenced by the fact that 75–80% of production cost is due to feed (Iheukwumere, 2008).

A common solution cost is by using locally available and cheap sources of feed ingredients, particularly those that do not involve competition between humans and livestock. One of the problems with the use of legumes is the presence of anti-nutritional factors (ANF). *Adenanthera pavonina* seeds have been reported to contain anti-nutrients such as taninns, phenols, cyanogenic glycosides, saponins, trypsin inhibitors and haemagglutinins (Umezuruike, 2006). Most of the processing methods employed to improve the feed value of alternative feedstuff do not completely eliminate the anti-nutrients, but only reduce their concentration to a tolerable level in the feedstuff (Akinmutimi, 2004).

Adenanthera pavonina, commonly called coral bean tree or red sandal wood, belongs to the family *Fabaceae*, sub-family *Mimisoideae*. The seed of *Adenanthera pavonina* contains appreciable amounts of protein, crude fat and minerals compared to commonly consumed staples (Ezeagu et al., 2004). It has also been reported that 25% of the seed weight is oil, with a high digestibility in animals and humans. The roasted seeds are eaten by humans while the leaves are used as fodder for animals. Apart from that, it is also used for medicinal, timber and ornamental purposes (Orwa et al., 2009). There is an active search for alternatives to conventional feed raw materials in animal feeding. This includes evaluation of leguminous seeds (Camara et al., 2003; Kwari et al., 2011; Emiola et al., 2013;Agbabiaka et al., 2013; Ukpabi et al., 2015), leaf meal (Esonu et al., 2006; Madubuike and Ekenyem, 2006; Ukpabi et al., 2009; Obikaonu et al., 2012; Onunkwo and George, 2015;) and by-products from food and ethanol industries (Amaefule et al., 2006; Alu et al., 2013; Adeyemo and Sani, 2013). The raw seed of *Adenanthera pavonina* had been reported to negatively affect the haematological and serum biochemistry of finisher broilers at levels exceeding 5% in the diet (Ukpabi et al., 2015).

Haematological and biochemical indices are essential indicators of health status in animals and have been indispensable in the diagnosis and treatment of many diseases (Emiola et al., 2013). Haematology and blood chemistry are also important tools for assessing the quality of feed and the health status of animals

that are placed on experimental diets (Merck, 2010). Thus, the aim of this study was to examine the effect of toasted *Adenanthera pavonina* seed meal (TAPSM) on the haematology and blood chemistry of finisher broilers.

Materials and Methods

Experimental site

The experiment was carried out at the Livestock Unit of the Faculty of Agriculture Teaching and Research Farms, Abia State University, Umudike Campus, Nigeria. Umudike has coordinates of 7°31¹ East and 5°28¹ North, and lies at an altitude of 122 meters above sea level (Adiele et al., 2005).

Procurement of feed ingredients

Adenanthera pavonina seeds were gathered from the premises of the Abia State University, Umudike Location and the National Institute for Horticulture (NIHORT), Mbato in Okigwe, Imo State. Other feed ingredients were purchased from a commercial feed shop in Umuahia.

Processing of *Adenanthera pavonina* seeds

The raw seeds were sorted and toasted for 15 minutes at a temperature range of 60−65°C, and the timing started immediately when the seeds were poured into the toaster. The *Adenanthera pavonina* seeds were cooled before being milled with a hammer mill. The toasted APSM was then weighed according to formulation before being incorporated in the diets.

Experimental design

A total of eighty-four, 5-week old Anak broiler chickens were used for the experiment. The birds were divided into four groups of twenty-one birds each and assigned to the four treatment diets in a completely randomized design (CRD). Each group was further sub-divided into three replicates of seven birds each. Feed and water were offered *ad libitum* and the feeding trial lasted 5 weeks.

Experimental diets

Four experimental diets were formulated by incorporating TAPSM at 0, 10, 20 and 30% dietary levels for T_1, T_2, T_3 and T_4, respectively. The gross composition of the experimental diets is shown in Table 1. The experimental diets and TAPSM

were subjected to proximate analyses following the procedure of A.O.A.C. (2006) and are presented in Table 2.

Table 1. Composition of the experimental diets (%).

Ingredients	TAPSM inclusion levels in diets (%)			
	T_1 (0)	T_2 (10)	T_3 (20)	T_4 (30)
Maize	50.00	45.00	40.00	35.00
Soybean meal	25.00	20.00	15.00	10.00
TAPSM	0.00	10.00	20.00	30.00
Blood meal	2.00	2.00	2.00	2.00
Palm kernel cake	14.00	14.00	14.00	14.00
Fish meal	4.00	4.00	4.00	4.00
Palm oil	1.00	1.00	1.00	1.00
Bone meal	3.00	3.00	3.00	3.00
Premix*	0.30	0.30	0.30	0.30
Salt	0.50	0.50	0.50	0.50
Methionine	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00
Calculated composition				
Crude protein (%)	23.03	23.37	21.71	21.05
ME (MJ/kg)	12.43	12.02	11.61	11.19

*Vitamin mineral premix provides per kg diet: vit. A, 13.340 iu, vit. D_3 2680 iu, vit. E iu, vit. K, 2.68 iu, Calcium panthonate, 10.68 mg, vit. B_{12} 0.022 mg; Folic acid, 0.668 mg; Choline chloride 400 mg; Chlorotetracycline, 26–28 mg; Manganese, 133.34 mg; Iron, 66.68 mg; Zinc, 53.34 mg; Copper, 3.2 mg; Iodine, 1.86 mg; Cobalt, 0.268 mg; Selenium, 0.108 mg. ME = Metabolizable energy (MJ/kg), calculated according to Pauzenga (1985) as ME (MJ/kg) = 37 × % CP + 81 × % EE + 35.5 × % NFE (Folorunso et al., 2016) TAPSM = Toasted *Adenanthera pavonina* seed meal.

Table 2. Determined proximate composition of experimental diets and toasted *Adenanthera pavonina* (L) seed meal (TAPSM).

Parameter	TAPSM inclusion levels in diets (%)				TAPSM
	T_1 (0)	T_2 (10)	T_3 (20)	T_4 (30)	
Dry matter	94.16	94.18	94.20	94.20	92.00
Crude protein	21.05	20.04	20.02	20.00	20.08
Crude fibre	11.00	11.16	13.04	15.00	14.00
Ether extract	9.12	8.66	8.65	8.16	9.04
Ash	9.52	9.04	8.56	8.51	3.10
NFE	43.47	45.28	44.16	42.92	45.78
ME (MJ/kg)*	12.84	12.79	12.77	12.84	13.00

TAPSM = Toasted *Adenanthera pavonina* seed meal; NFE = Nitrogen-free extracts; *ME = Metabolizable energy, calculated according to Pauzenga (1985) as ME (MJ/kg) = 37 × % CP + 81 × % EE + 35.5 × % NFE (Folorunso et al., 2016).

Blood sample collection and analysis

At the end of the experiment, one bird per replicate was randomly selected making a total of 12 birds. Blood samples (7.0 ml) for analysis were collected from under the wing veins of birds using 10-ml plastic disposable syringes for which 2ml were collected into a bijour bottle treated with ethylene diamine tetra acetic acid (EDTA) for the haematological assay (packed cell volume, haemoglobin, white blood cell and red blood cell) and 5mls of blood each were collected into EDTA-free bottles for serum biochemistry (total protein, blood glucose, blood urea and serum enzymes). Haematological indices were determined by the methods of Jain (1986). These are the Wintrobe'smicrohaematocrit kit for packed cell volume (PCV), the cyanmethaemoglobin method for haemoglobin (Hb), an improved Neubaur haemacytometer for red blood cells (RBCs) and white blood cells (WBCs) while mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration were calculated from PCV, RBC and Hb. Standard methods of serum separation were used to obtain sera which were used to determine urea, creatinine, total protein, glucose, cholesterol and serum enzymes (Kohn and Allen, 1995)

Data analysis

Data obtained were subjected to statistical analysis using a one-way analysis of variance (ANOVA) as outlined in Steel and Torrie (1980). The Duncan's multiple range test was used to separate significant treatment means where they occurred (Obi, 1990).

Results and Discussion

The haematological parameters of finisher broilers fed diets containing different levels of TAPSM are presented in Table 3. The values for total red blood cell, total white blood cell, Hb, PCV and MCH showed significant ($P<0.05$) differences among treatment means.

The PCV ranged from 28.33% in T_1 (0%) to 25.20% in T_4 (30%). The values of PCV obtained fall within the recommended range of 23−55% for healthy birds (Maxwell et al., 1990; Banerjee, 2005). The results of PCV in this study were in line with the results of Alibi et al. (2011) who reported 25−29.00% PCV in finisher broiler chickens. Low PCV in birds is an indication of iron deficiency (Iyayi et al., 2006). Since the values obtained were normal for healthy birds, it suggested that the experimental birds were not anaemic.

The WBC values obtained from this study ranged from 71.76 ($x10^3/\mu l$) in T_1 to 76.17 ($x10^3/\mu l$) in T_4. There were significant ($P<0.05$) increases in the WBC as

the level of TAPSM increased in the diet. A similar trend of a significant increase ($P<0.05$) in WBC with an increase in the level of *O. gratissium* in broiler chickens has been reported (Odoemelam et al., 2014). The increase in WBC as inclusion of TAPSM increased in the diet shows that the principal function of phagocytes which is to defend against invading microbes will be enhanced (Adedapo et al., 2012) or that the birds were reacting to inflammatory conditions resulting from the diets (Agbalaya et al., 2017).

Table 3. Haematological parameters of finisher broilers fed graded levels of toasted *Adenanthera pavonina* seed meal.

Parameter	TAPSM inclusion levels in diets (%)				SEM
	T_1 (0)	T_2 (10)	T_3 (20)	T_4 (30)	
PCV (%)	28.33[a]	26.10[b]	25.60[c]	25.20[c]	3.52
WBC ($x10^3$/µl)	71.76[b]	73.4[b]	75.07[a]	76.17[a]	4.25
RBC ($x10^6$/µl)	4.23[a]	4.00[a]	3.99[b]	3.99[b]	0.85
Hb (g/dl)	10.20[a]	9.03[b]	8.60[c]	7.63[d]	0.44
MCV (fl)	66.97[a]	65.25[a]	64.16[b]	63.15[b]	0.86
MCH (pg)	24.11[a]	22.57[b]	21.55[c]	19.12[d]	0.70
MCHC (%)	35.66[a]	34.59[b]	33.59[c]	30.27[d]	1.55

[a, b, c, d] Means in the same row with different superscripts differed significantly ($P<0.05$). TAPSM – Toasted *Adenanthera pavonina* seed meal, SEM – The standard error of the means, PCV = Packed cell volume, WBCs = White blood cells, RBCs = Red blood cells, Hb = Haemoglobin, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration.

Red blood cell values for birds on T_1 were significantly ($P<0.05$) different and higher than for those on diets T_2, T_3 and T_4. The red blood cells (RBCs) are responsible for the transportation of oxygen and carbon dioxide in the blood, hence higher values indicate a greater potential for this function and a better state of health (Olugbemi et al., 2010). A low value of RBC observed in birds fed diets containing 30% TAPSM may be attributed to a high level of TAPSM in the diet or due to low quality of feed and protein deficiency (Awoniyi et al., 2000). The values for RBC obtained ranged from 4.23 ($\times10^6$/µl) in T_1 to 3.99 ($\times10^6$/µl) in T_4. The values obtained in this study were within the normal reference values of 2.0–4.0 reported for chickens (Banerjee, 2005).

The haemoglobin values obtained ranged from 7.63g/dl in T_4 to 10.20g/dl in T_1. Treatment T_1 had the highest value which was significantly ($P<0.05$) different from T_2, T_3 and T_4. Haemoglobin values decreased as the level of TAPSM in the diet increased. The results fall within the recommended haemoglobin concentration of 7−18.6g/dl for healthy birds (Pellet and Young, 1980). A reduction in the haemoglobin values obtained in birds fed diets 3 (20% TAPSM) and 4 (30%TAPSM) indicated a possibility of poorer transportation of oxygen from the respiratory organs to peripheral tissues and carbon dioxide for excretion (Murray, 2009).

Mean corpuscular volume (MCV) values obtained were: 6.97 fl for T_1, 65.25 fl for T_2, 64.16 fl for T_3 and 63.15 fl for T_4. There was no significant ($P>0.05$) difference between T_1 and T_2 but they differed significantly ($P<0.05$) from T_3 and T_4 which were similar ($P>0.05$). The values obtained were higher than the values reported in literature (Oko et al., 2011).

Mean corpuscular haemoglobin (MCH) values showed significant ($P<0.05$) differences among the treatments. The values obtained decreased as the level of TAPSM in the diets increased from 0 to 10%. Values of MCH in this study were below the range of 53−97 pg reported in literature (Orwa et al., 2009). Since MCH is an indicator of the oxygen carrying ability of the RBC (Ugwuene, 2011), the blood of the birds fed diets 1 (0%) and 2 (10%) may be more efficient than the blood of others in performing respiratory functions.

The mean corpuscular haemoglobin concentration (MCHC) values were 35.66% for T_1, 34.59% for T_2, 33.59% for T_3 and 30.27% for T_4. The values decreased significantly ($P<0.05$) as the level of TAPSM increased in the diet, which is an indication of the presence of anti-nutrients in the experimental diets, which invariably had an adverse effect on blood formation.

Biochemical parameters of finisher broilers fed graded levels of TAPSM are presented in Table 4. Serum biochemistry is a generalized medium of assessing the health status of animals (Frandson, 1981). There were significant differences ($P<0.05$) in the biochemical parameters measured in this study.

Table 4. Biochemical parameters of finisher broilers fed diets containing graded levels of TAPSM.

Parameter	Level of inclusion of TAPSM (%)				
	T_1 (0)	T_2 (10)	T_3 (20)	T_4 (30%)	SEM
Urea (mg/dl)	4.00^b	5.00^b	6.67^b	10.00^a	1.86
Creatinine (mg/dl)	0.33^b	0.25^c	0.43^a	0.46^a	0.04
Cholesterol (mg/dl)	149.67^a	135.67^b	113.67^c	102.67^d	9.87
Total protein (mg/dl)	3.33^a	3.43^a	2.97^b	2.50^c	0.15
Glucose (mg/dl)	146.14^b	208.49^a	179.66^a	135.33^b	8.53
ALP (iu/*l*)	105.65^d	111.20^c	132.67^b	145.00^a	2.85
AST (iu/*l*)	68.67^c	70.00^c	78.00^b	85.67^a	3.99

$^{a, b, c, d}$ Means in the same row with different superscripts differed significantly ($P<0.05$). TAPSM – Toasted *Adenanthera pavonina* seed meal, SEM – The standard error of the means, ALP – Alkaline phosphatase, AST – Aspartate transaminase.

The values obtained for urea in T_1, T_2 and T_3 were similar ($P>0.05$), but differed significantly ($P<0.05$) from T_4. High blood urea concentrations recorded in the birds as the level of TAPSM in the diets increased and as in T_4 may be due to the protein quality of TAPSM (Kecceci et al., 1998).

Creatinine is a waste product of muscle metabolism and a good measure of kidney function (Ologhobo et al., 1993). Creatinine values ranged from 0.46 mg/dl

in T_4 to 0.25 mg/dl in T_2 with T_4 having the highest value. The values of creatinine obtained in the present studies were lower than 1−2 mg/dl reported for chickens (Reece and Swenson, 2004), which therefore suggested that there was no muscle wastage and birds did not survive at the expense of body reserves (Akinmutimi, 2004).

Cholesterol values ranged from 149.67 mg/dl in T_1 to 102.67 mg/dl in T_4. The treatments differed significantly ($P<0.05$), the values obtained in birds fed diets T_1 (0%TAPSM) and T_2 (10% TAPSM) were within the normal range of 125−200 mg/dl reported for healthy birds (Bolu and Adelakun, 2013) while the values obtained in birds fed diets T_3 and T_4 were below the normal range of 125−200 mg/dl reported for healthy birds. *Adenanthera pavonina* seed meal contains high crude fibre and this factor may have accounted for the depressed cholesterol level obtained in birds fed diets T_3 and T_4. Intakes of 9−16.5 g/day of varieties of soluble fibres have been shown to produce net reductions in serum cholesterol levels in humans (Reece and Swenson 2004), and may also be applicable to birds.

Total protein values ranged from 3.43 mg/dl in T_2 to 2.50 mg/dl in T_4. There were significant ($P<0.05$) differences among the treatment means. The values obtained in T_1 and T_2 were within the normal range (4.0–5.2 mg/dl) reported for chickens (Reece and Swenson, 2004). Since total protein is usually a reflection of the protein quality fed, it would seem that the significant decrease with an increase in the level of TAPSM is an indication of the presence of a poor quality protein and poor protein utilization of test diets possibly caused by anti-nutritional factors in TAPSM.

Glucose values ranged from 135.33 mg/dl in T_4 to 208.49 mg/dl in T_2. The values recorded fall within the recommended range of 130–270 mg/dl for chickens (Reece and Swenson, 2004; Bolu and Adelakun, 2013). The values were also in line with the recommended range of 230–250 mg/100ml for birds (Rubin, 2011). The values obtained differed significantly ($P<0.05$) between treatments. Generally, birds can maintain a high and relatively constant blood sugar level even in low feed intake (Olomu, 1995)

Serum enzyme activities have been used as indices of toxicity as well as for monitoring protein quality. Alkaline phosphatase (ALP) values ranged from 145.00 iu/l in T_1 to 105.65 iu/l in T_4. There were significant ($P<0.05$) differences among the treatment means. The values increased as the level of TAPSM increased in the diets. ALP is predominantly found in the liver, kidney, intestine and placenta (Shipman et al., 2013). Aspartate transaminase (AST) values ranged from 68.67 iu/l in T_1 to 85.676 iu/l in T_4. The values increased significantly ($P<0.05$) among treatments, which is an indication that TAPSM had a negative effect on the functioning of the liver and the kidney (Tennant, 1997).

Conclusion

The results obtained from this study indicated that finisher broilers could tolerate the 10% TAPSM inclusion level in the diet without any negative effect on the haematology and serum biochemistry. Feeding of TAPSM to broiler chickens is safe as no mortality was recorded throughout the period of the experiment. *Adenantherapavonina* seed toasted for fifteen minutes at 60–65^{o}C did not satisfactorily eliminate the anti-nutrients in the seeds. Increased values of alkaline phosphatase in the blood across treatments suggested an increased activity of the liver due to the presence of toxic factors.

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UTICAJ SAČME TOSTIRANOG SEMENA BILJKE *ADENANTHERA PAVONINA* NA HEMATOLOŠKE I BIOHEMIJSKE PARAMETRE BROJLERA U ZAVRŠNOM TOVU

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R e z i m e

Ispitivan je uticaj različitih količina tostiranog semena biljke *Adenanthera pavonina* (engl. *toasted Adenanthera pavonina seed meal*– TAPSM) na hematološke i biohemijske indekse brojlera, u petonedeljnom ogledu sa 84 pilića (starih pet nedelja) raspoređenih u četiri grupe po 21 jedinke. Svaka grupa je imala tri ponavljanja sa 7 brojlera. Četiri ogledna obroka bila su formulisana tako da sadrže 0, 10, 20 odnosno 30% TAPSM, koji su predstavljeni kao T_1, T_2, T_3 odnosno T_4. Po modelu potpuno slučajnog plana, predviđena su četiri obroka za ptice, koje su bile smeštene u objektu sa dubokom prostirkom. Hrana i voda su pilićima bili ponuđeni *ad libitum*. Hematološki parametri su ukazali na značajan ($P<0,05$) pad hemoglobina (10,20, 9,03, 8,60 i 7,63 g/dl), hematokrita (28,33, 26,10, 25,60 i 25,20%), ukupnih eritrocita (4,23, 4,00, 3,99 i 3,99 $\times 10^6/\mu l$), prosečne zapremine eritrocita (66,97, 65,25, 64,16 i 63,15 fl), prosečne mase hemoglobina po eritrocitu (24,11, 22,57, 21,55 i 19,12pg) i srednje koncentracije hemoglobina u eritrocitu (35,66, 34,59, 33,59 i 30,27%) sa porastom nivoa TAPSM u obrocima. Ukupni leukociti (71,76, 73,40, 75,07 i 76,17 $\times 10^3/\mu l$) povećavali su se značajno ($P<0,05$) kako se nivo TAPSM povećavao. Druge vrednosti su bile kao što sledi: urea (4,00, 5,00, 6,67 i 10,00 mg/dl), kreatinin (0,33, 0,25, 0,43 i 0,46mg/dl), holesterol (149,67, 135,67, 113,67 i 102,67 mg/dl), ukupni proteini (3,33, 3,43, 2,97 i 2,50mg/dl), glukoza (146,14, 208,49, 179,66 i 135,33 mg/dl), alkalna fosfataza (105,65, 111,2, 132,67 i 145,00 iu/*l*) i aspartat transaminaza (68,68, 70,00, 78,00 i 85,67 iu/*l*). Da zaključimo, 10% TAPSM u obrocima za brojlere najpovoljnije je uticao na hematološke i biohemijske parametre.

Ključne reči: *Adenanthera pavonina*, tostiran, brojleri u završnom tovu, hematološki parametri, biohemijski parametri.

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ANALIZA SADRŽAJA MAKRO- I MIKROELEMENATA U VODI ZA PIĆE IZ VODOVODNE MREŽE GRADA POŽAREVCA

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Sažetak: Predmet ispitivanja bila je voda za piće iz vodovodne mreže Grada Požarevca, sa aspekta fizičko-hemijske i mikrobiološke ispravnosti i prisustva makro- i mikroelemenata. Ispitivanja su ukazala na povećanu koncentraciju Ca u vodi za piće kao posledica položaja Požarevca na sedimentu rečne terase akumulativnog karaktera t1, koja je pretežno sagrađena od *kvarcita*. Kao posledica povećane koncentracije Ca, voda iz vodovodne mreže je alkalnog karaktera („kalcijumova", „biokarbonatna voda") i veoma tvrda. Osim Ca, K i Al prevazilaze maksimalno dozvoljene koncentracije, dok se Pb i Cd nalaze na samoj granici. Proračunati podaci pokazuju rizik od unošenja elemenata koji se u vodi iz vodovodne mreže Grada Požarevca nalaze u vrednosti višoj od dozvoljene pravilnikom, ni za jedan metal ne postoji kratkoročni zdravstveni rizik po zdravlje ljudi. Analiza podataka o dugoročnom zdravstvenom riziku ukazuje da jedina realna opasnost postoji od prisustva Cd u vodi za piće. Rizik od pojave kancera prisutan je kod 202 stanovnika od 1000 stanovnika koji koriste ovu vodu za piće.

Ključne reči: voda za piće, kamenac, kalcijum, teški metali, Požarevac.

Uvod

Voda iz javnih vodovoda koristi se u različite svrhe: za piće, pripremu hrane, održavanje lične higijene i za druge potrebe u domaćinstvima, zatim za komunalne i industrijske potrebe i dr. Potrošnja vode za piće i pripremu hrane je mala u odnosu na ostale potrebe, ali to je voda za koju se mora obezbediti najbolji kvalitet, izuzimajući neke industrije koje zahtevaju isti takav kvalitet ili čak i bolji od njega.

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Međutim, brojnost potrošača vode za piće i moguć uticaj na njihovo zdravlje jesu razlozi zbog kojih se moraju postavljati uslovi za kvalitet vode u vodovodu u odnosu na zdravstvene efekte (Milojević, 2001).

Na području Grada Požarevca postoje tri načina vodosnabdevanja stanovništva (Rajković et al., 2015): 1. Centralno vodovodsko snabdevanje u gradovima Požarevac i Kostolac (od 1980. godine) i seoskim naseljima Ćirikovac, Knenovik, Stari Kostolac, Drmno i Brodarac (seoski vodovod koje Vodovod održava); 2. Snabdevanje iz lokalnih vodnih objekata (javne česme), i 3. Individualno snabdevanje iz sopstvenih bunara u ostalim naseljima.

O prečišćavanju i distribuciji vode u Požarevcu brine se JKP „Vodovod i kanalizacija", Požarevac (od 1961. godine), a u okviru ovog preduzeća sektor Vodovod. Delatnosti sektora Vodovod su distribucija (merenje i registrovanje isporučenih količina) vode od izvorišta do potrošača i proizvodnja vode, kao i kontrola kvaliteta vode: fizičko-hemijska i mikrobiološka analiza, kao i adekvatan izbor parametara kvaliteta (WHO, 2011; Rekalić, 1998). Vodovod se stara i o ispiranju vodovodne mreže, izradi i sprovođenju planova vodosnabdevanja, a sistem vodovoda eksploatiše u optimalnom režimu vodeći računa o gubicima.

Fizičko-hemijska i mikrobiološka ispravnost vode za piće u vodovodnoj mreži koja se isporučuje potrošačima redovno se ispituje i prati, dok se periodično ispituje i sadržaj toksičnih elemenata. Sadržaj teških (i toksičnih) metala nije parametar koji se određuje u vodi za piće, jer se teški metali nalaze u veoma niskim koncentracijama za koje se smatra da nemaju (negativan) uticaj na korisnike.

U vodi za piće nalaze se mnogobrojne neorganske supstance koje doprinose tvrdoći vode i utiču na njen kvalitet i higijensku ispravnost (Rajković, 2003). Postojeće metode za ispitivanje sadržaja (ili tragova) metala ne mogu detektovati prisustvo metala u niskim koncentracijama u vodi za piće, pa je zbog toga predložena nova metoda određivanja sadržaja metala koja je pokazala svoju potpunu primenljivost (Rajković et al., 2008a; Rajković et al., 2008b; Rajković et al., 2009). Suština metode je da se za ispitivanje kvaliteta vode iz vodovodne mreže koristi depozit (*kamenac*, suvi ostatak) koji se izdvaja na grejaču kućnog bojlera prilikom zagrevanja vode, a koji nastaje taloženjem neorganskih nevolatilnih supstanci koje se nalaze u vodi za piće. Pošto sastav kamenca potiče od prisutnih neorganskih jedinjenja u vodi, ovom metodom je moguće da se pouzdano, određivanjem elementarnog sastava kamenca, a zatim preračunavanjem na sadržaj u vodi odrede masene koncentracije metala koji se nalaze u vodi za piće.

Cilj ovoga rada bio je da se metoda indirektnog određivanja elemenata upotrebi za procenjivanje kvaliteta vode za piće sa aspekta sadržaja makro- i mikroelemenata tokom upotrebe vode za piće iz vodovodne mreže Grada Požarevca, u periodu 2012–2017. godine.

Materijal i metode

Uzorkovanje, metode ispitivanja i tumačenje rezultata urađeni su u skladu sa Pravilnikom o higijenskoj ispravnosti vode za piće (Rajković et al., 2008b; Sl.list SRJ br. 42/98 i 44/99; Sl.list SCG br. 53/05; Rajković, 2010). Uziman je zahvaćen uzorak vode u plastične boce, pri čemu uzorci nisu konzervirani (Rajković et al., 2012). Na samom mestu uzorkovanja merena je temperatura i provodljivost uzoraka vode (Sl.list SRJ br. 42/98). Radila se fizičko-hemijska analiza vode koja obuhvata sledeće parametre: miris, boju, pH vrednost, utrošak $KMnO_4$, mutnoću, nitrite, nitrate, amonijak, hloride, elektroprovodljivost, kao i mikrobiološka analiza (Rajaković-Ognjanović, 2016).

Analitičke metode i granica detekcije za sve ispitivane parametre prikazane su u tabeli 1 (EPA, 2015).

Tabela 1. Analitičke metode primenjene u radu i njihova granica detekcije.
Table 1. Analytical methods used in the paper and their detection limit.

Pokazatelj *Parameter unit*	Merna jedinica *Measurement unit*	Analitička metoda *Analytical methods*	Granica detekcije *Detection limit*	MDK vrednost *Maximum acceptable concentration* (MAC)
Amonijak	mg/dm^3	spektrofotometrijski sa Neslerovim reagensom		0,50
Boja	mg/dm^3 Pt/Co skale	spektrofotometrijski: kolorimetrijski pomoću Pt/Co skale	-	20
Elektroprovodljivost	µS/cm na 20ºC	konduktometrijski	-	2500
Hloridi, kao Cl^-	mg/dm^3	jon-selektivna elektroda (ISE)	0,01	250
Kolimorfne bakterije	broj/100 cm^3			0
pH vrednost	-	potenciometrijski, ISE, pH-metar CONSORT C830	-	6,50–8,00
Miris	-	organoleptički	-	bez
Mutnoća	NTU jedinica	metoda A: turbidimetrijski sa silikatnom zemljom metoda B: nefelometrijski prema standardnom formazinskom polimeru	-	4
Oksidativnost	mg O_2/dm^3	potrošnja $KMnO_4$, titracijom prema Kubel-Tijemanu		do 8 mg/dm^3
Ukus	-	organoleptički	-	bez
Nitrati, kao NO_3^-	mg/dm^3	jonska hromatografija (IC)	0,01	0,5
Nitriti, kao NO_2^-	mg/dm^3	jonska hromatografija (IC)	0,01	0,005
Arsen, kao As	mg/dm^3	AAS hidridna tehnika	0,01	0,01
metali: Ca, Mg, Fe, Mn, Al, Pb, Zn, Cu, Ni, Cd, Cr	mg/dm^3	atomska apsorpciona spektrofotometrija (AAS)	0,01	0,01
Temperatura vode	°C	živin termometar		
Mineralizacija (ili *suvi ostatak* na 180°C) je ukupni maseni sadržaj svih prisutnih materija u vodi.	g/dm^3	Na analitičkoj vagi se izmeri čaša iz koje će uparavati uzorak. Uparavanje 100 cm^3 uzorka se vrši na vodenom kupatilu do suva. Potom se ostatak u čaši suši 8h u sušnici na 180°C. Čaša se hladi i meri.		

Depozit (*kamenac*, suvi ostatak) koji je ispitivan u radu nastao je iz vode koja se nalazi u vodovodnoj mreži Grada Požarevca (44°36′33″ N, 21°10′34″ E), tokom vremenskog perioda od 2012. do 2017. godine.

Kamenac dobijen je tako što je 1 dm^3 vode za piće (sakupljene na mesečnom nivou) zagrevan do ključanja i uparavan do suva. Sastav tako dobijenog kamenca određen je upotrebom atomskog apsorpcionog spektrofotometra Perkin Elmer *AAnalyst* 300 Atomic Absorption Spectrometer, prema standardu SRPS B.B8.070:1970 (dopunjen sa SRPS B.B8.070:1982) (Rajković et al., 2004; Rajković et al., 2008a; Rajković et al., 2009).

Na osnovu sadržaja elemenata u kamencu, koji je dobijen iz vode iz vodovodne mreže Grada Požarevca, izvršen je proračun njihove koncentracije u vodi za piće.

Rezultati i diskusija

Geološka karta Grada Požarevca

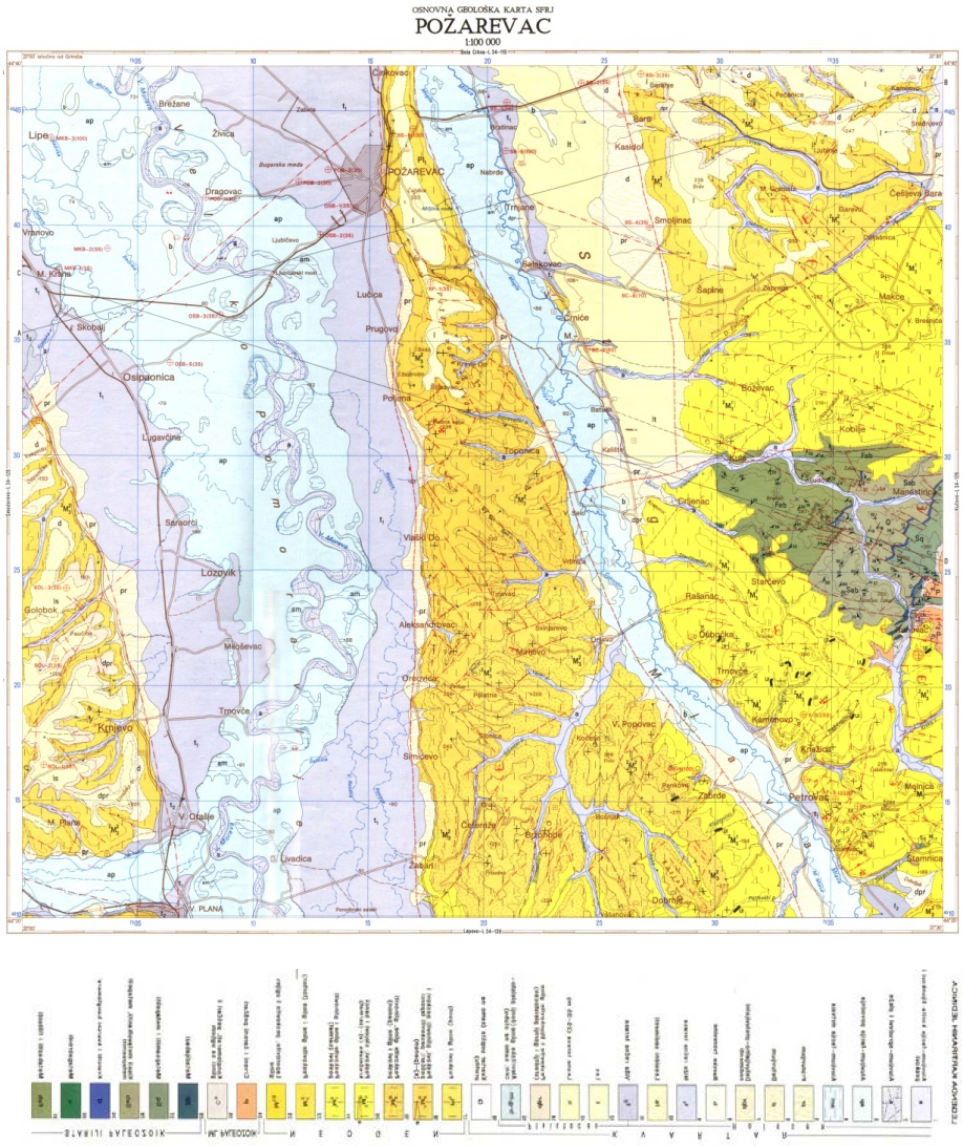
Na slici 1 prikazana je geološka karta i legenda Grada Požarevca (Rakić, 1984; Jelenković, 1999). Kao što se može videti sa slike 1, Grad Požarevac leži na sedimentima rečne terase t1, a desno su stene pontske serije (Rakić, 1984; Jelenković, 1999).

Formiranje **niže rečne terase (t1)** započelo je najverovatnije krajem najmlađeg pleistocena, a glavno formiranje je izvršeno u toku starijeg holocena. Terasa je akumulativnog karaktera i izgrađena je od dva dela. U donjem delu nalaze se peskoviti šljunkovi i šljunkoviti peskovi sa valucima veličine i do 5 cm u prečniku. Petrografska proučavanja utvrdila su pretežno prisustvo *kvarcita* i *rožnaca*. Među mineralima teške frakcije preovlađuju metalični minerali (22−50%), zatim amfiboli (14−26%) i granati (oko 14%), dok su ostali zastupljeni sasvim malo. Kod lake frakcije najčešći su kvarc, feldspat i alterisana mineralna zrna.

Gornji deo terase predstavljen je povodanjskom facijom koja je sastavljena od alevritskih peskova, peskovito-glinovitih alevrita i peskovitih glina. Imaju istu mineralnu asocijaciju kao i sedimenti facije korita. Na površini terase, mestimično uz njen obod, javljaju se **lesoidni sedimenti** kao produkti deluvijalnih spiranja kopnenih lesova na obodu doline. Debljina terase je različita: na potezu Žabari − Donja Livadica iznosi oko 12 m, a nizvodno od Goloboka i Poljane ona se naglo povećava i iznosi oko 35 m.

Tvorevine **PONTA (Pl1)** otkrivene su na severnom delu Požarevačke grede, na ukupnoj površini oko 15 km^2. Leže preko panonskih sedimenata, iz kojih se postepeno i razvijaju. Osnovni sastav ponta čine: peskovi, peskovite i ugljevite gline, retkih proslojaka peščara i ugljeva. Najzastupljeniji su srednjezrni i sitnozrni,

liskunoviti, sivo-žuti peskovi koji se javljaju i u setovima od nekoliko desetina metara debljine.



Slika 1. Geološka karta Grada Požarevca sa legendom.
Figure 1. Geological map of the city of Požarevac with the legend.

Sedimenti ponta blago tonu u pravcu SSZ gde ih u dolini Velike Morave pokrivaju debele fluvijalne akumulacije. Padni uglovi se kreću u granicama od 2 do 8 stepeni. Istražnim bušenjima utvrđeno je prisustvo pontijskih sedimenata i istočno od Požarevačke grede, u dolini reke Mlave. Debljina pontijskih sedimenata iznosi oko 150 m. Ovi sedimenti predstavljaju podinsku seriju glavnom, trećem ugljenom sloju „kostolačke produktivne serije". Sadrže, pored karakteristične ostrakodske vrste, i bogat palinološki spektar karakterističan za donji pliocen.

Rezultati ispitivanja vode za piće iz vodovodne mreže Grada Požarevca

U tabeli 2 prikazani su rezultati fizičko-hemijskog ispitivanja, a u tabeli 3 mikrobiološkog ispitivanja vode za piće iz vodovodne mreže Grada Požarevca.

Proračunate koncentracije elemenata u vodi za piće

Rezultati ispitivanja kamenca nastalog u periodu 2012−2017. godine, dobijenog iz vode koja se nalazi u vodovodnom sistemu Grada Požarevca prikazani su u tabeli 4. Na osnovu rezultata ispitivanja kamenca, proračunate su koncentracije elemenata u vodi za piće i upoređene sa pravilnikom Republike Srbije i standardom EU i WHO, što je prikazano u tabeli 5.

U pravilnicima nije definisana maksimalno dozvoljena količina za HCO_3^-, budući da nije utvrđen negativni uticaj na zdravlje čoveka.

Tabela 2. Rezultati fizičko-hemijskog ispitivanja vode za piće iz vodovodne mreže Grada Požarevca.
Table 2. Results of the physical and chemical analysis of drinking water from the Požarevac city public water supply.

Broj uzorka *Number of samples*	Miris *Smell*	Ukus *Taste*	Mutnoća *Turbidity*	Boja *Colour*	pH vrednost *pH value*	Nitrati *Nitrates*	Nitriti *Nitrites*	Amonijak *Ammonia*	Hloridi *Chlorides*	Utrošak KMnO4 *Consumption of* KMnO4	Elektroprovodljivost *Electroconductivity*
MDK (*MAC*)	-	-	NTU	Co-Pt	-	mg/dm^3	mg/dm^3	mg/dm^3	mg/dm^3	mg/dm^3	µS
	bez	bez	5	5	6,8−8,5	50,0	0,03	1,0	200,0	8,0	do 1000
uzorak 1 *sample 1*	bez	-	<0,10	<5	7,18	44,60	<0,005	<0,050	22,70	1,93	861
uzorak 2 *sample 2*	bez	-	<0,10	<5	7,20	44,80	<0,005	<0,050	27,10	2,18	866

Tabela 3. Rezultati mikrobiološkog ispitivanja vode za piće iz vodovodne mreže Grada Požarevca.
Table 3. Results of microbiological analysis of drinking water from the Požarevac city public water supply.

Parametar *Parameter*	Ukupan broj aerobnih mezofilnih bakterija u 1cm^3 vode *Total number of aerobic mesophilic bacteria in 1 cm^3 of water*	Ukupne koliformne bakterije u 100 cm^3 vode *Total coliform bacteria in 100 cm^3 of water*	Koliformne bakterije fekalnog porekla *Coliform bacteria of faecal origin*	Streptokoke fekalnog porekla u 100 cm^3 vode *Faecal streptococci in 100 cm^3 of water*	Proteus vrste u 100 cm^3 vode *Proteus species in 100 cm^3 of water*	Pseudomonas aeruginosa u 100 cm^3 vode *Pseudomonas aeruginosa in 100 cm^3 of water*	Sulfidoredukujuće klostridije u 100 cm^3 vode *Sulfidoreduced clostridia in 100 cm^3 of water*
MDK (*MAC*)	100	10	0	0	0	0	0
uzorak 1 *sample 1*	<1	<1	<1	-	-	-	<1
uzorak 2 *sample 2*	<1	<1	<1	-	-	-	<1

Na osnovu podataka iz tabele 4, može se zaključiti sledeće: proračunata pH vrednost za piće veoma je visoka (9,07) i prevazilazi vrednosti predviđene našim pravilnicima (Sl.list SRJ br. 42/98 i 44/99; Sl.list SCG br. 53/05). Podaci o sadržaju kalcijuma u vodi za piće ukazuju da se radi o alkalnoj vodi (što je vidljivo i iz pH vrednosti). Sadržaj Ca^{2+}: 245,86 mg/dm^3 govori da se radi o „kalcijumovoj vodi" (za vrednosti Ca^{2+} >150 mg/dm^3), dok sadržaj HCO_3^-: 994,24 mg/dm^3 govori da se radi o „bikarbonatnoj vodi" (za vrednosti HCO_3^- >600 mg/dm^3) (Petrović et al., 2012).

Rezultati dobijeni preračunavanjem masene koncentracije u vodi za piće na osnovu sastava u kamencu pokazali su da ispitivana vode pripada kategoriji vode: vrlo *tvrde* (u vodi se nalazi 613,92 mg/dm^3 $CaCO_3$). Takođe, na osnovu tvrdoće vode (stara oznaka tvrdoće vode u nemačkim stepenima tvrdoće, °D) od 34,4, voda pripada kategoriji vrlo tvrde vode (>30°D) (Rajković, 2007).

Podaci o mineralizaciji, prikazani u tabeli 5, govore da se radi o malomineralnoj vodi, odnosno o vodi do 1 g/dm^3, koje se zahvataju pretežno iz karbonatnih stena – *krečnjaka* ($CaCO_3$) i *dolomita* ($MgCO_3 \cdot CaCO_3$). Podatak o elektroprovodljivosti (tabela 3) govori da je voda za piće iz vodovodne mreže Grada Požarevca bogata rastvorenim materijama (što odgovara podacima iz tabele 5).

Tabela 4. Koncentracija makro- i mikroelemenata u vodi za piće iz vodovodne mreže Grada Požarevca upoređene sa pravilnikom Republike Srbije i standardima SZO i EU.

Table 4. Concentrations of macro- and microelements in drinking water from the Požarevac city public water supply compared to the Rulebook of the Republic of Serbia, and WHO and EU Standards.

Parametar *Parameter*	Pronađeno u kamencu *Scale sample (% by mass)*	Izračunata masena koncentracija u vodovodnoj mreži Grada Požarevca *Calculated mass concentration in drinking water from the Požarevac city public water supply*	Jedinica *Unit*	Pravilnik o higijenskoj ispravnosti vode za piće *Rulebook on hygienic quality of drinking water* (Sl. list SRJ br. 42/98 i 44/99)	
				MDK za parametre u vodi za vodosnabdevanje *MAC for parameters in water for water supply*	MDK za parametre u oligomineralnoj flaširanoj vodi *MAC for parameters in oligomineral bottled water*
Kalcijum	53,17%, kao CaO	**245,86**	mg/dm^3	200	100
Magnezijum	1,87%, kao MgO	7,30	mg/dm^3	50	30
Ca/Mg		33,68		3–4	
Natrijum	0,017%, kao Na_2O	0,082	mg/dm^3	150	20
Kalijum	0,0031%, kao K_2O	0,017	mg/dm^3	12	10
Gvožđe	0,042%, kao Fe_2O_3	190	µg/dm^3	300	50
Silicijum	0,92%, kao SiO_2	**2,78**	mg/dm^3	–	–
Aluminijum	0,16%, kao Al_2O_3	**540**	µg/dm^3	200	0,05
Titan	<0,003%, kao TiO_2	**11,63**	µg/dm^3	–	–
Mangan	16,99 ppm	10,99	µg/dm^3	50	20
Olovo	14,99 ppm	**9,70**	µg/dm^3	10	–
Kobalt	15,49 ppm	**10,02**	µg/dm^3	0	0
Cink	219,88 ppm	142,26	µg/dm^3	3.000	100
Bakar	103,44 ppm	66,93	µg/dm^3	2.000	100
Nikal	14,99 ppm	9,70	µg/dm^3	20	10
Kadmijum	4,50 ppm	**2,91**	µg/dm^3	3	5
Cr (ukupni)	3,5 ppm	2,26	µg/dm^3	50	50
Gubitak žarenjem	43,77%				
H_2O	0,95%				
Σ	99,99%	263,17		255,80	130,42
Suvi ostatak		647	mg/dm^3		
HCO_3		994,24	mg/dm^3	–	–
pH		**9,07**		6,80–8,50	6,80–8,50

Tabela 4. Nastavak.
Table 4. Continued.

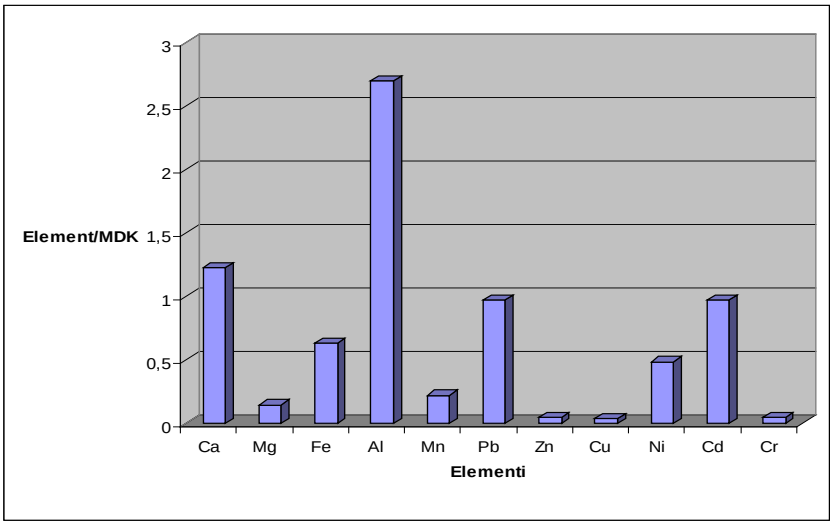
Pravilnik o kvalitetu i dr., zahtevima za prirodnu mineralnu, izvorsku i stonu vodu *Rulebook on quality, etc., requirements for natural mineral, spring and table water* (Sl. list SCG br. 53/05)	Direktiva EU *EU Directive*			
	1998/83/EC Voda za piće *Drinking water*	2003/40/EC Mineralna voda *Mineral water*	2009/54/EC Prirodna mineralna voda *Natural mineral water*	SZO voda za piće *WHO drinking water*
150	–	–	<150	–
50	–		<50	50
200	200	–	<200	200
–	–	–	–	–
200	200	–	–	300
–	–	–	–	–
200	200	-	–	200
–	–	–	–	–
50	50	500	–	400
10	10	10	–	10
–	–	–	–	–
–	–	–	–	3.000
2.000	2.000	1000	–	2.000
20	20	20	–	70
3	5	3	–	–
50	50	50	–	50
202,73				
600	–		<600	–
-	≥6,50–≤9,50	-	–	-

Na osnovu geološke analize sastava zemljišta na teritoriji Grada Požarevca, može se objasniti prisustvo karbonata i Si u vodi za piće iz lesnih sedimenata, u kojima ima kalcita. Kvarciti i rožnaci su SiO_2 po hemijskom sastavu, što objašnjava prisustvo Si u vodi za piće.

Unos elemenata u čovekov organizam konzumiranjem vode za piće

Na osnovu podataka iz tabele 5, može se videti da je sadržaj pojedinih elemenata viši u odnosu na MDK vrednost koja je predviđena pravilnicima (Sl.list SRJ br. 42/98 i 44/99; Sl.list SCG br. 53/05), pa je zbog toga izračunavan odnos sadržaj elemenata u vodi za piće/MDK vrednost, što je grafički prikazano na slici 2.

Na osnovu podataka sa slike 2, može se videti da *zemnoalkalni element* Ca (245,86 prema 200 mg/dm^3), *alkalni element* K (16,65 prema 12 mg/dm^3), od predstavnika **s-elemenata**, i Ti (11,63 prema 0 μg/dm^3) i Co (10,02 prema 0 μg/dm^3), od predstavnika **d-elemenata**, svojim sadržajem prevazilaze vrednosti koje su dozvoljene pravilnicima (Sl.list SRJ br. 42/98 i 44/99; Sl.list SCG br. 53/05). Od predstavnika **p-elemenata**, Al značajno prevazilazi dozvoljenu vrednost (540 prema 200 μg/dm^3) i Si (2,78 prema 0 mg/dm^3). Takođe, kritične su i vrednosti za Cd i Pb, upravo zbog velike toksičnosti i kancerogenosti ovih elemenata, a čije su vrednosti svega 3% niže od dozvoljenih.



Slika 2. Sadržaj makro- i mikroelemenata koji se nalaze u vodi za piće u vodovodnoj mreži Grada Požarevca u poređenju sa dozvoljenim koncentracijama, saglasno Pravilnicima.

Figure 2. The contents of macro- and microelements in drinking water from the Požarevac city public water supply, compared to the allowed concentrations, according to the Rulebooks.

Iz tih razloga izvršena je analiza uticaja korišćenja vode za piće iz vodovodne mreže Grada Požarevca sa sadržajem makro- i mikroelemenata na zdravlje ljudi (tabela 4), konzumiranjem u dužem vremenskom intervalu.

Unos elemenata u čovekov organizam konzumiranjem vode za piće izračunat je na osnovu podataka da *zapremina jedne čaše* iznosi 200 cm^3 (0,2 dm^3), a da *dnevni unos* pretpostavlja da čovek normalno unosi 10 čaša vode (2 dm^3 vode). Proračun je zasnovan na osnovu izračunatog sadržaja odgovarajućeg elementa na osnovu *suvog ostatka*. *Mesečni unos* baziran je na 30 dana, a *godišnji unos* baziran je na 12 meseci (365 dana).

Unošenje elemenata (u mg) u čovekov organizam konzumiranjem vode za piće iz vodovodne mreže Grada Požarevca na dnevnom, mesečnom i godišnjem nivou prikazano je u tabeli 5.

Podaci iz tabele 5 govore da je unos *zemnoalkalnih metala* u čovekov organizam tokom konzumiranja vode za piće na godišnjem nivou u ispitivanom vremenskom intervalu (u %) dominantan. Vodom za piće najviše se unose *zemnoalkalni metali* (182,28 g/dm^3 ili 96,02%), **više od 24 puta od svih ostalih elemenata zajedno**, što nedvosmisleno govori o velikoj tvrdoći vode za piće iz vodovodne mreže Grada Požarevca, što je zapaženo i prikazano u tabeli 4.

Tabela 5. Unošenje elemenata u čovekov organizam konzumiranjem vode za piće iz vodovodne mreže Grada Požarevca na dnevnom, mesečnom i godišnjem nivou.
Table 5. Daily, monthly and annual human intakes of elements from drinking water of the Požarevac city public water supply.

Elemenat *Element*	Trivijalni naziv grupe elemenata u PSE *The trivial name of the group of elements in PSE*	Unošenje elemenata u čovekov organizama konzumiranjem vode za piće *The intake of elements in human organism based on the consumption of drinking water*				
		1 čaša *One glass of water*	Dnevni unos *Daily intake*	Mesečni unos *Monthly intake*	Godišnji unos* *Annual intake*	Uneta masa elemenata *, u % *Mass of element intake, in %*
Na	*alkalni metali*	0,016 mg	0,16 mg	4,92 mg	59,04 mg	0,04
K		3,4 µg	0,03 mg	1,02 mg	12,24 mg	
Mg	*zemnoalkalni metali*	1,46 mg	14,6 mg	438 mg	5,26 g	96,0
Ca		49,17 mg	0,49 g	14,75 g	177,02 g	
Ti	*teški metali*	2,33 µg	23,26 µg	697,8 µg	8,37 mg	2,68
Cr (ukupni)		0,45 µg	4,52 µg	135,6 µg	1,63 mg	
Mn		2,2 µg	0,02 mg	0,66 mg	7,92 mg	
Fe		0,038 mg	0,38 mg	11,4 mg	0,14 g	
Co		2 µg	20,04 µg	0,60 mg	7,21 mg	
Ni		1,94 µg	19,4 µg	582 µg	6,98 mg	
Cu		1,34 mg	13,38 mg	401,4 µg	4,82 g	
Zn		0,03 mg	0,28 mg	8,4 mg	100,8 mg	
Cd		0,58 µg	5,82 µg	174,6 µg	2,08 mg	
Pb		1,94 µg	19,4 µg	0,58 mg	6,98 mg	
Al	*amfoterni metali*	0,11 mg	1,08 mg	32,4 mg	388,8 mg	≈ 0,21
Si	*polumetali*	0,56 mg	5,56 mg	166,8 mg	2,00 g	1,05
		Σ			189,83	≈ 100

*Godišnji unos je proračunat na osnovu mesečnog unosa, budući da se od proračuna na godišnji unosu na osnovu dana razlikuju za 1,37%.
**The annual intake is calculated on the basis of the monthly intake, as they differ by 1.37% from the annual intake based on the day.*

Podatak da je koncentracija Ca daleko iznad maksimalno dozvoljene koncentracije (≈ 1,23 puta viša), ipak ne mora da bude alarmantan, jer se na taj način dnevno, preko vode, unosi 49,17 mg Ca (oko 0,05 g), što je daleko ispod preporučene dnevne potrebe za Ca od 0,5 g.

Pošto se tri elementa nalaze u koncentracijama koje su iznad MDK vrednosti: K, Ca i Al, u daljem tekstu prodiskutovaće se njihov uticaj na zdravlje ljudi.

U organizmu odraslog čoveka nalazi se 0,2% **kalijuma** (oko 250 g). K zadržava vodu u organizmu, reguliše osmotski pritisak, učestvuje u održavanju kiselo-bazne ravnoteže, i reguliše potencijal ćelijske membrane. K se prvenstveno nalazi unutar ćelije (95%), a u međućelijskoj tečnosti ga ima oko 5%. K, koji se nalazi u tečnosti izvan ćelija, utiče na aktivnost mišića srca, pa je uloga K zbog toga veoma značajna. Učestvuje u funkcionisanju niza enzima, a naročito je važan za metabolizam ugljenih hidrata, za građenje puferskih sistema, procesa pojačanog izlučivanja Na i dr. K se lako resorbuje, ali se i vrlo lako izlučuje iz organizma. MDK vrednost za K u vodi za piće je oko 10% od vrednosti za Na (12 mg/dm^3), jer se unosi u velikim količinama i hranom, pa se relativno lako zadovoljavaju potrebe čoveka koje iznose 2−4 g/dan.

U vodi za piće iz vodovodne mreže Grada Požarevca nađeno je ≈ 1,4 puta više, što može prouzrokovati tegobe kod konzumenata. Prekomerna količina K u krvi (*hiperkalijemija*) izaziva slabu podražljivost, a time i slabost srčanog i ostalih mišića u telu. A zbog slabosti rada srčanog mišića i neadekvatnog pumpanja krvi kroz pluća nastaje otežano disanje, gušenje vodom u plućima, i životna ugroženost. Mišićna slabost, paralize, mučnina i zamor samo su prateći fenomeni glavnih, tj. kardiovaskularnih poremećaja. Pri manjem porastu K srčani ritam se usporava, a pri većem postaje nepravilan i na kraju ekstremno brz, a srce slabo i neefikasno (Pizent and Butković, 2010).

Kalcijum se u organizmu čoveka nalazi u velikim količinama, 1,5−2% telesne mase čoveka, npr. čovek telesne mase od 70 kg sadrži 1,2 kg Ca, a od toga 99% se nalazi u skeletu, kostima i zubima (tzv. koštana masa) (oko 80% kalcijuma se nalazi u obliku $Ca_3(PO_4)_2$, a 13% u obliku $CaCO_3$), i u obliku hidroksiapatita (Rajković, 2002). Ostatak od 1% Ca nalazi se u telesnim tečnostima i mekim tkivima, delimično u obliku jona, delimično vezan za proteine (albumine). Ca^{2+}-jon ima važnu ulogu u koagulaciji krvi (u suprotnom bolest *hemofilija*), permeabilnosti ćelijskih membrana, osetljivosti srca, mišića i nerava. Ca takođe igra značajnu ulogu u kontrakciji mišića, otpuštanju hormona i osiguravanju pravilnog funkcionisanja mozga i živaca. Baš kao što je moguće patiti zbog nedostatka Ca u telu, tako je moguće susresti se i sa njegovim viškom (*hiperkalcemija*).

Resorbuje se samo 10−30% Ca iz hrane, a resorpcija se vrši u gornjim delovima crevnog trakta. MDK vrednost za Ca u vodi za piće je najveća i iznosi 200 mg/dm^3 a uslovljena je pre svega vrstom vode, da li je tvrda ili meka, ali

stepen usvojenosti Ca od strane organizma zavisi od odnosa kalcijum:fosfor, koji mora biti 2:1, jer pri nižem odnosu fosfor vezuje Ca za sebe.

Osim preterane konzumacije Ca i sunčevog vitamina, do ovog stanja može doći usled preterane aktivnosti paratiroidne žlezde, raka i drugih bolesti. Najčešći simptom previše Ca je izuzetna letargija. Među ozbiljnije simptome *hiperkalcemije* spada nepravilan ritam otkucaja srca i vrlo nizak pritisak**.**

Aluminijum je sveprisutan u prirodi (najčešći metal u Zemljinoj kori) i prirodno se pojavljuje u većini prehrambenih namirnica i u vodi, a dnevna izloženost putem hrane je 3−10 mg. Al može reagovati sa niskim dozama fluora u vodi, dovodeći do toga da sve više Al prolazi krvno-moždanu barijeru stvarajući naslage u mozgu. Postoji veliki broj studija koje se tiču uticaja aluminijuma na zdravlje ljudi. Ove studije su odavno pokazale vezu između unošenja Al i neurološke demencije kod bubrežnih bolesnika, a studije novijeg datuma (iz poslednjih 10−15 godina, inače perioda kada se „zvanično" prestalo sa posmatranjem Al kao toksičnog) pokazuju njegov loš uticaj na ljudsko zdravlje, posebno naglašavajući njegovo učešće kod Alchajmerove bolesti, Parkinsonove bolesti i amiotropne lateralne skleroze (Lu Gerigova bolest), koje postaju uobičajene među starijim ljudima. Povišeni nivoi Al u mozgu nekih pacijenata od Alchajmerove bolesti su od nepoznate uzročno-posledične važnosti, pa je, radi zaključivanja povezanosti Al i Alchajmerove bolesti, potrebno dodatno istraživanje (Becaria, Campbell i Bondy, 2002).

Procena kratkoročnog i dugoročnog potencijalnog kancerogenog rizika

Na osnovu rezultata dobijenih ispitivanjem sadržaja mikrokomponenata u analiziranim uzorcima vode, može se uočiti da je u nekoliko uzoraka zabeležena povećana koncentracija pojedinih metala: Ca, K, Al, dok je kod Cd i Pb, ta vrednost jako blizu maksimalno dozvoljenim koncentracijama, u poređenju sa vrednostima propisanim Pravilnikom o higijenskoj ispravnosti vode za piće (tabela 5).

Da bi se utvrdila (eventualna) opasnost od prisustva toksičnih elemenata u koncentracijama višim od vrednosti dozvoljene Pravilnikom o higijenskoj ispravnosti vode za piće, urađena je procena **kratkoročnog** i **dugoročnog potencijalno kancerogenog rizika** (Rajković, Stojanović i Milojković, 2017a).

Kratkoročni rizik

Unos toksičnih elemenata i rizik po zdravlje ljudi, koji je prouzrokovan konzumiranjem vode za piće, određeni su na nedeljnom nivou (**kratkoročni rizik**), preko *procenjenog nedeljnog unosa vode* (**PNU**) i *koeficijenta rizika* po zdravlje ljudi (**KR**).

Ovi koeficijenti određeni su na osnovu sledećih jednačina (Lin et al., 2015):

$$\mathbf{PNU} = \frac{PPV \cdot c \cdot 7}{PTM} \qquad \mathbf{KR} = \frac{PNU}{TNU}$$

gde je: **PPV** – *prosečna potrošnja vode* po stanovniku (2 dm^3 dnevno) (Papić et al., 2012), *c* – *koncentracija elemenata* u ispitivanim uzorcima vode izražena u μg/dm^3, **PTM** – *prosečna telesna masa stanovnika* koja iznosi 75,65 kg (Pavlica et al., 2010), a **TNU** je *tolerantni nedeljni unos toksičnih metala* izražen kao μg/kg telesne mase.

Pri proceni kratkoročnog rizika po ljudsko zdravlje, smatra se da visok rizik postoji ukoliko je koeficijent rizika (KR) za neki elemenat veći od 1 (Leung et al., 2008; Kostić et al., 2016).

Dugoročni, potencijalno kancerogeni, rizik po zdravlje ljudi

Osim kratkoročnog rizika, prouzrokovanog konzumiranjem vode sa povišenim sadržajem toksičnih elemenata, moguće je odrediti i **dugoročni**, **potencijalno kancerogeni**, **rizik po zdravlje ljudi** (Wu i Sun, 2015).

Kao parametri za procenu ovog tipa rizika, određeni su *unos toksičnih elemenata oralnim putem* (konzumiranjem vode za piće), $\mathbf{U_{oral}}$, kao i *koeficijent rizika izazvan oralnim unosom toksičnih elemenata*, $\mathbf{KR_{oral}}$, preko sledećih jednačina:

$$\mathbf{U_{oral}} = \frac{PPV \cdot c \cdot 365 \cdot 30}{PTM \cdot 10950} \qquad \mathbf{KR_{oral}} = \frac{U_{oral}}{RfD_{oral}}$$

gde je: $\mathbf{RfD_{oral}}$ *referentna vrednost za unos kancerogenih i potencijalno kancerogenih kontaminenata oralnim putem* propisana od strane američke Agencije za zaštitu životne sredine (engl. *Environmental Protection Agency* [*EPA* ili USEPA]) (Momot i Synzynys, 2005; CHMP, 2008; Kostić et al., 2016), a skraćenice **PPV** i **PTM** su već objašnjene.

Procena kratkoročnog zdravstvenog rizika izražena preko tolerantnog nedeljnog unosa (TNU), procenjenog nedeljnog unosa (PNU) i koeficijenta rizika (KR) za potencijalno toksične metale prikazana je u tabeli 6. Pri proceni kratkoročnog rizika po ljudsko zdravlje smatra se da visok rizik postoji ukoliko je koeficijent rizika za neki elemenat veći od 1 (WHO, 2011; Rajković et al., 2004; Kostić et al., 2016b; WHO, 1998; ATSDR, 1997).

Na osnovu podatka o gustini metala, prikazane u tabeli 6, u teške metale ubrajaju se: Cd, Cu, Pb i Fe (jer im je gustina > 5 g/cm^3) (Rajković, 2002).

Na osnovu podataka za kratkoročni zdravstveni rizik i koeficijenata rizika za navedene elemente koji su pronađeni u povećanim koncentracijama, može se zaključiti da ni od jednog elementa ne preti kratkoročni zdravstveni rizik, jer su sve vrednosti KR daleko ispod 1. S obzirom na to da za Ca ne postoji podatak o

vrednosti za tolerantni nedeljni unos (TNU), jer se ne smatra toksičnim metalom (a nije ni teški metal), nije izračunata vrednost za koeficijent rizika.

Tabela 6. Kratkoročni zdravstveni rizik izražen kroz tolerantni nedeljni unos (TNU), procenjeni nedeljni unos (PNU) i koeficijent rizika (KR) za toksične metale.
Table 6. Short-term health risks expressed through tolerable weekly intake (TWI), estimated weekly intake (EWI) and risk coefficient (RC) for toxic metals.

		Kratkoročni rizik *Short-term risk*		
Elemenat *Element*	Gustina *Density* (g/cm^3)	Tolerantni nedeljni unos (TNU) *Tolerable weekly intake* (TWI) (µg/kg)	Procenjeni nedeljni unos (PNU) *Estimated weekly intake* (EWI)	Koeficijent rizika (KR) *Risk coefficient* (RC)
Ca	1,55	–	–	–
Cd	8,65	7	0,539	0,077
Cu	8,96	3500	12,38	$3{,}54\cdot10^{-3}$ ili << 1
Pb	11,34	25	1,795	0,072
Fe	7,86	5600	35,16	$6{,}28\cdot10^{-3}$ ili << 1
Al	2,702	7000	99,93	0,014

Procena dugoročnog zdravstvenog rizika izražena kroz oralni unos (U_{oral}), i koeficijenta rizika unosa (KR_{oral}) za potencijalno toksične metale prikazana je u tabeli 7.

Tabela 7. Dugoročni zdravstveni rizik izražen kroz oralni unos (U_{oral}) i koeficijent rizika unosa oralnim putem (KR_{oral}) za odabrane toksične metale.
Table 7. Long-term health risks expressed through oral intake (U_{oral}) and risk coefficient of oral intake (KR_{oral}) for selected toxic metals.

		Dugoročni rizik *Long-term risk*	
Elemenat *Element*	Referentna vrednost za unos oralnim putem *Reference value for oral intake* RfR_{oral} (mg/dan/kg)	Oralni unos *Oral intake* (U_{oral})	Koeficijenat rizika unosa oralnim putem *Risk coefficient of oral intake* (KR_{oral})
Cd	0,38	0,077	0,202
Cu	0,05	$1{,}77\cdot10^{-3}$	0,035
Pb	0,0085	$2{,}56\cdot10^{-4}$	0,030
Fe	– *	–	–
Al	– *	–	–

*Ne postoji RfD_{oral} za Fe i Al.
**There is no information of RfD_{oral} for Fe and Al.*

Na osnovu rezultata dobijenih za dugoročni potencijalni rizik za pojavu i razvoj kancerogenih oboljenja, mogu se uočiti razlike za ispitivane metale, u zavisnosti od procenjenog unosa oralnim putem (RfD_{oral}), kao i propisanih

referentnih vrednosti (ATSDR, 1997; EPA, 2009). U slučaju Fe i Al, nije moguće odrediti dugoročni rizik s obzirom na to da se ovi elementi ne nalaze na EPA listi potencijalno kancerogenih supstanci (ne postoji RfD_{oral} za Fe i Al), pošto nije potvrđeno postojanje kancerogenog rizika od strane EPA, a Al je klasifikovan kao GRAS (engl. *Generally Regarded As Safe*).

Analiza podataka o dugoročnom zdravstvenom riziku ukazuje da jedina realna opasnost postoji od prisustva Cd, Cu i Pb u vodi za piće. Analiza podataka pokazuje da je najmanji rizik od pojave kancera prisutan od Pb (kod 30 stanovnika od 1000 stanovnika) i Cu (kod 35 stanovnika od 1000 stanovnika) koji koriste ovu vodu za piće.

Realno najveći dugoročno zdravstveni rizik preti od prisustva Cd u vodi za piće: kod 202 stanovnika od 1000 stanovnika (svaki peti stanovnik). Kako je na osnovu naših istraživanja (Rajković, Stojanović, Milojković, 2017b) već utvrđena direktna povezanost prisustva toksičnih elemenata (Pb, Cd, Cr, Si, U) u vodi za piće i pojave porasta hroničnih nezaraznih oboljenja, kao što je hronična bubrežna insuficijencija (HBI) i bolesti mokraćnog sistema sa 84,06%, naročito kod žena doba starosti preko 15 godina, koja je zabeležena u Braničevskom okrugu i čija stopa iznosi 126,3/1000 (Zavod za javno zdravlje Požarevac, 2012), ovaj rad i ove vrednosti za Cd su još jedna potvrda realne opasnosti koja postoji od njegovog prisustva u vodi za piće.

Zaključak

U radu je ispitivan kamenac koji je nastao iz vodovodne mreže Grada Požarevca, tokom vremenskog perioda 2012−2017. godine. Na osnovu dobijenih rezultata ispitivanja, mogu se izvesti sledeći zaključci:

Fizičko-hemijska i mikrobiološka analiza uzoraka vode za piće iz vodovodne mreže Grada Požarevca u Braničevskom okrugu pokazala je da je voda kvaliteta pogodnog za humanu upotrebu i da odgovara kvalitetu preporučenom pravilnikom;

Prema količini suvog ostatka (do 1 g/dm^3), voda se ubraja u malomineralne vode. Radi se o vodi koja se zahvata pretežno iz karbonatnih stena – *krečnjaka* ($CaCO_3$) i *dolomita* ($MgCO_3 \cdot CaCO_3$);

Podatak o elektroprovodljivosti govori da je voda za piće iz vodovodne mreže Grada Požarevca bogata rastvorenim materijama;

Na osnovu sadržaja $CaCO_3$ u dm^3 od 613,92 mg/dm^3 voda za piće iz vodovodne mreže Grada Požarevca nalazi se u kategoriji *tvrde vode*. Na osnovu geološke analize sastava zemljišta na teritoriji Grada Požarevca, može se objasniti prisustvo karbonata i silicijuma u vodi za piće iz lesnih sedimenata, u kojima ima kalcita;

Od makroelemenata određen je sadržaj metala: Ca, Mg, Na i K. Sadržaj ovih elemenata je u skladu sa Pravilnikom o higijenskoj ispravnosti vode za piće, osim Ca čija je vrednost viša od maksimalno dozvoljene koncentracije. Zabrinjava i

odnos Ca/Mg koji je, umesto 3−4, više od deset puta viši, što ukazuje na nizak sadržaj Mg u vodi;

Od mikroelemenata određen je sadržaj metala: Zn, Mn, Fe i Cu. Sadržaj Zn, Cu i Mn je u skladu sa Pravilnikom o higijenskoj ispravnosti vode za piće;

Od ostalih elemenata određen je sadržaj Pb, Cd i Al u vodi za piće. Sadržaj Cd i Pb je na samoj granici dozvoljenih koncentracija, dok je sadržaj Al 2,7 puta viši od maksimalno dozvoljene koncentracije;

Na osnovu ovih istraživanja uočena je povezanost prisustva toksičnih elemenata (Pb, Cd, Al) u vodi za piće i pojave porasta hroničnih nezaraznih oboljenja kao što je hronična bubrežna insuficijencija (HBI) i bolesti mokraćnog sistema sa 84,06%, naročito kod žena doba starosti preko 15 godina, koja je zabeležena u Braničevskom okrugu i čija stopa iznosi 126,3/1000.

Proračunati podaci ukazuju da rizik od unošenja elemenata koji se u vodi za piće iz vodovodne mreže Grada Požarevca nalaze u vrednosti višoj od one dozvoljene pravilnikom, ni za jedan metal ne pokazuje kratkoročni zdravstveni rizik po zdravlje ljudi.

Analiza podataka o dugoročnom zdravstvenom riziku ukazuje da jedina realna opasnost postoji od prisustva Cd u vodi za piće. Rizik od pojave kancera prisutan je kod svakog petog stanovnika koji koristi ovu vodu za piće.

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ANALYSIS OF MACRO- AND MICROELEMENTS IN DRINKING WATER FROM THE POŽAREVAC CITY PUBLIC WATER SUPPLY SYSTEM

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A b s t r a c t

This study aims at analysing physical, chemical and microbiological properties, and content of macro- and microelements in the water from the Požarevac city public water supply system. Analysis shows an increased content of Ca in drinking water, as a result of the position of Požarevac on the sediments of river terrace of accumulative character of t1 type, predominantly consisting of quartzite. The water from the public water supply is alkaline ('calcic', 'bicarbonate water') and very hard, due to the increased Ca content. Beside Ca, potassium and aluminium exceed the maximum contaminant level, while Pb and Cd are at the very limit. Calculated data show that there are no short-term health risks regarding elements exceeding allowed concentrations found in the public water supply system. Data analysis regarding long-term health risks shows that Cd present in drinking water poses the only relevant threat to human health. Cancer risk is present in 202 out of 1000 inhabitants using this water.

Key words: drinking water, scale, calcium, heavy metals, Požarevac.

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ENVIRONMENTAL ASSESSMENT OF THE GREENHOUSE GASES EMISSION FROM POULTRY PRODUCTION IN RUSSIA'S CENTRAL REGION

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Abstract: With an estimated rise in poultry production and consumption of chicken meat in Russia by 9% up to 2022, as well as development of self-sustainable poultry production, the need has arisen for environmental assessment of this production, and within it especially greenhouse gases (GHGs) emission assessment. The goal of this work is to show a calculation procedure for obtaining estimations for the carbon footprint of the 1 kg of live chicken at the farm gate, taking into account regional typological features of agricultural production in agro-ecosystems. The methodology of carbon footprint (CF) calculation is based on the life cycle assessment (LCA) methodology, and on IAGRICO$_2$ calculator, developed for agriculture products. Results have shown that in modern technology of poultry farming, 5.79 kg CO_2 e was emitted on average per kg of body mass, and that about 47% of emission was from manure, around 27.5% from crop production (fuel and fertiliser) and 25.5% from fuel and energy needed for heating, sanitation and feeding of chickens. The main distinction of Central Russia is low efficiency of the fertiliser application on crop fields and manure management, storage and utilisation, which has as a result high emissions of the nitrous oxide. This is the field where the implementation of the intensive technologies of precise farming, manure handling, utilisation and management will significantly decrease GHG emission, with preserving yield of crops and quantity and quality of chicken meat.

Key words: environmental assessment, greenhouse gases, poultry, manure, energy, fertilisers, agro-ecosystems, carbon footprint.

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Introduction

Ever-increasing human population represents a major challenge for modern society, and anthropogenic pressure on ever decreasing natural resources is one of the major problems of environmental science, and anthropogenic greenhouse gases (GHGs) emission is one of the most prominent ecological issues within it. In addition, this population boom is setting the task to the agriculture: production of sufficient quantities of safe food for the constantly growing number of humans, with the efficient use of the limited quantity of natural resources (IPCC 2007, 2013). To fulfil this task, agriculture increased both intensity of production as well as arable land area, which increased the GHG emission from land use change and agricultural procedures, and resulted in modern agriculture participating in the global GHG emission with 16%, which could be compared to other sectors of human activity (energy generation – 26%, industry – 19%, transport – 13%) (IPCC, 2007).

Not all agricultural products are of the same biological value for human nutrition, because humans are in need of high quality proteins in the diet for normal growth, development and sustenance of life. Basically, the main source of these proteins is the meat, which is produced from domestic animals, and because of that livestock sector is producing more GHGs than other sectors of food production, mainly methane and nitrous oxide (IPCC 2007; Popp et al., 2010).

For the purpose of providing needed quantity of meat for human consumption, the more intensive technologies in animal production are becoming increasingly interesting because resources are more efficiently used in more intensified system, which results in the cheapest unit price of the final product. Poultry raising is the most intensive branch of the animal husbandry, and the chicken meat is the most widely distributed and accessible type of meat both in quantity and in price, not only in Russia but also in the world (Figure 1).

FAO is predicting that in Russian Federation consumption of meat in 2022 will increase total meat consumption by 11.8 kg per capita, comparing to 2012, with the poultry meat share of 56.8% in this increase (Figure 2). Because of its livestock development program and increase in the production of meat, Russia should have a clear idea about the allocation of greenhouse gas emissions at each phase of the poultry production.

The goal of this work is to show a calculation procedure for obtaining estimations for the carbon footprint of an agricultural product, namely 1 kg of live chicken at the farm gate, taking into account regional typological features of agricultural production in agro-ecosystems. The carbon footprint (CF) represents the amount of GHGs released during production of unit of some goods or services, represented in the kg CO_2 equivalent (kg CO_2 e), and it is calculated by multiplying the amount of specific gas with corresponding global warming potential of a given gas (1 for CO_2, 23 for CH_4 and 296 for N_2O) (FAO, 2006).

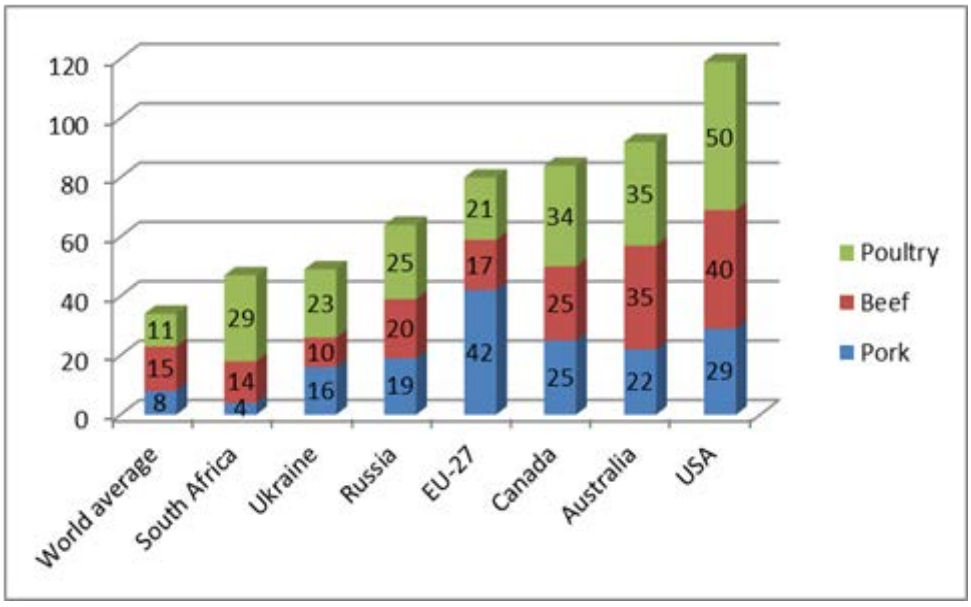

Figure 1. Meat consumption in 2009 (kg per capita). (http://www.fao.org/faostat/en/#data)

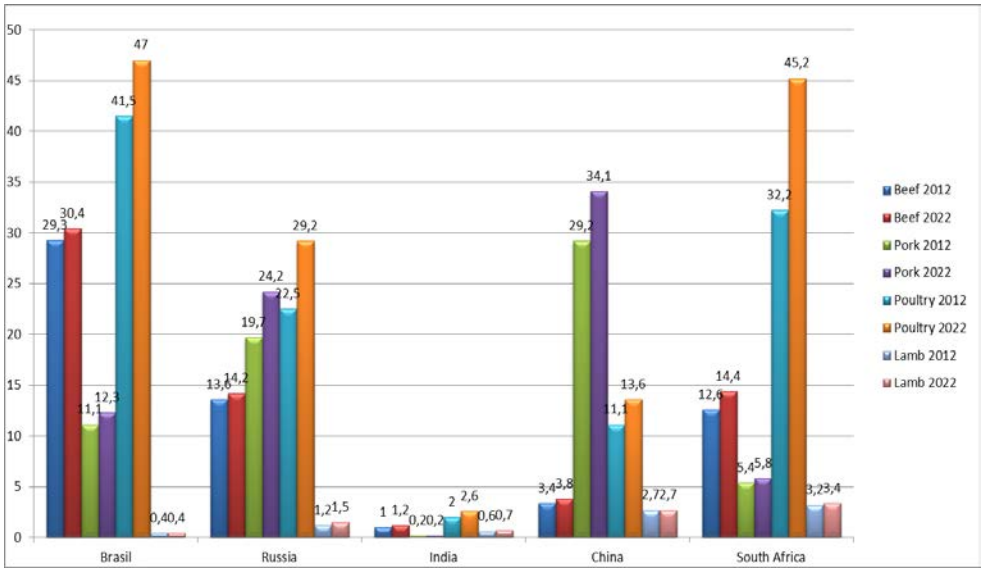

Figure 2. Estimation of the meat consumption in BRICS countries in 2012 and 2022 (kg per capita). (http://www.fao.org/faostat/en/#data)

Materials and Methods

Data used in this paper were obtained through research on experimental training farms "Mummovskoe" (Saratov region, Russian Federation) and "Druzhba" (Yaroslavl' region, Russian Federation), in the period from 2011 to 2014. In addition, complex data were obtained through LAMP field experiments in the Kursk region as well as data obtained through LISSOZ software application.

The methodology of a carbon footprint (CF) calculation is based on the life cycle assessment (LCA) methodology, i.e. the calculation of emissions that take place throughout the life cycle of a product from the production of the raw materials up to the disposal (from cradle to grave). The calculation takes account of each stage and includes the transport within the production chain from the first step up to the defined border of the system (the end of the chain or the end of the chain segment)(Samardžić et al., 2014).

LCA in poultry and chicken meat production can be divided into 5 principal phases:

- Phase 1: Feed and crop production;
- Phase 2: Poultry production;
- Phase 3: Meat processing;
- Phase 4: Chicken meat retail;
- Phase 5: Consumption and waste management.

This paper will focus on the first two phases. The methodology described in this article is based on IAGRICO$_2$ (Castaldi, 2013).

Results and Discussion

There are two main technologies of poultry production in Russian Federation, based on the length of the growth period: the first technology with the growth period of 42 days, and with a medium terminal weight of 1900 g and the second technology with the growth period of 56 days, and with a medium terminal weight of 3300 g. The first technology is more intensive one, which can be measured by feed conversion (the amount of feed needed for 1 kg of body mass gain), because of more efficient nutrient usage in the earlier stage of life and balanced mix of feed inputs (Table 1). In the following text, the focus will be on the more intensive technology.

Calculation of CF in the phase of feed production: GHG emissions in this phase are dominated by CO_2 from fuel consumption, and N_2O emissions as a result of the fertiliser production and application as well as transformation of the ammonia from the applied manure to nitrates followed by processes of denitrification (Figure 3).

Table 1. Differences of the feed conversion in two main technologies of poultry production in Russia.

Type of technology	Bodyweight at the end of growth (kg)	Feed conversion (kg feed/kg growth)
42 days	1.9	1.76
56 days	3.3	2.1

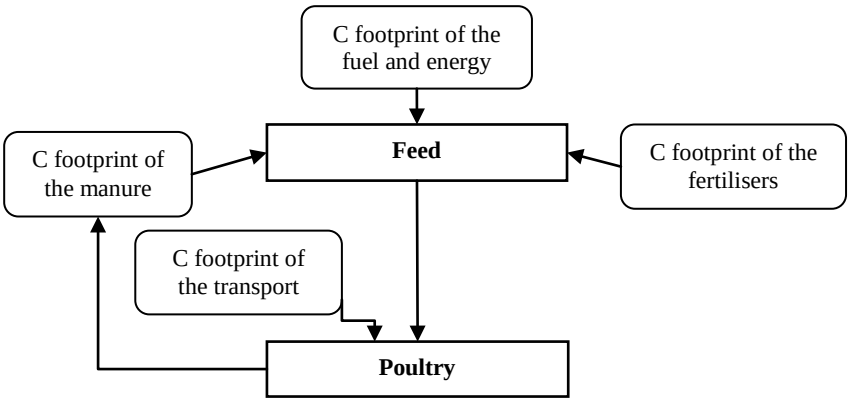

Figure 3. Diagram of the greenhouse gas emissions in the feed and crop production phase.

Individual components of complex concentrated feed have different CFs, and birds are not consuming an equal amount of each component. To calculate CF of feed, it is necessary to determine quantities of consumed components throughout lifetime (Table 2) and the amount of fuel and fertiliser used in the specific crop production process and their representative CF (Table 3) (Hillier et al., 2009), as well as the amount of N_2O of fertiliser origin emitted from soil (FAO, 2001, 2006; IPCC, 2006, 2013).

Table 2. Quantities of feed components needed for the growth of the birds to the slaughtering weight.

Crops	Quantity (kg)
Maize	1.5
Wheat	0.7
Barley	0.4
Soya	0.8

Table 3. Carbon footprint of specific feed components.

Crops	Yield (t ha^{-1})	Applied per hectare		GHG emissions per hectare		GHG emissions per kg of crop yield		Carbon footprint of feed component (kg CO_2 e)
		Nitrogen (kg)	Fuel (litres)	Nitrogen (kg CO_2 e)	Fuel (kg CO_2 e)	Nitrogen (kg CO_2 e)	Fuel (kg CO_2 e)	
Maize	5	130	120	1,269	316.2	0.32	0.06	0.38
Wheat	6	120	73.52	1,756	194.1	0.29	0.03	0.32
Barley	6	220	69.05	2,147	182.3	0.36	0.03	0.39
Soya	3	228	65	2,225	171.6	0.74	0.06	0.8

Carbon footprint of feed and crop production can be calculated by the following equation:

$$1.5\times0.38+0.7\times0.32+0.4\times0.39+0.8\times0.8=1.59 \text{ kg } CO_2 \text{ e.}$$

Calculation of CF in the phase of poultry production: Concerning poultry as a source of the GHG emission, the main sources at this phase are energy consumption for feeding and accommodation of the animals and manure management (Figure 4).

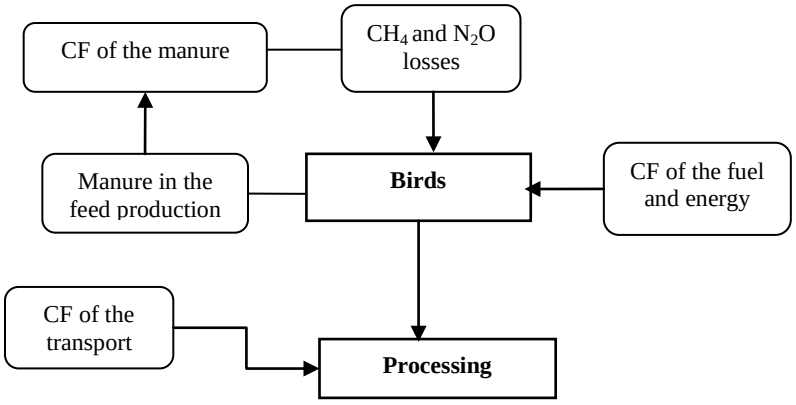

Figure 4. Diagram of the greenhouse gas emissions in the poultry production phase.

Fuel consumption for feeding, manure handling and internal farm transport for the poultry was 0.005 litres of diesel per bird, which is equal to the 0.0132 kg CO_2 e; the energy needed for ventilation and heating had CF of 1.46 kg CO_2 e, which resulted in CF of energy equal to 1.47 kg CO_2 e. One bird produced approximately 3.9 kg of manure during lifetime, with N content of 0.195 kg. Losses of N as a consequence of bad manure managing practices were 40% and the amount of lost N transformed to N_2O was 7.5%.

To calculate CF from manure, we needed to multiply the amount of N_2O with its global warming potential (296 for N_2O):

$$0.195 \times 0.4 \times 0.075 \times 296 = 1.73 \text{ kg } CO_2 \text{ e.}$$

CF of the poultry production phase was:

$$1.47 + 1.73 = 4.2 \text{ kg } CO_2 \text{ e.}$$

Carbon footprint of poultry production at the farm gate was equal to:

$$1.59 + 4.2 = 5.79 \text{ kg } CO_2 \text{ e.}$$

From the given results, it is evident that the GHG emissions in the phase of feed production amounted to 27.46% of total emissions from poultry production. In this phase, dominant greenhouse gases were CO_2 from fuel consumption, and N_2O emissions as a result of the fertiliser production and application, as well as transformation of the ammonia from the applied manure to nitrates followed by processes of denitrification (calculation of fertiliser production CF [6.8 kg CO_2 e kg^{-1} N in fertiliser] (Cederberg et al., 2009) and the amount of N_2O of fertiliser origin emitted from soil). From Table 3, it is evident that around 75% of all GHG emissions in the feed and crop production phase were emitted as a consequence of fertiliser application. Using precision farming methods there could be achieved a reduction in the quantity of applied fertiliser (and consequently GHG emission) up to 40% without a decrease in crop yield.

In Russia's conditions, the poultry sector has reached production intensity equal to the production level of developed regions in the world (EU, USA), but manure handling practices are not developed enough, which results in high losses of ammonia and consequently, in the greater GHG emission from manure. Moreover, 35% of GHG emissions from poultry production phase are a consequence of fuel and energy use, and 65% from manure management, which gives a possibility of GHG emission mitigation through improved manure storage and handling practices.

Conclusion

According to the performed analysis of the basic sources of GHG emissions in the life cycle of the poultry meat, it is concluded that the most efficient means for the greenhouse gases emission evaluation and assessment was an integral algorithm of GHG emission calculation, which was divided into 5 phases of the LCA: (1) feed and crop production, (2) poultry production, (3) meat processing, (4) chicken meat retail, (5) consumption. Every phase was characterised by specific emission factors. Regulation of those emission factors can provide means for a reduction of this specific anthropogenic impact on the environment.

The first phase was connected with analysis of the applied fodder technologies in the concrete soil, climate and agroecological conditions. Those conditions were

defined by maximum essential spatial variability and temporal changes, which determined priorities of their research in the conditions of the central regions of European part of Russia (CRER). Differences between traditional and modern ways of the tillage and their corresponding GHG emission must be taken into consideration.

The second phase was characterised by a high level of unification of applied zootechnologies, with dominating contrast variants of high intensity poultry business (imported bird varieties and hybrids as well as housing and feeding technology) with ever reducing segment of extensive technologies of poultry business in the conditions of CRER. Conducted analyses show intensive lowering of the CF with the replacement of the older technologies with modern ones, chiefly by decreasing growth time and improvement of the feed conversion (42 days vs. 56 days of growing, 1.76 kg of feed vs. 2.1 of feed per 1 kg of weight), which should be included in the efficiency assessment of the modernisation projects of the poultry farms.

The main distinction of CRER is low efficiency of the manure utilisation, which has as a result high emissions of the nitrous oxide. This is the field where the implementation of the intensive technologies of manure handling, utilisation and management will significantly decrease GHG emissions.

Acknowledgements

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EKOLOŠKA OCENA EMISIJE GASOVA STAKLENE BAŠTE IZ PROIZVODNJE BROJLERA U CENTRALNOM REGIONU RUSIJE

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R e z i m e

Sa očekivanim porastom proizvodnje u živinarstvu i povećanjem korišćenja pilećeg mesa u Rusiji od 9% do 2022. godine, kao i sa državnom politikom Ruske Federacije o kompletnoj samodovoljnosti u proizvodnji hrane, a naročito živinskog mesa, nastala je potreba za ocenom uticaja živinarstva na životnu sredinu, a posebno emisiju gasova staklene bašte. Cilj ovog rada je prikazati proceduru izračunavanja ugljenikovog otiska (engl. *carbon footprint*) za 1 kg žive mase na kraju tova brojlera, uzimajući u obzir regionalne tipološke osobine poljoprivredne proizvodnje u agroekosistemima. Metodologija proračuna ugljenikovog otiska bazirana je na metodologiji ocene životnog ciklusa (engl. *Life Cycle Analysis* – LCA), i na kalkulatoru IAGRICO$_2$, prilagođenom poljoprivrednim proizvodima. Rezultati su pokazali da se u modernoj tehnologiji živinarstva, u proseku emituje 5,79 kg CO_2 ekvivalenta po kg telesne mase, te da je oko 47% emisije poreklom iz stajnjaka, oko 27,5% od proizvodnje useva (upotreba goriva i đubriva) i 25,5% od goriva i energije potrebne za grejanje, čišćenje i hranjenje pilića. Glavna odlika centralnog regiona evropske Rusije je niska efikasnost primene azotnih đubriva na poljima, kao i upravljanje skladištenjem i primenom stajnjaka, što ima za posledicu velike količine emitovanog azot-suboksida. Ovo predstavlja polje u kojem bi implementacija intenzivnih tehnologija precizne poljoprivrede i skladištenja i primene stajnjaka mogla značajno smanjiti emisiju gasova staklene bašte, sa očuvanjem prinosa poljoprivrednih kultura i količine i kvaliteta pilećeg mesa.

Ključne reči: ekološka ocena, gasovi staklene bašte, živina, stajnjak, energija, đubriva, ugljenikov otisak.

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ANTIFUNGAL ACTIVITY OF CHITOSAN AND ITS QUATERNIZED DERIVATIVE IN GEL FORM AND AS AN EDIBLE COATING ON CUT CHERRY TOMATOES

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Abstract: The antifungal activities of medium molecular weight chitosan and its hydrosoluble derivative salt *N*,*N*,*N*-trimethylchitosan were examined as both gel and as a solid protective coating against three common food spoilage fungi (*Penicillium*sp., wild *Aspergillus*sp. and one standard strain of *Aspergillusflavus*). The salt derivative is characterized by having permanent positive charges and is expected to have a higher antimicrobial activity than commercial chitosan. In gel form, the minimum inhibitory concentration (MIC) resulted in the same value for both polymers against all tested fungi (> 2.0 gl^{-1}). The derivative presented a significant fungistatic action against the *Penicillium* strain within the concentration range of 0.2 to 0.6 gl^{-1}. When applied as protective coatings on freshly cut cherry tomatoes, the commercial chitosan appeared to be more effective in forming stable films and preventing fungal infestation than its derivative. Less than 20–25% of samples were infected after one week of incubation when compared to control (uncoated) and chitosan treated samples.

Key words: chitosan, antifungal activity, edible coatings, minimally processed tomatoes.

Introduction

Tomato (*Lycopersicum esculentum* M.) is the second most consumed vegetable in the world after potato, being widely accepted in all cuisines and cultures. Despite the availability of industrially processed products, the worldwide

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consumption of fresh tomatoes continues to increase, accounting for about 74% of the total tomato market (FAOSAT, 2013).

Mature fresh tomatoes, however, have a short postharvest life. Due to its high water content and low mechanical resistance, the fruit is quite susceptible to diseases and damages, particularly contamination with spoilage microorganisms. *Alternaria, Fusarium, Aspergillus, Mucor, Rhizopus* and *Penicillium* are the most common fungi species that can occur in tomatoes after harvesting.

Several techniques can be applied in order to minimize fresh tomato degradation such as temperature and humidity control during storage, modified atmosphere packaging, ozone washing and applications of chemical, biocidal or sanitary irradiation. The use of protective edible coatings is a simple alternative, although a promising approach. A variety of natural materials including chitosan (El Ghaouth et al., 1992), corn zein proteins (Park et al., 1994), arabic gum (Ali et al., 2010) and carnauba wax (Avina et al., 2011) have been tested to form protective coatings on tomatoes. Amongst them, chitosan is one of the most studied due to its broad antimicrobial activity (Goy et al., 2009) and effective action in stimulating host-defense responses (Bautista-Baños et al., 2006).

Chitosan is a copolymer with the chemical structure composed of2-acetamido-2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose (D-glucosamine) units linked by O-glycosidicsβ (1-4) bonds. It has been widely tested as an edible coating and its chemical transformation to a salt derivative (*N,N,N*-trimethylchitosan) is reported to enhance the polymer activity against several bacteria and fungi that often infect fruit and vegetables (Qin et al., 2006; Belalia et al., 2008).

This derivative is characterized by having permanent positive charges distributed along the polymeric backbone due to the quaternization of primary amino groups of the parent chitosan. This feature gives the polymer a cationic response independent of the solvent pH. Since the most acceptable mechanism of the antimicrobial activity of chitosan is based on its cationic nature (Goy et al., 2009), it is expected that the derivate resulted in an improved bioactivity. It is also worth highlighting that, unlike chitosan, the trimethylated salt is not a commercial product and its potential applications in food are still in the initial stages of investigation.

The aim of this study is to assess and compare the antifungal capacity of parent commercial chitosan and its derivative *N,N,N*-trimethylchitosan, *in vitro* in diluted form (gel) and *in vivo* as protective coatings on cut cherry tomatoes, against intentional inoculation of *Penicillium* and *Aspergillus* species.

Materials and Methods

Polymers

Chitosan (here identified as Chit) in powder (medium molecular weight with 80% deacetylated units) was purchased from Sigma Aldrich (St. Louis, USA) and used as a reference and to synthesize the quaternized derivative *N,N,N*-trimethylchitosan (TMC). The TMC was obtained by reaction at 70 °C, comprising a suspension of 1.0 g of chitosan (0.005 mol) in 4 mL of deionized water with additions of 16 mL of dimethylsulfate (Synth, R. Janeiro, Brazil) and 1.2 g of NaOH (0.015 mol) plus 0.88 g of NaCl (0.015 mol). The resulting derivative was dialyzed in a cellophane membrane (cut-off of 12000–14000 $gmol^{-1}$) and the final product obtained by precipitation, followed by washing with acetone. Details of the methylation process and TMC full characterization are available within the literature (Britto and Assis, 2007; Britto et al., 2011). Chitosan gel (used as a reference) was prepared by dissolution in 1.0% acetic acid aqueous solution (pH 4.3) and TMC gel was obtained by direct solubilization in distilled water (pH 6.6), both at a concentration of 2.0 gl^{-1}. Therefore, samples were homogenized for 2 hours under magnetic stirring.

Fungal strains and cultures

Three food spoilage fungi were used for testing: *Penicillium* sp. originally isolated from decayed apples; *Aspergillus* sp. originally isolated from coffee beans and one standard strain of *Aspergillus flavus* (ATCC 14108), which was used for comparison. The wild fungal cultures were provided by the Culture Collection of Federal University of São Carlos and previously identified at the genus level in accordance with Singh et al. (1991). The use of wild fungi was intended to evaluate the general action of the polymers and to compare the efficiency against a control strain. The chosen fungi release toxins, mainly the *Aspergillus* sp. which are considered the largest producers of aflatoxins, carcionogenicmycotoxins that commonly contaminate agricultural commodities.

The fungi were incubated in Petri dishes (potato dextrose agar medium) for seven days at 35°C before testing.

Minimum inhibitory concentration (MIC) determination

For the MIC determination of the gels, the average concentration of inoculum was standardized as follows: the fungi colonies grown were picked up and transferred into BHI (brain heart infusion) broth and again incubated at 35°C. After the mycelial growth was visually confirmed, the formed conidal mass was removed and transferred to 10 mlof saline solution at 90%. Each suspension was

homogenized using a vortex and the inoculum separated by filtration through glass wool. The number of colonies in suspension was determined by UV spectroscopy (UV-vis Spectrum series SP-2000UV, Shanghai, China), correlating the absolute absorbance at 530 nm with a suspension corresponding to approximately 104 CFU ml^{-1} (EUCAST-AFTS, 2008). To achieve this concentration, several dilutions were necessary in an RPMI-160 medium.

The antifungal activity of the Chit and TMC was performed using 96-well cell microliter culture plates (Fisher Scientific, Atlanta, USA) by a standard dilution method. 100 µlof RPMI-160 medium was added to all wells, with exception of those in column 3 for which 3 wells (3A, 3B and 3C) were filled with Chit gel, then wells 3D and 3E were filled with acetic acid at 1.0% (Chit solvent), and 3F, 3G and 3H were filled with the TMC derivative gel.

The first row (1A−1H) was marked as a negative control without any inocula. The second row (2A−2H) served as a positive control (no polymers). From the fourth column on, each well was filled with an additional 100 µg of polymer gels (Chit and TMC, separately) in serial two-fold dilutions (from 2.0gl^{-1} to 7.8 µgml^{-1}). The inoculum was added (100 µlof fungal suspension) in these wells. The plates were stored for 7 days at 37 °C under aerobic conditions. The lowest concentrations without detectable change in turbidity were defined as MICs. All tests were run in triplicate.

Coatings

Cherry tomatoes (*Solanum lycopersicum* var. cerasiforme) were acquired at a local market. The fruits were picked from the same lot on the same day after harvest. The samples were sorted by similar size (average diameter of 2.8 cm), mass (approximately 20 grams) and appearance. The tomatoes were washed and sanitized by immersion in a 200 ppm sodium hypochlorite solution for 10 min, then rinsed, dried spontaneously and sliced along the equator into approximately 5 g pieces. Groups of 15 slices were separately dipped for 3 min in each coating solution (Chit, TMC and into acetic acid at 1.0%, Chi solvent) for comparison. Excess gel was allowed to drain off and samples were allowed to dry at room temperature. Fifteen slices of non-treated samples were considered as a control. All treatments were performed in triplicate. Table 1 summarizes the tested treatments.

Table 1. Treatments applied on tomatoes (*Solanum lycopersicum*).

Sample identification	Treatment	Number of surfaces analyzed*
T_{ctr}	Control (no coating)	45
T_{chit}	Chit gel coating**	45
T_{tmc}	TMC gel coating**	45

*triplicate of 15 samples per treatment; ** concentration of 2gl^{-1}.

Antifungal activity of Chit and TMC on cut tomatoes

For *in vivo* evaluation, the samples were transferred to Petri dishes (9-cm diameter) and each surface was inoculated with a suspension of 10^4 conidia ml^{-1}. All strains were assayed on coated and non-coated tomatoes. The samples were kept at 30 °C and evaluations of rot incidence were scored daily by visual observation over 7 days. Samples were considered infected when the development of fungal mycelia was clearly visualized on cut surfaces. The data was considered as a percentage of infected samples according to the relation: (%) of infection = (number of infected samples/total number of samples) X 100.

Statistical analyses

The experimental results were evaluated by one-way analysis of variance (ANOVA) and the means were compared by Tukey's test considering a significance level of $p \leq 0.05$, using the software Statistica 8.0 (StatSoftInc, Tulsa, USA).

Results and Discussion

In vitro analyses

The antifungal activity of Chit and its derivatives is usually expressed by the ability to prevent spore germination and to temporarily inhibit fungi growth. As to whether chitosan possesses fungistatic or fungicidal properties, the literature reports it to be dependent on several physical-chemical factors (Qiu et al., 2014). There is evidence that Chit has fungistatic rather than fungicidal activity against most of the fungi species (Barka et al., 2004). Our results from an *in vitro* assay showed that, in gel form, concentrations lower than 2.0 gL^{-1} were not effective in reducing the fungal growth, i.e., the tested strains were not sensitive to the medium with low contents of Chit and TMC. This would indicate that the presence of dissolved polymers, as higher than 2.0 gl^{-1}, is necessary to achieve a satisfactory response.

These results should be taken with some caution due to lack of previously reported literature. There is generally an absence of numerical data concerning determination of MIC for polyelectrolytes like Chit and its derivatives. Additionally, the analytical antifungal essays are largely randomized and do not follow a standardized procedure with regards to fungi strains, culture medium, pH, incubation temperature or polymer characteristics such as molar weight, purity or degree of acetylation. Experimental conditions are therefore difficult to verify or replicate (Llop et al., 2000; Balouiri et al., 2016).

Despite this uncertainty, it is possible to find some numerical values relating to the activity of different types of chitosans against certain fungi. For example, Tsai et al. (2002) reported the same value i.e. MIC's > 2.0 gl^{-1} when assaying commercial Chit against fungi from *Aspergillus* family. Similar values (1.0−2.0 gl^{-1}) were also reported by Li et al. (2008) and Souza et al. (2013), who presented the MIC of 4.0 gl^{-1} against *A. flavus* in similar analysis. Conversely, Santos et al. (2012) described the necessity of 10 gl^{-1} of Chit to overcome one resistant strain of *A. flavus* and Lopes (2013) cited values as high as 13 gl^{-1} for MIC of Chit on *A. flavus.*

The activity of quaternized Chit derivatives is much less reported than the activity of the mother polymer Chit, with little numerical information available. TMC, however, is generally described as more effective than Chit against various fungi strains (Sajomsang et al., 2012).

Generally, *Aspergillus* species are reported to present some resistance to the toxic effect of Chit-based polymers, attributed to two possible properties: i) the previous natural existence of glucosamines and chitosan as structural components in cell walls of some *Aspergillus* strains, which prevents and reduces the severity of wall damages (Bartnicki-Garcia, 1968), and ii) a defense response by producing chitosanase enzymes that degrade the Chit and reduce the intensity of activity over time (Cheng and Li, 2000). Besides the *Aspergillus* family, several other fungi may induce the production of chitosan which enhances the tolerance and intensifies the degenerative effect on Chit-based polymers.

Very little data has been published for Chit and its derivatives against *Penicillium* sp. Liu et al. (2007) suggested that Chit concentrations were superior to 1.0 gl^{-1} for attaining an effective inhibition of spore's germination, whilst Wang et al. (2014) indicate a MIC of 5.0 gl^{-1} for a complete interruption of *Penicillium* sp. growth. No previous data could be found in the literature for TMC activities on *Penicillium* sp.

In the present study, the MIC values against *Penicillium* sp. were the same as those measured against *A. flavus* (> 2.0 gl^{-1}) for both tested materials (Chit and TMC). It is noteworthy that for *Penicillium*, in the medium amended with TMC, the analysis by UV spectroscopy showed a significant reduction in fungus colonies (though without a complete inhibition) for concentrations between 0.2 and 0.6 gl^{-1}, with a maximum activity at a concentration of 0.4 gl^{-1} (Figure 1).

This can be interpreted as the fungistatic activity of TMC when dispersed in low concentrations. The activity of Chit-based polymers against fungi is relatively well reported where microscopic observations provide evidence of damage in the hyphal structure (Cota-Arriola et al., 2011). The exact mechanism behind this activity, however, remains uncertain, although it is generally accepted that charges present in Chit amino groups and in the *N*-quaternized moieties in the TMC backbone play an important role in the electrostatic interaction between positively

charged polymers and oppositely charged functionalities present in the cell walls of fungi (Goy et al., 2009).

Previous studies have reported that the derivative TMC is more likely to be able to penetrate through the cell walls, causing structural damages and fluid imbalances that would inhibit sporulation, conidial germination and mycelial growth (Tan et al., 2013).

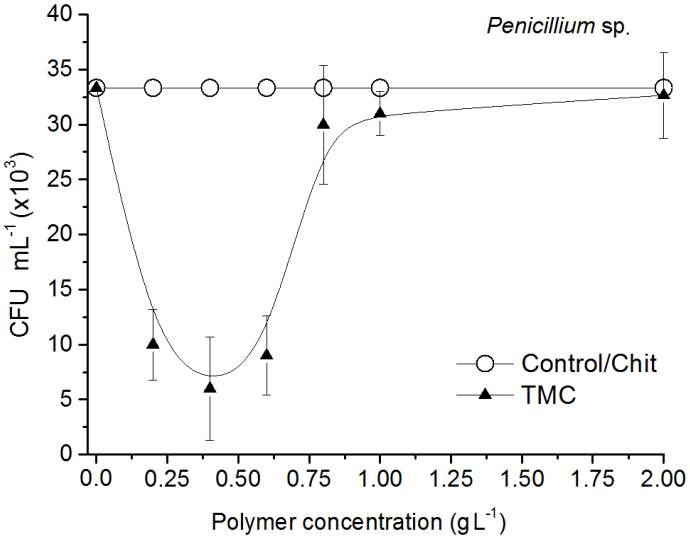

Figure 1. Colony forming units (CFUs) of fungi related to polymer concentration in the gel, as measured by UV spectroscopy. For other strains, no activity was observed in this range.

The reduction of the antifungal inhibition as TMC concentration increases in the medium, as observed against the *Penicillium* sp. (Figure 1), can be understood in terms of two interrelated processes:

i) The spatial arrangement of the polymer chains. TMC is a reactive polymer and at low diluted concentrations, a small number of primary inter-chain interactions are established, so the TMC charged sites available for external coupling are maximized. As the polymeric concentration increases, there is an increase in the hydrodynamic volume per unit of molecular weight. Such an increase of mass favors interactions between ionic groups located in the same or in different chains leading to the formation of coils densely overlapped (Freitas et al., 2010). The extensive chain entanglement causes the polymer to collapse in a smaller configuration (Wyatt et al., 2011), reducing the overall interaction to fungal surface. This effect was also observed when assaying TMC against bacteria (Goy and Assis, 2014);

ii) The gradual weakening as the concentration increases has also been interpreted as a consequence of the elution of chitonolytic enzymes that hydrolyze Chit-based polymers into reduced sugars and D-glucosamine unities (Wang et al., 2008). Such fractions can be further utilized as nutriments accelerating microorganism growth. Li et al. (2008) report such behavior in assaying Chit against *Aspergillus niger*, whose maximum activity was at a concentration of 1.0 to 2.0 gl^{-1} followed by a gradual fungal growth as the polymer concentration increases. Further investigation is needed to better understand this behavior.

No significant reduction in fungal growth was observed when using only acetic acid, indicating that the solvent had little or no effect on the measured antimicrobial activity.

In vivo analyses

In solid form, as edible coatings on cut cherry tomatoes, both polymers exerted inhibitory activity against the inoculated fungi (at 2.0 gl^{-1}). In Table 2, the mean values of proportional infected samples are listed as recorded every day, in each group, for one week.

The chitosan coating (Chit) acted more effectively than TMC with a proportionally reduced number of infected samples for the inoculated fungus.

Table 2. Fractions of infected samples, as recorded each day for one week, in function of the coating material and inoculated fungus.

Aspergillus flavus ATCC 14108			
	Infected samples (%)		
Day	Control*	TMC	Chit
1	2 ± 1.47^{a}	0^{a}	0^{a}
2	91 ± 4.72^{a}	37.67 ± 4.04^{b}	8.34 ± 4.04^{c}
3	95.34 ± 5.16^{a}	57.67 ± 4.04^{b}	15.34 ± 5.14^{c}
4	97.67 ± 4.12^{a}	75.34 ± 5.14^{b}	17.67 ± 8.08^{c}
5	97.67 ± 4.04^{a}	84.34 ± 7.50^{b}	24.34 ± 7.33^{c}
6	100^{a}	95.35 ± 3.74^{a}	24.33 ± 7.50^{b}
7	100^{a}	95.34 ± 3.97^{a}	35.34 ± 4.04^{b}
Aspergillus flavus (wild strain)			
	Control	TMC	Chit
1	2 ± 2.00^{d}	0^{d}	0^{d}
2	91 ± 3.47^{d}	29.00 ± 3.72^{e}	4.00 ± 3.46^{f}
3	95.34 ± 5.16^{d}	51.00 ± 3.47e	8.34 ± 4.04^{f}
4	97.67 ± 4.14^{d}	89.34 ± 4.02e	10.67 ± 4.08^{f}
5	97.67 ± 3.93^{d}	93.34 ± 6.50^{d}	17.67 ± 4.74^{e}
6	100^{d}	95.33 ± 3.04^{d}	17.67 ± 3.98^{e}
7	100^{d}	97.67 ± 4.74^{d}	24.67 ± 4.12^{e}

Table 2. Continued.

	Penicillium sp (wild strain)		
	Control	TMC	Chit
1	2 ± 1.64^{g}	4 ± 3.47^{g}	2 ± 1.64^{g}
2	91 ± 3.07^{g}	57.67 ± 8.08^{h}	8.33 ± 4.02^{i}
3	95.67 ± 4.04^{g}	71.34 ± 7.54^{h}	17.67 ± 4.24^{i}
4	97.67 ± 4.14^{g}	71.34 ± 7.08^{h}	17.67 ± 4.02^{i}
5	97.67 ± 3.93^{g}	82.34 ± 4.74^{h}	24.67 ± 4.50^{i}
6	100^{g}	82.34 ± 3.98^{h}	26.67 ± 6.50^{i}
7	100^{g}	87 ± 3.46^{h}	31 ± 3.46^{i}

*non-coated samples. Means in the same line with different letters are statistically different at $p<0.05$.

The daily evolution in the number of infected samples is best visualized in graphical form, as shown in Figure 2, on control and on coated surfaces inoculated with *Aspergillus* (standard (a) and wild strain (b)). The fungal inhibition was similar against both strains, indicating the same mechanism of action, and changes in the kinetic models promoted by the coatings can be clearly observed in all tested samples. Control (non-coated) tomatoes resulted in an exponential growth with almost all samples infected by the second to third day after inoculation. The TMC coating promoted a reduction, following a quasi-exponential mode with an inferior number of samples infected over time. Finally, the Chit-coating resulted in a linear relationship with a significant reduction in the number of samples infected (more than 60% at the end of seven days). In short, the TMC coating provided a measurable reduction in fungal spreading, mainly in the first four to five days, though the Chi coatings were more efficient in limiting overall contamination.

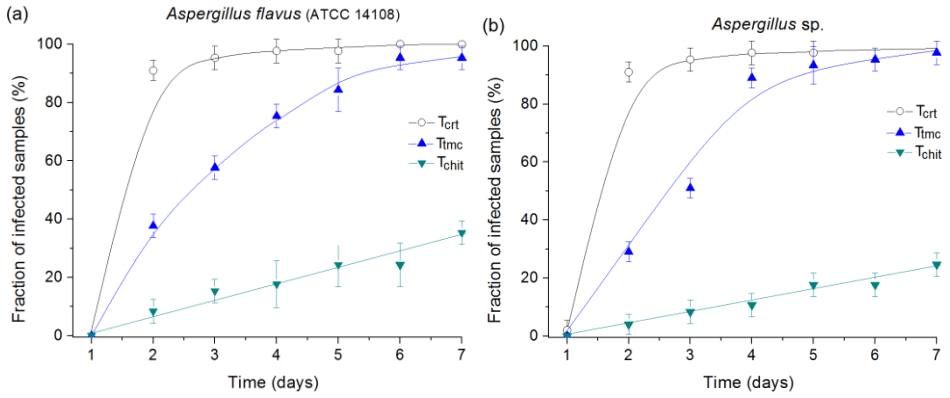

Figure 2. Fractions of infected samples by *Aspergillus flavus* (a) and *Aspergillus* sp. (b) as measured on cut tomato surfaces uncoated and coated with Chi and TMC (2.0 gl^{-1}) as a function of the storage time at 30°C (identification of samples as in Table 1).

Similar behavior was observed in samples inoculated with *Penicillium* sp., as shown in Figure 3. For this strain, the TMC coating reduced approximately 20% of the total of infected samples by the end of one week when compared to uncoated samples.

The Chit, however, was highly effective. Figure 4 illustrates the aspects of some samples with TMC and Chit after seven days of *Penicillium* inoculation, confirming the better antifungal properties of Chi gel as a coating.

This higher protection of Chit when compared to the TMC coating could not have been predicted by the *in vitro* analyses. Several factors, however, can be identified that directly or indirectly must be considered in interpreting this result. Firstly, the degree of quaternization of TMC derivative deeply alters the polymeric water affinity. As the degree of quaternization increases, the water retention will be higher in the matrix, and thus surface hydrophilicity and gas permeability.

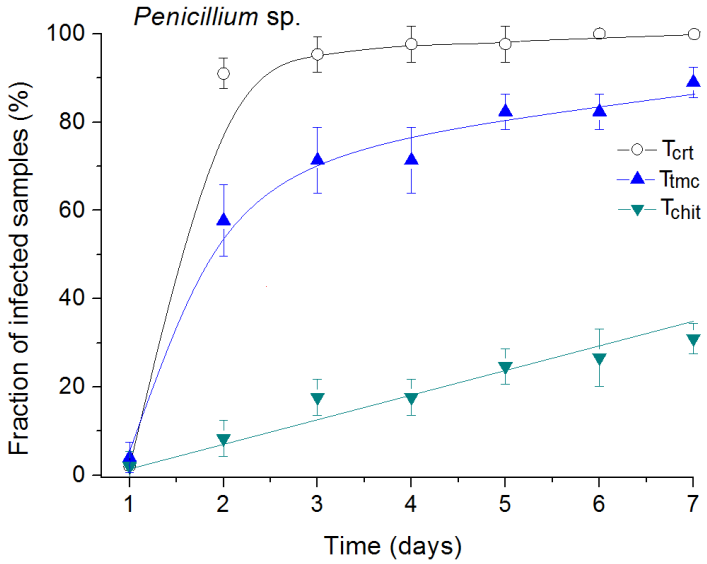

Figure 3. Fraction of infected samples by *Penicillium* sp., as measured on cut tomato surfaces uncoated and coated with Chi and TMC (2.0 gl^{-1}) as a function of the storage time at 30°C (identification of samples as in Table 1).

In the synthesis conducted in this work, the average degree of quaternization was around 35% (Britto and Assis, 2007), i.e. at least 35% of the primary amino groups of the precursor Chit polymer were converted to quaternary amines. Such an increase of charges favors the electrostatic repulsions between chains, contributing to the formation of a less compact network (Huei and Hwa, 1996). Less dense films lead to matrix instability by facilitating water uptake, causing

swelling, and consequently increase the diffusion of water molecules through the coating network (Uragami et al., 2002).

Secondly, the quaternization reaction occurs by electrophilic substitution of nitrogen, liberating H^+ as a byproduct. An excess of H^+ causes breaking of the glycosic bonds resulting in a derivative with low molecular weight. Commercial chitosan has a molecular weight of 400,000 g mol^{-1}, while for the TMC the average weight is around 55,000 g mol^{-1} (Britto et al., 2011). As characterized by Bof et al. (2015), regarding Chit films, the higher the molecular weight, the lower will be the water vapor permeability, since higher molecular weight leads to a more effective polymer chain arrangement with fewer interstitial spaces.

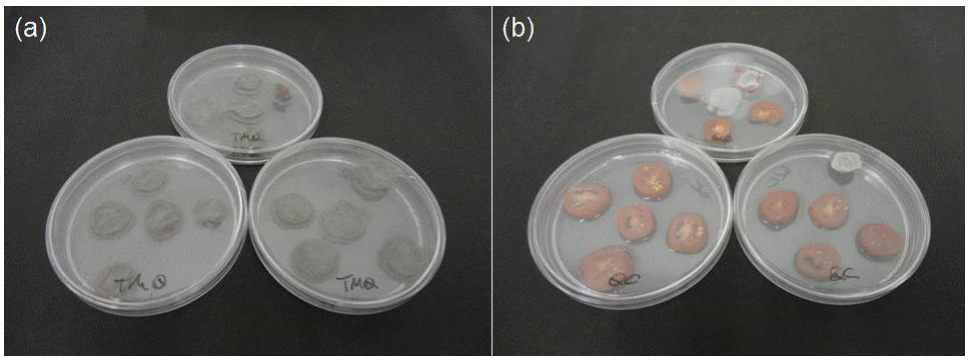

Figure 4. Visual aspect of samples coated with TMC (a) and Chit (b) after seven days of *Penicillium* incubation at 30 °C. It is possible to observe the effectiveness of the Chit coating in reducing fungal infestation. By this time, all control samples were also infected.

TMC films are also characterized by having a high degree of wetting (elevated surface hydrophilicity) than chitosan films (Britto and Assis, 2010). This also contributes to maintaining the relative physiological conditions, i.e., water in surface is favorable for spore germination and fungi growth. It should be observed that there is a direct relationship between the amount of available water in one surface and the average growth rate of *Aspergillus* and *Penicillium* species (Ayerst, 1969).

Such features, which are only detectable in solid state (coating format), differ from the properties initially assessed in gel form, in which Chit and TMC present similar antifungal activities. TMC coatings have been reported to be successfully used in the protection of vitamins (Britto et al., 2012), as a carrier system of vaccines (Nnamani et al., 2011) and for general drug delivery (Mourya and Inamdar, 2009). However, for an effective application on food products, the association of TMC with other less hydrophilic polymers, such as PLA or even

non-quaternized chitosan, can be an alternative to overcome the negative effects caused by its high solubility.

Conclusion

The antifungal tests (under the present experimental conditions) did not identify a clear difference between commercial chitosan (Chit) and its quaternized derivative *N,N,N*-trimethylchitosan (TMC), when in gel form, in inhibiting the germination of *Aspergillus* and *Penicillium* species. The MIC stipulated for both polymers in BHI culture medium was greater than 2.0 gl^{-1}. Of interest is that the diluted TMC (in concentrations between 0.2 and 0.6 gl^{-1}) demonstrated fungistatic activity against *Penicillium* sp., although with no activity outside this range. When gels at a concentration of 2.0 gl^{-1} were applied to form edible coatings on cut cherry tomatoes, both polymers demonstrated activity against inoculated *Aspergillus* and *Penicillium*. The derivative TMC slowed the kinetics of growth, mainly in the first four days of storage, but with steady growth on subsequent days. Chit coatings performed better by reducing significantly the number of infested samples (to 20% for the tested microorganisms in the same period assessed). This is understood to be due to terms of polymer physical-chemical interaction in film forming.

In conclusion, commercial chitosan with medium molar weight, despite its low solubility and low number of charged sites, was effective in controlling fungal infections on cut cherry tomatoes. The feasibility of using Chit in forming protective coatings is confirmed for commercial application in processed tomatoes during storage and/or marketing.

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ANTIGLJIVIČNO DEJSTVO HITOZANA I NJEGOVOG KVATERNIZOVANOG DERIVATA U OBLIKU GELA I KAO JESTIVE PREVLAKE NA ISEČENOM ČERI PARADAJZU

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R e z i m e

Antigljivična dejstva hitozana srednje molekulske mase i njegovog derivata soli rastvorljivog u vodi *N,N,N*-trimetilhitozan ispitivana su kao gel i kao čvrsta zaštitna prevlaka u odnosu na tri uobičajene gljive koje izazivaju kvarenje hrane (*Penicillium* sp., *Aspergillus* sp. i jedan standardni soj *Aspergillus flavus*). Derivat soli poseduje stalno pozitivno naelektrisanje kao i očekivano veće antimikrobno dejstvo nego kod komercijalnog hitozana. U obliku gela, minimalna inhibitorna koncentracija (engl. *minimum inhibitory concentration*− MIC) rezultirala je istom vrednošću za oba polimera u odnosu na testirane gljive (> 2,0 gl^{-1}). Derivat je pokazao značajno fungistatičko dejstvo u odnosu na soj *Penicillium* u okviru opsega koncentracije od 0,2 do 0,6 gl^{-1}. Kada se upotrebi kao zaštitna prevlaka na sveže isečenom paradajzu, komercijalni hitozan se pokazao efikasnijim u stvaranju stabilnih filmova i sprečavanju gljivične infekcije od svog derivata. Manje od 20−25% uzoraka bili su zaraženi posle jednonedeljne inkubacije u poređenju sa kontrolom (neobloženi uzorci) i uzorcima koji su tretirani hitozanom.

Ključne reči: hitozan, antigljivično dejstvo, jestive prevlake, minimalno obrađen paradajz.

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A COMPARATIVE STUDY ON THE NUTRITIONAL AND MICROBIAL SAFETY OF FRESH 'WARA' HAWKED IN ILORIN AND OGBOMOSO TOWNS

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Abstract: Malnutrition resulting from low protein intake is one of the nutritional problems facing most developing countries including Nigeria. Most proteinaceous food sources are costly and in short supply. 'Wara' is a proteinaceous ready to eat food product made by curdling milk. It does not normally undergo any further safety treatments before consumption. Frequent hawking on our major streets and roads calls for determination of the safety of these products. 'Wara' samples sourced from four different locations each at Ilorin, Kwara State and at Ogbomoso, Oyo State respectively, were analysed for nutritional and microbial safety. Proximate composition of the samples over the period of storage showed that moisture content and carbohydrates increased from 59.69% to 72.00% and from 2.39% to 11.39% respectively, while protein, fat and ash contents reduced from 22.20% to 10.80%, 15.80% to 3.62% and from 2.99% to 0.25%, respectively. Microbial and fungal counts ranged from 2.0 X 10^2cfu to 6.3 X 10^5cfu and from 2.0 X 10^2cfu to 7.1 X 10^5 cfu, respectively. *Klebsiella*and *Salmonella species*, *Escherichia coli* and some fungiwere isolated. The study revealed that some of the hawked cheeses were not safe for consumption. Attributable reasons were unhygienic practices of the hawkers or producers and/or lack of requisite preservatives.

Key words: 'wara', quality, safety, hawking, preservation, pathogens.

Introduction

Food is a biological material consumed to provide nutritional support for the body (provide energy, maintain life and stimulate growth). It is usually of plant and animal origin and contains essential nutrients such as fat, protein, vitamins and minerals etc. In modern times, ready to eat food is usually supplied by food industries (Jango-cohen, 2005).

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Animals are used as food either directly or indirectly, mostly after processing. Animal foods include milk, which is obtained from the mammary glands of mammals, e.g. cow milk, which in many cultures is drunk or processed into various other dairy products (Curry, 2013). Livestock farming in general and milk production in particular still play an important socio-economic role in many developing countries, Nigeria inclusive. In Nigeria, the Fulani pastoralists process their surplus fresh milk into various products like 'warankasi', 'nono' (fermented skimmed milk) and 'mai-shani' (meaning milk fat in the Hausa language) (Belewu et al., 2005; Ashaye et al., 2006). Milk, an extremely nutritious food, is a good source of rich nutrients and an excellent medium for microbial growth (Akinyele et al., 1999; Adesokan et al., 2009; Sangoyomi et al., 2010), hence a highly perishable commodity.

Cheese, on its own, is a concentrated dairy commodity produced by acid or rennet coagulation or curdling of milk, stirring and heating of the curd, draining off the whey, collecting and pressing the curd. The cheese is ripened, cured, or aged to develop the flavour and texture (Raheem et al., 2009; Beresford et al., 2001). The manufacture of 'wara' cheese is widespread in Nigeria and a similar cheese called 'wogachi' is made in Northern provinces of Benin Republic, a French speaking country, to the western part of Nigeria. The Fulanis of northern Nigeria are traditionally cattle rearers and have access to fresh milk. 'Wara' cheese making is thought to have started in the Northern region and as a result of their nomadic lifestyle, and this lifestyle has spread to other parts of Nigeria, such as Kwara, Oyo, and Ondo States (Bamidele, 2006), and according to FDA (2003), two criteria, moisture and the milk fat contents, were used to define cheeses.

Cheese can be classified into two groups depending on raw material, texture, type, interior or exterior characteristics, and composition. Cheese flavour and texture are overwhelmed by fatty acid composition of milk and the firmness up to 24 hours. Cheese, however, has a shelf life from 4–5 days up to 5 years depending on the variety. The West African soft cheese which is a special type of cheese found in Nigeria is believed to normally have a shelf life of around 2 days when immersed in its whey, because of its fresh nature and how it has been handled. 'Wara' has a relatively short shelf life due to the presence of some food borne microbial flora comprising bacteria and fungi (Belewuet al., 2005).

Various preservation methods used for the preservation of cheese have been well documented (Aworh and Egounlety, 1985). Joseph and Akinyosoye (1997), in their report, used 0.8% propionic acid and 0.8% sodium benzoate to preserve cheese for 8 days. Cheese is equally an excellent source of protein, fat and minerals such as calcium, iron and phosphorus, vitamins and essential amino acids, and, therefore, is an important food in the diet of both young and old people (Tona et al., 2013).

Local cheeses are hawked in almost all the major streets of the states in Nigeria, mainly by the Fulani tribes. Previous research reported that hygienic standards in the preparation of locally fermented cereal and dairy foods are very poor (Omemu and Aderoju, 2008; Olasupo et al., 2002). For example, Olasupo et al. (2002) isolated *Staphylococcus aureus* and *Klebsiella species* from 'wara',while *Escherichia coli, Salmonella* and *Klebsiella species* were isolated from 'nono', a fermented milk product. Cheese is highly perishable and must be handled properly for extended shelf life. Hence, in this study, the safety of the cheeses hawked in Ilorin town, Kwara State and Ogbomoso town, Oyo State was determined.

Materials and Methods

Cheese samples were obtained from the northern, southern, western and eastern parts of both Ilorin and Ogbomoso towns directly from the local producers. The samples were collected aseptically immediately after production into a clean white covered container and conveyed to the laboratory for immediate analyses.

The proximate analysis of the samples was determined in triplicate in accordance with the procedure described by AOAC (2005).

Total bacterial and fungal counts as well as isolation of pathogenic organisms were determined by the established methods of Fawole and Oso (2007).

Results and Discussion

Physical properties of stored cheese samples

The collected cheese samples were analysed over a 7-day storage period. During this period, it was observed that the cheese samples developed a bad odour, loss of curd by the fresh samples, i.e. the fresh cheeses were no longer firm, they were out of shape and became slippery. These observations suggest degradation in some quality parameters, as a result of microorganisms and/or enzymes naturally present in the cheese samples. The changes noticed started manifesting prominently from the third day of storage.

Proximate composition of stored cheese samples

There was a significant difference ($p<0.05$) in the proximate composition of the cheese measured over five days (Tables 1, 2 and 3). Moisture and carbohydrate contents of the cheese were increasing during storage. But the protein, fat and ash contents were decreasing accordingly. This scenario signifies a reduction in the nutrients as the storage period progresses. According to FAO (2003), the moisture and milk fat play an active role in the quality of cheeses. The loss of fat, protein and ash contents during storage could have made the cheese lose its firmness,

flavour and texture. The increase in the moisture content could have supported the fact stated earlier, that is, an increase in the microbial activities eventually leads to nutrient loss and reduction in consumer acceptance. High moisture content creates a favourable environment for the growth of microorganisms (Belewu et al., 2005).

Table 1. Proximate composition of fresh cheese samples in (%) on day one.

Sample	Moisture	Fat	Protein	Ash	Carbohydrate
A	64.00±0.00^{b}	9.26±0.01^{g}	21.80±0.00^{b}	1. 89±0.00^{c}	3.05±0.05
B	67.01±0.01^{a}	9.20 ±0.00^{g}	19.80± 0.00^{d}	1.59 ±0.00^{c}	2.40±0.00^{d}
C	59.99±0.00^{f}	9.90±0.01^{f}	22.00±0.00^{a}	2.00 ±0.00^{b}	6.11±0.00^{a}
D	60.01±0.01^{e}	15.80±0.00^{a}	20.20±0.01^{c}	1.60±0.00^{e}	2.39±0.01^{d}
E	62.00±0.00^{c}	10.62±0.00^{e}	21.60± 0.00^{b}	1.78±0.00^{d}	4.00±0.00^{c}
F	60.01 ±0.01^{e}	11.10±0.00^{d}	22.20±0.00^{a}	1.74±0.00^{d}	4.95±0.05^{b}
G	61.79 ±0.00^{d}	11.67 ±0.00^{b}	19.90±0.00^{d}	1.69±0.00^{e}	4.95±0.05^{b}
H	59.69±0.01^{f}	11.63±0.00^{c}	20.70±0.00^{c}	2.99±0.00^{a}	4.99±0.00^{b}

Mean ± SD. Means with different superscripts along the column are significantly different (p<0.05).A: Fresh Oja-Oba sample (Ilorin); B: Fresh Asa dam sample (Ilorin); C: Fresh Tanke sample (Ilorin); D: Fresh Kulende sample (Ilorin); E: Fresh Ogbomoso sample (Aroje); F: Fresh Ogbomoso sample (Taki); G: Fresh Ogbomoso sample (Owode), and H: Fresh Ogbomoso sample (Gambari).

Table 2. Proximate composition of fresh cheese samples in (%) on day three.

Sample	Moisture	Fat	Protein	Ash	Carbohydrate
A	67.15±0.02^{b}	9.00±0.05^{c}	19.00±0.00^{b}	1.65±0.00^{b}	3.20±0.00^{f}
B	70.88±0.01^{a}	5.51±0.00^{f}	16.00±0.00^{d}	0.88±0.00^{g}	6.73±0.00^{d}
C	62.29±0.01^{c}	9.32±0.00^{ab}	19.70±0.00^{a}	1.45±0.00^{d}	7.24±0.00^{b}
D	64.79±0.00^{e}	9.30±0.00^{ab}	18.80±0.00^{c}	1.11±0.00^{f}	6.00±0.00^{de}
E	67.69±0.00^{b}	7.37±0.00^{e}	16.40±0.00^{e}	1.50±0.00^{c}	7.04±0.00^{bc}
F	64.00±0.00^{d}	8.70±0.00^{d}	18.60±0.00^{c}	1.29±0.00^{e}	7.41±0.00^{b}
G	66.89±0.00^{c}	9.99±0.00^{a}	15.70±0.00^{e}	1.42±0.00^{d}	6.00±0.00^{de}
H	63.27±0.00^{f}	9.96±0.00^{a}	16.60±0.00^{d}	2.02±0.00^{a}	8.15±0.05^{a}

Mean ± SD. Means with different superscripts along the column are significantly different (p<0.05).A: Fresh Oja-Oba sample (Ilorin); B: Fresh Asa dam sample (Ilorin); C: Fresh Tanke sample (Ilorin); D: Fresh Kulende sample (Ilorin); E: Fresh Ogbomoso sample (Aroje); F: Fresh Ogbomoso sample (Taki); G: Fresh Ogbomoso sample (Owode), and H: Fresh Ogbomoso sample (Gambari).

The proximate composition of the cheese samples was similar to that reported by Alalade and Adeneye (2006) and Ojedapo et al. (2014). The rapid rate of deterioration observed in the samples could be attributed to lack of standardisation in the production methods being used by the local producers, environmental influence and lack of good hygienic practices (Adetunji, 2011; Adetunji et al., 2008). The sample sourced from Aroje area of Ogbomoso had the highest protein content of 22.20% of all the samples, while the sample sourced from Asa dam area of Ilorin had the least protein (19.80%). Regarding fat, the sample sourced from Kulende area of Ilorin had the highest content (15.80%), and the sample from Asa

dam, the least (9.20%). It was noticed that none of the sample sourced from a particular area had the best quality overall. This could be attributed to many factors ranging from cow's variety, feeding habits, age of the animal, condition of the animal during milking, time of milking, weather conditions, health status of the animals etc. that influence quality. The high moisture content noticed in all the samples could have been responsible for the less firmness of the cheese samples as compared to that of cheddar cheese, which normally ranges from between 33% and 36% (Smith, 1995; David, 2007).

Table 3. Proximate composition of fresh cheese samples in (%) on day five.

Sample	Moisture	Fat	Protein	Ash	Carbohydrate
A	71.09 ± 0.01^{b}	3.62 ± 0.00^{f}	14.00 ± 0.00^{c}	1.39 ± 0.00^{a}	9.90 ± 0.00^{d}
B	72.00 ± 0.00^{a}	3.85 ± 0.00^{d}	16.70 ± 0.00^{b}	0.31 ± 0.00^{e}	10.14 ± 0.00^{f}
C	68.48 ± 0.00^{c}	5.09 ± 0.00^{c}	16.90 ± 0.00^{a}	1.09 ± 0.00^{c}	8.44 ± 0.00^{e}
D	70.80 ± 0.00^{c}	5.75 ± 0.00^{b}	12.70 ± 0.00^{d}	0.25 ± 0.00^{f}	10.50 ± 0.00^{c}
E	71.00 ± 0.00^{b}	5.50 ± 0.00^{b}	11.30 ± 0.00^{f}	1.21 ± 0.00^{b}	10.99 ± 0.01^{b}
F	70.01 ± 0.01^{c}	3.77 ± 0.00^{e}	16.40 ± 0.00^{b}	0.75 ± 0.00^{d}	9.07 ± 0.00^{d}
G	71.00 ± 0.00^{b}	6.52 ± 0.00^{a}	10.80 ± 0.00^{e}	0.89 ± 0.00^{d}	10.79 ± 0.00^{b}
H	69.88 ± 0.01^{f}	5.88 ± 0.00^{b}	12.30 ± 0.00^{d}	0.55 ± 0.00^{e}	11.39 ± 0.00^{a}

Mean ± SD. Means with different superscripts along the column are significantly different ($p<0.05$).A: Fresh Oja-Oba sample (Ilorin); B: Fresh Asa dam sample (Ilorin); C: Fresh Tanke sample (Ilorin); D: Fresh Kulende sample (Ilorin); E: Fresh Ogbomoso sample (Aroje); F: Fresh Ogbomoso sample (Taki); G: Fresh Ogbomoso sample (Owode), and H: Fresh Ogbomoso sample (Gambari).

Microbial population of stored cheese samples

There are significant differences among the samples for the bacterial and fungal counts (Tables 4 and 5), with high microbial loads noticed in the stored samples. All the samples showed a progressive increase in their microbial load over the period of storage. This could be attributed to the high moisture content of the samples, which invariably may have been favourable for the growth of microorganisms, the reduction rates and shorter shelf life noticed in the nutritional content of the cheese samples respectively. On day one, the microbial load of sample F was the highest, while that of sample A was the highest on day seven of storage (Table 4). For the fungal counts, samples A, B, D and E were significantly different from each other, and were with higher fungal loads, while sample A had the highest fungal load on day seven of storage (Table 5). The unhygienic nature or practices of the local producers and/or hawkers coupled with the high ambient temperature of the environment could have equally contributed to the high microbial load recorded. Lack or absence of a known preservative could have also contributed to the reduced shelf life. The reduction in the protein, fat and ash contents of the cheese samples (Tables 2 and 3) may be explained by the proliferation of the microorganisms (Tables 4 and 5), which may have fed on the

nutritional components. The increase in microbial population may have possibly contributed to the reduction in the shelf life of the samples, and the corresponding loss of flavour, texture and firmness. Previous studies similarly found significant reductions in the fatty acid composition of the milk, protein and ash contents of Africa soft cheese during storage (Belewu et al., 2005).

Table 4. Total bacteria counts of the cheese samples (in cfu/g).

Sample	Day 1	Day 3	Day 5	Day 7
A	$2.2\pm2.0^{b}x10^{2}$	$2.0\pm2.2^{a}x10^{4}$	$5.1\pm3.1^{b}x10^{4}$	$6.1\pm3.2^{a}x10^{5}$
B	$2.0\pm1.3^{c}x10^{2}$	$1.7\pm1.8^{b}x10^{4}$	$4.4\pm2.4^{c}x10^{4}$	$5.6\pm2.6^{c}x10^{5}$
C	$2.1\pm1.7^{b}x10^{2}$	$1.7\pm1.8^{b}x10^{4}$	$4.2\pm2.3^{c}x10^{4}$	$5.8\pm2.3^{c}x10^{5}$
D	$2.1\pm1.7^{b}x10^{2}$	$1.8\pm2.1^{b}x10^{4}$	$4.9\pm2.2^{b}x10^{4}$	$6.0\pm2.0^{b}x10^{5}$
E	$2.2\pm2.0^{b}x10^{2}$	$1.9\pm2.5^{b}x10^{4}$	$5.0\pm2.9^{b}x10^{4}$	$6.0\pm2.0^{b}x10^{5}$
F	$2.4\pm2.1^{a}x10^{2}$	$2.1\pm2.3^{a}x10^{4}$	$5.3\pm2.8^{a}x10^{4}$	$6.3\pm2.5^{a}x10^{5}$
G	$2.0\pm1.3^{c}x10^{2}$	$1.6\pm1.4^{c}x10^{4}$	$5.1\pm3.1^{b}x10^{4}$	$5.4\pm2.9^{c}x10^{5}$
H	$1.8\pm2.1^{d}x10^{2}$	$1.3\pm2.0^{d}x10^{4}$	$5.4\pm3.0^{a}x10^{4}$	$5.0\pm3.1^{d}x10^{5}$

Mean ± SD. Means with different superscripts along the column are significantly different ($p<0.05$). A: Fresh Oja-Oba sample (Ilorin); B: Fresh Asa dam sample (Ilorin); C: Fresh Tanke sample (Ilorin); D: Fresh Kulende sample (Ilorin); E: Fresh Ogbomoso sample (Aroje); F: Fresh Ogbomoso sample (Taki); G: Fresh Ogbomoso sample (Owode), and H: Fresh Ogbomoso sample (Gambari).

Table 5. Total fungal counts of the cheese samples (in cfu/g).

Sample	Day 1	Day 3	Day 5	Day 7
A	$3.0\pm2.8^{a}x10^{2}$	$2.6\pm1.4^{a}x10^{4}$	$3.1\pm1.5^{b}x10^{4}$	$7.1\pm2.4^{a}x10^{5}$
B	$2.8\pm2.0^{a}x10^{2}$	$1.7\pm1.1^{c}x10^{4}$	$2.8\pm2.1^{c}x10^{4}$	$6.2\pm2.5^{c}x10^{5}$
C	$2.7\pm2.3^{b}x10^{2}$	$1.7\pm1.1^{c}x10^{4}$	$2.1\pm2.2^{e}x10^{4}$	$6.0\pm2.3^{c}x10^{5}$
D	$2.9\pm1.6^{a}x10^{2}$	$1.8\pm1.7^{c}x10^{4}$	$3.3\pm1.8^{b}x10^{4}$	$6.1\pm1.6^{c}x10^{5}$
E	$2.9\pm1.6^{a}x10^{2}$	$1.9\pm2.1^{c}x10^{4}$	$3.1\pm1.5^{b}x10^{4}$	$6.3\pm1.2^{c}x10^{5}$
F	$2.4\pm2.1^{c}x10^{2}$	$2.2\pm2.0^{b}x10^{4}$	$2.4\pm2.0^{d}x10^{4}$	$6.7\pm1.8^{b}x10^{5}$
G	$2.6\pm1.2^{b}x10^{2}$	$1.6\pm1.2^{d}x10^{4}$	$3.8\pm1.4^{a}x10^{4}$	$5.9\pm2.0^{d}x10^{5}$
H	$2.0\pm1.4^{d}x10^{2}$	$1.1\pm1.3^{e}x10^{4}$	$3.6\pm1.7^{a}x10^{4}$	$5.2\pm2.1^{e}x10^{5}$

Mean ± SD. Means with different superscripts along the column are significantly different ($p<0.05$).A: Fresh Oja-Oba sample (Ilorin); B: Fresh Asa dam sample (Ilorin); C: Fresh Tanke sample (Ilorin); D: Fresh Kulende sample (Ilorin); E: Fresh Ogbomoso sample (Aroje); F: Fresh Ogbomoso sample (Taki); G: Fresh Ogbomoso sample (Owode), and H: Fresh Ogbomoso sample (Gambari).

Microbial isolates from stored cheese samples

Having considered the shortcomings, the presence of pathogenic organisms in the hawked samples was investigated. Different types of organisms were isolated from the cheese samples (Table 6). Organisms such as *Escherichia coli* were noticed in three of the eight samples. Other organisms isolated from the samples include *Aspergillusniger*, *Aspergillusflavus*, *Rhizopus species*, *Salmonella species*, *Lactobacillus acidophilus*, and *Klebsiellaspecies*. The presence of these organisms

suggests that some of the 'wara' samples hawked on major streets in Ogbomoso and Ilorin towns may have been contaminated. The contamination noticed could be due to unhygienic conditions under which the cheeses were produced, or probably, the unsterilised nature of the equipment and materials (Adetunji et al., 2007). The contamination could have equally emanated from the raw milk or its sources, which may have been contaminated (Ibrahim and Falegan, 2013).

Table 6. Isolated and identified organisms from the cheese samples.

Organism isolated\samples	A	B	C	D	E	F	G	H
Klebsiella species	-	+	+	+	+	-	+	+
Salmonella species	-	+	+	+	-	+	+	+
Lactobacillus acidophilus	+	+	+	+	+	-	-	+
Escherichia coli	+	-	-	-	-	-	+	+
Aspergillusniger	+	+	+	+	+	+	+	+
Aspergillusflavus	+	-	-	+	+	+	+	+
Rhizopus species	+	-	+	-	+	+	+	+

Fungal species; + (present); - (absent). A: Fresh Oja-Oba sample (Ilorin); B: Fresh Asa dam sample (Ilorin); C: Fresh Tanke sample (Ilorin); D: Fresh Kulende sample (Ilorin); E: Fresh Ogbomoso sample (Aroje); F: Fresh Ogbomoso sample (Taki); G: Fresh Ogbomoso sample (Owode), and H: Fresh Ogbomoso sample (Gambari).

Conclusion

Stored cheese samples showed a progressive reduction noticed in its nutritional value, which could be due to the progressive increase in the microbial load. The high microbial load could have been caused by the high moisture content and the action of the identified pathogenic organisms. All the shortcomings noticed could be traced to the behavioural lifestyle of the processors, hawkers and/or other handlers who may have handled the productor its raw materials in an unhygienic way. The health of consumers of the hawked cheeses is at risk, as a result of the unsafe nature of the hawked cheeses. The regulatory body saddled with the responsibility of overseeing the quality of food in the country should seriously look into the quality of local cheeses being hawked around.

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KOMPARATIVNO ISTRAŽIVANJE HRANLJIVE VREDNOSTI I MIKROBIOLOŠKE BEZBEDNOSTI SVEŽEG SIRA "WARA" KORIŠĆENOG U GRADOVIMA ILORIN I OGBOMOSO

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R e z i m e

Neuhranjenost koja je rezultat niskog unosa proteina, jedan je od problema ishrane sa kojim se suočava većina zemalja u razvoju uključujući Nigeriju. Većina proteinskih izvora hrane je skupa i deficitarna. 'Wara' je proteinski prehrambeni proizvod gotov za upotrebu, koji se pravi podsirivanjem mleka. Pre upotrebe uglavnom ne podleže nikakvim daljim bezbednosnim tretmanima. Česta prodaja na našim glavnim ulicama i putevima zahteva određivanje bezbednosti ovih proizvoda. Uzorci sira 'wara' sa četiri različite lokacije u Ilorinu, državi Kvara odnosno u Ogbomosu, državi Ojo analizirani su radi provere hranljive vrednosti i mikrobiološke bezbednosti. Neposredni sastav uzoraka tokom perioda skladištenja pokazao je da su se sadržaj vlage i ugljeni hidrati povećali sa 59,69% na 72,00% odnosno sa 2,39% na 11,39%, dok su se sadržaji proteina, masti i pepela smanjili sa 22,20% na 10,80%, 15,80% na 3,62% odnosno sa 2,99% na 0,25%. Broj bakterija i gljiva kretao se od 2,0 X 10^2cfu do 6,3 X 10^5cfu odnosno od 2,0 X 10^2cfu do 7,1 X 10^5 cfu. Izolovane su vrste *Klebsiella* i *Salmonella*, *Escherichia coli* i neke gljive. Istraživanje je pokazalo da neki od korišćenih sireva nisu bezbedni za upotrebu. Navedeni razlozi su bili nehigijenska praksa trgovaca ili proizvodjača i/ili nedostatak potrebnih sredstava za zaštitu.

Ključne reči: 'wara', kvalitet, bezbednost, prodaja (na ulici), čuvanje, patogeni.

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THE EFFECT OF CROPPING PATTERN ON THE PROFITABILITY OF LIQUID FERTILIZER USAGE IN DRY SEASON VEGETABLE PRODUCTION IN THE SOUTHERN GUINEA SAVANNAH ZONE OF NIGERIA

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Abstract: Liquid fertilizers in dry season vegetable production are applied using different cropping patterns with little or no empirical evidence on which pattern is the most profitable. This study, therefore, investigated the effect of cropping patterns on the profitability of liquid fertilizer usage in dry season vegetable production. Specifically, the study identified the various vegetable enterprises, assessed the inputs and outputs of the different vegetable enterprises and estimated the profitability of the vegetable enterprises. A multi-stage random sampling procedure was used to select 309 farmers in the Southern Guinea Savannah zone. Pretested and structured interview schedules were used for data collection. Descriptive statistics and partial budgeting techniques were used for data analysis. Twelve different vegetable enterprises were identified in the study. Sixty percent of users of liquid fertilizer cultivated only fruit vegetables such as okra and peppers. The usage of the combination of both liquid and non-liquid fertilizers in mixed vegetable production yielded the highest quantity of output of about 1374kg/ha. However, usage of sole liquid fertilizer on exotic vegetables gave the highest profitability of 323 percent on the rate of return to capital investment. The study has concluded that the use of liquid fertilizer increases profitability and therefore recommends the formulation and implementation of policies that will encourage liquid fertilizer usage among the farmers.

Key words: liquid fertilizer, dry season, vegetable enterprise, profitability, fertilizing, crop production.

Introduction

The Southern Guinea Savannah zone is characterized by low rainfall and long dry periods which make it an excellent location for vegetable production. Dry season vegetable production is an important component of the farming systems in

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this zone. This is because it provides an opportunity for diet improvement and serves as a source of income and employment to the farmers. Vegetables are usually planted as sole crops or intercropped with staple crops like rice, maize or tubers (James et al., 2010). Farmers frequently intercrop vegetables on the same bed, so that a single bed can hold as many as five different vegetables (Ogunyinka et al., 2004). Intercropping is economically more profitable than sole cropping (James et al., 2010). This is because it increases the farmers' income per unit of land and labor; helps maintain good soil structure, and the incidences of pests and diseases are reduced.

Fertilizers are needed in vegetable production due to the poor health conditions of the soils used for production in Nigeria. In the absence of the regular chemical fertilizers, farmers are left with no choice than to look for alternative means of fertilizing their soils. Commercial liquid fertilizer was first introduced into the country in 2003. Even though it had been around for over a decade, very little is known about it, especially with regards to its usage in dry season vegetable production. These liquid fertilizers act fast, and studies have shown that apart from an increase in output, crops grown with liquid fertilizer are more nutritious (Agbulu and Idu, 2008; Criollo et al., 2011). Its use has been associated with superior quality as well as the quantity of crops. There is also the advantage of improved efficiency in the application of liquid fertilizer. This is because it can be done alongside irrigation (drip or sprinkler) and pesticide application thereby saving time and resources (Finck, 1992; Dittmar, 2007).

Since vegetables are usually intercropped, an assessment of the cropping pattern of vegetable production on the profitability of liquid fertilizer usage will bring to limelight the interaction of the different cropping patterns, farm resources, and farm enterprises. This will enable the farmers to make the best decision as to what vegetables to grow, the quantity of inputs required as well as the output they stand to get.

In view of the foregoing, the study sought to: (i) identify the different vegetable enterprises in the study; (ii) assess the inputs and outputs of the different vegetable enterprises; and (iii) estimate the profitability of the vegetable enterprises in the study.

Materials and Methods

Study area, sampling technique, and sample size

This study was carried out in the Southern Guinea Savannah zone of Nigeria. The rainfall in this zone shows two peaks in July and September (Ogundare et al., 2012). Despite the fact that this is the most luxuriant of the savannah vegetation belts in Nigeria, the soils are low in organic matter and chemical fertility.

The population for the study comprised all dry season vegetable farmers in the study area. Locations, where dry season vegetable production was predominantly carried out, were identified from the 2012 Crop Area Yield Survey (CAYS) manual of the zone. Twenty-five percent of the identified locations in the zone were randomly selected. This gave a total of seventeen locations. Next, the different farmer groups in each of the selected locations were identified. A list of all dry season vegetable farmers was obtained from the leader of each of the groups. From those lists, another list was compiled to give the total number of vegetable farmers in that location irrespective of the group they belonged to. From the compiled list, twenty-five percent of the listed vegetable farmers were randomly selected from each location to give a sample size of 317 vegetable farmers who were interviewed for the study. Data for only 309 farmers were eventually useful for analysis due to insufficient information given by the remaining eight.

Method of data collection

Data for the study were collected for the 2013/2014 dry season vegetable production using a well-structured interview schedule administered to vegetable farmers. Focus Group Discussion (FGD) was also organized with the local leaders of the vegetable farmer groups to supplement the data obtained from the interview schedule, and pretesting was done with 30 vegetable growers.

Analytical techniques

Descriptive statistics which include measures of central tendencies such as frequency distribution, mean, mode and percentages were used to identify the various vegetable enterprises encountered in the study, while the mean was used to assess the input and output of the different vegetable enterprises. The study also adopted the use of partial budgeting to calculate the profitability of liquid fertilizer usage for the vegetable enterprises. The gross margin (GM) was used specifically to estimate the costs and returns of the vegetable enterprises. GM analysis enables the comparison of the relative performances regarding returns of similar enterprises directly. Since GM is not a measure of farm profit per se because it does not include capital or fixed cost, profitability indices such as the operating ratio (OR) and return to capital invested (RCI) which can be calculated from the gross margin, and net profit were, therefore, employed in this study to show the profitability of the vegetable enterprises.

Mathematically,

$$GM = GVO - TVC \quad (1)$$

$$GVO = P \times Q \quad (2)$$

Net profit = GM – TFC (3)
where,
GVO = Gross value of output;
TVC = Total variable cost;
P = Unit price of each vegetable; Q = Quantity of vegetable output; and
TFC = Total fixed cost.

TVC was then computed by summing up all the cost incurred for labor and purchased inputs for the production season while the TFC was computed by depreciating the fixed cost components. The straight line method of depreciation was used, and this is given as $\frac{Costofitem-salvagevalue}{Usefullife}$ (4)

For this study, the salvage value was assumed to be zero because the vegetable farmers rarely sell off their equipment and machines. They use them until the value is completely or almost completely lost.
Profitability indices used were given as:
OR = TVC/GVO (5)
RCI = Net profit / Total cost (TC) (6)
where TC = TFC + TVC.

GM is best calculated on a per hectare basis. This allows for easy projection/estimation of figures based on the actual land size intended for use in vegetable production. Consequently, the analysis was therefore done on a per plot basis. Thus, the 309 sampled farmers had a total of 448 plots.

Results and Discussion

The results obtained from the data analysis and the corresponding discussions are presented in this section.

Dry season vegetable production according to vegetable enterprises

The classes of vegetables cultivated by farmers and the different categories of fertilizers used in the study are presented in Table 1.

Table 1. Distribution of farms based on the category of fertilizer usage and class of vegetables.

Category of fertilizer usage/ class of vegetables	Sole fruit vegetables	Sole leafy vegetables	Sole exotic vegetables	Mixed vegetables	Total
Liquid fertilizer only	40	7	4	2	53
Liquid with non-liquid fertilizer	35	21	10	7	73
Non-liquid fertilizer	112	133	13	64	322
Total	187 (41.70)	161 (35.90)	27 (6.10)	73 (16.30)	448 (100.00)

Source: Field survey, 2015; *Figures in parentheses are in percentages.

The vegetables encountered on the field during the survey were amaranthus, bitter leaf, celosia, corchorus, kenaf, pumpkin, scent leaf and waterleaf (classified as leafy vegetables). The fruit vegetables were garden egg, okra, onion, sweet pepper, hot pepper, long pepper, green pepper, and tomatoes. Others were cabbage, cucumber, lettuce, and carrots and these were classified as exotic vegetables.

Table 1 shows that the modal class of vegetables planted was the sole fruit, accounting for about 42 percent of total plots for dry season vegetable production. Almost 60 percent of plots where liquid fertilizer was used, either solely or with non-liquid fertilizers, also had sole fruit vegetables planted on them. This was contrary to expectation. This was probably due to the fact that the information gathered during the pilot survey showed that liquid fertilizers were mainly used for leafy vegetables because they made the leaves of the vegetables very green. The reason for this unexpected trend may be because the vegetable farmers in the study area are fruit vegetable experts. It may also be because the returns for fruit vegetables are higher during the dry season than those for leafy vegetables. Profitability analysis will help to throw more light on the latter reason. It may also be because the liquid fertilizers simply work better with the fruit vegetables compared with the leafy vegetables. Liquid fertilizers were mostly used for okra, followed by garden egg, hot pepper, and sweet pepper among the fruit vegetables. Overall, the dominant leafy vegetables planted by the farmers were amaranthus and corchorus while it was okra and peppers generally for the fruit vegetables. Four different classes of vegetables were identified as well as three fertilizer categories, making a total of twelve (12) vegetable enterprises identified in the study. These are presented in Table 2.

Table 2. Distribution of farms according to vegetable enterprises.

Vegetable enterprise	Frequency	Percentage
Liquid only on sole fruit vegetables (E_1)	40	8.93
Liquid only on sole leafy vegetables (E_2)	7	1.56
Liquid only on sole exotic vegetables (E_3)	4	0.89
Liquid only on mixed vegetables (E_4)	2	0.45
Liquid with non-liquid on sole fruit vegetables (E_5)	35	7.81
Liquid with non-liquid on sole leafy vegetables (E_6)	21	4.68
Liquid with non-liquid on sole exotic vegetables (E_7)	10	2.23
Liquid with non-liquid on mixed vegetables (E_8)	7	1.56
Non-liquid only on sole fruit vegetables (E_9)	112	25.00
Non-liquid only on sole leafy vegetables (E_{10})	133	29.70
Non-liquid only on sole exotic vegetables (E_{11})	13	2.90
Non-liquid only on mixed vegetables (E_{12})	64	14.29
Total	448	100.00

Source: Field survey, 2015.

As shown in Table 2, plots, where non-liquid fertilizers were used on sole leafy vegetables (E10), were the modal class of vegetable enterprises. Plots where liquid fertilizer was used accounted for 28 percent of the total number of plots ($E_1 - E_8$).

Input-output analysis of the different vegetable enterprises

The physical quantities of liquid and non-liquid fertilizers used as well as other inputs used in vegetable production for the different enterprises are presented in this sub-section. These are presented in Table 3.

Table 3. Summary of physical inputs and outputs per hectare for the vegetable enterprises.

Variables	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8	E_9	E_{10}	E_{11}	E_{12}	Total
No. of plots	40	7	4	2	35	21	10	7	112	133	13	64	448
Average farm size (ha)	0.76	0.82	1.03	0.5	0.86	0.64	0.37	0.94	0.71	0.56	0.29	0.69	0.66
Total farm size (ha)	30.3	5.75	4.1	1.0	30.18	13.5	3.65	6.6	79.09	74.70	3.82	44.17	296.85
Qty of output (kg)	433.99	104.68	293.71	721.59	763.54	281.75	300.19	1374.21	438.06	176.73	174.33	253.53	350.52
Qty of labor (man-day)	126.15	162.51	161.72	296.63	198.84	208.17	130.48	235.24	286.65	482.44	188.87	359.7	320.21
Qty of liquid fert. (litres)	3.95	2.71	6.92	4.00	3.22	2.82	3.51	1.35	0	0	0	0	3.41
Qty of non-liq.fert. (kg)	0	0	0	0	214.98	162.62	175.83	274.29	348.43	219.08	398.95	284.63	278.8
Qty of seed (kg)	0.10	0.07	0.01	0.15	0.17	0.11	0.02	0.05	0.20	0.51	0.03	0.28	0.27
Qty of herbicide (litres)	2.10	1.86	2.50	1.00	2.79	0.81	0.90	4.14	1.87	0.74	0.92	1.29	1.48
Qty of pesticide (litres)	3.44	1.78	8.17	3.00	5.91	3.08	8.33	7.48	7.10	4.31	9.65	5.71	5.48
Qty of fuel for irrigation (litres)	216.39	226.92	217.44	207.69	199.52	248.46	72.31	211.15	235.46	252.4	140.77	179.66	227.80
Qty of water for irrigation (Ha cm^3)	61.12	113.75	90.7	68.04	60.7	77.54	43.66	94.16	127.92	118.50	58.59	69.83	95.38

Source: Field survey, 2015.

Table 3 shows that users of sole liquid fertilizers on exotic vegetables (E_3) had the largest average plot size, while users of sole non-liquid fertilizers on the same exotic vegetables (E_{11}) had the smallest. Regarding total farm size, users of sole non-liquid fertilizers on fruit vegetables (E_9) had the largest total farm size, while users of sole liquid fertilizers on mixed vegetables (E_4) had the smallest. Users of both liquid and non-liquid fertilizers on mixed vegetables (E_8) had the highest output, measured in grain equivalent, while users of sole liquid fertilizers on leafy vegetables (E_2) had the least. This trend was, however, contrary to expectation.

The results on the quantity of labor used in dry season vegetable production show that users of sole non-liquid fertilizer on leafy vegetables (E_{10}) used the highest quantity of labor, and users of sole liquid fertilizers on fruit vegetables (E_1) used the least. Users of sole liquid fertilizers on exotic vegetables (E_3) used the highest quantity of liquid fertilizer, while users of both liquid fertilizers and non-liquid fertilizers on mixed vegetables (E_8) used the least quantity. Similarly, users of sole non-liquid fertilizers on exotic vegetables (E_{11}) used the highest quantity of non-liquid fertilizers while users of both liquid and non-liquid fertilizer on leafy vegetables (E_6) used the smallest quantity. Analysis on the quantity of seed used in the study shows that users of sole liquid fertilizers on exotic vegetables (E_3) used the least quantity of seeds while users of sole non-liquid fertilizers on leafy vegetables (E_{10}) used the highest quantity. This may have been due to the very tiny nature of the seeds of the exotic vegetables which made them almost weightless. Also, most of the farmers planted an improved variety of the exotic vegetables and so did not need to sow more than the recommended seed rate because a hundred percent germination rate was almost guaranteed. The same could not be said for farmers who planted leafy vegetables. They used more of the local varieties and so had to sow more than the recommended seed rate to ensure a relatively high germination rate. The healthy looking vegetable plants are usually left on the field after germination, while the rest are weeded out.

On the average, users of both liquid and non-liquid fertilizers on mixed vegetables (E_8) used the highest quantity of herbicides. This may have stemmed from their relatively larger plot sizes, and as such, use of herbicides especially during land preparation may have been cheaper. Users of sole non-liquid fertilizers on leafy vegetables (E_{10}), on the other hand, used the least quantity of herbicides. Again, this may have stemmed from their relatively small plot sizes, so that clearing the weed with manual labor was more cost-effective, especially if the source of the labor was the family. In the same vein, users of sole liquid fertilizers on leafy vegetables (E_2) used the least quantity of pesticides while users of sole non-liquid fertilizers on exotic vegetables (E_{11}) used the highest quantity.

Users of sole non-liquid fertilizers on leafy vegetables (E_{10}) used the highest quantity of fuel for irrigation while users of both liquid and non-liquid fertilizers on exotic vegetables (E_7) used the least quantity. The same trend was noticed for the

quantity of water used for irrigation. This may have been because more than half of the farmers in this group (E_7) irrigated the land manually; hence, this could have contributed to the low usage of fuel and water for irrigation.

Profitability of liquid fertilizer usage in dry season vegetable production

This sub-section presents the results obtained from the profitability analysis of liquid fertilizer usage among the vegetable farmers in the study. These are presented in Table 4.

Table 4. Profitability analysis of the vegetable enterprises (₦/ha).

Variables	E_1	E_2	E_3	E_4	E_5	E_6	E_7
Gross value of output (A)	517,313	176,843	1,030,250	804,500	675,158	388,972	401,925
Rent on land	10,154	0	0	0	13,780	7,500	21,000
Cost of hired labor & imputed family labor	92,369	55,808	143,233	130,700	172,573	74,701	93,758
Cost of liquid fertilizers	9,164	6,614	14,833	11,000	8,372	4,718	7,919
Cost of non-liquid fertilizers	0	0	0	0	37,501	15,353	20,792
Cost of seeds	11,236	8,029	28,290	12,500	20,356	12,120	9,810
Cost of herbicides	3,513	2,257	7,767	2,000	3,891	2,222	2,883
Cost of pesticides	3,714	1,800	8,167	3,200	6,720	3,214	8,658
Cost of fueling and maintenance of pumps	33,355	19,857	28,092	24,000	23,162	24,590	10,817
Total variable cost (B)	163,505	94,365	230,382	183,400	286,355	144,418	175,637
Total fixed cost (C)	13,421	10,285	13,059	10,498	13,691	4,338	12,649
Gross margin (D = A–B)	353,808	82,478	799,868	621,100	388,803	244,554	226,288
Net profit (E = D–C)	340,387	72,193	786,809	610,602	375,112	240,216	213,639
Operating ratio (B/A)	0.32	0.53	0.22	0.22	0.42	0.37	0.44
Return to investment (E/B+C)	1.92	0.69	3.23	3.15	1.25	1.61	1.13

Table 4. Continued.

Variables	E_8	E_9	E_{10}	E_{11}	E_{12}	Total
Gross value of output (A)	326,743	531,431	249,881	293,105	330,089	396,690
Rent on land	14,500	10,022	11,450	10,000	12,600	12,334
Cost of hired labor & imputed family labor	35,078	106,602	65,297	65,120	73,985	89,569
Cost of liquid fertilizers	3,369	0	0	0	0	8,866
Cost of non-liquid fertilizers	46,275	51,718	26,581	30,566	30,876	33,550
Cost of seeds	10,985	18,447	15,489	12,883	13,760	15,465
Cost of herbicides	4,228	3,590	1,218	4,467	2,179	2,666
Cost of pesticides	9,771	7,521	4,486	8,878	5,602	5,739
Cost of fueling and maintenance of pumps	23,086	22,558	21,267	30,566	30,876	24,352
Total variable cost (B)	147,742	220,458	145,770	162,480	169,878	192,541
Total fixed cost (C)	12,680	12,247	7,379	5,630	8,216	9,866
Gross margin (D = A–B)	179,001	310,973	104,111	130,625	160,211	204,149
Net profit (E = D–C)	166,321	298,726	96,732	124,995	151,995	194,283
Operating ratio (B/A)	0.45	0.41	0.58	0.55	0.49	0.49
Return to investment (E/B+C)	1.04	1.28	0.63	0.74	0.85	0.96

Table 4 confirms that dry season vegetable production was profitable for all the 12 enterprises. This agrees with the findings by Nwanchukwu and Onyenweaku (2007), Iwuchukwu and Uzoho (2009), Enete and Okon (2010), Ogunniyi (2011), Tsoho and Salau (2012) that dry season vegetable production is a profitable enterprise. Table 4 also shows that except for enterprise two (E_2), users of liquid fertilizers either solely or with non-liquid fertilizers had higher net profit than users of non-liquid fertilizers when comparison is done based on the class of vegetables planted (i. e. compare E_1 and E_5 with E_9; E_3 and E_7 with E_{11}; and E_4 and E_8 with E_{12}). Users of sole liquid fertilizers on exotic vegetables (E_3) had the highest net

profit. This was followed by users of sole liquid fertilizers on exotic vegetables (E_4), then by users of both liquid fertilizers and non-liquid fertilizers on fruit vegetables (E_5), and then by users of sole liquid fertilizers on fruit vegetable (E_1). As earlier hypothesized, the result of cost and returns clearly shows that cultivation of fruit vegetables was more profitable than the cultivation of leafy vegetables.This is because, in all three fertilizer use categories, only the fruit vegetable class (E_1, E_5, E_9) had higher net profit than the average recorded for the study which was calculated to be ₦194,283.

Enterprises E_3, E_4, and E_1, in that order, had the highest rate of return to capital invested and the lowest operating ratio, while enterprises E_{10} and E_{11}, in that order, had the lowest rate of return to capital invested and the highest operating ratio.

Conclusion

The study concluded that there were four different classes of vegetables and three different fertilizer usage categories, thus making a total of 12 vegetable enterprises identified in the study. Users of sole liquid fertilizers on leafy vegetables (E_2) had the least cost of production, while users of sole liquid fertilizers on exotic vegetables (E_3) had the highest gross margin and net profit, and consequently, the highest return to capital invested. Based on the findings, the study concludes that usage of liquid fertilizers whether solely or in combination with non-liquid fertilizers was more profitable for the dry season vegetable production. The study, therefore, recommended the use of sole liquid fertilizers for dry season vegetable production in the study area.

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UTICAJ SISTEMA GAJENJA NA PROFITABILNOST UPOTREBOM TEČNOG ĐUBRIVA U POVRTARSKOJ PROIZVODNJI TOKOM SUŠNE SEZONE U SAVANSKOJ ZONI JUŽNE GVINEJE U NIGERIJI

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R e z i m e

Tečna đubriva se u povrtarskoj proizvodnji tokom sušne sezone primenjuju korišćenjem različitih sistema gajenja sa malo ili bez empirijskih dokaza o tome koji je oblik najprofitabiliji. Ovim se istraživanjem, stoga, ispituje uticaj sistema gajenja na profitabilnost upotrebe tečnog đubriva u povrtarskoj proizvodnji tokom sušne sezone. Naime, u ovom istraživanju identifikovana su različita povrtarska preduzeća/gazdinstva, procenjena su ulaganja i prinosi različitih povrtarskih kultura i procenjena je njihova profitabilnost. Za odabir 309 proizvođača u savanskoj zoni južne Gvineje korišćena je višestepena procedura slučajnog uzorkovanja. Za prikupljanje podataka korišćeni su predtestirani i strukturirani intervjui prema rasporedu za prikupljanje podataka. Za analizu podataka korišćene su deskriptivna statistika i tehnike delimičnog budžetiranja. Za istraživanje je indentifikovano dvanaest različinih povrtarskih kultura. Šezdeset procenata korisnika tečnog đubriva uzgajalo je samo plodovito povrće, kao što su bamija i paprika. Upotreba kombinacije i tečnih i čvrstih đubriva u mešovitoj povrtarskoj proizvodnji dala je najviši prinos od 1.374 kg/ha. Međutim, najveća profitabilnost u visini od 323 procenata po stopi povraćaja na kapitalna ulaganja ostvarena je prilikom upotrebe isključivo tečnog đubriva u proizvodnji egzotičnog povrća. Ovim istraživanjem se zaključuje da upotreba tečnog đubriva povećava profitabilnost i stoga se preporučuje formulacija i sprovođenje politika koje će podsticati korišćenje tečnog đubriva od strane poljoprivrednih proizvođača.

Ključne reči: tečno đubrivo, sušna sezona, povrtarsko preduzeće/gazdinstvo, profitabilnost, đubrenje, ratarska proizvodnja.

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COMPARATIVE ANALYSIS ON THE PROFITABILITY OF SOLE MAIZE CROPPING AND MAIZE/MELON INTERCROP IN OSUN STATE, NIGERIA

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Abstract: Profitability is a motivational factor in any enterprise. The study compared the profitability of sole maize and maize/melon intercrop in Osun State. A purposive sampling technique was used and primary data collected with the aid of a structured questionnaire. Descriptive statistics, budgetary technique, inferential statistics and regression techniques were used to analyse the data collected. The majority of the respondents were active, male, had formal education and had less than 21 years of experience in cropping systems. The estimated net return to management was ₦59,323.83 per sole maize farmer or ₦37, 548.75 per hectare per year and ₦175,178.68 per farmer or ₦102, 832.17 per hectare for maize/melon. Budgetary analysis results showed that both sole maize and maize/melon intercrop were profitable. The multiple regressions for maize/melon intercrop revealed that 94.2% of variation in profit was obtained by independent variables in the model. The multiple regressions for the sole maize profit function revealed that 62.3% of variation in profit was obtained by independent variables in the model. The costs of input used, labour employed and quantity sold were the major determinants of profitability. The appropriate policies to enable the farmers to have access to inputs at a subsidised rate should be put in place.

Key words: profitability, sole cropping, maize/melon intercrop, multiple regressions.

Introduction

Maize (*Zea mays L.*), based on the area cropped and quantity produced, is the third most important cereal grown in Nigeria after sorghum and millet (Olaniyan, 2015). It comes after wheat and rice in terms of world production. Maize is an annual cereal plant of the *Poaceae* family and native of Mexico (Hugar and Palled, 2008). It is grown for its grain which contains 65% of carbohydrate, 10−12% of protein and 4−8% of fat (Iken and Amusa, 2004). The crop also contains the

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vitamins A, B, C and E, including mineral salts and essential trace elements such as carotene, thiamine, ascorbic acid and tocopherol (Groote, 2002). Low capitalisation, price fluctuation, disease and pest, poor storage facilities and inefficiency or resource utilisation are the identified problems in maize production in Nigeria (Ojo, 2000). "Egusi" melon (*Citrullus lunatus* Thunb.) is among the most popular African indigenous vegetable crops produced in Nigeria on a large scale. Egusi melon is a member of the family *Cucurbitaceae* (Ojieh et al., 2008). The edible seed/kernel of melon contains approximately 46% of oil and 36% of protein (Ogbona and Obi, 2010). Olufemi and Salami (2006) have stated that melon is easily identifiable with the complex traditional mixed cropping systems of the humid and sub-humid tropical zone of Nigeria, as the trailing nature of its vines, alternately arranged and pinnately dissected leaves allow interplanting at distances dictated by number, sequence, type and combination of crops in the mixture.

Mixed cropping is practised to ensure food security against total crop failure or with intent to maximise yield and profit by making use of the same labour (Yusuf et al., 2008). According to Javanmard et al. (2009), intercropping is popular because of its advantages over sole cropping which include security of returns and higher profitability due to higher combined returns per unit area of land. Poggio (2005) reported that farmers intercropped for varied reasons, including insurance against crop pests, yield increment, weed control and high monetary returns. Intercropping encourages a higher nutrient uptake than in sole cropping and water use efficiency is high because of the inter-cooperative interaction between the intercrops. Intercropping is done with crop rotation to break weed, diseases and pests' cycles and it also provides complementary fertilisation to crops in sequence with each other (Ibeawuchi, 2007).

Researchers have worked on cereal based intercropping, such as maize/bean, maize/potato, maize/cassava, maize/yam, maize/soybean, and maize/groundnut, amongst many others (Jiao et al., 2008; Ijoyah et al., 2012). However, there is little work on maize/melon intercropped. About 70% of cassava, 73% of maize and 55% of egusi melon grown in Nigeria are produced under intercropping system (Iken and Amusa, 2004; Ogbona and Obi, 2010; Ijoyah et al., 2012). The incorporation of melon into maize/cassava intercrop at the right time has been reported to be more profitable and more environmentally friendly (Ogunremi, 2005).

Maize/melon intercrop is a farming practice gaining momentum in Osun State, Nigeria, with the choice being motivated by the economic objective of producing maximum output to earn a positive economic return (profit). The yield advantage may be in terms of higher yield or higher net income. There is, therefore, the need to examine the differences in profitability of sole maize cropping and maize/melon intercrop in Osun State. Specifically, the study compares the socio-economic characteristics of the sole maize farmers and the maize/melon intercrop farmers. It

also compares the profitability of sole maize with maize/melon intercrop with a view to determining their relative profitability. Knowledge of this research will help farmers to obtain empirical information about the profitability and its determinants of sole maize and maize/melon intercrop farming practices in order to make a pre-informed farming decision on maize production.

Materials and Methods

The study was conducted in Osun State, Nigeria. It is located in the south-west of Nigeria. The state has thirty Local Government Areas (LGAs). The major occupation of the people in the area is farming (NgeX, 2013). Osun State is bounded in the North by Kwara State, in the East by Ekiti State, in the West by Oyo State and in the South by Ogun and Ondo States. Osun State (7.5º N, 4.5º E) is an inland State in south-western Nigeria with Osogbo as its capital. It occupies a land mass of approximately 8,602 square kilometres with a population of 3,416,959 people (NBS, 2012). A total of 94 farmers were purposively sampled with the assistance of extension agents from Osun State Agricultural Development Programme (OSSADEP) from the two LGAs, namely: Atakunmosa West and Iwo based on the predominance of maize and maize/melon intercrop in these LGAs relative to the rest. Forty-seven questionnaires were administered to each of sole maize farmers and maize/melon intercrop farmers.

The primary data were collected using a pre-tested and validated questionnaire. The variables observed were: the socio-economic characteristics of the respondents; quantities and prices of inputs and outputs in the area during the 2015/2016 farming season. Descriptive statistics, budgetary technique, inferential statistics, and multiple regressions were used to analyse data collected.

The specific type of budgetary technique used was the gross margin analysis as well as the net farm income. The model is stated as follows:

GM = GI – TVC (1)

where: GM = Gross margin; GI = Gross income; TVC = Total variable cost,

NFI = GM – TFC (2)

where: NFI = Net farm income; GM = Gross margin; TFC = Total fixed cost.

The model used for estimating net farm income can be expressed by the equation:

$$NFI = \Sigma_{i=1\rightarrow 2}^{n} P_{yi} Y_i - \Sigma_{i=1\rightarrow 2}^{n} P_{xj} X_{ij} - \Sigma_{k=1}^{k} F_k \quad (3)$$

where:

Y_i = Enterprise's product(s) (where i = 1, 2 products); P_{yi} = Unit price of the product,

X_j = Quantity of the variable inputs (where j = 1, 2, 3…… m variable inputs),

P_{xj} = Price per unit of variable inputs; F_k = Cost of fixed inputs; Σ = Summation (addition) sign.

The total variable cost (TVC) includes items such as total cost of labour, transportation, fertiliser and seed. The total fixed cost (TFC) includes the depreciation on farm tools such as hoes and cutlasses and the cost of renting land.

Multiple regression models were used to find out the factors determining the profitability of the two distinct groups of cropping systems.

The regression model is specified as follows:

Profit margin = f (X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}) + e (4)

X_1 = Implement cost, X_2 = Years of experience, X_3 = Years of education, X_4 = Cost of inputs, X_5 = Level of education, X_6 = Age of respondents, X_7 = Labour cost, X_8 = Transportation cost, X_9 = Land value/rent, X_{10} = Quantity consumed, X_{11} = Quantity sold, e = Error term.

The functions that were tried include linear and Cobb-Douglas functions. The best fit was selected on the basis of the coefficient of multiple determination (R^2), the 't' and the F ratio and the responsiveness of the magnitude of the coefficient.

Linear function: $P = b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7 + b_8X_8 + b_9X_9 + b_{10}X_{10} + b_{11}X_{11} + e$ (5)

Cobb Douglas: $LogP = Logb_o + b_1LogX_1 + b_2LogX_2 + b_3LogX_3 + b_4LogX_4 + b_5LogX_5 + b_6LogX_6 + b_7LogX_7 + b_8LogX_8 + b_9LogX_9 + b_{10}LogX_{10} + b_{11}LogX_{11} + e$ (6)

where "P" = profit margin, b_o, b_1, b_2... b_{11} = coefficients of the parameters to be estimated.

Hypothesis of the study

Null hypothesis (Ho)

Ho: there will be no differences in the profitability of sole maize compared to the profitability of maize/melon intercrop.

Results and Discussion

Socio-economic characteristics of farmers

Regarding the sole maize farmers, the modal age brackets were between 46 and 55 years, which constituted 57.5% of the sole maize farmers interviewed (Table 1a). The mean age was 47. The majority (93.6%) of the respondents were male since it is common knowledge that the work of farming is a vocation that requires strength as practised in the study areas and such requirement can only be met by the male. All the respondents were married, with an average family size of 6.4. A large proportion (85.1%) of respondents had either primary or secondary education. Only 42.5% of the respondents had both primary and secondary education. More than half (53.2%) of the sole maize respondents had between 0 and 6 years of formal primary education.

Table 1a. Percentage distribution of respondents according to their socio-economic characteristics.

Characteristics	Sole maize		Maize/melon	
	Frequency	Percentage	Frequency	Percentage
Age of farmers (years)				
20–35	5	10.6	5	10.6
36–45	10	21.3	10	21.3
46–55	27	57.5	31	66.0
56–65	5	10.6	1	2.1
Gender of farmers				
Male	44	93.6	39	83
Female	3	6.4	8	17
Total	47	100	47	100
Marital status				
Single	0	0	1	2.1
Married	47	100	46	97.9
Family size				
1–4	10	21.3	10	21.2
5–8	32	68.1	31	66.0
9 and above	5	10.6	6	12.8
Educational level				
Primary	20	42.6	13	27.7
Secondary	20	42.5	25	53.2
Tertiary	2	4.3	3	6.3
No formal education	5	10.6	6	12.8
Years of formal education				
0–6	25	53.2	15	31.9
7–12	20	42.6	26	55.4
13–18	1	2.1	5	10.6
19–22	1	2.1	1	2.1
Membership of farmers' cooperatives				
Yes	15	31.9	13	26.7
No	32	68.1	34	72.3
Years of experience in type of cropping systems				
1–15	32	68.1	35	74.5
16–20	11	23.4	9	19.1
21–25	3	6.4	1	2.1
26–30	1	2.1	2	4.3
Hectares used for cropping				
1–2	42	89.4	46	97.9
3–4	5	10.6	1	2.1
Source of land used for cropping				
Gift	6	12.8	4	8.5
Rent	32	68.1	18	38.3
Inheritance	9	19.1	22	46.8
Purchase	0	0.0	3	6.4

Source: Field survey, 2016.

The majority (68.1%) of them were not members of the cooperative society. The average years of experience were 14.7 years meaning that the respondents were not new in the farming activity. The average farm size was 1.68 ha and about 68.1% of farmers acquired their land through rent. The majority (59.6%) of the respondents used personal savings as a source of funds (Table 1b). More than three quarters (76.6%) of sole maize respondents procured their inputs at Osun State Agricultural Development Programme (OSSADEP) input office. These findings support the results of Oladejo and Adetunji (2012), where it was reported that the mean age for sole maize farmers was 45.8 years with more than half of them being literate while the major source of finance for the farmers was personal savings and the mean land area cultivated was 2.2 ha.

Table 1b. Percentage distribution of respondents according to their socio-economic characteristics.

Characteristics	Sole maize		Maize/melon	
	Frequency	Percentage	Frequency	Percentage
Source of fund				
Personal saving only	28	59.6	26	55.3
Friends & relatives	0	0.0	2	4.3
Formal financial institutions	3	6.4	2	4.3
Personal savings and friends & relatives	7	14.9	8	17.0
Personal savings and formal financial institutions	8	17.0	6	12.8
Personal savings, friends& relatives and formal financial institutions	1	2.1	3	6.3
Land use security				
Very high	26	55.3	22	46.9
High	11	23.4	9	19.1
Low	6	12.8	16	34.0
Very low	4	8.5	0	0.0
Sources of input distribution				
Coop. society	2	4.3	6	12.8
Open market	5	10.6	3	6.4
Friends and family	1	2.1	1	2.1
OSSADEP	36	76.6	36	76.6
Coop. society and OSSADEP	3	6.4	1	2.1

Source: Field survey, 2016.

The modal age bracket for the maize/melon intercrop farmers was between the ages of 46 and 55 years (Table 1a). The mean age was 45, which indicated that

farmers were mostly middle-aged the same as the sole maize cropping farmers. Both groups of farmers were still active and energetic to meet the rigour of farming. Similarly, there was a positive effect on their managerial skills and ability which in turn affected their profitability. The majority (83%) of the respondents were male, with a mean family size of 6.5. A substantial proportion (80.9%) of respondents had basic primary and secondary education. Less than half (31.9%) of the respondents had between 0 and 6 years of formal education, 55.4% had 7–12 years of education. The majority (72.3%) opted not to join the cooperative society while only 26.7% of farmers belonged to the farmers' cooperative society. The average years of experience in maize/melon farming were 13.1 years, with the average farm size of 1.54 ha. About 46.8% of farmers acquired their land through inheritance while 38.3% of the farmers acquired their land through rent. More than half (55.3%) of the farmers depended solely on their personal savings (Table 1b). The majority (66%) of the farmers were sure of their continued use of the land, while 34% had very low land use security. The majority (76.6%) of respondents procured their inputs at OSSADEP.

There was no significant difference between the mean ages, family sizes; years of experience and farm size of sole maize and maize/melon intercrop farmers.

Budgetary analysis

The mean farm size was 1.54 ha and the average revenue per hectare for sole maize was ₦76, 070.71 per hectare. The total cost of production was ₦61, 744.47 per farmer or ₦38, 521.97 per hectare. Cost of labour (51.0%) had the largest share of the total cost, followed by cost of consumable farm inputs (28.5%). This corroborates the finding of Chukwuji (2008), who reported that labour constituted the single most important cost item on the average. The net return to management was ₦59, 323.83 per farmer or ₦37, 548.75 per hectare. The results showed that sole maize farming was profitable. Findings by Oladejo and Adetunji (2012) also showed that maize farming was profitable as respondents made ₦70, 325.74 of profits per hectare of maize produced during the year of survey.

For the maize/melon intercrop, the mean farm size was 1.68 ha and the average revenue per hectare was ₦158, 094.35. The total cost per hectare was ₦55, 262.18. Like sole maize farmers, cost of labour (44.1%) had the largest share of total cost. This is in tandem with the position of Abdulsalam et al. (2012), whose results showed that mixed cropping was profitable. Likewise, Yusuf et al. (2008) revealed that the average net farm income per hectare for melon in an intercrop was ₦915.77. Cost of consumable farm inputs, which comprise seeds, fertilizer/manure, herbicides and pesticides, which serve as indicators of the level of technology, had the second largest share of about 43.9 percent. The estimated net return to management was ₦175, 178.68 per farmer or ₦102, 832.17 per hectare per year.

Table 2. Average costs and returns (₦) of sole maize and maize/melon intercrop farmers in Osun State.

Items	Sole maize cropping (n=47)			Maize/melon intercrop (n=47)		
Farm size	1.54 ha	1.0 ha	Cost as % of TC	1.68 ha	1.0 ha	Cost as % of TC
Yield (kg)	4,321.28			6223.11		
Total revenue	117,148.90	76,070.71		265,598.50	158,094.35	
Rent on land	2,329.79	1,512.85	3.9	2,046.81	1,218.34	2.2
Cost of labour	30,236.17	19,633.88	51.0	40,940.43	24,369.30	44.1
Consumable farm inputs	16,919.36	10,986.60	28.5	40,717.66	24,236.70	43.9
Transport	3,808.51	2,473.06	6.4	3,414.89	2,032.67	3.7
Depreciation on tools	6,030.00	3,915.58	10.2	5,720.67	3,405.16	6.2
Total cost	61,744.47	38,521.20		90,419.82	55,262.18	
Return to management	59,323.83	37,548.74		175,178.68	102,832.17	

Source: Computed from survey data; ha – hectare.

From the foregoing, it is clear that there was a significant difference in the profits realised by sole maize cropping and maize/melon intercropping farmers per hectare which were ₦37, 548.75 and ₦102, 832.17 at the 5% significance level. This is due to intercropping of maize and melon on the same plot of land. This significance complies with the position of Abdulsalam et al. (2012), whose study showed that mixed enterprises were generally more profitable compared to the sole enterprises. This disagrees with the findings of Law-Ogbomo and Ekunwe (2011), who reported similar increases in the economic yield of sole maize compared to maize/melon under intercropping system. Costs of planting, weeding and fertiliser application for sole maize were lower and statistically different at the 5 per cent significance level from those for maize/melon intercrop. This is partly explained by the high cost of manual weeding associated with reduced spacing in the intercrop. Cost of pesticide application was significantly higher for maize/melon intercrop than sole maize enterprise at the 10% significance level. Cost of drying and sorting for maize/melon was significantly different and greater than that of sole maize (at the 1% significance level) due to the melon fruit being processed by depulping, drying and sorting.

All the consumable farm input costs for maize/melon, i.e. cost of maize and melon seeds, fertilisers, herbicides and pesticides, were significantly different and greater than those for sole maize at the 1% significance level. At the 1% significance level, the total input cost for maize/melon intercrop was higher and significant than the total input cost for sole maize.

Table 3. T-ratios for the tests of the hypothesis about the costs and returns vis-à-vis profitability per farmer regarding sole maize and maize/melon intercrop farmers in Osun State.

Items	Sole maize	Maize/melon	T-ratio	Significance
Farm size (ha)	1.54	1.68	4.251	0.001*
Revenue from maize	117,148.90	195,462.33		
Revenue from melon	0.00	70,136.17		
Total revenue	117,148.90	265,598.50	-3.747	0.000*
Variable cost				
Labour cost on:				
Land preparation	8,074.47	8,178.72	0.105	0.917
Heaping	1,382.98	1,961.70	0.552	0.583
Planting	3,485.11	6,885.11	2.571	0.013**
Weeding	2,927.66	8,119.15	2.518	0.015**
Fertiliser application	2,508.51	5,561.70	2.554	0.014**
Pesticide application	142.55	434.04	1.705	0.095***
Harvesting	9,512.77	9,800.00	0.127	0.900
Drying and sorting	0.00	2,202.13	-3.417	0.001*
Security	0.00	0.00		
Total labour cost	30,236.17	40,940.43	1.654	0.105
Consumable farm inputs:				
Maize seeds	4,004.89	6,551.49	3.183	0.003*
Melon seeds	0.00	2,420.64	-3.616	0.001*
Fertiliser/manure	10,729.79	22,255.32	4.068	0.001*
Herbicides	2,072.34	9,017.02	3.580	0.001*
Pesticides	112.34	473.19	3.056	0.004*
Total input cost	16,919.36	40,717.66	4.013	0.001*
Transportation	3,808.51	3,414.89	-0.428	0.671
Fixed cost				
Annual depreciation on:				
Cutlass	524.82	989.36	2.981	0.005*
Hoes	653.90	515.25	-2.547	0.014**
Sprayer/knapsack	3,819.15	3,009.86	-0.548	0.587
Baskets/bags	1032.13	1160.11	0.462	0.647
Farm coat	0.00	14.19	1.000	0.323
Boots	0.00	31.92	1.000	0.323
Wheel barrow	0.00	0.00		
Farm building	0.00	0.00		
Rent on land	2,329.79	2,046.81	-0.436	0.665
Total costs	61,744.47	90,419.82	2.609	0.012**
Return to management	59,323.83	175,178.68	-2.615	0.012**

Source: Field survey, 2016. ₦ (Nigerian currency).
*Significant at $P<0.01$, **Significant at $P<0.05$, ***Significant at $P<0.1$.

Depreciation cost of using a cutlass in maize/melon intercropping was statistically different and greater than that of sole maize (the 1% significance level) because clearing and weeding were better done with a cutlass in the maize/melon

intercrop as against sole maize because of the high population density of maize in the intercrop. As for the cost of hoes, there was a significant difference between the two cropping systems (at the 5% significance level). However, the total cost for maize/melon intercrop was significant (the 5% significance level) and higher than the total cost for sole maize thereby complying with *a priori* expectation.

Results of regression analysis/Sole maize enterprise

The linear function was chosen as the lead equation because of the relative larger adjusted R^2. The regression model had an adjusted R^2 of 0.942, which indicates that 94.2% of the variation in the profitability of sole maize cropping was jointly explained by the independent (explanatory) variables included in the model and this is a good indicator that the included explanatory variables had a very good influence on the profitability (Table 4). The model had an F-value of 69.102 which was significant at the 1% level meaning that the model has a good fit. Out of the eleven explanatory variables included in the model, three were significant. They are: cost of inputs, labour cost and total quantity sold. The Durbin-Watson value showed that there was no serial correlation among the explanatory variables.

Table 4. Regression results of the determinants of profitability of sole maize.

Independent variables	Coefficients	T-value
Constant	152914.366	0.316
Implement cost (X_1)	10.471	0.135
Years of experience (X_2)	-1966.935	0.497
Years of education (X_3)	-642.369	0.852
Cost of inputs (X_4)	-2.383	0.012**
Level of education (X_5)	-7486.708	0.639
Age of respondents (X_6)	-1557.055	0.588
Labour cost (X_7)	-2.193	0.001***
Transportation cost (X_8)	2.685	0.666
Land value/rent (X_9)	15.395	0.137
Quantity consumed (X_{10})	-110.243	0.110
Quantity sold (X_{11})	71.144	0.001***
Adjusted R^2	0.942	
F-value	69.102	
Durbin-Watson	1.991	

Source: Data analysis, 2016.
*** = Significant at $P<0.01$; ** = Significant at $P<0.05$ and * = Significant at $P<0.1$.

Cost of inputs

The coefficient of this variable carried a negative sign and was also statistically significant at the 5% level of significance. This shows that an increase in input costs would lead to a reduction in the profitability of sole maize farming (Table 4).

Labour cost

This variable had an expected coefficient that was negative and significant at the 1% level of significance implying that the higher the labour cost, the lower the profitability.

Total quantity sold

The coefficient of the variable was both positive and significant at the 1% level of significance implying that the higher the quantity sold, the higher the profitability. This agrees with the findings of Oladejo and Adetunji (2012), whose regression analysis has shown that significant relationships exist between maize production costs and returns to maize farmers in the study area.

Maize/melon enterprise

The regression model had an adjusted R^2 of 0.623, which indicates that 62.3% of the variation in the profitability of maize/melon intercrop was jointly explained by the independent (explanatory) variables included in the model and this is a good indicator that the included explanatory variables had a very good influence on the profitability (Table 5). The model had an F-value of 5.254 which was significant at the 1% level meaning that the model has a good fit. Out of the eleven explanatory variables included in the model, two were significant. They are: the input cost and the total quantity sold. The Durbin-Watson value showed that there was no serial correlation among the explanatory variables.

Table 5. Regression results of the determinants of profitability of maize/melon.

Independent variables	Coefficients	T-value
Constant	95105.052	0.236
Implement cost (X_1)	-1.429	0.223
Years of experience (X_2)	-1931.752	0.262
Years of education (X_3)	-912.611	0.679
Cost of inputs (X_4)	-2.222	0.035**
Level of education (X_5)	-3745.769	0.730
Age of respondents (X_6)	-1223.237	0.464
Labour cost (X_7)	-0.427	0.675
Transportation cost (X_8)	-0.019	0.995
Land value/rent (X_9)	3.704	0.450
Quantity consumed (X_{10})	-30.324	0.636
Quantity sold (X_{11})	82.980	0.001***
Adjusted R^2	0.623	
F-value	5.254	
Durbin-Watson	2.214	

Source: Data analysis, 2016.
*** = Significant at $P<0.01$; ** = Significant at $P<0.05$ and * = Significant at $P<0.1$.

Input cost

As expected, this variable had a coefficient that was negative and significant at the 5% level of significance. This implies that the higher the input cost, the lower the profitability.

Total quantity of produce sold

The coefficient of this variable carried a positive sign and was also statistically significant at the 1% level of significance. This shows that an increase in total quantity of output sold increased the profitability directly.

Conclusion

The study performed a comparative analysis of the profitability of sole maize cropping and maize/melon intercrop in Osun State. Both enterprises were profitable in the study area, but there was a significant difference in the profitability of the two cropping systems practised as the maize/melon intercrop was more profitable. The intercrop is, therefore, recommended for the farmers since it is more profitable and provides a variety of income generation for the farmers and with planned planting, farmers can make more money throughout the year, thereby ensuring food security and income security.

Cost of input and total quantity sold were the major variables that affected the profitability of maize/melon intercrop in the study area while input cost, labour cost and the total quantity of produce sold were the major determinants of the profitability of sole maize cropping in the study area.

The appropriate policies to enable the farmers to have access to inputs at a subsidised rate should be put in place.

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KOMPARATIVNA ANALIZA PROFITABILNOSTI KUKURUZA U MONOKULTURI I ZDRUŽENOG USEVA KUKURUZA I DINJE U DRŽAVI OSUN U NIGERIJI

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R e z i m e

Profitabilnost je motivacioni faktor u bilo kom preduzeću/gazdinstvu. Istraživanjem se poredila profitabilnost gajenja kukuruza u monokulturi i združenih useva kukuruza i dinje u državi Osun. Korišćena je tehnika ciljanog uzorkovanja i prikupljeni su primarni podaci uz pomoć strukturiranog upitnika. Za analizu prikupljenih podataka korišćene su deskriptivna statistika, tehnika budžetiranja, statistička inferencija i tehnike regresije. Većina ispitanika su aktivni muškarci, sa formalnim obrazovanjem i manje od 21 godine iskustva u bavljenju ratarstvom. Procenjeni neto povraćaj kapitala za upravljanje bio je ₦59.323,83 po poljoprivredniku koji je uzgajao kukuruz u monokulturi ili ₦37.548,75 po hektaru po godini i ₦175.178,68 po poljoprivredniku ili ₦102.832,17 po hektaru za združene useve kukuruza i dinje. Rezultati budžetske analize pokazali su da su i kukuruz u monokulturi i združeni usevi kukuruza i dinje profitabilni. Višestruka regresija za združene useve kukuruza i dinje pokazala je da je 94,2% varijacije profita dobijeno nezavisnim varijablama u ovom modelu. Višestruka regresija za funkciju profita kukuruza u monokulturi pokazala je da je 62,3% varijacije profita postignuto nezavisnim varijablama u ovom modelu. Troškovi inputa, radne snage i količine prodatog proizvoda su glavne determinante profitabilnosti. Trebalo bi uspostaviti odgovarajuće politike koje bi omogućile poljoprivrednicima da imaju pristup subvencionisanim inputima.

Ključne reči: profitabilnost, kukuruz u monokulturi, združeni usevi kukuruza i dinje, višestruka regresija.

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Navodi se pun naziv i adresa ustanove u kojoj je autor zaposlen. Ispisuje se neposredno nakon imena autora, centrirano. Ukoliko su autori iz različitih institucija brojčanom oznakom u superskriptu ispred institucije označava se ustanova u kojoj je zaposlen svaki od navedenih autora.

Sažetak
Sažetak je kratak informativni prikaz sadržaja članka koji čitaocu omogućava da brzo i tačno odredi njegovu relevantnost. U interesu je autora da sažetak sadrži termine koji se koriste za indeksiranje i pretraživanje. Sažetak ne sme da sadrži reference. Sastavni delovi sažetka su cilj istraživanja, metode, rezultati i zaključak. Sažetak treba da ima od 100 do 250 reči. Reč „Sažetak“ piše se boldovano i uvlači jednim tabulatorom, nakon čega slede dve tačke, a zatim tekst sažetka.

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.

Rezultati i diskusija
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.

Zaključak
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.

Zahvalnica
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.

Literatura
Poglavlje „Literatura“ treba da sadrži samo radove citirane u glavnom tekstu. Rad citiran u tekstu treba da sadrži prezime autora i godinu. Ako citat obuhvata jednog autora on se navodi kao Jalikop (2010) ili (Jalikop, 2010). Kada citat obuhvata dva autora on se navodi kao Sadras i Soar (2009) ili (Sadras i Soar, 2009). Ako se u tekstu citiraju više od dva autora posle prezimena prvog autora navodi se skraćenica „et al.“, a zatim godina. Ovakav citat navodi se kao Lehrer et al. (2008) ili (Lehrer et al., 2008). Ako se za određeni problem istovremeno citira više radova onda se oni hronološki nabrajaju. Odvajanje većeg broja citiranih radova van zagrade vrši se zarezom (,) a u zagradi tačkom i zarezom (;). Ako se citiraju dva ili više rada istog autora oni moraju biti poređani prema hronološkom redu (1997, 2002, 2006, itd.). Ukoliko se određeni autor pojavljuje nekoliko puta u istoj godini, dodaju se slova (2005a, b, c, itd.). Citate ličnih komunikacija i neobjavljenih podataka treba izbegavati, osim ako je to apsolutno neophodno. Takvi citati bi trebali da se pojave samo u tekstu (npr. Brown, lična komunikacija), ali ne i u spisku referenci.

Literatura koja je citirana u tekstu navodi se u spisku referenci u originalnom obliku, po abecednom redu, bez numeracije. Ako se citira veći broj radova istog autora najpre se navode radovi kada je autor sam, a zatim kada su prisutna dva i više autora. Ako se u nekoj od ovih kategorija javlja veći broj radova, treba ih hronološki srediti po godinama (1997, 2002, 2006, itd.), a ako se u istoj godini javlja veći broj radova dodaju se slova (2005a, 2005b, 2005c, itd.). Literaturni podatak treba da sadrži: prezime autora, početno slovo imena, godinu izdanja u zagradi, naslov rada, naziv časopisa, volumen i broj stranica (prva-poslednja). Prilikom citiranja knjiga navodi se izdavač i mesto izdavanja. Redovi svake reference posle prvog reda moraju biti uvučeni. U časopisu se koristi APA - Publication Manual of the American Psychological Association citatni stil.

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

Rezime
Rezime na srpskom jeziku (za radove napisane na engleskom jeziku) ili na engleskom jeziku (za radove napisane na srpskom jeziku) navodi se na kraju rada i treba da ima od 100 do 250 reči. Ispred osnovnog teksta rezimea, navodi se naslov rada, puno ime, srednje slovo i prezime svih autora i naziv i adresa ustanove autora. Naslov „Rezime“ piše se razmaknuto i centrirano. Nakon naslova sledi jedan razmak, a zatim tekst rezimea, uvučen jednim tabulatorom. Neposredno nakon teksta rezimea, navode se ključne reči, sa tačkom na kraju. E-mail adresa autora za kontakt navodi se ispod crte, pri dnu stranice.

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.

Nomenklatura
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.

Nakon objavljivanja rada, autoru za kontakt će biti poslat jedan primerak časopisa.
Mole se svi budući saradnici da rad pripreme prema datom uputstvu, kako bi olakšali rad redakcije časopisa. Ukoliko se rad ne pripremi po navedenom uputstvu neće biti prihvaćen za objavljivanje.

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