ISSN 1581-9175

Acta agriculturae Slovenica

Letnik 90, številka 1
Volume 90**,** Number 1

90-1	*Acta agriculturae Slovenica*	str. 1–66	Ljubljana, november 2007

Acta agriculturae Slovenica, **90(november 2007)1**

Acta agriculturae Slovenica

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Razmnoževanje	ROTOSI d.o.o., Tomačevo 19, SI-1000 Ljubljana, v 450 izvodih
Naslov uredništva	Groblje 3, SI-1230 Domžale, tel.: 01 7217 800, telefaks: 01 7241 005
E-pošta	peter.dovc@bfro.uni-lj.si
Domača stran	http://aas.bf.uni-lj.si/
Letna naročnina	25,04 EUR, za tujino 30 EUR
Posamezna številka	16,69 EUR, za tujino 20 EUR
Imetnik računa	UL, Biotehniška fakulteta, Jamnikarjeva 101, SI-1111 Ljubljana
Banka	BANKA SLOVENIJE, Slovenska 35, SI-1505 Ljubljana
Račun	01100-6030707410, sklic na številko 40-521-200341
Sofinancira	Javna agencija za raziskovalno dejavnost Republike Slovenije
Zbornik redno selektivno zajemajo	AGRIS, CAB Abstracts, COBISS in FSTA
Dokumentacijska obdelava	**Mednarodna:** Slovenski nacionalni center AGRIS **Domača:** INDOK Oddelka za zootehniko
Publikacije v zameno za Zbornik pošljite na naslov	Centralna knjižnica Biotehniške fakultete Univerze v Ljubljani, Jamnikarjeva 101, SI-1111 Ljubljana, p.p. 2995

UDK 57/63 ISSN 1581-9175

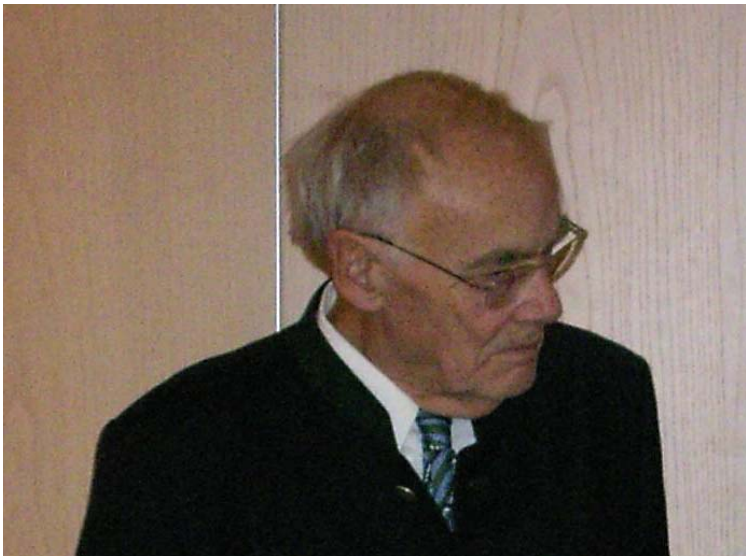
Acta agriculturae Slovenica

Letnik 90 Ljubljana, november 2007 Številka 1

VSEBINA / CONTENTS

Acta agriculturae Slovenica, 90(november 2007)1, 3–4.

Prof. Dr. Franz PIRCHNER CELEBRATED HIS 80th ANNIVERSARY



The Editorial Board of *Acta agriculturae Slovenica* (AAS) would like to congratulate Prof. Dr Franz Pirchner on his 80th birthday which he celebrated on 7 January this year. We are grateful for his help and support as a member of the Editorial Board of AAS since 1990.

Prof. Pirchner was born in Imst, Innsbruck, Tirol where he first came in contact with agriculture at the family farm. After obtaining degrees at Veterinary School (1950) and Agricultural University (1952) of Vienna, he joined J.L. Lush at Iowa Stete University from which he received his PhD in 1957. After few years of work at research station Wieselburg and in a poultry breeding company in USA he has held a professorship since 1964 first at Veterinary School of Vienna and than from 1970 until 1995 at Technical Faculty of Munich, Weihenstephan. Prof. Pirchner guided a large number of doctoral students, among them a fair number of international students. His enormous knowledge of literature inspired profound and fruitful discussions often setting new trends in animal breeding. He was a member of the Committee for Genetical-Statistical Methods of the German Society of Animal Breeding, the Executive Editor of the *Journal of Animal Breeding and Genetics* between (1975–1998), he chaired the Genetics Comission of the EAAP (1966–1972), International Permanent Committee of WCGALP (1988–1992) and German Society of Animal Science (1984–1988). For his scientific contribution to the field he obtained *doctor honnoris causa* from the University of Ghent, Belgium and from ETH Zürich, Switzerland. From the German Society of Animal Breeding he received the Herman Nathusius Medal in 1998. In addition to more than hundred scientific papers he wrote also a standard text book *Population Genetics in Animal Breeding*.

Editor
Peter DOVČ

Prof. dr. Franz PIRCHNER
OSEMDESETLETNIK

Uredniški odbor *Acte agriculturae Slovenice* (AAS) se pridružuje čestitkam ob 80. rojstnem dnevu prof. dr. Franza Pirchnerja, ki ga je praznoval 7. januarja letos. Hvaležni smo mu za pomoč in podporo v vlogi člana Uredniškega odbora AAS od leta 1990 naprej.

Prof. Pirchner je bil rojen v Imstu pri Innsbrucku na Tirolskem, kjer se je prvič srečal s kmetijstvom na domači kmetiji. Po diplomah na Veterinarski fakulteti (1950) in Kmetijski univerzi (1952) na Dunaju, je pod vodstvom J.L. Lush-a doktoriral na Iowa State University leta 1957. Po nekajletnem delu na raziskovalni postaji Wieselburg in v perutninarski firmi v ZDA je sprejel profesuro najprej na veterinarski fakulteti na Dunaju (1964), nato pa na Tehniški univerzi v Münchnu, Weihenstephan (1970–1995). Prof. Pirchner je bil mentor številnim doktorandom, med njimi tudi velikemu številu mednarodnih študentov. Njegovo izredno široko poznavanje strokovne literature je spodbujalo poglobljene in plodne diskusije, ki so pogosto pomenile začetek novih trendov v živinoreji. Prof. Pirchner je bil član odbora za genetsko statistične metode pri nemškem živinorejskem društvu, odgovorni urednik *Journal of Animal Breeding and Genetics* med leti 1975 in 1998, predsedoval je Komisiji za genetiko pri EAAP (1966–1972), Mednarodnemu stalnemu odboru WCGALP (1988–1992) in nemškemu društvu za znanost o živalih (1984–1988). Za njegov znanstveni doprinos na področju znanosti o živalih je prejel častna doktorata Univerze v Ghentu, Belgija in ETH v Zürichu, Švica. Nemško živinorejsko združenje mu je leta 1998 podelilo medaljo Hermanna Nathusiusa. Ob več kot sto znanstvenih člankih je prof. Pirchner napisal tudi standardni učbenik populacijske genetike v živinoreji - *Population Genetics in Animal Breeding*.

Urednik
Peter DOVČ

Acta agriculturae Slovenica, 90(november 2007)1, 5–16.

http://aas.bf.uni-lj.si

Agris category codes: Q04 COBISS Code 1.01

RELATION OF MYOFIBRIL FRAGMENTATION TO TEXTURAL AND CHEMICAL PARAMETERS OF AGED PORK *Longissimus dorsi* *

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Received February 16, 2006, accepted March 30, 2007.
Delo je prispelo 16. februarja 2007, sprejeto 30. marca 2007.

ABSTRACT

The relation of myofibrilar fragmentation (length of myofibrilar fragments, myofibrilar fragmentation index) to textural (Warner-Bratzler share force) and chemical parameters (non-protein nitrogen changes) of pork *Longisimus dorsi* muscles (acquired 24 h *post mortem*, normal meat quality) were investigated over a 16-day ageing period at 2 °C (± 1 °C). Ageing time significantly affected all parameters at the 1% level or less. The pH value for 16-day aged samples was slightly higher; the average values being 5.61 for nonaged and 5.67 for aged samples. Length of myofibrilar fragments being in average the highest (73 μm) for nonaged samples, and the lowest (15.7 μm and 12.4 μm) for 11 and 16 days aged ones. Myofibrilar fragmentation index increases significantly with storage: for nonaged samples below 30, after a 2-day ageing about 50, and after 5-day ageing 63.7 (determined as described by Olson *et al*., 1976), or after 11-day ageing 56.9 (determined as described by Hopkins *et al.*, 2004) Indexes for Hopkins procedure were about 7% lower ($P \leq 0.001$) compared to those for Olson procedure. Non-protein nitrogen after 11 and 16 days of storage was higher (10.78% and 10.93% of total nitrogen) compared to the nonaged pork (9.39% of total nitrogen). Warner-Bratzler share force was markedly affected by 16-day ageing (nonaged 51.3 N, 16 days 29.2 N). On the basis of instrumentally measured texture differences in thermally treated aged pork we concluded that myofibrilar fragmentation index was a suitable proteolysis rate pointer already from the second day on. The increase in non-protein nitrogen content indicates a release of free amino acids; so, it is a suitable measure of proteolysis after 5 days of ageing.

Key words: pigs / meat / ageing / myofibrilar fragments / length / myofibrilar fragmentation index / non-protein nitrogen / Warner-Bratzler shear force

POVEZAVA MED MIFIBRILARNO FRAGMENTACIJO, TEKSTRURNIMI IN KEMIJSKIMI PARAMETRI ZORENE PRAŠIČJE MIŠICE *Longissimus dorsi* †

IZVLEČEK

Namen raziskave je bil ugotoviti vpliv miofibrilarne fragmentacije (dolžina miofibrilarnih fragmentov, indeks miofibrilarne fragmentacije) na teksturne (Warner-Bratzler strižna trdnost) in

* This article is part of a dissertation thesis 'Effects of quality, proteolysis and degree of doneness on heterocyclic aromatic amines formation in thermal aged pork *Longissimus dorsi*', issued by Dejan Došler, supervisor Assoc. Prof. Lea Gašperlin, Ph.D.

† Prispevek je del doktorske disertacije Dejana Došlerja z naslovom 'Vpliv kakovosti, proteolize in stopnje pečenosti na nastanek heterocikličnih aromatskih aminov v dolgi hrbtni mišici prašiča', mentor doc. dr. Lea Gašperlin.

kemijske parametre (neproteinski dušik) mišice *Longissimus dorsi* (LD) prašiča. V poskus so bile 24 ur *post mortem* vključene leve in desne LD normalne kakovosti šestih prašičev. Mišice smo razdelili na 3 dele, jih vakuumsko embalirali in zoreli 1-, 2-, 3-, 5-, 11- in 16 dni pri temperaturi 2 °C (± 1 °C). Čas zorenja je značilno ($P \leq 0{,}001$) vplival na vse parametre. Vrednost pH se je v 16-ih dneh nekoliko povečala, in sicer je bila povprečno: pri nezorenih mišicah 5,61 in pri zorenih 5,67. Dolžina miofibrilarnih fragmentov je bila v povprečju največja (73 μm) pri nezorenih vzorcih, medtem ko je bila pri 11- in 16 dni zorenih vzorcih značilno manjša (15,7 μm in 12,4 μm). Indeks miofibrilarne fragmentacije se z zorenjem značilno poveča, in sicer je v povprečju: pri nezorenih vzorcih pod 30, pri 2 dni zorenih pod 50 in pri 5 dni zorenih 63,7 (določen po Olsonu in sod., 1976) oziroma pri 11 dni zorenih 56,9 (določen po Hopkinsu s sod.). Indeks določen po Hopkinsu (2004) je okrog 7 % ($P \leq 0{,}001$) nižji glede na indeks določen po Olsonu. Neproteinski dušik je pri 11- in 16 dni zorenih vzorcih večji (10,78 % in 10,93 %) od nezorenih vzorcev (9,39 % od celokupnega dušika). Warner-Bratzler strižna trdnost se v 16-ih dnevih zorenja značilno ($P \leq 0{,}001$) spremeni. Povprečna vrednost nezorenih vzorcev je 51,3 N in pri 16 dni zorenih vzorcih 29,2 N. Na osnovi instrumentalno izmerjene teksture termično obdelanih vzorcev lahko zaključimo, da je indeks miofibrilarne fragmentacije že po drugem dnevu zorenja ustrezni pokazatelj mikrostrukturnih proteolitičnih sprememb, in da je vsebnost neproteinskega dušika ustrezni pokazatelj biokemičnih proteolitičnih sprememb šele po petem dnevu zorenja, ker so povečane vrednosti neproteinskega dušika posledica sproščanja prostih aminokislin.

Ključne besede: prašiči / meso / zorenje / miofibrilarni filamenti / dolžina / miofibrilarna fragmentacija / indeks / neproteinski dušik / Warner-Bratzler strižna trdnost

INTRODUCTION

Important changes in chemical composition and structure of muscle tissues take place during ageing. It is known that the process of meat ageing differs for different muscles of the same animal, for different animal species and even for various meat qualities (Devine, 2004; Čandek-Potokar *et al.*, 1999).

The influence of protein proteolysis stages during ageing of beef meat on the increase in tenderness *(*Davey and Gilbert, 1969; Dransfield, 1994; Olson and Parrish, 1977; Olson *et al.*, 1976), and the improvement of its taste and aroma (Nishimura *et al.*, 1988; Smith *et al.*, 1978) have been extensively studied, and recently, interest in different animal species such as pork, is increasing (Okumura *et al.,* 2003). The ageing (conditioning) indicators for tenderness evaluation of aged beef have been reported. The sarcomere length in miofibrils (Strydom *et al.,* 2005), the shear force value, the myofibrillar fragmentation index (MFI) (Olson *et al.*, 1976), the 30 kDa component (Koohmaraie, 1994), phosphorylase b, creatine kinase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Okumura *et al.,* 2003*)* were correlated with the tenderness of bovine muscles. In the case of pork, stored without as well as with vacuum packaging at low temperature, Okumura *et al.* (2003) have found some useful ageing indicators, such as MFI, the 32-kDa components, peptides P1 and P2 as well as GAPDH.

In the present work, the myofibril fragmentation was examined in the pork *Longisimus dorsi* muscle (LD) stored under vacuum packaging at 2 °C for 16 days *post mortem*, and was compared with the results of texture evaluation. However, we anticipated that the myofibrilar fragment length (MFL) and Warner-Bratzler share force (WBSF) would decrease rapidly, while the MFI, the content of non-protein nitrogen fraction (NPN) would increase with ageing. On the other hand, as a contribution to the methodology of proteolysis evaluation we also wanted to compare two methods for MFI determination.

MATERIAL AND METHODS

Animals and tissue sampling

A total of six crossbred (Swedish Landrace × Large White × Duroc × Hampshire) barrows were included in the study. They were commercially slaughtered; their weight being between 80 and 85 kg (warm carcass weight), they contained 55–60% of lean meat, and had an ultimate pH (24 h *post mortem)* between 5.4 and 5.8. The carcasses were stored for 24 h at 4 °C (± 1 °C). Left and right LD muscles between 4th thoracic and the last lumbar vertebrae were removed from carcasses and used in further study.

Left and right LD muscles were cut into six samples and than vacuum packaged in polyethylene bags. Random sampling provided the part of muscle for a defined time of ageing; thus, the effect of sample location in the muscle was eliminated. After 1, 2, 3, 5, 11 and 16 days of ageing in the refrigerator at 2 °C (± 1 °C) each of 36 samples was divided into three sub-samples. On the first sub-sample (25 mm thick) thermal treatment was performed (grilling at 165 °C to the internal temperature of 70 °C (± 1 °C)). Grilled steaks were prepared for instrumental measuring of the texture (Warner-Bratzler Shear Force − WBSF) after 24 hours of cooling at 4 °C (± 1 °C). The second sub-sample was cut and immediately used for MFI determination. The third sub-sample was homogenized in a blender, repacked into polyethylene bags, frozen at –21 °C (± 1 °C), and was used for myofibrilar length (MFL) and non-protein nitrogen measurements, as well as for determination of water, protein, fat and ash content (carried out as well on nonaged samples − 24 h *post mortem*). Most of analyses were carried out in duplicate; WBSF was measured seven times and MFL 100 times on each sample.

Determination of water, total protein, intramuscular fat and ash content

The water content was determined on samples of 5 g of minced meat. Samples were dried in the oven at 105 °C according to AOAC 950.46 (Official Methods of Analysis, 1997). Total protein content (crude protein, N × 6.25) was assessed by the Kjeldahl method according to AOAC 928.08 (Official Methods of Analysis, 1997). The ash content was determined by mineralization of samples at 550 °C according to AOAC 920.153 (Official Methods of Analysis, 1997). Intramuscular fat content was determined by the method described in AOAC Official Method 991.36. Fat (Crude) in Meat and Meat Products (Official Methods of Analysis, 1997). The total lipids were extracted by hot treatment with petroleum ether as solvent.

Determination of pH value

pH value was measured directly using a spear combined glass-gel electrode type 03 (Testo pH electrode) with thermometer (type T, Testo penetration temperature probe) connected to pH meter (Testo 230, Testo). The pH meter was calibrated using pH = 4 and pH = 7 buffers and recalibrated after every 20 readings. Accuracy of reading was ± 0.01 pH unit. pH was measured six times per muscle; at day 1 (nonaged sample) and at 2, 3, 5, 11 and 16 days *post mortem* (aged samples).

Determination of MFL

Myofibrilar fragment suspension was prepared as outlined by Hopkins *et al.* (2000). A minced 0.5 g sample was placed in ice-cold vessels for homogenisation in 30 mL of ice-cold buffer. Homogenisation (Ultra-turrax T 25 with dispersing element S 25 N – 18 G at 15,000 rpm) was performed by two bursts of 30 s with a 30-s break on ice between them. The buffer

was 0.1 M KCl (Kemika, 112097), 1 mM EDTA (Merck, 1.08418), 1 mM NaN_3 (Merck, 1.06688), 7 mM KH_2PO_4 (Merck, 1.05108) and 18 mM K_2HPO_4 (Kemika, 1116108). Myofibril suspensions were filtered (1.0 mm mesh strainers) to remove connective tissue.

An aliquot (a drop) of the myofibril suspension was placed on a microscopic slide and was examined microscopically (Nikon Microphot-FXA, 20×10 magnify) using a camera (Sony DXC-930P) and a picture analysing programme (LUCIA_MTM). For each sample 6 different areas were chosen and 100 fragments were measured. MFL was expressed in μm.

Determination of MFI

MFI was determined according to the methods described by Olson *et al.* (MFI-Olson) (Olson *et al.,* 1976) and Hopkins *et al.* (MFI-Hopkins) *(*2000) using a UV/VIS spectrophotometer (Ultrospec 2000, Pharmacia Biotech). It was expressed as absorbance of a myofibril protein solution (concentration 0.5 mg mL^{-1}) at 540 nm multiplied by 100.

Determination of total nitrogen and non-protein nitrogen

All nitrogen contents were measured using Kjeldahl's method according to AOAC 928.08 (Official Methods of Analysis, 1997). Total nitrogen (TN) was measured on minced 1 g samples. Method for determination the non-protein nitrogen (NPN) was as fallows: a minced 5 g sample was homogenized with 40 ml of 3% (w/w) trichloroacetic acid (Ultra-turax T 25 with dispersing element S 25 N – 18 G, 120 s at 20,000 rpm). Then, the mixture was passed through a Sartorius no. 388 filter paper. Filtrate was analysed by BÜCHI Kjeldahl Line acording to AOAC 928.08 Kjeldahl method (Official Methods of Analysis, 1997). NPN was expressed as percent of TN.

Determination of WBSF

Steak (25 mm thick) was grilled at 165 ºC to 70 °C (± 1 °C) internal temperature and cooled for 24 h at 4 °C (± 1 °C). Seven cylinders (diameter 12.7 mm) were removed parallel to the longitudinal orientation of the muscle axis. Each cylinder was shared at the centre with a Warner-Bratzler shear 'V' slot blade (thickness of 3.0 mm and a triangular aperture of 60°) using a TA.XT plus texture analyser (Stable Micro Systems). The crosshead speed was 3.3 10^{-3} m s^{-1}. Newtons (N) being the units of the measurement.

Data analysis

For statistical evaluation of experimental data, the computer program SAS/STAT (SAS Software, 1999) was used. Basic statistical parameters were calculated by the MEANS procedure. Data were tested for normal distribution and analysed by the GLM (General Linear Model) and TTEST paired procedures. For data analyses two statistical models were used. For analysing the data for pH value, WBSF, MFL, MFI and NPN the statistical model [1] was used. The statistical model [2] for MFI included the effect of analytical method. The models were described by the following equations:

$$y_{ijk} = \mu + AT_i + A_j + e_{ijk} \quad [1]$$

where y = the observation parameter, μ = general mean, AT_i = effect of i^{th} ageing time (i = 1, 2, 3, 5, 11 and 16 days *post mortem*), A_j = effect of j^{th} animal (j = l to 6), and e = residual random term with variance σ^2_e.

$$y_{ijk} = \mu + AT_i + M_j + e_{ijk} \quad [2]$$

where y = the observation parameter, μ = general mean, AT_i = effect of i^{th} ageing time (i = 1, 2, 3, 5, 11 and 16 days *post mortem*), M_j = effect of j^{th} analytical method (j = Olson *et al*. (1976), Hopkins *et al.* (2000)) and e = residual random term with variance σ^2_e.

Least square means for experimental groups were obtained using the LSM procedure and were compared at the 5% probability level. Relations between instrumental and chemical parameters were assessed by Pearson correlation coefficients using the CORR procedure.

RESULTS AND DISCUSSION

Proving the homogeneity of the samples

Basic statistical parameters for chemical composition of raw pork LD muscle are shown in Table 1, the data clearly show the homogeneity of the samples.

Table 1. Basic statistical parameters for chemical composition of pork LD day 1 *post mortem* (N = 6)

Preglednica 1. Osnovni statistični parametri za kemijsko sestavo nezorene (prvi dan *post mortem*) prašičje LD mišice (N = 6)

Parameter/(g/100 g)	$\bar{x}$	Min.	Max.	SD	CV/%
Water Voda	74.2	73.2	75.6	0.83	1.11
IMF IMM	1.47	0.80	1.80	0.32	21.75
Protein Beljakovine	23.1	21.5	24.1	0.69	2.30
Ash Minerali	1.12	1.05	1.22	0.04	3.95

N – number of observations / število vzorcev, $\bar{x}$ – mean / povprečje, Min. – minimal value / minimalna vrednost, Max. – maximal value / maksimalna vrednost, SD – standard deviation / standardni odklon, CV (%) – coefficient of variation / koeficient variabilnosti, IMF – intramuscular fat / IMM – intramuskularna maščoba.

The average pH value of all our measurements 24 h *post mortem* was 5.61 ± 0.19; colour of muscles was appropriate for normal muscle quality. pH value as physicochemical criteria showed pork meat quality to be normal. pH_{24} values were comparable to pH_{24} from pigs of different age at slaughter and different feed restriction investigated by Čandek-Potokar *et al.* (1998) or those from pigs being slaughtered without or under minimal stress investigated by Henckel *et al.* (2000).

It is also known that pH increases for some tenths of a pH-unit due to ageing. In this study, an approximately 0.06-unit increase of pH value was determined after 16-days of ageing (Table 2).

Myofibril fragmentation

Meat tenderness is related also to structural (and biochemical) properties of skeletal muscle fibres, especially those of myofibrils and intermediate filaments. Histological studies dealt with

myofibrils breaking into shorter segments during *post mortem* storage of muscle, this phenomenon is called myofibril fragmentation. It is considered a useful ageing indicator of aged meat (Veiseth *et al.*, 2001). Myofibril fragmentation can be estimated by different methods: by homogenization of muscle, determination of protein content and measurement of the turbidity of samples adjusted to a standard protein concentration, by examination of myofibrils under a light microscope (Takahashi *et al.*, 1967; Moller *et al.,* 1973), by passing homogenized muscle through a filter system and recording the weight of the sample product removed (Reagan *et al.*,1975; Purchas *et al.*,1997) or by measuring of myofibrilar fragment length (Fernandez and Tornberg, 1994). Positive correlation between the rate of myofibrilar fragmentation and the tenderness of the meat is well known.

Table 2. Effect of ageing (1, 2, 3, 5, 11 and 16 days at 2 °C (± 1 °C)) on pH value, myofibrilar length, myofibril fragmentation index determined according to the methods described by Olson *et al.* (1976) and by Hopkins *et al.* (2000), non-protein nitrogen content, and Warner-Bratzler Shear Force of pork LD muscle (Model [1], N = 36)

Preglednica 2. Vpliv časa zorenja na vrednost pH, dolžino miofibrilarnih fragmentov, indeks miofibrilarne fragmentacije (določen po Olsonu s sod., 1976 in Hopkinsu s sod., 2000), neproteinski dušik in Warner-Bratzler strižno trdnost prašičje LD mišice (Model [1], N = 36)

Effect of: Vpliv:	Ageing/days Zorenje/dnevi						SE	P value
	1	2	3	5	11	16		
pH value Vrednost pH	5.62^{b}	5.61^{b}	5.52^{c}	5.62^{b}	5.64^{ab}	5.68^{a}	0.02	<0.001
MFL/μm DMF/μm	73.0^{a}	53.1^{b}	31.8^{c}	24.7^{d}	15.7^{e}	12.4^{e}	1.62	<0.001
MFI-Olson IMF-Olson	29.4^{c}	52.8^{b}	56.7^{b}	63.7^{a}	62.5^{a}	63.2^{a}	1.92	<0.001
MFI-Hopkins IMF-Hopkins	28.5^{d}	44.8^{c}	45.3^{c}	50.5^{b}	56.9^{a}	60.0^{a}	1.60	<0.001
NPN/(% of TN)	9.39^{d}	9.57^{cd}	9.73^{c}	10.12^{b}	10.78^{a}	10.93^{a}	0.10	<0.001
WBSF/N WBSS/N	51.3^{a}	37.9^{b}	35.8^{b}	32.7^{c}	31.5^{cd}	29.2^{d}	0.98	<0.001

N – Number of observations. SE – standard error. Least squares means with a different superscript within rows differ significantly ($P \leq 0.05$). Levels of significance: statistically significant: $P \leq 0.05$; highly statistically significant: $P \leq 0.001$. MFL – myofibrilar fragment length. MFI-Olson – myofibril fragmentation index (Olson *et al.,* 1976). MFI-Hopkins – myofibril fragmentation index (Hopkins *et al.*,2000). NPN – non-protein nitrogen. TN – total nitrogen. WBSF – Warner-Bratzler Shear Force.

N – število obravnavanj. SE – standardna napaka ocene. Pričakovane srednje vrednosti z različnimi nadpisanimi črkami ^{a,b,c,d,e} se statistično značilno ($P \leq 0.05$) razlikujejo. Stopnja značilnosti: statistično značilna: $P \leq 0.05$, statistično visoko značilna: $P \leq 0.001$. DMF – dolžina miofibrilarnih fragmentov. IMF-Olson – indeks miofibrilarne fragmentacije (1976). IMF-Hopkins – indeks miofibrilarne fragmentacije (2000). NPN – neproteinski dušik. TN – celokupni dušik. WBSS – Warner-Bratzler strižna trdnost

Generally, ageing can and does affect the MFL of pork meat (Table 2). The average MFL was 73 μm for day 1 *post mortem* and decreased significantly with the time of ageing. After 11 days

(15.7 μm) and 16 days (12.4 μm) of ageing the lowest MFL was measured, however the difference (between 15.7 μm and 12.4 μm) was not statistically significant. Therefore we assume that a great part of fragmentation measured occurred within day 3 and day 5 of ageing. The coefficient of variation for MFL was above 130%. This high variation in myofibril length was probably due to different activity of enzymes as well as due to nonenzyme process of decomposition (e.g. by calcium ions).

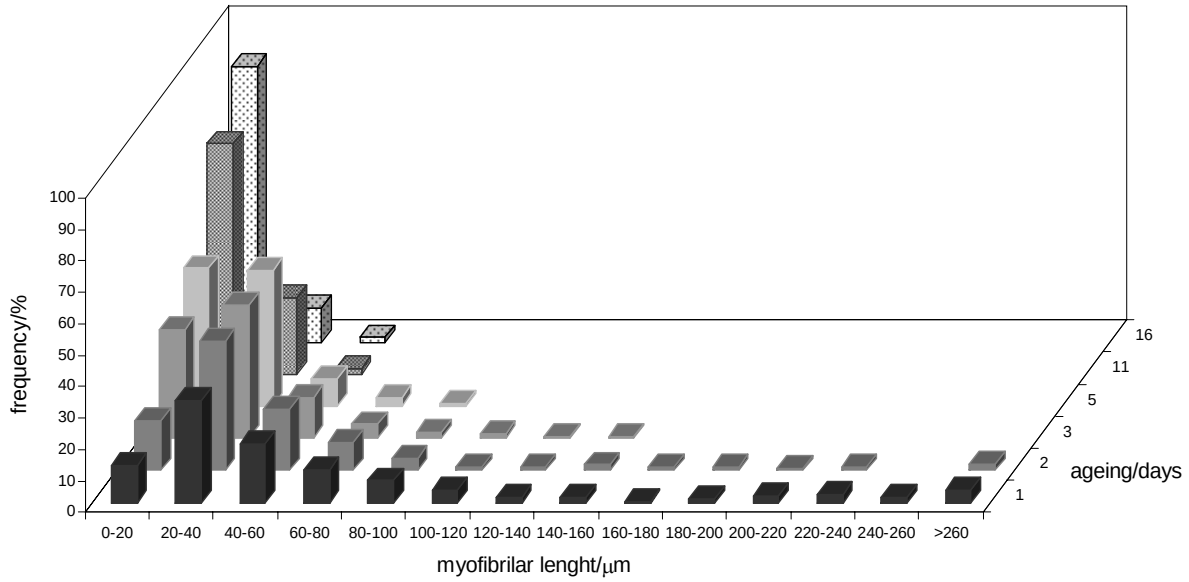

Figure 1. Relative frequency distribution of myofibrilar length of pork LD muscles as a function of ageing time.

Slika 1. Relativna frekvenca porazdelitve dolžine miofibrilarnih fragmentov prašičje mišice LD v odvisnosti od zorenja.

Myofibril breaking into shorter fragments during *post mortem* ageing of muscle is presented in Figure 1. In nonaged samples the size of fragments being between 0 and 20 μm (12%), 20 and 40 μm (33%), 40 and 60 μm (19%), 60 and 80 μm (10%), some of them being longer (28%). Generally, fragments isolated from samples 2, 3 and 5 days *post mortem* have the size of 0 to 20 μm, 20 to 40 μm and 40 to 60 μm. In samples at 11 and 16 days of ageing the average myofibrilar fragment length is in the range between 0 and 20 μm (73% and 88% of all measured fragments, respectively). The relative frequency in the first class (between 0 and 20 μm) progressively increases with *post mortem* time (Figure 1).

Direct comparison of these data with those from literature is difficult, since the myofibril fragment length depends on ageing condition, species and process of homogenising. Early as well as recent studies dealt with the changes in sarcomere length (Herring *et al.,* 1965; Rees *et al.,* 2002) or myofibril fragment length for beef, lamb or chicken muscles. Myofibril fragmentation of beef and pork LD, as Strydom *et al.* (2005) and Čandek-Potokar *et al.* (1998) emphasize, was significantly influenced by ageing (beef: 2 days 34.2 μm, 14 days 24.7 μm; pork: 1 day 19.4 μm, 4 days 9.77 μm). Our results agree with their data in spite of the fact that measured lengths acquired on pork LD after 2 days of ageing are noticeably longer (53.1 μm). These differences are probably due to the use of different extraction procedure and different homogenisers (Olson *et al.,* 1976; Hopkins *et al.,* 2000; Culler *et al.,* 1978).

The fragmentation of myofibrils has been observed during post-mortem ageing for 16 days and its index increased ($P < 0.001$) until day 16 (Table 2). The present study shows MFI values

ranging from less than 30 to more than 60. The MFI values of nonaged samples were below 30, values about 50 have been reached at day 2 of ageing. Increase after day 5 of ageing in MFI-Olson was hardly found. MFI-Hopkins after day 11 of ageing remained unchanged.

Absolute MFI values for 3-day aged pork LD acquired in this study are somewhat lower compared to those of Veiseth *et al*. (2001) for pork as well as those of Bruas-Reignier and Brun-Bellut (1996) for bulls reported for at the same *post mortem* time, but on the other hand are comparable to MFI for 2 to 20-day aged pork loins as investigated by Okumura *et al.* (2003). These differences are probably due to the use of different myofibril preparation, such as speed/time of homogenization and type of homogenizer particularly the blade type, used scaling factor 200, 150 or 100 and the state of the sample (fresh or frozen and thawed) (Hopkins *et al.,* 2000; Hopkins *et al.,* 2004), anatomical parts of the pigs taken as samples, days *post mortem*, etc. It should be emphasized that the difference between MFI determined on fresh and frozen muscles was not significant (Veiseth *et al.*, 2001; Hopkins *et al.*, 2000). Comparisons of the data in Table 2 with other published reports indicate that at day 1 *post mortem* ovine muscle exhibits 2 times higher MFI values (Hopkins *et al.,* 2000) compared to pork LD muscle in this study.

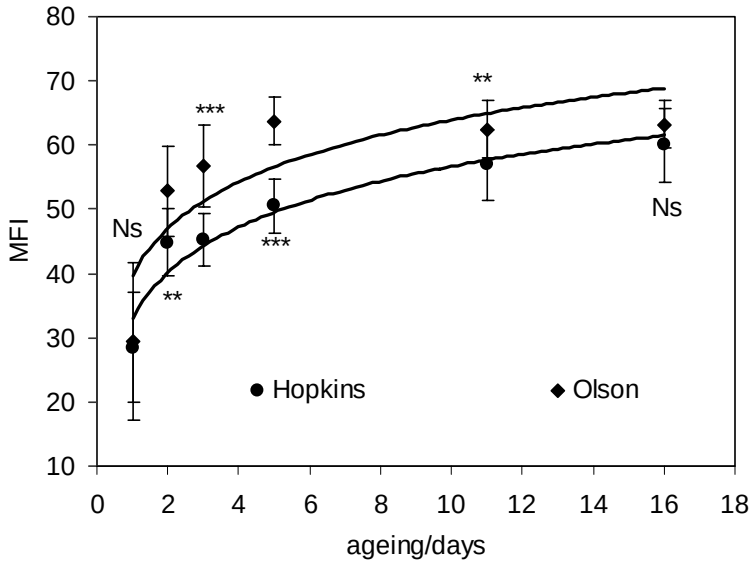

Figure 2. Comparison of MFI (myofibril fragmentation index) examined as described by Olson *et al.* (1976) and Hopkins *et al.* (2000), from aged pork LD muscles (mean ± standard deviation) (Model [2], Levels of significance: not significant: Ns – $P > 0.05$, statistically significant: ** $P \leq 0.01$, statistically highly significant: *** $P \leq 0.001$).

Slika 2. Primerjava IMF (indeks miofibrilarne fragmentacije) prašičje mišice LD, določenega po Olsonu s sod. (1976) in Hopkinsu s sod. (2000) (srednja vrednost ± standardni odklon) (Model [2], stopnja značilnosti: neznačilna: Ns – $P > 0{,}05$, statistično značilna: ** $P \leq 0{,}01$, statistično visoko značilna: *** $P \leq 0{,}001$).

Figure 2 shows the changes in MFI examined as described by Olson *et al.* (1976) and Hopkins *et al.* (2000), and prepared from pork LD muscles stored at 2 °C for 1–16 days after slaughter. Main differences between these two methods are in the amount of meat sample, volume of isolating medium (buffer) per mass unit of meat sample, the sequence of centrifugations and filtrations, time of homogenization and number of washings. Increases in MFI-Olson and MFI-Hopkins were continuously observed during storage for 16 days; on the average, the values ($P < 0.001$) for MFI-Hopkins are about 7% lower compared to those for

MFI-Olson, with the exceptions for the nonaged and 16-day aged samples, similar values were obtained.

Repeatability of both MFI methods was established by analysing the same sample in six replicates, the coefficient of variation for MFI-Olson being 4.6% and that for MFI-Hopkins 9.0%.

Textural and chemical parameters

Table 2 shows also the effect of ageing time on textural properties of roasted pork meat. The WBSF values were significantly different for different ageing times. Significantly the highest values (51.3 N) across the fibres were determined 1 day *post mortem*. After 2 or 3 days samples were significantly tenderer, the lowest values (31.5 N and 29.2 N) were observed after 11 and 16 days of ageing. According to the statement of Van Oeckel *et al*. (1999), pork meat (stored for 48 h at 4 °C, frozen stored during several months at –18 °C and grilled until an internal temperature of 74 °C, followed by cooling in tap water for 40 min) had a WBSF value of 35.5 N. Results of our study (37.9 N) are in slight discrepancies with their data, since lower values were determined, very probably due to different genotypes, different cooling and different parts of LD (*thoracis vs. lumborum*) muscles used. On the other hand, Fortin *et al.* (2005) determined higher values (62.9 N) on grilled loins (2 days *post mortem*, internal temperature of 72 °C, cooled in an ice/water bath, chilled for 24 h). Significantly lower absolute values in our study could be explained mainly due to smaller diameter (12.7 mm *vs.* 19 mm) of cylinders.

The non-protein nitrogen fraction (NPN) corresponds to the muscle's soluble non-protein compounds containing nitrogen (creatine, creatine phosphate, nucleotides, urea etc., amino acids and small peptides derived from the protein metabolism *post mortem*) (Bruas-Reignier and Brun-Bellut, 1996; Mikami *et al.*, 1991). Significant increase in NPN content during days 3–11 of ageing (Table 2, Figure 3) is in agreement with conclusions of Bruas-Reignier and Brun-Bellut (1996), who claimed that the increase in NPN can be considered an indicator of beef meat proteolysis. NPN content reached the highest value at about day 11 *post mortem*; by this time the maximum release of amino acids and small peptides has probably occurred.

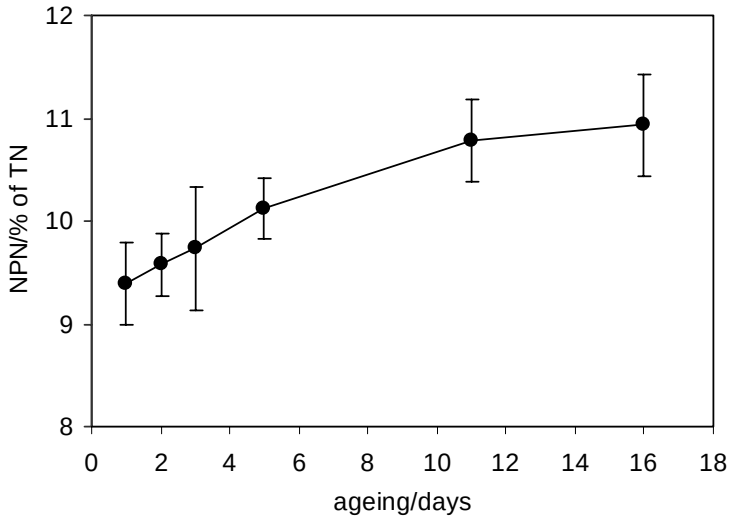

Figure 3. Effect of ageing time on non-protein nitrogen content (NPN) (% of total nitrogen − % of TN) in aged pork LD muscles (mean ± standard deviation).

Slika 3. Vpliv časa zorenja na vsebnost neproteinskega dušika (NPN) (% od celokupnega dušika − % od TN) v prašičjih mišicah LD (srednja vrednost ± standardni odklon).

Correlations

Correlation coefficients between instrumental values and physicochemical proteolysis parameters of pork LD muscles are shown in Table 3.

WBSF is associated with the MFL, strong positive correlation between the parameters was observed ($R = 0.82^{***}$). Furthermore, the WBSF was negatively correlated with variations in MFI values, MFI-Olson ($R = –0.82^{***}$) and MFI–Hopkins ($R = –0.77^{***}$), which is in agreement with the results of Culler *et al*. (1978). These results further on substantiate the fact that the usual term for the state of tenderness, namely, the expression myofibril fragmentation tenderness, is an appropriate one to describe tenderness of a conventionally aged pork LD muscle.

Table 3 further on shows that the increase in the NPN content is not related to the extent of the myofibril fragmentation. The increase in MFI or decrease in MFL and WBSF, which occurred mainly during the first 5 days *post mortem*, are not directly related to the increase in amino acid content and small peptide content, which increased from day 5 to day 11 of pork ageing.

Table 3. Pearson correlation coefficients between textural, chemical and myofibril fragmentation parameters of pork LD muscles (N = 36)

Preglednica 3. Pearsonovi korelacijski koeficienti med teksturnimi, kemijskimi in fragmentacijskimi parametri prašičje mišice LD (N = 36)

Parameters Parametri	WBSF WBSS	MFI-Olson IMF-Olson	MFI-Hopkins IMF-Hopkins	NPN
MFL DMF	0.82^{***}	$–0.84^{***}$	$–0.87^{***}$	$–0.65^{***}$
WBSF WBSS	1.00	$–0.82^{***}$	$–0.77^{***}$	$–0.58^{***}$
MFI-Olson IMF-Olson		1.00	0.86^{***}	0.58^{***}
MFI-Hopkins IMF-Hopkins			1.00	0.67^{***}

Levels of significance: statistically significant: * $P \leq 0.05$ and ** $P \leq 0.01$; highly statistically significant: *** $P \leq 0.001$. WBSF – Warner-Bratzler Shear Force. MFL – Myofibrilar length. MFI-Olson – myofibril fragmentation index (1976). MFI-Hopkins – myofibril fragmentation index (2000). NPN – non-protein nitrogen.
Stopnja značilnosti: statistično značilna: * $P \leq 0.05$ in ** $P \leq 0.01$, statistično visoko značilna: *** $P \leq 0.001$. WBSS – Warner-Bratzler strižna trdnost. DMF – dolžina miofibrilarnih fragmentov. IMF-Olson – indeks miofibrilarne fragmentacije (1976). IMF-Hopkins – indeks miofibrilarne fragmentacije (2000). NPN – neproteinski dušik.

CONCLUSIONS

Generally, 16-day ageing of pork meat does affect the microstructure, texture and non-protein nitrogen content. Myofibrilar fragmentation increases significantly from the first day *post mortem* during the entire ageing period. Up to 60% decrease of MFL occurs within the first 3 days and up to 50% increase of MFI within the first 2 days of ageing. We assume that a great part of fragmentation occurred between day 2 and day 5 of ageing. It can be concluded that under the conditions of this experiment, 60% of tenderisation of pork LD occurs within 2 days *post mortem* and 84% within 5 days. WBSF is associated with the MFL ($R = 0.82^{***}$), MFI-Olson ($R = –0.82^{***}$) and MFI-Hopkins ($R = –0.77^{***}$). The increase in the NPN content was not related to the extent of myofibril fragmentation. Myofibrilar fragmentation, which occurred

mainly during the first 5 days *post mortem*, was not directly related to the increase in amino acid content and small peptide content, which both increased also after 5 days of ageing.

As far as methods for MFI determination are concerned, it can be concluded that on the average the values for MFI-Hopkins are about 7% lower compared to those for MFI-Olson.

ACKNOWLEDGEMENTS

This research was financed by the Slovene Ministry of Education, Science and Sport (J4-6475-0481-04/4.02).

We want to express our gratitude to Milica Kač, Ph. D. for her valuable comments on an earlier draft of this paper.

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Acta agriculturae Slovenica, 90(november 2007)1, 17–24.

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Agris category codes: L20 COBISS Code 1.02

PHENOTYPIC HETEROGENEITY IN BACTERIAL POPULATIONS

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Received January 17, 2007, accepted March 30, 2007.
Delo je prispelo 17. januarja 2007, sprejeto 30. marca 2007.

ABSTRACT

Genetically uniform bacterial cells exhibit heterogeneity such as intrapopulation differences in metabolism as well as variation in growth rate. Additionally, phenotypic heterogeneity in more complex developmental processes where a portion of a population performs specialized functions has been described. Heterogeneity within populations of bacterial cells ensures that a small fraction of the population is prepared to survive adverse environmental conditions. Phenotypic heterogeneity is mediated by two mechanisms: (i) genotypic alterations such as, mutations and rearrangements of specific DNA fragments or (ii) epigenetic phenomenon. Here examples of genotypic as well as epigenetically regulated phenotypic heterogeneity from several bacterial species are presented.

Key words: microbiology / bacteria / phenotypic heterogeneity / genotype / epigenetic regulation

FENOTIPSKA HETEROGENOST V BAKTERIJSKIH POPULACIJAH

IZVLEČEK

Genetsko enake bakterijske celice izkazujejo heterogenost kot sta npr., znotraj populacijske razlike v metabolizmu in hitrosti rasti. Poleg tega so opisani primeri fenotipske heterogenosti zapletenih procesov razvoja pri katerih del populacije vrši posebne naloge. Heterogenost znotraj populacij bakterijskih celic zagotavlja, da je majhen del populacije pripravljen na morebitne neugodne pogoje okolja. Fenotipsko heterogenost posredujeta dva mehanizma: (i) genotipske spremembe kot so mutacije in prerazporeditve določenih fragmentov DNA ali (ii) epigenetski pojavi. V pričujočem prispevku so opisani primeri genotipske kakor tudi epigenetsko uravnane fenotipske heterogenosti nekaterih vrst bakterij.

Ključne besede: mikrobiologija / bakterije / fenotipska heterogenost / genotip / epigenetsko uravnavanje

INTRODUCTION

Genetic differences and environmental influences are the basis of variation among individuals. However, variation is also observed in genetically identical organisms and cells under the same environmental conditions. The advent and increased use of flow cytometry and fluorescence microscopy to analyse individual cells has revealed a number of examples of heterogenous gene expression in genetically uniform populations of bacteria. Microorganisms have to survive and multiply in often rapidly changing and hazardous environments. Alterations in availability of nutrients, temperature, salinity, osmolarity and pH are frequent. Additionally, microorganisms are repeatedly confronted by adverse conditions such as, antibiotics, toxins, mutagens, bacteriophage and radiation. It is presumed that phenotypic heterogeneity ensures that some bacterial cells are better prepared if a sudden change in environmental conditions occurs.

Phenotypic heterogeneity can arise due to genotypic alterations such as genome rearrangements or mutations. Additionally, phenotypic heterogeneity can also be mediated by heterogeneity in expression of individual genes due to epigenetic phenomenon where no genotypic alterations are involved and growth conditions are homogenous. Such heterogeneity arises due to "noise", random fluctuations in rates of protein synthesis and degradation. Two types of noise are distinguished: intrinsic and extrinsic (Swain *et al*., 2002). Intrinsic noise is a reflection of random bursts of activity of a promoter while extrinsic noise reflects cell to cell variation in activity of proteins regulating expression of a given gene. Noise can sometimes lead to bistability when a population of genetically identical cells forms two subpopulations. Individual cells in such populations follow or do not follow a specific developmental pathway.

In the presented review examples of phenotypic heterogeneity within bacterial populations are presented and some of the pathways responsible for heterogeneity are discussed. Cases of phenotypic heterogeneity based on genotypic modifications as well as on epigenetic phenomenon are presented. Some are well documented while others have received only recent attention.

GENOTYPIC MODIFICATIONS AND PHENOTYPIC HETEROGENEITY

Phase and antigenic variation

Phase variation involves reversible alterations in specific genomic loci. The best characterized are those engaged in the synthesis of antigenic surface structures such as outer lipopolysaccharides (LPS), pili and flagella (Henderson *et al*., 1999; Hallet 2001). Phase variation assists bacteria in evading host immune defences and colonisation of new ecological niches. To meet this end bacteria have evolved a number of molecular mechanisms. Generally, some act by turning individual genes "on" or "off", while others enable expression of multiple phenotypes via rearrangements of DNA sequences. Phase variation has been described in a number of bacterial pathogens namely, *Salmonella thyphimurium*, *Neisseria gonorrhea, Neisseria meningitidis*, *N. gonorroehae*, *Haemophilus influenzae* and *Escherichia coli* (Dybvig *et al*., 1993). Phase variation can be mediated by reversible changes in the length of short DNA sequence repeats associated with genes for surface structures. Nucleotide sequences are gained or lost by slipped-strand mispairing (SSM) (van Belkum *et al*., 1998), which can take place during chromosomal replication as well as DNA repair or recombination. Changes in DNA repeat length can result in translation frameshift mutants.

Phase variation can also be mediated by inversion of a DNA fragment belonging to a specific locus via recombination (Hallet and Sheratt, 1997). For example *Salmonella typhimurium*, by inverting a promoter with respect to the structural gene switches expression of alternative alleles for flagellin, the flagellum protein. Alternatively, some pathogens, such as *N. gonorroehae* exhibit antigenic variation of pilin, the structural protein of pili, which is mediated by intramolecular recombination between variable silent DNA casettes and expre ssed loci (Howell-Adams and Seifert, 2000). Recombination between silent and expressed genes has also been demonstrated for variable surface lipoproteins and proteins in *Borrelia burdorferi* and *B. hermsii*, respectively (Casjens *et al*., 2000; Barbour and Restrepo, 2000). Additionally, in species exhibiting natural competence for transformation, such as *Neisseria meningitidis,* intergenomic recombination to yield phase variation occurs between cells (Swartley *et al*., 1997).

Mutators

Another example of genotypic heterogeneity is the appearance of bacterial cells designated mutators. Under constant environmental conditions mutation rates are low as most are deleterious. However, when bacterial populations are faced with variable environmental conditions higher mutation rates are detected (Ishii *et al*., 1989; Travis and Travis 2002). Mutators, bacterial cells with high mutation rates relative to the wild type have been described within different bacterial species and have been detected at high frequencies among pathogenic bacteria (LeClerc *et al*., 1996; Oliver *et al*., 2000; Richardson *et al*., 2002).

In laboratory strains of *E. coli* and *Salmonella typhimurium,* mutators arise at frequencies of 10^{-5} to 10^{-6} (LeClerc *et al*., 1998, Boe *et al*., 2000,) while in natural isolates mutators occur at much higher frequencies of approximately 1–5% (LeClerc *et al*., 1996). Commonly, mutators in natural strains have defects in genes involved in DNA repair, such as the methyl-directed mismatch repair pathway (MMR) genes, *mutS*, *mutL*, *mutH* and *uvrD* (LeClerc *et al*., 1996, Matic *et al*., 1997). The MMR pathway is a DNA repair system that corrects base mismatches in newly replicated DNA and also represents the main barrier preventing recombination of mismatched heteroduplexes. Defects in MMR thus allow a broader range of interspecies DNA exchange which implicates that horizontal gene transfer influences survival and growth. Additionally, evidence also suggests that mutators could play an important role in the evolution of resistance to antibiotics.

EPIGENETIC REGULATION AND PHENOTYPIC HETEROGENEITY

The *lac* network

A classical example of epigenetic heterogeneity is the utilization of lactose as a source of carbon and energy (reviewed in Miller and Reznikoff, 1978). The protein products of the *lac* structural genes *lacZYA,* enable uptake and utilization of β-galactosides. *lacZ* encodes the enzyme β-galactosidase which breaks a β-galactoside, such as lactose, into glucose and galactose; *lacY*, encodes a permease responsible for the transport of lactose into the cell and *lacA* encodes a β-galactoside transacetylase that transfers an acetyl group from acetyl-CoA to β-galactosides (Figure 1). A separate operon encodes the repressor LacI which prevents expression of the three structural genes. LacI is in turn inhibited by allolactose. As early as 1957, Novick and Weiner showed that an *E. coli* population with a low level of *lac* operon induction yields two populations of cells, one with high *lac* expression and the other non *lac* expressing. The *lac* system has subsequently been very well characterized (Ozbudak *et al*., 2004). In the absence of lactose cells harbor only very few molecules of β-galactosidase. Immediately following the addition of lactose only a few cells will be able to utilize lactose. In these lactose utilizing cells random, noninduced transcription of the *lac* operon occurs as the repressor is present in only approximately 10 molecules. It follows that enzyme levels within the bacterial population are heterogeneous. Cells with high levels of permease accumulate lactose more rapidly and further in these cells, due to higher levels of β-galactosidase, transglycosylation proceeds to form the inducer, allolactose. This in turn leads to increased transport of lactose, transglycosylation, induction and production of galactose and glucose as sources of carbon and energy. Subsequently, variability decreases as the lactose metabolizing cells become predominant.

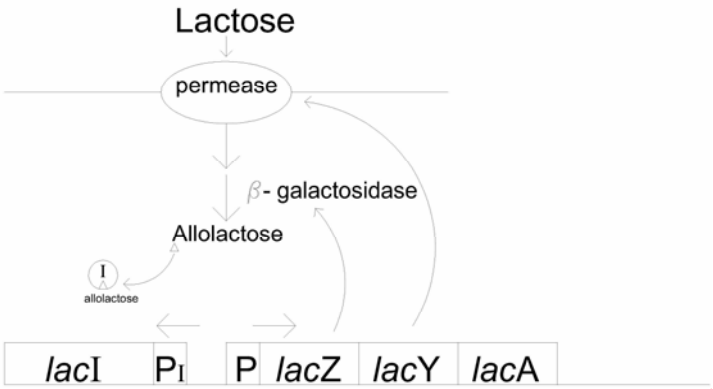

Figure 1. Induction of the *lac* operon.
Slika 1. Indukcija operona *lac*.

Lysogeny versus the lytic cycle

Another example of heterogeneity is the outcome of infection of *Eschericiha coli* cells by bacteriophage λ (Campbell 1996). Infection can result in either lysis or lysogeny (integration of bacteriophage DNA into the bacterial chromosome) due to competition between the cI repressor and Cro protein for the operators of the two divergent early promoters P_L and P_R. The operators consist of three binding sites which have different orders of binding for cI and Cro. In a lysogenic cell, cI has gained occupancy of the operators however, if it is replaced by Cro, the lytic cycle follows (Folkmanis *et al*., 1977) (Figure 2). The first genes transcribed from the two early promoters P_L and P_R are leftward *N* and rightward *cro*. *N* is an antiterminator of transcription which allows expression of the downstream *cII*. The cII protein is labile due to protease degradation however, if sufficient concentrations are available, it will together with the cIII protein activate the *cI* repressor gene, switching off expression of *cro* which is crucial for the lytic cycle. Again a key regulator the cI repressor, is present in only a few copies per cell. Thus, lysogeny is maintained by the continuous production of the repressor as well as by its adequate partitioning to the two daughter cells at cell division (Dodd *et al*., 2005).

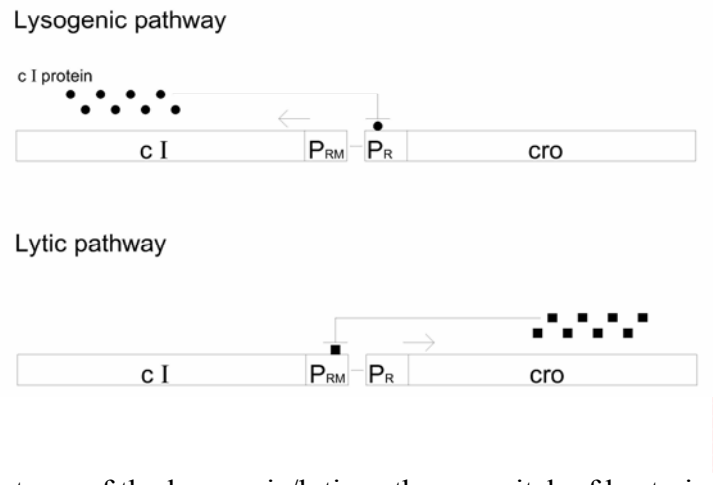

Figure 2. The key features of the lysogenic/lytic pathway switch of bacteriophage λ.
Slika 2. Poglavitne značilnosti preklopa lizogene/litične poti bakteriofaga λ.

Persisters

In 1944, Bigger discovered that even though the addition of penicillin to staphylococci induced lysis, a small fraction of the cells remained viable even after prolonged incubation and resumed growth when the antibiotic was removed. The surviving cells were named persisters. Subsequently, persisters were described in various bacterial species following treatment with antimicrobials (Brooun *et al*., 2000; Keren *et al*., 2004). Recently, biofilm resistance to antimicrobials has been shown to be based on the presence of persisters (Spoering *et al*., 2001). The proportion of persister cells has been shown to be higher in a stationary phase population, with a pronounced increase at mid to late-exponential phase in *E. coli*, *P. aeruginosa* and *Staphylococcus aureus* (Keren *et al*., 2004). Persisters exhibit a state of reduced growth which is entered into spontaneously and is a pre-existing characteristic and not produced in response to antibiotic treatment (Balaban *et al*., 2004). As yet only the *hipAB* operon, encoding a toxin-antitoxin module, has been identified as affecting development of a persister state. HipA is a toxin inhibiting macromolecular synthesis and promoting persistence by protecting the cells from the damaging effects of antibiotics that act in a growth-dependent manner (Keren *et al*., 2004a).

Regulation of competence in Bacillus subtilis

A well characterized example of bistability is the development of competence for transformation in B. subtilis. Transformation comprises the uptake of naked DNA from the environment and subsequent recombination which incorporates the DNA into the bacterial genome.

Competence development in *B. subtilis* is expressed as a response to nutrient limitation and quorum sensing. Even under optimal conditions only a small fraction of the cells in a culture will develop competence (reviewed in Dubnau 1999; Hamoen *et al*., 2003). ComK is the key transcription factor regulating transformation which includes DNA-binding, uptake and recombination. Regulation of *comK* expression is complex and involves autoregulation. However, even under conditions optimal for competence development only up to 10% of the cells will synthesize ComK and develop competence. Expression of ComK is tightly regulated and is induced in response to nutrient limitation and quorum sensing, the ability of bacteria to communicate and coordinate behaviour via signaling molecules. Binding of ComK as a tetramer composed of two dimers induces *comK* transcription. An autostimulatory loop of *comK* expression is the only factor required for bistability of *comK* expression (Figure 3). In some cells, due to noise in expression, the concentration of *comK* exceeds a threshold, activating the positive loop and subsequently competence development.

Figure 3. Positive autoregulation of *comK* is central for competence development in *B. subtilis*.
Slika 3. Pozitivno samo uravnavanje je ključno za razvoj kompetence pri *B. subtilis.*

On the other hand in *Streptococcus pneumoniae,* natural competence is presumed to be developed by the large majority of the population under inducing conditions. However, it has recently been shown that DNA is actively released by competence-induced lysis of a subfraction of the cells (Steinmoen *et al.*, 2002). Cell lysis and DNA release is brought about by lysins such as LytA synthesized by competent cells and which are most probably attached to the cell surface (Steinmoen *et al*., 2003).

Sporulation

Sporulation, the formation of a dormant spore which exhibits extreme resistance to environmental conditions is another example of bistability in *B. subtilis*. The key transcriptional regulator of sporulation is Spo0A and its activity is controlled by phosphorylation mediated by a phosphorelay system (Burbulys *et al*., 1991). Under nutrient limiting conditions only some cells activate Spo0A. Sporulation is energy consuming and is irreversible following an early stage. Similarly as for ComK, a positive feedback loop involving transcription of *spo0A* and its phosphorylation controls bistability of sporulation gene expression. A threshold level of active Spo0A has to be reached in order to initiate sporulation (Chung *et al*., 1994). Additionally, sporulating cells can produce a killing factor which destroys nonsporulating cells (Gonzalez-Pastor *et al*., 2003). Nutrients that are released by the non sporulating cells can be used by the sporulating cells. Further, bistability of entry into sporulation allows remaining cells which had not sporulated to resume growth upon sudden nutrient availability.

Cell lysis in Pseudomonas aeruginosa biofilm development

Bacteria often grow in biofilms where they undergo complex differentiation (Stoodley *et al.*, 2002). Microcolony formation within a biofilm is a coordinated, adaptive response that facilitates biofilm development and dispersal. Quorum sensing has been shown to be involved in microcolony development in some organisms for example: P. aeruginosa (Davies *et al.*, 1998), Burkholderia cepacia (Huber *et al.*, 2001) and Aeromonas hydrophila (Lynch *et al.*, 2002).

Recently, cell death – killing inside microcolonies has been demonstrated in wild-type *P. aeruginosa* biofilms (Webb *et al*., 2003). Prophage-mediated cell death and lysis inside microcolonies have been linked with the accumulation of reactive oxgygen species (ROS). Prophage - mediated cell death benefits a subpopulation of surviving cells and has an important role in subsequent biofilm differentiation and the dispersal of surviving biofilm cells. The dispersing cells have been shown to be more similar to planktonic cells than to mature biofilm cells, indicating that dispersing biofilm cells revert to the planktonic mode of growth (Sauer *et al*., 2002).

Colicin production

Colicins are plasmid-encoded bacteriocins, synthesized by and active against cells of Escherichia coli and sometimes related species such as Shigella and Salmonella spp. Colicin-producing strains are found with high frequency among natural isolates and have been demonstrated to play a role in intraspecies population dynamics (Kirkup and Riley, 2004). Production of colicins is characteristically encoded by a cluster of three genes: a gene encoding the colicin activity protein; the immunity gene encoding the immunity protein which potects the producing strain and a lysis gene, encoding the lysis protein. Regulation of colicin K is induced primarily by an increase in ppGpp due to nutrient depletion (Kuhar and Žgur-Bertok, 1999; Kuhar *et al.*, 2001). Furthermore, colicins are released semispecifically, by cell lysis and the colicin K activity gene cka is expressed in only 3% of the bacterial population upon induction by nutrient starvation. On the other hand the immunity gene is expressed in the large majority of

cells. The LexA protein has been shown to play a key role in establishing differential expression of colicin synthesis at the level of transcription (Mulec *et al.*, 2003).

CONCLUSIONS

Bacteria have evolved molecular mechanisms which allow divergence into populations of phenotypically separate subpopulations. Heterogeneity within bacterial populations can be mediated by specific genotypic modifications or alternatively, by epigenetic mechanisms. In this review several examples have been presented however, it is quite likely that such phenomenon are very widespread. As bacteria evolved these mechanisms to survive adverse environmental conditions understanding the molecular basis of bacterial heterogeneity has important implications for the food preservation industry and for antimicrobial chemotherapy.

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Acta agriculturae Slovenica, 90(november 2007)1, 25–31.

http://aas.bf.uni-lj.si

Agris category codes: L20 COBISS Code 1.01

THE RELATIONSHIP BETWEEN TOTAL AND CULTURABLE BACTERIA IN COLD SOILS

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Received December 20, 2006, accepted January 30, 2007.
Delo je prispelo 20. decembra 2006, sprejeto 30. januarja 2007.

ABSTRACT

Cold ecosystems are essential to global ecology. Microbial activities are predicted to increase in low temperature regions due to global warming thus further affecting the green gas emissions and stored carbon overturn. This all has led to increased awareness how minute is our understanding of bacterial assemblages in the cold soils and also other environments, spurring the idea of standardization of research protocols. This work focused on comparison of recently published data on culturability of bacteria from 23 cold soils in the frame of International polar Year 2007 (IPY 2007). The results show that linear relationship exists between direct counts and numbers of cultured bacteria. 11 environmental parameters were reported in these studies. However, only two categories were present in all, preventing attempt to identify governing environmental factors. As there is such heterogeneity in reporting and performing research in microbial ecology, standardization of approaches and protocols in microbial ecology could improve comparability of results substantially.

Key words: microbiology / bacteria / microbial ecology / cold soil / direct count / cultivation

ODNOS MED SKUPNIM ŠTEVILOM IN ŠTEVILOM GOJENIH BAKTERIJ IZ HLADNIH TAL

IZVLEČEK

Hladni ekosistemi so ključni za globalno ekologijo. Mikrobne aktivnosti naj bi se v teh področjih zaradi globalnega segrevanja povečale in tako dodatno vplivale na povečanje emisij toplogrednih plinov in presnove organskega ogljika v teh tleh. To je povečalo zavedanje o pomankljivem razumevanju bakterijskih združb v hladnih teh in drugih ekosistemih ter sprožilo prizadevanja po standardizaciji raziskovalnih protokolov mikrobne ekologije. V tem delu je prikazana primerjava pred kratkim objavljenih podatkov o stopnji uspešnosti gojenja bakterij iz 23 hladnih tal v okviru mednarodnega polarnega leta 2007 (IPY 2007). Rezultati kažejo linearni odnos med skupnim številom bakterij iz direktnega štetja in iz gojitvenih eksperimentov. Od 11 parametrov okolja objavljenih v teh študijah sta bili le dve kategoriji prisotni v vseh objavah. To je onemogočilo identifikacijo ključnih faktorjev okolja. Zaradi prisotne raznolikosti v poročanju in opravljanju raziskav v mikrobni ekologiji bi standardizacija pristopov in protokolov v veliki meri izboljšala primerljivost raziskovalnih rezultatov.

Ključne besede: mikrobiologija / bakterije / mikrobna ekologija / hladna tla / direktno štetje / gojitvene tehnike

INTRODUCTION

Arctic, alpine and other cold ecosystems are critical to global ecology as they represent almost a third of Earth's terrestrial surface. These soils store a third of Earth’s soil carbon, are

large sources of atmospheric methane, are very sensitive to climate change, and are already changing rapidly. The intention of International Polar Year 2007 (http://www.ipy.org/) is to strengthen the awareness of scientific community about the importance of cold regions of the world and to increase our understanding of microbiological components of these vast ecosystems. So far, the basic responses of microorganisms to environmental factors in cold ecosystems have been identified. To survive at low temperature, microbes can reduce their cell size and their capsular polysaccharide coat thickness, as well as they can change their fatty acid and phospholipid composition. Moreover, they can decrease the fractional volume of cellular water, increase the fraction of ordered cellular water and extract energy by catalyzing redox reactions of ions in aqueous veins in ice or in thin aqueous films covering solid particle surface (Price and Sowers, 2004). The unfrozen microsites are surrounded by ice and therefore have limited gas exchange. According to Christensen and Tiedje (1990) and Christiensen and Cristiensen (1991), the availability of labile carbon in this water film is high as a consequence of microorganisms being killed by freezing or hygroscopic effects, and from organic matter from broken aggregates. Further, nutrient concentrations in the liquid water film also increase due to ion exclusion from the growing ice grid (Edwards and Cresser, 1992; Stahli and Stadler, 1997). The process of ion exclusion therefore contributes significantly to the establishment of nutrient enriched microsites covered with liquid water at temperatures below zero. As the cold soils are dominated by strong gradients in environment structure and extreme variation within the course of a single year these ecosystems are one of the most challenging on earth for terrestrial life. While a number of groups around the world are contemplating complex research questions, each study is focused on a single local area, usually on a specific subset of the microbial community, and usually uses a suite of analytical techniques. The aim of present study was to gain insight into basic relationship between total and culturable bacteria in various cold soils from recent publications and to correlate the findings to reported environmental parameters.

MATERIALS AND METHODS

Data selection

Literature on enumeration and cultivation of bacteria in cold soils was explored using available public databases: Medline (http://www.ncbi.nlm.nih.gov/PubMed), ScienceDirect (http://www.sciencdirect.com) and ASM (http://aem.asm.org/searchall). The following criteria for literature exploration and selection were adopted: **(i)** publication should be less than ten years old, **(ii)** it should report on more than two cold soil samples, **(iii)** the methodological approaches to direct enumeration of bacteria should be comparable, **(iv)** the methodological approaches to cultivation should be comparable, **(v)** the data on environmental parameters should be provided. The following publications reporting on 23 cold soil samples were selected, focusing on various soils from Antarctica (Zdanowski *et al.*, 2001; Aislabie *et al.*, 2006) and young glacial forefield soils from Switzerland (Sigler *et al.*, 2002).

Data analysis

The environmental data on 23 cold soils were explored. The data categories on various soil properties shared among studies were selected. Two relationships were explored: first, the relationship between environmental data categories, and second, the relationship between environmental parameters and biological variables. The analyses were performed using simple linear regression using MS Excel.

The data on cold soil total and culturable bacteria was compiled and the values were log10 transformations of the original values obtained from the literature. The relationship between total and culturable bacteria in cold soils was analyzed by linear regression of the abundance data according to Lemke and Leff (2006).

RESULTS AND DISCUSSION

In the present study elucidation of basic relationship between environmental parameters and total and culturable bacteria counts was attempted based on recently published data on 23 cold soils.

The relationship between the only two environmental parameters shared among studies, total carbon and total nitrogen, appears to be linear, $R^2 = 0.8321$ (Fig. 1). In case the soil samples with higher carbon content (> 3%) are removed, the goodness of fit remains basically unaffected ($R^2 = 0.824$). This correlation increases substantially ($R^2 = 0.9875$) if one single data point of soil 1 from Antarctica is omitted from analysis. This tight correlation of carbon and nitrogen is in accordance with previously published literature, however, the fractions of various forms of carbon and nitrogen play more crucial role in survival strategies of microbial communities than total abundance per se (Stenstrom *et al.*, 2001). In addition, the majority of the cold environments analyzed in this study are not rich in carbon as 82% (19 of 23) have less than 0.7% of carbon, indicating the oligotrophic nature of cold soils.

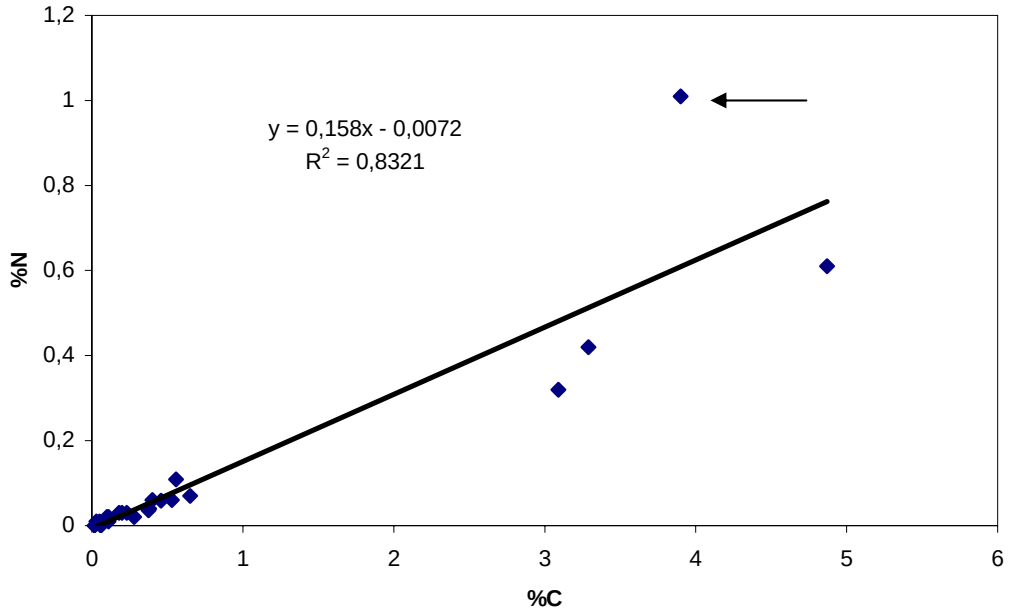

Figure 1. The linear relationship between total carbon (% C) and total nitrogen (% N) present in the 23 cold soils. The data were adopted from Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006). When the data of soil 1 from Antarctica (marked by arrow) is omitted the correlation increases to R2 = 0.9875.

Slika 2. Linearni odnos med deležem skupnega ogljika (% C) in dušika (% N) v 23 hladnih tleh. Podatki so iz naslednjih virov: Zdanowski in sod. (2001), Sigler in sod. (2002) in Aislabie in sod. (2006). Pri analizi brez podatkov za tla 1 Antarktike (označeno s puščico) je korelacija $R^2 = 0{,}9875$.

The studies of Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006) reported altogether on 11 soil properties: soil particle size distribution, pH, C:H:N:S ratio, total C, total N,

C/N ratio, soil moisture, soil temperatures, total phosphorus, electrical conductivity, nitrate concentration. The fact that only two categories (total C, total N) were present in all three studies is alarming. This illustrates the diversity of research approaches in microbial ecology. All the published data presented here are based on single measurements in time. The analysis of only one sampling point is not terribly informative from ecological perspective aiming at resolving questions as: What organisms are found in cold soils? Are there local endemic species and communities? How widely distributed are those species and communities? How do they vary across the gradients? Do communities actually vary in their composition and size seasonally or does the growing-season-active community just get frozen in at the onset of winter period? In this respect, there is a true need for standardization of methodological approaches. In addition, reporting on standard set of environmental parameters measured using rigorous approaches of soil chemistry should be adopted in everyday microbial ecology practice.

Out of 11 categories only two of them could be used for analyses. Soil total carbon and total nitrogen are linearly correlated and are thus inefficient in explaining the differences observed in bacterial abundances (figure 2). Various functions could be fitted to the data, however, with very little or no ecological relevance (data not shown). This highlights the importance of reporting environmental attributes thus facilitating identification of governing environmental factors shaping microbial communities. On the other hand, modeling of environmental gradients in controlled laboratory experiments could give better clues on environmental factors shaping microbial communities at hand.

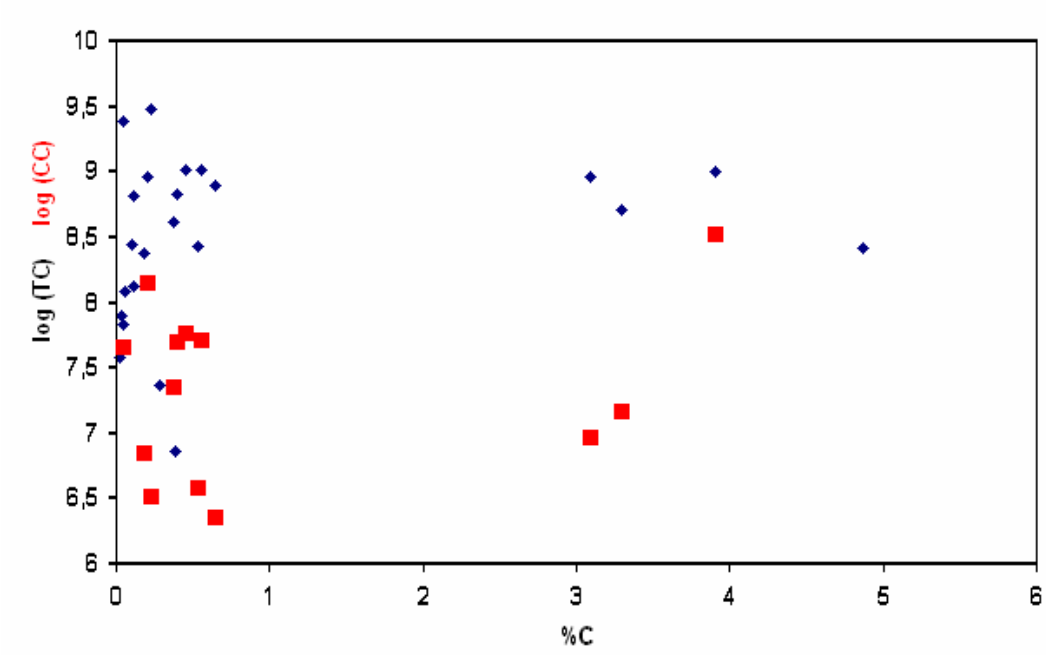

Figure 2. Total (TC) (♦) and culturable (CC) (■) bacteria as a function of total organic carbon (%C) present in 23 cold soils. Values of abundance data were log 10 transformations of values obtained from Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006).

Slika 2. Skupno število (TC) (♦) in število vzgojenih celic (CC) (■) kot funkcija skupnega organskega ogljika (%C) v 23 hladnih tleh. Prikazane so logaritmirane vrednosti podatkov o skupnem številu in številu vzgojenih celic iz literature: Zdanowski in sod. (2001), Sigler in sod. (2002) in Aislabie in sod. (2006).

The relationship between total and culturable bacteria in 23 cold soils is presented in figure 3. Two interesting features can be observed. First, total abundance of bacteria spans from 10^7 to 10^9 per g of cold soil, thus giving rise to comparable abundance of bacteria as in other temperate soils (Curtis *et al.* 2002; Torsvik *et al.* 2002). Second, a linear relationship exists between total bacterial number in cold soils and the number of culturable bacteria, in spite of differences in counting and cultivation approaches used to collect the data. The linear correlation coefficient ($R^2 = 0.64$) between total and culturable bacteria is comparable to recent study conducted on sediment and stream water ($R^2 = 0.76$) that included data on nine previously published samples (Lemke and Leff, (2006)). This demonstrates that the percentage of cells that are culturable is consistent across different cold soil environments.

Some of the variation in the number of culturable bacteria may be in general accounted for by a wide range of culturing factors like buffer used in preparation of soil suspension, media type, temperature of incubation, duration of incubation, humidity, gas composition and oxygen saturation level, used by different investigators. However, differences among soils are also likely to play part, especially as media composition were very similar in the studies of Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006). All investigators used media derivatives of nutrient broth as a carbon source solidified with agar.

From regression results, one would predict that average percent of culturable bacteria from a cold soil would be 0.1% that is approximately six times less than predicted for oligotrophic water streams and sediments (Lemke and Leff, 2006).

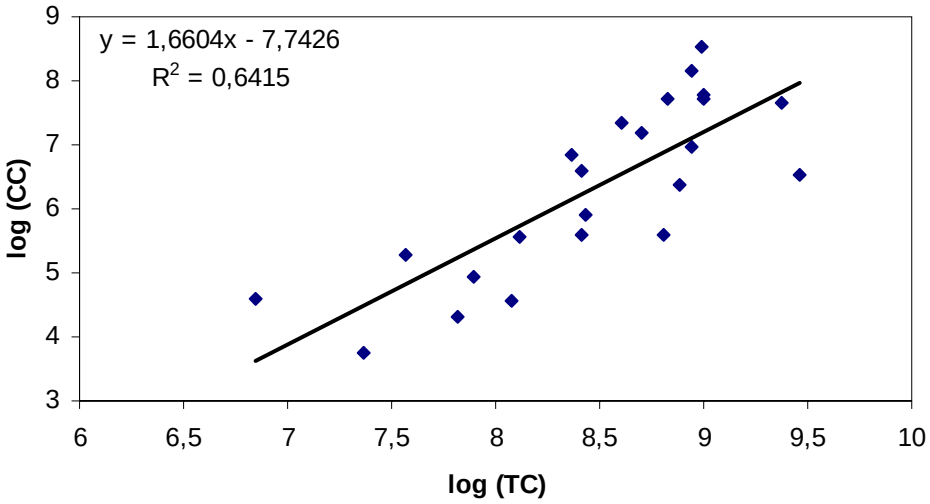

Figure 3. Relationship between total (TC) and culturable (CC) bacteria in 23 cold soils. Values were log 10 transformations of values obtained from literature: Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006).

Slika 3. Odnos med skupnim številom (TC) in številom kultiviranih bakterij (CC) v 23 hladnih tleh. Prikazane so logaritmirane vrednosti podatkov iz literature: Zdanowski in sod. (2001), Sigler in sod. (2002) in Aislabie in sod. (2006).

Based on relatively low success of cultivation approaches, bacterial communities appear to be numerically dominated by bacteria in viable but not culturable (VBNC) state. This suggests that the pool of nonculturable cells includes representatives of species that are under some circumstances culturable, as well as other types of bacteria that resist cultivation under the current conditions of cultivation. There is lack of data on how a wider variety of species divide, grow, reproduce, mature and die off, as prokaryotic life cycles have been described for a few species only (Colwell and Grimes, 2000). In general, it appears that few free-living populations

possess the energy and resources to reproduce constantly, and therefore, in the case of bacteria, will all surely not always be culturable.

Because a substantial number of "nonculturable" cells retain measurable metabolic activities (Creach *et al.*, 2003; Kell *et al.*, 1998) a reproductive phase seems to represent only a brief part of the life cycle carried out by a small portion of the population, usually not exceeding 1% of population. However, Stenström *et al.* (2001) reported on reversible transition between active and dormant microbial states in soil upon glucose addition and consumption, where the respiration rate of growing microorganisms represented 5–20% of overall microbial community respiration.

CONCLUSIONS

The results of this study showed that percentage of culturable cells is consistent across 23 different cold soil environments despite differences in techniques used. Using culturing approaches adopted by Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006) it appears to be possible to cultivate only 0.1% of total microbial community. However, more elaborated and imaginative approaches to culturing were already shown to be well suited to yield much higher numbers of cultivated bacteria from temperate soils. Bakken and Olsen (1984) and Lindahl and Bakken (1995) have shown that using appropriate techniques it is possible to retrieve up to 40% of total bacterial counts from soil. Unfortunately, none of these approaches have been tested on cold soils so far.

While microbial ecology is well poised to make a major leap in understanding microbial life, it is constrained by the lack of coordinated study (Morris *et al.* 2002). Thus, a key to this would be to start with coordinating the development of the protocols from sampling up to molecular analyses as this would give strong grounds for intercomparison of results. In addition, this would enable researchers to perform new analyses from different perspectives using collections of already published data to gain additional insights. However, this would constitute the first really coordinated study in microbial ecology in the world and International Polar Year 2007 (http://www.ipy.org/) constitutes a remarkable opportunity to accomplish this task.

ACKNOWLEDGMENTS

I acknowledge the support of late Ivan Mahne, who directed my attention to this topic.

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Acta agriculturae Slovenica, 90(november 2007)1, 33–43.

http://aas.bf.uni-lj.si

Agris category codes: F30 COBISS Code 1.01

GENETIC VARIABILITY OF SOME QUALITY TRAITS IN *Lathyrus* spp. GERMPLASM

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Received September 27, 2007, accepted October 15, 2007.
Delo je prispelo 27. septembra 2007, sprejeto 15. oktobra 2007.

ABSTRACT

Sixty-six accessions representing eighteen species of the genus *Lathyrus* collected from different geographic regions were evaluated for variations of quality traits (100 seeds weight, ash, total seed proteins and 3-(-N-oxayl)-L-2,3 diaminopropoinc acid – ODAP contents). High variability of ODAP levels was exhibited at both inter-specific and intra-specific levels. This variability was attributed to genetic and environmental factors. No significant correlation was found between ODAP and each of total protein content, ash content and 100 seeds weight. Cluster analysis of C.V. (Coefficient of variance) values for each accession identified the sixty-six accessions into eight groups. The most promising accession for breeding programs was *L. sativus* from Tunisia. This accession has good grain quality due to relatively low ODAP level and high protein content. The variations of protein content, ash content and 100-seeds weight were also discussed.

Key words: legumes / *Lathyrus sativus* / ODAP / genetic variability / quality traits / germplasm

GENETSKA VARIABILNOST NEKATERIH PARAMETROV KAKOVOSTI SEMENA *Lathyrus* spp.

IZVLEČEK

Ocenili smo variabilnost kakovosti (masa 100 semen, pepel, skupne beljakovine in vsebnost 3-(-N-oksail)-L-2,3 diaminoproposke kisline – ODAP) 66 akcesij 18 vrst rodu *Lathyrus*, ki smo jih zbrali z različnih geografskih regij. Ugotovili smo visoko stopnjo variabilnosti vsebnosti ODAP med vrstami in znotraj njih. To variabilnost povzročajo tako genetsko kot okoljski dejavniki. Nobene značilne povezave nismo našli med vsebnostjo ODAP in vsebnostjo celokupnih beljakovin, vsebnostjo pepela in maso 100 semen. Klasterska analiza variacijskega koeficienta (C.V.) je 66 akcesij razdelila v osem skupin. Najbolj obetavna akcesija za nadaljnje žlahtnjenje je bila akcesija *L. sativus* iz Tunizije. Ta akcesija ima dobro kakovost zrna, ki je posledica nizke vsebnosti ODAP in visoke vsebnosti beljakovin. Presojali smo tudi variabilnost vsebnosti beljakovin, pepela in mase 100 semen.

Ključne besede: stročnice / *Lathyrus sativus* / ODAP / genetska variabilnost / lastnosti / kakovost semena

INTRODUCTION

Lathyrus (*Leguminosae*; *Papilionoideae*) is the largest genus in tribe *Vicieae* and has an importance as traditional foodstuffs in many cultures worldwide **(**Kenicer *et al.,* 2005). It is a very popular crop in many Asain and African countries where it is grown either for stockfeed or human consumption. Kislev (1989) reported that the domestication of *Lathyrus* began in the Balkan Peninsula as a consequence of the Near East agriculture expansion into the region. Now

the cultivation of *Lathyrus* spread to include margainal lands in Syria, Lebanon, Egypte, Libya, Alegria, Morocco, France and Spain, the Mediterranean basin.

The most importance traits of *Lathyrus* consists of drought tolerance, resistance of stored grains to pests, adaptability to nearly all type of soils as well as to adverse climatic conditions and low input enviroment (Abdel Moneim *et al*., 1999; Hanbury *et al*., 1999; Sharma *et al*., 2000; Granati *et al*., 2003)

In spite of the importance of *Lathyrus* for human and animal, it has a limited uses due to the presence of the neurotoxic compound 3-(-N-oxayl)-L-2,3 diaminopropoinc acid – ODAP contents. The other forms of the toxic substances in *L. sativus* are BOAA (Beta-N-oxalyl amino-L- Alanine) (Smartt *et al*., 1994; Williams *et al*., 1994). The neurotoxin compound causes an irreversible paralysis of the lower limbs in human and the four limbs in animal and is known as Lathyrism (Spencer *et al*., 1986; Williams *et al*., 1994). Lathyrism has been known to occur in grasspea areas of the world for a long time. The disease was recorded first in the Narowal area of district Sialkot.

Neurotoxin concentration is lower in *L. cicera* than in *L. sativus*. In *L. cicera* it ranges from 0.04–0.76%. These values are genotype-dependent and show a little environment interaction (Hanbury *et al*., 2000). Campbell and Briggs (1987) reported that *L. sativus* Var. 8246 has low ODAP content ranging from 0.0259 to 0.0401% (w/w) of dry seed. The safe content of ODAP for human consumption is lower than 0.2% (Abdel Moneim *et al*., 1999).

Due to the neurtoxin presence, *Lathyrus* product has been banned in many countries. However, due to the importance of this crops in developing countries, these countries has established of breeding programmes mainly focused on getting a genotype with high seed yeild and low toxicity. Hanbury *et al.* (1995) and Granati *et al.* (2003) found a considerable variation in the neurotoxin content in *L. sativus* germplasm of different origin.

There are two ways to have a genotype with high seed yield and low toxicity, first is using the genetic engineering to produce transgenic plants (Hanbury *et al.*, 1999; Hanbury *et al.*, 2000). The second way is through eliminate the toxic substance by careful selection so far and through hybridization between low and high toxin varieties (Qayyum *et al*., 2001; Ben Brahim *et al*., 2001). To follow such way, enough information about genetic diversity and genetic resources of *Lathyrus* germplasm around the world is needed. This can be done through studying the variations in many genetically based traits among *Lathyrus* populations such as morphology, taxonomy and molecular markers.

A series of studies adovcated to ascertain the genetic variability present in *Lathyrus* germplasm of different origin (Infantino *et al*., 1994; Granati *et al*., 2000; Alba *et al*., 2001; Polignano *et al*., 2001). What has been done of the collected germplasm around the world is not a heck of a lot. This work, therefore, advocated to evaluate the genetic variability of 100 seeds weight, and total seed protein, ash and ODAP contents in 66 accessions belong to 18 species of different origins. The selection for recovering the accessions with superior quality traits introduces a valuable genetic material for local or national breeding programmes

MATERIAL AND METHODS

Material

Seeds of *Lathyrus spp* were obtained as a donation from International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria and germplasm collection of the USDA, ARS, WRPIS Washington State University, Regional Plant Introduction Station, 59 Johnson Hall, P.O. 646402 Pullman, Washington, United States, 99164-6402.

Methods

Quantitative estimation of total seed proteins

Total proteins were extracted separately from 20 mg air-dried defatted seed meals in 1000 µl extraction buffer (0.125 µ Tris borate pH 8.9, 1% SDS) for 24 hours at –4 °C. After that time, the extracts were centrifuged for 10 minutes at 1000 g. Quantitative estimation of the total seed proteins was made according to Bradford (1976). Three replicas were made for each sample.

Ash content and 100 seeds-weight

The ash content was determined by combusting seeds in silica crucibles in a muffle furnace at 550 °C for 6 h, with 50% of the ash free mass being regarded as carbon content (Allen *et al*., 1974). In addition, for each accession 100-seeds weight (g) was recorded.

ODAP content

Neurotoxin level was analyzed following a modified Rao's procedure (Rao, 1987). The seeds were finely milled and 100 mg of the grass pea flour were extracted for 5 h with 10 ml ethanol 60% (v/v). The suspension was then centrifuged and 75 µl of the supernatant were added to 92 µl of distilled water and 0.33 ml of 3N KOH. The sample (4 replica/genotype) was kept in a boiling water bath for 30 min (alkaline hydrolysis to convert from ODAP to DAP which can be determined) and then brought to 1 ml with water.

To detect ODAP, OPT (Ortho-phathalaldehyde) reagent was used, it was composed of 100 mg of OPT, 1ml 95% ethanol, 0.2 ml of mercaptoethanol and 99 ml of potassium tetra borate buffer (0.05 M in distilled water, pH 9.9). This reagent freshly made and used for 3 days long only. OPT reagent (2 ml) was added to the sample and absorbance of resulting yellow solution was measured after 30 min using a spectrophotometer set at $\lambda = 420$ nm . The results obeyed Beer's law.

$$\mathbf{A = C\ \varepsilon\ L}$$

(A) absorbance, (C) concentration, (ε) extinction co-efficient absorbitinty is constant and (L) is the path length which always be 1 cm.

Data analysis

The genetic diversity among the populations was evaluated by the Jaccard similarity index, Regression analysis, co-efficient of variance (CV). Multivariate analysis (factor analyses and cluster analysis) were made using the software package »SYSTAT for Windows«, Version 7.0 copyright (C) 1997, SPSS INC. A dendrogram was constructed through the complete linkage-joining rule.

RESULTS

Means, standard errors, range of variation estimated for each trait in all accessions are reported in Table 1. Protein content ranged from 22.6 to 49.3% with mean value of 35.4%. Extreme ODAP levels were 0.19 and 6.2% showing mean value of 1.05%. One hundred seeds weight was more variable, ranging from 1.35 to 31.5 g. On the contrary, the ash content showed variation ranging from 1.2 to 8.6%.

Table 1. Mean values, standard error (SE), ranges (Max, Min) and, coefficients of variation (C.V.) observed in 66 accessions belonging to 18 species of *Lathyrus* spp.

Preglednica 1. Srednje vrednosti, standardna napaka (SE), interval (maksimum, minimum) in koeficient variabilnosti (C.V.) pri 66 akcesijah 18 vrst *Lathyrus* spp.

Trait	Mean ± SE	Max	Min	C.V.
Protein content, mg g^{-1} dry matter)	354.166 ± 53.091	493.680	226.440	0.150
Ash content, %	5.147 ± 8.089	8.6000	1.200	1.571
100 Seeds Weight, g	8.472 ± 6.019	31.530	1.354	0.710
ODAP content, %	1.051 ±1.036	6.230	0.190	0.985

K-means clustering separated the 66 accessions into 8 clusters (Table 2). Cluster memberships, means, minimum and maximum values for each cluster are presented in Table 2. Clusters included different proportions of accessions belong to different species and from different countries, except cluster 2, 5 and 6 were homogenous in terms of the species they belong to. In particular, cluster 1 included accessions belong to *L. aphacce, L. sphaerius, L. hirsutus, L. sylvestris, L. hierosolymitan, L. gorgoni* and *L. inconspicuos* and showed the highest mean value for the total protein content. Clusters 2 and 6, the smallest clusters contained one accession each, included accession of *L. sylvestris* originated in Germany and accession of *L. sativus* originated in Tunisia respectively. It was interesting to note that cluster 2 presented the highest mean value for ash content and ODAP, and cluster 6 the highest mean value for 100 seed weight. Cluster 7, the most interesting one, showed the lowest mean value for ODAP (0.54) and intermediate values for the other traits. The low ODAP accessions were represented by the accession of *L. inconspicuos* collected from Turkey.

Table 2. Cluster memberships, cluster mean, coefficient of variation, and minimum and maximum values

Preglednica 2. Sestava klastrov, srednje vrednosti, koeficient variabilnosti ter najmanjše in največje vrednosti

Sample	TPC, mg g^{-1}	Ash content, %	Wt 100 seed, g	ODAP, %
L. aphacce Syria	364.100	3.300	3.552	0.3
L. aphacce Iran	416.160	4.500	1.525	0.24
L. sphaerius Syria	369.240	1.200	2.242	0.32
L. sphaerius Turky	363.120	1.900	1.767	0.35
L. hirsutus Egypt	399.840	4.300	2.900	0.320
L. hirsutus France	471.240	4.800	2.151	0.685
L. hirsutus Turky	412.060	3.800	2.264	0.470
L. sylvestris USA	493.680	5.400	4.117	6.230
L. sylvestris Yugoslavia	424.320	4.800	3.350	3.580
L. sylvestris Kazakhstain	440.640	5.500	3.975	3.970
L. hierosolymitan Turky	371.280	2.400	4.014	0.259
L. gorgoni Turky	367.200	4.500	2.893	0.335
L. inconspicuos Yughoslvia	252.960	1.800	1.354	0.3
Mean ± SE	395.83 ± 60.13	3.71 ±1.45	2.78 ± 0.97	1.34 ± 1.95
Min	252.96	1.20	1.35	0.24
Max	493.68	5.50	4.12	6.23
C.V	0.152	0.390	0.348	1.455

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Sample	TPC, mg g^{-1}	Ash content, %	Wt 100 seed, g	ODAP, %
L. sylvestris Germany	391.68	7.40	3.427	2.070
Mean ± SE	391.68	7.40	3.43	2.070
Min	391.68	7.40	3.43	2.070
Max	391.68	7.40	3.43	2.070
C.V	0.00	0.00	0.00	0.00
L. sativus Ethiopia	291.720	5.800	7.720	1.400
L. sativus Bangladish	346.800	3.900	8.139	0.560
L. sativus India	365.160	3.700	7.970	1.460
L. clymenum AUS	367.200	5.270	7.666	1.31
L. clymenum Portugal	361.080	4.080	7.632	0.82
L. clymenum Turky	363.120	3.100	7.307	0.33
L. latifolius Netherland	401.880	3.600	5.845	0.3
L. tingitanus Portugal	373.320	7.010	9.443	0.28
L. annus Turky	395.800	3.060	6.479	1.64
L. sylvestris Moroco	422.280	8.600	8.452	0.820
L. hierosolymitan Plastine	344.760	2.200	7.036	0.270
L. gorgoni Jordon	373.320	4.200	6.142	0.396
L. articulatus Grc	352.920	3.900	7.308	0.830
L. articulatus Frc	354.960	1.900	7.000	0.890
L. articulatus Austalia	361.080	1.700	7.063	0.600
L. mamoratus Turky	367.200	2.900	7.300	0.612
L. ciceria Pakistan	361.080	4.900	7.541	1.790
L. ciceria Portugal	242.760	6.500	8.819	0.94
Mean ± SE	358.14 ± 39.29	4.24 ± 1.84	7.49 ± 0.89	0.85 ± 0.49
Min	242.76	1.70	5.84	0.27
Max	422.28	8.60	9.44	1.79
C.V	0.110	0.349	0.120	0.576
L. sativus Egypt	340.680	3.900	14.180	0.850
L. sativus Ussr	316.187	3.300	13.630	1.410
L. sativus Afghanstan	318.240	2.600	11.980	1.060
L. sativus Germany	330.480	3.900	16.090	0.330
L. sativus Italy	408.000	4.200	19.880	0.830
L. sativus Yughoslavia	346.800	3.400	13.390	1.380
L. sativus Canda	328.440	6.900	17.240	0.914
L. ochrus Portugal	336.600	3.600	15.426	1.400
L. pseudocicer Jordon	269.280	3.040	9.974	0.910
L. blepharicar Turky	275.400	3.300	9.872	0.304
Mean ± SE	327.01 ±38.64	3.81 ± 1.18	14.17 ± 3.15	0.94 ± 0.40
Min	269.28	2.60	9.87	0.30
Max	408.00	6.90	19.88	1.41
C.V	0.12	0.310	0.222	0.425
L. sativus Libia	373.320	2.800	23.400	1.530
L. sativus Spain	336.600	5.400	24.930	1.320
L. sativus Hungery	334.560	2.300	20.980	0.860
Mean ± SE	348.16 ± 21.81	3.50 ± 1.66	23.10 ±1.99	1.24 ± 0.34
Min	334.56	2.30	20.98	0.86
Max	373.32	5.40	24.93	1.99
C.V	0.060	0.474	0.086	0.274
L. sativus Tunisia	320.280	3.254	31.530	0.560
Mean ± SE	320.28	3.25	31.53	0.56
Min	320.28	3.25	31.53	0.56
Max	320.28	3.25	31.53	0.56
C.V	0.00	0.00	0.00	0.00

continued overleaf / nadaljevanje na naslednji strani

Sample	TPC, mg g–1	Ash content, %	Wt 100 seed, g	ODAP, %
L. sativus Pakistan	352.920	8.900	5.750	1.37
L. aphacce GRC	348.840	5.600	1.801	0.35
L. latifolius Netherland	373.320	10.800	3.589	0.51
L. annus Syria	373.320	7.200	3.224	0.39
L. hirsutus Tunisia	416.380	6.200	2.786	0.609
L. hirsutus USA	410.040	8.100	2.611	0.590
L. mamoratus Syria	401.880	7.800	1.468	0.350
L. inconspicuos Turky	244.800	3.700	2.730	0.19
Mean ± SE	365.19 ± 54.80	7.29 ± 2.16	2.99 ± 1.31	0.54 ± 0.36
Min	244.80	3.70	1.47	0.19
Max	416.38	10.80	5.75	1.37
C.V	0.150	0.296	0.438	0.666
L. sativus Sudan	314.160	4.400	11.150	3.260
L. sativus Iran	332.520	3.100	9.460	1.640
L. sativus Turky	367.200	4.600	11.507	1.280
L. ochrus Cyprus	342.720	2.800	10.337	1.960
L. ochrus India	330.480	2.700	10.995	1.670
L. ochrus Iran	367.200	2.800	11.738	1.28
L.ciceria Syria	361.080	3.300	8.915	0.500
L. ciceria Turky	389.640	2.780	9.324	1.020
L. ciceria Cyprus	263.160	1.700	7.063	0.800
L. ciceria Norway	226.440	3.340	8.420	0.79
L. pseudocicer Turky	244.800	1.400	7.103	0.502
L. blepharicar Syria	295.880	4.100	8.287	0.609
Mean ± SE	319.61 ± 52.25	3.09 ± 0.97	9.52 ±1.63	1.28 ± 0.79
Min	226.44	1.40	7.06	0.50
Max	389.64	4.60	11.74	3.26
C.V	0.163	0.255	0.171	0.617

Table 3. Principal component analysis in 66 accessions of *Lathyrus* spp., Eigen-values and percent of variation accounted by the first two principal components (PRIN1 and PRIN2)

Preglednica 3. Analiza glavnih component 66 akcesij *Lathyrus* spp., Eigen-vrednosti in odstotek variabilnosti, ki ga pojasnjujeta prvi komponenti (PRIN1 in PRIN2)

Trait	PRIN1	PRIN2
Protein content	0.731	–0.305
Ash content	0.632	0.289
100 Seeds Weight	–0.309	0.861
ODAP content	0.727	0.421
Eigen – value	1.557	1.095
Variation, %	38.925	27.370
Variation accumulated, %		66.295

Table 3 gives the results of principal component analysis for the studied quality traits. The first two principal components account for 66.295% of the total variance of all traits, indicating a high degree of correlation among the characters for the accessions analyzed. Separate percentages of variation attributable to the first two components by decreasing order were 38.925 and 27.370%. By examining the eigenvectors of individual components, indications may be obtained about their levels of association with the original traits. Protein, ash contents and ODAP showed higher coefficients in the first component (PRIN1), while 100 seeds weight was a primary source of variation with the largest coefficient (0.861) in second principal component

(PRIN2). According to these results, the first two components in the principal component analysis were only considered.

Cluster analysis based on coefficient of variances derived by K-means clustering gave eight clusters (Figure 1), accounting for a 77% share of original variation. Clusters 8, 4, 6 were most similar and also 5, 3, 7 were most similar. Cluster 2 was the most distant and consequently, the least similar one and cluster 1 stands in an intermediate position between cluster 2 and other clusters. 18 accessions were grouped in the largest cluster (cluster 3), while cluster 1, 2, 4, 5, 6, 7 and 8 include 13, 1, 10, 3, 1, 8 and 12 accessions, respectively.

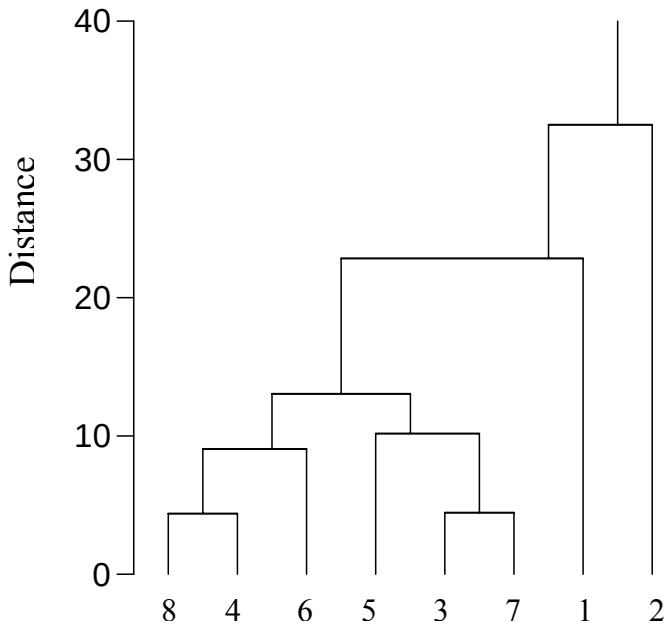

Figure 1. Dendrogram from cluster analysis of coefficient of variances derived from K-means clustering for four quality traits (total protein and ash contents, 100 seeds weight and ODAP percent) of 66 accessions of *Lathyrus* spp.

Slika 1. Dendrogram klastrske analize koeficientov variabilnosti, ki smo jih dobili iz povprečij za štiri lastnosti kakovosti (vsebnost celokupnih beljakovin in pepela, masa 100 semen in odstotek ODAP) 66 akcesij *Lathyrus* spp.

The means of the total proteins, ash content, 100 seeds weight and ODAP percent for each cluster were shown in Table 3. In this table, clusters 6 and 7, grouped the accessions with lower ODAP values and cluster 6 included the accession, which had the highest 100 seeds weight. The total protein contents varied between 395.83 mg g^{-1} in cluster 1 and 319.61 mg g^{-1} in cluster 8.

Regression analysis between ODAP and each of total protein content – TPC, ash content and 100 seeds weight for the studied accessions gave positive non significant correlation; R^2 equals 0.1381, 0.0139 and 0.0029 respectively. The results of variance analysis performed for individual trait showed that differences among accessions were statistically significant for all traits.

Table 4. Mean values of TPC, ash content %, 100 seeds wt and ODAP% for each of the eight groups

Preglednica 4. Srednje vrednosti vsebnosti celokupnih proteinov, odstotek pepela, masa 100 semen in odstotek ODAP za vsako od osmih skupin

Sample	TPC, mg g^{-1}	Ash content, %	Wt 100 seed, g	ODAP, %
1	395.83	3.71	2.78	1.34
2	391.68	7.400	3.427	2.070
3	358.14	4.24	7.49	0.85
4	327.01	3.81	14.17	0.94
5	348.16	3.50	23.10	1.24
6	320.28	3.25	31.53	0.56
7	365.19	7.29	2.99	0.54
8	319.61	3.09	9.52	1.28

DISCUSSION

Among the accessions analyzed none was classified as lacking the neurotoxin. In general the lowest β-ODAP levels (< 0.2%) were recorded in seeds of *L. inconspicuous* from Turkey, the highest value (> 6%) was recorded in seeds of *L. sylvestris* collected from USA. *L. sativus* accessions showed neurotoxin levels varying between 0.33% and 3.26. Our results indicated a high variability of level of β-ODAP at interspecific and intraspecific levels. Such variability of the level of β-ODAP in seeds of *L. sativus* accessions was in agreement with similar previous report by Polignano *et al*. (2005) who reported that the percent of β-ODAP varied from 0.24% to 0.64%. The content of β-ODAP in the examined accessions ranged between 0.19–6.23 percent. Although the extreme values of variation was similar to the data reported by Polignano *et al.* (2005), it was higher than the levels recorded by Urga *et al.* (2005), Yigzaw *et al.* (2001) and Urga *et al.* (1995). The variation in β-ODAP content between the different sets of data might be attributed to the method of analysis used (Tavoletti et al., 2005), environmental conditions and genetic factors (Dahiya, 1986; Barat *et al.,* 1989; Siddique *et al.,* 1996). The β-ODAP was determined by colorimetric and capillary electrophoresis analyses. A high positive correlation between the two methods was found (r= 0.83), but the colorimetric values showed, on average, significant 14% lower ODAP values (Tavoletti *et al.*, 2005). In this piece of work, the colorimetric method was used to determine β-ODAP in the accessions of *Lathyrus* spp.

Regression analysis between ODAP and each of total protein content, ash content and 100 seeds weight for our materials was non significant, indicating that these traits did not have any significant correlation. The same conclusion was deduced by Roy and Rao (1978) in their work on twenty nine varieties of *L. sativus* seeds. However, Urga *et al.* (2005) found a significant positive correlation between β-ODAP and crude protein content. The discrepancy between the data of Urga *et al*. (2005) and ours was attributed to the difference between crude protein and total protein. Whereas the crude protein was estimated from measuring the total nitrogen content, coming from both protein and non-protein nitrogen sources, the total protein reflects on the nitrogen associated with protein not include the nitrogen from non-protein source.

The results of variance analysis performed for individual trait showed that differences among accessions were significant for all traits. ODAP showed the highest coefficient of variation (CV) (96.5%), on the contrary protein content showed lowest coefficient variation (15%). Intermediate values of variation were shown by 100-seeds weight (71.9%) and ash content (46.6%). The

highest variability in β-ODAP and 100-seeds weight were attributed to the different taxa used in this study and to the different geographical regions and habitats the taxa collected from.

It was reported that *L. sativus* and *L. cicera* included the most interesting entries concerning low ODAP level (Granati *et al.,* 2001). Our results showed that this finding cannot stand up. It was found that accessions belonging to *L. inconspicuous, L. aphaca, L. hierosolymitan; L. tingitanus* had an amount of β-ODAP less than that found in *L. saivus* and *L. cicera.*

The variation in β-ODAP between the accessions of the same taxa was due to the variation in water stress. It was found that the severely stressed plants had significantly higher β-ODAP concentration than unstressed plants (Swarup and Lai, 1993), it was also found that ODAP concentration is affected by phenology as well as by both genetic control and environmental conditions (Lambein *et al.,* 1990). Our alternative explanation is that the lower concentration found in good environment is the result of toxin dilution, suggesting a restricted "pool" of toxin is distributed over a large number and weight of seeds.

It was suggested that β-ODAP accumulation in grass pea might be related to the level of total free nitrogenous compounds and that nitrogen and phosphate may be crucial nutrient factors influencing β-ODAP content under field conditions (Jiao *et al*., 2006). Thus the application of appropriate nitrogen and phosphorus fertilizers to the soil may decrease the content of β-ODAP in the seeds and leaves of grass pea.

Protein content in the seeds of *Lathyrus spp* ranged from 22.6 to 49.3 with mean value 35.4%. The highest content was recorded in *L. sylvestris* from USA (49.36%) and the lowest in *L. cicera* from Norway (22.6). As far as known, majority of study on total protein content in genus *Lathyrus* was directed to *L. sativus*. That was the interpretation for the contradictory between our results and that reported by Hove and King (1978), Shobhana *et al.*, (1976), Urga *et al.*, (2005), Granati *et al.*,(2001), Roy and Rao (1978) who reported a protein content ranked between 23 and 31% with mean value 29.7%.

The ash content assessed in 66 accessions in *Lathyrus spp* showed variation ranging from 1.2 to 8.6%. One hundred seed weight was more variable and ranged from 1.35 to 31.5 g. The largest was found in accessions of *L. sativus*. This data was in agreement with the result of Granati *et al*. (2001) and Della and Polignano (2002). Since *L. sativus* has grown in a good environment as a cultivated plant and there was a negative relationship between seed yield components and stresses, the highest 100-seeds weight of *L. sativus* can be justified. However, there was no positive correlation between 100-seed and β-ODAP within the accessions of the other taxa. In the light of the suggestion that seed weight was negatively correlated with stresses and β-ODAP content (Urga *et al*., 2005), and the contradictory trend found in our results, we inferred that the relationship between seed weight and β-ODAP couldn't be simplified in stresses only. It might be influenced by both environmental and genetic factors, but the mechanism by which the environment acts is not clear.

Multivariate analysis (principal co-ordinate analysis), showed a sort of association between protein, ash content and β-ODAP. This association can be interpreted in terms of the role of (1) mineral (Zinc, Calcium, Phosphorus and Molybdenum) which is the main component of the ash in stimulating β-ODAP accumulation in the seeds and (2) the crucial role of free nitrogenous compounds in influencing β-ODAP content under the field. It was found that the decrease in free nitrogenous compounds in the developing seed was accompanied by a rapid accumulation of protein (Jiao *et al*., 2006), and that the free nitrogenous compounds such as glutamine and serine as well as nucleotide nitrogen, all significantly enhanced the accumulation of β-ODAP in young seedling (Lambein *et al.*, 2007).

Based on cluster of C.V values for each accession, eight groups are identified; accounting for a 77% share of original variation, the most promising accessions was *L. sativus* accessions collected from Tunisia. This accession grouped in cluster 6, being good accession for grain quality, due to their relatively low ODAP level and high protein content.

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Acta agriculturae Slovenica, 90(november 2007)1, 45–56.

http://aas.bf.uni-lj.si

Agris category codes: E10 COBISS Code 1.01

OPTIMISATION OF PRODUCTION ACTIVITIES ON INDIVIDUAL AGRICULTURAL HOLDINGS IN THE FRAME OF DIFFERENT DIRECT PAYMENTS OPTIONS

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Received March 06, 2007, accepted July 24, 2007.
Delo je prispelo 06. marca 2007, sprejeto 24. julija 2007.

ABSTRACT

Linear programming model has been developed and applied to the hypothetical agricultural holding in the hilly part of Slovenia in order to find optimal production plans by maximizing total gross margins. The model covers especially those sectors of Slovenian agriculture, for which the most drastic changes due to the actual reform of CAP in the field of direct payments – in Slovenia implemented in 2007 – are anticipated. On the basis of developed model the economic impacts of CAP reform and importance of agri-environmental measures have been evaluated. Model results lead to the conclusion that the reform will have the most unfavorable impacts for agricultural holdings with intensive production practice, especially those with animal production activities which are under the standard scheme eligible for relatively high production coupled direct payments (up to 70% of achieved total gross margin). Negative impacts of the reform can be mitigated by combining different production activities and technologies. Economic results markedly improve (up to 28%) if farming management complies with agri-environmental measures.

Key words: agriculture / linear programming / CAP reform / direct payments / Slovenia

OPTIMIRANJE PROIZVODNIH DEJAVNOSTI NA KMETIJSKEM GOSPODARSTVU Z VIDIKA MOŽNIH KOMBINACIJ NEPOSREDNIH PLAČIL

IZVLEČEK

Razvili smo model na podlagi linearnega programa, s katerim smo na hipotetičnem kmetijskem gospodarstvu, iz gričevnatega predela Slovenije, iskali optimalen proizvodni načrt na podlagi maksimalnega skupnega pokritja. Model zajema predvsem tiste sektorje znotraj kmetijstva, pri katerih bo prišlo do korenitejših sprememb, predvsem na področju neposrednih plačil, z aktualno reformo SKP – v Sloveniji vpeljana v letu 2007. S pomočjo razvitega modela smo ocenili kakšne bodo ekonomske posledice reforme in kakšen je pomen okoljskih plačil. Na podlagi dobljenih rezultatov smo ugotovili, da bo reforma najhuje prizadela kmetijska gospodarstva z intenzivno proizvodnjo, zlasti živinorejsko usmerjena, katera so bila po standardni shemi upravičena do relativno visokih proizvodno vezanih neposrednih plačil (do 70 % doseženega pokritja). Negativne učinke reforme bo možno ublažiti s kombiniranjem različnih usmeritev in tehnologij. Ekonomski rezultati se prav tako pomembno izboljšajo (do 28 %) v primeru gospodarjenja v skladu s kmetijsko okoljskimi ukrepi.

Ključne besede: kmetijstvo / linearno programiranje / skupna kmetijska politika / CAP / reforme / direktna plačila / Slovenija

INTRODUCTION

Successful farmers can not manage their holdings just on the basis of technology and production results. They must understand and also be able to use knowledge of economics, marketing, production and finances in the meaning of planning and also in the meaning of leading (Boehlje and Eidman, 1984). At the first step they should define the aim of farming. The most important aims are economic ones, especially when agriculture is the only source of income. Kavčič (1996) mentions besides economics aims also a group of personal aims like independence in decision making, spare time and reputation in the local area.

Direct payments are important element of modern agricultural policy which could significantly influence on decision making process at the agricultural holdings level. After accession to European Union direct payments became also one of the most important income sources for farmers in Slovenia (Volk *et al.*, 2006). Policy changed significantly in year 2007 as result of the implementation of 2003 reform of the Common Agricultural Policy (CAP) direct payment scheme (Rednak *et al.*, 2005) which also should have impact on the decision making process on the agricultural holdings in Slovenia.

Changing environment leads Slovenian farmers to make new decisions about which sector to choose, what to produce, by which technology in which period of the year and finally in what quantities to produce (Hazell and Norton, 1986). There exist many techniques of decision making that could help farmers to solve such problems (Boehlje and Eidman, 1984). One of them is indubitably linear programming that basis on mathematic techniques for solving optimization problems.

The paper has objective to shortly to present developed linear programming model that can be utilized on concrete Slovene agricultural holding with aim to find optimal production plan on economics basis (maximal total gross margin). We are going to represent how important is CAP in Slovene agriculture area with its measures and consequently also the impact of current reform in different agricultural sectors. New economic conditions, caused by reform will also face farm managers with new range of solutions by improving financial results.

The paper is structured as follows. The first part is short review of literature in connection with mathematical programming stressed on linear programming, with some examples of its application in agricultural sector. In followed methodological chapter, we shortly present the structure of developed model with all included activities and constraints. Then we describe basic characteristics of analyzed farm and eight presumed policy scenarios. In the next chapter we summarize the results with discussion. The paper is concluded with short conclusion in English and Slovene language.

REVIEW OF LITERATURE

Mathematical and linear programming

Gallenti (1997) said that mathematical programming is actually a method, which finds a solution that satisfies all constraints of the analyzing problem. It chooses between farm enterprises (activities) on the bases of objective function with respect to a set of fixed farm constraints. In other words, objective function represents the preferences of the agricultural holding. One of the most commonly applied methods from mathematical programming assortment in agricultural sector is linear programming. The basic concept of linear programming procedure is to maximize or minimize a specific outcome (objective function). A set of mathematical rules known as simplex algorithm is used to solve optimization models with constraints.

In the past linear programming was already proven as a very useful tool in planning farm business. Boehlje and Eidman (1984) pointed out this technique as applicable to almost any resource allocation problem faced by decision maker on the farm. Besides that it is capable to handle more complex problems than other more simple methods (for example budgeting and marginal analysis) also used in farm management. Calculated optimal solution presents the best production-marketing-financial plan, based on efficient (optimal) resources allocation. By-product of linear programming is information on resources that are limiting for further growth of potential income of the farm operation, which resources are in excess and therefore not totally used, and also how much is worth to destine for additional units of the limiting resources. For relevance of the farm plan obtained it is also important its stability or sensitivity. This is another attribute of using linear programming in farm management analysis. It could be evaluated how the results would be changed if deviations occurred in prices or technical proportions. This is also called parameter programming or sensitivity analysis (Zadnik Sitrn, 2001b)

Mathematical formulation of linear programming

Linear program can be written in mathematical form as shown in the equation [1] and un-equations [2] and [3]. In those equations an example is shown an example where the objective function has to be maximized. If we handle with opposite problem where objective function has to be minimized, we just multiply the vector c_j (coefficient in the objective function) with –1.

$$\max Z = \sum_{j=1}^{n} c_j X_j , \quad \text{such that} \qquad [1]$$

$$\sum_{j=1}^{n} a_{ij} X_j \le b_i , \qquad \textit{all } i = 1 \textit{ to } m \qquad [2]$$

$$X_j \ge 0 \qquad \textit{all } j = 1 \textit{ to } n \qquad [3]$$

Meanings of notations:

Z objective function (total gross margin)
c_j coefficients of objective function (total gross margin of one unit of *j-th* activity)
a_{ij} the quantity of the *i-th* resource (requirements of kilogram fodder, hours of labor) required to produce one unit of the *j-th* activity (technological coefficients)
X_j the level of the *j-th* farm activity (acreage of maize, wheat, number of animals)
b_i extent *i-th* available resource (hectare of field, capacity of stable)

Equation [1] defines maximum of objective function (total gross margin) as the sum of products of total gross margin (c_j) per single activity with its level included into solution (X_j). Un-equation [2] requires that solution with achieved maximal total gross margin does not violate any of the fixed resource constraints. Practical fact that solution can not be negative is considered in un-equation [3]. Problem defined by equations [1] to [3] is known as the primal linear programming problem (Hazell and Norton, 1986). Such problems are usually solved with set of mathematical rules also known as simplex algorithm, developed by Dantzig in 1947 (Zadnik Stirn, 2001a).

Application of linear models in agricultural sector

In the past linear programming was very commonly used for solving problems in agriculture. Winston (2004) mentions that in one survey of 500 firms in USA it is found out that 85% of them used linear programming in operation researches. If we focus only on using it in agriculture

sector, we can find out that in spite of complexity of agricultural problems it is still very commonly used. But many researchers also expose (Zadnik Stirn, 2001b) that in spite of rough reduction of real optimization problems many cases can not be solved with linear programming. In such examples one should use another technique of mathematical programming (nonlinear programming, integer programming) or even another method.

Linear programming techniques and farm optimization models have also been successfully used in recent years for estimation of potential impact in changing agricultural policy. Majewski and Was (2005) exposed some analyses based on this method that had been created in connection with current CAP reform, focusing mainly on farm economic situation and their production structure. Such models could be found for Germany (Kleinhans *et al*., 2000, cited by Majewski and Was, 2005), Ireland (O'Connell, 1998, cited by Majewski and Was, 2005) and Poland (Berg *et al*., 1999) in the last case the linear model has been used to assess the impact of implementing CAP in this new member state.

Also for Slovenian agriculture sector some researches based on linear programming have been performed. Jerič (1990) applied a linear program to find the optimal production plan at the farm level by maximizing total gross margin. Model is developed for farm holdings situated in flat and hilly part of Slovenia. It is tied to the households where their production is predominantly based on tillage. Pajntar (1991) used linear programming model to optimize production with emphasis on employment optimization and maximization of income at the farm gate. Developed model enables to find the optimal employment structure within all included activities. For evaluation of manager decisions on farm holdings, Udovč (1992) modulated a simulation model. It is based on income criterion. Model is constructed on four sub-models (crop production, animal production, labor resource and resources for production) that together represent the whole farm system. By the animal sub-model optimal herd size was searched in dependence on home produced forage. Rozman *et al.* (2002) developed an optimization model to find the optimal steer feeding ratio. Linear method has shown as useful also in searching for optimal 'trade niche' for ecological products (Ulamec, 2005). They tried to reduce distance (physical, economics and recognition) between consumers and suppliers of ecological products.

MATERIAL AND METHODS

The model has been developed in Microsoft's Excel framework. In its basic version it includes a macro called solver that is capable to solve linear and also non-linear problems. If we presume linearity the optimizer employs the simplex to find an optimal solution and sensitivity information. The "free" bundled version of the Excel Solver supports just up to 200 decision variables (Microsoft Excel…, 1999). This is the main reason why we have chosen only a few activities from the numerous possible in Slovenian agricultural sector. We decided to focus on those sectors in Slovene agriculture where we can expect significant impacts of actual CAP reform in the field of direct payments. Previous researches (Rednak *et al.*, 2005) have shown that this reform will have the most significant impact in cattle sector.

Developed linear model has quite complex structure. Different calculations with corresponding data are placed on separate sheets. Such structure enables easier overview and any further control including simulation or completion is much easier. Another reason to put emphasis to this complex structure lies in user-friendly input for analyzing individual farm case. The most elegant way to solve such problem is to gather all input data on one sheet and make links to each calculation. This makes analysis for different agricultural holdings simple and fast. Consequently also possibility for mistakes is much reduced.

Included activities and restrictions

Important step in modeling is to define activities (processes) with technological (input-output) coefficients. Boehlje and Eidman, (1984) define a process as a method of transforming resources or inputs into a specific output. Graphically it can be represented as a single linear ray that is defined by two variables: factor and final product. This means that process can vary only in size and scale, any changes in input-output proportions result in a new activity. This leads to essential increase in number of included activities, which makes model more complex. Depending on used basic platform (in our case Excel) the complexity of the model is constrained. For this reasons the spectrum of included activities in comparison with possible activities in Slovene agricultural sector is limited. Consequently the model is useful only for agricultural holdings dealing with activities included in the model. The main part of model database, especially input-output coefficients, is taken from Gross Margin Catalogue (Jerič, 2001). Since this catalogue considers prices from the year 2001 they are updated to 2005 values. We apply average prices and costs that are annually calculated for the needs of model-calculations (KIS, 2006).

Included activities could be distributed in four groups:

- In the first group we can place livestock activities including different types of cattle and sheep breeding.
- Second group includes forage production on arable and grass land.
- Very comprehensive part presents crop production activities. Their main purpose is in covering livestock nutrients needs and only minor part for selling on market.
- In the last group we can classify all other activities (purchase, commodity selling, hiring and transfers within farm household). This group is the most heterogeneous, because it connects and completes all other three groups at different stages.

Basis for sensible result interpretation is good determination of units' measurement. This is very simple in livestock activities where breeding results are annual and the units of measurement are simply an animal. Much more complicated is in the case when period of breeding is not exactly 1 year (beef fattening). Quite simple is for activities that include arable land, where the basic unit of measure is a hectare of land. More attention is also needed by activities like balance of fertilizers and forage where different units of measurement are used (kilogram, hundred kilograms and tones).

To get more realistic model we decided to sub-divide production activities according to possible technologies and consequently also to different potential achieved harvests. One part of production activities is divided into sale and production (field harvests and hay). Just the opposite is in animal-breeding activities where selling is presumed. Program is organized in the way that only one technology could be selected at once. So in the first place developed program is not meant for searching the best technology or the optimal intensiveness, but to find the optimal solution within pre-selected activities.

Different production intensity does not result only in different amounts of product and needed inputs, but also in different costs and incomes. In other words, corresponding gross margin varies. For activities with wide possible range of intensity we simplified model in the way that we didn't take into consideration function relation but we just separated them into classes (linear sections). With this step we made some mistake, but the model is much simpler.

Developed model among livestock activities includes only cattle and sheep sector. Cattle sector is presented by activities of dairy cows, suckler cows, beef and veal production. The main two reasons why we also included sheep production is that in the last few years we can notice significant increase of sheep breeding especially in the hilly parts of Slovenia and because the 2003 CAP reform is going to have also some impacts on its economic situation.

The second activities group joins all kinds of fodder conservation like grazing, preparing hay, silage etc on arable and grass land. Several technologies of cereals production like maize, corn

and barley are joined in the third group. As said, the last group is the most heterogeneous. It includes buying and selling produced fodder, labor hiring, arable and grass land renting, storehouse balance, demand and supply of milk quota and of several premium rights.

The model includes only the most important constraints that must be satisfied to find the optimal solution. We can separate them into four major groups:

- zootechnical constraints (herd size, animal nutrition needs, maximal livestock density)
- agrotechnical constrains (arable land surface, grass land surface, pasture surface, crops rotation, mineral nutrition balance, share of cultivation)
- policy (milk quota, premium rights for suckler cows, premium rights for sheep)
- specific farm constraints (labor capacities, harvesting technology, storehouse capacity)

For all crop and animal products we assumed their full utilization. In reality this assumption is not realistic. But we still think that this simplification does not cause significant errors since we analyze farms that already sale their products.

Characteristics of analyzed farm household

Developed linear model is capable to analyze different types of farm households (specialized or mixed) within included sectors and activities. It is also capable to analyze effects of variation in production factors, within each farm plan, on the final residual – different production plans. All necessary and available data are collected in a linear programming matrix that can be used to evaluate the optimal production – organization. At the beginning user defines constraints within which linear model search for the optimal solution. By description of farm characteristics constrains are focused on labor resources, arable and grass land surface, stables' capacities and potential activities on individual farm.

We tested developed model on hypothetical farm, situated in the hilly part of Slovenia possessing 5 hectares of arable land and 10 hectares of grassland. Half of total area belongs to less favoured area. On this land farm produces forage, mainly for their own herd and in the case of overproduction also for sale. By searching for the optimal crop production on the arable land also crop rotation was considered (maize up to 70%, cereals 60% and at list 20% clovers). We assumed that farm was specialized in dairy and suckler cows. The farm owns 120 tones of milk quota and 20 premium rights for suckler cows. In searching for new production plan it is also possible to include other animal production activities (beef, calves and sheep). The labor available is estimated on 1.6 annual working units (1 AWU equals to 1800 hours). When additional labor is necessary it is possible to hire it.

With developed model we tested what kind of economic result is possible to achieve on the farm according to different specializations and policy scenarios. Since production factors were assumed to be fixed in all plans, excessive production could be sold (in many cases this enables linear program to find possible solution). Six different types of specialization were observed: dairy cows, bulls fattening, suckler cows, calves fattening, dairy sheep and fattening sheep.

Scenario analysis

Developed model includes three different direct payments' schemes: (i) until 2006 valid standard scheme assuming 100% level of payments, (ii) combined scheme to be implemented in the period 2007 to 2013 and (iii) regional scheme that is likely to follow after 2013. According to given conditions and constraints of each scheme we analyzed their effects on optimal production plans. It was taken into consideration that within each scheme it is possible to combine different types of CAP measures dependent on livestock density. On the basis of these conditions (types of subsidies and livestock density) eight different policy scenarios were analysed (Table 1).

Except in the forth scenario (KP0) where no budgetary support is assumed, all other scenarios envisage also payments for less favor areas (LFA).

Table 1. Scenarios analysed
Preglednica 1. Proučevani scenariji

Scenario abbrev. Oznaka scenarija	Scenario specification (type of direct payments and inclusion into agri-environmental measures (SKOP)*) Opis scenarija (vrsta neposrednih plačil in vključenost v SKOP plačila*)	Livestock units (GLU ha^{-1}) ** Obtežba (GVŽ ha^{-1})**
SSOS	Until 2006 implemented standard scheme; farm not included in Slovene agri-environmental scheme (SKOP) Do leta 2006 veljavna standardna shema, kmetija ni vključena v SKOP	2.5
SSSKOP	Standard scheme; farm included in SKOP Standardna shema, kmetija je vključena v SKOP	1.9
SSSEKP	Standard scheme; farm included in SKOP; farm eligible for extensification premiums Standardna shema, SKOP plačila, kmetija je upravičena do ekstenzifikacijske premije	1.4
KP0	Liberal-market (no subsidy is in place) Liberalno-tržna shema (ni nobenih proračunskih podpor)	No restriction
RK	Combined scheme, implemented during 2007–2013; farm not included in SKOP V obdobju 2007–2013 veljavna kombinirana shema, kmetija ni vključena v SKOP	2.5
RKSKOP	Combined scheme; farm included in SKOP Kombinirana shema in SKOP plačila	1.9
RR	Regional scheme with single area payment; farm not included in SKOP Regionalna shema z enotnimi plačili na površino, kmetija ni vključena v SKOP	2.5
RRSKOP	Regional scheme; farm included in SKOP Regionalna shema in SKOP plačila	1.9

* Model includes agri-environmental payments (SKOP) for the period 2004–2006
** Maximal livestock density per hectare of agricultural land (for some payments utilized agricultural area, for the other agricultural land for forage production)

RESULTS AND DISCUSSION

Developed linear programming model was employed to find optimal production plan under different conditions for analysed hypothetical specialized farm. Results are summarized in table 2.

The highest total gross margin is attainable with dairy production. This seems logical because predominant part of utilized area is grassland where farm can produce only voluminous forage. Optimal solution under standard scheme (SSOS) includes 33 dairy cows, while their number is reduced proportionally with livestock density constraints in scenarios SSSKOP and SSSEKP. Almost the same herd size and slight financial improvement in all reform scenarios (RK, RKSKOP, RR and RRSKOP) show that economic interest on the analyzed farm will not significantly change under the assumption of constant commodity and input prices. Stability of this solution is mostly dependent on achieved milk price and price for purchased milk quota. Because of its abolition we took into consideration lower price in all four reform scenarios. This presumption improves the result for 1,300 or 700 EUR dependent on purchased milk quota.

Significant financial improvement is noticeable in all schemes if farm enters in agri-environmental scheme (SKOP) and just the opposite holds for farming without any subsidy (KP0).

Already on the basis of area available we can expect that bulls fattening is not competitive to dairy production on analyzed farm, except this is an additional activity on the holding (therefore farming does not represent the main source of income). Since under beef production physical resources are only partly utilized, financial result could be quite interesting for additional activity. For the optimal feed ration of animals essentially higher percentage of arable land would be necessary on the farm (current share only 33%). Since this share on hypothetical farm is assumed to be fixed, it could be expected that herd size is more or less the same for all scenarios. The number of fattened bulls is reduced only in the third scenario of standard scheme (SSSEKP), where the reduction is imposed by lower livestock density (1.4 LSU). In this case extensification premiums efficiently compensate deficit of revenue caused by lower livestock density.

Bulls fattening is one of those sectors, where CAP reform will have the most negative impacts on economic outcome (RK, RKSKOP, RR and RRSKOP). This is the consequence of total or partial reduction of production coupled direct payments. Almost 4,000 EUR better financial results are obtained under combined scheme compared to regional scheme, since in the former one part of payments remains coupled and another one in form of historical payments. The same tendency in all reform scenarios can be observed also in gross margin per working hour.

Suckler cows optimal herd size is more or less constant in all standards (SSOS, SSSKOP and SSSEKP) and combined scheme (RK and RKSKOP) scenarios. Slight decrease in number of suckler cows is indicated in KP0 and both regional scheme (RR and RRSKOP) scenarios, where no coupled payments are in place. Economic outcome in comparison with dairy and meat production is not simulative, but it has to be taken into consideration that extensive organization in this case brings lower harvests and consequently also lower labor demand. Suckler cows specialization seems interesting especially when farming represent only a supplementary source of disposable agricultural household' income.

Under standard scheme farm could improve financial result with involvement into agri-environmental measures and managing under limits of 1.4 livestock units per hectare to get additional payments (extensification premiums). From 2007 it is undoubtedly sensible to adapt agricultural practice in compliance with CAP rural development program conditions (LFA and egri-environmental payments), which will help farmers to improve financial result. In the analyzed case this means up to 4,000 EUR increase of total gross margin. The importance of subsidies confirms also the fourth scenario (KP0) where financial result (gross margin, not income!) in general is halved compared with actual policy environment.

Even though calves fattening is not very frequent specialization on Slovene farms, we simulate it. What is interesting in this sector is that breeding is actually not connected with land, because all forage is possible to purchase. Linkage to land is required through allowed livestock density. In all scenarios with exception of KP0 (where the main limited factor is forage), area is the most limiting factor. Except smaller amounts of hay all other farm harvests are sold. In standard scheme scenarios (SSOS and SSSKOP) high level of direct payments are considered, especially slaughtered payments that are going to be cancelled after CAP reform. This fact will not have an important impact on the optimal herd size, but in worsening financial situation of the sector.

Sheep specialization was also tested with the model. If we focus on sheep for milk production with further milk processing and direct sale of dairy products at farm gate. It demands very high labor input. This leads to lack of labor supply and consequently in all scenarios labor force is hired (more than half of needs). Consequently is expected gross margin per hour is decreased, but it has to be taken into consideration that all hired force was paid (4 EUR/hour).

Table 2. The main results for different specializations of analyzed farm household
Preglednica 2. Pomembnejši rezultati za različne primere specializacije na analiziranem kmetijskem gospodarstvu

	Agricultural policy scenarios / Scenariji kmetijske politike							
	SSOS	SSSKOP	SSSEKP	KP0	RK	RKSKOP	RR	RRSKOP
Specialization (GLU) Specializacija (GVŽ)								
Dairy cows Krave molznice	33	28	20	33	33	28	33	29
Bulls fattening Pitanje bikov	16	16	14	16	16	16	16	16
Suckler cows Krave dojilje	19	19	19	12	19	19	12	17
Calves fattening Pitanje telet	38	29	14	55	37	28	37	28
Sheep breeding - milk Ovce za prirejo mleka	15	15	9	15	15	15	15	15
Sheep breeding - meet Ovce za prirejo mesa	21	21	14	21	21	21	21	20
Total gross margin (EUR) Skupno pokritje (EUR)								
Dairy cows Krave molznice	29,791	33,321	27,925	20,677	31,673	35,507	29,661	33,433
Bulls fattening Pitanje bikov	22,509	23,727	23,794	9,765	18,499	19,592	14,138	15,315
Suckler cows Krave dojilje	14,501	18,628	20,560	5,654	12,748	16,875	10,320	14,751
Calves fattening Pitanje telet	23,869	21,224	15,636	15,089	21,532	20,385	17,581	16,433
Sheep breeding - milk Ovce za prirejo mleka	27,120	29,644	27,491	20,614	23,744	26,281	25,138	27,704
Sheep breeding - meet Ovce za prirejo mesa	16,203	18,716	16,199	7,833	11,830	14,342	12,999	15,482
Gross margin per hour (EUR/hour) Pokritje/uro (EUR/uro)								
Dairy cows Krave molznice	8.64	10.90	12.14	6.00	9.19	11.62	8.60	10.94
Bulls fattening Pitanje bikov	21.91	23.10	19.11	9.51	18.01	19.07	13.76	14.91
Suckler cows Krave dojilje	14.08	18.09	19.96	7.79	12.38	16.38	14.20	15.14
Calves fattening Pitanje telet	12.24	12.94	13.52	6.00	11.05	12.43	9.02	10.02
Sheep breeding - milk Ovce za prirejo mleka	3.57	3.90	5.72	2.77	3.13	3.46	3.38	3.64
Sheep breeding - meet Ovce za prirejo mesa	11.13	12.85	13.54	5.38	8.12	9.85	8.93	10,82

In all scenarios herd size is the same, except in the third scenario (SSSEKP) with more restricted livestock density. The reason is not alone in available land, but in the fact that farm

applies for direct payments for cereals which are otherwise taken into account for GLU constraint. Anyhow, adapting management to conditions of SSSEKP scenario would be irrational since no extra payments are on disposal for sheep. The optimal financial plan would be achieved with involvement into agri-environmental measures (SSSKOP). Comparing with other livestock sectors this is the only one where regional scheme would lead to better financial result. Difference between combined and regional scheme is approximately 850 EUR and both results can be improved for 2,500 EUR by agri-environmental payments.

Less intensive in terms of working hours is lamb production. Scenario results assume only around 37% of available labor resources. This percentage includes all working hours necessary for animal and crop production. Farm would improve obtained result by substitution of 5 hectares of fields for meadows. As we already found out for sheep milk production, scenario SSSEKP has no sense also for lamb production. Even though farm has to purchase individual premium rights in scenarios SSOS, SSSKOP, RK and RKSKOP, herd size does not reduce compared to other scenarios. From this fact we can conclude that the most limiting factor for herd size increase is forage produced on grassland. Regional scheme gives improved economic outcome compared to combined scheme. One reason can be found in very low livestock density achieved which makes regional (totally decoupled) payments more efficient in comparison with premium rights and partial decupled payments that are presumed in combined scheme. Another reason lies (860 EUR) in purchased premium rights that are in conditions of regional scheme not presumed. But in both reform scenario financial results deteriorate for 3,000 to 4,000 EUR compared with standard scheme. In all four reform scenario cases deterioration for 2,500 EUR is caused by expected lover price, because of quota system abolition, for sold milk quota (120 tones). So this result doesn't reflect general position of the sheep sector after reform but includes links with analysed farm.

CONCLUSIONS

Model results confirm the hypothesis that the reform will have negative economic impacts on farms with intensive production practice, especially those with high livestock density. But in many cases it is possible to improve economic outcome of farming just with more efficient production plan.

In analyzed livestock sectors high importance of subsidies is shown, ranged between 23% and 73% of total farm gross margin. In both CAP reform schemes this percentage is reduced. In combined scheme it remains between 26 and 60% dependent on farm' involvement in agri-environmental measures. Compared to combined scheme under regional scheme drastic change in achieved total gross margin is noticed. Nevertheless, the share of subsidies remains comparable to those in combined scheme or decreases only for few percentages. Model results confirm that calves fattening specialization is most dependent on subsidies (in standard scheme) and consequently this sector experiences the highest shock. Just the opposite holds for dairy farming – both cows and sheep –, where share of subsidies in farm gross margin will remain stable. Budgetary support will remain at the highest level in suckler cows sector (65–82% of gross margin).

Model results also confirm growing importance of CAP pillar II payments, among them particularly agri-environmental support. In all three schemes observed direct payments enable farmers to improve financial results and in both reform schemes they alleviate economic impacts of CAP reform.

POVZETEK

Cilj raziskovalne naloge je bil razviti orodje za optimiranje proizvodne usmeritve na podlagi skupno doseženega pokritja na konkretnem kmetijskem gospodarstvu. V ta namen smo uporabili pristop linearnega programiranja. S pomočjo rezultatov razvitega orodja smo poizkušali odgovarjati na vprašanja konkretnih kmetijskih gospodarstev, še zlasti z vidika reforme skupne kmetijske politike v letu 2003, ki bo v Sloveniji izpeljana v letu 2007.

Model smo razvili v Excelu, ki že v osnovni različici vključuje makro za reševanje linearnih problemov. Orodje je zasnovano tako, da je na preprost način možno spreminjati nabor vključenih aktivnosti in pogoje ter na ta način prilagajati matriko analiziranemu problemu, za katerega z linearnim modelom iščemo optimalno rešitev. Glavnino podatkov in tehnoloških parametrov smo povzeli po Katalogu kalkulacij (Jerič, 2001). Osredotočili smo se na tiste živinorejske sektorje, znotraj katerih po izvedeni reformi SKP pričakujemo najbolj drastične spremembe. Poleg neposrednih plačil smo v modelu zajeli tudi izravnalna plačila iz drugega stebra SKP (OMD in SKOP).

Razviti model smo testirali na hipotetičnem kmetijskem gospodarstvu. Predpostavili smo, da leži v gričevnatem predelu Slovenije in razpolaga z 1,6 PDM (polno vrednih delovnih moči), ki so na voljo za gospodarjenje na 15 ha površin – od teh je 2/3 travnikov, preostali del pa njive. Analizirali smo različne proizvodne načrte, ki se v grobem razlikujejo v različnih proizvodnih možnostih. Z vidika živinoreje jih lahko razdelimo na specializirane in mešane. V tem prispevku smo se omejili le na proizvodne načrte specializacije. Obravnavali smo šest tipov specializacije: prirejo mleka, prirejo govejega mesa, pitanje telet, rejo krav dojilj ter rejo drobnice za meso in mleko.

Poleg obsega vključenih aktivnostih smo spremljali vrednost in strukturo doseženega pokritja. Vse proizvodne načrte smo analizirali v pogojih različnih ukrepov, ki jih ponuja SKP pred in po reformi. Glede na dovoljene obremenitve površin z organskim gnojem in aktualne ukrepe neposrednih in izravnalnih plačil smo jih združili v osem scenarijev kmetijske politike (SSOS, SSSKOP, SSSEKP, KP0, RK, RKSKOP, RR in RRSKOP), pri čemer je scenarij KP0 povsem hipotetičen, saj predvideva ukinitev vseh proračunskih plačil. Z njim smo testirali, kako bi se spremenila optimalna rešitev, če bi se država umaknila iz kmetijskega sektorja in bi hkrati prenehale vse pravne omejitve, znotraj katerih so kmetje dolžni gospodariti. S scenarijsko analizo smo skušali odgovoriti na vprašanje, kakšna je optimalna rešitev pri različnih shemah SKP, ki se spreminja in tako ugotoviti, kaj prinaša njena postopna liberalizacija na konkretnih kmetijskih gospodarstvih.

Dobljeni rezultati veljajo le za analizirano kmetijo, ugotovljene spremembe pa se da v veliki meri posplošiti. Vseeno bi pri drugačnih pogojih (zlasti z vidika dosežene intenzivnosti in osnovnih proizvodnih virov) lahko dobili povsem drugačne rešitve, a bi te po vsej verjetnosti kazale podobne zakonitosti.

Izmed vseh analiziranih tipov se je kot najzanimivejša izkazala reja krav molznic, kjer po reformi SKP lahko pričakujemo celo rahlo izboljšanje doseženega pokritja (predvsem na račun predvidene cenejše mlečne kvote, kar je posledica napovedane ukinitve kvotnega sistema). Nasprotno smo potrdili, da pri pitanju bikov lahko pričakujemo poslabšanje rezultatov. Ta usmeritev sicer ni najbolj primerna za analizirano kmetijo, saj razmerje med njivskimi in travnatimi površinami zanjo ni ugodno. V primeru, da imamo možnost zaposlitve v izven-kmetijski dejavnosti, se reja krav dojilj izkaže kot zanimiva alternativa. Specializacija kmetije v pitanje telet ne bi bila najboljša odločitev, ekonomski rezultati pa se z letošnjo reformo še poslabšajo. Specializirana reja drobnice za prirejo mleka in predelavo tega na kmetij bi bila zanimiva v primeru možnosti najema cenene, a zanesljive delovne sile. Za prirejo jagnjet za meso velja obratno, saj bi bila zanimiva le pri manjši zaposlitvi delovne sile za delo na kmetiji.

Zanimal nas je tudi pomen proračunskih plačil v doseženem - optimalnem - rezultatu gospodarjenja. Deleži se med posameznimi sektorji zelo razlikujejo. Pri standardni shemi neposrednih plačil se gibljejo med 23 in 73 % skupnega pokritja, odvisno tudi od tega, ali kmetija uveljavlja plačila tudi iz drugega stebra SKP. Finančni rezultat se pri obeh reformnih shemah - z izjemo specializacije v prirejo mleka pri kombinirani shemi - večinoma močno poslabša, delež proračunskih plačil v doseženem pokritju pa je bolj stabilen. Pri kombinirani shemi se ti deleži gibljejo med 26 in 60 %, pri specializirani reji krav dojilj celo do 82 %. Ob bistveno slabšem ekonomskem rezultatu večine specializacij pri regionalni shemi se delež proračunskih plačil v pokritju tudi pri tej shemi ohranja ali pade le za nekaj odstotkov.

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http://aas.bf.uni-lj.si

UDK 57/63 ISSN 1581-9175

Acta agriculturae Slovenica

Letnik 90 Ljubljana, november 2007 Številka 1

SUBJECT INDEX BY AGROVOC DESCRIPTORS

PREDMETNO KAZALO PO DESKRIPTORJIH AGROVOC

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Acta agriculturae Slovenica, 90(november 2007)1, 59.

http://aas.bf.uni-lj.si

UDK 57/63 ISSN 1581-9175

Acta agriculturae Slovenica

Letnik 90 Ljubljana, november 2007 Številka 1

SUBJECT INDEX BY AGRIS CATEGORY CODES

VSEBINSKO KAZALO PO PREDMETNIH KATEGORIJAH AGRIS

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Acta agriculturae Slovenica, 90(november 2007)1, 61.

http://aas.bf.uni-lj.si

UDK 57/63 ISSN 1581-9175

Acta agriculturae Slovenica

Letnik 90 Ljubljana, november 2007 Številka 1

ABECEDNO KAZALO AVTORJEV

AUTHOR'S INDEX

Acta agriculturae Slovenica, 90(november 2007)1, 63–64.

http://aas.bf.uni-lj.si

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Sprejemamo izvirne znanstvene članke, predhodne objave in raziskovalne notice s področja zootehnike (genetika, mikrobiologija, imunologija, prehrana, fiziologija, ekologija, etologija, mlekarstvo, ekonomika, živalska proizvodnja in predelava živalskih proizvodov, tehnologija in dokumentalistika) v slovenskem in angleškem jeziku, znanstveno pregledne članke samo po poprejšnjem dogovoru. Objavljamo tudi prispevke, podane na simpozijih, ki niso bili v celoti objavljeni v zborniku simpozija. Če je prispevek del diplomskega, magistrskega ali doktorskega dela, navedemo to in tudi mentorja na dnu prve strani. Navedbe morajo biti v slovenskem in angleškem jeziku.

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Hvelplund, T. Protein evaluation of treated straws. V: Evaluation of straws in ruminant feeding (ur.: Chenost, M./ Reiniger, A.). London, Elsevier Applied Science, 1989, 66–74.

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Acta agriculturae Slovenica, 90(november 2007)1, 65–66.

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Fliegerová, K./ Pažoutová, S./ Hodrová, B. Molecular genotyping of rumen fungi based on RFLP analysis. Zb. Bioteh. Fak. Univ. Ljubl., Kmet. Zooteh., 72(1998), 95–98.

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Proof Reading	Vanda ŠUŠTERŠIČ
Printed by	ROTOSI d.o.o., Tomačevo 19, SI-1000 Ljubljana, Slovenia, in 450 copies
Address of Editor	Groblje 3, SI-1230 Domžale, Slovenia, Tel.: +386 1 7217 800, Telefaks: +386 1 7241 005
E-mail	peter.dovc@bfro.uni-lj.si
Home page	http://aas.bf.uni-lj.si/index-en.htm
Annual subscription	25,04 EUR, for foreign countries 30 EUR
Individual issue	16,69 EUR, for foreign countries 20 EUR
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