ISSN 1581-9175

Acta agriculturae Slovenica

Letnik 88, številka 1
Volume 88**,** Number 1

88-1	*Acta agriculturae Slovenica*	str. 1–62	Ljubljana, november 2006

Acta agriculturae Slovenica, **88(november 2006)1**

Acta agriculturae Slovenica

Izdaja	Biotehniška fakulteta Univerze v Ljubljani, Jamnikarjeva 101, SI-1111 Ljubljana. Letno izhajata dva letnika vsak z dvema številkama.
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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 **Domača stran**	peter.dovc@bfro.uni-lj.si http://aas.bf.uni-lj.si/
Letna naročnina **Posamezna številka**	6 000 SIT (25,04 EUR)*, za tujino 30 EUR 4 000 SIT (16,69 EUR)*, za tujino 20 EUR
Imetnik računa **Banka** **Račun**	UL, Biotehniška fakulteta, Jamnikarjeva 101, SI-1111 Ljubljana BANKA SLOVENIJE, Slovenska 35, SI-1505 Ljubljana 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

* Za izračun cen v EUR je uporabljen centralni paritetni tečaj, ki znaša 1,00 EUR = 239,640 SIT.

Acta agriculturae Slovenica, 88(november 2006)1, 1–2.

UDK 636/641 ISSN 1581-9175

Acta agriculturae Slovenica

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

Agris category codes: L53 COBISS Code 1.01

ABSOLUTNA IN RELATIVNA PLODNOST, MASA IKER TER VPLIV MASE IKER NA MASO LARV IN ZARODA PRI KALIFORNIJSKI POSTRVI *(Onchorynchus mykiss)* V PRVI IN DRUGI DRSTI

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Delo je prispelo 25. novembra 2005, sprejeto 13. junija 2006.
Received November 25, 2005, accepted June 13, 2006.

IZVLEČEK

Od kalifornijskih postrvi iste plemenske jate smo pridobili ikre v prvi in drugi drsti. Za vsako samico smo pri vsaki od drsti določili maso telesa (M_{Plm}) ter absolutno plodnost (P_{Abs}). Na podlagi teh podatkov smo izračunali relativno plodnost (P_{Rel}). Od vsake plemenke smo individualno stehtali 50 iker v fazi iker z očmi, ter tri vzorce po 50 potomcev v različnih fazah rasti. Potomce smo tehtali 11 dni po izvalitvi (T1), nato pa še 6 tednov (T2) ter 10 tednov po izvalitvi (T3). Na ta način smo dobili podatke o povprečni masi iker (M_{Iker}) ter podatke o masah potomcev pri tehtanju T1, T2 in T3 (M_{Rib1}, M_{Rib2} in M_{Rib3}). Na podlagi teh podatkov smo s statističnim modelom parcialne regresije, ki je kot neodvisni spremenljivki vključeval relativno plodnost in povprečno maso iker, ocenili vpliv M_{Iker} na M_{Rib1}, M_{Rib2} in M_{Rib3}. Rezultati kažejo, da se iz težjih iker izvalijo težje larve. Vpliv M_{Iker} je relativno kratek, saj parcialni regresijski koeficient M_{Iker} na M_{Rib3} ni statistično značilen ne pri ikrah iz prve ne pri ikrah iz druge drsti. Rezultati kažejo, da je za produkcijo smiselno uporabljati tudi ikre iz prvi drsti, čeprav so majhne. Deset tednov po izvalitvi se povprečna masa rib, izvaljenih iz majhnih iker, pridobljenih pri prvi drsti, ne razlikuje bistveno od povprečne mase rib, izvaljenih iz večjih iker, pridobljenih v drugi drsti.

Ključne besede: akvakultura / ribe / postrvi / kalifornijska postrv / *Onchorynchus mykiss* / reprodukcija / ikre / larve / masa

ABSOLUTE AND RELATIVE FECUNDITY, EGG WEIGT AND INFLUEBCE OF EGG WEIGHT ON BODY WEIGHT OF LARVAE AND ALEVINS IN RAINBOW TROUT *(Onchorynchus mykiss)* AT FIRST AND SECOND SPAWNING

ABSTRACT

From a certain number of females, eggs were collected during their first and second spawning. Body mass at spawning (M_{Plm}) and absolute fecundity (P_{Abs}) was registered for each female. On the basis of these data the relative fecundity was calculated (P_{Rel}). For each female 50 eyed eggs as well as 50 offspring at different stages of growth were individually weighted. Weightings were done 11 days (T1), 6 weeks (T2) and 10 weeks (T3) after hatching. By such procedure mean weight of the eggs (M_{Iker}) and the mean body mass of alevins attained at T1, T2 in T3 (M_{Rib1}, M_{Rib2} and M_{Rib3}) were obtained. The statistical model of partial regression including P_{Rel} and M_{Iker} as independent variables was used to evaluate the influence of M_{Iker} on M_{Rib1}, M_{Rib2} and M_{Rib3}. Results indicate that from heavier eggs heavier larvae are hatched. Nevertheless, the influence of egg weight on body mass is only short termed. Partial regression coefficient of M_{Iker} on M_{Rib3} is not statistically significant regardless whether relation between eggs and alevins at first or second spawning is observed. According to our results it is suggested that eggs collected

at first spawning should not be discarded even they appear to be too small for production. At age of 10 weeks after hatching, average size of alevins hatched from small eggs collected at first spawning did not to essentially differ from average size of alevins hatched from large eggs collected at second spawning

Key words: aquaculture / fish / trouts / rainbow trout / *Onchorynchus mykiss* / reproduction / eggs / size / larvae / mass

UVOD

Od samic kalifornijskih postrvi lahko pridobimo ikre s postopkom, ki ga imenujemo smukanje, samo enkrat na leto. V večini primerov lahko samice prvič osmukamo že pri starosti dveh let. Ikre, pridobljene od prvesnic, so manjše in lažje od iker, ki jih dobimo od starejših živali. Na splošno velja, naj v proizvodnih pogojih, iker, osmukanih od prvesnic, ne bi uporabljali. (Skalin, 1993). Ker ikre od prvesnic zavržemo, se stroški za reprodukcijo povečajo. Če izvajamo selekcijo, je zaradi tega letni genetski napredek manjši, saj se generacijski interval podaljša z dveh na tri leta.

Podatki o tem, v kakšni meri velikost oziroma masa iker vpliva na velikost oziroma maso, ki jo ribe, izvaljene iz teh iker, dosežejo v kasnejših fazah rasti, so v literaturi redki in se med različnimi avtorji razlikujejo. V literaturi nismo našli podatkov, ki bi poročali o spremembi povprečne mase iker iste plemenske jate v zaporednih drsteh. Prav tako nismo našli podatkov o tem ali se pri istih plemenkah med prvo in drugo drstjo spremeni vpliv mase iker na kasneje dosežene teže rib.

PREGLED LITERATURE

Absolutna plodnost, merjena kot število iker, ki jih osmukamo od posamezne živali, se pri salmonidih povečuje s starostjo in velikostjo (maso) plemenk. S starostjo in velikostjo plemenk naj bi se povečala tudi povprečna velikost oziroma masa iker, kar je bilo ugotovljeno tako za kalifornijsko postrv (Bromage in Camaratung, 1988; Bromage in sod., 1992) kot za potočno postrv (Ojanguren in sod., 1996) pa tudi za atlantskega lososa (Thorpe in sod, 1984; Heinimaa in Heinimaa, 2004). Potrebno je poudariti, da sta Heinimaa in Heinimaa (2004) ugotovila, da je absolutna plodnost odvisna od mase plemenk, vpliv velikosti plemk na povprečno velikost iker pa je bil zelo majhen. Do podobnih ugotovitev je pri kalifornijski postrvi prišel tudi Kortoglu s sedelavci (1998). Barnes s sod. (2000) pri eni izmed vrst tihoocenskaga lososa (*Ocorhynchus tshawytscha*) ni našel povezave med velikostjo plemk in velikostjo osmukanih iker.

Glede na to, da imajo ribe t.i. nedeterminirano rast (Weatherley in Rogers, 1978), so lahko prvesnice v času drsti enako velike in težke kot ribe, ki se drstijo drugič ali celo tretjič. Tako je zelo težko ločiti vpliv mase plemenk na povprečno maso iker od vpliva starosti oziroma zaporedne drsti.

Springate in Bromage (1984) zagovarjata stališče, da je pri kalifornijskih postrveh s stališča ekonomike reprodukcije bolj smiselno uporabljati za pridobivanje iker manjše in mlajše ribe, ker se relativna plodnost, merjena kot število iker na enoto telesne mase, s starostjo in velikostjo rib zmanjšuje. V nasprotju s tema avtorjema Estay in sodelavci (1994) niso našli povezave med telesno maso in relativno plodnostjo.

Berg in sod. (2001) pri atlantskem lososu ugotavljajo, da se večina variabilnosti v velikosti iker, ki jo opazimo v populaciji, pojavlja kot komponenta variance med živalmi in le manjši delež kot komponenta variance znotraj živali. To pomeni, da so ikre, ki jih dobimo od posamezne samice, zelo izenačene.

Avtorji za različne salmonidne vrste navajajo, da se iz večjih iker izvalijo večje larve (Pitman, 1997; Springate in Bromage, 1985; Ojanguren in sod. 1996; Heath in sod., 1999; Berg in sod.,

2001; Pakkasmaa in Jones, 2002; Einum, 2003; Bascinar in Okumus 2004). Mnenja o tem kdaj ta vpliv izgine, so različna. Zelo redki so podatki o statističnih parametrih, s katerimi bi lahko kvantitativno vrednotili vpliv mase iker na težo rib, izvaljenih iz teh iker, ki jo živali dosežejo pri določeni starosti.

MATERIAL IN METODE DELA

Material

V poskusu smo proučevali vpliv mase iker na maso larv ter zaroda izvaljenega iz teh iker pri isti populaciji rib, osmukani v dveh zaporednih drsteh. Poskus je potekal na ribogojnici Pšata, ki jo napaja izvirska voda s stalno temperaturo 10 ºC. Oskrba poskusnih živali je bila enaka standardni oskrbi plemenskih živali.

Ob prvi drsti so bile ribe stare dve leti. Osmukali smo 145 prvesnic. Po smukanju smo ribe individualno označili in jih vzrejali v istem bazenu skupaj z drugimi plemenskimi živalmi. Leto dni kasneje, ko so bile ribe stare tri leta, smo lahko na osnovi oznak zanesljivo identificirali 33 rib, ki smo jim določili proučevane parametre že prejšnje leto. Te ribe smo osmukali in pri njih določili iste parametre kot pri prvem smukanju.

Poskusne živali smo v času drsti tedensko pregledovali, da bi ugotovili, pri katerih živalih je že nastopila ovulacija. Vse ikre vsake od samic, pri kateri je že nastopila ovulacija, smo z abdominalno masažo iztisnili iz trebušne votline v posodo, kjer smo jih oplodili z mešano spermo večjega števila samcev. Vsaki osmukani samici smo po smukanju individualno določili telesno maso (M_{Plm}). Po oploditvi in nabrekanju smo prešteli ikre, ki smo jih dobili od vsake samice, in določili absolutno plodnost vsake plemenke (P_{Abs}). Na osnovi mase plemenke in absolutne plodnosti smo izračunali tudi relativno plodnost (P_{Rel}) kot število iker na 100 g telesne mase. Od vsake samice smo naključno odbrali 400 iker in jih ločeno inkubirali v kalifornijskih valilnikih. Iz njih izvaljene larve in zarod smo ločeno vzrejali v individualnih bazenih.

Ko so ikre dosegle fazo razvoja, ki jo poznamo kot »fazo z očmi«, smo izmed iker vsake od samic naključno odbrali 50 iker, jih individualno stehtali in izračunali povprečno maso ikre vsake od plemenk (M_{Iker}). Na 11. dan po izvalitvi, 6 tednov po izvalitvi in 10 tednov po izvalitvi smo iz vsake skupine naključno odbrali vzorec 50 rib in vsak osebek individualno stehtali. Povprečno maso rib, izračunano za prvo tehtanje (T1), drugo tehtanje (T2) in tretje tehtanje (T3), smo označili kot M_{Rib1}, M_{Rib2} in M_{Rib3}.

Odrasle živali smo tehtali na mehanični tehtnici; v prvem letu do 5 gramov, v drugem pa do 10 gramov natančno. Ikre, larve in zarod smo tehtali na elektronski laboratorijski tehtnici do tisočine grama natančno.

Statistične metode

Za statistično vrednotenje podatkov o vplivu mase iker na maso larv in zaroda smo uporabili naslednji model:

$$y_i = \mu + b_1(x_i - \bar{x}) + b_2(z_i - \bar{z}) + e_i$$

kjer je:

y_i – povprečna masa osebkov, izvaljenih iz iker i-te plemenke, dosežena pri T1, T2 in T3

μ – srednja vrednost

x_i – relativna plodnost i-te plemenke

$\bar{x}$ – povprečna relativna plodnost

z_i – povprečna masa iker i-te plemenke

$\bar{z}$ – povprečna masa vseh iker

b_1 – parcialni regresijski koeficient povprečne mase rib, dosežene pri T1, T2 in T3, na relativno plodnost

b_2 – parcialni regresijski koeficient povprečne mase rib, dosežene pri T1, T2 in T3, na maso iker

e_i – ostanek

Enostavne korelacijske koeficiente med M_{Iker}, in M_{Rib1}, M_{Rib2} , M_{Rib3} smo izračunali po naslednji formuli:

$$r_{xy} = \frac{\mathrm{cov}_{yx}}{\sigma_x \cdot \sigma_y}$$

kjer je:

r_{xy} – korelacijski koeficient med paroma spremenljivk (M_{Iker} in M_{Rib1}, M_{Rib2}, M_{Rib3})

cov_{xy} – kovarianca med paroma spremenljivk (M_{Iker} in M_{Rib1}, M_{Rib2}, M_{Rib3})

σ_{xy} – standardni odklon prve spremenljivke (M_{Iker})

σ_{xy} – standardni odklon druge spremenljivke (M_{Rib1}, M_{Rib2} in M_{Rib3})

REZULTATI IN DISKUSIJA

V preglednici 1 so prikazani osnovni statistični parametri za lastnosti, ki smo jih merili pri prvesnicah, in sicer: telesna masa, absolutna plodnost, relativna plodnost, masa iker v fazi »z očmi« in masa larv in zaroda na enajsti, dvainštirideseti in sedemdeseti dan po izvalitvi.
Enaki statistični parametri izmerjeni pri ribah pri drugi drsti, so prikazani v preglednici 2.

Preglednica 1. Osnovni statistični parametri za maso plemenk (M_{Plm}), absolutno plodnost (P_{Abs}), relativno plodnost (P_{Rel}), povprečno maso iker (M_{Iker}) ter povprečno maso rib pri prvem, drugem in tretjem tehtanju (M_{Rib1}, M_{Rib2}, M_{Rib3}) pri prvem smukanju

Table 1. Basic statistical parameters for body weight of females (M_{Plm}), absolute fecundity (P_{Abs}), relative fecundity (P_{Rel}), average weight of eyed eggs (M_{Iker}) and weight of alevins at first, second and third weighting (M_{Rib1}, M_{Rib2}, M_{Rib3}) at first spawning

Spremenljivka Variable	Število skupin Number of groups	Srednja vrednost Mean value	Standardni odklon Standard deviation	Minimalna vrednost Minimal value	Maksimalna vrednost Maximal value	Koeficient variabilnosti, % Coefficient of variation, %
M_{Plm}, g	145	1134,31	165,99	785	1540	14,63
P_{Abs}	138	2745,73	597,66	1497	4614	21,77
P_{Rel}	138	244,64	56,22	134,29	405,98	22,98
M_{Iker}, mg	88	42	4	30	52	11,20
M_{Rib1}, mg	81	64	7	43	82	11,88
M_{Rib2}, mg	45	214	31	143	291	14,72
M_{Rib3}, mg	36	562	87	366	742	15,49

Podatki iz preglednice 1 kažejo, da se je število živali, pri katerih smo lahko opravili določene meritve, zmanjševalo. Če smo lahko določili število iker pri 138 prvesnicah, je bilo mogoče določiti povprečno maso iker samo pri 88 živalih, maso zaroda pri starosti sedemdeset dni pa le

pri 36 živalih. Takšno zmanjševanje je posledica smrtnosti skupin, ki se je pojavila med poskusom.

Preglednica 2. Osnovni statistični parametri za maso plemenk (M_{Plm}), absolutno plodnost (P_{Abs}), relativno plodnost (P_{Rel}), povprečno maso iker (M_{Iker}) ter povprečno maso rib pri prvem, drugem in tretjem tehtanju (M_{Rib1}, M_{Rib2}, M_{Rib3}) pri drugem smukanju

Table 2. Basic statistical parameters for body weight of females (M_{Plm}), absolute fecundity (P_{Abs}), relative fecundity (P_{Rel}), average weight of eyed eggs (M_{Iker}) and weight of alevins at first, second and third weighting (M_{Rib1}, M_{Rib2}, M_{Rib3}) at second spawning

Spremenljivka Variable	Število skupin Number of groups	Srednja vrednost Mean value	Standardni odklon Standard deviation	Minimalna vrednost Minimal value	Maksimalna vrednost Maximal value	Koeficient variabilnosti, % Coefficient of variation, %
M_{Plm}, g	33	2730,45	587,00	1170	4010	21,49
P_{Abs}	32	4916,61	1306,30	3020	8835	26,57
P_{Rel}	32	182,73	39,85	166,15	289,67	21,93
M_{Iker}, mg	30	72	9	56	99	13,40
M_{Rib1}, mg	30	100	14	68	129	14,15
M_{Rib2}, mg	27	281	51	194	385	18,23
M_{Rib3}, mg	25	644	176	296	946	27,14

Pri drugi drsti smo lahko proučili parametre pri manjšem številu rib, ker so v času od prve do druge drsti nekatere ribe poginile, nekatere pa so izgubile značko.

Do največjega zmanjšanja skupin je prišlo pri prvesnicah v času od drsti do faze iker z očmi, ko se je število skupin zmanjšalo od 138 na 88, pa tudi v času med enajstim in dvainštiridesetim dnevom po izvalitvi, ko se je število skupin zmanjšalo od 81 na 45. Prvo zmanjšanje lahko pripišemo nizki stopnji oploditve iker v posameznih skupinah, drugo pa nesposobnosti sprejemanja eksogene hrane.

Pri ribah v drugi drsti je zmanjšanje števila skupin v posameznih obdobjih bistveno manjše. Na podlagi tega podatka bi lahko sklepali, da je oploditvena sposobnost iker pridobljenih v drugi drsti večja, prav tako je mogoče zaključevati tudi, da se je pri manjšem odstotku skupin kot pri prvi drsti ob prehodu na eksogeno hrano pojavila visoka smrtnost pri določenih skupinah. Manjšo smrtnost pri prehodu na eksogeno hrano bi lahko razložili z dejstvom, da so bile ikre, pridobljene pri drugi drsti, večje kot v prvi. Heath in sod., (1999) namreč ugotavljajo, da je preživitvena sposobnost v zgodnjih fazah rasti pozitivno povezana z velikostjo iker.

Primerjava povprečnih mas rib ob prvi in drugi drsti kaže, da se je v enem letu masa rib več kot podvojila, kar pa seveda ne velja za število iker. Relativna plodnost je bila po teh podatkih v drugem letu nižja in je znašala 182 iker na 100 g telesne mase, medtem ko je pri prvesnicah znašala relativna plodnost 245 iker na 100 g telesne mase. Naši rezultati so v skladu s podatki iz literature. Tako Kurtoglu in sod. (1998) navajajo, da je povprečna relativna plodnost v populaciji kalifornijskih postrvi, ki so jo proučevali, znašala 1364 iker na kg telesne mase, Springate in Bromage (1984), pa ugotavljata, da imajo večje ribe manjšo relativno plodnost.

Masa iker v fazi z očmi je pri drugi drsti za več kot 70 % večja kot masa iker, dobljenih od iste populacije rib pri prvi drsti. Seveda ni mogoče trditi, da gre za iste ribe, saj je število rib v drugi drsti le vzorec rib, ki smo jih proučevali, ko so bile prvesnice. Vendar ni nobenega razloga, da ne bi predpostavljali, da so ribe, ki smo jih proučevali pri drugi drsti, naključen vzorec rib, ki smo jih proučevali pri prvi. Da gre dejansko za naključni vzorec, vsaj glede mase rib in povprečne mase iker, bi lahko sklepali na podlagi dejstva, da se koeficient variabilnosti za obe

lastnosti pri populaciji rib pri drugi drsti ni zmanjšal glede na koeficient variabilnosti za ti dve lastnosti pri prvesnicah.

Direktna primerjava podatkov o masi larv in zaroda iz našega poskusa s podatki drugih avtorjev ni mogoča, ker mase nismo določali ob enaki starosti kot drugi avtorji. Še najbolj ustrezna je primerjava naših podatkov s podatki, ki jih navajajo Kurtuglu in sod. (1998). Avtorji pišejo, da so njihove larve 40 dni po izvalitvi tehtale v povprečju 305 mg (+/– 56 mg), medtem ko smo v našem poskusu ugotovili, da so 42 dni po izvalitvi tehtale larve, ki so se izvalile iz iker prvesnic, 214 mg, larve, izvaljene iz iker, dobljenih v drugi drsti, pa 281 mg.

Iz primerjave podatkov, prikazanih v preglednici 1 in preglednici 2, lahko ugotovimo, da se razlika v masi larv in zaroda, izvaljenega iz iker, pridobljenih iz iker rib iz iste populacije v prvi in drugi drsti, s časom zmanjšuje. Razlika v povprečni masi zaroda enajst dni po izvalitvi znaša 36 mg, kar pomeni, da je pri tej starosti zarod, izvaljen iz iker druge drsti, več kot 50 odstotkov težji od zaroda, izvaljenega iz iker prve drsti. Ta razlika znaša 10 tednov po izvalitvi 78 mg, kar pomeni, da je zarod, izvaljen iz iker druge drsti, manj kot 15 odstotkov težji od zaroda, izvaljenega iz iker prve drsti.

Ta podatek že nakazuje, da se vpliv velikosti oziroma mase iker na maso larv in zaroda s časom zmanjšuje.

Podatke o vplivu mase iker na maso larv in zaroda na enajsti dan ter štiri in deset tednov po izvalitvi, izračunane po statističnem modelu prikazanem v prejšnjem poglavju, prikazujemo za prvo drst v preglednici 3, v preglednici 4 pa za drugo drst.

Preglednica 3. Srednja vrednost (μ) in parcialni regresijski koeficienti relativne plodnosti (P_{Rel}) in povprečne mase iker (M_{Iker}) pri prvem smukanju (b_1, b_2) na povprečno maso (mg) rib pri prvem, drugem in tretjem tehtanju (M_{Rib1}, M_{Rib2}, M_{Rib3})

Table 3. Mean value (μ) and partial regression coefficient of relative fecundity (P_{Rel}) and average weight of eyed eggs (M_{Iker}) at first spawning (b_1, b_2) on weight (mg) of alevins at first, second and third weighting (M_{Rib1}, M_{Rib2}, M_{Rib3})

Lastnost Trait	Statistični parametri Statistical parameters		
	μ, mg	b_1	b_2
M_{Rib1}	65,155	–0,011	1,313*
M_{Rib2}	212,881	0,168	3,063*
M_{Rib3}	558,789	0,411	4,849

* – $p < 0,0001$

Preglednica 4. Srednja vrednost (μ) in parcialni regresijski koeficienti relativne plodnosti (P_{Rel}) in povprečne mase iker (M_{Iker}) pri drugem smukanju (b_1, b_2) na povprečno maso (mg) rib pri prvem, drugem in tretjem tehtanju (M_{Rib1}, M_{Rib2}, M_{Rib3})

Table 4. Mean value (u) and partial regression coefficient of relative fecundity (P_{Rel}) and average weight of eyed eggs (M_{Iker}) at second spawning (b_1, b_2) on weight (mg) of alevins at first, second and third weighting (M_{Rib1}, M_{Rib2}, M_{Rib3})

Lastnost Trait	Statistični parametri Statistical parameters		
	μ, mg	b_1	b_2
M_{Rib1}	100,657	–0,062	1,236*
M_{Rib2}	281,394	0,215	0,711
M_{Rib3}	558,789	0,407	–3,723

* – $p < 0,0001$

Kot je razvidno iz preglednice 3 in preglednice 4, na maso larv in zaroda, doseženo v različnih obdobjih po izvalitvi, ne vpliva relativna plodnost plemenk, saj se parcialni regresijski koeficienti, ki merijo, koliko se spremeni povprečna masa larv, če se spremeni relativna plodnost statistično značilno ne razlikujejo od nič. Povprečna masa iker pa vpliva na povprečno maso larv enajst dni po izvalitvi pri prvi in drugi drsti. Oba parcialna regresijska koeficienta se statistično značilno razlikujeta od nič. Parcialni regresijski koeficient povprečne mase iker na povprečno maso rib 6 tednov po izvalitvi se statistično značilno razlikuje od 0 pri prvi drsti in znaša 3,063, pri drugi drsti pa se vpliv mase iker na maso zaroda pri starosti štirih tednov izgubi. Na maso rib, doseženo enajst tednov po izvalitvi, povprečna masa iker ne vpliva več.

Povezava med povprečno maso iker in maso rib v kasnejših obdobjih rasti prikazujemo v preglednici 5, z enostavnimi korelacijskimi koeficienti.

Preglednica 5. Enostavni korelacijski koeficienti med povprečno maso iker (M_{Iker}) in maso rib doseženo pri prvem drugem in tretjem tehtanju (M_{Rib1}, M_{Rib2}, M_{Rib3})

Table 5. Simple correlation coefficients between weight of eyed eggs (M_{Iker}) and weight of alevins at first, second and third weighting (M_{Rib1}, M_{Rib2}, M_{Rib3})

	Korelacijski koeficient / correlation coefficient		
	M_{Iker} x M_{Rib1}	M_{Iker} x M_{Rib2}	M_{Iker} x M_{Rib3}
Prva drst First spawning	0,8679	0,5071	0,2789
Druga drst Second spawning	0,8863	0,1015	0,2865

Podatki kažejo, da obstaja zelo močna povezava med maso larv, doseženo 11 dni po izvalitvi, in maso iker. Povezava med maso zaroda 10 tednov po izvalitvi in maso iker pa je zelo šibka. To velja tako za prvo kot tudi za drugo drst. V obeh premerih je korelacija med M_{Iker} x M_{Rib1} statistično značilno različna od nič, korelacija med M_{Iker} x M_{Rib3} pa se ne razlikuje statistično značilno od vrednosti 0.

Rezultati kažejo, da je povezava med velikostjo iker in maso iz teh iker izvaljenega zaroda na začetku rasti visoka in predstavlja tipičen maternalni vpliv. Ta maternalni vpliv pa se zelo hitro izgubi. Dva meseca po izvalitvi masa zaroda ni več pod vplivom velikosti iker, iz katerih so se ribe izvalile. Pri živalih, ki imajo tako imenovano determinirano rast, kar pomeni, da ne rastejo celo življenje, pač pa dosežejo končno velikost, je povsem razumljivo, da se vpliv »rojstne« teže na težo v kasnejših obdobjih izgubi. V to skupino spadajo praktično vse domače živali. Pri postrveh, za katere je značilno, da imajo nedeterminirano rast, pa bi lahko razlika v »rojstni« teži, ki bi nastala zaradi tega, ker so se določene ribe izvalile iz večjih iker, pomenila, da se bo ta razlika v času rasti še povečevala. Vendar naši rezultati govorijo prav nasprotno.

ZAKLJUČKI

Naši rezultati kažejo, da je nesmiselno za produkcijske namene ikre prvesnic zavreči. Ugotovili smo namreč, da je bil deset tednov po izvalitvi zarod, ki se je izvalil iz iker, pridobljenih v prvem smukanju, le malo lažji kot zarod, izvaljen iz iker, pridobljenih pri drugem smukanju, čeprav so bile ikre, pridobljene pri drugem smukanju, precej večje.

Čeprav stroški za proizvodnjo iker ne predstavljajo v celotni kalkulaciji stroškov tako velikega deleža, kot je delež za reprodukcijo pri drugih domačih živalih, je kljub temu vredno uporabljati že ikre prvesnic in na ta način stroške za reprodukcijo znižati. Z uporabo iker, pridobljenih od prvesnic, bi lahko skrajšali generacijski interval pri postrveh od treh na dve leti,

torej za tretjino. To pomeni, da bi se genetski napredek lahko povečal za enak delež. Prav dolg generacijski interval pri selekciji postrvi predstavlja omejitveni dejavnik za hitrejši genetski napredek.

VIRI

Barnes, M.E./ Hanten, R.P./ Cordes, R.J./ Sayler, W.A./ Carreiro J. Reproductive performance of inland fall chinook salmon. North American journal of aquaculture, 62(2000), 203–211.

Bascinar, N./ Okumus, I. The early development of brook trout Salvelinus fontinalis (Mitchill): Survival and growth rates of alevins. Turkish journal of veterinary & Animal Science, 28(2004), 297–301.

Berg, O.K./ Hendry, A.P./ Svendsen , B./ Bech, C./ Arnekleiv, J.V./ Lohrmann, A. Maternal provisioning of offspring and the use of those resources during ontogeny: variation within and between Atlantic salmon families. Functional ecology, 15(2001), 13–23.

Bromage, N./ Camaratung, R. Eggs production in the rainbow trout. V: Recent advantage in aquaculture. (ur.: Muir, J.F./ Roberts, R.J.). London, Croom helm, Portland, Timebre Press, 1988, 63–138.

Bromage, N./ Jones, J./ Randall, C./ Thrush, M./ Davies, B./ Springate,J./ Duston, J./ Barker, G. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Onchorynchus mykiss*). Aquaculture, 100(1992), 141–166

Einum, S. Atlantic salmon growth in strongly food-limited environments: Effects of egg size and paternal phenotype? Environmental biology of fishes, 67(2003), 263–268.

Estay, F./ Diaz, N.F./ Neira, R./ Fernandez, X. Analysis of reproductive-performance of rainbow trout in a hatchery in Chile. Progressive fish culturist, 56(1994), 244–249.

Heath, D.D./ Fox, C.W./ Heath, J.W. Maternal effects on offspring size: Variation through early development of chinook salmon. Evolution, 53(1999), 1605–1611.

Heinimaa, S./ Heinimaa, P. Effect of the female size on egg quality and fecundity of the wild Atlantic salmon in the sub-arctic River Teno. Boreal Environment Research, 9(2004), 55–62.

Kurtoglu, I.Z./ Okumus, I./ Celikkale, M.S. Analysis of reproductive performance of rainbow trout (*Oncorhynchus mykiss*) broodstock in a commercial farm in Eastern Black Sea Region. Turkish journal of veterinary & Animal Science, 22(1998), 489–496.

Ojanguren, A.F./ Reyesgavilan, F.G./ Brana, F. Effect of egg size on offspring development and fitness in brown trout, *Salmo trutta* L. Aquaculture, 147(1996), 9–20.

Pakkasmaa, S./ Jones, M. Individual-level analysis of early life history traits in hatchery-reared lake trout. Journal of fish biology, 60(2002), 218–225.

Pitman, R.W. Effects of female age and egg size on growth and mortality in rainbow trout. The progressive Fish-culturist, 51(1997)4, 204–205.

Skalin, B. Ribogojstvo. Ljubljana, ČZP Kmečki glas, 1993.

Springate, J./ Bromage, N. Egg size and number – it's a »trade-off«. Fish farmer, 7(1984), 12–13.

Springate, J. R.C./ Bromage, N. R. Effects of egg size on early growth and survival in rainbow trout (*Salmo gairdneri* Richardson). Aquaculture, 47(1985), 163–172.

Thorpe, J.S./ Miles, M.S./ Keay, D.S. Development rate, fecundity and egg size in Atlantic salmon, *Salmo salar* L., Aquaculture, 42(1984), 289–305.

Weatherly, A.H./ Rogers, S.C. Some aspects of age and growth. V: Ecology of freshwater fish production. (ur.: Gerkin, S.D.). Oxford, Blackwell Publishing, 1978.

Acta agriculturae Slovenica, 88(november 2006)1, 11–18.

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

Agris category codes: L10 COBISS Code 1.01

EFFECT OF *PPARGC-1* GENE ON BACKFAT THICKNESS IN PIGS *

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Received July 27, 2006, accepted November 20, 2006.
Delo je prispelo 27. julija 2006, sprejeto 20. novembra 2006.

ABSTRACT

PPARGC-1 gene is assumed to be a candidate gene with a major effect on fatness and meat quality. In this study, frequency of T/A substitution at position 1378 in *PPARGC-1* gene in pigs was examined in boars of 4 different breeds. Frequency of genotypes and alleles were compared between animals with the thinnest and with the thickest backfat. Differences in genotype frequency between groups were significant in dam line of Slovenian Landrace (SL11) and sire line of Large White breed (LW66). Allele A was predominantly present in animals with thick backfat in SL11 (71.05%), LW66 (58%) and in sire line of Slovenian Landrace (67.65%). Differences in allele frequency were significant in SL11. We found a significant effect on phenotypic and breeding values for backfat in population of Large White breeds, where homozygotes TT had the thickest backfat, and heterozygotes the thinnest. In population of Slovenian Landrace breed the thickest backfat had homozygotes AA and the thinnest homozygotes TT. Similar results were obtained by analysis of gene effect on breeding values. Inconsistency could be explained by different background of allele, epistasis and intensity of selection.

Key words: pigs / backfat / genetics / *PPARGC-1* gene / polymorphism

POVEZAVA GENA *PPARGC-1* Z DEBELINO HRBTNE SLANINE †

IZVLEČEK

Gen za *PPARGC-1* je kandidatni gen z velikim učinkom na zamaščenost in kvaliteto mesa. V prispevku smo preučevali frekvence točkovnih mutacij A → T na mestu 1378 v 8. eksonu gena za *PPARGC-1* pri merjascih štirih različnih linij dveh pasem. Frekvence genotipov in alel smo primerjali med merjasci s tanjšo (skupina A) ter merjasci z debelejšo hrbtno slanino (DHS), (skupina B). Razlike v frekvencah genotipov so bile značilne med skupinama merjascev pri maternalni liniji pasme slovenska landrace (SL11) ter pri terminalni liniji pasme large white (LW66). Alela A je bila pogostejša pri živalih z debelejšo DHS pri SL11 (71,05 %), LW66 (58 %) ter pri terminalni liniji pasme slovenska landrace (67,65 %). Razlike v frekvencah alel so bile značilne v populaciji SL11. Genotip je značilno vplival na DHS v populacijah LW11 in LW66, kjer so imeli debelejšo slanino homozigoti TT in tanjšo heterozigoti. V populacijah SL11 in SL55 so imeli najdebelejšo slanino genotipi AA in najtanjšo slanino homozigoti TT.

* This article is part of a graduation thesis ´Effect of polymorphism T1378A in *PPARGC-1* gene on fattening and growth rate traits in pigs´, issued by Tina Flisar, supervisor prof. Peter Dovč, Ph.D.
† Prispevek je del diplomskega dela Tine Flisar z naslovom ´Vpliv polimorfizma T1378A v genu za *PPARGC-1* na pitovne lastnosti pri prašičih´, mentor prof. dr. Peter Dovč.

Nasprotujoči rezultati so lahko posledica genetskega ozadja alel, epistaze in intenzivnosti selekcije.

Ključne besede: prašiči / debelina hrbtne slanine / genetika / gen *PPARGC-1* / polimorfizem

INTRODUCTION

Although most genetic progress in pigs has been made by selection on predictor of breeding values, information, acquired from molecular genetics, can clarify some genetic factors with effect on production traits. Due to quantitative nature of production traits, the majority of genetics is oriented to identification and mapping quantitative trait locus (QTL) with effect on economically important traits. One of the most important traits is also meatiness, because in Slovenia the carcasses are still paid regarding the lean meat content.

Growth of adipose tissue is a quantitative trait regulated by many transcription factors, included in the process of adaptive thermogenesis and biosynthesis of mitochondria (Rosen *et al.*, 2000). One of them is protein *PPARGC-1* (peroxisome proliferator-activated receptor-gamma coactivator-1), which is a transcriptional coactivator of the nuclear receptor PPARγ (peroxisome proliferator-activated receptor-gamma). It is an important factor in the process of adipocyte differentiation and muscle fibre type determination (Lin *et al.*, 2002; Rosen *et al.*, 2000). *PPARGC-1* stimulates mitochondrial biogenesis and respiration in muscle cells. It induces activity of the genes included in adaptation of organism on temperature and nutritional changes (Knutti and Kralli, 2001; Puigserver *et al.*, 1998). The mechanism of adipogenesis regulation and the role of PPARγ and *PPARGC-1* were also described by Milosevic Berlic *et al.* (2004).

The porcine *PPARGC-1* gene (GenBank acc. no. AY346131) is located on chromosome 8 (Milosevic Berlic, 2002; Jacobs *et al.*, 2006). The sequencing, polymorphisms analysis and mapping of porcine *PPARGC-1* gene were reported by Jacobs *et al.* (2006). Substitution T/A at nucleotide position 1378 causes amino acid substitution (Cys→Ser) at position 430. According to Jacobs *et al.* (2006) Cys/Ser substitution could have major effects because of influences on disulfide bridges that might be present in the protein.

Frequency distribution of mutation A1378T in *PPARGC-1* was studied in Chinese and Western pig breeds because of remarkable differences in fatness (Kunej *et al.*, 2005). Allele T was associated with fat type Chinese pig breeds, whereas the A allele was more frequent in lean type Western pigs (Kunej *et al.*, 2005). Jacobs *et al.* (2006) studied the *PPARGC-1* gene effect on performance in Meishan x White Composite resource population and the study did not reveal the effect of A1378T on performance. They found a significant correlation between SNP in exon 9 (A1747C) and leaf fat weight, backfat, and weight of the belly. Several QTLs with effect on backfat, located on chromosome 8 were reported (Bidanel and Rothschild, 2002; Rohrer, 2000; Bidanel *et al.*, 2001). Some of these regions correspond to the location of *PPARGC-1*. *PPARGC-1* is assumed to be a candidate gene with a major effect on backfat and other characteristic associated with fatness. This gene can be also considered as a candidate gene for meat quality (Knutti and Kralli, 2001).

The purpose of this study was to find the frequency of mutation of *PPARGC-1* gene in boars of different phenotype for backfat. The effect of *PPARGC-1* gene on phenotypic and breeding values was analyzed using a linear model.

MATERIAL AND METHODS

Data Preparation

In this study, boars of dam and sire line of Slovenian Landrace (SL11 and SL55) and Large White (LW22 and LW66) breed were included. Boars were selected at intermediate selection stage in the year 2003 in the performance test and finished the test at 100 kg. Animals were divided in two groups based on backfat thickness (Table 1). Animals of each breed with the thinnest backfat were assigned to group A and those with the thickest backfat to group B. The total number of genotyped animals was 166. The differences between groups are evident (Table 1).

Table 1. Statistics for backfat in group A and B
Preglednica 1. Osnovna statistika za debelino hrbtne slanine v skupinah A in B

	A				B			
Breed Pasma	n	$\bar{x}$	min	max	n	$\bar{x}$	min	max
LW22	25	7.97	6.67	8.67	21	12.48	11.33	15.00
LW66	19	7.51	6.33	8.67	25	10.29	9.33	12.67
SL11	22	7.55	6.00	8.00	19	13.26	12.33	16.67
SL55	18	8.63	7.67	9.33	17	15.86	14.67	17.67

n – number / število; $\bar{x}$ – mean / povprečje; min – minimum / najmanj; max – maximum / največ;

PCR-RFLP analysis

Genomic DNA was isolated from skin tissue samples by standard phenol-chloroform-isoamyl alcohol (25:24:1) extraction (Ausubel *et al.*, 2000). The 200-bp fragment of *PPARGC-1* gene was amplified with PCR reaction, which was carried out in a volume of 10 µl: 2 µl of template DNA, 1 x PCR buffer (Fermentas, Vilnius, Lithuania), 1mM $MgCl_2$, 200 µM dNTP, 0.5 U *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania) and 5 pmol of each primer. The primers used for amplification of target sequences were:

PPARGC-1 – SSCP.F (5'- TAAAGATGCCGCCTCTGACT - 3')
PPARGC-1 – SSCP.R (5'- CTGCTTCGTCGTCAAAAACA - 3').

The following amplification parameters were applied: 95 °C for 5 min followed by 31 cycles: 95 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min. The reaction was completed by the final synthesis at 72 °C for 7 min. The PCR products were digested with 2U of *Alu*I (Fermentas, Vilnius, Lithuania) at 37 °C overnight and analyzed on 2% agarose gels.

Statistical Analysis

Frequency distribution of genotypes and alleles were calculated. The statistical package SAS was used (SAS Inst. Inc., 2001). Genotype and allele frequencies between groups were tested using the multinomial model in proc logistic (SAS Inst. Inc., 2001).

The effect of *PPARGC-1* genotype on phenotypic values was analyzed with the following statistical model:

$$y_{ijk} = \mu + G_i + S_j + b(x_{ijk} - \bar{x}) + e_{ijk} \quad [1]$$

For analysis the effect of PPARGC-1 genotype on breeding values the following statistical model was used:

$$y_{ij} = \mu + G_i + e_{ij} \quad [2]$$

where y_{ijk} is the phenotypic respectively breeding value for backfat, μ is intercept, G_i is PPARGC-1 genotype (i=1, 2, 3), S_j is the season of selection at intermediate selection stage in performance test (j=1, 2, 3, …, 11, 12), b is the regression coefficient for body weight at the end of performance test, and e_{ijk} is a residual.

RESULTS AND DISCUSSION

Following digestion of PCR product with *Alu*I, the T allele was cut into fragments of 121, 27, 31 and 21 bp (Figure 1). Allele A had one restriction site more at position 1379 and 121 bp band was cleaved into 61 and 60 bp band, consecutive the allele A had: 61, 60, 27, 31 and 21 bp bands. On agarose gels were defined: genotype AA had ≈60 and ≈30 bp, genotype TT 121 and ≈30 bp and heterozygote AT had 3 bands: 121, ≈60 and ≈30 bp.

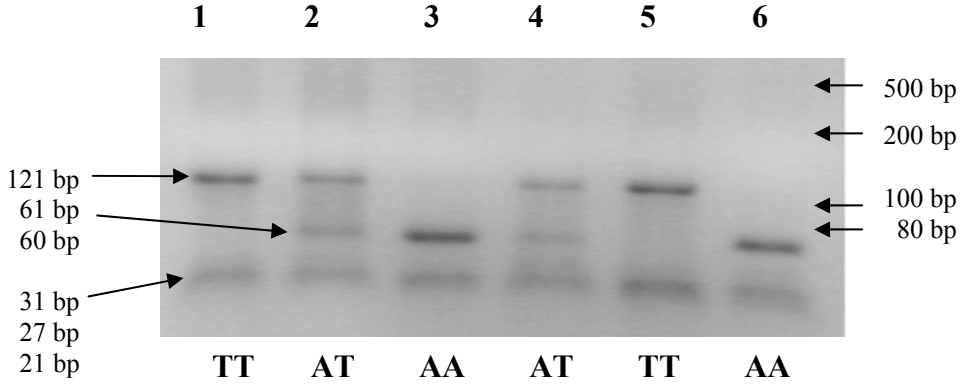

Figure 1. Restriction patterns of samples.
Slika 1. Restrikcijska analiza vzorcev.

Three genotypes were identified in the studied populations: AA, TT and AT (Table 2). The distribution of *PPARGC-1* genotypes frequencies demonstrated significant differences between group A and group B in dam line of Slovenian Landrace (SL11) and sire line of Large White breed (LW66). In the populations of SL11 the frequency of heterozygote was 54.5% in group A and 57.9% in group B. The frequency of TT was 22% in group A, whereas no genotype TT was found in group B. In populations of LW66, the difference of frequency of AT between groups was larger (78.9% in group A and 44% in group B). In group B genotype TT was present in 20%, whereas in group A in 5.3%.

Group A and B of dam line of Large White (LW22) had a similar distribution of genotypes (Table 2). In both groups genotype AA was the most frequent (80 and 81%) and the frequency of heterozygote was similar (20% and 18%). In populations of SL55 the most frequent was genotype AT (50% and 64.7%). In group A the frequency of genotype TT was 11%, whereas in group B was not present.

Table 2. Frequency of genotypes of *PPARGC-1* gene and test of differences between groups
Preglednica 2. Frekvence genotipov gena *PPARGC-1* in test razlik med skupinama

Breed Pasma	Group Skupina	Total	AA		AT		TT		Test
		n	n	frequency %	n	frequency %	n	frequency %	P-value
LW22	A	25	20	80.0	5	20.0	0	0.0	1.0000
	B	21	17	81.0	3	18.0	1	1.0	
LW66	A	19	3	15.8	15	78.9	1	5.3	0.0772
	B	25	9	36.0	11	44.0	5	20.0	
SL11	A	22	5	22.7	12	54.5	5	22.7	0.0472
	B	19	8	42.1	11	57.9	0	0.0	
SL55	A	18	7	38.9	9	50.0	2	11.0	0.8330
	B	17	6	35.3	11	64.7	0	0.0	

Table 3 Frequency of alleles of *PPARGC-1* gene and test of differences between groups
Preglednica 3 Frekvenca alelov gena *PPARGC-1* in test razlik med skupinama

Breed Pasma	Group Skupina	Total	Allele A		Allele T		Test
		n	n	frequency %	n	frequency %	P-value
LW22	A	50	45	90.00	5	10.00	0.7703
	B	42	37	88.10	5	11.90	
LW66	A	38	21	55.26	17	44.74	0.7974
	B	50	29	58.00	21	42.00	
SL11	A	44	22	50.00	22	50.00	0.0550
	B	38	27	71.05	11	28.95	
SL55	A	36	23	63.89	13	36.11	0.7407
	B	34	23	67.65	11	32.35	

Allele A was predominantly present in animals with thick backfat (group B) in SL11 (71.05%), LW66 (58%) and in 67.65% was present in SL55 (Table 3). In LW22 allele A was more frequent in animals with thin backfat. Differences in frequency of alleles were significant in SL11.

The analysis revealed significant differences in frequency distribution of genotypes and alleles of *PPARGC-1* gene between animals with different phenotype for backfat in populations

of SL11 and LW66. These results support the assumption that T/A substitution at 1378 nucleotide position in *PPARGC-1* gene influences the fatness characteristics (Kunej *et al.*, 2005).

Table 4 Least square means (LSM) and standard errors (SE) for phenotypic values for backfat for different breeds

Preglednica 4 Ocenjene srednje vrednosti (LSM) in standardne napake (SE) za debelino hrbtne slanine (v mm) po pasmah in genotipih

Breed Pasma	Trait Lastnost	Analysis Analiza	AA	AT	TT	P-value
LW22	BF-2	1	10.34 ± 0.39	8.94 ± 0.82	10.95 ± 2.54	0.3006
		2	10.30 ± 0.38	8.89 ± 0.82		0.1339
	BF-3	1	10.15 ± 0.36	8.62 ± 0.76	10.17 ± 2.35	0.2104
		2	10.11 ± 0.35	8.58 ± 0.76		0.0809
LW66	BF-2	1	10.20 ± 0.59	8.67 ± 0.34	10.40 ± 0.70	0.0248
		2	10.00 ± 0.67	8.46 ± 0.34		0.0735
	BF-3	1	9.92 ± 0.54	8.46 ± 0.32	10.13 ± 0.65	0.0184
		2	9.73 ± 0.61	8.46 ± 0.34		0.0615
SL11	BF-2	1	11.16 ± 0.83	9.92 ± 0.66	8.38 ± 1.30	0.1624
		2	11.13 ± 0.86	9.93 ± 0.68		0.2666
	BF-3	1	11.24 ± 0.84	9.91 ± 0.66	8.44 ± 1.32	0.1603
		2	11.22 ± 0.88	9.92 ± 0.69		0.2370
SL55	BF-2	1	11.30 ± 1.34	11.73 ± 1.13	7.30 ± 2.67	0.2928
		2	11.38 ± 1.41	11.70 ± 1.16		0.8754
	BF-3	1	11.56 ± 1.39	12.17 ± 1.17	7.60 ± 2.77	0.3010
		2	11.67 ± 1.46	12.12 ± 1.20		0.8281

LW22 – dam line of Large White breed; LW66 – sire line of Large White; SL11 – dam line of Slovenian Landrace; SL55 – sire line of Slovenian Landrace; BF–2 – average of two measurements of backfat; BF-3 – average of 3 measurements of backfat; Analysis 1 – least square means for different genotypes AA, AT, and TT; Analysis 2 – comparison between homozygotes AA and heterozygotes AT;
LW22 – ženska linija pasme Large White; LW66 – moška linija pasme Large White; SL11 – ženska linija slovenske landrace; SL55 – moška linija slovenske landrace; BF–2 – povprečje dveh meritev hrbtne slanine; BF-3 – povprečje treh meritev hrbtne slanine; Analiza 1 – povprečja najmanjših kvadratov za različne genotipe AA, AT in TT; Analiza 2 – primerjava homozigotov AA in heterozigotov AT;

Results of analysis of the effect of PPARGC-1 genotype are shown as the least square means (Table 4). The analysis 1 was done to compare all three genotypes (AA, AT, and TT), whereas in the analysis 2 homozygotes TT were excluded because of the low number of observations and the estimates of mean values were less reliable. In population of Large White boars the thinnest backfat had heterozygotes, what could be the consequence of non-additive effects. The thickest backfat had homozygotes TT, what was in agreement with results obtained by Kunej *et al.*

(2005). In population of Slovenian Landrace boars the homozygotes had the thinnest backfat, whereas the homozygotes AA had the thickest backfat. A significant effect of PPARGC-1 genotype was found in population of Large White boars.

The estimation of *PPARGC-1* gene effect on breeding values revealed similar results (Table 5). The heterozygotes had the smallest predicted breeding values in Large White populations but in population of Slovenian Landrace boars the best predicted breeding values had homozygotes TT. We found a significant effect of *PPARGC-1* gene on breeding values for backfat in population of sire line of Large White breed.

Table 5 Least square means (LSM) and standard errors (SE) for breeding values for backfat for different breeds and genotypes

Preglednica 5 Ocenjene srednje vrednosti (LSM) in standardne napake (SE) za plemenske vrednosti za debelino hrbtne slanine po pasmah in genotipih

Breed Pasma	Analysis Analiza	AA	AT	TT	P-value
LW22	1	–1.0987 ± 0.1586	–1.6916 ± 0.3411	0.0580 ± 0.9648	0.1374
	2	–1.0987 ± 0.1586	–1.6916 ± 0.3411		0.1223
LW66	1	0.4397 ± 0.1846	–0.1128 ± 0.1254	0.2308 ± 0.2611	0.0491
	2	0.4398 ± 0.1864	–0.1128 ± 0.1266		0.0192
SL11	1	–1.9898 ± 0.4490	–2.7588 ± 0.3376	–3.8344 ± 0.7241	0.0980
	2	–1.9898 ± 0.4742	–2.7589 ± 0.3565		0.2036
SL55	1	–0.5046 ± 0.5650	–0.2923 ± 0.4555	–3.0280 ± 1.4403	0.2097
	2	–0.5046 ± 0.5739	–0.2923 ± 0.4627		0.7752

LW22 – dam line of Large White breed; LW66 – sire line of Large White; SL11 – dam line of Slovenian Landrace; SL55 –sire line of Slovenian Landrace; Analysis 1 – least square means for different genotypes AA, AT, and TT; Analysis 2 – comparison between homozygotes AA and heterozygotes AT;
LW22 – ženska linija pasme Large White; LW66 – moška linija pasme Large White; SL11 – ženska linija slovenske landrace; SL55 – moška linija slovenske landrace; Analiza 1 – povprečja najmanjših kvadratov za različne genotipe AA, AT in TT; Analiza 2 – primerjava homozigotov AA in heterozigotov AT;

CONCLUSIONS

The comparison of the genotype frequency between groups of the thinnest (group A) and the thickest (group B) revealed differences in population of sire line Large White breed and dam line of Slovenian Landrace breed, but the distribution of allele differed between group only in population of dam line of Slovenian Landrace breed. The estimation of *PPARGC-1* gene effect showed a significant effect of *PPARGC-1* gene on phenotypic values in population of Large White boars, but the analysis of gene effect on breeding values revealed a significant effect only in sire line of Large White breed. Such conflicting results may be the consequences of different background of allele, epistasis, intensity of selection, and deficiency of experiment design. Although these results did not confirmed the effect of allele A to be involved in inhibited fat deposition in both populations this type of analysis (especially as data size increases) with porcine candidate genes provides useful information for better understanding of the process of fat deposition. Further evaluations and researches of effect of T1378A substitution in porcine

PPARGC-1 gene should be studied on performance traits in different commercial pig populations.

REFERENCES

Ausubel, F.M./ Brent, R./ Kingston, R.E./ Moore, D.D./ Seidman, J.G./ Smith, J.A./ Struhl, K. Current protocols Mo. Vol I. section 2.1.3. New York, John Wiley and sons. Inc., 2000.

Bidanel, J.P./ Milan, D./ Iannuccelli, N./ Amigues, Y./ Boscher, M.Y./ Bourgeois, F./ Caritez, J.C./ Gruand, J./ Le Roy, P./ Lagant, H./ Quintanilla, R./ Renard, C./ Gellin, J./ Ollivier, L./ Chevalet, C. Detection of quantitative trait loci for growth and fatness in pigs. Genet. Sel. Evol., 33(2001), 289–309.

Bidanel, J.P./ Rothschild, M. Current status of quantitative trait locus mapping in pigs. Pig News Inform., 23(2002), 39N–54N.

Jacobs, K./ Rohrer, G./ Van Poucke, M./ Piumi, F./ Yerle, M./ Barthenschlager, H./ Mattheeuws, M./ Van Zeveren, A./ Peelman, L.J. Porcine *PPARGC1A* (peroxisome proliferative activated receptor gamma coactivator 1A): coding sequence, genomic organization, polymorphism and mapping, Cytogenet. Genome Res., 112(2006), 106–113.

Knutti, D./ Kralli, A. PGC-1. versatile coactivator. Trends Endocrin. Met., 8(2001)12, 360–365.

Kunej, T./ Wu, X.L./ Berlic Milosevic, T./ Michal, J.J./ Jiang, Z./ Dovc, P. Frequency distribution of a Cys430Ser polymorphism in peroxisome proliferator-activated receptor-gamma coactivator-1 (*PPARGC-1*) gene sequence in Chinese and Western pig breeds. J. Anim. Breed. Genet., 122(2005), 7–11.

Lin, J./ Wu, H./ Tarr, P.T./ Zhang, Z.Y./ Wu, Z./ Boss, O./ Michael, L.F./ Puigserver, P./ Isotani, E./ Olson, E.N./ Lowell. B.B./ Bassel-Duby, R./ Spiegelman, B.M. Transcriptional co-activator PGC-1α drives the formation of slow-twich muscle fibres. Nature, 418(2002), 797–801.

Miloševič Berlič, T. Molekulski mehanizmi uravnavanja tvorbe maščobnega tkiva pri prašiču (*Sus scrofa*). Doktorska disertacija. Univerza v Ljubljani. Slovenija, 2002, 142 p.

Miloševič Berlič, T./ Dovč, P. Transkripcijsko uravnavanje adipogeneze in vloga koaktivatorja PGC-1α. *Acta agriculturae Slovenica*, 84(2004)2, 97–107.

Rohrer, G.A. Identification of quantitative trait loci affecting birth characters and accomulation of backfat and weight in a Meishan – White composite resource population. J. Anim. Sci., 78(2000), 2547–2553.

Rosen, E.D./ Walkey, C.J./ Puigserver, P./ Spiegelman, B.M. Transcriptional regulation of adipogenesis. Gene. Dev. 14(2000), 1293–1307.

SAS Inst. Inc. The SAS System for Windows. Release 8.02. Cary. NC. SAS Institute, 2001.

Acta agriculturae Slovenica, 88(november 2006)1, 19–27.

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

Agris category codes: L01, Q04 COBISS Code 1.01

VPLIV SESTAVE KRME IN SPOLA PRAŠIČEV NA KAKOVOST SUŠENIH VRATIN *

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Delo je prispelo 13. junija 2006, sprejeto 20. novembra 2006.
Received June 13, 2006, accepted November 20, 2006.

IZVLEČEK

Namen članka je predstaviti vpliv spremenjene sestave krme in spola prašičev na senzorične in instrumentalne lastnosti sušenih vratin. V raziskavo je bilo vključenih 12 kastratov in 12 svinjk. Razdelili smo jih v štiri skupine glede na različne dodatke h krmi (laneno seme, ogrščično seme, ogrščično seme+vit. E in kontrolna mešanica). V vseh 24 vzorcih sušenih vratin smo določili skupno količino maščob, jih senzorično ocenili ter instrumentalno določili barvo (CIE -L, -a, -b) in teksturo (strižna trdnost – Kramerjeva celica). Rezultati so pokazali, da različni dodatki h krmi ne vplivajo na skupno vsebnost maščob, medtem ko ima spol statistično značilen vpliv. Priokusi se pojavijo pri skupinah, krmljenih z dodatkom lanu in ogrščice. Dodatek vitamina E poslabša senzorične lastnosti (vonj, aroma, tekstura) in da mesu najsvetlejšo barvo. Tekstura je najčvrstejša pri krmljenju z dodatkom ogrščice. Sušena vratina kastratov je temnejše barve in čvrstejše teksture kot vratina svinjk.

Ključne besede: prašiči / prehrana živali / krma / krmi dodatki / spol / meso / sušena vratina / kakovost / senzorične lastnosti / instrumentalne lastnosti

THE INFLUENCE OF PIG FODDER COMPOSITION ON QUALITY OF DRIED PORK NECK †

ABSTRACT

The aim of the article is to show the influence of diet composition and sex on sensory and instrumental quality of dried pork neck. For the research we used 12 hogs and 12 sows. They were separated into 4 groups regarding different food mixture (flaxseed, rapeseed, rapeseed+vit.E and control mixture). All 24 samples of dried neck were analyzed on fat content. Sensory analysis of dried necks and instrumental measurement of colour (CIE -L, -a, -b) and texture (Kramer shear cell) were performed. The results showed that different fodder composition had no influence on fattiness of the product, while sex had a significant influence. Aftertaste was perceived by feeding with addition of flaxseed and rapeseed. Added vitamin E decreased sensory characteristics (smell, aroma, texture) and gave lighter colour. The firmer

* Prispevek je del diplomskega dela Marjete Furman z naslovom ´Vpliv sestave krme prašičev na kakovost sušenih vratin´, mentor prof. dr. Božidar Žlender.

† This article is part of a graduation thesis ´The influence of pig fodder composition on quality of dried neck´, issued by Marjeta Furman, supervisor prof. Božidar Žlender, Ph.D.

texture was shown by feeding with rapeseed. Neck from hogs had darker colour and firmer texture than neck from sows.

Key words: pigs / animal nutrition / feed / feed additives / sex / meat / dried pork neck / quality / sensory properties / instrumental properties

UVOD

Kakovost mesnih izdelkov je zelo širok pojem. Vključuje lastnosti, ki prispevajo k prehranski vrednosti izdelkov, oblikujejo gastronomski učinek mesnin in zagotavljajo »varnost« zdravju porabnika. Prehransko vrednost mesnih izdelkov ugotavljamo s kemijskimi metodami, senzorično kakovost pa s senzorično analizo (Rajar, 2002). Na kakovost surovine lahko vpliva prehrana živali, spol, genotip, starost in masa živali. Na sušeno meso pa poleg naštetih dejavnikov pomembno vpliva še obdelava mesa (Berčič, 2004; Žlender, 1997).

Vpliv krme

Način prehrane je ključni dejavnik poteka rasti in posledično vpliva na klavno kakovost. Prehrana živali je najodločilnejši dejavnik nivoja maščob v klavnih trupih oziroma mesu. Pri pitanju živali za prirejo mesa želimo prirediti največjo možno količino najkakovostnejšega mesa ob primerni stopnji zamaščenosti, kar nam pogosto ne uspe zaradi nepravilne prehrane. Pogosto energijsko prebogat obrok (prehrana po volji), zlasti ob zaključku pitanja, povzroči prekomerno nalaganje maščob, kar negativno vpliva na kakovost oz. komercialno vrednost klavnih trupov (Čepin in Žgur, 2000).

Krma živali, obogatena z različnimi prehransko pomembnimi maščobnimi kislinami, lahko izboljša prehransko vrednost maščob živali in posredno zagotovi boljšo preskrbljenost človeka z esencialnimi maščobnimi kislinami. Povečanje vsebnosti večkrat nenasičenih maščobnih kislin v krmi lahko povzroči priokus po ribah, ki je posledica žarkih olj v prehrani živali (Scaife in sod., 1994).

Dodatek lanu h krmi živali ima precejšen vpliv na kakovost mesnih izdelkov. Znano je, da laneno seme vsebuje veliko nenasičnih maščobnih kislin, med katerimi sta v največji količini zastopani ravno esencialni maščobni kislini (linolna in α-linolenska). Linolne kisline vsebuje laneno seme okoli 16 utežnih odstotkov, medtem ko se delež trikrat nenasičene α-linolenske kisline povzpne kar na 53 ut. % (Otrhofer, 1996). Zaradi vsebnosti velike količine večkrat nenasičenih maščobnih kislin obstaja nevarnost, da bi v izdelkih, pridobljenih iz živali, krmljenih z veliko količino lanenega semena prišlo do oksidacije nestabilnih maščobnih kislin. Take spremembe bi se lahko kazale v določenih senzoričnih lastnostih, kot so vonj in priokus po žarkem. Hoz in sod. (2004) navajajo, da se sušeni mesni izdelki, pridobljeni iz živali, krmljenih s krmo, obogateno z n-3 maščobnimi kislinami in α-tokoferolom, razlikujejo v maščobnokislinski sestavi. Pri krmljenju s krmo, obogateno z lanenim semenom dobimo končne produkte z več nenasičenimi maščobnimi kislinami in ugodnejšim razmerjem n-6/n-3, pod 4 (Enser in sod., 2001). Navaja tudi, da ob dodatku lanenega semena in α-tokoferola v krmo živali ni zaznati opaznih razlik v senzoričnih lastnostih, kot so vonj, barva, tekstura, sočnost in okus sušenih mesnin. Pri dodatku lanu brez α-tokoferola pa je prisoten nezaželen odtenek žarkega okusa. Tudi Deckel in sod. (1999) so ugotovili, da pri dodatku α-linolenske kisline v krmo ni zaznanih bistvenih razlik v senzoričnih lastnostih mesa kastratov in svinjk.

Ogrščično seme prav tako vsebuje večje količine nenasičenih maščobnih kislin. Med njimi je največ enkrat nenasičene oleinske (47 ut. %) in dvakrat nenasičene linolne, ki je je okoli 31 ut. % (Orthofer, 1996). Precejšen delež (10 ut. %) je tudi α-linolenske kisline (Shahidi, 1990). Takšna maščobnokislinska sestava ogrščičnega semena bi lahko pomenila tudi nekoliko boljšo oksidacijsko stabilnost izdelkov, pridobljenih iz živali, krmljenih z ogrščičnimi semeni kot pri

krmljenju z lanom. Corino in sod. (2002) navajajo, da dodatek ogrščičnega semena v krmo za prašiče vpliva na porast α-linolenske kisline v mesu, oksidativna stabilnost se nekoliko poslabša po daljšem skladiščenju. Senzoričnih sprememb pri takem mesu ni zaznati.

Vitamin E deluje kot antioksidant in ščiti tkiva pred neencimsko oksidacijo, predvsem oksidacijo večkrat nenasičenih maščobnih kislin. Ena molekula α-tokoferola lahko zaščiti 100 in več molekul večkrat nenasičenih maščobnih kislin (VNMK) tako, da reagira s peroksi radikali, ki nastanejo pri oksidaciji VNMK. Razmerje med α-tokoferolom in VNMK je pomemben kriterij, ki vpliva tako na optimalno oksidacijsko zaščito kot tudi na toksikološko neoporečnost maščobe (Kitts, 1996). Tudi Rey in sod. (2003) so ugotovili izboljšano lipidno stabilnost pri dodatku α-tokoferola. Med dodanim lanenim oljem in α-tokoferolom ni bilo interakcij. Vitamin E naj bi torej vplival na boljšo stabilnost maščob in s tem pripomogel, da se senzorične lastnosti izdelkov, pridobljenih iz živali, krmljenih z dodatki večkrat nasičenih maščobnih kislin, ne bi občutno poslabšale. O'Sullivan in sod. (2002) so ugotovili, da je meso prašičev, krmljenih z vitaminom E, bolj rdeče in svetlejše v primerjavi z drugimi vzorci.

Vpliv spola

Nekastrirane moške živali v primerjavi z ženskimi živalmi so veliko manj nagnjene k zamastitvi (Čepin in Žgur, 2000), saj imajo moške živali iste pasme ob enaki prehrani manj maščob v sestavi trupov kot ženske. Kastrirane moške živali imajo praviloma vmesne vrednosti, pri prašičih in ovcah pa so kastrati celo močneje zamaščeni kot ženske živali. V deležu mesa in kosti so razlike med spoloma veliko manjše. Tudi Čandek in Šegula (1996) sta prišla do podobnih ugotovitev, da se živali različnega spola razlikujejo v rastnosti in telesni sestavi. Kastrati rastejo hitreje od svinjk, a manj učinkovito izkoriščajo krmo in imajo slabšo mesnatost. Armero in sod. (1999) opažajo, da imajo svinjke večja stegna, močnejši zadnji del, manj hrbtne slanine, merjene na vratu, in manjši trebuh glede na kastrate.

Beltram (2000) ugotavlja, da spol značilno vpliva na mesnatost prašičev, marmoriranost mišic, na delež intramuskularne maščobe v mišicah in L- a- in b- vrednosti instrumentalno izmerjene barve. Svinjke so v povprečju težje in tudi bolj mesnate kot kastrati, medtem ko imajo kastrati v povprečju debelejšo hrbtno slanino kot svinjke. Spol vpliva tudi na marmoriranost mišic, saj je pri vseh mišicah senzorična in kemijska analiza marmoriranosti pokazala večjo zamaščenost kastratov v primerjavi s svinjkami. Instrumentalno izmerjena barva mišic kastratov je nekoliko temnejša, manj rdeča in rumena glede na mišice svinjk. V nasprotju z opisanim pa Skvarča (2001) navaja, da na barvo mesa posredno vpliva tudi zamaščenost, saj je marmorirana mišičnina svetlejša. Latorre in sod. (2003) pa so v skladu z ugotovitvijo Beltram (2000) opazili pri svinjkah bolj intenzivno barvo (bolj rdečo) kot pri kastratih.

V članku želimo predstaviti ugotovitve, kako spol in različni dodatki h krmi (lan, ogrščica in ogrščica v kombinaciji z vitaminom E) vplivajo na skupno vsebnost maščob ter na senzorično in instrumentalno ocenjene parametre sušenih vratin.

MATERIAL IN METODE

Material

V poskus je bilo vključenih 24 prašičev istega genotipa – hibrida 12 (križanci med slovensko landrace, linija 11 in large white). Vsaka skupina je zajemala tri kastrate in tri svinjke. Tekače so pri 25 kg razdelili v štiri skupine in jih pričeli krmiti z osnovno krmno mešanico (kontrolna skupina; skupina 1) ter dodatki lanu (skupina 2), ogrščice (skupina 3) in ogrščice+vitamina E (skupina 4). Pri skupini 1, smo v obrok dodali 3 ut. % lanu, pri skupini 2 smo dodali 15 ut. %

ogrščice, medtem ko je pri skupini 3 bilo zraven 15 ut. % ogrščice dodanega še 50 mg vit.E / kg krmne mešanice. Vse krmne mešanice so bile izohranilne, kar pomeni, da so imele enako ali vsaj približno enako metabolno energijo in vsebnost surovih beljakovin. Do mase 60 kg so jih krmili z eno vrsto krmne mešanice, potem je bila sestava osnovne krmne mešanice spremenjena, brez spremenjene količine dodatkov.

Metode

Zakol 6 mesecev starih in v povprečju 105 kg težkih prašičev je potekal po ustaljenih postopkih. Po hlajenju polovic 24 h *post mortem* in razseku so surove vratine zamrznili, da bi preprečili zorenja mesa. Po mesecu in pol so vratine odtajali, stehtali in suho solili. Nanesli so mešanico morske soli (2,5 %), sofosalta (2,5 %), izomiksa, popra in česna. Razsoljevanje je trajalo 10 dni, nato še 6 dni tako imenovano počivanje (riposo), oboje pri temperaturi okoli 3 ^{o}C. Po razsoljevanju so spremembo mase tedensko spremljali. Vratine so se sušile 100 dni pri temperaturi med 15 in 18 °C. Relativna vlaga na začetku sušenja je znašala 85–90 %, nato je postopoma padla na 65–70 %. Vseh 24 vzorcev sušenih vratin je bilo do analiz vakuumsko zapakiranih in shranjenih pri –30 °C.

Sušene vratine smo odtajali in odstranili embalažo vakuumskega pakiranja. Za določanje vsebnosti skupnih maščob smo približno 150 g vsakega vzorca sesekljali s kuhinjskim nožem na drobne koščke in jih zmleli s kuhinjskim mlinčkom. Tako homogenizirane vzorce smo s čimmanj prisotnega kisika zapakirali v polietilenske vrečke in jih shranili v zamrzovalni komori pri temperaturi –21 ^{o}C do nadaljnjih analiz. Za senzorično in instrumentalni analizi smo odvzeli rezine vzorcev primerne debeline za vsako analizo.

Vsebnost skupne maščobe v vzorcih smo določili z metodo po Weibullu in Stoldtu (AOAC 991.36, 1997). Vzorec smo kuhali s HCl, da so se beljakovine popolnoma razkrojile. Izločeno mast smo odfiltrirali in ekstrahirali z organskim topilom (petroleter) v Soxhletovem aparatu. Po končani ekstrakciji smo ohlajeno bučko z mastjo stehtali in izračunali vsebnost maščobe (g) tako, da smo odšteli maso prazne bučke. Tako smo dobili vsebnost intramuskularne maščobe v odstotkih (%).

Senzorično oceno nekaterih lastnosti sušenih vratin smo opravili na svežem prerezu vratin. Za degustacijo smo za vsakega ocenjevalca odvzeli dve rezini vzorca debeline 2 mm. Senzorično ocenjevanje je opravila štiri članska degustacijska komisija, sestavljena iz izkušenih ocenjevalcev. Pri senzorični analizi smo uporabili deskriptivni test z nestrukturirano točkovno lestvico, kjer se senzoričnim parametrom po pravilu dodelijo ocene od 1 do 7 točk, pri čemer višja ocena pomeni bolj izraženo lastnost. Tekstura je ocenjena z lestvico 1-4-7 točke, pri čemer 4 točke pomenijo optimalno oceno. Na svežem prerezu vratine smo ocenili odtenek, intenzivnost, enakomernost barve prereza mišičnine, odtenek barve maščobnega tkiva. Na rezinah vratine smo ocenili vonj, tuje vonje, aromo, priokuse in teksturo.

Za instrumentalno analizo barve smo uporabili kromometer Minolta CR 200b, ki ima priključen računalnik DATA DP 100. Barvo smo izmerili kot vrednosti -L, -a in -b na približno 5 cm debelem kosu suhe vratine. Pri vsakem vzorcu smo opravili po štiri meritve na svežem prerezu na različnih mestih mišičnine izdelka.

Teksturo sušene vratine smo izmerili s Texture Analyser TA.XT plus aparatom, zmogljivosti 500 N. Za kontaktni nastavek smo uporabili Kramerjevo celico, opremljeno s 5 rezili (HDP/KS5). Za vzorec smo vratinam odvzeli 3 rezine, debeline 3 mm, in jih oblikovali na dolžino 8 cm in širino 2,5 cm. Pri vsakem vzorcu smo izmerili silo v Newton (N) in delo v Joule (J) so sledile tri ponovitve.

Podatke, zbrane v tem poskusu, smo statistično obdelali s proceduro GLM (General Linear Models) v programu SAS (Software Version 8.01, 1999). Statistični model je vključeval vpliv krme živali (K) in spola (S):

$$y_{ijk} = \mu + K_i + S_j + e_{ijk}$$

kjer je y_{ijk} opazovana vrednost, μ povprečna vrednost, K_i vpliv krme oziroma skupine (i=1 skupina 1, i=2 skupina 2, i=3 skupina 3, i=4 skupina 4); S_j vpliv spola (j=1 kastrat, j=2 svinjka) in e_{ijk} ostanek.

REZULTATI Z RAZPRAVO

Vpliv krme

Na vsebnost skupnih maščob različni dodatki h krmi (ogrščica, lan in vitamin E) nimajo značilnega vpliva (preglednica 1). Rezultat je pričakovan, saj so vse krmne mešanice izohranilne, torej je bila s krmo živali vnesena energija enaka. Povprečen delež maščobe v sušeni vratini znaša 28,2 %, kar se ujema s podatkom Berčič (2004), da 100 g sušene vratine vsebuje 26 – 28 g maščob.

Preglednica 1. Vpliv dodatkov v krmo na vsebnost maščob in senzorične lastnosti sušenih vratin
Table 1. Effect of pig diet composition on fat content and sensory characteristics in dried neck

	Kontrola Control LSM ± SE	Lan Flax LSM ± SE	Ogrščica Rapeseed LSM ± SE	Ogrščica + vitamin E Rapeseed + vitamin E LSM ± SE	Znač.
Vsebnost maščobe / Fat content	29,86 ± 1,04a	27,65 ± 1,04a	28,84 ± 1,06a	26,99 ± 1,04a	nz
Odtenek barve mišičnine Shade of muscle colour	6,00 ± 0,00a	6,00 ± 0,00a	6,00 ± 0,00a	6,00 ± 0,00a	nz
Intenzivnost barve mišičnine Intensity of muscle colour	5,81 ± 0,09a	5,88 ± 0,09a	5,71 ± 0,09ab	5,54 ± 0,09b	nz
Enakomernost barve mišičnine Equability of muscle colour	5,44 ± 0,12a	5,25 ± 0,12a	5,28 ± 0,12a	4,88 ± 0,12b	**
Odtenek barve maščobnega tkiva Shade of fat tissue colour	5,23 ± 0,10a	5,21 ± 0,10a	5,36 ± 0,11a	5,35 ± 0,10a	nz
Vonj / Odour	5,88 ± 0,08a	5,67 ± 0,08ab	5,83 ± 0,08ab	5,63 ± 0,08b	*
Tuji vonji / Foreign odour	1,17 ± 0,08a	1,40 ± 0,08a	1,25 ± 0,08a	1,38 ± 0,08a	nz
Aroma / Aroma	5,83 ± 0,07a	5,48 ± 0,07b	5,73 ± 0,08a	5,48 ± 0,07b	**
Priokusi /Aftertaste	1,15 ± 0,08b	1,44 ± 0,08a	1,35 ± 0,08a	1,40 ± 0,08ab	*
Tekstura / Texture	4,46 ± 0,08ab	4,29 ± 0,08b	4,60 ± 0,08a	4,63 ± 0,08a	**
Skupni vtis / Common effect	5,81 ± 0,08a	5,52 ± 0,08a	5,62 ± 0,08ab	5,40 ± 0,08b	**

LSM – tehtane srednje vrednosti; SE – standardna napaka ocene; ** – $p \leq 0,01$; * – $p \leq 0,05$; nz – statistično neznačilen vpliv ($p > 0,05$); skupine z enako črko v indeksu se med seboj statistično značilno ne razlikujejo
LSM – least square means; SE – standard error; ** – $P \leq 0,01$; * – $P \leq 0,05$; nz – no significant influence ($P > 0.05$); no difference between group with the same index

Na odtenke in intenzivnost barve mišičnine dodatki h krmi ne vplivajo, le enakomernost barve mišičnine se ob dodatku vitamina E poslabša. Tudi na vonj in tuje vonje krma nima vpliva, kar se ujema z ugotovitvami Hoz in sod. (2004), da pri dodatku lanu in α-tokoferola v krmo živali ni značilnega vpliva na senzorično ocenjen vonj in barvo sušenih mesnin. Zanimivo je, da je tudi intenzivnost barve maščobnega tkiva ostala nespremenjena, saj bi lahko med sušenjem vratine prišlo do oksidacije večkrat nenasičenih maščobnih kislin in s tem do spremenjenega odtenka barve maščob pri določenih dodatkih h krmi.

Aroma je slabše ocenjena pri skupina 2 in 4. Lan vsebuje veliko α-linolenske kisline, ki lahko oksidira in s tem prispeva k poslabšanju arome. Naše ugotovitve se ne skladajo s Hoz in sod. (2004), saj le-ti med drugim navajajo, da ob dodatku lanenega semena in α-tokoferola v krmo živali ni zaznati opaznih razlik v sočnosti in okusu sušenih mesnin. Najbolje ocenjeno aromo pri kontrolni skupini bi morda lahko povezali tudi s 3 % večjo vsebnostjo maščobe kot pri skupini z dodatkom vitamina E (preglednica 1), vendar ta razlika ni značilna.

Priokusi so najbolj zaznavni pri skupinah lan in ogrščica. Dodatek α-tokoferola priokuse nekoliko zmanjša, kar bi lahko povezali z njegovo antioksidativno sposobnostjo, preprečitvijo oksidacije maščobnih kislin in s tem ublažitev priokusov. Naši rezultati se ujemajo z ugotovitvijo Hoz in sod. (2004), da je pri dodatku lanu brez α-tokoferola prisoten nezaželen žarek okus. Najmanj priokusov je bilo zaznati pri kontrolni skupini, saj le-ta ne vsebuje dodatkov z nenasičenimi maščobnimi kislinami in tako oksidacija ni prisotna v tolikšni meri, da bi lahko zaznali značilen priokus po žarkem.

Tudi na teksturo ima krma precejšen vpliv. Najbližje optimalni je bila tekstura sušene vratine, pridobljene iz mesa prašičev, krmljenih z dodatkom lanu. Prav tako sta skupini lan in kontrola najbolje ocenjeni za skupni vtis. Tudi Deckel in sod. (1999) navajajo, da pri dodatku α-linolenske kisline v krmo ni zaznati bistvenih razlik v senzoričnih lastnostih mesa kastratov in svinjk. Corino in sod. (2002) pa so ugotovili, da pri dodatku ogrščičnega semena h krmi prašičev ni zaznati senzoričnih sprememb v mesu, vendar se oksidativna stabilnost tako pridobljenih izdelkov nekoliko poslabša po daljšem skladiščenju. Tudi v našem primeru so skupni vtis in priokusi slabše ocenjeni pri skupini 3 kot pri kontrolni skupini. Spremembe v kakovosti maščob bi lahko pripisali postopku sušenja.

Preglednica 2. Vpliv dodatkov h krmi na instrumentalno izmerjeno barvo in teksturo sušenih vratin

Table 2. Effect of diet composition on instrumental measurement of colour and texture of dried neck

	Kontrola Control LSM ± SE	Lan Flax LSM ± SE	Ogrščica Rapeseed LSM ± SE	Ogrščica + vitamin E Rapeseed + vitamin E LSM ± SE	Znač.
Barva (Minolta) / Colour (Minolta)					
Vrednost -L / Value -L	32,53 ± 0,56c	34,25 ± 0,56ab	32,99 ± 0,57bc	35,04 ± 0,56a	**
Vrednost -a / Value -a	11,37 ± 0,30a	11,35 ± 0,30a	11,94 ± 0,31a	12,05 ± 0,30a	nz
Vrednost -b / Value -b	4,87 ± 0,28b	5,50 ± 0,28ab	5,76 ± 0,28a	5,82 ± 0,28a	*
Tekstura (Kramer) / Texture (Kramer)					
Sila (N) / Force (N)	169,48 ± 11,89bc	147,26 ± 11,89c	233,51 ± 12,06a	189,36 ± 11,89b	***
Delo (J) / Work (J)	538,24 ± 33,07bc	462,79 ± 33,07c	666,62 ± 33,54a	572,99 ± 33,07ab	**

LSM – tehtane srednje vrednosti; SE – standardna napaka ocene; *** – $p \leq 0,001$; ** – $p \leq 0,01$; * – $p \leq 0,05$; nz – statistično neznačilen vpliv ($p > 0,05$); skupine z enako črko v indeksu se med seboj statistično značilno ne razlikujejo

LSM – least square means; SE – standard error; *** – $P \leq 0,001$; ** – $P \leq 0,01$; * – $P \leq 0,05$; nz – no significant influence ($P > 0.05$); no difference between group with the same index

Najtemnejša barva (najvišje vrednosti –L) sušenih vratin se pokaže pri prašičih, krmljenih s kontrolno mešanico in ogrščico (preglednica 2). Suha vratina ima najsvetlejšo barvo v primeru

dodanega vitamina E, kar se ujema z ugotovitvijo O'Sullivan in sod. (2002), ki navajajo, da je meso prašičev, krmljenih z dodatkom vitamina E, svetlejše. Navajajo še, da je tako meso bolj rdeče, česar pa naše ugotovitve niso potrdile, saj krma ni pokazala značilnega vpliva na vrednost -a.

Najbolj čvrsto teksturo, to je največjo silo oziroma največ potrebnega dela za pretrganje vzorca, smo zaznali pri sušeni vratini, pridobljeni iz prašičev, krmljenih z dodatkom ogrščice (preglednica 2), najmanjšo pa pri skupini, krmljeni z lanom. To se ujema z našo ugotovitvijo, da je vpliv lanu na teksturo sušene vratine najbližje optimalnemu, medtem ko je pri skupinah 3 in 4 zaznana bolj čvrsta tekstura (preglednica 1).

Vpliv spola

Sušena vratina kastratov vsebuje 3 % več maščobe ($p < 0,01$) kot vratina svinjk (preglednica 3). To se ujema z rezultati Beltrama (2000), da je mišična zamaščenost kastratov večja v primerjavi s svinjkami. Tudi Latorre in sod. (2003) navajajo, da so kastrati ob enakem krmljenju debelejši in imajo več mišične maščobe kot svinjke.

Preglednica 3. Vpliv spola na senzorične lastnosti sušenih vratin
Table 3. Effect of sex on sensory characteristics of dried neck

	Kastrati / Hogs LSM ± SE	Svinjke / Sows LSM ± SE	Znač.
Vsebnost maščobe / Fat content	29,64 ± 0,71a	26,53 ± 0.76b	**
Odtenek barve mišičnine / Shade of muscle colour	6,00 ± 0,00a	6,00 ± 0,00a	nz
Intenzivnost barve mišičnine / Intensity of muscle colour	5,62 ± 0,06b	5,86 ± 0,07a	*
Enakomernost barve mišičnine / Equability of muscle colour	5,18 ± 0,08a	5,25 ± 0,09a	nz
Odtenek barve maščobnega tkiva / Shade of fat tissue colour	5,26 ± 0,07a	5,31 ± 0,08a	nz
Vonj / Odour	5,78 ± 0,06a	5,72 ± 0,06a	nz
Tuji vonji / Foreign odour	1,30 ± 0,06a	1,30 ± 0,06a	nz
Aroma / Aroma	5,61 ± 0,05a	5,66 ± 0,06a	nz
Priokusi /Aftertaste	1,33 ± 0,06a	1,33 ± 0,06a	nz
Tekstura / Texture	4,49 ± 0,05a	4,50 ± 0,06a	nz
Skupni vtis / Common effect	5,55 ± 0,06a	5,63 ± 0,06a	nz

LSM – tehtane srednje vrednosti; SE – standardna napaka ocene; ** – $p \leq 0,01$; * – $p \leq 0,05$; nz – statistično neznačilen vpliv ($p > 0,05$); skupine z enako črko v indeksu se med seboj statistično značilno ne razlikujejo;
LSM – least square means; SE – standard error; ** – $P \leq 0,01$; * – $P \leq 0,05$; nz – no significant influence ($P > 0.05$); no difference between group with the same index;

V senzoričnih lastnostih sušenih vratin med kastrati in svinjkami ni razlik (preglednica 3). Izjema je le večja intenzivnost barve mišičnine pri svinjkah kot pri kastratih ($p<0,05$), kar se sklada z ugotovitvijo Latorre in sod. (2003).

Vratina kastratov je značilno temnejša (nižje vrednosti-L) kot vratina svinjk (preglednica 4). Vzorci svinjk in kastratov se med seboj ne razlikujejo v odtenkih rdeče (vrednost -a) in rumene (vrednost -b) barve. Tudi Teixeira in sod. (2005) menijo, da ima meritev -L pri ženskem spolu višjo vrednost kot pri samcih, hkrati pa navajajo, da spol živali nima vpliva na vrednosti -a in -b. Beltram (2000) ugotavlja podobno, da je barva mišic kastratov nekoliko temnejša od mišic svinjk, hkrati pa ugotavlja tudi značilen vpliv spola na rdeč (vrednost -a) in rumen (vrednost -b) odtenek barve.

Ugotovitev, da je sušena vratina kastratov bolj zamaščena (preglednica 1) vendar temnejša v primerjavi z vratino svinjk (preglednica 2 in preglednica 3), se ne sklada s trditvijo Skvarča (2001), da na barvo posredno vpliva tudi zamaščenost mesa in je marmorirana mišičnina svetlejša.

Instrumentalno izmerjena tekstura sušenih vratin kastratov je bolj čvrsta (višje vrednosti za silo in delo) od vratin svinjk (preglednica 4), kar se ne sklada s senzorično oceno teksture, ki med spoloma ni pokazala razlik. (preglednica 3).

Preglednica 4. Vpliv spola, na instrumentalno izmerjeno barvo in teksturo sušenih vratin
Table 4. Effect of sex on instrumental measurement of colour and texture of dried neck

	Kastrati / Hogs LSM ± SE	Svinjke / Sows LSM ± SE	Znač.
Barva (Minolta) / Colour (Minolta)			
Vrednost -L / Value -L	33,03 ± 0,38b	34,37 ± 0,42a	*
Vrednost -a / Value -a	11,45 ± 0,21a	11,90 ± 0,22a	nz
Vrednost -b / Value -b	5,28 ± 0,19a	5,70 ± 0,20a	nz
Tekstura (Kramer) / Texture (Kramer)			
Sila (N) / Force (N)	204,63 ± 8,12a	161,58 ± 8,83b	*
Delo (J) / Work (J)	569,26 ± 22,58a	549,41 ± 24,57a	nz

LSM – tehtane srednje vrednosti; SE – standardna napaka ocene; * – $p \leq 0,05$; nz – statistično neznačilen vpliv ($p > 0,05$); skupine z enako črko v indeksu se med seboj statistično značilno ne razlikujejo

LSM – least square means; SE – standard error; * – $P \leq 0,05$; nz – no significant influence ($P > 0.05$); no difference between group with the same index

SKLEPI

Prehrana prašičev z izohranilno krmno mešanico in različnimi dodatki za izboljšanje maščobnokislinske sestave mišičnih lipidov ne vpliva na vsebnost skupnih maščob v izdelkih sušenih vratin. Bolj so zamaščene sušene vratine, pridobljene iz kastratov, kot iz svinjk.

Senzorično ocenjen skupni vtis sušenih vratin je najbolje ocenjen pri skupinah, krmljenih s kontrolno mešanico in z dodatkom lanu. Enakomernost barve mišičnine se v skupini z dodatkom vitamina E občutno poslabša. Najslabše ocenjena aroma je pri skupinah z dodatkom lanu in vitamina E. Največ prisotnih priokusov je zaznati pri sušenih vratinah prašičev, krmljenih z lanom in ogrščico, dodatek vitamina E k ogrščici pa te priokuse nekoliko ublaži.

Instrumentalne meritve so pokazale najčvrstejšo teksturo sušenih vratin skupine z dodatkom ogrščice in najsvetlejšo barvo skupine z dodatkom vitamina E. Barva sušene vratine svinjk je intenzivnejša vendar svetlejša od barve vratine kastratov. Sušena vratina kastratov je čvrstejša od vratine svinjk.

SUMMARY

Feeding with different diet to improve fatty acid composition of muscles lipids do not have an influence on fat contain. There is more fat found in dried neck of hogs than sows.

Sensory estimate common effect of dried neck has the highest mark at the group feeding by control and with adding flax. Adding of vitamin E in pig fodder make equability of colour worse. Aroma was the worst by the diet with flax and vitamin E. The most of aftertaste are known by feeding with addition of flax and rapeseed. Diet with rapeseed and vitamin E alleviate aftertaste.

Instrumental measurements have shown the strongest texture of dried neck by feeding with adding of rapeseed. The lighter colour is notice by adding of vitamin E. Colour of dried neck of sows is more intensely but lighter then colour of hogs. Hogs have more tightly dried neck as sows.

VIRI

AOAC Official Method 991.36 Fat (Crude) in Meat and Meat Product. V: Official methods of analysis of AOAC International. 16th ed. (Ed.: Cunnif, P.). Washington, AOAC International, 1997, 39–2.

Armero, E./ Flores, M./ Toldra, F./ Barbosa, J.A./ Olivet, J./ Pla, M./ Baselga, M. Effect of pig sire type and sex on carcass traits, meat quality and sensory quality of dry – cured ham. Journal of the Science of Food and Agriculture, 66(1999), 297–282.

Beltram, B. Kakovost mišičnine prašičev različnih genotipov. Diplomska naloga. Ljubljana, Biotehniška fak., Odd. za živilstvo, 2000, 40–44.

Berčič, T. Kraški zašink. Meso in mesnine, 5(2004)2, 38–40.

CIE. Supplement No. 2 to CIE Publication No. 15 (E-1.3.1.) 1978, 1971/(TC-1-3). Recommendations on uniform color spaces-color difference equations, Psychometric Color Terms. Commission Internationale de l'Eclairage, Paris, 1976.

Corino, C./ Magin, S./ Pagliarini, E./ Rossi, R./ Pastorelli, G./ Chiesa, L.M. Effects of dietary fats on meat quality and sensory characeristics of heavy pig lions. Meat Science, 6(2002)1, 1–8.

Čandek-Potokar, M./ Šegula, B. Klavna kakovost prašičev. Tehnološki list. Ljubljana, Kmetijski inštitut Slovenije, 1996, 3–16.

Čepin, S./ Žgur, S. Možnosti zmanjševanja maščob in holesterola v prireji mesa. V: Meso in mesnine za kakovostno prehrano. 2. posvet o vlogi in pomenu mesa v normalni – zdravi in dietni prehrani, Portorož, 2000-02-10/11, (ur.: Žlender, B./ Gašperlin, L.). Ljubljana, Biotehniška fak., Odd. za živilstvo, 2000, 49–65.

Deckel, M.J./ Casteels, M./ Warnants, N./ Damme, L./ Boucque, C.V. Omega-3 fatty acids in pig nutrition: Implications for the intrinsic and sensory quality of the meat. Meat Science, 44(1999)1/2, 55–63.

Enser, M./ Scollan, N./ Gulati, S./ Richardson, I./ Nute, G./ Wood, J. The effect of ruminally-protected dietary lipid on the lipid composition and quality of beef muscle. Proceedings 47th International Congress of Meat Science and Technology, Krakow, Poland, 2001, 186–187.

Hoz, L./ Arrigo, M.D./ Cambero, I./ Ordonez, J.A. Development of an *n*-3 fatty acid and *α*-tocopherol enriched dry fermented sausage. Meat Science, 67(2004)3, 485–495.

Kitts, D. Toxicity and safety of fats and oils. V: Bailey's industrial oil and fat products. Vol 1. (ur.: Hui, Y.H.). New York, John Wiley & Sons, 1996, 226–229.

Latorre, M.A./ Lazaro, R./ Gracia, M.I./ Nieto, M./ Mateos, G.G. Effect of sex and terminal sire genotype on performance, carcass characteristics, and meat quality of pigs slaughtered at 177 kg body weight. Meat Science, 65(2003)4, 1369–1377.

Orthofer, F.T. Vegetable oils. V: Bailey's industrial oil and fat products. Vol 1. (ur.: Hui, Y.H.). New York, John Wiley & Sons, 1996, 19–29.

O'Sullivan, M.G./ Byrne, D.V./ Stagsted, J./ Andersen, H.J./ Martens, M. Sensory colour assessment of fresh meat from pigs supplemented with iron and vitamin E. Meat Science, 60(2002)3, 253–265.

Rajar, A. Senzorične lastnosti mesnih izdelkov. Meso in mesnine, 3(2002)1, 50–50

Rey, A.I./ Lopez-Bote, C.J./ Kerry, J.P./ Lynch, P.B./ Buckley, D.J./ Morrissey, P.A. Modification of lipid composition and oxidation in porcine muscle and muscle microsomed as affected by dietary supplementation of n-3 with either n-9 or n-6 fatty acids and α-tocopheryl acetate. Animal Feed Science and Technology, 113(2003)1/4, 223–238.

Scaife, J.R./ Moyo, J./ Galbraith, H./ Michine, W./ Campbell, V. Effect of different dietary supplemental fats and oils on the tissue fatty acid composition and growth of female broilers. British Poultry Science, 35(1994), 107–118.

Shahidi, F. North America production of canola. V: Canola and rapeseed (ur.: Shahidi, F.). New York, Memorial University of Newfouland, Departmement of Biochemistry, 1990, 16–16.

Skvarča, M. Senzorične lastnosti mesa. Sodobno kmetijstvo, 34(2001)3, 126–130.

Teixeira, A./ Batista, S./ Delfa, R./ Cadavez, V. Lamb meat guality of breeds with protected origin designation. Influence of breed, sex and live weight. Meat Science, 71(2005)3, 530–536.

Žlender, B. Sestava in prehranska vrednost mesa in mesnih izdelkov. V: Meso v prehrani in zdravje. Posvet posvečen 50. obletnici Biotehniške fakultete, Radenci, 1997-11-20/21 (ur.: Žlender, B./ Gašperlin, L.). Ljubljana, Biotehniška fak., Odd. za živilstvo, 1997, 95–102.

Acta agriculturae Slovenica, 88(november 2006)1, 29–36.

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

Agris category codes: L50 COBISS Code 1.01

MHS STATUS AND SALIVARY CORTISOL CONCENTRATION IN INDIVIDUALLY HOUSED PIGS *

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Received October 24, 2006, accepted November 20, 2006.
Delo je prispelo 24. oktobra 2006, sprejeto 20. novembra 2006.

ABSTRACT

Salivary cortisol was evaluated as stress measure in pigs of two malignant hyperthermia (MH) genotypes (NN and Nn), housed individually in metabolic cages and in comfortable large pens with straw. Three replicates were done, each including 8 German Landrace barrows, four (2 NN, 2 Nn) housed in pens and four (2 NN, 2 Nn) in metabolic cages. Altogether there were 24 animals included in the experiment. Saliva samples of all animals were collected simultaneously every 15 minutes between 8.00 and 11.00 a.m. on days 8, 22 and 36 of the experiment. Pigs in more stressful conditions (metabolic cages) had higher salivary cortisol values than pigs in pens, indicating that salivary cortisol might be a suitable indicator of stress in pigs. Higher salivary cortisol values in NN- in comparison to Nn- pigs indicated stronger response to stressful conditions in NN-genotype.

Key words: pigs / animal physiology / endocrinology / malignant hyperthermia / saliva / plasma / cortisol

MHS – STATUS IN KONCENTRACIJA KORTIZOLA V SLINI PRI INDIVIDUALNO UHLEVLJENIH PRAŠIČIH †

IZVLEČEK

Kortizol v slini je bil ovrednoten kot pokazatelj stresa pri prašičih dveh MH – genotipov (NN in Nn) v dveh individualnih uhlevitvah (metabolne kletke in prostorni boksi z nastilom). V vsaki od treh ponovitev je bilo 8 kastratov pasme nemška landrace. Štiri živali (2 NN, 2 Nn) so bile uhlevljene v bokse, štiri (2 NN, 2 Nn) pa v metabolne kletke. Skupno je bilo v raziskavo vključenih 24 živali. Vzorci sline so bili odvzeti istočasno od vseh živali, vsakih 15 minut med 8. in 11. uro, in sicer 8., 22. in 36. dan poskusa. Živali v bolj stresnem okolju (metabolne kletke) so imele višjo koncentracijo kortizola v slini kot živali v boksih, kar nakazuje, da je kortizol v slini primeren pokazatelj stresa pri prašičih. Višje koncentracije kortizola pri živalih NN – genotipa v primerjavi z genotipom Nn kažejo na močnejši odziv na stresne razmere pri genotipu NN.

Ključne besede: prašiči / fiziologija živali / endokrinologija / maligna hipertermija / slina / plazma / kortizol

* The paper presents a part of the results from the Ph.D. thesis ´The estimation of the degree of stress in pigs with the evaluation of cortisol concentration in plasma, urine and saliva´, issued by Nataša Siard, supervisor prof. Ivan Štuhec, Ph.D.

† V prispevku je predstavljen del rezultatov doktorske disertacije Nataše Siard ´Ocenjevanje stopnje stresa pri prašičih z določanjem koncentracije kortizola v krvni plazmi, urinu in slini´, mentor prof. dr. Ivan Štuhec.

INTRODUCTION

The adrenocortical hormone cortisol measured in plasma is often used as a stress indicator in pigs. However, the procedures for obtaining blood can, in itself, stimulate cortisol release, especially in case of venipuncture. The use of vein catheters is considered less stressful, but health problems can occur and only a very limited number of animals can be included in the experiment. In addition, insertion of catheters is a skilled technique and catheters are often difficult to maintain over longer periods. Measurement of cortisol in saliva is a noninvasive way of sampling. Saliva samples can be obtained also on farms, without any health risk for the animals. Furthermore, since there is no question of catheter functioning, the experiments can last for a longer period.

Salivary cortisol measurement was found to be a suitable replacement for plasma cortisol measurement in various species. In man, Vining *et al*. (1983) described the correlation between salivary and serum cortisol as excellent. High correlations were found in different situations: in patients with adrenal insufficiency and in healthy people, in tests of adrenal function (dexamethasone suppression, ACTH stimulation) and circadian variation as well as in random samples. The results of Laudat *et al*. (1988) confirmed excellent correlation in normal people and in patients with adrenal disfunction. According to Fell *et al*. (1985) and Fell and Shutt (1986), salivary cortisol was a reliable indicator of stress in sheep (transport and confinement) and calves (transport, castration) as well as in goats during transport (Greenwood and Shutt, 1992). Steinhardt and Thielscher (2000) confirmed good agreement between plasma and salivary cortisol in calves. Patzl *et al*. (1992) tested reliability of salivary cortisol in dogs in non-stressful conditions. Results were in good agreement with plasma cortisol. For horses, van der Kolk *et al.* (2001) found high correlations between total plasma and salivary cortisol, while Elsaesser *et al*. (2001) did not find salivary cortisol a reliable marker of the training status and fitness in horses. The results for pigs are unclear. In prepubertal boars and gilts housed in metabolic cages the response to ACTH stimulation was lower in salivary than in plasma cortisol, indicating that salivary cortisol was less sensitive indicator of adrenal activity than plasma cortisol. However, it was concluded, that salivary cortisol is of use for stress assessment in intensively housed pigs (Parrott *et al*., 1989). Other research confirmed the usefulness of salivary cortisol as stress indicator in pigs: in prepubertal boars, stress (transport, mixing) resulted in salivary cortisol levels similar to those seen after ACTH stimulation (Parrott and Misson, 1989). On the other hand, Blackshaw and Blackshaw (1989) did not find salivary cortisol useful in the assessment of cortisol secretion, because the correlations between salivary and either total or free plasma cortisol in different categories of pigs housed in intensive conditions were low. The research of Cook *et al*. (1996) gave different results. High correlations were found between serum and salivary cortisol values following ACTH stimulation and restraint in female pigs. In the study of Bushong *et al.* (2000), the correlation between total cortisol concentration in plasma and saliva was affected by the duration of ACTH stimulation.

Despite unclear results, salivary cortisol is frequently used as a stress measure in pigs; for example, in the evaluation of different treatments of pregnant sows (Spoolder *et al*., 1996) and in the investigation of welfare of the pigs during transport (Bradshaw *et al*., 1996; Geverink *et al*., 1998). More recently, salivary cortisol was used as a stress measure in mixing of unfamiliar pigs (de Groot *et al*., 2001), as a physiology measure of coping styles in gilts (Geverink *et al*., 2002; Ruis *et al*., 2002) and as a measure of individual responses to weaning (van Erp-van der Kooij *et al*., 2003; Mason *et al*., 2003). O′Connell *et al*. (2003) used salivary cortisol as a stress measure in sows of different social status.

Salivary cortisol was used also in the evaluation of housing conditions in pigs. Space allowance, social contacts and control of the environment are important factors in adaptation to the environment. Ekkel *et al*. (1995) compared so called Specific-Stress-Free (SSF) housing

system to a conventional housing system and found lower cortisol concentrations in SSF pigs. In the study of Broom *et al*. (1995), pregnant sows in three housing systems did not show clear differences in salivary cortisol responses on different environment, while in the study of de Jong *et al*. (1998) enriched-housed growing pigs had higher salivary cortisol values than poor-housed pigs. The results of de Groot *et al*. (2000) are even more complex. Enriched-housed pigs showed higher concentrations of cortisol in saliva than barren-housed pigs, but only in the light period of the day. In the dark period of the day salivary cortisol concentrations were low in both housing conditions. Klont *et al*. (2001) compared the reactions to transport between poor- and enriched-housed pigs. Pigs from the barren environment had higher increase in salivary cortisol from farm to slaughter. De Leeuw *et al*. (2003) found higher salivary cortisol values in individually housed gilts with no substrate in comparison with those on wood shavings litter spread on the foraging area.

Adrenocortical response to stressful conditions is one important aspect, the other interesting one is the possible connection between malignant hyperthermia syndrome (MHS) and adrenocortical action. MHS is a reaction to acute stressors, like transport, high ambient temperature, mixing with unknown animals. It is inherited by a single recessive gene (n) and characterised by hyperthermia and muscle rigidity. These symptoms are often followed by sudden death within minutes of acute stress. Two malignant hyperthermia (MH) genotypes (NN and Nn) differed in adrenocortical response to stressful conditions. NN animals had higher cortisol concentration in plasma (Siard *et al*., 2003a) and urine (Siard *et al*., 2003b). To our knowledge, no comparisons between different MH genotypes have been made for salivary cortisol concentration in pigs.

The aim of the study was to evaluate two individual housing conditions of fattening pigs, pens with straw (enriched individual housing system) and metabolic cages (very barren environment), with salivary cortisol monitoring. Further aim was to assess the relationship between two MH genotypes (NN and Nn) and salivary cortisol concentration.

MATERIAL AND METHODS

The experiment was done in three replicates, each including eight German Landrace barrows housed individually, four in large pens with litter (1.98 x 1.93 m) and four in metabolic cages with wire mesh floor. Altogether, there were 24 animals included in the experiment. Pens bedded with straw were separated by solid wooden partitions. In the metabolic cages without straw, animals were able to stand up and lie down, but not to turn around.

The MH genotype was determined by a DNA-based test (Fujii *et al*., 1991). Altogether, 95 animals were checked. There were only six animals with nn-genotype and they were unevenly spred over the three replicates. Thus, it was not possible to include nn animals into the experiment. Therefore, half of the pigs in each housing condition were dominant homozygous (NN) and the other half were heterozygous (Nn).

Pigs were housed in the experimental environment 14 days prior to the experiment. The animals had no previous experience with the two housing conditions. The health condition of the animals was followed by daily measurement of body temperature. Dry meal and water were available *ad libitum*. The illumination and ventilation were natural.

Pigs were made familiar with chewing the cotton buds for saliva collection in a 14-day period prior to the experiment. There were three sampling days, in-between each day of collection there was a 14-day interval. Saliva samples were therefore collected on days 8, 22 and 36 of the experiment. On each sampling day the samples were collected simultaneously from all animals between 8.00h and 11.00h in 15 min. intervals. The collection of saliva did not take more than one minute; no restraint was needed. During the sampling period the water was withdrawn. Pigs

were always handled by a familiar person. They chewed the buds until they were thoroughly moistened. The buds were then fitted into glass centrifuge tubes. The saliva was extracted from the buds by centrifugation. All samples were centrifuged immediately at 2500 x g for 15 minutes and stored at –20 °C.

The volumes of saliva samples were not larger than 300 µl. Due to low volumes, salivary cortisol was determined by chemiluminescence method (kit provided by Nichols Institute Diagnostica). The intra-assay variability was up to 10%.

Salivary cortisol data had to be logarithmically transformed ($\mathbf{y_{ijklmn}}$) to achieve normality. The following fixed effects were included in the model: the three replicates ($\mathbf{R_i}$), the two housing conditions ($\mathbf{H_j}$), the two MH genotypes ($\mathbf{G_k}$), the individual animals ($\mathbf{A_{ijkl}}$) and the sampling day ($\mathbf{D_{im}}$). Possible changes of cortisol concentration within sampling day (trial) were described by the linear regression ($\mathbf{b_{im}}$). The model also included two and three level interactions, which showed the significant effects in the preliminary analysis:

$$y_{ijklmn} = \mu + R_i + H_j + G_k + A_{ijkl} + D_{im} + b_{im}(x_{imn} - 5.5) + RH_{ij} + RG_{ik} + RHG_{ijk} + e_{ijklmn}$$

RESULTS

Salivary cortisol values for individual pig from the three replicates are presented in Figure 1. Considerable variability between and within the animals was observed in all replicates (Figure 1). The average values per animal were ranging from 3.3 to 36.0 ng ml^{-1}. Standard deviation within animal was high. However, the average cortisol concentration in saliva was the smallest in replicate three (in the first replicate the average ± SD was 17.3 ± 11.7 ng ml^{-1}, in the second 16.8 ± 7.9 ng ml^{-1} and in the third replicate 8.9 ± 6.3 ng ml^{-1}). Transformed data from the model included also the extreme values from Figure 1, which presents untransformed data.

Table 1. Statistically significant effects on salivary cortisol concentration
Preglednica 1. Statistična značilnost vplivov na koncentracijo kortizola v slini

Effects Vplivi	Degrees of freedom Stopinje prostosti	P-value p-vrednost
Replicate Ponovitev	2	0.0001
Animal Žival	12	0.0001
Housing condition Uhlevitev	1	0.0001
MH genotype MH - genotip	1	0.01
Sampling day Dan odvzema vzorca	6	0.0001
Trial – linear regression Odvzem – linearna regresija	9	0.0001
Replicate × housing Ponovitev × uhlevitev	2	0.002

High variability of salivary cortisol values between and within the animals (Figure 1) was reflected in the high significance of animal and replicate. The effects of housing condition and MH genotype were significant (Table 1). Pigs in metabolic cages had higher cortisol values than

pigs in individual pens. In addition, NN animals had higher cortisol values than Nn animals (Table 2). The effect of sampling day within the replicate was highly significant. There was no trend up or down in the 4-week period within the replicates. The effect of trial (3-hr period of sampling within the sampling day) was also highly significant. The possible reason for the significant effect of interaction between replicate and housing (Table 1) is again high variability in cortisol values between and within the animals.

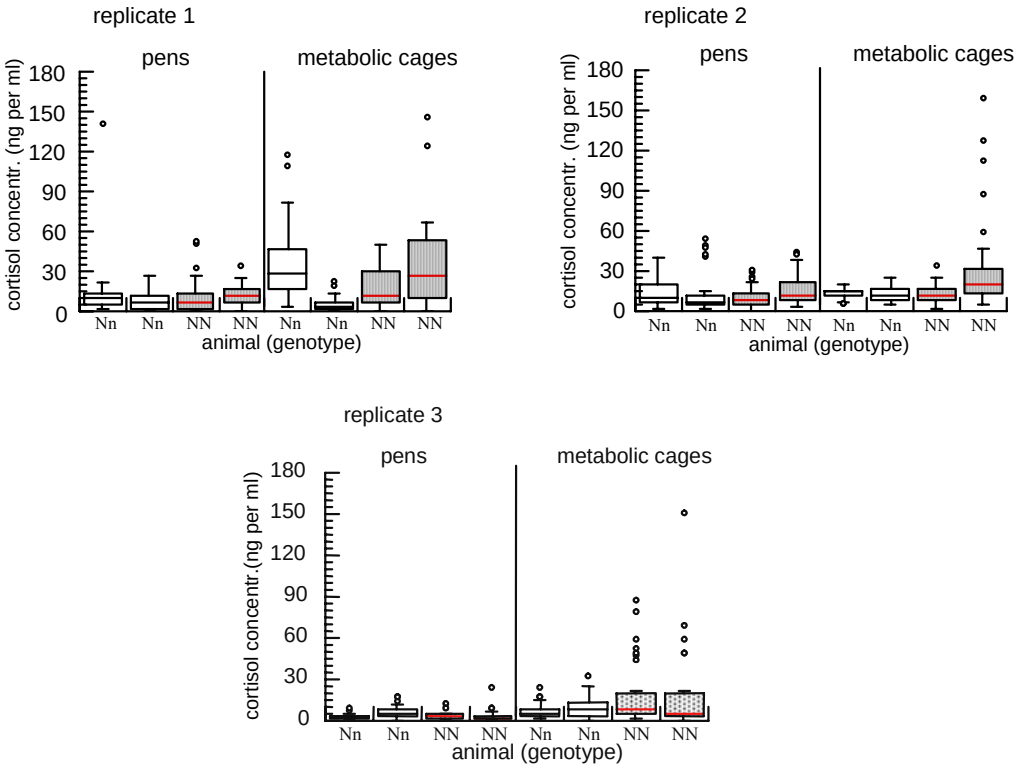

Figure 1. Salivary cortisol values for each pig respectively in the three replicates. Boxes represent the second and the third quartile and bars represent the first and the fourth quartile. The lines in the boxes are medians, the points are extreme values (by default the values larger than 3 SD).

Slika 1. Koncentracija kortizola v slini pri posameznih živalih v treh ponovitvah. Boksi predstavljajo drugi in tretji kvartil, navpične črte pa prvi in četrti kvartil. Prečne črte v boksih so mediane, točke pa ekstremne vrednosti (večje od treh standardnih odklonov).

Table 2. Least square means (LSM) and standard errors (s.e.) for salivary cortisol in two housing conditions and in two MH genotypes

Tabela 2. Vrednosti LSM in s.e. za koncentracijo kortizola v slini pri različnih uhlevitvah in MH – genotipih

Effects Vplivi	LSM ± S.E.	Difference
Housing / Uhlevitev		
Individual pens / Individualni boksi	0.78 ± 0.021	–0.244 ± 0.029
Metabolic cages / Metabolne kletke	1.03 ± 0.020	
MH genotype / MH-genotip		
NN	0.94 ± 0.020	0.069 ± 0.028
Nn	0.87 ± 0.020	

DISCUSSION

High variability in salivary cortisol values between and within the animals was found. That was observed also in plasma (Siard *et al*., 2003a) and urine (Siard *et al*., 2003b). High variability is reflected in the significant effects of replicate and housing, and presumably also in the effect of interaction between replicate and housing (Table 1).

The animals in metabolic cages had higher salivary cortisol values than pigs in pens (Table 2). This is in line with the results for plasma cortisol (Siard *et al*., 2003a) and with the results of de Leeuw *et al*. (2003), who found higher salivary cortisol values in individually housed gilts with no substrate in comparison with those on substrate. However, considering other studies in which they evaluated different housing conditions with salivary cortisol, the picture is not clear. In the study of de Jong *et al.* (1998), pigs in enriched, presumably less stressful environment, had higher salivary cortisol values than pigs in poor environment. They found these results surprising and they tried to explain them with the possible underlying physiological mechanisms. One of the possible explanations was flattened circadian rhythm in stressed animals. De Groot *et al.* (2000) indeed found blunted circadian rhythm of salivary cortisol secretion in more stressful conditions (small pens with no straw in comparison to large pens with bedding). However, in their study the samples were taken every two hours and that is quite a long interval. Considering our results, where the linear regression within 3-hr period was significant (Table 1), the 2-hr interval might have not reflected adequately the circadian rhythm of cortisol secretion. The effect of sampling day was also significant (Table 1), however, no trend was observed in a 4-week period.

NN animals had higher cortisol values than Nn animals. This is in line with plasma (Siard *et al.,* 2003a) and urine (Siard *et al.,* 2003b) cortisol results. To our knowledge, no studies were done on the relationship between salivary cortisol concentration and MH genotypes. However, results from this study and from the previous ones on plasma and urine cortisol with relation to MH status, show higher adrenocortical activity in stress resistant NN-animals. These results are comparable with the results of Mitchell and Heffron (1981). They found higher plasma cortisol values in stress resistant pigs (the animals were classified into resistant and susceptible groups by the halothane test). They suggested that reduced cortisol secretion contributed to the activation of MHS. Higher ability of adrenal cortex reactivity is perhaps good for better acute stress coping in NN genotype.

CONCLUSIONS

The animals in metabolic cages (more stressful environment) had higher salivary cortisol values than pigs in large individual pens with straw. This indicates that saliva cortisol might be, like plasma cortisol, a suitable indicator of stress in pigs. This is important, because saliva sampling can be done in practice, while blood samples can only be taken in research conditions. Furthermore, contrary to blood sampling, saliva collection is not an invasive method. Higher cortisol concentration in saliva in NN- in comparison to Nn- pigs indicates stronger response to stressful conditions in stress resistant NN-genotype. Presumably that is in favour for better acute stress coping in this genotype.

ACKNOWLEDGEMENTS

We would like to thank K. Weirauch and his colleagues in Trenthorst for cortisol analyses, prof. dr. M. Kovač for help in statistical analyses and colleagues from Zootechnical Department in Domžale for technical assistance.

REFERENCES

Blackshaw, J.K./ Blackshaw, A.W. Limitations of salivary and blood cortisol determinations in pigs. Veterinary Research Communications, 13(1989), 265–271.

Bradshaw, R.H./ Parrott, R.F./ Goode, J.A./ Lloyd, D.M./ Rodway, R.G./ Broom, D.M. Behavioural and hormonal responses of pigs during transport: effect of mixing and duration of journey. Animal Science, 62(1996), 547–554.

Broom, D.M./ Mendl, M.T./ Zanella, A.J. A comparison of the welfare of sows in different housing conditions. Animal Science, 61(1995), 369–385.

Bushong, D.M./ Friend, T.H./ Knabe, D.A. Salivary and plasma cortisol response to adrenocorticotropin administration in pigs. Laboratory Animals, 34(2000), 171–181.

Cook, N.J./ Schaefer, A.L./ Lepage, P./ Morgan Jones, S. Salivary vs. serum cortisol for the assessment of adrenal activity in swine. Canadian Journal of Animal Science, 76(1996), 329–335.

de Groot, J./ Ruis, M.A.W./ Scholten, J.W./ Koolhaas, J.M./ Boersma, W.J.A. Long-term effects of social stress on antiviral immunity in pigs. Physiology and Behavior, 73(2001), 145–158.

de Groot, J./ de Jong, I.C./ Prelle, I.T./ Koolhaas, J.M. Immunity in barren and enriched housed pigs differing in baseline cortisol concentration. Physiology and Behavior, 71(2000), 217–223.

de Jong, I.C./ Ekkel, E.D./ van de Burgwal, J.A./ Lambooij, E./ Korte, S.M./ Ruis, M.A.W./ Koolhaas, J.M./ Blokhuis, H.J. Effects of strawbedding on physiological responses to stressors and behavior in growing pigs. Physiology and Behavior, 64(1998), 303–310.

de Leeuw, J.A./ Ekkel, E.D./ Jongbloed, A.W./ Verstegen, M.W.A. Effects of floor-feeding and the presence of a foraging substrate on the behaviour and stress physiological response of individually housed gilts. Applied Animal Behaviour Science, 80(2003)4, 297–310.

Ekkel, E.D./ Van Doorn, C.E.A./ Hessing, M.J.C./ Tielen, M.J.M. The Specific-Stress-Free housing system has positive effects on productivity, health, and welfare of pigs. Journal of Animal Science, 73(1995), 1544–1551.

Elsaesser, F./ Klobasa, F./ Ellendorff, F. ACTH Stimulationstest und Bestimmung von Cortisol im Blut und Speichel zur Bewertung des Trainings-zustands/der Kondition beim Warmblutpferd. Deutsche tierärztliche Wochenschrift, 108(2001)1, 31–36.

Fell, L.R./ Shutt, D.A. Use of salivary cortisol as an indicator of stress due to management practices in sheep and calves. Proceedings of the Australian Society for Animal Production, 16(1986), 203–206.

Fell, L.R./ Shutt, D.A./ Bentley, C.J. Development of a salivary method for detecting changes in plasma “free” cortisol arising from acute stress in sheep. Australian Veterinary Journal, 62(1985), 403–406.

Fujii, J./ Otsu, K./ Zorzato, F./ de Leon, S./ Khanna, V.K./ Weiler, J.E./ O`Brien, P.J./ Maclennan, D.H. Identification of mutation in Porcine Ryanodine Receptor associated with malignant hyperthermia. Science, 253(1991), 448–451.

Geverink, N.A./ Bradshaw, R.H./ Lambooij, E./ Wiegant, V.M./ Broom, D.M. Effects of simulated lairage conditions on the physiology and behaviour of pigs. Veterinary Record, 143(1998), 241–244.

Geverink, N.A./ Schouten, W.G.P./ Gort, G./ Wiegant, V.M. Individual differences in aggression and physiology in peri-pubertal breeding gilts. Applied Animal Behaviour Science, 77(2002), 43–52.

Greenwood, P.L./ Shutt, D.A. Salivary and plasma cortisol as an index of stress in goats. Australian Veterinary Journal, 69(1992), 161–163.

Klont, R.E./ Hulsegge, B./ Hoving-Bolink, A.H./ Gerritzen, M.A./ Kurt, E./ Winkelman-Goedhart, H.A./ de Jong, I.C./ Kranen, R.W. Relationships between behavioural and meat quality characteristics of pigs raised under barren and enriched housing conditions. Journal of Animal Science, 79(2001), 2835–2843.

Laudat, M.H./ Cerdas, S./ Fournier, R.C./ Guiban, C./ Guilhaume, B./ Luton, J.P. Salivary cortisol measurement: a practical approach to assess pituitary-adrenal function. The Journal of Clinical Endocrinology and Metabolism, 66(1988), 343–348.

Mason, S.P./ Jarvis, S./ Lawrence, A.B. Individual differences in responses of piglets to weaning at different ages. Applied Animal Behaviour Science, 80(2003)2, 117–132.

Mitchell, G./ Heffron, J.J.A. Plasma cortisol levels in pigs susceptible and resistant to malignant hyperthermia. Journal of the South African Veterinary Association, 52(1981), 109–112.

O'Connell, N.E./ Beattie, V.E./ Moss, B.W. Influence of social status on the welfare of sows in static and dynamic groups. Animal Welfare, 12(2003)2, 239–249.

Parrott, R.F./ Misson, B.H. Changes in pig salivary cortisol in response to transport simulation, food and water deprivation, and mixing. British Veterinary Journal, 145(1989), 501–505.

Parrott, R.F./ Misson, B.H./ Baldwin, B.A. Salivary cortisol in pigs following adrenocorticotrophic hormone stimulation: comparison with plasma levels. British Veterinary Journal, 145(1989), 362–366.

Patzl, M./ Mostl, E./ Bamberg, E. Determination of cortisol in saliva of dogs. In: 5th Congress of ISACB, Parma, 1992-09-02/06, 337–339.

Ruis, M.A.W./ Te Brake, J.H.A./ Engel, B./ Buist, W.G./ Blokhuis, H.J./ Koolhaas, J.M. Implications of coping characteristics and social status for welfare and production of paired growing pigs. Applied Animal Behaviour Science, 75(2002), 207–231.

Siard, N./ Kovač, M./ Ladewig, J./ Štuhec, I. Relationship between MHS status and plasma cortisol concentration in individually confined pigs. Czech Journal of Animal Science, 48(2003a)7, 265–270.

Siard, N./ Kovač, M./ Ladewig, J./ Štuhec, I. Urine cortisol in pigs of different MH-genotypes. Research Reports, Agriculture, Zootechny, 82(2003b)1, 47–55.

Spoolder, H.A.M./ Burbidge, J.A./ Edwards, S.A./ Simmins, P.H./ Lawrence, A.B. Effects of food level and straw bedding during pregnancy on sow performance and responses to an ACTH challenge. Livestock Production Science, 47(1996), 51–57.

Steinhardt, M./ Thielscher, H.H. Transportbelastung bei Milchrindern. Effekte von Aufzuchtbedingungen auf metabolische und hormonelle Variablen. Tierärztliche Umschau, 55(2000), 22–28.

van der Kolk, J.H./ Nachreiner, R.F./ Schott, H.C./ Refsal, K.R./ Zanella, A.J. Salivary and plasma concentration of cortisol in normal horses and horses with Cushing´s disease. Equine Veterinary Journal, 33(2001)2: 211–213.

van Erp-van der Kooij, E./ Kuijpers, A.H./ van Eerdenburg, F.J.C.M./ Dieleman, S.J./ Blankenstein, D.M./ Tielen, M.J.M. Individual behavioural characteristics in pigs – influences of group composition but no differences in cortisol responses. Physiology and Behavior, 78(2003)3, 479–488.

Vining, R.F./ McGinley, R.A./ Maksujtis, J.J./ Yho, K. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. Annals of Clinical Biochemistry, 20(1983), 329–335.

Acta agriculturae Slovenica, 88(november 2006)1, 37–51.

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Agris category codes: Q03 COBISS Code 1.01

THE PRESENCE OF SOME PATHOGEN MICRO ORGANISMS, YEASTS AND MOULDS IN CHEESE SAMPLES PRODUCED AT SMALL DAIRY-PROCESSING PLANTS

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Received September 26, 2006, accepted October 03, 2006.
Delo je prispelo 26. septembra 2006, sprejeto 03. oktobra 2006.

ABSTRACT

The presence of pathogenic and some indicator micro organisms was studied in 40 samples of cheese comprising 14 curd samples, 13 samples of soft ripened salted or non-salted cheese and 13 samples of semi-hard cheese manufactured at five small dairy-processing plants. The mean number of coagulase-positive staphylococci in all tested samples was 2.5×10^4 cfu* g^{-1}, while the number of *E. coli* bacteria was 1.4×10^6 cfu g^{-1}. In 20.0% out of 40 samples tested, the number of coagulase-positive staphylococci exceeded the prescribed regulations, particularly in soft cheese (12.5%) and curd (7.5%). About 17.5% of samples were contaminated with *E. coli* in higher concentrations than national valid regulations allowed. The number of *E. coli* was mostly exceeded in soft cheese and curd in 12.5% and 5.0% of all examined samples, respectively. One sample of semi hard cheese was contaminated with sulphite-reducing clostridia. *Proteus* was detected in 3 samples (7.5%) and *L. grayi* in 1 (2.5%) sample. *Salmonela* and *L. monocytogenes* were not detected. According to the valid regulations 9 (22.5%) samples in our investigation did not reach the adequate microbiological quality. Both, yeasts and moulds were isolated from 60% of tested cheese samples with average concentrations of 5.8×10^4cfu $g^{-1}$and 2.0×10^4cfu g^{-1}, respectively. The genera *Geotrichum* (91.9%), *Moniliella* (5.4%) and *Aspergillus* (2.7%) were the most frequently isolated strains from examined cheese samples. The *Aspergillus* strains did not belong to the species *A. flavus* or *A. parasiticus* and did not produce aflatoxins.

Key words: milk products / cheese / microbiology / pathogen micro-organisms / yeasts / moulds / aflatoxins

PRISOTNOST NEKATERIH PATOGENIH MIKROORGANIZMOV, KVASOVK IN PLESNI V VZORCIH SIRA, PROIZVEDENIH V MALIH MLEKARSKO-PREDELOVALNIH OBRATIH

IZVLEČEK

Proučevali smo prisotnost patogenih in nekaterih indikatorskih mikroorganizmov v 40 vzorcih sira, od tega v 14 vzorcih skute, 13 vzorcih soljenega ali nesoljenega svežega (mehkega) sira in 13 vzorcih poltrdega tipa sira, proizvedenih v petih malih mlekarsko-predelovalnih obratih. Povprečno število koagulaza-pozitivnih stafilokokov v vseh vzorcih je znašalo $2,5 \times 10^4$ KE† g^{-1}, medtem ko je bilo število bakterij vrste *E. coli* $1,4 \times 10^6$ KE g^{-1}. Izmed 40 vzorcev je v 20.0 % število koagulaza- pozitivnih stafilokokov presegalo slovenske veljavne normative, posebno v vzorcih mehkega sira (12,5 %) in skute (7,5 %). Okrog 17,5 % vzorcev je bilo okuženo z

* Cfu: colony forming units
† KE: kolonijske enote

bakterijami *E. coli* v višjih koncentracijah, kot to dovoljujejo slovenski veljavni predpisi. Število bakterij *E. coli* je bilo previsoko v vzorcih mehkega sira (12,5 %) in skute (5,0 %). En vzorec poltrdega sira je vseboval sulfid-reducirajoče klostridije. Vrste rodu *Proteus* smo ugotovili v 3 (7,5 %) in *L. grayi* v 1 (2,5 %) vzorcev. *Salmonella* in *L. monocytogenes* nista bili ugotovljeni v nobenem izmed preiskanih vzorcev. Izmed 40 preiskanih vzorcev jih 9 (22,5 %) ni ustrezalo veljavnim slovenskim normativom glede njihove mikrobiološke kakovosti. Tako kvasovke kot plesni so bile izolirane iz 60 % preiskanih vzorcev. Povprečno število kvasovk je bilo $5{,}8 \times 10^4$ KE g^{-1} in plesni $2{,}0 \times 10^4$ KE g^{-1} vzorca. Najpogosteje so bile izolirane plesni iz rodov *Geotrichum* (91,9 %), *Moniliella* (5,4 %) in *Aspergillus* (2,7 %). Sev iz rodu *Aspergillus* ni pripadal vrstama *A. flavus* /*parasiticus* in tudi ni tvoril aflatoksinov.

Ključne besede: mlečni izdelki / siri / mikrobiologija / patogeni mikroorganizmi / kvasovke / plesni / aflatoksini

INTRODUCTION

The number and types of micro-organisms present in milk and dairy products depends on the microbial quality of milk used, heat treatment of milk, the conditions in which the products are manufactured, the temperatures and duration of storage, feeding of the animals, season, area, general sanitation in the plant, quality of starter cultures, occurrence of phages, quality of rinsing water, etc. (Bramley, 1990; Anonim., 1994; Harkye and Ebenezer, 2002).

Examination for the presence and number of specific micro-organisms is, therefore, an integral part of any quality control or quality assurance plan and it may be applied to a number of areas: raw materials, intermediate samples, finished products, or environmental/equipment sites. The most common spoilage micro-organisms in milk and dairy products are *Pseudomonas*, coliforms, *Bacillus* spp, *Clostridium* spp, lactic-acid producing bacteria, yeasts and moulds, enterococci, etc. On the other hand, milk-borne and milk-product borne outbreaks, caused mostly by cheeses, represent 2–6% of bacterial food-borne outbreaks reported by surveillance systems from several countries. The cheeses represent a large risk of bacterial food-borne outbreaks because of pathogen micro-flora, divided into pathogens of current concern (*Salmonella* spp., *Campylobacter* spp., coagulase-positive staphylococci, *Listeria monocytogenes* etc.), and those which cause disease only occasionally (*Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens*, etc.) (De Buysier *et al*., 2001).

L. monocytogenes is a food-borne pathogen that can contaminate dairy products (Menendez *et al*., 2001). It is a psychrotroph and can grow on contaminated cheese at low temperatures. This bacteria is fairly heat-tolerant, widely distributed in dairy farms, and frequently found in raw milk. Some strains can survive pasteurization, and adapt to acidic environments. The most commonly occurring species in food are *L. innocua* and *L. monocytogenes*. Although *Listeria* is mostly inactivated under normal conditions of pasteurization (Farkye and Vedamuthu, 2002), problems can arise from post-pasteurisation contamination. A seasonal effect (with peaks in winter) was observed. The farm milk contamination is, most often, a sporadic event. The number of bacterial cells of *Listeria* was also very low which are very likely to be due to environmental contamination (Meyer Broseta *et al*., 2003).

The contamination of raw milk with *Salmonella* usually occurs as a result of transfer of faeces from an animal to the milk via unclean teats and udders. Such contamination can pass into milk during milking and, once present on milking parlour equipment, it can then readily proliferate and spread if such equipment is not adequately cleaned and sanitised. Its growth in milk should be limited by effective refrigeration (<8 ºC). Effective milking parlour hygiene (cleaning and disinfection of udders and teats), cleaning and sanitisation of milking equipment and subsequent milk storage systems are essential elements in preventing the spread of this organism (McManus and Lanier, 1987).

Sulphite-reducing clostridia (mostly *Cl. perfringens*) are spore-forms that are present in sediment of various types and in the intestinal tracts of man and animals. They gain entry to milk

via faeces, soil and feedstuff, especially silage. Strains may be psychrotrophic, mesophilic or thermophilic. Since most strains are strictly anaerobic, they have the greatest potential importance as spoilage organisms of cheese and canned milk products. They produce a number of soluble toxic substances (Gilmour and Rowe, 1990).

Examination of the presence of *E. coli* as an indicator of faecal contamination and/or poor hygienic practices has traditionally been done in dairy plants. It is well known that some strains might be enteropathogenic or enterotoxigenic. Both of these groups have been responsible for outbreaks of diseases involving cheese and milk (Anonim., 1994).

Coagulase-positive staphylococci (*Staphylococcus aureus* and related species) may cause human disease through the production of toxins. The formation of effective levels of toxin requires a high number of micro-organisms (approximately 10^5–10^6 micro-organisms per gram of food) at a pH value greater than 5 and so the presence of coagulase-positive staphylococci at a low level does not necessarily constitute a hazard. Dairy-related outbreaks of staphylococcal intoxication have been attributed to raw milk, dried milk, cheese and ice cream. Coagulase-positive staphylococci may be present in raw milk from the udder and teat canals of a cow, particularly if lesions are present. Also, the nasal area and hands of humans are recognised sites of contamination: poor personal hygiene can result in the contamination of milk and dairy products. Essential to the production of toxin is the growth of micro-organisms. In general, *Staphylococcus aureus* and the related species *Staphylococcus intermedius* and *Staphylococcus hyius* do not multiply at temperatures below 8 ºC while 10 ºC is the minimum for toxin production. These micro-organisms are, however, resistant to salt. Pasteurization will be effective against them but if toxins are present, they will not be inactivated. Therefore, toxins may be present in the absence of viable micro-organisms. The higher counts of *Staphylococcus* recorded in spring, when milk yields are at their peak, are a cause for concern and mammary infections (Anonim., 1994).

Pasteurization cannot guarantee the absence of pathogenic micro-organisms when they are present in large numbers in raw milk or due to post-pasteurization contamination (Salmeron *et al*., 2002).

The production of milk products should be in accordance with legal regulations for good sanitary practice on removal from the processing establishment. The microbiological criteria for cheese are *L. monocytogenes* and *Salmonella* (absent in 25 g or 1 g of sample), *S. aureus* (m=1 000, M=10 000, n=5, c=2, for fresh cheese: m=10, M=100, n=5, c=2), *E. coli* (m=10 000, M=100 000, n=5, c=2, for soft cheese m=100, M=1000, n=5, c=2) and coliforms at 30 ºC for soft cheese (m=10 000, M=100 000, n=5, C=2) (Off. J. of the European Communities, 1992, consolidated in 2004; Pravilnik…., Ur. l. RS, 2004). The microbiological criteria for foodstuffs according the Regulation EU no. 2073, (Off. J. of the European Communities, 2005) are more strict for *E. coli* in cheese (m=100, M=1000, n=5, c=2). The allowed number of *S. aureus* depends on the type of examined cheese and on the thermization of the milk, used for cheese production. These criteria are designed for the food products during or at the end of the production process, except for the presence of *Salmonella*, which should be estimated in milk products on the market.

Yeasts and moulds in some cheese types can periodically cause problems, both economic and sensory. Usually present in raw milk, they do not survive pasteurization; their presence in pasteurized milk and other milk products is caused by re-infection during manufacturing (Nelson, 1987; Jodral *et al.*, 1993). The contamination of milk products, particularly cheeses is due to yeasts and moulds presence in environment of cheese factories, like walls and shelves of ripening rooms, air, equipment, water, milk, brine, etc. (Chapman and Sharpe, 1990; Jay, 1992).

Yeasts themselves are not commonly the cause of defect in dairy products unless they ferment lactose. In this case they can grow rapidly and produce a typical yeasty or fruity flavour and obvious gas (Davis and Wilbey, 1990).

On the other hand, some specific strains in a starter cultures for soft cheese production or in the maturation and aroma formation play an important role in many cheese varieties, contributing with their metabolic properties to the ripening process. They metabolize lactic acid in the cheese, and raise the pH of the microenvironment in the area adjacent to the surface, and allow good growth and metabolic activities of the bacteria in the smear. Additionally, the lysis of yeast cells liberates vitamins and amino acids, which stimulate the bacteria, and provide flavour precursors. They also produce metabolites, e.g. short-chain fatty acids and other compounds, with known toxic effects against undesired micro-organisms in the intestinal tract (Jakobsen and Narvhus, 1996).

Although moulds have little practical importance in raw milk, they are important in pasteurized milk, particularly when it is used for the manufacture of cheese and other dairy products. The characteristic feature of some mould-ripened cheese types is extensive proteolysis and lipolysis. The presence of wild types of moulds is undesirable as they may influence the organoleptic characteristics of the cheeses, they can produce mycotoxins and represent a potential health risk (Jodral *et al.*, 1993; Wouters, *et al.*, 2002). The major toxigenic species of fungi belong to genera *Aspergillus, Fusarium, Acremonium* and *Phomopsis* (D'Mello and Macdonald, 1997).

For this purpose we wanted to find out the presence of pathogens and indicator micro-organisms including yeasts and moulds in 40 samples of curd, soft and semi-hard cheese from individual small dairy-processing plants that sold their products on the market. The objective of this study was also the identification of isolated mould strains and detection of their aflatoxin production.

MATERIAL AND METHODS

Sampling

A total of 40 samples of cheese comprising 14 curd samples, 13 samples of soft salted or non-salted cheese and 13 samples of semi-hard cheese were collected from November 2004 to January 2005. The cheeses were manufactured at five individual small dairy-processing plants that sold their products on the market.

The samples were taken in accordance with the instructions given in ISO/DIS 707, (1995).

Methods

Media

For the detection of *L. monocytogenes* according to EN ISO 11290-1 (1996), *Listeria* Enrichment Broth (Biokar Diagnostics, France) as pre-enrichment (inc. 30 ºC/24–48 h) and 1 Fraser broth (Merck, Germany) as enrichment broth (inc. 37 ºC/24–48 h) were used. Palcam (Biokar Diagnostics, France), Oxford (Biokar Diagnostics, France) and ChromAgar Listeria (Mast Diagnostica, Germany) were used for isolation. The immunological method Tecra Unique Listeria (Tecra, Australia) and API Listeria strips (Biomerieux, France) were used for confirmation and identification.

For the detection of *Salmonella* in cheese samples Buffered peptone water (Biokar Diagnostics, France) as a non-selective pre-enrichment medium and Selenite cystein buffer (Biokar Diagnostics, France) as an enrichment medium (ISO 6579, 2002) were used. XLD agar (Biokar Diagnostics, France), BSA agar (Biokar Diagnostics, France) and Rambach agar (Merck, Germany) were used for isolation. The immunological method Tecra Unique Salmonella (Tecra, Australia) and API 10 S strips (Biomerieux, France) were used for confirmation and identification.

Detection of *Proteus* spp. was carried out with inoculation of the sample into Nutrient broth (Biokar Diagnostics, France (inc. 37 ºC/24 h), spreading the colonies on the Brilliant Green Agar according to Edel and Kampelmacher (Biokar Diagnostics, France), typical colonies were confirmed and identified on a Kligler iron slant agar (Merck, Germany) and with API 10 S strips (Biomerieux, France).

For the enumeration of bacteria *Escherichia coli* the chromogenic medium COLI ID (Biomerieux, France) (inc. 37 ºC/24 h) and identification of the selected colonies with API 10 S strips (Biomerieux, France) were used.

For the enumeration of coagulase positive staphylococci (*Staphylococcus aureus* and other species) the Baird Parker with RPF supplement agar (Biokar Diagnostics, France) was used (SIST EN ISO 6888-2, 1999). The Petrifilm^{TM} Staph Express Count System (3 M^{TM}, USA) was used for confirmation.

The presence of sulphite-reducing clostridia spores was detected after heating the samples at the temperature of 80 ºC/10 minutes, inoculating the SPS agar according to Angelotti, (1962) (Merck, Germany) and incubation in anaerobic conditions (inc. 35 ºC/24–48 h).

The number of yeasts and moulds in samples the yeast-extract-glucose-chloramphenicol agar (YGC) (Merck, Germany) was used. Yeast and mould colonies growing on the plates were counted after 5 days incubation at 25 ºC. (ISO 6611/IDF 94, 2004).

Inoculation

For the enumeration of yeasts, moulds, coagulase-positive staphylococci and *E. coli* the primary suspensions of cheese samples were prepared with 3–5 min homogenization of 10 g of the sample in 90 ml of sterile 20% di-potassium hydrogen phosphate (Merck, Germany) using Bagmixer® 400 (Interscience, France). The primary suspensions of cheese samples were prepared with quarter-strength Ringer's solution and inoculated by pouring the plates with chosen medium (EN ISO 8261 (E), 2001). For detection of *Listeria* spp., *Salmonella* spp. and *Proteus* spp., after the previous pre-enrichment, the culture has been spread out onto the solid selective media and later the identification took place.

Moulds isolation and identification

Each morphologically different mould colony from the plates was picked up, transferred on to the YGC and AFPA (Oxoid, England) mediums and incubated at 25 ºC/5 days and 30 ºC/42 hours, respectively.

Primary classification of colonies from solid mediums YGC and AFPA was based on colony characteristics (pigmentation, shape, background colour and on microscopic examination of moulds using immersion objective magnified 100/1.30 (160/0.17), according to Malloch and Samson and Hoekstra (2000). The identification of strains *A. flavus* and *A. parasiticus* was confirmed by reverse, yellow to orange pigmentation on AFPA medium (Pitt, 1983).The reference strains *A. flavus* EXF 523 and *A. flavus* EXF438 were kindly given as a gift from the University of Ljubljana, Biotechnical Faculty, Department for Biology.

Statistical analyses

For statistical analyses the SAS/STAT (SAS/STAT User's Guide, 2000) and Excel XP were used.

Descriptive statistics including average, standard deviation, minimum and maximum were calculated.

The CORR Procedure was taken for calculating the correlation coefficients between variables log number of yeasts, moulds, coagulase-positive staphylococci and *E. coli* in different types of products and in products offered by different producers.

RESULTS

Pathogen micro-organisms in cheese samples

The mean number of coagulase-postive staphylococci in all tested samples was 2.5 x 10^4 cfu g^{-1}, while the number of bacteria *E. coli* was 1.4 x 10^6 cfu g^{-1}. Because the samples were collected in one instead of in five units (n=1 instead n=5), we estimated that the product was unsuitable according regulations, when the number of micro-organisms exceed the maximum value M. In 20% out of 40 samples tested the number of coagulase-positive staphylococci exceeded the prescribed regulations (Pravilnik…., 2004), particularly in soft cheese (12.5%) and curd (7.5%). About 17.5% of samples were contaminated with *E. coli* in higher concentrations than regulations according Pravilnik…., (2004) allowed. The number of *E. coli* was mostly exceeded in soft cheese (12.5%) and in curd (5.0% of samples) (Tables 1 and 2, Figure 1). According the regulations ES 2073/2005, the number of *E. coli and S. aureus* exceeded in 37.5% and 20% of samples, respectively. One sample of semi-hard cheese was contaminated with sulphite-reducing clostridia. *Proteus* sp. was detected in 3 samples (7.5%) and *L. grayi* in 1 (2.5%) sample. *Salmonela* sp. and *L. monocytogenes* were not detected in any sample.

Table 1. The number of coagulase-positive staphylococci in cheese samples (in cfu g^{-1})
Preglednica 1. Število koagulaza-pozitivnih stafilokokov v vzorcih sira (v KE g^{-1})

Statistical parameters Statistični parametri	Samples Vzorci			
	Curd Skuta	Soft cheese Mehki sir	Semi-hard cheese Poltrdi sir	Mean (all samples) Povprečje (vsi vzorci)
Mean / povprečje	1.4×10^2	7.2×10^4	5.0×10^1	2.5×10^4
Min	≤10	≤10	≤10	≤10
Max	1.3×10^3	1.0×10^6	3.0×10^1	1.0×10^6
Sd	3.6×10^2	2.7×10^5	9.6	1.6×10^5

Sd – standard deviation / standardni odklon; Min – minimal values / minimalne vrednosti; Max – maximal values / maksimalne vrednosti;

Table 2. The number of *E.coli* in cheese samples (in cfu g^{-1})
Preglednica 2. Število *E. coli* v vzorcih sira (v KE g^{-1})

Statistical parameters Statistični parametri	Samples Vzorci			
	Curd Skuta	Soft cheese Mehki sir	Semi-hard cheese Poltrdi sir	Mean (all samples) Povprečje (vsi vzorci)
Mean / povprečje	1.6×10^6	2.2×10^6	1.0×10^3	1.4×10^6
Min	≤10	≤10	≤10	≤10
Max	2.3×10^7	1.5×10^7	9.1×10^3	2.3×10^7
Sd	6.1×10^6	5.2×10^6	2.6×10^3	4.7×10^6

Sd – standard deviation / standardni odklon, Min – minimal values / minimalne vrednosti, Max– maximal values / maksimalne vrednosti

The correlation coefficient between the number of coagulase-positive staphylococci and the number of *E. coli* in curd is r=0.93 (P<0.01** significant) in soft cheese r=0.64 (P<0.01** significant) and in hard cheese r=–0.16 (P>0.05, not significant).
The correlation between the number of coagulase-positive staphylococci in all three types of cheese samples is negative as well as between the number of *E. coli*, except between the number of *E. coli* in soft and semi-hard cheese (r=0.59, P<0.05 * significant).

Yeasts and moulds in cheese samples

The yeasts in concentration above 10 cells per gram of the sample were determined in 24 (60%) out of 40 samples. In this concentration yeasts were present in 9 (64.3%) out of 14 of curd samples, in 11 (84.6%) out of 13 soft cheese samples and in 4 (21.4%) out of 13 semi-hard cheese samples. The statistical parameters are represented in Table 3.

Moulds were present in the number up to 10 per gram of the sample in 24 (60%) out of 40 tested samples. In this concentration moulds were found in 10 (71.4%) out of 14 of curd samples, in 9 (69.2%) out of 13 soft cheese samples and in 5 (38.4%) out of 13 semi-hard cheese samples. The statistical parameters are presented in Table 4.

Table 3. The number of yeasts in cheese samples (in cfu g^{-1})
Preglednica 3. Število kvasovk v vzorcih sira (v KE g^{-1})

Statistical parameters Statistični parametri	Samples Vzorci			
	Curd Skuta	Soft cheese Mehki sir	Semi-hard cheese Poltrdi sir	Mean (all samples) Povprečje (vsi vzorci)
Mean / povprečje	1.4×10^5	1.5×10^4	1.3×10^4	5.8×10^4
Min	≤10	≤10	≤10	≤10
Max	7.8×10^5	1.5×10^5	1.6×10^5	7.8×10^5
Sd	2.8×10^5	3.9×10^4	4.4×10^4	1.8×10^5

Sd – standard deviation / standardni odklon, Min – minimal values / minimalne vrednosti, Max– maximal values / maksimalne vrednosti

Table 4. The number of moulds in cheese samples (in cfu g^{-1})
Preglednica 4. Število plesni v vzorcih sira (v KE g^{-1})

Statistical parameters Statistični parametri	Samples Vzorci			
	Curd Skuta	Soft cheese Mehki sir	Semi-hard cheese Poltrdi sir	Mean (all samples) Povprečje (vsi vzorci)
Mean / povprečje	2.1×10^4	4.0×10^4	5.8×10^1	2.0×10^4
Min	≤10	≤10	≤10	≤10
Max	1.2×10^5	4.7×10^5	3.8×10^2	4.7×10^5
Sd	4.2×10^4	1.2×10^5	1.1×10^2	7.8×10^4

Sd – standard deviation / standardni odklon, Min – minimal values / minimalne vrednosti, Max– maximal values / maksimalne vrednosti

The correlation coefficient between number of yeasts and moulds in curd is r=0.55 (P<0.05* statistically significant) in soft cheese r=0.10 (P>0.05 statistically not significant) and in hard cheese r=–0.24 (P>0.05, statistically not significant).

The comparison between the numbers of micro-organisms in different cheese samples is represented in Figure 1.

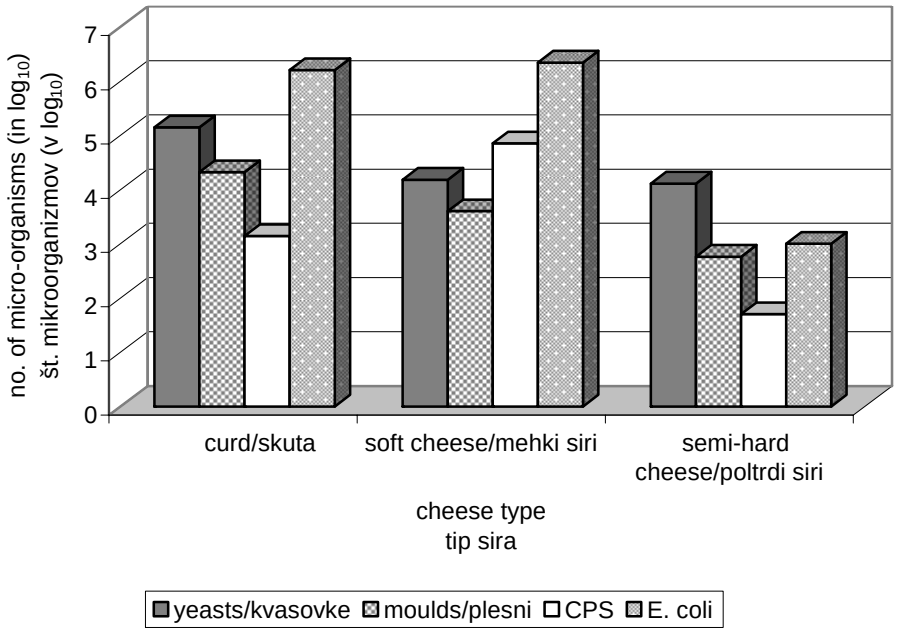

Figure 1. The $\log_{10}$ values of the mean number of yeasts, moulds, coagulase-positive staphylococci (CPS) and *E. coli* per g (g^{-1}) of different types of cheese samples.

Slika 1. Log_{10} vrednosti povprečnega števila kvasovk, plesni, koagulaza-pozitivnih stafilokokov (CPS) in *E. coli* v gramu (g^{-1}) vzorca različnih tipov sira.

The microbiological quality of samples from different small food-processing plants

The samples of cheese were collected from five small food-processing plants that offered their milk products on the market. At sampling we tried to take approximately the same number and type of product at every plant. But some of them are specialized on specific types of cheeses. In spite of this we compare the microbiological quality of samples between plants. The results of the comparison are expressed in the Figure 2.

The samples collected from plants A and B were particularly most often highly contaminated (in 43% of cases), while plant C offered faultless products. Only one sample of the plant D and plant E, respectively did not correspond to valid regulations, because they contained very high number of *E. coli*. The samples collected at plant E contained also *Proteus* sp. and *L. grayi,* while the number of other micro-organisms was very low.

From 24 cheese samples we isolated 37 mould strains that mostly belonged to genus *Geotrichum* (91.9%) as found out after morphological and microscopic examinations. Only 2 (5.4%) isolates were classified in genus *Moniliella* and 1 strain (2.7%) in genus *Aspergillus*.

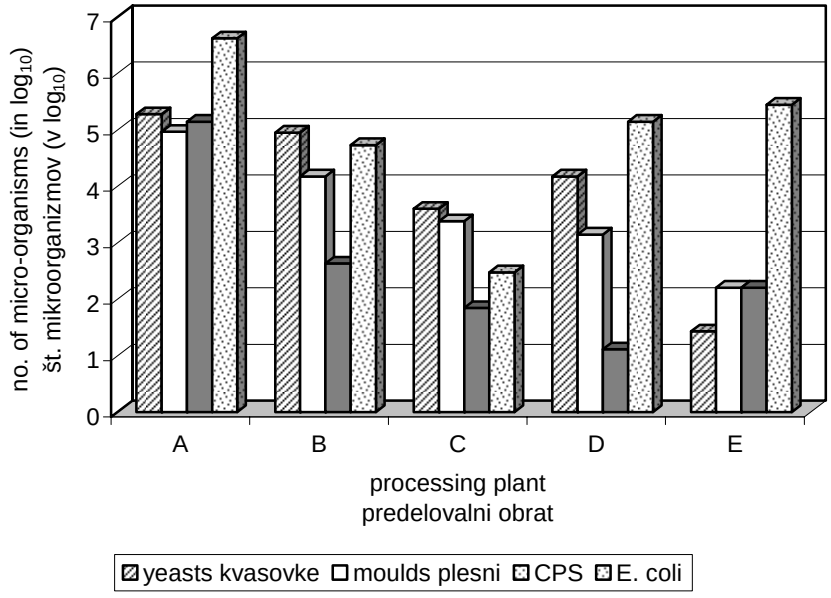

Figure 2. The $\log_{10}$ values of the mean number of yeasts, moulds, coagulase-positive staphylococci (CPS) and *E. coli* per g (cfu g^{-1}) of cheese samples produced by small dairy-processing plants (signed from A to E).

Slika 2. Log_{10} vrednosti povprečnega števila kvasovk, plesni, koagulaza-pozitivnih stafilokokov (CPS) in *E. coli* na gram(KE g^{-1}) vzorcev sirov, proizvedenih v malih mlekarsko-proizvodnih obratih (označenih od A do E).

The growth on AFPA medium and aflatoxin production

All isolated mould strains including standard strains *A. flavus* were inoculated on *A. flavus* and *A. parasiticus* agar (AFPA). Isolated *Aspergillus* strain grew on the medium, but it did not produce a distinctive bright orange yellow reverse colour on AFPA medium after 42–48 h incubation at 30 ºC (Frädberg, *et al.*, 2003) as it was detected at standard strains.

Only about 70% out of 24 *Aspergillus* sp. strains, growing on YGC medium, were able to form the colonies on YES medium supplemented with methyl-β-cyclodextrin and 0.6% sodium deoxycholate. On these mediums none of isolated strains could produce colonies with typical white under UV light fluorescent zone.

DISCUSSION

About 40% of all cow milk produced in Slovenia each year is processed into different sorts of cheese (Valjavec, 2000). There is not so many data about the microbiological quality of cheeses which are not processed in larger dairies. Therefore we evaluated the presence of pathogen micro-organisms, yeasts and moulds in 40 samples of cheese comprising 14 curd samples, 13 samples of soft salted or non-salted cheese and 13 samples of semi-hard cheese, which were manufactured at individual small dairy-processing plants selling their artisan products on the market.

Pathogen micro-organisms in cheese samples

Coagulase-positive staphylococci (*S. aureus*) and *E. coli* in cheese are frequently used as indicators of hygienic quality and show lack of microbiological safety (IFST, 1998). In our study

the number of coagulase positive staphylococci and *E. coli* was rather high (2.5 x 10^4cfu g^{-1} and 1.4 x 10^6 cfu g^{-1}, respectively). The samples of curd and particularly soft cheese were contaminated most often in the range from 10^2 to 10^4 cfu g^{-1} for staphylococci and in the range of about 10^6 cfu

g^{-1} for *E. coli*. The samples of semi-hard cheese were less contaminated with *E. coli* (10^3 cfu g^{-1}) and staphylococci (10^1 cfu g^{-1}) and fulfilled the criteria of valid Slovenian regulations (Pravilnik….Ur.l., 2004), but one sample was contaminated with clostridia and one with *Proteus*. *Salmonella* and *L. monocytogenes* were not detected in any of cheese samples tested.

According Pravilnik.., Ur.l. RS (2004) nine (22.5%) samples in our investigation did not reach the adequate microbiological quality, while the normatives EU (ES 1073, 2005) of the number of coagulase-positive staphylococci and *E. coli* were exceeded in 37.5% of samples.

Sk *et al.*, (2004) reported about 24% of white pickled cheese samples where the number of *S. aureus* (coagulase-positive staphylococci) was unacceptable according to Turkish microbial standards. These numbers are higher than our results.

Higher results were reported also by Borges *et al.* (2003) who evaluated the hygienic-sanitary quality of 43 Coalho cheese samples produced in different regions of Brazil. *E. coli* was confirmed in 93.1% of samples. Coagulase-positive staphylococci were observed in 93.1% of samples ranging from 1.0 x 10^1 to 2.0 x 10^9 cfu g^{-1}. The presence of *Salmonella* was confirmed in 34.9% and *Listeria* sp. in 6.9% of cheese samples (Borges *et al.*, 2003).

Very close to our results is the number of coagulase positive staphylococci according to the study of Brindani *et al.* (2001) where only one out of 50 samples of Ricotta cheese was contaminated with coagulase-positive staphylococci (4.0 x 10^4 cfu g^{-1}), but *Salmonella* was present in one sample and *L. monocytogenes* in two samples.

On the other hand, Menendez *et al*. (2001) established low average numbers of *S. aureus* (<61 per g of cheese) and *E. coli* (<52 per g of cheese) in 24 Tetilla cheese samples. *L. monocytogenes* was detected in two of the 24 samples. None of the samples yielded *Salmonella* spp.

The reasons for higher contamination of soft cheeses lay in their relatively high moisture and low acid content, which makes them easily susceptible to microbiological spoilage. The high salt content of brine and its dehydrating effect provide a few more days of shelf life, but the coagulase-positive staphylococci are, however, resistant to salt. (Anonim., 1994; Farkye and Vedamuthu, 2002; Tekinşen and Őzdemir, 2005).

There were differences in microbiological quality of cheese samples offered by different processing plants. These differences were not only the consequence of the offering the different types of cheese with different quality, but also of the hygienic conditions at milking process and cheese production.

The best way to ensure good shelf life of soft cheeses is to observe strict sanitary practices throughout the milking, manufacture steps, and post-manufacture handling. All the equipment used should be properly cleaned and sanitized. The quality and the pH of water used for washing the curd are very important. The water should be of potable quality and pH preferably slightly acidic or neutral (Farkye and Vedamuthu, 2002).

Yeasts and moulds in cheese samples

The samples of cheese were obtained in autumn and winter season, when the pasture or the hay was replaced by conserved or ensiled feed. Many authors reported on the higher number of yeasts, moulds and consecutively the higher concentration of mycotoxins in ensiled feed which is used mostly in winter season (Blanco, *et al.*, 1988; Lopez; *et al.*, 2003; Kamkar, 2005). For the needs of Slovenian cattle-breeding, about a half of the whole required voluminous forage should be conserved (Babnik and Verbič, 2003), but data on its contamination with yeasts and moulds are rare. The contamination of the feed also varies according to the area, moisture, climatic

differences and temperature (Jay, 1992; Sassahara, 2005). Some moulds like *A. flavus* and *A. parasiticus* can easily grow in feed having moisture between 13% and 18% and environmental moisture between 50% and 60%, furthermore they can produce toxin (Jay, 1992).

Beside the feed, the main sources of contamination of milk and milk products with yeasts and moulds are also environment and, particularly, the air (Finne Kure, *et.al*., 2004)

It is documented that yeasts occur in raw milk at insignificant numbers (Fleet, 1996) probably due to competitive utilization for the growth substrates by psychrotrophic bacteria of milk or owing to inhibition by metabolites excreted by bacteria (Viljoen, 2001).

Yeasts were found in our study in 60% of tested cheese samples in the mean number of 5.8 x 10^4 (4.7 $\log_{10}$) cfu g^{-1}. More contaminated cheese samples were offered by plants A and B. Producers A and B processed mostly more contaminated curd and soft cheese, while the plant E more low contaminated semi-hard cheese (Figure 2). The soft cheese samples were contaminated more frequently (84.6%), while the semi-hard cheese samples only in 21.4%. The mean values of yeasts were in samples of curd a bit higher (5.1 $\log_{10}$) than in samples of soft cheese and semi hard-cheese (4.1 $\log_{10}$). The higher contamination was probably the consequence of improper hygienic conditions during processing. Robinson and Tamime (2002) explicitly reported that yeasts as spoilage organisms generally enter in milk and cheese as contaminants from the air, or from improperly stored containers used for packaging the product. Brines are a potent source of yeasts too. The manufacturing process, the pH and a_w values, ripening time, etc., might also be the reasons of differences in number of yeasts in various types of cheese. Var *et al.* (2006) reported on the increase of yeast number between the 15^{th} to the 30^{th} day of ripening of the Kashar cheese and the decrease was observed after 30 to 60 day of ripening, depending on the antimicrobial agent or packaging material used. In the further ripening the number of yeasts was not changed drastically. Our results are in range or even lower than data obtained in other studies. For example, the number of yeasts in Spanish Armanda cheese samples after 1 month-ripening counts 10^6 cfu g^{-1} (Tornadijo *et al.*, 1998). Mean yeast counts of artisan Portuguese ewes' cheese ranged from 2.7 to 6.4 $\log_{10}$ cfu g^{-1} (Pereira-Dias *et al*., 2000) and yeast counts from 10^3 in curd and 10^5 cfu g^{-1} in one month old cheese were shown by Hatzikamari *et al.* (1999). Borges *et al.* (2003) reported that all (43) tested Coalho cheese samples from Brazil were contaminated with yeasts and moulds in from 1.7 x 10^4 to 1.6 x 10^9 cfu g^{-1}.

About 60% of cheese samples were contaminated with moulds in concentration of about 2.0 x 10^4 cfu g^{-1}, which is probably the consequence of contamination from the environment. Higher number of yeasts and moulds in cheese samples were also obtained in one of our previous studies (Godič Torkar and Golc Teger, S., 2004). The contamination with moulds in samples of curd and soft cheese was in the range of 10^4 cfu g^{-1} and was much higher than the contamination level of semi-hard cheese samples (mean value 58 cfu g^{-1}). For this reason the number of moulds in the samples collected from plant E that offered mostly semi-hard cheese was lower than in samples offered by other producers. The non-contaminated samples were available mostly at plants D and E. The samples from plant C were of the worst quality and were highly contaminated with yeasts and moulds.

We advised the producers that a lot of attention should be focused on the hygiene during cheese processing. Finne Kure *et al.*, (2004) also think that it is important to have focus on the air to reduce mould growth on the cheese. He recommended treating the mouldy cheese carefully. The level of mould spores that are able to grow on cheese will increase dramatically in the air if the mouldy cheese is not handled with care. The workers handling mouldy cheese should not enter the production rooms after this operation. The ventilation system may also contain a lot of mould spores and it is, therefore, necessary to have maintenance of this system in order to reduce spreading of spores from the ventilation systems (Finne Kure *et al*., 2004).

It was not surprising that 91.9% of strains isolated from cheese samples in our investigation were classified as *Geotrichum* spp., because Chapman and Sharpe (1990) reported that the high

moisture soft cheeses, cottage cheese and cream cheese were subject to spoilage by species *Geotrichum*. A member of the genus *Geotrichum*, *G. candidum*, also called *Oospora lactis*, is the most important species in foods (Jay, 1992). It is recognised as real milk mould, which is very often isolated from milk and, therefore, it is not surprising that this fungus plays also a role with *Penicillium* and *Mucor* in the manufacturing of some dairy products (Wouters *et al*., 2002). *G. candidum* inhibits the growth of *Listeria monocytogenes*, produces several enzymes for the breakdown of protein and fat and plays a key role in the ripening of camember cheese (Wouters *et al.*, 2002). It also plays an important role in competition with undesirable contaminants in the cheese (Nielsen *et al.*, 1998). Its lipases and proteases release fatty acids and peptides that contribute to the development of distinctive flavour and aroma in cheese (Tornadijo *et al*., 1998).

Only 2 (5.4%) isolates were classified in genus *Moniliella* and 1 strain (2.7%) in genus *Aspergillus*. Surprising, *Penicillium* spp. was not isolated from samples in spite of Scott (1989), and Fine Kure and Skaar (2000) reported that genus *Penicillium* is most frequent mould in cheese, followed by *Aspergillus*, *Cladosporium*, *Geotrichum* and *Mucor*. In our samples of cheese the genus *Aspergillus* was rare too, probably owing to low temperatures of milk chilling and cool season. Equaly Bullerman (1981) suggests that *Aspergillus* species, in contrast to *Penicillium* species, cannot grow at low temperatures.

In cheese samples there are only a few different sorts of moulds because the pasteurisation reduces the presence of contaminants from the group of yeasts and moulds. At the same time the environmental factors in winter are not advantageous for secondary contamination during cheese production (Scott, 1989, Fine Kure and Skaar, 2000).

A. flavus is not a common species on cheese too. Most studies showed that aflatoxins could only be produced on cheese at temperatures higher than 10ºC and a limiting a_w of 0.79 was found for growth of *A. flavus* and aflatoxin production (Scott, 1989).

In our study we found out that only one strain of the genus *Aspergillus*, but it was not identified as *A. flavus* or *A. parasiticus* after typical growth on AFPA medium. This strain did not produce the aflatoxin detected on YGC or YES medium supplemented with methyl-β-cyclodextrin.

CONCLUSIONS

- In our study the coagulase-positive staphylococci (*S. aureus*) and *E. coli* present in samples of cheese were most often the problem of hygienic quality and show lack of microbiological safety.
- The samples of curd and particularly soft cheese were contaminated most often in the range from 10^2 to 10^4 cfu g^{-1} for staphylococci and in the range of about 10^6 cfu g^{-1} for *E. coli*. The samples of semi–hard cheese were less contaminated with *E.coli* and staphylococci and fulfilled the criteria of valid regulations.
- *Salmonella* and *L. monocytogenes* were not detected in any of the cheese samples examined.
- According to the valid regulations 9 (22.5%) samples in our investigation did not reach the adequate microbiological quality.
- About 60% of samples were contaminated with yeasts and moulds, which is probably the consequence of the contamination from the environment.
- The contamination with moulds in samples of curd and soft cheese was in the range of 10^4 cfu g^{-1} and was much higher than the contamination level of semi–hard cheese samples (mean value 58 cfu g^{-1}).
- There were differences in microbiological quality of cheese samples offered by different processing plants.

- The samples from the plant C were of the worst quality and they were highly contaminated with yeasts and moulds.
- The samples collected from the plants A and B were particularly most often highly contaminated (in 43% of cases).
- 91.9% of mould strains isolated from cheese samples in our investigation were classified as *Geotrichum* spp.
- We advised the producers that a lot of attention should be focused on the hygienic conditions during milking and cheese processing.

POVZETEK

V Sloveniji je kar nekaj individualnih majhnih živilsko–predelovalnih obratov, kjer proizvajalci mleko predelajo v mlečne izdelke, zlasti v različne tipe sirov in skuto. Te izdelke prodajajo na tržnicah v večjih mestih. Higienska kakovost in zdravstvena ustreznost proizvedenih sirov in skute je odvisna od ustrezne mikrobiološke kakovosti namolzenega mleka kot surovine, kakovosti molže, razmer, v katerih se mleko predeluje v sire, temperature hranjenja mleka in sirov, krme, sezone, uporabe različnih starterskih kultur, higiene mlekarskega pribora, okolja, ne nazadnje tudi vode, ki se uporablja pri predelovalnem procesu.

Preverjanje prisotnosti in števila specifičnih, posebno patogenih in potencialno patogenih mikroorganizmov, je pomemben dejavnik pri kontroli in sistemu zagotavljanja kakovosti proizvodnje in samih izdelkov.

Namen našega dela je bil ugotoviti prisotnost nekaterih patogenih in potencialno patogenih mikroorganizmov v 40 vzorcih skute in sirov, vzorčenih pri petih malih mlekarsko–predelovalnih obratih, ki ponujajo svoje izdelke na eni izmed slovenskih tržnic. Ugotavljali smo tudi število in prisotnost kvasovk in plesni, saj zlasti plesni rodu *Aspergillus*, ki kot proizvajalec aflatoksinov povzročajo tudi zdravstveno rizičnost živila.

Največji problem je predstavljalo povišano število koagulaza–pozitivnih stafilokokov (*S. aureus*) in *E. coli*. Najpogosteje so bili kontaminirani vzorci skute in zlasti mehkih sirov v območju od 10^2 do 10^4 KE g^{-1} stafilokokov in okrog 10^6 KE g^{-1} za *E. coli*. Vzorci poltrdega sira so bili manj pogosto kontaminirani in so vsi izpolnjevali zahteve veljavnih predpisov. *Salmonella* in *L. monocytogenes* nista bili ugotovljeni v nobenem izmed preiskanih vzorcev. Po veljavnih mikrobioloških kriterijih (Pravilnik…, Ur.l., 2004) je bilo 9 (22.5 %) preiskanih vzorcev neustreznih. Okrog 60 % vzorcev je vsebovalo kvasovke in plesni. Koncentracija plesni v skuti in mehkih sirih se je gibala v območju 10^4 KE g^{-1} in je bila veliko višja kot koncentracija v poltrdih sirih (povprečje 58 KE g^{-1}), medtem ko se je število kvasovk v vseh vzorcih gibalo v območju od 10^4 do 10^5 KE g^{-1}. Ugotovili smo razlike v mikrobiološki kakovosti vzorcev sira pri različnih proizvajalcih.

Kar 91,9 % izoliranih sevov plesni je pripadalo rodu *Geotrichum* spp. Samo en sev je pripadal rodu *Aspergillus*, ki pa ga nismo mogli uvrstiti v vrsti *A. flavus/parasiticus* in ni tvoril aflatoksinov.

REFERENCES

Angelotti, R./ Hall, H.E./ Foter, M.J./ Lewis, K.M. Quantitation of *Clostridium perfringens* in Foods. Appl. Microbiol., 10(1962), 193–199.

Anonimus. Recommendations for the hygienic manufacture of milk and milk based products, appendix A.1 Spoilage and pathogenic bacteria in milk based products. Bulletin of the International Dairy Federation, IDF 292/1994, Brussels, Belgium, (1994), 28–32.

Babnik, D./ Verbič, J. The effect of long-term grass silage storage on its nutritive value and quality. Zb. Bioteh. Fak. Univ. Ljublj., Kmet. Zooteh., 82(2003)2, 109–117.

Blanco, J.L./ Dominguezs, L./ Gomez-Lucia, E./ Garayzabal, J.F.F./ Garcia, J.A./ Suarez, G. Presence of aflatoxin M_1 in commercial ultra-high temperature treated milk. Applied Environmental Microbiology, 56(1988), 1622–1623.

Borges, M. de F./ Feitosa, T./ Nassu, R. T./ Muniz, C.R./ Azevedo, E.H.F.de/ Figueiredo, E.A.T.de. Pathogenic and indicator micro organisms isolated from "coalho" cheese produced in the Ceara State, Brazil. Boletim do Centro de Pesquisa e processamento de Allimentos, 21(2003)1, 31–40.

Bramley, A.J./ McKinnon, C.H. The Microbiology of Raw Milk. In: Dairy Microbiology, I. (Ed.: Robinson, R.K.). London, New York, Elsevier Applied Science, 1990, 171.

Brindani, F./ Pizzin, G./ Bonardi, S./ Bacci,C. Tradittional Ricotta cheese produced in the Parmesan-Reggiano district: evaluation of its microbiological profile. Annali della Facolta di Medicina Veterinaria, Universita di Parma, 21(2001), 283–292.

Bullerman, L.B. Public health significance of molds and mycotoxins in fermented dairy products. J. Dairy Sci., 64(1981), 2439–2452.

Chapman, H.R./ Sharpe, M.E. Microbiology of Cheese. In: Dairy Microbiology (Ed.: Robinson, R.K.). London and New Yersey, Applied Science Publishers, 1990, 203–290.

Davis, J.G./ Wilbey, R.A. Microbiology of Cream and Dairy Desserts. In: Dairy Microbiology (Ed.: Robinson, R.K.). London and New Yersey, Applied Science Publishers, 1990, 41–108.

De Buyser, M.L./ Dufour, B./ Maire, M./ Lafarge, V. Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. J. Food Microbiol., 67(2001)1–2, 1–17.

De Man, J.D./ Rogosa, M.A./ Sharpe, M.E. Medium for the cultivation of lactobacilli. J. Appl. Bact., 23(1960)1, 130–135.

D'Mello, J.P.F./ Macdonald, A.M.C. Mycotoxins. Animal Feed Sci. Techn., 69(1997), 155–166.

EN ISO 11290-1(E). Microbiology of food and animal feedeing stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1: Detection method (ISO 11290-1, 1996). International Organization for Standardization, Genova, Switzerland, 1996, 1–16.

EN ISO 4833. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of micro organisms-Colony-count technique at 30 ºC. International Organization for Standardization, Genova, Switzerland, (2003), 1–9.

Farkye, N.Y./ Vedamuthu, E.R..Microbiology of soft cheese. In: Dairy Microbiology Handbook, Third Edition, (Ed.: Robinson, R.K.). New York, Wiley Interscience, 2002, 479–514.

Finne Kure, C./ Skaar, I./ Brendehaug, J. Mould contamination in production of semi-hard cheese. Intern. J. Food Microbiol., 93(2004), 41–49.

Finne Kure, C./ Skaar, I. Mould growth on the Norwegian semi-hard cheeses Norvegia and Jarlsberg. Intern. J. Food Microbiol., 62(2000), 133–137.

Fleet, G.H. Yeasts in dairy products. J. Appl. Bacteriol., 68(1990), 199–211.

Gilmour, A./ Rowe, M. T. Micro-organisms Associated with Milk. In: Dairy Microbiology, I. (Ed.: Robinson, R.K.). London, New York, Elsevier Applied Science, 1990, 37–76.

Godič Torkar, K./ Golc Teger, S. The microbiological quality of some critical control points in the cheese production of individual slovenian cheese-makers. *Acta agriculturae Slovenica*, 84(2004)1, 43–61.

Hatzikamari, M./ Litopoulou,-Tzanetaki, E./ Tzanetakis, N. Microbiological characteristics of Anevato: a traditional Greek cheese. J. Appl. Microbiol., 87(1999), 595–601.

IFST, Food safety and and cheese. Food Science and Technology Today, 12(1998)2, 117–122.

ISO/DIS 707. Milk and milk products-Guidence on sampling. International Organization for Standardization, Genova, Switzerland, 1995, 1–41.

ISO 6611/IDF. Milk and milk products-Enumeration of colony-forming units of yeasts and/or moulds-Colony-count technique at 25ºC, ISO, FIL/IDF, International Organization for Standardization, Geneva, Switzerland, International Dairy Federation, Brussels, Belgium, 2004, 8 p.

ISO/FDIS 8261 (E). Milk and milk products – General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination. International Organization for Standardization, Genova, Switzerland, 2001, 1–12.

ISO 6579. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. (ISO 6579:2002). International Organization for Standardization, Genova, Switzerland, 2002,1– 27.

Jacobsen, N./ Narvhus, J. Yeasts and their possible beneficial and negative effects on the quality of dairy products. Int. Dairy J., 6(1996), 755–768.

Jay, J. M. Modern Food Microbiology, 4th edition. New York, Chapman&Hall, 1992, 720 p.

Jodral, M./ Liñan, E./ Acosta, I./ Gallego, C./ Rojas, F./ Bentabol, A. Mycoflora and toxigenic *Aspregillus flavus* in Spanish milk. Inter. J. Food Microbiol., 18(1993)2, 171–174.

Kamkar, A. A study on the occurence of aflatoxin M_1 in Iranian Feta cheese. Food Control, 17(2006)10, 768–775.

Lopez, C.E./ Ramos, L.L./ Ramadán, S.S./ Bulacio, L.C. Predsence of aflatoxin M_1 in milk for human consumption in Argentina. Food Control, 14(2003)1, 31–34.

Malloch, D. http://www.botany.utoronto.ca/ResearchLabs/MallochLab/Malloch/Moulds/Examination.html;

McManus C./ Lanier, J.M. *Salmonella*, *Campylobacter jejuni* and *Yersinia enterocolitica* in raw milk. J. Food Prot., 50(1987)1, 51–55.

Menendez, S./ Godinez, R./ Centeno, J.A./ Rodriguez-Otero, J.L.. Microbiological, chemical and biochemical characteristics of Tetilla raw cows-milk cheese. Food Microbiol., 18(2001)2, 151–158.

Meyer Broseta, S./ Diot, A./ Bastian, S./ Riviere, J./ Cerf, O. Estimation of low bacterial concentration: *Listeria monocytogenes* in raw milk. Int. J. Food Microbiol., 80(2003.)1, 1–15.

Nelson, F.E. The microbiology of Markel Milk. In: Dairy Microbiology (Ed.:Robinson, R.K.) The microbiology of milk. London and New Jersey, Applied Science Publishers, 1981, 165–207.

Officional Journal of the European Communities.Council directive 92/46/EEC, of 16 June 1992 , laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products, ANEX A: Requirements relating to the acceptance of raw milk at treatment and/or processing establishments, ANEX C, Chapter II: Microbiological criteria for milk-based products and drinking milk No L 268(1992), 1–19, 26–28. Consolidated Consleg: 1992L0046-EN-01/05/2004, Office for Official Publications of the European Communities, 2004, 1–40.

Officional Journal of the European Communities. Council directive 2073/2005, of November 15. 2005 about microbiological criteria for foodstuffs, L 338/8–338/19.

Pereira-Dias, S./ Potes, M.E./ Marinho, A./ Malfeito-Ferreira, M./ Loureiro, V. Characterisation of yeast flora isolated from an artisanal Portuguese ewe's cheese. Int. J. Food Microbiol., 60(2000), 55–63.

Pravilnik o veterinarsko-sanitarnem nadzoru živilskih obratov, veterinarsko-sanitarnih pregledih ter o pogojih zdravstvene ustreznosti živil in surovin živalskega izvora. Uradni list RS št. 28(2004), 3183–3197.

Robinson, R.K./ Tamime, A.Y. Maintaining a clean working environment. In: Dairy Microbiology Handbook, Third Edition (Ed.: Robinson, R.K.). New York, Wiley Interscience, 2002, 561–592.

Salmeron, J./ De Vega, C./ Perez-Elortondo, F.J./ Albisu, M./ Barron, L.J.R. Effect of pasteurization and seasonal variations in the microflora of ewe's milk for cheesemaking. Food Microbiol., 19(2002), 2–3, 167–174.

SAS/STAT User's Guide, 2000. SAS Online Doc® , Version 8, Vol. 2, SAS Institute Inc. Cary NC., USA.

Sassahara, M./ Pontes Netto, D./ Yanaka, E.K. Aflatoxin occurence in foodstuff supplied to dairy cattle and aflatoxin M_1 in raw milk in the North of Paraná state. Food and Chem. Toxicol., 43(2005), 981–984.

Scott, P.M. Mycotoxigenic fungal contaminants of dairy products: In: Mycotoxins in dairy products (Ed.:Van Egmont, H.P), New York, Elsevier Science Publishers ltd, 1989, 194–235.

SIST EN ISO 6888-2. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) Part 2: Technique using rabbit plasma fibrinogen agar medium. International Organization for Standardization, Genova, Switzerland, 1999, 1–7.

Sk, B./ Kucukcetn, A./ Yaygn, H. Physicocchemical quality and occurrence of bacterial pathogens in white pickled cheese. Milchwissenschaft, 59(2004)11/12, 636–637.

Tekinşen, K.K./ Őzdemir, Z. Prevalence of foodborne pathogens in Turkish Van otlu (Herb) cheese. Food Control, 17(2006)9, 707–711.

Tornadijo, M.E./ Fresno, J.M./ Sermiento, R.M./ Carballo, J. Study of the yeasts during the ripening process of Armada cheeses from raw goat's milk. Lait 78(1998), 647–659.

Valjavec, I. Slovene dairies. Cattle Bells, Periodical of Cattle Breeders' Association of Slovenia, 5(2000), 1–2, 39–43.

Var, I./ Erginkaya, Z./ Güven, M./ Kabak, B. Effects of antifungal agent and packaging material on microflora of Kashar cheese during storage period. Food Control, 17(2006)2, 132–136.

Viljoen, B.C. The interaction between yeasts and bacteria in dairy environments. Int. J. Food Microbiol., 69(2001), 37–44.

Wouters, J.T.M./ Ayad, E.H.E./ Hugenholtz, J./ Smit, G. Microbes from raw milk for fermented dairy products. Int. Dairy J., 12(2002), 91–109.

Acta agriculturae Slovenica, 88(november 2006)1, 53.

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

UDK 636/641 ISSN 1581-9175

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Acta agriculturae Slovenica, 88(november 2006)1, 55.

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

UDK 636/641 ISSN 1581-9175

Acta agriculturae Slovenica

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Acta agriculturae Slovenica, 88(november 2006)1, 57.

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

UDK 636/641 ISSN 1581-9175

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NAVODILA AVTORJEM

Prispevki

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.

Pri prispevkih v slovenskem jeziku morajo biti preglednice, grafikoni, slike in priloge dvojezični, povsod je slovenščina na prvem mestu. Naslovi grafikonov in slik so pod njimi. Slike in grafikoni so v besedilu. Priloženi morajo biti tudi jasno označeni izvirniki slik (fotografije ali ločene grafične datoteke). Na avtorjevo željo jih vračamo. Grafikoni morajo biti črno-beli, brez rastrov. Dovoljeni so vzorci v črno-beli kombinaciji. Latinske izraze pišemo ležeče. V slovenščini uporabljamo decimalno vejico, v angleščini decimalno piko. Prispevki v angleščini morajo imeti povzetek v slovenščini in obratno.

Prispevki naj bodo strnjeni, kratki, največ 12 strani. Uporabljamo Microsoft Word 97 ali novejšo verzijo (Windows); pisava v besedilu in preglednicah je Times New Roman, velikost črk 12, v obsežnih preglednicah je lahko 10, pisava v grafikonih in slikah je Ariel, velikost črk najmanj 9, pisava za primerjave nukleotidnih in aminokislinskih zaporedij je Courier; zunanji rob 2,0 cm, notranji 2,5 cm, zgoraj živa *pagina* v eni vrstici, velikost črk 10 z avtorjem oz. avtorji in naslovom prispevka, zaključenim s piko. Če je naslov daljši, ga smiselno okrajšamo. Primera: Štuhec, I. in Siard, N. Obnašanje prašičev. Stibilj, V. in sod. Določitev maščobno-kislinske sestave … vzorcev mleka v Sloveniji.

Prva stran

Na prvi strani prispevka na desni strani označimo vrsto prispevka v slovenščini in angleščini, sledi naslov prispevka, pod njim avtorji. Ime avtorjev navedemo v polni obliki (ime in priimek). Vsak avtor naj bo označen z indeksom, ki ga navedemo takoj pod avtorji, in vsebuje polni naslov ustanove ter znanstveni in akademski naslov; vse v jeziku prispevka. Navedemo sedež ustanove, kjer avtor dela. Če je raziskava opravljena drugje, avtor navede tudi sedež te inštitucije. Na željo avtorjev bomo navedli naslov elektronske pošte.

Pod naslovi avtorjev je datum prispetja in datum sprejetja prispevka, ki ostaneta odprta. Sledi razumljiv in poveden izvleček z do 250 besedami. Vsebuje namen in metode dela, rezultate, razpravo in sklepe. Sledijo ključne besede.

Izvlečku v jeziku objave sledi naslov in izvleček s ključnimi besedami v drugem jeziku.

Predlogo za pomoč pri oblikovanju prve strani prispevka najdejo avtorji na domači strani: http://aas.bfro.uni-lj.si/predloga-aas.dot.

Viri

V besedilu navajamo v oklepaju avtorja in leto objave: (priimek, leto). Če sta avtorja dva, pišemo: (priimek in priimek, leto), če je avtorjev več, pišemo: (priimek in sod., leto). Sekundarni vir označimo z »navedeno v« ali »cv.«. Seznam virov je na koncu prispevka, neoštevilčen in v abecednem redu. Vire istega avtorja, objavljene v istem letu, razvrstimo kronološko z a, b, c. Primer: 1997a. Navajanje literature naj bo popolno: pri revijah letnik, leto, številka, strani; pri

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Fraser, A.F./ Broom, D.M. Farm animal behaviour and welfare. London, Bailliere Tindall, 1990, 437 str.

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.

Stekar, J.M.A. Vsebnost makro elementov v slovenski mrvi. V: Posvetovanje o prehrani domačih živali »Zadravčevi-Erjavčevi dnevi«, Radenci, 1997-10-27/28. Murska Sobota, Živinorejsko-veterinarski zavod za Pomurje, 1997, 105–117.

Stekar, J.M.A./ Golob, A./ Stibilj, V./ Koman Rajšp, M. Sestava in hranilna vrednost voluminozne krme v letu 1990. Zb. Bioteh. Fak. Univ. Ljubl., Kmet. Živin., 58(1991), 149–155.

Stekar, J.M.A./ Pen, A. Sadržaj natriuma, cinka i mangana u stočnoj hrani sa travnatih površina. Agrohemija, 21(1980)1–2, 7–15.

Oddaja

Avtorji prispevke oddajo v dveh izvodih, enega z dvojnim razmikom med vrsticami in največ 35 vrstic na strani, in na disketi. Priložijo tudi izjavo s podpisi vseh avtorjev, da avtorske pravice v celoti odstopajo reviji.

Prispevke recenziramo in lektoriramo. Praviloma pošljemo mnenje prvemu avtorju, po želji lahko tudi drugače. Če urednik ali recenzenti predlagajo spremembe oz. izboljšave, vrne avtor popravljeno besedilo v 10 dneh v dveh izvodih, enega z dvojnim razmikom. Ko prvi avtor vnese še lektorjeve pripombe, odda popravljeno besedilo v enem izvodu in na disketi ter vrne izvod z lektorjevimi popravki.

Prispevke sprejemamo vse leto.

Acta agriculturae Slovenica, 88(november 2006)1, 61–62.

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Fraser, A.F./ Broom, D.M. Farm animal behaviour and welfare. London, Bailliere Tindall, 1990, 437 p.

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

Ristič, M./ Klein, F.W. Schlachtkoerperwert von Broilern verschiedener Herkunfte. Mitteilungsblatt der Bundesanstalt fuer Fleischforschung, Kulmbach, 101(1988), 8045–8051.

Stekar, J.M.A. Silage effluent and water pollution. In: 6th International Symposium "Animal Sciences Days", Portorož, 1998-09-16/18, Slovenia. Zb. Bioteh. Fak. Univ. Ljubl., Kmet. Supl., 30(1998), 321–325.

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Acta agriculturae Slovenica

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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	6 000 SIT (25,04 EUR)*, for foreign countries 30 EUR
Individual issue	4 000 SIT (16,69 EUR)*, for foreign countries 20 EUR
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