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Prof. ddr. Franc LOČNIŠKAR, dipl. inž.agr. (1923–2010)

Od prof. ddr. Franca Ločniškarja, zaslužnega profesorja Univerze v Ljubljani in častnega člana Slovenskega genetskega društva, smo se poslovili 14. oktobra 2010.

Rodil se je 23. aprila 1923 na Turjaku kot peti otrok v učiteljski družini. Po gimnazijskih letih v Zavodu sv. Stanislava v Šentvidu in maturi na Klasični gimnaziji v Ljubljani se je leta 1945 vpisal na Kmetijsko-gozdarsko fakulteto v Zagrebu, kjer je decembra 1949 diplomiral. V tem času je na njegov nadaljnji razvoj pomembno vplival akademik prof. dr. Alojz Tavčar, ki ga je navdušil za genetiko, ki je postala osrednja tema njegovega kasnejšega raziskovalnega dela. Prof. Ločniškar je bil prvi doktorand Biotehniške fakultete v Ljubljani (1959), leta 1960 pa je s temo s področja kvantitativne genetike doktoriral tudi na Georg-August Univerzi v Göttingenu v Nemčiji. Kot profesor je vse svoje delovno obdobje deloval na Biotehniški fakulteti Univerze v Ljubljani, katere dekan je bil v letih 1979–1981. Univerza v Ljubljani mu je po upokojitvi leta 1991 za izjemne zasluge na pedagoškem in raziskovalnem področju podelila naziv zaslužni profesor. Za svoje delo je prof. Ločniškar prejel številne nagrade, med drugim tudi nagrado Sklada Borisa Kidriča (1959), Jesenkovo priznanje (1974), medaljo dela z zlatim vencem (1979) ter Svečano listino in zlato plaketo Univerze v Ljubljani (1984).

Raziskovalno delo prof. Ločniškarja obsega raziskave na področju populacijske genetike in genetike kvantitativnih lastnosti, kjer se je ukvarjal z oceno genetske in okoljske komponente variance in kovariance proizvodnih lastnosti. Med prvimi v Evropi je določil dednostni delež za pomembne gospodarske lastnosti prašičev. V sedemdesetih letih prejšnjega stoletja se je začel ukvarjati s citogenetiko. Po prvem opisu kromosomske translokacije pri prašiču (1974), je v sodelovanju z I. Gustavssonom, M. Hageltornom in L. Zech z Univerze v Uppsali na Švedskem leta 1976 objavil svoj najodmevnejši članek "Cytological origin and points of exchange of a reciprocal chromosome translocation (1p-; 6q+) in domestic pig" v reviji Hereditas. Na tem področju so nato sledili še opisi mozaicizma spolnih kromosomov in avtosomov ter Ro-

bertsonove fuzije pri govedu, izdelava kariotipov postrvi in lipana ter kariotipov za medvrstne hibride.

Prof. Ločniškarja so dolga leta raziskovalno zaposlovali učinki inbridinga in križanja pri selekciji domačih živali. Z izvajanjem dvosmerne selekcije pri kokoših je vzpostavil pomemben genetski model, ki ga raziskovalci na Oddelku za zootehniko Biotehniške fakultete še vedno s pridom uporabljajo. Prof. ddr. Franc Ločniškar je tudi pomembno prispeval k razvoju animalne biotehnologije v Sloveniji.

In Memoriam
Prof. Dr. Franc LOČNIŠKAR, Ph.D. (1923–2010)

Prof. Dr. Franc Ločniškar, Ph.D, a honorary professor of the University of Ljubljana and honorary member of the Slovenian Genetics Society was buried at Ljubljana cemetery Žale on October 14. 2010.

He was born on April 23. 1923 in Turjak as a fifth child in a teacher's family. After his high school years at Diocesan Classical Gymnasium at St. Stanislav's Institution he entered the study of Agriculture at the Faculty for Agriculture and Forestry in Zagreb in 1945, from which he graduated in 1949. During his study period in Zagreb was of tremendous importance for his further development his contact to the Academy member Prof. Dr. Alojz Tavčar, who initiated his enthusiasm for Genetics which remained the central topic of his research interest for his entire career. Prof. Ločniškar was the first Ph.D. student who obtained his Ph.D. from the Biotechnical Faculty at the University of Ljubljana in 1959. In 1960 he received his second Ph.D. for his thesis in quantitative genetics from the Georg-August University in Göttingen, Germany. Professor Ločniškar spent his entire teaching career at Biotehcnical Faculty, University of Ljubljana, where he served as a Dean of the Faculty from 1979 to 1981. At the occasion of his retirement in 1991 he received the title of honorary professor of the University of Ljubljana. For his research work he got numerous awards: Boris Kidrič Foundation research award (1959), Jesenko award (1974), State medal with golden wreath (1979) and Golden plaque of the University of Ljubljana (1984).

Prof. Ločniškar started his research work in the area of population and quantitative genetics with estimations of environmental and genetic components of variances and covariances for production traits. He was among first geneticists in Europe who estimated heritabilities for economically important traits in pigs. In the nineteen seventies, his research interest focused on cytogenetics. After first description of chromosomal translocation in pig (1974), he published in collaboration with I. Gustavsson, M. Hageltorn and L. Zech from the University in Uppsala his most important article "Cytological origin and points of exchange of a reciprocal chromosome translocation (1p-; 6q+) in domestic pig" in the journal Hereditas in 1976. In the following years his work was focused on mosaicism of sex chromosomes and autosomes, detection of Robertson's fusion in cattle and karyotyping of trout, grayling and interspecies hybrids.

Prof. Ločniškar also studied effects of inbreeding and specific crossing combinations in selection of domestic animals. He established divergently selected chicken and created an important experimental model which is still frequently used by the researchers at the Department of Animal Science. Prof. Ločniškar's contribution to the development of animal biotechnology in Slovenia was also significant.

Prof. dr. Peter Dovč

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SIMPLE REPARAMETERIZATION TO IMPROVE CONVERGENCE IN LINEAR MIXED MODELS

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Simple reparameterization to improve convergence in linear mixed models

Slow convergence and mixing are one of the main problems of Markov chain Monte Carlo (McMC) algorithms applied to mixed models in animal breeding. Poor convergence is to a large extent caused by high posterior correlation between variance components and solutions for the levels of associated effects. A simple reparameterization of the conventional model for variance component estimation is presented which improves McMC sampling and provides the same posterior distributions as the conventional model. Reparameterization is based on the rescaling of hierarchical (random) effects in a model, which alleviates posterior correlation. The developed model is compared against the conventional model using several simulated data sets. Results show that presented reparameterization has better behaviour of associated sampling methods and is several times more efficient for the low values of heritability.

Key words: statistics / mixed model / Bayesian analysis / McMC / reparameterization / convergence

Enostavna reparametrizacija za izboljšanje konvergence linearnih mešanih modelov

Počasna konvergenca je eden največjih problemov uporabe metode Monte Carlo z Markovimi verigami (McMC) za mešane modele na področju genetike in selekcije domačih živali. Slaba konvergenca je v veliki meri posledica visoke posteriorne korelacije med komponentami variance in rešitvami za ravni pripadajočih vplivov. Predstavljamo enostavno reparametrizacijo običajnega modela, ki izboljša lastnosti metode McMC in daje enake posteriorne porazdelitve parametrov modela kot standardni pristop. Reparametrizacija temelji na standardizaciji hierarhičnih (naključnih) vplivov v modelu, kar posledično spremeni posteriorne korelacije med parametri. Oba pristopa smo primerjali na večjem setu simuliranih podatkov. Rezultati kažejo, da reparametrizacija vodi do bolj učinkovitih metod McMC vzorčenja in je nekajkrat bolj učinkovita za analizo lastnosti z nizko heritabiliteto.

Ključne besede: statistika / mešani model / bayesovska analiza / McMC / reparametrizacija / konvergenca

1 INTRODUCTION

Mixed models are abundantly used in the field of animal breeding and genetics with the aim to infer genetic values of animals given some phenotypic and pedigree information (Henderson, 1984). In it simplest form the mixed model can be written as:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}, \quad (1)$$

where **y** is a vector of phenotypes, **b** is a vector of effects like sex, breed, age, etc., **a** is a vector of individual additive genetic effects and **e** residual, $p\left(\mathbf{e} \mid \sigma_e^2\right) \sim N\left(\mathbf{0}, \mathbf{I}\sigma_e^2\right)$, while **X** and **Z** are design matrices linking effects to phenotypic records. Pedigree information is included in the model hierarchically with prior distribution of individual additive genetic values, $p\left(\mathbf{a} \mid \mathbf{A}, \sigma_a^2\right) \sim N\left(\mathbf{0}, \mathbf{A}\sigma_a^2\right)$. Henderson (1972) developed the so called mixed model equations (2) to efficiently obtain joint solutions for **b** and **a**, where $\mathbf{G} = \mathbf{A}\sigma_a^2$ and $\mathbf{R} = \mathbf{I}\sigma_e^2$:

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$$\begin{pmatrix} \mathbf{X}^T\mathbf{R}^{-1}\mathbf{X} & \mathbf{X}^T\mathbf{R}^{-1}\mathbf{Z} \\ \mathbf{Z}^T\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}^T\mathbf{R}^{-1}\mathbf{Z}+\mathbf{G}^{-1} \end{pmatrix} \begin{pmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{a}} \end{pmatrix} = \begin{pmatrix} \mathbf{X}^T\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{Z}^T\mathbf{R}^{-1}\mathbf{y} \end{pmatrix} \quad (2)$$

Use of mixed model equations assumes known variance components σ_a^2 and σ_e^2. Standard procedure is to estimate these variance components using restricted maximum likelihood method (REML; Patterson and Thompson, 1971) and to use these estimates in mixed model equations (2) ignoring the error of estimation in variance components.

Another approach to statistical inference, Bayesian approach, treats inference of all model parameters jointly. Although conceptually very appealing, Bayesian approach leads to formulas that are computationally intractable. This can be avoided by sampling methods such as Markov chain Monte Carlo (McMC; e.g., Gelman, *et al.*, 2004). Wang *et al.* (1993) showed how McMC methods can be used with linear mixed models in animal breeding applications. In the case of linear mixed models all McMC computations follow from the posterior distribution (3):

$$p\left(\mathbf{b},\mathbf{a},\sigma_a^2,\sigma_e^2 \mid \mathbf{y}\right) \propto \left|\mathbf{R}\right|^{-\frac{1}{2}} \exp\left(-\tfrac{1}{2}(\mathbf{y}-\mathbf{X}\mathbf{b}-\mathbf{Z}\mathbf{a})^T\mathbf{R}^{-1}(\mathbf{y}-\mathbf{X}\mathbf{b}-\mathbf{Z}\mathbf{a})\right) \times \left|\mathbf{G}\right|^{-\frac{1}{2}} \exp\left(-\tfrac{1}{2}\mathbf{a}^T\mathbf{G}^{-1}\mathbf{a}\right), \quad (3)$$

where prior distributions for and both variance components σ_a^2 and σ_e^2 were assumed uniform (e.g., Gelman *et al.*, 2004). Given that σ_a^2 and **a** are a *priori* correlated due to the prior definition of **a**, the a *posteriori* correlation between them is expected to be high. This leads to high autocorrelation between consecutive samples, making McMC method inefficient. Autocorrelations can be really problematic with low or near zero values for some variance components (e.g. additive genetic variance). This is caused by the shrinkage of **a** towards zero and in a next round of sampling variance component will again be close to zero, which can make the sampler stuck for quite some time at the values near zero (Gelman *et al.*, 2004).

Chib and Carlin (1999) proposed block sampling of some parameters in (2) to improve convergence. Autocorrelation has also been alleviated by the use of centered models (Gelfand *et al.*, 1995), parameter expanded models (Liu and Wu, 1999; Gelman *et al.*, 2003; Gelman, 2004) and data augmentation based models (Meng and van Dyk, 1997; van Dyk and Meng, 2001). These methods have been applied both to accelerate the Expectation-Maximization (EM) algorithm and to alleviate the autocorrelation of McMC algorithms. In this work a reparameterization will be employed where additive genetic values will be a *priori* uncorrelated with σ_a^2. This approach will be compared against the conventional model of Wang *et al.* (1993).

2 METHOD

Let us consider a simple animal model **y** = **Xb** + **Za** + **e** with the following distributional assumptions:

$$\begin{aligned} p\left(\mathbf{y} \mid \mathbf{b},\mathbf{a},\sigma_e^2\right) &\sim N\left(\mathbf{X}\mathbf{b}+\mathbf{Z}\mathbf{a},\mathbf{I}\sigma_e^2\right), \\ p\left(\mathbf{a} \mid \mathbf{A},\sigma_a^2\right) &\sim N\left(\mathbf{0},\mathbf{A}\sigma_a^2\right), \\ p\left(\mathbf{e} \mid \sigma_e^2\right) &\sim N\left(\mathbf{0},\mathbf{I}\sigma_e^2\right). \end{aligned} \quad (4)$$

For this particular case and assuming uniform priors for **b** and both variance components, $p(\mathbf{b}) \propto const.$, $p\left(\sigma_a^2\right) \propto const.$, and $p\left(\sigma_e^2\right) \propto const.$, the equation (3) becomes:

$$p\left(\mathbf{b},\mathbf{a},\sigma_a^2,\sigma_e^2 \mid \mathbf{y}\right) \propto \left(\sigma_e^2\right)^{-\frac{n}{2}} \exp\left(-\frac{(\mathbf{y}-\mathbf{X}\mathbf{b}-\mathbf{Z}\mathbf{a})^T(\mathbf{y}-\mathbf{X}\mathbf{b}-\mathbf{Z}\mathbf{a})}{2\sigma_e^2}\right) \times \left(\sigma_a^2\right)^{-\frac{q}{2}} \exp\left(-\frac{\mathbf{a}^T\mathbf{A}^{-1}\mathbf{a}}{2\sigma_a^2}\right), \quad (5)$$

where n is the number of records and q the number of animals. Full conditionals of the posterior (5) can be sampled using the coefficient (left hand side) matrix of the mixed model equations (2), sums of squares, normal and scaled central χ^{-2} deviates (Wang *et al.*, 1993).

Here another approach is proposed, which alleviates the autocorrelation of samples from (5). It is based on the reparameterization of the model in the terms of a new augmented variable **u**, **a** = **u**σ_a. Such a model has been already proposed by Foulley and Quaas (1995) in a heterogeneous variance EM-REML context. To simplify the notation, σ_a is used instead of $\sqrt{\sigma_a^2}$, but the model is still considered written in terms of σ_a^2. The model is now **y** = **Xb** + **Zu**σ_a + **e**, with the following distributional assumptions:

$$\begin{aligned} p\left(\mathbf{y} \mid \mathbf{b},\mathbf{u},\sigma_a^2,\sigma_e^2\right) &\sim N\left(\mathbf{X}\mathbf{b}+\mathbf{Z}\mathbf{u}\sigma_a,\mathbf{I}\sigma_e^2\right), \\ p\left(\mathbf{u} \mid \mathbf{A}\right) &\sim N\left(\mathbf{0},\mathbf{A}\right), \\ p\left(\mathbf{e} \mid \sigma_e^2\right) &\sim N\left(\mathbf{0},\mathbf{I}\sigma_e^2\right). \end{aligned} \quad (6)$$

The joint posterior distribution, assuming again uniform priors on **b** and both variance components, is:

$$p\left(\mathbf{b},\mathbf{u},\sigma_a^2,\sigma_e^2 \mid \mathbf{y}\right) \propto \left(\sigma_e^2\right)^{-\frac{n}{2}} \exp\left(-\frac{(\mathbf{y}-\mathbf{X}\mathbf{b}-\mathbf{Z}\mathbf{u}\sigma_a)^T(\mathbf{y}-\mathbf{X}\mathbf{b}-\mathbf{Z}\mathbf{u}\sigma_a)}{2\sigma_e^2}\right) \times \exp\left(-\frac{\mathbf{u}^T\mathbf{A}^{-1}\mathbf{u}}{2}\right), \quad (7)$$

Note that in (7) variance component σ_a^2 drops out from the last part, but σ_a comes in the sum of squares of residuals. The full conditional distributions for the levels of both **b** and **a** are univariate normal distributions as in the conventional model, but considering **a** = **u**σ_a:

$$p\left(b_i \mid \mathbf{b}_{-i},\mathbf{u},\sigma_a^2,\sigma_e^2,\mathbf{y}\right) \sim N\left(\frac{s_i-\sum_{j\neq i}c_{i,j}b_j-\sum_k c_{i,k}u_k}{c_{i,i}},\frac{\sigma_e^2}{c_{i,i}}\right), \quad (8)$$

$$p\left(u_i \mid \mathbf{u}_{-i}, \mathbf{b}, \sigma_a^2, \sigma_e^2, \mathbf{y}\right) \sim N\left(\frac{s_i - \sum_j c_{i,j} b_j - \sum_{k \neq i} c_{i,k} u_k}{c_{i,i}}, \frac{\sigma_e^2}{c_{i,i}}\right), \quad (9)$$

where both s_i and $c_{i,j}$ are closely related with the conventional mixed model (2) but modified as:

$$\mathbf{C} = \begin{pmatrix} \mathbf{X}^T\mathbf{X} & \mathbf{X}^T\mathbf{Z}\sigma_a \\ \mathbf{Z}^T\mathbf{X}\sigma_a & \mathbf{Z}^T\mathbf{Z}\sigma_a^2 + \mathbf{A}^{-1}\sigma_e^2 \end{pmatrix}, \quad \mathbf{S} = \begin{pmatrix} \mathbf{X}^T\mathbf{y} \\ \mathbf{Z}^T\mathbf{y}\sigma_a \end{pmatrix}. \quad (10)$$

The full conditional distribution of σ_e^2 can be sampled from scaled inverted chi-square distribution with $n - 2$ degrees of freedom as in the conventional model:

$$p\left(\sigma_e^2 \mid \mathbf{b}, \mathbf{u}, \sigma_a^2, \mathbf{y}\right) \sim (\mathbf{y} - \mathbf{Xb} - \mathbf{Zu}\sigma_a)^T(\mathbf{y} - \mathbf{Xb} - \mathbf{Zu}\sigma_a)\chi_{n-2}^{-2}. \quad (11)$$

After some algebra the full conditional of σ_a^2 is:

$$p\left(\sigma_a^2 \mid \mathbf{b}, \mathbf{u}, \sigma_e^2, \mathbf{y}\right) \propto \exp\left(-\frac{-2\frac{\mathbf{u}^T\mathbf{Z}^T(\mathbf{y}-\mathbf{Xb})}{\mathbf{u}^T\mathbf{Z}^T\mathbf{Zu}}\sigma_a + \sigma_a^2}{2\frac{\sigma_e^2}{\mathbf{u}^T\mathbf{Z}^T\mathbf{Zu}}}\right), \quad (12)$$

from which a truncated normal distribution can be recognized when presented in terms of σ_a with mean $\frac{\mathbf{u}^T\mathbf{Z}^T(\mathbf{y}-\mathbf{Xb})}{\mathbf{u}^T\mathbf{Z}^T\mathbf{Zu}}$, variance $\frac{\sigma_e^2}{\mathbf{u}^T\mathbf{Z}^T\mathbf{Zu}}$, and truncation point at 0:

$$p\left(\sigma_a \mid \mathbf{b}, \mathbf{u}, \sigma_e^2, \mathbf{y}\right) \sim TN\left(\frac{\mathbf{u}^T\mathbf{Z}^T(\mathbf{y}-\mathbf{Xb})}{\mathbf{u}^T\mathbf{Z}^T\mathbf{Zu}}, \frac{\sigma_e^2}{\mathbf{u}^T\mathbf{Z}^T\mathbf{Zu}}, 0\right). \quad (13)$$

When the full conditional distribution of σ_a^2 does not involve the neighbourhood of zero, it is a scaled noncentral χ^2 distribution with 1 degree of freedom, with a scale parameter $\frac{\sigma_e^2}{\mathbf{u}^T\mathbf{Z}^T\mathbf{Zu}}$ and noncentrality parameter

$$\lambda = \frac{\mathbf{u}^T\mathbf{Z}^T(\mathbf{y}-\mathbf{Xb})(\mathbf{y}-\mathbf{Xb})^T\mathbf{Zu}}{2\mathbf{u}^T\mathbf{Z}^T\mathbf{Zu}\sigma_e^2}.$$

$$p\left(\sigma_a^2 \mid \mathbf{b}, \mathbf{u}, \sigma_e^2, \mathbf{y}\right) \sim \frac{\sigma_e^2}{\mathbf{u}^T\mathbf{Z}^T\mathbf{Zu}}\chi^2(1, \lambda). \quad (14)$$

For cases where the posterior distribution of σ_a^2 is close to zero, the Metropolis-Hastings algorithm with positive proposal can be implemented, where the natural logarithm of the conditional density derived from (12) is:

$$\ln\left(p\left(\sigma_a^2 \mid \mathbf{b}, \mathbf{u}, \sigma_e^2, \mathbf{y}\right)\right) = -\frac{-2\tau\sigma_a + \sigma_a^2}{2\rho}, \quad (15)$$

where τ represents mean and ρ variance from (13).

3 APPLICATION

Seven simulated datasets were used to compare the length of burn-in period and Monte Carlo variance of the model $\mathbf{y} = \mathbf{Xb} + \mathbf{Zu}\sigma_s + \mathbf{e}$ against the conventional sire model $\mathbf{y} = \mathbf{Xb} + \mathbf{Zs} + \mathbf{e}$. All datasets consisted of 10,000 records, 100 herds (**b**) and 500 unrelated sires (**s**). Records were randomly assigned to herds and sires, i.e., having on average 100 records per herd and 20 records per sire. True phenotypic variance was 100 and sire variances for each simulated case were: 0.25, 0.5, 1.25, 2, 3.75, 5, and 10.

Markov chain Monte Carlo method was implemented using Gibbs sampler for the full conditional distributions described in (8, 9, and 11), while Metropolis sampler was used for sampling from (15). The length of burn-in period was determined by the use of coupling argument (Johnson, 1996; García-Cortés *et al.*, 1998), where the tolerance of difference between two chains for the sire variance component was set to 10^{-4}. After the burn-in period, chains with 20,000 samples were produced. Monte Carlo error was calculated empirically after 50 replicates for each simulated dataset. Presented results show the rate of convergence in the terms of burn-in period (Table 1) and after burn-in period (Table 2) for the conventional model (4) and the new reparameterized model (6).

Table 1: *Average (± standard deviation obtained empirically from 50 replicates) burn-in length by model and true heritability (h^2)*
Preglednica 1: *Povprečna (± standardni odklon, pridobljen empirično iz 50 ponovitev) dolžina ogrevalne faze glede na model in dejansko vrednost heritabilitete (h^2)*

True h^2	Conventional model	Reparametrized model
0.01	569.6 ± 266.1	9.8 ± 6.4
0.02	332.7 ± 165.2	8.4 ± 3.9
0.05	173.9 ± 37.1	7.8 ± 2.6
0.10	162.4 ± 41.2	7.8 ± 2.9
0.15	55.1 ± 5.8	6.8 ± 2.4
0.20	42.6 ± 2.7	7.4 ± 2.2
0.40	25.2 ± 3.6	8.4 ± 3.5

Reparameterization of the model resulted in substantial reduction in burn-in phase of McMC procedure (Table 1), especially with the low values of heritability. Inspection of trace plots (not shown) showed that in the case of low heritability values for additive genetic variance were very close to zero as well as individual additive genetic values, which is expected. However, conventional model was prone to stuck in that configuration, while reparameterized model more easily explored wider pa-

Table 2: *Posterior mean (± standard deviation obtained empirically from 50 replicates) for the component of variance between sires by model and true heritability (h^2)*
Preglednica 2: *Posteriorno povprečje (± standardni odklon, pridobljen empirično iz 50 ponovitev) komponente variance med očeti glede na model in dejansko vrednost heritabilitete (h^2)*

True σ_s^2	True h^2	Conventional model	Reparametrized model
0.25	0.01	0.39 ± 0.03	0.38 ± 0.01
0.50	0.02	0.91 ± 0.03	0.98 ± 0.01
1.25	0.05	1.45 ± 0.02	1.44 ± 0.01
2.50	0.10	1.69 ± 0.02	1.69 ± 0.01
3.75	0.15	4.39 ± 0.01	4.39 ± 0.01
5.00	0.20	6.02 ± 0.01	6.03 ± 0.01
10.00	0.40	13.03 ± 0.01	13.05 ± 0.02

rameter space, which in turn leads to faster convergence to stationary distribution (e.g., Gelman *et al.*, 2004).

Both models gave the same posterior mean on average (Table 2) for variance between sires. Only results for this effect are reported as this is one of the parameters that are hard to accurately estimate in linear mixed models (e.g., Gelman *et al.*, 2004). Posterior means for variance between sires were larger than the true value. This can be attributed to skewed posterior distributions for this effect. Monte Carlo variance obtained after 50 replicates of conventional analysis was sensitive to the value of the true heritability, while this was not the case for reparameterized model. In addition, Monte Carlo variance was higher with conventional model for heritabilities up to 0.1. More stable behaviour of reparameterized model was due to the possibility of easier escape from the neighbourhood of zero value for variance between sires. This means that reparameterized model is of a great value when traits with low heritability are analysed.

4 DISCUSSION

The new data augmentation scheme resulted in an algorithm faster than the conventional Gibbs sampler for linear mixed models. Estimates for variance components do not suffer from getting stuck when visiting values close to zero and then the rate of convergence does not depend on the true value of heritability. When new model was applied to data sets with small heritability, Monte Carlo variance was around five times smaller. Therefore, the new model needs about twenty five times shorter chains to get the same Monte Carlo variance as the conventional model of Wang *et al.* (1993). The new model can be easily implemented in existing programs for the conventional model – slightly modifying the mixed model equations according to (10) and using the Metropolis algorithm to sample from the full conditional density of σ_a^2.

Our procedure is very similar to the parameter expanded models presented in (Liu and Wu, 1999; Gelman *et al.*, 2003; Gelman, 2004) among others for both the most frequent EM and Bayesian McMC. Their approach also standardizes the additive genetic values, but in terms of $\mathbf{a} = \mathbf{u}\alpha$, where α represents an extra augmented variables in the model, while our approach standardizes breeding values with its hyper-parameter, i.e., σ_a. The data augmentation scheme presented here can be understood as a particular case of that presented in van Dyk and Meng (2001), which is based on linear transformations of random variables, such as $\mathbf{y} = \mathbf{Xb} + \mathbf{Zp} + \mathbf{e}$, where $\mathbf{p} = \mathbf{Yu} + \gamma$. In our case $\mathbf{Y} = \mathbf{I}\sigma_a^{-1}$ and $\gamma = 0$, is the simplest case having a significant reduction of the Monte Carlo variance.

Reparameterized model has been tested with a sire model example. Further research is necessary for animal models or multiple trait models (Henderson, 1984), where the amount of missing information may be higher causing more stringency in standard McMC samplers. In such cases reparameterization in terms of **u** is expected to provide even better results than presented here.

5 CONCLUSION

In summary, reparameterization of hierarchical effects resulted in a feasible Markov chain Monte Carlo algorithm that accelerates the convergence of the conventional sampling methods for Bayesian analysis of linear mixed models. This procedure requires a little programming effort for implementation by researchers who have experience with the conventional sampling methods.

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MULTIVARIATE ANALYSIS OF PHENOTYPIC DIFFERENTIATION IN BUNAJI AND SOKOTO GUDALI CATTLE

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Multivariate analysis of phenotypic differentiation in Bunaji and Sokoto Gudali cattle

The study aimed at examining morphometric differentiation in two Nigerian breeds of cattle using multifactorial discriminant analyses. Ten morphological traits (withers height, rump height,chest circumference, body length, face length, tail length, rump length, head width, rump width and shoulder width) of 224 Bunaji and 87 Sokoto Gudali cattle were measured. The animals, which were aged 2.5–3.6 years, were subjected to extensive management system. The linear type traits of Sokoto Gudali cattle were significantly ($P < 0.05$) higher than those of their Bunaji counterparts, with the exception of body length and face length respectively. The stepwise discriminant analysis gave a better resolution as only three variables, rump width, withers height and face length were more discriminating in separating the two cattle breeds. The Mahalanobis distance (7.19) between the two cattle populations was high and significant, which is an indication that they belong to genetically different groups. This was complemented by the result of the Nearest Neighbour Discriminant Analysis, where 85.48% of Bunaji cattle were classified into their source population while 96.55% of their Sokoto Gudali counterparts were correctly assigned into their source genetic group. The present phenotypic information will be the basis for the establishment of further characterization, conservation and selection strategies for the two Nigerian breeds of cattle.

Key words: cattle / breeds / morphological traits / discriminant analysis / characterization / Nigeria

Multivariatna analiza fenotipskih razlik med "Bunaji" in "Sokoto Gudali" govedom

V študiji smo z multivariatno diskriminantno analizo proučevali morfometrične razlike med dvema nigerijskima pasmama goveda. Merili smo deset morfoloških lastnosti (višina vihra, višina trupa, obseg prsi, dolžina telesa, dolžina glave, dolžina repa, dolžina trupa, širina glave, širina trupa in širina pleč) pri 224 živalih pasme "Bunaji" in 87 živalih pasme "Sokoto Gudali". Živali so bile v ekstenzivni reji, stare med 2,5 ter 3,6 leti. Izmerjene vrednosti za linearne lastnosti živali pasme "Sokoto Gudali" so bile statistično značilno večje ($P < 0,05$) kot pri živalih pasme "Bunaji", izjema sta bila le dolžina telesa in dolžina glave. Za doseganje boljše resolucije smo uporabili postopno diskriminantno analizo, ker so le tri spremenljivke, širina telesa, višina vihra in dolžina glave, omogočile zanesljivo ločevanje obeh pasem. Mahalanobijeva distanca (7,19) med obema pasmama je bila visoko statistično značilna, kar nakazuje, da populaciji pripadata različnim pasemskim skupinam. Te rezultate potrjuje tudi diskriminantna analiza najbližjih sosedov, kjer je bilo 85,48% "Bunaji" goveda razvrščenega v izvorno populacijo, medtem, ko je bil ta odstotek pri "Sokoto Gudali" pasmi še višji (96,55). Tako pridobljene fenotipske informacije bomo uporabili za še natančnejši opis , zaščito in oblikovanje rejske strategije obeh nigerijskih pasem goveda.

Ključne besede: govedo / pasme / morfološke lastnosti / diskriminantna analiza / karakterizacija / Nigerija

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1 INTRODUCTION

The wide range of breeds and species that have evolved in various environments represent unique sets of genetic diversity. Genetic diversity has been defined as the variety of alleles and genotypes present in a population, and this is reflected in morphological, physiological and behavioural differences between individuals and populations (Frankham *et al*., 2002). It is generally accepted that the highest amount of genetic diversity in the populations of livestock is found in the developing world where record keeping is poor, and the risk of extinction high and on the increase. Recently, loss of genetic diversity within indigenous livestock breeds has been a major concern (Kastelic *et al*., 2005). Every year many species and breeds of animals become extinct thereby decreasing the biodiversity and genetic variation of populations. Thus, breeds and species that have a tradition of breeding for many a centuries, a unique genotype and aesthetic and cultural value are being lost (Macijauskiene and Juras, 2003; Adamczyk *et al*., 2008). Hence, need for sustainable management and conservation strategies for these animal genetic resources. Since the breed is the operational unit for the assessment of livestock diversity all over the world (Duchev and Groeneveld, 2006), contributions to characterization of local domestic animal populations are of major importance in developing countries.

Characterization of livestock breeds is the first approach to a sustainable use of its animal genetic resources (Lanari *et al*., 2003). The first step of the characterization of local genetic resources is based on the knowledge of variation in the morphological traits (Delgado *et al*., 2001). Morphometric measurements have been used to evaluate the characteristics of various breeds of animals, and could provide useful information on the suitability of animals for selection (Nesamvuni *et al*., 2000; Rastija *et al*., 2004; Araujo *et al*., 2006; Mwacharo *et al*., 2006; Martins *et al*., 2009; Yakubu, 2010). The outcome of genetic improvement programmes could also be evaluated on morphological basis (Riva *et al*., 2004). Although recent analyses have focused on molecular techniques, most mammalian species and subspecies originally were described on the basis of morphological characteristics (Feldhamer *et al*., 2004). Previous efforts on the phenotypic characterization of breeds of livestock have been restricted to the use of analysis of variance, whereas the current trend in livestock classification involves the use of multivariate statistical tools (Traore *et al*., 2008; Yakubu and Akinyemi, 2010). This is because univariate statistical analysis, according to Dossa *et al*. (2007), analyze each variable separately and do not explain how the populations under investigations differ when all measured morphological variables are considered jointly. Multifactorial discriminant analyses have been found to be more suitable in assessing variation within a population and can discriminate different population types when all measured morphological variables are considered jointly.

Cattle are the single most important livestock species in Nigeria in terms of animal protein, value and biomass (Tewe, 1998). However, information is scanty on the morphological characteristics of indigenous cattle especially the Bunaji and Sokoto Gudali which constitute 37.2 and 31.6% of the Nigerian cattle herd of 13,770,641 (RIM, 1992). The research questions are: How morphologically heterogeneous are Nigerian breeds of cattle? And has the classification of Nigerian cattle into different breeds any scientific support? The general objective of the study is to characterize two indigenous cattle breeds of Nigeria based on morphological variation using multivariate discriminant analyses, which could help in proper management, conservation and genetic improvement of the local stock.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMALS AND LOCATION OF STUDY

The experiment made use of a random sample of 211cattle of both sexes, comprising 124 Bunaji and 87 Sokoto Gudali, respectively. The animals were 2.5–3.6 years old as determined by dentition. They were reared through the extensive management system and originated from different herds sampled in Nasarawa state, north central Nigeria. Efforts were made to restrict sampling to phenotipically pure Bunaji and Sokoto Gudali cattle respectively by measuring only those that conformed to the classification descriptors of both breeds.

2.2 MEASURED TRAITS

Ten morphometric traits were measured on each animal. The body parameters were withers height (WH), rump height (RH), chest circumference (CC), body length (BL), face length (FL), tail length, rump length (RL), head width (HW), rump width (RW) and shoulder width (SW). Anatomical reference points were as earlier described (Yakubu *et al.*, 2009). The height measurement (cm) was done using a graduated measuring stick. To achieve this, animals were placed on a flat ground and held by two field assistants. The length and circumference measurements (cm) were effected using a tape rule while the width measurements (cm) were taken using a calibrated wooden calliper. All measurements were car-

Table 1: *Descriptive statistics of morphological traits of Bunaji and Sokoto Gudali cattle*
Preglednica 1: *Opisna statistika morfoloških lastnosti "Bunaji" in "Sokoto Gudali" goveda*

	Bunaji			Sokoto Gudali		
Traits	Mean ± SE	SD	CV	Mean ± SE	SD	CV
Withers height	111.84 ± 0.98[b]	10.87	9.72	127.50 ± 0.53[a]	4.97	3.90
Rump height	120.34 ± 1.01[b]	11.20	9.31	149.53 ± 1.55[a]	14.43	9.65
Chest circumference	141.94 ± 1.62[b]	18.07	12.73	181.15 ± 1.92[a]	17.89	9.88
Body length	175.29 ± 2.25[a]	25.04	14.28	179.02 ± 1.55[a]	14.41	8.05
Face length	52.88 ± 0.49[a]	5.48	10.36	53.28 ± 0.34[a]	3.19	5.99
Tail length	76.81 ± 0.97[b]	10.80	14.06	84.27 ± 0.41[a]	3.87	4.59
Rump length	39.06 ± 0.42[b]	4.73	12.11	42.17 ± 0.31[a]	2.91	6.90
Head width	15.54 ± 0.14[b]	1.60	10.30	21.15 ± 0.41[a]	3.80	17.97
Rump width	33.32 ± 0.44[b]	4.95	14.86	50.43 ± 1.02[a]	9.47	18.78
Shoulder width	28.94 ± 0.43[b]	4.77	16.48	31.79 ± 0.28[a]	2.58	8.12

SE – Standard error, SD – Standard deviation, CV – Coefficient of variation.
Means in the same row with different superscripts are significantly different ($P < 0.05$)

ried out by the same person in order to avoid between-individual variations.

2.3 STATISTICAL ANALYSIS

The morphological traits were subjected to analysis of variance to determine genotype effect using the MEAN procedure of SPSS (2001). Means were separated using the two-tailed, two-sample t-test of the same statistical package. Stepwise discriminant procedure (SAS, 1999) was applied using PROC STEPDISC to determine which morphological traits have more discriminant power than others. The relative importance of the morphometric variables in discriminating between the two cattle populations was assessed using the level of significance, partial R^2 and F-statistic. The CANDISC procedure was used to perform univariate and multivariate one-way analysis that calculated the Mahalanobis distance between the two cattle breeds. The ability of these canonical functions to assign each individual animal to its breed was calculated as the percentage of correct assignment to each genetic group using the DISCRIM procedure (Nearest Neighbour Discriminant Analysis).

3 RESULTS AND DISCUSSION

Descriptive statistics of the morphological traits of Bunaji and Sokoto Gudali cattle are presented in Table 1. Generally, the linear body measurements of Sokoto Gudali were significantly ($P < 0.05$) higher than those of the Bunaji cattle with the exception of body length and face length respectively. Comparative measurements of morphometric traits can provide evidence of breed relationships and size. The considerable variation in body dimensions of the two cattle breeds might not be unconnected with individual breed's potential and peculiarities. While the Bunaji cattle is noted for milk production, their Sokoto Gudali counterparts which rank second in milk production produce more meat and appear to have more draught power than the former. The estimates obtained for height at withers of adult cattle in this study are comparable to those of the Nandi (110–122 cm), Mongalla (100–110 cm) (Rege, 1999), Mexican Criollo Chinampo (101–117 cm) (Espinoza *et al.*, 2009) and Sudan Baggara (115.9–148.80 cm) (Alsiddig *et al.*, 2010) cattle, respectively. The chest circumference values are, however, higher than the range of 122–127 cm reported for North Bengal Grey cattle in Bangladesh (Al-Amin *et al.*, 2007).

Table 2: *Summary of stepwise selection of traits*
Preglednica 2: *Povzetek postopnega izbora lastnosti*

Step	Variables entered	Partial R^2	F-value	Pr > F	Wilk's Lambda	Pr < lambda	Average squared canonical correlation	Pr > ASCC
1	RW	0.5824	291.54	< 0.0001	0.417550	< 0.0001	0.582	< 0.0001
2	WH	0.0948	21.67	< 0.0001	0.362555	< 0.0001	0.637	< 0.0001
3	FL	0.0408	8.85	0.0033	0.400504	< 0.0001	0.599	< 0.0001

RW – rump width, WH – withers height, FL – face length.

The stepwise discriminant analysis showed that rump width, withers height and face length were the most discriminating variables between Bunaji and Sokoto Gudali cattle (Table 2). Their respective partial R^2 and F-values were 0.5824, 0.0948 and 0.0408; 291.54, 21.67 and 8.85 with high significant values ($P < 0.01$–$P < 0.0001$). Morphological variables are easy to monitor and may facilitate the use of ethnological characterization and at the same time institute reliable racial discriminants (Herrera *et al*., 1996). The three morphological variables obtained in the present study are more important and informative, and could be used to assign the two cattle breeds into distinct populations, thereby reducing the errors of selection in future breeding and selection programmes.

Table 3: *Mahalanobis distance between Bunaji and Sokoto Gudali cattle*
Preglednica 3: *Mahalanobijeve distance med "Bunaji" in "Sokoto Gudali" govedom*

Breed	Bunaji	Sokoto Gudali
Bunaji	0	7.19
Sokoto Gudali	7.19	0

The Mahalanobis distance matrix is given in Table 3. The pairwise distance (7.19) between the two cattle breeds was highly significant ($P < 0.001$). This was substantiated by the classification result (posterior probability of membership in each population). While 85.48% of Bunaji cattle were classified into their source population, 96.55% of their Sokoto Gudali counterparts were correctly assigned into their source genetic group (Table 4). The high morphological distance between the two cattle populations coupled with high correct assignment to source genetic groups is an indication that they belong to different breeds. This could have been facilitated by the fact that measurements were restricted to phenotypically pure animals. The use of multivariate discriminant analyses therefore could be successfully used in morphometric differentiation. This is similar to the reports of previous workers on goats (Dossa *et al*., 2007, Yakubu *et al*., 2010a,b and c), sheep (Traore *et al*. 2008; Yakubu and Akinyemi, 2010), cattle (Ndumu *et al*., 2008) and buffalo (Johari *et al*., 2009) respectively.

Table 4: *Percent (%) of individual cattle classified into breed*
Preglednica 4: *Odstotek (%) osebkov, razvrščenih v posamezno pasmo*

Breed	Bunaji	Sokoto Gudali
Bunaji	85.48	14.52
Sokoto Gudali	3.45	96.55
Error level	0.15	0.03
Priors	0.50	0.50

The general aim of genetic conservation is to maintain within and across breed diversity, where within breed diversity refers to the genetic management of one population and the across breed diversity implies the genetic management of many populations. Within breed diversity it is needed for the breed to genetically adapt to changes in the production and economic environment, and to avoid inbreeding problems. Across breed diversity is needed to provide alternatives if a breed happens to run into genetic problems due to genetic drift or changes in the production systems (Meuwissen, 2009). Population studies which elucidate the relationship existing between the different breeds of a given species may offer useful information for the conservation and management of animal genetic resources (AnGR) such as the evolution of the breeds, the development of gene pools and the magnitude of genetic differentiation. According to Mariante *et al*. (2008), national AnGR conservation programmes should use the association of phenotypic data, molecular polymorphisms and adequate statistical methods which reflect the real condition of a population. This was buttressed by Berthouly *et al*. (2010) who studied genetic diversity of Vietnamese H'mong cattle using multivariate analysis on morphometric and genetic data. The present information on the phenotypic differentiation of Bunaji and Sokoto Gudali could therefore be exploited in designing appropriate strategies for their management and conservation. However, there is a need for a genetic study using protein and DNA microsatellite markers to complement the results arisen from morphometric differentiation of the two most populous Nigerian breeds of cattle.

4 CONCLUSIONS

This study showed that Sokoto Gudali had higher mean values in withers height, rump height, chest circumference, tail length, rump length, head width, rump width and shoulder width compared to their Bunaji counterparts. The two cattle breeds were not significantly different in body length and face length respectively. However, rump width, withers height and face length were found to be the most discriminating variables to assign Bunaji and Sokoto Gudali cattle into distinct genetic groups. However, the present information on the morphometric differentiation of Bunaji and Sokoto Gudali breeds of cattle could be complemented with genetic characterization using biochemical and DNA markers.

This could aid field assessment, management and conservation of the two cattle populations, where the goal is to obtain phenotypically pure local genetic resources for future selection and breeding improvement strategies.

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ANTIBIOTIC-RESISTANT SOIL BACTERIA IN HIGH-ALTITUDE (5000–6000 m) SOILS OF THE HIMALAYA

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Antibiotic-resistant soil bacteria in high-altitude (5000–6000 m) soils of the Himalaya

In this study, low-carbon soils collected from an altitude transect from 5000 m to 6000 m were adopted as a simple model system with lower interaction complexity. This could help disentangle the basic environmental factors shaping the abundance and distribution of expressed resistance traits in culturable portion of fast growing heterotrophic strains. Improved plate counts were performed at 4 °C using 0.01 diluted nutrient broth supplemented with cold soil extract as a general media and additionally supplemented with antibiotics Ampicillin, Erythromycin, Kanamycin and Tetracyclin. A number of colonies (500) isolated from six locations were also tested separately for their antibiotic resistance. The results show that these high-altitude cold soils contained bacterial populations culturable at 4 °C in the range of 106 cells / g that were resistant to the four antibiotics and their various combinations tested in this study. The highest prevalence of resistance was observed in vegetated soils, whereas almost two orders of magnitude lower abundance of resistant cells was cultured from barren soils. Redundancy analysis showed that vegetation, soil carbon and pH were successful in explaining the interaction between environmental parameters and various culturable fractions of cold soil bacteria used in this study.

Key words: microbiology / bacteria / antibiotics / resistance / high-altitude / soil / interaction model

Na antibiotike odporne bakterije v visokogorskih tleh Himalaje (5000–6000 m)

V študiji sem uporabil vzorce tal z nizko vsebnostjo organskega ogljika iz višinskega transekta 5000 m–6000 m kot poenostavljen modelni sistem z nizko kompleksnostjo interakcij. Ta bi lahko pomagal razumeti osnovne okoljske dejavnike, ki uravnavajo porazdelitev in obseg izraženih rezistenčnih lastnosti gojljivega dela hitro rastočih heterotrofnih sevov. Izboljšano štetje na ploščah sem izvedel pri 4 °C na 0,01 koncentriranem hranilnem bujonu, dopolnjenim s hladnim ekstraktom tal, kot splošnim gojiščem, ki sem ga dopolnil z posameznimi antibiotiki (ampicilin, eritromicin, kanamicin in tetraciklin). Večje število izolatov (500) iz šestih lokacij sem prav tako testiral ločeno na njihovo odpornost na antibiotike. Ugotavljal sem tudi povezavo med okoljskimi dejavniki ter porazdelitvijo odpornih sevov in splošnega gojljivega deleža talnih bakterij. Rezultati kažejo, da visokogorska hladna tla vsebujejo pri nizkih temperaturah gojljive bakterijske populacije (106 / g), ki so odporni na posamezne antibiotike in razne njihove kombinacije, uporabljene v tej študiji. Poraščena tla imajo največji delež odpornih bakterij, skoraj dva reda manjši pa je prisoten v golih tleh. Statistična analiza je pokazala, da vegetacija, organski ogljik ter pH uspešno razložijo interakcijo med okoljskimi dejavniki in posameznimi gojenimi deleži bakterij, izoliranih iz hladnih tal.

Ključne besede: mikrobiologija / bakterije / antibiotiki / rezistenca / visokogorje / tla / model interakcij

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1 INTRODUCTION

The influence of the wide spread and long term use of antibiotics on the prevalence of resistance traits in the environment is still poorly understood although antibiotic resistance has been recognized as a global public health concern (for review see e.g. Allen *et al.*, 2010; Nwosu, 2001). In this respect, understanding the role of environmental bacteria as resistance gene reservoir is one of the key problems (Demaneche *et al.*, 2008; Riesenfeld *et al.*, 2004; Nwosu, 2001). Many studies have shown the existence of a considerable pool of resistance genes in agricultural soils, fish farm sludges and waters, in isolated human populations even in the absence of an obvious selection pressure (Allen *et al.*, 2010). In addition, metagenomic studies have identified novel resistance genes, a much wider diversity of known genes belonging to various resistance gene families and novel genes coding molecules and enzymes involved as potentiators of microbial resistance (Demaneche *et al.*, 2008; Riesenfield *et al.*, 2004; Allen *et al.*, 2010).

A simple model system composed of low-carbon soil altitude transect from 5000 m to 6000 m was adopted. Low plant diversity and short growing season in one of the most remote and human least directly impacted regions served as a system with lower interaction complexity that could help disentangle the basic environmental factors shaping the distribution and abundance of expressed resistance traits in culturable portion of fast growing heterotrophic strains. Improved plate counts for this environment were performed at 4 °C using 0.01 diluted nutrient broth supplemented with cold soil extract as a general media. In addition, a number of colonies (500) isolated from six locations were tested for their antibiotic resistance. The distribution of antibiotic strains was correlated to environmental parameters recorded and described before (Stres *et al.*, 2010).

2 MATERIALS AND METHODS

2.1 GENERAL CULTURABILITY

The soils and physical-chemical and various biological characteristics of the six soil samples were described before (Stres *et al.*, 2010). Shortly, soils were collected on the south facing slope of high alpine ridge descending from Drohmo peak (6980 m), the Kanchenjunga Himal, Nepal. The abundance of the culturable fraction of heterotrophic microbial community was assessed using plate counts according to approach described by Hashimoto and Hattori (1989) and Janssen *et al.* (2002) and modified as described below. The soil dilution series was prepared in 1 g / L MgSO4 buffer and three replicates per dilution were plated on the following three different oligotrophic complex media for each sample: 0.01 strength Nutrient Broth (Difco) in salt solution solidified with 1% agar (NB-A) supplemented with cold soil extract (Ley *et al.*, 2001; Janssen *et al.*, 2002; Olsen and Bakken, 1987). A colony – forming curve (CFC) (Hashimoto and Hattori, 1989) was generated for each soil by counting newly visible colonies over a 14 week incubation period at 4 °C and plotting the cumulative number of colonies at each time point. Only the counts after three weeks were used for calculations in the present work.

2.2 GENERAL RESISTANCE TO ANTIBIOTIC COMPOUNDS.

The 10 g portions of soil samples were resuspended in total volume of 100 mL of sterile salt solution (Ley *et al.*, 2001) and the cells were stripped from soil particles at 200 rpm for 20 min. Decimal serial dilutions were prepared and 100 µL were inoculated on NB-A plates supplemented with one of the four antibiotic compounds (Ampicillin (50 µg / mL), Tetracycline (20 µg / mL), Kanamycin (20 µg / mL), Erythromicin(15 µg / mL) and incubated at 4 °C. Plates were inspected for well – spaced colonies (distance > 5 mm) after 3 weeks as no additional colonies appeared after 4 week incubation and 95% confidence intervals were calculated.

To verify the antibiotic resistance of the strains appearing after longer incubation periods, a subset of randomly selected colonies (n = 50) from each sample was restreaked on the same plates supplemented with single antibiotic compound.

2.3 ANTIBIOTIC RESISTANCE OF ISOLATED STRAINS

In order to obtain a more conservative estimate on resistant fraction within the culturable portion of bacteria, strains obtained from the first cultivation experiment without antibiotic compounds were tested separately for their antibiotic resistance. Cultures were plated on the same media they were isolated on, but supplemented with one of the four antibiotics as above.

2.4 STATISTICAL ANALYSES

The antibiotic resistance of isolated strains obtained in this study and environmental parameters (Stres *et al.*, 2010) served as input data in linear constrained ordina-

tion, redundancy analysis (RDA) with forward selection that was used to create an environmental model explaining the variability in response variables (antibiotic resistance patterns, abundance of resistant colonies, general abundance of culturable cells). The Monte Carlo permutation test (999 permutations) was applied to compute the significance of hypothetical relations using CANOCO V 4.5 (Biometris) (Leps and Smilauer, 2002).

3 RESULTS AND DISCUSSION

The abundance of resistant CFU to four antibiotics used ranged from lowest 10^2 to 10^6 CFU / g soil in barren and plant covered soils, respectively. Antibiotic resistant CFU determined at 4 °C were almost 100 times more abundant in plant covered (5200 m, 5400 m, 5600 m) than in barren soils (5000 m, 5800 m, 6000 m), despite rather similar number of culturable cells in these soils (Fig. 1).

There was no discernible effect of particular antibiotic compound on the abundance of resistant CFU within particular soil sample, however, the levels of resistant colonies were significantly different ($P < 0.01$) between barren and vegetated soils. The percentages of resistant bacteria varied from 0.01 to 15% in barren soils, median 2%. Surprisingly, the number of antibiotic resistant and the number of culturable bacteria appeared to be equal in plant covered soils, suggesting that all culturable bacteria were also resistant to antibiotics. This is surprising as the values reported in this study are one to two orders of magnitude higher than those reported for transgenic and control corn fields for Ampicillin resistance. In addition, the prevalence of Ampicillin resistant bacteria in undisturbed prairie soil ranged from 54.4% to 69.9% (Demaneche *et al.*, 2008), representing half of the prevalence found in this study. The results of the two studies could represent a simple gradient from intensive agricultural practice through undisturbed prairie to simplified more extreme natural vegetated environment where antibiotic resistance could represent a novel competitive advantage. However, whether these strains are more exposed to antibiotic producing strains or are only exposed to better conditions for gene exchange can not be resolved. Seemingly the question, whether the antibiotics used in this study serve as activators of specific biochemical path-

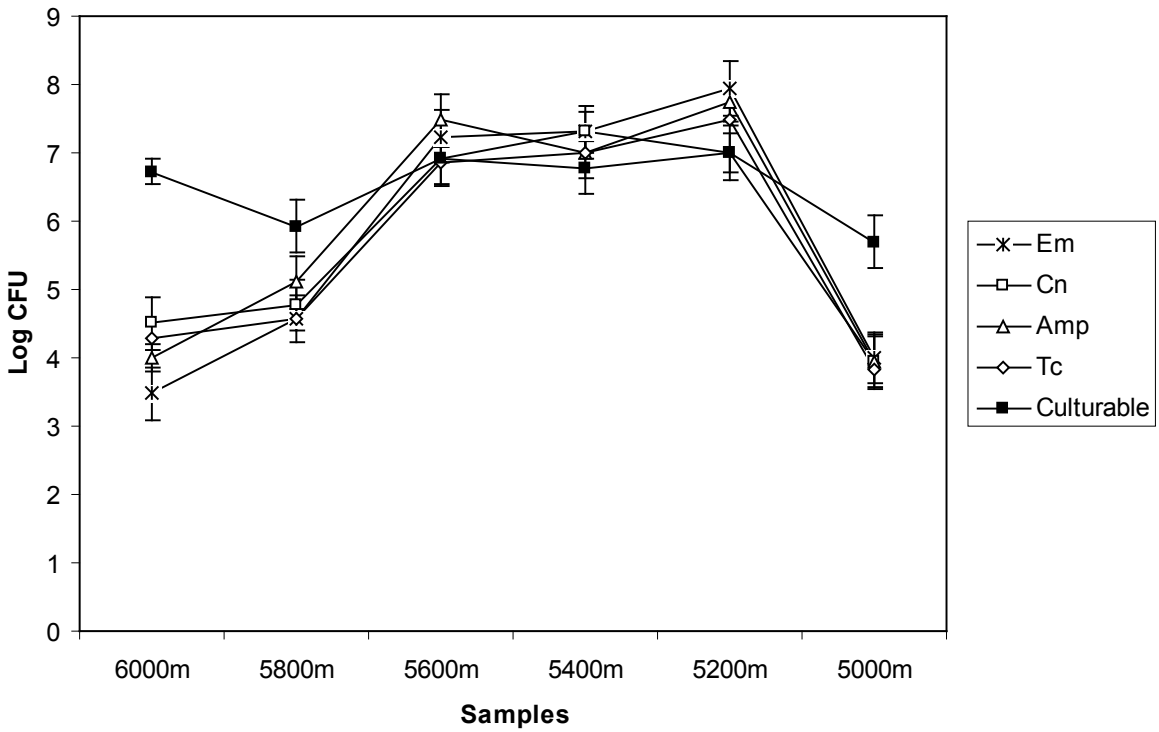

Figure 1: *Colony counts of heterotrophic and antibiotic resistant strains emerging on 0.01 NB-A plates supplemented with single antibiotic compound at 4 °C. Error bars represent 95% confidence intervals. Amp – Ampicillin; Em – Erythromycin; Kn – Kanamycin; Tc – Tetracycline.*

Slika 1: *Število heterotrofnih in na antibiotike odpornih konij, ki so zrasle na gojišču 0,01 NB-A s posameznimi antibiotiki pri 4 °C. Oznake napak predstavljajo 95 % intervale zaupanja. Amp – ampicilin; Em – erithromicin; Kn – kanamicin; Tc – tetraciklin.*

ways, signaling molecules in quorum sensing or just as a simple carbon source can not be answered at this time.

The technical limitations and differences in approaches could be also limiting the comparability of the results between studies, as different approaches to cell stripping, temperature and time of incubation were used next to different carbon source. This highlights the profound inconsistencies in the approaches used to monitor the resistance properties of environmental bacteria as these approaches are not standardized and the data are produced on a range of media, antibiotic concentrations, temperatures and incubation periods (Nwosu, 2001; D'Costa *et al.*, 2006; Allen *et al.*, 2010).

However, it is tempting to speculate that these results indicate that antibiotic resistance is a common trait in this high altitude environment and that plant presence significantly increased the frequency of antibiotic resistance to one and combinations of multiple antibiotics. It also seems that these resistance traits are acquired through different mechanisms than human application and indicate that cold soil bacteria are an important reservoir of antibiotic resistance genes potentially entering water flows during enhanced percolation during snow thaw.

Further, the testing of the strains isolated on NB-A-CSA plates without antibiotic compounds revealed that the vast majority of resistant strains were resistant to three antibiotics, Ampicillin, Kanamycin and Erythromycin (Fig. 2). This is congruent with the recent findings of D'Costa *et al.* (2006) that environmental strains are resistant to multiple antibiotics and also suggests that the distribution of resistance determinants is rather similar among the antibiotic resistance strains from the six samples of the high-altitude cold soils. In addition, the overall abundance of resistant population appears to be modulated exclusively by the presence of plants, despite the similar abundances of culturable bacteria in other barren samples, differences in soil chemistry and plant species (Poa sp., mosses or combination of the two) covering vegetated soils (Stres *et al.*, 2010).

In order to establish which environmental parameters were significantly associated with the observed patterns in antibiotic resistance patterns in the cold soils, RDA analysis was conducted. Environmental characteristics (soil physical and chemical parameters) reported in

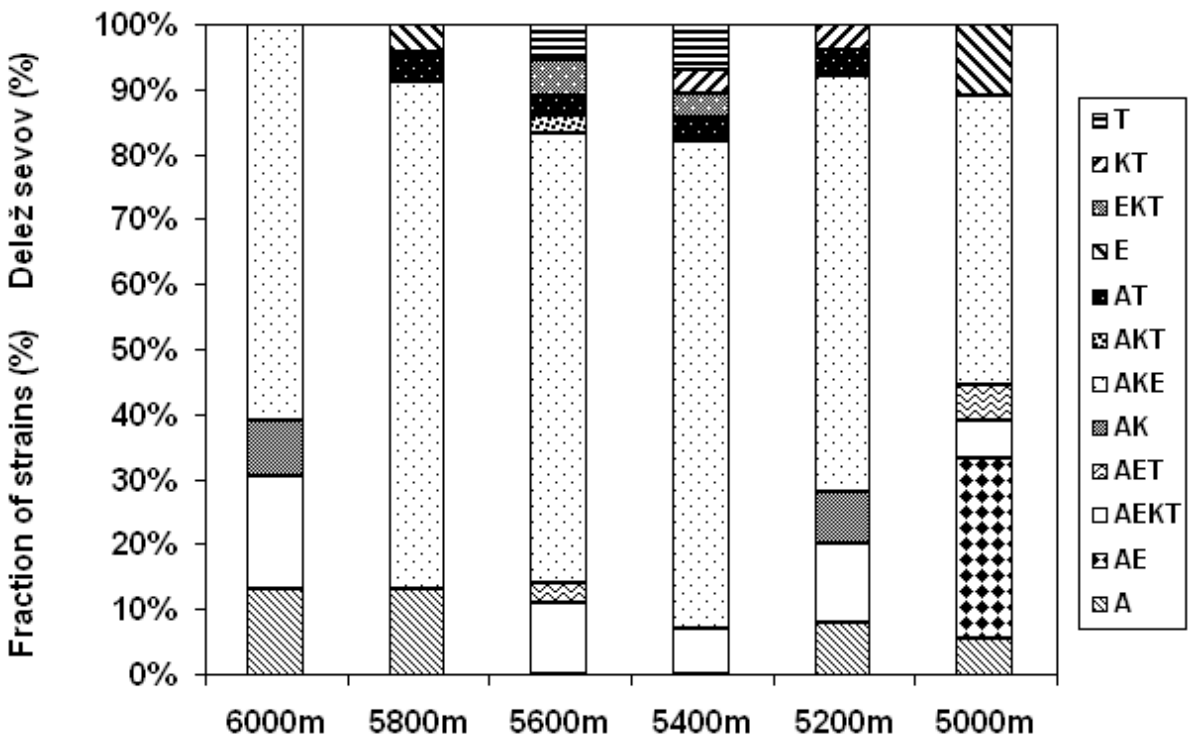

Figure 2: *The distribution of antibiotic resistance determinants in analyzed strains isolated from six composite high-altitude cold-soil samples from 5000 – 6000 m altitude transect. The letters designate the antibiotics and their combinations. A- Ampicillin; E – Erythromycin; K – Kanamycin; T – Tetracycline.*

Slika 2: *Porazdelitev determinant odpornosti analiziranih sevov izoliranih iz šestih visokogorskih hladnih tal iz transekta med 5000–6000 m nadmorske višine. Oznake napak predstavljajo antibiotike ali njihove kombinacije: A – ampicilin; E – erithromicin; K – kanamicin; T – tetraciklin.*

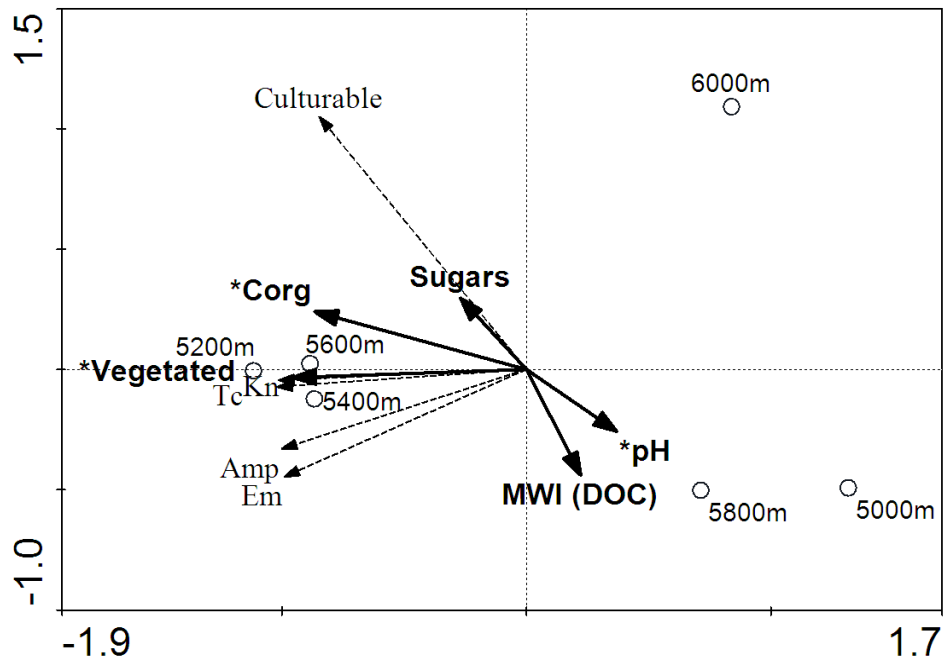

Figure 3: *Results of redundancy analysis (RDA) describing general cultivation success and the abundance of various resistant populations (the response variables) in relation to the sampling localities (empty circles) and environmental parameters (bold arrows). The significant environmental parameters are marked with asterisk (*). Corg – organic carbon; MWI(DOC) – molecular weight index of dissolved organic carbon; Amp – Ampicillin; Em – Erythromycin; Kn – Kanamycin; Tc – Tetracycline.*
Slika 3: *Rezultati statistične analize (RDA) prikazujejo uspešnost gojenja heterotrofnih mikroorganizmov ter različnih odpornih populacij (odzivne spremenljivke – črtkane puščice) v odvisnosti od mest vzorčenja (krožci) ter okoljskih dejavnikov (poudarjene puščice). Signifikantni okoljski dejavniki so označeni z zvezdico (*). Corg – organski ogljik; MWI(DOC) – indeks velikosti molekul raztopljenega organskega ogljika; Amp – ampicilin; Em – erithromicin; Kn – kanamicin; Tc – tetraciklin.*

Stres *et al.* (2010) served as explanatory variables whereas the general culturability and abundance of resistant CFU to each antibiotic (Fig. 1) served as response variables. RDA showed that only three out of 20 measured environmental parameters could explain significantly the variability in the measured abundances of culturable cells and resistant populations. The best predictor of variability in the high-altitude microbial abundance at 4 °C was vegetation, organic carbon and pH, explaining 87.1%, 9.1% and 3% of the data variability (P = 0.002; P = 0.026; P = 0.01), respectively. This environmental model explained 99.2% of variability in abundance of the various culturable fractions explored in this study and 99.7% of species – environment relations. Other environmental parameters tested in this study (Stres *et al.*, 2010) did not produce significant effects and were omitted from Fig. 3 with two exceptions (MWI(DOC), sugars).

Interestingly, the soil content of reductive sugars (Stres *et al.*, 2010) was directly correlated to general culturability of soil bacteria, however, this correlation was not found statistically significant. This finding is interesting in its own right in understanding of environmental parameters that enable recovery of larger proportions of culturable bacteria from the environmental samples (Janssen *et al.*, 2002; Davies *et al.*, 2005). This approach could provide a different strategy in cultivation approaches, first by analyzing the environmental conditions in various samples and pinpointing the environmental parameters correlated to increased culturabiltiy of microorganisms, with efforts mostly directed to various organic species, which is now much more easily achievable through the use of GC-MS or MALDI-TOF MS. On the other hand, the molecular weight index describing the size of complex organic substances was inversely proportional to general culturability. This is also interesting as the size of this index is inversely proportional to molecular weight, suggesting that the general measure of an average molecular weight in dissolved organic carbon fraction offers a too low resolution to be of any particular value in such cases. On the other hand, RDA showed no significant correlation between patterns of antibiotic resistance (Fig. 2) and environmental parameters. This suggests that the distribution of antibiotic resistance determinants did not differ significantly from a random pattern. Alternatively, the presence of other factors and processes that shape the distribution of particular anti-

biotic patterns, not recorded in this study, could explain these observations.

4 CONCLUSIONS

The high-altitude cold-soils contain at 4 °C culturable bacterial populations that are resistant to the four antibiotics tested in this study. The highest prevalence of resistance to antibiotics was recorded for plant covered soils, where all culturable cells exhibited resistance to antibiotics. On the contrary, almost two orders of magnitude lower abundance of resistant cells was cultured in barren soils. Redundancy analysis showed that vegetation, soil carbon and pH were successful in explaining the interaction between environmental parameters and culturable fractions of cold soil bacteria used in this study.

5 ACKNOWLEDGMENTS

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METHYLMERCURY INHIBITS GROWTH AND INDUCES MEMBRANE CHANGES IN *Pseudomonas putida*

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Methylmercury inhibits growth and induces membrane changes in Pseudomonas putida

A bacterial model system (*Pseudomonas putida* DSM 50026) was used in this research to assess toxicity of the environmentally relevant concentrations of mercury species (MeHg and Hg(II)) that represent important pollutants of aquatic ecosystems at sites of industrial or mining activities. In addition to direct monitoring of bacterial growth, we also analyzed fatty acid profiles of exposed and non-exposed cultures to determine possible toxic effects manifested on membrane level. The results showed that exposure of *P. putida* to Hg(II) in concentrations of 0.2–200 µg/L did not have any significant effects on growth nor fatty acid composition of exposed bacterial culture. On the other hand, when bacteria were exposed to up to 1600-times lower concentrations of MeHg (0.12–**12.5 µg/L), growth inhi**bition as well as significant changes in fatty acid composition were detected. Observed adaptive membrane changes due to MeHg exposure were similar to those associated with responses to organic solvents and some other membrane-disrupting compounds.

Key words: microbiology / environmental protection / bacteria / *Pseudomonas putida* / aquatic ecosystems / pollution / mercury / methylmercury / growth inhibition / membrane adaptation / *cis-trans* isomerization

Metil živo srebro inhibira rast in povzroča spremembe v membranah bakterije Pseudomonas putida

V raziskavi smo na bakterijskem modelu (*Pseudomonas putida* DSM 50026) analizirali strupenost okoljskih koncentracij anorganske (Hg(II)) in organske (MeHg) oblike živega srebra, ki predstavljata pomembna vira onesnaženja vodnih ekosistemov v bližini nekaterih industrijskih in rudarskih območij. Poleg neposrednega spremljanja bakterijske rasti smo analizirali tudi maščobnokislinske profile izpostavljenih bakterijskih kultur in jih primerjali s tistimi, ki živosrebrovima spojinama niso bili izpostavljeni. Rezultati so pokazali, da izpostavitev *P. putida* Hg(II) v koncentracijah med 0,2 in 200 µg/L ne inhibira rasti, niti ne vpliva na maščobnokislinsko sestavo bakterijskih membran. Nasprotno pa je izpostavitev celic do 1600-krat nižjim koncentracijam MeHg povzročila tako upočasnitev rasti kot tudi prilagoditvene spremembe na membranskem nivoju. Slednje so bile podobne kot tiste, opažene ob izpostavitvi bakterij organskim topilom in nekaterim drugim spojinam, ki motijo integriteto membran.

Ključne besede: mikrobiologija / varstvo okolja / bakterije / *Pseudomonas putida* / vodni ekosistemi / onesnaževanje / živo srebro / metil živo srebro / inhibicija rasti / membranska adaptacija / *cis-trans* izomerizacija

1 INTRODUCTION

In the past few decades, environmental pollution has become one of the world's major concerns. Heavy metals are a group of pollutants representing environmental problem in most parts of the world. One of the most toxic metals is certainly mercury, which represents a significant concern especially in aquatic ecosystems at sites with industrial or mining activities. Mining operations in areas rich in cinnabar ore may represent strong sources of Hg for many years even after mining has been discontinued (Benoit *et al.*, 1994). One of mercury (Hg) affected sites also lies in Western part of Slovenia where

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lies the second largest Hg mine in the world. The Idrija Mine operated for 500 years until its closure in 1994, but mercury laden tailings still line the banks and the system is a threat to the Idrija River and water bodies downstream, including the Soča / Isonzo River and the Gulf of Trieste in the northern Adriatic Sea, which are therefore subjects to continuous environmental monitoring (Hines *et al.*, 2000; Faganeli *et al.*, 2003).

Mercury can cause acute as well as chronic poisoning in animals and humans (Sweet & Zelnikoff, 2001; Crespo-Lopez *et al.*, 2007; Han *et al.*, 2008). The most toxic forms of mercury are usually considered to be organic compounds, such as methylmercury (MeHg) and dimethylmercury (Me_2Hg), which have the tendency to accumulate in hydrophobic environments such as cell membranes (Mason *et al*, 1996; Girault *et al.*, 1997). Bioaccumulation on different trophic levels leads to biomagnification effect in natural ecosystems, which means that even low levels of organic mercury compounds in the environment may have detrimental effect on organisms at the end of the food chain (Mason *et al.*, 1996). However, not all the mercury that is present in natural ecosystems is bioavailable and therefore harmful to the living organisms (Golding *et al.*, 2007). Standard chemical analytical methods do not have the power to discriminate between bioavailable and fixed forms of mercury in environmental samples and therefore need to be complemented with methods, based on responses of living (micro)organisms for proper risk assessment (Farre *et al.* 2005). Living cells (organisms) can be used as bioindicators, as well as *test-species* in bioassays. By recent establishment of modern 3R concept (reduction, replacement, refinement), the development and application of bioassays based on microbial cells is being promoted, due to their simple cultivation in axenic cultures and lack of ethical issues usually present when using higher organisms (Marinšek Logar and Vodovnik, 2007).

Cell membrane as the first barrier separating cellular interior from its environment represents a primary defense line against unfavorable environmental impacts and therefore appears to be a good target for ecophysiological as well as toxicological studies. Bacteria are known to react to several environmental triggers by modifying fatty acid composition of their membranes, predominantly by changing the ratio of saturated to unsaturated fatty acids (Cronan 2002). However, several strains of ubiquitous bacterium *Pseudomonas putida* have been shown to use at least three adaptation mechanisms at membrane level which apply to different types of environmental stressors: (1) changes in the overall degree of saturation of fatty acids (Loffhagen *et al.* 2004), (2) the formation of cyclopropane fatty acids (Härtig *et al.* 2005) and (3) *cis-trans* isomerization (von Wallbrun *et al.* 2003). Meanwhile the first two responses are mainly associated with temperature stress and starvation, *cis-trans* isomerization, appears to be involved in toxic stress defence (Heipieper *et al.* 1995; Heipieper *et al.* 1996). It has been shown in solvent-tolerant strains of *P. putida* that toxicity and concentration of organic solvents in their membrane correlate with increase in *trans/cis* fatty acid ratio (Heipieper *et al.* 1992; Heipiepper *et al.* 1994; Weber *et al.*, 1996). Moreover, several heavy metal ions, namely Zn^{2+}, Cd^{2+}, Cr^{3+}, Co^{2+}, Cu^{2+} and Ni^{2+} have also been shown to induce adaptive changes resulting in increased accumulation of *trans* fatty acids (Heipieper *et al.* 1996). However, there are so far no reports on effects of mercury (Hg) species on *P. putida* (or other bacterial) membranes, which is the objective of this article. Our hypothesis was that organic (MeHg), as well as inorganic (Hg(II)) form of mercury may influence the membrane (fatty acid profile) of *P. putida*. However, due to its hydrophobic nature, the degree to which membranes are affected was expected to be larger in case of MeHg.

2 MATERIALS AND METHODS

2.1 MICROORGANISM

P. putida DSM 50026 cells were purchased in freeze-dried form from Deutsche Sammlung von Mikroorganizmen und Zellkulturen GmbH (DSMZ, 1998).

2.2 CHEMICALS

General reagents dimethylsulfoxide (DMSO), methanol, n-hexane and glycerol as well as both mercury species: MeHg in the form of CH_3HgCl and Hg(II) in the form of $HgCl_2$ were purchased from Sigma (St. Luis, MO, USA) or Merck (Darmstadt, Germany). Bacteriological agar and peptone were purchased from Biolife (Milan, Italy) and meat extract from Becton Dickinson (New Jersey, NJ, USA). Standard calibration mixture of bacterial FAME in methyl-caproate (BAME standard) was purchased from Sigma (St. Luis, MO, USA) and standard calibration mixture of bacterial FAMEs in hexane (MIDI standard) was purchased from Hewlett Packard (USA).

2.3 CULTURE CONDITIONS

P. putida DSMZ 50026 was cultivated for 20 hours in medium described by DSMZ (1998) containing 3 g of meat extract and 5 g of bacteriological peptone per

Table 1: *Concentrations of tested mercury species*
Preglednica 1: *Koncentracije testiranih živosrebrovih spojin*

Tested compound	Concentrations (µg/L)			
$HgCl_2$ (Hg (II))	200	20	2	0.2
CH_3HgCl (MeHg)	12.5	1.25	0.12	-

1000 ml of distilled water (dH_2O). Cells were grown in 10 ml test-tubes, at 27 °C (without shaking).

2.4 EXPOSURE CONDITIONS

After 20 hours incubation, selected environmentally relevant concentrations (Quiu *et al.*, 2006) of tested mercury species (Table 1) were added to the cultures. MeHg, which is water insoluble, was dissolved in 50% DMSO instead of distilled water before added to the culture medium. Negative controls for those samples were performed with the addition of an adequate amount of DMSO as well. Cells exposed to tested concentrations of mercury species were incubated for another 24 hours at 27 °C. During incubations, growth was followed by measuring optical density at 654 nm by Novaspec II Visible Spectrofotometer. Cells were harvested by centrifugation (3000 rpm, 4 °C, 10 min). Pellets were resuspended in sterile double distilled water (1 mL), frozen (–20 °C) and freeze-dried.

2.5 LIPID EXTRACTION AND TRANSESTERIFICATION

Bacterial lipids were extracted from freeze dried samples and transesterified using modified HCl/methanol procedure that has already been described before (Ivancic *et al.*, 2009).

2.6 GAS CHROMATOGRAPHY

Fatty acid methyl esters (FAMEs) extracts in hexane were analyzed on gas chromatograph Shimadzu GC-14A equipped with flame ionization detector (FID). Capillary column (Equity-1; Supelco, 28046-U) with non-polar stationary phase (100% poly-dimethyl-siloxane) was used. The analysis followed the temperature program: temperature gradient from 150 to 250 °C at 4 °C min^{-1}. The flow rate of carrier gas (He) was 30 ml min^{-1}. The injector temperature was held at 250 °C and detector at 280 °C. The results were registered on Chromatopac C-R6A integrator. Relative proportions of fatty acids between C10 and C20 were calculated from peak areas. Identification was done either directly by comparison of retention times of unknown peaks with standard fatty acid calibration mixtures (BAME, MIDI; SIGMA-Aldrich) or indirectly by equivalent chain length (ECL) factors calculation (Mjøs, 2003).

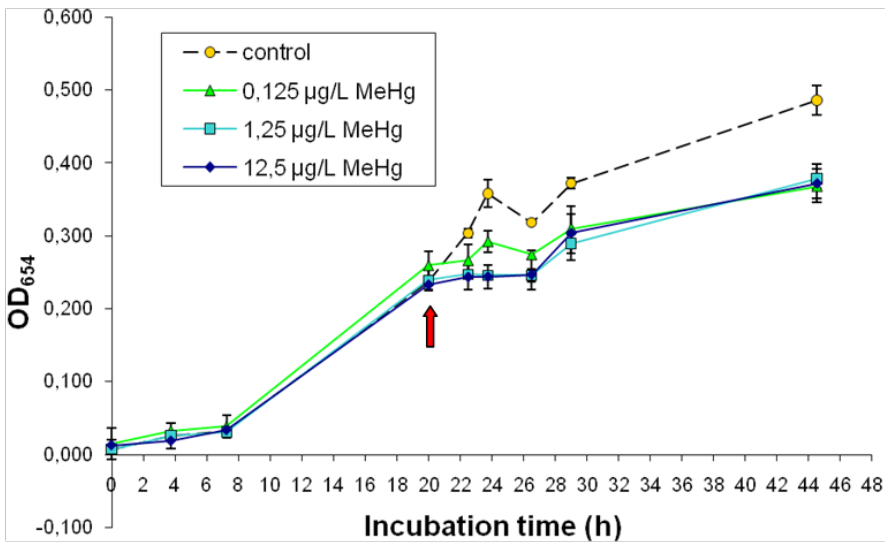

Figure 1: *Growth curves of P. putida DSM 50026 culture exposed to MeHg in concentrations from 0.12 to 12.5 µg/L in comparison to non-exposed cells (control). The time of MeHg addition is marked by arrow.*
Slika 1: *Rastne krivulje kulture P. putida DSM 50026 izpostavljene MeHg v koncentracijah od 0,12 do 12,5 µg/L v primerjavi s kontrolno (neizpostavljeno) kulturo. Začetni čas izpostavitve MeHg je označen s puščico.*

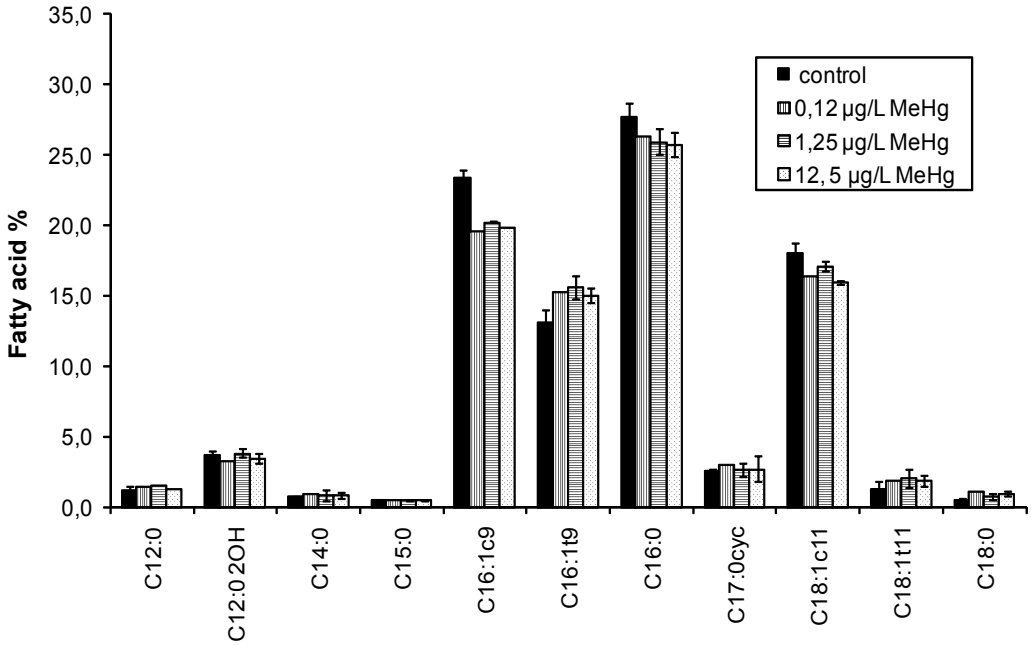

Figure 2: *Changes in fatty acid profile of P. putida DSM 50026 culture exposed to MeHg in concentrations from 0.12 to 12.5 µg/L.*
Slika 2: *Spremembe v maščobnokislinskem profilu kulture P. putida DSM 50026 ob izpostavitvi MeHg v koncentracijah od 0,12 µg/L do 12,5 µg/L.*

2.7 CALCULATIONS

Membrane fatty acids which were present in less than 0.5% of total fatty acids were signed as fatty acids in traces and were not considered for further interpretation.

Trans/cis ratio of unsaturated fatty acids was calculated according to Heipieper *et al.*, 1995.

2.8 STATISTICAL ANALYSIS

All the exposures were performed in 4 parallel samples. The data was statistically analyzed using Student`s t-test with significance level of 0.05.

3 RESULTS

Our experiments showed that exposure *of P. putida* DSM 50026 to $HgCl_2$ in concentrations of 0.2–200 µg/L did not result in any significant effects on growth nor fatty acid composition of exposed bacterial culture (results not shown). However, when bacteria were exposed to organic mercury in form of CH_3HgCl (from 0.12–12.5 µg/L) growth inhibition as well as significant changes in fatty acid composition were observed (Figures 1, 2). Most significant (dose-effect) inhibition of cell growth occurred in the first 4–6 hours after exposure of cells to MeHg. After 8 hours of growth in MeHg supplemented medium, cell culture appeared to grow with approximately the same (attenuated) rate, regardless of mercury concentration. The behavior of growth curves that can be observed in Fig. 1 suggests the possibility of adaptive changes of microbial cells, enabling the culture to continue growing under the changed conditions. Since the membrane represents the primary barrier between cells and the environment and is responsible to regulate the flow of molecules into (and out of) the cell, we decided to focus our research on possible adaptive changes that may be detected on lipid level. Our results show that exposure of *P. putida* to MeHg in concentrations between 0.12–12.5 µg/L significantly influences fatty acid profile of tested bacteria, resulting in increase of *trans/cis* fatty acid ratio from 0.35 ± 0.04 (in non-exposed cells) to 0.47 ± 0.02 (in cells exposed to 0.12 µg/L or 1.25 µg/L MeHg) or 0.48 ± 0.03 (in cells exposed to 12.5 µg/L). The observed shift in *trans/cis* ratio is mainly associated with statistically significant decreases in C16:1*cis*9 and C18:1*cis*11 fatty acids, accompanied by increase in C16:1*trans*9.

4 DISCUSSION

Little is known about the molecular mechanisms controlling (methyl)mercury uptake and toxicity so far. The primary targets of both, $CH_3Hg(II)$ as well as inorganic Hg(II), are considered sulfhydril-containing macromolecules (especially of various molecular weight thiol-containing proteins). Covalent binding of mercury compounds to proteins acting as antioxidants (i.e. glutathione) or components of electron transport chains appears to be associated with free radical accumulation, leading to oxidative damage of macromolecules and lipid peroxidation (Patrick, 2002; Han *et al.*, 2008). Despite the generally recognized common molecular targets, the levels of mercury species inducing toxicity usually differ. It is generally assumed that MeHg is the most toxic Hg species, which is often ascribed to its higher lipid solubility (Sweet, 2001). However, the octanol/water partition coefficients (K_{ow}) of uncharged $HgCl_2$ and CH_3HgCl species do not differ significantly (3.3 and 1.7 respectively) (Broniatowski, 2009). These data suggest that the actual interaction of the Hg species with the cell membranes is very much dependent on the environmental factors influencing their ionization as well as membrane charge (especially pH and the types, as well as concentrations of ions present in the solution) (Sweet, 2001).

Only few studies on methylmercury binding to biomembrane lipids have been reported. Early "*in vitro*" studies suggested a direct mechanism of $CH_3Hg(II)$ action on selected lipids. Segal & Wood (1974), for example, performed an NMR study which showed that MeHg can react both catalytically and directly with plasmalogens (a group of phospholipids which are important in a membrane structure for cells of the central nervous system of higher organisms). They showed that MeHg ion is soluble in phospholipids and catalyses rapid hydration and hydrolysis of the vinyl ether linkage to give a mixture of palmitic and stearic aldehydes plus the linolenic monoglyceride product (Segal and Wood, 1974). Furthermore, studies performed by LeBlanc *et al.* (1984) revealed a pH-dependent binding of MeHg to acidic phosphatidylserine (PS) and phosphatidylinositol (PI) phospholipids, but not to zwitterionic phosphatidilcholine (PC) and sphingomielin (SM). The most extensive study on MeHg interaction with phospholipid membranes is probably the one performed by Girault *et al.*(1997), in which the authors used three complementary approaches: (i) ^{199}Hg-NMR which quantitatively describes MeHg mobility and complexation, both in solution and at the membrane interface, (ii) fluorescence polarization which reveals dynamic changes of the hydrophobic interior and (iii) solid state ^{31}P-NMR which is indicative of the phosphate group structure and mobility and allows detection of non-bilayer phases. The study revealed that $CH_3Hg(II)$ interactions with membrane lipids are electrostatic in nature and primarily depend on the polar head groups negative charges (phosphate moiety), which is not the case with $HgCl_2$(Delnomdedieum *et al.*, 1992; Girault *et al.* 1997). Extensive metal binding (up to three MeHg molecules per lipid) induces limited membrane destabilization, which may, in some cases, be associated with loss of its integrity (Girault *et al.*, 1997).

Our results confirmed the hypothesis that effects of mercury compounds on *P. putida* cells essentially depend on their chemical structures. Meanwhile chosen concentrations of inorganic mercury in form of $HgCl_2$, did not inhibit growth nor induced any adaptive changes in bacterial membranes the opposite was the case with its methylated form. MeHg exhibited toxicity that reflected at both levels (culture growth as well as membrane changes) at concentrations up to 1600-times lower that the highest Hg(II) concentration tested. Most significant inhibition of cell growth occurred in the first 4–6 hours after exposure to MeHg. After 8 hours of growth in MeHg supplemented medium, cell culture appeared to grow with approximately the same (slightly attenuated) rate, regardless of methylmercury concentration. The lack of dose-effect inhibition at this stage may indicate that differences in chosen concentrations were too small to inhibit significantly different proportions of cells that would be observable by spectrophotometric measurements. However, the behavior of growth curves that can be observed in Fig. 1 suggests the possibility of adaptive changes in certain number of microbial cells that have survived the MeHg presence, enabling the cultures to continue growing under the changed conditions.

Observed membrane changes associated with MeHg exposure resulted in overall increase in *trans/cis* fatty acid ratio, indicating the prevalent isomerization of *cis-* to *trans-* unsaturated fatty acids. This adaptive response is known to be associated with decrease in membrane fluidity, enabling *Pseudomonas* strains to grow in the presence of membrane-disrupting compounds (Von Wallbrun *et al.*, 2003; Härtig *et al.*, 2005). The same type of response has already been described when selected *P. putida* strains were exposed to toxic concentrations of toluene (Weber *et al.*, 1994; Heipieper *et al.*, 1994), phenol (Heipieper *et al.*, 1992), ethanol (Heipieper *et al.*, 1994) and six different heavy metals, namely Zn^{2+}, Cd^{2+}, Cr^{3+}, Co^{2+}, Cu^{2+} and Ni^{2+} (Heipieper *et al.*, 1996). The degree of isomerization was shown to depend on the toxicity and the concentration of membrane-affecting agents. The described way of membrane adaptation is performed by *cis–trans*-isomerase (Cti), a constitutively expressed periplasmic enzyme that, to exert its action, necessitates neither ATP nor other cofactors, and consistently, is in-

dependent of de novo synthesis of lipids. Due to its direct correlation with toxicity, *cis–trans*-isomerization is a potential biomarker for recording solvent stress or changes of other environmental conditions (Bernal *et al.*, 2007; Heipieper *et al.*, 2010). The question that needs to be addressed at this point is how do *P. putida* cells detect the presence of membrane disrupting compounds like MeHg, which leads to activation of protective mechanism(s). In the presence of organice solvents, the detection and activation appears to be directly associated with detected increase in membrane microviscosity caused by changes of the acyl chain order (Killian *et al.*, 1992). According to Neumann *et al.*, the hydrophilic structure and periplasmic location of Cti supports the assumption that the enzyme can only reach its target (the double bonds of unsaturated fatty acids that are located at a certain depth of the membrane) when the membrane is destabilized (i.e. the fluidity at certain regions is increased) by environmental factors (Neumann *et al.*, 2003; Härtig *et al.*, 2005). Since direct effect on membrane fluidity has also been observed in the case of MeHg (Girault *et al.*, 1997; Schara *et al.*, 2001), the abovementioned mechanism may apply here as well.

5 CONCLUSIONS

In our research a bacterial model has been used to assess toxicity of two mercury species that represent important pollutants of aquatic ecosystems at sites of industrial or mining activities.

The results showed different toxicities of Hg(II) and MeHg to (bacterial) cells. Meanwhile inorganic form, Hg(II) did not influence the growth nor induce any significant changes in fatty acid profile of *P. putida*, exposure to methylated form of mercury resulted in partial growth inhibition, which appears to be balanced by adaptive membrane changes. We showed that changes in fatty acid profile of *P. putida* resulting from MeHg exposure are similar to those observed as a response to organic solvents, as well as some other membrane-disrupting compounds, and are associated with (adaptive) decrease in membrane fluidity.

Despite the fact that response of *P. putida* to MeHg is not specific, these bacteria might possibly be used to develop a bioassay, used to indicate the potential presence of toxic bioavailable concentrations of MeHg in environments where mercury represents the major pollutant (i.e. Idrijca river, where MeHg also accumulates in freshwater fish and crabs). Neverthless, more research needs to be done to assess the influence of different physico-chemical parameters (like pH, ionic strength etc.) as well as other potentially interfering compounds on the responsiveness of the system.

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VARIATIONS IN THE FATTY ACID COMPOSITION AND NUTRITIONAL VALUE OF ADRIATIC SARDINE (*Sardina pilchardus* Walb.) THROUGH THE FISHING SEASON [1]

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Variations in the fatty acid composition and nutritional value of Adriatic sardine (Sardina pilchardus Walb.) through the fishing season

We investigated the chemical composition, in terms of water, protein, ash, total fat and fatty-acid composition, of sardine meat, and estimated its nutritional value. The samples originated from Adriatic sardines (*Sardina pilchardus* Walb.) that were collected in the north Adriatic Sea through the winter, spring, summer and autumn seasons. The content of 20 fatty acids was determined by *in-situ* transesterification and capillary column gas-liquid chromatography, using nonadecanoic acid (19:0) as internal standard. The mean contents of the sardine meat were: 70.8% water, 21.0% protein, 2.5% ash and 6.4% fat. For the fatty-acid composition, 18.0% were mono-unsaturated, 42.6% polyunsaturated and 39.8% saturated. The total-fat content increased through the year, from winter to autumn (0.69 to 18.15 g/100 g meat). The fatty-acid composition in the sardine meat varied significantly, with the levels of the polyunsaturated fatty acids (4.6 g/100 g meat), and especially eicosapentaenoic acid (20:5*n*-3, 0.98 g/100 g meat) and docosahexaenoic acid (22:6*n*-3, 1.9 g/100 g meat), being the highest in autumn, before spawning. The *n*-6/*n*-3 ratio (0.13) and P/S ratio (7.6) show that sardine meat can and should be included in a balanced human diet. Considering the recommended daily intake of *n*-3 polyunsaturated fatty acids is 0.45 g per day for a healthy population, this would be consumed as 10 g sardine meat collected in the autumn or 100 g sardine meat collected in the winter.

Key words: human nutrition / food / fish / Adriatic sardine / *Sardina pilchardus* Walb. / composition / fatty acids / nutritional value / season

Maščobnokislinski profil in prehranska vrednost jadranske sardele (Sardina pilchardus Walb.) v odvisnosti od sezone ulova

Raziskovali smo kemijsko (vsebnost vode, beljakovin, maščob in mineralnih snovi) in še posebej maščobno kislinsko sestavo mesa sardel ter določili njeno prehransko vrednost. Jadranske sardele (*Sardina pilchardus* Walb.) so bile ujete v severnem Jadranskem morju v štirih različnih lovnih sezonah: pozimi, spomladi, poleti in jeseni. Z metodo *in situ* transesterifikacije in določitve na plinsko tekočinskem kromatografu smo določili 20 maščobnih kislin. Meso sardin povprečno vsebuje 70,8 % vode, 21,0 % beljakovin, 2,5 % mineralnih snovi in 6,4% maščob; od skupnih maščobnih kislin je 18,0 % enkrat nenasičenih, 42,6 % večkrat nenasičenih ter 39,8 % nasičenih. Vsebnost maščob je močno nihala med sezonami (naraščala od zime proti jeseni, od 0,69 % do 18,15 %) in statistično značilno vplivala na maščobnokislinsko sestavo mesa. Največ večkrat nenasičenih maščobnih kislin (skupaj 4,6 g/100 g mesa), predvsem eikozapentaenojske (20:5*n*-3, 0,98 g/100 g mesa) in dokozaheksaenojske (22:6*n*-3, 1,9 g/100 g mesa) so vsebovale sardele jesenskega ulova (pred drstenjem). Razmerja *n*-6/*n*-3 (0,13) in P/S (7,6) kažeta, da imajo sardine visoko prehransko vrednost. Priporočen dnevni vnos večkrat nenasičenih maščobnih kislin (0,45 g/dan za zdravo populacijo) dosežemo že z dnevnim zaužitjem 100 g mesa sardel, ujetih pozimi, in 10 g mesa sardel, ujetih jeseni.

Ključne besede: prehrana ljudi / živila / ribe / jadranska sardela / *Sardina pilchardus* Walb. / sestava / maščobne kisline / prehranska vrednost / letni čas

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1 INTRODUCTION

The Adriatic sardine (*Sardina pilchardus* Walb.) is a widespread fish along the European and African coasts of the Mediterranean Sea. It is also found in the English Channel, and in the Black Sea and North Sea. This pelagic oily fish was, and still is, an important species for the fish industry in Mediterranean countries.

Evidence suggests that fish consumption decreases the risk of cardiovascular disease, cancers and asthma, and particularly consumption of the oily fish that contain high levels of polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA, 22:6*cis*-4,7,10,13,16,19) and eicosapentaenoic acid (EPA, 20:5*cis*-5,8,11,14,17). Fish consumption also has positive influences on infant neurodevelopment, along with many other reported benefits (Simopoulos, 2002; FSA, 2004). However, although all fish are generally considered to be of similar nutritional value, it has been recognized that the PUFA composition can vary across different fish species. Therefore, when fish is suggested as a means of improving health, both the species and the PUFA composition should be taken into account (Osman *et al.*, 2001).

The role of lipids in nutrition is manifold. They are the carriers of the energy value and contain the essential FAs and PUFAs, and they are the precursors for the biosynthesis of the eicosanoids, which have important functions in the human body (Tapiero *et al.*, 2002; Nelson and Cox, 2005). Indeed, the most important value of fish meat and lipids are the PUFAs, and especially DHA and EPA, both of which are found mainly in fish lipids and in trace amounts in food of animal and plant origin.

Studies of the FA composition of fish are important when related to the above-mentioned benefits. From the nutritional point of view, there are different important indices, such as the P/S ratio, *n*-6/*n*-3 ratio and atherogenic index. The recommended ratio of PUFA to saturated FAs (P/S) should be above 0.4, with the normal P/S ratio for meat at around 0.1 (Wood *et al.*, 2003). Simopoulos (2002) concluded that the optimal ratio of *n*-6/*n*-3 varies from 1:1 to 4:1, depending on disease under consideration. A low ratio of *n*-6/*n*-3 PUFAs is more desirable to reduce the risks of many of chronic diseases of high prevalence in both Western societies and developing countries, which are being exported to the rest of the World. The World Health Organisation has recommended similar values for the *n*-6/*n*-3 PUFA ratio, as 5:1 to 10:1 (WHO, 1994). Attempts to develop a better index of the potential health attributes of foods containing a mixture of FAs have been reported by Ulbricht and Southgate (1991), with their indices of atherogenicity and thrombogenicity. The atherogenic index is recommended as between 0.70 and 0.72 (Ulbricht and Southgate, 1991), although Salobir (1997) recommended even lower levels, of under 0.5.

In view of these details, we have here followed the need for further studies of the nutritional value and lipid profiles of the most commonly consumed fish in Mediterranean countries throughout the four seasons of the year.

2 MATERIAL AND METHODS

The sardines (*Sardina pilchardus*) used in this study were caught in the north Adriatic Sea, off Slovenia and north Croatia, through the four seasons of the year: winter, spring, summer and autumn. Three samples were taken during each season, each of which consisted of eight sardines. The fish were prepared as ready-to-eat: they were beheaded, gutted and frozen (–80 °C), for a maximum period of one month before sampling.

The chemical composition of these sardines was determined at the end of the sample collection, in terms of their ash, water, proteins and fat content. The water content was determined on 5 g samples of the minced meat. These samples were dried in an oven at 105 °C according to the AOAC 950.46 (1997) recommendations. The total protein (crude protein, N × 6.25) content was determined by the Kjeldahl method, according to the AOAC 928.08 (1997) standard methodology. The ash content was determined by mineralisation of the samples at 550 °C, according to the AOAC 920.153 (1997) standard method.

The FA compositions of the samples were determined by gas-liquid chromatography (GLC). The method chosen was *in-situ* transesterification (Park and Goins, 1994). The contents of the FA methyl esters (FAMEs) were determined by GLC using an Agilent Technologies 6890 gas chromatograph with a flame ionisation detector and an SP™-2380 capillary column (Supelco, Cat.No. 24111) (60 m × 0.25 mm × 0.2 μm). The separation and detection were performed under the following conditions: temperature programme, 170 °C (hold 5 min); 4 °C/min to 250 °C (25 min); injector temperature, 250 °C; detector temperature, 280 °C; injector: split:splitless, 1:30, volume 1 μL; carrier gas: He, 1.0 mL/min; make-up gas: N_2, 45 mL/min; detector gases: H_2, 40 mL/min; synthetic air (21% O_2) 450 mL/min.

The FAMEs were determined through comparison with the retention times of the FAMEs from standard mixtures (Supelco, 37-component FAME ester mix; Cat. No. 18919-1AMP; Supelco, PUFA No.1: Animal source, Cat. No. 47015-U; Supelco, Linoleic Acid Methyl Ester *cis*/*trans* Isomer Mix, Cat. No. 47791; Supelco, *cis*-7-octadecenoic methyl ester, Cat. No. 46900-U; Supelco, *cis*-11-

octadecenoic methyl ester, Cat. No. 46904; Fluka, methyl stearidonate, Cat. No. 43959; natural ASA CLA 10t, 12c in CLA 9c, 11t; NuChek standards: GLC-68D, GLC-85, GLC-411 and GLC-546). As an internal standard, 100 µL of a solution of nonadecanoic acid in hexane (10 g/L; C19:0) (Sigma, N4129) was added to the samples before saponification. The NuChek GLC-68D and GLC-85 standard mixtures were used to determine the response factor, Rf_i, for each FA. The weight portion of each FA in the sample was determined using Rf_i and the transformation factor of FA content from the FAME content. The reliability and accuracy of the analytical methods for the detection of the FAs was ensured by the use of the certified CRM 163 reference matrix (blended beef-pork fat; BCR), and these were in good agreement with the certified values. The FAs are expressed in % of total FAs (w/w) and as mg FA in 100 g of edible fish.

The data for the chemical composition of sardine meat were analysed by least squares analysis using the GLM procedure (SAS, 1999). The statistical model included the effects of season (S) and fish (F):

$$y_{ijk} = \mu + S_i + F_j + e_{ijk},$$

where y_{ijk} = the ijkth observation, μ = the general mean, S_i = the effect of the ith catching season (winter, spring, summer, autumn), F_j = the effect of the jth fish (1–24 fish), and e_{ijk} = the residual random term with a variance of σ^2_e.

The means of the experimental groups were obtained using the Duncan test, with relationships between the parameters assessed by Pearson correlation coefficients, using the CORR procedure (SAS, 1999).

3 RESULTS AND DISCUSSION

The fat content and the FA profiles of the sardine meat varied significantly according to the seasons of capture ($P \leq 0.001$). Table 1 gives the mean values for the chemical compositions, which are similar to other data reported in the literature (Castrillón *et al.*, 1997; Macciola *et al.*, 2003). The ash content was higher (2.5%) in comparison with other meats. The main reason for this was in the preparation of the samples, as they were prepared ready-to-eat: beheaded and gutted, but without the fish bones being removed as they are edible after cooking. The amount of total fat varied over the period of a year, in the range of 0.7% to 18.2% (Table 2).

Table 1: *Chemical composition of the sardine meat*
Preglednica 1: *Kemijska sestava mesa sardin*

Chemical composition, %	N	Mean ± SD	Min	Max	CV (%)
Protein Beljakovine	96	20.96 ± 0.98	19.62	22.63	4.68
Water Voda	96	70.79 ± 5.54	61.05	76.62	7.82
Fat Maščobe	96	6.42 ± 6.16	0.69	18.15	95.98
Ash Pepel	96	2.50 ± 0.25	2.13	2.83	9.86

N – number of observations / število vzorcev, – mean / povprečje, SD – standard deviation / standardni odklon, Min. – minimal value / minimalna vrednost, Max. – maximal value / maksimalna vrednost, SD – standard deviation / standardni odklon, CV (%) – coefficient of variation / koeficient variabilnosti.

Changes in fat content vary as the sardines drain or replenish their fat reserves in response to the availability of food, their spawning cycles and other factors in the sea (Hardy and Keay, 1972). Adriatic sardines spawn from the end of autumn to the end of winter, when little food is available, and therefore their fat stores are used up during this period. The sardines accumulate fat in their tissues for spawning and wintering in temperate seasons (summer, autumn), as is seen by the data given in Table 2.

Zlatanos and Laskaridis (2007) reported that sardines collected during winter had the highest lipid content (10.6%). Our findings are not in agreement with their results, whereby our data show that the sardines collected at the end of winter, and at the end of spring to the beginning of summer, had the lowest fat content (Table 2). The highest fat content was seen in sardines collected in late summer and autumn, and the values were much higher than those reported for the Greek study (8.3% to 15.1%, *vs.* 5.9% to 8.5%). Similar results for the fat content of marinated sardines (highest in late summer and autumn,

Table 2: *Fat content (%) of the sardine meat (N = 24) with respect to catching season*
Preglednica 2: *Vsebnost maščob (%) v mesu sardin v odvisnosti od sezone ulova*

Parameter, %	Winter /Zima	Spring / Pomlad	Summer / Poletje	Autumn / Jesen	P-value / p-vrednost
Fat / Maščoba	0.97 ± 0.22^{c}	1.26 ± 0.43^{c}	8.33 ± 2.38^{b}	15.11 ± 3.08^{a}	< 0.0001

Mean values ± standard deviation. *N* – number of sardines in each season. ^{a,b,c} – mean values of seasons with different letters are statistically significant different ($P < 0.05$)
Povprečne vrednosti ± standardni odklon. *N* – število vzorcev v vsaki sezoni. ^{a,b,c} – srednje vrednosti z različnimi nadpisanimi črkami se statistično značilno ($p \leq 0.05$) razlikujejo.

and lowest in winter and spring) were reported by Macciola *et al.*, (2003), while Hardy and Key (1972) saw lower levels of fat during spawning, due to the fat mobilisation associated with gametogenesis.

The FA composition of the fish investigated in the present study are given in Table 3. The data show remarkable and significant changes ($P \leq 0.05$ or less) in the individual FAs during this one-year period.

On average, the sardine meat contained 6.4% fat, and for the FA composition, 18.0% was mono-unsatu-

Table 3: *Fatty acid composition (% of total fatty acid) of the sardine meat (N=24) with respect to catching season*
Preglednica 3: *Maščobnokislinski profil (% od skupnih maščobnih kislin) maščobe v mesu sardin v odvisnosti od sezone ulova*

FA / % total FAs MK / % skupnih MK	Winter Zima	Spring Pomlad	Summer Poletje	Autumn Jesen	Mean ± SD Povprečje ± so	P-value p-vrednost
12:0	0.03 ± 0.01^{c}	0.06 ± 0.01^{b}	0.07 ± 0.01^{b}	0.08 ± 0.00^{a}	0.07 ± 0.01	< 0.0001
14:0	1.42 ± 0.07^{c}	5.67 ± 1.03^{b}	6.68 ± 0.30^{ab}	7.26 ± 0.37^{a}	5.94 ± 1.65	< 0.0001
15:0	0.46 ± 0.01^{c}	0.87 ± 0.09^{b}	0.83 ± 0.06^{b}	1.01 ± 0.04^{a}	0.86 ± 0.15	< 0.0001
16:0	23.13 ± 0.76^{c}	27.48 ± 1.37^{a}	24.73 ± 1.18^{bc}	26.30 ± 1.07^{ab}	26.24 ± 1.85	0.0057
16:1*cis*-9	1.36 ± 0.04^{c}	4.45 ± 1.07^{ab}	4.18 ± 0.14^{b}	5.39 ± 0.21^{a}	4.37 ± 1.22	0.0009
17:0	0.79 ± 0.01^{c}	0.95 ± 0.06^{b}	1.09 ± 0.04^{a}	1.14 ± 0.07^{a}	1.01 ± 0.12	< 0.0001
18:0	6.08 ± 0.39^{a}	4.99 ± 0.36^{b}	4.92 ± 0.24^{b}	5.79 ± 0.30^{a}	5.24 ± 0.53	0.0002
18:1*trans*-9	0.13 ± 0.03^{c}	0.14 ± 0.02^{c}	0.25 ± 0.01^{b}	0.36 ± 0.03^{a}	0.22 ± 0.09	< 0.0001
18:1*cis*-9	4.97 ± 0.01^{d}	6.87 ± 0.62^{c}	11.77 ± 1.54^{b}	14.38 ± 0.90^{a}	9.59 ± 3.56	< 0.0001
18:2*cis*-9,12	2.62 ± 0.02^{b}	3.03 ± 0.34^{a}	2.49 ± 0.07^{b}	2.99 ± 0.10^{a}	2.86 ± 0.33	0.0028
18:2 CLA	0.96 ± 0.01^{c}	1.99 ± 0.16^{b}	2.17 ± 0.04^{a}	2.04 ± 0.05^{ab}	1.96 ± 0.32	< 0.0001
18:3*cis*-9,12,15	0.73 ± 0.00^{c}	2.39 ± 0.28^{b}	3.52 ± 0.13^{a}	3.74 ± 0.06^{a}	2.83 ± 0.89	< 0.0001
20:1*cis*-11	0.50 ± 0.13^{d}	2.73 ± 0.23^{c}	4.03 ± 0.25^{a}	3.12 ± 0.19^{b}	2.95 ± 0.91	< 0.0001
22:1*cis*-13	1.82 ± 0.17^{a}	0.91 ± 0.11^{b}	0.59 ± 0.11^{c}	0.96 ± 0.15^{b}	0.92 ± 0.32	< 0.0001
18:4*cis*-6,9,12,15	0.85 ± 0.17^{c}	1.16 ± 0.08^{a}	1.04 ± 0.04^{ab}	0.95 ± 0.03^{bc}	1.06 ± 0.13	0.0027
20:5*cis*-5,8,11,14,17 (EPA)	6.94 ± 0.07^{b}	8.92 ± 1.23^{a}	7.78 ± 0.66^{ab}	7.23 ± 0.63^{b}	8.12 ± 1.21	0.0692
22:3*cis*-13,16,19	0.62 ± 0.04^{c}	1.63 ± 0.15^{a}	1.20 ± 0.11^{b}	1.03 ± 0.09^{b}	1.31 ± 0.35	< 0.0001
22:4*cis*-10,13,16,19	1.52 ± 0.03^{a}	0.95 ± 0.13^{b}	0.77 ± 0.10^{bc}	0.74 ± 0.08^{c}	0.90 ± 0.23	< 0.0001
22:5*cis*-7,10,13,16,19	1.04 ± 0.06^{a}	1.03 ± 0.07^{a}	1.03 ± 0.11^{a}	1.13 ± 0.12^{a}	1.05 ± 0.10	0.2041
22:6*cis*-4,7,10,13,16,19 (DHA)	44.03 ± 0.56^{a}	23.77 ± 4.40^{b}	20.88 ± 2.61^{b}	14.39 ± 1.39^{c}	22.50 ± 8.04	< 0.0001
SFA / nasičene MK	31.92 ± 1.24^{c}	40.02 ± 2.31^{ab}	38.32 ± 1.59^{b}	41.58 ± 1.79^{a}	39.36 ± 3.11	0.0007
MUFA / enkrat nenasičene MK	8.78 ± 0.27^{d}	15.10 ± 1.50^{c}	20.82 ± 1.76^{b}	24.20 ± 1.20^{a}	18.03 ± 4.88	< 0.0001
PUFA / večkrat nenasičene MK	59.30 ± 0.96^{a}	44.88 ± 3.50^{b}	40.87 ± 3.23^{b}	34.22 ± 1.99^{c}	42.60 ± 7.12	< 0.0001
ΣEPA+DHA	50.97 ± 0.63	32.69 ± 5.63	28.66 ± 3.27	21.62 ± 2.02	30.62 ± 9.25	< 0.0001
Σ*n*-3	55.73 ± 0.93^{a}	39.85 ± 3.82^{b}	36.31 ± 3.32^{b}	29.20 ± 2.07^{c}	37.78 ± 7.46	< 0.0001
Σ*n*-6	3.58 ± 0.03^{c}	5.02 ± 0.32^{a}	4.66 ± 0.10^{b}	5.02 ± 0.11^{a}	4.83 ± 0.46	< 0.0001
n-6/*n*-3	0.07 ± 0.00^{c}	0.13 ± 0.02^{b}	0.13 ± 0.01^{b}	0.17 ± 0.02^{a}	0.13 ± 0.03	< 0.0001
P/S	9.23 ± 0.28^{a}	7.26 ± 0.29^{c}	7.28 ± 0.21^{c}	7.92 ± 0.29^{b}	7.57 ± 0.62	< 0.0001
IA	0.43 ± 0.02^{b}	0.86 ± 0.11^{a}	0.86 ± 0.06^{a}	0.98 ± 0.07^{a}	0.85 ± 0.16	< 0.0001

Mean value ± standard deviation. N – number of sardines in each season. a, b, c, d – mean values of seasons with different letters are statistically significant different ($p < 0.05$). Σn-3 – sum of 18:3cis-9,12,15, 18:4cis-6,9,12,15, 20:5cis-5,8,11,14,17, 22:3cis-13,16,19, 22:4cis-10,13,16,19, 22:5cis-7,10,13,16,19 and 22:6cis-4,7,10,13,16,19. Σn-6 – sum of 18:2cis-9,12 and 18:2 CLA. IA – index of atherogenicity = (12:0 + 4 × 14:0 + 16:0)/(Σ(n-6) + Σ(n-3) + 18:1cis-9 + other MUFA) (Ulbricht and Southgate, 1991).

Povprečje ± standardni odklon. N – število vzorcev v vsaki sezoni. a,b,c,d – srednje vrednosti z različnimi nadpisanimi črkami se statistično značilno ($p \leq 0.05$) razlikujejo. Σn-3 – vsota 18:3cis-9,12,15, 18:4cis-6,9,12,15, 20:5cis-5,8,11,14,17, 22:3cis-13,16,19, 22:4cis-10,13,16,19, 22:5cis-7,10,13,16,19 in 22:6cis-4,7,10,13,16,19. Σn-6 – vsota 18:2cis-9,12 in 18:2 CLA. IA – indeks aterogenosti = (12:0 + 4 × 14:0 + 16:0)/(Σ(n-6) + Σ(n-3) + 18:1cis-9 + druge MUFA) (Ulbricht in Southgate, 1991).

rated, 42.6% polyunsaturated, and 39.4% saturated. The lipids of this sardine meat contained large proportions of palmitic acid (16:0; 26.2%) and DHA (22.5%). Oleic acid (18:1*cis*-9; 9.6%), EPA (8.1%), myristic acid (14:0; 5.9%), stearic acid (18:0; 5.2%), palmitooleic acid (16:1*cis*-9; 4.4%), α-linolenic acid (18:3*cis*-9,12,15; 2.8%) and docosapentaenoic acid (22:5*cis*-7,10,13,16,19; 1.1%) were all present as minor components.

The saturated FAs in the sardine fat ranged from 31% to 45%; the highest proportions being seen as palmitic (26.2%) and myristic (5.9%) acids. In spring, summer and autumn, the fat had a significantly higher weight percentage in the 16:0 palmitic acid than in winter ($P < 0.05$), presumably due to the spawning season and wintering. The content of the 14:0 myristic acid increasing during the year by more than five-fold, with its peak in autumn. In our study, these weight percentages of the 14:0 and 16:0 FAs were more variable in comparison with the values reported by Bandarra *et al.* (1997) and Zlatanos and Laskaridis (2007), which were seen to be particularly constant throughout the year and did not appear to be influenced by the diet of the sardines. This phenomenon could be explained according to the sea temperature and its oscillations: in the north Adriatic Sea, the temperatures oscillate from 8 °C in winter to 29 °C in summer and at the beginning of autumn. More food is available in the warmer periods, which the sardines accumulate as fat for the colder periods. In warmer seas, food is more uniformly available and therefore less fat accumulation is needed, which would explain the more constant saturated FA composition seen by others.

The mono-unsaturated FAs in the lipids ranged from 8.8% to 24.2%; with the highest proportions seen for oleic acid (18:1*n*-9C). Here, these 18:1*n*-9C levels (5.0% to 14.4%) were similar than those reported by Bandarra *et al.* (1997) in their Portugese study (7.4% to 14.3%) and Zlatanos and Laskaridis (2007) in a Greek study (3.5% to 10.6%).

The PUFAs in the sardine lipids ranged from 34.2% to 59.3%, with the highest proportions seen for DHA (mean, 22.5%) and for EPA (mean, 8.1%). The DHA content decreased from winter (44%) to autumn (14%), and generally the EPA content increased significantly during spring and summer, when compared to the winter-autumn period (8.9% and 7.8% *vs.* 6.9% and 7.2%, respectively).

Our data for the *n*-3 PUFAs are in agreement with the literature (Bandarra *et al.*, 1997), although they were higher (mean, 37.8%) than those reported by Zlatanos and Laskaridis (2007) in the Greek study (35.3%) and much higher than those reported by Luzia, Sampaio, Castellucci, and Torres (2003) in the Brazilian study (13.4% in summer). In the present study, there was a negative correlation between the fat content and that of the *n*-3 PUFAs: the *n*-3 PUFAs were low during the months with a high fat content ($R = -0.84$, $P = 0.0001$) (not presented in tables). In contrast, the saturated FA content increased during the months with a high fat content, in summer and autumn ($R = 0.81$, $P = 0.0001$). These data are in agreement with other studies (Bandarra *et al.*, 1997; Macciola *et al.*, 2003; Zlatanos and Laskaridis, 2007).

From the nutritional point of view, the P/S ratio, *n*-6/*n*-3 ratio and the atherogenic index were calculated. The P/S ratio was high (7.6 in average) due to presence of large content of DHA (22:6*cis*-4,7,10,13,16,19) and EPA (20:5*cis*-5,8,11,14,17) (Table 3). The *n*-6/*n*-3 ratio in the present study (mean, 0.13) was favourable mainly because of the low *n*-6 FA content. This ratio is considered to be a risk factor in cancers and coronary heart disease, and it is recommended that it is less than 10.0, and even less than 4.0 (WHO, 1994; Simopoulos, 2002; FSA, 2004).

The introduction of a correct combination of sardines and other food into the diet can assure a good balance for human nutrition. On the other hand, in the present study, the atherogenic index values of the sardine varied, from 0.43 to 0.98 (mean, 0.85), which is higher than recommended (lower than 0.72). The main reason for these high atherogenic index values appears to lie in the presence of 14:0 myristic acid. The myristic acid value according to the atherogenic index is enhanced because of the large influence of cholesterol in the blood (Ulbricht and Southgate, 1991). Aside from the relatively high atherogenic index, there can be further benefits from the high amounts of *n*-3 PUFAs that are seen, because they balance the *n*-6/*n*-3 ratio, which in a modern western diet is generally greater than 15:1 to 16.7:1 (Simopoulos, 2002).

The Food Standards Agency (FSA, 2004) has published recommendations for the daily intake of *n*-3 PUFAs: 0.45 g daily for the protection of the adult population. Chapkin (1992) recommends 0.8 g of EPA and DHA daily for a healthy adult population, while Simopoulos (2002) recommends 0.65 g of EPA and DHA daily (calculated on a 8,400 kJ diet). This should be increased twofold or more for the *n*-3 PUFA intake for a population with cardiovascular disease: from 0.9 g to 1.5 g of *n*-3 PUFAs (FSA, 2004), and up to 2 g to 4 g of EPA and DHA daily (AHA, 2003).

As can be seen from the present study, a healthy adult can satisfy their daily *n*-3 PUFA intake (0.45 g; FSA, 2004) by eating only 10 g of the sardine meat collected in the autumn, or 100 g of the sardine meat when the sardines are lean. Similarly, people with different cardiovascular diseases can cover the recommended amounts of the *n*-3 PUFAs by eating just 30 g of the sardine meat collected in autumn, and up to 330 g of the sardine meat

Table 4: *Mean FA levels (mg FA/100 g meat) in the sardine meat (N = 24) with respect to catching season*
Preglednica 4: *Povprečne vrednosti maščobnih kislin (mg FA/100 g mesa) v mesu sardine (N = 24) v odvisnosti od sezone ulova*

FA / mg FA/100 g meat MK / mg MK/100 g mesa	Winter Zima	Spring Pomlad	Summer Poletje	Autumn Jesen	Mean Povprečje
12:0	0 ± 0	1 ± 0	5 ± 0	11 ± 0	4 ± 1
14:0	12 ± 1	64 ± 12	501 ± 23	988 ± 50	343 ± 95
15:0	4 ± 0	10 ± 1	63 ± 5	137 ± 5	50 ± 9
16:0	202 ± 7	312 ± 16	1855 ± 88	3578 ± 145	1516 ± 107
16:1*cis*-9	12 ± 0	51 ± 12	314 ± 10	733 ± 28	252 ± 71
17:0	7 ± 0	11 ± 1	82 ± 3	155 ± 9	58 ± 7
18:0	53 ± 3	57 ± 4	369 ± 18	787 ± 41	303 ± 30
18:1*trans*-9	1 ± 0	2 ± 0	18 ± 1	49 ± 5	12 ± 5
18:1*cis*-9	43 ± 0	78 ± 7	883 ± 115	1956 ± 122	554 ± 206
18:2*cis*-9,12	23 ± 0	34 ± 4	187 ± 5	406 ± 13	165 ± 19
18:2 CLA	8 ± 0	23 ± 2	163 ± 3	277 ± 7	113 ± 19
18:3*cis*-9,12,15	6 ± 0	27 ± 3	264 ± 10	509 ± 8	164 ± 52
20:1*cis*-11	4 ± 1	31 ± 3	302 ± 19	424 ± 25	170 ± 53
22:1*cis*-13	16 ± 1	10 ± 1	44 ± 8	130 ± 20	53 ± 19
18:4*cis*-6,9,12,15	7 ± 1	13 ± 1	78 ± 3	129 ± 4	61 ± 7
20:5*cis*-5,8,11,14,17 (EPA)	61 ± 1	101 ± 14	584 ± 49	983 ± 85	469 ± 70
22:3*cis*-13,16,19	5 ± 0	19 ± 2	90 ± 8	140 ± 13	76 ± 20
22:4*cis*-10,13,16,19	13 ± 0	11 ± 2	58 ± 8	100 ± 11	52 ±13
22:5*cis*-7,10,13,16,19	9 ± 1	12 ± 1	77 ± 8	154 ± 16	61 ± 6
22:6*cis*-4,7,10,13,16,19 (DHA)	385 ± 5	270 ± 50	1566 ± 196	1957 ± 189	1300 ± 464
SFA / nasičene MK	279 ± 11	455 ± 26	2874 ± 119	5656 ± 244	2275 ± 179
MUFA / enkrat nenasičene MK	77 ± 2	172 ± 17	1561 ± 132	3291 ± 163	1042 ± 282
PUFA / večkrat nenasičene MK	519 ± 8	510 ± 40	3065 ± 243	4654 ± 270	2462 ±412
ΣEPA+DHA	446 ± 6	371 ± 64	2150 ± 245	2940 ± 274	1769 ± 534
Σ*n*-3	487 ± 8	453 ± 43	2723 ± 249	3971 ± 282	2183 ± 431
Σ*n*-6	31 ± 0	57 ± 4	349 ± 8	683 ± 16	279 ± 26
ΣFA/100 g meat / ΣMK/100 g mesa	874 ± 23	1137 ± 134	7500 ± 581	13602 ± 797	5778 ± 1273
g fat/100 g meat / g maščobe/100 g mesa	0.97 ± 0.03	1.26 ± 0.15	8.33 ± 0.65	15.11 ± 0.89	6.42 ± 1.41

Mean value ± standard deviation. N – number of sardines in each season. Σn-3 – sum of 18:3cis-9,12,15, 18:4cis-6,9,12,15, 20:5cis-5,8,11,14,17, 22:3cis-13,16,19, 22:4cis-10,13,16,19, 22:5cis-7,10,13,16,19 and 22:6cis-4,7,10,13,16,19. Σn-6 – sum of 18:2cis-9,12 and 18:2 CLA.
Povprečje ± standardni odklon. N – število vzorcev v vsaki sezoni. Σn-3 – vsota 18:3cis-9,12,15, 18:4cis-6,9,12,15, 20:5cis-5,8,11,14,17, 22:3cis-13,16,19, 22:4cis-10,13,16,19, 22:5cis-7,10,13,16,19 in 22:6cis-4,7,10,13,16,19. Σn-6 – vsota 18:2cis-9,12 in 18:2 CLA.

collected in spring. Also, for the DHA and EPA intake for a healthy population, 20 g of the sardine meat collected in autumn or 220 g of the sardine meat collected in winter would cover the daily recommendations, with higher amounts of the sardine meat covering the EPA and DHA recommendations for cardiovascular patients. The full data for the analysis of the individual FAs (expressed as mg FA/100 g edible sardine meat) are given in Table 4.

4 CONCLUSIONS

Although *n*-3 PUFAs are not only found in fish, as they are also present in flax seeds and nuts in particular, the EPA and DHA from fish fat are much more efficiently incorporated into the human body. α-linolenic acid, as a short *n*-3 PUFA, must be converted in the body to DHA and EPA, a process that is not particularly efficient in many people (and especially not so in the elderly), thus indicating that the direct consumption of DHA and EPA

is preferable. In conclusion, due to the increasing importance of the *n*-3 FAs for our health, the aim of this study was achieved: the definition of the FA composition of sardine collected in the Adriatic sea throughout the year, to provide this specific information for food specialists to include sardines in their menus for different kind of diets. The differences in the FA composition through the different periods of the year should also be taken into account in these diets.

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FORAGING BEHAVIOUR OF SHEEP AT PASTURE WITH DIFFERENT TYPES OF VEGETATION IN A PADDOCK [1]

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Foraging behaviour of sheep at pasture with different types of vegetation in a paddock

This experiment was designed to study the foraging behaviour of ewes on a pasture with paddocks with three different types of vegetation, herbaceous (n = 3), woody (n = 2), and semi open (n = 1). Forty sheep were bred on a farm in the Karst region of Slovenia. Ten sheep were focally observed during day light (5 a.m.–9 p.m.). Ewes were observed for 2 days in each paddock with one rotation, so 12 days in total. Grazing time, circadian rhythm of grazing, drinking frequency, and frequency of salt consumption were the observed behaviours. On average, ewes grazed for 10.5 hours a day (mean ± SD = 626.2 ± 47.2 min), with a significant difference between individual variation ($P < 0.001$). Sheep grazed the most in herbaceous paddocks ($P < 0.001$), with lower yet similar levels observed in woody and semi open paddock. The frequency of drinking and salt consumption was low. Individual grazing sheep would drink slightly less than once per day, while consuming salt on average 1.25 times per day. Drinking frequency was the highest in the semi open paddock with some trees and bushes, whereas salt consumption was most frequently observed in the woody paddocks.

Key words: sheep / animal behaviour / ethology / grazing / pastures / paddocks / vegetation / Karst / drinking / salt consumption / Slovenia

Obnašanje ovc na kraškem pašniku z različno vegetacijo

Poskus je bil zastavljen z namenom proučiti obnašanje ovc na pašniku s čredinkami, v katerih so obstajale tri različne vrste vegetacije: travna ruša (n = 3), gozdna (n = 2) in delno zaraščena z drevesi in grmovjem (n = 1). Trop 40 ovc se je pasel na kmetiji na kraškem svetu v Sloveniji. Deset individualnih ovc je bilo direktno opazovanih v času dnevne osvetlitve (od 5. do 21. ure). Ovce so bile opazovane 2 zaporedna dneva v posamezni čredinki z eno ponovitvijo, torej skupaj 12 dni. Opazovana je bila dolžina zauživanja zelinja (+ listje iz grmovja in dreves), dnevni ritem zauživanja travne ruša, pogostost pitja in zauživanja soli. Na dan so se ovce pasle povprečno 10,5 ur (povprečje ± SD = 626,2 ± 47,2 min), vendar so bile značilne razlike med ovcami ($P < 0,001$). Najdaljši čas za pašo so ovce imele v čredinkah s travno rušo ($P < 0,001$). Podoben čas paše je bil opazovan v gozdnih čredinkah in pol odprti čredinki. Pogostost pitja in zauživanja soli je bila nizka. Živali so pile malo manj kot enkrat na dan, medtem ko so zaužile sol 1,25 krat na dan. Pogostost pitja je bila največja v pol odprti čredinki, kjer so bila prisotna tudi drevesa in grmovje, medtem ko je bila največja pogostost zauživanja soli v gozdnih čredinkah.

Ključne besede: ovce / obnašanje živali / etologija / paša / pašniki / čredinke / vegetacija / Kras / pitje / zauživanje soli / Slovenija

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1 INTRODUCTION

Small ruminant social environment varies widely from very intensive feeding with no grazing opportunities to more extensive grazing areas with high grazing opportunities. Behavioural constraints are different and more diversified at pasture than indoors. As the human population becomes more aware of the quality of food, maximising forage utilisation through grazing is an increasingly important tool in animal production. Allowing the animals to perform more natural behaviour while being outside in the open can also improve their welfare (Špinka, 2006).

In Slovenia, sheep production is the most widespread form of extensive animal production. In Karst, western part of Slovenia that occupies almost half of the territory (SURS, 2002), 85% of the countries sheep and goats are reared (SURS, 2002). This area is difficult to cultivate by agricultural machinery and is classified as inappropriate for agricultural production. In 1998, between 120.000 and 150.000 ha of agricultural land in the Karst region was abandoned and overgrown with shrubs, trees, and brushwood (Cunder, 1998). Woody plants are a common component of the overgrowth. However, small ruminants can keep the pastures and farm land clear of ingrown woods through their capacity to graze. Small ruminants can also contribute to the safeguarding of agricultural functions, like care and preservation of the landscape, through maintaining grasslands and preventing land from bush encroachment and fires.

Grazing is defined as the time spent each day in grazing activity, that is, prehension and mastication (Woodward, 1997). It is well documented that sheep and goats show selective grazing and select for a high quality, nutritionally balanced diet. Grazing duration and rhythm is often related to specific forage characteristics (Baumont *et al.*, 2000) due to different dietary choices (Morand-Fehr, 2003). This is partly the reason for keeping sheep and goats together at pasture. They have different preferences for feeds and the area is therefore more intensely grazed. At pasture, two main grazing periods usually occur at sunrise and sunset, which are also the preferred drinking times in both sheep (Rook and Penning, 1991) and goats (Rossi and Scharrer, 1992). However, drinking frequency can differ greatly between individuals, partly as a result of differences in social hierarchies (Milinski and Parker, 1991) and space availability around the drinking troughs (Ehrlenbruch *et al.*, 2010).

In a heterogeneous environment, the management of the grazing circuit has become an important factor. An understanding of sheep behaviour in a complex environment is therefore essential for optimizing the management of sheep and goat flocks in unfavourable areas, such as the Karst region in Slovenia. Studying more feeding behaviour of ruminants may provide a firm knowledge on ethological traits of animals and a better understanding of how to achieve a good economical production, good animal welfare, and at the same time preserve the semi open landscape as best as possible.

The aim of the study was to investigate how forage characteristics and type of vegetation influence foraging behaviour during sheep grazing. Sheep were observed at three different types of vegetation in a paddock in the Karst region of Slovenia.

2 MATERIAL AND METHODS

2.1 MATERIAL

The experiment was carried out during the summer time on a farm in the hilly Karst region of Slovenia called Vremščica (altitude of 900 – 1000 m a.s.l.). Two days before the onset of an experimental procedure, 40 ewes of Istrian Pramenka and 10 goats cross breeds were mixed and released into the same foraging area in order to get familiar with each other. The animals were reared on that farm and thus used to the area. The animals were of similar age to prevent any effects of age on different flocking behaviour and handling responses (Hargreaves and Hutson, 1997). The area was fenced and for the purpose of the experiment divided into 6 paddocks of similar size (approx. 400 m^2). The shape of paddocks depended on the structure of the ground and the type of vegetation. There were 3 paddocks covered with only grass sward (herbaceous paddock), 1 partly covered with trees and bushes (semi open paddock) and 2 fully overgrown paddocks with hazel and beech trees (woody paddock) (Fig. 1). Thus, 50 animals were grazing in six paddocks with three different types of vegetation for a period of 6 weeks. Animals stayed at the pasture for 24 hours. At each paddock there was one drinking trough and one wooden trough with salt. Therefore, animals had the possibility to both feed and drink. The average ambient temperature during the observation period of 12 days was 14.1 °C, ranging from 9.2 °C to 18.5 °C.

2.2 METHODS

2.2.1 BEHAVIOURAL OBSERVATIONS

For the purpose of this experiment ten young sheep were directly observed during foraging in the flock of 50 animals. Each of the 10 ewes was marked with a different combination of stripes on the back using red, black,

a) b) c)

Figure 1: *Paddock with three different types of vegetation (a: herbaceous paddock, b: semi open paddock with some trees and bushes, c: woody paddock).*
Slika 1: *Čredinke z različno vegetacijo (a: travnata čredinka, b: delno zaraščena čredinka z grmovjem in drevesi, c: gozdna čredinka).*

or green water resistant colour spray. The observations lasted 12 days during a 6 week period, with six interrupted intervals each lasting two consecutive days. Animals were 5 or 6 days at a paddock, depending on the feeds availability, and afterwards moved to another paddock. During the experiment the animals were rotated between all 6 paddocks in such a way that they were observed twice at the same paddock. Two observers, situated on a raised platform, began to observe the animals two days after moving the sheep into a paddock. Animals were observed inside (woody paddock) or outside the paddock (herbaceous paddock, semi open paddock). When observed outside, observers sat in a caravan 300 m distanced from the pasture, using binoculars. Before the observations started in the woody paddocks, the observers had spent 2 days at the pasture together with the sheep, so that the animals got used to their presence. The observers always wore the same working coat that was familiar to the animals. Observers started with the observations on the third day after moving the animals into a specific paddock. Observations started at 5 a.m. and finished at 9 p.m. Only one observer per time was observing the animals, 2 hours in a row, and then the observers were changed. Daily observation time was 16 hours. Activities of an individual sheep were scored on sheets of paper. Recordings were made for the following foraging activities:

- grazing (duration, daily rhythm),
- drinking water,
- salt consumption.

Grazing was recorded every 5 min during 16 hours of observation using instantaneous sampling. The number of drinking and salt consumption bouts was scored within the same time period but using continuous sampling.

2.2.2. STATISTICAL ANALYSIS

We prepared data with Microsoft Excel for Windows and analysed them using statistical package SAS/STAT (SAS, 2008). The general linear model (GLM) was used to determine the effects of normally distributed data. The daily values of data were tested for normality. All the tests were two-tailed and the significant level was set at P≤0.05. Data for grazing was normally distributed and the model shown in equation [1] was developed using three fixed effects and an independent variable. The effect of breed had been tested but later omitted from the model as it described only 0.000126% of the variability.

$$y_{ijk} = \mu + P_i + D_j + A_k + b_i(t_{ijkl} - \bar{t}) + e_{ijk}, \quad (1)$$

where P_i is the effect of paddock ($_i$ = 1–6), D_j is the effect of day ($_j$ = 1–2), A_k is the effect of animal ($_k$ = 1–10), $b_i(t_{ijkl} - \bar{t})$ is the effect of averaged daily temperature, and e_{ijk} is a random error.

In the case of other activities, drinking frequency and salt intake, data were not normally distributed even after transformation. The number of drinking and salt consumption bouts was very low; therefore, these behaviours are presented in a descriptive manner only.

3 RESULTS

3.1 GRAZING

Sheep were free at pasture for 24 hours and it was observed that during the afternoon heat sheep moved into the shade, if available. This suggests shade should be made available to animals in pastures. At pasture, sheep could develop their own foraging strategy. They spent on average 10.5 hours grazing during light hours (mean ± SD = 626.2 ± 47.2 min). The maximum duration of graz-

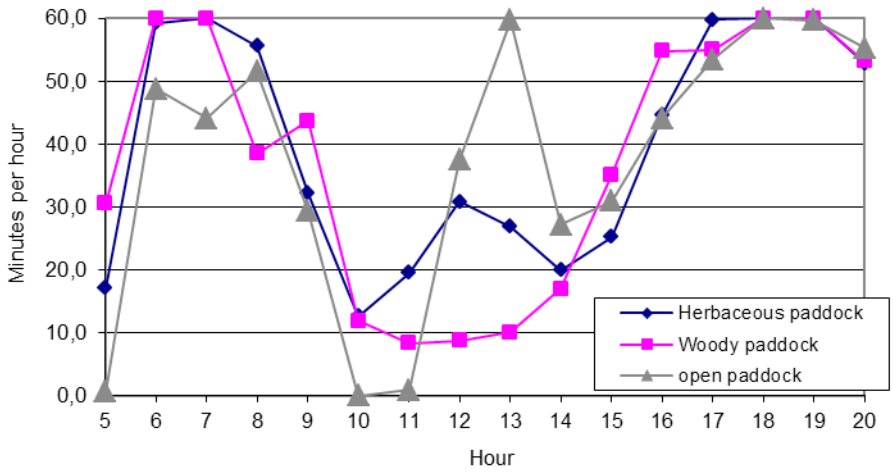

Figure 2: *Daily grazing rhythm at herbaceous paddock, woody, and semi open paddock with some trees and bushes.*
Slika 2: *Dnevni ritem paše v travni čredinki, gozdni čredinki in delno zaraščeni čredinki z grmovjem in drevesi.*

ing per day was a bit less than 12 hours (713.3 min). The similar duration of grazing was observed in the study of Lynch *et al.* (1992). They reported 8–9 hours of grazing a day with a maximum of around 13 hours when the feed supply was limited. This means that the broad diversity of feeds in our study motivated ewes to graze. The beginning of the grazing was synchronous in our study. If one animal started to graze, it was followed by the others. Such behaviour is species specific, and with sheep being social animals, they tend to be synchronous in their starting of grazing bouts (Champion *et al.*, 1994).

The circadian rhythm of grazing was significantly different during the day (Fig. 2). Grazing was the most intense in the morning with the peak between 6–8 a.m. when animals would spend from 45–60 min per hour grazing, whereas in the afternoons between 6–7 p.m., animals would graze during the entire observational period. After 5 p.m., the amount of time grazing increased with animals spending more than 50% of their time on that activity. This trend was also reported by Shinde *et al.* (1997) where grazing was generally observed at any time of day or night, but was most intensive in the morning and late afternoon until dusk. Lynch *et al.* (1992) explained that in continental areas grazing activity is concentrated to 4 hours after dawn and in the last 4 hours around sunset, but can easily start before dawn and extend long into the dark.

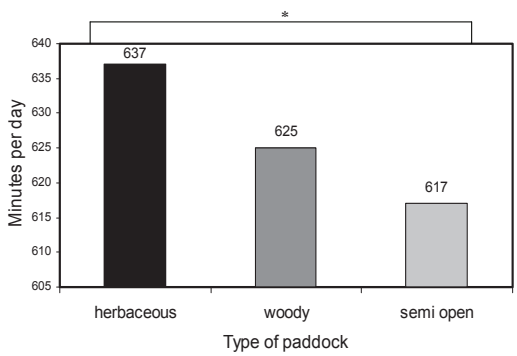

Figure 3: *Grazing time in different types of paddock. Difference between bars:* $F_{2,60} = 5.23$; $*P < 0.001$.
Slika 3: *Čas paše v različnih vrstah čredink. Razlika med stolpci:* $F_{2,60} = 5,23$; $*P < 0.001$.

As shown in Fig. 3, we found significant differences in the grazing duration in different types of paddock ($P < 0.001$). Sheep spent the most time grazing at the herbaceous paddock whereas at the woody and semi open paddock the grazing was reduced to a similar level. This means that soil (grass) and aerial (woody) feeding behaviour differed. According to Vidrih *et al.* (1996) and Baumont *et al.* (2000), differences in herbage composition between types of paddock can affect grazing duration, and may explain the differences observed in our study. There was an additional effect of the individual on grazing duration ($P < 0.001$; Fig. 4). The variation in the average grazing duration over the 12 observed days ranged between 592 and 662 min.

The temperature did not significantly affect the grazing time ($P > 0.1$), but it affected the circadian rhythm of grazing ($P < 0.05$; Fig. 5). When temperature was below 15.4 °C, sheep grazed more during 9 a.m. and 4 p.m. than when above 15.4 °C. This is a predictable result since ruminants tend to avoid grazing during the hottest part of the day and thus reduce their daily grazing time. To avoid thermal stress ruminants find shade and spend more time resting (Shinde *et al.*, 1997).

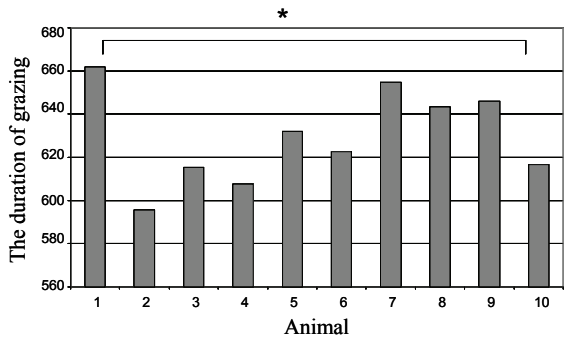

Figure 4: *The average grazing time of an individual sheep. Difference between bars:* $F_{9,60} = 4.58$; *$P < 0.001$.
Slika 4: *Povprečen čas paše za posamezno žival. Razlika med stolpci:* $F_{9,60} = 4{,}58$; *$P < 0.001$.

On the first observed day, sheep spent less time grazing compared to the second day ($F_{1,60} = 122.33$; $P < 0.001$; Table 1). Sheep thus showed different foraging strategies between days due to shortages in herbage availability during the second day. This is based on the conclusions of Baumont *et al.* (2000), where it is suggested that one of the limiting factors of grazing is the herbage availability and growth stage of vegetation.

3.2 DRINKING

Animals had free access to water. On the basis of visual observations, it can be stated that ewes approached the water trough very suddenly and they would always run towards it. It was observed that when one or two animals started to approach the watering point, the other animals followed. According to this, we support the conclusion that drinking behaviour by sheep is socially facilitated (Forkman, 1996) and a synchronised behaviour (Rook and Penning, 1991), with similar findings in Vidrih *et al.* (1996). As all the animals arrived at the drinking source at approximately the same time, competition for water was most probably high (Ehrlenbruch *et al.*, 2010). When sheep are housed indoors, an increased number of ewes per nipple drinker may lead to an increase in total drinking time and number of displacements (Bøe, 1998).

On some days some ewes were not observed to drink during the observational period. However, Lynch *et al.* (1992) concluded that during summer sheep should drink at least once a day, otherwise they tend to reduce grazing time in the heat and increase grazing at night or early in the morning when dew is on the grass. According to our results, it can be concluded that in the case of a small flock of grazing sheep, enough space for drinking water should be available at the pasture, so that the

Table 1: *Differences in the grazing duration between 2 observed days (variables are given as mean min ± SD)*
Preglednica 1: *Razlike med časom paše v dveh opazovanih dneh (spremenljivke so podane kot min ± SD)*

	Day			
Behaviour	1	2	F-value	P-value
Grazing time	589.25 ± 33.75	663.1 ± 23.99	122.33	< 0.001

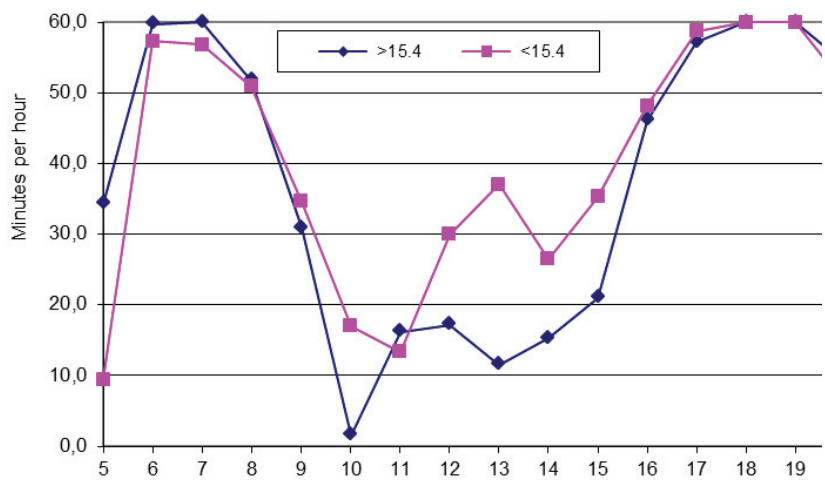

Figure 5: *Grazing rhythm at temperatures above and below the average daily temperature. Difference between the lines:* $F_{1,60} = 4.08$; $P < 0.05$.
Slika 5: *Pašni ritem nad in pod povprečno dnevno temperaturo. Razlika med nad in pod povprečno dnevno temperaturo:* $F_{1,60} = 4.08$; $P < 0.05$.

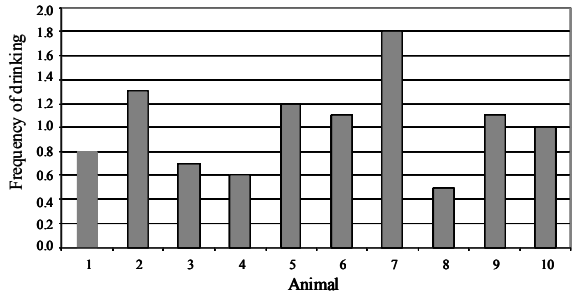

Figure 6: *Frequency of drinking of an individual sheep.*
Slika 6: *Pogostnost pitja pri posamezni ovci.*

majority of animals have access to the water at the same time.

The drinking frequency was low during the observation period (Table 2). The average drinking frequency during this time was 0.99 per animal. The maximum number of drinking bouts per observation period was two. The animals drank the most frequently in the morning between 8 a.m. and 9 a.m., and between 3 p.m. and 7 p.m. in the afternoon. The water usage differed between the types of paddock. Sheep drank the most in the semi open paddock, but in the herbaceous and the woody paddock the frequency was lower, yet the same

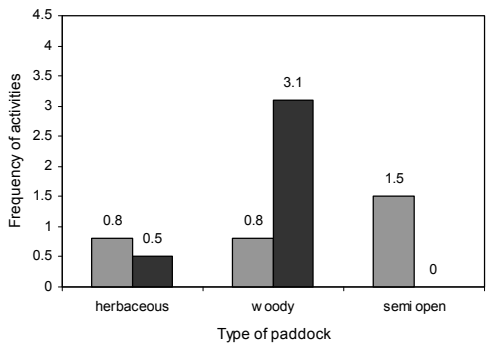

Figure 7: *Frequency of drinking (grey bar) and salt consumption (black bar) in paddock with different types of vegetation.*
Slika 7: *Pogostnost pitja (sivi stolpec) in konzumacija soli (črn stolpec) v čredinkah z različno vegetacijo.*

(Fig. 7). Climate conditions affected drinking behaviour as well. When the temperature was higher, there was a greater need for water. Water usage was different among animals, showing genetic influence on the behaviour expressed. The animal that drank the most often was one of the two ewes that spent the most time grazing. The lowest frequency among ewes was 0.5 and observed by animal 8.

Table 2: *Drinking frequency and salt intake frequency between the observation days*
Preglednica 2: *Pogostnost pitja in konzumacije soli med opazovanimi dnevi*

Observation days	Drinking	Salt intake
1	1.1	0.0
2	1.0	0.1
3	0.9	0.0
4	0.9	0.0
5	1.4	2.2
6	1.4	2.5
7	0.8	1.3
8	0.4	0.5
9	2.1	4.5
10	0.9	3.1
11	0.3	0.3
12	0.7	0.5
Mean	0.99	1.25

3.3 SALT CONSUMPTION

Salt appetite or sodium hunger is a motivational state in which animals seek out and ingest substances containing sodium (Johnson and Thunhorst, 1997). Sheep in our study had access to feed on leaves from bushes and trees. The expected result is that the frequency of salt intake was the highest at the woody paddock, with a lower value for the herbaceous paddock. At the semi open paddock animals were not seen to consume salt during the observation period (Fig. 7). Salt consumption occurred mainly in the morning between 6 a.m. and 8 a.m., and between 5 p.m. and 9 p.m. in the afternoon. The average frequency of salt consumption per day was 1.25 (Table 2). However, individual variation existed between the animals for salt consumption (Fig. 8). This may show that sheep differ in their taste preference of feed found in the environment (Baumont *et al.*, 2000). Vidrih *et al.* (1996) analysed the concentration of particular minerals in the leaves of hazel and beech tree, with leaves containing 0.15–1.17 g so-

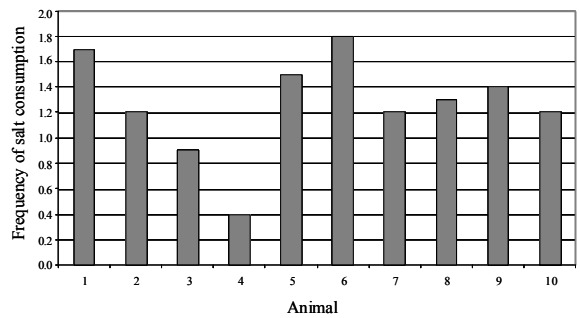

Figure 8: *Frequency of salt consumption of an individual sheep.*
Slika 8: *Pogostnost konzumacije soli pri posamezni ovci.*

dium/kg of dry matter. Sheep weighing less than 50 kg have a nutritional requirement of 1.5 g of sodium in dry matter per day to maintain optimum health (Vidrih *et al.*, 1996). It was observed that animals would chew the bark off trees or wood at the woody paddock. This might be a consequence of the lack of sodium (Kermauner, 1996). However, further study of the nutritional value of forages is required.

4 CONCLUSIONS

For sheep, time spent grazing was on average 10.5 hours per day during the light hours of 5 a.m. to 9 p.m. The type of paddock influenced the grazing duration and daily rhythm. The frequency of water drinking was overall low with animals drinking less than once per day. The highest water usage was recorded at the semi open paddock. It can be concluded that enough space for drinking should be available on the pasture, especially at semi open paddock, since sheep are showing synchronised drinking behaviour. The frequency of salt consumption was the highest at the woody paddock, which can be explained by the lack of sodium in the leaves and branches that are often eaten. It is advised to provide additional sodium, in the form of salt, under such environmental conditions. In conclusion, foraging behaviour under grazing conditions is greatly influenced by differences between individual ewes and forage conditions.

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FLUCTUATING ASYMMETRY IN DIPLOID FEMALE AND STERILE TRIPLOID RAINBOW TROUT (*Oncorhynchus mykiss*)

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Fluctuating asymmetry in diploid female and sterile triploid rainbow trout (Oncorhynchus mykiss)

Viability of an organism and possibility to survive in natural environment could be judged by the magnitude of fluctuating asymmetry (FA) which is defined as random deviation from perfect symmetry of an organism. In order to estimate if there is the difference in FA between diploid female and sterile triploid rainbow trout (*Oncorhynchus mykiss)* the number of rays in pelvic and pectoral fins was determined on both sides of body in 150 individuals from two populations which were of the same genetic origin and were reared under same farm conditions. Units of asymmetry were determined as the absolute value of difference between counts on both sides of body. Results indicate that diploids exhibit larger FA than triploids in both traits; however the difference between both populations is statistically significant only if the number of units of asymmetry for both traits for each fish is summed up. The need to estimate the viability of these two populations on the basis of other traits is discussed and the necessity to use the metric traits to determine FA is stressed out.

Key words: fish / rainbow trout / *Oncorhynchus mykiss* / viability / fluctuating asymmetry / developmental stability / sterile triploids / diploid females

Fluktuacijska asimetrija pri diploidnih in sterilnih triploidnih samicah kalifornijske postrvi (Oncorhynchus mykiss)

Vitalnost nekega organizma in verjetnost, da preživi v naravi, je mogoče presojati na podlagi velikosti fluktuacijske asimetrije (FA). Ta je določena s tem, koliko telo določenega osebka odstopa od popolne simetrije. Da bi ocenili ali se samice in sterilni triploidni osebki kalifornijske postrvi (*Oncorhynchus mykiss)* med seboj razlikujejo v FA, smo pri 150 osebkih iz obeh populacij določili število plavutnic v prsnih in trebušnih plavutih na obeh straneh telesa. Kot mero za asimetrijo smo uporabili absolutno razliko v številu plavutnic v prsnih in trebušnih plavutih, na vsaki strani telesa. Rezultati kažejo, da je za obe lastnosti FA večja pri diploidnih osebkih; razlika med populacijama je dovolj velika, da jo lahko štejemo kot statistično značilno le, če obe lastnosti obravnavamo združeno. Delo problematizira možnost presojanja sposobnosti preživetja le na osnovi FA in poudarja, da bi bilo nujno za določanje FA uporabiti metrične lastnosti.

Ključne besede: ribe / kalifornijska postrv / *Oncorhynchus mykiss* / vitalnost / fluktuacijska asimetrija / razvojna stabilnost / sterilni triploidni organizmi / diploidne samice

1 INTRODUCTION

Fluctuating asymmetry (FA) is the phenomenon observed in organisms which are bilaterally symmetrical. It occurs when the trait on one side of body differs in a random way from the same trait on the other side. It can be expressed as a difference of the trait observed on the left and right sides; the difference can be expressed as difference in number, size, shape or some other feature. FA is a measurement of developmental stability; developmental homeostasis keeps FA at a low level. Increased level of FA reflects the fact that development of an organism was instable. This instability could be caused either by genetic factors or by environment. The number of studies investigating the impact of miscellaneous environmental stressors on developmental stability is rather high and the variety of organisms studied is wide (Eriksen *et al*., 2008). Two opposite hypotheses exist regard-

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ing the relationship between genetic factors and FA; the first one claims that more heterozygous individuals have greater developmental stability which results in lower FA, while second one believes that when genes complexes which ensure developmental stability are disrupted (for instance by hybridization which increases heterozygotity) stability decreases and FA is elevated (Wilkins *et al*, 1995).

In fish it is possible to experimentally manipulate the chromosome sets. By different techniques individuals of the same species with different sets of chromosomes originating from both parents or from one parent only could be produced (Komen and Thorgaard, 2007). In fish crossing of individuals from different species many times results in viable offspring. Fishes and specifically salmonids were therefore used to study the relationship between heterozygosity and developmental stability for types of heterozygotity introduced by manipulating chromosome sets or hybridization which could be found only exceptionally in natural populations (Leary *et al*., 1985; Wilkins *et al*., 1995; Young *et al*., 1995; Vøllestad and Hindar, 2001).

Populations of rainbow trout where all individuals are either females (resulting from fertilizing ova of "normal" females with sperm of individuals which are genetically females but produce sperm) or sterile triploids (resulting from retention of second polar body in such ova induced by thermal or pressure shock) are widely used in aquaculture practice due to apparent advantage of such populations over populations of mixed sexes in production traits. In order to prevent reproduction of introduced species which could endanger native population it is also suggested that only such populations should be used for restocking waters where introduced species is not native (Aprehamian *et al*., 2001). The question of interest for aquaculturists and fish managers is whether sterile triploids exhibit larger viability than females. According to Leary *et al*. (1984) viability of an organism and possibility to survive in natural environment could be judged by the magnitude of FA. The aim of our study was therefore to compare the difference in FA between two populations of rainbow trout; namely population of all-females and population of sterile triploids.

2 MATERIALS AND METHODS

Number of pectoral and pelvic fin rays on left and right side of 150 females and 150 sterile triploids was determined by counting. After the fish were euthanized by electric shock the pectoral and pelvic fins on both sides were cut off and put for one minute into 50% solution of NaOH. The macerated skin and muscle tissue adjoining rays was removed by tweezers; the fin was put on blotting paper to dry up. The number of rays was established when pulled out from fin base one by one. All the counting was done by same person. For a batch of fish treated at the same day, the counting was consecutively done for each fish first on one side and then on the other side in order to avoid bias which could result from knowing the count on one side of individual fish while counting the number on the other side. For each fish and for each fin asymmetry was determined by subtracting the count on one side from the count on the opposite side.

Both populations from which fish were sampled were of the same age (at the beginning of sampling 240 days after hatching) and reared under same condition at Fish research station Pšata which belongs to Biotechnical faculty of University of Ljubljana. Each day approximately 20 fish from both groups were sampled and treated.

Both groups were of the same genetic origin. Eggs stripped from broodstock kept at Fish research station Pšata were fertilized by sperm obtained from sex-inverted females using the methodology described by Ingram (1986). After fertilization half of eggs were put directly in incubation trays for incubation while half of eggs were firstly exposed to temperature shock and after that treated the same way as the eggs not exposed to temperature treatment. The heat shock was done according to slightly adapted method described by Ingram (1986). By heat shock triploid induction could range from 10 to 100% (Solar *et al*., 1984). Only those fish from second group were used for FA in which examination of gonads revealed sterility.

3 RESULTS

The average, the lowest and the highest number of rays in pectoral and pelvic fins in both groups is shown in Table 1. The average number of pectoral rays is higher than average number of pelvic rays. The same was observed by Leary *et al*. (1985). The lowest number of pelvic and pectoral rays observed in diploids is lower than in triploids, while highest number does not differ between two groups. The average number of observed pectoral rays and pelvic rays found in our population is similar to numbers given by Leary *et al*. (1985). The difference in the average number of pelvic and pectoral rays between two groups is not statistically significant.

The numbers obtained by subtracting the count of rays on one side of body from the count on the opposite side is shown in Table 2. These numbers are the measure of asymmetry. If the count for specific trait was the same on both sides, such fish was not considered to be asymmetric for the trait under consideration. As it could

Table 1: *Average, minimum and maximum number of rays for pelvic and pectoral fins in diploid and triploid animals*
Preglednica 1: *Povprečno ter najnižje in najvišje število plavutnic v prsnih in trebušnih plavutih diploidnih in triploidnih živali*

Number of rays	Diploids			Triploids		
	Min	Max	Average	Min	Max	Average
Pectoral fins	24	32	28.56	26	32	28.81
Pelvic fins	16	23	20.94	19	23	21.04

be seen from the numbers in this table, fishes could exhibit the difference of 1, 2, 3 or 4; the latest number is the maximum difference in count for pectoral fin rays as well as pelvic fin rays. For both traits under consideration the majority of fish in both groups were not asymmetric; for pelvic fins around 80% of all fish did not exhibit asymmetry, for pectoral fins more than 60% of the fish were symmetric. It is somehow surprising that pelvic fins which on average have lower number of rays are more asymmetric than pectoral fins. Figures indicate that the percentage of asymmetric individuals is higher in the population of diploids that in the population of triploids. The difference between two populations is 4.7% for pectoral fins and 4.3% for pelvic fins. χ^2 – test revealed that difference in numbers of individuals exhibiting 0, 1, 2, 3 or 4 disparity in counts for each trait in two populations is not large enough to be considered statistically significant.

On the basis of the same test done after pooling all individuals which were not symmetric into one group and comparing the numbers of animals in this group with the numbers of animals which were symmetric conclusion was alike even the test was done with lower degrees of freedom. For pectoral fins there were 84 symmetric versus 66 asymmetric individuals in group of diploids and 91 symmetric versus 59 asymmetric individuals in group of triploids. For pelvic fins the numbers of symmetric and asymmetric fish were 111 versus 39 for diploids and 119 versus 31 for triploids. The difference was too small to be statistically significant.

Since we were measuring the magnitude of asymmetry (the number of units by which the left and right side differed) in two traits, we were able to summing the number of units of asymmetry for both traits for each fish. These numbers presented in Table 3 characterize the total magnitude of asymmetry for individual fish.

By such methods the magnitude of asymmetry was increased; there were fishes for which the summed number of units by which the left and right side differed in both traits was as much as 6. χ^2 – test revealed that difference in numbers of individuals exhibiting 0, 1, 2, 3, 4, 5 or 6 disparity in pooled counts for both traits in two populations is large enough to be considered statistically significant at P = 0.050. (calculated χ^2 value was 12,762).

The asymmetry measured by magnitude defined in such a way was increased; the numbers of symmetric and asymmetric fish were 65 versus 85 for diploids and 69 versus 81 for triploids.

4 CONCLUSIONS

Our results demonstrate that sterile triploid rainbow trout exhibit lower asymmetry than diploid females. The percentage of fish which were asymmetric was larger in the group of diploid females than in the group of sterile triploid for both traits investigated. Even the samples were rather large, these differences were not statistically significant when each trait was considered separately; the difference in pooled counts for both traits exhibited significance. Compared with the samples of similar experiment found in literature, samples used in our experiment were rather large. Wilkins *et al.* (1995) compared FA in diploids and triploids of Atlantic salmon (*Salmo salar*) and hybrids between Atlantic salmon and brown

Table 2: *Number and percentage of diploid and triploid animals exhibiting (a)symmetry for pectoral and pelvic fins*
Preglednica 2: *Število in odstotek diploidnih in triploidnih živali, ki kažejo (a)simetrijo v prsnih in trebušnih plavutih*

Asymmetry measured as L-R ray count (absolute value)	Pectoral fins				Pelvic fins			
	Diploids		Triploids		Diploids		Triploids	
	Number of animals	%	Number of animals	%	Number of animals	%	Number of animals	%
0	84	56.0	91	60.7	111	74.0	119	79.3
1	14	9.3	19	12.7	15	10.0	18	12.0
2	46	30.7	39	26.0	23	15.3	13	8.7
3	1	0.7	0	0.0	0	0.0	0	0.0
4	5	3.3	1	0.7	1	0.7	0	0.0
Total	150	100.0	150	100.0	150	100.0	150	100.0

Table 3: *Number and percentage of diploid and triploid animals exhibiting (a)symmetry for pectoral and pelvic fins*
Preglednica 3: *Število in odstotek diploidnih in triploidnih živali, ki kažejo (a)simetrijo v prsnih in trebušnih plavutih*

Units of asymmetry after summing L-R absolute difference for both traits	Diploids		Triploids	
	Number of animals	%	Number of animals	%
0	65	43.3	69	46.0
1	18	12.0	30	20.0
2	45	30.0	43	28.7
3	9	6.0	3	2.0
4	9	6.0	5	3.3
5	1	0.7	0	0.0
6	3	2.0	0	0.0
Total	150	100.0	150	100.0

trout (*Salmo trutta*). They collected data from 40 diploid salmon, 19 triploidised salmon, 41 hybrids and 41 triploidised hybrids. Their results indicate that triploidised salmon had FA values which were very similar to those of diploid salmon. There were 21% of triploids and 18% of diploids asymmetric in pectoral fin rays. The percentage of asymmetric individuals in pelvic fins was 11% and 21% respectively. None of differences between two groups was statistically significant. In our experiment the percentage of asymmetric individuals for both traits under consideration was higher.

The direct comparison of our results with these results is not possible. Species examined by Wilkins *et al.* (1995) and species examined in our research belong two different groups of salmonids. Our results can also not to be directly compared to results of Leary *et al.* (1985) even they studied the same species as we did since the main goal of their research was to compare developmental stability of gynogenetic diploid and triploid rainbow trout. Nevertheless, to some extend their results could be used for a comparison with ours as in addition to data collected on experimental population of triploids they also presented data on population of diploids collected in fish from commercial farms. These data show that triploids and diploids do not differ in absolute numbers of rays in pectoral and pelvic fins. For them this is one of the indicators that morphology of the triploid is similar to that of their diploid counterparts. The same can be concluded from our results shown in Table 1. The difference between diploids and triploids regarding these traits and the absolute numbers which were found for both traits in our research are similar to the results presented by them. Their conclusion about FA is the same as the one which can be done on the basis of our results: triploids had less FA than diploids. The mean magnitude of asymmetry which is the sum of the absolute values of the left minus right counts for all traits per individual were 1.67 and 1.76 for two strains of triploids compared to 2.08 and 1.90 for diploids of same strains. In our research these values were 0.97 for triploids and 1.29 for diploids. The difference in absolute numbers resulted from the fact that in our research two meristic traits (pelvic and pectoral fins) were used to calculate this value while Leary *et al.* (1985) used five meristic counts. In research done on salmon and trout (Wilkins *et al.* 1995) values of mean magnitude of asymmetry were 0.90 for diploid and 0.79 for triploid salmon. Three meristic traits were used: number of gill rackers, number of rays in pelvic fins and number of rays in pectoral fins.

In the focus of the question of interest for aquaculturists and fish managers whether sterile triploids exhibit larger viability than females if they exhibit lower FA it is worth to mention the view of Leary *et al.* (1984) that lower FA in triploids does not indicate that triploidity has no deleterious effects on the development of rainbow trout. Therefore the comparisons of viability of two populations should be done on the basis of other traits. The most appropriate would be to use traits which are important when fish is grown for human consumption or for restocking, like growth rate, survival, resistance to diseases.

Majority of studies investigating FA in salmonids were based on meristic traits. (Young *et al.*, 1995; Sánchez-Gálan *et al.* 1998; Young *et al.*, 2009; Skog Eriksen *et al.*, 2008). The metric traits were used only in few studies. Vøllestad *et al.* (1998) for example measured the diameter of left and right eye as well as upper and lower left and right jaw length in grayling (*Thymallus thymallus*).

General conclusion about methods used either for performing counts of meristic traits or measuring metric traits is that they are characterized as tedious tasks with low precision. Therefore it would be of great benefit for further research in this area to find method which could

be used to count or measure traits with higher precision and speed.

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OCENA PARAMETROV DISPERZIJE ZA LASTNOSTI ZUNANJOSTI PRI KONJIH HAFLINŠKE PASME

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Ocena parametrov disperzije za lastnosti zunanjosti pri konjih haflinške pasme

V naši raziskavi smo pri konjih haflinške pasme v Sloveniji za lastnosti zunanjosti ocenjevali komponente (ko)varianc. V podatkovni zbirki je bilo skupaj 3371 živali, od tega smo jih v raziskavo vključili 600 (15 žrebcev in 585 kobil). Živali, ki smo jih vključili v analizo, so imele zapise desetih ocen in/ali devetih meritev ter znanega vsaj enega od staršev. Model je za vse ocenjene in izmerjene lastnosti vseboval leto ocenjevanja in/ali merjenja kot sistematski vpliv in naključni vpliv živali. Uporabili smo metodo omejene največje zanesljivosti (REML) v programu VCE. Pozitivno definitne matrike smo dobili s pomočjo postopka, ki se imenuje ukrivljanje matrik (*ang. bending*). Za ocenjene lastnosti so znašale heritabilitete od 0,40 za prednji del trupa do 0,78 za pasemsko značilnost. Heritabilitete za izmerjene lastnosti so se gibale med 0,20 za globino prsi in 0,62 za višino vihra, merjeno s palico. Genetske korelacije so bile v večini pozitivne. Najvišja genetska korelacija pri ocenjenih lastnostih je 0,92 med skupno oceno in zadnjim delom trupa. Med oceno pasemske značilnosti in oceno prednjih nog korelacije ni bilo. Pri izmerjenih lastnostih so bile genetske korelacije ocenjene od 0,38 med dolžino trupa in obsegom prsi do 0,95 med višino vihra, merjeno s palico in višino vihra, merjeno s trakom.

Ključne besede: konji / pasme / haflinška pasma / haflinger / lastnosti zunanjosti / selekcija / genetski parametri / Slovenija

Estimation of dispersion parameters for linear type traits in the Haflinger horses

The covariance components for exterior traits were estimated on Haflinger horses in Slovenia. There were 3371 data included in the database. Data from 600 animals (15 stallions and 585 mares) with known pedigree were analysed. For each horse, at most ten traits were scored and nine traits were measured. The fixed part of the model included only the year when horse was scored or measured and animal was treated as random effect. Genetic and environmental parameters for exterior traits were estimated by the restricted maximum likelihood method (REML) as implemented in the program package VCE. To make matrices positive definite we used a statistic method commonly known as 'bending'. Heritabilities for the scored traits were estimated between 0.40 for front body part and 0.78 for the breed type. For measured traits the heritabilities were between 0.20 for chest depth and 0.62 for withers height (measuring stick). Genetic corelations were in most cases positive. The highest genetic corelation for scored traits was 0.92 between total score and rear body part. There was no corelation betwen breed type and front legs. Genetic corelations for measured traits were from 0.38 between body lenght and chest size to 0.95 betwen withers hight measured with stick and measured with tape.

Key words: horses / breeds / Haflinger / exterior traits / selection / genetic parameters / Slovenia

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1 UVOD

Haflinška pasma konj je nastala na Južnem Tirolskem in je razširjena v več kot petdesetih državah po vseh kontinentih (Viliani, 2008). Konj haflinške pasme je majhnega okvirja z značilno barvo lisjaka in s plavo grivo in repom. Povprečna višina vihra konj haflinške pasme je med 140 in 155 cm. Kobile so nekoliko nižje od žrebcev. Telesna masa pri odrasli živali je okoli 500 kg. Konjev hafinške pasme ne uvrščamo niti med toplokrvne niti med hladnokrvne. V Sloveniji se ta pasma vzreja kot tradicionalna pasma konj (Pravilnik o ohranjanju ..., 2004) in se razvija v tipu ljubiteljskega, jahalnega konja (Rus, 2005). Odbira živali poteka na podlagi desetih ocen in/ali devetih meritev, ki so opisane v rejskem programu za konje haflinške pasme (Rus, 2005).

Napovedovanje plemenske vrednosti konj poteka na osnovi ocen in meritev, ki se opravljajo direktno na živali (Arnason in Van Vleck, 2000). Pri izboru lastnosti, ki so pomembne za selekcijo, je potrebno upoštevati dednost, merljivost, gospodarski in biološki pomen lastnosti. Pri različnih pasmah konj po svetu se plemenske vrednosti napoveduje predvsem za delovne in tekmovalne sposobnosti (Langlois in Blouin, 2004). Pri tem se vključuje podatke z ocenjevanja in merjenja konj ter njihovo poreklo.

Za napovedovanje plemenske vrednosti v konjereji najbolj razširjena metoda mešanih modelov. Metoda omogoča hkratno ocenjevanje genetskih in okoljskih vplivov in je postala standardna metoda za genetsko vrednotenje konj (Arnason, 1996). V tujini pri sestavljanju modela pri različnih populacijah upoštevajo veliko različnih vplivov. Kot vplive v modelih uporabljajo spol, starost, rejca, popisovalca in ocenjevalca, geografsko regijo, genetsko skupino in kondicijo (van Bergen Henk in van Arendonk, 1993; Koenen in sod., 1995; Dolvik in Klemetsdal, 1999). V Italiji s to metodo napovedujejo plemensko vrednost konjem haflinške pasme za linearne lastnosti telesnega ustroja (Samoré in sod., 1997), na Islandiji vsem konjem, ki so vključeni v podatkovno zbirko (Huganson, 1994), na Poljskem konjem arabske pasme (Sobczyinska in Kownacky, 1996), kasačem v Franciji (Langlois in Vrijenhoek, 2004) ter kasačem na Norveškem (Arnason, 1996). Parametre disperzije za ocenjene lastnosti zunanjosti so ocenili pri konjih haflinške pasme v Italiji (Samoré in sod., 1997), za izmerjene lastnosti pri haflinških konjih pa bomo rezultate predstavili prvi, saj jih za tuje populacije haflinških konj v literaturi nismo zasledili.

Za ocenjevanje lastnosti zunanjosti se v Sloveniji uporablja skala z opisnimi ocenami od 1 do 10 brez vmesnih ocen (Rus, 2005). Ocena 0 pomeni, da žival ni bila ocenjena za to lastnost in ima manjkajočo vrednost. Posamezne ocene za lastnosti zunanjosti, ki predstavljajo število točk, se seštejejo v skupno oceno in na podlagi skupne ocene se živali razvrščajo v šest kakovostnih razredov (1a, 1b, 2a, 2b, 3a, in 3b). Znotraj razredov se pri mejnih vrednostih pripiše oznaka plus (+) pri zgornji meji ali minus (–) pri spodnji meji. V Italiji (Samoré in sod., 1997) linearno ocenjujejo pri konjih haflinške pasme 10 sklopov lastnosti, znotraj katerih je skupaj 26 posameznih lastnosti ocenjenih z ocenami od 0 do 10 na pol točke natančno. Pri andaluzijskih konjih v Španiji (Molina in sod., 1999) linearno ocenjujejo 11 lastnosti, ki so razporejene v dveh sklopih: morfološke lastnosti in ocene pasemskega tipa. Pri šetlandskih ponijih (van Bergen Henk in van Arendonk, 1993) smo lahko zasledili 28 ocen lastnosti zunanjosti in pri nizozemskem toplokrvnem konju 26 lastnosti, ki so ocenjene na linearni točkovni skali z vrednostimi od 0 do 40.

Pri konjih haflinške pasme v Sloveniji merimo največ osem lastnosti zunanjosti (Rus, 2005). V Braziliji so na konjih panteniro pasme izvajajo 14 meritev (Miserani in sod., 2002) in na nizozemskih vlečnih konjih kar 31 meritev (Druml in sod., 2008). V Sloveniji je minimalna višina vihra, merjena s palico, za vpis žrebcev v elitno knjigo 142 cm in 140 cm za vpis žrebcev v knjigo žrebcev II in kobil v glavno knjigo kobil. Višina 138 cm je minimalna pri vpisu kobil v splošno rodovniško knjigo in kobile morajo dosegati vsaj 136 cm višine vihra, merjeno s palico, za vpis v evidenčno knjigo.

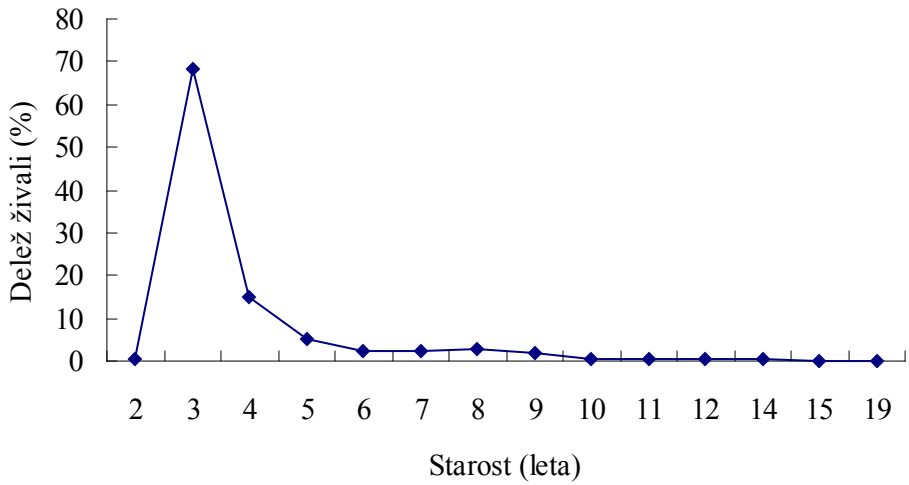
Pri odbiri konj je pomembna tudi informacija o oceni delovne sposobnosti konj (Rus, 1996). Preizkus delovnih sposobnosti haflingerjev je v tujini določen z rejskim programom. Preizkus delovnih sposobnosti za toplokrvne konje v Sloveniji se ni opravljali do leta 1996, predvsem zaradi majhnega števila konj in slabe kakovosti plemenskega materiala (Rus, 1996). Po letu 1996 se je povečal interes za rejo toplokrvnih konj. Kakovost plemenskega materiala se je izboljšala in preizkus delovnih sposobnosti je tudi pri nas postal pomemben vir informacij za napoved plemenske vrednosti.

Namen našega dela je bil proučiti lastnosti zunanjosti pri konjih haflinške pasme in postaviti večlastnostni statistični model, primeren za napovedovanje plemenske vrednosti v Sloveniji.

2 MATERIAL IN METODE

2.1 MATERIAL

Podatke o konjih haflinške pasme v Sloveniji smo dobili z Inštituta za zdravstveno varstvo kopitarjev na Veterinarski fakulteti, kjer opravljajo identifikacijo, registracijo in vpis živali v rodovniško knjigo. Skupno število živali v podatkovni zbirki je bilo 3371. V statistične analize za oceno parametrov disperzije so bili vključeni



Slika 1: *Porazdelitev živali glede na starost ob ocenjevanju in/ali merjenju.*
Figure 1: *The distribution of animals according to age at evaluation and / or measurement.*

podatki konj haflinške pasme, ki so bili vneseni in urejeni v podatkovni zbirki do marca 2008.

Podatke iz podatkovne zbirke smo uredili s programskim jezikom SQL, pripravili smo datoteko z ocenjenimi in izmerjenimi lastnostmi ter datoteko s poreklom živali. Iz podanih posameznih ocen o pasemskih značilnosti, vratu, glavi, prednjem, srednjem in zadnjem delu trupa, prednjih in zadnjih nogah ter pravilnosti in izdatnosti hodov smo izračunali skupno oceno za živali, ki so imele podane vseh deset ocen. Na živalih so bile opravljene naslednje meritve: višina vihra, merjena s trakom in palico, višina križa, merjena s trakom, obseg prsi in piščali, širina prsi in križa, globina prsi in dolžina trupa. V obdobju od leta 1990 do marca 2008 je tako bilo ocenjenih 600 živali. Število ocenjenih živali se je med leti spreminjalo. V povprečju je bilo na leto ocenjenih in/ali merjenih 31,6 živali. Največ (64) jih je bilo ocenjenih v letu 2005. Starost živali je bila od dveh pa do 19 let. Kar 68,5 % živali je bilo ocenjenih in/ali merjenih pri starosti treh let (slika 1). Živali, ki so bile stare med pet in vključno devet let, so predstavljale 9,6 % vseh ocenjenih in/ali merjenih živali. Kar 1,7 % živali je bilo starih deset let ali več. V letu 2008 je bilo do marca ocenjenih in/ali merjenih 13 živali, starih med sedem in deset let.

Pripravljeno poreklo je zajemalo skupaj 1956 živali z globino osem generacij (pregl. 1). Prvo generacijo so predstavljale živali, ki so imele vsaj pet ocen in/ali meritev. V predhodne generacije so bili uvrščeni njihovi predniki. V poreklu je imelo 80,57 % živali znanega vsaj enega starša, oba starša sta bila znana pri 79,47 % živalih.

Preglednica 1: *Struktura porekla*
Table 1: *The origin structure*

Generacija	Št. živali	Oba starša znana	Znan oče	Znana vsaj en starš	Neznani starši
1	600	559	1	600	-
2	280	280	-	280	-
3	227	227	-	227	-
4	294	270	5	275	19
5	338	148	5	153	185
6	173	25	6	31	142
7	35	6	2	8	27
8	9	1	1	2	7
Skupaj	1956	1556	20	1576	377
Delež (%)	199	79,55	1,02	80,57	19,27

Osnovno oziroma izhodiščno populacijo so predstavljale živali z neznanimi starši (377 oziroma 19,27 %). Med konji haflinške pasme, ki smo jih vključili v analizo, je bilo 71 različnih očetov in 363 mater. V povprečju je bilo na vsakega očeta skoraj 28 potomcev in na vsako mater 5 potomcev.

2.2 METODE

Podatkovno zbirko za kopitarje v Sloveniji vzpostavljamo iz starih zapisov. Nabor možnih vplivov, ki bi jih vključili v model, je bil v primerjavi s tujo literaturo manjši. Pri izboru končnega modela smo upoštevali statistično značilnost vplivov (p-vrednost), koeficient determinacije (R^2) in število stopinj prostosti za posamezne vplive in za model v celoti. Razlike med spoloma niso bile statistično značilne, kar je verjetno posledica neugodne strukture podatkov po spolu. Žrebci so bili premalo zastopani, ocenjevalna skala pa že upošteva spolni dimorfizem. Starost, ki je predstavljala razliko med letom ocenjevanja in letom rojstva, se je pri nekaterih lastnostih pokazala za značilno, vendar pa bi vključitev vpliva v model le malo doprinesla k pojasnjeni varianci.

Po predhodnih statističnih analizah smo uporabili enostavni model (enačba 1), ki je vključeval samo leto ocenjevanja in/ali merjenja (Li) kot sistematski vpliv. Naključni vpliv je predstavljala žival oziroma aditivni genetski vpliv (aij).

$$y_{ij} = \mu + L_i + a_{ij} + e_{ij} \quad (1)$$

Pri napovedovanju plemenskih vrednosti se lahko uporabi samo pozitivno definitne matrike genetskih varianc in kovarianc (Hayes in Hill, 1981). Pozitivna definitnost matrik je bil zato naš kriterij za izbor lastnosti zunanjosti pri konjih haflinške pasme. Pozitivno definitnost matrik smo preverjali s Cholesky razčlenitvijo (Hayes in Hill, 1981; Jorjani in sod., 2003). Ker matrika merjenih lastnosti ni bila pozitivno definitna, smo naredili ukrivljanje matrike (bending).

Razvoj sistematskega dela modela smo opravili po metodi najmanjših kvadratov s proceduro GLM v statističnem paketu SAS (SAS Inst. Inc., 2001). Parametre disperzije smo analizirali z metodo omejene največje zanesljivosti (REML) v programu VCE (Kovač in sod., 2002). Podatke smo za ta program predhodno pripravili s programom PEST (Groeneveld in sod., 1990).

3 REZULTATI IN RAZPRAVA

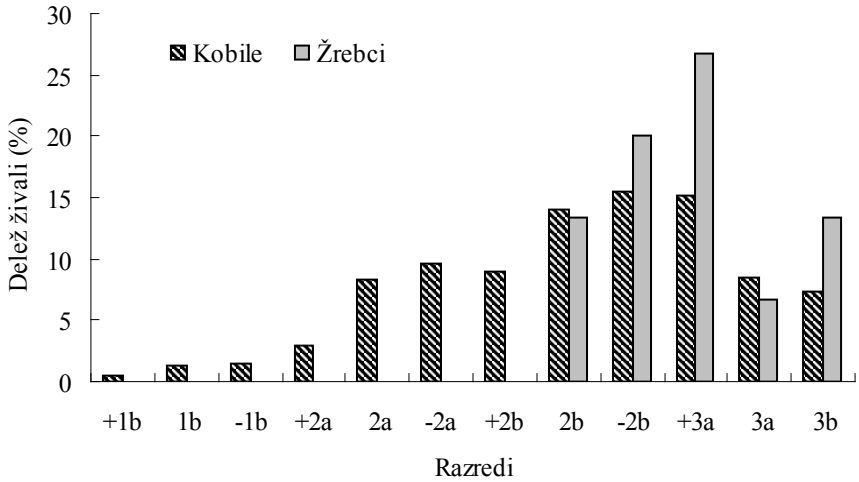
V genetsko analizo smo vključili 600 živali, ki so imele podatke o ocenjevanju in merjenju. Rezultate smo razdelili v dva sklopa. V prvem delu predstavljamo fenotipske vrednosti in v drugem koeficiente determinacije.

3.1 FENOTIPSKE VREDNOSTI ZA LASTNOSTI ZUNANJOSTI

Pri ocenjenih lastnostih zunanjosti je bilo največ ocenitev (600) opravljenih za pasemsko značilnost, glavo in vrat ter najmanj ocen (561) za izdatnost hodov (pregl. 2). Najvišje povprečje je bilo pri ocenah za prednji del trupa, kjer je bila povprečna ocena 7,6 in najmanjše za lastnost zadnjih nog, kjer je bila povprečna ocena 6,7. Povprečje skupne ocene je bilo dobrih 71 točk. Pri konjih haflinške pasme v Italiji so se povprečne vrednosti ocen gibale med 4,5 in 5,5 (Samoré in sod., 1997), kar bi lahko

Preglednica 2: *Opisna statistika ocen za lastnosti zunanjosti*
Table 2: *Descriptive statistics for estimated traits*

	Št. ocen	Povprečje	Standardni odklon	Modus	Minimum	Maksimum
Skupna ocena	561	71,4	4,72	69	58	87
Pasemska značilnost	600	7,3	0,70	7	6	10
Glava	600	7,2	0,82	7	5	9
Vrat	600	7,3	0,77	7	6	9
Prednji del trupa	599	7,6	0,68	8	5	9
Srednji del trupa	599	7,1	0,74	7	5	9
Zadnji del trupa	599	7,3	0,67	7	5	9
Prednje noge	598	6,8	0,92	7	4	9
Zadnje noge	598	6,7	0,73	7	4	9
Pravilnost hodov	574	6,9	0,80	7	4	9
Izdatnost hodov	561	7,4	0,77	7	5	10



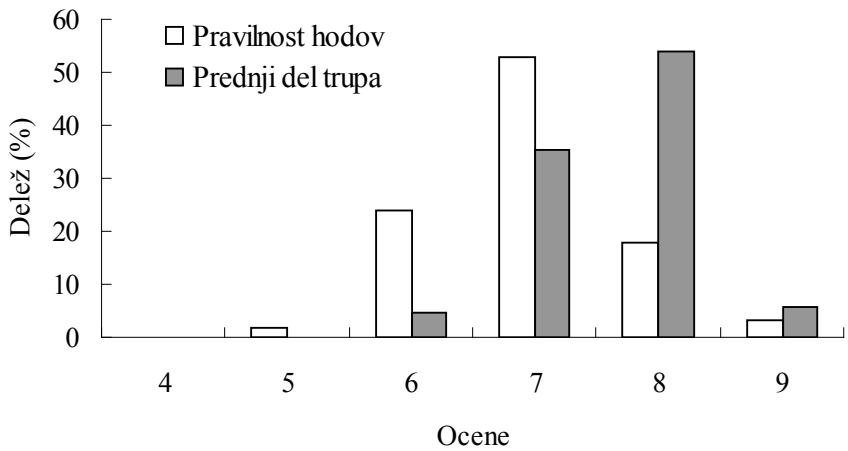
Slika 2: *Porazdelitev živali v kakovostne razrede ločeno po spolu.*
Figure 2: *Animals in classes by gender.*

pripisali večjemu številu ocenjenih živali in uporabi celotne skale za ocenjevanje. Med posameznimi lastnostmi je največji standardni odklon v našem primeru imela ocena prednjih nog (0,9) in najmanjši standardni odklon ocena prednjega dela trupa (0,7). Standardni odkloni so bili v raziskavi, ki so jo opravili Samoré in sod. (1997), med 0,9 za poslušnost in 2,0 za barvo grive. Standardni odkloni v našem primeru pri nobeni posamezni lastnosti ne dosežejo pričakovane vrednosti, ki znaša 1,66. Modus za vse ocenjene lastnosti, z izjemo ocen za prednji del trupa, je znašal 7 (pregl. 2).

Razpon ocen na ocenjevalni skali je od 1 do 10, vendar v praksi vidimo, da je bila najmanjša podeljena ocena 4 (pregl. 2) pri oceni nog in hodov. Od vseh 5928 dodeljenih ocen v naši raziskavi je bila najbolj pogosta ocena 7, uporabljena 2921-krat, kar predstavlja polovico vseh podeljenih ocen. Razpon med ocenami za lastnosti je bil največ šest točk.

Na podlagi skupne ocene se konji razvrstijo v kakovostne razrede. Nobena žival v našem poskusu ni presegla 87 točk, zato je 1a razred v našem primeru prazen, saj je zanj potrebnih 90 točk in več (slika 2). Večina živali je bila razvrščena med +3a in +2b kakovostnim razredom. V najslabši razred 3b je bilo razvrščenih 45 živali (7,5 %).

Podatki, ki smo jih obdelali, nakazujejo možnost, da je bila prva odbira opravljena pred ocenjevanjem. Odbiro opravijo že sami rejci, ki konje s slabšimi lastnostmi ne pripeljejo na ocenjevanje. Porazdelitve ocen za posame-



Slika 3: *Porazdelitev ocen za lastnosti pravilnost hodov in prednji del trupa.*
Figure 3: *Distribution of accuracy of walk and the front of body.*

Preglednica 3: *Opisna statistika za izmerjene lastnosti*
Table 3: *Descriptive statistics for measured traits*

	Št. meritev	Povprečje	Standardni odklon	Minimum	Maksimum
Višina vihra – palica (cm)	593	139,34	3,21	132	152
Višina vihra – trak (cm)	592	150,20	4,27	140	167
Obseg prsi (cm)	593	177,24	8,56	152	218
Globina prsi (cm)	451	65,29	2,74	50	72
Širina prsi (cm)	454	41,96	3,67	30	64
Obseg piščali (cm)	586	19,31	0,93	17	22
Višina križa (cm)	455	140,67	4,71	131	151
Širina križa (cm)	450	52,75	3,23	34	88
Dolžina trupa (cm)	450	150,82	5,41	135	169

zne lastnosti naj bi bile simetrične. Ocene za pravilnost hodov so v primerjavi z ostalimi lastnostmi porazdeljene dokaj simetrično (slika 3). Zajete so ocene od 4 do 10, najpogostejša vrednost pa je 7. Za primerjavo prikazujemo še porazdelitev ocen za prednji del trupa, kjer so bile uporabljene le štiri ocene na intervalu od 5 do 9 z najpogostejšo oceno 8. Opisna skala je naravnana tako, da olajša odbiro, ni pa najbolj primerna za obdelavo podatkov.

Pri konjih haflinške pasme v Sloveniji (pregl. 3) je bilo največ meritev (593) opravljenih za višino vihra, merjeno z merilno palico, ter obseg prsi in najmanj (450) za dolžino trupa in širino križa. V povprečju je bila višina vihra, merjena s palico, 139,3 cm s standardnim odklonom 3,2 cm. Meritev vihra s trakom je bila za 10,9 cm višja. V povprečju bi se lahko vse živali vpisale v splošno rodovniško knjigo, kjer je pogoj minimalna višina vihra merjena s palico 138 cm. Živali, ki so imele višino vihra nižjo od 136 cm, niso zadostile pogojem za vpis v evidenčno knjigo. Višina križa je bila v povprečju za 1,33 cm višja kot višina vihra, merjena s palico.

Obseg piščali je bil v povprečju 19,31 cm in obseg prsi 177,24 cm, kjer je bil tudi največji standardni odklon, ki je znašal 8,56 cm. Variabilnost merjenih živali je bila majhna. Kadar je majhna variabilnost posledica predhodne odbire, otežuje selekcijsko delo in zmanjšuje učinkovitost rejskega dela.

Preglednica 4: *Ocena heritabilitet (diagonala), kovarianc (nad diagonalo) za aditivni genetski vpliv , genetske korelacije (pod diagonalo) ter ocena fenotipskih varianc (zadnji stolpec) za ocenjene lastnosti*
Table 4: *Estimates of heritability (diagonal) covariance (above diagonal) for additive genetic effects, genetic correlations (below diagonal) and phenotypic variance estimate (last column) for the evaluated traits*

	S	PZ	GL	VR	PDT	SDT	ZDT	PN	ZN	PH	IH	Fenotip. var.
S	0,64	1,55	−0,10	1,53	1,33	1,07	2,26	1,86	1,74	2,01	2,23	24,27
PZ	0,53	0,68	−0,12	0,24	0,14	0,11	0,30	0,00	0,11	0,05	0,16	0,82
GL	−0,03	−0,21	0,78	−0,09	−0,05	0,12	−0,03	−0,09	−0,16	−0,15	−0,11	0,73
VR	0,65	0,51	−0,18	0,52	0,23	0,21	0,20	−0,04	0,13	0,07	0,20	0,76
PDT	0,81	0,45	−0,15	0,87	0,40	0,13	0,17	0,08	0,13	0,14	0,20	0,44
SDT	0,42	0,22	0,25	0,52	0,49	0,61	0,06	−0,09	−0,05	−0,02	0,17	0,68
ZDT	0,92	0,65	−0,07	0,51	0,65	0,16	0,53	0,31	0,29	0,29	0,27	0,73
PN	0,63	0,00	−0,16	−0,08	0,24	−0,18	0,66	0,52	0,39	0,47	0,25	1,08
ZN	0,67	0,22	−0,32	0,29	0,47	−0,11	0,72	0,79	0,65	0,34	0,14	0,66
PH	0,75	0,11	−0,29	0,16	0,78	−0,04	0,69	0,91	0,77	0,62	0,34	0,74
IH	0,74	0,28	−0,19	0,42	0,63	0,36	0,57	0,44	0,27	0,65	0,53	1,09

S – skupna ocena, PZ – pasemska značilnost, GL – glava, VR – vrat, PDT – prednji del trupa, SDT – srednji del trupa, ZDT – zadnji del trupa, PN – prednje noge, ZN – zadnje noge, PH – pravilnost hodov, IH – izdatnost hodov

3.2 OCENE (KO)VARIANC ZA LASTNOSTI ZUNANJOSTI

3.2.1 OCENJENE LASTNOSTI

V analizo parametrov disperzije smo zajeli vse ocenjene lastnosti. Fenotipske variance so se gibale med 0,44 in 1,09 z izjemo skupne ocene, kjer je fenotipska varianca 24,27 (pregl. 4). Genetska varianca je bila pričakovano največja (15,63) pri seštevku ocen. Pri posameznih lastnostih je najmanjša genetska varianca (0,14) bila pri oceni za srednji del trupa. Aditivne genetske variance so Albertsdóttir in sod. (2007) v raziskavi, kjer so ocenjevali genetske parametre za lastnosti zunanjosti in rezultate tekmovanj pri islandskih konjih, ocenili med 0,06 in 0,94. Od primerljivih lastnosti imajo najvišje (0,11) ocenjeno varianco za aditivni genetski vpliv lastnosti kakovosti nog (v našem poskusu je bila ocenjena nad 0,40) in najnižje (0,06) ocenjeno stojo nog. Aditivno genetsko varianco za glavo in vrat so ocenili na 0,07 ter za zadnji del trupa 0,08, kar je precej manj, kot je bilo v našem primeru. Razlike v ocenah genetskih varianc so lahko posledica razlik v pasmi in različnih ocenjevalnih skal.

Heritabilitete pri haflinških konjih v Sloveniji so se gibale med 0,40 za prednji del trupa in 0,78 za oceno glave (pregl. 4). V primerjavi z našimi rezultati so nižje heritabilitete za lastnosti zunanjosti ocenili pri konjih haflinške pasme v Italiji (Samoré in sod., 1997). Za lastnost vratu so heritabiliteto izvrednotili na 0,04 in za lastnosti nog med 0,10 in 0,17. Pri nizozemskih toplokrvnih konjih (Koenen in sod., 1995), kjer je skala z ocenami od 0 do 40, so bile heritabilitete za ocenjene lastnosti med 0,09 in 0,28. Pri šetlandskih ponijih (van Bergen Henk in van Arendonk, 1993) so heritabiliteto za izdatnost hoda ocenili na 0,35 (pri nas 0,53) in za zadnje noge 0,07 (pri nas 0,65). Pri islandskih konjih (Albertsdóttir in sod., 2007) so za lastnosti glave, nog in hoje podali nekoliko nižje rezultate. Heritabiliteto za prednji del trupa pri populaciji vlečnih konj na Nizozemskem so Druml in sod. (2008) ocenili na 0,16, v našem primeru smo jo na 0,40. Suontama in sod. (2009) so heritabiliteto za lastnosti nog ocenili na 0,13 in za lastnosti gibanja 0,17.

Genetske korelacije so bile v večini pozitivne. Negativne korelacije smo izračunali med oceno za glavo in ostalimi lastnostmi. Genetska korelacija med prednjimi in zadnjimi nogami je ocenjena na kar 0,79. Genetsko korelacijo med prednjimi in zadnjimi nogami so Samoré in sod. (1997) ocenili na 0,06. Najvišjo genetsko korelacijo smo ocenili na 0,92 med skupno oceno in zadnjim delom trupa. Samoré in sod. (1997) so močne genetske korelacije ocenili med lastnostmi vratu (0,94) in med posameznimi lastnostmi glave (0,99). Višje ocenjene genetske korelacije so bile tudi pri nizozemskih konjih (Koenen in sod., 1995). Šibko genetsko korelacijo (0,07) so Druml in sod. (2008) ocenili med glavo in pravilnostjo hodov. Do razlik med ocenami parametrov disperzije lahko prihaja zaradi različnih pasem, zaradi postopka ocenjevanja in ocenjevalne skale. Največjo težavo pri primerjavi predstavlja opisna skala, ki se uporablja pri ocenjevanju konj v Sloveniji. V tujini se za ocenjevanje konj uporabljajo linearne skale.

3.2.2 PARAMETRI DISPERZIJE ZA IZMERJENE LASTNOSTI

Fenotipske variance so bile med 18,06 cm^2 za višino vihra, merjeno s palico, in 0,76 cm^2 za obseg piščali (pre-

Preglednica 5: *Ocena heritabilitet (diagonala), kovarianc (nad diagonalo) za aditivni genetski vpliv , genetske korelacije (pod diagonalo) ter ocena fenotipskih varianc (zadnji stolpec) za izmerjene lastnosti*
Table 5: *Estimates of heritability (diagonal) covariance (above diagonal) for additive genetic effects, genetic correlations (below diagonal) and phenotypic variance estimate (last column) for the measured traits*

	VVT	VVP	GP	ŠP	OP	OPI	VK	ŠK	DT	Fenotip. var.
VVT	0,57	7,17	6,39	2,67	2,63	0,80	4,92	1,86	5,44	8,90
VVP	0,95	0,62	10,50	3,55	4,45	1,10	7,54	3,59	8,37	18,06
GP	0,73	0,81	0,20	4,48	5,08	1,23	6,69	5,38	8,64	7,70
ŠP	0,68	0,61	0,66	0,43	1,50	0,50	1,98	1,39	3,39	7,08
OP	0,62	0,70	0,69	0,46	0,29	0,39	2,42	2,41	2,31	12,55
OPI	0,72	0,67	0,65	0,59	0,42	0,32	0,76	0,46	1,22	0,76
VK	0,92	0,94	0,72	0,48	0,54	0,65	0,51	2,08	5,92	11,09
ŠK	0,53	0,68	0,89	0,51	0,81	0,59	0,56	0,24	3,04	10,38
DT	0,74	0,77	0,68	0,60	0,38	0,76	0,76	0,60	0,36	11,99

VVT – višina vihra, merjena s trakom, VVP – višina vihra, merjena s palico, GP – globina prsi, ŠP – širina prsi, OP – obseg prsi, OPI – obseg piščali, VK – višina križa, ŠK – širina križa, DT – dolžina trupa

gl. 5). Genetske variance pri izmerjenih lastnostih so bile ocenjene dokaj majhne (pregl. 5). Genetska varianca za višino vihra, merjeno s palico, je bila ocenjena na 11,23 cm^2, za dolžino trupa 10,58 cm^2 in za globino prsi na 15,08 cm^2. Najnižja genetska varianca je bila pri obsegu piščali, kjer je bila ocenjena na 0,24 cm^2. Genetske variance za merjene lastnosti so ocenjevali na nizozemskih toplokrvnih konjih (Dolvik in Klemetsdal, 1999). Za višino vihra, merjeno s trakom, sta variance ocenila na 12,25 cm^2 in za obseg piščali 0,38 cm^2.

Za izmerjene lastnosti so ocene heritabilitet presegale 0,20 (pregl. 5). Heritabilitete so bile visoke za višino vihra, merjeno s palico (0,62) ali trakom (0,57) ter višino vihra (0,51). Najnižjo (0,20) heritabiliteto je imela v našem primeru globina prsi. Pri norveških hladnokrvnih konjih (Dolvik in Klemetsdal, 1999) je bila heritabiliteta za višino vihra, merjeno s palico, ocenjena na 0,73, pri islandskih konjih na 0,67 (Albertsdóttir in sod., 2007) in pri andaluzijskih konjih v Španiji na 0,50 (Molina in sod., 1999) oziroma 0,60 (Gómez in sod., 2009). Primerljiva je bila ocena heritabilitete za višino vihra, merjeno s palico, pri konjih panteneiro pasme v Braziliji, ki je bila ocenjena na 0,61 (Miserani in sod., 2002).

Pri konjih panteneiro pasme v Braziliji (Miserani in sod., 2002) je bila heritabiliteta za širino prsi ocenjena na 0,51, v Španiji pri andaluzijskih konjih (Molina in sod., 1999) na 0,56 oziroma 0,42 (Gómez in sod., 2009) in v našem primeru na 0,43. Na andaluzijskih konjih v Španiji (Molina in sod., 1999; Gómez in sod., 2009) so heritabiliteto za dolžino trupa ocenili na 0,72 oziroma 0,49 (v našem poskusu 0,36) in za obseg piščali na 0,35 oziroma 0,51. Ocene raziskav v Španiji so primerljive z ocenami za populacijo haflinških konj pri nas. Višje ocenjene heritabilitete za lastnosti dolžine trupa (0,72) in obseg piščali (0,53) so imeli tudi andaluzijski konji v Španiji (Miserani in sod., 2002).

Vse genetske korelacije so bile v našem primeru pozitivne in ocenjene med 0,38 in 0,95. Genetske korelacije lahko ocenimo kot močne. Visoke genetske korelacije so bile predvsem med višinama vihra in višino križa. Podobne rezultate so dobili Suontama in sod. (2009) pri finskih kasačih, kjer je bila genetska korelacija med višino vihra in višino križa ocenjena na 0,98 ter med višino vihra in dolžino trupa na 0,84. Molina in sod. (1999) so genetske korelacije ocenili med 0,20 in 0,60.

4 SKLEPI

V raziskavi smo proučevali lastnosti zunanjosti pri konjih haflinške pasme v Sloveniji in analizirali podatke, ki so bili zajeti v obdobju zadnjih 19 let. Povprečna starost živali je bila 3,82 leta. Vse ocenjene in/ali izmerjene živali so imele znanega vsaj enega starša in poreklo je bili sestavljeno iz 8 generacij. Najnižja podeljena ocena za lastnosti zunanjosti je bila 4 in maksimalna 10. Povprečje za skupno oceno je bilo 71,7 točk. Nobena žival se ni uvrščala v najboljši 1a kakovostni razred.

Razvili smo enostaven model z enim sistematskim in enim naključnim vplivom. Podatki niso omogočali proučevanja drugih vplivov, kot so spol in starost. V statistični model bi bilo potrebno vključiti več sistematskih vplivov, ki pa morajo biti znani pri večini živali. Za vključitev vplivov bi bilo potrebno izboljšati kakovost podatkov.

Naši rezultati so primerljivi z nekaterimi raziskavami, ki so jih opravili na haflinških konjih v Italiji in na nizozemskih toplokrvnih konjih. Najnižja heritabiliteta (0,20) je bila ocenjena za globino prsi in najvišja (0,78) za oceno pasemske značilnosti. Za lažjo primerjavo in natančnejše ocene parametrov disperzije bi bilo potrebno v analize vključiti več podatkov, uporabljati bi bilo potrebno celotno ocenjevalno skalo ter ocenjevati oziroma meriti bi bilo potrebno večji delež živali v populaciji, tudi nezaželene. Vredno bi bilo razmisliti tudi o linearni ocenjevalni skali.

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

VIRI

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ODDAJA

Avtorji prispevke oddajo v natisnjenem in elektronskem izvodu. 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 natisnjenem in elektronskem izvodu. Ko prvi avtor vnese še lektorjeve pripombe, odda popravljeno besedilo v natisnjenem in elektronskem izvodu.

Pri oddaji končne verzije avtor priloži jasno označene izvirnike slik (ločene grafične datoteke ali fotografije). Datoteke slik poimenuje enako kot v tekstu (npr. Slika1.jpg, Slika2.eps, Slika3.bmp). Originalne fotografije na avtorjevo željo vrnemo. Vektorske slike sprejemamo samo v eps (Encapsulated Postscript) formatu, s tekstom, ki je spremenjen v krivulje. Rasterske slike morajo biti v enem od običajnih formatov (npr. tiff, jpg, bmp). Ločljivost naj bo vsaj 300 dpi.

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NOTES FOR AUTHORS

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The papers should be condensed, short and should not exceed 12 pages, editted with word processor and submitted as doc or rtf file (Windows). Text formatting should be as simple as possible, without headers and footers. Font Times New Roman, size 12 should be used for text and tables (in large tables size 10 is allowed), Ariel should be used for graphs and figures (letter size at least 8) and Courier for nucleic- and amino acid sequence alignments. Right margin is 2.0 cm, left margin 2.5 cm

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Under the address of the authors some space for dates of arrival and acceptance for publishing should be left. A comprehensive and explicit abstract up to 250 words follows indicating the objective and methods of work, results, discussion and conclusions. Key words follow the abstract.

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Simončič M., Horvat S., Stevenson P.L., Bünger L., Holmes M.C., Kenyon C.J., Speakman J.R., Morton N.M. 2008. Divergent physical activity and novel alternative responses to high fat feeding in polygenic fat and lean mice. Behavior Genetics, 38, 3: 292–300

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

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

Žgajnar J., Kermauner A., Kavčič S. 2007. Model za ocenjevanje prehranskih potreb prežvekovalcev in optimiranje krmnih obrokov. In: Slovensko kmetijstvo in podeželje v Evropi, ki se širi in spreminja. 4. konferenca DAES, Ljubljana, 8–9 sep. 2007. Kavčič S. (ed.). Domžale, Društvo agrarnih ekonomistov Slovenije: 279–288

ISO 5534 / IDF 4. Cheese and processed cheese – Determination of the total solids content – Reference method. 2004: 1–7

Frajman P., Dovč P. 2004. Milk production in the post-genomic era. *Acta agriculturae Slovenica*, 84, 2: 109–119. http://aas.bf.uni-lj.si/zootehnika/84-2004/PDF/84-2004-2-109-119.pdf (15. mar. 2009)

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