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Effect of magnetic field on chicken hatching

Účinok magnetického poľa na liahnutie kurčiat

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ABSTRACT: The effect of magnetic field with the intensity 0.07 T on hatching of ROSS 208 chickens was studied. The hatchability of eggs that were influenced by magnetic field during the storage of hatching eggs (20–40 minutes) increased in comparison with eggs that were not exposed to magnetic field ($P \leq 0.05$). Hatchability from eggs that were influenced by magnetic field during their incubation (experimental groups) decreased to $66.19 \pm 4.51\%$ and $67.58 \pm 3.62\%$ as compared with control groups ($89.68 \pm 1.54\%$ and $94.87 \pm 5.83\%$). The difference was significant ($P \leq 0.001$). The negative effect of magnetic field was manifested by lower weight of hatched chickens in experimental groups (34.58 ± 1.54 g and 36.81 ± 1.04 g). The results were significant ($P \leq 0.05$) in comparison with the control groups with the average weight of hatched chickens 41.43 ± 0.92 g, and 43.75 ± 1.50 g.

Keywords: chickens; hatching; hatchability; magnetic field; weight of chickens

ABSTRAKT: V práci sme sledovali vplyv magnetického poľa s intenzitou 0,07 T na liahnutie kurčiat hybridu ROSS 208. Pri pôsobení magnetického poľa počas uskladnenia vajec (20–40 minút) sa u takto ošetrovaných násadových vajec zvýšila liahnivosť v porovnaní s násadovými vajcami na ktoré počas uskladnenia nepôsobilo magnetické pole ($P \leq 0,05$). Pri pôsobení magnetického poľa počas inkubácie vajec liahnivosť v pokusných skupinách klesla na $66,19 \pm 4,51$ % resp. $67,58 \pm 3,62$ % v porovnaní s kontrolnými skupinami ($89,68 \pm 1,54$ % resp. $94,87 \pm 5,83$ %) bol rozdiel štatisticky preukazný ($P \leq 0,001$). Negatívny vplyv magnetického poľa sa prejavil v zníženej hmotnosti vyliahnutých kurčiat v pokusných skupinách ($34,58 \pm 1,45$ g resp. $36,81 \pm 1,04$ g). Výsledky boli preukazné ($P \leq 0,05$) v porovnaní s kontrolnými skupinami s priemernou hmotnosťou $41,43 \pm 0,92$ g resp. $43,75 \pm 1,50$ g.

Kľúčové slová: kurčatá; liahnutie; liahnivosť; magnetické pole; hmotnosť kurčiat

INTRODUCTION

Magnetic field is a permanent component of the living environment of animals. It can be divided into a permanent magnetic field and a temporary one (Bearly, 1992). Every animal cell consists of molecules. When a cell is placed into a magnetic field, all molecules get magnetised (Tanokura and Suzuki, 1999). Stocker and van Gunsteren (2000) claim that animal cells create changing electric fields as part of their living processes, which then influence the creation of a temporary magnetic field of the cells. Neher (1982) proved that the application of magnetic field to the animal cells results in changes in permeability of cell membranes, in the increase of thrombotisation, and the occurrence of vasodilatation and anti-inflammatory effects. It can also have a spasmolytic effect on the smooth muscular tissue as well as support the metabolism of mineral substances. Several authors discussed the effect of mag-

netic field on the embryonic development of birds (Pan, 1996; Behr *et al.*, 1991; Blackman *et al.*, 1988) as well as on the embryonic development of mammals (Eckert, 1976).

The aim of this article was to determine the effect of temporary magnetic field on the hatching parameters in broiler chickens.

MATERIAL AND METHODS

A total of 1 560 hatching eggs of broiler chickens of ROSS 208 hybrid aged 44–61 weeks were used for an experiment. The eggs were divided according to their weight into two groups. In the 1st control and experimental groups, the eggs weighing 56–60 g were included, while in the 2nd control and experimental groups, the eggs weighed 61–65 g. The eggs included in control groups were not exposed to any magnetic field during their stor-

age and incubation. The experimental groups of eggs were exposed to a magnetic field with the induction of 0.07 T during their storage and incubation.

The study was carried out in two experiments

In the 1st experiment we tried to determine the effect of magnetic field on the hatchability of chickens during the storage of hatching eggs. The storage time was 2 days – at 8–12°C and 60–70% of relative air humidity. The magnetic field was applied for 20–40 minutes.

In the 2nd experiment we tried to determine the effect of magnetic field on the chicken hatching during incubation. The eggs were hatched in BIOS MONO 06 hatcheries. The magnetic field was applied to the chicken embryos for 10 minutes daily during their whole incubation time. The following indicators were monitored during the incubation: beginning of pecking, pecking time, hatching time, hatchability and weight of the hatched chickens.

The results are based on the six consecutive experiments. They served as a basis for the calculation of the basic variation-statistical indicators. The differences between groups were tested by Student's *t*-test.

RESULTS AND DISCUSSION

The results of the experiment testing the effect of magnetic field applied during the egg storage time showed that hatchability was higher in the experimental groups (Table 1). The 56–60 g hatching eggs exposed to the magnetic field for 20 minutes during the storage time showed the highest hatchability (91.25 ± 4.21%). If compared with the respective control group (with the hatchability of 85.73 ± 2.11% only), the results were not statistically significant. In the group with the 61–65 g hatching eggs, the highest hatchability (92.41 ± 4.18%) was recorded for the eggs that were exposed to a tem-

porary magnetic field for 30 minutes during the storage. In the control group with no magnetic field exposure the hatchability was lowest – it reached only 83.21 ± 1.85% ($P \leq 0.05$). Similar results were reported by Ottová *et al.* (1981).

Evaluation of the effect of temporary magnetic field applied to the eggs during their incubation (Table 2) on the component traits (beginning of pecking, pecking and hatching times) did not reveal any greater differences between control and experimental groups. These results do not correspond with the findings of Veterány *et al.* (1998). In the experimental groups with chicken embryos exposed to a temporary magnetic field, a higher embryonic mortality was recorded, and consequently, also a decrease in hatchability (66.19 ± 4.51% or 67.58 ± 3.62%) was found. In comparison with the hatchability in control groups (89.68 ± 1.54% and 94.87 ± 5.83%, respectively), the differences were highly significant ($P \leq 0.001$). Pan (1996) also reported similar results. The application of an ultrasound during the incubation of chicken embryos also significantly decreased their hatchability (Veterány *et al.*, 2000). Moreover, we think that, among other things, the magnetic field slows down the blood flow. It was also confirmed by Holan *et al.* (1982). The slowing down of blood flow belongs to the rheological factors causing the origin of the thrombosis (Vašků *et al.*, 1984). According to Ganong (1993), the slowing down of the blood flow in the veins can cause the concentration of blood clots, which are not washed off fast enough and, consequently, may result in the formation of thrombosis. In our experiment we recorded an increase in the occurrence of thromboses in the heart of almost all sacrificed chickens. This could be related to the embolism of the great blood circulation (Boďa and Surynek *et al.*, 1990). Neher (1982) arrived at similar conclusions. The chickens hatched in the experimental groups had lower weight (34.58 ± 1.45 g and 36.81 ± 1.04 g, respectively) than the chickens from control groups without exposure to a temporary magnetic field during incubation (41.43 ± 0.92 g and 43.75 ± 1.50 g, respectively).

Table 1. Effect of magnetic field during storage of eggs on hatchability (means ± standard errors)

Parameters	1st control group C ₁	1st experimental group E ₁	2nd control group C ₂	2nd experimental group E ₂
Weight of eggs (g)	56–60	56–60	61–65	61–65
Total number of stored eggs	105	315	105	315
Average number of stored eggs per experiment	17.50 ± 3.15	17.50 ± 3.15	17.50 ± 3.15	17.50 ± 3.15
Hatchability without exposure to magnetic field (%)	85.73 ± 2.11	–	83.21 ± 1.85	–
Hatchability with magnetic field exposure lasting:				
20 min (%)	–	91.25 ± 4.21	–	C ₂ : E ₂ ⁺ 90.57 ± 3.14
30 min (%)	–	90.08 ± 3.52	–	C ₂ : E ₂ ⁺ 92.41 ± 4.18
40 min (%)	–	89.54 ± 5.06	–	87.81 ± 1.58

* $P \leq 0.05$

Table 2. Effect of magnetic field during incubation on hatching traits (means ± standard deviation)

Parameters	1st control group C ₁	1st experimental group E ₁	2nd control group C ₂	2nd experimental group E ₂
Weight of eggs (g)	56–60	56–60	61–65	61–65
Total number of incubated eggs	180	180	180	180
Average number of incubated eggs per experiment	30.00 ± 0.00	30.00 ± 0.00	30.00 ± 0.00	30.00 ± 0.00
Beginning of pecking (h)	493.41 ± 1.87	488.27 ± 2.18	490.94 ± 2.82	491.38 ± 3.15
Pecking time (h)	13.64 ± 2.25	13.68 ± 2.04	14.82 ± 1.52	15.04 ± 1.31
Hatching time (h)	507.05 ± 2.88	501.95 ± 2.58	505.76 ± 3.56	506.42 ± 1.47
Hatchability (%)	89.68 ± 1.54	C ₁ : E ₁ ⁺⁺⁺ 66.19 ± 4.51	94.87 ± 5.83	C ₂ : E ₂ ⁺⁺⁺ 67.58 ± 3.62
Body weight of chicks hatched (g)	41.43 ± 0.92	C ₁ : E ₁ ⁺ 34.58 ± 1.45	43.75 ± 1.50	C ₂ : E ₂ ⁺ 36.81 ± 1.04

*P ≤ 0.05, ***P ≤ 0.001

The differences are statistically significant ($P \leq 0.05$). Garcia-Peres *et al.* (1999) claim that magnetic field facilitates the transfer of the electric charge in the organism and thus influences the metabolism. Our conclusions correspond with the conclusions of Varga and Oblyvač (1979), who prove that the decrease in body weight is one of the effects on a live organism which have been thoroughly discussed.

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Estimation of genetic parameters for milk production traits in Czech dairy cattle populations

Odhad genetických parametrů pro znaky mléčné užitkovosti českých populací dojeného skotu

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ABSTRACT: Multiple-trait analyses treating the yields in the first three lactations as different traits were performed for milk, fat and protein yield for the populations of the Holstein and Czech Pied breeds kept in the Czech Republic. Data sets from the national milk recording data base were used. Five subsets of data were formed for each breed because the overall data sets were very large. The total number of animals with lactation records was 738 488 for Holstein and 1 016 388 for Czech Pied breed. Variance and covariance components were estimated with VCE using REML under an animal model. The model for the analyses included the factors herd-year-season (fixed), animal (random), calving age, days open and calving interval (covariables). The estimated heritabilities for milk yield were higher for Holstein (0.28 to 0.30) than for Czech Pied cattle (0.21 to 0.26). They were slightly higher than the heritabilities for fat and protein yield. A genetic correlation near one was found between the yields for the second and third lactations.

Keywords: cattle; milk production traits; genetic parameters; multi-trait animal model; REML

ABSTRAKT: Byly provedeny víceznakové analýzy (jako znaky vystupovaly užitkovosti v první, druhé a třetí laktaci) pro produkci mléka, tuku a bílkovin pro populaci holštýnského a českého strakatého skotu v České republice. K analýze byly využity údaje z národní databáze kontroly užitkovosti. Vzhledem k rozsáhlosti databáze bylo vytvořeno pět podsouborů pro každou populaci. Celkový počet zvířat s užitkovostí byl pro plemeno holštýnské 738 488 a pro plemeno české strakaté 1 016 388. K odhadu složek rozptylu byl použit program VCE a metoda REML pro víceznakový animal model. Použitý model zahrnoval pevný efekt stáda-roku-období, náhodný efekt jedince a jako regresní proměnné věk při otelení, servis periodu a mezidobí. Vyšší koeficienty dědivosti pro množství mléka byly nalezeny u holštýnské populace (0,28 až 0,30), nižší u českého strakatého skotu (0,21 až 0,26). Dědivosti zjištěné pro produkci tuku a bílkovin byly nižší. Pro všechny znaky byla odhadnuta genetická korelace blízka jedné mezi produkcí v druhé a třetí laktaci.

Klíčová slova: skot; mléčná užitkovost; genetické parametry; víceznakový animal model; REML

INTRODUCTION

Dairy cattle evaluation in the Czech Republic is carried out using Best Linear Unbiased Prediction (BLUP) and Animal Model (AM). Milk production traits are evaluated for the first three lactations using three-parity models for milk, fat and protein yields in the first three lactations. Until recently, only (co)variance estimates from small data sets have been available (Kučera, 1997; Čermák, personal communication).

Methods to determine genetic variances have been greatly improved over the last three decades. Maximum Likelihood based methods have been introduced (Patterson and Thompson, 1971) making use of mixed models (Henderson, 1984). The animal model makes it possible to use all available information. Restricted Maximum Likelihood (REML) estimates from the animal model are therefore expected to be unbiased by selection (Swalve and Van Vleck, 1987; Reents *et al.*, 1994).

For dairy as well as dual-purpose cattle breeds, (co)variance estimates from REML-AM analyses have been reported by several authors. Swalve and Van Vleck (1987) and Albuquerque *et al.* (1994) analysed the milk yield in the first three lactations. Van Vleck and Dong (1988), Dong *et al.* (1988), Reents *et al.* (1994) and Albuquerque *et al.* (1995) performed a multivariate analysis of milk, fat and protein yield in the first lactation. Visscher and Thompson (1992) reported results from univariate and multivariate analyses for milk production traits in the first three lactations.

The aim of the present study was to estimate genetic parameters for milk, fat and protein yields in the first three lactations for the dairy (Holstein) and dual-purpose (Czech Pied breed) cattle populations in the Czech Republic using large data sets from the national milk recording data base and REML for a multi-trait AM analysis.

MATERIAL AND METHODS

Milk performance data of Holstein (H) and Czech Pied (C) cattle were analysed. The data were recorded from

Table 1. Number of records in the first three lactations and number of animal effects in the model for milk yield (1st line), milk fat yield (2nd line) and milk protein yield (3rd line)

Data set	Number of animal effects	Number of records for lactation		
		1	2	3
H1	154 117	65 997	32 421	15 462
	154 117	65 997	32 421	15 462
	150 663	63 364	32 349	15 454
H2	111 638	50 572	27 743	15 152
	111 634	50 574	27 745	15 152
	111 242	50 031	27 699	15 145
H3	129 000	56 842	30 049	15 424
	129 000	56 842	30 049	15 424
	127 712	55 523	30 045	15 433
H4	211 786	94 945	49 319	24 729
	211 786	94 945	49 320	24 725
	211 525	94 679	49 273	24 716
H5	131 947	53 507	26 717	13 005
	131 947	53 507	26 717	13 004
	130 018	51 931	26 577	12 984
Holstein total	738 488	321 863	166 249	83 772
	738 484	321 865	166 252	83 767
	731 160	315 528	165 943	83 732
C1	124 188	47 247	27 885	19 257
	124 187	47 247	27 885	19 257
	122 344	45 589	27 831	19 158
C2	212 820	87 533	53 811	39 099
	212 789	87 533	53 732	39 067
	211 019	86 024	53 476	38 181
C3	213 005	87 564	55 762	41 236
	213 005	87 564	55 762	41 236
	209 780	85 550	55 545	40 098
C4	261 920	111 837	68 481	49 013
	261 922	111 836	68 481	49 013
	261 822	111 753	68 359	48 922
C5	204 455	80 434	51 468	38 223
	204 454	80 433	51 468	38 223
	202 342	79 275	51 359	37 580
Czech Pied cattle total	1 016 388	414 615	257 407	186 828
	1 016 357	414 613	257 388	186 796
	1 007 307	408 191	256 570	183 939

H1–H5 = data sets for Holstein cattle

C1–C5 = data sets for Czech Pied cattle

1991 to 1999. The pedigree was traced back to cows born in 1984; all available information was used for sires. Traits analysed were 305-day lactation milk, fat and protein yields.

Five subsets of data (H1 to H5 for Holstein, C1 to C5 for Czech Pied cattle) were formed for each breed because the complete data sets were too large for (co)variance component estimation. The subdivision was carried out on a territorial principle. The subsets covered more than 75% of the total amount of data. The numbers of observations of the individual data sets and the numbers summed

up within the breeds are given in Table 1. The phenotypic means and standard deviations for the three milk production traits included in the analyses are presented in Table 2. Higher mean values were usually accompanied by higher standard deviations. The milk yield of the Holstein breed was about 1 000 kg higher than that of the Czech Pied cattle.

Altogether, 30 three-parity models (3 traits \times 10 data sets) were calculated, considering the same trait in another lactation as a different trait. The model equations for

Table 2. Phenotypic means and standard deviations for milk yield in kg (1st line), milk fat yield in kg (2nd line) and milk protein yield in kg (3rd line) in the first three lactations

Data set	Mean \pm standard deviation for lactation		
	1	2	3
H1	4 728 \pm 1 376 200.8 \pm 60.3 155.5 \pm 46.6	5 460 \pm 1 707 232.3 \pm 74.3 179.7 \pm 57.2	5 821 \pm 1 807 248.4 \pm 78.0 191.0 \pm 60.8
H2	4 451 \pm 1 253 187.2 \pm 56.1 143.2 \pm 42.4	5 125 \pm 1 507 217.4 \pm 68.5 166.8 \pm 50.6	5 450 \pm 1 506 232.3 \pm 68.9 176.8 \pm 50.4
H3	5 027 \pm 1 271 211.3 \pm 54.8 163.6 \pm 42.2	5 740 \pm 1 542 242.2 \pm 67.1 187.6 \pm 50.7	6 099 \pm 1 584 257.9 \pm 68.9 198.4 \pm 52.3
H4	4 953 \pm 1 351 207.2 \pm 57.6 160.0 \pm 46.0	5 611 \pm 1 578 234.8 \pm 68.1 182.9 \pm 53.3	5 974 \pm 1 598 251.8 \pm 68.3 193.5 \pm 52.9
H5	4 826 \pm 1 357 204.3 \pm 58.5 158.0 \pm 46.5	5 521 \pm 1 563 234.1 \pm 67.7 182.0 \pm 52.6	5 868 \pm 1 580 249.9 \pm 69.0 191.9 \pm 53.2
Holstein weighted means	4 820 \pm 1 328 203.0 \pm 57.6 156.7 \pm 45.0	5 509 \pm 1 583 232.7 \pm 69.2 180.3 \pm 53.1	5 858 \pm 1 618 248.5 \pm 70.5 190.7 \pm 54.0
C1	3 822 \pm 937 169.7 \pm 48.8 129.7 \pm 32.6	4 387 \pm 1 102 197.4 \pm 54.0 149.0 \pm 38.5	4 545 \pm 1 135 203.9 \pm 56.4 153.3 \pm 39.4
C2	3 706 \pm 866 159.3 \pm 41.0 123.8 \pm 30.1	4 256 \pm 1 019 184.2 \pm 48.9 143.3 \pm 36.0	4 439 \pm 1 047 192.0 \pm 50.3 149.0 \pm 36.8
C3	3 994 \pm 877 172.3 \pm 41.5 134.4 \pm 30.2	4 539 \pm 1 055 196.6 \pm 48.9 153.7 \pm 36.3	4 746 \pm 1 079 205.5 \pm 50.4 159.9 \pm 37.0
C4	4 105 \pm 963 172.1 \pm 43.7 137.4 \pm 33.8	4 724 \pm 1 147 202.9 \pm 52.1 159.5 \pm 40.3	4 933 \pm 1 166 211.2 \pm 53.2 165.4 \pm 40.6
C5	4 092 \pm 946 176.2 \pm 43.5 137.6 \pm 32.5	4 681 \pm 1 129 201.7 \pm 51.7 157.9 \pm 38.9	4 885 \pm 1 158 209.4 \pm 53.1 163.8 \pm 39.6
Czech Pied weighted means	3 963 \pm 919 170.0 \pm 43.3 133.1 \pm 31.9	4 541 \pm 1 093 196.8 \pm 50.9 153.4 \pm 38.1	4 739 \pm 1 118 204.8 \pm 52.4 159.2 \pm 38.7

H1–H5 = data sets for Holstein cattle

C1–C5 = data sets for Czech Pied cattle

the milk (fat, protein) yield (y_{ijk}) of animal j in lactation k and herd-year-season class i was:

$$y_{ijk} = HYS_{ik} + \text{animal}_{jk} + b_{1k}AC + b_{2k}AC^2 + b_{3k}DO + b_{4k}DO^2 + b_{5k}CI + b_{6k}CI^2 + e_{ijk} \quad (1)$$

where: HYS_{ik} – the fixed effect of the i^{th} herd-year-season of the k^{th} lactation
 animal_{jk} – the random effect of the j^{th} animal in the k^{th} lactation
 b_{1k} to b_{6k} – regression coefficients for the k^{th} lactation
 AC – the age at calving
 DO – days open
 CI – the calving interval
 e_{ijk} – the random residual effect for the k^{th} lactation

The regression on the calving interval and the calving interval squared is omitted in 1st lactation. Three seasons were defined (February to April, May to September, October to January).

Model (1) including the definition of the seasons is essentially the same as the model used for routine genetic evaluation of dairy cattle in the Czech Republic. But in the routine genetic evaluation pre-corrections are carried out before running the animal and the animal model does not contain any regression terms.

Model (1) can be rewritten in matrix terms as follows:

$$y = Xb + Za + e$$

where: y – the vector of observations ordered by traits within animals

X – the known incidence matrix for fixed effects

b – the unknown vector of fixed effects

Z – the known incidence matrix for animal effects

a – the unknown vector of animal effects and e is the vector of residual effects

The joint distribution of y , a and e is assumed to be multivariate normal with expectation and variance:

$$E \begin{bmatrix} y \\ a \\ e \end{bmatrix} = \begin{bmatrix} Xb \\ 0 \\ 0 \end{bmatrix}, \quad \text{var} \begin{bmatrix} y \\ a \\ e \end{bmatrix} = \begin{bmatrix} V & ZG_A & R \\ G_A Z' & G_A & 0 \\ R & 0 & R \end{bmatrix}$$

with

$$V = R + ZG_A Z', \quad G_A = A \otimes G_{A0}, \quad R = \bigoplus_n R_{0n}$$

where: V, G_A, R – the covariance matrices for y, a and e , resp.

A – the numerator relationship matrix between animals

G_{A0} – the 3×3 genetic covariance matrix between traits

R_{0n} – the residual covariance matrices between traits

The concrete form of R_{0n} depends on the fact if and which observations (lactations) are missing for the given animal.

The estimation method was REML (Patterson and Thompson, 1971) using a quasi Newton algorithm with

analytical gradients, described by Neumaier and Groeneveld (1998). The software used was VCE 4.2 (Groeneveld and Garcia Cortés, 1998) running on a LINUX operating system. The overall means of the (co)variance components were calculated by weighting the estimates of the individual data sets by the numbers of observations.

RESULTS

Similar results were achieved for all data sets within a breed. Therefore, the results for the individual data sets will be presented in tables only and will not be commented in further detail. The presentation of the results will be focused on the average values of estimates for (co)variances, heritabilities and genetic correlations for the two breeds. The estimates of genetic and residual variances are given in Table 3. Several similar trends were found in both breeds. For all analysed traits, the value of the genetic as well as the residual variances increased with parity. The residual variances were higher than the genetic variances. For the genetic as well as residual variances, a higher relative increase occurred from 1st to 2nd lactation than from 2nd to 3rd lactation. This trend was more clearly expressed in the residual variances than in the genetic variances.

The highest genetic as well as residual covariances were estimated between the 2nd and 3rd lactations (Table 4). Within the analysed cattle populations, the genetic covariances between the 1st and 2nd and between the 1st and 3rd lactations achieved similar values. But a different behaviour of the residual and genetic covariances was observed. In both breeds, the residual covariances between the 1st and the 2nd lactations tended to be higher than the residual covariances between the 1st and the 3rd lactations, while the genetic covariances between the 1st and the 2nd lactations inclined to be lower than those between the 1st and the 3rd lactations.

Concerning the differences between the two breeds, the values of genetic variances and covariances calculated for Czech Pied cattle were about one half of those calculated for Holstein breed or even less (Tables 3 and 4). Similarly, the residual variances and covariances for Czech Pied cattle were generally lower than these estimates for Holstein cattle. Nevertheless, the differences in the residual variances between the analysed populations were not so striking as the differences in the genetic variances.

Furthermore, differences between the two breeds were found in the way of relative increase of genetic variances with parity (Table 3). In Holstein, the relative increase of the genetic variances with parity was almost the same as that of the residual variances, i.e. higher increase from 1st to 2nd lactation than from 2nd to 3rd lactation. On the contrary, in Czech Pied cattle the genetic variances showed nearly the same relative increase from 1st to 2nd as from 2nd to 3rd lactations. This relative increase was lower

Table 3. Genetic and residual variances for milk yield (1st line), milk fat yield (2nd line) and milk protein yield (3rd line) in the first three lactations. All numbers are in kg

Data set	Genetic variance for lactation			Residual variance for lactation		
	1	2	3	1	2	3
H1	185 211 267.9 155.1	300 937 440.5 277.3	391 033 614.2 372.0	503 342 1034.3 535.6	730 687 1521.4 771.4	866 764 1843.9 921.2
H2	155 247 234.9 137.4	217 238 406.0 204.2	249 098 426.8 228.2	385 809 785.0 385.3	548 091 1 125.8 560.0	651 128 1 405.4 673.1
H3	232 260 348.6 189.7	292 620 500.9 269.4	387 029 552.3 346.1	472 910 915.7 476.0	724 461 1 444.4 731.8	827 125 1 789.5 868.1
H4	217 587 283.5 158.4	287 989 432.6 238.3	333 625 596.2 290.2	465 078 888.2 477.2	692 399 1352.8 716.5	820 658 1612.1 849.3
H5	212 843 328.5 156.7	262 187 477.0 225.2	351 055 552.8 306.5	507 657 953.7 534.2	759 439 1467.3 789.5	877 843 1792.2 920.9
Holstein weighted means	202 956 291.6 159.6	275 398 449.2 243.7	341 471 554.1 306.9	468 931 917.7 483.5	692 353 1382.8 715.5	808 573 1678.1 845.3
C1	106 954 189.8 87.5	121 933 227.3 109.4	133 680 277.7 119.6	316 632 771.0 349.0	447 162 1142.5 500.5	514 610 1294.7 568.1
C2	89 962 147.2 67.8	87 023 152.6 74.7	110 272 210.3 107.1	287 524 633.7 314.9	393 937 889.6 436.5	431 548 979.7 476.6
C3	102 580 174.8 78.1	120 937 221.7 110.1	135 498 273.8 129.3	309 800 679.6 332.2	442 091 1000.0 481.9	484 697 1125.9 533.9
C4	118 597 197.1 93.6	134 098 244.2 123.0	175 043 308.5 163.1	325 982 671.8 350.3	471 004 986.3 518.4	514 662 1101.6 566.4
C5	123 059 222.7 99.3	136 942 273.2 133.2	159 527 313.2 155.0	335 780 704.5 354.3	477 191 1038.9 513.4	537 569 1186.1 583.2
Czech Pied weighted means	108 708 186.0 85.3	120 657 224.2 110.7	145 322 278.1 137.9	315 281 683.1 339.7	447 284 996.5 490.5	495 336 1118.7 544.3

H1–H5 = data sets for Holstein cattle

C1–C5 = data sets for Czech Pied cattle

than in Holstein. Concerning the estimates of covariance (Table 4), in Holstein the genetic covariances were generally larger than the residual ones, whereas in Czech Pied cattle the situation was *vice versa*.

Table 5 presents the calculated heritabilities and genetic correlations. The approximate standard errors of the heritabilities were in the range from 0.006 to 0.017 for milk yield, from 0.005 to 0.016 for fat yield and from 0.006 to 0.016 for protein yield. The approximate standard errors of genetic correlations ranged from 0.001 to 0.025 for milk

yield, from 0.006 to 0.024 for fat yield and from <0.001 to 0.032 for protein yield.

The heritabilities were found higher for Holstein than for Czech Pied breed. In Holstein as well as in Czech Pied cattle, the highest heritabilities were obtained for the milk yield. The heritabilities in Holstein were around 0.25 for fat and protein yields and near 0.30 for milk yield, while in Czech Pied cattle the heritabilities reached values very close to 0.20 for fat and protein yield and between 0.18 and 0.27 for milk yield.

Table 4. Genetic and residual covariances for milk yield (1st line), milk fat yield (2nd line) and milk protein yield (3rd line) in the first three lactations. All numbers are in kg

Data set	Genetic covariance between lactations			Residual variance between lactations		
	land 2	1 and 3	2 and 3	land 2	1 and 3	2 and 3
H1	209 818	246 709	341 448	197 876	158 339	281 724
	305.4	377.2	508.6	365.3	283.2	539.0
	184.9	218.2	319.5	212.2	157.7	309.7
H2	168 263	176 717	231 212	125 208	123 735	192 339
	282.2	293.1	415.5	235.5	222.0	374.9
	152.3	155.9	214.4	128.0	118.6	208.3
H3	240 385	275 981	335 672	203 069	172 488	279 144
	389.5	404.1	522.5	350.2	331.6	552.7
	211.1	236.0	303.1	199.9	169.3	304.4
H4	227 026	238 388	300 261	181 400	180 263	261 313
	321.6	367.5	499.9	334.7	310.7	486.0
	178.1	184.5	258.7	273.2	179.5	289.4
H5	223 824	260 394	303 344	205 520	201 541	309 447
	375.9	392.9	511.9	349.9	361.6	568.3
	176.3	205.0	262.7	224.7	198.2	344.2
Holstein weighted means	216 091	240 051	302 372	183 786	168 569	263 360
	333.1	367.7	492.3	330.7	302.5	500.7
	180.9	198.9	270.7	192.8	166.2	289.7
C1	109 319	110 142	120 564	131 569	126 103	194 403
	198.0	207.6	236.5	294.1	261.1	451.1
	94.2	93.0	107.6	146.0	126.3	217.1
C2	80 263	86 363	97 589	121 454	116 385	165 572
	133.0	150.9	178.7	247.9	227.8	330.3
	63.5	72.1	89.0	138.3	120.1	187.3
C3	102 948	105 797	126 931	139 459	128 378	193 302
	178.6	196.0	243.1	285.4	256.7	412.3
	84.0	87.1	118.0	152.6	135.3	218.8
C4	117 362	128 177	150 786	148 100	128 291	208 727
	201.0	215.1	268.0	273.2	229.1	392.2
	99.2	107.0	138.7	158.8	126.9	233.2
C5	119 914	121 900	146 241	158 001	140 887	222 280
	224.9	225.6	289.7	305.3	268.4	434.0
	105.1	103.9	141.3	166.2	138.8	243.2
Czech Pied weighted means	106 064	111 379	130 342	140 687	128 102	197 587
	186.2	198.8	245.0	279.0	246.1	398.3
	89.1	93.3	121.2	153.2	129.6	220.9

H1–H5 = data sets for Holstein cattle

C1–C5 = data sets for Czech Pied cattle

The estimates of genetic correlations between parities showed very similar values for milk, fat or protein yields. The correlations between 1st and 2nd and between 1st and 3rd lactations were close to a value of 0.90. The correlations between the 1st and 3rd lactations seemed to be somewhat lower than the correlations between the 1st and the 2nd lactations, especially in the Czech Pied breed. A correlation of unity or nearly unity was found between the 2nd and 3rd lactations.

DISCUSSION

The estimated heritabilities were in good agreement with the results from the literature. For Holstein, Van Vleck and Dong (1988) using REML methods with an animal model reported heritabilities of 0.36, 0.35 and 0.33 for milk, fat and protein yield in the 1st lactation. Similarly, Albuquerque *et al.* (1994) found heritability estimates of 0.34, 0.31, 0.27 for milk yield, 0.35, 0.33, 0.31 for fat yield and 0.40,

Table 5. Heritabilities and genetic correlations for milk yield (1st line), milk fat yield (2nd line) and milk protein yield (3rd line) in the first three lactations

Data set	Heritability for lactation			Genetic correlation between lactation		
	1	2	3	1 and 2	1 and 3	2 and 3
H1	0.27	0.29	0.31	0.89	0.92	1.00
	0.21	0.23	0.25	0.89	0.93	0.98
	0.23	0.26	0.29	0.89	0.91	1.00
H2	0.29	0.29	0.28	0.92	0.90	0.99
	0.23	0.27	0.23	0.91	0.93	1.00
	0.26	0.27	0.25	0.91	0.88	0.99
H3	0.33	0.29	0.32	0.92	0.92	1.00
	0.28	0.26	0.24	0.93	0.92	0.99
	0.29	0.27	0.29	0.93	0.92	0.99
H4	0.32	0.29	0.29	0.92	0.86	0.98
	0.24	0.24	0.27	0.92	0.89	0.98
	0.25	0.25	0.26	0.92	0.86	0.98
H5	0.30	0.26	0.29	0.95	0.95	1.00
	0.26	0.25	0.24	0.95	0.92	1.00
	0.23	0.22	0.25	0.94	0.94	1.00
Holstein mean values ¹	0.30	0.28	0.30	0.91	0.91	0.99
	0.24	0.25	0.25	0.92	0.91	0.99
	0.25	0.25	0.27	0.92	0.90	0.99
C1	0.25	0.21	0.21	0.96	0.92	0.94
	0.20	0.17	0.18	0.95	0.90	0.94
	0.20	0.18	0.17	0.96	0.91	0.94
C2	0.24	0.18	0.20	0.91	0.87	1.00
	0.19	0.15	0.18	0.89	0.86	1.00
	0.18	0.15	0.18	0.89	0.85	1.00
C3	0.25	0.22	0.22	0.92	0.90	0.99
	0.21	0.18	0.20	0.91	0.90	0.99
	0.19	0.19	0.20	0.91	0.87	0.99
C4	0.27	0.22	0.25	0.93	0.89	0.98
	0.23	0.20	0.22	0.92	0.87	0.98
	0.21	0.19	0.22	0.93	0.87	0.98
C5	0.27	0.22	0.23	0.92	0.87	0.99
	0.24	0.20	0.21	0.91	0.85	0.99
	0.22	0.21	0.21	0.91	0.84	0.98
Czech Pied mean values ¹	0.26	0.21	0.23	0.93	0.89	0.98
	0.21	0.18	0.20	0.91	0.87	0.98
	0.20	0.18	0.20	0.92	0.86	0.98

¹calculated from the weighted means of the variance and covariance components

H1–H5 = data sets for Holstein cattle

C1–C5 = data sets for Czech Pied cattle

0.39, 0.37 for protein yield in 1st, 2nd and 3rd lactations, respectively.

The REML sire model gave usually lower estimates (Dong *et al.*, 1988) than the animal model, as only a part of relationships is included in the sire model. Teepker and Swalve (1988) found heritabilities of 0.29, 0.23, 0.25 for milk yield, 0.27, 0.22, 0.23 for fat yield and 0.21, 0.17, 0.19 for protein yield in 1st, 2nd and 3rd lactations, respectively. They stressed that the magnitude of the estimates depended on the estimation method and data selection.

Meyer (1984) reported heritabilities of 0.34, 0.35, 0.28 for milk yield, 0.32, 0.33, 0.23 for fat yield and 0.24, 0.29, 0.12 for protein yield in 1st, 2nd and 3rd lactations, respectively.

In Fleckvieh, Alps (1971) estimated the following heritabilities without taking into account the relationship matrix: 0.25, 0.20, 0.19 for milk yield and 0.21–0.24, 0.16–0.19, 0.15–0.18 for fat yield in 1st, 2nd and 3rd lactations, respectively. Later estimates from Karras and Schlote (1982) and Alps *et al.* (1984) are similar. Using a simple ANOVA techniques without taking into account the

relationship matrix, Kučera (1997) reported relatively high values of heritabilities (0.32 to 0.36) for milk yield traits for Czech Pied cattle.

In Montbéliarde cattle, Beaumont (1989) reported heritability estimates of 0.27, 0.24, 0.27 for milk yield, 0.26, 0.20, 0.24 for fat yield and 0.18, 0.18, 0.22 for protein yield in the 1st, 2nd and 3rd lactations, respectively, from a REML sire model. Sölkner (1989) using the same techniques found heritabilities of 0.31, 0.30, 0.25 for milk yield, 0.30, 0.27, 0.22 for fat yield and 0.26, 0.26, 0.22 for protein yield in 1st, 2nd and 3rd lactations, respectively.

Comparable heritability estimates were published for other cattle breeds. In Swedish Red and White, Strandberg and Danell (1989) received the following REML sire model estimates of heritability: 0.29, 0.27, 0.24 for milk yield and 0.24, 0.20, 0.21 for fat yield in the 1st, 2nd and 3rd lactation, respectively. For Dutch Red and White, Van Veldhuizen *et al.* (1991) estimated heritabilities of 0.31, 0.37, 0.34 for milk, fat and protein yield, respectively, in 1st lactation. Low values (0.23, 0.19, 0.16 for the 1st lactation milk, fat and protein yields, respectively) were found by Liinamo *et al.* (1999) for Finnish Ayrshire. Using Henderson's method III, Soliman *et al.* (1990) reported high values of heritabilities (0.40, 0.39, 0.41) for the 1st lactation milk, fat and protein yields, respectively, for Pinzgauer cattle in Austria.

The finding that the heritability of milk yield is lower in 2nd lactation than in 1st and 3rd lactations was confirmed by Meyer (1984), Simianer (1986), Strandberg and Danell (1989), Swalve and Van Vleck (1987), Teepker and Swalve (1988) and Beaumont (1989). The authors mentioned above explained this fact mainly by the different behaviour of the residual and the genetic variances. Whereas a large increase in the residual variance in the 2nd lactation compared with the 1st lactation was observed, the appropriate increase in the genetic variance was considerably smaller. This applies to the present results as well. Other studies reported a continuous decrease in heritabilities with parity (Alps, 1971; Romberg *et al.*, 1983; Reents *et al.*, 1998). Albuquerque *et al.* (1994) found the largest estimate of genetic variance in the 2nd lactation for all analysed production traits. The estimates of residual variances increased with parity. The heritability estimates decreased with the order of lactation mainly as a result of increased environmental variances. Beaumont (1989) reported that the genetic variances remained nearly constant from the 1st to 2nd lactation and the genetic variance of the 3rd lactation was the highest. He concluded that the phenotypic variances increased with lactation number probably as a result of the scale effect.

A lower heritability in the 2nd lactation than in the 1st and 3rd lactations for fat yield was found by Meyer (1984), Strandberg and Danell (1989) and Beaumont (1989). On the contrary, Romberg *et al.* (1983) and Teepker and Swalve (1988) reported fat heritabilities for 2nd and 3rd lactations to be of the same magnitude or lower than those for 1st

lactation. The latter is in agreement with our findings for fat and protein yield in Holstein and the former agrees with the present results for Czech Pied cattle.

Beaumont (1989) suggested that the decrease in the heritability in the 2nd lactation observed by most authors might be due to selection bias. Because of the selection at the end of 1st lactation, methods as Henderson III underestimate the heritabilities for later lactations. The methods based on an animal model may better take into account selection since all relationships can be included in the model and thus no decrease in the heritability for the 2nd lactation is observed (Swalve and Van Vleck, 1987). According to Beaumont (1989), another reason may be, at least partly, due to sampling errors. The genetic determinism of 2nd lactation may also be slightly different because the 2nd lactation performance depends both on the dairy value of the animal and on its ability to recover from both growth and 1st lactation.

In the literature in agreement with the present findings, slightly lower estimates were reported for heritabilities of fat and protein yield compared with heritabilities of milk yield (Romberg *et al.*, 1983; Boichard and Bonafati, 1987; Jakobsen *et al.*, 2000). Hill *et al.* (1983) found that protein yield has a slightly lower heritability than fat yield. This was explained by the lower genetic correlation between milk yield and protein content that is more negative than that between milk yield and fat content.

The genetic correlations between milk yield of the 1st, 2nd and 3rd lactations estimated in the present study were almost identical to those published by Beaumont (1989) and Reents *et al.* (1998). They were slightly higher than the estimates reported by Romberg *et al.* (1983), Swalve and Van Vleck (1987), Teepker and Swalve (1988), Strandberg and Danell (1989), Visscher and Thompson (1992) and Albuquerque *et al.* (1994). The general conclusion from these cited papers that the lowest genetic correlation is between the 1st and 3rd lactations and the highest correlation between the 2nd and 3rd lactations is in good agreement with the results of the present study.

Strandberg and Danell (1989) and Teepker and Swalve (1988) concluded that the very high genetic correlation between the 2nd and 3rd lactations confirms the hypothesis that 2nd and 3rd lactation yields can be considered as the same trait.

Visscher and Thompson (1992) and Albuquerque *et al.* (1994) estimated higher (co)variance components and heritabilities than were estimated in the present paper for the same (Holstein) breed. These differences might be explained at least partly by a scale effect associated with the larger means in the herds investigated by these authors. The relations of the (co)variance components between the lactations were very similar in the paper of Visscher and Thompson (1992) and in the present study.

A scale effect in the estimation of the heritability was observed by Hill *et al.* (1983), De Veer and Van Vleck (1987), Van Vleck *et al.* (1988) and Carabaño *et al.* (1989). There

may be several reasons for the scale effect. The cow may be allowed to "express their potential" at the higher level, the pedigree records may be better, furthermore lower disease incidence and more individual feeding are expected in high production herds.

When comparing the estimates of heritability based on the 305-day production and those based directly on the test-day production, some studies found heritabilities for test-day yield to be lower than those for the complete lactation yield (Danell, 1982; Gengler *et al.*, 1999).

CONCLUSIONS

The (co)variance matrices estimated in the presented study are in good agreement with literature data. They should be used in the national genetic evaluation of the Holstein and Czech Pied cattle in the Czech Republic.

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Weight coefficients of lactations in animal model

Váhové koeficienty laktací v animal modelu

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ABSTRACT: Dairy cattle are selected for an average of lifetime milk production. Breeding value is estimated using MT-AM <multi-trait animal model>, (each lactation as separate trait). For each order of lactation discounted interest values are calculated, dependent on age structure and calving interval. The first lactation out of 10 lactations has a relative importance of the whole number of lactations. In the case of a 0% interest rate it is 0.34, with a 5% interest rate it is 0.37 and with a 10% interest rate it is 0.40. The average lifetime production is predicted on the basis of the early lactations. If the interest rate is 5%, the breeding value for the first lactation will predict the average lifetime production with reliability over 0.90, breeding values for the first two lactations with reliability over 0.98 and for the first three lactations with reliability over 0.99. The age structure does not have a great influence on the reliability of prediction. Optimal weight in the index for partial breeding values, in case of the first lactation only, is approximately 1. In case of the first two lactations approximately 0.34 and 0.63, in case of the first three lactations 0.31, 0.11 and 0.52. If all the three lactations in the index have the same weight, i.e. 0.33, 0.33 and 0.33, the lifetime average production is predicted practically with equal reliability as in the optimal index. It is caused by high genetic correlations between lactations.

Keywords: dairy cattle; milk production; breeding value; animal model; selection index; weights of lactations

ABSTRAKT: Skot je šlechtěn na průměr celoživotní mléčné užitkovosti. Plemenná hodnota je odhadována pomocí MT-AM (jednotlivé laktace jako odlišné vlastnosti). Pro jednotlivá pořadí laktací jsou stanoveny odúrokované hodnoty, které závisí na věkové struktuře a délce meziodobí. Při úroku 0 % mají první laktace relativní důležitost z celkového počtu 10 laktací 0,34, při úroku 5 % 0,37 a při úroku 10 % 0,40. Průměrná celoživotní užitkovost je předpovídána na základě prvních laktací. Za podmínky úroku 5 %, plemenná hodnota pro první laktaci předpovídá celoživotní užitkovost se spolehlivostí nad 0,90, plemenné hodnoty pro první dvě laktace se spolehlivostí nad 0,98 a plemenné hodnoty pro první tři laktace se spolehlivostí nad 0,99. Věková struktura má malý vliv na spolehlivost předpovědi. Optimální váha v indexu pro díleč plemenné hodnoty je v případě pouze první laktace přibližně 1, v případě prvních dvou laktací přibližně 0,34 a 0,63, v případě prvních třech laktací 0,31, 0,11 a 0,52. Je-li v případě třech laktací dána v indexu všem laktacím stejná váha, t.j. 0,33, 0,33 a 0,33, je průměrná celoživotní užitkovost předpovídána prakticky se stejnou spolehlivostí jako v optimálním případě. Je to dáno vysokými genetickými korelacemi mezi laktacemi.

Klíčová slova: skot; mléčná užitkovost; plemenná hodnota; animal model; selekční index; váhy laktací

INTRODUCTION

The estimation of breeding value is presently carried out frequently by means of repeatability animal models, when the milk production in different lactations is considered as a repetition of the same trait. Repeatability and multi-lactation (multi-trait) animal models for breeding value for milk production estimation have some presumptions (Henderson, 1987). The first type of model is useful if genetic correlation between all pairs of lactation is 1,

phenotype correlation between all pairs of lactation is constant and variability of all lactations is constant. Milk production fulfils none of these presumptions. Calculation is more complicated in the second type of model, and fewer contemporaries for each parity in a herd are available which reduces reliability. Both methods were compared on simulated data by Přibyl and Přibyl (1992). Multi-lactation model is theoretically better, but repeatability model allows a higher number of contemporaries within herd. Differences of breeding value estimations

according to both models depend on differences in real genetic parameters.

Strabel and Misztal (1999) analysed heritability and genetic correlation during the first and second lactations. Based on test days they found higher heritability during the second lactation. Genetic correlation between the same stage of the neighbouring lactations varies from 0.52 to 0.90 according to test day, with maximum in the middle of lactation. Strandén *et al.* (2000) in comparison test days in the first, and average of later lactations found the genetic correlations only 0.66 to 0.79. Dědková and Wolf (2001) found high genetic correlation between the second and third lactation, but the first lactation to the second and third lactation is less correlated, for different milk traits practically did not exceed 0.90.

As the actual genetic correlation between lactations does not equal 1 and especially relation of the first lactation to the others differs, in a number of countries therefore a theoretically better “multi-lactation” model is used. In this model the single lactations are considered as different traits (Interbull Bulletin No.24, 2000). In this case the selection index is used and partial breeding values for single lactations are given weights according to their importance, which fluctuates between countries. Breeding value estimation according to the first lactation only, or first three lactations was compared by Jagusiak and Żarnecki (1998). Correlation between both evaluations was high, when looking at top animals, only 48 to 79 % of the best animals were found in both ranks, depending on the intensity of selection. In connection with international comparison (Interbull), Canavesi *et al.* (1999) preferred to use the first three lactations for breeding value estimation rather than all the lactations.

The aim of selection is the maximum possible production on average for lactation during the lifetime of the cow. Single lactation manifestations of production do not occur with the same frequency. Each consecutive lactation is delayed by a calving interval compared with the previous lactation. McClintock and Cunningham (1974) introduced “discounted expression per cow” which takes into consideration both number of realisation units and discounting of values in time and allows for a more objective comparison. In those years several authors came with the discounting methodology in application to animal breeding (Brascamp, 1975; Hill, 1974; Niebel, 1974; Petersen *et al.*, 1974), which results in the G-flow method for simulation of animal breeding procedures (Brascamp, 1978). Newly this methodology used Filistowicz *et al.* (1996). For estimation of the breeding value, the first few lactations are decisive, on which bases the lifetime production is predicted. The weights of lactations in the reduced index based on the first three lactations were introduced by Dempfle and Ponzoni (1986). They took into consideration several variants of the age structure of the herd, discounting and genetic correlation between lactations. The weight of the first lactation was practically

twice as much as that of second one. The weight of the third lactation reached, depending on the entry parameters, 70% to 361% of the first lactation weight. They do not recommend to use constant weights for all lactations, but to determine weight coefficients for every case separately.

The principle of using the BLUP method in prediction of the breeding value based on the correlated traits for the traits which are not directly observed, was generalised by Schneeberger *et al.* (1992). The weights of observed traits in the index (combination of breeding values from a multidimensional evaluation) are determined on the basis of the economic weights and genetic covariance matrices.

Together with the development of the population, also genetic parameters have been changed which influences the methods of the breeding value estimation, construction of selection indices and a consequent strategy of selection. Genetic parameters of the milk production for national dairy cattle populations have been newly set by Dědková and Wolf (2001).

The aim of the present work is to determine the optimum weight coefficients of breeding values in the first three lactations for selection on the average lifetime milk production, based on age structure and genetic parameters in local populations of dairy cattle.

MATERIAL AND METHODS

Databases of milk recording of the Czech Pied and Holstein cattle for the 1980–1997 period have been used for determination of the age structure of cows (PL). Discounted methodology is adapted from Brascamp (1978). Based on the length of calving interval equal to 398 days (the present average value) and 365 days (ideal length) and the interest rates of 0%, 5% and 10%, there have been determined discounted values (OH) of single orders of lactations.

$$OH_i = PL_i / (1 + f)^{(i \cdot m - m)} \quad (1)$$

where: OH_i – discounted value of lactation i
 PL_i – proportion of cows in lactation i
 f – interest rate
 i – lactation order 1 to 10
 m – duration of calving interval

The economic value of lactation was set as a proportion of the discounted value to the total sum of discounted values.

$$EH_i = OH_i / \sum OH_i \quad (2)$$

Based on the standard methodology of construction of selection indices and consequently, by using of the economic value and genetic covariance matrices, there have been determined the weight coefficients for breeding values of the first lactations, determined by means of MT-

AM, with the aim to select on the average of lifetime milk production.

$$G_I b = G_Y EH \quad (3)$$

where: G_I – genetic covariance matrix of lactation in the index (multi-lactation AM)

b – vector of weights in the index

G_Y – genetic covariance matrix of lactation in the index to all lactations

EH – vector of economic values

Reliability of prediction of the average lifetime milk production on the basis of partial index is determined according to:

$$r^2 = (b' G_Y EH)^2 / ((b' G_I b) (EH' G_C EH)) \quad (4)$$

where: G_C – genetic covariance matrix of all lactations

Genetic parameters for the first three lactations have been taken over from Dědková and Wolf (2001). The others are, based on variability in population, modelled in two variants (Table 1). In the first variant, genetic correlations decrease with the lactation distance. In the second one, genetic correlations between the second and further lactations are constant with the exception of neighbouring two lactations. The second variant has on average higher genetic correlations between lactations.

RESULTS

Age

Age structure was determined on the basis of 9.8 million of Czech Pied and 5.1 million of Holstein lactations. The data set consists of cows calved between 1980 and

Table 1. Genetic parameters – genetic correlations between lactations in % (above the diagonal variant 1), below the diagonal variant 2) and genetic standard deviation (Czech Pied – C, Holstein – H)

Lactation	1	2	3	4	5	6	7	8	9	10	$\sigma_g C$	$\sigma_g H$
1		91	90	85	80	75	70	65	60	55	324	485
2	91		98	95	90	85	80	75	70	65	372	589
3	90	98		98	95	90	85	80	75	70	387	586
4	90	95	98		98	95	90	85	80	75	388	544
5	90	95	95	98		98	95	90	85	80	386	526
6	90	95	95	95	98		98	95	90	85	385	495
7	90	95	95	95	95	98		98	95	90	377	487
8	90	95	95	95	95	95	98		98	95	373	468
9	90	95	95	95	95	95	95	98		98	361	450
10	90	95	95	95	95	95	95	95	98		361	446

Table 2. Age structure of dairy cows in %

Lactation order	Calving period 1980–1997				Calving period 1996–1997			
	Czech Pied		Holstein		Czech Pied		Holstein	
	%	cumulative frequency	%	cumulative frequency	%	cumulative frequency	%	cumulative frequency
1	33.9	33.9	33.2	33.2	29.0	29.0	34.3	34.3
2	21.3	55.2	22.1	55.3	22.3	51.3	24.0	58.3
3	15.5	70.7	16.0	71.3	16.0	67.3	15.6	73.9
4	11.0	81.7	11.2	82.5	11.8	79.1	11.0	84.9
5	7.5	89.2	7.4	89.9	8.6	87.7	7.0	91.9
6	4.8	94.0	4.7	94.6	5.6	93.3	4.1	96.0
7	2.9	96.9	2.7	97.4	3.4	96.7	2.2	98.2
8	1.6	98.5	1.5	98.8	1.8	98.5	1.0	99.2
9	0.8	99.4	0.7	99.5	0.9	99.4	0.5	99.7
10	0.4	99.8	0.3	99.8	0.4	99.8	0.2	99.9
11	0.2	99.9	0.1	99.9	0.1	99.9	0.1	100.0
12	0.1	100	0.0	100	0.0	100.0	0.0	100.0

1997, with majority of observations from the 1984–1996 period (Table 2). The first lactations represent 1/3 of all lactations, while the first three lactations more than 70% of all lactations. The twelfth and further lactations account only for up to 0.1% of all lactations. In an analysis of longer time sequences, the age structure for both breeds is practically the same. The age structure of cows on the basis of the most recent calving periods in 1996 and 1997 (667 thousand lactations of Czech Pied and 322 thousand lactations of Holstein cattle) is different for breeds. Holstein breed participated in the first two lactations by 7% more lactations than Czech Pied breed in comparison of the corresponding lactation orders (in both breeds). Holstein breed accounts for 1.18 times higher number of cows in the first lactations and for 1.08 times more in the second lactations than in Czech Pied breed. These figures may be connected with the change of longevity in both breeds, but also with the intentional intervention of breeders following the numerical change in cattle populations. For the basis of the following calculations we use the average age structure resulting from a long-term analysis with reduction to 10 lactations (10th and higher lactations).

Total variability of milk production for closed lactations exceeding 200 days and 1 700 kg milk for the last period is given in Table 3. The difference in production between orders of lactations is connected with maturity and realised genetic trend. The maximum production is, from this viewpoint, in Czech Pied breed between the 3rd and 5th lactation, in Holstein breed in the 2nd and 3rd lactations. With lactation order, Holstein breed shows lower production growth and probably the change of production type is more manifested in younger individuals due to selection. With the order of lactation, the differences between breeds decrease. The changed average is related to the change of variability, which is the highest in the same lactations.

Discounted values

As it follows from the table of age structure, the first lactations are the most important. The economic value of single lactations in the total index is given by the age structure with consideration of the interest rate which takes into consideration delayed expression of production compared with the initial investments into selection. This results in a further disadvantage for the later lactations. For the purposes of mutual comparability, we discounted the frequency of later lactations to the first lactation. Table 4 gives the discounted economic values in dependence on the calving interval in the long-term age structure of the 1980–1997 period. Consideration of the interest rates increases the importance of the first lactations. At a 0% interest rate, the first lactation has a 33.9% relative importance, at 5% interest rate, the importance of the first lactation increased to 37% and at a 10% interest rate, it reaches 40% of all the observed lactations (1.18 multiple compared with the variant without the discounted interest rate). At a 5% interest rate, the importance of two first lactations is 59% in the sum and of the first three lactations it is 74% of the total number of the observed sum of lactations. At a 10% interest rate, these values are approximately 62% and 77%. Both in ideal calving interval equal to 365 days and the actual calving interval, the discounted economic values are practically the same. It is because majority of lactations are discounted within the range of a few years from the first calving and therefore the difference in the calving period duration did not play an important role in discounting.

At the age structure of the most recent calving periods in 1996 and 1997, the discounted values dependent on the breeds are shown in Table 5. Based on age structure, the economic values in Czech Pied breed are lower both in comparison with a long-term structure and with Holstein breed. The total of economic values for the period of two

Table 3. Variability of milk production in dependence on age. Calving period 1996–1997

Lactation order	Czech Pied			Holstein		
	average (kg)	proportion	σ	average (kg)	proportion	σ
1	4 082	1.00	1 024	5 064	1.00	1 346
2	4 633	1.13	1 176	5 742	1.13	1 634
3	4 830	1.18	1 223	5 780	1.14	1 625
4	4 828	1.18	1 226	5 567	1.10	1 510
5	4 812	1.18	1 222	5 424	1.07	1 458
6	4 785	1.17	1 217	5 236	1.03	1 374
7	4 713	1.15	1 192	5 074	1.00	1 351
8	4 623	1.13	1 180	4 875	0.96	1 297
9	4 496	1.10	1 141	4 691	0.93	1 225
10	4 397	1.08	1 141	4 550	0.90	1 236
11	4 366	1.07	1 135	4 404	0.87	1 212
12	4 170	1.02	1 105	4 491	0.89	1 223

Table 4. Discounted economic values of lactations, dependent on the interest rate and calving interval, at a longterm age structure for the 1980–1997 period

Lactation order	0%	Calving interval of 365 days		Calving interval of 398 days	
		5%	10%	5%	10%
1	0.339	0.369	0.396	0.371	0.401
2	0.213	0.221	0.226	0.221	0.227
3	0.155	0.153	0.150	0.153	0.149
4	0.110	0.103	0.097	0.103	0.095
5	0.075	0.067	0.060	0.067	0.059
6	0.048	0.041	0.035	0.040	0.034
7	0.029	0.024	0.019	0.023	0.019
8	0.016	0.012	0.010	0.012	0.009
9	0.010	0.007	0.005	0.007	0.005
10	0.005	0.003	0.002	0.003	0.002
Total	1	1	1	1	1

Table 5. Discounted economic values of lactations, dependent on the interest rate and calving interval, at the age structure for the 1996–1997 period

Lactation order	Czech Pied					Holstein				
	0%	calving interval				0%	calving interval			
		of 365 days		of 398 days			of 365 days		of 398 days	
	5%	10%	5%	10%	5%	10%	5%	10%		
1	0.290	0.318	0.344	0.320	0.349	0.343	0.370	0.396	0.373	0.400
2	0.223	0.233	0.241	0.234	0.242	0.240	0.247	0.252	0.247	0.252
3	0.160	0.159	0.157	0.159	0.156	0.156	0.153	0.149	0.152	0.148
4	0.118	0.112	0.105	0.111	0.104	0.110	0.103	0.095	0.102	0.094
5	0.086	0.078	0.070	0.077	0.068	0.070	0.062	0.055	0.061	0.054
6	0.056	0.048	0.041	0.047	0.040	0.041	0.035	0.029	0.034	0.028
7	0.034	0.028	0.023	0.027	0.022	0.022	0.018	0.014	0.017	0.014
8	0.018	0.014	0.011	0.014	0.010	0.010	0.008	0.006	0.007	0.006
9	0.009	0.007	0.005	0.006	0.005	0.005	0.003	0.003	0.004	0.003
10	0.006	0.003	0.003	0.005	0.004	0.003	0.001	0.001	0.002	0.001
Total	1	1	1	1	1	1	1	1	1	1

first lactations are by 6.5% higher for Holstein breed than for Czech Pied breed. Only the economic values for the ideal calving interval were included into the following calculations.

Weights of lactations in the index

Into the index there are incorporated partial breeding values for the early lactations determined by means of a multi-lactation model. Using the index, selection is done for the lifetime production on average for the period of 10 lactations. Alternatively there are included into the index first 1, 2 or 3 lactations. The indices are determined in

dependence on the economic values (Tables 4 and 5) and two variants of genetic parameters (Table 1). The results for the indices, only with the first lactation are shown in Table 6, while Table 7 gives indices with two first lactations and Table 8 with three first lactations. The weights are determined for various variants of indices, dependent on the breed and age structure, the interest rate and genetic parameters.

The total sum of weights in the index for separate lactations comes close to the value of 1. The age structure is manifested in weight coefficients of single lactations, but in the total reliability of production prediction it is reflected only a little. In the indices with two lactations, the second lactation has almost a double weight com-

Table 6. Weights of the 1st lactations in the index and reliability of prediction of breeding values of the average lifetime production

Index variant	Genetic parameters 1		Genetic parameters 2	
	b_1	r^2	b_1	r^2
C, age 80/97, interest rate 0%	1.000	0.885	1.040	0.921
C, age 96/97, interest rate 0%	0.998	0.868	1.044	0.911
H, age 80/97, interest rate 0%	0.988	0.891	1.024	0.922
H, age 96/97, interest rate 0%	1.001	0.896	1.000	0.922
C, age 80/97, interest rate 5%	1.005	0.898	1.039	0.927
C, age 96/97, interest rate 5%	1.003	0.881	1.042	0.917
H, age 80/97, interest rate 5%	0.997	0.902	1.027	0.928
H, age 96/97, interest rate 5%	1.008	0.906	1.032	0.927
C, age 80/97, interest rate 10%	1.008	0.908	1.037	0.932
C, age 96/97, interest rate 10%	1.007	0.892	1.040	0.922
H, age 80/97, interest rate 10%	1.004	0.912	1.029	0.933
H, age 96/97, interest rate 10%	1.013	0.914	1.034	0.932

Table 7. Weights of the first two lactations in the index and reliability of prediction of breeding value of the average lifetime production

Index variant	Genetic parameters 1			Genetic parameters 2		
	b_1	b_2	r^2	b_1	b_2	r^2
C, age 80/97, interest rate 0%	0.306	0.664	0.974	0.419	0.594	0.989
C, age 96/97, interest rate 0%	0.253	0.713	0.968	0.379	0.636	0.987
H, age 80/97, interest rate 0%	0.311	0.613	0.977	0.412	0.553	0.990
H, age 96/97, interest rate 0%	0.320	0.616	0.982	0.410	0.562	0.992
C, age 80/97, interest rate 5%	0.342	0.634	0.979	0.441	0.572	0.991
C, age 96/97, interest rate 5%	0.287	0.686	0.974	0.398	0.616	0.989
H, age 80/97, interest rate 5%	0.346	0.589	0.982	0.435	0.536	0.992
H, age 96/97, interest rate 5%	0.352	0.594	0.986	0.430	0.545	0.993
C, age 80/97, interest rate 10%	0.374	0.607	0.983	0.460	0.552	0.992
C, age 96/97, interest rate 10%	0.318	0.660	0.979	0.416	0.597	0.991
H, age 80/97, interest rate 10%	0.377	0.567	0.986	0.455	0.519	0.993
H, age 96/97, interest rate 10%	0.381	0.572	0.988	0.450	0.528	0.994

pared with the first one. The variant of genetic parameters No.1 presumes the decrease of genetic correlations with the more distant lactation orders. No. 2 variant of genetic parameters (Table 1) is with higher values of genetic correlations between lactations (constant value of genetic correlations between the second and higher lactations). This results in the increased weights in the index for the first lactation at the expense of the following lactations.

In the second variant, the first lactation in the index with three lactations has the weight between 0.357 to 0.442. In the first variant of genetic parameters the first lactation has the weight between 0.215 to 0.356. In the indices with three lactations, the second lactations have considerably

lower weights than the third one, which is connected with prediction of the following lactations.

The above mentioned weight coefficients are in accordance with the data given by Dempfle and Ponzoni (1986).

Reliability of prediction of lifetime production according to the first three lactations in the index reaches more than 99%. In selection based only on the first lactation in the index, the reliability reaches according to the index variant only the values between 87 to 93%.

The second variant of genetic parameters shows a marginally higher reliability of prediction of the average lifetime production.

Here it is to be taken into consideration that prediction of the breeding value for a particular lactation, dependent

Table 8. Weights of the first three lactations in the index and the reliability of prediction of breeding value of the average lifetime production

Index variant	Genetic parameters 1				Genetic parameters 2			
	b1	b2	b3	r ²	b1	b2	b3	r ²
C, age 80/97, interest rate 0%	0.272	0.072	0.607	0.992	0.399	0.236	0.368	0.996
C, age 96/97, interest rate 0%	0.215	0.063	0.667	0.990	0.357	0.252	0.394	0.995
H, age 80/97, interest rate 0%	0.279	0.095	0.555	0.993	0.392	0.230	0.347	0.996
H, age 96/97, interest rate 0%	0.291	0.139	0.511	0.995	0.390	0.248	0.337	0.997
C, age 80/97, interest rate 5%	0.311	0.101	0.548	0.994	0.421	0.237	0.345	0.997
C, age 96/97, interest rate 5%	0.252	0.095	0.606	0.992	0.377	0.254	0.372	0.996
H, age 80/97, interest rate 5%	0.317	0.120	0.503	0.995	0.416	0.231	0.327	0.997
H, age 96/97, interest rate 5%	0.325	0.161	0.465	0.997	0.412	0.250	0.317	0.998
C, age 80/97, interest rate 10%	0.345	0.122	0.498	0.996	0.442	0.236	0.324	0.997
C, age 96/97, interest rate 10%	0.286	0.121	0.553	0.994	0.396	0.256	0.351	0.997
H, age 80/97, interest rate 10%	0.351	0.139	0.460	0.996	0.437	0.232	0.308	0.998
H, age 96/97, interest rate 10%	0.356	0.177	0.423	0.997	0.433	0.252	0.297	0.998
*C, age 80/97, interest rate 5%	0.333	0.333	0.333	0.992	0.333	0.333	0.333	0.995
*C, age 96/97, interest rate 5%	0.333	0.333	0.333	0.988	0.333	0.333	0.333	0.995
*H, age 80/97, interest rate 5%	0.333	0.333	0.333	0.993	0.333	0.333	0.333	0.996
*H, age 96/97, interest rate 5%	0.333	0.333	0.333	0.995	0.333	0.333	0.333	0.996

*all three lactations in the index equal weights

on the amount and distribution of records, reaches only a certain reliability (R). The total reliability of prediction of the lifetime production therefore is

$$r^2_T = R * r^2 \quad (5)$$

where: R – reliability of isolated breeding value estimation for the particular lactation

r^2 – reliability of prediction of lifetime production from the breeding value of the particular lactation

From which follows that even in the most accurate prediction of the breeding value for the first lactation, reliability of prediction of the average of the lifetime production cannot practically exceed the level around 90%. If the higher lactations are also included in the index, the reliability increases.

The procedure shown in the equations (3), (4) and (5) is general and it is valid also for the multi-lactation model, where the breeding value of the early lactations (first three lactations) is, in gradually available information, determined only on the basis of the 1st lactation. The lifetime production is predicted in two steps, i.e. from the 1st lactation to the first three, and in the second step from first three lactations to all the others. The final reliability is the same, corresponding with the expression (5).

In case we substitute to the index with three lactations the same weight to all lactations (last lines of the Table 8), reliability of prediction of the lifetime production is not practically changed, compared with the optimal index. It is caused by a high genetic correlation between the sec-

ond and all further lactations. In the case where the genetic correlations between lactations were low, bigger changes could be expected.

Breeding value estimation in different countries is done according to different number of lactations per cow, different methodology and different weighting of lactations (Interbull Bulletin No. 24, 2000). The majority of countries use the repeatability animal model, and evaluation according to the first three to five lactations. According to our analysis (Table 2) first three lactations represent about 70% of all observations and first five lactations about 90% of all observations. Some countries use only the first lactation, some countries all lactations. Under this condition the breeding value for the same trait (milk production) in different countries cannot be considered as the same parameter and ranking of the animals in the international evaluation differs. Repeatability model produces the breeding value for an average of known observations, which, according to the number of evaluated lactations, has a different relation to the average of lifetime production. In multi-lactation (multi-trait) evaluation, the prediction of the average of lifetime lactation depends on weights in index. Weight factors differ according to countries.

The aim of similarity in the international evaluation motivated some countries to reduce the number of evaluated lactations (Canavesi *et al.*, 1999). This can yield higher correlation between countries in Interbull evaluation (similarity of procedures applied in different countries), but not to higher genetic progress in average of lifetime production.

CONCLUSIONS

1. Economic value of the 1st lactation increases with the interest rate up to 40% of all lactations.
2. Multi-lactation index of the animal model allows prediction of the lifetime breeding value of milk production based only on the 1st lactation with reliability around 90%. At the first two and more lactations, the conversion is within a 97–99% of reliability.
3. Total reliability of prediction is a multiple product of reliability of partial breeding value for concrete lactations and reliability of conversion from the given lactations for the lifetime production.
4. In the optimal index with three lactations, the weight of the first lactations is between 0.23 and 0.44 dependent on the interest rate and genetic parameters (from 23 to 44% of the total of weights of all three lactations). The weight of the third lactations exceeds significantly the second lactations.
5. A higher interest rate, as well as higher genetic parameters, increase the importance of the 1st lactation at the expense of the later lactations.
6. The differences in age structure according to breeds have a small influence on total reliability of breeding value prediction.
7. Substitution of the same weight to all the lactations into the index with three first lactations did not influence reliability of conversion compared with the optimal index.
8. Different variants of genetic parameters influenced reliability of prediction of the lifetime production only if it was estimated according to the 1st lactation alone. If more parities per animal are available, the influence of difference between genetic parameters decreases.

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The effect of age of pre-ruminant calves on starch digestibility

Vliv věku telat na stravitelnost škrobu

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ABSTRACT: Apparent starch digestibility (ASD) was studied in an experiment with five male calves of Czech Red Pied breed at the age of 9 to 91 days. Animals were fed on a milk replacer containing 5% of wheat starch. Digestibility was determined in 23 three-day balance periods using the chromic oxide indicator method. The average estimated value of ASD was 0.934 ± 0.00536 . Starch digestibility changed highly significantly ($P < 0.01$) with the age of calves. The dependence of determined values (Y) on age in days (X) was expressed by the 2nd degree parabola equation with a maximum on Day 54. The most marked increase was observed in the first weeks of the experiment, from Day 9 to Day 28, by 0.0064 per day ($P < 0.05$). It is of interest that even in very young animals starch was more digestible than it was expected.

Keywords: calves; effect of age; starch digestibility

ABSTRAKT: Vliv věku na bilanční stravitelnost škrobu byl sledován u pěti býčků českého strakatého plemene od 9. do 91. dne věku. Stravitelnost byla zjišťována indikátorovou metodou s oxidem chromitým ve 23 třídních bilančních periodách. Telata byla krmena mléčnou krmnou směsí obsahující 5 % pšeničného škrobu. V pokusu byla zjištěna průměrná stravitelnost 0.934 ± 0.00536 . Bilanční stravitelnost škrobu se s věkem telat vysoce průkazně měnila ($P < 0.01$). Závislost naměřených hodnot (Y) na věku ve dnech (X) byla vyjádřena rovnicí paraboly 2. stupně s maximem v 54. dni věku. Nejvýraznější vzestup byl zaznamenán v prvních týdnech pokusu, od 9. do 28. dne věku denně o 0.0064 ($P < 0.05$). Škrob v použité dávce byl poměrně dobře stravitelný i u velmi mladých zvířat.

Klíčová slova: telata; vliv věku; stravitelnost škrobu

INTRODUCTION

In calves, the digestibility of nutrients is influenced by a number of factors. The starch digestibility is probably limited by pancreatic production of amylase which increases with the age of calves during the first weeks of their life. The total production of this enzyme in milk-fed calves highly significantly increased ($P < 0.001$) in the study of Ternouth *et al.* (1971), in twelve-hour monitoring intervals from 9.4 mg to 57.5 mg and 240.0 mg on Days 7, 24 and 63 of age, respectively. In calves fed on milk replacer, the daily increase in twelve-hour amylase production was 0.972 mg in the period starting on Day 16 and ending on Day 37 ($P < 0.001$). Edwards and Barre (1977) fed wheat starch (200 g per 1 kg of milk replacer) to calves older than 17 days. They found that the values of starch digestibility

were 0.627–0.707 and 0.711–0.792 in 4th–5th and 9th–10th weeks of age, respectively. Toullec *et al.* (ex Assan and Thivend, 1976) mentioned that from birth to the age of 8 weeks, calves were able to digest starch from the milk replacer fed in the amount of 100–150 g/kg of dry matter. Veira *et al.* (ex Assan and Thivend, 1976) found in an experiment with weaned calves of the age 9–12 weeks fed on the maize-soybean meal diet that the values of starch digestibility ranged from 0.863 to 0.897. In older animals, microbial digestion of starch takes place above all in the proventriculum and caecum. Streeter *et al.* (1989) determined in heifers that 82.7, 2.9 and 5.7% of starch were digested in rumen, small intestine and large intestine, respectively. In ruminants, the capacity to digest starch in the intestine ranged from 45 to 85% of starch entering the duodenum; this capacity was apparently limited by

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the supply of pancreatic amylase (Huntington, 1997). The pancreatic secretion of amylase can be influenced by feed intake. The quantity of the pancreatic secretion was markedly lower when the calves were fed only once, not twice a day (Ternouth *et al.*, 1977).

The objective of this experiment was to determine how the digestibility of starch from the milk replacer varied in the course of fattening of pre-ruminant calves. To evaluate the effect of age as exactly as possible, it is necessary to carry out a great number of estimations in short time intervals during a longer period of life.

MATERIAL AND METHODS

Effect of age on the apparent digestibility of starch was studied within 23 three-day balance periods from Day 9 of age to Day 91 using five male calves of Czech Red Pied breed. Two subsequent three-day balance periods were followed by one-day pause every week. Calves were fed on a milk replacer containing spray-dried skim milk, buttermilk and whey, soya-protein concentrate HP 100, fat, feed additive premix and wheat starch. Milk replacer contained 206 g crude protein, 154 g crude fat, 8 g crude fibre, 558 g nitrogen-free extract, 51 g starch, 7.8 g Ca, 7.3 g P, 1.4 g Mg, 18.7 g K and 4.4 g Na per 1 kg of dry matter. The milk replacer was diluted with water in ratios of 1 : 9, 1 : 8, 1 : 7 and 1 : 6 till Days 18, 36, 71 and 91 of age, respectively. Milk drink of the temperature of 38–40°C was supplied *ad semi-libitum* three times a day.

The digestibility was estimated using the chromic oxide indicator method. The calves received 1 gelatine capsule (size 20 mm, diameter 6 mm; INTERCAPS, Ltd. Zlín) containing 1 gram of chromic oxide just before every feeding. Excrements were collected every 24 hr and samples were prepared from the pooled excrements collected within a period of 3 days. The content of chromic oxide in freeze-dried faeces was determined by the atomic absorption spectrophotometry (Williams *et al.*, 1962). The content of starch in feed and freeze-dried faeces was estimated polarimetrically after hydrolysis with hydrochloric acid and

removal of proteins using Carres' agents. Optical turn, measured in the apparatus Polamat (Carl Zeiss Jena), was corrected for the content of optically active substances soluble in the mixture ethylalcohol-water (Decree No. 222/1996 of the Czech Ministry of Agriculture).

The regression analysis of determined values was performed according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The average value of starch digestibility in the trial (114 metabolic experiments) was 0.934 ± 0.00536 (mean \pm standard error of the mean). We determined higher starch digestibility than expected during the whole trial. The values measured in our experiment were higher than those published by other authors (Edwards and Barre, 1977; Veira *et al.*, 1980). This can be caused by a relatively low starch content in the milk replacer used in our experiment. Digestibility varied highly significantly ($P < 0.01$) with the age of calves. The dependence of starch digestibility (Y) on age in days (X) was expressed by means of linear and 2nd degree parabola regression equations. The reduction in the sum of squares of deviations was tested against the mean square remaining after curvilinear regression by F -test (Snedecor and Cochran, 1967) and was highly significant in all calves ($P < 0.01$). Parameters a , b and c , correlation index, F -values and extreme coordinates of parabola equations are presented in Table 1. When involving the values measured in all calves into the mean, the apparent digestibility of starch varied in the period of Day 9 to Day 91 by the linear equation

$$Y = 0.908 + 0.00053 X; r = 0.215; P < 0.05$$

and parabola equation

$$Y = 0.756 + 0.0082 X - 0.00007567 X^2; r = 0.674; P < 0.01; X_{\text{extr.}} = 53.9; Y_{\text{extr.}} = 0.976$$

with a maximum on Day 54 (Figure 1). The parabolic expression described the examined dependence significantly more accurately than the linear regression ($P < 0.01$).

Table 1. Effect of age on apparent digestibility of starch

Calf No.	Mean \pm standard error of the mean	$Y = a + bX + cX^2$						
		a	b	c	r	P	$X_{\text{extr.}}$	$Y_{\text{extr.}}$
1	0.942 \pm 0.0123	0.781	0.0074	-0.00006883	0.590	< 0.01	53.8	0.980
2	0.944 \pm 0.0065	0.845	0.0046	-0.00004408	0.710	< 0.01	52.5	0.966
3	0.915 \pm 0.0181	0.613	0.0138	-0.00012667	0.833	< 0.01	54.4	0.988
4	0.926 \pm 0.0114	0.763	0.0081	-0.00007976	0.721	< 0.01	50.9	0.969
5	0.945 \pm 0.0073	0.823	0.0053	-0.00004695	0.734	< 0.01	56.2	0.972
1–5	0.934 \pm 0.0054	0.756	0.0082	-0.00007567	0.674	< 0.01	53.9	0.976

X – age in days

r – correlation coefficients

a , b , c – parameters of equation

Y – apparent digestibility of starch

P – significance of the deviation from linearity

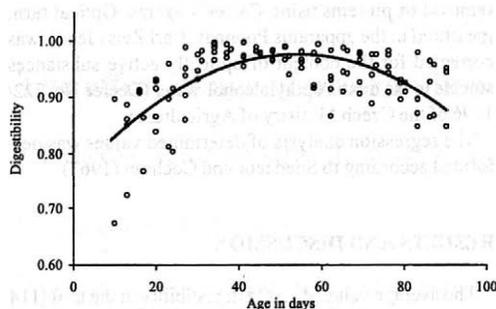


Figure 1. Apparent digestibility of starch

The most marked increase was observed within the first weeks of life. The apparent starch digestibility in the period of Day 9 to Day 28 was expressed by means of linear regression equation

$$Y = 0.751 + 0.0064 X; r = 0.486; P < 0.05$$

The daily increase of 0.0064 was significant ($P < 0.05$). This could be caused by an increase in the production of pancreatic amylase (Ternouth *et al.*, 1971). Wheat starch in the amount of 50 g per kg of milk replacer was digested well even by very young calves. Assan and Thivend (1976) also considered wheat starch as a suitable source of energy even for very young calves.

In older calves, the apparent starch digestibility decreased highly significantly after the 54th day ($P < 0.01$) of our trial. When expressed by means of a linear regression equation the starch digestibility decreased highly significantly within the period of Day 54 to Day 91; this was described by the equation

$$Y = 1.045 - 0.0015 X; r = 0.418; P < 0.01$$

For calves feeding only on milk replacer till the age of 3 months is not physiological. Under natural conditions the calf starts with roughage intake rather early and the major part of starch is digested by the rumen microbial population (Streeter *et al.*, 1989). The capacity to digest starch in the intestine is probably limited by the level of pancreatic amylase (Huntington, 1997).

The average values of starch digestibility in the periods from Day 9 to Day 28 and from Day 54 to Day 91 were

0.875 ± 0.0142 and 0.936 ± 0.0054 , respectively. In our trial starch was more digestible in the older calves; however, it was more digestible than expected even in very young animals.

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Frequency of feeding with TMR in dairy cows in summer season

Frekvence krmení dojnic směsnou krmnou dávkou (TMR) v letním období

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ABSTRACT: The observation was focused on the effect that a feeding frequency using TMR twice or three times a day has on the parameters of performance, dry matter intake (DMI), rumen fluid composition, chewing activity and behaviour of animals if they were given feeds in a summer season. 13 Holstein and 5 Czech Red Pied dairy cows, which were on average 70.5 days after calving, were used in a periodical experiment. If a feeding ration with a considerable ratio of corn starch was given three times a day, statistically significantly higher DMI ($P < 0.05$) by 1 310.74 g/head/day and higher daily milk yield by 1.24 kg/head (= 1.33 kg of FCM) were reached. Differences in milk protein and milk fat contents were minimal (0.02 and 0.04%, respectively). Differences in rumen fluid parameters and chewing activity were also statistically insignificant ($P > 0.05$).

Keywords: dairy cows; feeding frequency; DM consumption; milk yield; physiological parameters

ABSTRAKT: V periodickém pokusu na 18 dojnících holštýnského (13 ks) a českého strakatého plemene (5 ks), v průměru 70,5 dne po otelení, byl sledován vliv frekvencí krmení TMR 2× a 3× denně v letním období na parametry užitkovosti, příjem sušiny, složení bacherové tekutiny, žvýkačí aktivitu a chování zvířat při krmení. Krmná dávka byla tvořena vojtěškovou siláží, silážovanou drtí kukuřičných palic, silážovanou drtí pšenice a hrachu, lučným senem, pivovarským mlátem, sušenými cukrovarskými řízky, sójovým extrahovaným šrotem a jadrnou směsí pro dojnice (DO). Při zkrmování dávky s výrazným podílem kukuřičného škrobu bylo při frekvenci krmení 3× denně dosaženo statisticky průkazně ($P < 0,05$) vyššího příjmu sušiny (o 1 310,74 g/ks/den) a vyšší průměrné denní produkce mléka o 1,24 kg (1,33 kg FCM). Rozdíly v obsahu mléčné bílkoviny a mléčného tuku byly minimální (0,02, resp. 0,04 %). Při krmení 3× denně dosáhla průměrná denní užitkovost 33,45 kg, zatímco při frekvenci krmení 2× denně to bylo pouze 32,21 kg. Zjištěné hodnoty pH bacherové tekutiny se v průběhu pokusu pohybovaly v rozpětí 6,03–6,50, při krmení 3× denně bylo v průběhu dne pH vyrovnanější (6,08–6,38). Obsah amoniaku v bacherové tekutině byl v rámci fyziologických hodnot nejvyšší většinou při večerním odběru. Celková doba žvýkání za den při krmení 2× denně byla o 23,41 minut delší, než při krmení 3× denně. Vzhledem ke zvýšenému příjmu sušiny a vyšší mléčné užitkovosti je podle našich výsledků výhodnější krmit vysokoužitkové dojnice TMR v letním období 3× denně, než 2× denně.

Klíčová slova: dojnice; frekvence krmení; spotřeba sušiny; mléčná užitkovost; fyziologické parametry

INTRODUCTION

A feeding technique can influence the feed intake, milk yield and health condition of dairy cows in an expressive way. The feeding frequency and the way of feed administration are among the most important factors of feeding technique. Opinions on the number of feedings in the course of one day varied. The feeding system twice a day is used most frequently in regular practice. If we were to decide on the number of feedings during one day, apart from other things, it is necessary to take into consideration the com-

position of feeding ration, quality of feeds and performance of the barn. According to the contemporary conception and in view of the maximum feed intake, i.e. of DMI – it is necessary that the dairy cow may eat whenever it feels like eating. It could be said that more feedings during one day which often involve the accumulation of offered feeds, meet these demands. On the other hand, it is necessary to improve labour productivity and so possibly to reduce feeding to once a day system. The once a day variant makes it possible to use a “total mixed ration” (TMR) as a homogeneous mixture of all feeds that are chosen.

Kaufmann (1976) states that the higher feeding frequency facilitates higher intake of concentrates without a decrease in the rumen pH value, and the ratio of acetic to propionic acid is 3 : 1, and no decrease in milk fat occurs. If the automatic dosing systems of concentrates and higher frequency of feeding were used, it was possible to increase milk performance without a decrease in milk fat and an increase in metabolic disorders. Johnson *et al.* (1966) did not find any differences in milk yield and feed intake that would be caused either by changes in the feeding frequency or by similar differences in the forage intake of dry cows. Due to the unusually hot weather (26.7°C) in summer time the feed intake and milk yield were reduced approx. by 10%. Nocek and Braund (1968) fed TMR of the constant composition at the following frequencies: 1-, 2-, 4- and 8- times a day. They found if the dairy cows standing in the first lactation period received TMR *ad libitum* at different frequencies, the consumption of dry matter and water differed, nevertheless the course of changes in the rumen fluid composition was similar. DMI was higher by 3% and milk performance by 2% in the cows fed once a day in comparison with the animals fed four times a day. The higher feeding frequency in the case of feeding ration with a low portion of forage increased the milk fat production in experiments of Sutton *et al.* (1985). Similarly, Johnson (1979) reported that if the feeding ration was given five times a day, there was a higher percentage of milk fat in comparison with feeds administered just twice. The effect of the concentrate feeding frequency on pH level, volatile fatty acids production and performance was also investigated (Kaufmann *et al.*, 1980). Gill and Castle (1983) confronted the feeding of protein concentrate 2-, 4- or 22- times a day. The feeding frequency did not influence either the intake of silage, total intake of nutrients, milk performance or live body weight in that experiment. If the feeding frequency was risen, the concentration of milk fat rose up too, but the animal's behaviour concerning the time of eating and chewing of dairy cows was not influenced. Different variants of the feeding frequency of hay before or after concentrate administration were investigated by Macleod *et al.* (1994). Shabi *et al.* (1999) evaluated the effect of two feeding rations – both were given at two feeding frequencies (2- and 4- times a day) in Israel. Li (1999) found as the optimal feeding frequencies to administer forage 5 times a day and concentrates 6 times a day.

Possible physiological problems which have to do with separate feeding of higher portions of concentrates are solved in such a way that the concentrates and roughage are fed together in form of TMR. McCullough (1994) stated that there is a uniform progress of fermentation inside the rumen as a result of several daily feeding rations, and in this time there occurs an increase in DMI, better utilisation of energy and proteins, elimination of digestive problems and improvement of milk fat percentage.

MATERIAL AND METHODS

A periodical experiment with 13 Holstein and 5 Czech Red Pied dairy cows (7 of them were primiparous) was carried out in a summer season to find out the effect of changes in the TMR feeding frequency in the course of one day on feed intake, milk yield and some physiological parameters. Dairy cows were on average 70.5 days after calving at the beginning of the experiment and their average body weight was 625.9 kg. The following feeding frequencies were investigated while the cows were given TMR *ad libitum*:

- 1st period – twice a day
- 2nd period – three times a day
- 3rd period – twice a day

Each period lasted for 21 days and the first 7 days were as a preparatory period when the animals were adapted to a new frequency of feeding. The feeding ration (Table 1) consisted of alfalfa silage, silage from ground corn cobs, silage from a ground mixture of wheat and pea, meadow hay, draff, dried sugar beet pulps, extracted soybean meal and concentrate mixture for high-yielding dairy cows (CM). CM contained extracted soybean meal again, corn grain, fish meal, dicalcium phosphate, salt, limestone, magnesium dioxide and mineral-vitamin supplement VPDz.

Administration of feeds in form of TMR was carried out from a mixing wagon *Luclar* at 6:45 AM and 16:45 PM if fed twice a day, but if fed three times a day, another TMR was given at 12:00 AM. The values of consumption were taken from a list of values which were put down by a supported software. This software controlled troughs standing on tensiometric scales. Samples of rumen fluid were taken with a gullet probe twice for 6 animals, namely at 2½, 6 and 10½ hrs after morning feeding during the 1st and 3rd period and the same was done four times for the same animals and at the same time during the 2nd period.

Table 1. Composition of feeding ration

Feeds	% of DM
Alfalfa silage	18.11
Ground corn cob silage	34.48
Ground mixture of wheat and pea	5.71
Meadow hay	7.92
Fresh draff	4.98
Dried sugar beet pulps	4.03
Extracted soybean meal	16.82
Corn grain	2.36
Fish meal	3.18
Dicalcium phosphate	0.39
Salt	0.30
Limestone	1.07
Magnesium dioxide	0.40
Mineral-vitamin supplement VPDz	0.25

The cows that were selected for rumen fluid samplings got feed in the evening before rumen fluid sampling. pH value, NH_3 content and contents of single and total fatty acids were determined in the rumen fluid.

Chewing activity was examined in 10 animals in the course of one day at each period. Total time of chewing, length of chewing periods and their number during 24 hrs were also monitored.

Cows were milked twice a day in a milking parlour with 10 milking boxes, at the same time – in the morning between 4 and 5 o'clock and in the afternoon between 16 and 17 o'clock. They were stalled in a fre barn with sleeping boxes. Milk draw was measured every day. Samples of milk were taken once a day and contents of milk fat, milk proteins and lactose were determined. Dairy cows were weighed at the beginning and at the end of each period.

All results of measurements and analysis were processed by common statistical methods while – after basic array and arrangement in alphabetical order of a data file – Charlier's test for correctness of putting of values and partial calculations was used. Then two tests for elimination of possible extreme values (Grubbs' and Dixon's) followed and at the end some statistical parameters were calculated (e.g. arithmetic means, standard errors, range of statistical classes and their character and so on). Then these parameters were used in *F*-test for testing the significance of differences between mean squares of data files. In order to test the results of the previous test one of *t*-tests was chosen, by means of which the significance of possible differences in means of these two monitoring data files was tested. The results of testing were used to express the level of significance of differences or conformity between two monitoring data files.

RESULTS AND DISCUSSION

Effects of experimental intervention – the change in the feeding frequency – on the intake of dry matter (DMI) and simple organic and inorganic nutrients are shown in Table 2. While in the 1st and 3rd periods, when fed twice a day, dairy cows took 23 206.27 g of DM/head/day, in the

2nd period (the feeding frequency three times a day) DM consumption amounted to 24 517.01 g/head/day. The established difference of 1 310.74 g/head/day was statistically significant ($P < 0.05$) and it points to more frequent feeding of TMR to high-yielding dairy cows in the summer season. DMI in the 2nd period matched up to 3.83% of body weight, whereas this value matched up to 3.59% of body weight in the control period. Average temperature in the periods of our experiment was 20.04°C, and the maximum temperatures reached 31.9°C, 27.2°C and 27.7°C, respectively, at the relative humidity of 82.75, 88.62 and 96.24%, respectively. Although no changes occurred in the feed intake in some experiments with different feeding frequency (Klusmeyer *et al.*, 1990; Macleod *et al.*, 1994), in other experiments (Shabi *et al.*, 1999), if the feeding frequency increased, there occurred an increase in DMI, namely if feed rations with the higher portion of starch were used likewise in our experiment. Shabi *et al.* (1999) considered more frequent feeding of this type of feeding ration as more advantageous because of increasing the postprandial digestibility of organic substances (by 28.9%), nonstructural hydrocarbons and crude protein. More frequent feeding, in this case, was more advantageous also for the increase in milk yield and milk composition. Ruiz and Mowat (1987) found in the cows fed *ad libitum* only minimum differences in the intake and conversion of feeds for the feeding frequency once and four times a day, whilst if the consumption was reduced to the level 90% of the maximum, more frequent feeding was a reason for the increase in DMI, digestibility of organic substances and nitrogen retention.

The content of protein (nitrogenous substances – NS) was 19.45 or 20.14% (on the basis of feed dry matter). Chamberlain and Wilkinson (1996) recommended NS contents 17.5–18% (on the basis of feed dry matter) for performance of 40 l/day and 18–19% for performance of 50 l/day. If the cows consumed more than 200 g of crude protein/kg DM, these authors concluded that a considerable amount of proteins is deaminized, which can damage the liver function to such an extent that the fertility is decreased. The intake of P, including the values of PDI, was also higher than the benchmark recommendation of Som-

Table 2. Average consumption of organic nutrients and macro-elements (g/head/day)

Period	Nutrients							
	DM	P	PDIE	PDIN	NEL (MJ)	crude fibre	ADF	NDF
2nd	24 517.01	4 770.29	2 532.65	2 976.72	149.09	4 031.62	4 844.66	7 533.34
(1st+3rd)/2	23 206.27	4 673.76	2 437.07	2 916.53	140.22	4 017.94	4 545.62	7 350.19
	Macro-elements							
	Ca	P	Na	K	Mg			
2nd	244.2	108.18	72.33	239.71	87.61			
(1st+3rd)/2	226.96	106.6	70.02	235.08	86.13			

mer *et al.* (1994). The difference in NS-intake during the assessed period was 96.53 g and was related – similarly like the higher intake of some other nutrients (NEL, mineral elements) – to higher DMI if the cows were given meal three times a day. The energy intake during our experiment was higher, according to the recommendation of Sommer *et al.* (1994). The content of crude fibre was otherwise higher than the recommendations mentioned above, but, in terms of its percent of feed dry matter, it was basically at its optimum level (16.44% during the 2nd period and 16.38% on average for the 1st and 3rd period). The ADF rate (19.77% and 19.58% DM of feed ration) was optimal for the attained lactation level, analogous to NDF contents. The content of calcium (Ca) was higher than the recommendation of Sommer *et al.* (1994) while the content of phosphorus (P) was within the recommended values. Ca : P ratio was 2.26 : 1 in the 2nd period and average ratio for both control periods was 2.10 : 1. Potassium (K) to sodium (Na) ratio was approximately 3.3 : 1, and the intake of mineral elements was roughly the same for all investigated periods.

The milk yield is shown in Table 3. During the 2nd period when the cows received feed three times a day, the

daily milk yield (33.45 kg) was higher by 1.24 kg compared with the average of both control periods (32.21 kg). The difference is statistically significant ($P < 0.05$), analogous to the difference (1.33 kg) in FCM production ($P < 0.05$). The improvement of milk production (feeding three times a day) could be explained by the increased DMI. It did not happen in experiments of Macleod *et al.* (1994) when the frequency of concentrate feeding was increased. The results of experiments in which the effect of higher feeding frequency on performance of dairy cows was studied are often inconsistent (Gibson, 1984). Sutton *et al.* (1985) expected the effect on milk performance, and especially on milk fat content, in the case of feed rations with low fibre concentration. Percentual content of milk protein rose up during progressive lactation from 3.27% in the 1st period to 3.56% in the 3rd period, while the difference of 0.02% between the experimental period and the average of both control periods was not statistically significant in our experiment ($P > 0.05$). Continuously rising values were also recorded in the percentual content of milk fat, but with the insignificant difference between experimental and both control periods. Due to higher daily milk yield there was also statistically significantly ($P < 0.05$) higher absolute

Table 3. Average values of milk performance parameters

Parameters	Period			Mean (1st+3rd)/2	Difference 2nd – (1st+3rd)/2
	1st	2nd	3rd		
Milk (kg/day)	33.22	33.45	31.2	32.21	1.24
FCM (kg/day)	29.33	30.28	28.57	28.95	1.33
Protein (%)	3.27	3.43	3.56	3.41	0.02
Protein (kg/day)	1.09	1.15	1.11	1.1	0.05
Fat (%)	3.22	3.37	3.46	3.33	0.04
Fat (kg/day)	1.07	1.13	1.08	1.07	0.06
Lactose (%)	4.99	4.98	5.03	5.01	-0.03
Lactose (kg/day)	1.66	1.67	1.57	1.61	0.06

Table 4. Average composition of rumen fluid (nmol/l)

Parameter	Time of sampling	pH	NH ₃	Acid					
				fatty	lactic	acetic	propionic	butyric	valeric
Morning	1st period	6.39	18.31	127.26	0.71	71.50	36.07	13.55	5.44
	2nd period	6.34	17.25	136.70	0.23	80.31	35.04	15.86	5.44
	3rd period	6.41	20.60	129.96	0.00	76.55	33.39	14.20	5.82
	(1st+3rd period)/2	6.40	19.46	128.61	0.35	74.03	34.73	13.87	5.63
Midday	1st period	6.26	18.15	130.89	0.18	73.47	39.05	13.61	5.09
	2nd period	6.18	19.61	141.56	0.09	82.13	37.64	16.43	5.86
	3rd period	6.30	18.34	122.69	0.22	72.60	32.42	13.03	4.41
	(1st+3rd period)/2	6.28	18.25	128.79	0.20	73.04	35.74	13.32	4.75
Evening	1st period	6.03	24.28	139.51	0.96	76.35	41.54	22.92	5.62
	2nd period	6.38	22.27	129.21	0.32	74.39	33.97	15.34	5.20
	3rd period	6.50	20.31	112.73	0.10	66.90	29.07	12.12	4.54
	(1st+3rd period)/2	6.27	22.29	126.12	0.53	71.63	35.31	17.52	5.08

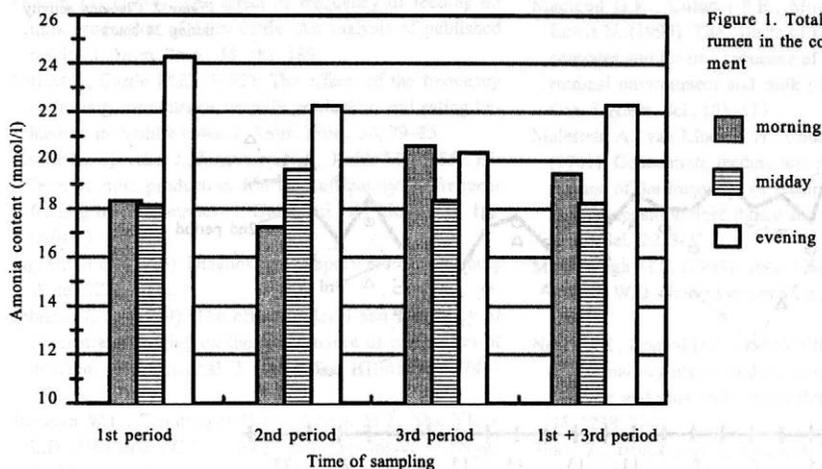


Figure 1. Total contents of ammonia in rumen in the course of the whole experiment

daily milk protein and milk fat production when the cows were given meals three times a day.

Mean values of rumen fluid parameters are shown in Table 4. The values of pH ranged from 6.03 to 6.50, whereas the range for the 2nd period was between 6.18 and 6.38 (difference of 0.2) and for the feeding frequency twice a day the lowest mean value during the evening feeding was 6.03 (difference of 0.47). During morning and midday samplings the reaction of the rumen fluid was more acidic for the three-times feeding frequency, whereas the same effect could be observed in the twice a day feeding frequency during the evening sampling. The above differences as well as differences in other parameters were not statistically significant ($P > 0.05$). Similarly in a number of other experiments no large changes in pH of the rumen fluid, fatty acids content, including filling of simple fatty acids were observed if different feeding frequencies were used, including the feeding frequency of concentrates (Malestein *et al.*, 1981; Hardie *et al.*, 1985 and others).

The content of total fatty acids was slightly above the reference values, as indicated in Jagoš (1985), which gave evidence about the enhanced intake of carbohydrates in feeding rations.

The content of total volatile fatty acids (VFA) was statistically insignificantly ($P > 0.05$) higher during the 2nd period (feeding three times a day) than the average of the 1st and 3rd periods. Mainly the levels of acetic and propionic acid increased. Content of butyric acid exceeded the limits recommended by Jagoš *et al.* (1985) just in one event (the evening, 1st period). Increased content of propionic acid confirmed an assumption about the higher intake of concentrates, but simultaneously the high content of acetic acid demonstrated that the feeding ration contained enough fibre in its structural form. The concentrates that were included in TMR, above all *high moisture shell corn* (LKS), did not exert any adverse effect in this sense. Sutton *et al.* (1985) reported that the effect of the higher feeding frequency on the molar proportion of VFA inside the

Table 5. Chewing activity of dairy cows

Category (number of cows)	Index	Period			
		1st	2nd	3rd	(1st+3rd)/2
Primiparous (5)	average time of chewing (24 hrs)	08:17:36	07:59:43	07:42:48	08:00:24
	number of chew. periods (24 hrs)	15.00	14.00	12.80	13.90
	duration of chewing period	00:33:10	00:34:16	00:36:09	00:37:17
Multiparous (5)	average time of chewing (24 hrs)	08:51:49	07:32:01	07:34:00	08:12:54
	number of chew. periods (24 hrs)	14.60	14.30	15.00	14.80
	duration of chewing period	00:36:26	00:32:45	00:30:16	00:33:18
Total (10)	average time of chewing (24 hrs)	08:34:42	07:45:52	07:38:24	08:09:33
	number of chew. periods (24 hrs)	14.80	14.15	13.90	14.35
	duration of chewing period	00:34:47	00:32:55	00:32:59	00:34:07

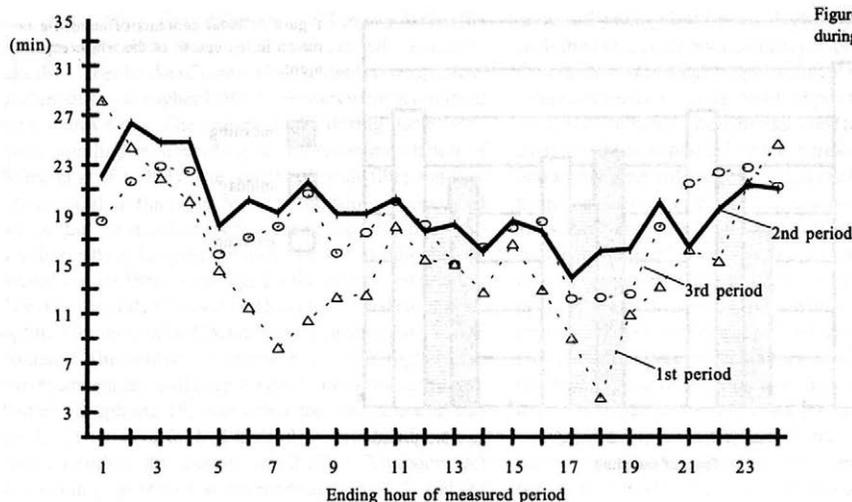


Figure 2. Chewing activity during 24 hours

rumen is plain and insignificant although there was an apparent tendency to increase the portion of acetic acid and to decrease the portion of propionic acid. Content of ammonia in the rumen fluid (Figure 1) was basically within the reference values, the highest values being found mostly in evening samples. Its well-balanced level was during midday sampling.

The process of changes in live weight (1st period – 636.69 kg, 2nd period – 640.01 kg, 3rd period – 655.00 kg) upheld a gradual pass to positive energy balance. In the course of the 3rd period, in which there was a decrease not only in DMI but also in milk yield, there was an increase in live weight by 14.99 kg in comparison with the 2nd period.

Chewing activity parameters in the experimental dairy cows are shown in Table 5. No significant differences between them were found. By feeding twice a day (1st and 3rd period) chewing duration was about 8 hours, therewith it passed on average from 12.8 to 15 chewing periods, which continued from 00:30:16 to 00:36:26 hrs. The period of chewing was generally by 00:23:41 hrs longer by twice a day feeding than by three times a day feeding, and the number of chewing periods, which were longer by 00:01:52 hrs, was higher by 0.2 for 24 hrs. If primiparous and multiparous dairy cows are compared, there were not any substantial differences in the chewing activity, but primiparous cows had less chewing periods, however these periods were longer. Per 1 kg of ingested dry matter, by feeding twice a day (1st + 3rd period) the time of chewing was on average 00:21:09 and by feeding three times a day (2nd period) only 00:19:00 hrs, which considering the probably lower production of saliva, could have the effect on the above mentioned lower pH of the rumen fluid during the 2nd period. The highest chewing activity took place during the larger part of the experiment between 22:00 and 4:00 hrs (Figure 2). The feeding frequency was

not probably decisive for the chewing activity, but the physical and chemical parameters of the feeding ration which were approximately the same all the time. A relatively slight response to changes in the feeding frequency is coincident with conclusions of other authors, e.g. Gill and Castl (1983), as well as Macleod *et al.* (1994). In view of the total time of chewing and duration of the chewing period Brydl (1998) found similar values in experimental dairy cows, but in an experiment of Shaver *et al.* (1984) where long hay was fed, the time of chewing was 704, for chopped hay 668 and pelleted hay 219 minutes/day, whereas the rumen pH values were 6.3, 6.4 and 5.6, respectively.

CONCLUSION

In the summer season there was a statistically significant increase in DMI and also in milk yield if dairy cows were given TMR *ad libitum* three times a day, in comparison with the feeding frequency twice a day. On the other hand, no significant differences were found in the composition of milk, rumen fluid quality or in the chewing intensity. With respect to higher DMI and higher milk production it is better, according to our results, to feed high-yielding dairy cows with TMR in the summer season three times a day, not twice a day.

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Intensive rearing of the common barbel (*Barbus barbus* L.) larvae using dry starter feeds and natural diet under controlled conditions

Intenzivní odkrm larev parmy obecné (*Barbus barbus* L.) při použití startérových krmných směsí a živé potravy v laboratorních podmínkách

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ABSTRACT: In an experiment we carried out intensive rearing of the fry of common barbel (*Barbus barbus* L.) up to the age of 25 days from the beginning of exogenous nutrition. The fish were fed 3 types of dry diets (recommended for initial feeding of aquarium, freshwater and sea fish) containing different levels of nutrients (proteins 48–62%, fat 7–12%) and live food (*Artemia salina* nauplii). We studied the survival rate, individual weight (w), total body length (TL), specific weight growth rate (SWGR) and condition of the fry (condition coefficient according to Fulton – C_F). The results were processed statistically using the method of one-way analysis of variance (ANOVA). The highest survival rate (99.7%) and the statistically significant ($P < 0.01$) highest growth rate were achieved in the barbel larvae fed live diet (TL = 25.7 mm, w = 144.5 mg, respectively). The cumulative survival rate of larvae fed dry diets was 73.8–97.6%. The most satisfactory results of dry-diet-based feed were obtained when feeding starter feeds for sea fish fry (TL = 21.0 mm, w = 101.6 mg). The C_F data for barbel fry initially fed dry diets were statistically highly significantly higher ($P < 0.01$) than for larvae fed live diets (i.e. 0.95–1.02 and 0.85, respectively). These results showed that intensive initial feeding of barbel fry under controlled conditions could be successful if suitable starter feeds were applied.

Keywords: barbel; larvae; initial feeding; growth; survival

ABSTRAKT: V experimentu jsme prováděli intenzivní odkrm plůdku parmy obecné (*Barbus barbus* L.) do věku 25 dnů od zahájení exogenní výživy. Použitými krmivy byly tři typy suchých diet (doporučené pro rozkrm akvarijních, sladkovodních a mořských druhů ryb) odlišného nutričního složení (N-látky 48–62 %, tuk 7–12 %) a živá potrava (*Artemia salina* v naupliovém stadiu). Sledovanými ukazateli byly úroveň přežití, individuální hmotnost (w), celková délka těla (TL), specifická rychlost hmotnostního růstu (SWGR) a kondiční stav plůdku – koeficient vyživenosti dle Fultona (K_F). Zjištěné výsledky byly statisticky vyhodnoceny metodou jednocestné analýzy variance (ANOVA). Vysoké úrovně přežití 99,7 % a statisticky vysoce průkazně ($P < 0,01$) nejvyššího růstu u larev parmy (TL = 25,7 mm, w = 144,5 mg) bylo dosaženo při aplikaci živé potravy. Při použití suchých krmných směsí činila úroveň kumulativního přežití larev 73,8–97,6 %. Nejpriznivější produkční výsledky rozkrmu na bázi suchých diet byly zaznamenány při použití startérové směsi pro plůdek mořských druhů ryb (TL = 21,0 mm, w = 101,6 mg). Plůdek parmy, rozkrmený suchými dietami, vykázal statisticky vysoce průkazně ($P < 0,01$) vyšší hodnoty K_F (0,95–1,02) v porovnání s larvami rozkrmenými živou potravou ($K_F = 0,85$). Dosažené výsledky ukazují, že při použití vhodného startérového krmiva lze úspěšně realizovat intenzivní rozkrm plůdku parmy v kontrolovaných podmínkách.

Klíčová slova: parma; plůdek; rozkrm; růst; přežití

INTRODUCTION

Common barbel (*Barbus barbus* L.) is an important element of the ichthyofauna of the running sections of rivers with stony bottom. In the past, this species was an

important component of the fish community under suitable conditions and was also a very popular object of interest for anglers (Baruš *et al.*, 1995). Anthropogenic effects (stream regulation, segmentation of rivers by waterworks, worse quality of the water, modern methods of

angling etc.) considerably reduced the abundance, or they completely eliminated the common barbel in many of its original biotopes (Bless, 1978; Penczak *et al.*, 1998).

The reduction of the common barbel population in most of the Central European rivers gradually resulted in the development of technologies of stripping and rearing early age categories, which were then let out in suitable sections of running rivers. In the Czech Republic (1963), Krupka (1987) and Lusk and Krčál (1988) studied this issue.

Intensive rearing of fry and stock of higher weight per individual is a promising method of increasing the numbers of the common barbel in running waters (Philippart, 1987). Philippart (1982) published experiments dealing with initial feeding of barbel larvae in water of a temperature of 18–20°C based on a live diet. Wolnicki and Górný (1993) carried out a similar experiment in water of a temperature of 22–28°C. An important condition for the successful mastering the technology of intensive rearing of the common barbel in controlled conditions is to specify the dietetic requirements of the respective age categories. In his study Pedersen (1997) focused on the nutritional and energetic requirements of the early fish fry. Labatzki and Fuhrmann (1992) applied a diet combined of artemia and starter feed for the initial feeding of barbel larvae. Wolnicki and Górný (1995) published results of the application of dry and combined diets in the intensive rearing of barbel larvae. The objective of our experiment was to compare the survival rate, growth rate and condition of the larval and juvenile stage of the common barbel intensively fed complete dry starter feed and natural feed.

MATERIAL AND METHODS

In an experiment we studied the intensive feeding of the larval and juvenile stage of the common barbel from the 1st to the 25th days of uptake of exogenous nutrition. They received 3 types of dry feed mixtures with different levels of nutrients (proteins and lipids) and live food *Artemia salina* (further only artemia).

The barbel fry, obtained by stripping of generation fish from the river Jihlava in the Třebíč locality, was partly warmed up and at the age of 5 days after hatching was placed in a non-flowing-through tank. Within 36 hours the temperature in the tank was gradually increased from 19.5°C to 25.5°C and in this time interval the barbel embryos finished the stage of warming up. Fry at the age of 7 days after hatching was adapted in the rearing tanks and the experiment began on the following day.

The experiment was conducted in 8 flow-through 9-litre glass aquaria, each with 180 warmed up fry (the stock density was 20 fry/l). The rearing tanks, placed in a common tempered tank, were parts of a closed re-circulation system. The water flow of 0.2 l/min and O₂ saturation of 95–100% ensured that the entire content of the experimental tanks was exchanged in 45-minute intervals. The re-circulation facility contained a sprinkling biological filter placed in a separate sedimentation tank. The basic physical parameters were controlled every day (Table 1 shows the average values, Figure 1 shows the curves of the values).

Table 1. Basic hydro-chemical parameters of culture conditions during 25 days of barbel larvae rearing

Parameters	M ± S _D
Water temperature (°C)	26.1 ± 0.3
O ₂ content (mg/l)	6.58 ± 0.2
O ₂ saturation (%)	80.4 ± 1.5
pH	7.42 ± 0.3

M = mean, S_D = standard deviation

In the experiment we established 4 feeding variants in two replications. In variant A we used commercial flaked feed with a proportion of dried artemia, recommended by the producer for aquarium fish. In variant B a starter mixture was fed, recommended by the producer for initial feeding of salmonids. The barbel fry in variant C was fed a dry

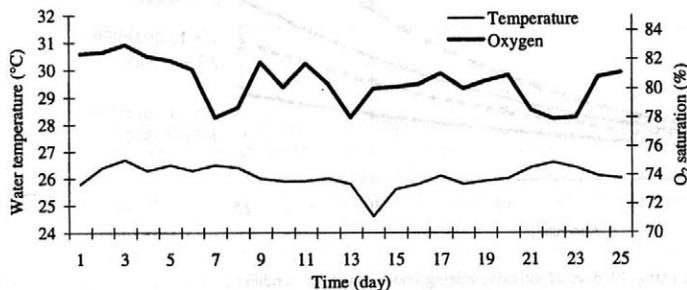


Figure 1. Water temperature and oxygen saturation level during 25 days of intensive barbel larvae rearing

Table 2. Nutritional characteristics and calculated total energy content of dry diets used during 25 days of barbel larvae rearing

Variables	A	B	C
Crude protein (%)	48.0	50.0	62.0
Fat (%)	7.0	12.0	11.0
Carbohydrates (%)	22.5	19.8	6.2
Dry weight (%)	92.0	92.0	90.0
Fibre (%)	7.0	2.2	0.8
Ash (%)	7.5	8.0	10.0
Total energy* (MJ/kg)	18.1	20.0	20.2

*Total energy content was calculated according to the coefficient of Jobling (1994)

starter mixture recommended by the producer for initial feeding of sea fish. In variant D the fry was initially fed live artemia in the nauplius stage (Table 2 gives the basic characteristics of the dry feed).

The dry feed was applied manually from 8 a.m. to 8 p.m. every two hours *ad libitum*. Retroactive calculations of the daily feed rations of starter feeds during the experiment ranged between 9.2 and 7.3% of the immediate total weight of the fish of the respective variants. The artemia were also fed from 8 a.m. to 8 p.m. but in 3-hour intervals. The daily feed ration decreased during the experiment from 50% to 25% of the immediate total weight of the fish of this variant.

In the course of the experiment, 30 fishes from each variant (containing 15 individuals from both replications) were sampled and fixed in 5-day intervals to define the growth rate. The fry samples were taken in the evening, 2 hours after feeding. The samples were exposed to a 4% solution of formaldehyde for 4 months and then the length and weight of the larvae, total body length (TL; measured to the nearest ± 0.25 mm) and individual weight ($w_i \pm 0.1$

mg) were determined. For orientation purposes the weight of larvae of the fish of variant D (artemia diet) was assessed immediately after killing to update the daily feed ration. The following calculations were used for the assessment of selected parameters:

Condition coefficient according to Fulton

$$C_F = \frac{w}{TL^3} \times 100$$

Specific weight growth rate

$$SWGR = \left[\left(\frac{w_2}{w_1} \right)^{\frac{1}{t}} - 1 \right] \times 100$$

The data on the morphometric parameters of the fish of the individual variants were statistically processed using the method of one-way variance analysis ANOVA.

RESULTS

The growth rate of larvae of the common barbel fed dry diets (SWGR = 5.98–8.67%/d) ranged between 60% and 85% of the value achieved with live food (SWGR = 10.22% per day, variant D). The parameters of the other studied production factors were similar and are given in Figure 2.

When live food was available (variant D), the survival rate of the barbel fry was high (99.7%) and a similar value was also recorded in variant A (97.6%). The survival rate of the other experimental variants was about 25% lower (73.8 and 77.9%, respectively – Table 3). Published data include only the natural mortality of larvae and do not include fish sampling. The mortality of the reared fry was observed from the 8th to the 24th day of the experiment (Figure 3). The daily mortality was the highest in variants B and C (3.7%/d) on the 15th and 16th day from the beginning of the uptake of dry feed.

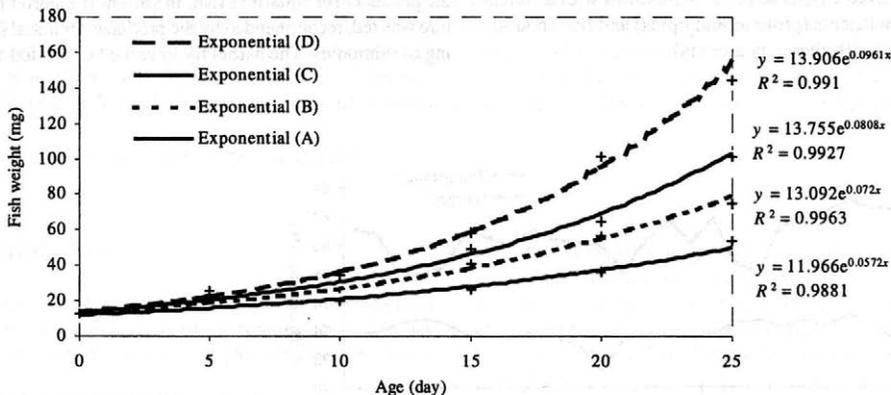


Figure 2. Intensive growth rate of barbel larvae during 25 days of intensive rearing under controlled conditions

Table 3. Values of selected parameters during a 25 days study period of intensive rearing of barbel larvae under controlled conditions

Variables	A	B	C	D
Beginning date – 7. 6. 1999				
Number of fish	360	360	360	360
Stocking weight (g)	3.81	3.81	3.81	3.81
Mean weight (mg)	12.7	12.7	12.7	12.7
Mean of total length (mm)	12.9	12.9	12.9	12.9
Termination date – 1. 7. 1999				
Number of fish	233	168	179	239
Stocking weight (g)	19.13	21.54	30.38	51.88
Mean weight (mg)	54.2	74.8	101.6	144.5
Mean of total length (mm)	17.8	19.6	21.0	25.7
Duration of experiment (day)				
	25	25	25	25
Survival rate (%)	97.6	73.8	77.9	99.7
Weight increment (mg)	41.5	62.1	88.9	131.8
Daily weight increment (mg/d)	1.66	2.48	3.56	5.27
Relative daily weight increment (%/d)	13.07	19.56	28.00	41.51
FCR	1.42	1.39	1.17	2.69
SWGR (%/d)	5.98	7.35	8.67	10.22

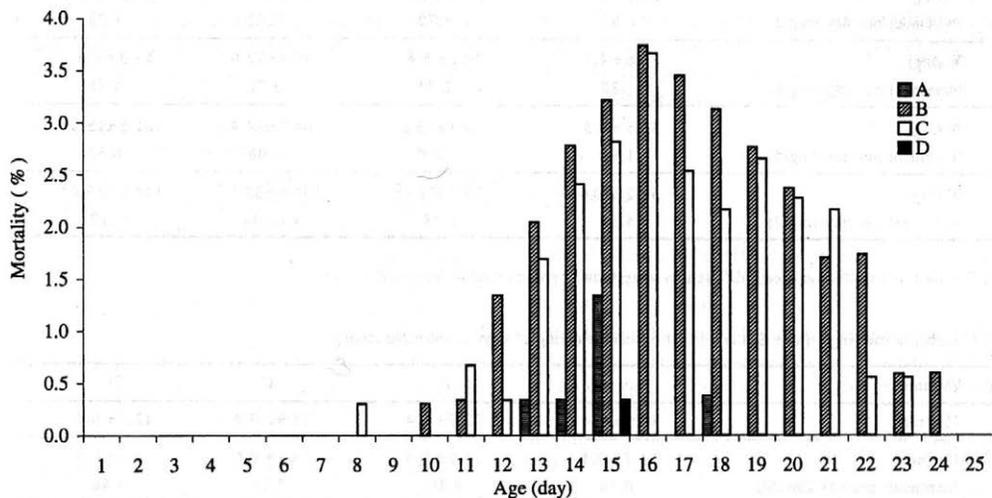


Figure 3. Relative mortality rate of barbel larvae during 25 days of intensive rearing using live food and dry diets

Tables 4–6 show the values of the basic morphometric data of the barbel larvae, including their statistical evaluation. Fish fed live artemia (variant D) showed the highest individual weight ($w = 144.5$ mg) and total body length (TL = 25.7 mm), but the lowest value of $C_f = 0.85$ (a highly significant difference in all parameters, $P < 0.01$). Comparisons of dry-diet-based feed variants showed that

fry of variant C (feed for sea fish) had the highest value of w (101.6 mg – statistically highly significant difference, $P < 0.01$), TL (21.0 mm) and C_f (1.02). Table 4 shows the length and weight parameters of barbel larvae defined at the beginning of the experiment and at its end. Tables 4 and 5 show data achieved in the course of the experiment.

Table 4. Basic length, weight parameters and Fulton coefficient of barbel larvae at the beginning of larval period and after 25 days of intensive rearing ($M \pm S_D$, V_x)

Variables		A	B	C	D
Beginning of experiment		End of experiment			
w (mg)	12.7 ± 0.9	54.2 ± 10.4 ^C	74.8 ± 21.4 ^C	101.6 ± 35.7 ^B	144.5 ± 19.0 ^A
V_x	7.1	19.2	28.7	33.0	13.1
TL (mm)	12.9 ± 0.4	17.8 ± 0.9 ^C	19.6 ± 1.5 ^B	21.0 ± 2.1 ^B	25.7 ± 1.2 ^A
V_x	2.8	4.8	7.7	11.7	4.6
C_F	0.59 ± 0.04	0.95 ± 0.07 ^B	0.96 ± 0.09 ^{AB}	1.02 ± 0.08 ^A	0.85 ± 0.04 ^C
V_x	6.1	7.9	9.5	8.0	4.3

$n = 30$

$M = \text{mean}$, $S_D = \text{standard deviation}$, $V_x = \text{coefficient of variation}$

A, B, C = no statistically significant differences were found between similar letters ($P < 0.01$)

Table 5. Barbel larvae weight ($M \pm S_D$) and weight increment during 25 days of intensive rearing

Day	Variables	A	B	C	D
1	W (mg)	12.7 ± 0.9	12.7 ± 0.9	12.7 ± 0.9	12.7 ± 0.9
5	W (mg)	16.0 ± 1.6	18.6 ± 2.0	21.3 ± 2.8	25.7 ± 2.0
	Increment per day (mg/d)	0.66	1.18	1.72	2.60
10	W (mg)	20.1 ± 1.7	27.2 ± 4.7	32.9 ± 6.6	34.6 ± 4.5
	Increment per day (mg/d)	0.82	1.72	2.32	1.78
15	W (mg)	26.6 ± 4.7	40.9 ± 8.4	49.4 ± 12.0	58.9 ± 6.3
	Increment per day (mg/d)	1.30	2.74	3.30	4.86
20	W (mg)	36.5 ± 7.5	56.4 ± 15.0	64.7 ± 24.4	101.5 ± 15.6
	Increment per day (mg/d)	1.98	3.10	3.06	8.52
25	W (mg)	54.2 ± 10.4 ^C	74.8 ± 21.4 ^C	101.6 ± 35.7 ^B	144.5 ± 19.0 ^A
	Increment per day (mg/d)	5.52	6.78	10.44	17.12

A, B, C = no statistically significant differences were found between similar letters ($P < 0.01$)

Table 6. Barbel larvae length ($M \pm S_D$) and length increment during 25 days of intensive rearing

Day	Variants	A	B	C	D
1	TL (mm)	12.9 ± 0.4	12.9 ± 0.4	12.9 ± 0.4	12.9 ± 0.4
5	TL (mm)	13.7 ± 0.4	13.9 ± 0.4	14.3 ± 0.6	15.2 ± 0.4
	Increment per day (mm/d)	0.16	0.20	0.28	0.46
10	TL (mm)	14.3 ± 0.5	15.3 ± 0.7	15.9 ± 0.7	17.1 ± 0.5
	Increment per day (mm/d)	0.12	0.28	0.32	0.38
15	TL (mm)	15.1 ± 0.6	16.7 ± 0.8	17.6 ± 0.9	19.6 ± 0.6
	Increment per day (mm/d)	0.16	0.28	0.34	0.50
20	TL (mm)	16.3 ± 0.8	18.2 ± 1.3	18.8 ± 1.8	22.3 ± 1.0
	Increment per day (mm/d)	0.24	0.30	0.24	0.54
25	TL (mm)	17.8 ± 0.9 ^C	19.6 ± 1.5 ^B	21.0 ± 2.1 ^B	25.7 ± 1.2 ^A
	Increment per day (mm/d)	0.54	0.58	0.68	1.22

A, B, C = no statistically significant differences were found between similar letters ($P < 0.01$)

DISCUSSION

The growth rate of fry during rearing in controlled conditions is primarily influenced by the water temperature (Jobling, 1985). The present results were achieved in water of a temperature of 26–28°C, in conditions that Wolnicki and Górný (1993) considered to be optimal for intensive initial feeding of barbel. If live food was used (variant D), the average individual weight of barbel after 15 days of initial feeding ($w = 58.9$ mg) was comparable with the weight published by Wolnicki and Górný (1993), i.e. $w = 66.3$ mg, achieved in water of a temperature of 26°C, but lower than Wolnicki and Górný (1995) achieved in similar conditions, i.e. $w = 95$ mg. The difference was probably caused by a lower intensity of feeding in our experiment. Here the values of the daily feed ration after re-calculation did not exceed 50% of the weight of the stock compared with the feed ration of 80% of the weight of the stock applied by the above-mentioned authors. The low mortality rate of barbel in the larval stage of ontogenesis when feeding natural feed (0.3%, $n = 360$ individuals) corresponds with literary data (Wolnicki and Górný, 1993, 1995).

The average individual weight of the fry during initial feeding based on dry feed ranged between 26.6 and 49.4 mg after 15 days of rearing. In the same period Wolnicki and Górný (1995) fed ALLER Krystal 1 and Ekvizo 1 and reported similar results ($w = 34$ and 36 mg, respectively) and the survival rate of larvae 73 and 99%, respectively. In our experiments the survival rate of barbel larvae fed a dry diet was 80–98% during 25 days. Fry mortality was monitored from the 8th to the 24th day from the beginning of feeding dry diets and culminated on the 15th and 16th days. In starving barbel fry reared at a temperature of 20–22°C Peňáz (1971) found that larval mortality began on the 9th day and the total mortality of larvae on the 21st day of the larval period of ontogenesis (achieving 563 D^o). From this point of view it appears that mortality should be monitored at least until the 25th day after the beginning of exogenous feeding of the fry when it is quite evident that all endogenous reserve substances have been resorbed.

An important factor affecting the growth rate of fish larvae fed on the basis of dry diets is the content of proteins in the feed ration (Wilson, 1989). In our experiment the highest SWGR (i.e. 8.67%/d) was achieved in the variant where a feed ration containing 62% of proteins was fed. A similar proportion of proteins (61%) is contained in the dry matter of live diets of the firm O.S.I Marine Lab., Inc. The growth rate of the barbel was 30% and 15% lower if the protein content in dry diets was 48% and 50%, respectively.

The C_F value of the barbel fry fed dry diets was statistically highly significantly higher ($P < 0.01$) than that of fry fed live diets (0.95–1.02 and 0.85, respectively). The level of metabolised energy of the feed apparently affected the

condition coefficients of the barbel fry. Paffenhöfer (1967) observed that the energy value of live artemia was lower than dry starter feeds (Jobling, 1994). The energy content of live food is insufficient to ensure a high growth rate of rheophile fish species under intensive initial feeding of the fry. Labatzki and Fuhrmann (1992), who used combined diets of live artemia and a starter feed ration in extensive initial feeding of barbel fry, confirmed this opinion. In their experiment in 21°C water the individual weight of barbel larvae at the age of 19 days was 220 mg.

Our present and previously published results proved that barbel fry could be successfully fed dry diets during intensive rearing. At the beginning of the larval stage, the barbel shows signs of a relatively advanced ontogenetic development (compared to other members of the Cyprinidae family) and its organism accepts the commercial starter feed of suitable size and composition.

In the case of intensive rearing of barbel fry based on a dry diet in water of more than 25°C, feed rations containing approximately 60% of proteins should be fed. In this water temperature the lower protein content in the diet could depress the growth of the barbel larvae. It is however necessary to further increase and verify information about the optimal content and suitable form of specific effective substances in the recommended dry starter feeds. The solution of this issue will result in a lower mortality rate of the barbel fry in the crucial period between the 10th and 20th day of the larval period of ontogenesis when rearing barbel fry on the basis of complete dry diets.

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Influence of dietary vitamin E supplementation on antioxidative status in muscle and meat quality of pigs

Vplyv prídavku vitamínu E v krmive na antioxidačnú kapacitu svalu a kvalitu mäsa ošípaných

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ABSTRACT: The effect of feeding a high level of vitamin E on the antioxidative status of *longissimus dorsi* muscle and meat quality of pigs was investigated. Treatments consisted of supplementation of vitamin E (200 mg/kg diet) for the last 60 days to finishing pigs before slaughtering. *Longissimus dorsi* (LD) muscle was examined after 2 and 5 days of storage for colour (reflectance), drip loss and shear force. α -Tocopherol level, total protein, intramuscular fat and pH (45 min *post mortem*) were also determined. Lipid peroxidation (thiobarbituric acid reactive substances – TBARS – measured as malondialdehyde equivalents) in 5 and 6 days after thawing of muscle samples and rate of oxidation by stimulation with Fe^{2+} /ascorbate were estimated in LD samples. α -Tocopherol level was higher ($P < 0.05$) in LD from pigs treated with higher vitamin E than in controls (1.71 vs. 2.73 mg/kg). There were observed tendencies to lower drip losses and higher shear force in LD pigs supplemented with vitamin E. Inhibition of TBARS during storage (frozen and thawed) and rate of oxidation were positively influenced by vitamin supplementation. We concluded that dietary vitamin E supplementation (200 mg/kg diet) to pigs during the last 60 days of finishing increases the α -tocopherol concentration of tissue, reduces lipid peroxidation and improves the antioxidative status of muscle tissue.

Keywords: pigs; vitamin E; α -tocopherol; lipid peroxidation; meat quality

ABSTRAKT: Cieľom práce bolo zistiť vplyv prídavku vitamínu E (α -tokoferol acetát) v krmive na obsah α -tokoferolu vo svalu, antioxidáciu a kvalitu mäsa ošípaných. Heterozygotné krížence biela mäsová \times pietrain na výskyt malígnej hypertermie (DNA test) sa rozdelili na skupinu kontrolnú ($n = 12$) a pokusnú ($n = 10$) s rovnakým zastúpením prasničiek a bravcov. Aplikácia vitamínu E (200 mg/kg) v krmive sa robila 60 dní pred zabitím. Po zabití sa vo vzorkách svalu (*m. longissimus dorsi* – LD) stanovili hodnoty pH (45 min), farby, straty odkvapom a strižnej sily (2. a 5. deň). Ďalej sa vo vzorkách LD stanovil obsah celkových bielkovín, intramuskulárneho tuku (Infratec) a obsah α -tokoferolu (HPLC). Peroxidačná stabilita (obsah látok typu malondialdehyd po reakcii s kyselinou tiobarbiturovou) sa zisťovala na 5. a 6. deň z homogenátu rozmrazeného svalu LD po stimulovaní oxidácie inkubáciou v zmesi Fe^{2+} /askorbát. Zistili sme vyšší ($P < 0.05$) obsah α -tokoferolu (1,71 vs. 2,73 mg/kg) v LD ošípaných kŕmených so zvýšeným prídavkom vitamínu E. Zistené tendencie nižších hodnôt strát odkvapom a vyšších hodnôt strižnej sily v LD ošípaných so zvýšeným prídavkom vitamínu E poukazujú na stabilizujúci efekt vitamínu E na membrány svalového tkaniva. Významné zníženie ($P < 0.05$) obsahu látok reagujúcich s kyselinou tiobarbiturovou (vyjadrených ako obsah malondialdehydu) v homogenáte svalu LD po stimulácii oxidácie s Fe^{2+} /askorbát poukázalo na zvýšenie antioxidácie kapacity svalu. Aplikácia zvýšeného prídavku vitamínu E (200 mg/kg) do krmiva 60 dní pred zabitím ošípaných má pozitívny vplyv na zvýšenie obsahu α -tokoferolu vo svalu (*m. longissimus dorsi*), zníženie oxidácie lipidov a zvýšenie antioxidácie kapacity svalu.

Kľúčové slová: ošípané; vitamín E; α -tokoferol; peroxidácia lipidov; kvalita mäsa

INTRODUCTION

Dietary supplementation of vitamin E increases the concentration of α -tocopherol in muscle and reduces the susceptibility of the muscle to lipid oxidation (Buckley *et al.*, 1995). The recommended level of α -tocopheryl acetate supplementation in growing pigs is 15 to 40 mg/kg of feed (Albers *et al.*, 1984), however, some meat quality parameters and oxidative stability improve when dietary α -tocopheryl acetate levels are higher (200 mg/kg) as was shown by several researchers (Asghar *et al.*, 1989; Buckley *et al.*, 1995; Monahan *et al.*, 1992; Turek *et al.*, 1999; Lahučký *et al.*, 2000). Lipid oxidation may be delayed by the addition of antioxidants. Vitamin E is accepted as an effective lipid-soluble free-radical scavenger in biological systems (Morrissey *et al.*, 1994). Inclusion of polyunsaturated fatty acids (PUFA) in diets increases the risk for lipid oxidation in the muscle and the carcass (Jakobsen, 1995). Oxidation of PUFA in cell membranes leads to disruption of normal membrane structure and function (Storrey, 1996). Vitamin E and antioxidant enzymes present in skeletal muscles are able to inhibit lipid oxidation in muscle foods. During the post-slaughter metabolism of muscles the process of lipid oxidation need no longer be tightly controlled due to weakness of the antioxidative defence system, and this may affect meat quality traits (Lauridsen *et al.*, 1999).

The aim of this study was to examine the effects of dietary α -tocopheryl acetate supplementation on α -tocopherol content, antioxidative status and meat quality in pigs.

MATERIAL AND METHODS

Twenty-two Slovak White Meaty \times Pietrain crosses were used in this experiment. Control group C ($n = 12$) and experimental group E ($n = 10$) were heterozygotes on malignant hyperthermia with equal number of gilts and castrates. The genotype (mutation on ryanodine gene) was determined by a DNA based test described by Lahučký *et al.* (1997). The pigs were penned in double boxes at Institute (RIAP) facilities to minimise the influence of stress. Control group was fed a diet supplemented with basal level of α -tocopherol (Tables 1 and 2). Experimental group

Table 1. Gross composition of diet (%)

Item	(%)
Wheat middlings	35
Barley	32
Soybean meal	16
Oat	8
Wheat meal	4
Mineral and vitamin mix	3
Meat and bone meal	2

Table 2. α -Tocopherol (mg/kg) content and chemical composition (g/kg) of diet

α -Tocopherol	37.9
Crude protein	175
Lipids	23
Crude fiber	46.2
Ash	82.1
Lysine	9.1
Methionine	2.8
Treonine	6.4
Tryptophane	2.2

received a supplemental level of α -tocopherol (200 mg/kg) for 60 days before slaughter. Vitamin E was provided by Slovakofarma company (Hlohovec, Slovakia).

Animals were stunned, slaughtered and exsanguinated in the slaughter house of RIAP Nitra (transportation about 200 m) with average live weight of 113 kg. *Longissimus muscle* samples were collected immediately after exsanguination, frozen in liquid nitrogen and stored at -70°C until used for estimation of oxidative stability. After slaughter, the carcasses were chilled at 4°C for 24 h, then *longissimus dorsi* (LD) muscle was removed from each carcass. A portion of the sample was used immediately (48 h) and the remaining sample was wrapped in aluminium film and stored in a refrigerator at 4°C for 5 days. A portion of LD was also frozen in liquid nitrogen and stored until analysed.

The concentration of vitamin E (α -tocopherol) in muscle was measured by HPLC (Berlin *et al.*, 1994) earlier described in detail (Lahučký *et al.*, 2000). Lipid oxidation was assessed by the 2-thiobarbituric acid method of Salih *et al.* (1987). For evaluating the peroxidative stability of *longissimus* homogenates the determination of thiobarbituric acid reactive substances (TBARS) was used. TBARS were expressed in terms of malondialdehyde, a breakdown product formed during peroxidation stimulated by Fe^{2+} /ascorbate. Protein content of homogenates was estimated by a modified biuret method.

The pH value of the carcass (*longissimus* – between 13th and 14th rib) was determined in 45 min post mortem using the combined pH electrode (Ingold). Colour changes after refrigerated storage were measured on the freshly cut surface of the sample by means of spectrophotometer (Specol, Germany) at 580 nm as external reflectance. Total protein and intramuscular fat were measured by the Infracal-Analyser. Drip loss analysis was made according to Honikel (1998). Shear force was determined with Warner-Bratzler (W-B) apparatus. The experiments were in accordance with the institutional guidelines for animal care (Research Institute of Animal Production, Nitra, 1999).

Statistical analyses were calculated as mean values and standard deviations and differences were evaluated by

t-test. Regressions were calculated between vitamin E level and the values of malondialdehyde in muscle homogenate after peroxidation stimulation.

RESULTS AND DISCUSSION

The content of vitamin E (α -tocopherol) of the *longissimus dorsi* muscles was determined and the results are presented in Table 3. The levels of vitamin E in muscles were found to be higher in the group of pigs supplemented with vitamin E ($P < 0.05$). The amount of vitamin E in *longissimus dorsi* in the present study when pigs received vitamin E 200 mg/kg feed is comparable with previously reported results (Buckley *et al.*, 1995; O'Sullivan *et al.*, 1997; Wen *et al.*, 1997; Honikel *et al.*, 1998) but higher as was reported earlier (Lahučký *et al.*, 2000) when the basal level of vitamin E in feed of control group was lower (10 mg/kg). It is believed that incorporating vitamin E into subcellular membranes increases the antioxidative status of the system and possibly also increases their physical stability (Buckley *et al.*, 1995).

The effect of vitamin E supplementation on meat quality values depends on the muscle concerned (Den Hertog-Meischke *et al.*, 1997). The pH value in *longissimus dorsi* muscle as follows from Table 3 was not influenced, which is in agreement with others (Honikel *et al.*, 1998; Turek *et al.*, 1999; Lahučký *et al.*, 2000). There was a tendency of improving drip loss in *longissimus dorsi* in 24 h post mortem but the differences were not significant ($P > 0.05$). Using a higher level of vitamin E supplementation (500 mg/kg diet) administered for 46 days could reduce a drip loss in unfrozen *longissimus thoracis* in heterozygotes and in normal on malignant hyperthermia pigs as was shown by Cheah *et al.* (1995). However, there are contra-

dictory results about positive effects of vitamin E supplementation on drip loss (Buckley *et al.*, 1995), or the amount of fluid in muscles (Lauridsen *et al.*, 1999). Some researchers were not able to find any significant differences between the drip loss of supplemented and control porcine muscle (Jensen *et al.*, 1997; Honikel *et al.*, 1998) as was also discussed earlier (Lahučký *et al.*, 2000). The tendency of a higher level of shear force value in pigs supplemented with vitamin E as follows from our results (Table 3) could influence the aging time of meat. There were reported contradictory results on the shear force value (tender-

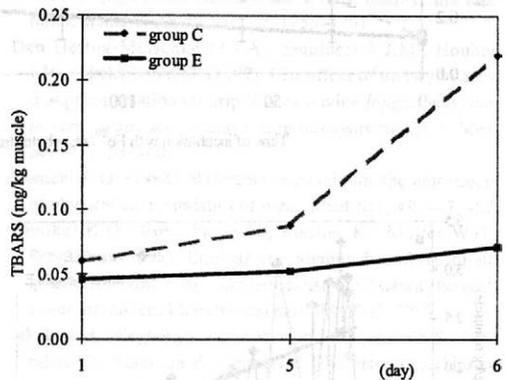


Figure 1. Effect of dietary vitamin E supplementation on thiobarbituric acid reactive substances (TBARS) during storage of *longissimus dorsi* at 4°C

Group C = control group (vitamin E 37.9 mg/kg diet)

Group E = group with vitamin E supplementation (vitamin E 200 mg/kg diet)

Table 3. α -tocopherol content and biophysical traits of *longissimus dorsi* muscle

Trait	Group C (n = 12)		Group E (n = 10)		
	mean	SE	mean	SE	
α -Tocopherol (mg/kg)	1.71	0.32	2.73 ⁺	0.26	
pH	45 min	6.18	0.17	6.22	0.11
Reflectance (580 nm)	24 h	24.57	1.68	23.83	1.84
Total protein (%)	24 h	23.69	0.22	23.49	0.33
Intramuscular fat (%)	24 h	1.84	0.54	1.64	0.26
Drip loss (%)	24 h	4.98	1.19	4.17	0.73
Shear force (N)	24 h	5.30	0.88	6.05	0.53
Reflectance (580 nm)	5 day	25.14	2.23	26.08	4.24
Drip loss (%)	5 day	8.94	1.40	8.48	1.61
Shear force (N)	5 day	4.51	1.06	5.20	0.98

⁺P < 0.05

Group C = control group (vitamin E 37.9 mg/kg diet)

Group E = group with vitamin E (supplementation vitamin E 200 mg/kg diet)

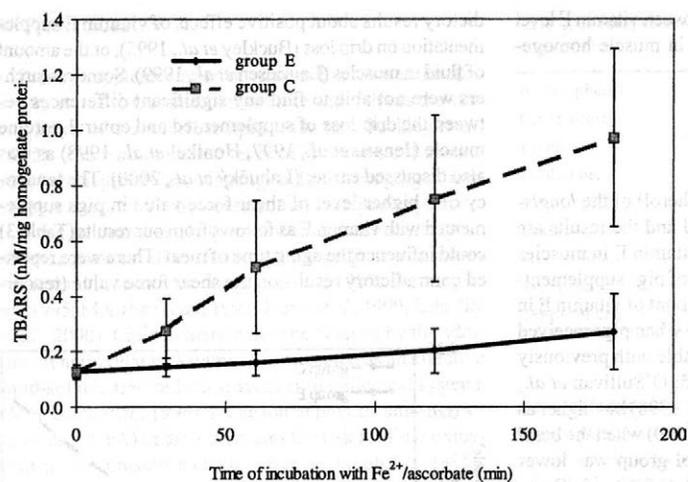


Figure 2. Effect of dietary vitamin E supplementation on the antioxidant stability of *longissimus dorsi* (incubation of protein homogenate with Fe²⁺/ascorbate)

Group C = control group (vitamin E 37.9 mg/kg diet)
 Group E = group with vitamin E supplementation (vitamin E 200 mg/kg diet)

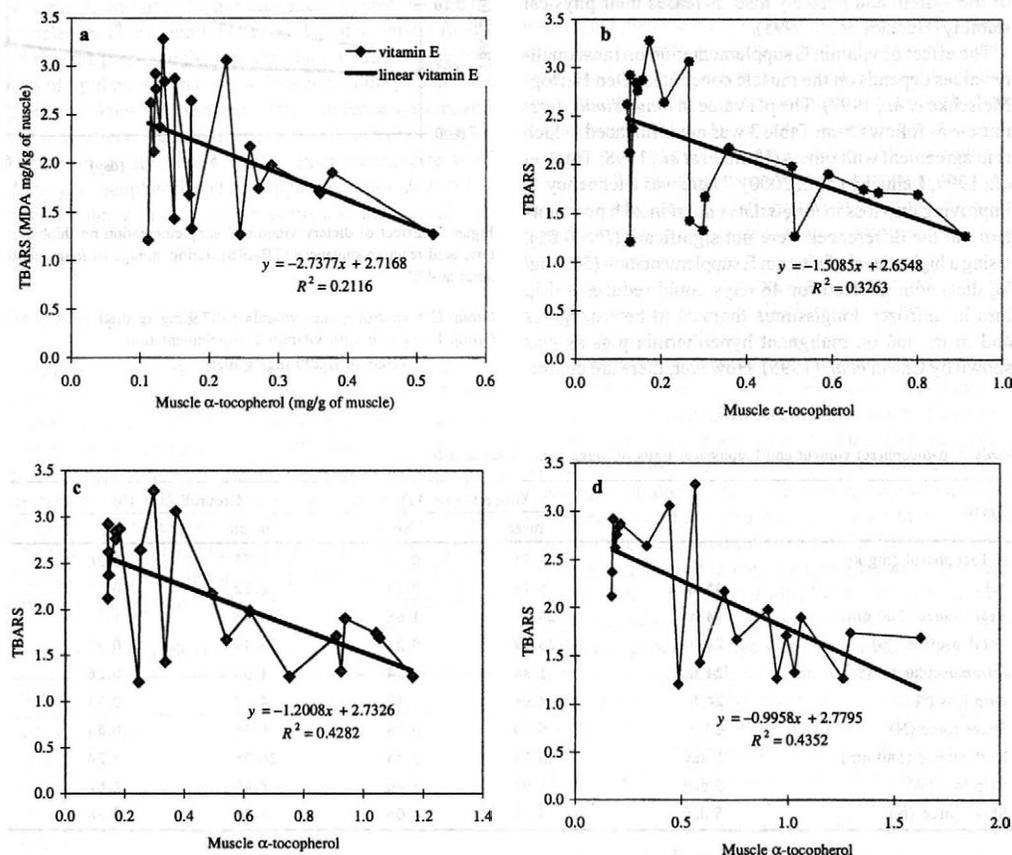


Figure 3. Relationship between muscle α -tocopherol and TBARS in 30 (a), 60 (b), 120 (c), 180 (d) minutes of incubation with Fe²⁺/ascorbate

ness of meat) after application of vitamin E (Cannon *et al.*, 1996; Mitsumoto *et al.*, 1995; Maiorano *et al.*, 1999). Recently, Montgomery *et al.* (2000) reported that dietary vitamin D₃ given daily for 9 days before slaughter improved the tenderness (lower W-B shear force values) of 14-days post mortem aged beef, and in order to optimise the “colour-tenderness relationship” of beef, supplementation of both vitamin E and D₃ to finishing cattle is recommended (Vargas *et al.*, 1999).

Another finding was that vitamin E supplementation at the levels 200 mg/kg of diet improved the carcass oxidation stability (Figures 1 and 2). After 6 days of storage (at 4°C) of thawed muscle samples, thiobarbituric acid reactive substances (TBARS) expressed as malondialdehyde value (mg/kg of muscle) were reduced in the experimental group (Figure 1), which agreed with several studies (Monahan *et al.*, 1992; Corino *et al.*, 1999). It is generally thought that the incorporation of α -tocopherol via the diet is probably due to the stabilisation of membrane lipids (Monahan *et al.*, 1992). It follows from Figure 2 that the rate of iron-induced lipid oxidation of LD homogenate was strongly influenced by dietary vitamin E, which agreed with earlier results (Lauridsen *et al.*, 1999; Nürnberg *et al.*, 2000; Krška *et al.*, 2001). There was an inverse relationship between TBARS levels in iron-induced muscle homogenate and α -tocopherol in muscle (Figure 3). The highest values ($R^2 = 0.436$) were obtained at 180 min incubation time. The results are comparable with findings of correlation between α -tocopherol in muscle and TBARS values after 6 days of storage reported by Corino *et al.* (1999). The authors showed a higher correlation (0.66) between TBARS and plasma α -tocopherol. We concluded that dietary vitamin E supplementation (200 mg/kg diet) to pigs during the last 60 days of finishing increases the α -tocopherol concentration of tissue, reduces lipid peroxidation and improves the antioxidative status of muscle tissue.

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