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Dynamics of biochemical indices of blood plasma in adolescent male breeder turkeys

Změny biochemických ukazatelů krevní plazmy u plemenných krocanů v období pohlavního dospívání

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ABSTRACT: Investigations into dynamics of biochemical indices, carried out in 24 male breeder turkeys (LW hybrids), covered the period from 10th to 30th week of life. The results, summarised in tabular form, indicate variations in mean blood plasma concentrations of protein in the range 33.87–40.40 g/l ($P \leq 0.01$) and of glucose in the range 14.96–17.50 mmol/l ($P \leq 0.01$), and increases in concentrations of cholesterol from 2.29 to 3.91 mmol/l ($P \leq 0.01$) and of calcium from 2.40 to 2.75 mmol/l ($P \leq 0.01$). Concentration of phosphorus in blood plasma decreased from 3.10 to 1.57 mmol/l ($P \leq 0.01$) while concentrations of magnesium were relatively stable varying between 0.88 and 0.80 mmol/l.

Keywords: turkey; sexual maturation; blood plasma; biochemical indices

ABSTRAKT: Práce shrnuje výsledky biochemického vyšetření souboru 24 klinicky zdravých krocanů hybridní kombinace LW v období pohlavního dospívání. Závěry práce poukazují na skutečnost, že s věkem plemenných krocanů kolísala průměrná hladina plazmatické bílkoviny od 33,87 do 40,40 g/l ($P \leq 0{,}01$), plazmatické glukózy z 14,96 mmol/l na 17,50 mmol/l ($P \leq 0{,}01$) a vzrůstala hladina plazmatického cholesterolu z 2,29 mmol/l na 3,91 mmol/l ($P \leq 0{,}01$). Z ukazatelů minerálního metabolismu se s věkem plemenných krocanů snižovala hladina plazmatického fosforu z 3,10 na 1,57 mmol/l ($P \leq 0{,}01$) a zvyšovaly se hladiny plazmatického vápníku z 2,40 mmol/l na 2,75 mmol/l ($P \leq 0{,}01$–$0{,}05$). Jako nejstabilnější se jevily hladiny plazmatického hořčíku, které se v průběhu sledovaného období pohybovaly v rozmezí od 0,88 do 0,80 mmol/l.

Klíčová slova: krocan; pohlavní dospívání; biochemické ukazatele krve

INTRODUCTION

Results of comprehensive studies of haematological and selected biochemical indices of blood plasma during the rearing and laying periods of Japanese quail were published by Straková *et al.* (1994).

Haematological and biochemical investigations of blood and blood plasma in chicken broilers during the fattening period were carried out by Straková *et al.* (1993).

Haematological changes in male breeder turkeys during the period of sexual maturation were described by Straková *et al.* (1996).

Suchý *et al.* (1995) summarised results of biochemical tests in clinically normal female turkeys during the fattening period.

Relationships between biochemical indices, nutrition, age, and performance were studied, among other authors, by Kredatus and Valenta (1983), Aletor (1989) and Pisarskij (1988).

The objective of our study, which covered the period of sexual maturation, was to extend and complete the current knowledge of the basic biochemical indices in male breeder turkeys.

MATERIAL AND METHODS

The investigations were carried out on 24 clinically normal male Large White hybrids with approximately the same live weights. The birds were kept on litter of hardwood shavings and had free access to the complete diet KR2 intended for turkey rearing and fattening, which contained 220 g crude protein and 12 MJ metabolisable energy per 1 kg.

Blood samples for biochemical tests were collected from vena basilica into heparinised test tubes at 5-week intervals from the 10th to the 30th week of life always between 08.00 and 09.00 a.m.

Analyses for blood plasma concentrations of total protein, glucose, and cholesterol were done using the automatic analyser COBAS MIRA S (La Rocha); calcium and magnesium concentrations were determined by atomic absorption using the Atomspek apparatus (Hilger).

The results were processed using the Student's test as described by Venčikov and Venčikov (1977).

RESULTS

All results of chemical analyses are summarised in Table 1. Mean total protein concentrations in blood plasma varied during the period of sexual maturation between 33.87 g/l (week 20) and 40.40 g/l (week 25). All between-week differences were highly significant ($P \leq 0.01$).

Mean glucose concentrations in blood plasma varied during the whole observation period between 14.96 mmol/l (week 25) and 17.50 mmol/l (week 10). Highly significant differences ($P \leq 0.01$) were observed between weeks 20 and 25 (17.04 vs. 14.96 mmol/l) and between weeks 25 and 30 (14.96 vs. 16.45 mmol/l).

Mean cholesterol concentrations in blood plasma increased from 2.29 mmol/l (week 10) to 3.91 mmol/l (week 25) and subsequently decreased to 3.42 mmol/l (week 30). All between-week differences were highly significant ($P \leq 0.01$).

Mean phosphorus concentration in blood plasma decreased during the observation period from 3.10 mmol/l (week 10) to 1.57 mmol/l (week 30). Between-week differences were significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$).

Table 1. The values of total plasma protein, glucose, cholesterol, phosphorus, calcium and magnesium including statistical processing

Index	Age (weeks)	A	X	s_{n-1}	S_X	v	T_d
Total protein (g/l)	10	24	37.22	5.557	1.134	14.93	
	15	24	38.37	4.133	0.844	10.77	0.814
	20	24	33.87	4.301	0.878	12.70	3.695**
	25	24	40.40	5.345	1.091	13.23	4.663**
	30	24	33.99	3.427	0.699	10.08	4.947**
Glucose (mmol/l)	10	24	17.50	1.378	0.281	7.87	
	15	24	16.48	2.558	0.522	15.52	1.720
	20	24	17.04	1.669	0.341	9.79	0.898
	25	24	14.96	1.616	0.330	10.80	4.383**
	30	24	16.45	1.317	0.269	8.01	3.500**
Cholesterol (mmol/l)	10	24	2.29	0.417	0.085	18.21	
	15	24	2.77	0.448	0.091	16.17	3.855**
	20	24	3.15	0.478	0.098	15.21	2.841**
	25	24	3.91	0.676	0.138	17.29	4.490**
	30	24	3.42	0.296	0.060	8.65	3.256**
Phosphorus (mmol/l)	10	24	3.10	0.873	0.178	28.16	
	15	24	2.23	0.237	0.048	0.63	4.719**
	20	24	2.10	0.201	0.041	9.52	2.059*
	25	24	1.81	0.209	0.043	11.55	4.881**
	30	24	1.57	0.102	0.021	6.50	5.015**
Calcium (mmol/l)	10	24	2.40	0.220	0.045	9.17	
	15	24	2.52	0.084	0.017	3.33	2.494*
	20	24	2.67	0.142	0.029	5.32	4.462**
	25	24	2.75	0.055	0.011	2.00	2.579*
	30	24	2.75	0.068	0.014	2.47	0.000
Magnesium (mmol/l)	10	24	0.88	0.098	0.020	11.14	
	15	24	0.88	0.069	0.014	8.85	4.096**
	20	24	0.80	0.066	0.013	8.25	1.047
	25	24	0.83	0.073	0.015	8.80	1.511
	30	24	0.83	0.059	0.012	7.11	0.000

*$P \leq 0.05$, **$P \leq 0.01$

On the other hand, mean calcium concentrations in blood plasma of male breeder turkeys increased from 2.40 mmol/l (week 10) to 2.75 mmol/l (week 25). Between-week differences were significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$). No change was observed between weeks 25 and 30.

Mean concentrations of magnesium in blood plasma of male turkeys varied in a very narrow range of 0.80 mmol/l (week 20) to 0.88 mmol/l (weeks 10 and 15).

DISCUSSION

The results of biochemical studies of the blood of male breeder turkeys during the period of sexual maturation demonstrated age-dependent changes in some of the indices under study. Accuracy of the results is warranted by the number of birds in each age group.

Mean concentrations of total blood plasma proteins varied highly significantly in the range 33.87 to 40.40 g/l during the period of sexual maturation. The most marked increase (to 40.40 g/l) was observed in week 25. Similar protein concentrations in blood plasma of turkeys were reported by Suchý *et al.* (1995).

Mean concentrations of glucose in blood plasma were markedly higher than those found by Suchý *et al.* (1989) in adolescent male breeder turkeys.

Mean cholesterol concentrations in blood plasma of adolescent turkeys increased significantly from 2.29 to 3.91 mmol/l. We assume that this increase was associated with the development of gonadal organs and onset of sexual activity. Our results are consistent with those reported by Straková *et al.* (1993) for chicken broilers.

The obtained mean phosphorus concentrations in blood plasma indicate a significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$) decrease from 3.10 mmol/l (week 10) to 1.57 mmol/l (week 30). On the other hand, mean calcium concentrations increased significantly ($P \leq 0.05$) or highly significantly ($P \leq 0.01$) from 2.40 mmol/l (week 10) to 2.75 mmol/l (weeks 25 and 30) and mean magnesium concentrations varied in a very narrow range of 0.80–0.88 mmol per l. The latter data are consistent with the results reported by Suchý *et al.* (1995) for fattened female turkeys (Ca 2.47 to 3.40 mmol/l; Mg 0.69 to 0.88 mmol/l). Higher calcium and magnesium concentrations were found by Straková *et al.* (1994) in Japanese quail during the rearing (Ca 2.51 to 3.58 mmol/l; Mg 0.93 to 1.36 mmol/l) and laying (Ca 5.61 to 7.80 mmol/l; Mg 1.48 to 1.82 mmol/l) periods.

Our investigations demonstrated changes in some biochemical indices of blood plasma in male breeder turkeys during the period of sexual maturation, yielded valuable information on the metabolic profile in individual weeks of adolescence, which is one of the most significant periods of the development of male breeder turkeys, and revealed a close similarity of the biochemical indices under study between poultry species.

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Effect of selected factors on sports performance of the Czech warm-blooded horse

Působení vybraných faktorů na sportovní výkonnost českého teplokrevníka

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ABSTRACT: Altogether 145 409 results of 6 846 horses from jumping tournaments taking place in riding seasons 1991–1998 were used to evaluate the sports performance of the Czech warm-blooded horse breed. Their jumping performance was expressed on the basis of the total sum of penalty points received in each tournament. Selected fixed effects – sex, age, year of start, degree of difficulty and random effects – competition, breeder, type of stud, rider and permanent environment on the sports performance of the Czech warm-blooded horse breed were evaluated using a linear model equation. The proportion of explained variability of the respective factors was 23.8% for the effect of sex, 14.5% for age, 36.4% for the degree of difficulty of the competition, 23.7% for the competition, 0.5% for the breeder, 0.0% for the type of stud, 6.8% for the rider and 1.5% for permanent environment.

Keywords: horse; Czech warm-blooded horse; jumping performance; BLUP animal model

ABSTRAKT: Pro posouzení míry vlivu vybraných efektů na sportovní výkonnost českého teplokrevníka bylo použito 145 409 výsledků 6 846 koní ze skokových soutěží jezdeckých sezon 1991 až 1998. Skoková výkonnost byla vyjádřena počtem získaných trestných bodů v soutěžích. V modelové rovnici byly zohledněny vlivy zvolených pevných efektů – pohlaví, věku, roku startu a stupně obtížnosti soutěže a náhodných efektů – soutěže, chovatele, typu chovu, jezdce a trvalého prostředí na sportovní výkonnost českého teplokrevníka. Procento vysvětlené proměnlivosti sledovaných faktorů je 23,8 pro efekt pohlaví, 14,5 pro věk, 36,4 pro stupeň obtížnosti soutěže, 23,7 pro soutěž, 0,5 pro efekt chovatele, 0,0 pro typ chovu, 6,8 pro efekt jezdce a 1,5 pro efekt trvalého prostředí.

Klíčová slova: kůň; český teplokrevník; skoková výkonnost; BLUP animal model

INTRODUCTION

The main breeding objective of the Czech warm-blooded horse breed is to produce sports horses with good performance, above all in jumping. Similarly like other types of performance jumping capabilities are also influenced by a complex of factors of both genetic and non-genetic nature. All authors interested in methods of breeding value estimation study characteristics of fixed and random systematic environmental factors influencing performance of horses in tournaments and try to eliminate effects of non-genetic nature. Veldhuizen (1997) involved fixed effects of age and sex in his model equation while Foran *et al.* (1995) considered fixed effects of performance class and sex; effect of age was evaluated as linear regression and those of animal and environment as random. Tavernier (1987) specified effects of age, sex, year of start, maternal effect and common effect of environment that involved, among others, also the effect of rider. Janssens *et al.* (1998) studied effects of age, sex, year of start, tournament and permanent environment. Bruns (1981) followed fixed effects of the year of father's birth, breeding organisation, age, sex, year of start and competition category while Hassenstein *et al.* (1996) studied fixed effects of tournament, sex, age, rider category, tournament category, and number of starts in the tournament as well as random effects of environment and animal. Similarly, Meinardus and Bruns (1989) and Bruns (1990) analysed fixed effects of sex, age, year of start (riding season), region and rider category.

Jiskrová (1996), Jiskrová and Misař (1997) investigated effects of selected factors on sports performance of Czech

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warm-blooded horses and characterised effects of sex, breeding organisation, breeding group (defined on the basis of pedigree analysis), and number of starts (linear regression) and tried to estimate their effects on performance of horses. This analysis involved 3 628 sports horses starting in Czech tournaments in the period of 1985–1994. All effects under study showed a statistically significant effect on sports performance of horses.

Pellarová (1986) elaborated an official system of evaluation of sports performance of horses in the Czech Republic. She based her calculation on the number of penalty points obtained in tournaments and converted them by means of a matrix involving the coefficient of tournament difficulty to the so-called ancillary points. These ancillary points were thereafter divided by the number of starts so that the effects of the number of starts and degree of difficulty of the tournament could be eliminated. Using the average of ancillary points per one start of horse (Average of Ancillary Points – AAP) a ladder table of results of sports horses has been issued every year (Pellarová and Dyková, 1999). These AAP are also used for the progeny test of stallions which enables to define the so-called Absolute and Relative Value of Sports Performance of each stallion (AVSP, RVSP). Both of them are based on a comparison of progeny performance.

In 1986, a system of evaluation of sports performance of Czech horses was introduced and its d-base enabled to increase the scope of followed factors (Jiskrová, 1996). The evaluation of these factors is dealt with in this paper within the framework of the grant project GAČR, reg. number 523/98/P009.

MATERIAL AND METHODS

The d-base used in this study involved results of jumping tournaments from the seasons 1991–1998. For each horse, the following factors were used: name, number of licence; year of birth; sex (1 – stallion, 2 – mare, 3 – gelding); pedigree (sire, dam, dam's sire); year of start (riding seasons); number of tournaments; degree of difficulty (Z – 1 m, ZL – 1.1 m, L – 1.2 m, S – 1.3 m, ST – 1.4 m, T – 1.5 m, TT – 1.6 m); name of breeder; number of stud (1 – English Thoroughbred, 2 – breeding stud, 3 – propagation stud, 4 – individual breeders, 5 – private horse, 6 – unknown, 7 – import from Western countries, 8 – import from Eastern countries, 9 – pony and small horses); number of rider's licence; and number of penalty points obtained in tournaments. Breeders of imported horses were characterised by an abbreviation of the country of origin. As far as the age was concerned, all horses were grouped as follows: 1 – four-years-old; 2 – five-years-old; 3 – six-years-old; 4 – seven-to-ten-years-old; 5 – eleven-to-fifteen-years-old and 6 – over sixteen years.

Official numbers of sports licences of the Czech Riding Federation were used to identify both the horses and the riders. Horses without their own sports results and representing the group of ancestors of sports horses were classified in corresponding places of the kinship matrix under their own names.

All data were analysed using programmes SAS (SAS Institute Inc., 1988) and JAA 20 (Misztal, 1993). Analysis was performed at two levels depending on the method of classification of performance factors:

a) For fixed factors *sex, age, year of start, degree of tournament difficulty* and *type of stud* using the least squares method and GLM procedure according to the equation:

$$y_{ijklm} = \mu + s_i + a_j + r_k + o_l + t_m + e_{ijklm},$$

where: y_{ijklm} – evaluated factor
μ – total average
s_i – fixed effect of *i*-th sex (i = 1, 2, 3)
a_j – fixed effect of *j*-th age (j = 1, ... 6)
r_k – fixed effect of *k*-th year of start (k = 1, ... 8)
oj – fixed effect of *l*-th degree of tournament difficulty (l = 1, ...7)
t_m – fixed effect of *m*-th type of stud (m = 1, ... 9)
e_{ijklm} – residual effect

b) For a more detailed classification of factors, including the random effect of factors *type of stud, breeder, rider, tournament, permanent environment, individuality of horse* the BLUP model was used. The importance of effects was evaluated on the basis of explained variability. Developed were 9 models and used for breeding value predictions by means of the following equations:

$$1)\ y_{ijk\,lmnopqu} = \mu + s_i + o_j + a_k + t_l + b_m + r_n + c_o + pe_p + h_q + e_{ijklmnopqu},$$

where: $y_{ijklmnopqu}$ – evaluated factor (number of penalty points)
μ – total average
s_i – fixed effect of *i*-th sex (i = 1, 2, 3)
o_j – fixed effect of *j*-th degree of tournament difficulty (j = 1, ... 7)
a_k – fixed effect of *k*-th age (k = 1, ... 6)
t_l – random effect of *l*-th type of stud (l = 1, ... 9)
b_m – random effect of *m*-th breeder (m = 1, ... 1 515)
r_n – random effect of *n*-th rider (n = 1, ... 5 651)
c_o – random effect of *o*-th tournament (o = 1, ... 6 245)
pe_p – random effect of permanent environment of *p*-th animal (p = 1, ... 6 846)
h_q – random effect of animal (q = 1, ... 13 366)
$e_{ijklmnopqu}$ – residual effect

Gradually, the following factors were eliminated from the model:

$$2)\ y_{jklmnopqu} = \mu + o_j + a_k + t_l + b_m + r_n + c_o + pe_p + h_q + e_{jklmnopqu},$$

$$3)\ y_{iklmnopqu} = \mu + s_i + a_k + t_l + b_m + r_n + c_o + pe_p + h_q + e_{iklmnopqu},$$

$$4)\ y_{ijlmnopqu} = \mu + s_i + o_j + t_l + b_m + r_n + c_o + pe_p + h_q + e_{ijlmnopqu},$$

$$5)\ y_{ijkmnopqu} = \mu + s_i + o_j + a_k + b_m + r_n + c_o + pe_p + h_q + e_{ijkmnopqu},$$

$$6)\ y_{ijklnopqu} = \mu + s_i + o_j + a_k + t_l + r_n + c_o + pe_p + h_q + e_{ijklnopqu},$$

7) $y_{ijklmopqu} = \mu + s_i + o_j + a_k + t_l + b_m + c_o + pe_p + h_q + e_{ijklmopqu}$,

8) $y_{ijklmnpqu} = \mu + s_i + o_j + a_k + t_l + b_m + r_n + pe_p + h_q + e_{ijklmnpqu}$,

9) $y_{ijklmnopqu} = \mu + s_i + o_j + a_k + t_l + b_m + r_n + c_o + h_q + e_{ijklmnopqu}$

The residual variance was calculated for each model. The lowest value of residual variance was calculated for the model involving all factors under study (Equation 1); this model explained the highest proportion of variability. Based on a comparison with final variances of all other models it was possible to obtain differences determining the effects of individual factors under study (i.e. of those that were eliminated from the model).

When using this method, no direct solution to a set of equations was carried out so that the statistical significance of individual effects was not known and for that reason it was evaluated only indirectly on the basis of differences in residual variance and the number of cases on the individual levels of effects under study.

RESULTS AND DISCUSSION

Method (a) was used to evaluate the effects of *sex, age, year of start, degree of tournament difficulty* and *type of stud*. Significance of effects of these factors on the performance of sports horses, as estimated by GLM method, is presented in Table 1.

All effects under study were highly significant. This finding corresponded with results obtained by Jiskrová (1996) and Jiskrová and Misař (1997). This means that they cannot be neglected and must be used in the model of breeding value prediction.

Table 1. Statistical significance of effects of factors under study on performance of sporthorses in jumping tournaments

Effect	df	Significance level
Sex	2	$P < 0.0001$
Age	5	$P < 0.0001$
Year of start	7	$P < 0.0001$
Degree of difficulty	6	$P < 0.0001$
Type of stud	8	$P < 0.0001$

Method (b) was used for a more detailed classification of factors with a high number (i.e. thousands) of levels on the basis of the analysis of residual variance. The results demonstrated that fixed effects showed a higher proportion of explained variability than random ones (with internal variability). Results of this analysis are presented in Table 2.

As shown in Table 2, factors *breeder* and *type of stud* showed a very low to zero effect on the explainable variability. Jiskrová (1996) and Jiskrová and Misař (1997) calculated $r^2 = 0.013$ for the factor *type of stud*; this means that this factor contributed only 1.3% to the total explainable variability. Bruns (1990) mentioned that he did not use the effect of *breeder* when using the BLUP model of calculation and reasoned that the influence of breeder on the horse and its performance is very small, namely till the weaning of colts and/or till the end of the second year of

Table 2. Analysis of residual variance

Number of model equation	Factors under study (*missing factors*)	Residual variance	Difference between the residual variance of model 1 and the other models	Difference (%)
1	sex, degree of tournament difficulty, age, type of stud, breeder, rider, tournament, permanent environment, animal (–)	683.8	–	–
2	degree of tournament difficulty, age, type of stud, breeder, rider, tournament, permanent environment, animal (*sex*)	846.8	163.0	23.8
3	sex, age, type of stud, breeder, rider, tournament, permanent environment, animal *(degree of tournament difficulty)*	931.5	247.7	36.4
4	sex, degree of tournament difficulty, type of stud, breeder, rider, tournament, permanent environment, animal (*age*)	782.9	144.1	14.5
5	sex, degree of tournament difficulty, age, breeder, rider, tournament, permanent environment, animal (*type of stud*)	683.8	0.0	0.0
6	sex, degree of tournament difficulty, age, type of stud, rider, tournament, permanent environment, animal (*breeder*)	687.5	3.7	0.5
7	Sex, degree of tournament difficulty, age, type of stud, breeder, tournament, permanent environment, animal (*rider*)	730.1	46.3	6.8
8	sex, degree of tournament difficulty, age, type of stud, breeder, rider, permanent environment, animal (*tournament*)	845.6	161.8	23.7
9	sex, degree of tournament difficulty, age, type of stud, breeder, rider, tournament, animal *(permanent environment)*	694.3	10.5	1.5

its life, i. e. before the beginning of training period. In his opinion, later non-genetic effects (training and rider) influenced horse's performance much more than the breeder. These factors are a part of the *permanent environment* effect. Tavernier (1987) concluded that the factor of *permanent environment* (partly environment, partly rider) represented as much as 20% of variability in performance, i. e. nearly the same percentage as the genetic value of the individual. In our study the effect of *permanent environment* on the explained variability was much lower; this could be caused by the construction of model equation. The permanent environment effect was held for random with a high degree of variability. It can be said that the fixed effects contributed at most to the explanation of total variability. Moreover, various interrelationships may exist between the individual factors under study. The existence of a hierarchic relationship between type of stud and breeder is very probable.

Sex

Mares were the most frequent sex group (3 427 individuals with 67 198 starts), followed by geldings and stallions (2 552 and 867 horses with 57 837 and 20 374 starts, respectively). Using the least squares method (GLM procedure) a significant effect of sex on jumping performance of horses was demonstrated (Table 1). When calculating the BLUP model, sex was used as a fixed effect. Results of the residual variance analysis also demonstrated the significance of the sex effect because the value of residual variance was the second highest, viz. 163, among all calculated results. Mares had not only the highest number of starts but also the highest average of penalty points. The average of penalty points of geldings was 17.85. The highest performance was found in stallions (the average score of penalty points was 13.89). However, their better performance in tournaments need not be influenced by sex only. As compared with the two other groups, the group of stallions was selected with a relatively high strictness prior to the beginning of their sports career: preliminary selection to the hundred-day test and hundred-day test itself. Such a selection was not done in the two other groups. It is also probable that the training of stallions and their management in tournaments could be done with a higher degree of responsibility due to the fact that the potential quality of sires should be demonstrated. Thus, reduced penalisation and lower degree of variance in performance of stallions need not explicitly be caused by sex but due to a synergic effect of sex and selection. This fact does not suppress but, quite on the contrary, supports the use of calculation of sex effect when predicting the breeding value of animals.

Degree of tournament difficulty

Of the total number of 145 409 starts of the horses under study in jumping tournaments altogether 47 220 were in Z tournaments; 46 300 in ZL tournaments, 31 561 in L tournaments; 16 514 in S tournaments; 3 330 in ST tournaments, 426 in T tournaments and 58 in TT tournaments. In lower competitions (Z, ZL, L), the penalisation of starting horses was lower (average score of penalty points ranged from 17.05 to 17.61) than in tournaments of the grade S and higher (penalty points 18.59–21.80).

Age

Age categories of sports horses were pooled into groups characterising individual periods of their performance and assuring a sufficient number of starts (four-years-old horses with 18 467 evaluated events; five-years-old with 27 403; six-years-old with 25 138; seven-to-ten-years-old with 54 387; eleven-to-fifteen-years-old with 17 823 and sixteen-to-twenty-two-years-old with 2 191 evaluated starts). The highest numbers of starts were recorded in the category of young horses and horses up to the age of 10 years. Age was the fixed effect showing statistical significance in both models of evaluation. Young horses up to the age of 10 years received the lowest score of penalty points; as compared with both groups of older sports horses (with 19.21 and 19.80 penalty points) they received only 15.75 to 18.03 penalty points. However, this could not be caused by better results of young sports horses in tournaments but, more probably, by more frequent starts of younger horses in less difficult tournaments where the penalisation is generally lower.

Type of stud and breeder

Horses imported from Western countries belonged to the group with the best results: their average score was 10.77 penalty points. In this group, breeds selected for sports performance for a long time predominated. There is no doubt that the relatively low penalisation of horses from breeding farms was also very interesting (their average score of penalty points was 14.55) and the results of horses from former State Stud Farms (Netolice, Albertovec, Kladruby n. L. with a herd of Czech warm-blooded horses) surely contributed to these results due to high quality of elite herds, selection of horses for sports performance, organisation of breeding and methods of training. Horses imported from Eastern countries showed a similar performance level. The result of this group was influenced above all by horses imported from the former USSR. A part of them represented the production of important Russian, Ukrainian and Belorussian stud farms and their performance was influenced by similar factors like that of sports horses from breeding farms. The average penalisation of sports horses from private farms was relatively high (on average 20.45 penalty points). This could be caused by a different level of quality of private farms because the majority of them came to the existence within the last decade and their standard of organisation and management is very different.

Rider

There were 5 651 riders in the set under study with altogether 145 409 starts on 6 846 horses. There was a problem with riders in this study because the rider's name was not mentioned in 14 884 starts (9.55%). This meant that the factor *Rider* represented the group with the highest frequency of unknown values in our d-base. This, unfortunately, was the cause of inaccuracy in our calculations that could not be eliminated. The factor *Rider* was classified as a random effect with high internal variability because riders behaved differently in individual tournaments. For that reason the difference between the residual variance of model 7 and model 1 is only 46.3 (Table 2); however, in our opinion it is always necessary to involve this factor into the animal model. Meinardus and Bruns (1987) and Hassenstein *et al.* (1996) evaluated this factor as a random effect of rider's category. Tavernier (1990) studied in detail the problem of the effect of factor *Rider* and mentioned that, in France, only 45% riders had only one horse and this could be a source of inaccuracy in the animal model. Besides, there is also a correlation between the effects *Rider* and *Genetic Value* so that the model with a random effect is not quite suitable because it would be necessary to record numbers of different horses starting under one rider. Meinardus (1988) used the factor *Rider* as a fixed effect in the animal model. This author grouped riders on the basis of their results with horses in all tournaments so that it was possible to reduce internal variability of the effect of factor *Rider*. This method could also be used when predicting the breeding value of the Czech warm-blooded horses. In the Czech Republic, there are altogether 1.2 horses per one rider. This means that we have the same problem as that mentioned by Tavernier (1990). Provided that the factor *Rider* would be used as a fixed effect, the value of explained variability could be higher. In this way it would be possible to eliminate differences in sports performance of riders that undoubtedly exist. For that reason it would be much suitable to characterise the effect of rider on the basis of his performance category under Czech conditions (i. e. similarly like Meinardus and Bruns, 1987 and Hassenstein *et al.*, 1998). Until now, performance categories of riders have not been recorded in the d-base of the Czech Riding Federation but there are serious considerations to re-introduce this practice so that it would be possible to use these data in the animal model.

Tournament

The factor *Tournament* was used as a random effect with low internal variability. It was expected that all horses had equal chances in individual tournaments because it was necessary to observe technical parameters defined in the Rules of Riding Sport (height, type and distribution of obstacles) and because of environmental conditions of each tournament (type, quality and configuration of the terrain). On the other hand, however, natural conditions (above all weather) may be considerably changing even during a single tournament. In this factor the proportion of explained variability was relatively high (161.8). It means that this factor was of cardinal importance because it classified horses into individual groups enabling to compete under relatively equal conditions and in the same time interval so that the contemporaries could be compared. The factor *Tournament* involves place, date, season, year of start, etc. For that reason the factor *Year of Start* was not involved into the model.

Permanent environment

This effect was discussed in detail in the first part of this chapter.

CONCLUSIONS

It results from data above that it is necessary to respect the characterisation of fixed and random systematic effects when developing BLUP models. The accuracy of calculation is affected not only by the precondition of a sufficient number of horses (necessary for the development of kinship matrix) but also by the influence of fixed and random factors on sports performance of horses, by proper interpretation of selected factors and by consideration of interrelationships (correlations) existing between the individual effects under study.

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Enzyme activities and milk performance by clusters of growth hormone gene (GH) and β-casein (β-cN) in German Holsteins

Enzymové aktivity a mléčná užitkovost ve vztahu ke genotypům růstového hormonu (GH) a β-kazeinu (β-CN) u německého holštýnského skotu

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ABSTRACT: The examined enzyme activities show the same trend as milk yield. The enzyme activities for combined aminopeptidases increased nonlinearly to a higher milk performance. Lower physiological load contributes to better fertility. The increasing enzyme activity of lysosomal esterase is connected with a higher ATP need, the protein synthesis increases simultaneously. The genetic effects of milk protein genotypes β-casein^{A2A2} are superior to those of β-casein^{A1A1}. The effect of days open shows the same trend in milk production and fertility. A high milk performance could be combined with high fertility on a genetic basis.

Keywords: cattle; enzyme; fertility; milk performance; milk protein genotypes

ABSTRAKT: Byl sledován vliv genotypu mléčných bílkovin, růstového hormonu a aktivity vybraných enzymů na mléčnou užitkovost německého holštýnského skotu. Analyzované enzymové aktivity vykázaly týž směr, jako dojivost. Enzymové aktivity kombinovaných aminopeptidáz se zvyšovaly nelineárně s vyšší mléčnou užitkovostí. Nižší fyziologická zátěž přispívá k vyšší plodnosti. Stoupající enzymová aktivita lysosomální esterázy je spojena s vyšší potřebou ATP, simultánně se zvyšuje proteosyntéza. Genetické efekty genotypu β-kazeinu^{A2A2} převyšují genetické efekty β-kazeinu^{A1A1}. Vysoká mléčná užitkovost může být geneticky spojena s dobrou plodností.

Klíčová slova: skot; enzymy; plodnost; mléčná užitkovost; genotypy mléčných proteinů

INTRODUCTION

The changes in the phenotypic production capacity of cows and of the exterior are connected with the changes in the genetic structure and interior. A high milk yield is connected with good health regarding metabolism, and a sufficient fertility in dairy cows depends on a well balanced distribution of energy in the body.

The anabolic and katabolic balance between protein synthesis and proteolysis in relation to lipogenesis and lipolysis is influenced by degradative enzymes. There is hardly any information found in literature about lysosomal activities of enzymes in cattle or about genetic aspects of the proteolytic activities of lysosomal enzymes in the plasma or leukocytes. Intracellular proteins are liable to a constant turnover. The main place for the breakdown of proteins is the lysosome compartment which contains about 20 endo- and exopeptidases. The lysosomal proteolysis depends on the type of cell and varies between 30 and 100% of total proteolysis in the cell. The lysosomal breakdown is rather specific and prefers proteins of a longer half-life. Proteins with high turnover rates usually underlie the ubiquitin-dependent degradation. Various degradation pathways are regulated in connection with each other. There are close kinetic relations between the amount of proteins in the cell and their constant turnover rates. The breakdown rates of proteins seem to determine the final concentration of these biomolecules. The individual breakdown of proteins is quite different. The mechanism of breakdown happens in three different ways (Bienkowski, 1983), the basal degradation in the endosomal system, the intralysosomal proteolysis and the ubiquitin-dependent degradation (Sommer and Seufert, 1992) in the cell cytoplasm. It can be assumed that the intracellular proteins are liable to a constant turnover and that this process is of important physiological meaning. In comparison to the present knowledge concerning the molecular mechanisms in the protein synthesis, the biochemical processes of the protein breakdown are less known (Panicke *et al.*, 1996). The turnover of the individual proteins is kinetically described as a result of two converse reactions (Millward, 1978; Amenta and Brocker, 1981). Concerning the degradative processes, this means the breakdown rates of a certain protein in a tissue or a cell-population are proportional to the concentration of this protein. Furthermore, all present molecules of this protein in the cell have the same probability of underlying to the degradation. An important conclusion of this process is the same dimension of proteolysis at all times in all cells of the tissue (Amenta and Brocker, 1981). Thus the degradation of various protein molecules regulates an important function of the cell like the structure and control of many key enzymes and other bioregulators. Not less important are special degradations of multicomplexes like the ribosomes and mitochondria.

Proteolytic activities of lysosomal enzymes in cattle

The performance of cattle includes growth, milk yield, fertility and health. These charecteristics are determined by the genotype and the environment. The genetic and physiological consideration of the protein and energy metabolism has a central position for the assessment of these parameters. By the provision of energy the fat and protein metabolism is controlled and balanced anabolically by lipogenesis and protein synthesis and catabolically by lipolysis and proteolysis. Knap (1995) simulated in a computer model the relation between both of them with assumed starting values without experiment. Differences in the protein yield as milk or growth were proved between the lactation and age periods and also hormonally explained (Purchas *et al.*, 1971; Pritchard *et al.*, 1972; Amir, 1974; Sejrsen *et al.*, 1982; Day *et al.*, 1986; Friedel *et al.*, 1986; Panicke, 1987, 1991; Staufenbiel, 1993; Lachmann, 1994; Panicke *et al.*, 1995). Protein yield is the result of biosynthesis as proteosynthesis and of the degradative breakdown as proteolysis. The degradation also determines the protein level in the biological units. Panicke *et al.* (1999a, b) proved the relation between the lysosomal enzyme activities and the milk yield in cows. A reduced proteolysis in the cell, which can be measured by the proteolytic activity of the enzymes, is also considered by Lobley (1998) as a possible characteristic for selection.

The intralysosomal proteolytic degradation includes the breakdown of most proteins. It plays a priority part in the regulation of the protein level in the cell. According to Pfeifer (1981), there is a close correlation between the activity of lysosomal enzymes and the level of protein in the cell. The proteolytic activity is inhibited in all cells of the growing organism, simultaneously the protein yield increases (Schmidt *et al.*, 1992, 1993; Krol *et al.*, 1994). Therefore, the proteolytic activity of the lysosomal enzymes would be a possible parameter for the explanation and assessment of different protein yields as well as for the stability of performance.

The activities of the lysosomal enzymes in particular allow to draw conclusions on the level of protein synthesis. The aim of this contribution is the consideration of the proteolytic activities of lysosomal enzymes and their relation to milk yield.

MATERIAL AND METHODS

Blood samples for determination of enzyme activities were taken in close relation to official milk recording. Blood was taken from the *vena jugularis* of female cattle and preserved with heparin. The isolation of leucocytes from bovine blood proceeded according to Zeman's *et al.* (1988) improved method of isolation. It is a simple and quick method for the simultaneous separation of lymphocytes and granulocytes in the peripheral blood. The plasma and leucocyte fractions are taken out of the blood by the following method using density gradient centrifugation with Gradisol G. Afterwards they are preserved by deep-freezing until the time of investigation:

- application of 1.5 ml gradisol in a siliconized 12.5 ml centrifugal tube at a temperature of 22 to 25°C
- 2.5 ml heparinised blood was layered on the top
- centrifugation for 25 minutes at 400 × G in a swing-out centrifuge
- taking off the clear phase (plasma)
- taking off the cloudy phase with a Pasteur pipette (mixed population of lymphocytes and granulocytes)
- washing of the mixed phase with 5 ml 0.9% NaCl
- suspension of leucocytes in 3 ml 0.1% Triton X-100 and freezing
- after thawing centrifugation of the leucocytes for 20 minutes at 20 000 × G
- utilization of the clear phase for enzymatic analysis

The enzyme activities were determined fluoro-spectrophotometrically and the values in leucocytes were expressed in nmol/mg protein/h and those in plasma in nmol/l plasma/h as a mean value of double determinations for the following enzymes in Table 1. Aminopeptidases Arginyl- (ARG/EC.3.4.11.6), Alanyl- (ALA/EC.3.4.11.14) and Leucyl- (LEU/EC.3.4.11.2) were analyzed in the bovine plasma using Ala- and Arg-Naphthylderivates (Barett and Heath, 1977). The sum of their enzyme activities is SAAL = ARG + ALA + LEU (Table 1). For the determination of lysosomal esterase (LE/EC.3.1.1.2) *p*-nitrophenol was me-

Table 1 Examined enzymes, genes and markers

	Investigation	
Lysosomal enzyme activities in leucocytes (nmol/mg protein/h), in plasma (mmol/l plasma/h)		
ENZ	ARG = arginylaminopeptidase	(EC.3.4.11.6)
	ALA = alanylaminopeptidase	(EC.3.4.11.14)
	LEU = leucylaminopeptidase	(EC.3.4.11.2)
	AGLD = α-glukosidase	(EC.3.2.1.20)
	EL = lysosomale esterase	(EC.3.1.1.2)
	SAAL = ARG + ALA + LEU	
Growth hormone gene (GH)		
GH	LL = leucine/leucine	
	VV = valine/valine	
	LV = leucine/valine	
Milk protein polymorphisms (MPP)		
MPP	α_{S1}-casein = A, B, C	
	β-casein = A1, A2, A3, B, C	
	κ-casein = A, B, C, E	
	β-lactoglobulin = A, B, C, D	

asured released from nitrophenyl palmitate. The measurement error was 4.4%. Genetic variants of growth hormone gene (GH) (L = leucine, V = valine) and milk protein genes (MPP) (α_{S1}-, α_{S2}-, β-, κ-casein and β-lactoglobulin) were determined at the same time. Phenotyping of milk samples was carried out by isoelectric focusing (IEF) in ultrathin polyacrylamide gels with carrier ampholytes. This method allows the simultaneous separation and phenotyping of all known variants within European cattle breeds at the protein level (Erhardt, 1989). The genotyping of the growth hormone variants *L* and *V* was carried out by PCR/RFLP method using primers according to Schlee *et al.* (1994) 5′-GCTGCTCCTGAGGGCCCTTCG-3′ and 5′-GCGGCGGCACTTCATGACCCT-3′ with 1.5 mM $MgCl_2$ and annealing temperature 60°C. The amplified fragment DNA of 223 bp was restricted by *AluI* at 37°C for 3 hours, the fragments were electrophoresed on 3.5% agarose gel with ethidium bromide.

The results of milk recording with the last monthly control (LK) one week before taking the blood sample, and the completed 305-days lactation record were taken from the VIT Verden Paretz:
– milk yield in kg (MK)
– fat yield in kg (FK)
– protein yield in kg (EK)
– fat level in % (FP)
– protein level in % (EP)

The program PEST (Groeneveld *et al.*, 1992; Groeneveld, 1993) was used to estimate the response of the systematic factors. The estimation was based on the following single trait model:

$$Y_{iklmn} = m + UG_i + BG_j + GT_k + LG_l + KM_m + A_n + e_{ijklmn}$$

where: Y – individual performance
μ – general mean
UG_i – i-th time of investigation (9)
BG_j – j-th farm (3)
GT_k – k-th part population: SMR (2) HF
LG_l – l-th lactation group (4)
1/A = 1. lactation beginning to 150th lact. day
1/E = 1. lactation end after 150th lact. day
2/A = 2. lactation beginning to 150th lact. day
2/E = 2. lactation end after 150th lact. day
KM_m – m-th birth month (8)
A_n – random response of the n-th cow
e_{ijklmn} – random residual response

The proteolytic activities of lysosomal enzymes are influenced by systematic environmental factors as well as the milk yield (Panicke *et al.*, 1999c). At the test station, they were considered by the simultaneousness of the cows. 357 cows were examined simultaneously in three test groups (TG) with 30, 141 and 186 cows. In the focus of the test were 186 cows of test group TG 407 (Table 2).

Table 2. Sample size (4) of cows in the investigation groups (TG)

TG No.	Polymorphism growth hormone	Polymorphism milk protein	Milk performance	Enzyme activities
405	–	–	30	30
406	–	–	141	141
407	186	186	186	186

TG – test groups

The milk yield and enzyme activities as well as the milk protein polymorphisms (MPP) and the growth hormone gene polymorphism (GH) of these cows were examined.

RESULTS

The performance of test group 407 was approximately 600 kg fat and protein yield, and 7 415 kg milk yield per lactation (Table 3). The variation coefficients were about 15% for milk, fat and protein yield, 12% for fat content, and 6% for protein content. The fat content was 4.5% and protein content 3.5%.

The phenotypic variation of the proteolytic activities of enzymes within the groups, which are measured in nmol/l plasma/h (Table 4), has a variation coefficient of 18 up to 25% in the same order of quantitative characteristics for the milk performance with 22 up to 28%.

The sum of enzyme activity SAAL = ARG + ALA + LEU increases nonlinearly with milk performance per lactation

Table 3. Means ($\bar{x}$) and variation coefficients (s %) of milk performance (TG 407, n = 186)

Traits	In lactation		Test day	
	$\bar{x}$	s (%)	$\bar{x}$	s (%)
Milk (kg)	7 415	15.9	27.5	28.1
Fat (%)	4.55	11.8	4.56	16.8
Fat (kg)	335	15.1	1.2	25.2
Protein (%)	3.49	6.4	3.38	11.4
Protein (kg)	258	14.5	0.9	21.5
Fat + protein (kg)	593	14.3	2.1	22.6

TG = test group

Table 4. Means ($\bar{x}$) and variation coefficients (s %) of lysosomal enzyme activities in plasma (nmol/l plasma/h) and in leukocytes (nmol/mg protein/h) of cows (TG 407, n = 186)

Enzyme	Leukocytes		Plasma	
	$\bar{x}$	s (%)	$\bar{x}$	s (%)
ARG	90	65.9	2421	25.6
ALA	172	74.3	3407	20.0
LEU	121	71.2	2951	18.8
SAAL	382	51.9	8780	14.8
EL	206	59.3	833	31.2

TG = test group

in three different years (Table 5). The enzyme activity measured by SAAL increased with milk performance in different GH genotypes. Under inclusion of β-casein variants (β-CN), the sum of enzyme activity SAAL of β-CN^{A2A2} genotypes decreased to 8 486 nMol/l/h under similar performances of β-CN^{A1A1} and β-CN^{A2A2}, which is almost half of standard deviation (s nearly 15%).

Cows with β-CN^{A2A2} genotype showed with higher performance around –2.5 days better days open (service period) and around –0.1 better insemination index (number of inseminations per conception (Table 6). The effect of milk protein genotype β-casein^{A2A2} is superior compared to β-casein^{A1A1} approximately by 200 kg milk and 10–12 kg of fat and protein with respect to actual estimations of other animals (Panicke *et al.*, 1998; Freyer *et al.*, 1999). κ-Casein^{BB} affects the fat and protein level positively (Table 7). Cows with κ-casein^{AB} show heterotic effects of 4.8 kg (fat and protein). Examinations in different populations of dairy cattle lead to similar results regarding the quantity and quality of estimated effects. The differences in protein genotypes show the same trend in milk yield and fertility (Table 8). Milk production and fertility show both positive differences by β-CN^{A2A2} and κ-CN^{AA} in the investigated sample size. It may be concluded that an energy supply in accordance with the demand leads to lower production stress and to stabilized fertility.

Table 5. Genotypes, enzyme activities and fat and protein performance and enzyme activities of different genotypes

Group	Genotype		Number	Performance	Enzymes	
	GH***	β-casein	n	fat + protein (kg)	SAAL (nmol/l/h)	EL (nmol/l/h)
405	all	all	30	513	6 637	436
405	all	all	141	576	8 213	700
407	all	all	186	593	8 780	823
407	VV	all	9*	563	8 394	764
407	LV	all	44*	584	8 536	777
407	LL	all	133*	597	8 888	857
407	LL	A2A2	23**	617	8 486	866
407	LL	A1A1	23**	613	9 020	815
407	LL	A1A2	48**	582	8 897	842

*total = 186 cows; **the remaining 133 are other genotypes; ***this part of research was supported by CEZ J06/98/122200004

Table 6. Genotypes of β-casein, milk performance and days open (n = 984) (Panicke *et al.*, 1998)

Milk protein genotype frequencies*		Milk (kg)	Fat (kg)	Protein (kg)	Fat (%)	Protein (%)	Days open	Insemination index
A1A1	0.23	–84^{a}	–1.83	–2.15	0.033^{a}	0.014	4.0^{a}	0.12^{a}
A1A2	0.46	2^{b}	–0.07	0.01	–0.001^{b}	–0.003	–3.0^{b}	–0.11
A2A2	0.18	124^{b}	3.81	3.68	–0.028^{b}	–0.018	1.5^{b}	0.03^{b}
Difference A2A2–A1A1		208	5.64	5.83	–0.061	–0.032	–2.5	–0.09

* not all genotypes; a, b – statistical differences

Table 7. Genotypes of k-casein, milk performance and days open (n = 984) (Panicke *et al.*, 1998)

Milk protein genotype frequencies*		Milk (kg)	Fat (kg)	Protein (kg)	Fat (%)	Protein (%)	Days open	Insemination index
AA	0.52	–15	–1.31	–1.25	–0.010[a]	–0.008[a]	–2.4	–0.05[a]
AB	0.37	33	1.81	1.82	0.004[b]	0.008[b]	0.0	0.05
BB	0.06	–88	–0.59	0.74	0.063[b]	0.050[b]	8.2	0.22[b]
Difference BB–AA		–73	0.72	1.99	0.073	0.058	10.6	–0.27

* – not all genotypes; a, b – statistical differences

Table 8. Genotypes, milk performance and enzyme activities of dairy cows (TG 407, n = 186)

Genotype			n	Milk performance						Enzyme activity	
GH	β-casein	κ-casein		milk (kg)	fat (%)	fat (kg)	protein (%)	protein (kg)	fat + protein (kg)	SAAL (nmol/l/h)	EL (nmol/l/h)
all			186	7 415		335		258	593	8 780	823
VV			9	7 323	4.41	318	3.37	245	563	8 394	764
LV			44	7 319	4.53	328	3.51	256	584	8 536	777
LL			133	7 453	4.57	338	3.49	259	597	8 888	857
LL	A2A2		23	7 857	4.44	346	3.46	271	617	8 486	866
LL	A1A1		23	7 622	4.61	349	3.48	264	613	9 020	815
LL	A1A2		48	7 173	4.63	329	3.55	253	582	8 897	842
LL	A2A2	AA	21	7 995	4.41	350	3.44	274	624	8 378	841
LL	A2A2	AB	2	6 406	4.80	308	3.68	236	544	9 523	1 041

DISCUSSION

The examined enzyme activities show the same trend as the milk yield (Table 5). Genetically determined physiological values should neither be maximized nor minimized, but optimized. This optimization should be quantified. Lower enzyme activities for combined aminopeptidases SAAL connected with high milk performance presume that the protein synthesis is increasing with a reduced proteolysis rate. Lower physiological load is connected with better fertility. A high milk performance could be combined with high fertility on a genetic basis.

The enzyme activity of lysosomal esterase (EL) increases with the performance of the cows. This points to an increasing hydrolysis of triglycerids (fats) and an increasing release of fatty acids, which are a substrate for the ATP-production in the citrate cycle. This corresponds to the rising ATP-consumption in an increasing protein synthesis.

Significant effects of milk protein genotypes on milk production and fertility traits have been estimated. The casein genes are located on bovine chromosome 6 and several groups already showed QTL's on this chromosome for milk production traits (Kühn *et al.*, 1996; Mosig *et al.*, 1998; Freyer *et al.*, 1998; Kalm, 2000). The proved effects contribute to the explanation of relations between considered production traits expressed by correlation coefficients or by means of the direct and indirect selection success. They may show the possibilities and limits of selection. A summarized contemplation suggests an equal trend of milk production and fertility. This is realizable because the milk production in a biological sense is necessary for the nutrition of the offsprings. Both milk production and fertility are influenced positively by β-CN^{A2} and by κ-CN^{A} connected with a lower content of fat and protein. It follows from the fact that a supply which corresponds to the demand for energy of genetically high-yielding cows may result in good fertility. The positive differences of β-$casein^{A2A2}$ on the days open, insemination index and milk yield may indicate this. High milk performance could be combined with high fertility on a genetic basis.

CONCLUSION

The proteolytic activities of chosen lysosomal enzymes in the plasma and leukocytes of growing female cattle of the Holstein Friesian population were examined in 357 cows in the present contribution, the results are summarized in Table 8. The following conclusions can be drawn, regarding all the results:

1. The phenotypic variation of proteolytic activities of the enzymes within the groups, which are measured in nmol/l plasma/h, has a variation coefficient of 18 up to 25% in the same order of quantitative characteristics for the milk performance with 22 up to 28%.

2. The plasma enzymes are more suitable for the investigations than the enzyme activities in leukocytes because other factors affect particularly aminopeptidases in leukocytes. They increase the variation coefficients to 65 up to 70%. Particularly the immune response to subclinic diseases, infection and stress are to be mentioned here.

3. The summary combination of the three aminopeptidase activities as a common value SAAL = ARG + ALA + LEU seems to be justified and decreases the variation coefficient to 15%.

4. The examined enzyme activities in plasma increase nonlinearly with the milk yield. Physiological characteristics are to be optimized. They should not be maximized nor minimized.

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The use of mRNA to study the polymorphism of goat alpha S1 casein in males

Využití mRNK ke studiu polymorfismu kozího alfa S1 kazeinu u samců

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ABSTRACT: mRNA of alpha S1-Cn gene was found in the lymphocytes of buck blood. 25 male goats were examined, using RT-PCR technique. Six different genotypes, created by the combinations of alleles: *A* (group of strong alleles *A*, *B*, *C*), *D* and *F*, were detected. "Male" alpha S1-Cn mRNA was positively isolated from buck blood during nine months corresponding to the goat reproductive season (September to May).

Keywords: alpha S1 casein; polymorphism; *Capra hircus*; bucks; mRNA

ABSTRAKT: V lymfocytech krve kozlů jsme zjišťovali mRNK genu alfa S1-Cn. Pomocí metody RT-PCR bylo vyšetřeno 25 kozlů. Zjistili jsme šest rozdílných genotypů vytvořených kombinacemi alel: *A* (skupina silných alel *A*, *B*, *C*), *D* a *F*. mRNK „samčího" S1-Cn jsme spolehlivě izolovali z krve kozlů v průběhu devíti měsíců, které odpovídaly reprodukčnímu období koz (září až květen).

Klíčová slova: alfa S1 kazein; polymorfismus; *Capra hircus*; kozli; mRNK

INTRODUCTION

Alpha S1 casein (alpha S1-Cn) is one of four caseins synthesised by the mammary gland during lactation. In goat species, it is a highly polymorphic protein, encoded by at least seven autosomal alleles: *A*, *B*, *C*, *D*, *E*, *F* and *0* (null allele) with a few subtypes (*B*1, *B*2, *F*1 –*F*9) (Leroux *et al.*, 1992; Martin, 1993; Grosclaude *et al.*, 1994). Recent works also show that there exist more alpha S1-Cn alleles, called G, H, L and I, in some goat populations (Chianese *et al.*, 1997).

Differences between alleles appear on the level of both DNA and protein (Tokarska *et al.*, 2001). Genetic variants of alpha S1-Cn are associated with some milk characteristics and its processing features; the strongest alleles (*A*, *B*, *C*) are the most desirable in milk production, however they are also associated with lower milk yield (Martin, 1993).

Goat alpha S1-Cn gene consists of 19 rather short exons (mean length: 24–63 bp) while introns are relatively long: 100–1 500 bp. The last two exons (18 and 19) are not translated into amino acid sequence (Perez *et al.*, 1994). Alleles differ in several point mutations in DNA sequence but, in variant E, there exists a large insertion of LINE sequence (457 bp) in exon 19 (Perez *et al.*, 1994). Allele *0* has a deletion of 8 kb that can cause the loss of its activity (Martin and Addeo, 1995). In the remaining alleles, DNA mutations lead to amino acid substitutions (variants *A*, *B*, *C*) or some defects during transcription that cause wrong splicing. Exons 9–11 in allele F and exon 9 in allele *D* are defectively removed due to point mutations in DNA sequence (Mahe and Grosclaude, 1989; Brignon et al., 1990).

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All mentioned changes are responsible for well-detectable differences in the length of each allele transcript (mRNA).

Because of the effect of alpha S1-Cn type on milk characteristics, the information about the genotype has been proposed to be included into a selection scheme for dairy goats (Manfredi *et al.*, 1998; Strzałkowska *et al.*, 1998) and it is important to create a simple test for goat genotyping that could be used for animals of either sex in a large scale. The study undertaken in our laboratory was aimed at finding an easy but reliable method for alpha S1-Cn allele identification both in female and male dairy goats.

MATERIAL AND METHODS

In the first experiment (Tokarska *et al.*, 2001), the presence of alpha S1-Cn mRNA was discovered in the lymphocytes of buck's blood. Blood lymphocytes were used as a source of total RNA. Blood samples were collected (in 10 ml tubes covered with EDTA) several times from each animal. 25 bucks of dairy breeds (Table 1) were included in the experiment: 23 were from the Silesia region of Poland and two were male kids at the age of 3 months. Total RNA was isolated according to TRIZOL method described by Chomczynski and Sacchi (1987). mRNA was reversed into cDNA with reverse transcriptase PCR (RT-PCR) with Stratagene RT-PCR kit on MJ Research 100 cycler. Alpha S1-Cn cDNA was amplified with PCR performed with the pair of primers complementary to fragments common for all alleles, flanking almost all cDNA of this gene (from exon 1 to exon 19). PCR reaction was carried out in 25 μl volume containing 25 ng caprine cDNA, 1 U of Taq DNA polymerase, 10 pmol of each primer, 200 μM of each dNTP, 1.5 mM $MgCl_2$ in standard PCR buffer. Primers were designed from mRNA sequences of alpha S1-Cn variants reported in GenBank (Accession No: X59836) and were as follows:

F (forward): 5' - GAC AAC CAT GAA ACT TCT CAT CC - 3'

R (reverse): 5' - CTT ACA GGA GAG GTG ATT CAA AG - 3'

The primers were also checked not to be complementary to any other sequence in caprine genomic DNA. PCR was done in MJ Research 100 cycler under these conditions: 96°C/2 min (96°C/30 sec; 68°C/30 sec; 72°C/1 min) × 40 and finally 72°C/7 min. PCR products were separated by electrophoresis in 2% agarose gel. The gels were analysed using GelDoc 2000 System (BioRad).

The lengths of caprine alpha S1-Cn cDNA expected after PCR with primers F/R were as follows: *A*, *B*, *C* (1 001 bp), *D* (968 bp), *E* (1458 bp), *F* (890 bp) and *G* (965 bp).

RESULTS AND DISCUSSION

The group of strong alleles (called *A*), allele *D* and *F* were detected in the studied population of bucks (Table 1, Figure 1). It is possible that also allele *G* was present among examined samples but its length would be very similar to the length of allele *D* and the proposed test would not distinguish these two variants. However, alleles *D* and *G* encode the same levels of alpha S1-Cn in goat milk. Strong alleles (*A*) were found to be present in 19 bucks but mostly as heterozygotes: *AD* or *AF*. Only three animals were homozygotes *AA* (Table 1). All the results were compared with those obtained for female goats. The length of the PCR products corresponded to that expected for alpha S1-Cn cDNA fragment and related to female

Table 1. Distribution of alpha S1-Cn genotypes in bucks of different breeds

Breed	*n*	Genotypes					
		AA	*AD*	*AF*	*DD*	*DF*	*FF*
Czech White Improved	2		1	1			
French Alpine	4	1	1	2			
Saanen	5	1	3	1			
Polish White Improved × Saanen	2			2			
Polish White Improved × Alpine	5	1	1	1		1	1
Polish White Improved × Syrian	1					1	
Syrian	2			1			1
Polish Coloured	4			2	2		
Total	25	3	6	10	2	2	2

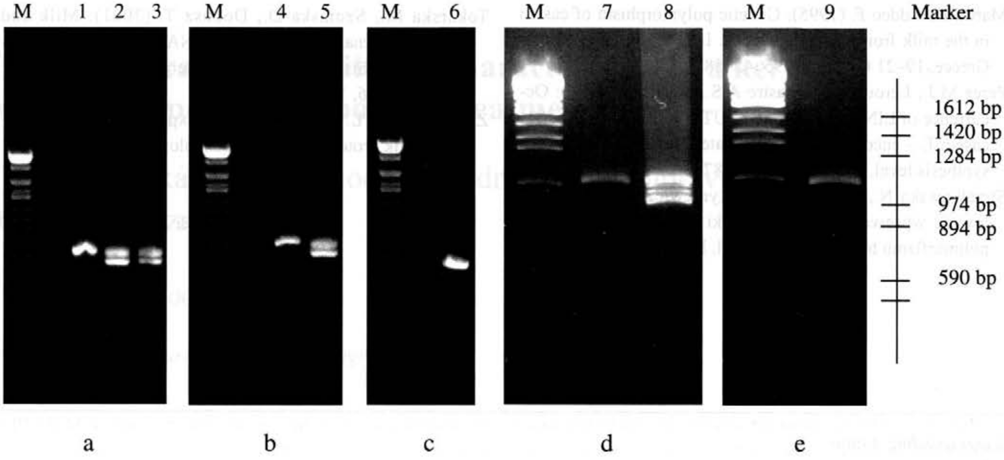

Figure 1. PCR fragments of goat alpha S1-Cn cDNA amplified with primers F/R. M – Lambda DNA/Eco47I (AvaII) Marker 13 (MBI Fermentas). (a) Fragments obtained from female cDNA; (b–e) "male" fragments. Lines: 1, 4, 7 – genotypes *AA*; 2, 3, 8 – genotypes *AF*, 5 – genotype *DF*, 9 – genotype *DD*. Allele "*A*" means "strong" alleles (*A*, *B*, *C*)

fragments amplified by the described method (Figure 1a). Partial sequencing of amplified fragments confirmed that they came from alpha S1-Cn cDNA (Tokarska *et al.*, 2001).

Blood samples from one adult animal were collected several times in different months and from young bucks – only once (3 months of age). Alpha S1-Cn cDNA was positively transcribed only when blood samples were collected from September to May. This fact suggests that mRNA of alpha S1-Cn gene is present seasonally in the male organism. Additionally, this season corresponds to the season of reproduction in goat species. It could lead to the conclusion that alpha S1-Cn gene is being activated by some hormones (like prolactin), whose level significantly increases in male blood during this period (Kosowska and Zdrojewicz, 1996). Because alpha S1-Cn cDNA was obtained also from male kids, the growth hormone (GH) could be responsible for casein gene activation in this case. Both conclusions would be in accordance with data on the hormone role in casein transcription regulation (Zwierzchowski, 1996).

The problem mentioned above needs further investigations. Although such genes as alpha S1-Cn gene are thought to be specifically activated depending on the sex, it is possible that they play another, unknown role both in female and male organisms.

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Technological and nutritional characteristics of a kernel of maize exposed to "cooking treatment"

Technologická a nutriční hodnota hydrotermicky upraveného kukuřičného zrna

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ABSTRACT: The aim of this investigation was to establish the sustainability of the digestibility of a kernel of maize by subjecting it to treatment before drying. According to the "cooking treatment" used in this study, a kernel of maize was "cooked" immediately after it had been harvested, and then dried. The treatment involved one drying only. This paper compares the dynamic resistance of a kernel of maize after the "cooking treatment" with that of a kernel of maize which had not undergone such a treatment. The research findings indicate that a kernel treated with heat did not take much longer to dry than a natural kernel. The dynamic characteristics and the sedimentation of a kernel treated with heat were weakened by its exposure to the "cooking treatment" (the rate of broken kernels increased from 26.6 to 59.8%). The amount of gluten in the kernel treated with heat increased from 21.6 to 60.9%. It can be concluded on the basis of these findings that a kernel of maize can be exposed to heat treatment immediately after it has been harvested.

Keywords: "cooking treatment"; kernel; digestibility; sedimentation; dynamic characteristics

ABSTRAKT: Cílem řešení bylo zjistit permanenci stravitelnosti živin kukuřičného zrna, které bylo před sušením hydrotermicky upraveno. Použitá úprava spočívala v hydrotermickém ošetření kukuřičného zrna hned po sklizni a v jeho sušení. V této práci se srovnává dynamická rezistence kukuřičného zrna po hydrotermické úpravě s dynamickou rezistencí neupraveného kukuřičného zrna. Z dosažených výsledků je zřejmé, že sušení hydrotermicky upraveného zrna netrvalo déle, než u zrna bez úpravy. Hydrotermická úprava oslabila dynamické vlastnosti a sedimentaci upraveného zrna (zvýšil se podíl zlomkového zrna z 26,6 na 59,8 %). Množství glutenu v hydrotermicky ošetřeném zrnu vzrostlo z 21,6 na 60,9 %. Na základě těchto poznatků lze konstatovat, že kukuřičné zrno může být hydrotermicky upraveno hned po sklizni.

Klíčová slova: hydrotermická úprava; kukuřičné zrno; stravitelnost živin; sedimentace; dynamické vlastnosti

INTRODUCTION

As one of the physical methods for the transformation of different substances, heat treatment includes the following processes: drying, toasting, internal heating (infrared and microwave radiation) and compressing (dry extruding, expanding, pelletising, roller pressing). Moreover, combinations of individual treatments, such as wet pressing in screw-presses (extrusion with the addition of steam) and infrared radiation with subsequent roller pressing (micronising), also constitute heat treatments for raw materials (Putier, 1993). One of the tasks of all technological methods, including physical ones, is to improve the digestibility of certain ingredients of foodstuffs, of carbohydrates in particular. The digestibility of carbohydrates in a naturally dried kernel ranges from 40 to 50%. Heat treatment increases the degree of gelatinisation and digestibility, which may reach 80% depending on the treatment applied. Naturally dried cereals contain around 20% gelatinised starch. After their exposure to hot-air drying, the degree of gelatinisation increases to 30%. Toasting and roller pressing increase the degree of gelatinisation to 35%, micronising to 30% and extruding to 62%. A cooking treatment increases the degree of gelatinisation to 70% (Katić *et al.*, 1993). In a similar way, the dynamic resistance of the treated kernel is also modified by new methods of treatment applied with the aim to increase digestibility (Katić *et al.*, 1994). Due to the high degree of mechanisation of some industrial operations, kernels are often damaged during transportation (Gunasekaran *et al.*, 1985; Gunasekaran and Paulsen, 1985; Pliestić and Krička, 1993). The consumption of energy plays a significant role in all heat treatments of kernels. Depending on the heat treatment applied, the energy consumption may range from 2 to 4 kg of petroleum per 100 kg of dried kernels (Katić, 1997). For all these reasons, the objective of this study was to establish whether it was possible to maintain the digestibility of a maize kernel by treating it before it was dried. Therefore, the ultimate goal was to omit one drying treatment by cooking a maize kernel immediately after it had been harvested and, following this, to dry it. The nutritive value of a kernel is indicated by the degree of starch gelatinisation and by the levels of available starch and protein (Orthoefer, 1994). Gelatinisation, i.e. the breakdown of starch during the treatment of cereals and leguminous plants rich in starch, is significant for two reasons:

- gelatinisation prepares starch for the enzyme-driven breakdown during digestion,
- gelatinisation helps to achieve a higher degree of utilisation.

This is particularly important for young animals (e.g. hogs), whose enzyme activity for the breakdown of starch is not well developed, or for animals whose dietary starch cannot be exploited to a greater extent because of a short digestion process (e.g. cats, fish, furry animals). Fodder-producing technology involves physical and chemical transformations of different substances. The applied physical methods include heat treatments and mechanical treatments. These help to improve the physical characteristics of products and raw materials with the aim to achieve the desired level of such physical characteristics.

MATERIAL AND METHODS

At the Zagreb Faculty of Agriculture, a device was designed for the steaming of kernels – a process known as the "cooking treatment". The "cooking treatment" was carried out by using a pressure vessel instead of a cooking column. The basic difference is that in the process of treating a maize kernel the steam does not come from the outside. A kernel is steamed by water vapour originating from the water which lies on the bottom of the vessel and which does not come into contact with the kernel. This vessel contains another cylindrical vessel with a perforated bottom, beneath which there is a ceramic plate with drill holes of 16 mm in diameter to avoid direct conductive contact between the kernel and the metal bottom and linings of the pressure vessel, and to avoid convection contact with water. The water vapour can pass freely and treat the kernel. The pressure vessel is heated by an electric heater and the temperature is regulated by a voltage regulator with a variable resistor. The vessel contains a thermometer and a manometer to control the temperature and the pressure inside the vessel, a valve for the release of surplus steam and a safety valve. The cooking treatment was applied to a kernel sample of 1 500 g which had previously been rehydrated to 31.6% of humidity. For each treatment, 500 ml of distilled water was poured into the pressure vessel. The kernels were treated for 10 minutes at a temperature higher than 100°C and at a steam pressure of 0.5 bars. During the entire treatment, the readings of the temperature and the pressure inside the vessel were checked every minute. After the cooking treatment, the kernels were dried in a laboratory model drier. The laboratory drier was constructed to be kinetically similar to a real-life industrial drier. Before the drying procedure, the humidity of the samples was determined and their total mass was weighed. To check the decrease in humidity, the samples were weighed every two minutes during the drying process until their humidity reached 13.5%. The temperature and the speed of air-flow were also controlled. The air temperature for the drying procedure was kept at 80°C and the speed of the air flowing through the layer of kernels was maintained at 1.0 m/s. The entire process was repeated 20 times. The study of the dynamic characteristics of a maize kernel was carried out on manually harvested and husked maize samples of the Bc 492 hybrid in order to avoid possible initial damage inflicted by machines which could affect the measurement readings. The resistance of a maize kernel to the dynamic load was measured by a centrifugal drum, also designed at the Zagreb Faculty of Agriculture. The housing of the centrifugal drum is made of aluminium sheet and placed on rubber cushions in order to damp any oscillations, sounds and blows. The lower part of the centrifugal drum contains an electric motor with a voltage of 220 V, a frequency of 50 Hz and a nominal power of 300 W. The internal disk (the rotating part) is fixed to the vertical central axis. The kernel falls on the disk through a tube situated in the centre of the drum and at this moment, ideally, it has zero movement – it is suspended. In order to establish the kinetic energy of the collision (clash) with the rigid, immovable drum wall, it is necessary to establish the absolute and relative speed of the kernel moving in the drum.

The nutritive value of the kernel was established through:

The degree of gelatinisation. An *F*-test was used to establish the degree of gelatinisation. The *F*-test consists of: the preparation of trials F and E for an enzyme-driven hydrolysis, followed by the actual enzyme-driven hydrolysis, sedimentation, and sedimentation (E, F and S trials) and oxidation of the decreasing sugar.

The degree of gelatinisation is calculated according to the following formula:

$$\text{Degree of gelatinisation (\%)} = \frac{\text{gelatinised starch} - \text{decreasing sugar}}{\text{total starch} - \text{decreasing sugar}} \times 100$$

The starch level. The starch level was established on the basis of a modified method according to Ewers.

The quantity of the entire starch was established according to the following formula:

$$\text{\% of starch} = \frac{a \times 100 \times 100}{\text{(a) D20} \times \text{I} \times \text{weighed amount}}$$

After the study was completed, the measurement results were statistically processed. An analysis of variance was made and coefficients of correlation, variation and regression were established. The drying rate of the maize kernel was mathematically presented by polynomial equations.

RESULTS AND DISCUSSION

While conducting the research on the drying rate of a maize kernel, the temperature and the relative air humidity in the laboratory were measured. The average temperature in the laboratory during the investigation amounted to t_0 = 19.80°C while the relative air humidity in storage amounted to an average of 75.1%. The obtained figures relating to the drying rate of a maize kernel were mathematically processed with polynomial equations. Table 1 shows the drying rates of a kernel exposed to the cooking treatment and of a kernel that was not exposed to the cooking treatment.

Temperatures are very important throughout the cooking treatment, especially when they exceed 100°C. According to the figures obtained during the study, the average temperature at which a maize kernel entered the drying process was 108.59°C. Under such conditions, the humidity of the kernel exposed to the cooking treatment increased to 39.54%. After the drying process, the dynamic characteristics of the maize kernel were examined (Table 2).

The data in the above Table indicate that the ratio between whole kernels and broken kernels amounted to 73.34 vs. 26.63 for the drying treatment only, and to 40.13 vs. 59.87 for the cooking and drying treatments. At the same time, the sedimentation rate was analysed in the particles of both the ground maize which was only dried and the ground maize which was exposed to the heat treatment before drying (Table 3).

After having been exposed to drying or cooking treatments, all samples were examined for the levels of digestible proteins, starch and gluten. In order to compare the levels of digestible proteins, starch and gluten, an analysis was made of the maize kernel which was exposed to drying only. The obtained data indicate that the kernel which was only dried contained 6.30% digestible protein, 21.60% gluten and 62.13% starch. Table 4 illustrates the figures relating to the maize kernel exposed to the heat (cooking) treatment.

Table 1. The equation of the drying process of a maize kernel not exposed to heat treatment (only drying) and the equation of the drying process of a maize kernel exposed to heat treatment (cooking + drying)

Treatment	t_1 (°C)	w (%)	Drying equation	r	r^2
Drying	77.86	31.67	$w = 31.114 - 0.201\tau + 0.001\tau^2$ $dw/d\tau = 0.002\tau - 0.201$	0.98	0.95
Cooking + drying	77.20	39.54	$w = 35.560 - 0.220\tau + 0.0001\tau^2$ $dw/d\tau = 0.002\tau - 0.220$	0.95	0.91

Table 2. Dynamic characteristics of the maize kernel dried and exposed to heat treatment

Treatment	Drying only (%)	Cooking + drying (%)
Whole kernel	73.34	40.13
Mean rate of broken kernels	26.63	59.87
S. D.	1.567	13.45
C.V.	5.885	30.203
Min.	24.73	21.2
Max.	28.52	63.34

Table 3. Quantity of sediment during sedimentation (%)

Treatment	w (%)	After 1 hour		After 24 hours	
		HCl	H_2O	HCl	H_2O
Drying	13.87	62.50	66.30	61.80	66.00
Cooking + drying	13.92	48.00	50.00	46.00	48.00

Table 4. Levels of protein, gluten and starch of a maize kernel after the cooking treatment

	Digestible protein (%)	Levels of gluten (%)	Levels of starch (%)
Mean levels	6.75	60.9	64.08
S. D.	0.39	6.55	2.07
C.V.	5.77	10.76	3.23
Min.	6.09	39.42	59.28
Max.	8.16	79.10	68.97
n	0.10	1.63	0.53
95% lower	6.65	59.27	63.56
upper	6.85	62.53	64.61

The technological treatment normally applied to kernels which are used as cattle feed involves drying, heat treatment, drying and utilisation. The objective of the above experiment was to establish whether the high level of digestibility of a maize kernel can be maintained if a maize kernel is exposed to a cooking treatment immediately after it has been harvested, and then dried. Such a procedure which excludes one drying treatment would reduce costs and speed up the process. If the equations relating to the time required for the drying of treated kernels are compared, it follows that the cooking treatment does not significantly affect the drying rate. Therefore, some 2 to 4 kg of petroleum could be saved per 100 kg of maize. However, the dynamic characteristics of maize kernels were undermined by the heat treatment, increasing the rate of broken kernels from 26.63 to 59.87%. These results are in agreement with the results of Pliestić and Krička (1993). Accordingly, the cooking treatment does have a negative effect on the dynamic characteristics of a maize kernel. An analysis was made of the sedimentation of a maize kernel exposed to the cooking treatment and drying, and of a kernel exposed to drying only, where both had the same humidity level. It showed that the sedimentation of the samples which were not exposed to the cooking treatment was 32.6% higher after 1 hour of settling in the water and 30.2% higher after one hour of settling in the acid. After 24 hours of immersion in the water and in the acid, the sedimentation of the samples which were not exposed to the cooking treatment was 37.5% higher in the water and 34.3% higher in the acid, compared to the samples which were exposed to the cooking treatment.

However, when comparing a dried maize kernel and a maize kernel treated with heat, a significant difference can be seen in the levels of gluten and digestible protein. Namely, the cooking treatment increases the levels of gluten and digestible protein. The difference between the levels of gluten in the dried kernel ($x = 21.60\%$) and in the kernel treated with heat ($x = 60.9\%$) is large and clearly indicates that cooking prior to drying increases the level of gluten. Moreover, the level of gluten is roughly the same in the maize kernel which is dried, treated with heat and then dried again. According to professional sources, the quantities of gluten can increase by up to 70%. In this particular case, the increase amounted to 60.9%. These results are in agreement with the results of Katić *et al.* (1994).

CONCLUSION

In the process of drying, the maize kernel treated by cooking behaved almost identically to the one that was not treated in this way. However, its dynamic features changed during the treatment and the rate of broken kernels increased from 26.63 to 59.87%. The level of the sedimentation of a maize kernel treated with heat and immersed for one hour in water decreased by 32.6%, and that of a kernel immersed in acid by 30.2%. After 24 hours, the level of sedimentation was even worse as the decrease amounted to 37.5% for the kernel immersed in water and 34.2% for the kernel in HCl. The nutritive value, i.e. the rate of gelatinisation, increased with the cooking treatment from 21.6 to 60.9%. On the basis of a comparison between the figures obtained in the process of exposing a kernel to the treatments of drying – cooking – drying – utilisation, and the figures obtained in this study, it can be established that the drying process prior to the cooking treatment can be omitted, as the nutritive value of the kernel will remain the same and the cost of the entire treatment will be reduced.

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Chemical monitoring of three water-supply reservoirs, using fish as bioindicators

Chemický monitoring tří vodárenských nádrží s použitím ryb jako bioindikátorů

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ABSTRACT: Biological indication of exposure of the aquatic environment to inorganic and organic pollutants was tested in the Kružberk, Šance and Morávka water-supply reservoirs in the basin of the Oder (Czech Republic) in 1996 to 1998. Muscle of 90 fishes of 14 species served as the indication matrix. The species included *Esox lucius*, *Stizostedion lucioperca*, *Perca fluviatilis*, *Aspius aspius*, *Anguilla anguilla*, *Oncorhynchus mykiss*, *Salmo trutta* m. *fario*, *Thymallus thymallus*, *Rutilus rutilus*, *Abramis brama*, *Cottus gobio*, *Tinca tinca*, *Leuciscus cephalus*, *Alburnus alburnus*, representing the ichthyofauna of predatory, zooplanktonophagous, omnivorous and benthophagous fishes of two non salmonid-type hydroecosystems (Šance, Kružberk) and one salmonid hydroecosystem (Morávka). Mercury was the most dangerous pollutant of the spectrum of metals: admissible tolerance of mercury (0.1 mg/kg for non-predatory fish and 0.5 mg/kg for predatory fish) was exceeded in the muscle of 78% of the fish. In all the reservoirs, the greatest accumulation of mercury was recorded in *Esox lucius* (0.985 ± 0.4227 mg/kg) (mean ± SD), *Perca fluviatilis* (0.872 ± 0.255 mg/kg), *Anguilla anguilla* (0.857 ± 0.1933 mg/kg) and *Stizostedion lucioperca* (0.715 ± 0.2867 mg/kg). A significant ($P = 0.05$) ascending tendency of mercury accumulation depending on age and weight was recorded in *Esox lucius* and a significant to highly significant ($P = 0.01$) ascending tendency of mercury content was recorded in heavier and older individuals of omnivorous fish (*Leuciscus cephalus*). The muscle of two nine-year old specimens of *Esox lucius*, one from Kružberk, the other from the Šance reservoir, had a hygienically inadmissible level of contamination by cadmium (0.110 mg/kg and 0.120 mg/kg, respectively). Values exceeding the admissible tolerance of lead were found only in a three-year old *Stizostedion lucioperca* (0.560 mg/kg) and a seven-year old *Abramis brama* (0.530 mg/kg) from Kružberk. The accumulation of the persistent organochlorine contaminants – PCBs (polychlorinated biphenyls), expressed as the sum of indicator congeners 28, 52, 101, 118, 138, 153, 180 – was the highest in the fish from the Kružberk reservoir (muscle of *Anguilla anguilla*: 1.801 mg/kg; muscular fat of *Aspius aspius* 17.055 mg/kg). The sum of planar PCB congeners 77, 126 and 169 in the muscular fat of the fish ranged in all reservoirs from 0.018 (*Cottus gobio*) to 0.272 mg/kg (*Aspius aspius*).The accumulation of DDT in fish was similar to that of PCBs. The highest concentration of DDT and its metabolites, exceeding the 0.5 mg/kg maximum tolerance of residues (MTR) in the edible portion, was found in *Anguilla anguilla* from Kružberk (0.638 to 1.485 mg/kg). The obtained values of DDT with a prevailing proportion of the high-stability metabolite, p,p′-DDE, resulted from chronic exposure of the fish to these slowly degrading substances and provided evidence of the persistent presence of this insecticide in the reservoirs. The organochlorinated pesticides HCB (hexachlorobenzene) and HCH (hexachlorocyclohexane) also contributed to the contamination of the fish. The MTR of hexachlorobenzene in the edible portion (0.05 mg/kg) was almost trebled in the muscle of two specimens of *Anguilla anguilla* (0.149 and 0.179 mg/kg) from Kružberk, which also had the highest deposits of HCB in muscular fat (0.606 to 0.831 mg/kg).

Keywords: water-supply reservoirs Kružberk, Šance, Morávka; muscle tissue; metals; PCB; DDT; HCB; HCH

ABSTRAKT: V podmínkách vodárenských nádrží Kružberk, Šance a Morávka (povodí řeky Odry) byla v letech 1996 až 1998 provedena bioindikace stupně zátěže vodního prostředí anorganickými a organickými cizorodými látkami. Jako indikační matrice sloužila svalovina 90 ks ryb náležejících k 14 druhům (*Esox lucius, Stizostedion lucioperca, Perca fluviatilis, Aspius aspius, Anguilla anguilla, Oncorhynchus mykiss, Salmo trutta* m. *fario, Thymallus thymallus, Rutilus rutilus, Abramis brama, Cottus gobio, Tinca tinca, Leuciscus cephalus, Alburnus alburnus*) representujících ichtyofaunu dravých, zooplanktonofágních, omnivorních a bentofágních ryb dvou hydroekosystémů nelososovitého typu (Šance, Kružberk) a jednoho hydroekosystému lososovitého typu (Morávka). K nejrizikovějším prvkům ze spektra kovů (tab. 1) patřila rtuť, která kontaminovala svalovinu 78 %

ryb nad přípustné množství (PM), které bylo stanovené pro nedravé druhy ryb hodnotou 0,1 mg/kg a pro dravé ryby hodnotou 0,5 mg/kg. Nejvyšší akumulace rtuti byla ve všech nádržích zjištěna u *Esox lucius* (0,985 ± 0,4227 mg/kg) (průměr ± SD), *Perca fluviatilis* (0,872 ± 0,255 mg/kg), *Anguilla anguilla* (0,857 ± 0,1933 mg/kg) a *Stizostedion lucioperca* (0,715 ± 0,2867 mg/kg). V souboru analyzovaných ryb byla zjištěna signifikantní (P = 0,05) stoupající akumulace rtuti v závislosti na věku a hmotnosti u *Esox lucius* (obr. 1 a 2) a signifikantní až vysoce signifikantní (P = 0,01) zvyšování obsahu rtuti u hmotnostně těžších a věkově starších omnivorních ryb (*Leuciscus cephalus*) (obr. 3 a 4). Hygienicky nepřípustná kontaminace svaloviny ryb kadmiem byla u dvou devítiletých *Esox lucius* z Kružberka (0,110 mg/kg) a Šancí (0,120 mg/kg) a hodnoty převyšující PM olova byly zaznamenány pouze u tříletého *Stizostedion lucioperca* (0,560 mg/kg) a sedmiletého *Abramis brama* (0,530 mg/kg) z Kružberka. Bioakumulace perzistentními organochlorovými kontaminanty PCB (polychlorované bifenyly), vyjádřených sumou indikátorových kongenerů 28, 52, 101, 118, 138, 153, 180 (tab. 2) byla nejvyšší u ryb z Kružberské nádrže (*Anguilla anguilla*: 1,801 mg/kg), v tukové komponentě pak u *Aspius aspius* (17,055 mg/kg). Suma planárních kongenerů PCB 77, 126 a 169 v tuku svaloviny se ve všech nádržích pohybovala od 0,018 (*Cottus gobio*) do 0,272 mg/kg (*Aspius aspius*). Hydroekosystém monitorovaných nádrží byl rovněž rezervoárem insekticidu 1,1,1-trichloro-2,2-bis(4-chlorfenyl)ethanu (DDT) a jeho metabolitů. Nejvyšší koncentraci DDT a jeho metabolitů ve svalovině převyšující maximální limit reziduí (MLR) v jedlém podílu 0,5 mg/kg měly *Anguilla anguilla* z Kružberka (0,638 až 1,485 mg/kg). Zjištěné hodnoty DDT s převládajícím zastoupením vysoce stabilního metabolitu p,p'-DDE byly výsledkem chronické expozice ryb těmto pomalu degradujícím látkám a současně důkazem dlouho přetrvávajících depozit tohoto insekticidu v nádržích. Na kontaminaci tuku DDT měly největší zásluhu především metabolity p,p'-DDE a p,p'-DDD (tab. 3). Podíl p,p'-DDE na sumě DDT je nejlépe vystižný na obr. 6 až 8. K pozitivní kontaminaci ryb patřily i organochlorové pesticidy HCB (hexachlorbenzen) a HCH (hexachlorcyklohexan). Téměř trojnásobné překročení MLR hexachlorbenzenu v jedlém podílu (0,05 mg/kg) bylo evidováno ve svalovině dvou *Anguilla anguilla* (0,149 a 0,179 mg/kg) z Kružberka i s nejvyššími depozitami HCB v tukové komponentě svaloviny (0,606–0,831 mg/kg) (tab. 4).

Klíčová slova: vodárenské nádrže Kružberk, Šance, Morávka; svalovina ryb; kovy; PCB; DDT; HCB; HCH

INTRODUCTION

Provision of good-quality drinking water from water-supply reservoirs is an issue of a growing economic importance. It is especially so in the industrialised conurbation around the city of Ostrava, where the high concentration of mining, metallurgical and chemical industries implies a great demand for water. The intensive utilisation of this region in North Moravia affected water quality not only in water courses but also in stagnant waters in the dam reservoirs. The effort to achieve the best possible quality of raw water to be treated for the production of drinking water has led to the use of biological methods, including special-purpose fish stock management. A balanced stock of predatory and zooplanktonophagous fish, which must be in a good condition and good health, is among the prerequisites for such a system, leading to a reduction of phytoplankton. Systematic identification of the factors influencing the fish health in the ecosystem concerned requires good knowledge of the distribution of pollutants whose presence (in terms of both quality and quantity) must also be investigated from the viewpoint of the hygienic value of fish flesh as the methods of regulation of the biomass and abundance of the special-purpose fish stocks include angling.

Biological monitoring focused on the Kružberk, Šance and Morávka water-supply reservoirs will contribute to assessment of long-term exposure of the fish bodies to pollutants. At the same time it will help to extend the knowledge of the state of pollution of the Moravice, Morávka and Ostravice rivers in the basin of the Oder (Czech Republic). The information acquired by investigations into the health of fish from the upstream and downstream parts of these reservoirs was among the factors that drew attention to the need for ecotoxicological review of these parts of the reservoirs in order to explain the aetiopathogenesis of certain findings suggesting suspicion of toxic etiology (cholangiofibrosis, morphological anomalies in the blood cell nuclei), as also pointed out by Myers *et al.* (1987, 1991) and Carrasco *et al.* (1990).

Kružberk was the only reservoir of those referred to above in which the presence of pollutants had been investigated (Pavelka *et al.*, 1986) – and even there the investigation had only been focused on mercury and cadmium contamination of fish.

The purpose of this study was to determine the level of contamination of the aquatic ecosystems by inorganic and organic pollutants: this was the first partial objective of the research project focused on reviewing the degree of hazard to the health of fish stocks under chronic exposure to pollutants. The results of the chemical and toxicological investigation of the fish flesh for the contents of metals, polychlorinated biphenyls, DDT-based pesticides, hexachlorobenzene and hexachlorocyclohexane are synthesised and analysed in this report.

MATERIAL AND METHODS

All samples of the fish were collected by means of bulk fishing gear, i.e. seines, gill nets and basket traps. The fish were identified in terms of species and sex, and their scales were sampled for determination of age. Age was not determined for the eel and bullhead. Then, upon determination of the basic conformation traits (body weight and length), the fish were subjected to pathological and anatomic post-mortem examination and to collection of tissue and blood for histological and haematological examination. The fish samples had been kept in a freeze box at –24°C until the flesh was subjected to chemical analysis. The fish were selected for analyses so as to provide a representative sample of zooplanktonophagous, benthophagous, omnivorous and predatory fishes of different age and sex, with regard to the autochthonous fish population, with minimum effect of introductions. The analyses were performed separately for each fish, except the bullheads where a mixed sample of whole fish including viscera and a mixed sample of whole fish excluding viscera were analysed.

The chemical and toxicological analysis included the determination of metals (mercury, lead, cadmium, chromium, copper, zinc, arsenic, nickel); polychlorinated biphenyls (PCBs) and their indicator congeners 28, 52, 101, 118, 138, 153, 180 and planar (toxic) congeners 77, 126, 169; pesticides on the basis of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its metabolites o,p′-DDE, p,p′-DDE, o,p′-DDD, p,p′-DDD, o,p′-DDT, p,p′-DDT; hexachlorocyclohexane (HCH) and its alpha, beta, gamma and delta isomers; and hexachlorobenzene (HCB). When evaluating the degree of contamination of fish muscle by these substances in relation to health safety for consumption, we respected the hygienic limits set out in Ministry of Health Decree no. 298 of 1997 as the admissible tolerance (AT) and maximum tolerance of residues (MTR). On the whole, we analysed 90 specimens belonging to 14 species: northern pike (*Esox lucius*), pike-perch (*Stizostedion lucioperca*), perch (*Perca fluviatilis*), asp (*Aspius aspius*), eel (*Anguilla anguilla*), asp (*Oncorhynchus mykiss*), brown trout (*Salmo trutta* m. *fario*), grayling (*Thymallus thymallus*), roach (*Rutilus rutilus*), bream (*Abramis brama*), bullhead (*Cottus gobio*), chub (*Leuciscus cephalus*), tench (*Tinca tinca*) and bleak (*Alburnus alburnus*).

Chlorinated pesticides and PCBs were determined by means of the HP 5890 chromatograph with an ECD detector. Mercury was detected using the AMA 254 analyser, and the remaining metals were determined by means of the PE 3100 atomic absorption spectrophotometer, following dry mineralisation. Toxicological examination for the presence of arsenic was performed by the hydride technique.

Owing to the complexity of the issue under study, it will be useful, first, to point out certain basic hydrological characteristics.

Kružberk

The reservoir was built in the barbel zone of the Moravice river at Kerhartice and Kružberk in 1955. The reservoir's basin is 567 km² and its total volume is 35.5 million m³, storage volume 24.6 million m³, retention volume 6.9 million m³ and permanent volume 4.0 million m³. The backwater area is 280 ha, length 9.0 km and width 0.5 km. The average annual flow rate is 5.93 m³/s and hundred-year flow rate 282 m³/s. The depth of the reservoir at its downstream end is 24.5 m.

Šance

The reservoir was built on the upper course of the Ostravice (near its spring area) in 1971. Its basin is 146.4 km² in size. Its total volume is 61.8 million m³, storage volume 44.2 million m³, retention volume 15.1 million m³ and permanent volume 2.5 million m³. The backwater area is 337 ha, length 7.6 km and width 0.6 km. The average annual flow rate is 3.11 m³/s and hundred-year flow rate 320 m³/s. The maximum depth at the dam is 55.5 m.

Morávka

The reservoir was built on the trout brook Morávka in 1967. Its basin is 60.3 km² in size. Its total volume is 11.3 million m³, storage volume 4.4 million m³, retention volume 6.5 million m³ and permanent volume 0.4 million m³. The backwater area is 79.5 ha, length 2.8 km and width 0.2 km. The average annual flow rate is 1.76 m³/s and hundred-year flow rate 222.0 m³/s. At the dam the depth is 36.8 m.

RESULTS

1. Metals

The contents of metals in the muscle of the fish from the different reservoirs are shown in Table 1.

MERCURY

Kružberk: The total number of analysed fish was 24, including 17 predators and 7 non-predators. Mercury contamination of as many as 20 of these fish (83%) exceeded the AT of 0.1 mg/kg for non-predatory fish and 0.5 mg/kg for predators. The range of values for the predatory fish was 0.289–1.560 mg/kg and for the non-predatory fish 0.154–0.360 mg/kg. The highest mercury level was found in the northern pike (0.309–1.560 mg/kg) and its accumulation in the fish muscle grew linearly with age ($r = 0.805$*, Figure 1); contamination exceeding the AT first occurred in five-year old pike, pike-perch and asp.

Šance: The total number of fish analysed was 20, including 19 predators. Mercury contamination above AT was recorded in 19 of these fish (95%). In all cases the mercury

Table 1. Content of metals in the muscle of fish in the Kružberk, Šance and Morávka dam reservoirs (mg/kg)

Fish species	*n*	Age (year)	Weight (g)	Hg	Cd	Pb	Cu	Zn	As	Cr
Kružberk										
Esox lucius	7	3–9	680–9 700	0.309–1.560	0.010–0.110	<0.010–0.440	0.230–3.960	3.57–12.15	< 0.050–0.063	< 0.100
Stizostedion lucioperca	3	3–8	1 090 – 5 610	0.289–1.097	0.020	0.320–0.560	1.520–2.630	11.35–12.98	< 0.050–0.056	< 0.100
Perca fluviatilis	2	5	240, 379	0.540, 0.650	0.010, 0.020	0.320, 0.450	1.950, 4.100	5.56, 13.50	–	< 0.100
Aspius aspius	2	4 and 5	443, 1 010	0.362, 0.630	0.010	0.360	3.630	10.66	0.070	< 0.100
Anguilla anguilla	3	–	1 130–1 280	0.732–1.080	0.010–0.040	0.330–0.420	2.760–3.210	12.31–14.20	0.053–0.270	< 0.100
Abramis brama	3	5–7	435–510	0.190–0.230	0.020–0.060	0.380–0.530	2.130–2.980	8.43–13.98	<0.050–0.071	< 0.100
Rutilus rutilus	3	5–7	150–173	0.202–0.360	0.040	0.380	3.230	12.43	0.058	< 0.100
Alburnus alburnus	1	6	70	0.1540	–	–	–	–	–	–
Šance										
Esox lucius	6	3–9	1 200–5 150	0.531–1.900	0.010–0.120	< 0.100	0.100–0.290	2.95–11.05	< 0.050–0.014	< 0.100–0.110
Stizostedion lucioperca	2	4 and 8	800 a 1 720	0.740, 0.743	< 0.010, 0.010	< 0.100	< 0.100, 0.530	6.72, 8.95	< 0.050, 0.010	< 0.100, 0.120
Perca fluviatilis	3	10–14	670–1 230	1.018–1.125	< 0.010–0.010	< 0.100	0.200–0.440	7.54–10.81	< 0.050	< 0.100
Leuciscus cephalus	8	5–14	280–1 610	0.141–1.112	< 0.010–0.020	< 0.100	0.200–0.420	4.46–11.60	< 0.010–0.100	< 0.100–0.100
Abramis brama	1	8	1 140	0.13	< 0.010	< 0.100	0.325	3.81	0.086	–
Morávka										
Oncorhynchus mykiss	2	3	194, 400	0.082, 0.100	< 0.010, 0.020	< 0.100, 0.230	0.120, 0.340	2.78, 10.36	0.050, 0.090	< 0.100
Salmo trutta m. *fario*	3	4–6	280–580	0.180–0.310	< 0.010–0.030	< 0.100–0.220	0.170–0.460	3.07–10.12	0.050–0.060	< 0.100
Thymallus thymallus	1	4	505	0.230	0.030	0.240	0.130	0.84	< 0.050	< 0.100
Perca fluviatilis	2	9, 11	434, 600	0.750, 0.870	< 0.010	< 0.100	0.200, 0.270	10.78,10.87	< 0.050, 0.060	< 0.100
Tinca tinca	1	6	540	0.500	< 0.010	< 0.100	0.400	10.52	0.050	< 0.100
Rutilus rutilus	2	7	379, 400	0.130, 0.280	0.030, 0.040	0.290, 0.300	0.090	2.28 a 3.31	< 0.050	< 0.100
**Cottus gobio*	30	–	–	0.027 (0.025)	0.020 (0.060)	0.155 (0.160)	0.530 (0.660)	15.17 (13.35)	0.072 (0.056)	< 0.100

*Mixed sample of 30 individual fishes

Shown in parentheses is the analytic value in the whole fish body

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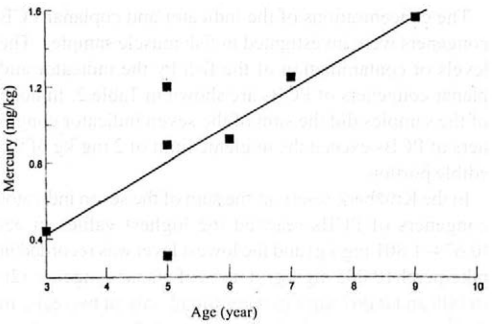
level in the predatory fish exceeded 1 mg/kg (1.018–1.900 mg/kg). The only non-predatory fish caught in the Šance reservoir had a mercury level of 0.130 mg/kg. The highest mercury concentration was recorded in a nine-year old pike (1.900 mg/kg). Mercury accumulation grew linearly with weight ($r = 0.874^*$, Figure 2) in the pike and chub ($r = 0.847^*$, Figure 3); in chub, mercury accumulation also grew highly significantly with age ($r = 0.831^{**}$, Figure 4). Mercury contamination above the AT first occurred in pike at an age of three years and in chub at an age of more than eight years.

Morávka: The analysis was performed in 11 individual fishes plus one mixed sample of bullhead. The AT limit was exceeded in 50% of the fish, the range for the predatory fish being from 0.082 mg/kg (rainbow trout) to 0.870 mg/kg (perch) and for the non-predatory fish from 0.024 mg/kg (bullhead) to 0.500 mg/kg (tench). The low levels recorded in brown trout (age 5+) were surprising.

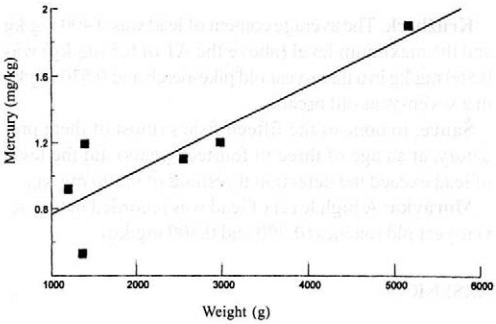
CADMIUM

Kružberk: Only one nine-year old pike of the 18 fishes was contaminated above the AT (0.1 mg/kg), and the cadmium level grew linearly with the age of this species. In the remaining fish – irrespective of whether predatory or non-predatory – the cadmium level ranged from 0.010 mg/kg (perch, asp, eel) to 0.060 mg/kg (bream).



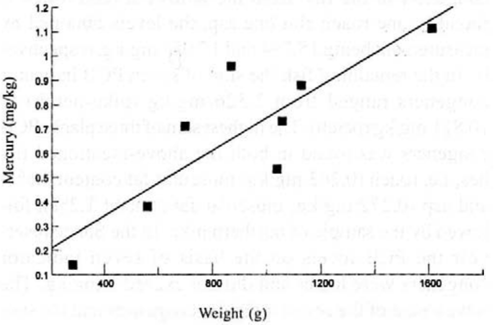
R2 = 0.64999634 SE = 0.28960529
C0 = −0.1508 C1 = 0.19064

Figure 1. Mercury content in the muscle of pike in the Kružberk reservoir, depending on age



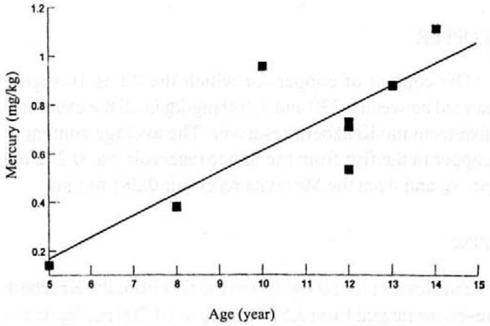
R2 = 0.763659835 SE = 0.243523836
C0 = 0.50778827 C1 = 0.00025916362

Figure 2. Mercury content in the muscle of pike in the Šance reservoir, depending on weight



R2 = 0.717108667 SE = 0.182660222
C0 = 0.0760464828 C1 = 0.00066376295

Figure 3. Mercury content in the muscle of chub in the Šance reservoir, depending on weight



R2 = 0.690657258 SE = 0.191009134
C0 = −0.278373171 C1 = 0.0891463415

Figure 4. Mercury content in the muscle of chub in the Šance reservoir, depending on age

Šance: Like in Kružberk, the highest cadmium concentration was recorded in a nine-year old pike (0.120 mg/kg). In the remaining pike at an age of three to seven years the maximum level of cadmium was at the threshold of detectability, 0.010 mg/kg. In chub, pike-perch and perch the cadmium level did not go beyond 0.020 mg/kg. In one chub at an age of eight years and weight of 1.140 g the cadmium content was below the threshold of measurability.

Morávka: The total number of analysed fish was 12, belonging to seven species. The cadmium levels in these fish ranged from 0.020 mg/kg (rainbow trout, brown trout) to 0.060 mg/kg (bullhead). A cadmium level below the threshold of measurability was recorded in a nine-year old and eleven-year old perch and six-year old tench.

LEAD

Kružberk: The average content of lead was 0.400 mg/kg and the maximum level (above the AT of 0.5 mg/kg) was 0.560 mg/kg in a three-year old pike-perch and 0.530 mg/kg in a seven-year old bream.

Šance: In none of the fifteen fishes (most of them predatory, at an age of three to fourteen years) did the level of lead exceed the detection threshold of 0.100 mg/kg.

Morávka: A high level of lead was recorded in two seven-year old roaches (0.290 and 0.300 mg/kg).

ARSENIC

Kružberk: In most of the fish caught, the recorded values were deep below the AT of 1 mg/kg and ranged from 0.050 to 0.071 mg/kg, the highest value being obtained in one of the three eels caught (0.270 mg/kg).

Šance: In most of the fish caught, the recorded arsenic values were below the measurable level (0.010 and 0.050 mg/kg).

Morávka: The arsenic level was 0.090 mg/kg at the maximum (rainbow trout).

COPPER

The content of copper for which the AT is 10 mg/kg ranged between 0.230 and 4.100 mg/kg in all the examined fish from the Kružberk reservoir. The average content of copper in the fish from the Šance reservoir was 0.272 mg per kg and from the Morávka reservoir 0.281 mg/kg.

ZINC

Zinc levels (AT 50 mg/kg) in the fish from the Kružberk reservoir ranged from 3.570 mg/kg to 14.200 mg/kg, in the fish from the Šance reservoir 2.950 to 11.600 mg/kg and in the fish from the Morávka reservoir from 0.840 (grayling) to 13.350 mg/kg (bullhead).

CHROMIUM

Chromium levels in the fish from the Kružberk and Morávka reservoirs were below the threshold of measurability (0.100 mg/kg). In the Šance reservoir they ranged from 0.200 to 0.440 mg/kg in perch, from 0.100 to 0.290 mg/kg in pike and from 0.200 to 0.420 mg/kg in chub.

NICKEL

The content of nickel in the muscle tissue of the fish in all reservoirs was below the detection threshold of 0.100 mg/kg.

2. Polychlorinated biphenyls

The concentrations of the indicator and coplanar PCB congeners were investigated in fish muscle samples. The levels of contamination of the fish by the indicator and planar congeners of PCBs are shown in Table 2. In none of the samples did the sum of the seven indicator congeners of PCBs exceed the hygienic limit of 2 mg/kg of the edible portion.

In the Kružberk reservoir the sum of the seven indicator congeners of PCBs reached the highest values in eel (0.674–1.801 mg/kg) and the lowest level was recorded in pike-perch (0.005 mg/kg). Traces of planar congener 126 (0.006 and 0.007 mg/kg) were found only in two eels. In the Šance reservoir the highest sum of the seven indicator congeners of PCBs was found in a nine-year old pike (0.237 mg/kg) but in the remaining fish this sum was below 0.055 mg/kg. In the fish from the Morávka reservoir the sum of the seven PCB indicator congeners was 0.037 mg/kg at the maximum (brown trout).

However, the detection of the indicator and planar congeners in muscular fat gave a somewhat different picture. The highest concentration of the seven PCB indicator congeners in the fish from the Kružberk reservoir was found in one roach and one asp, the levels obtained by measurement being 15.794 and 17.055 mg/kg, respectively. In the remaining fish the sum of seven PCB indicator congeners ranged from 2.526 mg/kg (pike-perch) to 10.811 mg/kg (perch). The highest sum of three planar PCB congeners was found in both the above-mentioned fishes, i.e. roach (0.263 mg/kg; muscular fat content 0.6%) and asp (0.272 mg/kg; muscular fat content 1.2%), followed by the sample of northern pike. In the Šance reservoir the PCB levels on the basis of seven indicator congeners were lower and did not exceed 5 mg/kg. The lowest sum of the seven indicator congeners and the sum of the three planar congeners of PCBs was found in an eight-year old bream (0.582 and 0.025 mg/kg). The highest PCB contamination by both indicator and planar congeners in the Morávka reservoir was recorded in brown trout

Table 2. Contamination of the fish muscle with PCB indicator congeners 28, 52, 101, 118, 153, 138, 180 and planar congeners 77, 126, 169

Fishspecies	n	Age (year)	Weight (g)	Sum of the indicator congeners of PCBs in mg/kg of muscle	Sum of the indicator congeners of PCBs in mg/kg in fat of muscle	Sum of the planar congeners of PCBs in mg/kg in fat of muscle
Kružberk						
Esox lucius	5	5–9	2 730–9 700	0.005–0.275	3.548–10.228	0.054–0.607
Stizostedion lucioperca	3	3–8	1 090–5 610	< 0.005–0.005	2.526–4.022	0.059–0.127
Perca fluviatilis	2	5	240, 379	0.024, 0.052	4.424, 10.811	0.084, 0.177
Aspius aspius	1	5	1 010	0.205	17.055	0.272
Anguilla anguilla	3	–	1 130–1 280	0.674–1.801	3.300–8.332	0.026–0.055
Abramis brama	3	5–7	435–510	< 0.005–0.149	3.268–6.371	0.047–0.200
Rutilus rutilus	1	5	163	0.090	15.794	0.263
Šance						
Esox lucius	4	3–9	1 370–5 150	< 0.005–0.237	1.492–3.769	0.033–0.176
Stizostedion lucioperca	2	4, 8	800, 1 720	< 0.005	1.346, 2.476	0.031, 0.060
Perca fluviatilis	3	10–14	670–1 230	0.009–0.055	1.798–4.083	0.060–0.106
Leuciscus cephalus	5	8–14	560–1 610	< 0.005–0.029	0.579–4.814	0.027–0.147
Abramis brama	1	8	1 140	0.033	0.582	0.025
Morávka						
Oncorhynchus mykiss	2	3	194, 400	< 0.005, 0.018	1.284, 1.672	0.069, 0.071
Salmo trutta m. *fario*	3	4–6	280–580	< 0.005–0.037	1.397–4.021	0.050–0.126
Thymallus thymallus	1	4	505	0.034	3.015	0.099
Perca fluviatilis	2	9, 11	434, 600	0.007–0.034	1.481, 2.037	0.036, 0.077
Tinca tinca	1	6	540	0.022	1.050	0.037
Rutilus rutilus	2	7	379, 400	0.018, 0.024	0.715, 1.105	0.023, 0.032
**Cottus gobio*	–	–	–	0.006 (0.017)	0.541 (0.794)	0.017 (0.018)

*Mixed sample of 30 individual fishes

Shown in parentheses is the analytic value in the whole fish body

in which the sum of seven PCB indicator congeners amounted to 4.021 mg/kg and the sum of three PCB planar congeners amounted to 0.126 mg/kg. One analysed grayling had a sum of seven PCB indicator congeners of 3.015 mg/kg and a sum of three PCB planar congeners of 0.099 mg/kg.

3. DDT and its metabolites

The sum of DDT in the muscle of the fish was first of all represented by the highly stable metabolite, p,p´-DDE, whose concentration dominated the whole metabolite spectrum. The MTR of the DDT sum in the edible portion is 0.5 mg/kg. In the Kružberk reservoir, the highest DDT contamination was found in the three eels examined (body weights of 1.130 to 1.280 g). The sum of DDT and the metabolites thereof ranged between 0.638 and 1.485 mg/kg and was three times as high as the MTR. In the remaining fish, the concentration of DDT and its metabolites ranged between 0.006 and 0.072 mg/kg. The largest amount of DDT and its metabolites (0.233 mg/kg) was found in a nine-year old pike weighing 5 150 g, out of the five fish species caught in the Šance reservoir. In the remaining fish the contamination ranged from 0.008 to 0.038 mg/kg. In the 12 fishes (of seven species) caught in the Morávka reservoir, the sum of DDT and its metabolites did not go beyond 0.059 mg/kg.

The analysed fish showed a high contamination of muscular fat with DDT-based substances. The levels of DDT and its metabolites, as organochlorinated pesticides, are summarised in Table 3. The p,p´-DDE and p,p´-DDD metabolites were the most amply represented compounds within the derivative spectrum. As to the highly stable metabolite, p,p´-DDE, its proportion in the total DDT sum and its metabolite sum are best illustrated by the curves in Figures 6 to 8. The average sum of DDT and its metabolites in the fish from the Kružberk reservoir was at its highest level in perch (5.087 mg/kg; fat content in muscle 0.47 to 0.63%), followed by pike (4.727 mg/kg, fat content in muscle 0.3 to 1.4%) and eel (4.573 mg/kg, fat content in muscle 20.0 to 24.3%); the lowest average level of DDT and its metabolites was found in pike-perch (2.014 mg/kg, fat content in muscle 0.13 to 0.3%). What was surprising was the high concentration of DDT and its metabolites in one roach: it was five times as high as the average level for the other fish. In the Šance reservoir the highest level of DDT and its metabolites was found in pikes, perches and chubs. The maximum values of p,p′-DDE and the DDT sum were recorded in a nine-year pike weighing 9.700 g (p,p′-DDE 2.790 and the DDT sum 3.888 mg/kg; fat content in muscle 1.79%). Like in the case of PCBs, the levels of DDT metabolites in the fish from the Morávka reservoir reached its peak in brown trout (7.410 mg/kg) and grayling (5.326 mg/kg). The mixed sample of bullhead had a minimum content of DDT and its metabolites (0.085 mg/kg).

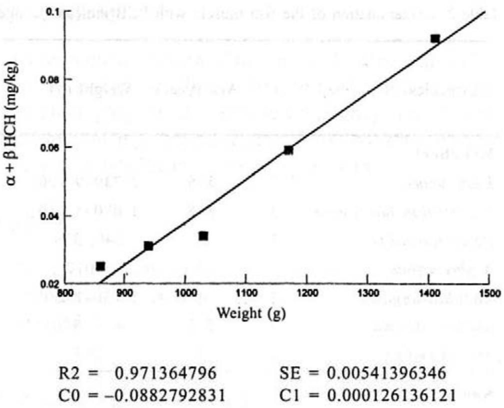


Figure 5. Sum of the α + β HCH in the muscular fat of chub in the Šance reservoir, depending on weight

4. HCB and HCH isomers

Positive findings of these xenobiotics were recorded in only several specimens in two reservoirs. The MTR in muscle is 0.05 mg/kg in HCBs whereas for alpha + beta HCH the MTR is 0.02 mg/kg and for lindane (HCH gamma isomer) it is 0.05 mg/kg. In the Kružberk reservoir, high HCB levels in muscle were found in two eels (0.149 and 0.179 mg/kg). A pike weighing almost ten kg, caught in the same reservoir, had an HCB concentration of 0.010 mg/kg. In the Šance reservoir a pike of the same age but with a lower weight had a HCB concentration of 0.005 mg/kg.

The levels of the organochlorinated pesticides HCB and HCH (including HCH isomers) in the muscular fat of the fish are shown in Table 4. The highest HCB levels were found in the eel from the Kružberk reservoir (0.606 to 0.831 mg/kg). In the remaining fish the residues were much smaller, ranging from 0.011 to 0.373 mg/kg. In the Šance and Morávka reservoirs, HCB contamination of the fish fat was lower by an order. In chub from the Šance reservoir, the sum of the α + β HCH isomers grew with the weight of the fish (Figure 5).

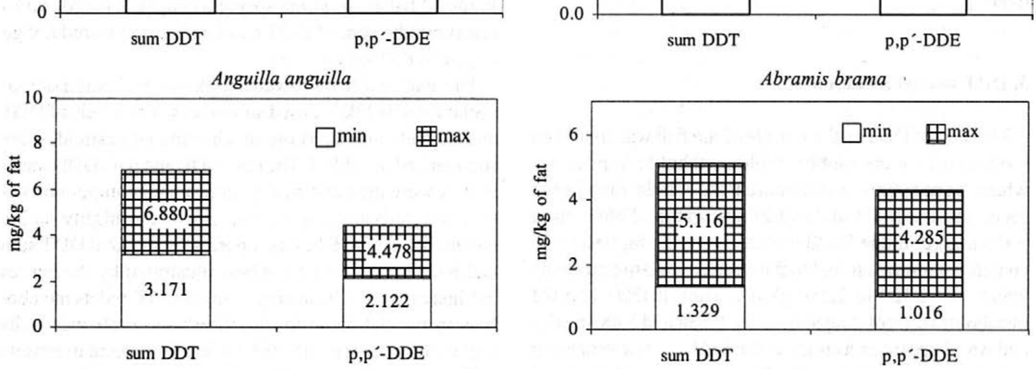


Figure 6. The contribution of the p,p′-DDE metabolite in the muscular fat of the fish to the DDT sum and its metabolites as expressed by the minimum and maximum values for certain fishes in the Kružberk reservoir

Table 3. Content of the organochlorinated pesticide DDT and the metabolites thereof in the muscular fat of the fish (mg/kg)

Fish species	*n*	Age (year)	Weight (g)	o,p´-DDE	p,p´-DDE	o,p´-DDD	p,p´-DDD	o,p´-DDT	p,p´-DDT	Sum DDT
Kružberk										
Esox lucius	5	5–9	2 730–9 700	< 0.005–0.114	2.264–5.120	0.142–0.491	0.302–1.038	0.026–0.373	0.060–0.157	2.835–7.179
Stizostedion lucioperca	3	3–8	1 090–5 610	0.034–0.044	1.011–1.849	0.106–0.118	0.180–0.203	0.030–0.037	0.051–0.452	1.466–2.288
Perca fluviatilis	2	5	240, 379	0.056, 0.064	2.598, 4.660	0.205, 0.286	0.622, 1.105	0.050, 0.072	0.178, 0.277	3.709, 6.464
Aspius aspius	1	5	1 010	0.144	4.845	0.690	0.763	0.093	0.252	6.787
Anguilla anguilla	3	–	1 130–1 280	0.024–0.103	2.122–4.478	0.091–0.311	0.683–1.557	0.023–0.059	0.228–0.372	3.171–6.880
Abramis brama	3	5–7	435–510	0.052–0.110	1.016–4.285	0.092–0.233	0.110–0.466	0.018–0.045	0.041–0.091	1.329–5.116
Rutilus rutilus	1	5	163	0.174	9.467	0.678	0.993	0.010	0.245	11.567
Šance										
Esox lucius	4	3–9	1 370–5 150	< 0.005–0.011	1.002–2.790	0.062–0.256	0.090–0.403	0.035–0.236	0.040–0.203	1.237–3.888
Stizostedion lucioperca	2	4, 8	800, 1 720	0.014, 0.022	0.008, 0.627	0.053, 0.058	0.055, 0.080	0.030, 0.043	0.031, 0.058	0.239, 0.840
Perca fluviatilis	3	10–14	670–1 230	0.023–0.051	0.607–2.336	0.076–0.176	0.176–0.447	0.043–0.085	0.022–0.068	0.992–3.089
Leuciscus cephalus	5	8–14	560–1 610	< 0.005–0.093	0.040–2.462	0.025–0.081	< 0.005–0.047	0.011–0.051	0.007–0.039	0.339–2.623
Abramis brama	1	8	1 140	< 0.005	0.136	0.009	0.035	0.012	< 0.005	0.192
Morávka										
Oncorhynchus mykiss	2	3	194, 400	0.011, 0.033	0.862, 0.923	0.034, 0.053	0.121, 0.142	0.009, 0.016	0.088, 0.093	1.147, 1.238
Salmo trutta m. *fario*	3	4–6	280–580	0.009 a 0.055	0.559–4.625	0.046–0.093	0.069–0.470	0.006–0.084	0.023–3.170	0.785–7.410
Thymallus thymallus	1	4	505	0.080	4.238	0.072	0.363	0.048	0.525	5.326
Perca fluviatilis	2	9, 11	434, 600	0.014, 0.028	0.261, 1.063	0.046, 0.079	0.152, 0.615	0.013, 0.015	0.024, 0.071	0.510, 1.871
Tinca tinca	1	6	540	0.044	0.255	0.041	0.119	0.007	0.017	0.483
Rutilus rutilus	2	7	379, 400	< 0.005, 0.009	0.411, 0.497	0.027, 0.061	0.147, 0.195	0.010, 0.032	0.017, 0.018	0.613, 0.811
**Cottus gobio*		–	–	–	0.025 (0.027)	0.011 (0.018)	0.007 (0.006)	0.012 (0.012)	0.042 (0.022)	0.097 (0.085)

*Mixed sample of 30 individual fishes
Shown in parentheses is the analytic value in the whole fish body

Table 4. Content of the organochlorinated pesticides HCB and HCH isomers in the muscular fat of the fish (mg/kg)

Fish species	*n*	Age (year)	Weight (g)	HCB	HCH				
					alpha	beta	gamma	delta	Sum alpha + beta
Kružberk									
Esox lucius	5	5–9	2 730–9 700	0.017–0.373	< 0.005–0.019	0.007–0.041	< 0.005–0.040	< 0.005–0.043	0.009–0.060
Stizostedion lucioperca	3	3–8	1 090–5 610	0.022–0.092	< 0.005–0.109	< 0.005–0.019	0.007–0.191	< 0.005–0.013	0.007–0.109
Perca fluviatilis	2	5	240, 379	0.011, 0.183	0.014, 0.060	< 0.005, 0.008	< 0.005, 0.016	0.005, 0.010	0.014, 0.069
Aspius aspius	1	5	1 010	0.138	0.012	0.026	0.057	0.007	0.038
Anguilla anguilla	3	–	1 130–1 280	0.606–0.831	< 0.005–0.009	0.018–0.024	0.008–0.033	< 0.005–0.007	0.018–0.032
Abramis brama	3	5–7	435–510	0.039–0.315	< 0.005–0.038	0.015–0.020	0.014–0.049	< 0.005–0.016	0.015–0.058
Rutilus rutilus	1	5	163	0.29	< 0.005	0.016	0.021	< 0.005	0.016
Šance									
Esox lucius	4	3–9	1 370–5 150	< 0.005–0.084	< 0.005–0.021	< 0.005–0.044	0.009–0.041	< 0.005–0.036	0.015–0.065
Stizostedion lucioperca	2	4, 8	800, 1 720	0.018, 0.021	< 0.005, 0.005	< 0.005, 0.026	0.016, 0.023	< 0.005, 0.015	0.031
Perca fluviatilis	3	10–14	670–1230	< 0.005–0.028	0.008–0.086	< 0.005–0.014	0.009–0.029	< 0.005–0.027	0.022–0.086
Leuciscus cephalus	5	8–14	560–1 610	< 0.005–0.028	< 0.005–0.013	< 0.005–0.106	0.007–0.035	< 0.005–0.031	0.006–0.119
Abramis brama	1	8	1 140	0.013	0.026	0.009	0.014	0.011	0.035
Morávka									
Oncorhynchus mykiss	2	3	194, 400	0.027, 0.037	< 0.005, 0.005	< 0.005	0.021, 0.025	< 0.005	0.005
Salmo trutta m. *fario*	3	4–6	280–580	0.031–0.081	0.010–0.013	0.007–0.025	< 0.005–0.058	< 0.005–0.015	0.017–0.038
Thymallus thymallus	1	4	505	0.010	<0.005	0.013	0.011	< 0.005	0.013
Perca fluviatilis	2	9, 11	434, 600	0.012, 0.020	<0.005,0.007	0.006, 0.007	0.019, 0.020	< 0.005	0.006, 0.014
Tinca tinca	1	6	540	0.014	0.006	0.006	0.020	0.009	0.012
Rutilus rutilus	2	7	379, 400	0.006, 0.009	<0.005	0.007, 0.014	0.006, 0.015	< 0.005, 0.009	0.007, 0.014
**Cottus gobio*		–	–	0.013 (0.014)	0.014 (<0.005)	0.005 (0.007)	0.010 (0.011)	0.013 (0.009)	0.014 (0.007)

*Mixed sample of 30 individual fishes

Shown in parentheses is the analytic value in the whole fish body

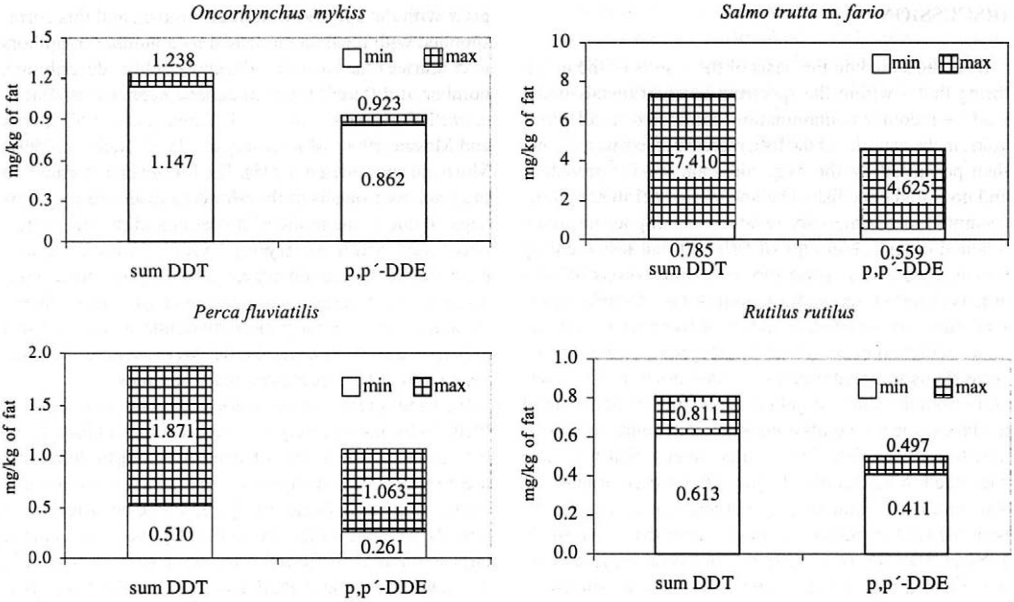


Figure 7. The contribution of the p,p'-DDE metabolite in the muscular fat of the fish to the DDT sum and its metabolites as expressed by the minimum and maximum values for certain fishes in the Morávka reservoir

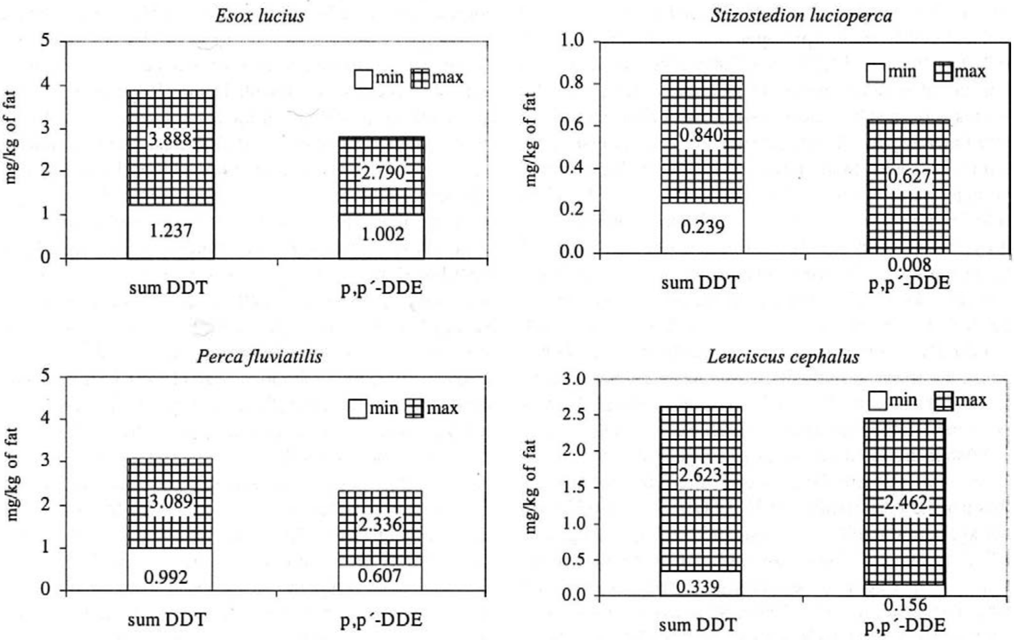


Figure 8. The contribution of the p,p'-DDE metabolite in the muscular fat of the fish to the DDT sum and its metabolites as expressed by the minimum and maximum values for certain fishes in the Šance reservoir

DISCUSSION

It can be stated on the basis of the results of the monitoring that – within the spectrum of eight metals under study – mercury contamination dominated in all reservoirs: in the majority of the fish, mercury levels were higher than permitted by the hygienic standard for predatory and non-predatory fish. The analyses confirmed that the accumulation of mercury residues was higher in predators and older age groups of fish and that less mercury was accumulated in predators with omnivorous behaviour, as found in the rainbow trout in the Morávka reservoir. The mercury levels found in the fish muscle prove an undesirable exposure of the hydrosphere of the studied ecosystems to contaminants washed down to the reservoirs from the land around them; the diffuse pollution of the landscape with emissions is another source of contamination of the fish. The results suggest that the same sites need to be examined again after a certain interval. This can be documented by comparison of our results with the studies performed in the Kružberk reservoir in 1984 by Pavelka *et al.* (1986). For pikes, these authors documented that mercury content directly depended on age: the average concentration of 0.065 mg/kg was reached at an age of 6.5 years and the highest concentration recorded by them was 0.140 mg/kg. These levels are far below the range recorded by us in the fish of the same age. Mercury contamination twice as high as the maxima recorded by Pavelka *et al.* (1986) were found in five-year old and eight-year old pike-perches within our samples: 0.710 vs. 0.310 and 1.097 vs. 0.500 mg/kg. Our conclusions can also be compared, to a certain extent, with the results published by Čelechovská *et al.* (1995), who monitored mercury levels in fourteen water-supply reservoirs within the basin of the Morava river. Within these results, younger pikes (1+ to 3+) had mercury levels of 0.013 to 0.317 mg/kg whereas in two eels, also in a younger category, the mercury levels were much lower (0.086 and 0.521 mg/kg); a five-year old asp from the Koryčany reservoir had the highest mercury contamination (1.348 mg per kg). Compared with the data by Svobodová *et al.* (1988), a higher mercury exposure was found in the Želivka reservoir (basin of the Elbe), as suggested by mercury levels in the muscle of pike of almost the same age. Mercury content in the muscle of the fish exceeded the levels published by Svobodová *et al.* (1987) mainly in the predatory fish from the Podhora water-supply reservoir (basin of the Vltava) (pike: 0.531–1.900 vs. 0.159–0.375 mg per kg; perch: 0.540–1.125 vs. 0.221–0.777 mg/kg; eel: 0.732–1.080 vs. 0.130–0.734 mg/kg). Compared with roach from the Lučina water-supply reservoir (basin of the Vltava) (Svobodová *et al.*, 1997), we found an almost equal contamination of muscle with mercury (0.130–0.360 vs. 0.120–0.489 mg/kg). Like Hejtmánek *et al.* (1975), Svobodová and Hejtmánek (1985), Svobodová *et al.* (1988, 1999), we found that the level of mercury in fish muscle tissue grew with the age and weight of the fish, and this corresponded with the data published by a number of authors in countries other than the Czech Republic, describing a number of different types of aquatic ecosystems (Bache *et al.*, 1971; Cross *et al.*, 1973; Luten *et al.*, 1987; Barak and Mason, 1990; Monteiro *et al.*, 1991; Jackson, 1991; Morrison and Thérien, 1995). The low level of mercury in the rainbow trout from the Morávka reservoir may perhaps be due to the mode of life of this salmonid, preferring food such as flying insects, plankton and macrozoobenthos, as documented by the post-mortem findings in the digestive tract. Residues of benthic fauna, sometimes with a large proportion of mat algae which had perhaps been eaten while the fish were seeking the organisms living there, prevailed in the samples.

We used a range of indicator congeners to determine PCBs as the most closely watched xenobiotics for a detailed review of the extent of exposure; and to determine them in view of assessment of toxic risks, we used three planar congeners. These slowly degrading persistent compounds, showing a high affinity for fat, were found in the largest amounts in the fish from the Kružberk reservoir. The sum of indicator PCB congeners in the tissue reached its marked peak levels in eel, whereas the sum of indicator congeners in fat reached its peak in pike and perch. The relatively high concentrations of PCB and DDT in roach and asp show how important it is to subject the widest possible spectrum of fish to chemical and toxicological analysis which is to afford a sufficiently detailed view of the given area's exposure to pollutants as needed for objective assessment and for focusing future investigation. This is also documented by the lower level of PCB and DDT in the eels from the same location: these eels had much more fat than other fish so that, as can be concluded, fat is not always the key requisite for the occurrence of both pollutants. The much lower sum of PCB congeners in one of the eels appears to suggest that the sources of these organochlorinated compounds are irregularly distributed in the studied ecosystem (this assumption is supported by the high migration activity of eel). The presence of PCBs in the organs and muscle of the fish in the water-supply reservoirs Lučina, Podhora and Želivka is also described by Svobodová *et al.* (1989). Using technical mixtures as standards, PCB levels in muscle were found to range from 0.1 mg/kg (Lučina) to 0.5 mg/kg (Podhora, Želivka). Studies based on congener analysis showed a greater exposure of fish muscle to PCBs compared to that described by Svobodová *et al.* (1997) for the perch and roach living in the Lučina reservoir and bream and roach in the Římov reservoir (basin of the Vltava). The sum of PCB indicator congeners in the roach from the Morávka and Kružberk reservoirs was higher by an order than that in the roach from the Lučina reservoir (0.0069 vs. 0.018–0.090 mg/kg). A similar picture was obtained in the bream from the Římov reservoir, compared with the bream from the Kružberk reservoir (0.018 vs. 0.149 mg/kg). What is also surprising is the contamination of the fish with DDT-group substances, especially in a reservoir with a drainage basin as small as the Morávka's. The recorded DDT levels with a prevailing proportion of the persistent metabolite, p,p′-DDE, are due to chronic exposure of the fish to these slowly degrading substances; at the same time, they prove that this insecticide which was banned in 1975 still persists in the environment. The work contributed additional findings to the knowledge of the distribution of heavy metals, polychlorinated biphenyls and other persistent chlorinated contaminants in fish in the territory of Moravia (eastern part of the CR) where these issues were studied by Hajšlová *et al.* (1997) in the Mušov dam reservoir (the Nové Mlýny Dam System) on the Dyje (Thaya) River and also by Spurný and Mareš (1991), who monitored heavy metals in the fish living in the Dalešice and Mohelno dam reservoirs on the Jihlava River (the Danube drainage area).

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Changes in fatty acid pattern, composition and technological parameters of milk in dairy cows fed heat-treated rapeseed cakes in the first stage of lactation

Změny zastoupení mastných kyselin, složení a technologických vlastností mléka v průběhu první fáze laktace u dojnic krmených upravenými řepkovými výlisky

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ABSTRACT: Two groups of 8 dairy cows each (Czech Red-pied × Ayrshire × Red Holstein) were fed basal diet and concentrate mixture with either heat-treated rapeseed cakes (HRC group) or soybean meal (SBM group) within the first hundred days of lactation. Milk constituents (solids, fat, solids-non-fat, crude protein, casein, whey protein, urea, lactose, calcium, citric acid), fatty acid pattern of milk fat, and technological traits (freezing point, acidity, heat stability, rennetability) were determined in milk samples taken on 21st, 42nd and 100th day of lactation. Neither yield of milk and yield of any milk constituent, nor the content of fat, total protein, casein, whey protein and calcium, respectively, differed ($P > 0.05$) between HRC and SBM groups. On the other hand, solids-non-fat and lactose content was higher ($P < 0.05$) in HRC-milk as compared to SBM-milk. Content of saturated fatty acids was lower ($P < 0.01$) and content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) higher ($P < 0.01$) in milk of dairy cows fed the diet with HRC. The ratio of total C16/C18 fatty acids was by 30% lower ($P < 0.01$) in HRC-milk in comparison with SBM-milk. Content of myristic acid increased ($P < 0.01$) and content of stearic acid decreased ($P < 0.05$) in milk fat with increasing day of lactation irrespective of the feeding intervention. Content of linoleic acid, total PUFAn6 and PUFA/MUFA ratio increased ($P < 0.05$) with increasing day of lactation within the HRC group. Neither rennetability nor heat stability of milk was negatively influenced by the presence of HRC in the diet. It was concluded that inclusion of heat-treated rapeseed cakes in the diet for dairy cows instead of SBM did not influence negatively either composition or the technological quality of milk, and on the other hand improved its nutritive value from the viewpoint of more favourable fatty acid spectrum of milk fat.

Keywords: rapeseed cakes; fatty acids; milk composition; rennetability; heat stability; lactation

ABSTRAKT: Dvě skupiny osmi dojnic (kříženek plemen české strakaté × Ayrshire × Red Holstein) byly krmeny základní krmnou dávkou a doplňkovou směsí obsahující buď tepelně upravené řepkové výlisky (pokusná skupina, HRC), nebo sojový extrahovaný šrot (kontrola, SBM). Složení doplňkových směsí je uvedeno v tabulce 1, jejich živinové složení, včetně zastoupení kvantitativně nejvýznamnějších mastných kyselin a obsahu glukosinolátů v tabulce 2. Ve vzorcích mléka, které byly odebírány 21., 42. a 100. den laktace, byl stanoven obsah sušiny, tuku, tukuprosté sušiny, celkových bílkovin, kazeinu, syrovátkových bílkovin, močovinového dusíku, laktózy, vápníku a citrátů, dále zastoupení mastných kyselin v mléčném tuku a konečně vybrané technologické ukazatele (bod mrznutí, kyselost, alkoholové číslo a syřitelnost). Dojivost ani produkce mléčného tuku, celkových bílkovin a laktózy se mezi skupinami HRC a SBM nelišila ($P > 0,05$; tabulka 3), stejně jako obsah tuku, celkových bílkovin, kazeinu, syrovátkových bílkovin a vápníku v mléce (tabulka 4). Mléko HRC dojnic mělo vyšší ($P < 0,05$) obsah laktózy a tukuprosté sušiny (tabulka 3). V mléčném tuku dojnic ze skupiny HRC bylo zjištěno nižší ($P < 0,01$)

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zastoupení nasycených mastných kyselin a vyšší ($P < 0,01$) zastoupení mononenasycených (MUFA), resp. polynenasycených (PUFA) mastných kyselin (tabulka 5). Poměr celkových C16/C18 mastných kyselin byl v mléčném tuku pokusné skupiny (HRC) o 30 % nižší ($P < 0,01$) ve srovnání s kontrolou (SBM). Zastoupení kyseliny myristové v mléčném tuku se zvyšovalo ($P < 0,01$) a zastoupení kyseliny stearové snižovalo ($P < 0,05$) s rostoucím dnem laktace bez ohledu na krmný zásah. V rámci SBM skupiny dojnic se s rostoucím dnem laktace zvyšovalo ($P < 0,05$) zastoupení kyseliny linolové, celkových PUFA a poměr PUFA/MUFA (tabulka 6). Ani syřitelnost, ani termická stabilita mléka nebyla přítomností HRC v krmné dávce negativně ovlivněna (neprůkazné rozdíly), v těchto ukazatelích mezi HRC a SBM skupinou, $P > 0,05$; tabulka 7). Z výsledků práce plyne, že zařazení tepelně upravených řepkových výlisků do krmné dávky dojnic místo sojového extrahovaného šrotu nemělo za následek jakékoliv negativní ovlivnění obsahových nebo technologických ukazatelů mléka a naopak pozitivně ovlivnilo nutriční hodnotu mléka z hlediska příznivějšího zastoupení mastných kyselin v mléčném tuku.

Klíčová slova: řepkové výlisky; mastné kyseliny; složení mléka; syřitelnost; termická stabilita; laktace

INTRODUCTION

The use of rapeseed in the diets for dairy cows increased recently in some European countries after introduction of the "00" cultivar. Rapeseed cakes are cheaper than soya or cereal concentrates, and feeding costs are lower (Ammann, 1996). Rapeseed in the form of meal or cakes with varying fat content became an usual component of the dairy cows diets (Jahreis *et al.*, 1996). However, as compared to soybean, rapeseed has a lower content of crude protein, and, in addition, high crude protein degradability. Therefore a proper heat treatment during oil extraction and feed processing is desirable for reduction of nitrogen degradability (Daccord, 1996). Despite the above treatment some authors expect insufficient synthesis of casein and whey proteins in dairy cows fed the diets based on rapeseed in comparison with soybean meal (Khorasani *et al.*, 1991). Actually Komprda *et al.* (2000) found lower content of casein ($P < 0.05$) and whey protein ($P < 0.01$), respectively, in milk of dairy cows fed the diet with heat-treated rapeseed cakes as compared to soybean meal (SBM). Also, according to Mustafa *et al.* (1997), diets based on canola meal produced milk with a lower protein content in comparison with the diets based on SBM. On the other hand, there are numerous papers suggesting that inclusion of rapeseed in the dairy cow diet does not decrease milk protein content (Tymchuk *et al.*, 1998; Wiesen *et al.*, 1990; Šimek *et al.*, 2000).

Also the content of milk fat can be negatively influenced by using rapeseed with too high crude fat content in the dairy cow diets (Kennely and Glimm, 1998). Therefore Pajtáš *et al.* (1975) recommended the maximal fat content in the dairy cow diet 5% of the dry matter.

As far as the fatty acid spectrum of milk fat is concerned, the main interest is to decrease the ratio of total C16/ total C18 fatty acids, because, according to the current state of knowledge, the effect of stearic acid (C18 : 0) is analogous to that of oleic acid (C18 : 1) regarding the decrease of the plasma cholesterol level in humans (Kennely and Glimm, 1998). Rapeseed, with its high content of oleic and linoleic acid, is suitable feed component to achieve the above mentioned improvement of the milk nutritive value (Focant *et al.*, 1998).

The data regarding the effect of rapeseed in the dairy cow diet on technological traits of milk are not easy to find in current available literature. Within these traits, freezing point is relatively more frequently mentioned, but rather from the viewpoint of the general aspects of nutrition. Some aspects of an inadequate nutrition regarding freezing point of cow's milk were discussed by Wiedemann *et al.* (1993).

The objective of the present study was – in continuation of the previous experiment (Komprda *et al.*, 2000) – to compare milk produced using heat-treated rapeseed meal or soybean meal in the course of the first hundred days of lactation, from the viewpoint of yield and composition, fatty acid spectrum of milk fat, and technological traits.

MATERIAL AND METHODS

Experimental animals, diets and taking milk samples

The experiments were carried out at the dairy co-operative farm Žichlínek, Czech Republic, in the course of October 1998 to February 1999, with twenty-four dairy cows, Czech Red-pied × Ayrshire × Red Holstein crossbreds. Dairy cows were kept individually and fed the basal diet calculated to produce 8 l of milk per day (maize silage 18 kg, red clover haylage 12 kg, meadow hay 0.5 kg), and the concentrate mixture at the rate 0.4 kg/l of milk produced above 8 l/day. Two groups of twelve dairy cows each were formed. Control group received concentrate mixture with soybean meal (SBM) and experimental group concentrate mixture with heat-treated rapeseed cakes (HRC). Ingredients and nutrient composition (including quantitatively most important fatty acids and glucosinolate content) of both concentrate mixtures are presented in Table 1 and 2, respectively. The average live weight within the group of dairy cows fed the SBM diet was 634 and 639 kg at the beginning and at the end (100th day of lactation) of the experiment, respectively. Corresponding values wi-

Table 1. Formulation of the production mixtures for dairy cows

Ingredient (%)	Production mixture	
	HRC[1)]	SBM[2)]
Wheat	30.5	29.6
Maize	19.0	19.0
Linseed	/	2.0
Wheat bran	/	10.0
Soybean meal	10.0	25.0
Heat-treated rapeseed cakes	27.0	/
Malt sprouts	10.0	10.0
Feed salt	0.9	0.9
Ground limestone	1.8	2.0
MgO	0.2	0.3
Dicalcium phosphate	/	0.6
Premix of biofactors	0.6	0.6

1) with heat-treated rapeseed cakes
2) with soybean meal

Table 2. Nutrient content of the production mixtures, including quantitatively most important fatty acids, and glucosinolates

Nutrient (g/kg of fresh matter)	Production mixture	
	HRC[1)]	SBM[2)]
Crude protein	206	221
Crude fat	46	25
Crude fibre	54	43
Crude ash	72	69
Nitrogen free extract	517	529
Organic matter	822	818
Oleic acid[3)]	39	20
Linoleic acid[3)]	34	42
α-linolenic acid[3)]	12	15
Dry matter (%)	89.4	88.6
Glucosinolates (mmol/kg)	1.17	< 0.05

1) with heat-treated rapeseed cakes
2) with soybean meal
3) percentage of total determined fatty acids

thin the group of dairy cows fed the diet with HRC were 633 and 642 kg.

Milk samples were taken when particular dairy cows of a given group reached 21st, 42nd and 100th day of lactation, respectively. An average milk sample from each dairy cow was taken during milking at the amount of 1 l. Samples were cooled to the temperature 4°C and transported to the laboratory. Content parameters, including fat, and technological traits were determined within 24 hours after transport. Extracted milk fat was stored at –20°C in dark powder bottles until successive analyses of fatty acids.

Rapeseed cakes treatment

Untreated rapeseed cakes contained in one kg of fresh matter (dry matter 91.4%) 288 g of crude protein, 88 g of crude fibre, 143 g of crude fat, 340 g of nitrogen free extract and 55 g of ash. The treatment (combination of heat, pressure, chemical substances and water) is mentioned in greater detail in the paper of Komprda *et al.* (2000).

Milk fat and fatty acid (FA) pattern were determined according to Komprda *et al.* (2000) with the following exceptions. GLC analysis of FA methyl esters (FAMEs) was performed on HRGC 5300 MEGA chromatograph (CE Instruments, Italy) using the capillary column with PEG bound phase 25 m × 0.32 mm × 0.2 μm (CP-WAX 52 CB, Chrompack, Netherlands).

Determination of other parameters of milk composition

The following traits were determined: solids (gravimetrically; ČSN 570530), solids-non-fat (calculated from solids and fat content), crude protein, casein and whey proteins (spectrophotometrically, using the Pro-Milk apparatus, Foss Electric, Denmark), urea nitrogen (spectrophotometrically at the wavelength 420 nm after the reaction with *p*-dimethylaminobenzaldehyde; Gajdůšek *et al.*, 1996), lactose (polarimetrically; ČSN 570530), Ca (by the complexometric titration using fluorexon indication; ČSN 570530), citric acid (on Milkoscan 4000 apparatus, Foss Electric).

Determination of technological traits

Active acidity was measured by pH meter (WTW 95 with SenTix 97 electrode) according to Černá and Cvak (1986). Acidity was determined as a consumption of 0.25 mol/l NaOH solution (factor 0.992) per 100 ml of milk with phenolphtalein as an indicator. Freezing point was measured on the Cryoskop 4D2 apparatus (Foss Electric).

Heat stability was expressed as a consumption of 96% ethanol (in ml) needed to produce flakes of precipitate in 5 ml of milk (Černá and Cvak, 1986).

Rennetability was assessed as a time interval needed for precipitation of the milk sample by rennet. One ml of diluted (2%) rennet solution was added to 100 ml of milk in Erlenmeyer flask (at the constant temperature 35°C and under the continuous stirring), and the time (in minutes) was measured when first flakes of curdle appeared (Teplý *et al.*, 1976).

In addition, somatic cell count was measured on Fossomatic apparatus (Foss Electric)

Statistical evaluation

Both the effect of the inclusion of SBM or HRC in the diet and the effect of the stage of lactation on each trait was tested using one-way classification of the variance ratio test. Duncan's test was used for evaluation of differences between particular subsets when the effect of the stage of lactation was considered. In case that significant differences were found between the stages of lactation, polynomial regression was calculated as a dependence of a given trait on the day of lactation (significance of linear or quadratic term was tested). Correlation analysis was used to test relationships between particular traits. All calculations were performed using statistical package Unistat, version 4.53 (Unistat Ltd., London, England 1999).

RESULTS AND DISCUSSION

Health status of dairy cows

One dairy cow from the control group died within the experiment due to the abomasoenteritidis and sepsis. Two dairy cows from this group were discarded because of a sharp decrease of milking within the experiment, and one due to the acidosis and worsened function of the digestive tract. Within the experimental group (production mixture based on rapeseed cakes) only one dairy cow was discarded due to the kidney disorders.

It is apparent from this comparison that inclusion of rapeseed cakes in the diet had no adverse effect on the health of dairy cows. This is in agreement with the statement of Ahlin *et al.* (1994) that the feeding of rapeseed products from certified double-low varieties of *Brassica napus* to adult dairy cows in amount up to 3 kg rapeseed meal per cow and day would not have any negative effects on animal health or fertility. According to Mawson *et al.* (1994), sows' fertility may be impaired by the intake of 8 mmol of glucosinolates per day, but ruminants tolerate a higher level in the long-term experiments. Total amount of glucosinolates in the daily ration for the HRC group was below 9 mmol in the present experiment (due to the rapeseed cake treatment by heat, pressure and chemical substances), as follows from the description of the feeding scheme and the data of Table 2.

In addition, only the samples that did not exceed the limit of somatic cell count 3×10^5/ml of milk were included in the evaluation. Eight dairy cows from each group fulfilled these criteria in each of the three tested periods of lactation.

Yields

Yield of milk and yield of any basic milk constituent (in kg per day) did not differ ($P > 0.05$) between dairy cows fed the diet with SBM or HRC (Table 3). These data, with the exception of milk fat, are in agreement with our previous experiment regarding the effect of heat-treated rapeseed cakes in dairy cow diet (Komprda *et al.*, 2000). However, absolute values of yields are substantially higher in the present experiment (e.g. as far as milk is concerned by 25%). The possible reason is that in the present experiment only samples from dairy cows which were in a such health status to be able to produce milk with somatic cell count less than 3×10^5/ml of milk within the whole tested period were taken into account.

Table 3. Yield of milk and basic milk constituents. One-way classification of the variance ratio test, $n = 24$

Trait (kg/day)	Feeding intervention (mean ± standard error of the mean)		Significance
	HRC[1)]	SBM[2)]	
Milk	27.4 ± 0.98	27.4 ± 0.89	$P > 0.05$
Milk fat	0.94 ± 0.02	0.97 ± 0.03	$P > 0.05$
Total protein	0.91 ± 0.03	0.86 ± 0.03	$P > 0.05$
Lactose	1.41 ± 0.02	1.37 ± 0.02	$P > 0.05$

[1)] heat-treated rapeseed cakes

[2)] soybean meal

Crude protein yield only tended to be higher in milk produced using heat-treated rapeseed cakes in the present experiment. Similarly to our results Šimek *et al.* (2000) did not find any difference in yield of total protein between dairy cows fed the diet with rape cakes or control mixture with soybean meal. The results of the present experiment are also confirmed by the data of Michalcová and Benczová (1995) and Tymchuk *et al.* (1998) as far as the yield of milk protein is concerned. On the other hand, when replacing SBM by rapeseed meal or full-fat rapeseed in the dairy cow diet, Emanuelson *et al.* (1993) found higher yield of total milk protein.

A lower yield of milk fat is sometimes reported in dairy cows receiving the diets based on rapeseed cakes as compared to soybean meal: e.g. Michalcová and Benczová (1995), Komprda *et al.* (2000). However, these findings were not confirmed in the present experiment (Table 3), which is in accordance with the data of Šimek *et al.* (2000).

Milk constituents

Basic parameters of milk composition are presented from the viewpoint of differences between feeding interventions and between days of lactation in Table 4. Content of fat, crude protein, casein, whey protein and calcium, respectively, in milk of dairy cows fed the diet containing HRC did not differ ($P > 0.05$) from milk produced using SBM. Similarly Munger (1996) found no differences in milk composition between the groups of dairy cows fed a diet with either rapeseed meal or soybean oilmeal. However, the above results are contrary to the experiment of Komprda *et al.* (2000), where inclusion of HRC in the production mixture for dairy cows instead of SBM resulted

Table 4. Constituents of milk produced using the production mixture with HRC[1)] or SBM[2)]. One-way classification of the variance ratio test

Milk component (%)	Feeding intervention (n = 24) (mean ± standard error of the mean)		Day of lactation (means; n = 8)					
	HRC[1)]	SBM[2)]	HRC[1)]			SBM[2]		
			21st	42nd	100th	21st	42nd	100th
Milk fat	$3.4^{A} \pm 0.08$	$3.5^{A} \pm 0.08$	3.35^{a}	3.47^{a}	3.43^{a}	3.22^{a}	3.64^{a}	3.56^{a}
SNF[3]	$9.1^{B} \pm 0.47$	$8.8^{A} \pm 0.36$	8.87^{abc}	9.06^{bcd}	9.31^{d}	8.69^{ab}	8.65^{a}	9.13^{cd}
Lactose	$5.0^{B} \pm 0.23$	$4.9^{A} \pm 0.25$	4.99^{b}	5.03^{b}	5.03^{b}	4.92^{b}	4.70^{a}	4.99^{b}
TP[4)]	$3.4^{A} \pm 0.23$	$3.2^{A} \pm 0.29$	3.41^{b}	3.39^{b}	3.28^{ab}	3.28^{ab}	3.31^{a}	3.42^{b}
Casein	$2.6^{A} \pm 0.22$	$2.5^{A} \pm 0.26$	2.59^{a}	2.57^{a}	2.50^{a}	2.51^{a}	2.49^{a}	2.60^{a}
WP[5)]	$0.8^{A} \pm 0.13$	$0.8^{A} \pm 0.09$	0.82^{a}	0.82^{a}	0.78^{a}	0.75^{a}	0.71^{a}	0.82^{a}
Urea[6)]	$1.3^{A} \pm 0.34$	$1.4^{A} \pm 0.51$	1.19^{a}	1.47^{a}	1.27^{a}	1.36^{a}	1.39^{a}	1.54^{a}
Ca	$1.2^{A} \pm 0.18$	$1.2^{A} \pm 0.2$	1.2^{a}	1.2^{a}	1.3^{a}	1.2^{a}	1.2^{a}	1.3^{a}
Citric acid[7)]	$0.16^{A} \pm 0.12$	$0.15^{A} \pm 0.03$	0.16^{a}	0.15^{a}	0.15^{a}	0.16^{a}	0.17^{a}	0.14^{a}

[1)] heat-treated rapeseed cakes
[2)] soybean meal
[3)] solids-non-fat
[4)] total protein
[5)] wheat protein
[6)] in mg/100 ml of milk
[7)] in g/100 ml of milk

A, B – means with different superscripts in lines differ significantly (P < 0.05; Duncan's test)
a ,b, c, d – means with different superscripts in lines differ significantly (P < 0.05; Duncan's test)

in lower percentage of fat, total protein, whey protein ($P < 0.01$) and casein ($P < 0.05$).

The possible explanation of different results presented in the paper of Komprda *et al.* (2000) and in the present paper regarding milk fat content, is crude fat and crude fibre content of the diet. Milk fat content is influenced among other things mainly by dietary fibre (Kennely and Glimm, 1998) and by dietary fat (Jahreis *et al.*, 1995). More acetate is produced in the rumen at the expense of propionate with consequent increase of milk fat concentration when the content of dietary fibre is higher. Similarly, growth restriction of rumen microorganisms and therefore the restriction of the acetate production follows from a higher concentration of dietary fat, especially when fat is given in a non-protected form and has a high ratio of unsaturated fatty acids (Jahreis *et al.*, 1996). Crude fat content in the production mixture with HRC used in our previous experiment (Komprda *et al.*, 2000) was more than six times higher in comparison with mixture based on SBM, and crude fibre content was lower. Production mixture with HRC used in the present experiment contained only 1.8 times more crude fat and more crude fibre in comparison with SBM-mixture (Table 2). In accordance with the present experiment Šimek *et al.* (2000) reported practically the same ($P > 0.05$) fat content in milk of dairy cows fed the diet with cold or hot pressed rape cakes as compared to the diet with SBM.

As far as protein content is concerned, Šimek *et al.* (2000) found in the above mentioned experiment only a tendency ($P > 0.05$) to lower total protein content in milk of dairy cows fed rape cakes as compared to SBM. We found the same tendency ($P > 0.05$) regarding the comparison of total protein content between the whole sets of dairy cows (first part of Table 4). This tendency had strengthened in the significant ($P < 0.05$) difference between total protein content of HRC-milk and SBM-milk only within the second part of the tested lactation stage (42nd day of lactation; second part of Table 4). Moreover, there are numerous reports confirming that inclusion of the rapeseed based ingredient in the dairy cow diet does not influence milk protein content (Tymchuk *et al.*, 1998; Wiesen *et al.*, 1990; Munger, 1996). On the other hand, Mustafa *et al.* (1997) reported a lower protein content in milk produced with the diets based on canola meal in comparison with the diets based on SBM.

Kennely and Glimm (1998) report the content of dietary protein as one of the main factors influencing positively the per cent of milk protein. Protein content in production mixture with HRC was only by 7% and 1–2% lower as compared to the mixture based on SBM in the present experiment and in the experiment of Šimek *et al.* (2000), respectively, but nearly by 22% lower in our previous experiment (Komprda *et al.*, 2000).

We were not able to find any difference ($P > 0.05$) in casein content between HRC-milk and SBM-milk in the present experiment (Table 4), contrary to the results of Šimek *et al.* (2000) and our previous experiment (Komprda *et al.*, 2000). Milk produced using the diet with rapeseed had lower casein content in comparison with SBM in both above quoted papers.

Contrary to our previous experiment (Komprda *et al.*, 2000), lactose content in HRC-milk was higher ($P < 0.05$) than in milk produced with SBM (Table 4). However, similarly to the results of the present experiment Jahreis *et al.* (1993) observed a significant increase of lactose content in milk of dairy cows fed the diet with full-fat rapeseed meal as compared to the diet without rapeseed.

Also the content of urea and citric acid was the same ($P > 0.05$) in HRC-milk and SBM-milk in the present experiment.

The content of solids-non-fat was higher ($P < 0.05$) in HRC-milk (Table 4). Different results were reported by Šimek *et al.* (2000), who did not find any difference in solids-non-fat content in milk produced using rape cakes and soybean meal, respectively.

Also the effect of the stage of lactation manifested itself most conspicuously in the case of solids-non-fat, whose content (Y; g/kg of milk) increased in the present experiment (irrespective of the use of HRC or SBM in the diet) with the increasing day of lactation (X; days) according to the equation $Y = 86.4 + 0.033X$ ($R^2 = 0.19$, $P < 0.01$). Similarly to our results the content of solids-non-fat increased in milk of dairy cows fed the diets with rape cakes in the experiment of Šimek *et al.* (2000) until the 100th day of lactation.

Fatty acid pattern

The effect of inclusion of HRC or SBM in the dairy cow diet on fatty acid spectrum in milk fat is apparent from Table 5. As far as fatty acids, whose content in milk fat is important, are concerned, the inclusion of HRC instead of SBM in the diet decreased myristic acid (C14 : 0; $P < 0.05$) and palmitic acid (C16 : 0; $P < 0.01$) content, and increased stearic (C18 : 0; $P < 0.05$), oleic (C18 : 1; $P < 0.01$), linoleic (C18 : 2n6; $P < 0.01$) and α-linolenic (C18 : 3n3; $P < 0.01$) acid content. With the exception of α-linolenic acid, these results are in full agreement with our previous experiment (Komprda *et al.*, 2000). As far as α-linolenic acid is concerned, only a tendency to higher content of this acid in HRC-milk in comparison with SBM-milk was observed in the experiment of Komprda *et al.* (2000). The literature data confirm our above findings regarding myristic, palmitic and stearic acid (Jahreis *et al.*, 1996), oleic acid (Jahreis *et al.*, 1995; Emanuelson *et al.*, 1993; Wiesen *et al.*, 1990), or linoleic acid (Wiesen *et al.*, 1990).

In milk of dairy cows fed HRC content of total saturated FA (SFA) was lower ($P < 0.01$) and content of total monounsaturated FA (MUFA) and total polyunsaturated FA (PUFA; Table 5) was higher ($P < 0.01$). This is also in full agreement with our previous results (Komprda *et al.*, 2000). However, despite the fact that the PUFAn6/PUFAn3 ratio was – similarly like in this and previous experiment (Komprda *et al.*, 2000) – higher ($P < 0.05$) in milk produced with HRC-based production mixture as compared to SBM-mixture, the absolute value of this ratio was lower by 40–50% both in HRC-milk and SBM-milk (more favourable from the viewpoint of human nutrition) in the present experiment. This fact is possible to explain by the lower ratio of linoleic/α-linolenic acid in both the experimental and the control production mixture in the present experiment. The percentage of HRC in the experimental mixture was nearly by one third lower, and the control mixture contained linseed (with relatively high content of α-linolenic acid) in the present experiment as compared to the previous one (Komprda *et al.*, 2000).

The ratio of total C16/C18 fatty acids was by 30% lower ($P < 0.01$) in HRC-milk in comparison with milk of dairy cows fed the diet with SBM (Table 5). This effect of inclusion of rapeseed-based ingredients in dairy cows diets is generally recognized (Jahreis *et al.*, 1995; Emanuelson *et al.*, 1993).

Apart from comparison of the feeding interventions (HRC vs. SBM) Table 5 contains also the data regarding changes in fatty acid spectrum in the course of the first hundred days of lactation. In fatty acids in whose content significant differences due to the stage of lactation were found (21st vs. 42nd vs. 100th day of lactation; using the Duncan's test at the significance level 95%), the dependences on the day of lactation were tested. The results for those cases when the coefficient of the linear term was significant ($P < 0.05$; inclusion of the quadratic term was not significant in any case) are presented in Table 6.

Irrespective of the feeding intervention, with increasing day of lactation the content of myristic acid increased ($P < 0.01$) and the content of stearic acid in milk fat decreased ($P < 0.05$). Within the group of dairy cows fed using HRC there was an increase in the content of linoleic acid ($P < 0.05$), content of total PUFAn6 ($P < 0.05$), and the ratio of PUFA/MUFA ($P < 0.05$; Table 6).

Technological traits

Within the set of any tested technological trait the differences between milk produced with SBM or HRC were not found (Table 7). However, tendency to a lower value of freezing point in HRC milk was observed. Significantly ($P < 0.05$) lower value of the freezing point of HRC-milk as compared to SBM-milk was observed within the second part (42nd day) of the tested lactation period. Freezing point depends, among other things, on lactose content and solids-non-fat content (Mitchell, 1989). Both these parameters were significantly higher ($P < 0.05$) in milk of dairy cows fed the diet with HRC as compared to the control group (Table 4). Coefficient of correlation between freezing point on the one hand and lactose content or solids-non-fat on the other hand was significant in both cases ($r = -0.33$, and $r = -0.23$, respectively, $P < 0.05$; calculated within the whole tested lactation period). In

Table 5. Fatty acid pattern of milk produced using the production mixture with HRC[1)] or SBM[2)]. One-way classification of the variance ratio test

Fatty acid (% of total measured fatty acids)	Feeding intervention (n = 24) (mean ± standard error of the mean)		Day of lactation (means; n = 8)					
	HRC[1)]	SBM[2)]	HRC[1)]			SBM[2]		
			21st	42nd	100th	21st	42nd	100th
C4 : 0	$5.4^{A} \pm 0.3$	$5.7^{A} \pm 0.2$	5.9^{a}	4.6^{a}	5.8^{a}	5.8^{a}	5.8^{a}	5.7^{a}
C6 : 0	$2.6^{A} \pm 0.1$	$2.8^{A} \pm 0.1$	2.7^{a}	2.6^{a}	2.5^{a}	2.8^{a}	2.7^{a}	2.8^{a}
C8 : 0	$1.3^{A} \pm 0.1$	$1.4^{A} \pm 0.1$	1.4^{a}	1.4^{a}	1.8^{a}	1.4^{a}	1.3^{a}	1.4^{a}
C10 : 0	$3.0^{A} \pm 0.2$	$3.1^{A} \pm 0.2$	3.0^{a}	3.1^{a}	2.9^{a}	2.8^{a}	3.2^{a}	3.4^{a}
C12 : 0	$3.1^{A} \pm 0.2$	$3.5^{A} \pm 0.1$	3.0^{a}	3.2^{a}	3.2^{a}	3.7^{a}	3.5^{a}	3.8^{a}
C13 : 0	$0.1^{A} \pm 0.0$	$0.1^{A} \pm 0.0$	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}
C14 : 0	$10.2^{A} \pm 0.3$	$11.1^{B} \pm 0.3$	9.3^{a}	10.4^{ab}	10.8^{abc}	10.3^{ab}	11.1^{bc}	12.0^{c}
C16 : 0	$25.4^{A} \pm 0.4$	$30.4^{B} \pm 0.7$	24.1^{a}	26.3^{ab}	25.7^{a}	28.7^{bc}	30.2^{cd}	32.1^{d}
C18 : 0	$11.5^{A} \pm 0.6$	$9.7^{A} \pm 0.4$	12.6^{b}	11.3^{b}	10.6^{ab}	10.4^{ab}	10.5^{ab}	8.2^{a}
C20 : 0	$0.2^{A} \pm 0.0$	$0.2^{B} \pm 0.0$	0.2^{b}	0.2^{b}	0.2^{b}	0.2^{ab}	0.2^{ab}	0.1^{a}
C22 : 0	$0.1^{A} \pm 0.0$	$0.1^{A} \pm 0.0$	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}
C24 : 0	$0.0^{A} \pm 0.0$	$0.0^{A} \pm 0.0$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
C10 : 1	$0.2^{A} \pm 0.0$	$0.2^{B} \pm 0.0$	0.1^{ab}	0.2^{a}	0.2^{ab}	0.2^{ab}	0.2^{b}	0.3^{c}
C12 : 1	$0.1^{A} \pm 0.0$	$0.1^{B} \pm 0.0$	0.0^{a}	0.0^{a}	0.1^{b}	0.1^{a}	0.1^{a}	0.2^{c}
C14 : 1	$0.6^{A} \pm 0.0$	$0.8^{B} \pm 0.1$	0.5^{a}	0.6^{a}	0.6^{a}	0.7^{a}	0.7^{a}	1.1^{b}
C16 : 1	$1.7^{A} \pm 0.1$	$1.8^{A} \pm 0.1$	1.9^{a}	1.5^{a}	1.6^{a}	1.6^{a}	1.7^{a}	1.9^{a}
C18 : 1	$28.0^{B} \pm 0.7$	$23.6^{A} \pm 1.0$	29.4^{c}	27.1^{bc}	27.4^{bc}	26.0^{bc}	23.4^{ab}	21.4^{a}
C20 : 1	$0.2^{B} \pm 0.0$	$0.2^{A} \pm 0.0$	0.2^{c}	0.2^{c}	0.3^{c}	0.2^{bc}	0.2^{a}	0.2^{ab}
C18 : 2n6	$3.8^{B} \pm 0.1$	$3.1^{A} \pm 0.1$	3.4^{a}	3.7^{ab}	4.2^{b}	3.3^{a}	2.9^{a}	3.2^{a}
C20 : 2n6	$0.0^{A} \pm 0.0$	$0.0^{A} \pm 0.0$	0.0^{ab}	0.0^{ab}	0.0^{b}	0.1^{b}	0.0^{a}	0.0^{ab}
C20 : 3n6	$0.1^{A} \pm 0.0$	$0.1^{A} \pm 0.0$	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}
C20 : 4n6	$0.3^{A} \pm 0.0$	$0.2^{A} \pm 0.0$	0.2^{a}	0.2^{ab}	0.3^{b}	0.2^{ab}	0.2^{ab}	0.2^{ab}
C22 : 2n6	$0.1^{A} \pm 0.0$	$0.1^{A} \pm 0.0$	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}
C18 : 3n3	$1.3^{B} \pm 0.1$	$1.0^{A} \pm 0.0$	1.1^{a}	1.3^{ab}	1.4^{b}	1.0^{a}	1.0^{a}	1.1^{a}
C20 : 5n3	$0.1^{A} \pm 0.0$	$0.1^{A} \pm 0.0$	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}
C22 : 5n3	$0.4^{A} \pm 0.0$	$0.5^{A} \pm 0.0$	0.4^{a}	0.4^{a}	0.5^{a}	0.4^{a}	0.4^{a}	0.5^{a}
C22:6n3	$0.0^{A} \pm 0.0$	$0.0^{A} \pm 0.0$	0.0^{ab}	0.0^{a}	0.0^{a}	0.0^{ab}	0.1^{b}	0.0^{a}
ΣSFA[3)]	$62.9^{A} \pm 0.8$	$68.0^{B} \pm 1.1$	62.4^{a}	63.2^{a}	63.0^{a}	65.7^{ab}	68.7^{b}	69.7^{b}
ΣMUFA[4)]	$30.7^{B} \pm 0.8$	$26.7^{A} \pm 1.0$	32.1^{c}	29.7^{abc}	30.1^{bc}	28.7^{abc}	26.3^{ab}	25.0^{a}
ΣPUFA[5)]	$6.2^{B} \pm 0.2$	$5.3^{A} \pm 0.2$	5.5^{ab}	6.2^{bc}	6.8^{c}	5.5^{ab}	5.7^{a}	5.3^{ab}
ΣPUFAn3	$1.8^{A} \pm 0.1$	$1.7^{A} \pm 0.1$	1.7^{a}	1.7^{a}	2.0^{a}	1.7^{a}	1.6^{a}	1.7^{a}
ΣPUFAn6	$4.3^{B} \pm 0.2$	$3.7^{A} \pm 0.2$	3.8^{ab}	4.4^{bc}	4.8^{c}	3.9^{ab}	3.4^{a}	3.7^{ab}
PUFAn6/n3	$2.4^{B} \pm 0.1$	$2.3^{A} \pm 0.1$	2.4^{a}	2.6^{a}	2.4^{a}	2.5^{a}	2.2^{a}	2.3^{a}
PUFA/SFA	$0.10^{B} \pm 0.0$	$0.08^{A} \pm 0.0$	0.09^{abc}	0.11^{c}	0.11^{bc}	0.08^{ab}	0.07^{a}	0.08^{a}
PUFA/MUFA	$0.20^{A} \pm 0.0$	$0.20^{A} \pm 0.0$	0.18^{a}	0.20^{ab}	0.23^{b}	0.20^{ab}	0.20^{ab}	0.22^{ab}
MUFA/SFA	$0.49^{B} \pm 0.0$	$0.40^{A} \pm 0.0$	0.53^{c}	0.47^{abc}	0.48^{bc}	0.45^{ab}	0.39^{abc}	0.36^{a}
C16/C18	$0.61^{A} \pm 0.0$	$0.88^{B} \pm 0.0$	0.56^{a}	0.65^{ab}	0.63^{ab}	0.76^{bc}	0.86^{c}	1.03^{d}

1) heat-treated rapeseed cakes

2) soybean meal

3) saturated fatty acids

4) monounsaturated fatty acids

5) polyunsaturated fatty acids

A, B – means with different superscripts in lines differ significantly ($P < 0.05$; Duncan's test)

a, b, c, d – means with different superscripts in lines differ significantly ($P < 0.05$; Duncan's test)

Table 6. Effect of day of lactation (X, days[1)]) on the content of selected fatty acids in milk fat (Y; % of the sum of fatty acids). Coefficients of linear regression ($Y = A + BX$)

Y	A	B	R^2	P
C14 : 0 (%)[2)]	9.6	0.012	0.14	0.04
C18 : 0 (%)[2)]	12.0	−0.027	0.12	0.02
C18 : 2n6 (%)[3)]	3.2	0.010	0.21	0.02
PUFAn6 (%)[3)]	3.7	0.013	0.14	0.04
C18 : 3n3 (%)[3)]	1.1	0.003	0.15	0.04
Σ PUFA (%)[3)]	5.3	0.016	0.21	0.02
PUFA/MUFA[3)]	0.2	0.001	0.23	0.01
C16/C18[4)]	0.7	0.003	0.27	0.01

[1)] measured within the interval 21st–100th day

[2)] total set (HRC + SBM), n = 48

[3)] set of dairy cows fed the diet with heat-treated rapeseed meal (HRC), n = 24

[4)] set of dairy cows fed the diet with soybean meal (SBM), n = 24

R^2 – coefficient of determination

P – significance level

this point our results are in agreement with the data of Elschner *et al.* (1997). We also found a significant relationship between freezing point and heat stability both within the set of experimental group (dairy cows receiving production mixture with rapeseed cakes; $r = -0.45$, $P < 0.01$) and within the whole set of dairy cows ($r = -0.34$, $P < 0.01$).

We did not observed any worsening of rennetability of milk produced using HRC-mixture as compared to SBM-milk ($P > 0.05$). Moreover, the tendency to lower rennetability was found in the HRC-milk (Table 7). According to Kratochvíl and Kohout (1993) feeding of oilseed rape meal to dairy cows significantly improved souring properties of milk.

Within the set of technological traits, stage of lactation influenced only the heat stability of milk (Y; ml of 96% CH_3OH/5 ml of milk), which increased with the increasing day of lactation (X; days) according to the equation $Y = 7.5 + 0.034X$ ($R^2 = 0.20$, $P < 0.01$; inclusion of the quadratic term was not significant).

CONCLUSIONS

The amount of rapeseed cakes that was fed daily per cow was on average slightly more than 2 kg, when the composition of production mixture (Table 1) and milk yield (27.4 kg/day; Table 3) are taken into account. This amount corresponds to 270 g of rapeseed oil (fat content in heat-treated rapeseed cakes was 130 g/kg) per cow and day. It follows from the results of the present experiment that this amount of HRC did not influence negatively either health status of dairy cows or composition of milk or technological traits of milk. On the other hand, the inclusion of HRC in the dairy cow diet improved nutritive value of milk, namely from the viewpoint of fatty acid pattern of milk fat: decreased content of SFA, increased content of MUFA and PUFA, and substantially decreased total C16/C18 fatty acids ratio.

Table 7. Technological traits of milk produced using the production mixture with HRC[1)] or SBM[2)]. One-way classification of the variance ratio test

Trait	Feeding intervention (n = 24) (mean ± standard error of the mean)		Day of lactation (means; n = 8)					
	HRC[1)]	SBM[2)]	HRC[1)]			SBM[2]		
			21st	42nd	100th	21st	42nd	100th
FP[3)] (°C)	$-0.525^{A} \pm 0.00$	$-0.522^{A} \pm 0.01$	-0.523^{a}	-0.527^{a}	-0.526^{a}	-0.524^{a}	-0.517^{b}	-0.527^{a}
pH	$-6.57^{A} \pm 0.09$	$6.58^{A} \pm 0.09$	6.59^{a}	6.57^{a}	6.56^{a}	6.57^{a}	6.62^{a}	6.57^{a}
SH[4)]	$7.5^{A} \pm 0.65$	$7.3^{A} \pm 0.91$	7.6^{a}	7.7^{a}	7.3^{a}	7.4^{a}	7.2^{a}	7.3^{a}
HS[5)]	$9.7^{A} \pm 2.7$	$9.0^{A} \pm 2.8$	8.5^{ab}	9.5^{ab}	10.8^{b}	7.4^{a}	8.9^{ab}	10.6^{b}
RA[6)] (s)	$100^{A} \pm 41.6$	$129^{A} \pm 40.1$	91^{a}	115^{a}	95^{a}	88^{a}	185^{a}	114^{a}

[1)] heat-treated rapeseed cakes

[2)] soybean meal

[3)] freezing point

[4)] milk acidity (Soxhlet-Henkel method; ml of 0.25 mol/l NaOH/100 ml of milk)

[5)] heat stability of milk (ml of 96% CH_3OH/5 ml of milk)

[6)] rennetability

A – means with the same superscript in lines do not differ significantly ($P > 0.05$)

a, b – means with different superscripts in lines differ significantly ($P < 0.05$; Duncan's test)

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