CZECH ACADEMY OF AGRICULTURAL SCIENCES

Czech Journal of ANIMAL SCIENCE

ŽIVOČIŠNÁ VÝROBA

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

1

VOLUME 47
PRAGUE 2002
ISSN 1212-1819

CZECH JOURNAL OF ANIMAL SCIENCE

An international journal published under the auspices of the Czech Academy of Agricultural Sciences and financed by the Ministry of Agriculture of the Czech Republic

Mezinárodní vědecký časopis vydávaný pod záštitou České akademie zemědělských věd a s finanční podporou Ministerstva zemědělství České republiky

For information on Czech J. Anim. Sci. and full papers from Vol. 47 visit http://www.cazv.cz

Aim and scope: The journal publishes scientific papers and reviews dealing with the study of genetics and breeding, physiology, reproduction, nutrition and feeds, technology, ethology and economics of cattle, pig, sheep, goat, poultry, fish and other farm animal management.
The journal is cited in the bibliographical journal Current Contents – Agriculture, Biology and Environmental Sciences and abstracted in Animal Breeding Abstracts. Abstracts from the journal are comprised in the databases: Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Environmental Sciences, Czech Agricultural Bibliography, Food Science and Technology Abstracts, Toxline Plus.

Periodicity: The journal is published monthly (12 issues per year). Volume 47 appearing in 2002.

Acceptance of manuscripts: Two copies of manuscript should be addressed to: Ing. Zdeňka Radošová, Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic, tel.: + 420 2 27 01 03 52, fax: + 420 2 27 01 01 16, e-mail: edit@uzpi.cz.

Subscription information: Subscription orders can be entered only by calendar year (January–December) and should be sent to: Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic. Subscription price for 2002 is 214 USD.

Cíl a odborná náplň: Časopis publikuje původní vědecké práce a studie typu review z oblasti genetiky, šlechtění, fyziologie, reprodukce, výživy a krmení, technologie, etologie a ekonomiky chovu skotu, prasat, ovcí, koz, drůbeže, ryb a dalších druhů hospodářských zvířat.
Časopis je citován v bibliografickém časopise Current Contents – Agriculture, Biology and Environmental Sciences a v časopise Animal Breeding Abstracts. Abstrakty z časopisu jsou zahrnuty v těchto databázích: Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Environmental Sciences, Czech Agricultural Bibliography, Food Science and Technology Abstracts,Toxline Plus.

Periodicita: Časopis vychází měsíčně (12× ročně), ročník 47 vychází v roce 2002.

Přijímání rukopisů: Rukopisy ve dvou kopiích je třeba zaslat na adresu redakce: Ing. Zdeňka Radošová, Ústav zemědělských a potravinářských informací, Slezská 7, 120 56 Praha 2, Česká republika, tel.: + 420 2 27 01 03 52, fax: + 420 2 27 01 01 16, e-mail: edit@uzpi.cz.

Informace o předplatném: Objednávky na předplatné jsou přijímány pouze na celý rok (leden–prosinec) a měly by být zaslány na adresu: Ústav zemědělských a potravinářských informací, vydavatelské oddělení, Slezská 7, 120 56 Praha 2. Cena předplatného pro rok 2002 je 1176 Kč.

F 4886

Metabolic effects of chromium administration to dairy cows in the period of stress

Vliv podávání chromu na metabolismus dojnic v období zvýšené zátěže

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ABSTRACT: In a herd of Czech Red Pied cattle we studied the influence of chromium (Cr) administration on the metabolism and changes in metabolism during the period of transfer from a stanchion barn into loose boxes. The ration fed to dairy cows was supplemented with Cr-enriched yeast (Co-Factor III Chromium Yeast, Alltech, 0.1% Cr^{3+}) at 8 mg Cr per animal per day starting one week before the transfer. Dairy cows were blood sampled 7 days before and on days 1, 3, 7, and 14 after the transfer. The administration of Cr was manifested as lower cortisol on day 3 after the transfer (26.8 ± 4.83 nmol/l vs. 43.22 ± 13.01 nmol/l; $P < 0.05$) and significantly higher numbers of lymphocytes (5 399 ± 1 401 . 10^6/l vs. 3 841 ± 172 . 10^6/l; $P < 0.05$) on day 14 after the transfer in Cr-supplemented cows. There were no significant between-group differences in the other parameters studied. The transfer to a new barn resulted in a moderate stress in most dairy cows, they adapted to the new environment quickly and there were no serious disorders of metabolism.

Keywords: cattle; stress; transfer; haematology; biochemistry

ABSTRAKT: V chovu dojnic červenostrakatého skotu byl studován vliv dotace chromu (Cr) na metabolismus a změny metabolismu dojnic v období přesunu z vazné stáje do stáje s volným ustájením. Bylo sledováno šest dojnic skupiny kontrolní a sedm dojnic skupiny pokusné, kterým byl dotován Cr v dávce 8 mg/kus/den ve formě kvasinek (Co-Factor III Chromium Yeast, Alltech, 0,1% Cr^{3+}) od jednoho týdne před přesunem. Dojnicím byla odebrána krev sedm dní před přesunem a dále 1., 3., 7. a 14. den po přesunu. Dotace Cr se projevila na nižších hodnotách kortizolu v krevním séru dojnic pokusné skupiny ve srovnání se skupinou kontrolní, signifikantní rozdíl byl zjištěn tři dny po přesunu (26,8 ± 4,83 nmol/l vs. 43,22 ± 13,01 nmol/l; $P < 0,05$). Dále byl zjištěn ve skupině pokusné 14. den po přesunu signifikantně vyšší počet lymfocytů (5 399 ± 1 401 . 10^6/l vs. 3 841 ± 172 . 10^6/l; $P < 0,05$) ve srovnání se skupinou kontrolní. U ostatních sledovaných ukazatelů energetického (glukóza, ketolátky, neesterifikované mastné kyseliny, cholesterol, triacylglyceroly), dusíkového (celková bílkovina, močovina), enzymatického (aspartátaminotransferáza, gama-glutamyltransferáza, kreatinkináza, laktátdehydrogenáza, alkalická fosfatáza) a hematologického profilu (hematokrit, hemoglobin, počet erytrocytů, počet leukocytů) nebyly signifikantní rozdíly mezi skupinami zjištěny. V souvislosti s přesunem dojnic byly zjištěny změny ve vybraných parametrech charakterizujících energetický a dusíkový metabolismus, v katalytické aktivitě enzymů i parametrech hematologických. Statistická významnost změny jednotlivých parametrů v průběhu pokusu je uvedena v tabulkách. Přesun do nové stáje představoval pro většinu dojnic pouze mírnou stresovou zátěž, dojnice se rychle adaptovaly na nové prostředí a nedošlo u nich k vážnějšímu narušení metabolismu.

Klíčová slova: skot; stres; přesun; hematologický profil; biochemický profil

The technology of housing represents a very important factor in the husbandry of all farm animals. Problems of the housing technology have been paid greater attention lately together with the endeavour to achieve the highest possible production and animal welfare standards. The change of housing aimed at getting nearer to natural conditions is a prerequisite to high yields in animals, so in the husbandry of cattle we

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Research Project No. 161700002).

see a gradual change from tie-stall housing systems to the loose ones. The change in the technology may, however, result in some stress in the animals accompanied by low yield and health problems. It is the aim of animal keepers to minimise all stressful factors for the animals kept or otherwise lower the susceptibility of animals to such stressors. It is in this context when the possibility of chromium (Cr) administration is evaluated (Lindemann, 1996).

Chromium as a trace element is responsible for the metabolism of carbohydrates, proteins and lipids and is an active component of the glucose tolerance factor. The level of Cr in animals is influenced by stress of physiological, pathological and nutritional type. It is e.g. exertion and trauma (Anderson, 1994) that increase the excretion of Cr in urine and may thus contribute to the Cr deficiency. Symptoms of the Cr deficiency become worse after feeding a low-protein diet, exertion, blood loss, and infections (Mertz, 1992). In spite of the lack of sufficient data on the feeding ration for farm animals with respect to the Cr content, we can suppose that even the ration containing enough Cr for most of the year may be Cr deficient in periods of stress. Such periods include late pregnancy, parturition and lactation onset in dairy cows, calf weaning, transfer and transit of animals (Mordenti *et al.*, 1997).

Our experiment was based on the supposition that the transfer of dairy cows into a new barn of a quite different technology of housing, feeding, milking and the necessity of new ethological rank formation in the herd represent a considerable stress for all the animals. According to published data, this period of higher stress should result in higher Cr demand and, thus, its administration should be reflected in milder health condition alterations.

MATERIAL AND METHODS

The experiment was performed in a herd of dairy cows of Czech Red Pied cattle with average milk yield of 4 500 l per lactation. Dairy cows were kept in tie-stalls till the transfer in a barn of 178 animal capacity and on-site milking. Dairy cows were transferred into a new barn with loose housing and a herringbone milking parlour, where they were divided into 4 groups according to the yield and reproduction cycle. In the group of dairy cows in the second month of lactation we selected individuals with milk yield above 20 l and divided them into control (C – 6 individuals, milk yield of 21.49 ± 1.07 l) and experimental (E – 7 individuals, milk yield of 22.53 ± 1.22 l) sub-groups. Experimental dairy cows were administered Cr in the dose of 8 mg pro toto a day in the form of yeast (Co-Factor III Chromium Yeast, Alltech, 0.1% Cr^{3+}) mixed together with feeding flour. Cr administration started one week before and finished 4 weeks after the transfer into the new barn. Blood was collected from *v. jugularis* 7 days prior to and on days 1, 3, 7, and 14 after the transfer.

Blood plasma was used to determine levels of glucose, urea, triacylglycerols (TAG), aspartate aminotransferase (AST), gamma glutamyltransferase (GMT), creatine kinase (CK), lactate dehydrogenase (LD), and alkaline phosphatase (ALP). The following parameters were determined from blood sera: total proteins, total bilirubin (Bil), total cholesterol (Chol), non-esterified fatty acids (NEFA) and cortisol. Concentrations of ketone bodies, erythrocytes, leukocyte numbers and differential count, haemoglobin, and haematocrit were determined in whole blood.

The following parameters were measured using the automatic analyser Cobas Mira and the tests given in parentheses: urea (Urea UV KIN 4 × 50, Cat. No. 1307017), total bilirubin (BIL 100, Cat. No. 1105309), triacylglycerols (TGL 4 × 100, Cat. No. 1312983), GMT (GMT KIN 100, Cat. No. 1302082), CK (CK NAC 7 × 15, Cat. No. 1303801; the sets supplied by Lachema), glucose (L-Glukosa, Cat. No. 11601), total protein (L-Protein celkovy (biuret), Cat. No. 12751), cholesterol (L-Cholesterol, Cat. No.10851), AST (L-AST, Cat. No. 10351), LD (L-LDH, Cat. No. 12352), ALP (L-Alkalicka fosftasa, Cat. No.10061; the sets supplied by BioVendor), NEFA (NEFA, Cat, No. FA 115; set supplied by RANDOX). Total ketone bodies (acetone, acetacetic acid, isopropanol, β-hydroxybutyric acid) were determined by gas chromatography as described by Hradecký *et al.* (1978). Cortisol (LKCO1; set supplied by BioVendor) was determined by the chemiluminiscence technique using the apparatus Immulite. Haematological parameters were determined by the Coulter Counter (Coulter Electronics, England).

The results were evaluated statistically using F-test and two-way Student's *t*-test for samples of equal and non-equal dispersal. The dynamics of individual parameters was evaluated by paired Student's *t*-test (EXCEL 7.0 program).

RESULTS AND DISCUSSION

Tables 1 and 2 present results of examinations for the metabolic profile in the dairy cow groups studied. No significant differences were found between the control and experimental group in the metabolic pro-

Table 1. Selected parameters of the energy and nitrogen metabolism in dairy cows of the experimental (E; $n = 7$) and control group (C; $n = 6$) starting one week prior to the transfer and two weeks following the housing technology change (x – mean, SD – standard deviation)

Days relative to transfer			–7	+1	+3	+7	+14
Glucose	E	x	2.76	3.63	3.45	3.27	3.76
		SD	0.77	0.46	0.10	0.43	0.34
	C	x	3.49	3.70	3.92	3.63	3.71
		SD	0.85	0.34	0.61	0.37	0.40
Total ketone bodies (mmol/l)	E	x	1.13	1.10	1.32	1.32	1.07
		SD	0.21	0.46	0.60	0.72	0.16
	C	x	0.97	0.98	1.02	0.93	1.01
		SD	0.17	0.18	0.16	0.31	0.31
Oxid. ketone bodies (μmol/l)	E	x	74.4	112.6	164.6	265.0	72.6
		SD	29.5	146.0	227.9	346.6	31.8
	C	x	48.7	41.0	53.3	45.2	49.8
		SD	10.2	8.6	11.7	9.1	12.5
NEFA (μmol/l)	E	x	51.4	216.7*	160.0	158.6	142.9
		SD	32.7	139.1	112.5	123.0	128.3
	C	x	54.0	134.0*	96.7	128.3	75.0
		SD	36.7	20.6	81.0	51.1	41.9
Total cholesterol (mmol/l)	E	x	4.79	4.62*	5.05	5.22	5.77
		SD	1.23	1.01	1.31	1.27	1.21
	C	x	5.88	5.21	5.70	6.19*	6.48
		SD	1.85	1.69	1.41	1.35	1.70
Triacylglycerols (mmol/l)	E	x	0.27	0.21*	0.13	0.18	0.34
		SD	0.04	0.05	0.04	0.07	0.31
	C	x	0.33	0.19**	0.15	0.36*	0.21
		SD	0.07	0.05	0.03	0.18	0.06
Total protein (g/l)	E	x	81.1	78.5	83.9	85.9	87.9
		SD	4.88	3.38	6.19	5.04	5.85
	C	x	82.3	75.1	82.0*	86.5*	87.1
		SD	9.76	7.45	6.07	4.46	7.66
Urea (mmol/l)	E	x	3.96	4.09	4.92*	5.82*	4.51**
		SD	0.67	0.84	1.17	0.91	1.26
	C	x	3.81	3.47	4.91**	4.92	4.16
		SD	0.72	0.73	0.39	0.80	1.30

* $P \leq 0.05$ compared to the previous sampling
** $P \leq 0.01$ compared to the previous sampling

file parameters studied except for cortisol, the level of which was significantly lower ($P < 0.05$) in the experimental group on day 3 after the transfer. The administration of Cr resulted in a lower cortisol increase in the blood serum during the first week after the transfer. As the stress, at least of short duration, is closely associated with increased levels of corticosteroids in the blood, these results indicate a drop in the susceptibility of animals to stress factors following Cr administration. Chang and Mowat (1992) found the level of cortisol to decrease by some 19 to 27% due to the stress after the transport/purchase of young cattle following Cr administration. Moonsie-Shager and Mowat (1993) also found decreased cortisol levels in calves 28 days

Table 2. Selected parameters of the enzymes and the concentration of total bilirubin and cortisol in dairy cows of the experimental (E; $n = 7$) and control group (C; $n = 6$) starting one week prior to the transfer and two weeks following the housing technology change (x – mean, SD – standard deviation)

Days relative to transfer			–7	+1	+3	+7	+14
AST	E	x	1.40	1.68*	1.54	1.50	1.57*
(μkat/l)		SD	0.18	0.32	0.19	0.13	0.16
	C	x	1.37	1.55	1.54	1.58	1.47
		SD	0.18	0.16	0.24	0.24	0.10
GMT	E	x	0.31	0.32	0.31	0.31	0.34**
(kat/l)		SD	0.06	0.06	0.06	0.07	0.07
	C	x	0.31	0.28	0.27	0.28	0.32*
		SD	0.02	0.05	0.04	0.03	0.04
ALP	E	x	1.42	1.67	1.45	1.44	1.50
(kat/l)		SD	0.96	1.29	1.14	1.01	0.99
	C	x	0.71	0.55	0.97*	1.08	1.38
		SD	0.40	0.24	0.60	0.52	0.89
LD	E	x	40.5	45.6	38.1	39.2	39.4
(μkat/l)		SD	6.57	8.37	2.89	5.57	6.98
	C	x	39.3	44.8	40.8	38.6*	39.9
		SD	4.52	6.78	2.56	1.31	3.36
	E	x	2.45	8.82	3.59	3.54	3.35
(μkat/l)		SD	1.30	7.22	1.83	1.96	1.04
	C	x	1.83	4.32	3.45	3.56	2.52
		SD	0.60	1.88	2.18	1.59	0.85
Bilirubin	E	x	1.93	3.08*	2.96	2.69	1.79
(μmol/l)		SD	0.43	1.04	0.97	1.74	0.33
	C	x	1.86	2.40**	2.52	1.44	2.03
		SD	0.23	0.35	0.45	0.89	0.25
Cortisol	E	x	25.42	31.14	[a]26.80	25.62	36.66
(nmol/l)		SD	1.00	8.56	4.83	1.25	18.58
	C	x	36.79	35.87	[a]43.22	29.43	37.71
		SD	26.73	15.93	13.01	4.69	14.40

*$P \leq 0.05$ compared to the previous sampling

**$P \leq 0.01$ compared to the previous sampling

[a] $P \leq 0.05$ comparing the experimental and control group

after the transfer into a new barn. Contrary to our results in dairy cows, the cortisol values in calves decreased only during the 4th week after the transfer. This difference was probably caused by the time of Cr administration because in the experiment Cr administration to calves started after the transfer, whereas we administered Cr 7 days prior to the transfer. The mechanism of the cortisol level decrease following Cr administration is not quite clear. Modification of biological responses to insulin is one of the possibilities because it was found that the biological response is the same at the lower level of insulin in Cr administered animals (Mallard and Borgs, 1997). There is a relation of antagonism between cortisol and insulin, which is evident especially during the action of stressors when an increase in cortisol directly causes a decrease in the insulin concentration. Cr administration may cause lower insulin concentrations producing normal biological responses and, if it is not acting only through the increase in the susceptibility of insulin receptors but also higher insulin release, the final effect may be that of cortisol decrease observed in Cr-supplied animals. This mechanism of action is supported by the study of Hübner *et al.* (1988) describing a stimulatory effect of Cr on the endocrine functions of pancreas in the rat under specific conditions of diet composition.

Changes in the energy and nitrogen metabolism and catalytic activity of enzymes were found in the context

Table 3. Selected parameters of haematology in dairy cows of the experimental (E; n = 7) and control group (C; n = 6) starting one week prior to the transfer and two weeks following the housing technology change (x – mean, SD – standard deviation)

Days relative to transfer			–7	+1	+3	+7	+14
Haemoglobin (g/l)	E	x	99.00	105.50	99.43 *	86.86	105.29
		SD	10.16	11.84	9.69	31.20	4.30
	C	x	97.75	103.60	99.17	97.83	99.83
		SD	10.94	13.81	8.86	15.18	16.79
Haematocrit (l/l)	E	x	34.71	35.67	35.14	31.14 *	32.29
		SD	2.76	2.69	3.80	1.25	1.28
	C	x	33.25	33.40	33.33	31.67	32.50
		SD	2.59	3.50	2.75	3.25	4.35
Erythrocytes (T/l)	E	x	6.48	6.91	6.73	6.65	6.92
		SD	0.63	0.68	0.76	0.43	0.33
	C	x	6.92	6.85	6.57	6.49	6.81
		SD	0.45	1.01	0.84	1.09	1.11
Leucocytes (G/l)	E	x	6.99	6.63	6.63	7.37	8.86
		SD	1.11	0.74	1.43	1.77	1.97
	C	x	6.97	7.16	7.85	6.62	8.95
		SD	1.15	1.08	2.43	1.81	3.33
Metamyelocytes (10^6/l)	E	x	67.3	156.8	19.0	35.3	69.4
		SD	88.9	141.2	26.4	43.7	131.4
	C	x	144.2	171.8	62.3	66.7	804.8
		SD	149.6	136.0	64.1	58.7	1 271.7
Neutrophils (10^6/l)	E	x	2 696.4	2 790.7	1 650.0	2 330.6	2 970.7
		SD	784.5	941.1	786.6	894.1	1 090.4
	C	x	2 656.2	2 468.8	2 961.3	2 091.5	3 749.0
		SD	1 110.7	264.0	1 168.2	964.4	1 855.1
Lymphocytes (10^6/l)	E	x	3 910.6	3 268.0	4 044.3**	3 950.6	[a]5 399.0*
		SD	630.1	234.3	524.3	874.5	1 401.1
	C	x	3 558.3	3 707.4	3 348.7	3 422.5	[a]3 841.0
		SD	874.8	802.0	1 344.1	726.9	171.6
Mononuclears (10^6/l)	E	x	29.0	83.8	16.3	74.1	0.14
		SD	46.5	99.7	39.9	95.1	0.35
	C	x	50.8	23.6	67.5	19.8	126.7
		SD	51.8	28.9	87.3	30.4	220.7
Eosinophils (10^6/l)	E	x	381.9	334.0	366.9	538.6	417.6
		SD	282.5	246.8	414.0	154.0	258.9
	C	x	557.2	388.4	543.5	826.3*	360.5
		SD	304.0	361.2	539.1	552.0	191.0
Basophils (10^6/l)	E	x	0	0	0	13.7	0
		SD	0	0	0	33.6	0
	C	x	0	0	0	6.5	51.3
		SD	0	0	0	14.5	114.8

*$P \leq 0.05$ compared to the previous sampling
**$P \leq 0.01$ compared to the previous sampling
[a]$P \leq 0.05$ comparing the experimental and control group

with the dairy cow transfer. Tables show the statistical significance of changes in individual parameters during the experiment. After the transfer there was an increase in concentrations of NEFA, urea, total protein, bilirubin, and catalytic activities of AST, CK. Triacylglycerol concentrations, however, decreased. The changes we found are similar to those mentioned by Nockels (1994) considering an increase in the levels of cortisol, glucose, CK, AST, urea, creatinine and fibrinogen to be characteristic of the development of stress. Changes in individual parameters were similar in both dairy cow groups, nevertheless, the degree of changes and their statistical significance differed. Disorders of metabolism in our study were only slight evidencing the fact that dairy cows adapted themselves quickly to the stress situation. The metabolic profile changes clearly document moderate catabolic processes characterised by an increase in the concentration of urea during the first two weeks after the transfer, increased levels of NEFA and ketone bodies in some dairy cows. These changes were associated with the increased stress in dairy cows and were caused by an increase in cortisol concentration in the blood and decreased susceptibility of cells to insulin as a primary reaction to the stress.

There were some differences between the groups in the concentration of total cholesterol in the blood which was gradually increasing during the experimental period and in the control group of dairy cows it was higher as compared with the experimental group. Differences between the groups, however, were not significant and, regarding the fact that higher values were already found in the control group at the beginning of the experiment, this tendency cannot be attributed explicitly to the effect of Cr administration.

In order to evaluate the energy metabolism, we checked the concentration of glucose, ketone bodies and NEFA. At the beginning of the experimental period there were some differences between the experimental and control group in the concentration of ketone bodies (total and oxidised ones, increased in the experimental group) and glucose (decreased in the experimental group). This difference between the groups was probably influenced by the distribution of dairy cows to the groups because the cows were not submitted to any screening examination of metabolism prior to it. They were distributed according to the stage of lactation, milk yield and clinical examination for the health condition only. Despite of the stress in the dairy cows of the control group, metabolism returned relatively quickly to normal, the concentration of ketone bodies decreased and the blood glucose got to normal physiological values. The transfer of dairy cows was accompanied by an increase in NEFA, which documents the onset of lipo-mobilisation due to the stress. NEFA, however, increased only moderately and did not exceed normal values of 35 μmol/l (Pechová *et al.*, 1997), i.e. the limit evidencing more severe metabolism disorders.

As a result of the transfer of dairy cows slightly higher catalytic activities of AST were found exceeding normal values 0.72–1.41 μkat/l (Pechová *et al.*, 1997); the values of GMT and total bilirubin also increased but neither of them increased above normal levels. Despite of the stress, there were no serious alterations of the liver parenchyma in the dairy cows studied. Higher levels of AST reflected its release from muscle cells following exercise during the transfer and catabolic processes. This presumption is also supported by the increased CK and LD activities. There is an analogy between our results and those reported by Benett *et al.* (1989) describing increased catalytic activities of AST and CK in the cattle in association with stress. Monitoring the catalytic activities of AST and CK at the time of muscle tissue alterations is not only of diagnostic but also of prognostic value because increased levels of AST remain for a longer time, whereas the CK value drops quickly (Kaneko *et al.*, 1997). The finding that CK values return to normal even though AST remains high can be interpreted as no further damage to the muscle tissue (Marco *et al.*, 1998) and as such is prognostically favourable. In our study we determined an increase in the CK catalytic activity in both groups of dairy cows immediately after the transfer with subsequent gradual decrease.

The haematological profile (Table 3) was evaluated with the aim to monitor the changes in dairy cows loaded by stress. No significant differences were found between the groups in the concentration of haemoglobin, haematocrit, and red and white blood cell counts during the experimental period. Counts of leukocytes were gradually increasing throughout the experiment; the increase, however, did not exceed normal values. The most marked changes concerned the leukogram. It was 14 days after the transfer that the count of lymphocytes significantly increased in the experimental group ($P < 0.05$) as compared with the control one whereas the count of neutrophils was lower; this difference not being significant due to the high standard deviation value. Certain differences were also found in the ratio of neutrophils/lymphocytes. On the first day following the transfer this ratio increased in the experimental group from 0.70 ± 0.23 to 0.86 ± 0.29, on the third day, however, it decreased significantly (0.40 ± 0.16; $P < 0.05$) and remained low (0.60 ± 0.21

and 0.58 ± 0.24 on day 7 and 14, respectively) till the end of the experimental period. There were large differences between individual dairy cows and samplings in the control group, which was reflected in the mean values and high standard deviations. The ratio of neutrophils/lymphocytes was higher in the control group than in the experimental one on most sampling occasions (1.03 ± 0.60 prior to the transfer, 0.71 ± 0.20, 1.76 ± 2.52, 0.60 ± 0.22 and 0.98 ± 0.50 on days 1, 3, 7, and 14, respectively). Lower changes in the leukogram and in the ratio of neutrophils/lymphocytes indicate lower susceptibility of Cr supplied dairy cows. Schaefer *et al.* (1997) found a significant increase in the neutrophils/lymphocytes ratio following the transit stress.

Transfer to a new barn represented only a moderate stress for most of the dairy cows. Considering individual stress phases, it was the first one – i.e. alarm reaction in the course of which dairy cows adapted themselves gradually to the new environment and no serious disorders of metabolism occurred. A relatively high individual variability in the response of dairy cows to the stress was found. Tadich *et al.* (2000) found analogous results with respect to the stress phases during a 36-hour transit. Cr administration was reflected in lower cortisol levels in dairy cows following the transfer, but it did not considerably influence other metabolic parameters.

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Received: 01–10–29
Accepted after corrections: 02–01–23

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The fate of female blastodermal donor cells in chimeric cockerels

Vývoj samičích blastodermálních dárcovských buněk u chimérních kohoutů

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ABSTRACT: The aim of this study was to assess the fate of female donor cells in male chicken chimeras, which were generated by the method of blastodermal cell transfer between the donor and acceptor embryos in embryonic stage X. In the experiments we tested 4 chimeric cocks carrying the W chromosome. In checking the progeny of these W positive chimera cocks with W chromosome-specific primers, two of these cocks had a normal ratio between male and female offspring. One cock had a ratio between males and females significantly shifted to females (1 : 1.49; $P < 0.01$). The results show that female donor cells can participate in the process of forming male reproduction organs of chimeric males.

Keywords: chicken chimeras; W chromosome; sex ratio; ejaculate

ABSTRAKT: Cílem této práce bylo prověření dalšího vývoje samičích blastodermálních buněk v samčích kuřecích chimérách, které byly vytvořeny přenosem blastodermálních buněk mezi embryem dárcem a příjemcem ve stadiu X. V experimentech byli testováni čtyři kohouti – chiméry, nesoucí W chromozóm. Při kontrole potomků W pozitivních chimérních kohoutů se zjistilo, že potomci dvou kohoutů vykazovali normální poměr samičího a samčího pohlaví vylíhlých kuřat. Jeden kohout měl poměr pohlaví u potomků statisticky průkazně posunut ve prospěch samičího pohlaví (1 : 1,49; $P < 0,01$). Výsledky naznačují, že slepičí donorové buňky se mohou podílet při formování samčích reprodukčních orgánů chimérních kohoutů.

Klíčová slova: kuřecí chiméra; W chromozom; poměr pohlaví; ejakulát

Production of chimeric chickens by the method of injection of blastoderm cells into the embryos of the same age is one of the most common techniques used in experiments aimed at production of transgenic chickens. It is possible to transfer foreign DNA into not differentiated early blastodermal cells and we could hope that in some cases, these cells could be incorporated into differentiating gonads and would form germinal cells. In most experiments these type donors and acceptors are not sexed before forming a chimera and so the mixed sex chimeras are also produced. We were interested in the fate of transferred cells that are of different sex from the acceptor and in a possibility to form a germinal chimera in this case.

In chickens the male has a homogametic (ZZ) and the female has a heterogametic (ZW) sex chromosome pattern. Molecular analysis of W chromosome has revealed that the majority of the W chromosome consists of repetitions of a member of the Xho I and EcoR I DNA family. This W chromosome property was used for embryo sexing (Uryu *et al.*, 1989; Mizuno *et al.*, 1992; Shaw *et al.*, 1992; Kagami and Tomita, 1992; Clinton, 1994; Simkiss *et al.*, 1996; Trefil *et al.*, 1999). It was considered that the genes on the W chromo-

Supported by Grant Agency of the Czech Republic (Project No. 523/98/1607) and National Agency for Agricultural Research in Prague (Grant No. QE 1001).

some might play an important role in sex determination (Thorne *et al.*, 1987).

In avian species, the developmental fate of different sex germ cells in the gonads is still unclear. The process of sexual differentiation in domestic fowl is a very complicated and still unclear process. There are only a few studies of this process available; most of them have used mixed sex chimeras. Kagami *et al.* (1995a) showed (in experiments in which the sex of the donor and recipient embryos was determined) that when cells from the male blastoderm (ZZ) were present in a genetically female chimera (ZZ/ZW), ZZ oogonia were induced within the ovarian follicles and genetically male oogonia were processed normally during meiosis. Following ovulation the chromosomally male driven ova were fertilised and produced normal male offspring.

When cells from a female embryo (ZW) were incorporated into a male chimera (ZW/ZZ), ZW spermatogonia entered meiosis I and produced functional Z spermatocytes that further differentiated into functional Z – bearing spermatozoa and produced offspring.

Only a very small number of W – bearing spermatozoa, differentiating from ZW spermatogonia, were identified and these were not functional.

Further Simkiss *et al.*(1996), Tagami *et al.* (1997) and Abinawanto *et al.* (1998) demonstrated the presence of spermatozoa containing female – specific W chromosomes in chimeric chickens. Shimada (2000) confirmed by FISH technique that meiotic division can yield spermatids and spermatozoa carrying the W chromosome, but that the number of spermatozoa in sex reversed females was in-sufficient for natural mating and fertilization.

The aim of this study was to assess the function of W-containing spermatozoa in male chicken chimeras which were generated by the method of blastodermal cell transfer between the donor and acceptor embryos of different sex at the embryonic stage X (Petitte *et al.*, 1990; Naito *et al.*, 1990, 1991; Kagami and Hanada, 1997; Thoraval *et al.*, 1994; Trefil *et al.*, 1995).

MATERIAL AND METHODS

Chimera production

Construction of chicken chimeras was made by the technique of transferring blastodermal cells, isolated from a freshly laid unincubated egg, into the second embryo according to the technique described by Trefil *et al.* (1998) – Figure 1.

Donor blastodermal cells were isolated from the embryos of developmental stage X by Eyal-Gilady and Kochav (1976). The embryos originated from mating male Black Minor (-ii, EE, b/b inbred line M) and female Barred Leghorn (ii,ee,B/- , line SH). Either of them is homozygous recessive (ii) at the dominant white locus (I). Barred gen B is a sex linked gene located on the non-homologous segment of Z chromosome.

Approximately 60 000 blastodermal cells were diluted in 100 µl of the medium M 199 (Sigma source). A small portion of these cells (about 400) was used for the identification of the W chromosome by PCR reaction (details see page 6), the other cells were directly used for the construction of chimeras.

Around 100 donor blastodermal cells were removed from the recipient White Leghorn embryo (F_1 cross of inbred lines CB and H6) blastodisc for identification of W chromosome in PCR reaction. Then approximately 200–400 donor blastodermal cells were inserted into the subgerminal cavity of the blastodisc of the White Leghorn (II) recipient embryo.

All manipulations were done using micromanipulator Narishige (Japan) and fine sharpened pipettes made from microcapillary pipettes (Sigma).

After 4 days of incubation under the standard conditions (37.7°C, 60% R.H., rotation once in a hour) in a table Curfew incubator the embryos were moved into other bigger (about 20% larger) egg shells and covered with cling film. Four days before hatching, the rotation of eggs was stopped and holes were made into the cling film to improve better hatching of the chickens.

In this model it was possible to identify directly the sex of donors on the basis of phenotypic manifestation of dark spots on the basic white plumage of the hatched chickens. The colour of the spots on hatched chimeric chickens indicated different sex genotypes of used donor cells (ZZ barred spots, ZW black spots).

For the experiments the following sex combinations were used:

donor cells	acceptor embryo	expected sex	number of used eggs
male	female	male or female	585
male	male	male	160
female	female	female	150
female	male	female or male	645

Altogether 1 540 treated eggs were used – i.e. verified on sex combinations via PCR for the presence of W chromosome in the donor and acceptor embryos.

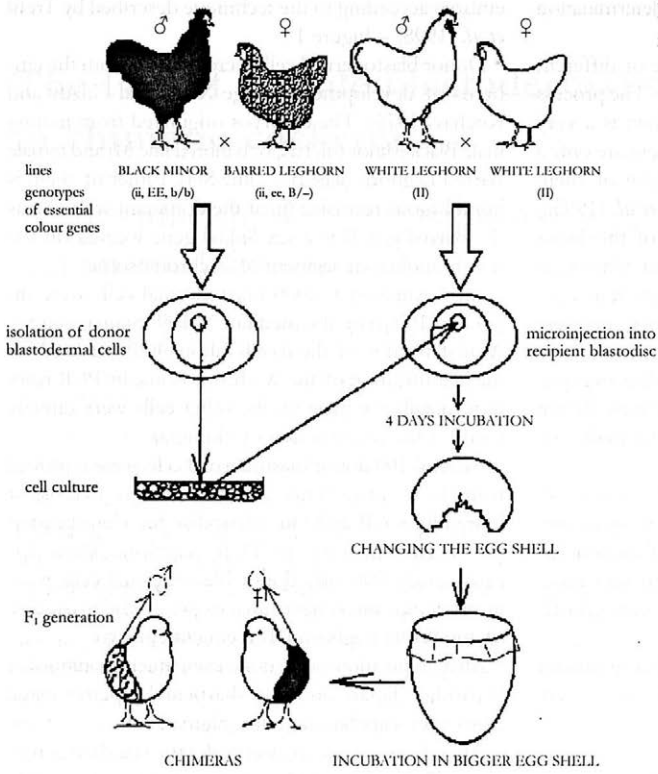

Figure 1. Model of the construction of chimeras

The hatched chimeras were reared under the standard technological conditions until adult. Immediately after hatching the chicks were assessed phenotypically for chimerism (presence of dark spots on plumage). The number of hatched chimeras is shown in Table 1. All hatched chimeras reached maturity and were used for further tests.

All male chimeras were tested for the presence of W chromosome in the cells from blood and ejaculates. The cocks with identified W chromosome in their ejaculate were tested in F_1 generation for possible transmission of this W chromosome. Ejaculates from these cocks were collected and artificial insemination of 10 hens (Barred Leghorns, ii,ee, B/-) per cock was

Table 1. Chimeras produced by different sex combinations of donors and acceptors

Group No.	Donor	Acceptor	Number of hatched chickens	Hatchability (%)	Number of hatched chimeras	Sex of hatched chimeras	Success of forming chimeras (%)	Statistical significance χ^2 value P
1^A	♀	♀	55	36.7^A	11	♀	20	
2^A	♂	♂	47	29.4^A	9	♂	19	23.95 < 0.01
3^B	♀	♂	200	31.0^A	9	3 ♀	1.5	
						6 ♂	3	
4^B	♂	♀	189	32.3^A	10	1 ♂	0.53	
						9 ♀	4.8	

Statistical significance (23.95; $P < 0.01$) was calculated for differences between group 1 and 2 (the same sex) and group 3 and 4 (different sex). χ^2 values for differences between group 1 and 3 and group 2 and 4 were 14.34 and 9.76, both significant on the level $P < 0.01$. Differences in hatchability between groups 1 and 2 and group 3 and 4 were not significant. Statistically significant differences are designated by different superscripts

performed. The control was normal cocks. Altogether 837 eggs were used. The control group consisted of normal cocks (not chimeras) without presence of the W chromosome in the ejaculate.

Hatched chickens were sexed phenotypically for the presence of W chromosome. Plumage colours were evaluated. Hatchability was made from settled eggs.

For statistical comparison of the ratio between males and females in the progeny after the W positive cocks the χ^2-test was used (Likeš and Machek, 1983).

DNA extraction for PCR analysis

Blastodermal cells. Five hundred µl of a suspension containing at least 300 blastodermal cells in PBS was subjected to centrifugation (12 000 g, 2 min) and the cell pellet was resuspended in 10 µl of cell lysis solution (10 mM Tris-HCl pH 7.6, 25°C, 10 mM EDTA, 50 mM NaCl and 0.2% SDS) to which proteinase K was added (final concentration of 0.1mg/ml). Incubation (2 hours) at 65°C followed and then proteinase K was inactivated at 95°C for 15 minutes. This solution was used for PCR reaction.

Blood cells. Blood samples were collected from the wing vein and diluted 1 : 4, in sodium citrate buffer pH 7.3, 25°C. Blood cells were pelleted by centrifugation and the plasma supernatant discarded. Subsequently, 5 l of the cell pellet was washed twice in 500 µl cell lysis solution (320 mM Sucrose, 5 mM $MgCl_2$, 10 mM Tris-HCl pH 7.6, 25°C and 1% of 100× Triton) and after centrifugation was re-suspended in 500 µl digest buffer (10 mM Tris-HCl pH 8.3, 25°C, 2.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml gelatine, 0.45% (w/v) Nonidet P40 and 0.45% (w/v) Tween 20) to which proteinase K was added (final concentration, 0.1 mg/ml). Incubation and inactivation conditions were identical to those used for blastodermal cells. Proteins were removed by phenol and phenol/chloroform (1 : 1 v/v) extraction. Residual phenol was removed by chloroform and DNA precipitated with ethanol. DNA was re-suspended in buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

Ejaculate. For ejaculate samples (diluted 1 : 5 in phosphate buffered saline (PBS) pH 7.3, 25°C), spermatozoa were pelleted by centrifugation (3 000 g, 5 min) and the supernatant discarded. Subsequently, the whole cell pellet was washed in 1 ml of lysis buffer (10 mM Tris pH 7.5, 10 mM EDTA, 50 mM NaCl, 0.2% SDS,) to which proteinase K was added (final concentration, 0.1 mg/ml). Incubation and inactivation conditions were identical to those used for blastodermal cells.

Proteins were removed by phenol and phenol : chloroform (1 : 1 v/v) extraction. Residual phenol was removed by chloroform and DNA precipitated with ethanol. DNA was resuspended in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

PCR conditions

Simkiss *et al.* (1996) showed that W chromosome specific primers are able to amplify a 447 bp fragment of the Eco RI 1.2 kb repeat (EMBL/Gen Bank no X57344). It was used:
forward (454-473) 5´-GCCTTTCTACCGCAAATAC-3´
reverse (900-882) 5´-AGGTGCTTTTTTTCTGGG-3´

DNA templates (containing 5 µl from blastodermal cells DNA solution, 0,5 ng/ml of DNA from red blood cells and 100 ng/ml from spermatozoa cells) were added to the prepared PCR master mixture (2 µl of 1mM dNTP mix, 1 µl of each primer at 20 pmol/µl and 0.2 µl (1 unit) of Taq polymerase (Top-Bio s.r.o., Prague), 5 µl concentrated buffer and water to give a final volume of 50 µl). This was followed by 25 cycles at 94°C (10 s), 55°C and 72°C (20 s each) with final extension at 72°C for 7 minutes in a Techne Cyclogene thermal cycler (Techne Ltd., Cambridge, U.K.). The reaction products (20 µl) were then loaded on to a 1.5% agarose – TBE gel for molecular sizing.

As a positive control, genomic DNA from a hen was used, for negative control genomic DNA from the normal cock.

RESULTS

Chimera production

In Table 1, there was practically no difference between all the groups in egg hatchability. Hatchability was very good in all groups with respect to the used technique of manipulation with embryos. The success in forming chimeras was much higher when donor cells as well as acceptor embryo were of the same sex (19.5%).

In case that the sex combination was incompatible, most of the hatched chimeras repeated the sex of the acceptor (15 hatched chimeras out of total 19).

Phenotypic sex of 4 chickens matched with the sex of donor cells. In three cases, hens originated from the combination of a female donor and male acceptor, one cock originated from the combination where the donor was male and the acceptor female.

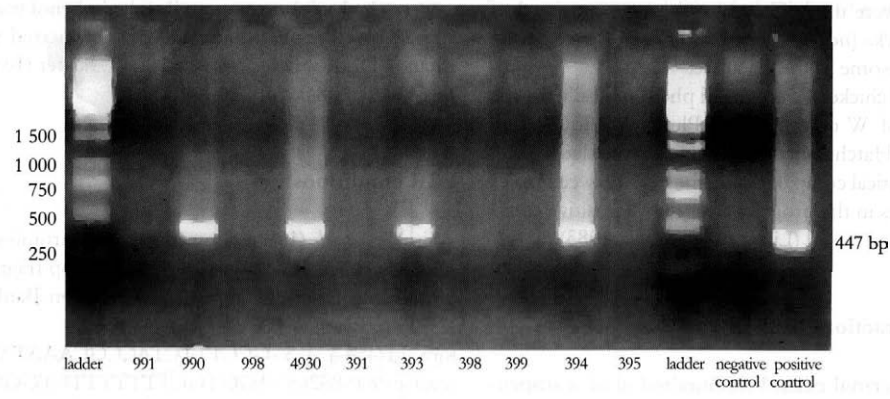

Figure 2. Presence of W chromosome in the spermatozoa of chimeric cocks

Eco RI PCR (25 cycles) of genomic DNA isolated from spermatozoa of chimeric cocks. Cocks No. 990, 393 and 394 (female donor, male acceptor), 4930 (male donor, female acceptor), show positive bands specific to W chromosome. Genomic DNA isolated from the hens blood was used for positive control, genomic DNA isolated from spermatozoa of normal cock was used as negative control. Ladder indicates size of fragments

Table 2. Sex ratio of the hatched progeny of W chromosome positive cocks

Origin of the cock	Cock No. (colour of spots)	Progeny		Ratio female/male	Hatchability (%)
		female	male		
♀ → ♂	990 (black)	128	86	$1.49 : 1^{B}$	74.82^{A}
♀ → ♂	393 (black)	13	9	$1.44 : 1^{A}$	44.00^{B}
♀ → ♂	394 (black)	57	56	$1.01 : 1^{A}$	67.66^{B}
♂ → ♀	4930 (barred)	53	52	$1.01 : 1^{A}$	69.76^{B}
	Control	98	102	$0.96 : 1^{A}$	82.40^{A}

From cock No. 990 (originated from the female donor and male acceptor), 214 progenies were obtained.
128 were females after the PCR test from blood cells, 86 were males. The sex ratio of progeny was 1.49 : 1 in favour of females. This shift in the sex ratio was significant on the level $P < 0.01$. The shift in the sex ratio was also found in the progeny of cock No. 393 (the same origin as cock No. 990), but it was not statistically significant for the small number of the tested progeny. We did not find a shift in the sex ratio in the progeny of cock No. 394 (originated from the same group as cock No. 990) nor of cock No. 4930 (originated from the male donor and female acceptor). χ^2-values for differences in hatchability (hatched progeny after the insemination with the control or chimeric cocks) between the control group and cocks 393, 394 and 4930 were significant ($P < 0.01$). Only the hatchability of chimeric cock 990 was not significant. Statistically significant differences are designated by different superscripts

Seven cocks originating from the groups where the donor and acceptor sex was different (groups 3 and 4) were tested for the presence of W chromosome in the ejaculate. Four of them were positive in this test – see Figure 2.

Cocks No. 990, 393 and 394 (donor female, acceptor male), 4930 (donor male, acceptor female) show positive bands specific to W chromosome.

These 4 cocks were tested for the progeny (Table 2).

Cock No. 990 originated from the female donor and male acceptor, phenotypically it looks like a male chimera with black spots identifying the female type of colouring. Together we obtained 214 progenies from this cock.

After the PCR test from blood cells, 128 were females, 86 were males. It means that the sex ratio of

progeny was 1.49 in favour of females. This shift in the sex ratio was significant on the level $P < 0.01$ as the value of χ^2 was 6.75.

A clear shift in the sex ratio was also found in the progeny of cock No. 393 (originated from the same group 3 as cock No. 990), but it was not statistically significant for the small number of the tested progeny.

We did not find a shift in the sex ratio in the progeny of cock No. 394 (originated from the same group as cock No. 990) as well as of cock No. 4930 (originated from the male donor and female acceptor, phenotypical male chimera with barred spots identifying the male type of colouring).

All progeny of these 4 cocks had the plumage colour of type Ii (white chicken with small dark spots). We did not find any evidence of participation of spermatozoa of donor origin in the reproduction process. If these donor cells participate in the process of fertilization, the dark chickens should be present in the progeny.

DISSCUSION

These results indicate that donor blastodermal cells from female embryos (ZW) were incorporated into male chimera (ZW/ZZ), confirming the results of Kagami *et al.*. (1995a). Contrary to Kagami *et al.* (1995b) we obtained different sex proportions of hatched chimeras. After the insertion of female blastodermal cells into the male recipient we also obtained female chimeras. We got a statistically significant difference ($P < 0.01$) in the success of forming chimeras between the group where cells with the same sex as the acceptor were transferred and the group where cells with different sex were transferred.

We assume as did Simkiss *et al.* (1996), Tagami *et al.* (1997), Abinawanto *et al.*. (1998) and Shimada (2000) that ZW and ZZ spermatogonia differentiated in the testes of chimeric cocks, followed by meiosis I. We suppose that functional ZZ spermatocytes as well as WW spermatocytes were formed there and after meiosis II they differentiated into functional Z and probably not functional W – bearing spermatozoa.

In our experiments from the statistical point of view cock No. 990 (originated from the female donor and male acceptor and identified by PCR for the presence of W chromosome in the spermatozoa) was able to change significantly ($P < 0.01$) the ratio of the progeny in favour of the females. On the other hand, all progeny of these 4 cocks (including cock No. 990) had the plumage colour of type Ii (white chicken with small dark spots) and we did not find any evidence of participation of spermatozoa of donor origin in the reproduction process. If these donor cells participate in the process of fertilization, dark chickens should be present in the progeny.

The only possible explanation is that W bearing spermatozoa were not functional and it is difficult to explain a statistically significant shift in the sex ratio of the progeny at this moment.

We only suggest that the transferred donor blastodermal cells (ZW) from the female embryo formed a part of the acceptor testes in the process of early development of testes and the whole process of sexual differentiation (through mitosis and meiosis) resulted in forming probably no functional W – bearing spermatozoa.

We get a shift in the sex ratio as well as in the progeny of cock No. 393 (originated from the same group as cock No. 990), but it was not statistically significant. We were not able to collect enough ejaculate for the insemination of hens. This cock produced ejaculate only from time to time and a very small amount so we could not verify a larger progeny. We do not know if this very bad reproduction ability was connected with its chimera status. We did not find a shift in the sex ratio in the progeny of cock No. 394 (originated from the same group as cock No. 990) nor of cock No. 4930 – originated from the male donor and female acceptor, phenotypical male chimera with barred spots identifying the male type of colouring. Hatchability (hatched progeny after the insemination with the control or chimeric cocks) between the control group and cocks 393, 394 and 4930 was significant ($P < 0.01$), only the hatchability of chimeric cock 990 was not significant.

Our results indicate that donor blastodermal cells from female embryos (ZW) are able to incorporate into male chimera (ZW/ZZ) to form a part of the testes and later, after the whole process of sexual differentiation, to produce spermatozoa carrying W chromosome. One cock with PCR identified chromosome was able to shift the progeny in favour of females. We were not able to explain the consequence of this shift in the sex ratio.

Acknowledgements

The authors would like to thank Dr. Pierrick Thoraval (died in August, 2000) for his sincerely appreciated discussion during research on this problem.

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Received: 01–10–11
Accepted after corrections: 02–01–21

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Intestinal digestibility of crude protein in concentrates determined by a combined enzymatic method

Intestinální stravitelnost dusíkatých látek u jadrných krmiv stanovená enzymaticky kombinovanou metodou

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ABSTRACT: The objective of the experiment was to test a combined enzymatic method of determination of intestinal digestibility of crude protein undegradable in the rumen of ruminants. The method was tested using a set of feeds that included concentrates and extracted meals. The advantage of this method consists in complete determination of values of intestinal digestibility of crude protein in the laboratory without using cannulated cows. The method is based on a modification of classical enzymatic method. The classical method uses a feed preincubated in the cow's rumen for 16 hours. In the combined enzymatic method feed preincubation in the rumen is replaced by preincubation with the proteolytic enzyme bromelain for a time interval of 1 or 16 hours, followed by the second phase of determination: incubation of the undegradable residue with the enzyme pancreatin for 24 hours. On the basis of comparison of the values with those obtained by a mobile bag method using cannulated animals, linear and polynomial regression equation, correlation coefficient, standard deviation, and parameters of regression equations for time intervals of 1 and 16 hours of enzymatic preincubation were calculated. Statistical analysis proved that the combined enzymatic method using 1-hour preincubation is more suitable for assessment of crude protein intestinal digestibility *in vitro*.

Keywords: ruminants; protein degradation; intestinal digestibility; *in vitro*; proteolytic enzymes

ABSTRAKT: Cílem experimentu bylo ověřit kombinovanou enzymatickou metodu stanovení intestinální stravitelnosti dusíkatých látek nedegradovaných v bachoru přežvýkavců. Metodou jsme testovali soubor krmiv (n = 9), který zahrnoval jadrná krmiva a extrahované šroty. Výhodou této metody je úplné stanovení hodnot stravitelnosti dusíkatých látek v laboratoři bez použití kanylovaných krav. Metoda je založena na modifikaci klasické enzymatické metody. Klasická metoda využívá krmivo preinkubované v bachoru krávy po dobu 16 hodin. U kombinované enzymatické metody jsme preinkubaci krmiva v bachoru nahradili preinkubací v proteolytickém enzymu bromelainu v časovém intervalu 1 nebo 16 hodin, na kterou pak navazovala druhá fáze stanovení – 24hodinová inkubace nezdegradovaného zbytku enzymem pancreatinem. Na základě porovnání s hodnotami získanými stanovením metodou mobile bag na kanylovaných zvířatech byla vypočítána lineární a polynomická regresní rovnice, hodnota korelačního koeficientu, směrodatná odchylka a parametry regresních rovnic pro časové intervaly 1 a 16 hodin. Hodnoty odpovídají souboru krmiv (n = 18), který jsme rozšířili, z důvodů větší přesnosti predikčních rovnic, o krmiva testovaná v předešlé práci (Tománková a Homolka, 1999). Polynomická regrese vykazuje vyšší stupeň těsnosti vztahu (R = 0,901) při hodinové preinkubaci jako i lineární regrese (R = 0,867), má příznivější parametry rovnic a směrodatné odchylky. Proto kombinovaná enzymatická metoda s hodinovou preinkubací je vhodnější než metoda s 16hodinovou preinkubací.

Klíčová slova: přežvýkavci; degradovatelnost proteinu; intestinální stravitelnost; *in vitro*; proteolytické enzymy

Supported by the Minstry of Agriculture of the Czech Republic (Project No. EP0960006339 and QD0176).

One of the preconditions of effective utilization of feed resources is a better understanding of digestive and metabolic processes and research on nutritional factors limiting the yield. These factors also include the quality of crude protein. Several systems of evaluation and determination of crude protein requirement in ruminants have been published in the last 20 years and they have replaced the older systems of feed evaluation. All these systems are based on separate evaluation of crude protein intake for rumen micro-organisms and for the host animal organism, and on degradability of protein in the rumen. In the Czech Republic, a PDI system is used that has been taken over from the French PDI system – Protéines vraies Digestibles dans l'Intestin (Vérité *et al.*, 1987). To determine PDI units, it is necessary to know nitrogen content, digestibility of organic matter, degradability of crude protein in the rumen and intestinal digestibility of crude protein undegradable in the rumen. *In vivo*, *in vitro* and *in situ* methods have been elaborated to determine degradability of crude protein in the rumen. *In vivo* methods are far from optimum because they are time-consuming and also due to technical difficulties. In the case of *in situ* methods, with correction as regards the rumen fractional outflow rate, cannulated animals (Orskov and McDonald, 1979) and, similarly to *in vivo* methods, standardized feeding conditions are necessary. Consequently, there is an effort to replace them by *in vitro* laboratory methods with simpler assurance of standard conditions of measurement. Rumen fluid enzymes were initially used in these methods (Waldo, 1979). Due to the non-standard character of rumen activity, application of commercial enzymes with better reproducibility was introduced. Aufrére *et al.* (1991) used *Streptomyces griseus* protease, Poos-Floyd *et al.* (1985) suggested bromelain. Like degradability, intestinal digestibility of crude protein undegradable in the rumen (DSI) can be determined using various methods: *in vivo*, by the mobile bag method using animals with rumen and duodenal cannula (Hvelplund *et al.*, 1992) and by *in vitro* methods using various proteolytic enzymes. Antoniewicz *et al.* (1992) used pancreatin for degradation of residues in the small intestine.

This work was preceded by research on *in vitro* methods aimed at selection of the most suitable enzyme and, in the case of mobile bag methods, at standardization of the method. On the basis of evaluation of conformity of *in vitro* and in sacco results, bromelain was selected as the most suitable enzyme for determination of protein degradability in the rumen (Tománková and Kopečný, 1995), and pancreatin was selected as the most suitable enzyme for intestinal digestibility of crude protein undegradable in the rumen (Kopečný *et al*, 1998). Comparison of *in vitro* methods with in sacco or mobile bag methods (Frydrych, 1992; Homolka *et al.*, 1996) enabled us to derive predictive equations.

The objective of this study was to test the combined enzymatic method using an extended set of concentrates, and to calculate a predictive equation for this set by comparison of its values with values determined using the mobile bag method. For more exact estimation of predictive equations these equations were calculated from a set of 18 feeds with 9 concentrated feeds (No. 1, 2, 3, 4, 5, 9, 10, 12 and 13) added from the previous work (Tománková and Homolka, 1999).

MATERIAL AND METHODS

In the experiment a set of feeds was tested that included concentrates and extracted meals. Table 1 shows tested feeds, crude protein, dry matter and nitrogen content in residues after preincubation. For comparison with the combined enzymatic method (CO), the values of crude protein intestinal digestibility determined by the mobile bag method (MB) and the classical enzymatic method (CL) are also given in Table 1.

Combined enzymatic method (CO)

It consists of two steps. The first step, which simulates degradation in rumen, is based on modification of our own methods (Tománková and Kopečný, 1995). The second step, which determines intestinal digestibility of crude protein, is based on modification of the classical enzymatic method (Antoniewicz *et al.*, 1992). The modification consisted in replacement of 16-hour preincubation of feed in the cow's rumen by preincubation of feed in an incubation solution with bromelain. The feeds were tested in time intervals of 1 and 16 hours. In the first step the feeds were preincubated in incubation solution with bromelain (Sigma, 2–4 U per mg protein) at a temperature of 39°C to obtain undegradable residues. The residues were dried at a temperature of 55°C for 24 hours. Nitrogen and dry matter content was determined in the residues. Subsequently, in the second step the residues were enzymatically degraded using pancreatin as described previously (Kopečný *et al.*, 1998). The second step consisted in incubation of the residue in an enzymatic solution with pancreatin (600 mg/l, 4 × UPS, Sigma). All samples were incubated three times and all measurements were done at least in triplicates.

Table 1. List of feeds, contents of crude protein, dry matter, nitrogen in residues and DSI values determined by combined enzymatic method, classical enzymatic method and mobile bag method

Feeds (meals)	CP (g/kg DM)	**CO/1** (%)	DM (g/kg DM)	N	**CO/16** (%)	DM (g/kg DM)	N	**CL** (%)	DM (g/kg DM)	N	**MB** (%)	DM (g/kg DM)	N
Wheat		**87.88**	891.3	10.2	**85.75**	911.9	8.1	**92.43**	923.9	31.7	**95.48**	923.9	31.7
Wheat	138	**81.15**	914.3	10.5	**70.85**	916.2	6.0	**87.45**	932.3	25.5	**94.58**	932.3	25.5
Barley	111	**84.59**	914.5	13.1	**71.99**	913.6	7.1	**87.92**	924.6	24.0	**95.47**	924.6	24.0
Oat	108	**67.82**	908.0	10.9	**64.59**	926.1	8.4	**73.68**	938.1	9.5	**72.02**	938.1	9.5
Rye	109	**73.65**	902.2	12.5	**75.66**	907.6	7.7	**84.15**	924.1	16.4	**86.49**	924.1	16.4
Maize	95	**82.57**	905.8	15.0	**82.04**	915.9	13.1	**89.85**	929.6	19.7	**92.28**	929.6	19.7
Rapeseed	421	**74.33**	906.8	64.6	**70.93**	913.5	55.0	**72.02**	919.8	72.2	**74.53**	919.8	72.2
Soybean	462	**93.29**	909.7	82.7	**89.99**	920.8	39.9	**96.55**	932.7	108.6	**97.33**	932.7	108.6
Sesame	443	**84.03**	919.6	48.2	**72.50**	932.3	32.3	**90.04**	938.0	81.3	**97.07**	938.0	81.3

CO/1 – combined enzymatic method using 1-hour preincubation
CO/16 – combined enzymatic method using 16-hours preincubation
CL – classical enzymatic method
MB – mobile bag method
DSI – intestinal digestibility of protein undegradable in rumen
DM – dry matter
CP – crude protein
N – nitrogen in residues

More detailed procedures of both steps of the combined method and concentrations of solutions were published in previous works by Tománková and Kopečný (1995) and Kopečný *et al.* (1998).

Classical enzymatic method (CL) – pancreatin method

For DSI determination by the classical enzymatic method feed preincubated in the rumen of a cow with rumen cannula for 16 hours was used (Kopečný *et al.*, 1998).

Mobile bag method (MB)

The procedure consists of three steps as described previously by Homolka *et al.*, 1996:

a) feed incubation in the rumen of cannulated cows
b) incubation of residues in artificial abomasum
c) determination of DSI in samples of feed in bags after their insertion into a duodenal cannula and passage through the digestive tract.

A relationship between the values obtained by the mobile bag method and combined enzymatic method was expressed by means of predictive equation for the set of tested feeds.

The regression of determined values was computed by SAS System (analysis of variance).

RESULTS AND DISCUSSION

Correlation between undegradable crude protein digestibility determined by the mobile bag method and by the combined enzymatic method in the given feed set was expressed using linear and polynomial regression. Correlation between MB method and CO/1 method with 1-hour preincubation is presented in Figure 1. Different DSI values of feeds in the set evoke a question whether the linear relation is correct. As a linear relation was presented in our previous works for other feed sets (roughage), we present this equation as well. Polynomial regression however shows a higher degree of relation tightness. Feed samples were tested in two time intervals of preincubation (1 hour and 16 hours) using the combined enzymatic method. The values of intestinal digestibility of rumen undegraded CP determined using CO/1 and CO/16 methods ranged from 66.85 to 93.29% ($\bar{x}$ = 80.27 ± 7.46), 57.80 to 89.99% ($\bar{x}$ = 74.00 ± 8.25), respectively. The method with 1-hour preincubation gave a higher correlation coefficient (R = 0.867 for linear regression, R = 0.901 for polynomial) than the method with 16-hour preincubation (R = 0.729 for linear regression, R = 0.764 for polynomial). On the contrary, standard deviations were lower for evaluation of the method with 1-hour preincubation for both regressions. The values of parameters of both predictive equations, correlation coefficients and standard deviations are given in Table 2. Enzymatic methods give lower DSI values than those determined

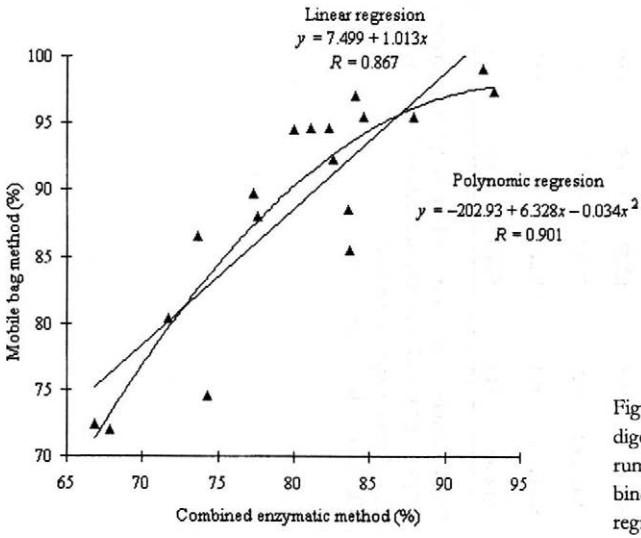

Figure 1. Comparison of the values of intestinal digestibility of undegradable crude protein in the rumen provided by mobile bag method and combined enzymatic method: linear and polynomial regression (1-hour preincubation)

Table 2. Regression of values determined by mobile bag method and combined enzymatic method for preincubation 1 and 16 hours and comparison of MB digestibility and enzymatic (CO/1, CO/16) intestinal digestibility of feed proteins

Types of regression	n	a	b_1	b_2	R	RSD	Method	$\bar{x} \pm SD$
Linear/1	18	7.499	1.013		0.867	4.47	MB	88.78 ± 8.71
Polynomial/1	18	–202.982	6.329	–0.033	0.901	4.02	CO/1	80.27 ± 7.46
Linear/16	18	31.822	0.769		0.729	6.14	CO/16	74.00 ± 8.25
Polynomial/16	18	–94.764	4.201	–0.023	0.764	5.99		

n – number of tested feeds R – correlation coefficient
a, b_1, b_2 – equation parameters RSD – residual standard deviation

by the mobile bag method (MB) (Table 1). This finding was also confirmed by White *et al.* (1999). They however pointed out the high correlation between both methods and, consequently, possible application of a suitable enzymatic method for DSI prediction. The values of classical enzymatic method (CL) are closest to those determined by the mobile bag method. They are lower and, like in other *in vitro* methods, they do not give the actual values found in animals. This is demonstrated more markedly by the combined enzymatic method (CO). Therefore it was necessary to calculate the predictive equations. It is obvious from Tables 1 and 2 that the combined method with 1-hour preincubation is more suitable for DSI determination.

The mean values of CP digestibility determined by CO/1 and MB method did not significantly differ ($P < 0.01$).

Antoniewicz *et al.* (1991) preincubated concentrates and feeds of animal origin in cannulated cow's rumen for 12 hours. Calsamiglia and Stern (1995) tested the effect of feed preincubation (12, 16 and 18 hours) in the rumen on DSI values using the classical method with pancreatin. They found that in the case of soybean meal the effect of rumen preincubation was small, which demonstrates that this feed is in fact digested in the small intestine. The time of feed preincubation affects DSI values at feeds of animal origin to a greater extent (Calsamiglia and Stern, 1995). DSI values estimated by most authors do not conform with each other completely. This may be due to differences in working procedures, mainly due to differences in the time course of degradation of the protein component of feeds. It may also be due to the fact that isolated proteases may differ from proteases of rumen microorganisms in their effect on the protein structure. Therefore an important precondition of application of various alternative methods summarized by Stern *et al.* (1997) is their correlation with the methods using experimental animals. Simple and rapid determination of feed DSI in laboratory by in vitro methods becomes necessary for practical evaluation of nutritive value and quality of feeds.

We can conclude that the combined enzymatic method using 1-hour preincubation with bromelain provides DSI values that are in good agreement with the values obtained by the mobile bag method. High correlation coefficient ($R = 0.867$) in linear regression (in polynomial regression $R = 0.901$) between the two methods suggests that the combined enzymatic method may be effectively used for estimation of intestinal digestibility of undegradable crude protein in the rumen in ruminants.

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Received: 01–10–31
Accepted after corrections: 02–01–04

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The profile of amino acids in intramuscular protein of bulls of milked and beef commercial types

Obsah aminokyselin ve svalovém proteinu býků dojených a masných užitkových typů

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ABSTRACT: The objective of analyses of the meat of bulls fattened under the same type of nutrition was to determine differences in the profile of 15 amino acids of selected commercial types of cattle. The amino acids were analysed in 224 meat samples – *musculus longissimus, pars thoracis*; significant differences in the content of the respective groups of amino acids (essential, semi-essential and non-essential) and particular amino acids were disclosed in 10 commercial types of cattle. In two experimental groups of bulls (dual-purpose – commercial dairy type and fattened – beef type) significant differences were recorded in the total content of essential amino acids ranging between 30.16% (Montbéliarde) and 34.50% (Maine-Anjou), and between 30.22% (Hereford) and 33.75% (Aberdeen-Angus). Within these significant differences between the commercial groups, the highest content was that of lysine, i.e. 7.85–8.73% and 7.76 to 8.75%, respectively. In the dual-purpose commercial types significant differences in the content of threonine, valine and isoleucine amino acids were disclosed. The average differences in valine, isoleucine and phenylalanine between the groups of fattened commercial cattle ($P < 0.05$) were found to be highly significant. Evaluation of the amino acid profile revealed that the highest number of significant differences between the fattened commercial cattle types was in the histidine level (3.86% – Limousine, 4.64% – Belgian White and Blue Pied). In the non-essential amino acids significant differences ($P < 0.05$) in the levels of glutamic acid, asparagine, proline, serine and alanine were found between the fattened cattle types.

Keywords: bulls; meat; commercial type; protein; amino acids

ABSTRAKT: Bylo analyzováno maso býků vykrmovaných při shodném typu výživy s cílem stanovit diference v profilu 15 aminokyselin zvolených užitkových typů skotu. Byla provedena analýza 224 vzorků masa – *musculus longissimus, pars thoracis*; u deseti užitkových typů skotu byly stanoveny signifikantní rozdíly ($P < 0,05$) u obsahu jednotlivých skupin (esenciální, semiesenciální a neesenciální) a jednotlivých aminokyselin. Ve dvou pokusných souborech býků (kombinovaný – dojený užitkový typ a výkrmový – masný typ) jsme zaznamenali signifikantní rozdíly celkového obsahu esenciálních aminokyselin s hodnotami od 30,16 % (montbéliarde) do 34,50 % (maine-anjou) a od 30,22 % (hereford) do 33,75 % (aberdeen-angus). Nejvyšší hodnoty při signifikantních diferencích mezi užitkovými skupinami jsme stanovili u obsahu lyzinu (7,85–8,73 %, resp. 7,76–8,75 %). Významné rozdíly hodnot jsme zaznamenali mezi užitkovými typy v rámci kombinovaných užitkových typů při hodnocení aminokyselin treoninu, valinu a izoleucinu. Nejvýznamnější rozdíly ($P < 0,05$) průměrů skupin u výkrmových užitkových typů skotu jsme zjistili u obsahu valinu, izoleucinu a fenylalaninu. Při hodnocení profilu aminokyselin bylo nejvíce signifikantních rozdílů hodnot stanoveno mezi výkrmovými užitkovými typy při sledování obsahu histidinu (3,86 % – limousine, 4,64 % – belgický modrobílý). Významnější diference v souboru neesenciálních aminokyselin jsme stanovili u výkrmových typů skotu při hodnocení obsahu kyseliny glutamové, asparaginu, prolinu, serinu a alaninu. Výsledky práce poukazují na významnost volby užitkového typu jatečných býků, který může ovlivnit i kvalitu svalového proteinu.

Klíčová slova: býci; maso; užitkový typ; protein; aminokyseliny

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Grant No. MSM 4321 0001).

It is important for the consumer to be confident that food safety and quality are guaranteed. In this connection the consumption of meat as an important component of the population's diet is subjected to increasing health and production pressures in the concrete time zones. The end result is a fluctuating consumption of meat and meat products, which is reflected in the consumers' demand and which considerably complicates the existence of slaughter cattle producers. In addition to food safety the general requirements for meat are affected by the nutritional and technological quality. For the majority of the population the optimal nutritional quality is based, first of all, on reduced fat content and increased content of full-value proteins. At the present time the above-mentioned requirements for the safety and quality of meat are reflected in beef production and to some extent they can be influenced by the producers of slaughter animals themselves. High-quality beef can also be produced by increasing the production of commercial beef cattle using various methods of crossing the dairy populations with bulls of specialised beef breeds. Therefore it appears necessary to map as many characters of meat efficiency as possible in the course of breeding, not only with respect to fattening performance but also to carcass value, including the parameters of meat quality.

Meat is a very complicated biological system and objective evaluation of its quality is very difficult. There is a higher demand on the market for meat and meat products of higher quality and they are more expensive. The market of meat and meat products has proved that consumers generally give preference particularly to the sensory aspects of meat quality, but also to the nutritional and other ones. The deterioration of several quality parameters or the serious downgrading of only one factor is very frequently the reason why the consumer's interest in meat declines. The consumer will tolerate the lower quality only if the price is markedly cut and, on the other hand, will appreciate the higher and reliable quality by buying it and being willing to pay an adequately higher price. This is associated with the production of meat; high production costs make it one of the most expensive foodstuffs. Beef quality is primarily based on the commercial type, sex and age category of slaughter cattle (Franc and Gayer, 1994; Teslík *et al.*, 1996). A number of authors involved in the evaluation of meat quality parameters often stated how difficult it was to characterise the chemical composition of meat and that the chemical composition of meat was primarily dependent on the method of fattening, age and sex of slaughtered animals. Šubrt and Schmidt (1994) reported that the protein and fat contents are the basic and characteristic traits of meat quality and that the protein content, in particular, is relatively stable in the muscles not only in commercial cattle, but in animals in general. Some authors evaluated the quality characteristics particularly of the *musculus longissimus*. In the *longissimus thoracis* of bulls of the Slovakian Pied cattle slaughtered when weighing 546 kg Palanská *et al.* (1986) found that the protein content ranged between 22.40 and 22.60%. In Hereford bullocks fattened by grazing on pasture Franc *et al.* (1978) found a 21.48% content of muscle proteins. Golda *et al.* (1989) specified basically the same concentration of proteins in meat, i.e. in Bohemian Pied bulls they detected 21.47%, in bulls from German Pied fathers 21.93% and in bulls from Montbéliarde fathers 20.99% of proteins. In recent experiments Bartoň *et al.* (1996) discovered a 19.81% protein content in the sirloin of the Czech Pied cattle and 18.95% in Black Pied bulls. Bartoň *et al.* (1998) reported a higher content of proteins in the sirloin of Czech Pied cattle, i.e. 21.09%.

There are various symptoms of protein insufficiency in the diet, such as retarded growth, extremely low body weight, extremely reduced fat in the body and muscle atrophy. Protein quality is primarily based on the profile of the respective amino acids. About 140 various amino acids have been isolated from biological systems until now. The proteins of the majority of organisms contain only 20 basic amino acids (Velíšek, 1999) and the proteins contain approximately 90% of the amino acids present in the organism. The other amino acids appear as intermediate products of the metabolism of proteins and some serve for further synthesis of various compounds (Šícho *et al.*, 1981). About 99% of the amino acids in most foodstuffs are bound in proteins and peptides and the rest (about 1%) are free amino acids. We usually find more free amino acids in foodstuffs since proteolysis occurs during production or storage, and these amino acids considerably affect the sensory properties of meat. Important aromatic and gustatory substances are frequently the products of amino acid reactions. Amino acids are also basic substances for the synthesis of a number of biologically active substances of non-protein character.

Based on their importance in human nutrition the coded amino acids are divided as follows: 1) essential – valine, leucine, isoleucine, threonine, methionine, lysine, phenylalanine and tryptophan, 2) semi-essential – arginine and histidine, 3) non-essential – the remaining amino acids. There are 8 essential amino acids for man and these amino acids are necessary because the human organism is not capable of synthesising car-

bonaceous skeletons. In rapidly growing organisms some non-essential amino acids the young organism is incapable of synthesising in sufficient amounts become essential amino acids. These amino acids are sometimes called semi-essential amino acids. The other amino acids that form the construction units of proteins but are not essential, are indicated as so-called relatively dispensable amino acids. The organism can synthesise them if it has enough essential amino acids. There are very few literary sources dealing with in the effect of breeding factors on the profile of amino acids. The majority of these published results of amino acid analyses are focused on the effect of animal nutrition and metabolic intermediate products when processing meat (Garber *et al.*, 1976; Griffith, 1977; Joseph and Griffith, 1986; Nguyen and Zarkadas, 1989; Perriello *et al.*, 1995; Feidt *et al.*, 1996). Most of the papers deal with studies how to influence the content of free amino acids in meat and their relationship to organoleptic characteristics of beef – the effect of meat electro-stimulation, etc. Zakhariev *et al.* (1980), Anderson (1988), Čítek and Košvanec (1990) studied the effect of different breeds on the content of amino acids in beef. Barabáš (1987) studied the biological value of proteins of various products, including beef proteins.

MATERIAL AND METHODS

The content of amino acids in meat was evaluated in 224 slaughter bulls, products mainly of commercial crossing (F_1 generation). The fattened bulls belong to 10 genotypes; their indications and numbers are listed in Table 1. The group of animals indicated as C > 76 represents the improved population with a more than 76% proportion of blood of the domestic Czech Pied dual-purpose cattle. The bulls of commercial cattle indicated as C 51–75 have a higher proportion of dairy breeds and they incline to the milked commercial cattle. In the group of Montbéliarde slaughter bulls we analysed the meat of purebred bulls; the other commercial

Table 1. Slaughter weight and content of total proteins in the *musculus longissimus dorsi*

Commercial type/ statistical value protein (%)		Slaughter weight (kg)	Total protein (%)	Commercial type/ statistical value		Slaughter weight (kg)	Total
C > 76	$\bar{x}$	576	21.05	Aberdeen-Angus	$\bar{x}$	557	20.35
(n = 32)	s	54.518	0.947	(n = 11)	s	36.563	0.868
	$V_{\%}$	9.46	4.50		$V_{\%}$	6.56	4.27
	min.	460	18.71		min.	485	19.19
	max.	679	22.72		max.	610	22.27
C 51–75	$\bar{x}$	637	20.92	Hereford	$\bar{x}$	564	21.56
(n = 10)	$s_{\bar{x}}$	45.309	0.29	(n = 8)	$s_{\bar{x}}$	27.333	0.37
	$V_{\%}$	7.10	4.42		$V_{\%}$	4.84	4.80
	min.	575	19.78		min.	513	19.63
	max.	710	22.84		max.	604	22.50
Montbéliarde	$\bar{x}$	536	21.59	Limousine	$\bar{x}$	605	21.80
(n = 27)	s	48.556	0.472	(n = 23)	s	70.514	2.020
	$V_{\%}$	9.06	2.19		$V_{\%}$	11.65	9.26
	min.	435	20.46		min.	457	19.17
	max.	630	22.20		max.	713	28.88
Maine-Anjou	$\bar{x}$	627	20.82	Belgian White	$\bar{x}$	555	21.37
(n = 5)	s	70.301	0.710	and Blue	s	36.512	1.070
	$V_{\%}$	11.22	3.41	(n = 10)	$V_{\%}$	6.58	5.01
	min.	577	19.78		min.	490	19.75
	max.	746	21.66		max.	605	22.91
Charolaise	$\bar{x}$	569	21.33	Blonde	$\bar{x}$	573	21.66
(n = 72)	s	43.176	0.975	d'Aquitaine	s	68.495	1.015
	$V_{\%}$	7.58	4.57	(n = 26)	$V_{\%}$	11.94	4.69
	min.	440	19.03		min.	380	18.90
	max.	650	23.60		max.	679	23.19

types are indicated according to the respective paternal breed, i.e. crosses of the F_1 generation of paternal breeds with the domestic improved population of dual-purpose efficiency.

The basic ingredient of the feed ration of all fattened animals was maize silage, lucerne hay and supplementary feed mixture of commercial or corporate origin. The animals were slaughtered according to the commercial cattle type at the weight of 550–630 kg (Table 1). The inter-group variability of slaughter weight was relatively high, but it corresponds to the total structure of weight of purchased slaughter bulls in the Czech Republic.

Meat samples for chemical analyses were taken from the sirloin muscle of the *musculus longissimus, pars thoracis* on the level of the 9th–11th thoracic vertebra and prior to laboratory analyses the covering fibrous tissue was removed from the muscles. The content of total proteins in the muscle sample was determined by traditional Kjeldahl method. The preparation of samples for the assessment of amino acids was based on the used method of hydrolysis. The chromatographic analysis of a hydrolysate was performed on the AAA T 339 apparatus (Fa Mikrotechna, Czech Republic) using sodium citrate buffers and ninhydrous detection.

Mathematical and statistical evaluation was conducted by means of the programme of the statistical package UNISTAT 5.0. The non-parametric test – Kruskal-Wallis analysis was used to test the differences between the respective sets. The differences were tested on a 95% probability level ($P < 0.05$).

RESULTS AND DISCUSSION

The difference in the content of total protein in the dry matter of meat between the groups of bulls was not significant (Table 1). The highest values were determined in bulls of the Limousine commercial type (21.80%) and they ranged between 19.17 and 28.88%. The meat quality of the Aberdeen-Angus commercial cattle was found to be lowest on the basis of the content of total protein in meat (20.35%). The content of protein was lower because of the relatively high content of intra-muscular fat. The variability of the content of total intra-muscular protein detected in the present study also corresponds with the values determined by Franc *et al.* (1978) in Hereford cattle. Palanská *et al.* (1986) reported higher protein content in the Slovakian Pied cattle (22.4–22.6%) and Bartoň *et al.* (1998) reported an insignificantly lower protein content in the meat of Czech Pied and Black Pied Lowland bulls.

Table 2. Essential amino acids in the meat of dual-purpose commercial bulls (%)

Amino acid/ statistical value		Breed of father – commercial type			
		C > 76 a	C 51–75 b	Montbéliarde c	Maine-Anjou d
Threonine	$\bar{x}$	4.04^{cb}	4.47^{ca}	3.79^{abd}	4.31^{c}
	s	0.526	0.268	0.383	0.296
	$V_{\%}$	13.02	6.00	10.10	6.86
	min.	3.00	4.10	3.03	3.93
	max.	4.91	4.92	4.52	4.74
Valine	$\bar{x}$	4.37^{b}	3.93^{ad}	4.24^{d}	4.76^{bc}
	s	0.619	0.546	0.494	0.365
	$V_{\%}$	14.17	13.88	11.64	7.67
	min.	2.86	3.28	3.50	4.45
	max.	5.55	4.91	5.48	5.39
Isoleucine	$\bar{x}$	4.10^{d}	3.90^{d}	4.01^{d}	5.00^{abc}
	s	0.625	0.645	0.466	0.483
	$V_{\%}$	15.26	16.53	11.61	9.66
	min.	2.61	2.96	3.34	4.64
	max.	5.25	4.90	5.18	5.77
Leucine	$\bar{x}$	7.05	7.36	6.83	8.18
	s	0.771	1.002	0.771	0.479
	$V_{\%}$	10.93	13.61	11.27	5.86
	min.	5.29	5.88	5.06	7.63
	max.	8.63	9.49	8.58	8.84
Phenyl-alanine	$\bar{x}$	3.35^{d}	3.43	3.44	3.75^{a}
	s	0.341	0.497	0.372	0.292
	$V_{\%}$	10.18	14.49	10.82	7.88
	min.	2.55	2.72	2.70	3.47
	max.	4.07	4.56	4.47	4.18
Lysine	$\bar{x}$	8.73^{c}	8.15^{c}	7.85^{ab}	8.50
	s	3.230	0.483	1.951	0.398
	$V_{\%}$	37.02	5.92	24.87	4.68
	min.	4.96	7.27	6.32	8.17
	max.	21.24	8.75	17.01	9.17
Total essential amino acids	$\bar{x}$	31.63^{cd}	31.25^{d}	30.16^{da}	34.50^{abc}
	s	4.79	2.81	3.63	2.01
	$V_{\%}$	15.14	8.99	12.04	5.79
	min.	24.09	26.97	26.36	32.61
	max.	46.38	36.54	43.62	37.52

a, b, c, d = $P < 0.05$

We studied the amino acid profile in the meat of slaughter bulls according to the important groups, i.e. essential, semi-essential and non-essential amino acids. The highest per cent proportion of essential amino acids (Tables 2 and 3) was that of lysine, in dual-

Table 3. Essential amino acids in the meat of fattened commercial bulls (%)

Amino acid/ statistical value		Breed of father – commercial type					
		Aberdeen -Angus e	Blonde d'Aquitaine f	Belgian White and Blue g	Hereford h	Charolaise i	Limousine j
Threonine	$\bar{x}$	4.26	4.18	3.96	4.22	4.13	4.16
	s	0.186	0.333	0.489	0.229	0.336	0.454
	$V_{\%}$	4.35	7.97	12.34	5.43	8.15	10.91
	min.	4.03	3.22	3.27	3.88	3.22	2.99
	max.	4.67	4.77	4.90	4.56	4.90	5.50
Valine	$\bar{x}$	4.70^{h}	4.64^{h}	4.54^{h}	3.72^{efgij}	4.77^{h}	4.50^{h}
	s	0.518	0.451	0.774	0.286	0.464	0.754
	$V_{\%}$	11.02	9.73	17.04	7.67	9.72	16.75
	min.	3.95	3.23	3.23	3.16	3.33	2.71
	max.	5.67	5.30	5.41	4.13	5.97	5.73
Isoleucine	$\bar{x}$	4.44^{gh}	4.32^{h}	4.31^{h}	3.83^{efgij}	4.44^{h}	4.25^{h}
	s	0.703	0.583	0.773	0.305	0.626	0.729
	$V_{\%}$	15.84	13.49	17.95	7.96	14.07	17.17
	min.	3.16	2.93	2.95	3.24	2.90	2.66
	max.	5.51	5.25	5.11	4.21	6.40	5.46
Leucine	$\bar{x}$	7.97^{hj}	7.95^{hj}	8.19	7.32^{efi}	7.82^{hj}	7.45^{efi}
	s	0.371	1.158	1.176	0.493	0.774	1.178
	$V_{\%}$	4.66	14.57	14.33	6.74	9.90	15.81
	min.	7.36	3.80	6.62	6.51	5.45	5.15
	max.	8.73	10.06	10.33	8.02	10.33	10.56
Phenylalanine	$\bar{x}$	3.88^{h}	3.75^{h}	4.10^{hj}	3.38^{efgi}	3.94^{hj}	3.55^{gi}
	s	0.399	0.334	0.471	0.260	0.400	0.615
	$V_{\%}$	10.30	8.90	11.49	7.68	10.15	17.33
	min.	3.48	2.80	3.62	2.97	3.07	2.41
	max.	4.87	4.22	5.11	3.77	5.11	4.82
Lysine	$\bar{x}$	8.49^{h}	8.31^{h}	8.75^{h}	7.76^{efgi}	8.36^{h}	8.13
	s	0.493	0.546	1.475	0.445	0.501	0.649
	$V_{\%}$	5.80	6.57	16.85	5.73	5.99	7.99
	min.	7.90	7.09	5.58	7.19	6.01	6.20
	max.	9.28	9.42	10.99	8.51	9.45	9.37
Total essential amino acids	$\bar{x}$	33.75^{hj}	33.15^{h}	33.85^{hj}	30.22^{efgij}	33.46^{hj}	32.04^{hegi}
	s	1.86	1.71	2.04	1.65	1.33	3.78
	$V_{\%}$	5.51	5.16	6.03	5.46	3.97	11.79
	min.	30.40	26.56	30.25	27.51	28.90	22.12
	max.	37.39	36.50	37.49	32.69	37.35	40.07

e, f, g, h, i, j = $P < 0.05$

purpose cattle it ranged between 7.85 and 8.73%, with significant differences ($P < 0.05$) between the Montbéliarde bulls and both groups of Czech Pied cattle. Zakhariev *et al.* (1980) reported similar results (7.77%) in the meat of Bulgarian Brown cattle. In the group of fattened commercial cattle we found significant inter-group differences ranging between 7.76 (Hereford) and 8.75% (Belgian cattle). The lowest significant content of lysine (below 8%) was detected in the meat of Montbéliarde slaughter bulls and crosses with Hereford. Compared with lysine, the content of leucine in the meat samples was insignificantly lower and the highest average content was observed in the group of crosses with Maine-Anjou (8.18%) and Belgian cattle (8.19%). Zakhariev *et al.* (1980) reported insignificantly higher values (8.42%). The content of leucine in the other groups of bulls ranged between 6.83% (Montbéliarde) and 7.97% (Aberdeen-Angus). The contents of threonine, valine and isoleucine made up 4% of the amino acid content. As for the inter-group values, threonine showed the best balanced values in the group of fattened slaughter cattle ($P < 0.05$). The content of threonine in the meat of the Belgian Blue and White bulls was close to 4%. We discovered significant differences in the content of threonine in the group of dairy commercial cattle, which was lowest in Montbéliarde. The variability in the content of threonine is in accordance with results published by Barabáš (1987) in meat. The amino acid valine contributes to the proper function of the nervous and muscular tissues. In the group of fattened commercial cattle the muscles of the Charolaise breed showed the highest

Table 4. Semi-essential amino acids in the meat of dual-purpose commercial bulls (%)

Amino acid/ statistical value		Breed of father – commercial type			
		C > 76	C 51–75	Montbéliarde	Maine-Anjou
		a	b	c	d
Histidine	x	3.75	3.64	3.96	3.85
	s	0.372	0.435	0.328	0.302
	$V_{\%}$	9.91	11.95	8.33	7.85
	min.	2.91	3.08	3.29	3.45
	max.	4.59	4.51	4.60	4.24
Arginine	x	5.67	5.52^{d}	5.68^{d}	6.62^{bc}
	s	0.746	0.694	0.802	0.828
	$V_{\%}$	13.15	0.126	14.11	12.50
	min.	4.42	4.14	4.59	5.40
	max.	6.97	6.28	8.01	7.55
Total semi-essential amino acid	x	9.43^{d}	9.17^{d}	9.64^{d}	10.47
	s	0.94	1.03	0.97	1.12
	$V_{\%}$	9.97	11.23	10.06	10.70
	min.	7.66	7.22	8.15	8.86
	max.	10.73	10.14	12.15	11.79

a, b, c, d = $P < 0.05$

Table 5. Semi-essential amino acids in the meat of fattened commercial bulls (%)

Amino acid/ statistical value		Breed of father – commercial type					
		Aberdeen-Angus	Blonde d'Aquitaine	Belgian White and Blue	Hereford	Charolaise	Limousine
		e	f	g	h	i	j
Histidine	x	4.39^{hj}	4.19^{ghj}	4.64^{fhji}	3.59^{efgi}	4.39^{hgj}	3.86^{efgi}
	s	0.301	0.539	0.213	0.247	0.537	0.709
	$V_{\%}$	6.86	12.87	4.68	6.88	12.25	18.47
	min.	3.80	3.08	4.24	3.36	3.36	2.59
	max.	4.90	5.07	5.04	4.08	6.94	5.16
Arginine	x	6.08	6.19	6.98^{h}	5.74^{g}	6.18	6.18
	s	0.307	0.664	1.207	0.405	0.627	0.992
	$V_{\%}$	5.04	10.72	17.30	7.05	10.14	16.06
	min.	5.60	4.78	5.55	5.20	4.66	3.38
	max.	6.67	7.95	8.99	6.43	7.95	7.95
Total semi-essential amino acids	x	10.48^{h}	10.38^{ghj}	11.62^{fhj}	9.34^{efgij}	10.57^{h}	10.03^{gh}
	s	0.54	0.92	1.24	0.63	0.88	1.42
	$V_{\%}$	5.15	8.86	10.67	6.75	8.33	14.15
	min.	9.40	7.86	10.01	8.56	8.45	5.97
	max.	11.57	12.43	13.61	10.51	13.74	12.47

e, f, g, h, i, j = $P < 0.05$

average content of valine (4.77%). The significantly lowest content of valine was detected in the meat of crosses with Hereford (3.72%). Velíšek (1999) reported that milk and egg proteins contain the highest amount of isoleucine (6–7%) while meat contains 4–5%. Our conclusions based on 10 commercial types of cattle (between 3.83% in hybrids with Hereford and 5.00% in hybrids with Maine-Anjou) correspond with these data. In the group of essential amino acids, the content of phenylalanine was lowest, ranging from 3.35 (C > 76) to 3.75% (Maine-Anjou) in the group of dual-purpose commercial slaughter bulls. Higher total contents of this amino acid were determined in the group of fattened commercial cattle and the highest content was found in the Belgian Blue and White bulls (4.10%).

In the group of semi-essential amino acids we examined the content of histidine and arginine (Tables 4 and 5). The contents of histidine and arginine were significantly higher in the meat of fattened commercial cattle, with the exception of hybrids with Hereford and Maine-Anjou. In the group of dual-purpose commercial cattle, the Maine-Anjou hybrids had the highest content of arginine (6.62%), which was significantly higher than in the other groups of slaughter bulls of this group of cattle. In the group of fattened commercial cattle the differences in the content of arginine between the Hereford bulls (5.74%) and Belgian Blue and White cattle (6.98%) were highly significant. The content of histidine and arginine in the meat of Bulgarian Brown cattle was 3.37 and 6.17%, respectively (Zakhariev *et al.*, 1980).

Of all amino acids the muscle proteins have the highest content of glutamic acid (Tables 6 and 7) (Dvořák, 1987). In beef Barabáš (1987) and Zakhariev *et al.* (1980) detected on average 15.5 and 13.3% of glutamic acid, respectively. In the analysed group of meat samples from slaughter animals the content of glutamic acid in dual-purpose cattle ranged between 14.58 and 16.77% ($P < 0.05$) and was highest in Maine-Anjou hybrids. In fattened commercial bulls we detected significant ($P < 0.05$) differences in average values from 14.42% (Limousine) to 16.24% (Blonde Aquitaine). The content of asparagine reached 8%, the differences in the average values being significant ($P < 0.05$) only in a part of the evaluated groups within the group of fattened bulls. The highest average content was determined in Aberdeen-Angus hybrids (8.73%) and the lowest in Hereford hybrids (7.49%). The lowest variability in the values of asparagine content with no significant differences was detected between the groups of fattened bulls. The absolutely lowest and balanced values for the groups of bulls of dual-purpose types

Table 6. Non-essential amino acids in the meat of dual-purpose commercial bulls (%)

Amino acid/ statistical value		Breed of father – commercial type			
		C > 76	C 51–75	Montbéliarde	Maine-Anjou
		a	b	c	d[c]
Asparagine	x	8.13	8.10	7.83	7.93
	s	0.792	0.785	0.733	0.499
	$V_{\%}$	9.73	10.69	9.36	6.28
	min.	6.78	7.09	6.79	7.34
	max.	9.67	9.93	9.50	8.42
Serine	x	3.37	3.20	3.20	3.28
	s	0.500	0.345	0.307	0.201
	$V_{\%}$	14.87	10.78	9.60	6.12
	min.	2.72	2.63	2.64	3.08
	max.	5.26	3.95	3.68	3.49
Glutamic acid	x	14.76	14.58[d]	14.70[d]	16.77[bc]
	s	1.54	2.12	1.51	1.48
	$V_{\%}$	10.45	14.51	10.28	8.81
	min.	12.05	11.34	12.18	15.96
	max.	17.66	19.06	18.01	19.40
Proline	x	3.79	3.44[c]	3.93[b]	3.78
	s	0.466	0.307	0.608	0.352
	$V_{\%}$	12.12	8.91	15.46	9.31
	min.	3.21	2.89	2.12	3.33
	max.	5.06	3.89	5.13	4.28
Glycine	x	3.83[cd]	3.91	3.53[ad]	4.24[ac]
	s	0.556	0.577	0.513	0.358
	$V_{\%}$	14.52	14.74	14.52	8.46
	min.	2.97	3.19	1.71	3.88
	max.	5.69	5.24	4.28	4.76
Alanine	x	4.81	4.55	4.85	4.76
	s	0.873	0.541	0.476	0.342
	$V_{\%}$	18.15	11.91	9.81	7.19
	min.	3.40	3.59	3.92	4.53
	max.	7.85	5.64	5.80	5.36
Tyrosine	x	3.66	3.51	3.79	3.73
	s	0.353	0.444	0.594	0.235
	$V_{\%}$	9.63	12.65	15.68	6.29
	min.	2.63	3.06	2.73	3.37
	max.	4.33	4.58	5.17	3.95
Total non-essential amino acids	x	42.35	41.29	41.83	44.49
	s	3.47	4.70	3.89	2.82
	$V_{\%}$	8.19	11.38	9.30	6.33
	min.	36.19	35.24	34.55	42.50
	max.	49.54	52.11	51.35	49.49

a, b, c, d = $P < 0.05$

Table 7. Non-essential amino acids in the meat of fattened commercial bulls (%)

Amino acid/ statistical value		Breed of father – commercial type					
		Aberdeen -Angus e	Blonde d'Aquitaine f	Belgian White and Blue g	Hereford h	Charolaise i	Limousine j
Asparagine	$\bar{x}$	8.73^{hj}	8.61^{hj}	8.24^{eh}	7.49^{efgj}	8.50^{h}	7.98^{efh}
	s	0.369	0.564	0.840	0.386	0.581	0.818
	$V_{\%}$	4.23	6.55	10.19	5.14	6.84	10.24
	min.	8.17	7.28	6.88	6.88	6.99	5.54
	max.	9.59	9.59	9.94	8.17	10.15	9.21
Serine	$\bar{x}$	3.51^{hij}	3.41^{h}	3.36^{h}	3.04^{efgi}	3.26^{he}	3.22^{e}
	s	0.326	0.344	0.389	0.188	0.372	0.412
	$V_{\%}$	9.29	10.10	11.56	6.19	11.39	12.79
	min.	2.80	2.78	2.86	2.74	2.51	2.13
	max.	3.93	4.27	4.05	3.26	4.13	4.31
Glutamic acid	$\bar{x}$	16.01^{hj}	16.24^{hig}	15.24^{f}	14.55^{efi}	15.93^{hj}	14.42^{efi}
	s	1.298	1.105	1.796	0.978	1.209	2.538
	$V_{\%}$	8.11	6.80	11.79	6.72	7.59	17.60
	min.	12.81	12.06	11.88	12.79	12.31	10.31
	max.	17.29	17.58	17.58	15.60	18.13	21.38
Proline	$\bar{x}$	4.01^{h}	3.94^{h}	4.07^{h}	3.33^{efgij}	3.91^{h}	4.09^{h}
	s	0.490	0.670	0.629	0.155	0.548	0.622
	$V_{\%}$	12.21	17.00	15.46	4.66	14.01	15.20
	min.	3.26	3.17	3.28	3.06	3.06	2.67
	max.	4.93	5.11	5.49	3.55	5.32	5.00
Glycine	$\bar{x}$	3.92	3.97	3.76	3.75	3.89	3.89
	s	0.297	0.342	0.464	0.202	0.323	0.612
	$V_{\%}$	7.57	8.60	12.35	5.39	8.29	15.75
	min.	3.36	3.13	2.93	3.46	3.12	2.75
	max.	4.47	4.63	4.41	4.04	4.67	5.59
Alanine	$\bar{x}$	5.31^{hj}	5.19^{hj}	5.44^{hj}	4.34^{efgij}	5.18^{hj}	4.74^{efghi}
	s	0.415	0.419	0.413	0.225	0.490	0.945
	$V_{\%}$	7.81	8.09	7.59	5.18	9.45	19.96
	min.	4.69	4.32	4.87	3.96	3.96	2.94
	max.	6.01	6.09	6.10	4.64	6.43	6.20
Tyrosine	$\bar{x}$	3.87^{gi}	3.97^{j}	4.22^{ej}	3.47	4.17^{ej}	3.54^{fgi}
	s	0.83	0.800	0.864	0.339	0.767	0.739
	$V_{\%}$	21.42	20.09	20.50	9.79	18.38	20.85
	min.	3.00	2.48	3.35	3.14	2.94	2.46
	max.	5.23	5.34	5.55	4.11	5.55	4.97
Total non-essential amino acids	$\bar{x}$	45.36^{hj}	45.32^{hj}	44.33^{h}	39.96^{feig}	44.85^{hj}	41.88^{fei}
	s	1.88	2.13	2.62	2.34	2.21	5.65
	$V_{\%}$	4.14	4.70	5.91	5.86	4.93	13.49
	min.	41.22	37.82	40.01	36.04	38.61	28.81
	max.	47.90	47.37	49.05	42.95	48.41	55.61

e, f, g, h, i, j = $P < 0.05$

were determined when evaluating the content of serine (i.e. 3.20–3.37%). In the group of beef bulls the most important significant difference was between the Hereford group (3.04%) and the Aberdeen-Angus (3.51%) group. In the group of bulls of the beef commercial type significant differences were also detected in the content of the other non-essential amino acids, i.e. alanine and tyrosine. The Limousine hybrids had the lowest content. Glycine contents were well balanced in the fattened groups of bulls, with no significant differences in group averages. Insignificant differences in the contents of alanine and tyrosine between the groups were detected in the experimental group of dual-purpose commercial cattle. In this group of cattle the most significant differences ($P < 0.05$) were discovered when evaluating glycine (4.24% Maine-Anjou, 3.53% Montbéliarde and 3.83% C > 76).

The analyses of the content of amino acids in the protein of beef (sirloin) indicated significant ($P < 0.05$) differences in their content between the commercial types of cattle divided into two groups – dual-purpose and beef commercial types produced by commercial crossing of the domestic population of Czech Pied cattle with specialised beef breeds. Due to the same type of nutrition and housing conditions of the fattened slaughter bulls, we can consider the differences in the resulting values of 15 amino acids divided into the groups of essential, semi-essential and non-essential amino acids as the effect of the commercial type.

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Received: 01–10–11
Accepted after corrections: 02–01–21

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Content of inorganic and organic pollutants in the fish from the Slezská Harta reservoir

Obsah anorganických a organických cizorodých látek v rybách v nádrži Slezská Harta

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ABSTRACT: In October 1996, in a reservoir with water level at 470.81 m above sea level (flooded area of the reservoir was 345 hectares), 33 fishes of 10 species (*Esox lucius, Perca fluviatilis, Oncorhynchus mykiss, Salmo trutta* m. *fario, Leuciscus cephalus, Rutilus rutilus, Abramis brama, Carassius carassius, Tinca tinca, Chondrostoma nasus*) were examined to record the starting levels of muscle contamination with metals (Hg, Cd, Pb, Cu, Zn, As, Cr, Al); polychlorinated biphenyls (PCBs) and their indicator congeners 28, 52, 101, 118, 138, 153, 180 and planar congeners 77, 126, 169; pesticides on the basis of 1,1,1-trichloro-2,2-bis(p-chlorphenyl)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). The multi-purpose reservoir of non-salmonid type had a drainage area of 464 km^2 and its ichthyobiomass represented at least 20 species with prevalence of cyprinids (*Rutilus rutilus, Abramis brama*). It was built on the Moravice river (basin of the Oder, Czech Republic) as a primary source of raw drinking water to feed the Kružberk water supply reservoir. Mercury was among the most hazardous elements of the spectrum of metals; mercury contaminated the muscle of 26 fishes (78%) above the admissible tolerance level (AT) of 0.1 mg/kg for non-predatory fish and 0.5 mg/kg for predatory fish. The highest mercury level was recorded in three- to six-year old *Perca fluviatilis* (0.637–1.170 mg/kg); in *Leuciscus cephalus*, the content of mercury increased with the weight of the fish (r = 0.945). In all the fish examined the sum of the indicator congeners of PCB in muscle remained under the maximum admissible tolerance (MAT) of 2 mg/kg of the edible component of the fish flesh. The data recorded for *Leuciscus cephalus* showed a very close dependence of the sum of the PCB indicator congeners (r = 0.971) and congeners PCB 138 (r = 0.933), 153 (r = 0.902) and 180 (r = 0.935) on the weight of the fish. PCB congener 153 contributed most significantly to the bioaccumulation of PCB indicator congeners in the muscle of *Esox lucius, Leuciscus cephalus, Rutilus rutilus* and *Abramis brama*. Contamination with the planar congeners of PCB, expressed by the sum of PCB congeners 77, 126 and 169, was highest in *Esox lucius*, PCB 126 being represented most significantly (56.94%). The bioaccumulation of DDT and its metabolites did not exceed the maximum tolerance of residues (MTR) of 0.5 mg/kg in the edible component. Contamination of the fat component of muscle with DDT and its metabolites was highest in *Leuciscus cephalus* (1.010 to 4.029 mg/kg) and *Esox lucius* (0.963–2.591 mg/kg). The sum of DDT and its metabolites in *Esox lucius* increased with the weight of the fish (r = 0.882). The p,p´-DDE metabolite contributed most significantly to the sum of DDT and its metabolites in muscle and fat. The HCB and HCH isomer levels in muscle were below the measurable value of 0.005 mg per kg. The HCB level in the fat component of the muscle was highest in *Leuciscus cephalus* (0.052–0.126 mg per kg) and lowest in tench. As to HCH isomers in the muscular fat, the proportion of beta isomer was highest: its relative concentration was 40.48% in *Esox lucius*, 43.06% in *Leuciscus cephalus* and 56.86% in *Abramis brama*. The results of the regression analyses of organic pollutant levels in relation to the weight of the fish as well as the results of the analyses of correlations between the individual PCB congeners and DDT metabolites, expressing their content in the muscular fat, suggest that causal relations should be sought between these phenomena.

Keywords: fish muscle tissue; mercury; cadmium; lead; copper; zinc; arsenic; chromium; aluminium; PCB; DDT; HCB; HCH

Supported by the Ministry of Agriculture of the Czech Republic and Ministry of Education, Youth and Sports of the Czech Republic (Project No. CEZ: J06/98: 126100003).

ABSTRACT: U 33 ks ryb náležejících k 10 druhům (*Esox lucius, Perca fluviatilis, Oncorhynchus mykiss, Salmo trutta* m. *fario, Leuciscus cephalus, Rutilus rutilus, Abramis brama, Carassius carassius, Tinca tinca, Chondrostoma nasus*) byla v říjnu roku 1996 při kótě hladiny 470,81 m n.m. a zatopené ploše 345 ha provedena revize výchozího stavu kontaminace svaloviny kovy (Hg, Cd, Pb, Cu, Zn, As, Cr, Al), polychlorovanými bifenyly (PCB) a jejími indikátorovými kongenery 28, 52, 101, 118, 138, 153, 180 a planárními kongenery 77, 126, 169, pesticidy na bázi 1,1,1-trichloro-2,2-bis(p-chlorphenyl)ethane (DDT) a jeho metabolity o,p´-DDE, p,p´- DDE, o,p´-DDD, p,p´-DDD, o,p´-DDT, p,p´- DDT, hexachlorcyklohexanem (HCH) a jeho alfa, beta, gama a delta isomery a hexachlorbenzenem (HCB). Víceúčelová nádrž nelosovitého typu na řece Moravici (povodí Odry) s plochou povodí 464 km^2 a s druhovou diversitou ichtyobiomasy představující minimálně 20 druhů s dominancí zástupců Cyprinidae (*Rutilus rutilus, Abramis brama*) vznikla jako předřazený zdroj surové pitné vody pro vodárenskou nádrž Kružberk. K nejrizikovějším prvkům ze spektra kovů patřila rtuť, která kontaminovala svalovinu 26 ryb (78 %) nad přípustné množství (PM) 0,1 mg/kg pro nedravé druhy ryb a 0,5 mg/kg pro dravé ryby. Nejvyšší hladina rtuti byla zjištěna u tříletých až šestiletých *Perca fluviatilis* (0,637–1,170 mg na kg) a u *Leuciscus cephalus* se obsah rtuti zvyšoval s hmotností ryb (r = 0,945). Suma indikátorových kongenerů PCB ve svalovině nepřekročila ani u jedné ryby maximální přípustné množství (MPM) 2 mg/kg jedlého podílu. U *Leuciscus cephalus* byla zjištěna vysoce těsná závislost sumy indikátorových kongenerů PCB (r = 0,971) a kongenerů PCB 138 (r = 0,933), PCB 153 (r = 0,902) a PCB 180 (r = 0,935) na hmotnosti ryb. Na bioakumulaci tukové složky svalové tkáně indikátorovými kongenery PCB se u *Esox lucius, Leuciscus cephalus, Rutilus rutilus* a *Abramis brama* podílel nejvyšší měrou kongener PCB 153. Kontaminace planárními kongenery PCB vyjádřena sumou kongenerů PCB 77, 126 a 169 byla nejvyšší u *Esox lucius* a podílel se na ni především kongener PCB 126 (56,94 %). Bioakumulace DDT a jeho metabolitů nepřekročila maximální limit reziduí (MLR) v jedlém podílu 0,5 mg/kg. Kontaminace tukové složky svaloviny DDT a jeho metabolity byla nejvyšší u *Leuciscus cephalus* (1,010–4,029 mg/kg) a *Esox lucius* (0,963–2,591 mg/kg). Suma DDT a jeho metabolitů se u *Esox lucius* zvyšovala s hmotností ryb (r = 0,882). Na sumě DDT a jeho metabolitů ve svalovině i v tuku svaloviny se nejvyšší měrou podílel metabolit p,p´-DDE. Obsah HCB a HCH izomerů ve svalovině byl pod měřitelnou hodnotu 0,005 mg na kg. Hladina HCB v tukové komponentě svaloviny byla nejvyšší u *Leuciscus cephalus* (0,052–0,126 mg/kg) a nejnižší u línů. Z izomerů HCH v tuku svaloviny kulminoval beta izomer, jehož koncentrace v relativním vyjádření představovala u *Esox lucius* 40,48 %, u *Leuciscus cephalus* 43,06 % a u *Abramis brama* 56,86 %. Výsledky regresních analýz obsahu organických polutantů ve vztahu k hmotnosti ryb, jakož i výsledky korelačních analýz mezi jednotlivými kongenery PCB a metabolity DDT vyjadřujících jejich obsah v tuku svaloviny motivují k hledání příčinných souvislostí mezi těmito jevy.

Klíčová slova: rybí svalovina; rtuť; kadmium; olovo; měď; zinek; arzén; chrom; hliník; PCB; DDT; HCB; HCH

The Slezská Harta reservoir is the eighth multipurpose dam reservoir in the relatively small territory of the Oder valley, involving considerable fish management challenges. The reservoir serves as an upstream source of raw drinking water for the Kružberk water supply reservoir. Its main purpose is to improve and stabilise water flow downstream from the Kružberk reservoir, reduce flood flow rates and minimise the fluctuation of water levels in that Kružberk. The Slezská Harta reservoir on the Moravice river is a relatively long reservoir of trough type. Its drainage area is 464 km^2, average depth 23 m and average flow rate Q_A 539 m^3. To support fish management in the reservoir, aimed at optimising the species composition and size of the stock, it appeared necessary to examine the initial state of health of the fish living in the reservoir at the beginning of its existence (at least 20 species). The knowledge of the state of health of the ichthyocoenosis also includes the qualitative and quantitative information on pollutants, particularly where the hydroecosystem concerned involves streams receiving cleaned municipal and industrial sewage. Later reviews of the distribution of pollutants will allow us to see how the pollutants are released from fields treated with fertilisers, combined with rainstorm washings from the areas around the reservoir. Knowledge of the contents of pollutants in the fish is also important for reasons of human health and food hygiene because it is planned that the reservoir is also to be used for angling.

The purpose of this study was to determine the initial levels of fish contamination with metals, polychlorinated biphenyls and pesticides on the basis of DDT, HCB and HCH. The study follows up with what was addressed in our previous paper (Řehulka, 2001) and enhances our knowledge of the occurrence of pollutants in the Moravice river upstream of the Kružberk reservoir.

MATERIAL AND METHODS

Fish

The fish were caught in dragnets and gillnets in October 1966 with water level at 470.81 m above sea

level, the water area of the reservoir being 345 hectares. The useful volume of water in the reservoir was reached in July 1997 when the water level was at 495.5 above sea level. After species and sex identification and scale sampling (to determine age), the fish were measured (total length, standard length) and weighed and then kept in a freeze box at –22°C until chemically analysed. Subject to individual analysis were 33 fishes of 10 species, including pike (*Esox lucius*), perch (*Perca fluviatilis*), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta* m. *fario*), chub (*Leuciscus cephalus*), roach (*Rutilus rutilus*), bream (*Abramis brama*), crucian carp (*Carassius carassius*), tench (*Tinca tinca*) and nase (*Chondrostoma nasus*).

Chemical analysis

The muscle of the fish was analysed to determine the content of metals (mercury, lead, cadmium, chromium, copper, zinc, arsenic, nickel, aluminium); polychlorinated biphenyls (PCBs) and their indicator congeners 28, 52, 101, 118, 138, 153, 180 and planar 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, delta isomers; and hexachlorobenzene (HCB).

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 mineralising. Examination for the presence of arsenic was performed by the hydride technique.

The standard reference materials used in the study were supplied by 2 Theta s.r.o. (with registered offices at Český Těšín). In respect of organic pollutants the results of the analyses were verified by the pilot laboratory of the Institute of Chemical Technology, Dept. of Food Chemistry and Analysis, Prague. In respect of metals they were verified by the pilot laboratory of the Ekocentrum Ostrava.

The Slezská Harta reservoir will develop into a large put and take non-trout fishery. Therefore, when evalu-

Table 1. Content of metals in the muscle of fish (mg/kg)

Fish species	*n*	Age (year)	Weight (g)	Hg	Cd	Pb
Esox lucius	6	2–7	470–4 190	0.4160–1.120	<0.010–0.060	<0.100–0.220
Perca fluviatilis	4	3–6	486–1 030	0.6370–1.170	<0.010–0.040	<0.100–0.350
Oncorhynchus mykiss	1	3	1 100	0.924	0.023	<0,100
Salmo trutta m. *fario*	1	4	810	0.697	<0.010	<0.100
Leuciscus cephalus	5	8–10	860–1 410	0.237–0.736	0.010–0.050	<0.100–0.580
Rutilus rutilus	4	7–12	184–630	0.181–0.337	<0.010–0.017	<0.100–0.155
Abramis brama	6	6–10	520–1 150	0.205–0.407	<0.010–0.021	<0.100
Carassius carassius	2	4, 12	372, 630	0.202, 0.423	0.010, 0.027	<0.100
Tinca tinca	3	5–8	423–70	0.163–0.217	<0.010–0.028	<0.100
Chondrostoma nasus	1	7	1 000	0.171	0.023	<0.100
Fish species	*n*	Cu	Zn	As	Cr	Al
Esox lucius	6	0.130–0.420	3.53–4.85	0.145–0.350	<0.100	<0.70–5.31
Perca fluviatilis	4	0.220–0.380	0.35–6.06	0.015–0.250	<0.100	<0.70–1.50
Oncorhynchus mykiss	1	0.360	3.16	0.075	<0.100	2.37
Salmo trutta m. *fario*	1	0.380	3.68	0.130	<0.100	<0.70
Leuciscus cephalus	5	0.190–0.540	0.43–4.99	<0.050–0.140	<0.100–0.120	0.70–1.40
Rutilus rutilus	4	0.200–0.300	6.27–12.35	0.051–0.150	<0.100–0.115	<0.70–2.60
Abramis brama	6	0.160–0.250	3.09–4.21	<0.050–0.074	<0.100–0.100	<0.70–2.70
Carassius carassius	2	0.480, 0.555	8.12, 11.27	<0.050, 0.052	<0.100, 0.110	<0.70, 3.60
Tinca tinca	3	0.245–0.315	3.81–8.15	<0.050–0.014	<0.100–0.135	<0.70–1.95
Chondrostoma nasus	1	0.380	8.21	<0.050	<0.100	4.70

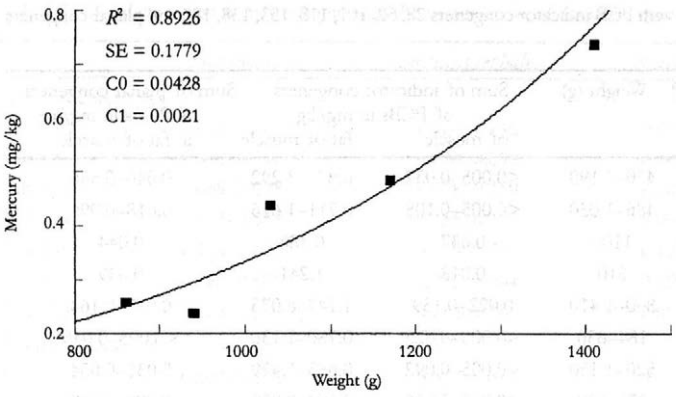

Figure 1. Mercury content in the muscle of chub, depending on weight

ating the level of contamination of the fish muscle with pollutants, we respected the hygienic limits determined in Decree No. 298/1997 of the Ministry of Health where the limits are defined as the admissible tolerance (AT), maximum admissible tolerance (MAT) and maximum tolerance of residues (MTR).

RESULTS

Metals

The contents of metals with indication of the number, age and weight of the fish are shown in Table 1.

Mercury

Of the 33 fishes analysed, 17 were predators and 16 non-predators. The number of fish contaminated above the AT (0.1 mg/kg for non-predatory fish and 0.5 mg/kg for predators) was 26, including 10 predators. Mercury contamination above the AT was recorded in pike as young as two years (0.964 mg/kg). The highest mercury level was found in perch: in 3-year-old perch the mercury level was 0.682 mg/kg and in 5-year-olds 1.170 mg/kg. As to chub old eight to ten years, only one fish was contaminated above the AT (0.736 mg/kg) and the mercury level increased with weight (r = 0.945) (Figure 1). In the remaining fishes the concentration of mercury amounted to 0.407 mg/kg (bream) and 0.423 mg/kg (crucian carp).

Cadmium

The cadmium AT of 0.1 mg/kg was not exceeded in any fish of the whole set of fish analysed. The highest cadmium concentrations were recorded in pike (0.060 mg/kg) and chub (0.050 mg/kg). In the remaining fishes the cadmium level was at 0.028 mg/kg.

Lead

Five fishes were analysed and only one (an 8-year-old chub) had a lead level above the AT (0.5 mg/kg).

Copper

The levels of copper were far below the AT (10 mg per kg), ranging between 0.160 and 0.555 mg/kg. There were no differences between predators and non-predatory fish.

Zinc

The levels of zinc (AT 50 mg/kg) ranged from 0.35 to 12.355 mg/kg, the highest values being recorded in roach and crucian carp.

Arsenic

The highest concentrations of this metal were recorded in pike (0.290 mg/kg), the average being about one-third of the MAT (1 mg/kg).

Chromium

In the majority of cases, chromium levels did not exceed the detection limit (0.100 mg/kg). The highest value of 0.135 mg/kg was recorded in an 8-year-old tench.

Aluminium

Aluminium content was highest in a 4-year-old pike (5.310 mg/kg) and nase (4.700 mg/kg). In the majority of cases, especially in roach, aluminium levels were below 0.7 mg/kg.

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

Fish species	*n*	Age (year)	Weight (g)	Sum of indicator congeners of PCBs in mg/kg of muscle	Sum of indicator congeners of PCBs in mg/kg fat of muscle	Sum of planar congeners of PCBs in mg/kg in fat of muscle
Esox lucius	6	2–7	470–4 190	<0.005–0.032	0.871–3.292	0.046–0.556
Perca fluviatilis	4	3–6	486–1 030	<0.005–0.108	0.711–1.616	0.018–0.096
Oncorhynchus mykiss	1	3	1100	0.037	0.788	0.044
Salmo trutta m. *fario*	1	4	810	0.018	1.241	0.039
Leuciscus cephalus	5	8–10	860–1 410	0.022–0.139	1.147–6.073	0.017–0.164
Rutilus rutilus	4	7–12	184–630	<0.005–0.025	0.780–1.136	<0.005–0.045
Abramis brama	6	6–10	520–1 150	<0.005–0.092	0.683–1.479	0.031–0.054
Carassius carassius	2	4, 12	372, 630	<0.005, 0.006	0.504, 0.839	0.008, 0.019
Tinca tinca	3	5–8	423–870	0.005–0.012	0.364–0.590	0.006–0.032
Chondrostoma nasus	1	7	1000	0.090	3.495	0.107

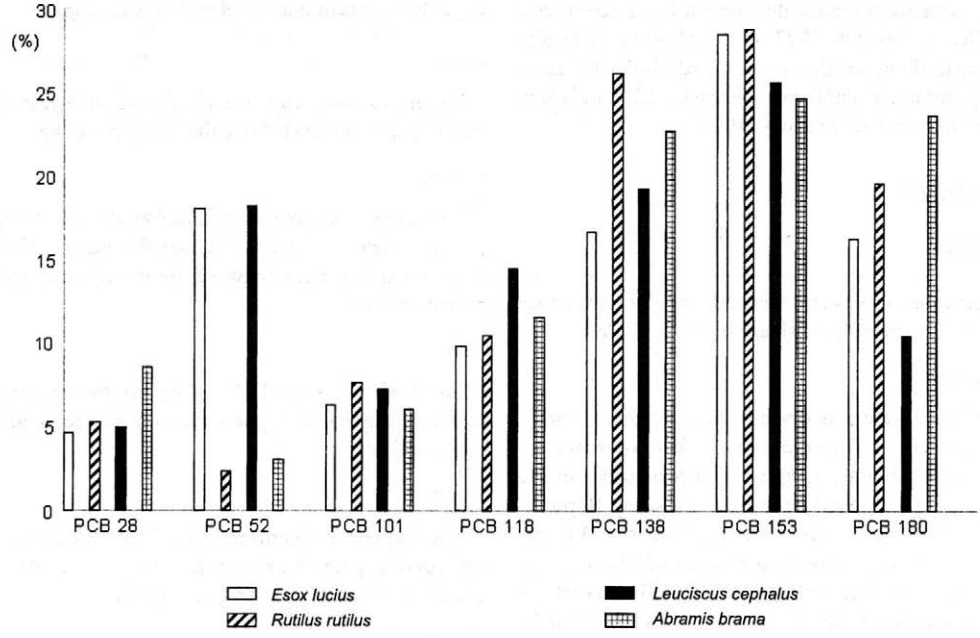

Figure 2. Contamination of the fat component of fish muscle with PCB indicator congeners, relatively expressed

Polychlorinated biphenyls

The totals for the seven PCB indicator congeners did not exceed the MAT of 2 mg/kg of the edible component in any fish, as indicated by the data in Table 2. The highest PCB indicator congener totals were recorded in chub, where the following congeners were represented: PCB 153 (0.008–0.044 mg/kg), PCB 138 (0.008–0.040 mg/kg) and PCB 180 (0.005–0.031 mg per kg). A significant dependence of the sum of the PCB indicator congeners (r = 0.971) and congeners PCB 138 (r = 0.933), PCB 153 (r = 0.902) and PCB

Table 3. PCB indicator and planar congeners in the muscular fat of four fish species (mg/kg)

	Esox lucius	*Leuciscus cephalus*	*Rutilus rutilus*	*Abramis brama*
Indicator congeners				
PCB 28	0.014–0.269	0.066–0.246	0.017–0.109	0.057–0.130
PCB 52	0.042–0.712	0.040–0.098	0.042–0.262	0.019–0.047
PCB 101	0.069–0.028	0.078–0.449	0.047–0.090	0.043–0.105
PCB 118	0.013–0.364	0.136–0.412	0.089–0.201	0.069–0.185
PCB 138	0.139–0.683	0.253–1.741	0.127–0.238	0.142–0.388
PCB 153	0.223–0.896	0.262–1.904	0.180–0.285	0.166–0.382
PCB 180	0.126–0.809	0.202–1.366	0.059–0.153	0.150–0.339
Planar congeners				
PCB 77	0.007–0.195	0.013–0.034	<0.005–0.016	0.007–0.016
PCB 126	0.014–0.346	<0.005–0.130	<0.005–0.033	0.010–0.042
PCB 169	<0.005–0.030	<0.005	<0.005–0.020	<0.005–0.022

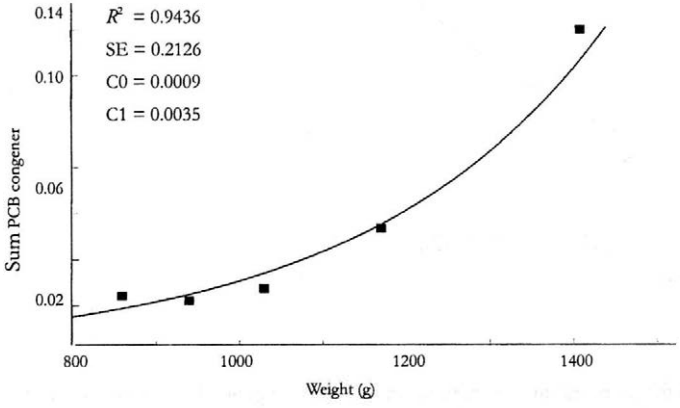

Figure 3. Sum of the indicator congeners of PCBs in muscle, depending on weight

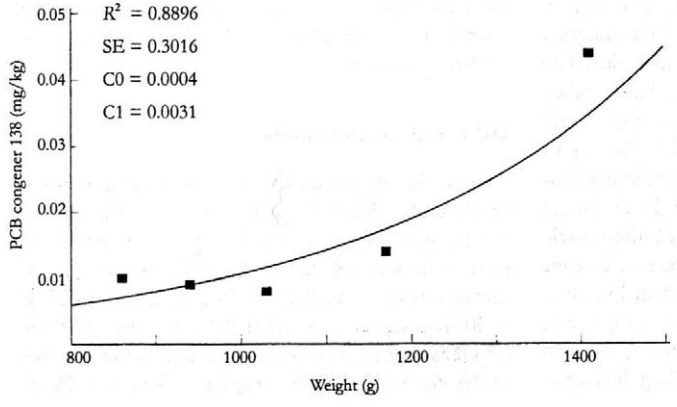

Figure 4. PCB congener 138 in the muscle of chub, depending on weight

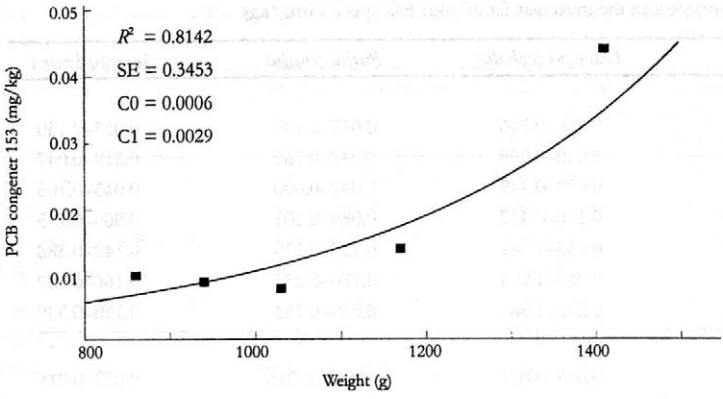

Figure 5. PCB congener 153 in the muscle of chub, depending on weight

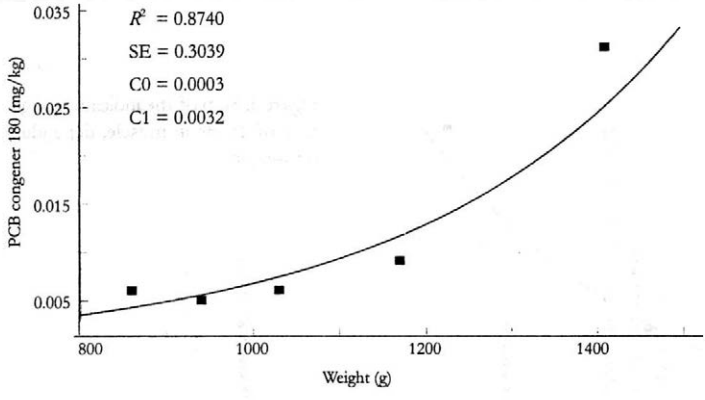

Figure 6. PCB congener 180 in the muscle of chub, depending on weight

180 ($r = 0.935$) on the weight of the fish was found in our study: it is shown by exponential functions in Figures 3 to 6.

In the fat component of the muscular tissue the levels of PCBs were entirely different. The highest bioaccumulation of PCB, represented by the indicator congener totals, was recorded in chub and pike. The levels of PCB indicator congeners in predators (pike), omnivores (chub), zooplanktonophages (roach) and benthophages (bream) are documented in Table 3. In all the mentioned species, the PCB 153 congener contributed most significantly to the sum of the PCBs: the contamination of the fat component of the muscle tissue by the individual PCB congeners in relative expression is clearly shown by the data in Figure 2. Contamination by the PCB planar congeners, expressed by the sum of the congeners 77, 126 and 169, was highest in pike (Table 2), including the largest contribution of PCB congener 126 (56.94%) and the smallest contribution of PCB congener 169 (9.03%) (Table 3). A highly significant correlation between certain PCB congeners in respect of their contents in muscular fat was found in pike, chub and bream. The correlation coefficients of these dependences are shown in Table 4.

DDT and its metabolites

As to the bioaccumulation of DDT in the muscle of the analysed fish, the highest bioaccumulation level was recorded in the case of the p,p´-DDE metabolite; only in bream did the total DDT also include the metabolites p,p´-DDD (0.010 mg/kg), o,p´-DDD (0.007 mg/kg) and o,p´-DDT (0.006 mg/kg). The sum of DDT and its metabolites reached its highest level in the chub (0.017–0.086 mg/kg), followed by bream

Table 4. Relationship between the PCB indicator congeners contained the muscular fat of some fishes, as described by correlation coefficients

	PCB 28		PCB 52		PCB 101		PCB 118		PCB 138		PCB 153		PCB 180	
	Corr	Prob	Corr	Prob	Corr	Prob	Corr	Prob	Corr	Prob	Corr	Prob	Corr	Prob
Esox Lucius														
PCB 28	****	****												
PCB 52	-0.5695	0.1191	****	****										
PCB 101	**0.8535**	0.0153	-0.2516	0.3153	****	****								
PCB 118	0.6528	0.0800	0.2233	0.3353	**0.8300**	0.0204	****	****						
PCB 138	**0.8680**	0.0125	-0.2239	0.3349	**0.9916**	0.0001	**0.8592**	0.0142	****	****				
PCB 153	0.2984	0.2828	0.5580	0.1250	0.6503	0.0810	**0.8643**	0.0132	0.6728	0.0715	****	****		
PCB 180	**0.9276**	0.0038	-0.2768	0.2977	**0.9329**	0.0033	**0.8228**	0.0222	**0.9605**	0.0012	0.6114	0.0986	****	****
Sum PCB	0.4741	0.1710	0.4241	0.2010	**0.7491**	0.0433	**0.9489**	0.0019	**0.7803**	0.0335	**0.9762**	0.0004	**0.7404**	0.0462
Leuciscus cephalus														
PCB 28	****	****												
PCB 52	0.1672	0.3940	****	****										
PCB 101	**0.9773**	0.0021	0.0613	0.4610	****	****								
PCB 118	0.6405	0.1222	**0.8322**	0.0402	0.5706	0.1575	****	****						
PCB 138	**0.9490**	0.0069	-0.0545	0.4653	**0.9799**	0.0017	0.4364	0.2313	****	****				
PCB 153	**0.9443**	0.0078	-0.0149	0.4905	**0.9736**	0.0026	0.4549	0.2207	**0.9976**	0.0001	****	****		
PCB 180	**0.9436**	0.0080	-0.0658	0.4582	**0.9677**	0.0035	0.4113	0.2457	**0.9979**	0.0001	**0.9970**	0.0001	****	****
Sum PCB	**0.9660**	0.0037	0.0315	0.4800	**0.9864**	0.0009	0.5090	0.1906	**0.9963**	0.0001	**0.9970**	0.0001	**0.9933**	0.0003
Abramis brama														
PCB 28	****	****												
PCB 52	0.5113	0.1500	****	****										
PCB 101	0.3126	0.2732	**0.8964**	0.0078	****	****								
PCB 118	0.3790	0.2294	**0.8389**	0.0184	**0.9648**	0.0009	****	****						
PCB 138	0.4883	0.1629	**0.9095**	0.0060	**0.9660**	0.0009	**0.9847**	0.0002	****	****				
PCB 153	0.6154	0.0967	**0.9170**	0.0050	**0.9042**	0.0067	**0.9441**	0.0023	**0.9814**	0.0003	****	****		
PCB 180	**0.7357**	0.0478	0.6392	0.0859	0.4552	0.1822	0.5937	0.1070	0.6566	0.0783	**0.7804**	0.0335	****	****
Sum PCB	0.6453	0.0832	**0.9020**	0.0070	**0.8765**	0.0110	**0.9300**	0.0036	**0.9683**	0.0007	**0.9975**	0.0000	**0.8188**	0.0231

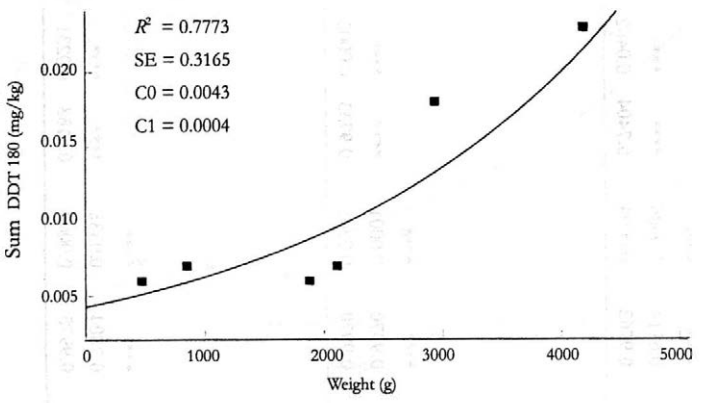

Figure 7. Sum of the DDT and its metabolites in the muscle fat of pike, depending on weight

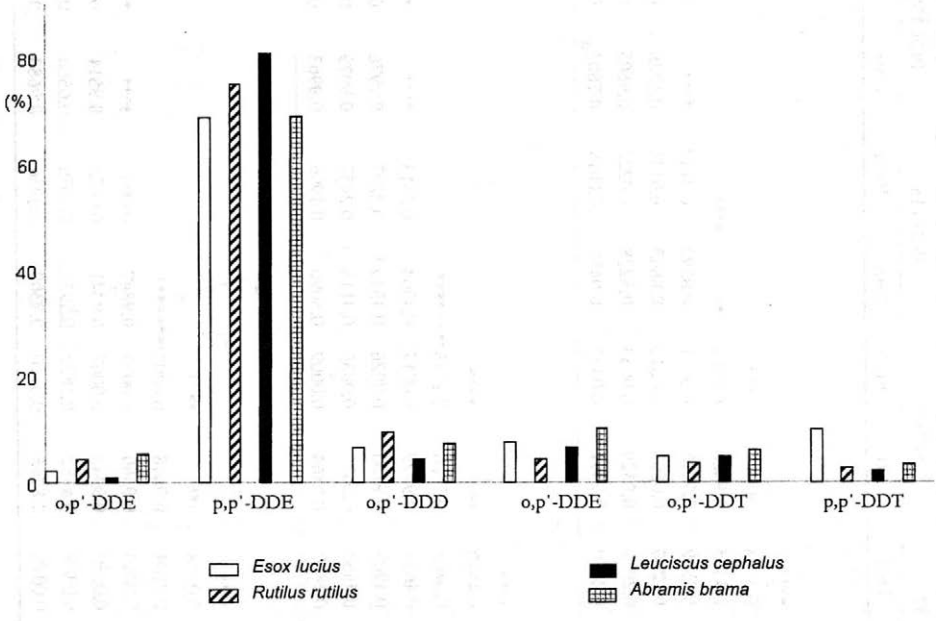

Figure 8. Contamination of the fat component of fish muscle with DDT metabolites, relatively expressed

(0.014–0.084 mg per kg), rainbow trout and nase (0.044 mg/kg), brown trout (0.038 mg/kg), roach (0.012–0.036 mg/kg), perch (0.005–0.035 mg/kg) and pike (0.006–0.023 mg/kg). The lowest levels were recorded in crucian carp and tench (0.006–0.015 mg/kg). The MTR for the sum of DDT in the edible component is 0.5 mg/kg.

The bioaccumulation of DDT and its metabolites in the fat component of muscle is shown in Table 5. The data in the table show that the chub and pike were the most DDT-contaminated species (1.010–4.029 mg/kg and 0.963–2.591 mg/kg, respectively). The sum of DDT and its metabolites in pike increased with the weight of the fish (r = 0,882) (Figure 7). What deserves mentioning is the comparatively high sum of DDT and its metabolites in nase. The highly stable metabolite p,p´-DDE contributed most significantly to the sum of DDT and its metabolites. The contributions of the

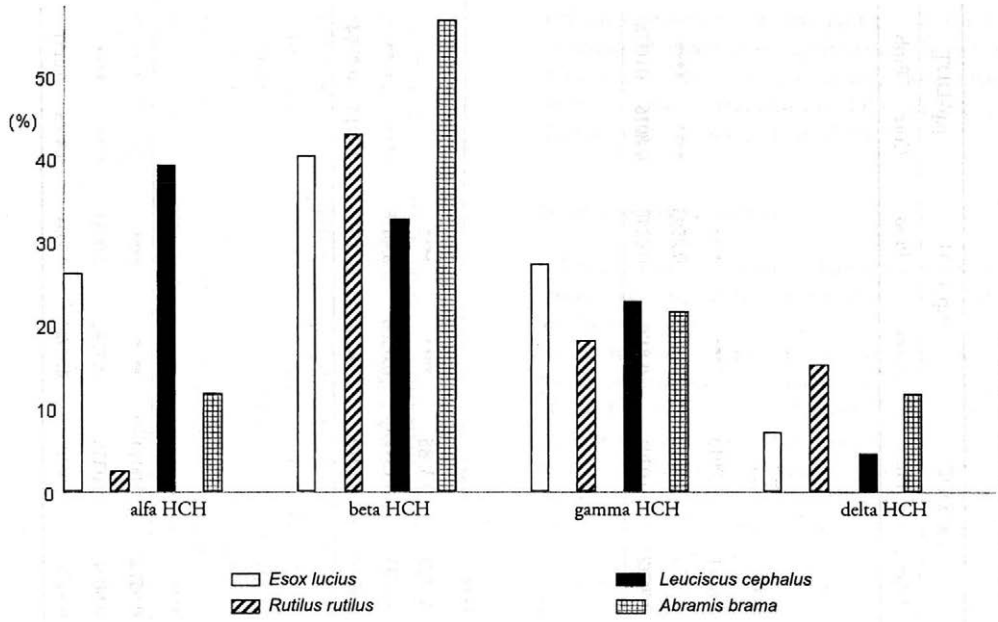

Figure 9. Contamination of the fat component of fish muscle with HCH isomers, relatively expressed

Table 5. Contents of the DDT organochlorinated pesticide and its metabolites in the muscular fat of fish (mg/kg)

Fish species	*n*	Age (year)	Weight (g)	o,p'-DDE	p,p'-DDE	o,p'-DDD
Esox lucius	6	2–7	470–4 190	0.042–0.120	0.638–1.904	0.042–0.244
Perca fluviatilis	4	3–6	486–1 030	0.031–0.080	0.589–1.136	0.044–0.201
Oncorhynchus mykiss	1	3	1 100	0.036	0.693	0.077
Salmo trutta m. *fario*	1	4	810	–	1.187	0.075
Leuciscus cephalus	5	8–10	860–1 410	0.038–0.197	0.645–3.046	0.110–0.414
Rutilus rutilus	4	7–12	184– 630	0.059	0.563–1.984	0.020–0.091
Abramis brama	6	6–10	520–1 150	0.034–0.067	0.375–0.975	0.014–0.109
Carassius carassius	2	4, 12	372, 630	0.028	0.381, 0.619	0.026, 0.038
Tinca tinca	3	5–8	423–870	0.025	0.292–0.295	0.011–0.044
Chondrostoma nasus	1	7	1 000	0.087	1.504	0.248
Fish species	*n*	Age (year)	p,p'-DDD	o,p'-DDT	p,p'-DDT	Sum DDT
Esox lucius	6	2–7	0.073–0.179	0.054–0.198	0.067–0.321	0.963–2.591
Perca fluviatilis	4	3–6	0.089–0.192	0.044–0.103	0.095–0.303	1.130–2.015
Oncorhynchus mykiss	1	3	0.143	0.052	0.197	1.198
Salmo trutta m. *fario*	1	4	0.149	0.084	0.312	1.807
Leuciscus cephalus	5	8–10	0.061–0.147	0.035–0.177	0.026–0.147	1.010–4.029
Rutilus rutilus	4	7–12	0.055–0.144	0.024–0.140	0.013–0.063	0.675–2.422
Abramis brama	6	6–10	0.046–0.151	0.032–0.090	0.009–0.049	0.476–1.425
Carassius carassius	2	4, 12	0.057, 0.075	0.024, 0.044	0.068, 0.082	0.584, 0.858
Tinca tinca	3	5–8	0.035–0.065	0.010–0.035	0.064–0.085	0.393–0.534
Chondrostoma nasus	1	7	0.154	0.118	0.088	2.199

Table 6. Relationship between the DDT metabolites contained the muscular fat of some fishes, as described by correlation coefficients

	o,p'-DDE		p,p'-DDE		o,p'-DDD		p,p'-DDD		o,p'-DDT		p,p'-DDT	
	Corr	Prob	Corr	Prob	Corr	Prob	Corr	Prob	Corr	Prob	Corr	Prob
Esox lucius												
p,p'-DDE			****	****								
o,p'-DDD			0.2600	0.3094	****	****						
p,p'-DDD			0.6187	0.0952	0.7147	0.0553	****	****				
o,p'-DDT			0.6895	0.0648	0.7239	0.0519	0.6271	0.0913	****	****		
p,p'-DDT			**0.9106**	0.0058	–0.0569	0.4574	0.4734	0.1715	0.3333	0.2593	****	****
Sum DDT			**0.9655**	0.0009	0.4997	0.1564	**0.7622**	0.0391	**0.8121**	0.0248	**0.8016**	0.0276
Leuciscus cephalus												
o,p'-DDE	****	****										
p,p'-DDE	**0.8384**	0.0380	****	****								
o,p'-DDD	**0.8983**	0.0192	0.6714	0.1073	****	****						
p,p'-DDD	**0.8614**	0.0303	0.6183	0.1332	0.6919	0.0978	****	****				
o,p'-DDT	0.2321	0.3536	0.6917	0.0978	–0.0414	0.4736	0.1760	0.3885	****	****		
p,p'-DDT	**0.8753**	0.0259	0.7361	0.0781	**0.9747**	0.0024	0.5703	0.1577	0.0506	0.4678	****	****
Sum DDT	**0.8854**	0.0229	**0.9948**	0.0002	0.7409	0.0760	0.6702	0.1079	0.6209	0.1318	0.7913	0.0554
Abramis brama												
o,p'-DDE	****	****										
p,p'-DDE	**0.8156**	0.0239	****	****								
o,p'-DDD	**0.9655**	0.0009	**0.7709**	0.0364	****	****						
p,p'-DDD	**0.7556**	0.0412	**0.9666**	0.0008	0.7253	0.0514	****	****				
o,p'-DDT	**0.8140**	0.0243	**0.9682**	0.0008	**0.8303**	0.0204	**0.9572**	0.0014	****	****		
p,p'-DDT	**0.8180**	0.0233	**0.8352**	0.0192	0.6958	0.0624	**0.8091**	0.0256	**0.7751**	0.0351	****	****
Sum DDT	**0.8734**	0.0115	**0.9923**	0.0000	**0.8387**	0.0185	**0.9638**	0.0010	**0.9768**	0.0004	**0.8545**	0.0151

Table 7. Content of the HCB and HCH organochlorinated pesticide isomers in the muscular fat of fish (mg/kg

Fish species	n	Age (year)	Weight (g)	HCB	HCH				
					alpha	beta	gamma	delta	Sum alpha + beta
Esox lucius	6	2–7	470–4 190	0.042–0.115	<0.005–0.042	0.007–0.083	0.011–0.042	<0.005–0.009	0.025–0.106
Perca fluviatilis	4	3–6	486–1 030	0.064–0.109	0.008–0.040	0.019–0.053	<0.005–0.032	<0.005–0.048	0.050–0.061
Oncorhynchus mykiss	1	3	1 100	0.043	<0.005	0.030	0.010	0.009	0.030
Salmo trutta m. *fario*	1	4	810	0.070	0.008	0.010	0.009	<0.005	0.018
Leuciscus cephalus	5	8–10	860 – 1 410	0.052–0.126	<0.005–0.069	0.023–0.051	0.005–0.028	<0.005–0.033	0.025–0.092
Rutilus rutilus	4	7–12	184–630	0.035–0.085	<0.005–0.038	0.014–0.032	0.011–0.016	<0.005–0.007	0.032–0.052
Abramis brama	6	6–10	520–1 150	0.037–0.116	<0.005–0.027	0.020–0.052	0.008–0.014	<0.005–0.011	0.020–0.059
Carassius carassius	2	4, 12	372, 630	0.034, 0.073	<0.005, 0.008	0.030, 0.037	0.008, 0.026	<0.005	0.038, 0.042
Tinca tinca	3	5–8	423–870	0.005–0.025	<0.005–0.022	0.016–0.019	0.005–0.012	<0.005–0.008	0.019–0.039
Chondrostoma nasus	1	7	1000	0.119	0.006	0.054	0.011	0.007	0.060

individual metabolites to the DDT total in relative expression are clearly shown by the data in Figure 8. Like in the case of PCBs we found very close relations between certain metabolites of DDT in respect of their contents in muscular fat (Table 6).

HCB and HCH isomers

The levels of these organo-chlorinated pesticides in muscle were below the measurable level of 0.005 mg/kg in all fishes. Contamination with HCB and HCH and their isomers in the fat component of muscle is described by the data in Table 7. The levels of HCB as a persistent and highly stable compound identified in all parts of the ecosystem did not vary much, the highest average contamination being recorded in chub (0.093 ± 0.0336 mg/kg) and the lowest in tench. The largest contribution to HCH bioaccumulation was made by the persistent beta isomer, whose level (relatively expressed) was 40.48% for pike, 43.06% for chub and 56.86% for bream (Figure 9). Only in roach were its absolute and relative levels exceeded by the alpha isomer (32.79 vs. 39.34%). In chub the level of the alpha + beta isomers increased with the weight of the fish (Figure 10).

DISCUSSION

The results of the chemical monitoring to which fish were subjected as bioindicators contributed to the determination of the exposure of the environment in the newly built reservoir to pollutants; in addition, they contributed to the verification of the toxic hazards represented by the pollutants for the state of health of the new fish population, and also to the evaluation of the hygienic and public health aspects relating to the consumption of the fish caught by anglers.

The important inorganic pollutants contaminating the aquatic environment include heavy metals, particularly mercury, cadmium and lead. Like in our previous report (Řehulka, 2001) 78% of the fish were contaminated above the AT and it was confirmed that mercury bioaccumulation increased in predators (perch) and decreased in the omnivores (chub). The mercury levels recorded in the analysed groups of pikes also exceeded the values indicated by Pavelka *et al.* (1986) (0.14 vs.1.12 mg/kg). Mercury as an element of a high cumulative capacity is among the best-mapped fish contaminants in the fish from both the flowing and stagnant hydroecosystems in the territory of the Czech Republic.

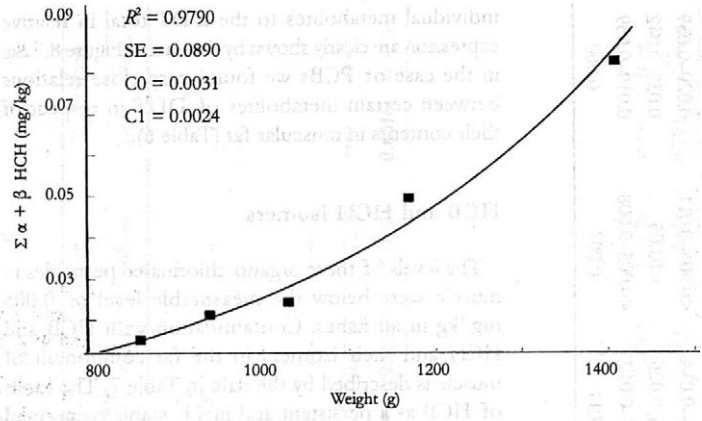

Figure 10. Sum of the alpha + beta HCH isomers in the muscular fat of chub, depending on weight

This is documented by a number of studies, referring to the basins of the Elbe, Vltava and Ohře, including Hejtmánek *et al.* (1975), Svobodová and Hejtmánek (1976), Svobodová *et al.* (1987, 1988, 1997, 1999), Lusková *et al.* (1997), Pružina *et al.* (1995) and also referring to the basin of the Morava river: Čelechovská *et al.* (1997) and Spurný and Mareš (1995). Like in our study conducted in the Kružberk and Šance water-supply reservoirs (Řehulka, 2001) and in keeping with the results published by Hejtmánek *et al.* (1975), Svobodová and Hejtmánek (1985), Svobodová *et al.* (1988, 1999), we found again that the content of mercury in the fish flesh increases with age and weight. This finding corresponds with the results published by a number of other authors, including (Bache *et al.*, 1971; Cross *et al.*, 1973; Luten *et al.*, 1987; Barak and Mason, 1990; Monteiro *et al.*, 1991).

Compared with the Kružberk reservoir, we did not observe any quantivative differences in mercury content. Taking into account the high pressure for the exploitation of these waters for the purposes of angling, it will be useful to examine the mercury levels again five years after the flooding of the reservoir: as suggested by numerous findings (Abernathy and Cumbie, 1977 and others), the total mercury level in fish from newly flooded reservoir increases. Cox *et al.* (1979) even recommend a temporary ban on the consumption of fish caught in newly flooded reservoirs. Morrison and Therien (1995) suggest that the mercury level should be investigated in fishes of different trophic nature: they used whitefish and pike as examples. Other authors, e.g. Verta *et al.* (1985), draw attention to the increased bioaccumulation of mercury in fish from waters with a high content of organic substances, which, as Akagi *et al.* (1979) assert, contribute to the intensification of the biochemical process of methylation of mercury and its penetration to the food chain, together with a higher concentration of humic substances (Surma-Aho *et al.*, 1985). Cadmium content in our fish did not differ much from that in the fish from Kružberk, but was higher than reported by Pružina *et al.* (1995) for the fish from the Vltava river near Prague and by Svobodová *et al.* (1994) for the fish also caught in the Elbe (near Opatovice) (0.001–0.004 resp. 0.003–0.005 mg/kg). Lead contamination of the fish was also higher by an order, compared with the levels recorded by Pružina *et al.* (1995) (0.007 to 0.019 mg/kg).

Samples of pike (which were relatively ample and had an adequate age distribution) allowed us, to a certain extent, to compare the exposure of fish to PCBs in the Slezská Harta reservoir and Kružberk reservoir. The sum of the indicator congeners of PCBs in muscle was lower in the muscle of the Slezská Harta pikes (< 0.005–0.032 vs. 0.005–0.275 mg/kg) and the same is true of the average sum of the PCB indicator congeners in muscular fat (2.001 vs. 6.856 mg/kg). A similar picture was obtained in the PCB planar congeners. The average sum of the planar PCB congeners was 1.5 times higher in the pikes from the Kružberk reservoir (0.217 vs. 0.144 mg/kg). Compared with Hajšlová *et al.* (1997), who studied the Mušov reservoir on the Dyje (Thaya in the basin of the Morava) and used bream, pike-perch and carp as bioidicators, we recorded a twice to five-times lower content of PCB indicator congeners in the muscular fat of bream (0.683–1.479 vs. 1.549–7.976 mg/kg). Like in the study by Hajšlová *et al.* (1997), PCB 153 made the largest

contribution to the sum of PCB indicator congeners in both the muscle and muscular fat of the fish.

Monitoring revealed the presence of the DDT insecticide and its metabolites. As distinct from Hajšlová *et al.* (1997), the exposure of the Slezská Harta reservoir to pesticides can be considered to be low. This can be best documented by comparison with the highly stable metabolite p,p´-DDE (1,1-dichloro-2,2-bis(4-chlorphenyl) ethylene) which showed the highest concentration of the whole spectrum of the metabolites under study. In our study, the average values in the muscular fat of bream were seven times lower (0.639 vs. 4.508 mg per kg) and in pike, unlike pike-perch, our values were almost five times lower (1.283 vs. 6.171 mg/kg).

Contamination of fish with isomers of HCH (as volatile, persistent and lipophilous compounds) was different from that in the Mušov reservoir. The average levels in the muscular fat of the gamma isomer, which used to be sold to farmers under the trade name Lindane, ranged from 0.011 to 0.023 mg/kg in our fish and from 0.048 to 0.125 mg/kg in the fish from the Mušov reservoir. In the samples of bream our values were eleven times lower (0.011 vs. 0.125 mg/kg). On the other hand, the bioaccumulation of the most persistent beta isomer was higher in our fish (0.020–0.038 vs. < 0.005–0.013 mg/kg). In bream the contamination was four times higher (0.029 vs. 0.007 mg/kg). The results of regression analyses of the levels of organic pollutants in relation to weight, as well as the results of the analysis of the correlations between the DDT metabolites and PCB congeners, suggest that the examinations should be performed again at a later date to find any causal relations between these phenomena.

Acknowledgement

The research project was conducted as part of the general assignment CEZ: J06/98: 126100003 with financial support of the Food Production Department of the Ministry of Agriculture of the Czech Republic under co-ordination by S. Hrabětová. The author wishes to thank the team of employees of the State Veterinary Institute at Opava (director J. Blahut), led by L. Látková, for the chemical analyses of the fish. Thanks also go to Mr. Jedlička with the team of the Fish Management Section of the Oder Valley Authority and B. Lojkásek, of the Ostrava University for the catching of the fish. Thanks also go to Prof. B. Minařík, of the Mendel University of Agriculture and Forestry at Brno, Department of Statistics, for the graphical processing of the results.

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Received: 01–10–11
Accepted after corrections: 02–01–21

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CZECH JOURNAL OF ANIMAL SCIENCE

Volume 47, No. 1 January 2002

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