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Viability of fresh and frozen transferred IVP bovine embryos

Přežívání čerstvých a zmrazených IVP embryí skotu po přenosu

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ABSTRACT: This study was aimed at evaluating pregnancy rates characterizing transfers of fresh and cryopreserved bovine embryos recovered from superovulated donors and produced *in vitro*. Various cryoprotectant agents and freezing protocols (10% v/v glycerol GLY, routine freezing method using three-step washing out of thawed embryos, 9% v/v ethylene glycol – EG, for direct transfer technology – DT, and one-step vitrification procedure) were studied. Embryos recovered from superovulated donors – FSH-PGF_{2α} regimen (control group) and embryos produced by *in vitro* procedure – IVP embryos (experimental group) were tested. Oocytes recovered from ovaries of slaughtered females were used for the production of IVP embryos. Fresh and frozen embryos were transferred nonsurgically to recipients synchronized by double PGF_{2α} dose (11 day-interval) on Day 7 of their cycles. Survival rates of transferred experimental embryos (IVP) are satisfactory as related to the rates found in control embryos (fresh and preserved *in vivo* produced embryos by superovulation of donors). The following pregnancy rates characterized transfers of *in vivo* and *in vitro* produced embryos: fresh embryos: 64.8% (245/378), 49.2% (29/59), resp., $P < 0.05$; total frozen embryos: 59.0% (125/212), 37.8% (159/421), resp., $P < 0.01$; embryos preserved by GLY procedure: 66.2% (47/71), 34.7% (129/372), resp.; embryos preserved by EG procedure: 53.1% (60/113), 72.0% (18/25), resp. ($P < 0.01$); embryos vitrified by one-step method: 64.3% (9/14) and 50.0% (12/24), resp. ($P > 0.05$, $P < 0.01$). The mentioned results document applicability of available cryopreservation procedures for preservation of IVP embryos.

Keywords: cattle; embryo; cryopreservation; IVM-IVF-IVC-ET; pregnancy rate

ABSTRAKT: Cílem práce bylo vyhodnotit zabřezávání přijemkyň po přenosu čerstvých a kryokonzervovaných embryí získaných od superovulovaných dárkyň a produkovaných v podmínkách *in vitro* postupem IVM-IVF-IVC-ET. K ochraně embryí před účinky nízkých teplot byla použita různá kryoprotektiva a zmrazovací protokoly – 10 % objemových glycerolu a rutinní metoda zmrazování s použitím třístupňového odmývání kryoprotektiva v médiu se sacharózou po rozmrazení, 9 % objemových etylenglykolu pro přímý přenos embryí bez odmývání kryoprotektiva a jednostepová vitrifikační metoda. Bylo testováno přežívání embryí získaných od dárkyň nechirurgickým výplachem po superovulaci režimem FSH-PGF_{2α} (kontrolní skupina) a embryí produkovaných v podmínkách *in vitro* – IVP (pokusná skupina). Oocyty získané od porážených zvířat byly použity pro produkci IVP embryí. Čerstvá

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a zmrazená embrya byla přenášena nechirurgicky 7. den pohlavního cyklu recipientkám synchronizovaným dvojitou aplikací PGF_{2α} v 11denním intervalu. Přežívání přenesených experimentálních embryí je uspokojivé v porovnání s přežíváním embryí vzniklých po superovulaci. Bylo zjištěno toto zabřezávání příjemkyň po přenosu *in vivo* a *in vitro* produkovaných embryí: čerstvá embrya celkem: 64,8 % (245/375), 49,2 % (29/59), $P < 0,05$; embrya konzervovaná celkem: 59,0 % (125/212), 37,8 % (159/421), $P < 0,01$; embrya konzervovaná v glycerolu: 66,2 % (47/71), 34,7 % (129/372), $P < 0,01$; embrya konzervovaná v EG pro přímý přenos: 53,1 % (60/113), 72,0 % (18/25), $P < 0,01$; embrya konzervovaná vitifikací: 64,3 % (18/28), 50,0 % (12/24) $P > 0,05$, $P < 0,01$). Získané výsledky dokumentují možnost použití testovaných postupů pro kryokonzervaci a uchování *in vitro* produkovaných embryí skotu.

Klíčová slova: skot; embryo; kryokonzervace; IVM-IVF-IVC-ET; zabřezávání

Complications associated with cryopreservation of IVP bovine embryos present serious problems in routine and commercial utilization of these embryos. At present, bovine embryos recovered from superovulated donors can be frozen without problems – Niemann (1991), Říha (1994), Říha *et al.* (1998) and others. Embryos prepared under laboratory conditions are more sensitive to freezing procedures – Leibo and Loskutoff (1995), Vajta *et al.* (1997), Ohboshi *et al.* (1997).

Lower frequency of morphologic changes was found in the case of *in vitro* embryos frozen by a classic freezing method. According to Khurana and Niemann (2000) survival rate can be improved by a more suitable cultivation method, by selection based on development kinetics of embryos, and by modification of cryopreserving procedures. Application of an exactly defined *in vitro* culture medium can improve the functional and morphologic quality of *in vitro* produced (IVP) embryos.

Some bibliographic references presenting new media mention improved survival rate of frozen-thawed IVP embryos – Vajta *et al.* (1996), Hoshi *et al.* (1998). According to Dinnyes *et al.* (1996) the time of the first division of fertilized zygotes and completion of blastocyst stage affect the survival rate of thawed embryos significantly. Modifications of routine slow-rate cryopreservation protocols are specified by Massip *et al.* (1986), Říha (1994), Mahmoudzadeh *et al.* (1995), Palasz *et al.* (1996), and others. Carvalho *et al.* (1996) used 1.5 M glycerol for freezing IVP embryos (blastocysts and morulae). Reexpansion was recorded in 33% of thawed and washed blastocysts. Development of morulae was negligible. Hoshi *et al.* (1998) tested ethylene glycol, glycerol, and propylene glycol in freezing procedures – good conception rates characterized the first two mentioned preparations, pro-

pylene glycol application resulted in significantly lower conception rate. New technologies characterized by extremely high freezing and thawing rate (up to 20 000–25 000°C/min) – Říha *et al.* (1991), Martino *et al.* (1996), Arav and Zeron (1997), Vajta *et al.* (1997, 1999) and completed to the form of one-step procedure – Říha (1994), Vajta *et al.* (1999) – are often used. Lewis *et al.* (1999) mention high conception rate associated with transfer of vitrified embryos – combination of ethylene glycol (EG) and dimethylsulphoxide (DMSO) in TCM 199 medium completed with 20% foetal calf serum as well as modified equilibration period were used in this study. Some other references also mention the usefulness of vitrification procedures in cryopreservation of IVP embryos – e.g. Vajta *et al.* (1996, 1999), Dinnyes *et al.* (1996). Agca *et al.* (1998) found the highest rate in the case of transferring fresh IVP embryos: transfer of vitrified and routinely frozen IVP embryos resulted in somewhat lower, and much lower conception rates resp. Yang *et al.* (1999) recommend the following freezing regimen for IVP blastocysts: 1.8 M EG, 0.3°C/min. Sucrose and trehalose addition improved neither blastocyst reexpansion nor development until hatching. Martinez *et al.* (1998) mention rates ranging from 35.2% to 43.7% characterizing transfers of embryos vitrified by different methods.

According to most references, developmental stage and morphologic quality of frozen embryos are the principal factors affecting the survival rate of thawed IVP bovine embryos.

Evaluation of survival rates characterizing transfer of fresh and cryopreserved bovine embryos by different methods recovered from superovulated donors (*in vivo* produced) – control group and produced *in vitro* (IVP) – experimental group were the principal objectives of this study.

MATERIAL AND METHODS

Oocyte donors, *in vitro* embryo production

Cows and heifers were used as oocyte donors. Methodical procedures of embryo production are specified by Pavlok *et al.* (1988), Říha *et al.* (1996). Ovarian oocytes were recovered from slaughtered females by puncturing follicles (2–6 mm diameter) with needle No. 18 or following dissection of the cortical section of ovaries. Maturation period (19 to 22 h) was followed by incubation with capacitated sperm cells of the chosen bull. Fertilization and cultivation were made on the monolayer of cumular cells until the 7th day post fertilization (until the stage of compact morula at minimum).

Superovulation of embryo donors

Embryos were recovered from sires' dams (dairy and beef females) superovulated by pFSH regimen (Follicotropin, Spofa Prague, doses: 500 I.U. *pro toto* in cows, 400 I.U. *pro toto* in heifers) – prostaglandin $F_{2\alpha}$ (Oestrophan, Léciva Prague, dose: 0.5 mg).

Embryo cryopreservation and embryo thawing

a) Embryos (produced *in vivo* and *in vitro*) characterized by good morphologic quality (grade 1 and grade 2) and corresponding developmental stage (compact morula up to blastocyst stages) were prepared for cryopreservation by 10–15 min equilibration in a complete medium with 10% v/v glycerol. Straws filled with embryos were immediately placed into the freezer chamber (up from -6°C to -7°C). Seeding with an undercooled forceps followed after 5 min. Embryos were frozen ($0.3^{\circ}\text{C}/\text{min}$) to -33°C after successive 5 min interval. Straws were then placed into liquid nitrogen. Straws with embryos were removed from liquid nitrogen, dipped into water bath (35°C) after 7-s interval in the air. This step was finished by the thawing of ice crystals. Glycerol was removed by three-step washing procedure in a complete medium with lowering glycerol concentration (presence of 0.3 M sucrose) for 5 min in every step. Embryos were washed out (3 times) in a fresh conditioned medium (Machatková *et al.*, 1988) with epithelial cells (M-199 or H-MEMD,

SEVAC, Prague, CR) and prepared for transfer to synchronized recipients – standard method.

b) Embryos showing good morphologic quality and corresponding developmental stage were prepared for cryopreservation by equilibration (10–15 min) in a complete medium with 9% v/v ethylene glycol. Regimens of seeding and freezing were the same as in a). Thawing (identical regimen) was followed by direct embryo transfer (within 3-min interval) to synchronized recipients – direct transfer.

c) Embryos showing good morphologic quality and corresponding developmental stage (Manual IETS, 1990) were preserved by one-step vitrification procedure for direct transfer described by Říha (1994) – one step method. Intact embryos were equilibrated in culture medium with 10% (v/v) glycerol for 10 min. Then they were transferred into a drop of vitrification medium (30% (v/v) glycerol, 20% (v/v) FCS, 50% (v/v) 2 M sucrose in tridistilled water) for 1–2 min and filled into 0.25 ml straws (IMV, France) in the column of vitrification medium between two parts (columns) of wash medium (0.8 M sucrose in culture medium). Straws were closed with warm forceps and dipped into LN_2 bath (plug upwards). Thawing was made by rubbing straws in hands; vitrification medium and wash medium crystals having been thawed, the straw content was mixed by shaking. Washing was done by turning the straw up several times. Embryos were immediately transferred nonsurgically into recipients on day 7 after synchronized estrus – vitrification method.

Cultivation of cryopreserved IVP embryos under *in vitro* conditions

Developmental competency of cryopreserved IVP embryos in a complete conditioned medium with 20% FCS (72 h-interval) in heat-sealed transparent straws was tested. Embryos released from the zona pellucida (i.e. hatched embryos) were classified as the developing ones.

Embryo transfer and pregnancy diagnosis

Morphological control of fresh and thawed embryos was followed by non-surgical transfer (Wörlein apparatus) into the ipsilateral uterine horn (to the ovary with functional CL) of heif-

ers synchronized with double dose of PGF_{2α} (Oestrophan, Léciva Prague) administered within 11-day interval. Embryos were transferred on Day 7 of the cycle. Fresh embryos produced by superovulation regimen were transferred directly in donor herds within 4 h post embryo recovery. Fresh embryos produced *in vitro* were transported (in straws with conditioned complete medium) from the laboratory to the donor herd within 3–6 h interval. Pregnancy diagnosis was made during the 3rd month post ET, embryo survival was confirmed by obstetrical examination.

Data processing

Chi-square test (Uni-stat version 6.0) was used for data analysis.

RESULTS AND DISCUSSION

Survival rate of fresh and frozen embryos

Pregnancy rate found in recipients of fresh embryos recovered from superovulated donors – 64.8% (245/378) – can be classified as satisfactory with regard to high genetic values of donors. This rate is significantly higher than the rate found in recipients of *in vitro* produced embryos – 49.2% (29/59), $P < 0.05$.

As for pregnancy rate recorded in recipients of frozen-thawed embryos, the following values characterized the particular groups: 59.0% (125/212) and 37.8% (159/421), resp., $P < 0.01$ (Table 1). The mentioned differences are statistically significant. Regarding bibliographic references – Khurana and Niemann (2000), Vajta (2000) – and the category of donors (sires' dams) the obtained re-

sults are relatively good. Non-significantly lower pregnancy rate characterizing recipients of frozen *in vivo* embryos as compared with the pregnancy rate found in recipients of *in vivo* produced fresh embryos corresponds with bibliographic references and with European and world statistical data – the difference between the mentioned rates amounts to 5.8% (Table 1) – Niemann (1991), Říha *et al.* (1998) and others.

The mentioned results document satisfactory mastering of technological procedures used for freezing embryos produced by superovulation. Transfer of fresh IVP embryos was associated with significantly lower pregnancy rate as compared with the transfer of fresh *in vivo* embryos ($P < 0.05$, 64.8% vs. 49.2%). Our previous results as well as bibliographic data confirm the mentioned tendency. Khurana and Niemann (2000) found significant effects of biochemical levels – differences in oxidoactive processes above all – monitored during *in vitro* cultivation. As for our study, effects of relatively prolonged transport of embryos from the laboratory to the herd could be important. The mentioned phenomenon could also contribute to the lower pregnancy rate characterizing recipients of IVP frozen embryos (37.8%) as related to the rate found in recipients of thawed embryos produced by superovulated donors (59.0%) – $P < 0.01$ (Table 1). Differences between survival rates of IVP fresh (49.2%) and IVP frozen (37.8%) embryos were insignificant ($P > 0.05$).

Development of preserved IVP embryos after *in vitro* cultivation

Developmental competency of thawed IVP embryos post cultivation amounted to 44.4–50.0% (as related to specific cryopreservation procedures).

Table 1. Development of bovine embryos produced under *in vivo* and *in vitro* conditions

	Fresh embryos			Frozen embryos		
	transferred <i>n</i>	pregnant recipients <i>n</i>	(%)	transferred <i>n</i>	pregnant recipients <i>n</i>	(%)
<i>In vivo</i>	378	245	(64.8) ^a	212	125	(59.0) ^a
<i>In vitro</i>	59	29	(49.2) ^b	421	159	(37.8) ^{ab}

^{a-b} values in the same column with different superscripts differ significantly ($P < 0.05$)

^{a-c} values in the same column with different superscripts differ significantly ($P < 0.01$)

^{b-c} insignificant difference ($P > 0.05$)

Table 2. Development of preserved IVP embryos post *in vitro* cultivation

Freezing procedure, cryoprotectants Embryo type	Cultivated embryos <i>n</i>	Development after 48h	
		<i>n</i>	(%)
Experimental – IVP			
a) 10% v/v glycerol	28	14	(50.0) ^c
b) 9% v/v ethylene glycol – DT	24	11	(45.8) ^c
c) vitrification	27	12	(44.4) ^c
Control	10	7	(70.0) ^a

Fresh embryos recovered from superovulated donors

^{a,c} values in the same column with different superscripts differ significantly ($P < 0.01$)

Table 3. Effect of cryopreservation on development of transferred embryos

Method	<i>In vivo</i> embryos			<i>In vitro</i> embryos		
	transferred	pregnant recipients		transferred	pregnant recipients	
	<i>n</i>	<i>n</i>	(%)	<i>n</i>	<i>n</i>	(%)
Standard	71	47	(66.2) ^a	372	129	(34.7) ^a
Direct	113	60	(53.1) ^a	25	18	(72.0) ^c
Vitrification	28	18	(64.3) ^a	24	12	(50.0) ^{ac}

^{a,c} values in the same column with different superscripts differ significantly ($P < 0.01$)

This value is significantly lower ($P < 0.01$) than developmental competency of fresh embryos recovered non-surgically from donors (70.0%) – Table 2. Similar results were found in the study of Carvalho *et al.* (1996) in embryos preserved by the method specified as the standard one in our study (1.5 M glycerol), by the method with ethylene glycol (Hochi *et al.* (1998), or by vitrification procedure – Arav and Zeron (1997), Vajta *et al.* (1997), Martinez *et al.* (1998), Vajta (2000) and others.

Development of preserved IVP embryos after ET

Survival rate of thawed embryos preserved in 10% v/v glycerol in complete conditioned medium (standard method) amounted to 66.2% (47/71). Survival rate of transferred IVP embryos preserved by the identical method amounted to 34.7%. The mentioned difference is not significant ($P > 0.05$), but relatively high from the technological standpoint.

High pregnancy rates characterized direct transfers of IVP embryos and *in vivo* embryos (produced by superovulation treatment) – 72.0% and 53.1%, resp. ($P < 0.01$). Pregnancy rates found in recipients of *in vivo* embryos preserved by vitrification and recipients of vitrified IVP embryos amounted to 50.0% (12/24) and to 64.3% (18/28), respectively (Table 3).

Pregnancy rates found in recipients of embryos (produced *in vivo* and *in vitro*) preserved by experimental procedures are higher than or comparable with the data presented in studies of the cited authors. The vitrification method was tested in a smaller embryo set, the results can be classified as promising ones similarly like in studies of Lazar *et al.* (2000), Vajta (2000). The vitrification procedure is suitable and utilizable for cryopreservation of oocytes and micromanipulated embryos – Nguyen *et al.* (2000), Dattena *et al.* (2000), Hurtt *et al.* (2000), Keskindepe *et al.* (2000), Dhali *et al.* (2000), Papis *et al.* (2000) and others. Its applicability and effectiveness are evident mainly in the case of small quantity of cryopreserved embryos

– Říha and Landa (1989), Říha *et al.* (1991), Říha (1994), Vajta (2000).

Conclusions and recommendations

According to bibliographic references and obtained results it is evident that routine cryopreservation procedures can serve as provisional methods for preservation of IVP embryos (produced by IVM-IVF-IVC method) in spite of the somewhat lower pregnancy rate of recipients as compared with the rate recorded in recipients of control fresh and preserved embryos.

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Single- and multi-trait animal model in the silver fox evaluation

Jedno- a víceznakový animal model pro hodnocení stříbrných lišek

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ABSTRACT: Data on 4 435 animals, collected on two fox farms in 1985–1999, were used in the estimation of genetic parameters and genetic trends for four traits evaluated according to Polish grading standard. The graded traits were: body size (BS), colour type (CT), purity of silvering and coat colour (PSCC), and coat quality (CQ). (Co)variance components were estimated by the restricted maximum likelihood method (REML) using the average information (AI) algorithm. A linear model that included herd, year of birth and birth season as fixed effects, and additive genetic as a random effect was used in both, single- and multi-trait analyses. The annual genetic trend of the studied traits was estimated as a regression of breeding value on time. Comparable estimates of heritability were derived using single- (ranging from 0.192 for CQ to 0.356 for CT) and multi-trait (ranging from 0.191 for CQ to 0.345 for CT) models. Quite high genetic correlations were found between CT and PSCC, and CT and CQ (0.47 and 0.43, respectively), whereas the correlations between other traits were low or moderate (ranging from 0.03 to 0.18). All phenotypic correlations were low ranging from -0.04 (between CQ and PSCC) to 0.15 (between CT and PSCC). The estimated genetic trends were positive for all the traits and ranged from 0.014 to 0.057 when single-trait analysis was used or they ranged from 0.017 to 0.057 when multi-trait analysis was applied.

Keywords: coat traits; genetic parameters; genetic trends; silver fox

ABSTRAKT: K odhadu genetických parametrů a genetických trendů u čtyř znaků, které byly hodnoceny podle polského klasifikačního standardu, jsme použili údaje o 4 435 zvířatech získané v letech 1985 až 1999 na dvou liščích farmách. Hodnocenými znaky byla velikost trupu (BS), typ zbarvení (CT), čistota stříbřitosti a zbarvení srsti (PSCC) a kvalita srsti (CQ). Odhad složek variance a kovariance jsem prováděli pomocí metody omezené maximální věrohodnosti (REML) s použitím průměrného informačního algoritmu (AI). Pro jedno- i víceznakové analýzy jsme použili lineární model, který jako pevné efekty obsahoval chov, ročník narození a sezónu narození a jako náhodný efekt aditivní genetiku. Odhad meziročního genetického trendu u sledovaných znaků vycházel z regrese plemenné hodnoty vzhledem k času. Pomocí jedno- (v rozpětí od 0,192 pro CQ do 0,356 pro CT) a víceznakového (v rozpětí od 0,191 pro CQ do 0,345 pro CT) modelu jsme odvodili srovnatelné odhady dědivosti. Zjistili jsme relativně vysoké genetické korelace mezi CT a PSCC a mezi CT a CQ (0,47 resp. 0,43), zatímco korelace mezi ostatními znaky byly nízké nebo střední (od 0,03 do 0,18). Všechny fenotypové korelace dosahovaly nízkých hodnot od $-0,04$ (mezi CQ a PSCC) do 0,15 (mezi CT a PSCC). Odhadnuté genetické trendy byly pro všechny znaky kladné; při použití jednoznakové analýzy se pohybovaly od 0,014 do 0,057 a při použití víceznakové analýzy od 0,017 do 0,057.

Klíčová slova: znaky srsti; genetické parametry; genetické trendy; stříbrná liška

The breeding value evaluation is the most important part of the multi-stage process aimed at improvement of the genetic merit of a population. The best method of breeding value evaluation that has widely been used in practice is the Best Linear Unbiased Prediction (BLUP) with the use of an animal model (AM) (Engel *et al.*, 1999). The BLUP AM incorporates all sources of genetic information into the prediction of genetic merit, improving the accuracy of breeding value predictors (Meyer, 1989).

Although in all modern breeding programmes the best linear unbiased predictions (BLUPs) of the breeding value are used when selecting animals, this method has not been introduced into Polish fur farming yet. Instead of BLUPs Polish fur farmers use as selection criteria the total score (sum of scores given to an individual during conformation and coat grading) or a simplified form of selection index (calculated as a sum of scores given for four sources of information) (Wierzbicki and Filistowicz, 2001). These methods, however, are not reliable enough since the subjectivity of grading, and construction of selection index without using all available sources of information on animal genetic merit lead to the low accuracy of selection and, as a result, to unsatisfactory genetic progress (Wierzbicki *et al.*, 2000). Disadvantages of selection schemes based on the total score as a selection criterion were reported by many authors (Reiten, 1977; Jeżewska and Maciejowski, 1983; Jeżewska *et al.*, 1994; Lohi, 1995; Lohi *et al.*, 1996; Rasmussen, 1996).

In Nordic countries (Finland, Norway, Denmark) the genetic merit of fur animal populations (mainly foxes and mink) is evaluated with the use of BLUP AM (Smëds, 1992; Johannessen *et al.*, 2000). As a consequence considerably higher accuracy of breeding value estimation is achieved, and the genetic gain is bigger (for example, reproduction results on Finnish farms applying BLUP AM have been 0.1–0.4 cubs/litter above the national average – www.stkl-fpf.fi).

BLUP AM was not introduced into fur animal breeding at the same time as in major species of livestock. In Finland and Norway this method was applied for the fox breeding value estimation in the 1990s (Smëds, 1992; Johannessen *et al.*, 2000). However, in both countries the systems of breeding value evaluation are different. In Finland the breeding value evaluation is carried out on farms using the SAMPO computer software distributed

by the Finnish Fur Breeders' Association. Breeding value of traits of economic importance (evaluated traits are selected by a breeder) is estimated using data collected on a farm (grading scores), data coming from skin auctions, or both sources of information are combined (Smëds, 1992; www.stkl-fpf.fi). In Norway, in contrast, the data are recorded on farms, and they are sent to the central data bank for processing. After the statistical analyses breeding values are estimated, combined selection index is calculated, and the results are sent back to the farmers (Johannessen *et al.*, 2000).

Although clear differences are seen in the methods and schemes used for breeding value estimation in Poland and Nordic countries (BLUP AM vs. phenotypic selection, centralised vs. non-centralised system), in all the countries the selection of foxes has always been oriented towards the improvement of conformation and coat traits as well as reproductive performance.

This paper presents the genetic parameters and genetic trends estimated in a population of silver fox (*Vulpes vulpes*) kept on Polish farms using the single- and multi-trait BLUP AM.

MATERIAL AND METHODS

Data on 4 435 silver foxes, collected in 1985 to 1999 on two farms were used in the estimation of genetic parameters and genetic trends for four traits evaluated according to Polish grading standard (CSHZ, 1997). The graded traits were: body size (BS), colour type (CT), purity of silvering and coat colour (PSCC), and coat quality (CQ). After grading the scores were summed up giving the total score that was the main selection criterion. The selection pressure put on each trait was different hence each trait had its own scale of scores. The animals were evaluated by a classifier when the fur coat was fully developed (in November each year). The data were collected in the database of the LISY computer system (Chudoba *et al.*, 1988). Description of the scale of scores and statistical information on the data set are given in Table 1.

Pedigrees were traced back up to two generations giving the pedigree file of 4 435 animals (3 582 with records). The pedigree structure is presented in Table 2.

The estimation of (co)variance components was performed using the single- and multi-trait animal models and the restricted maximum likelihood

Table 1. Statistical description of the data set

Trait	Scale of scores	No. of records	Mean	Standard deviation
Body size	0–6	3 582	5.04	1.35
Colour type	0–3	3 582	2.54	0.72
Purity of silvering and coat colour	0–5	3 582	4.33	0.71
Coat quality	0–6	3 582	5.01	0.52

Table 2. Pedigree structure

Number of base animals	849	Sires with progeny records	406
Animals with records	3 582	Dams with progeny records	663
with unknown sire	77	Grand sires with progeny records	257
with unknown dam	125	Grand dams with progeny records	330

(REML) method. The DMU 6.4 package (Madsen and Jensen, 2000) and the average information (AI) algorithm were used in the estimation. The following mixed model was applied in both, single- and multi-trait analyses:

$$X_{ijkl} = \mu + h_i + y_j + s_k + a_{ijkl} + e_{ijkl}$$

where: X_{ijkl} = the observation

h_i = fixed effect of herd ($i = 1, 2$)

y_j = fixed effect of the year of birth ($j = 1-15$)

s_k = fixed effect of birth season ($k = 1, 2, 3$)

a_{ijkl} = additive genetic effect of the animal

e_{ijkl} = residual effect

The effect of animal and residual effect were assumed random with $E(a) = 0$, $E(e) = 0$, $\text{var}(a) = A \cdot G_0$ and $\text{var}(e) = I \cdot R_0$, where A is additive relationship matrix, G_0 is additive genetic (co)variance matrix, I is identity matrix and R_0 is (co)variance matrix for residuals. The estimate standard errors were derived from the AI information matrix. Coefficients of determination (R^2) for the analysed traits, indicating the total variation explained by fixed effects were as follows: BS – 0.26, CT – 0.23, PSCC – 0.21, and CQ – 0.15.

After estimation of the (co)variance components, estimated breeding values (EBVs) for BS, CT, PSCC and CQ were computed using the BLUP

with the above animal model. The genetic trends for the studied traits were estimated as a regression of EBVs on time.

RESULTS AND DISCUSSION

The linear model used in the analyses did not include the joint herd-year-season (HYS) effect because the data were collected within the period of 14 years, and many very small classes herd-year-season were created (some of them were empty). Moreover, the data originated from 2 farms, but there were a few years when we had data from one (bigger) farm only.

Fixed effects fitted for the studied traits explained from 15% (CQ – $R^2 = 0.15$) to 26% (BS – $R^2 = 0.26$) of the total variation for these traits. Although the values of R^2 are quite low, they can be comparable to those reported by Hermes *et al.* (2000), who studied genetic parameters in Australian pigs. The coefficient of determination calculated for 4 performance traits ranged from 0.17 to 0.39, and for 8 carcass traits it ranged from 0.15 to 0.37.

The linear model fitted for all analysed traits included only direct effect of the animal. In the case of BS a maternal effect might have been included. However, in this study, which is a part of wider research, we decided to test one model for all the

Table 3. Estimates of variance components, heritability and annual genetic trends (ΔG) for studied traits derived by the single- and multi-trait animal model

Trait	σ_a^2	σ_e^2	σ_p^2	h^2	ΔG (point/year)	ΔG (% of mean)
Single-trait animal model						
Body size	0.364	0.995	1.359	0.268 (s.e. 0.034)	0.057	1.13
Colour type	0.149	0.270	0.419	0.356 (s.e. 0.037)	0.038	1.49
Purity of silvering and coat colour	0.103	0.291	0.394	0.261 (s.e. 0.033)	0.014	0.32
Coat quality ¹	0.047	0.198	0.245	0.192 (s.e. 0.031)	0.019	0.38
Multi-trait animal model						
Body size	0.358	1.000	1.358	0.264 (s.e. 0.034)	0.057	1.13
Colour type	0.144	0.274	0.418	0.345 (s.e. 0.036)	0.041	1.61
Purity of silvering and coat colour	0.103	0.291	0.394	0.262 (s.e. 0.033)	0.017	0.39
Coat quality	0.047	0.198	0.245	0.191 (s.e. 0.031)	0.021	0.42

Variance components: σ_a^2 – additive genetic; σ_e^2 – error variance; σ_p^2 – phenotypic variance ($\sigma_a^2 + \sigma_e^2$)

$$h^2 = \sigma_a^2 / \sigma_p^2$$

s.e. = standard error

traits. Furthermore, according to Meyer (1992) standard errors of heritability estimates can be 3 to 5 times larger with maternal effect model as compared with a model involving only direct effect.

The variance components and heritabilities of the studied traits are presented in Table 3. Regardless of the type of analysis (single- or multi-trait) the additive and error variance components and the estimates of heritability were comparable or identical. Slightly different were the estimates of additive genetic variance for BS and CT ranging from 0.364 to 0.358 and from 0.149 to 0.144, respectively. When the single- or multi-trait animal model was applied, the respective estimated heritabilities for these traits were 0.268 and 0.264 for BS and 0.149 and 0.144 for CT. For the remaining traits (PSCC and CQ) the variance components estimated using the single- and multi-trait analysis were identical giving similar heritabilities (PSCC – 0.261 and 0.262 when the single- or multi-trait model was used, respectively; CQ – 0.192 and 0.191, respectively).

In the earlier research Wierzbicki (2000) studied the effect of data transformation on the additive genetic and error variance components in a population of arctic fox (*Alopex lagopus*). He found similar heritabilities to those presented here, for BS (0.288, s.e. 0.047) and CT (0.342, s.e. 0.049). However, the heritabilities were estimated after application of the probit transformation of the data set. The estimates of heritability derived using untransformed data were markedly higher ranging from 0.461 for BS to 0.445 for CT. In contrast, Filistowicz *et al.* (1999, 2000) carried out studies in populations of silver and golden fox, respectively, and reported significantly lower heritabilities for CT (ranging from 0.08 to 0.168) and for BS (0.081). However, the estimates of heritability had high standard errors (from 0.07 to 0.14).

Heritabilities of the conformation traits (scored by a classifier) reported for other domesticated species were usually low or moderate (Manfredi *et al.*, 2001; Serenius *et al.*, 2001; Schaeffer *et al.*, 2001). Although the same traits of body conformation are

evaluated in different species (for example body size and general appearance), the genetic parameters estimated for these traits cannot be compared easily since evaluation standards differ between species, and are changed in the course of time. While continuous revision of the standards is desirable from a breed improvement standpoint, the consequences are that it is impossible to genetically compare individuals classified many years apart (Schaeffer *et al.*, 2001). According to Schaeffer *et al.* (2001) another aspect of subjectively evaluated traits is the fact that the animal cannot receive a higher score than the highest category allowed for the trait. The effect of this limit may shrink the genetic variability that is present in the population. This finding seems to be supported by the results of the present study (Table 1). Means of the traits are very close to maximal scores (upper limits) and their standard deviations (except BS) are small indicating that a narrow scale of scores is used when evaluating the traits.

The genetic and phenotypic correlations are presented in Table 4. Quite high genetic correlations were found between CT and PSCC (0.47), and between CT and CQ (0.43) indicating a strong association between the pools of genes that determine these two traits. The other genetic correlations were low (0.04 between BS and PSCC) or moderate (0.14 between BS and CT, and 0.18 between BS and CQ) and had high standard errors (from 0.092 to 0.107). Almost all phenotypic correlations were very low ranging from –0.02 between PSCC and BS to 0.10 between CQ and CT. The moder-

ate phenotypic correlation was found only between PSCC and CT (0.15).

Markedly different genetic and phenotypic correlations between conformation and coat traits in silver and golden fox populations were found by Filistowicz *et al.* (1999, 2000). The authors estimated genetic parameters for seven (report from 2000) or eight (report from 1999) traits scored according to the old grading standard replaced by the new one in 1997. The wide ranges of genetic (from –0.97 to 0.87) and phenotypic (from –0.96 to 0.82) correlations were found between investigated traits. The pronounced differences between the results reported by Filistowicz *et al.* (1999; 2000) and the results presented in this study may have been caused by (i) subjectivity of this type of evaluation, (ii) different standards of evaluation (old and new ones) used in both studies, (iii) different methods of (co)variance components estimation (animal model vs. sire and dam model). Moreover, the number of animals used in this study was higher (4 435 individuals) as compared with the study by Filistowicz *et al.* (1999; 2000) (2 072 and 1 013 foxes, respectively).

The estimated genetic trends were positive for all analysed traits (Table 3). In both types of analyses (single- and multi-trait animal model) markedly higher genetic trends were estimated for BS and CT. When the single-trait model was applied, the genetic trends for BS and CT reached 0.057 and 0.038, respectively (expressed as percentages of the means the trends were 1.13% for BS and 1.49% for CT). In the case of the multi-trait analysis, when covariances between traits were taken into account,

Table 4. Estimates of genetic (above diagonal) and phenotypic (below diagonal) correlations between analysed traits

Trait	BS	CT	PSCC	CQ
BS	–	0.14 (s.e. 0.092)	0.04 (s.e. 0.098)	0.18 (s.e. 0.107)
CT	0.03	–	0.47 (s.e. 0.081)	0.43 (s.e. 0.094)
PSCC	–0.02	0.15	–	0.03 (s.e. 0.108)
CQ	0.06	0.10	–0.04	–

BS – body size, CT – colour type, PSCC – purity of silvering and coat colour, CQ – coat quality
s.e. – standard error

the genetic trend estimated for BS did not differ from that estimated using the single-trait model, whereas the genetic trend for CT was higher and reached 0.041 (1.61%). In the remaining traits the genetic trends were lower when estimated using the single-trait model (0.014 and 0.019 for PSCC and CQ, respectively), whereas the estimation under the multi-trait model resulted in higher genetic trends (0.017 and 0.021 for PSCC and CQ, respectively).

The magnitude of genetic trends estimated for BS and CT was within the range (from 1% to 2% per year) comparable with that reported for other domesticated species (Falconer and Mackay, 1996). The lower genetic trends estimated for PSCC and CQ may have been brought about by measurement error introduced during scoring. Evaluation of these two traits is more subjective as compared with BS and CT, and causes more difficulties for classifiers. Low precision of this type of evaluation was indicated by Jeżewska and Maciejowski (1983), who found low repeatability of scores ranging from 0.25 to 0.66 in the populations of silver fox, arctic fox and mink. According to Jeżewska *et al.* (1994) only 21–54% of the total variation of scores was determined by animals, whereas up to 60% of the total variation was defined as an error variance.

Genetic trends in fur animals have not been estimated frequently. In populations of arctic fox the trends for conformation and coat traits were estimated by Socha (1996), Wierzbicki *et al.* (2000) and Wierzbicki and Filistowicz (2001). All these authors reported low or even negative genetic trends ranging from -0.0051 for body size to 0.073 for total score (Socha, 1996), or from -0.0004 for body size to 0.013 for colour purity (Wierzbicki and Filistowicz, 2001). However, these estimates cannot be considered as unbiased predictions of breeding values because of trait evaluation subjectivity (a completely objective conformation assessment system is difficult to find, or too costly to apply) (Schaeffer *et al.*, 2001).

CONCLUSIONS

Comparison of the single- and multi-trait animal model applied for genetic evaluation of silver foxes revealed small differences between the estimates derived by both methods. The estimates of heritability obtained by the single- and multi-trait analysis were comparable and had moderate values. Quite high

genetic correlations were found between CT and PSCC, and CT and CQ, whereas the other ones were low or moderate. Low values of the phenotypic correlation indicated weak phenotypic associations between the studied traits. The genetic trends estimated by the multi-trait animal model, when covariances between traits were incorporated into prediction of breeding value, were slightly higher than those estimated with the use of the single-trait animal model.

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Effect of copper sulphate supplementation on performance of broiler chickens, cholesterol content and fatty acid profile of meat

Vliv doplňků síranu měďnatého na užítkovost brojlerových kuřat, obsah cholesterolu a profil mastných kyselin v mase

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ABSTRACT: Six hundred day-old male Ross 208 chickens were fed on wheat/maize – soybean meal diet and divided into 4 groups. Chickens of the 1st and 2nd group were fed diets containing 13 and 35 mg Cu/kg, respectively. Chickens of the 3rd group were fed the feed with 126 mg Cu from the 1st to the 14th day of age, then the feed with 35 mg Cu/kg till the 41st day of age. Chickens of the 4th group were fed the former feed from the start to the end of the experiment. Copper was added to feed mixtures as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Higher concentrations of Cu significantly ($P < 0.05$) decreased weight gains (by 7.0 and 6.1% in the 3rd and the 4th group, respectively), and insignificantly reduced mortality of chickens (from 2.6 to 0.6–1.3%). Feed conversion was worse in chickens of the 3rd group than in other chickens ($P < 0.05$). In 8 chickens from each group lipids of breast muscles were analyzed. Total lipid and cholesterol concentration in breast muscles of chickens of the 3rd group was significantly ($P < 0.05$) lower than in control chickens by 30.3 and 20.2%, respectively. There was no important effect of Cu supplementation on the fatty acid profile of lipids of breast muscles, except minor changes in contents of some fatty acids. Thus the reduction of total muscle lipids and cholesterol content was the main effect of Cu supplementation in chickens.

Keywords: broiler; copper sulphate; performance; quality of meat; cholesterol; fatty acids

ABSTRAKT: K pokusu jsme použili 600 kohoutků Ross 208 ve věku jednoho dne. Kohoutci byli krmeni směsí, v níž největší podíl měla pšenice, kukuřice a sojový extrahovaný šrot. Kuřata jsme rozdělili do čtyř skupin. Obsah mědi v krmivu kuřat 1. skupiny (kontrolní) byl 13 mg/kg. V krmivu 2. skupiny byl zvýšen přidáním $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ na 35 mg/kg. Touto směsí byla krmena také kuřata 3. skupiny od 15. do 41. dne věku, předtím však směsí s obsahem 126 mg Cu/kg. Kuřata 4. skupiny byla krmena směsí obsahující 126 mg Cu/kg od začátku do konce pokusu. Vyšší koncentrace Cu signifikantně ($P < 0,05$) snížila hmotnostní přírůstky kuřat (o 7,0 a 6,1 % v 3. a 4. skupině). Měď přidaná ve vyšší koncentraci nesignifikantně snížila úhyn kuřat z 2,6 % na 0,6–1,3 %. U osmi kuřat z každé skupiny jsme analyzovali lipidy extrahované z prsních svalů. Kuřata 3. skupiny, mající nejhorší konverzi krmiva, měla nejnižší koncentraci tuku a cholesterolu v prsních svalech ($P < 0,05$). Rozdíl proti kuřatům kontrolní skupiny činil 30,3 % u obsahu tuku a 20,2 % u obsahu cholesterolu. Vliv doplňku Cu na zastoupení mastných kyselin a jejich skupin v lipidech prsních svalů nebyl výrazný, vyjma malých změn obsahu některých mastných kyselin. Lze konstatovat, že hlavním pozitivním účinkem doplňku Cu v našem pokuse bylo snížení obsahu tuku a cholesterolu v mase kuřat.

Klíčová slova: brojlerová kuřata; síran měďnatý; užítkovost; kvalita masa; cholesterol; mastné kyseliny

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The influence of higher levels of copper sulphate pentahydrate on growth and meat quality of broilers was tested by Tyleček *et al.* (1961). The susceptibility of meat lipids to oxidation depends on the concentration of unsaturated fatty acids, availability of antioxidants and the presence of transition metals such as Cu in meat. Copper promotes oxidation of low-density lipoproteins *in vitro* (Strain, 1994). Copper in its unbound form is a pro-oxidant (Diplock *et al.*, 1998). It is however also an essen-

tial component of superoxide dismutase, an enzyme defending living organisms against reactive oxygen species (Barman, 1974). Konjufca *et al.* (1997) observed that copper salt at a pharmacological dose decreased cholesterol 7 α -hydroxylase activity (involved in cholic acid formation). It has been found that dietary copper in excess of nutritional requirements reduces cholesterol content in meat of broilers (Bakali *et al.*, 1995) and in meat of rabbits (Skřivanová *et al.*, 2001). Lauridsen *et al.* (1999),

Table 1 Composition of experimental diets

Ingredients (g/kg)	Group			
	1	2	3	4
Wheat	290	290	290	290
Maize	290	290	290	290
Soybean meal	325	325	325	325
Fish meal	20	20	20	20
Rapeseed oil	40	40	40	40
Limestone	15	15	15	15
Dicalcium phosphate	12	12	12	12
Vitamin-mineral mix*	5	5	5	5
Sodium chloride	2	2	2	2
DL-methionine	0.9	0.9	0.9	0.9
CuSO ₄ ·5H ₂ O	–	0.13	0.49 ^a /0.13 ^b	0.49
Composition by analysis (g/kg)				
Dry matter		904.0		
Crude protein		224.0		
Fat		65.0		
Fibre		36.0		
Ash		58		
Ca		9.2		
P		5.6		
Cu	0.013	0.035	0.126 ^a /0.035 ^b	0.126
ME by calculation (MJ/kg)		12.62		

^a) from 1 to 14 d of age, ^b) from 15 to 41 d of age

* the vitamin-mineral premix provided per kg of diets: vitamin A – 12 000 i.u., vitamin D₃ – 500 i.u., vitamin E – 105 mg, vitamin K₃ – 3 mg, vitamin B₁ – 3 mg, vitamin B₂ – 5 mg, vitamin B₆ – 4 mg, vitamin B₁₂ – 0.04 mg, niacin amide – 40 mg, Ca pantothenate – 12 mg, biotin – 0.15 mg, folic acid – 1.5 mg, choline-Cl – 250 mg, ethoxyquin – 100 mg, Mn – 80 mg, Zn – 60 mg, Fe – 50 mg, I – 1 mg, Se – 0.25 mg

however, found that plasma cholesterol of pigs was not influenced by supplemental dietary copper. Komprda *et al.* (1999) found that cholesterol content and fatty acid composition of chicken tissues were influenced by the rate of growth. Cholesterol in breast and thigh muscles, however, tended to decrease with increasing growth rate.

Dietary saturated fatty acids are implicated as a risk factor for atherosclerosis. Clandinin *et al.* (1999) examined the effect of high vs. low palmitic acid intakes on plasma lipoprotein cholesterol levels and on rates of endogenous synthesis of cholesterol in healthy and hyperlipidemic subjects. Diets were formulated to provide combinations of 16 : 0 at two levels of 18 : 2_{n-6}. Serum total cholesterol and low density lipoprotein-cholesterol were not significantly affected by a high level of 16:0 when diets also contained a high level of 18 : 2_{n-6}. The results indicate that 16 : 0 has no effect on serum lipoprotein profiles in the presence of recommended intakes for 18 : 2_{n-6}. Changes in the profile of fatty acids of lipids of edible animal tissues have consequences for human health. It is important to know how to change the composition of fatty acids in animal lipids in a desirable way. Skřivan *et al.* (2000) observed that copper sulphate supplementation reduced the saturated fatty acid proportion in abdominal fat of broilers. The object of the present experiment was to determine the effects of copper supplementation on the performance and composition of broiler meat and fat.

MATERIAL AND METHODS

Diets and husbandry

Day-old broiler chickens Ross 208 were housed in pens on wood shavings in an environmentally controlled room. Six hundred male chickens were divided into 12 pens and 4 treatment groups. In each pen there were 50 chickens (150 chickens per group). The chickens had *ad libitum* access to water and experimental diets. The diets contained 40 g/kg rapeseed oil and 100 mg/kg vitamin E. Copper as copper sulphate pentahydrate was added to the feed of 2nd, 3rd and 4th group. The feed of chickens of the 2nd and the 4th group contained 35 and 126 mg Cu/kg, respectively. Chickens of the 3rd group received feed with higher Cu concentration from the 1st to the 14th day of age, and the feed with lower Cu concentration from the 15th to the 41st day of age (Table 1). During the experiment, food intake per pen and group, body weight and mortality were recorded. At the end of the trial, 8 male chickens of similar body weight from each group were killed, dissected and samples of breast and thigh muscles without skin and skin with subcutaneous fat alone were stored at -20°C until analysed.

Analyses

Total lipids were extracted from breast muscles with 2 : 1 chloroform-methanol according to the

Table 2. Effect of dietary copper supplementation on broiler performance (mean ± SE)

Parameter	Diet and dietary level of total copper (mg/kg)			
	1 (13)	2 (35)	3 (126/35)	4 (126)
Weight gain (g)				
1 to 14 d	242 ^a ± 4.32	217 ^b ± 4.69	243 ^a ± 4.42	217 ^b ± 4.44
15 to 41 d	1 783 ^a ± 16.98	1 683 ^b ± 27.65	1 814 ^a ± 27.70	1 667 ^b ± 25.43
1 to 41 d	2 024 ^a ± 20.11	1 900 ^b ± 31.15	2 056 ^a ± 31.04	1 883 ^b ± 28.54
Feed : gain (g/g)				
1 to 14 d	1.76 ^{ab} ± 0.12	1.69 ^{ab} ± 0.06	1.61 ^b ± 0.04	1.90 ^a ± 0.02
15 to 41 d	1.77 ^b ± 0.03	1.81 ^b ± 0.06	1.77 ^b ± 0.05	2.05 ^a ± 0.02
1 to 41 d	1.77 ^b ± 0.03	1.80 ^b ± 0.05	1.75 ^b ± 0.04	2.03 ^a ± 0.01
Mortality (%)	2.6	1.3	2.0	0.6

^{a, b} means with different superscripts in lines differ at $P < 0.05$

Table 3. Total lipid (%), cholesterol (mg/kg) and fatty acid pattern (g/100 g of total fatty acids) of breast meat samples (mean \pm SE)

Compound	Diet and dietary level of total copper (mg/kg)			
	1 (13)	2 (35)	3 (126/35)	4 (126)
Total lipids	8.87 ^a \pm 0.38	7.63 ^{ab} \pm 0.53	7.50 ^{ab} \pm 1.03	6.18 ^b \pm 0.62
Cholesterol	625 ^a \pm 20.54	602 ^a \pm 22.29	584 ^a \pm 19.61	499 ^b \pm 42.97
14 : 0	0.47 \pm 0.01	0.45 \pm 0.02	0.46 \pm 0.02	0.47 \pm 0.02
15 : 0	0.11 \pm 0.01	0.11 \pm 0.00	0.10 \pm 0.01	0.11 \pm 0.01
16 : 0	20.70 \pm 0.27	20.83 \pm 0.46	20.83 \pm 0.33	20.82 \pm 0.25
17 : 0	0.16 \pm 0.01	0.17 \pm 0.01	0.16 \pm 0.01	0.15 \pm 0.01
18 : 0	7.47 \pm 0.15	8.01 \pm 0.020	7.87 \pm 0.40	7.64 \pm 0.35
20 : 0	0.12 \pm 0.01	0.11 \pm 0.00	0.13 \pm 0.01	0.13 \pm 0.01
14 : 1	0.05 \pm 0.00	0.04 \pm 0.00	0.05 \pm 0.01	0.05 \pm 0.01
16 : 1	2.40 ^a \pm 0.09	2.05 ^{ab} \pm 0.15	2.21 ^{ab} \pm 0.19	1.92 ^b \pm 0.16
18 : 1	40.01 \pm 0.41	39.43 \pm 0.80	38.74 \pm 0.16	38.86 \pm 0.02
20 : 1	0.61 \pm 0.02	0.64 \pm 0.02	0.61 \pm 0.03	0.61 \pm 0.02
22 : 1	0.03 ^b \pm 0.00	0.03 ^{ab} \pm 0.00	0.04 ^{ab} \pm 0.03	0.04 ^a \pm 0.00
18 : 2 _{n-6}	17.14 \pm 0.33	16.50 \pm 0.23	16.29 \pm 0.39	17.10 \pm 0.46
18 : 3 _{n-6}	0.21 \pm 0.00	0.21 \pm 0.01	0.20 \pm 0.02	0.20 \pm 0.01
20 : 2 _{n-6}	0.36 ^b \pm 0.02	0.44 ^{ab} \pm 0.04	0.47 ^{ab} \pm 0.04	0.48 ^a \pm 0.04
22 : 2 _{n-6}	0.21 ^b \pm 0.03	0.22 ^b \pm 0.01	0.20 ^b \pm 0.03	0.32 ^a \pm 0.02
20 : 3 _{n-6}	0.53 \pm 0.02	0.63 \pm 0.04	0.57 \pm 0.05	0.58 \pm 0.04
20 : 4 _{n-6}	2.45 \pm 0.08	2.85 \pm 0.22	2.61 \pm 0.37	2.95 \pm 0.19
22 : 4 _{n-6}	0.53 ^b \pm 0.02	0.75 ^a \pm 0.02 ^a	0.62 ^{ab} \pm 0.08	0.69 ^a \pm 0.04
18 : 3 _{n-3}	2.55 \pm 0.08	2.31 \pm 0.07	2.28 \pm 0.18	2.39 \pm 0.11
20 : 3 _{n-3}	0.12 \pm 0.01	0.15 \pm 0.02	0.17 \pm 0.02	0.16 \pm 0.01
20 : 5 _{n-3}	0.58 \pm 0.03	0.60 \pm 0.03	0.71 \pm 0.07	0.64 \pm 0.05
22 : 5 _{n-3}	1.44 ^b \pm 0.05	1.83 ^a \pm 0.07	1.52 ^{ab} \pm 0.19	1.70 ^{ab} \pm 0.11
22 : 6 _{n-3}	1.68 ^b \pm 0.06	2.11 ^a \pm 0.07	1.75 ^b \pm 0.17	1.86 ^{ab} \pm 0.12
SFA	29.02 \pm 0.34	29.68 \pm 0.48	29.54 \pm 0.57	29.32 \pm 0.49
MUFA	43.10 \pm 0.48	42.20 \pm 0.90	41.65 \pm 0.25	41.48 \pm 0.14
PUFA	27.80 \pm 0.42	28.59 \pm 0.40	27.38 \pm 0.78	29.07 \pm 0.79
PUFA _{n-6}	21.43 \pm 0.33	21.60 \pm 0.37	20.96 \pm 20.96	22.32 \pm 0.66
PUFA _{n-3}	6.37 \pm 0.11	7.00 \pm 0.15	6.42 \pm 0.33	6.74 \pm 0.19
PUFA/SFA	0.96 \pm 0.02	0.96 \pm 0.02	0.93 \pm 0.03	0.99 \pm 0.03
n-6/n-3	3.37 \pm 0.05	3.10 \pm 0.09	3.31 \pm 0.14	3.32 \pm 0.09

^{a, b} means with different superscripts in lines differ at $P < 0.05$

method of Folch *et al.* (1957). The meat samples were finely ground before analysis. Alkaline trans-methylations of fatty acids was carried out according to ISO 5509 (1994). Gas chromatographic analysis of methyl esters was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a programmed HP-Innowa capillary column (180 to 240°C) and an FI detector. In order to determine cholesterol, lipids were saponified and the unsaponified matter was extracted according to Nolle (1996). Silyl derivatives were separated and quantified on the gas chromatograph equipped with a SAC-5 capillary column (Supelco), operated isothermally at 285°C. Copper was determined by atomic absorption spectrometry (Perkin Elmer, model 5000). Other analyses of feed were done by standard procedures. All analyses were carried out in the Research Institute of Animal Production at Uhřetíněves, Prague 10.

Statistical analyses

The data were analysed by one-way ANOVA. Significant treatment effects were detected by Duncan's multiple range test. Differences were considered significant at $P \leq 0.05$. Results were expressed as means with their standard errors.

Table 4. Carcass dissection (mean \pm SE)

Parameter	Diet and dietary level of total copper (mg/kg)			
	1 (13)	2 (35)	3 (126/35)	4 (126)
Live weight (g)	2 104 \pm 13.91	2 116 \pm 13.54	2 083 \pm 9.68	2 096 \pm 10.17
Dressing percentage	74.1 \pm 0.96	73.2 \pm 0.52	74.9 \pm 0.47	74.8 \pm 0.63
Carcass weight (g)	1 413 \pm 16.16	1 394 \pm 15.93	1 412 \pm 15.25	1 422 \pm 14.23
As (%) proportion of carcass weight				
Liver	2.91 ^{ab} \pm 0.06	3.09 ^b \pm 0.03	2.83 ^a \pm 0.02	2.77 ^a \pm 0.04
Abdominal fat	1.10 \pm 0.16	1.00 \pm 0.10	1.08 \pm 0.13	0.77 \pm 0.11
Breast meat	25.87 \pm 0.97	24.33 \pm 0.50	25.23 \pm 0.51	24.42 \pm 0.57
Thigh meat	22.86 \pm 0.71	22.96 \pm 0.58	21.78 \pm 0.51	22.80 \pm 0.50
Breast and thigh meat	48.73 \pm 1.10	47.29 \pm 0.94	47.01 \pm 0.75	47.22 \pm 0.78
Breast skin	2.00 ^a \pm 0.07	1.92 ^a \pm 0.04	1.78 ^{ab} \pm 0.09	1.59 ^b \pm 0.10
Thigh skin	3.29 ^a \pm 0.13	3.07 ^{ab} \pm 0.18	3.07 ^{ab} \pm 0.17	2.78 ^b \pm 0.13
Breast and thigh skin	5.29 ^a \pm 0.17	4.99 ^a \pm 0.21	4.85 ^{ab} \pm 0.19	4.37 ^b \pm 0.21

^{a, b} means with different superscripts in lines differ at $P < 0.05$

RESULTS AND DISCUSSION

Performance parameters are presented in Table 2. Higher concentration of copper sulphate significantly reduced weight gain of chickens. Feed consumption to gain ratios were significantly higher in broilers receiving the diet with 126 mg/kg total Cu between 1 and 14 d of age and 35 mg/kg during days 15 to 41 (group 3). The largest, but statistically insignificant effect of Cu supplements was a decrease in mortality. The growth-promoting effect of Cu in chickens was not confirmed in this study. Experiments of Lauridsen *et al.* (1999) with pigs showed that the addition of 175 mg copper/kg improved growth rate and feed intake for a short period but did not affect feed utilization. In the European Union, a copper supplementation at 175 mg per kg feed is permitted by 16 weeks of pig age, and from 17 weeks of age 35 mg copper per kg feed is allowed. An EU Directive, however, restricted the maximum of copper allowed in diets for poultry to 35 mg/kg.

Total lipid and cholesterol levels in breast muscle were suppressed by copper significantly in group 3 by 30% and 20% (Table 3). Previous results showed that dietary copper supplementation alters lipid metabolism and changes the fatty acid composition

of depot lipids of swine (Amer and Elliot, 1973; Lauridsen *et al.*, 1999). These studies are in agreement with results of our previous study (Skřivan *et al.*, 2000). On the other hand, palmitoleic acid was significantly decreased and myristic acid was insignificantly reduced too. The content of 126 mg Cu/kg in group 4 increased the concentration of long-chain DPA and DHA. Both effects were significant ($P < 0.05$). Many studies have examined the effects of dietary long-chain polyunsaturated fatty acids (PUFA) supplied especially as fish oil or fish meal on the FA composition of broiler carcass, to increase the human dietary intake of long-chain $n-3$ PUFA which have beneficial effects on human health and resistance to various inflammatory diseases (López-Ferrer *et al.*, 2001).

Carcass dissection (Table 4) demonstrates a significant decrease in breast skin and thigh skin with fat, as affected by copper supplementation.

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Comparison of digestive enzyme activities in the larvae of Siberian sturgeon (*Acipenser baeri* Brandt) and Russian sturgeon (*Acipenser gueldenstaedti* Brandt) back cross hybrids

Porovnání aktivity trávicích enzymů u larev recipročních zpětných hybridů jesetera sibiřského (*Acipenser baeri* Brandt) a jesetera ruského (*Acipenser gueldenstaedti* Brandt)

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ABSTRACT: The activities of digestive enzymes were measured in two reciprocal sturgeon hybrids: Siberian sturgeon (*Acipenser baeri* Brandt) × Russian sturgeon (*Acipenser gueldenstaedti* Brandt) × Siberian sturgeon (SR.S), and Siberian sturgeon (*Acipenser baeri* Brandt) × Russian sturgeon (*Acipenser gueldenstaedti* Brandt) × Russian sturgeon (SR.R) from 3 to 10 weeks of rearing. After a diet change in the 4th week from ASTA-AC to trout starter Aller-Aqua, both hybrids showed a decrease in the activity of lipolytic (about 20%) and amylolytic enzymes (2- and 5-times for SR.R and SR.S, respectively) in the 5th week. In both hybrids acidic proteases were more active than alkaline ones. The ratio of acidic/alkaline protease activities was considerably higher in SR.R than SR.S, beginning from the 4th week of rearing. Its highest values were observed in week 9 (3.43 for SR.R and 1.89 for SR.S). Such a pattern of enzymatic activities explains the higher growth rate of SR.R, compared with SR.S observed from the 5th week of experiment.

Keywords: *Acipenser baeri*; *Acipenser gueldenstaedti*; Acipenseridae; sturgeon hybrids; digestive enzymes

ABSTRAKT: Aktivita trávicích enzymů byla zjišťována u dvou recipročních zpětných hybridů jesetera sibiřského (*Acipenser baeri* Brandt) a jesetera ruského (*Acipenser gueldenstaedti* Brandt) – hybridů SR.S (*A. baeri* × *A. gueldenstaedti*) × *A. baeri* a SR.R (*A. baeri* × *A. gueldenstaedti*) × *A. gueldenstaedti* v průběhu od třetího do desátého týdne odchovu. Po změně diety ve čtvrtém týdnu z krmiva ASTA-AC na pstruží startér Aller-Aqua došlo u obou hybridů v pátém týdnu ke snížení aktivity lipolytických a glykolytických enzymů. U obou hybridů byla acidická proteáza aktivnější než alkalická proteáza. Poměr aktivity acido-bazické proteázy byl vyšší u SR.R než u SR.S od čtvrtého týdne odchovu. Tento charakter enzymatické aktivity se projevil zvýšením rychlosti růstu u SR.R ve srovnání s SR.S od pátého týdne experimentu.

Klíčová slova: *Acipenser baeri*; *Acipenser gueldenstaedti*; Acipenseridae; hybridy jesetera; trávicí enzymy

New species are introduced to the aquaculture in order to improve economic results of fish rearing. Over the last years much attention has been paid

to sturgeons (Bronzi *et al.*, 1999; Kolman, 1999). Among these fish, Siberian sturgeon became the most popular in many countries including Poland

(Kolman, 1999). At the same time, other species and various interspecific hybrids were tested (Kolman *et al.*, 1997; Szczepkowski *et al.*, 2000). The attempts at back cross hybridization were also done (Sadowski *et al.*, 2000). It is assumed that such a heterozygotic sturgeon hybrid offspring would not only inherit advantageous properties of both parental species, but also these properties would be enhanced. Such advantages include good survival, faster sexual maturation, and high growth rate (Kolman, 1999). The latter is related, among others, to efficient digestion and absorption of artificial feed, especially during the larval period. Feed quality and supply must be adjusted to the presence and activity of fish digestive enzymes.

In the present study the activities of digestive enzymes were compared in two reciprocal sturgeon hybrids obtained from fertilization of the eggs of hybrid females of Siberian sturgeon (*Acipenser baeri* Brandt) × Russian sturgeon (*Acipenser gueldenstaedti* Brandt) with sperm of Siberian (SR.S) or Russian sturgeon (SR.R).

MATERIAL AND METHODS

The material was obtained during comparative rearing of larvae of two reciprocal hybrids: Siberian sturgeon (*Acipenser baeri* Brandt) × Russian sturgeon (*Acipenser gueldenstaedti* Brandt) × Siberian sturgeon (SR.S), and Siberian sturgeon (*Acipenser baeri* Brandt) × Russian sturgeon (*Acipenser gueldenstaedti* Brandt) × Russian sturgeon (SR.R).

The fish were reared in water recirculation tanks with full water treatment, and control of basic water quality parameters. Water temperature was 16°C. Dissolved oxygen saturation did not decrease below 70%. Ammonia and nitrite concentrations did not exceed the toxicity thresholds for sturgeon larvae (Kolman, 1999).

Feeding started on day 11 post hatching. For the first 5 days the fish were fed ASTA-AC starter (Kolman *et al.*, 1996) supplemented with 10% of brine shrimp (*Artemia* sp.) nauplii. From day 23 to 27, ASTA-AC was gradually replaced by trout starter SGP-493, Aller Aqua. Feeding rates were calculated according to the feeding curves (Kolman *et al.*, 1996).

From day of rearing 21 to 70, 10 larvae were sampled weekly from each tank, anesthetized, weighed and frozen, and kept for further analyses at -18°C. After careful thawing, digestive tracts were isolated

using a preparatory needle, weighed and homogenized with 0.9% NaCl (1 : 4) using a Potter's glass homogenizer. The homogenates were centrifuged for 10 min at 2600 × g. Protein level was measured in the supernatant according to Spector (1978), and the activities of the following enzymes were evaluated: α-amylase, glucoamylase, maltase, saccharase, trehalase, and lactase, total activity of alkaline and acidic proteases, trypsin, chymotrypsin, pepsin, and triglycerol lipase.

Enzymatic analyses: The activity of α-amylase was measured according to Caraway (1959), and expressed in international units. Glucoamylolytic activity was measured as an amount of glucose released from glycogen during incubation of the mixture containing 0.1 ml of enzymatic extract, 0.1 ml of 1% glycogen solution, and 0.8 ml 0.15 M veronal-acetate buffer of pH 6.12. The activities of disaccharidases were measured according to Dahlqvist (1968), using 25 mM aqueous solutions of the following disaccharides: maltose, lactose, saccharose, and trehalose. The samples were incubated for 1 h. Concentrations of glucose were measured with glucose oxidase, using a Cormay Lublin kit. The activity of triacylglyceric lipase was measured using Cherry-Crandall method (according to Kłyszczko-Stefanowicz, 1999). Peptic activity was determined according to Ryle (1985), tryptic activity according to Geiger and Fritz (1985), and chymotryptic activity according to Geiger (1985), using synthetic chromogenic peptides by Sigma: N-acetyl-DL-phenylalanine-p-nitroanilide for pepsin, N-benzyl-DL-arginine-p-nitroanilide for trypsin, and N-succinyl-L-phenylalanine-p-nitroanilide for chymotrypsin.

The amount of enzyme producing 1 μM of product during 1 h incubation under optimum conditions was taken as a unit of enzymatic activity (u). All the values were calculated per 1 mg of enzymatic extract protein content. The results are shown as the averages of three replications.

RESULTS

In the initial phase of rearing, when the fish received ASTA-AC feed (until 23–27 day) SR.S hybrids grew faster than SR.R (Figure 1). Fish growth regression curves at that time were described by the equations: $Y = 0.0002X^2 + 0.0023X + 0.0221$ ($r > 0.99$) for SR.S, and $Y = 0.00007X^2 + 0.01870$ ($r > 0.99$) for SR.R. After the diet change,

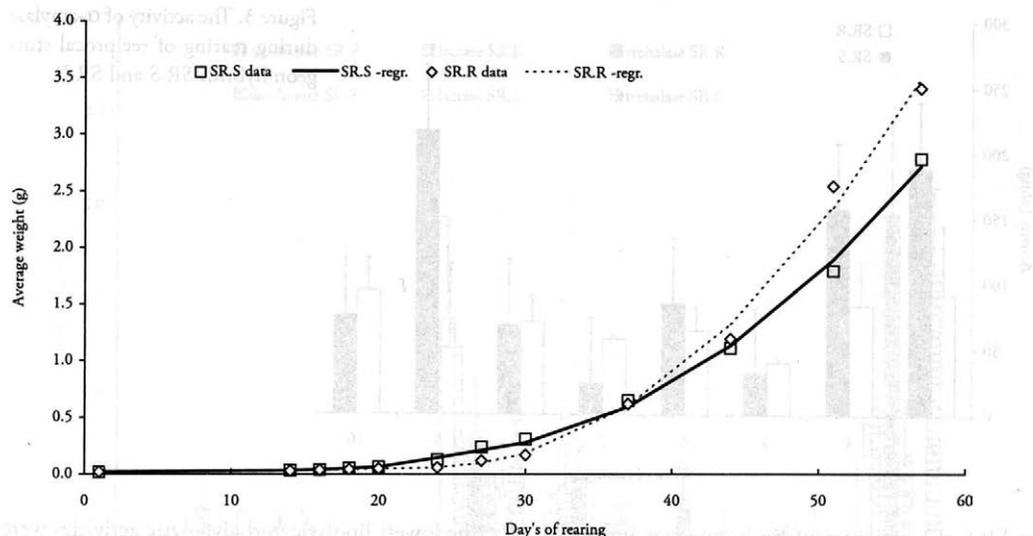


Figure 1. Body mass dynamics of reciprocal sturgeon hybrids SR.S and SR.R

SR.R growth rate quickly increased, and after 36 days of rearing they showed higher body mass in comparison with SR.S (Figure 1). At that time fish growth rates were described by the equations: $Y = 0.0031X^2 - 0.1498X + 1.8656$ (SR.R), and $Y = 0.0023X^2 - 0.1065X + 1.4393$ (SR.S), both at $r > 0.99$.

The analyses of enzymatic activity were carried out to determine their optimum pH. They did not differ between the hybrids, except for lactase. The pH optima were: 8.0 for lipase, 6.12 for α -amylase, glucoamylase, and maltase, 5.53 for saccharase and trehalase, 7.25 for alkaline proteases, 2.08 for acidic proteases, and 7.2 for trypsin. In the case of

lactase, optimum pH were 6.39 in SR.S, and 4.72 in SR.R.

At the beginning of rearing, in both 21 days old hybrids receiving ASTA-AC feed high activities of lipolytic and glycolytic enzymes were observed. SR.R hybrid showed much higher activity of lipase and glucoamylase in comparison with SR.S (Figures 2, 4) while the level of α -amylolytic activity (Figure 3) at that time was higher in SR.S. In the 4th week of rearing, when ASTA-AC feed was gradually replaced by trout starter, lipolytic activity decreased about 4.5 times in both hybrids. The activity of glucoamylase dropped 32 times, and that of maltase about 10 times in SR.R (Figure 4) while

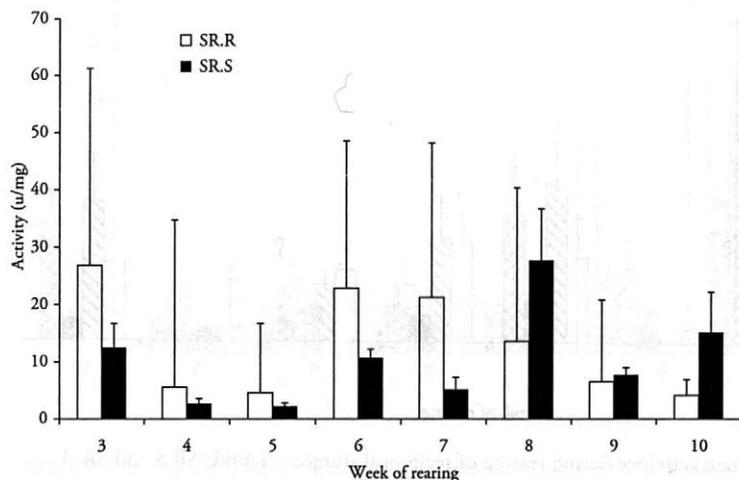


Figure 2. Lipase activity during rearing of reciprocal sturgeon hybrids SR.S and SR.R

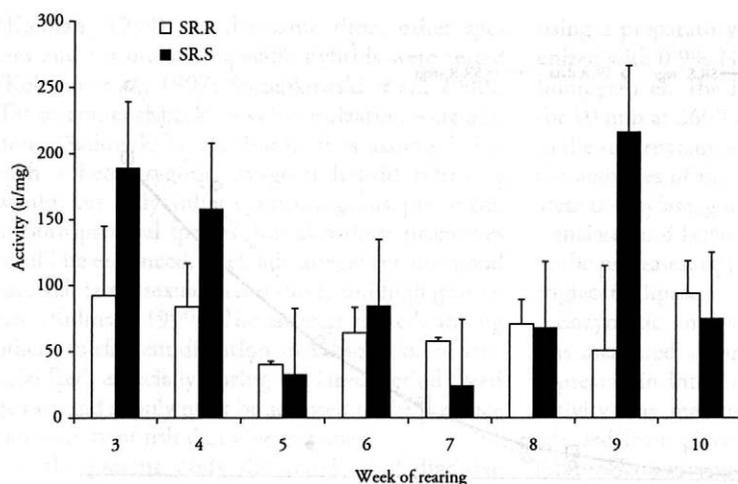


Figure 3. The activity of α -amylase during rearing of reciprocal sturgeon hybrids SR.S and SR.R

in SR.S the activities of both enzymes increased, especially that of glucoamylase (60%) (Figure 4). In the 4th week of rearing proteolytic activity also increased in both hybrids (Figures 6, 7), particularly in SR.S in which the activity of trypsin increased 2.5 times over the 4th week of the experiment (Figure 7).

The lowest lipolytic and glycolytic activities were noted in the 5th week of rearing (Figures 2–5). At that time no maltase activity was found in SR.S, and the activities of other disaccharidases were very low (Figure 5). Similarly low lipolytic and glycolytic activities were observed in the 9th week of rearing (Figures 2, 4, 5). In the 8th and 9th weeks

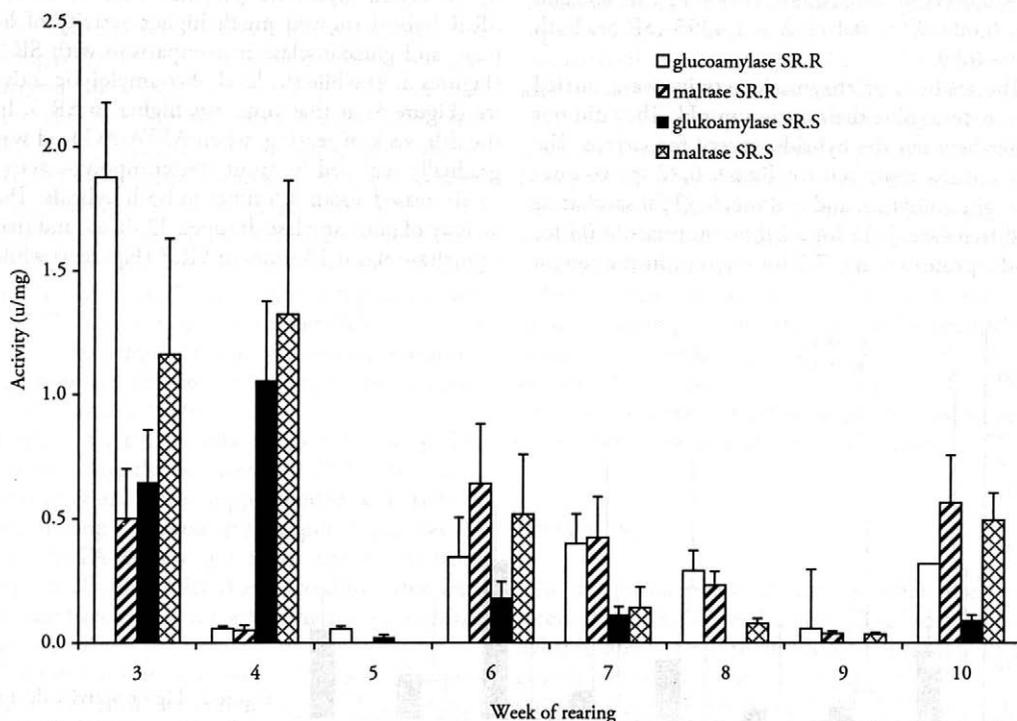


Figure 4. Glucoamylase and maltase activities during rearing of reciprocal sturgeon hybrids SR.S and SR.R

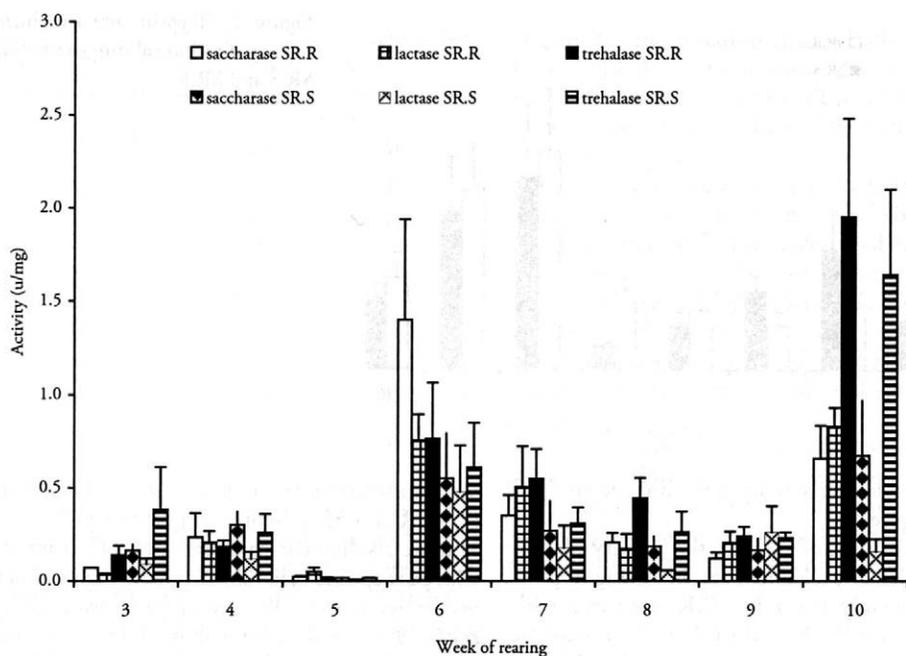


Figure 5. The activities of disaccharidases during rearing of reciprocal sturgeon hybrids SR.S and SR.R

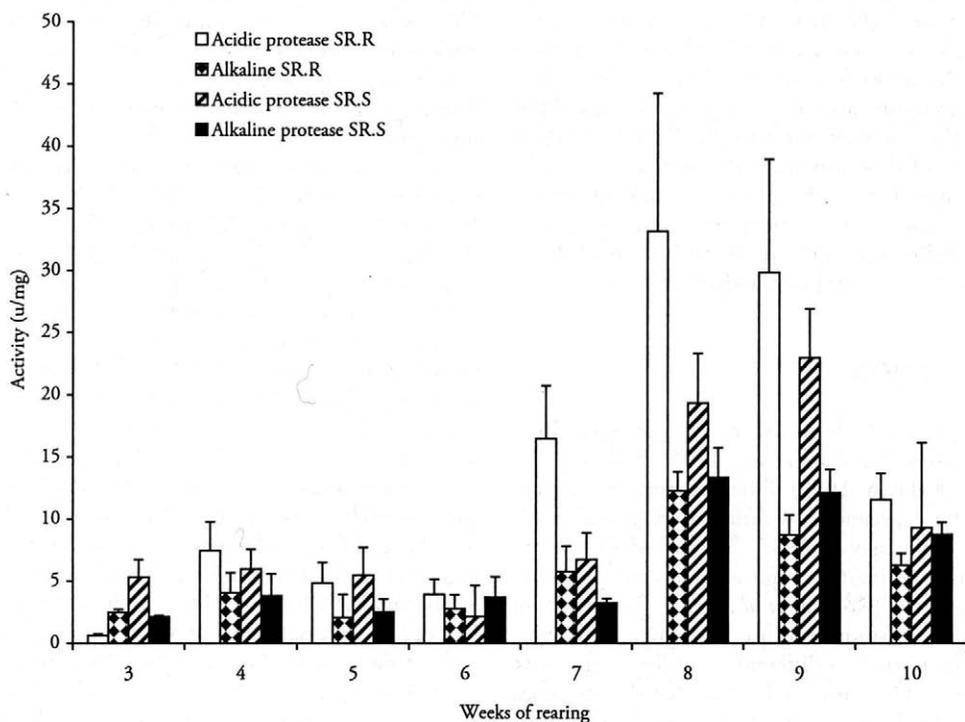


Figure 6. Protease activities during rearing of reciprocal sturgeon hybrids SR.S and SR.R

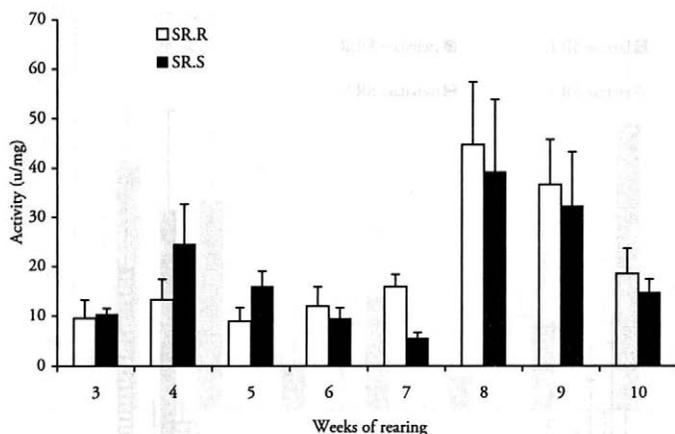


Figure 7. Trypsin activity during rearing of reciprocal sturgeon hybrids SR.S and SR.R

no glucoamylolytic activity was observed in SR.S hybrid (Figure 4).

In the 5th week of rearing, the activity of proteases (particularly alkaline ones, including trypsin) was considerably lower in SR.R compared with SR.S (Figures 6, 7). After the 6th week, proteolytic activities increased in both hybrids. The highest activity of acidic proteases was observed in the 9th week, and of alkaline ones – in the 8th week. At that time acidic proteases were more active in SR.R than in SR.S (Figure 6). On the contrary, alkaline proteases were more active in SR.S than in SR.R from the 8th week of rearing (Figure 6). The differences in tryptic activities were less pronounced but from the 6th week of rearing SR.R showed higher activity of that enzyme than SR.S (Figure 7). In both hybrids over the entire experimental period acidic proteases were more active compared with the alkaline ones (Figure 6). In both hybrids no peptic or chymotryptic activities were observed.

DISCUSSION

Due to the high growth rate under aquaculture conditions (Kolman *et al.*, 1996; Prokeš *et al.*, 1996; Kolman, 1999), Siberian sturgeon is often used for experimental hybridization (Ronay *et al.*, 1991; Kolman *et al.*, 1997). The hybrids differ from parental species in their growth rates and food conversion rates (Kolman *et al.*, 1997). The results of the present study indicate that these differences may be related to different activities of digestive enzymes. Optimum pH values for the enzymes, except for lactase, were similar for both hybrids, and considerably different from those observed in

Siberian sturgeon (Żółtowska *et al.*, 1999). Lipases of SR.R and SR.S hybrids digest triacylglycerides in more alkaline environment (pH 8.0) compared with pure species (pH 7.3). Similarly high pH optima for lipases were observed in *Pagrus major* (Iijima *et al.*, 1998). Both hybrids showed, however, lower (by 1.5) pH optima for α -amylase and saccharase while pH for maltase and trehalase were more alkaline. The pH optimum for SR.S lactase (6.39) was similar to that reported for Siberian sturgeon (6.6) (Żółtowska *et al.*, 1999), but in SR.R the same enzyme is most active at more acidic pH (4.72). That value is probably closer to lactase optimum pH in Russian sturgeon, no data were, however, found on the digestive enzymes of that species.

The activities of fish digestive enzymes depend on feeding preferences (Kuz'mina, 1996; Hidalgo *et al.*, 1999), and change with fish age (Buddington and Droshov, 1986; Gawlicka *et al.*, 1995; Martinez *et al.*, 1999). The effect of age on the digestive system development and enzymatic activity in Siberian sturgeon was described by Gisbert *et al.* (1999), Łopieńska *et al.* (1999), and Żółtowska *et al.* (1999). The activities of sturgeon digestive enzymes also depend on food composition (Lin *et al.*, 1997). In the present study, both fish age and diet composition affected the results obtained in the 4th and 5th week of rearing. In the 4th week fish diet was changed from carbohydrate-rich ASTA-AC to high-protein trout starter Aller-Aqua. Maľdov and Savushkina (1997) reported that high-protein feed induced an increase in neutral protease activity, and a slight decrease in the activity of acidic ones in Siberian sturgeon. The changes in activities of these enzymes were observed already after a week, and complete adaptation took 3 weeks. In the present

Table 1. The ratio of acidic/alkaline protease activity during rearing of reciprocal sturgeon hybrids

Weeks of rearing	Hybrids	
	SR.R	SR.S
3	0.25	2.51
4	1.83	1.57
5	2.34	2.18
6	1.43	0.58
7	2.87	2.09
8	2.71	1.45
9	3.43	1.89
10	1.84	1.06

study a considerable increase in both acidic and alkaline protease activities occurred after 3 weeks of feeding Aller-Aqua feed (Figure 6). It should be stressed that the initial proteolytic activities differed in both hybrids. In the 3rd week of rearing SR.R showed 9 times lower acidic protease activity compared with SR.S (Figure 6). The difference explains the lower SR.R growth rate at that time (Figure 1).

After the change in diet, SR.R showed a fast increase in acidic protease activity, typical of stomach digestion, and these enzymes predominated over the intestinal alkaline proteases (Figure 6). Also in SR.S acidic proteases were more active compared with the alkaline ones (Figure 6) but the ratio of acidic/alkaline protease activity was always higher in SR.R, beginning from the 4th week of study (Table 1). We suppose that beginning from the 4th week of rearing, stomach digestion played a more important role in SR.R hybrid than in SR.S, similarly like in young beluga (Belyayeva *et al.*, 1997). Moreover, from the 6th week trypsin activity was also higher in SR.R than in SR.S (Figure 7). Lemieux *et al.* (1999) reported that in Atlantic cod trypsin activity was a limiting factor of fish growth. Our observations of growth rates of both reciprocal sturgeon hybrids confirm that opinion. In the first two weeks of the experiment, trypsin activity was higher in SR.S hybrid, and so was the growth rate of that hybrid (Figures 1, 7). SR.S also showed higher activities of glycolytic enzymes: amylase, maltase, saccharase, and trehalase (Figures 3–5), which indicates better enzymatic adaptation to the feed. Such an adaptation allowed SR.S hybrid for more efficient utilization of ASTA-AC feed, and resulted in faster growth compared with SR.R hybrid

(Figure 1). The situation changed after transition from ASTA-AC to trout starter that was probably more efficiently digested by SR.R, which resulted in faster growth of that hybrid from the 5th week of rearing (Figure 1).

A considerable decrease in glycolytic and lipolytic enzyme activities observed in both hybrids in the 5th week of study (Figures 2–5) could be related to genetic factors. A similar pattern was observed in Siberian sturgeon (Żółtowska *et al.*, 1999) which confirms such a hypothesis. Peres *et al.* (1998) observed a similar, diet-independent reduction of amylase activity in 29 days old *Dicentrarchus labrax*.

The understanding of basic digestion physiology phenomena, including digestive enzyme characteristics, and their interspecific differences can help to develop physiologically balanced feeds for new sturgeon hybrids, important for efficient fish rearing.

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Development and behaviour of two reciprocal back cross hybrids of Siberian sturgeon (*Acipenser baeri* Brandt) and Russian sturgeon (*Acipenser gueldenstaedti* Brandt) during early ontogenesis sturgeon

Vývoj a chování dvou recipročních zpětných hybridů jesetera sibiřského (*Acipenser baeri* Brandt) a jesetera ruského (*Acipenser gueldenstaedti* Brandt) v průběhu rané ontogeneze

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ABSTRACT: A study of two reciprocal back cross hybrids of Siberian (*Acipenser baeri* Brandt) and Russian sturgeon (*Acipenser gueldenstaedti* Brandt) was undertaken to describe their early development, and to evaluate basic factors determining stock quality. Embryonic development of both hybrids was very similar. Distinct differences in morphology and body pigmentation became obvious just after hatching. Pre-larvae of SR.S (*A. baeri* × *A. gueldenstaedti*) × *A. baeri* were longer than SR.R ones (*A. baeri* × *A. gueldenstaedti*) × *A. gueldenstaedti* until the beginning of exogenous feeding. Also their behaviour was significantly different – SR.S pre-larvae showed strong phototaxis, settled on the bottom earlier (5 days post hatching – 5 D), and started to feed earlier (12 D) compared with SR.R. After the start of exogenous feeding, SR.S mortality was caused mainly by cannibalism attempts while in SR.R – by poor feeding that resulted in starvation. Both hybrids showed high growth rates – after 57 days of rearing their body mass amounted to 2.8 ± 0.5 g (SR.S) and 3.4 ± 1.3 g (SR.R), and survival was over 50%, which indicates that they are useful for aquaculture.

Keywords: *Acipenser baeri*; *Acipenser gueldenstaedti*; sturgeon hybrids; behaviour; ontogenesis

ABSTRAKT: Studie dvou recipročních hybridů jesetera sibiřského (*Acipenser baeri* Brandt) a jesetera ruského (*Acipenser gueldenstaedti* Brandt) podává popis raného vývoje a hodnotí základní faktory určující kvalitu násadového materiálu. Embryonální vývoj obou hybridů je velmi jednoduchý. Zřetelné rozdíly v morfologii a pigmentaci těla jsou zřejmé právě po vylíhnutí. Prelarvální stadia SR.S – (*A. baeri* × *A. gueldenstaedti*) × *A. baeri* byla delší než SR.S (*A. baeri* × *A. gueldenstaedti*) × *A. gueldenstaedti* na začátku exogenní výživy. Také jejich chování bylo průkazně rozdílné, prelarvální stadia SR.S vykazovala zápornou fototaxi, na počátku se vyskytovala na dně (5 dnů po vylíhnutí). První příjem potravy zahajovala 12. den ve srovnání se SR.S, kde nízký příjem potravy vyvolával hladovění. Obě hybridní formy prokázaly vysokou rychlost růstu. Za 57 dnů odchovu bylo dosaženo individuální hmotnosti 2,8 g (SR.S) a 3,4 g (SR.R) a přežití vyšší než 50 %. To indikuje jejich využitelnost v akvakultuře.

Klíčová slova: *Acipenser baeri*; *Acipenser gueldenstaedti*; hybridy jesetera; chování; vývoj

Information on body morphological and physiological development and behaviour during early ontogenesis is of key importance for advancement of fry rearing biotechnology. Such knowledge is necessary for assessment of environmental and feeding demands of sturgeon pre-larvae and larvae, and for evaluation of stocking material quality. These issues were studied on some sturgeon species (Detlaf *et al.*, 1981; Szczepkowski *et al.*, 2000) but the data for hybrids are missing.

The aim of the present study was to describe early development of two hybrids of Siberian (*Acipenser baeri* Brandt) and Russian sturgeon (*Acipenser gueldenstaedti* Brandt). The results, besides their scientific value, would be of practical importance since sturgeon hybrids are important aquaculture species in Poland and in other European countries (Hochleithner, 1991; Williot *et al.*, 1993; Kolman 1999).

MATERIAL AND METHODS

The eggs of Siberian and Russian sturgeon hybrid females were obtained in accelerated artificial breed-

ing (Kolman and Szczepkowski, 2001) and divided into two groups. One group of eggs was fertilized using Siberian sturgeon sperm, and the other – with Russian sturgeon sperm. That resulted in two reciprocal back cross hybrids: SR.S – (*A. baeri* × *A. gueldenstaedti*) × *A. baeri*, and SR.R (*A. baeri* × *A. gueldenstaedti*) × *A. gueldenstaedti*. Fertilized eggs were incubated in the Weiss apparatuses, at the average water temperature 14.5°C (Figure 1), dissolved oxygen concentration 9.3–10.5 mg/l, and pH 7.2–7.8. Water flow rate ranged from 0.032 to 0.068 l/s. Newly hatched larvae of both sturgeon hybrids were transferred to the separate plastic rotation tanks of 2 m × 2 m dimensions and 0.5 m depth, included into the water recirculation system. Each tank was stocked with 33.5 thousand fish.

From the beginning of exogenous feeding the fish were fed ASTA-AC feed, according to the feeding curves (Kolman *et al.*, 1996). For the first 5 days of feeding, small amounts of brine-shrimp (*Artemia* sp.) larvae were also provided. When the fish body mass amounted to 150 mg, a trout starter Nutra Amino Balance (54% of protein, 18% of fat) was used. The hatchery was continually lightened, and the light intensity (measured over the water

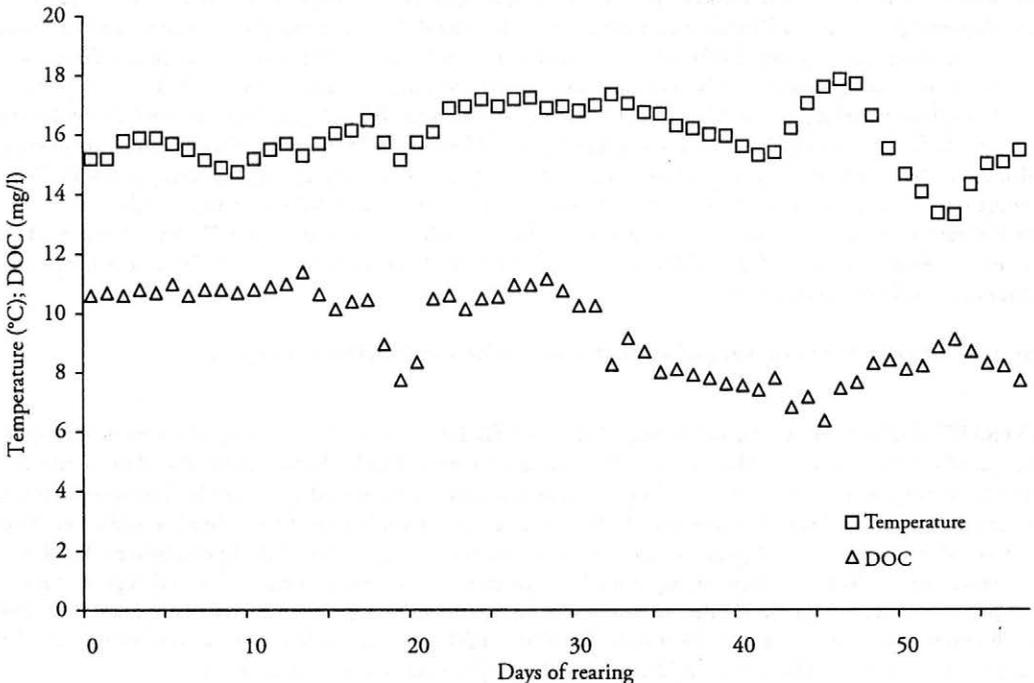


Figure 1. Water quality parameters of experimental sturgeon rearing

surface in various parts of the tank) ranged from 43 to 68 lx. Fish reaction to light was tested using a 60 W bulb placed in the corner of the adjacent tanks while the hatchery lights were off. The light intensity in the lighted corners was 110 lx in SR.R tank, and 113 lx in the SR.S one, whereas in the opposite corners – 1 and 0 lx, respectively.

Water temperature, dissolved oxygen concentration (Hanna HI 9143), pH (PM 600), and water flow were measured daily. Total ammonia nitrogen (TAN) was measured using direct Nessler method, and nitrite level (NO_2^-) with sulphanilic method (Hermanowicz *et al.*, 1999), both in 2–3 day intervals. Maximum of TAN and nitrite level were 0.28 mg/l and 0.17 mg/l, respectively.

Embryonic and postembryonic development was observed until the beginning of exogenous feeding. Fish growth rate, survival, and behaviour were measured for 57 days from hatching.

Observations of embryonic and postembryonic development were done on live and preserved fish, using a binocular Nikon SMZ 10A. The fish were fixed with Bouin solution, and washed with 70% ethanol. The samples of 10–30 fishes were taken every 1–8 h during embryonic development, and daily after hatching.

Until day 12 post hatching, total body length was measured using Carl Zeiss micrometer eyepiece, with 0.01 mm accuracy. When the fish started exogenous feeding, they were weighed and measured weekly, with 1 mg and 1 mm accuracy, respectively. Condition coefficients (Fulton's coefficient) were calculated according to the formula:

$$K = 100 * \frac{W}{L_T^3}$$

where: K = condition coefficient

W = body weight (g)

L_T = total body length (cm)

RESULTS

Embryonic and postembryonic development

Embryonic development of both sturgeon hybrids was similar, and all the stages occurred at the same time (Table 1). Egg pigmentation slightly differed (SR.R eggs were darker), and so did the head size (larger in SR.S). More pronounced differences between the hybrids were observed during postembryonic development (Table 2). Pre-larvae of SR.S

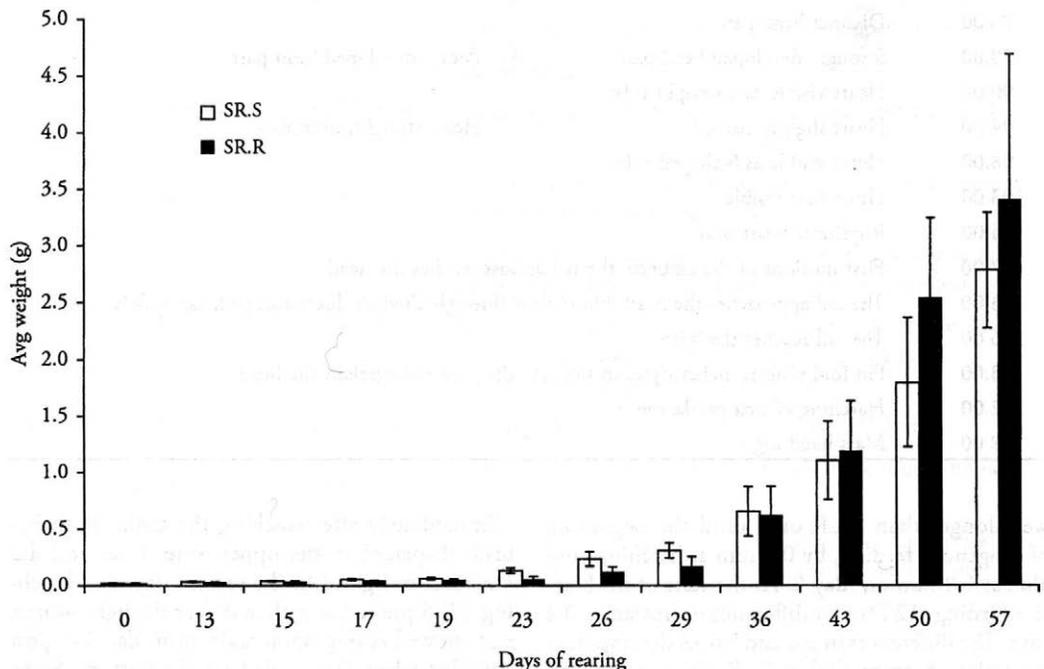


Figure 2. The increase of body mass of two reciprocal back cross hybrids of Siberian and Russian sturgeon

Table 1. Embryonic development of two reciprocal back cross hybrids of Siberian and Russian sturgeon

Time from fertilization (h)	SR.S hybrid	SR.R hybrid
0.00	Fertilization	
4.00	First cleavage fissure visible	
5.00	First cleavage fissure reaching the equator	
5.30	Start of second cleavage division, first fissure surpasses the equator	
6.30	First fissure intersects across the animal pole, the second one reaches the equator, perivitelline space clearly visible	
7.00	Start of third division	
26.30	Dark cell belt visible around the equator	
28.00	Start of gastrulation, small pit on the equator	
41.00	Stage of medium stopper	
47.00	Stage of small stopper (on vegetative pole), grey micromere aggregation on the animal pole, the egg looks as if it had two stoppers: black one at the bottom, and bigger grey one at the top	
52.00	Lower stopper entirely sealed, neural channel visible	
55.00	Neural channel reaches 1/3 of egg circumference, its anterior part enlarged, perivitelline space small	
67.00	Neural channel reaches almost 1/2 of egg circumference, germs of excretory organs visible in the anterior part, neural tube still open near the vegetative pole	
	Poorly pigmented egg	Strongly pigmented egg, pigment most concentrated at the embryo's sides, metameres visible
70.00	Distinct head part	
79.00	Strongly developed head part	Poorly developed head part
90.00	Heart visible as a straight tube	
94.00	Heart slightly curved	Heart straight, immobile
98.00	Heart visible as S-shaped tube	
103.00	Heart beat visible	
114.00	Rhythmic heart beat	
117.00	First motions of the embryo, the tail almost reaches the head	
123.00	The tail approaches the head, blood flow through Cuvier's ducts and yolk sac vessels	
126.00	The tail reaches the head	
138.00	Fin fold visible, melanocytes in the intestine, the tail overlaps the head	
152.00	Hatching of first pre-larvae	
168.00	Mass hatching	

were longer than SR.R ones until the beginning of exogenous feeding, by 0.7 mm at hatching, and almost 1.8 mm on day 8. At the start of SR.S active feeding (12 D) that difference decreased to 0.4 mm. The differences in eye and barbel development were also observed (Table 2). Body pigmentation developed much faster in SR.R while SR.S showed earlier reduction of embryonic fold.

Immediately after stocking the tanks, both hybrids dispersed in the upper water layer and did not react to light. On the second day after hatching, SR.S pre-larvae gathered near the light source, and showed strong phototaxis until day 5–7 post hatching when they settled on the bottom. Some SR.S pre-larvae showed no phototaxis on the second day post-hatching, and 10–20% of them

gathered in dark places. On subsequent days the share of photophobic fish increased. The negative phototaxis disappeared when the larvae settled on the bottom (7–9 day).

The beginning of exogenous feeding was also different in both hybrids. Ejection of melanin stoppers

from the digestive tract started on the 12th day in SR.S larvae, and in most fish ended on the 13th day. In SR.R that process started on the 13th day and ended on the 16th day post hatching.

During exogenous feeding, the SR.S larvae stayed all the time on the tank bottom, and only some fish

Table 2. Postembryonic development of two reciprocal back cross hybrids of Siberian and Russian sturgeon.

Time from hatching (h)	Total body length (mm)		SR.S hybrid	SR.R hybrid
	SR.S	SR.R		
0	10.08 ± 0.26	9.36 ± 0.12	Mass hatching, the larvae vigorously leave the Weiss apparatus. Mouth opening closed, slight invagination of olfactory opening	
1	11.15 ± 0.18	10.07 ± 0.20	Mouth opening starts to open, germs of pectoral fins visible	
			Clearly visible eye vesicle	Poorly visible eye vesicle
2	12.08 ± 0.14	10.89 ± 0.30	Four lumps near the upper lip, germs of barbels. Body segmentation fully developed	
			Eye vesicle distinct (round white spot)	Poorly developed eye vesicle
3	13.05 ± 0.24	11.90 ± 0.20	Mouth open, barbels start to grow	
4	14.31 ± 0.35	13.69 ± 0.50	Olfactory septum develops, dorsal fin rays visible, gill primary lamellae longer than opercula	
			Olfactory opening elongated	Olfactory opening shorter
5	14.68 ± 0.26	13.97 ± 0.16	Anal fin ray germs	
			Larvae migrate to the bottom	Larvae stay dispersed in the water
6	15.88 ± 0.34	15.13 ± 0.24	Start of ventilation, small ventral fins appear, olfactory septum fully developed in most individuals	
7	16.99 ± 0.58	15.21 ± 0.35	Pectoral fins translocate towards the lower part of yolk sac, dorsal fin distinctly developed, anal fin poorly developed	
			Middle and posterior body parts pigmented, in anterior part pigmentation poor	Larvae migrate to the bottom, entire body pigmented
8	17.73 ± 0.26	15.97 ± 1.02	Fin fold reduced	Fin fold still distinct
9	18.18 ± 0.24	16.05 ± 0.41	Dorsal fin rays distinct (about 20)	Poorly visible dorsal fin rays
10	18.20 ± 0.64	17.54 ± 0.54	Barbels elongated, reach the mouth opening	Barbels shorter and thick, do not reach mouth opening
12	18.67 ± 0.68	18.25 ± 0.40	Mass ejection of melanin stoppers	
14	–	–	Mass ejection of melanin stoppers	

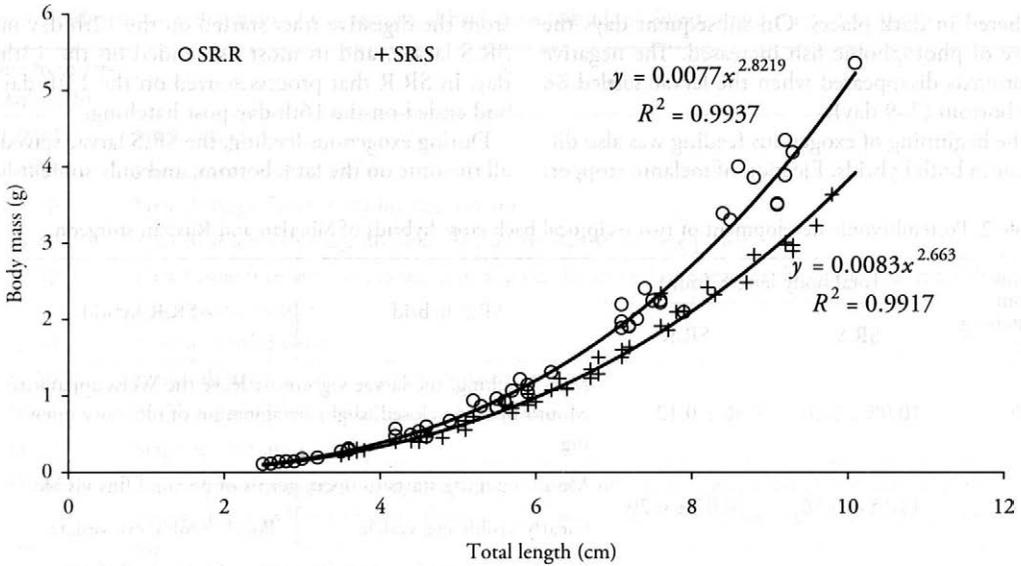


Figure 3. Comparison of relationships between body mass and total length in two reciprocal back cross hybrids of Siberian and Russian sturgeon

swam in the upper water layer. The SR.R larvae, after a short time of bottom dwelling, dispersed in entire water volume. In the case of both groups, open water individuals were deprived of food, and many of them starved. At the onset of exogenous feeding, cannibalism occurred. Examination of dead individuals done on the 15th day post hatching revealed severely damaged pectoral fins in 100% of SR.S fish, while in SR.R group only 62.6% of dead fish showed minor fin damage. At the same time, the live individuals of both hybrids also showed fin lesions – 30.7% and 11.3%, respectively.

Body weight and length increase, and fish survival

At the beginning of rearing, SR.S showed higher growth rate but the difference decreased with time, and from day 43 post hatching SR.R hybrids grew faster. At the end of the experiment (57 D), average body weight amounted to 3.4 ± 1.3 g in SR.R, and 2.8 ± 0.5 g in SR.S (Figure 2). Body length increase was faster all the time in SR.S that reached 89.3 ± 6.8 mm at the end of experiment, while SR.R – 85.7 ± 12.7 mm. The values of condition coefficient decreased with time, and were higher in SR.R

than in SR.S (Figure 3). The difference in condition coefficient between the hybrids increased with fish age – on the 29th day post hatching it was equal to 15.9% (0.68 in SR.R and 0.57 in SR.S), and on the 57th day it reached 32% (0.54 and 0.37, respectively). Survival of SR.S was 54.8%, and of SR.R hybrid – 52.8% compared with the initial stock number. The highest mortality of SR.R fish occurred at the onset of exogenous feeding, with the maximum on the 16th day post hatching (10.3%) (Figure 4). At the same time the highest mortality in SR.S was lower – max. 4.07% (15 D). The second mortality peak in SR.R group, equal to 10.3%, occurred between days 22 and 26.

DISCUSSION

Embryonic and postembryonic development of two Siberian and Russian sturgeon hybrids was similar like in other *Acipenseridae* (Detlaf *et al.*, 1981; Conte *et al.*, 1988; Szczepkowski *et al.*, 2000). The embryos of both hybrids attained successive stages at the same time, and slight differences were observed only in body pigmentation and head size. More pronounced differences occurred in postembryonic life, and concerned both

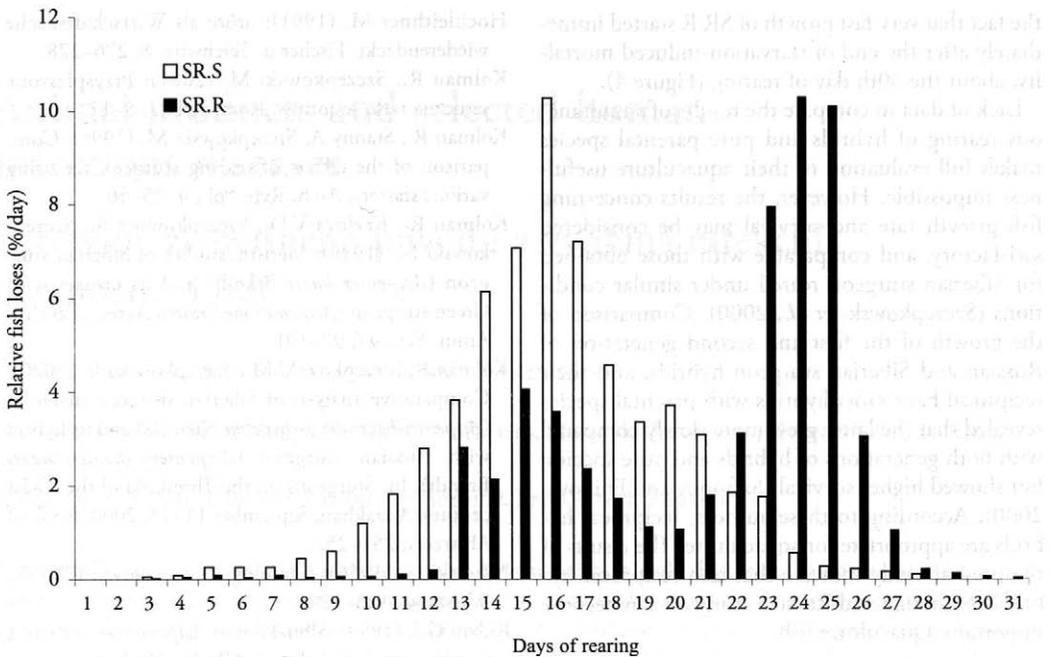


Figure 4. Fish mortality during the rearing of two reciprocal back cross hybrids of Siberian and Russian sturgeon

morphology and behaviour. Both hybrids were similar to the paternal species – SR.S resembled Siberian sturgeon, and SR.R – Russian sturgeon. Similarity to the paternal species was reported also for other sturgeon hybrids: Siberian and green sturgeon (*Acipenser medirostris* Ayres), or Siberian and Russian sturgeon (Kolman *et al.*, 1999, 2000).

Both hybrids also differed in terms of behaviour. Their reactions to light were different during early postembryonic development. Pre-larvae of SR.S showed strong phototaxis, similarly like Siberian sturgeon (Gisbert *et al.*, 1999; Szczepkowski *et al.*, 2000), while SR.R pre-larvae were indifferent or photophobic, which is typical of Russian sturgeon. That species is indifferent to light during the entire pre-larval period (Baburina, 1972), or, according to Nikolski (1956), shows negative phototaxis. The behaviour of hybrids after the beginning of exogenous feeding was also much the same as in parental species: SR.S searched for food primarily on the bottom, the same way as Siberian sturgeon (Gisbert *et al.*, 1999), while SR.R dispersed in open water like Russian sturgeon (own observation). Fish behaviour considerably affected the results of rear-

ing. In SR.S strong cannibalism and aggression resulted in severe fin damage, while SR.R hardly accepted artificial feed which led to starvation. These disturbances were probably enhanced by high stocking density (about 8 thousand larvae per m^2) since simultaneous rearing of the same hybrids at the density of 5 thousand per m^2 resulted in 10% lower fish loss.

The initially faster growth of SR.S hybrid resulted mainly from 2 days earlier start of active feeding compared with SR.R. The beginning of feeding of both hybrids was determined by water temperature (16°C) that was probably more appropriate for SR.S, since thermal optimum for early stages of this species is slightly higher than for Russian sturgeon (Ruban, 1999). Earlier start of feeding by SR.S could also result from the fact that they stayed on the bottom where food was abundant. Fish growth rate was also related to the differences in fish mortality. Mortality of SR.R was size-related since deaths of the smallest, starving fish were observed, while larger fish survived. In SR.S mortality was size-independent, fish died from injuries, and dead individuals were of various size. It is confirmed by

the fact that very fast growth of SR.R started immediately after the end of starvation-induced mortality, about the 30th day of rearing (Figure 4).

Lack of data to compare the results of simultaneous rearing of hybrids and pure parental species makes full evaluation of their aquaculture usefulness impossible. However, the results concerning fish growth rate and survival may be considered satisfactory, and comparable with those obtained for Siberian sturgeon reared under similar conditions (Szczepkowski *et al.*, 2000). Comparison of the growth of the first and second generation of Russian and Siberian sturgeon hybrids, and their reciprocal back cross hybrids with parental species revealed that the latter grew more slowly compared with both generations of hybrids and pure species, but showed higher survival (Safronov and Filipova, 2000). According to these authors, reciprocal hybrids are appropriate for aquaculture. The results of the present study indicate that also reciprocal hybrids of Siberian and Russian sturgeon can become important aquaculture fish.

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Animal products and selected human infectious diseases

Potraviny živočišného původu a vybraná infekční onemocnění lidí

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ABSTRACT: The developing of close contacts of humans with animals that served humans for meat and as guardians brought among others the exchange of microflora in both organisms. By inhalation of aerosol from animal's hair and scales, and especially by consumption of animal meat, eggs and milk, men became a part of natural circulation of animal microorganisms. Many of originally zoopathogenic agents did not survive in human hosts, other some got adapted and slowly became zoonthropopathogenic and cause typical human diseases e.g.: tuberculosis (TBC), Acquired Immunodeficiency Syndrome (AIDS), Ebola virus, Creutzfeldt-Jacob disease. From the epidemiological point of view the animals are an important source of non-typical human diseases. This leads to higher susceptibility and more severe symptoms of the disease in humans (rabies, tick-borne encephalitis). The most common zoonthroposes are acute alimentary infections and toxicosis. The authors aimed to review the epidemiology of bacterial infectious diseases that are transmitted through primary contaminated animal food (salmonellosis, campylobacteriosis, yersiniosis, *E. coli*-enteritidis, listeriosis). The primary and secondary prevention of the diseases both in human and veterinary medicine can limit the transmission and development of the infections. Making food safe in the first place is a major effort, involving the farm and fishery, the production plant or factory, and many other points from farm to table. For some particularly risky foods, even the most careful hygiene and sanitation are insufficient to prevent contamination, and a definitive microbe-killing step must be included in the process. Careful review of the whole food production process can identify the principal hazards, and the control points where contamination can be prevented, limited, or eliminated. A formal method for evaluating the control of risk in foods exists is called the Hazard Analysis Critical Control Point (HACCP) system.

Keywords: animal and food-related public health risks; bacterial contamination; mode of transmission; acute food-borne infection; preventive measures

ABSTRAKT: Postupné sblížení člověka se zvířaty a rozšíření chovu zvířat za účelem pomoci i obživy přineslo i vzájemnou výměnu fyziologické mikroflóry obou živočišných druhů. Při těsném soužití, při inhalaci aerosolu ze srsti a kožních šupin a zejména konzumací masa, vajec a mléka, se člověk stal náhodným článkem v přírodním koloběhu zvířecích mikroorganismů. Mnoho původně pouze zoopatogenních původců infekčních nemocí v prostředí lidského organismu nepřežilo. Jiné mikroorganismy se adaptovaly a postupně se staly zoonthropopatogenní či v současnosti vyvolávají typická lidská onemocnění včetně interhumánního přenosu, například TBC, AIDS, Ebola virus, Creutzfeld-Jacobova nemoc. Zvířata jsou z epidemiologického pohledu významným zdrojem pro člověka neobvyklých nákaz. Důsledkem je většinou vyšší vnímavost a výrazně těžší klinický průběh infekcí u člověka než u zvířat (klíšťová encefalitida, vzteklinka). Nejčastější zoonthropozózy jsou akutní alimentární infekce a toxikózy. Autoři sdělení se zabývají epidemiologií alimentárních infekcí bakteriálního původu, kdy vektorem přenosu pů-

vodců je primárně kontaminovaná živočišná potravina (salmonelózy, kampylobakteriόzy, yersiniόzy, enteritidy *E. coli*, listeriόzy). Primární a sekundární prevence zoonózních onemocnění jak v oblasti veterinární, tak i lidské medicíny mohou omezit jejich šíření a pozitivně ovlivnit aktuální epidemiologickou situaci. Na prvním místě se jedná o bezpečnou výrobu potravin jak na farmách a rybářských pracovištích, v rostlinných výrobnách, tak i v celém dalším výrobním procesu až ke stolu. Pro některé zvláště rizikové potraviny i nejpřísnější hygienické a sanitární podmínky v místě původu nestačí k zabránění kontaminace. Proto musí být zařazen kvalitní a bezpečný postup inaktivace mikroorganismů cíleně během zpracování potravin. Pečlivá analýza celého procesu výroby potravin může určit hlavní rizika a kontrolní body, kde kontaminaci potravin může být zabráněno, limitováno či eliminováno. Standardní metoda k prevenci rizika infekce z potravin se jmenuje Hazard Analysis Critical Control Point (HACCP).

Klíčová slova: živočišné potraviny; epidemiologické riziko; bakteriální kontaminace; cesta přenosu; akutní alimentární infekce; preventivní opatření

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1. INTRODUCTION

A large group of human infectious disease agents is characterized by originating in animal hosts – so called zoonoses. The first important familiar contacts of people with animals were recorded already in the Neolithic period. Such animals were wolves and dogs that consumed wastes outside the human homes. At the same time, they helped people to hunt and catch animals and served them as guardians. In those cases of intimate associations human microflora was exchanged with that of the animals. That brought a risk of new human infections. Typical examples are rabies, anthrax, tularemia, etc.

The domestication of other animal kinds meant new infections to human beings. Birds and pigs came up to people apparently also as searchers for wastes. Rats and mouse fed on wastes allured cats. Small mammals, rabbits and guinea pigs were caught and bred in captivity for meat. Four thousand years ago, horse, beef cattle, goats and sheep were held as draught animals and also for meat.

People became infected both by inhalation of animals' hair and scales, and by consumption of their meat, milk and eggs. Many etiologic agents

can not survive in the human body environment. Being natural agents of animal diseases, they are not transmitted under natural conditions from man to man. Of some of the causal agents of zoonoses, new types split during evolution getting more and more adapted to survival in man. They now cause typical human diseases, for example *Mycobacterium tuberculosis* evolved apparently from *M. bovis*, the causal agent of bovine tuberculosis. *M. tuberculosis* is now transmitted only from man to man. Similarly originated smallpox from cowpox, rickettsial typhus from murine typhus, etc. Human clinical symptoms of some infections are more serious as by animals (Bakoss, 1999).

From the epidemiological point of view zoonoses are also important as the main source of new, emerging infections. For example, recognized in the early 80s, was in genetic studies linked to an infection of apes caused by a similar retrovirus agent. In Africa, a new agent called Ebola virus was recently involved in at least four epidemics and numerous smaller outbreaks after its recognition in 1976. In 1986, a new disease in cattle, bovine spongiform encephalopathy, was recognized in Europe and has been associated in time and place with previously unknown variant of Creutzfeldt-Jacobs' disease.

We can divide zoonoses into several groups on the epidemiological basis of selected human infections attached to animal productions.

A large proportion of zoonoses are connected with preparation and consumption of primarily or secondarily contaminated food. Acute food-borne disease infections and intoxications are much more of a concern to governments and the food industry today than a few decades ago. Some of the factors that have led to this include: 1. the identification of new agents that have caused life-threatening conditions; 2. the finding that traditional agents are associated with foods that were of no concern previously; 3. an increasing number of large outbreaks being reported; 4. the impact of food-borne disease on children, the aging population and the immunocompromised; 5. migrant populations demanding their traditional foods in the countries of settlement; 6. the ease of world shipment of fresh and frozen food; and 7. the development of new food industries, including aquaculture (Todd, 1997).

In our paper we aimed to review selected bacterial infectious diseases transmitted by means of primarily contaminated animal products.

2. EPIDEMIOLOGY OF BACTERIAL ZOOANTHROPOSES TRANSMITTED BY PRIMARILY CONTAMINATED ANIMAL PRODUCTS

2.1. Salmonellosis

Salmonella has been known to cause illness for over 100 years. The first isolate was detected from pigs, later on from other kinds of animals. An American scientist Salmon, after whom they are named, discovered them. Salmonella is Gram negative rod-like bacteria.

In 1914, 12 serotypes of salmonella bacteria were described, by now 2375 of them are known. The distribution of salmonellosis is worldwide (Švrček, 1998).

Salmonellosis (A 02) is the most frequent alimentary infection in the Czech Republic. The number of illnesses from 1952 was slightly increasing with the peak in 1981 (146 patients/100 000 inhabitants). After a period with constant number of cases, in 1989 a pandemic occurred not only in our country (above 400 patients/100 000 inhabitants), but also in Western Europe, Great Britain and United States

of America, where it started in 1985. Such frequency of cases have lasted till now. The salmonella sepsis (A.02.1) occurred in 0.2–0.3 cases/100 000 inhabitants in the period from 1996–1999. The mortality of acute salmonellosis was 26 persons in 2000 (25 cases of *S. enteritidis*, 1 case of *S. montevideo*) (Beneš, 2001). Because many milder cases are not diagnosed or reported, the actual number of infections may be twenty or more times greater. Salmonellosis is more common in summer than in winter (Benenson and Chin, 1995; Berends *et al.*, 1998).

Salmonella can affect all kind of mammals, birds or cold blood animals; the most susceptible are the younger ones. The species often involved in clinical symptoms are by beef cattle – *S. dublin*, *S. typhimurium*, *S. enteritidis*, *S. newport*, by pigs – *S. typhimurium*, *S. typhisuis*, by sheep and goats – *S. typhimurium*, *S. anatum*, by horses – *S. typhimurium*, *S. enteritidis*, *S. anatum*, *S. newport*, *S. heidelberg*. The sources of infection are ill animals and carriers and they are the most important source of the disease.

Animals are often infected perorally; other modes of entrance (per nostril, per conjunctiva) are rare. Microorganisms rapidly reach the lymphoid tissue of intestine or regional lymphatic nodes. This results in enteritis; in the case of bacteremia, etiological agents are disseminated in the organism. Acute illness can pass to chronic infection (enteritis, arthritis, pneumonia). Persistence of salmonella bacteria is possible; mainly in lymphatic nodes, tonsils, intestine wall, parenchymatous organs and a biliary system (Morgan *et al.*, 1994; Suzuki, 1994; Humbert and Salvat, 1997).

Salmonella is usually transmitted to humans by eating contaminated foods that usually look and smell normal (Ferretti *et al.*, 2001). Contaminated foods are often of animal origin, such as beef, poultry, milk, or eggs or egg-containing food, but all foods may become contaminated (Urfer *et al.*, 2000). Many raw foods of animal origin are frequently contaminated. The unwashed hands of an infected food handler, who forgot to wash his hands, may also contaminate food or his hands with soap after using the bathroom (Berends *et al.*, 1998; Fell *et al.*, 2000).

Most persons infected with salmonella usually develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment. However, in some persons the diarrhea may be so severe that the patient needs to be hospital-

ized. In these patients, the salmonella infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics (Chambers and Aster, 1995). Antibiotics are not usually necessary unless the infection spreads from the intestines. Unfortunately, some salmonella bacteria have become resistant to antibiotics, largely as a result of the use of antibiotics to promote the growth of feed animals. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness. Persons with diarrhea usually recover completely, although it may be several months before their bowel habits are entirely normal. A small number of persons, who are infected with salmonella, will go on to develop pains in their joints, irritation of the eyes, and painful urination. This is called Reiter's syndrome. It can last for months or years, and can lead to chronic arthritis, which is difficult to treat. Antibiotic treatment does not make a difference in whether or not the person later develops arthritis.

Salmonella can be detected in the stools of an infected person. Once the bacteria are identified, further testing can determine its specific type and antibiotic sensitivity.

2.2. Campylobacteriosis

The second most common alimentary bacterial infection in the Czech Republic is campylobacteriosis (A 04.5) and its health and epidemiological significance grows up all the time – from 21.7 patients/100 000 inhabitants in 1993 to 163 patients/100 000 inhabitants in 2000 (Beneš, 2001). Higher incidence of campylobacteriosis can be connected among others with higher consumption of poultry. Some epidemiological parameters of salmonellosis and campylobacteriosis (e.g. mode of transmission, vectors of transmission) are similar (White *et al.*, 1977). Virtually all cases occur as isolated, sporadic events, not as a part of large outbreaks. Clinical symptoms occur without symptoms of sepsis. The main etiological agent is *Campylobacter jejuni*; in 2000 it was isolated from 98% of investigated stools in the Czech Republic.

Even though surveillance is very limited, over 10 000 cases are reported to the Centers for Disease Control and Prevention (CDC) each year, equaling approximately six cases per each 100 000 persons in the population. Many more cases go undiagnosed

or unreported, and campylobacteriosis is estimated to affect over 2 million persons every year, or 1% of the population. Campylobacteriosis occurs much more frequently in the summer months than in the winter. The organism is isolated from infants and young adults more frequently than from other age groups and from males more frequently than females (Benenson and Chin, 1995). Although campylobacter does not commonly cause death, it has been estimated that 500 persons with campylobacter infections may die each year (Roels *et al.*, 1998; Fields and Sverdlow, 1999).

The campylobacter organisms are actually a group of spiral-shaped bacteria. In animals the following agents are important: *C. cinadei*, *C. coli* (etiological agents of enteritis, diarrhea and colitis in small pigs), *C. concisus*, *C. cryaerophila*, *C. fenilliae*, *C. fetus*, subsp. *fetus* (sporadic aborticide for beef cattle and enzootic aborticide for sheep), *C. fetus* subsp. *veneralis* (causal organism of abortion of beef cattle), *C. hyointestinalis*, *C. jejuni* subsp. *jejuni* (etiological agents of hepatitis in birds and cofactor for winter diarrhea of beef cattle), *C. jejuni* subsp. *doyle*, *C. lari*, *C. mucosalis*, *C. nitrofigillius*, *C. sputorum* subsp. *sputorum* (causative agent of enteritis in young animals), *C. sputorum* subsp. *bubulus*, *C. sputoru* subsp. *fecalis*, *C. upsaliensis*.

Many chicken flocks are silently infected with campylobacter; that means the chickens are infected with the organism but show no signs of illness. *C. jejuni* can colonize the poultry intestinal tract and its subsequent shedding can result in environmental contamination and subsequently in an increased risk of infection for the rest of the flock (Achen *et al.*, 1998; Norton *et al.*, 2001). No evidence was found for vertical transmission from breeder flocks via the hatchery to progeny, nor for horizontal transmission from one broiler flock to the next via persistent contamination of the broiler house (Jacobs-Reitsm, 1977).

Campylobacter can be easily spread from bird to bird through a common water source or through contact with infected feces. When an infected bird is slaughtered, campylobacter can be transferred from the intestines to the meat. It is also present in the giblets and in porcine liver (Moore *et al.*, 1998). A match, case-control study identified tuna salad as the likely food item associated with a food borne outbreak of *C. jejuni* infection in a summer camp in Wisconsin (matched odds ratio = 22; 95% confidence intervals /CI/ = 3.6–908) (Roels *et al.*, 1998).

Unpasteurized milk can become contaminated if the cow has an infection with campylobacter in its udder or the milk is contaminated with manure. Surface water and mountain streams can become contaminated from infected feces from cows or wild birds. This infection is common in the developing world, and travelers to foreign countries are also at risk for becoming infected with campylobacter.

Campylobacter spp. has enabled investigation of the determinative epidemiology of these organisms in a variety of situations. However, the culture methodology, i.e. the use of direct plating or enrichment, affected this subtype distribution. Moreover, the number of isolates analyzed per sample was limited. The "fla typing" (PCR-restriction fragment length polymorphism analysis of the fla A and fla B) also indicated that some campylobacter subtypes survive poultry processing better than others do. The extent of resistance to the environmental stresses during processing varied between strains. The more robust subtypes appeared to contaminate the abattoir environment, surviving through carcass chilling, and even carrying over into subsequent flocks. It was confirmed that some campylobacter-negative flocks reach the abattoir but various campylobacter subtypes rapidly contaminate the carcasses from such flocks during processing.

C. jejuni grows best at the body temperature of a bird, and seems to be well adapted to birds, which carry it without becoming ill. The bacterium is fragile, it cannot tolerate drying and can be killed by oxygen. It grows only if there is less than the atmospheric amount of oxygen present. Freezing reduces the number of campylobacter bacteria present on raw meat (Fields and Sverdlow, 1999).

Most people who become ill with campylobacteriosis get diarrhea, cramping, abdominal pain, and fever within 2 to 5 days after exposure to the organism. The diarrhea may be bloody and can be accompanied by nausea and vomiting. The illness typically lasts one week. Some persons, who are infected with campylobacter have no symptoms at all. In persons with compromised immune systems, campylobacter occasionally spreads to the bloodstream and causes a serious life-threatening infection. Virtually all persons infected with campylobacter will recover without any specific treatment. Patients should drink plenty of fluids as long as the diarrhea lasts. In more severe cases, antibiotics such as erythromycin or a fluoroquinolone can be used, and can shorten the duration of symptoms if they are given early in the

illness. Rarely, some long-term consequences can result from a campylobacter infection. Some people may have arthritis following campylobacteriosis; others may develop a rare disease that affects the nerves of the body beginning several weeks after the diarrheal illness. This disease, called Guillain-Barré syndrome, occurs when a person's immune system is "triggered" to attack the body's own nerves, and can lead to paralysis that lasts several weeks and usually requires intensive care. It is estimated that approximately one in every 1 000 reported campylobacteriosis cases leads to Guillain-Barré syndrome. As many as 40% of Guillain-Barré syndrome cases in this country may be triggered by campylobacteriosis.

The bacteria can be detected in the stool of an infected person. Most cases of campylobacteriosis are associated with handling raw poultry or eating raw or undercooked poultry meat. A very small number of campylobacter organisms (fewer than 500) can cause illness in humans.

2.3. *Helicobacter pylori*

Spiral and curved bacteria were observed in gastric samples of humans already in 1874, followed by similar observations in animals and in patients with gastric carcinoma. These microaerophilic bacteria resembled campylobacters by light microscopy and the quanine plus cytonine content, and were thus named *Campylobacter pyloridis* later changed to *C. pylori*. However, later studies on the ultrastructure and fatty acid profile of *C. pylori* as well as the 16 subunit ribosomal RNA sequences of this organism clearly revealed that it did not belong to the genus *Campylobacter*, and thus the bacterium was renamed *Helicobacter pylori* (Goodwin *et al.*, 1989). The new name reflects the helical appearance of this organism *in vivo* as well as the most common isolation place – the pylorus of the stomach. Several urease-positive organisms have been isolated from the stomach of different animals like ferret, macaque monkey, baboon, cat, dog and pig (Paster *et al.*, 1991).

Since the first isolation in 1982, *H. pylori* has been isolated in all parts of the world. Furthermore, in both asymptomatic and symptomatic populations the prevalence of *H. pylori* antibodies is age-dependent.

The reservoirs and transmission of *H. pylori* are at present not known. Animals have been sug-

gested as possible reservoirs. Helicobacter-like organism in swine could possibly be transmitted to humans through under-cooked pork products but the considerable prevalence of the *H. pylori* infection among Moslem populations argues against this theory (Megraud *et al.*, 1989; Bohmler *et al.*, 1996). An environmental reservoir for *H. pylori* is possible too. A common water source appeared to be the source of infection in one study and there is evidence that *H. pylori* can survive in chilled river water for at least a week (Graham, 1990). As human gastric mucosa is a large reservoir of this bacterium, person-to-person as the fecal-oral transmission seems possible (Hook-Nikanne, 1991).

There is a very strong evidence connecting *H. pylori* infection with the presence of gastritis. Depending on the distribution of the organism in the stomach and the duodenum, *H. pylori* can give rise to antritis, corpusitis, pangastritis, or duodenitis. Most of these inflammatory states affecting the gastric mucosa have clinical equivalents, but the guidelines for the clinical and therapeutic management of the associated conditions have not been worked out fully yet.

Several studies have shown that the risk of gastric cancer is significantly higher in patients with *H. pylori* infection as compared to healthy controls (Kolářová *et al.*, 2001).

2.4. *Escherichia coli* diarrhea

E. coli was first recognized as the cause of illness during an outbreak of severe bloody diarrhea by Escherich in 1885; the outbreak was traced to contaminated hamburgers. Since then, most infections have come from eating undercooked ground beef.

The incidence of the disease was 1 189 cases in 2000 in our country (12 patients/100 000 inhabitants) and the distribution is through the whole year and is stable during the last 10 years. The age distribution of *E. coli* infection is the highest in newborns and sucklings, after the first years of life it drops and this trend continues in next years (Benenson and Chin, 1995; Todd, 1997). In contrast to salmonellosis, where the morbidity slowly increases during the first year of life and then it occurs in high numbers.

In some persons, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic

syndrome, in which the red blood cells are destroyed and the kidneys fail. About 2–7% of infections led to this complication. *E. coli* O157 : H7, which causes hemorrhagic enteritis, eventually hemolytic-uremic syndrome, was identified in about 100 cases annually in the Czech Republic (Beneš, 2001).

The combination of letters and numbers in the name of the bacterium refers to the specific markers found on its surface and distinguishes it from other types of *E. coli* (Lhotová, 1999). The most common serovars of *E. coli* are 026, 055, 0126 and 0127. *E. coli* O157:H7 is one of hundreds of strains of the bacterium *Escherichia coli*. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness (Timm *et al.*, 1999).

Pathogenous *E. coli* cause a typical animal disease called enteritidis *E. coli* in domestic animals. The younger animals are very susceptible to this infection, especially in their first days of life. The primary infection source are ill animals that excrete the bacteria during septicemia through urine, nasal and oral secretions. The infection is spread either by direct contact, either by contaminated material. Carriers are the reservoir of the infection, so that pathogen is circulating in the animal population. The development of infection is influenced by predisposing factors, such are the hygiene during eating. The morbidity in calf is about 30–75%, with mortality around 10–50%, in pigs the infection with *E. coli* kills about 50% from all ill animals with enteropathy. The most common symptoms in newborns are enteritic or septic form of the disease.

Four types of *E. coli* can cause enteral colibacillosis:

1. Enterotoxigenic strains *E. coli* (ETEC)
2. Enteropathogenic strains *E. coli* (EPEC)
3. Enteroinvasive strains *E. coli* (EIEC)
4. Enterohemorrhagic strains *E. coli* (EHEC)

The organism can be found in a small number on cattle farms and can live in the intestines of healthy cattle. Meat can become contaminated during slaughter, and organisms can be thoroughly mixed into beef when it is ground. Bacteria present on the cow's udders or on the equipment may get into raw milk.

Infection vectors for man are the eggs of ill poultry that are contaminated during collection followed by penetration of bacteria into the egg.

Eating meat, especially ground beef, that has not been cooked sufficiently to kill *E. coli* can cause infection. Although the number of organisms required to cause the disease is not known, it is suspected to be very small. Among others the known modes of infection transmission are consumption of sprouts, lettuce, salami, unpasteurized milk and juice, and swimming in or drinking sewage-contaminated water. Young children typically shed the organism in their feces for a week or two after their illness resolves. Older children rarely carry the organism without symptoms. *E. coli* infection often causes severe bloody diarrhea and abdominal cramps; sometimes the infection causes nonbloody diarrhea or no symptoms. Usually little or no fever is present, and the illness resolves in 5 to 10 days. Infection with *E. coli* is diagnosed by detecting the bacterium in the stool. All persons who suddenly have diarrhea with blood should get their stool tested for *E. coli*.

Most persons recover without antibiotics or other specific treatment in 5–10 days. There is no evidence that antibiotics improve the course of the disease, and it is thought that treatment with some antibiotics may precipitate kidney complications. Hemolytic uremic syndrome is a life-threatening condition usually treated in an intensive care unit. Blood transfusions and kidney dialysis are often required. With intensive care, the death rate for hemolytic uremic syndrome is 3–5%. Another 8% of persons with hemolytic uremic syndrome have other lifelong complications, such as high blood pressure, seizures, blindness, paralysis, and the effects of having part of their bowel removed. Persons who only have diarrhea usually recover completely.

2.5. Yersiniosis

The distribution of yersiniosis is worldwide. The agent was first isolated at the end of the 19th century by Benenson and Chin (1995), who first cultured *Yersinia pseudotuberculosis* from a man.

Relative yersinia morbidity in the Czech Republic is 2 cases/100 000 inhabitants. In 2000, 231 illnesses were registered. The highest incidence is in children until 4 years of age. The main etiologic agent is *Y. enterocolitica*. It is a relatively infrequent cause of diarrhea and abdominal pain. Children are infected more often than adults, and the infection is more common in the winter (Benenson and Chin, 1995).

Y. enterocolitica belongs to a family of rod-shaped bacteria. Other species of bacteria in this family include *Y. pseudotuberculosis*, which causes an illness similar to *Y. enterocolitica*, and *Y. pestis*, which causes plague. Only a few strains of *Y. enterocolitica* cause illness in humans.

The major animal reservoir for *Y. enterocolitica* strains that causes human illness is pigs, but other strains are also found in many other animals including rodents, rabbits, sheep, cattle, horses, dogs, and cats. In pigs, the bacteria are most likely to be found on the tonsils.

Infection is most often acquired by eating contaminated food, especially raw or undercooked pork products. The preparation of raw pork intestines (chitterlings) may be particularly risky. Drinking contaminated unpasteurized milk or untreated water can also transmit the infection. Occasionally *Y. enterocolitica* infection occurs after contact with infected animals. On rare occasions, it can be transmitted as a result of the bacterium passing from the stools or soiled fingers of one person to the mouth of another person. This may happen when basic hygiene and hand washing habits are inadequate. Rarely, the organism is transmitted through contaminated blood transfusion (Ostroff *et al.*, 1994).

Most human illness is caused by one species, *Y. enterocolitica*. Infection with *Y. enterocolitica* can cause a variety of symptoms depending on the age of the infected person. Infection with *Y. enterocolitica* occurs most often in young children. Common symptoms in children are fever, abdominal pain, and diarrhea, which is often bloody. Symptoms typically develop 4 to 7 days after exposure and may last 1 to 3 weeks or longer. In older children and adults, right-sided abdominal pain and fever may be the predominant symptoms, and may be confused with appendicitis. In a small proportion of cases, complications such as skin rash, joint pains, or spread of bacteria to the bloodstream can occur.

Y. enterocolitica infections are generally diagnosed by detecting the organism in the stools. The organism can also be recovered from other sites, including the throat, lymph nodes, joint fluid, urine, bile, and blood.

Uncomplicated cases of diarrhea due to *Y. enterocolitica* usually resolve on their own without antibiotic treatment. Most infections are uncomplicated and resolve completely. Occasionally, some persons develop joint pain, most commonly in the knees,

ankles or wrists. The joint pain usually develop about 1 month after the initial episode of diarrhea and generally resolve after 1 to 6 months. A skin rash, called "erythema nodosum", may also appear on the legs and trunk; this is more common in women. In most cases, erythema nodosum resolves spontaneously within a month.

2.6. Listeriosis

Listeriosis, a rare but a serious infection caused by eating food contaminated with the bacterium *Listeria monocytogenes*, has recently been recognized as an important public health problem. *L. monocytogenes* is an important pathogen in maternal-child medicine, newborns, and adults with weakened immune systems. The agent was first isolated in 1926 in rabbits and guinea-pig. It was first detected in Czechoslovakia in animals in 1951 and in humans in 1953.

Epidemics of this illness have been recognized since 1981; however, sporadic cases account for the majority of cases of listeriosis (CDC, 1992). Morbidity in the Czech Republic is low – about 10 cases per year (from 8 cases in 1994 to 23 cases in 2000); with relative morbidity 0.1 patients/100 000 inhabitants.

L. monocytogenes is short G + rods that are not forming capsules and spores and are not acidoreistant. They can be found in animal organisms, in soil and on plants. The temperature of 100°C kills them in 3–5 minutes, but they can survive in 5°C in soil for 5 years. Common disinfectants kill them in 10–20 minutes. Animals can carry the bacterium without becoming ill and can contaminate foods of animal origin e.g. meats and dairy products. The bacterium has been found in a variety of raw foods, such as uncooked meats and vegetables, as well as in processed foods that become contaminated after processing – such soft cheeses and cold cuts at the deli counter (Huss *et al.*, 2000). Unpasteurized (raw) milk or foods made from unpasteurized milk may contain the bacterium (Tkačikova *et al.*, 2000).

Men get infected with listeriosis by eating food contaminated with *Listeria* (Schlech *et al.*, 1983). Babies can be born with listeriosis if their mothers eat contaminated food during pregnancy. Although healthy persons may consume contaminated foods without becoming ill, those at increased risk for infection can probably get listeriosis after eat-

ing food contaminated with even a few bacteria. Persons at risk can prevent *Listeria* infection by avoiding certain high-risk foods and by handling food properly.

A person with listeriosis has fever, muscle aches, and sometimes gastrointestinal symptoms such as nausea or diarrhea. If infection spreads to the nervous system, symptoms such as headache, stiff neck, confusion, loss of balance, or convulsions can occur.

Infected pregnant women may experience only a mild, flu-like illness; however, infections during pregnancy can lead to premature delivery, infection of the newborn, or even stillbirth.

When infection occurs during pregnancy, antibiotics given promptly to the pregnant woman can often prevent infection of the fetus or newborn. Babies with listeriosis receive the same antibiotics as adults, although a combination of antibiotics is often used until physicians are certain of the diagnosis. Even with prompt treatment, some infections result in death. This is particularly likely in the elderly and in persons with other serious medical problems.

Recommendations for persons at high risk, such as pregnant women and persons with weakened immune systems, in addition to the general recommendations are:

- Avoid soft cheeses such as feta, brie, camembert, blue-veined, and Mexican-style cheese. (Hard cheeses, processed cheeses, cream cheese, cottage cheese, or yogurt need not be avoided).
- Left-over foods or ready-to-eat foods, such as hot dogs, should be cooked until steaming hot before eating.
- Although the risk of listeriosis associated with foods from deli counters is relatively low, pregnant women and immunosuppressed persons may choose to avoid these foods or thoroughly reheat cold cuts before eating.

2.7. *Staphylococcus aureus*

In recent years numerous *in vitro* studies have demonstrated that *Staphylococcus aureus* may be internalized and survive in a bovine mammary epithelial cell line. The results showed the presence of *Staphylococcus* DNA inside the two isolated populations of cells, confirming that *S. aureus* could penetrate alveolar cells and macrophages in chronically infected cow milk (Hebert *et al.*, 2000).

3. PREVENTION OF FOOD-BORNE INFECTIONS

Making food safe in the first place is a major effort, involving the farm and fishery, the production plant or factory, and many other points from farm to table. Many different groups in public health, industry, regulatory agencies, and academia have roles to play in making the food supply less contaminated (Altrock *et al.*, 2000). Consumers can promote general food safety with their dollars, by purchasing foods that have been processed for safety. For example, milk pasteurization was a major advance in food safety that was developed 100 years ago. Buying pasteurized milk rather than raw unpasteurized milk still prevents an enormous number of food-borne diseases every day. Now juice pasteurization is a recent important step forward that prevents *E. coli* O157:H7 infections and many other diseases. Consumers can look for and buy pasteurized fruit juices and ciders. In the future, meat and other foods will be available that have been treated for safety with irradiation. These new technologies are likely to be an important step forward as the pasteurization of milk.

Food-borne diseases are largely preventable, though there is no simple one-step prevention measure like a vaccine. Instead, measures are needed to prevent or limit contamination all the way from farm to table. A variety of good agricultural and manufacturing practices can reduce the spread of microbes among animals and prevent the contamination of foods. Careful review of the whole food production process can identify the principal hazards, and the control points where contamination can be prevented, limited, or eliminated. A formal method for evaluating the control of risk in foods exists and it is called the Hazard Analysis Critical Control Point (HACCP) system. This was first developed by NASA to make sure that the food eaten by astronauts was safe. HACCP safety principles are now being applied to an increasing spectrum of foods, including meat, poultry, and seafood.

For some particularly risky foods, even the most careful hygiene and sanitation are insufficient to prevent contamination, and a definitive microbe-killing step must be included in the process. For example, early in the century, large botulism outbreaks occurred when canned foods were cooked insufficiently to kill the botulism spores. After research was done to find out exactly how much heat was needed to kill the spores, the canning in-

dustry and the government regulators went to great lengths to be sure every can was sufficiently cooked. As a result, botulism related to commercial canned foods has disappeared in this country.

Similarly the introduction of careful pasteurization of milk eliminated a large number of milk-borne diseases. This occurred after sanitation in dairies had already reached a high level. In the future, other foods can be made much safer by new pasteurizing technologies, such as in-shell pasteurization of eggs, and irradiation of ground beef. Just as with milk, these new technologies should be implemented in addition to good sanitation, not as a replacement for it.

To conclude, it is up to the consumer to demand a safe food supply; up to industry to produce it; up to researchers to develop better ways of doing so; and up to government to see that it happens, to make sure it works and to identify problems still in need of solutions.

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