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Habilitační práce

Kvalita lipidů v mase kapra obecného (*Cyprinus carpio*)

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Prohlášení

Prohlašuji, že jsem předloženou habilitační práci "Kvalita lipidů v mase kapra obecného (*Cyprinus carpio*)" zpracoval samostatně s použitím publikací uvedených v seznamu literatury. K sestavení habilitační práce jsem využil vybrané originální výsledky, které jsem společně se spoluautory uvedenými v jednotlivých publikacích získal v průběhu své vědecké práce.

V Českých Budějovicích, dne 31. května 2017 Ing. Jan Mráz, Ph.D.

Obsah

1. Seznam použitých zkratek

AA	Kyselina arachidonová (20:4n-6)
ALA	Kyselina alfa linolenová (18:3n-3)
Δ	Delta
DHA	Kyselina dokosahexaenová (22:6n-3)
DPA	Kyselina dokosapentaenová (22:5n-3)
EPA	Kyselina eikosapentaenová (20:5n-3)
HUFA	Vysoce nenasycené mastné kyseliny (20 a více uhlíků a zároveň 3 a více dvojných vazeb)
LA	Kyselina linolová (18:2n-6)
MUFA	Mononenasycené mastné kyseliny (jedna dvojná vazba)
PUFA	Polynenasycené mastné kyseliny (2 a více dvojných vazeb)
SFA	Nasycené mastné kyseliny (bez dvojných vazeb)

2. Úvod

2.1. Lipidy a jejich vliv na kvalitu masa

Lipidy jsou „velmi heterogenní skupinou organických látek nacházejících se v organismech. Jsou omezeně rozpustné ve vodě a naopak dobře rozpustné v organických rozpouštědlech (definice podle vlastností)“ (Kodíček, 2007). Jsou hlavní zásobárnou energie v těle (obsahují 2,3× více energie než sacharidy a 1,7× více než bílkoviny) a základní složkou biologických membrán (především fosfolipidy). Dále mají funkci ochrannou (tepelnou a mechanickou) a regulační (steroidní hormony, eikosanoidy).

Jejich obsah a kompozice zásadně ovlivňují kvalitu masa ryb z hlediska senzorických a technologických vlastností, nutriční hodnoty i skladovatelnosti. Z hlediska senzorických vlastností jsou lipidy nositeli chutí a vůní (Dostálová, 2011; Kawai, 1996) a jejich obsah výrazně ovlivňuje texturu masa (Andersen et al., 1997; Masilko et al., 2015). Z nutričního hlediska je v mase ryb zásadní především kompozice mastných kyselin. Ryby jsou obecně bohatým zdrojem esenciálních polynenasycených mastných kyselin (PUFA) a n-3 vysoce nenasycených mastných kyselin (HUFA, 20 a více uhlíků a 3 a více dvojných vazeb). Ty jsou důležité pro prevenci a léčbu kardiovaskulárních onemocnění, rozvoj mozku, nervové soustavy, očí a kognitivních vlastností (Helland et al., 2003; Horrocks a Yeo, 1999; Innis, 2007; Lauritzen et al., 2001; Mozaffarian a Wu, 2011; Simopoulos, 2008) (více viz kapitola 2.4.). Lipidy také významně ovlivňují skladovatelnost rybího masa, především při skladování mražených ryb a rybích výrobků. Kvůli vysokému obsahu PUFA a HUFA jsou totiž ryby náchylné k autooxidaci a následnému vzniku nežádoucích pachů a pachutí (Jacobsen, 1999; Refsgaard et al., 2000; Venkateshwarlu et al., 2004). Produkty oxidace lipidů mohou také mít nepříznivý vliv na lidské zdraví a podle typu mohou být více či méně toxické (karcinogenní, mutagenní, hepatotoxické, cytotoxické, popř. přispívají k rozvoji aterosklerózy) (Billek, 2000). Obsah tuku také významně ovlivňuje technologické vlastnosti rybího masa. Například pro uzení makrely obecné (*Scomber scombrus*) je vhodný obsah tuku v mase alespoň 10 % (Keay, 2001) a naopak příliš vysoký obsah tuku může způsobovat její praskání a trhání při uzení (Ingr, 1994).

Obsah tuku a kompozice mastných kyselin v mase ryb je vysoce variabilní a je ovlivněn mnoha faktory, jako je druh ryby, výživa, teplota prostředí, pohlaví, věk, šlechtění atd. (Henderson a Tocher, 1987; Tocher, 2003). Díky tomu lze vhodnou kombinací některých technologických kroků

v průběhu chovu a zpracování ryb dosáhnout optimálních požadovaných hodnot (více viz kapitola 2.3.).

2.2. Lipidy v akvakultuře

Akvakultura je od roku 1950 nejrychleji rostoucí odvětví produkce živočišných potravin s ročním nárůstem okolo 8 %. V současnosti pokrývá 50 % potřeby ryb pro lidskou konzumaci (FAO, 2016). Díky tomuto prudkému růstu začal být celosvětově nedostatek tradičních krmných komponent pro výrobu rybích krmiv – rybí moučky a oleje. Ty jsou vyráběny především z mořských pelagických druhů ryb nevhodných pro lidskou spotřebu. Jejich zdroje jsou ale limitované a poptávka po nich již předběhla jejich nabídku. Díky tomu cena rybí moučky a oleje výrazně vzrostla a v současnosti se tak jedná o strategické komodity (Tacon a Metian, 2008). Jsou ceněné především pro vysokou stravitelnost a vyváženost živin a aminokyselin (rybí moučka) a vysoký obsah n-3 HUFA (rybí olej). Kvůli jejich nedostatku a vysoké ceně jsou producenti krmiv nuceni tyto ingredience nahrazovat jinými, déle udržitelnými alternativami (Tacon a Metian, 2008). Rybí moučka se nahrazuje především rostlinnými moučkami a proteinovými koncentráty a rybí olej se nahrazuje především rostlinnými oleji. To má za následek postupné snižování obsahu n-3 HUFA v mase chovaných ryb (Pickova a Morkore, 2007; Sprague et al., 2016). Od roku 2006 do roku 2015 například poklesl obsah n-3 HUFA v mase lososa obecného (*Salmo salar*) ve Skotsku na poloviční hodnotu a předpokládá se jejich další pokles (Sprague et al., 2016). To je pro producenty ryb negativní jev, protože tím mohou do budoucna ztratit svou velkou výhodou na trhu. Nahrazování rybí moučky a oleje rostlinnými alternativami může také mít u některých druhů ryb za následek snížení využitelnosti krmiva, či negativní vliv na welfare ryb a životní prostředí (Montero a Izquierdo 2010). Vyřešení těchto problémů je tedy jednoznačně považováno za klíčový bod dalšího dlouhodobě udržitelného rozvoje akvakultury.

Efektivní využívání či nahrazování rybí moučky a oleje jinými alternativami je intenzivně zkoumané téma. Jsou rozvíjeny různé krmné strategie (jako je např. technologie finishing feeding nebo používání směsí olejů), které mají za cíl efektivněji využít rybího oleje tak, aby byly energetické potřeby ryb pokryty jinými levnějšími alternativami a n-3 HUFA z rybího oleje byly v co největší míře uloženy v rybím mase (Bell et al., 2004; Jobling, 2004; Lane et al., 2006; Turchini et al., 2006). Jsou zkoumány různé alternativní krmné komponenty obsahující n-3 HUFA jako je např. krill, plankton, bakteriální a řasová biomasa či odpady ze zpracování ryb (Turchini et al., 2010). Intenzivně jsou zkoumány oleje z GMO rostlin (např. olej z lničky seté (*Camelina sativa*)), které jsou

geneticky upravené tak, aby obsahovaly velké množství n-3 HUFA (Betancor et al., 2016; Betancor et al., 2015; Tejera et al., 2016). Velká očekávání jsou dále vkládána do pochopení metabolismu biosyntézy n-3 HUFA v rybím těle. Předpokládá se, že jeho hlubším pochopením získáme možnosti pro ovlivnění rybího metabolismu ve prospěch větší biosyntézy n-3 HUFA z jejich rostlinných prekurzorů (šlechtěním, optimalizací krmiva, složením tuku, použitím bioaktivních látek či genetickým inženýrstvím) (Tocher, 2003; Zheng et al., 2005). Zajímavou oblastí výzkumu je studium vlivu různých bioaktivních látek, které ovlivňují biosyntézu n-3 HUFA. Bylo zjištěno, že existuje řada látek rostlinného původu, které snižují či zvyšují efektivitu této syntézy. Například přidáním lignanů ze sezamového oleje (sesamin a sesamin/episesamin) do krmiva pro pstruha duhového (*Oncorhynchus mykkis*) došlo k výraznému zvýšení obsahu kyseliny dokosahexaenové (DHA) proti kontrolní skupině (Trattner et al., 2008a). Pomocí radio značených mastných kyselin bylo v *In vitro* studii v lososích hepatocytech prokázáno, že k tomuto zvýšení došlo větší konverzí z kyseliny alfa linolenové (ALA) (Trattner et al., 2008b). Další z těchto bioaktivních látek je např. kyselina lipoová (Trattner et al., 2007) či genistein (Schiller Vestergren et al., 2011). Vliv těchto látek je druhově specifický a v tuto chvíli není jasné, za jakých podmínek a jakým způsobem přesně na rybí organismus působí. Jedná se však o slibnou oblast, která otevírá dveře pro další rozvoj.

Další opomíjenou alternativou je lepší využití přirozené potravy v rybnících a schopnosti sladkovodních ryb efektivněji syntetizovat n-3 HUFA z jejich rostlinných prekurzorů. Přirozená potrava ryb v rybnících (plankton a bentos) je totiž bohatá na n-3 HUFA (Bell et al., 1994; Domaizon et al., 2000) a vhodným rybničním managementem je možné tyto zdroje efektivně přetransformovat do rybího těla (Mraz et al., 2012a). Jak již bylo uvedeno, sladkovodní ryby jsou schopné syntetizovat n-3 HUFA z jejich rostlinných prekurzorů (Tocher, 2003). Nicméně při tradičním chovu kapra obecného (*Cyprinus carpio*), založeném na přikrmování obilovinami, není tato schopnost efektivně využita (Mráz et al., 2012a). Nabízí se tedy možnost použití doplňkového krmiva, které by obsahovalo ALA. Z dostupných a levných krmných zdrojů se pro tyto účely nabízí extrudované lněné semínko a řepkové výlisky. Je předpoklad, že díky velkému množství sladkovodních ryb, které se celosvětově v rybniční akvakultuře produkují, začnou být v budoucnu tyto alternativy důležitou součástí řešení problému nedostatku rybího oleje.

2.3. Obsah tuku a kompozice mastných kyselin v mase kapra

Obsah tuku v mase kapra je silně variabilní a může se pohybovat od 1 % až po více než 25 % (Masilko et al., 2016; Mraz et al., 2012a; Oberle et al., 1997; Urbanek et al., 2010). Jeho obsah v mase je ovlivněn především výživou (Oberle et al., 1997). Dva hlavní faktory, které ovlivňují obsah tuku v mase kapra, jsou poměr stravitelného proteinu k stravitelné energii a kompozice esenciálních aminokyselin v dietě (Becker et al., 1983; Bureau et al., 2002; Eckhardt et al., 1982). Pokud je stravitelné energie k stravitelnému proteinu v dietě málo, dochází k tomu, že jsou proteiny metabolizovány na energii. Tím dochází k nízkému využití krmiva, zvýšení krmných nákladů a vylučování amoniaku (Bureau et al., 2002). V rybníkářské praxi k tomuto scénáři dochází v případě, že je obsádka kapra chována pouze na přirozené potravě, popř. pokud je nedostatečně přikrmována obilovinami. Pokud je naopak stravitelné energie k stravitelnému proteinu nadbytek, dochází ke zvýšené tvorbě a ukládání tuku v mase kapra (Urbanek et al., 2010). V rybníkářské praxi k tomuto scénáři dochází, pokud je přirozené potravy nedostatek (např. rybník je přesazen a rybí obsádka svým vyžíracím tlakem utlumí rozvoj hrubého zooplanktonu nebo při přemnožení plevelných ryb, které konkurují kapru apod.) a ryby jsou intenzivně přikrmovány obilovinami.

Druhým faktorem, který významně ovlivňuje metabolizovatelnost krmiva a v důsledku pak obsah tuku v mase kapra je kompozice esenciálních aminokyselin (Bureau et al., 2002). Kapr stejně jako ostatní ryby a hospodářská zvířata není schopen syntetizovat esenciální aminokyseliny a musí je tedy pro svůj zdravý vývoj a růst získávat v dostatečném množství ze své potravy. Pokud kompozice aminokyselin v potravě kapra neodpovídá jeho nutričním požadavkům, tak se syntéza proteinů v těle zastaví v okamžiku, kdy dojde k vyčerpání první limitující aminokyseliny a všechny ostatní dostupné aminokyseliny jsou následně metabolizovány na energii. V důsledku toho dojde k nízkému využití krmiva, ke zvýšené tvorbě amoniaku a ke zvýšení dostupné energie, která v případě nadbytku vede ke zvýšené tvorbě a ukládání tuku v mase kapra (Bureau et al., 2002).

Dalšími faktory, které ovlivňují metabolizovatelnost krmiva a ukládání tuku jsou stres, podmínky prostředí, obsah a kvalita tuku v krmivu. Například dostatečné množství esenciálních mastných kyselin v dietě inhibuje *de novo* syntézu lipidů (Farkas et al., 1977, 1978).

To, jaký by měl být optimální obsah tuku v mase kapra, je subjektivní vjem a liší se jak mezi populacemi, tak uvnitř nich. Např. v Bavorsku je preferován kapr s nízkým obsahem tuku okolo 5 % (Oberle, ústní sdělení), zatímco v ČR je jako optimální vnímán obsah tuku kolem 8-10 % a ryby s obsahem tuku pod 5 % jsou vnímány jako suché a málo šťavnaté (Mráz, nepublikováno). Studie

Aas a Oberle (2009) ukázala, že to, jaký je optimální obsah tuku v mase kapra, se liší také tím, zda jej hodnotí lidé, kteří jí ryby často, zda se jedná o producenty ryb, restauratéry či běžnou populaci. V souhrnu lze uvést, že optimum leží mezi 5 a 10 % a za senzoricky akceptovatelnou hodnotu lze považovat maximálně obsah tuku do 15 %. V Bavorsku v oblasti Aishgrundska si jako normu pro kapra s chráněným zeměpisným označením Aishgrundský kapr stanovili, že musí mít obsah tuku do 10 % (Eatglobe, 2017). Distribuce tuku v mase kapra není rovnoměrná a tak může způsob zpracování či odběru vzorku významně ovlivnit výsledné množství tuku. Touto problematikou se blíže zabývá naše studie Mráz a Picková (2009).

Zatímco je kompozice aminokyselin v mase kapra geneticky kódována, a tak se za běžných podmínek nedá ovlivnit, tak kompozice mastných kyselin v mase ryb je silně variabilní a v podstatě na ni lze použít známe rčení „jste to, co jíte“. Kompozice mastných kyselin v mase do velké míry odráží kompozici mastných kyselin v dietě (Steffens, 1996, 1997). U kapra navíc významnou měrou dále přispívá *de novo* syntéza mastných kyselin z přebytečné metabolizovatelné energie (viz předchozí text). Stejně jako ostatní obratlovci, je karp schopen z přebytečné metabolizovatelné energie syntetizovat nasycené mastné kyseliny (SFA; především kyselinu palmitovou 16:0 a stearovou 18:0) a mononenasycené mastné kyseliny (MUFA; především kyselinu olejovou 18:1n-9). Postrádá ale enzymy (stejně jako ostatní obratlovci), které přeměňují kyselinu olejovou na kyselinu linolovou (LA, 18:2n-6) a ALA (18:3n-3) a ty jsou pro něj esenciální (Takeuchi a Watanabe, 1977). Jejich zdrojem jsou především rostlinné oleje a v případě kapra chovaného v rybnících pak přirozená potrava (plankton a bentos). Pokud je strava kapra deficitní na LA a ALA, dochází k tomu, že kapr ve snaze zachovat dostatečnou fluiditu buněčných membrán syntetizuje z kyseliny olejové PUFA řady n-9, především Meadovu kyselinu (20:3n-9) (Watanabe et al., 1975). Z kyselin LA a ALA je kapr pomocí enzymů desaturáz a elongáz schopen vytvářet další kyseliny řady n-3 a n-6 s delším uhlíkatým řetězcem a s více dvojnými vazbami. V řadě n-6 je metabolicky a nutričně velmi významná kyselina arachidonová (AA, 20:4n-6) a v řadě n-3 jsou to především kyselina eikosapentaenová (EPA, 20:5n-3), dokosapentaenová (DPA, 22:5n-3) a DHA (22:6n-3).

V rybníkářské praxi, v případě velkého množství metabolizovatelné energie (málo přirozené potravy, intenzivní přikrmování obilovinami), dochází k tomu, že z ní kapr syntetizuje a v tukových zásobách kumuluje velké množství MUFA (především kyseliny olejové), které může dosahovat 50-60 % všech mastných kyselin v mase (Csengeri, 1996). Pokud je ve stravě kapra velké množství přirozené potravy, nebo dostává doplňkové krmivo obsahující LA a ALA (např. řepkové semeno, některé doplňkové krmné směsi) je kompozice mastných kyselin v jeho mase bohatá na PUFA a

díky biosyntéze LA a ALA dochází též ke zvýšení HUFA (Csengeri, 1996; Mraz et al., 2012a; Steffens, 1997).

2.4. Konzumace ryb a lidské zdraví

Konzumace ryb je celosvětově považovaná za součást zdravého životního stylu. Ryby jsou dobrým zdrojem lehce stravitelných proteinů, n-3 mastných kyselin (zvláště pak EPA a DHA), vitamínů (především D) a minerálů (fosfor a vápník) (Mozaffarian a Rimm, 2006). EPA a DHA jsou důležité pro prevenci a léčbu kardiovaskulárních onemocnění, rozvoj mozku, nervové soustavy, očí a kognitivních vlastností (Helland et al., 2003; Horrocks a Yeo, 1999; Innis, 2007; Lauritzen et al., 2001; Mozaffarian a Wu, 2011; Simopoulos, 2008). Odborníci i mezinárodní zdravotnické organizace se shodují na tom, že bychom měli konzumovat ryby alespoň dvakrát týdně a že příjem EPA+DHA pro běžnou populaci by měl být alespoň 250 mg/osobu/den a příjem n-3 PUFA alespoň 2 g/osobu/den. Pro těhotné a kojící matky je doporučeno zvýšit příjem DHA ještě o dalších alespoň 100 mg/den (EFSA, 2010). Pro osoby s kardiovaskulárním onemocněním jsou doporučovány dávky EPA+DHA v řádech gramů/osobu/den (Mozaffarian a Rimm, 2006). Přehled nutričních doporučení pro příjem EPA+DHA a n-3 PUFA od různých zdravotnických organizací a pravděpodobné mechanismy pozitivního vlivu konzumace ryb na lidské zdraví jsou dobře shrnuty v práci autorů Mozzafarian a Wu (2011).

V poslední době se začínají objevovat také studie, které ukazují, že i rybí protein má podobný vliv na lidské zdraví jako rybí tuk. Bylo zjištěno, že rybí proteiny mají pozitivní vliv v prevenci a léčbě metabolického syndromu, zvyšují sensitivitu k insulinu, mají protizánětlivé efekty, působí preventivně proti obesitě či snižují hodnoty C reaktivního proteinu (Lavigne et al., 2001; Oullet, et al., 2008; Pilon et al., 2011). Mechanismy způsobující tyto efekty nejsou zatím plně objasněné. Každopádně je pravděpodobné, že pozitivní vliv konzumace ryb na lidské zdraví je kromě vysokého obsahu n-3 HUFA, ovlivněn celou řadou dalších látek a efektů.

Celosvětová spotřeba ryb od roku 1950 neustále stoupá, a zatímco dodávka ryb na osobu a rok činila v roce 1950 cca 6 kg, tak v roce 2016 již dosáhla cca 20 kg ekvivalentu živé hmotnosti ryb/osobu/rok (FAO, 2016). Oproti tomu, spotřeba ryb v ČR dlouhodobě stagnuje a je velmi nízká (pouze cca 5,5 kg/osobu/rok (Ženíšková a Gall, 2009)). Ostatní zdroje n-3 HUFA také nejsou ve stravě české populace běžné, a tak je celkový příjem n-3 HUFA v ČR nedostatečný (Hibbeln et al., 2006). Společnost pro výživu doporučuje, že bychom měli týdně konzumovat 400 g rybího masa

(Dostálová et al., 2012). To je v přepočtu 20,8 kg rybího masa na osobu a rok. Současná spotřeba rybího masa v ČR je tak ve srovnání s doporučenými hodnotami alarmující a je potřeba jí zvýšit na cca čtyřnásobek.

Pro prevenci rozvoje kardiovaskulárních onemocnění a zdravý vývoj dětí v ČR je kromě zmíněného doporučení zvýšení spotřeby ryb a rybích výrobků dále potřeba udržet (u ryb, které jsou krmeny krmivy s rybím olejem, např. lososovité) či zvýšit obsah n-3 PUFA a n-3 HUFA (u ryb, které nejsou běžně krmeny krmivy s rybím olejem, např. kaprovité) v mase ryb a rybích výrobcích.

2.5. Cíle práce

Cílem této práce je prozkoumat faktory ovlivňující obsah a kompozici tuku v mase kapra obecného (*Cyprinus carpio*) a vyvinout dlouhodobě udržitelnou technologii produkce kapra se zvýšeným obsahem n-3 mastných kyselin. Sekundárním cílem je zjistit, jakým způsobem působí maso kapra v sekundární prevenci kardiovaskulárních onemocnění a to, zda je tento dopad ovlivněn způsobem chovu kapra, potažmo kompozicí mastných kyselin ve svalovině.

Výsledky této práce jsou sumarizovány v následujících kapitolách a detailně popsány v publikovaných článcích, které jsou přílohou této habilitační práce.

3. Výsledky a diskuse

3.1. Faktory ovlivňující obsah tuku a kompozici mastných kyselin v mase kapra

(Mráz a Picková, 2011; příloha 1)

Jak již bylo uvedeno, je obsah tuku a kompozice mastných kyselin v mase kapra vysoce variabilní znak. Studie Mráz a Picková (2011; **Příloha 1**) sumarizuje poznatky z dostupné literatury i našeho dosavadního výzkumu o faktorech, které tyto kvalitativní parametry u kapra ovlivňují. V současné době je známo, že obsah tuku a jeho složení v mase kapra ovlivňuje několik environmentálních (výživa, hladovění, sádkování, teplota vody), interních (genetické pozadí, velikost a věk ryby, pohlaví a pohlavní dozrávání) a dalších faktorů (typ tkáně, zpracování a kuchyňská úprava).

Obecně má kapr z nutričního hlediska velmi příznivou kompozici mastných kyselin a měl by být považován za zdravý produkt. Nicméně jeho kvalitu lze díky výše zmíněným faktorům výrazně zlepšit i zhoršit. Mezi nejdůležitější faktory, které mohou kompozici mastných kyselin v mase kapra výrazně zlepšit, pak patří především velké množství přirozené potravy, vhodné doplňkové krmivo s vysokým obsahem ALA, vhodné zpracování a kuchyňská úprava. Další vylepšení by pak mohlo přijít s lepším pochopením biosyntézy HUFA, vlivu genetického pozadí a selekcí linií s větší schopností biosyntézy HUFA, stejně jako další rozvoj v oblasti studia bioaktivních látek, ovlivňujících metabolismus lipidů.

3.2. Bioaktivní látky

(Zajíc et al., 2016; příloha 2)

Sesamin je bioaktivní lignan ze sezamového oleje (Moazzami a Kamal-Eldin, 2006). Ve studiích s pstruhem duhovým a hypatocyty lososa obecného bylo zjištěno, že sesamin pozitivně ovlivňuje biosyntézu DHA z ALA (Trattner et al., 2008a; Trattner et al., 2008b). V naší studii Mráz et al. (2010) jsme testovali použití sesaminu v krmivu pro kapra obecného v tržní hmotnosti. Bohužel sesamin v této studii neměl na kompozici mastných kyselin v mase kapra pozitivní vliv. Ve studii Zajíc et al. (2016; **Příloha 2**) jsme chtěli zjistit, zda se pozitivní vliv sesaminu na kvalitu masa kapra projeví, pokud bude použit v chovu juvenilních ryb, kde je předpoklad většího obratu mastných kyselin, při nízké bazální koncentraci EPA a DHA v krmivu a různém poměru n-3 a n-6 kyselin. Ani tento experiment nepotvrdil, že by sesamin měl u kapra pozitivní vliv na syntézu DHA.

Nicméně rozdílný obsah LA a ALA v krmivu měl za následek zvýšení korespondujících HUFA v mase kapra. Tyto výsledky potvrzují předchozí studie (Tocher et al., 1989; Tocher a Dick, 1999), že sladkovodní ryby, včetně kapra, jsou schopny vytvářen n-3 HUFA z jejich prekurzorů, a předpoklad, že míra konverze je závislá na přítomnosti a množství těchto prekurzorů. Enzymy, které působí v dráze syntézy HUFA, jsou totiž pro obě série esenciálních mastných kyselin stejné a substráty obou sérií si tak vzájemně konkurují. Výsledky ukazují, že přebytek LA vede ke zvýšené produkci n-6 HUFA a přebytek ALA naopak vede ke zvýšené produkci n-3 HUFA. Toto zjištění je důležité v případě, že chceme maximálně využít kapacitu kapra syntetizovat n-3 HUFA z jejich rostlinných prekurzorů. Výsledky naznačují, že je vhodné použít dietu, kde bude převaha n-3 prekurzorů a pouze taková dávka n-6 mastných kyselin, která pokryje nutriční potřebu těchto esenciálních mastných kyselin.

3.3. Krmné strategie
(Mráz et al., 2012b; příloha 3)

Výživa a krmné strategie mají velký vliv na výslednou kvalitu rybího masa. Krmná strategie „finishing feeding“ (závěrečné krmení) spočívá v použití levnějších krmiv s rostlinnými oleji v průběhu většiny doby chovu ryb, se závěrečnou fází odchovu, kde je použito vysoce kvalitní krmivo s rybím olejem, s cílem dosáhnout požadované kvality masa ryb. Tato krmná strategie je především studována v chovu lososovitých ryb. Pomocí ředícího modelu zde lze s vysokou přesností předpovědět výslednou kompozici mastných kyselin v mase ryb v závislosti na délce závěrečné fáze (Bell et al., 2004; Jobling, 2004; Zajic et al., 2016b).

Ve studii Mráz et al. (2012b; **Příloha 3**) jsme krmnou strategii „finishing feeding“ testovali na kapru obecném. Experiment ukázal, že v chovu kapra lze tuto krmnou strategii úspěšně použít a dosáhnout tak požadované výsledné kompozice mastných kyselin v mase kapra. Ředící model poskytoval velice přesnou předpověď výsledné kompozice mastných kyselin (R^2 = 0,992-0,996). Pokud by byl např. požadavek, aby dvě porce kapřího filetu (2 × 200 g) týdně pokryly ze 100 % doporučenou denní dávku EPA+DHA (250 mg × 7 dní = 1750 mg/2 porce = 875 mg/porci), bylo by potřeba při krmné strategii „finishing feeding“ aplikovat 70 denní závěrečnou fázi výkrmu s testovaným krmivem s rybím olejem. Takováto strategie by mohla být např. aplikována v případě produkce kapra s definovanou kvalitou masa pro specifické nutriční potřeby skupin obyvatelstva (např. pro prevenci či léčbu některých kardiovaskulárních chorob). Tato krmná strategie by jednak

vedla k přesné předpovědi kvality masa a jednak k úspoře finančních prostředků a potřeby rybího oleje. Producenti kapra by díky ní mohli snadněji standardizovat a deklarovat kvalitu ryb.

3.4. Sádkování
(Zajíc et al., 2013; příloha 4)

Sádkování je běžnou součástí technologie chovu kapra před jeho dodáním na trh. Většinou se provádí v podzimním období mezi vlastním výlovem ryb z rybníků a jejich dodáním na vánoční trh. Ryby jsou po dobu několika týdnů drženy v sádkách s průtočnou čistou vodou bez přikrmování, za účelem jejich uchování do doby prodeje, vyprázdnění trávicího traktu a eliminaci případných pachutí. Tento proces vede jednak k celkovému vylehčení obsádky ryb a také k mobilizaci energetických rezerv (Einen et al., 1998; Palmeri et al., 2008). Lze tedy předpokládat, že se obsah tuku a kompozice mastných kyselin v mase kapra v průběhu sádkování bude měnit.

Studie Zajíc et al. (2013; **Příloha 4**) zkoumala vliv podzimního sádkování (70 dní) a předchozí výživy (přikrmování obilovinami, přikrmování doplňkovou směsí KP Len, pouze přirozená potrava) na změny obsahu tuku a kompozice mastných kyselin v mase tržního kapra (hmotnost 1,7-2,6 kg). Vedle očekávané ztráty hmotnosti došlo u přikrmovaných skupin k výraznému poklesu obsahu tuku (z původních cca 7,5 – 8,5 % na cca 3,5 %), zatímco skupina nepřikrmovaná měla obsah tuku v průběhu experimentu relativně stabilní. V přikrmovaných skupinách také docházelo k výrazným změnám kompozice mastných kyselin. Obě skupiny preferenčně metabolizovaly MUFA a tak relativní podíl PUFA, n-3 PUFA, EPA+DHA v průběhu experimentu stoupal. Nicméně po čase, při nižším obsahu tuku, začaly metabolizovat i PUFA. Na konci experimentu už měly všechny 3 skupiny velice podobné hodnoty obsahu tuku, SFA, MUFA i PUFA. Tyto výsledky naznačují, že kapr je schopen selektivně metabolizovat určité mastné kyseliny pro své energetické potřeby v případě, kdy je v dobrých podmínkách a má dostatečné rezervy tuku. Když má tukových zásob nedostatek, začne metabolizovat i ostatní typy mastných kyselin tak, aby si udržel specifickou kompozici, která mu zajistí ideální složení membrán a dalších metabolických funkcí.

Z praktického hlediska je možné doporučit, aby bylo delší sádkování aplikováno v případech, kdy má obsádka kapra příliš vysoký obsah tuku, který je již senzoricky nepříjemný (15 % a více) a došlo tak k jeho redukci a zároveň optimalizaci jeho kompozice. U kapra s optimálním množstvím tuku (5-10 %), popř. u kapra se zvýšeným obsahem n-3 PUFA je možné doporučit spíše kratší dobu sádkování tak, aby došlo k vyprázdnění trávicího traktu a zbavení se případných nežádoucích pachutí, ale zároveň, aby nedošlo k redukci žádoucích mastných kyselin n-3 PUFA a EPA+DHA.

Pokud je potřeba u těchto ryb aplikovat delší období sádkování, je vhodné, aby bylo prováděno pokud možno při nízkých teplotách, kdy je předpoklad nižší míry metabolismu, a tak i nežádoucí ztráty nutričně významných mastných kyselin.

3.5. In situ měření obsahu tuku
(Másílko et al., 2016; příloha 5)

Obsah tuku zásadně ovlivňuje kvalitu masa kapra a zároveň se jedná o velice variabilní parametr (Mraz a Pickova, 2011; Oberle et al., 1997). Existují různé metody, jak obsah tuku v mase ryb měřit. Asi nejrozšířenější metodou je měření obsahu tuku laboratorní analýzou pomocí extrakce tuku organickými rozpouštědly (Bligh a Dyer, 1959; Hara a Radin, 1978). Jedná se sice o přesnou metodu, ale její nevýhodou je vysoká cena, časová náročnost, potřebné laboratorní vybavení a nutnost destrukce vzorku. Pro běžnou rybníkářskou praxi popř. šlechtění ryb je potřeba použití metod, které budou rychlé, levné, jednoduché a pokud možno nedestruktivní.

Studie Másílko et al. (2016; **Příloha 5**) porovnávala použití dvou rychlých *In situ* metod stanovení obsahu tuku v mase kapra s laboratorní analýzou metodou Hara a Radin (1978). První metoda využívala přístroj Distell Fish Fatmeter. Jedná se o neinvazivní a nedestruktivní metodu, která může být použitá na živých rybách. Její princip je založen na měření obsahu vody na osmi bodech na těle kapra a jejím matematickém přepočtení na obsah tuku (S předpokladem, že mezi obsahem vody a tuku v mase ryb je těsná negativní korelace.) (Urbanek et al., 2010). Druhá metoda využívala měření výšky hřbetního tuku digitální šuplerou na skeletu po filetování (S předpokladem, že mezi výškou hřbetního tuku a obsahem tuku ve filetu je těsná pozitivní korelace.).

Obě testované *In situ* metody měly statisticky signifikantní pozitivní korelaci v porovnání s chemickou analýzou. Korelační koeficient mezi chemickou analýzou a hodnotami predikovanými metodou měření hřbetního tuku byl 0,76 (R^2 = 0,58) a hodnotami predikovanými metodou fatmetru byl 0,88 (R^2 = 0,78). Pro zpřesnění predikce obsahu tuku ve filetu pomocí těchto dvou *In situ* metod byly testovány multiregresní modely, které kromě naměřených hodnot zahrnovaly některé morfometrické charakteristiky a údaje o způsobu přikrmování ryb. Nejpřesnější model pak dosahoval při použití fatmetru R^2_{adj} = 0,88 (při zahrnutí parametrů: šířka těla, hmotnost vnitřností, hmotnost těla, hmotnost hepatopankreatu a hmotnost gonád) a při použití měření výšky hřbetního tuku dosahoval R^2_{adj} = 0,91 (při zahrnutí parametrů: hmotnost hepatopankreatu, šířka těla, výška těla, hmotnost gonád a použité doplňkové krmivo).

Obě metody byly vyhodnoceny jako použitelné pro rybníkářskou praxi. Jejich přesnost je sice o něco nižší, než chemická metoda, ale to je vyváženo jejich rychlostí, nízkými náklady, jednoduchostí a tím, že se jedná o nedestruktivní metody. Velkou výhodou fatmetru je možnost použití na živých rybách, což umožňuje použití při šlechtění či dalších typech studií, kde je nutné, aby ryba byla živá. Výhodou měření hřbetního tuku jsou především nízké pořizovací náklady.

V Aishgrundsku je hodnocení obsahu tuku pomocí fatmetru zohledněno při jednání o ceně kapra a pokud chce producent využívat ochrannou známku Aishgrundský kapr, tak nesmí obsah tuku v mase kapra překročit 10 %. Při rutinní kontrole by byla chemická analýza příliš drahá a pomalá, a tak je zde použití fatmetru velmi rozšířené (Oberle, ústní sdělení).

Legislativa (Nařízení evropského parlamentu a rady č. 1169/2011) nově (od 13.12.2016) vyžaduje povinné uvádění výživových údajů na potravinářských výrobcích. Jedná se o analýzu tzv. „BIG 7“, která zahrnuje obsah proteinů, lipidů, SFA, sacharidů, cukrů, vody a energetickou hodnotu ve výrobku. V rámci splnění těchto legislativních požadavků je u rybích výrobků právě obsah lipidů vzhledem k jejich vysoké variabilitě nejproblematičtější. Výše zmíněné *In situ* metody by tak mohly producentům rybích výrobků pomoci při rutinní kontrole kvality rybí suroviny.

3.6. Zpracování a kuchyňská úprava
(Sampels et al., 2014; příloha 6)

Jak bylo naznačeno v kapitole 3.1., má zpracování a kuchyňská úprava velký vliv na výslednou kvalitu rybího masa. Jako extrémní případ lze například uvést výrobek „rybí prsty obalované“ z Estonska, které u nás distribuuje firma Bidvest. Tento výrobek obsahuje pouze 22 % drceného rybího masa z tresky a zbytek tvoří pšeničná mouka, olej, hrachová vláknina a bílkovina, kalamáry, sůl, dextróza, mletá kurkuma, glutaman sodný E 621 a kypřicí látka E 503 (Novinky.cz, 2015). Zatímco maso tresky obsahuje velmi malé množství tuku (cca 0,5-1 %) má tento výrobek kolem 8 % tuku. Logicky je tak tuk obsažený v tomto výrobku tvořen více jak z 97 % tukem, který byl použit na jeho předsmažení. Často je používán na předsmažení těchto výrobků tuk palmový nebo slunečnicový. Například studie autorů Sampels et al. (2009) ukázala, že poměr n-6/n-3 mastných kyselin je v předsmažených rybích výrobcích až 400 násobně jiný, než v rybím mase, ze kterého je vyroben. Důležité je také si uvědomit, že tyto předsmažené rybí výrobky jsou často dále upravovány smažením, a tak jejich výsledný obsah tuku kuchyňskou úpravou ještě stoupne a kompozice mastných kyselin se dále změní v závislosti na použitém tuku. Výsledný produkt má pak už jen velmi málo společného s rybou a rybím tukem a je otázka, zda by mělo být možné takovéto

produkty dodávat na trh pod názvem „rybí“. Paradoxní je, že častým argumentem pro nákup těchto výrobků je jejich cena. Ve zmíněném případě je běžná cena tohoto výrobku na trhu 25 Kč za 250 g výrobku. Při obsahu deklarovaného obsahu rybího masa 22 % se v přepočtu jedná o cenu 454 Kč za kilogram „drceného“ rybího masa. To už je cena srovnatelná s vysoce kvalitním filetem z lososa, a je tak s podivem, za jakou cenu jsou zákazníci ochotni kupovat takto nekvalitní výrobek.

Nejběžnější úpravou kapra v České republice je vzhledem k vánoční tradici pravděpodobně smažení, a to buď v trojobalu či bez něj. Pro zjištění toho, jak tento typ kuchyňské úpravy ovlivní maso kapra, jsme provedli studii Sampels et al. (2014; **Příloha 6**). Porce kapřího filetu (cca 100 g) jsme připravili v trojobalu a bez něj, smažením na pánvi v malém množství tuku. Ke smažení byly vybrány 4 běžně používané druhy tuku v českých domácnostech: sádlo, máslo, řepkový a slunečnicový olej.

Jak bylo předpokládáno, smažení zvýšilo obsah tuku ve finálním výrobku z původních cca 4-5 % na dvojnásobek (v případě filetu bez trojobalu cca 10-11 %), až na trojnásobek, v případě filetu v trojobalu (cca 13-14 %). Stejně tak kompozice mastných kyselin ve finálním výrobku silně reflektovala použitý druh smažícího tuku a jeho nárůst. Filety smažené v trojobalu se tak kvůli většímu nárůstu obsahu tuku podobaly více použitému smažícímu tuku, v porovnání s filety bez trojobalu. Smažení v sádle výrazně zvýšilo obsah kyseliny palmitové a stearové, v másle obsah kyseliny myristové a palmitové, ve slunečnicovém oleji výrazně zvýšilo obsah kyseliny olejové a LA. Z nutričního hlediska pak měly nejlepší výslednou kompozici filety smažené v řepkovém oleji. Změny finální kompozice mastných kyselin byly vysoce blízké hodnotám predikovaným ředícím modelem ($p < 0{,}001$; $R^2 = 0{,}999$), což indikuje, že v našem případě nedocházelo k selektivním ztrátám či absorpci některých mastných kyselin ze smažícího oleje do finálního produktu a zpět. Z toho vyplývá, že v případě kapřích filet je možné s vysokou pravděpodobností matematicky předpovědět finální kompozici smažených výrobků. Tyto výsledky jsou významné pro nutriční terapeuty při výpočtu nutriční hodnoty pokrmů. Například studie Sampels et al. (2009) poukázala na to, že databáze potravin, často obsahují významně odlišné hodnoty tuku a kompozice mastných kyselin u rybích výrobků, než je tomu ve skutečnosti. Tyto databáze totiž často neobsahují informace o způsobu chovu ryb, ani o způsobu jejich zpracování. Vzhledem k zmíněné variabilitě obsahu i kompozici tuku v rybích výrobcích pak pravděpodobně může docházet k významnému zkreslení výživových studií, které často složení potravin neanalyzují, ale pouze vychází z databází složení potravin (např. Garemo et al., 2007; Matthys et al., 2006; Sioen et al., 2007).

Pro domácnosti a veřejné stravování pak lze na základě našich výsledků doporučit především metody šetrné kuchyňské úpravy kapra, kde nedochází k přidání dalšího tuku tak, aby byla co nejvíce zachována nutriční hodnota rybího masa. Z běžných kuchyňských úprav pak lze doporučit především toto pořadí: syrové > marinované > vařené v páře > vařené > pečené > smažené v malém množství tuku > fritované. V případě použití smažícího tuku pak lze doporučit smažení bez trojobalu a z běžně používaných tuků použití řepkového oleje, který obsahuje velmi malé množství zdravotně nepříznivých SFA, velké množství MUFA, PUFA a velmi dobrý poměr n-3 a n-6 kyselin.

Pro nutriční odborníky lze doporučit rozšíření databází složení potravin o informace o nutričním složení různých ryb a rybích výrobků pocházejících z různých chovných systémů popř. upravených různými způsoby. Ke zvážení je taktéž použití předsmažených rybích výrobků s nízkým obsahem rybího masa a vysokým obsahem tuku, barviv, stabilizátorů a ochucovadel (viz příklad produktu „rybí prsty předsmažené“) ve stravování předškolních a školních dětí. Takovéto produkty nemají s rybou prakticky skoro nic společného a v podstatě se jedná o předraženou smaženou strouhanku.

Jednoznačně je však potřeba iniciovat diskusi mezi producenty, zpracovateli ryb, nutričními specialisty, státní správou a veřejností a lépe komunikovat problematiku nutriční kvality ryb v rámci celého řetězce „od rybníka až po vidličku“.

3.7. Technologie produkce omega 3 kapra
(Mráz et al., 2017; příloha 7)

V rámci projektu NAZV QH92307 jsme vyvinuli technologii produkce kapra se zvýšeným obsahem omega 3 kyselin (dále jen omega 3 kapr) (Mráz et al., 2017; **Příloha 7**). Tato technologie je určena pro polointenzivní chov kapra v rybniční akvakultuře. Je založena na maximálním využití přirozené potravy (plankton a bentos), která je bohatým zdrojem n-3 HUFA (Mraz et al., 2012a; Mraz a Pickova, 2009; Mraz a Pickova, 2011), v kombinaci s doplňkovou krmnou směsí obsahující řepkové výlisky a extrudované lněné semínko. Tato krmná směs je bohatá na ALA (Mráz et al., 2011a,b; Zajic et al., 2013), která je pro kapra prekurzorem pro tvorbu n-3 HUFA (Farkas, 1984; Olsen et al., 1990; Tocher, 2003; Zheng et al., 2004). Při vývoji této technologie jsme vycházeli z toho, že chceme maximálně využít tradiční způsob chovu kapra v rybnících bez použití rybí moučky a oleje, které jsou dlouhodobě neudržitelné a celosvětově je nutné je v krmivech pro ryby nahrazovat udržitelnými alternativami (Pickova a Morkore, 2007; Tacon a Metian, 2008). Také jsme nechtěli jít cestou použití GMO či použití neekologických postupů. Důležitým parametrem

pro rozhodování byla též ekonomika chovu, protože jsme chtěli vyvinout produkt, který se dostane mezi lidi a nezůstane pouze zajímavou vědeckou publikací, či výsledkem tzv. „v šuplíku“.

Technologie chovu omega 3 kapra se skládá z těchto kroků:

1) Rybníky vhodné pro chov omega 3 kapra musí mít dostatečnou přirozenou produktivitu (alespoň 300 kg/ha) a jejich management je volen tak, aby maximálně podporoval rozvoj přirozené potravy a zároveň potlačoval rozvoj plevelných ryb.
2) Na jaře (v dubnu) jsou rybníky nasazeny kaprem o hmotnosti cca 1 kg ve vhodné hustotě obsádky tak, aby přirozený přírůstek tvořil 50 % celkového přírůstku (2-2,3× přirozená produktivita rybníka).
3) Kapři jsou v průběhu vegetační sezóny přikrmováni peletovanou krmnou směsí KP len, obsahující řepkové výlisky a extrudované lněné semínko (Mráz et al., 2011a,b).
4) Rybníky jsou loveny v období říjen-listopad a omega 3 kapři jsou sádkováni po dobu 2-4 týdnů pro vyčištění trávicího traktu, zbavení se možných pachutí a vylepšení profilu lipidů (Zajic et al., 2013).
5) Omega 3 kapři jsou filetováni a v případě velkého množství zásobního tuku (kapr nad 2,5 kg; obsah tuku nad 10 %) je odříznut proužek abdominální stěny, který obsahuje velké množství SFA a MUFA (Mraz a Pickova, 2009).
6) Před dodáním na trh je provedena kontrolní analýza obsahu tuku a kompozice mastných kyselin.

Technologie produkce omega 3 kapra je chráněna českým národním patentem č. 302744 (Mráz et al., 2011a) a složení krmné směsi KP Len je chráněno užitným vzorem č. 21926 (Mráz et al., 2011b). Omega 3 kapr je od roku 2011 k dostání na českém trhu pod označením ochranné známky (Jihočeská univerzita v Českých Budějovicích et al., 2011; Obr. 1).

Filety omega 3 kapra obsahují méně MUFA, více n-3 PUFA, n-3 HUFA a nižší poměr n-6/n-3 v porovnání s kaprem z tradičního chovu přikrmovaným obilovinami (Mraz et al., 2012a; Mraz et al., 2017; Zajic et al., 2013). Jedna porce omega 3 kapra (200 g) obsahuje alespoň 300 mg EPA+DHA a 1 g n-3 PUFA (obvykle 500 mg EPA+DHA a 1,5 g n-3 PUFA). Tyto hodnoty jsou blízké doporučovaným hodnotám pro prevenci kardiovaskulárních chorob (Mozaffarian a Wu, 2011) viz kapitola 2.4.

Obr. 1. Ochranná obrazová známka pro omega 3 kapra (Jihočeská univerzita v Českých Budějovicích et al., 2011).

Cena omega 3 kapra na trhu je cca o 15-20 % vyšší v porovnání s „normálním“ kaprem, což je dáno vyššími náklady na krmivo, management chovu, kontrolní analýzy kvality masa a licenční poplatek. Podle našich dosavadních zkušeností při vánočních prodejích je tato zvýšená prodejní cena pro zákazníky akceptovatelná. Jsme rádi, že se léta výzkumu zúročila a povedlo se omega 3 kapra dostat od prvotní myšlenky až na trh k zákazníkům. Jsme si vědomi toho, že v této oblasti jsme teprve na začátku a věříme, že se nám lepším porozuměním metabolismu mastných kyselin a konverze ALA na n-3 HUFA podaří obsah těchto látek v mase kapra v budoucnu ještě zvýšit.

3.8. Kapr v prevenci kardiovaskulárních chorob
(Adámková et al., 2011, příloha 8; Mráz et al., 2017 příloha 7)

Navzdory tomu, jak je oblast vlivu konzumace ryb a n-3 mastných kyselin na lidské zdraví intenzivně zkoumaným tématem, je publikací, které se zaměřují na vliv sladkovodních ryb v lidské výživě, velice málo. V dostupné literatuře jsme dokonce nenašli žádnou studii, která by se zabývala touto problematikou u kapra. Sladkovodní ryby jsou navíc často v odborné literatuře i dalších médiích neprávem přehlíženým zdrojem n-3 HUFA (Ackman, 2002). Výjimkou nejsou ani výroky typu „Ryby sladkovodní totiž nejsou zdrojem omega-3 mastných kyselin na rozdíl od ryb mořských.“ (Neuwirthová, 2017). Takové výroky jsou zavádějící a nezakládají se na pravdě. Naopak některé sladkovodní ryby mají až několikanásobně vyšší obsah n-3 HUFA než např. maso tresky obecné (*Gadus morhua*) (treska: 158 mg EPA+DHA/100 g vs. pstruh: 935 mg EPA+DHA/100 g) (Mozaffarian a Rimm, 2006).

V rámci studie Adámková et al. (2011; **Příloha 8**) jsme ukázali, že již malé zařazení kapra do jídelníčku (2 × 200 g kapřího masa týdně/po dobu 4 týdnů) mělo pozitivní vliv na vylepšení lipidů a markeru zánětu v plasmě pacientů, kteří prodělávali rekonvalescenci po operaci srdce v Lázních Poděbrady, v porovnání s kontrolní skupinou, která dostávala standardní dietu pro tyto pacienty. V druhé studii Mráz et al. (2017; **Příloha 7**) jsme tyto výsledky potvrdili a ukázali jsme, že míra tohoto efektu je ovlivněna tím, z jakého typu produkčního systému (tradiční způsob chovu s přikrmováním obilovinami vs. omega 3 kapr) kapr pochází. Vzhledem k tomu, že kapři z obou skupin měli podobnou hmotnost (2,3 kg) a obsah tuku (6,8 % kapr přikrmovaný obilovinami vs. 6,2 % omega 3 kapr) je pravděpodobné, že rozdíl mezi skupinami pacientů, kteří dostávali kapra, byl způsoben rozdílnou kompozicí mastných kyselin v jejich mase. Maso kapra přikrmovaného obilovinami bylo charakteristické vysokým obsahem MUFA (zvláště kyseliny olejové), zatímco maso omega 3 kapra mělo velké množství PUFA (3× více), n-3 PUFA (4× více) a n-3 HUFA (4,8× více). Navíc měl omega 3 kapr 5krát vyšší obsah příznivých EPA+DHA než kapr přikrmovaný obilovinami (106 vs. 524 mg EPA+DHA/200 g porci). I přes lepší výsledky při použití omega 3 kapra je nutné zmínit, že obě skupiny, které dostávaly v jídelníčku kapra, měly lepší výsledky, než kontrolní skupina.

Zajímavým zjištěním je také to, že všichni pacienti z naší studie, kteří v Lázních Poděbrady dostávali pokrmy z kapra, udávali, že byli mile překvapeni jejich dobrou chutí, variabilitou receptů a tím, že lze připravit kapra tzv. „bez kostí“. Dokonce ještě 5 let po naší studii 44 % pacientů v dotazníku uvedlo, že stále dodržuje doporučení jíst ryby alespoň 2krát týdně. Z toho lze usuzovat, že je zde velký prostor pro osvětu, aby se lidé v ČR naučili jíst kapra nejen na Vánoce, ale i v průběhu roku. Podle mého názoru je důležité to, aby se seznámili s tím, jak kapra připravit, či si ho koupit „bez kostí“, jak ho jednoduše kulinárně připravit na jiné způsoby, než jen „vánočního smaženého kapra v trojobalu“, a proč je pro jejich zdraví důležité jíst ryby.

Velikost efektu konzumace kapra v obou studiích již po tak krátké době (4 týdny) nás překvapila. Je nutné uvést, že takto velkých změn bylo dosahováno ve studiích s mořskými rybami či s kapslemi s rybím olejem, které obsahovaly mnohem vyšší dávky EPA+DHA podávané pacientům s vyšší frekvencí. Důvodů může být několik a pro jejich objasnění je nutné provést další detailnější studie. Dovolím si je tedy vyslovit pouze jako určité hypotézy k dalšímu zkoumání.

1) Lidé v ČR konzumují velice málo ryb (Ženíšková a Gall, 2009), a tak je pravděpodobné, že naši pacienti měli velký deficit n-3 PUFA a EPA+DHA. Tak i malé dávky mohli mít po krátké době silný efekt na jejich parametry lipidů v plazmě a marker zánětlivých procesů.
2) Zařazením kapra dvakrát týdně do jídelníčku pacientů došlo k nahrazení jiných jídel ze standardní diety. Výsledný efekt by tak mohl být kombinací vlivu kapra a vyloučení jiných jídel. Existují například studie, které ukazují, že červené maso může mít negativní vliv na rozvoj kardiovaskulárních onemocnění a vznik kolorektálních nádorů (Koeth et al., 2013; Oostindjer et al., 2014). Maso hospodářských zvířat také může obsahovat vyšší množství SFA (Dostálová, 2011; Wood et al., 2008), které jsou známé svým negativním vlivem na rozvoj kardiovaskulárních onemocnění (Lichtenstein et al., 2006).
3) Maso kapra pravděpodobně obsahuje i další pozitivní látky, než jen omega 3 mastné kyseliny. Například bylo zjištěno, že rybí proteiny a peptidy mají podobný pozitivní vliv jako omega 3 kyseliny (Lavigne et al., 2001; Ouellet et al., 2008; Pilon et al., 2011; Wergedahl et al., 2004). Dalších látek a molekul (např. proteiny, vitamíny, minerály, fosfolipidy…), které mohou společně s omega 3 kyselinami působit synergisticky, může být mnoho, a tak lze předpokládat, že mohly přispět k pozitivním výsledkům pozorovaným v našich studiích.
4) Většina studií, které byly doposud v této oblasti provedeny, používala mořské ryby či kapsle s jejich olejem (Mozaffarian a Rimm, 2006). Ten obsahuje z n-3 HUFA ve velké míře EPA+DHA (Gruger at al., 1964), a tak je většina nutričních doporučení směřována pouze k těmto kyselinám. Sladkovodní ryby obsahují i další kyseliny n-3 HUFA (jako je např. DPA), které by mohli být sami o sobě bioaktivními látkami, nebo mohou být v lidském těle efektivněji přeměněny na EPA a DHA, než je ALA. Dále má maso sladkovodních ryb vyvážený obsah n-3 a n-6 kyselin (Steffens, 1997). Tato oblast si zaslouží hlubší zkoumání a je možné, že současná nutriční doporučení budou v budoucnu rozšířena o další „rybí“ mastné kyseliny.

Závěrem lze uvést, že zařazení kapra do jídelníčku má pozitivní vliv na lidské zdraví a prevenci kardiovaskulárních onemocnění. Síla efektu konzumace kapra je ovlivněna tím, z jakého systému chovu kapr pochází a jakou má kompozici mastných kyselin. To, proč byly naše výsledky podobné, jako ve studiích s mnohem vyššími dávkami omega 3 kyselin není plně jasné, a tato oblast vyžaduje další podrobnější zkoumání. Z výsledků je také patrné to, že v ČR existuje velký prostor pro osvětu a významné zvýšení spotřeby sladkovodních ryb včetně kapra.

4. Závěry

V této práci byly nejprve shrnuty faktory ovlivňující obsah tuku a kompozici mastných kyselin v mase kapra obecného. Mezi nejdůležitější faktory, které mohou tyto kvalitativní parametry v mase kapra výrazně zlepšit, patří především velké množství přirozené potravy, vhodné doplňkové krmivo s vysokým obsahem ALA, vhodné zpracování a kuchyňská úprava.

Dále byl studován vliv sesaminu a dvou různých poměrů n-3 a n-6 kyselin v krmivu na kvalitu masa kapra. Sesamin neměl pozitivní vliv na syntézu HUFA. Rozdílný obsah LA a ALA v krmivu měl za následek zvýšení korespondujících HUFA v mase kapra. Výsledky naznačují, že pro maximální využití schopnosti kapra syntetizovat n-3 HUFA z jejich rostlinných prekurzorů, je vhodné použít dietu, kde bude převaha n-3 prekurzorů a pouze taková dávka n-6 mastných kyselin, která pokryje jeho nutriční potřebu.

V chovu kapra byla úspěšně otestována krmná strategie „finishing feeding“. Bylo zjištěno, že díky této strategii lze jednoduše dosáhnout požadované výsledné kompozice mastných kyselin v mase kapra, kterou lze navíc velmi přesně předpovědět pomocí ředícího modelu (R^2 = 0,992-0,996).

Byl zkoumán vliv podzimního sádkování a předchozí výživy na změny obsahu tuku a kompozici mastných kyselin v mase tržního kapra. V průběhu sádkování docházelo k výrazným změnám v obsahu tuku a kompozici mastných kyselin. Výsledky naznačují, že kapr je schopen selektivně metabolizovat určité mastné kyseliny pro své energetické potřeby v případě, kdy má dostatečné rezervy tuku. V případě nedostatku tukových zásob, pak metabolizuje i ostatní typy mastných kyselin tak, aby si udržel specifickou kompozici, která mu zajistí ideální složení membrán a dalších metabolických funkcí.

Bylo testováno použití dvou rychlých *In situ* metod stanovení obsahu tuku v mase kapra (použití fatmetru a měření výšky hřbetního tuku). Obě metody byly vyhodnoceny jako použitelné pro rybníkářskou praxi. Jejich přesnost je sice o něco nižší, než chemická analýza, ale to je vyváženo jejich rychlostí, nízkými náklady, jednoduchostí a tím, že se jedná o nedestruktivní metody. Jsou tedy vhodné především pro šlechtitelskou práci, kontrolu výživného stavu či pro rutinní kontrolu kvality rybí suroviny.

Zpracování a kuchyňská úprava má velký vliv na výslednou kvalitu masa kapra. Smažení kapřích filet zvyšuje obsah tuku na dvou až trojnásobek (v případě filetu v trojobalu). Kompozice mastných

kyselin ve finálním výrobku silně reflektuje použitý druh smažícího tuku a jeho nárůst. Z běžně používaných tuků ke smažení lze z nutričního hlediska doporučit především řepkový olej.

Byla vyvinuta patentovaná technologie produkce kapra se zvýšeným obsahem omega 3 kyselin (dále jen omega 3 kapr). Je založena na maximálním využití přirozené potravy (plankton a bentos) v kombinaci s doplňkovou krmnou směsí obsahující řepkové výlisky a extrudované lněné semínko. Jedna porce omega 3 kapra (200 g) obsahuje alespoň 300 mg EPA+DHA a 1 g n-3 PUFA (obvykle 500 mg EPA+DHA a 1,5 g n-3 PUFA). Tyto hodnoty jsou blízké doporučovaným hodnotám pro prevenci kardiovaskulárních chorob.

Bylo zjištěno, že již malé zařazení kapra do jídelníčku (2 × 200 g kapřího masa týdně/ po dobu 4 týdnů) mělo pozitivní vliv na vylepšení lipidů a markeru zánětu v plasmě pacientů, kteří prodělávali rekonvalescenci po operaci srdce v Lázních Poděbrady, v porovnání s kontrolní skupinou, která dostávala standardní dietu pro tyto pacienty. Bylo zjištěno, že velikost tohoto efektu je ovlivněna tím, z jakého typu produkčního systému (tradiční způsob chovu s přikrmováním obilovinami vs. omega 3 kapr) kapr pochází a jakou má kompozici mastných kyselin.

Závěrem lze uvést, že kapr má vysokou nutriční hodnotu, měl by být považován za zdravou potravinu a jeho zařazení do jídelníčku má pozitivní vliv na lidské zdraví a prevenci kardiovaskulárních onemocnění. Jeho nutriční kvalitu lze mnoha faktory ještě vylepšit. Tato habilitační práce přináší jednak souhrn těch, které nutriční kvalitu kapřího masa ovlivňují a zároveň přináší technologii chovu omega 3 kapra, která je již v současnosti zavedena do praktických podmínek.

5. Probíhající výzkum a další směřování

Ze zmíněných studií je zřejmé, že obsah tuku a kompozice mastných kyselin v mase kapra obecného jsou vysoce variabilní a je možné je ovlivnit mnoha faktory. V předložené habilitační práci byly tyto faktory sumarizovány a byla vyvinuta technologie produkce omega 3 kapra. Předpokládáme, že se nám na základě probíhajícího výzkumu podaří obsah n-3 HUFA v mase kapra dále zvýšit.

Jedním z hlavních faktorů, které obsah n-3 HUFA v mase ovlivňují, je množství přirozené potravy ve výživě kapra. V současné době je mnoho rybníků v ČR v eutrofním až hypertrofním stavu, díky čemuž mnoho zažitých rybníkářských pouček přestává platit a dochází ke stavu, kdy nejsou živiny a energie z primární produkce efektivně transformovány do ryb. V rámci probíhajícího projektu GAČR (17-09310S) společně s Hydrobiologickým ústavem a Přírodovědeckou fakultou JU studujeme chování rybničního ekosystému a retenci živin v rybách za různých podmínek. Cílem je najít efektivní způsob rybničního managementu, který pomůže k vyššímu přenosu živin a energie z primární produkce do ryb, a tím k lepšímu využití rybničního potenciálu. To by mělo vést k vyššímu podílu přirozené potravy ve stravě kapra a k následnému zvýšení n-3 HUFA v jeho svalovině.

Druhým důležitým faktorem je schopnost kapra syntetizovat n-3 HUFA z jejich rostlinných prekurzorů. Zde lze čerpat především ze studií, které se zabývají touto problematikou u lososovitých ryb. Z výzkumu vědeckého týmu z University of Stirling vedeného profesorem Tocherem vyplývá, že porozuměním procesu konverze ALA na n-3 HUFA a faktorům, které ji ovlivňují, lze dosáhnout vyššího stupně této konverze. Také se ukazuje, že se např. u lososa obecného jedná o vysoce dědivý znak (h^2 = 0,77) a existuje tak možnost v budoucnu šlechtit plemena či linie s vysokou schopností této syntézy (Leaver et al., 2011). Na rozdíl od lososa nejsou zatím u kapra nalezeny a funkčně charakterizovány všechny enzymy zapojené do syntézy n-3 HUFA. U kapra byla zatím funkčně charakterizována pouze Δ-6 desaturáza (Zheng et al., 2004). Ren et al. (2012) následně identifikoval dvě varianty Δ-6 desaturázy. Ty zatím nebyly funkčně charakterizovány a není tedy jasné, zda se skutečně jedná o dvě Δ-6 desaturázy, o bifukční Δ-6/Δ-5 desaturázy, či o jednu Δ-6 a jednu Δ-5 desaturázu. Watanabe et al. (2016) totiž ve své recentní studii ukázal, že změna byť jediné aminokyseliny v sekvenci desaturázy může změnit její substrátovou specificitu. V rámci probíhajícího GAJU projektu (095/2017/Z) chceme identifikovat

a funkčně charakterizovat enzymy, které se u kapra podílejí na konverzi ALA na n-3 HUFA. Předpokládáme, že to nám následně pomůže lépe porozumět tomu, jak různé environmentální, nutriční či genetické faktory ovlivňují tuto dráhu a podaří se nám nalézt nástroje, jak její efektivitu u kapra prakticky zvýšit.

V oblasti studia vlivu konzumace ryb na lidské zdraví se chceme ve spolupráci s Institutem klinické a experimentální medicíny zaměřit na objasnění hypotéz, které jsem naznačil v kapitole 3.8. a na vliv různých frakcí rybího masa na lidské zdraví.

Jelikož rybí maso rychle ztrácí kvůli mikrobiálním, autolytickým a autooxidačním procesům svou kvalitu zaměřujeme se také na to, jak ji udržet co možná nejdéle. V této oblasti vidím velký prostor pro zlepšení současné praxe pomocí optimalizace welfare ryb před zpracováním, způsobu zabití a vykrvení, zpracovatelských postupů, či použitím přírodních antimikrobiálních a antioxidačních látek (Lunda et al., 2016; Sternisa et al., 2016).

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7. Seznam prací autora, které jsou součástí habilitační práce

Součástí habilitační práce je celkem 8 publikovaných prací. Všechny práce byly publikovány v mezinárodních vědeckých časopisech s indikátorem IF databáze Web of Science společnosti Thomson Reuters. Práce jsou číslovány podle odkazů v jednotlivých kapitolách textu. Uvedené hodnoty IF u každé publikace jsou hodnotami v roce vydání popř. poslední dostupné v době sepsání této habilitační práce tj. IF v roce 2015.

Příloha č. 1: Mraz, J., Pickova, J., 2011. Factors influencing fatty acid composition of common carp (*Cyprinus carpio*) muscle. Neuroendocrinology Letters 32 (Suppl. 2), 3–8. (IF 2011 = 1.296)

Příloha č. 2: Zajic, T., **Mraz, J.**, Pickova, J., 2016. Evaluation of the effect of dietary sesamin on white muscle lipid composition of common carp (*Cyprinus carpio* L.) juveniles. Aquaculture Research 47, 3826–3836. (IF 2015 = 1.606)

Příloha č. 3: Mraz, J., Zajic, T., Pickova, J., 2012. Culture of common carp (*Cyprinus carpio*) with defined flesh quality for prevention of cardiovascular diseases using finishing feeding strategy. Neuroendocrinology Letters 33 (Suppl. 2), 101-108. (IF 2012 = 0.932)

Příloha č. 4: Zajic, T., **Mraz, J.**, Sampels, S., Pickova, J., 2013. Fillet quality changes as a result of purging of common carp (*Cyprinus carpio* L.) with special regard to weight loss and lipid profile. Aquaculture 400–401, 111–119. (IF 2013 = 1.828)

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8. Český abstrakt

Mráz, J., 2017. Kvalita lipidů v mase kapra obecného (*Cyprinus carpio*). Habilitační práce, Jihočeská univerzita v Českých Budějovicích, Fakulta rybářství a ochrany vod, Ústav akvakultury a ochrany vod: 40 s.

Předloženou habilitační práci tvoří komentovaný soubor 8 publikací zaměřených na obsah a kvalitu lipidů v mase kapra obecného (*Cyprinus carpio*) a na vliv konzumace kapřího masa v sekundární prevenci kardiovaskulárních onemocnění.

V první částí jsou sumarizovány faktory ovlivňující obsah tuku a kompozici mastných kyselin v mase kapra. Byl studován vliv bioaktivního lignanu sesaminu a dvou různých poměrů n-3 a n-6 kyselin v krmivu na kvalitu masa kapra. V chovu kapra byla úspěšně otestována krmná strategie „finishing feeding“. Byl zkoumán vliv podzimního sádkování a předchozí výživy na změny obsahu tuku a kompozice mastných kyselin v mase tržního kapra. Byly testovány dvě *In situ* metody (použití fatmetru a měření výšky hřbetního tuku) pro rychlé nedestruktivní stanovení obsahu tuku v mase kapra. Byl sledován vliv smažení kapřích filet ve čtyřech běžně používaných tucích na výslednou kvalitu masa.

V druhé části je popsána patentovaná technologie chovu kapra se zvýšeným obsahem n-3 mastných kyselin. Ta je založena na maximálním využití přirozené potravy (plankton a bentos) v kombinaci s doplňkovou krmnou směsí obsahující řepkové výlisky a extrudované lněné semínko.

Třetí část této habilitační práce pojednává o vlivu konzumace kapřího masa v sekundární prevenci kardiovaskulárních onemocnění. Zařazení kapra do jídelníčku (2 × 200 g kapřího masa týdně/ po dobu 4 týdnů) mělo pozitivní vliv na vylepšení lipidů a markeru zánětu v plasmě pacientů po operaci srdce. Velikost tohoto efektu je ovlivněna tím, z jakého typu produkčního systému kapr pochází a jakou má kompozici mastných kyselin.

Klíčová slova: kapr, kardiovaskulární onemocnění, lidské zdraví, mastné kyseliny, rybniční akvakultura, výživa

9. Anglický abstrakt

Mráz, J., 2017. Lipid Quality of Common Carp (*Cyprinus carpio*) Flesh. Habilitation thesis, University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Institute of Aquaculture and Protection of Waters: 40 pp. (in Czech)

The presented habilitation thesis consists of an annotated set of 8 publications focused on the content and quality of lipids in carp (*Cyprinus carpio*) flesh as well as on the influence of carp consumption on the secondary prevention of cardiovascular diseases.

The first part summarizes the factors influencing the fat content and the fatty acid composition in carp flesh. The effects of bioactive lignan sesamin and two different ratios of n-3 and n-6 fatty acids in the feed on the quality of carp were studied. The finishing feeding strategy was successfully tested in carp breeding. The effect of the autumn purging period and previous nutrition on the changes in the fat content and fatty acid composition in carp flesh was investigated. Two *In situ* methods (the use of a fat meter and the measurement of the dorsal fat height) were tested for rapid non-destructive determination of the fat content in carp flesh. The effect of frying in the 4 commonly used fats on the carp fillet quality was monitored.

In the second part, a patented carp breeding technology with an increased content of n-3 fatty acids is described. It is based on the maximal use of natural feed (plankton and benthos) in combination with a supplemental feed mixture containing rapeseed cake and extruded linseed.

The third part of this habilitation thesis deals with the influence of carp flesh consumption on the secondary prevention of cardiovascular diseases. The incorporation of carp in the diet (2 × 200 g carp flesh per week / for 4 weeks) had a positive effect on the improvement of lipids and the marker of inflammation in plasma of patients after heart surgery. The magnitude of this effect is influenced by a pond production system as well as the carp flesh fatty acids composition.

Key words: cardiovascular diseases, carp, fatty acids, human health, nutrition, pond aquaculture

10. Poděkování

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11. Přílohy

Příloha č. 1

Mraz, J., Pickova, J., 2011. Factors influencing fatty acid composition of common carp (*Cyprinus carpio*) muscle. Neuroendocrinology Letters 32 (Suppl. 2), 3–8. (IF 2011 = 1.296)

Factors influencing fatty acid composition of common carp (*Cyprinus carpio*) muscle

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Key words: **common carp; DHA; EPA; fatty acids; improvement; n-3 HUFA**

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Abstract

There is evidence that n-3 highly unsaturated fatty acids (n-3 HUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are beneficial for human health, especially for the cardiovascular system. The sources of n-3 HUFA, including EPA and DHA, are scarce in diet consumed by the Czech population. Thus, it would be beneficial to generally increase fish consumption and also to increase the content of the beneficial fatty acids (FA) in locally produced fish and other products. Therefore the overall aim of this paper was to review factors influencing lipid content and composition in common carp, which is the major cultured fish in the Czech Republic, and to identify long term sustainable ways for increasing the beneficial fatty acids in the carp flesh. We conclude that there are several ways to improve the FA composition of common carp in the traditional pond production. High amount of natural food, good supplemental diet containing high level of alpha-linolenic acid (ALA) and suitable processing and cooking were identified as the most important ones.

Abbreviations:

ALA	- alpha-linolenic acid
DHA	- docosahexaenoic acid
EPA	- eicosapentaenoic acid
FA	- fatty acids
HUFA	- highly unsaturated fatty acids
MUFA	- monounsaturated fatty acids
PUFA	- polyunsaturated fatty acids

INTRODUCTION

There is evidence that n-3 highly unsaturated fatty acids (n-3 HUFA; carbons ≥20, double bonds ≥3), especially eicosapentaenoic (20:5 n-3; EPA) and docosahexaenoic acid (22:6 n-3; DHA) are beneficial for human health. EPA and DHA have many different functions in human body, e.g.: influencing the physical nature of cell membranes, membrane-protein-mediated responses, being eicosanoid precursors, cell signalling and gene expression (Calder & Yaqoob 2009). EPA and DHA have been shown to have beneficial effect in a range of cardiovascular risk factors, which result in primary cardiovascular prevention, reduction

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in total and cardiovascular mortality (Calder & Yaqoob 2009).

Several studies showed that n-3 HUFA biosynthesis is limited in humans (Burdge & Calder 2005) and therefore it was proposed they should be consumed directly for optimal health status.

Today's western diet is generally deficient in n-3 fatty acids (FA) and excessive in n-6 FA resulting in a low n-3/n-6 ratio. It was proposed that we have been evolutionary adapted to a diet with the n-3/n-6 ratio close to 1:1 whereas in the western diet it exceeds 1:15 (Leaf & Weber 1987; Simopoulos 2008). This dietary change is associated with many life style diseases, including cardiovascular diseases, cancer, inflammatory and autoimmune diseases.

Currently, there are several dietary recommendations for n-3, n-6, EPA, DHA and fish intake around the world. The European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies proposed reference labelling intake values for general population: 250 mg EPA+DHA; 2 g alpha-linolenic acid (ALA; 18:3 n-3) and 10 g of linoleic acid (18:2 n-6) per day (EFSA 2009). WHO/FAO (2003) recommended that "regular fish consumption (1–2 servings per week) is protective against coronary hearth diseases and ischemic stroke". "The serving should provide an equivalent 200–500 mg EPA+DHA". The American Heart Association recommends for general population to "eat a variety of (preferably fatty) fish at least twice a week" (Kris-Etherton *et al.* 2002).

Generally, fish and fish products are not abundant in the food basket of the Czech population (5.5 kg of fish or fish products per capita per year (Ženíšková & Gall 2009). Other sources of n-3 HUFA, including EPA and DHA, are scarce in diet consumed by the Czech population (Hibbeln *et al.* 2006). Thus, it would be beneficial to generally increase fish consumption and also to increase the content of the beneficial FA in locally produced fish and other products.

Therefore the overall aim of this paper was to review factors influencing lipid content and composition in flesh of common carp (*Cyprinus carpio*), which is the major cultured fish in the Czech Republic and to identify long term sustainable ways for increasing the beneficial fatty acids in the carp flesh.

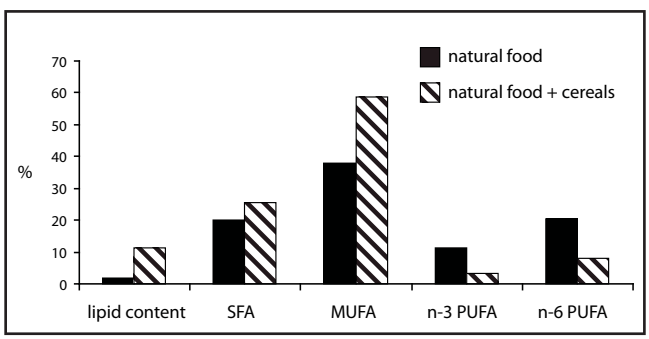

Fig. 1. Lipid content and fatty acid composition of muscle total lipids of carps cultured on natural food only (natural food; mean weight 0.8 kg) or supplemented with cereals (natural food + cereals; mean weight 1.6 kg) (Adapted from Vácha *et al.* 2007).

FACTORS INFLUENCING LIPID CONTENT AND COMPOSITION IN COMMON CARP FLESH

Lipid content and composition in carp flesh is influenced by several environmental and internal factors. The environmental factors are e.g. diet, starvation, and water temperature and the internal factors are e.g. genetic background, size, age, sex, maturation. When the carp flesh is prepared as a meal for consumers other factors further influence the final composition such as the part of the fish used, processing and preparation (cooking, frying, use of additional fat). The mentioned factors will be explained in detail in following chapters and potential for improvements will be discussed.

ENVIRONMENTAL FACTORS

"You are what you eat" – the nutrition has a major impact on the lipid content and composition in common carp. Generally, carp reflects the lipid pattern of its diet to a high extent (Steffens 1997; Steffens & Wirth 2007). Common carp is traditionally produced by semi-intensive techniques in earthen ponds. Carp nutrition is based on natural food zooplankton and zoobenthos (Adámek *et al.* 2003), and supplemental feeding to fulfill the energy requirements (Buchtová *et al.* 2007). The natural food (Bell *et al.* 1994; Bogut *et al.* 2007; Domaizon *et al.* 2000; Mráz & Pickova 2008) is rich in n-3 HUFA therefore the carps cultured on natural food only have high content of these beneficial FA in the flesh (Csengeri 1996). The cereal supplementation generally causes higher lipid content, high level of monounsaturated FA (MUFA) and low level of n-3 FA in the carp flesh (Csengeri 1996; Steffens 1997; Steffens & Wirth 2007; Vácha *et al.* 2007) (Figure 1).

Since carp is able to bio-convert ALA to n-3 HUFA (Tocher & Dick 1999; Tocher 2003) it might be possible to increase n-3 HUFA content in carp flesh by supplemental feeding containing ALA. Such supplemental pellets based on rapeseed cake and extruded linseed as a lipid source were developed and tested (Mráz *et al.* unpublished results). Fillets of carps supplemented by these pellets were characterized by lower level of MUFA, higher level of n-3 PUFA, n-3 HUFA and with lower n-6/n-3 ratio compared to the carps supplemented by cereals (Figure 2). One serving (200 g) of fillet from this carp contained 300 mg of EPA+DHA and 1 g of n-3 FA which is very close to the recommended values.

Generally, bioactive minor lipid compounds have been shown to be able to affect the lipid profiles in fish. Such compounds studied are e.g. conjugated linoleic acid, alpha-lipoic acid, tetradecylthioacetic acid and sesamin/episesamin. Inclusion of bioactive com-

pounds in the diet might be used to influence muscle lipid composition by alteration of the fish metabolism to synthesize or deposit more n-3 HUFA or to affect β-oxidation (Pickova *et al.* 2010). Alpha-lipoic acid is a potential bioactive compound which was observed to influence lipid composition in fish. It acts as an antioxidant, influences lipid homeostasis (Navari-Izzo *et al.* 2002) and was found to increase level of EPA in muscle polar lipids in South American pacu (*Piaractus mesopotamicus*) (Trattner *et al.* 2007). Conjugated linoleic acid and tetradecylthioacetic acid were proposed to have stimulatory effect on DHA synthesis in salmonids (Kennedy *et al.* 2007). Sesamin/episesamin was suggested to be a potent compound in lipid homeostasis showing effects in fatty acid composition of tissues (Trattner *et al.* 2008a; Trattner *et al.* 2008b). It was shown to increase DHA in white muscle of rainbow trout (*Oncorhynchus mykiss*) (Trattner *et al.* 2008a) and in Atlantic salmon (*Salmo salar*) hepatocytes (Trattner *et al.* 2008). However, such an effect was not seen in common carp (Mráz *et al.* 2010).

Purging of fish before slaughtering is a common practice in carp culture in order to remove possible off-flavors and eliminate undigested food from intestine (Vácha *et al.* 2007). This clean and off-flavours quality is achieved by keeping the fish in clean and cold running water without feeding of the fish for several weeks. It was proposed that this practice has a positive impact on the fish nutritional quality because it reduces excessive fat and increases percentage of n-3 HUFA (Einen *et al.* 1998; Palmeri *et al.* 2008). Csengeri (1996) observed that purging decreased level of oleic acid in the carp muscle whereas PUFA were protected (Figure 3).

Water temperature was shown to influence carp lipid metabolism. Carps increase activity of enzymes involved in FA desaturation with decreasing temperature to adapt the fluidity of membranes (Cossins *et al.* 2002). Increased formation of n-3 HUFA in liver of common carp exposed to low temperature was shown by Farkas (1984). This factor may play a role during the purging period when the carps are subjected to the decreasing temperature in combination with starvation.

INTERNAL FACTORS

Another important factor affecting lipid content and composition is the genetic background. It was shown that muscle lipid content is a highly heritable trait (>0.5) in common carp and that there is a relatively high positive genetic correlation between body size (standard length and body weight) and lipid content (0.71 and 0.59, respectively) (Kocour *et al.* 2007).

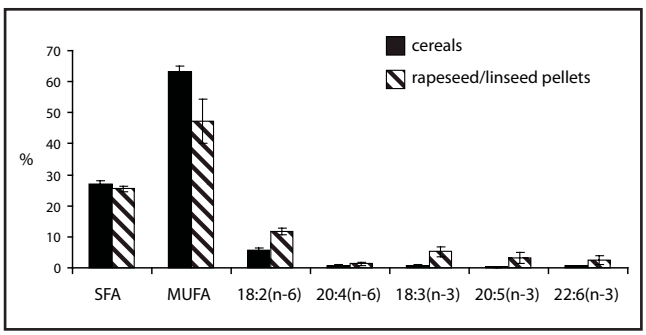

Fig. 2. Fatty acid composition of fillet total lipids of carps supplemented either by cereals (cereals; mean weight 1.7 kg; lipid content 6.8 %; n = 6) or pellets based on rapeseed cake and extruded linseed as a lipid source (rapeseed/linseed pellets; mean weight 2 kg; lipid content 6.2; n = 6) (Adapted from Mráz *et al.* unpublished results).

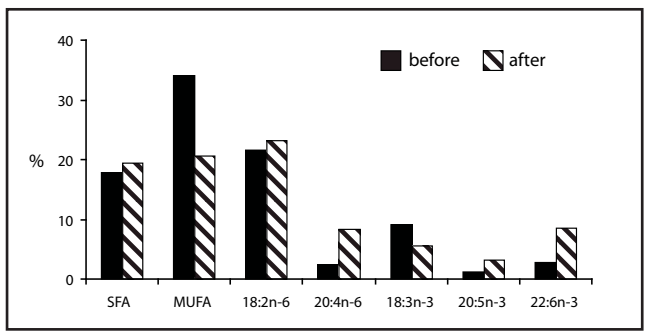

Fig. 3. Effect of 11-week starvation at 25 °C on the carp muscle total lipid fatty acid composition (weight of the fish 150–200 g). (Adapted from Csengery 1996).

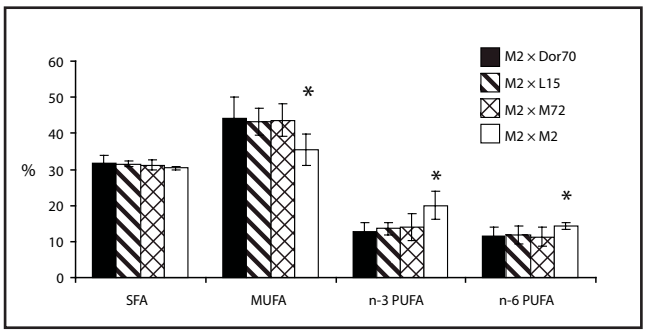

Fig. 4. Fatty acid composition of dorsal muscle total lipids from four different carp crossbreeds (n = 4) (Adapted from Mráz & Pickova 2008).

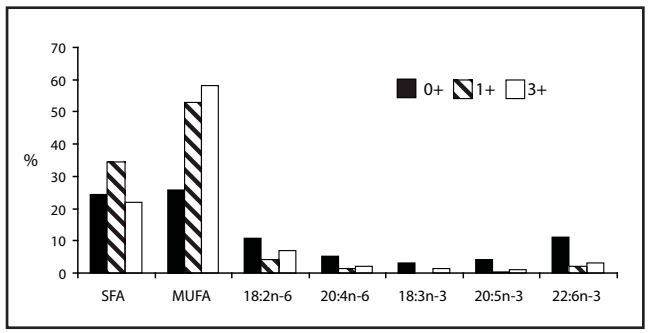

Fig. 5. Fatty acid composition of muscle total lipids in three age groups (mean weight: 0 + 15 g; 1 + 600 g; 3 + 2 600 g) of carp reared under pond conditions (Adapted from Csengery 1996).

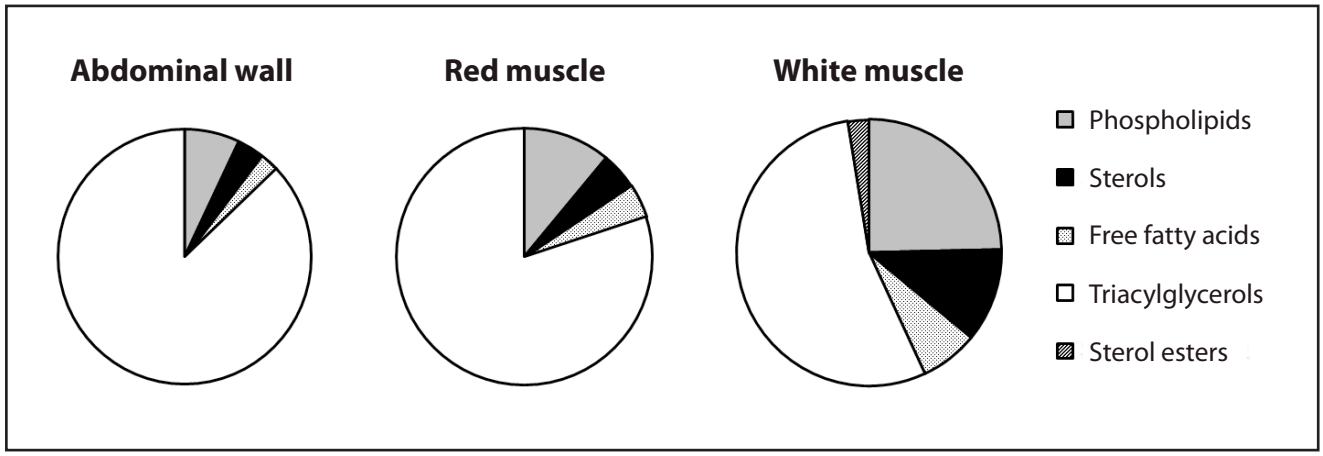

Fig. 6. Lipid class composition (as relative %) of three parts (white muscle, red muscle and abdominal wall) of common carp fillet (mean weight: 2 kg; n = 6) (Adapted from Mráz & Pickova 2008).

Leaver *et al.* (2011) analyzed flesh lipid parameters in 48 families of Atlantic salmon and showed that flesh n-3 HUFA composition is a highly heritable trait ($h^2 = 0.77 \pm 0.14$).

There are not many data available for carp about the effect of genetic origin on the lipid composition (Fauconneau *et al.* 1995). In a study with four carp crossbreeds, Buchtová *et al.* (2007) found that, the FA composition was not affected to any great extent by the hybrid type. Mráz & Pickova (2008) performed a similar study and found that the pure line of Hungarian mirror carp (M2 × M2) contained lower level of MUFA and higher level of PUFA compared to other three crossbreeds, when looking at the total lipid FA composition (Figure 4).

Nevertheless, there was no difference when phospholipid and triacylglycerol fractions were analyzed separately. So the differences seen in the total lipid FA composition were ascribed to differences in the lipid content and consequently to different proportion between the two lipid fractions and their different FA composition (Mráz & Pickova 2008).

Younger stages of carp are usually leaner and have higher percentage of phospholipids and consequently lower level of MUFA and higher percentage of n-3 HUFA compared to adult carps (Csengeri 1996) (Figure 5). Since the younger fish do not deposit high level of fat the FA turnover, selective storage and HUFA biosynthesis might play more important role compared to the adult fish.

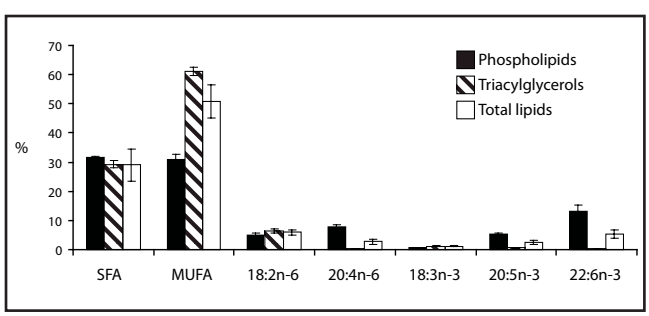

Fig. 7. Common carp white dorsal muscle fatty acid composition in total lipids, phospholipid and triacylglycerol fraction (mean weight: 2 kg; n = 6) (Adapted from Mráz & Pickova 2008).

Another factor with a strong effect on lipid content and composition in animals is sex or sexual maturation (De Smet *et al.* 2004; Nurnberg *et al.* 1998). Kocour *et al.* (2007) reported that females of Hungarian synthetic mirror carp were fattier than males probably due to later maturation. In study with four common carp hybrids, Buchtová *et al.* (2008) found only minor differences in lipid composition between males and females probably caused by different lipid content. Fajmonová *et al.* (2003) did not find any sexual dimorphism in lipid content and FA composition in three-year-old carps.

OTHER FACTORS

Fish fillet is highly heterogeneous and is composed from several different tissues, e.g., white muscle, red muscle, adipose tissue and skin. The tissues differ greatly in lipid content and therefore the lipids are not equally distributed in the fillet. In common carp, this factor was studied by Mráz & Pickova (2008) showing that there is a large difference in lipid content of white dorsal muscle (~1–2%), red muscle (16–17%) and the abdominal wall (~30%) reflected in the FA composition. The percentage of n-3 PUFA was negatively correlated with increasing fatness. This was suggested to be explained by the FA composition of the major lipid fractions and the relative contribution of these fractions to total lipids (Figure 6).

FA composition of phospholipid and triacylglycerol fraction was quite similar in saturated fatty acids (SFA) (~30%) but very different in MUFA and PUFA. Phospholipid fraction had lower level of MUFA (~30%) and higher level of PUFA (~40%), especially EPA and DHA compared with triacylglycerol fraction (MUFA 60%; PUFA 10%) (Figure 7). Thus the white muscle, being the leanest, was strongly influenced by phospholipids and had significantly higher proportion of

n-3 HUFA, EPA and DHA (2008). This was suggested to have implications for processing of products from carp.

The last but not the least factor influencing lipid content and composition is processing and cooking. It was proved that especially the quality of fats and oils added during processing has a very strong influence on lipid composition (Ansorena & Astiasaran 2004; Sampels *et al.* 2009). Sampels *et al.* (2009) found very high variation of n-6/n-3 ratio in fish products being up to 400 times higher than in the raw fish. It was concluded that fat used during the processing and preparation has the largest impact on the food FA content and composition and proposed that it should be declared on the product label (Pickova 2009; Sampels *et al.* 2009).

FUTURE PROSPECTIVE

As already mentioned the knowledge about the effect of genetic background of carp on muscle fatty acid composition is scarce and should be broaden. It is possible that carp lines with either higher capacity of HUFA biosynthesis or higher HUFA deposition will be identified in available stocks or achieved by selection from these which would enable their use in future carp culture.

The limitation of the studies carried on with carp so far is in genetical closeness of the lines studied. The other limitation is the use of semi-intensive technique for such experiments. The first reason is that it is not possible to separate between possible genetic differences in lipid metabolism and behavioral differences among the breeds as the behavior can cause different growth and fattening pattern. The second reason is that when using supplemental feed based on cereals the fish do not have enough ALA (n-3 HUFA precursors) in the diet to show any difference in ability to biosynthesize n-3 HUFA. In our on-going trial, we use carp lines which are genetically more distant, e.g.: the two subspecies of common carp *C.c. carpio* and *C.c. haematopterus*. The trial is performed under controlled conditions and the carps are fed a diet containing high level of ALA.

The research focused on the use of bioactive compounds to modulate fish lipid composition is also a promising area with potential to be used in future aquaculture. In spite of the positive experimental results with the above mentioned compounds the mechanisms behind these effects have not been fully understood yet. This aspect need to be addressed before the usage of such compounds in aqua-feed production. It is likely that in future some potent bioactive compound with positive effect on HUFA biosynthesis in common carp will be identified and used to increase n-3 HUFA content in carp flesh.

There is a general presumption that by understanding the molecular mechanisms behind the HUFA biosynthesis we might find tools enabling us to use more effectively the fish ability to produce n-3 HUFA (Zheng *et al.* 2004). Genes involved in the HUFA biosynthesis, except already characterized Δ-6 desaturase (Zheng *et al.* 2004), and the mechanisms of their regulation remains to be identified and characterized for common carp. The rapidly evolving "omics" techniques as well as the sequencing of common carp genome (Carp Genome Project – China) will probably help to address these questions. This might further improve the carp culture to become an important producer of n-3 HUFA for human nutrition.

CONCLUSIONS

To conclude that common carp muscle has favorable FA composition and should be regarded as a healthy product. However, there are several possibilities to further improve the lipid composition of cultured carp according to new research. High amount of natural food, good supplemental diet containing high level of ALA and suitable processing and cooking with use of healthy ingredients were identified as the most important ones. Further improvement might be reached in future by better understanding of HUFA biosynthesis, effects of genetic background and consequent selection of carps with higher ability of n-3 HUFA biosynthesis as well as the development in the area of bioactive compounds.

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Příloha č. 2

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Evaluation of the effect of dietary sesamin on white muscle lipid composition of common carp (*Cyprinus carpio* L.) juveniles

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Abstract

This study was focused on the clarification of the effect of dietary sesamin on fatty acids and the composition of different lipid fractions [phospholipids (PLs), cholesterol and triacylglycerols] in the white muscle of common carp (*Cyprinus carpio* L.) juveniles. Two different basic diets with defatted fishmeal as a protein source and either only linseed oil or a mixture of linseed and sunflower oil as a lipid source designed to have two different n-3/n-6 ratios (1.21 – CL group; 0.32 – CM group) were produced. Each diet was then used with or without added sesamin (0.58 g $100g^{-1}$). One hundred and forty-four individuals were fed in triplicated groups for 63 days until their weight had doubled. No influence of dietary sesamin on growth, mortality or on the white muscle lipid content of the fish was found. Added sesamin significantly decreased the content of PLs and increased the cholesterol content in the CM group. No effect was found in the total lipid fatty acid composition but there was found a significantly lower content of saturated fatty acids and 20:5n-3 in PLs and of 22:6n-3 in triacylglycerols in the sesamin supplemented CL group. These and other differences show either a tendency of lower long chain n-3 fatty acids biosynthesis or their higher use in β-oxidation in sesamin-supplemented groups. We conclude that sesamin in this experiment had no substantial positive impact on the lipid metabolism of juvenile carp.

Keywords: biosynthesis, carp, fatty acid, linseed oil, sesamin, sunflower oil

Introduction

The best sources of n-3 long chain polyunsaturated fatty acids (LC-PUFA) for human consumption are fish oil or fish muscle respectively; but fish consumption is still very low, especially in landlocked countries (Sargent, Henderson & Tocher 2002; Howe, Meyer, Record & Baghurst 2006; De Henauw, Van Camp, Sturtewagen, Matthys, Bilau, Warnants, Raes, Van Oeckel & De Smet 2007). For humans, the metabolically most important fatty acids from fish are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from the n-3 series. In addition, arachidonic acid from the n-6 series and the precursors of all of these LC-PUFA, alpha-linolenic acid (ALA-18:3n-3) and linoleic acid (LA-18:2n-6) have a major impact on various metabolic functions in the human body, i.e. inflammatory and anti-inflammatory functions of metabolic products of these acids (Simopoulos 2002).

Furthermore, it is known that the present use of fish oil as a dietary fat source for aquaculture feeds, as well as a source of n-3 LC-PUFA for human consumption pharmaceuticals and other uses, is not managed in a sustainable way (Turchini, Torstensen & Ng 2009; Tacon & Metian 2013). Within the next decade, some alternative ways for the feeding of aquaculture fish need to be

developed to counteract the increasing demand for other applications as well as the increasing costs for feeds containing fish oil. Most likely, land-based (plant) sources will be used for replacing traditional fish oil (Pickova & Mørkøre 2007). Many studies focused on fish oil replacement have been carried out both in freshwater and marine fish species (Bell, McEvoy, Tocher, McGhee, Campbell & Sargent 2001; Tocher 2003; Mourente, Good & Bell 2005; Schulz, Knaus, Wirth & Rennert 2005; Mørkøre 2006; Pettersson, Pickova & Brännäs 2009). As vegetable oils, such as linseed (flaxseed), rapeseed (canola) or sunflower oil contain mainly 18 carbons precursors of both n-3 and n-6 LC-PUFA, this will be reflected in the FA composition of the fish. However, some fish species, especially from freshwater, are able to convert the short chain precursors towards the LC-PUFA via several metabolic pathways (Tocher, Carr & Sargent 1989). In addition, earlier research has shown that there are some possibilities to increase this conversion in fish by dietary biological active compounds (Trattner, Pickova, Park, Rinchard & Dabrowski 2007). One good candidate of such compound is sesamin (Pickova 2009).

Sesamin is a natural lignan present in sesame seeds and oil in relatively high amounts (Moazzami & Kamal-Eldin 2006). The properties of sesamin as a lipid modulator have been investigated positively (increased of PUFA concentration) in mammalian systems, specifically in rats (Mizukuchi, Umeda-Sawada & Igarashi 2003; Ide, Hong, Ranasinghe, Takahashi, Kushiro & Sugano 2004) and in human lipid metabolism (Jeng & Hou 2005). The use of sesamin as a modulator of LC-PUFA composition in fish was tested successfully on rainbow trout (*Oncorhynchus mykiss*) (Trattner, Kamal-Eldin, Brannas, Moazzami, Zlabek, Larsson, Ruyter, Gjoen & Pickova 2008; Vestergren, Trattner, Pan, Johnsson, Kamal-Eldin, Brännäs, Moazzami & Pickova 2013). In these studies, dietary sesamin supplementation resulted in a significant increase of elongation and desaturation processes from ALA to DHA. Also, in an *in vitro* study on Atlantic salmon (*Salmo salar*) hepatocytes the effect of sesamin on elongation and desaturation has been proved by Trattner, Ruyter, Ostbye, Gjoen, Zlabek, Kamal-Eldin and Pickova (2008) and by Schiller Vestergren, Trattner, Mraz, Ruyter and Pickova (2011). Alhazzaa, Bridle, Carter and Nichols (2012) observed the positive influence of sesamin on total lipid (TL) n-3 LC-PUFA levels in early juvenile barramundi (*Lates calcarifer*). The effect of dietary sesamin in market common carp was studied by Mráz, Schlechtriem, Olohan, Fang, Cossins, Zlabek, Samuelsen and Pickova (2010) and on molecular level by Wagner, Zlabek, Trattner and Zamaratskaia (2013) or Wagner, Trattner, Pickova, Gómez-Requeni and Moazzami (2014) for common carp and Atlantic salmon respectively.

Composition of fatty acids in carp is very variable (Mráz & Pickova 2011), and its fat contains generally lower levels of n-3 LC-PUFA compared with e.g. salmon. However, there are ways to significantly increase the proportion of n-3 LC-PUFA in carp fillet (Mráz, Máchová, Kozák & Pickova 2012; Mráz, Zajic & Pickova 2012; Zajic, Mraz, Sampels & Pickova 2013). The effect of dietary sesamin on the fatty acid metabolism in adult common carp was found ineffective; however, an increased expression of genes related to lipid metabolism was observed (Mráz *et al.* 2010). However, the earlier studies on salmonids showing the positive effects of sesamin (Trattner, Kamal-Eldin *et al.* 2008) were carried out on juveniles. Carp juveniles have slightly different requirements on essential FA in the diet. While the recommended dosage (% of diet) for larval and early juvenile stages is no less than 0.25% LA and 0.75% ALA (Radunz-Neto, Corraze, Bergot & Kaushik 1996), the same recommendation for older juvenile and pre-adult fish is 1% LA and 0.5–1% LA respectively (Takeuchi 1996). Unlike some other widely farmed fish (i.e. salmonids), carp has no direct needs on LC-PUFA presence in the diet. Moreover, the natural diet of a given species can change substantially during development, so the ability to convert C18 PUFA to LC-PUFA can also differ (Sargent *et al.* 2002). Hence, our hypothesis was that due to possible differences in lipid metabolism of fish of different age (the metabolism of juvenile fish is known to be faster compared with adults), sesamin as a lipid modulator might mostly be effective in juvenile fish. In addition, the feeds used in a previous study (Mráz *et al.* 2010) contained some proportion of fish oil and we believe that an increased presence of the LC-PUFA might inhibit synthesis. Therefore, no fish oil or defatted fish meal were used in the present study. A third aspect influencing the metabolism might be the proportion of substrate and the ratio of n-3/n-6 in the diet, because it is known that the same desaturase and elongase enzymes are active on the n-3 and n-6 FA series, but due to competition between the n-3 and n-6 PUFA, a dietary excess

of ALA will depress the metabolism of LA, and vice versa (Tocher, Bell, McGhee, Dick & Fonseca-Madrigal 2003).

Therefore, the aim of this study was to investigate the effect of dietary sesamin on the amounts of LC-PUFA of juvenile carp fed a diet low in LC-PUFA and with two different n-3/n-6 ratios. In case we are able to increase LC-PUFA biosynthesis, sesamin could be used as an effective lipid modulator in feeds for sustainable farming of common carp with greater fillet quality for consumers.

Material and methods

Fish, diets and experimental design

The carps used in this experiment were taken from an experimental rearing system (Faculty of Fisheries and Protection of Waters, Vodňany, Czech Republic). One hundred and forty-four juveniles were divided into four triplicate groups (CLS, carp linseed oil+sesamin; CLC, carp linseed oil control; CMS, carp linseed/sunflower oil+sesamin; CMC, carp linseed/sunflower oil control) and distributed randomly into 12 aquaria (100 L) and 12 individuals per tank (8.3 L per fish). The composition of the experimental diets is presented in Table 1. Diet ingredients were mixed in a household food mixer. Oil was gradually added during mixing. Pellets were produced through a cold extruder and obtained strips cut manually. The proportion of used vegetable oils was calculated to achieve different n-3/n-6 ratio (0.32 in CM and 1.21 in CL group respectively). An adaptation period of 15 days was applied prior the experiment. The experiment was conducted for 63 days with the initial weight of fish 53.1 ± 0.3 g. Fish were fed by the four experimental diets. The feeding rate was maintained between 2.5% and 3.5% of the actual biomass (weighing regularly every 2 weeks) in the tanks and was divided into three parts during a day to ensure all the feed was eaten (fish were fed by hand). The specific growth rate (SGR) as well as the feed conversion ratio (FCR) were calculated for each aquarium (n = 3) in Table 4. A nutritional composition of diets is shown in Table 1; the initial fatty acid composition in white muscle of experimental fish in Table 2.

The experiment was conducted in a recirculation system with mechanical and biological filtration and UV lamps were used for disinfection. The water temperature was kept at 22.8 ± 0.7°C and the photoperiod was constantly 12 h light/12 h dark. The oxygen content and pH were measured every day. The NH_3/NH_4^+, NO_2^- and NO_3^- content were measured regularly once a week. The results never excluded a safe range. The FCR and SGR were calculated, see Table 4.

Table 1 Formulation and nutritional composition (% of dry matter) of the experimental diets (CL, CM) with or without sesamin addition, provided to juvenile common carp

Ingredients (%)	CLS	CLC	CMS	CMC
Casein*	16	16	16	16
Gelatine*	1	1	1	1
Fish meal (defatted)	28	28	28	28
Dextrin*	20	20	20	20
Ca_3Po_4*	4	4	4	4
Potato starch†	16.5	16.5	16.5	16.5
Linseed oil‡	13	13	10	10
Sunflower oil‡	0	0	3	3
Sodium alginate*	1	1	1	1
Vitamins + minerals§	0.5	0.5	0.5	0.5
Sesamin (0.58 g $100g^{-1}$)¶	0.58	0	0.58	0
Nutritional composition (% of dry matter)				
Crude protein	38.21			
Crude fat	13.84			
NFE**	37.29			
Ash	10.66			
Gross energy (MJ kg^{-1})††	20.89			

*Obtained from VWR International GmbH, Vienna, Austria.
†Obtained from Carl Roth GmbH, Karlsruhe, Germany.
‡Obtained from local market.
§Vitamins+minerals mixture obtained from Guyokrma, Prague, Czech Republic.
¶Sesamin (98% purity) obtained from Kebiotech, Beijing, China.
**Nitrogen-free extract, NFE = 100 − (protein + lipid + ash + fibre).
††Calculated assuming conversion factors of 23.6, 39.5 and 17.2 MJ kg^{-1} for protein, lipid and NFE respectively.
Abbreviations: CLS, linseed sesamin group; CLC, linseed control group; CMS, linseed/sunflower sesamin group; CMC, linseed/sunflower control group.

Sampling

At the start of the experiment, samples of diets were taken to determine the fatty acid composition (Table 3). Six individuals were sampled for the analyses of the initial white muscle fatty acid composition in TL, storage lipids (triacylglycerols, TAG) and membrane lipids (phospholipids, PL) respectively. At the end of the experiments, three carps per aquarium (total of nine individuals from each group) were sampled. Fish were anaesthetized by

Table 2 Initial white muscle fatty acid composition (% of identified) in total lipid, triacylglycerol and phospholipid fraction of juvenile carp (mean values ± SD, $n = 6$)

	Total lipid	Phospholipids	Triacylglycerols
SFA	30.5 ± 1.3	33.5 ± 0.5	28.6 ± 2.5
MUFA	31.8 ± 3.6	20.4 ± 0.6	45.5 ± 3.2
18:2 n-6	9.82 ± 1.0	5.17 ± 0.4	14.9 ± 1.5
18:3 n-6	ND	0.11 ± 0.0	0.44 ± 0.1
20:2 n-6	0.47 ± 0.0	0.64 ± 0.1	0.26 ± 0.0
20:3 n-6	0.83 ± 0.2	1.29 ± 0.1	0.24 ± 0.0
20:4 n-6	4.35 ± 1.1	8.29 ± 0.4	0.43 ± 0.1
22:4 n-6	0.36 ± 0.1	0.61 ± 0.1	ND
Σ n-6 PUFA	15.8 ± 0.4	16.1 ± 0.5	16.2 ± 1.6
18:3 n-3	0.85 ± 0.2	0.24 ± 0.0	1.54 ± 0.0
18:4 n-3	0.58 ± 0.2	0.14 ± 0.1	1.20 ± 0.3
20:4 n-3	ND	0.16 ± 0.0	ND
20:5 n-3	4.58 ± 0.5	5.26 ± 0.4	2.63 ± 0.6
22:5 n-3	1.68 ± 0.2	2.40 ± 0.1	0.68 ± 0.1
22:6 n-3	14.1 ± 2.1	21.9 ± 0.7	3.50 ± 0.9
Σ n-3 PUFA	21.8 ± 2.2	30.1 ± 0.2	9.6 ± 1.9
n-3/n-6	1.38 ± 0.1	1.87 ± 0.1	0.60 ± 0.2

SFA, saturated fatty acids incl.: 12:0, 14:0, 15:0, 16:0, 18:0, 20:0; MUFA, monounsaturated fatty acids incl.: 16:1n-7, 18:1n-9, 18:1n-7, 18:1n-5, 20:1n-9; PUFA, polyunsaturated fatty acids; ND, not detected.

clove oil, measured, weighed, killed and filleted. The left fillets were packed, marked and immediately frozen in liquid nitrogen. All collected samples were stored in −80°C until further analysis.

Lipid extraction and separation of lipid classes

The fillets were thawed, de-skinned, the belly flap as well as the red muscle strip was removed by a scalpel and the white muscle was briefly homogenized on a table cutter. Then 1 g of the representative sample was taken for analysis. The lipids were extracted with hexane-isopropanol (3:2) according to Hara and Radin (1978) with slight modifications described in Zajic *et al.* (2013).

The total fish lipids were fractionated on TLC silica coated plates 20 × 20 cm (MERCK, Darmstadt, Germany) by placing the plates with 2 mg lipid bands in hexane:diethyl ether:acetic acid (85:15:1, v/v/v) solution for 1 h. The retention distance of the lipid classes was detected by an external standard (Nu-Check Prep, Elysian, MN, USA). The extraction of different lipid classes was performed following the method of Dutta & Appelqvist (1989).

Lipid class composition was measured as described in Zajic *et al.* (2013). Samples were applied with Camag ATS 4 automatic TLC sampler (CAMAG, Muttenz, Switzerland), the lipid classes were separated with ADC 2 automatic developing chamber. Different classes of lipid (PLs, CHOL – cholesterol, MAG – monoacylglycerols and TAG) were identified with a TLC scanner 4 by comparison with standard mixture 18-5C (Nu-Check Prep).

Preparation of fatty acid methyl esters

The samples were methylated according to Appelqvist (1968). First, 0.01 M NaOH in dry methanol were added to each tube. Next, BF_3 (14% boron trifluoride–methanol complex) were added (esterification). Then, 20% NaCl and hexane were added. The fatty acid methyl esters phase was transferred into new test tubes and evaporated under nitrogen

Table 3 Fatty acid composition (% of identified) of experimental diets for juvenile carp containing different vegetable oils as the lipid sources (mean ± SD, $n = 3$/group)

	CL		CM	
	% of total fatty acids	% of diet	% of total fatty acids	% of diet
∑SFA	10.6 ± 1.10	1.19 ± 0.04	11.3 ± 0.60	1.27 ± 0.04
∑MUFA	21.5 ± 2.00	2.39 ± 0.07	24.1 ± 1.60	2.70 ± 0.07
18:2 n-6	30.7 ± 3.30	4.09 ± 0.12	48.9 ± 3.30	5.47 ± 0.14
18:3 n-3	36.7 ± 3.10	3.42 ± 0.09	15.3 ± 1.90	1.99 ± 0.20
20:5 n-3	0.21 ± 0.02	0.03 ± 0.01	0.24 ± 0.00	0.03 ± 0.00
22:6 n-3	0.44 ± 0.10	0.05 ± 0.01	0.48 ± 0.10	0.06 ± 0.01
∑PUFA	67.9 ± 5.10	7.56 ± 0,21	64.6 ± 4.70	7.23 ± 0.18
n-3	37.4 ± 2.80	3.47 ± 0.10	15.7 ± 1.10	1.76 ± 0.04
n-6	30.7 ± 3.30	4.09 ± 0.12	48.9 ± 4.00	5.47 ± 0.14
n-3/n-6	1.21 ± 0.10		0.32 ± 0.10	

SFA, saturated fatty acids incl.: 14:0, 16:0, 18:0, 20:0, 22:0; MUFA, monounsaturated fatty acids incl.: 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 22:1n-11; PUFA, polyunsaturated fatty acids; CL, group with linseed oil; CM, group with linseed/sunflower oil mixture.

gas. The dry samples were dissolved in hexane, vortexed and stored in the freezer at −20°C prior to GC (gas chromatographic) analyses.

Gas chromatography

Fatty acid methyl esters were analysed in a gas chromatograph Varian CP3800 (Stockholm, Sweden) equipped with a flame ionization detector and split injector and fitted with a 50 m length × 0.22 mm i.d. × 0.25 μm film thickness BPX 70 fused-silica capillary column (SGE, Austin, TX, USA). The samples were injected by a CP8400 auto sampler with a split ratio of 1:10. The column temperature was programmed to start at 158°C for 5 min, then increased by 2°C min^{-1} from 158 to 220°C and remained in 220°C for 8 min. The carrier gas was helium (22 cm s^{-1}, flow rate 0.8 mL min^{-1}). The makeup gas was nitrogen. The injector and detector temperatures were 230 and 250°C respectively. Fatty acids were identified and their retention times were determined by comparing the peaks to those of the standard sample GLC-68 A (Nu-Chek Prep). Peak areas were integrated using the Star chromatography workstation with software version 5.5 (Varian).

Statistical analysis

All statistical analyses were performed using the Statistica CZ 12.0 software package (StatSoft CR, Prague, Czech Republic). Data are presented as mean and SD. Significant differences between groups within the same diet lipid source were tested using the Student's *t*-test. One-way ANOVA followed by Tukey's HSD test was used where needed. Differences were assumed to be statistically significant at $P < 0.05$.

Results

Fish growth

Only minimal mortality was observed during the experiment, which was caused by the escape of one fish from the group CLC. Fish from the sesamin groups (CLS, CMS) showed on a trend ($P < 0.11$) towards higher SGR compared with those without sesamin (CLC, CMC), but these differences were not statistically significant. There were no differences in the final weight among the groups, neither due to the used vegetable oil (linseed or sunflower/linseed) nor to the added sesamin. Also, no significant difference in the final FCR was observed (Table 4).

Fat content and fatty acid composition

The lipid content in the white muscle was 0.78 ± 0.14% at the beginning of the experiment and the final content was 0.90 ± 0.14%, 0.89 ± 0.21%, 0.88 ± 0.16% and 0.96 ± 0.19% for CLS, CLC, CMS and CMC group respectively. No significant differences were seen. The different oil and the addition of sesamin to the feeds did not cause any changes in the white muscle lipid content of juvenile common carp. During the experiment, there was no evidence of any positive significant effect of added sesamin on the TL LC-PUFA content in the white muscle of experimental fish (Table 5). The influence of the dietary fat source and the n-3/n-6 ratio respectively was visible between the groups with different vegetable oil as a lipid source. The n-3/n-6 ratio in the diet was clearly reflected in the final ratio in the TL fraction of white muscle. The higher value of sunflower oil increased the LA and the total n-6 PUFA's content in the fillet. Simultaneously, there were no significant differences in the fatty acid composition of the TL fraction between the groups with the same lipid source with or without sesamin addition. The performed ANOVA and Tukey's test revealed the expected differences in content of linoleic and ALAs related to the lipid composition of the diets. However, Table 5 also shows some differences between CM and CL groups in the representation of fatty acids biosynthesized from C18 precursors. An increased ALA and LA content in

Table 4 Initial and final weight, SGR and FCR of juvenile common carp fed the different vegetable oils as the lipid source and with/without sesamin addition (mean ± SD)

	Initial weight (g)	Final weight (g)	SGR (% day^{-1})	FCR
CLS	53.2 ± 9.2	125.9 ± 29.3	1.35 ± 0.05	1.49 ± 0.11
CLC	53.0 ± 10.2	121.0 ± 23.2	1.25 ± 0.12	1.54 ± 0.13
CMS	52.8 ± 8.3	118.9 ± 22.5	1.35 ± 0.09	1.48 ± 0.09
CMC	53.2 ± 8.3	113.4 ± 24.9	1.18 ± 0.15	1.45 ± 0.12

For abbreviations of group names see Table 1. Specific growth rate (SGR) = (ln final weight − ln initial weight)/days*100; Feed conversion ratio (FCR) = (final weight − initial weight) *ingested feed.

Table 5 Fatty acid composition (% of identified fatty acid) in phospholipids and triacylglycerols of white muscle of juvenile common carp fed the diets with different vegetable oils as the lipid source and with/without sesamin addition (mean values ± SD, $n = 9$)

	Total lipids				**Phospholipids**				**Triacylglycerols**			
	CLS	**CLC**	**CMS**	**CMC**	**CLS**	**CLC**	**CMS**	**CMC**	**CLS**	**CLC**	**CMS**	**CMC**
ΣSFA	22.9 ± 1.5	23.1 ± 1.8	22.6 ± 2.3	22.5 ± 1.3	29.8 ± 1.0**aY**	28.4 ± 0.8**bX**	28.4 ± 0.7**ab**	28.1 ± 0.9**b**	15.9 ± 0.8	16.0 ± 0.9	15.5 ± 1.3	15.8 ± 0.5
ΣMUFA	24.0 ± 2.5	23.2 ± 3.0	23.5 ± 2.7	25.5 ± 2.1	17.2 ± 1.0	17.2 ± 1.1	16.6 ± 0.6	16.9 ± 0.8	35.0 ± 1.6	34.9 ± 3.1	35.6 ± 1.9	36.9 ± 1.8
18:2 n-6	18.3 ± 1.5**b**	18.2 ± 2.5**b**	24.0 ± 3.8**a**	23.9 ± 2.6**a**	11.0 ± 0.8**b**	11.5 ± 0.9**b**	14.0 ± 0.5**a**	14.3 ± 0.9**a**	27.3 ± 1.0**b**	27.0 ± 2.1**b**	35.1 ± 2.2**a**	33.6 ± 1.6**a**
18:3 n-6	0.35 ± 0.0**b**	0.33 ± 0.1**b**	0.55 ± 0.2**a**	0.47 ± 0.1**ab**	0.20 ± 0.1	0.21 ± 0.1	0.32 ± 0.1	0.25 ± 0.1	0.42 ± 0.1**b**	0.43 ± 0.1**b**	0.71 ± 0.2**a**	0.59 ± 0.2**ab**
20:2 n-6	0.71 ± 0.1**b**	0.69 ± 0.1**b**	0.85 ± 0.2**a**	0.88 ± 0.1**a**	1.05 ± 0.1**b**	1.03 ± 0.1**b**	1.31 ± 0.1**a**	1.40 ± 0.2**a**	0.38 ± 0.0**b**	0.32 ± 0.1**b**	0.44 ± 0.1**ab**	0.46 ± 0.1**a**
20:3 n-6	1.26 ± 0.2**b**	1.21 ± 0.2**b**	1.62 ± 0.3**a**	1.56 ± 0.2**a**	1.88 ± 0.1**b**	1.77 ± 0.2**b**	2.42 ± 0.1**a**	2.48 ± 0.4**a**	0.39 ± 0.0**bY**	0.34 ± 0.0**bX**	0.52 ± 0.1**a**	0.51 ± 0.1**a**
20:4 n-6	2.65 ± 0.9	2.36 ± 0.5	3.18 ± 0.7	2.96 ± 0.7	4.61 ± 1.1**b**	4.11 ± 0.2**b**	5.84 ± 0.5**a**	5.58 ± 0.8**a**	0.29 ± 0.1**b**	0.27 ± 0.0**b**	0.36 ± 0.1**ab**	0.41 ± 0.1**a**
22:4 n-6	0.05 ± 0.0	0.06 ± 0.0	0.10 ± 0.0	0.07 ± 0.0	0.26 ± 0.1	0.24 ± 0.0	0.36 ± 0.1	0.32 ± 0.1	ND	ND	ND	ND
Σn-6 PUFA	23.3 ± 1.1b	22.8 ± 1.9b	30.3 ± 2.8a	29.8 ± 1.8a	19.0 ± 1.4b	18.9 ± 0.8b	24.3 ± 0.6a	24.4 ± 0.9a	28.8 ± 1.0b	28.4 ± 2.1b	37.1 ± 2.2a	35.6 ± 1.5a
18:3 n-3	10.3 ± 1.7**a**	10.5 ± 1.9**a**	5.58 ± 1.4**b**	5.24 ± 0.8**b**	4.92 ± 0.5**a**	5.39 ± 0.7**a**	2.37 ± 0.1**b**	2.43 ± 0.1**b**	16.0 ± 1.4**a**	16.1 ± 1.9**a**	8.32 ± 0.8**b**	7.69 ± 0.8**b**
18:4 n-3	0.79 ± 0.2**a**	0.61 ± 0.2**a**	0.44 ± 0.2**b**	0.44 ± 0.2**b**	0.26 ± 0.2**a**	0.25 ± 0.1**a**	0.12 ± 0.1**ab**	0.06 ± 0.0**b**	0.89 ± 0.2**a**	0.97 ± 0.3**a**	0.65 ± 0.2**b**	0.68 ± 0.2**ab**
20:4 n-3	0.58 ± 0.1**a**	0.58 ± 0.1**a**	0.11 ± 0.0**b**	0.19 ± 0.2**b**	0.84 ± 0.1**a**	0.87 ± 0.2**a**	0.39 ± 0.2**b**	0.46 ± 0.1**b**	0.38 ± 0.1**a**	0.31 ± 0.2**a**	0.03 ± 0.0**bX**	0.13 ± 0.1**bY**
20:5 n-3	2.95 ± 0.4**ab**	3.23 ± 0.6**a**	2.34 ± 0.4**b**	2.33 ± 0.4**b**	4.09 ± 0.2**aX**	4.41 ± 0.3**aY**	3.25 ± 0.3**b**	3.23 ± 0.2**b**	0.90 ± 0.2**ab**	0.97 ± 0.1**a**	0.75 ± 0.2**bX**	0.90 ± 0.1**abY**
22:5 n-3	1.16 ± 0.2**ab**	1.21 ± 0.2**b**	1.01 ± 0.3**a**	0.98 ± 0.2**a**	1.75 ± 0.2	1.85 ± 0.1	1.65 ± 0.1	1.68 ± 0.1	0.29 ± 0.1	0.33 ± 0.0	0.26 ± 0.1	0.28 ± 0.1
22:6 n-3	14.0 ± 2.5	14.7 ± 3.2	14.17 ± 3.4	13.0 ± 2.7	22.2 ± 1.6	22.8 ± 1.3	23.0 ± 1.2	22.7 ± 1.4	1.47 ± 0.2**X**	1.64 ± 0.3**Y**	1.41 ± 0.3	1.46 ± 0.3
Σn-3 PUFA	29.8 ± 2.2a	30.9 ± 2.7a	23.6 ± 2.9b	22.2 ± 2.5b	34.1 ± 1.8a	35.6 ± 1.1a	30.7 ± 1.0b	30.6 ± 1.5b	19.9 ± 1.4a	20.2 ± 1.8a	11.4 ± 0.9b	11.1 ± 0.8b
n-3/n-6	1.28 ± 0.1a	1.36 ± 0.2a	0.78 ± 0.1b	0.74 ± 0.1b	1.80 ± 0.2a	1.89 ± 0.1a	1.27 ± 0.1b	1.25 ± 0.1b	0.69 ± 0.05a	0.71 ± 0.04a	0.31 ± 0.02b	0.31 ± 0.02b
n-3 LC-PUFA/18:3 n-3	1.90 ± 0.5**b**	1.96 ± 0.6**b**	3.56 ± 1.8**a**	3.30 ± 1.0**a**	5.93 ± 0.7**b**	5.67 ± 0.9**b**	12.0 ± 1.0**a**	11.6 ± 1.0**a**	0.19 ± 0.0**b**	0.20 ± 0.0**b**	0.29 ± 0.1**aX**	0.36 ± 0.1**aY**

For abbreviations see Table 1. SFA, saturated fatty acids incl.: 12:0, 14:0, 15:0, 16:0, 18:0, 20:0; MUFA, monounsaturated fatty acids incl.: 16:1n-7, 18:1n-9, 18:1n-7, 18:1n-5, 20:1n-9; PUFA, polyunsaturated fatty acids; n-3 LC-PUFA – ≥20 carbons and ≥3 double bonds; ND, not detected; capital letters (X, Y) indicate significant difference ($P < 0.05$) between the groups within the same lipid source and with/without sesamin (Student's *t*-test); different small letters within acids indicate significant difference between all experimental groups (Tukey's HSD test).

diets resulted in a higher biosynthesis of their corresponding LC-PUFA derivatives in TL, PLs as well as TAG of white muscle at the end of the experiment. These differences are namely in 18:3n-6, 20:2n-6 and 20:3n-6 from n-6 series and 18:4n-3, 20:4n-3, 20:5n-3 and 22:5n-3 from n-3 series respectively. However, there was no difference in the DHA content in TLs between CM and CL groups.

In the storage lipids (TAG) fraction (Table 5), the content of 20:4n-3, EPA as well as the desaturation index n-3 LC-PUFA/18:3n-3 decreased significantly in group CMS compared with CMC. On the contrary, we observed significantly higher representation of 18:3n-6 in CMS compared with CMC. In the groups fed linseed oil only, the DHA content was significantly lower in the sesamin group (CLS) compared with the group without sesamin supplementation (CLC). There was no difference in the content of SFA and MUFA, and there was a similar tendency for the LA and ALA derivatives in the TAG fraction as in TLs.

Very similar results were also found in the membrane lipids (PL), however with two exceptions. There was a significantly ($P < 0.05$) lower content of SFA in the CLC group compared with CLS and a higher representation of EPA in the CLC group. However, the first derivative (18:3n-6) from LA does not differ significantly and the same applies to the content of n-3 DPA.

Representation of lipid classes

Four different lipid classes could be identified in the white muscle of juvenile carp at the beginning of the experiment – PL (278 ± 17 mg $100g^{-1}$), CHOL (164 ± 5 mg $100g^{-1}$), MAG (15 ± 3 mg $100g^{-1}$) and TAG (318 ± 133 mg $100g^{-1}$). However, after 63 days of feeding only three classes could be separated and identified (PL, CHOL and TAG). We did not find any significant differences in the proportion of the TAG fraction between the different groups. However, there was a trend ($P < 0.12$ for CMS vs. CMC; $P < 0.14$ for CLS vs. CLC) for a higher proportion of TAG in both control groups compared with those fed sesamin. Student's *t*-test revealed a significantly lower proportion of PL in the CMS group compared with CMC. On the other hand, the white muscle of fish from the CMS group contained significantly more CHOL than the CMC group (Fig. 1).

Discussion

The present study describes the effects of dietary sesamin on FA composition of white muscle of juvenile common carp. The experimental carps originated from earth ponds with full access to natural feed (plankton and benthos). Therefore, their initial FA composition largely reflected the composition of natural feed (Domaizon, Desvilettes, Debroas & Bourdier 2000) and was altered by the

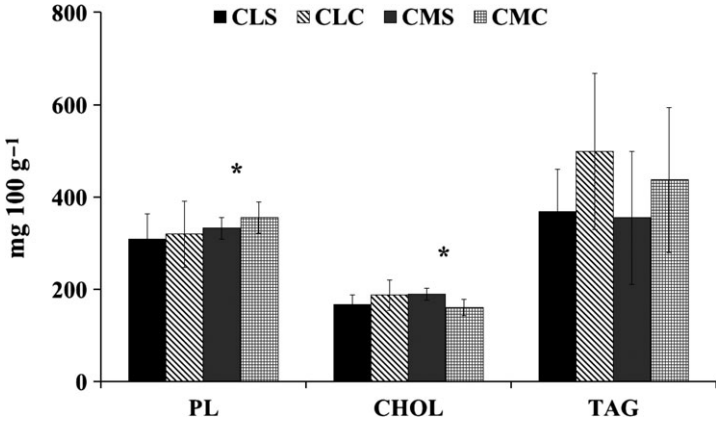

Figure 1 Lipid classes composition (mg $100g^{-1}$ white muscle) of juvenile common carp fed the diets with different vegetable oils as the lipid source and with/without sesamin addition (mean values ± SD, $n = 8$). Abbreviations: CLC, linseed oil group without sesamin; CLS, linseed oil group with sesamin; CMC, mixture of linseed/sunflower oil group without sesamin; CMS, mixture of linseed/sunflower oil group with sesamin; PL, phospholipids; CHOL, cholesterol; TAG, triacylglycerols; *significant difference ($P < 0.05$) between the groups with the same lipid source and with/without sesamin.

lipid source of the experimental feed (linseed or a mixture of linseed and sunflower oils).

The white muscle was studied to recognize possible changes in this tissue. The reason for this is its low and relatively stable lipid content due to the higher proportion of PL compared with the whole fillet with red muscle and belly flap respectively (Mráz & Pickova 2009). In addition, similar studies have been performed on white muscle (Trattner, Kamal-Eldin *et al.* 2008; Mráz *et al.* 2010; Vestergren *et al.* 2013). The juvenile fish used in the present study are not aimed at direct human consumption and therefore there is no direct need to analyse all the edible parts. Sesamin in the diet did not significantly affect the performance parameters (SGR, FCR), i.e. similar to Mráz *et al.* (2010) for carp or Alhazzaa *et al.* (2012) for barramundi. However, the FCR value was relatively high, probably due to the lab scale diet preparation. On the other hand, the experimental fish did not exhibit any health problems and the diet preparation was the same in all groups, hence it was taken as comparable.

Besides the effect of sesamin on FA composition, this experiment confirmed previous findings (Tocher *et al.* (1989) that carp is able to transform LA and ALA to a corresponding LC-PUFAs. The degree of this biosynthesis is dependent on the presence and amount of precursors in the diet. Simultaneously, the results show that the prevalent amount of LA causes an increased production of n-6 LC-PUFA and, vice versa, if the predominant precursor is ALA, then mainly n-3 LC-PUFA are formed. This is confirmed by the fact that white muscle of carp from CM groups contained significantly higher levels of LA products – 18:3n-6, 20:2n-6 and 20:3n-6 respectively (Table 5), in TL compared with the groups CL (whether with or without sesamin addition). On the other hand, in carps from CL groups (with higher levels of ALA in the diet), we detected significantly higher amounts of n-3 LC-PUFA – 18:4n-3 and 20:4n-3 in both CLC and CLS groups, as well as 20:5n-3 and 22:5n-3 in the CLC group. Similar results were achieved in both TAG and PL lipid fractions respectively.

An increase of LC-PUFA was observed despite the fact that carps had a significant excess of C18 precursors in the diet compared with minimal nutrition requirements (Radunz-Neto *et al.* 1996) which is inconsistent with Csengeri (1996), who stated that the rate of *de novo* fatty acid synthesis is inhibited at the substrate level by the administration of PUFA in the diet. In summary, as the minimal requirement for LA and ALA is 0.25% and 0.75% of the diet respectively (Radunz-Neto *et al.* 1996), even a lower amount of dietary C18 PUFA could be suggested. It is possible that the amount of both C18 PUFA as well as LC-PUFA has to be altered to obtain a higher conversion.

In addition, this study was designed to confirm or refute some of the hypothesis evolving from a previous experiment on common carp. Mráz *et al.* (2010) suggested that sesamin inefficiency in adult carp may be due to the several possible explanations:

(1) An evolutionary aspect, assuming that cyprinids, as herbivorous or omnivorous species, have some differences in their lipid metabolism compared with carnivore species and therefore the dietary sesamin has no effect on the enzymes involved in the LC-PUFA biosynthesis. Contrary to carp, salmonids or barramundi, where sesamin showed to enhance elongation and desaturation of 18:3n-3 (Trattner, Kamal-Eldin *et al.* 2008; Trattner, Ruyter *et al.* 2008; Alhazzaa *et al.* 2012), are carnivorous. The previous studies as well as our present results so far confirm this hypothesis and we believe that sesamin has only a little (non-significant) or no effect on white muscle FA composition of common carp.
(2) The presence of EPA and DHA in the diet may suppress the activity of the enzymes included in *de novo* biosynthesis of n-3 LC-PUFA. This hypothesis has been already confirmed, i.e. for Eurasian perch (*Perca fluviatilis*) (Bell *et al.* 2001; Blanchard, Makombu & Kestemont 2008). However, Mráz *et al.* (2010) used diets containing approximately 3.9% and 3.3% of EPA and DHA respectively. Therefore, we used defatted fish meal in the present study as a protein source in the experimental diets formulation in combination with linseed oil as a source of the n-3 LC-PUFA precursor ALA to verify this hypothesis. Nevertheless, the almost complete absence of EPA and DHA in the diet (approximately 0.2% EPA and 0.5% DHA respectively, see Table 3) did not seem to result in an increased synthesis towards LC-PUFA in this study. Either the amount of available LC-PUFA precursor or the ratio between n-6 and

n-3 FA did not affect the final LC-PUFA representation in juvenile carp. Based on our results, both the presence or absence of n-3 LC-PUFA in the diet as well as the proportion of precursor seem to be marginal in the case of common carp exposed to dietary sesamin.

(3) The effect of the age and size of the fish. Trattner, Kamal-Eldin *et al.* (2008) used rainbow trout of an average weight of 31–55 g, while Mráz *et al.* (2010) used carp in the market size of 800–1700 g in his experiment. On the other hand, Wagner *et al.* (2013) in their experiment studied the effect of sesamin in adult Atlantic salmon with an average weight 1660 g which is a totally comparable weight as used in the study of Mráz *et al.* (2010). However, to test the hypothesis of, and effect of age and size, we used juvenile carps with an average weight around 50 g in the present study. Contrary to the results of Trattner, Kamal-Eldin *et al.* (2008) or Alhazzaa *et al.* (2012), there was no major influence of dietary sesamin on the lipid metabolism of juvenile carp.

(4) The chemical structure of the sesamin. Trattner, Kamal-Eldin *et al.* (2008), Trattner, Ruyter *et al.* (2008) and Kushiro, Masaoka, Hageshita, Takahashi, Ide and Sugano (2002) used an equimixture of sesamin/episesamin, on rainbow trout, salmon and rats respectively, while in the previous study on carp (Mráz *et al.* 2010) and on rainbow trout (Schiller Vestergren, Wagner, Pickova, Rosenlund, Kamal-Eldin & Trattner 2012) as well as in the present study, only pure sesamin was used. However, the efficiency of the sesamin itself was demonstrated by Alhazzaa *et al.* (2012), who simultaneously refuted the hypothesis that sesamin could be active at low temperatures only, because their study was focused on warm-water barramundi and the dietary sesamin increased the amount of n-3 LC-PUFA in TLs up to 25%. Episesamin, which has been reported as a stronger lipid modulator compared with sesamin (Jeng & Hou 2005) should most likely be used in fish evolutionary resist (omnivorous and herbivorous), such as common carp.

In summary, our results in comparison with the available literature data thus far support the hypothesis of Mráz *et al.* (2010) that sesamin might affect the lipid metabolism only in carnivorous species, such as barramundi, as mentioned above. We conclude that dietary sesamin most likely does not affect the n-3 LC-PUFA biosynthesis in common carp. The rational is most likely the evolutionary efficiency of omnivorous fish species to convert essential PUFA into their higher homologues in normal conditions as well as a suboptimal fatty acid composition of the diets in this study.

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ORIGINAL ARTICLE

Culture of common carp (*Cyprinus carpio*) with defined flesh quality for prevention of cardiovascular diseases using finishing feeding strategy

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Abstract

OBJECTIVES: Fish is the major source of n-3 polyunsaturated fatty acids (n-3 PUFA) which are well known to have positive effects in prevention of cardiovascular diseases. This study investigated the possibility to produce common carp with defined flesh quality using finishing feeding strategy and predict changes of fillet FA by a dilution model.

METHODS: During the 110-day experiment, fish were fed diets with two different vegetable oils (rapeseed/linseed blend, VO; olive oil, OO) only, or with a subsequent fish oil (FO) finishing treatment for 30 or 60 days. Fillet FA composition was measured and data were compared to the ones predicted by the dilution model.

RESULTS: The FO finishing treatment resulted in the higher percentage of SFA (from 19.1% to 23.6%; $p<0.001$), MUFA (from 46.8% to 51.9%; $p<0.001$), n-3 PUFA (from 3.6% to 7.4%; $p<0.001$) and lower n-6 PUFA (from 30.5% to 16.9%; $p<0.001$) and n-6/n-3 ratio (from 8.7 to 2.3; $p<0.001$) in groups previously fed the VO diet and in lower MUFA percentage (from 67% to 63%; $p<0.001$) and n-6/n-3 ratio (from 8.2 to 2.8; $p<0.001$) and higher n-3 PUFA percentage (from 1.5% to 4.5%; $p<0.001$) in group previously fed the OO diet. The dilution model gave a good prediction for fillet FA changes (slope of the regression line 0.97–1.00; R^2 value of 0.992–0.996).

CONCLUSION: The finishing feeding strategy is suggested for production of common carp with a required flesh FA composition for purposes of special nutritional needs, especially for primary and secondary prevention of cardiovascular disease.

Abbreviations:

DHA	- docosahexaenoic acid (22:6n-3)
EPA	- eicosapentaenoic acid (20:5n-3)
FA	- fatty acids
FAME	- fatty acid methyl esters
FO	- fish oil
HUFA	- highly unsaturated fatty acids (20 ≥ carbons, 3 ≥ double bonds)
MUFA	- monounsaturated fatty acids
OO	- olive oil
PUFA	- polyunsaturated fatty acids
SFA	- saturated fatty acids
VO	- vegetable oil blend (rapeseed/linseed blend)

INTRODUCTION

The n-3 highly unsaturated fatty acids (n-3 HUFA; 20 ≥ carbons, 3 ≥ double bonds), especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are beneficial for human health (Mozaffarian & Rimm 2006). These fatty acids (FA) play an important role in biological functions, including brain development, inflammatory response, homeostasis and prevention of cardiovascular disease (Calder 2006; Calder & Yaqoob 2010). Fish is the major dietary source of n-3 HUFA and worldwide promoted as healthy and beneficial for human health, especially in prevention of cardiovascular diseases. Several specific dietary recommendations have been developed related to fish consumption and intake of n-3 FA by nutrition and health authorities. For the general population, two servings of fish per week or 250 mg of EPA and DHA per day are recommended (EFSA 2009). Patients with documented cardiac heart disease and hypertriglyceridemia are advised to have daily intake of EPA+DHA even higher up to 1 and 2–4 g, respectively (Kris-Etherton 2002). However, it is difficult for the public to meet the nutritional recommendations for the FA by a diet since the FA composition of fish flesh is highly variable and influenced by many factors, mainly by feeding (Mráz & Pickova 2011). Therefore it would be valuable if fish producers could produce fish with high and defined content of n-3 HUFA.

Feed sources rich in n-3 HUFA, such as fish oil, are becoming scarce, while algae and various microorganisms that supply n-3 HUFA are expensive and not yet available on a commercial scale. Therefore it would be of great economic and sustainability value if a feeding strategy could be devised where these feedstuffs are not used for the entire feeding period but only for the final part, thus saving resources. In line with this there is a need to be able to predict changes of fish FA composition during the course of feeding.

Such a finishing feeding strategy has been suggested and developed for carnivorous fish species, including medium fatty fish species such turbot (*Psetta maxima*) (Robin *et al.* 2003), fatty fish such as Atlantic salmon (*Salmo salar*) (Jobling 2003 and 2004b) and lean fish species such as Atlantic cod (*Gadus morhua*) (Jobling *et al.* 2008) and Murray cod (*Maccullochella peelii peelii*) (Turchini *et al.* 2006). The results so far have been promising and a finishing feeding strategy for commercial applications has been proposed. The overall conclusions from all these different studies are in agreement with a general dilution model suggested by Robin *et al.* (2003).

Common carp (*Cyprinus carpio*) is one of the most cultured fish species in the world (FAO 2008). Thus, from a worldwide nutrition perspective, a method to increase the amount of n-3 HUFA in carp fillet is valuable. Optimization of the FA composition of carp has been examined in previous studies (Domaizon *et al.* 2000; Chen *et al.* 2011; Mráz *et al.* 2012; Steffens 1997; Steffens & Wirth 2007). Mráz & Pickova (2011) concluded that adjusting the lipid composition in the feed is the most effective tool to achieve the desired n-3 HUFA content. However, previous studies have not investigated the response of muscle FA composition to dietary changes and the duration of finishing feeding period needed to achieve desired changes in n-3 HUFA content. Therefore the aim of this study was to examine the applicability of the finishing feeding strategy in common carp production with defined and tailored flesh quality for specific needs in human nutrition.

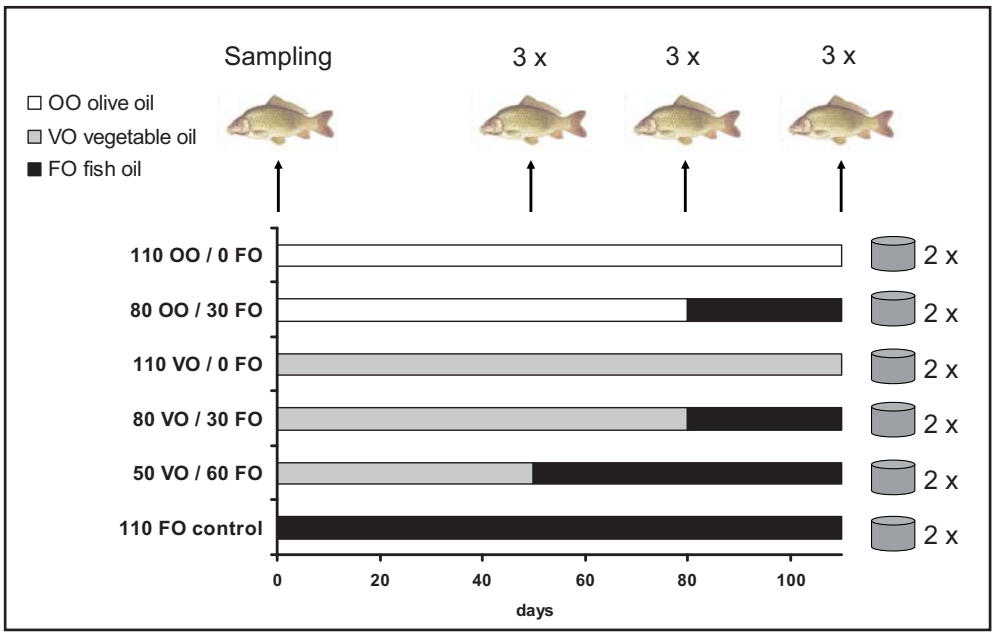

Fig. 1. Experimental design of the 110-day feeding trial. Fish (10 per tank, duplicate tanks per treatment) were fed one of three diets containing vegetable oil (VO), olive oil (OO) or fish oil (FO) alone for 110 days, or with a 30 or 60 day finishing period with FO. The different diets were: 110 OO/0 FO; 80 OO/30 FO; 80 OO/30 FO; 110 VO/0 FO; 110 VO/0 FO; 80 VO/30 FO; 50 VO/60 FO and 110 FO control. Fish were sampled at 0, 50, 80 and 110 days.

MATERIALS AND METHODS

Diets

The experimental diets used were based mainly on vegetable components (Table 1). No fish meal was used, in order to avoid high background levels of n-3 HUFA in the diet. The diets differed only in lipid source. The basal diet contained either a blend of vegetable oils (VO; rapeseed/linseed 3:2) or olive oil (OO) and the finishing feeding diet contained fish oil (FO). All diets were manufactured by extrusion. The proximate and FA composition of the experimental diets are listed in Table 1.

Tab. 1. Formulation (g 100g^{-1}), proximate composition (% on as is basis) and FA composition (% of identified FAs) of the experimental diets.

	VO Vegetable oil	OO Olive oil	FO Fish oil
Soybean meal	30	30	30
Wheat	18	18	18
Soycomil[a]	15.1	15.1	15.1
Maize	12	12	12
Fish oil	0	0	9
Linseed oil	3.6	0	0
Rapeseed oil	5.4	0	0
Olive oil	0	9	0
Corn gluten[b]	6	6	6
Wheat germ	5	5	5
Yeast vitex[c]	3	3	3
Aminovitan KPd[d]	0.6	0.6	0.6
Salt	0.5	0.5	0.5
Limestone	0.4	0.4	0.4
DL-methionine[d]	0.4	0.4	0.4
Dry matter	94.8	95.3	96.9
Protein	34.1	34.4	34.6
Fat	8.5	8.3	8.8
Fiber	3.5	3.1	3.7
Carbohydrates	44.1	44.8	44.2
Ash	4.6	4.7	5.6
SFA[e]	11.8	15.5	27.9
MUFA[f]	31.0	61.1	34.6
PUFA[g]	57.2	23.3	37.5
18:2n-6	53.0	21.7	16.2
20:2n-6	0	0	0.3
20:4n-6	0	0	0.3
18:3n-3	4.2	1.7	2.7
18:4n-3	0	0	2.2
20:5n-3	0	0	6.3
22:5n-3	0	0	0.6
22:6n-3	0	0	8.8
n-6/n-3	12.7	13.1	0.8

[a] ADM (Archer Daniels Midland Company), Olomouc, Czech Republic; [b] Bodit Tachov, s.r.o., Stribro, Czech Republic; [c] Biocel, a.s., Paskov, Czech Republic; [d] Zavod Biochemickych Sluzeb, s.r.o., Slusovice, Czech Republic; [e] SFA: saturated fatty acids; [f] MUFA: monounsaturated fatty acids; [g] PUFA: polyunsaturated fatty acids

Fish and experimental design

Two-year-old common carp (*Cyprinus carpio*) of the mirror scaly type with an average weight of 780g were used for the experiment. The fish were transferred from an earthen pond to the experimental facility at the Research Institute of Fish Culture and Hydrobiology in Vodnany, Czech Republic. Six fish were sampled to determine the initial lipid content and composition (data not shown). The fish were placed in tanks (1 m^3) and divided into 12 groups of 10 fish each. The tanks were supplied with oxygenated water from a recirculating system after mechanical and biological filtration (flow 0.1 l s^{-1}; dissolved oxygen 7–10 mg l^{-1}; temperature 20°C). The water level in the tanks was set to 0.4 m (tank volume 400 l). The fish were subjected to a light:dark (12h:12h) regime. During the 110-day experiment, the fish groups were fed only VO (110 VO/0 FO), only OO (110 OO/0 FO) or only FO (110 FO control), or VO or OO with a subsequent 30 or 60-day FO finishing treatment (80 OO/30 FO, 80 VO/30 FO, 50 VO/60 FO), with duplicate groups for each treatment. The experimental design is shown in Figure 1. The diets were supplied by automated continual feeders for 9 hours at a feeding ratio of 1.5% of current biomass. The fish stock biomass was determined every second week. Three fish were randomly sampled from each tank after 50, 80 and 110 days for lipid analysis. Samples of the fillets were immediately frozen in liquid nitrogen and stored at –80 °C until further analysis.

Lipid analysis

Lipid analyses were performed as described in detail by (Mraz & Pickova 2009). In brief, lipids from the fillet and feed samples were extracted with hexane and isopropanol according to Hara & Radin (1978). The FA were methylated (Appelqvist 1968) and the fatty acid methyl esters (FAME) were analyzed with a gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with flame ionization detector and split injector and fitted with a 50 m long × 0.22 mm i.d. × 0.25 µm film thickness BPX 70 fused-silica capillary column (SGE, Austin, TX, USA) according to (Fredriksson Eriksson & Pickova 2007). The FA were identified by comparison with a standard FA mixture (GLC standard 461, Nu-Chek Prep, Elysian, MN, USA) and specific retention times. Peak area integration was performed using Star chromatography workstation software version 5.5 (Varian AB, Stockholm, Sweden). The FA were quantified using internal standard methyl 15-methylheptadecanoate (Larodan Fine Chemicals AB, Malmö, Sweden).

Dilution model

The data obtained from lipid analyses of fillet tissues from groups 80 OO/30 FO, 80 VO/30 FO and 50 VO/60 FO were compared against predicted data calculated according the dilution model designed by Robin *et al.* (2003) and shown in Equation 1, as verified by Jobling (2004a).

$P_T = P_R + [(P_0 - P_R) / (Q_T/Q_0)]$ (Equation 1), where

P_T = Predicted percentage of a fatty acid at time T

P_R = Percentage of a fatty acid measured at time T in the fillet of control fish continuously fed the reference/finishing diet

P_0 = Percentage of a fatty acid in the fillet of tested fish at the beginning of finishing feeding period

Q_T = Quantity of total fatty acids in the tested fish at time T

Q_0 = Quantity of total fatty acids in the tested fish at the beginning of the finishing feeding period.

The predicted percentage of specific FA, e.g. EPA at a specific time point (P_T) (in this case the end point after 110 days), was calculated by taking the percentage of the specific FA measured at time T (110 days) in fish continuously fed the finishing diet (P_R = value for 110 FO control at 110 days) and the corresponding percentage in the other experimental groups (80 OO/30 FO, 80 VO/30 FO or 50 VO/60 FO) at time point P_0 (50 or 80 days), directly before the finishing feeding period. Q_0 was taken as the average total FA content (lipid content × body mass) of the experimental fish before the finishing feeding period and Q_T as the final total FA content of fish from the corresponding group at the end of the experimental period. All values represent the mean of six replicates (three fish per duplicate tank).

Statistical analysis

Where applicable (n>2), all data are presented as mean values ± standard deviation (SD) and differences were regarded as significant at $p<0.05$. The SAS General Linear Model (GLM), Tukey´s test (SAS Institute Inc., Cary, NC, USA, version 9.2) was used to compare fillet FA composition among the dietary treatments.

RESULTS

Survival, growth and feed conversion data for the different groups are presented in Table 2. The fillet lipid content was not affected by any dietary treatment over the course of the feeding trial ($p>0.05$). The final fillet lipid content varied between 9–10% at the end of the trial.

Replacing OO or VO with FO as the lipid source in the diet of common carp resulted in fillets with clearly different FA profiles (Figure 2). In the groups previously fed the VO diet the percentage of SFA (from 19.1% to 23.6%; $p<0.001$), MUFA (from 46.8% to 51.9%; $p<0.001$), n-3 PUFA (from 3.6% to 7.4%; $p<0.001$), EPA (from 0.36% to 1.53%; $p<0.001$) and DHA (from 0.71% to 3.16%;$p<0.001$) were positively correlated to the length of the FO finishing feeding period while the percentage of n-6 PUFA (from 30.5% to 16.9%; $p<0.001$) and the n-6/n-3 ratio (from 8.7 to 2.3; $p<0.001$) were negatively correlated. In the group previously fed the OO diet, the finishing treatment resulted in a lower percentage of MUFA (from 67% to 63%; $p<0.001$), a lower n-6/n-3 ratio (from 8.2 to 2.8; $p<0.001$) and a higher percentage of n-3 PUFA (from 1.5% to 4.5%; $p<0.001$), EPA (from 0.24% to 0.84%; $p<0.001$) and DHA (from 0.49% to 1.54%; $p<0.001$). The percentages of EPA and DHA both increased linearly with cumulative FO consumption (R^2 value >0.99) (Figure 3).

Although the fillet FA composition (Figure 2) changed significantly in response to the dietary FA composition (Table 1), the FA composition in the fillet did not reach that in the feed. The most obvious differences were seen in percentage of MUFA and PUFA (Figure 2 and Table 1). The percentage of MUFA in the VO and FO diet was 31% and 35%, respectively. The percentage of MUFA was considerably higher in the fillet, varying between 47% and 54% ($p<0.001$). The PUFA content in the VO and FO diet was 57% and 38%, respectively, but the corresponding values in the fillet were significantly lower and varied from 20% to 34% ($p<0.001$).

At the end of the experiment, the observed FA composition in the fillet samples from fish receiving the finishing feed (80 OO/30 FO, 80 VO/30 FO and 50 VO/60 FO) was compared against the values predicted using the dilution model designed by Robin *et al.* (2003) (Figure 4). The data showed that the dilution model gave a good prediction for the 10 most important FA or FA groups in the fillet of common carp, with a slope of the regression line close to 1 (0.97, 0.99 and 1.00, respectively) and with an R^2 value of 0.996, 0.993 and 0.992, respectively. Similar regression statistics were obtained for all FA identified (data not shown).

DISCUSSION

This study investigated possibility to produce common carp with defined flesh quality (high content of n-3 PUFA, EPA, DHA) for prevention of cardiovascular

Tab. 2. Fish performance (data presented are mean of duplicate values).

	Survival (%)	Final body weight (g)	Feed conversion (kg feed kg yield^{-1})
110 VO/0 FO	100	1610	1.76
80 VO/30 FO	95	1466	1.96
50 VO/60 FO	100	1407	1.73
110 OO/0 FO	100	1648	1.57
80 OO/30 FO	100	1491	1.74
110 FO control	95	1624	1.69

Abbreviations: VO, vegetable oil mixture; FO, fish oil; OO, olive oil

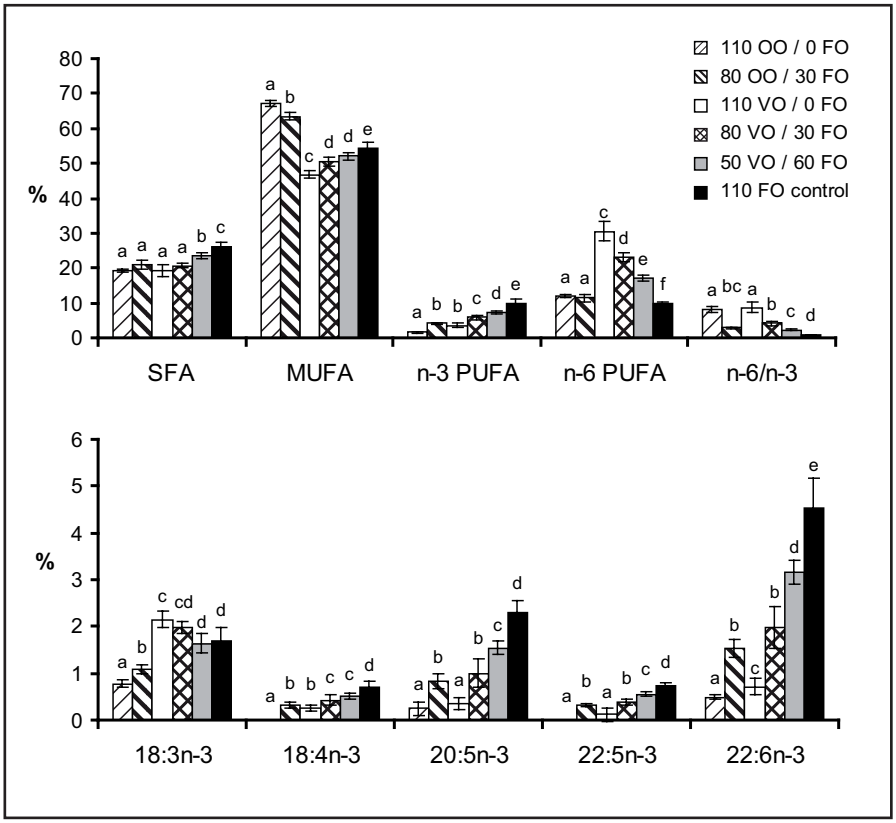

Fig. 2. Fatty acid (FA) composition (% of identified) in the fillet of the experimental fish at the end of the experiment (n=6; mean ± SD). Different letters indicate significant difference among the treatments. Abbreviations: SFA = saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA.

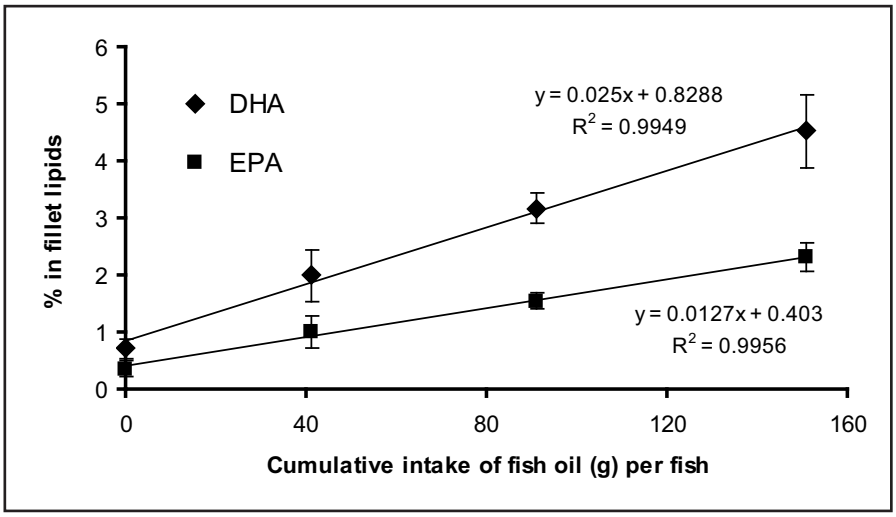

Fig. 3. Percentage of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (% of total identified FA) in fish fillet lipids in relation to the cumulative intake of fish oil (g) per fish (n=6; mean ± SD).

diseases using finishing feeding strategy and predict changes of fillet FA by a dilution model.

In the present study, the fillet FA composition highly reflected the FA composition of the diet and was significantly correlated to the length of the feeding period. This agrees with previous findings that it is possible to boost the content of beneficial EPA and DHA in fish fillet by n-3 HUFA supplementation prior to harvest (Bell *et al.* 2004; Torstensen *et al.* 2005, re Atlantic salmon; Benedito-Palos *et al.* 2009, re gilthead sea bream; Steffens 1997; Steffens & Wirth 2007, re common carp; Turchini *et al.* 2006, re murray cod).

However, when the dietary composition was used as the reference value for prediction of the FA composition in the groups continuously fed the same diet (110 FO control, 110 VO/0 FO and 110 OO/0 FO; Figure 5) the observed percentage of MUFA were significantly higher than the predicted values ($p<0.001$) while percentage of PUFA were significantly lower ($p<0.001$). This could indicate that the carp synthesized a significant amount of MUFA de novo from excess energy, or that MUFA are the preferred FA group for storage in common carp. This is supported by the fact that regardless of the low amount of MUFA in the natural and supplemented diet

of carp, MUFA is the major FA group stored in carp fillet and is probably produced from energy obtained from cereals (Buchtová *et al.* 2010, Mráz *et al.* 2012).

Using the dilution model proposed by Robin *et al.* (2003) showed to give an excellent prediction of the FA composition in fillet of carp of marketable size. This confirms previous findings for carnivorous fatty fish species such as Atlantic salmon (Jobling 2003), where the lipids are predominantly represented by storage fat (triacylglycerols). The dilution model is clear in its straightforwardness and can therefore be applicable for fish farmers, enabling production of high quality fish as well as minimizing the use of expensive feed. A small disadvantage is that the model does not account for the FA composition of the feed used, but for the FA composition in the fillet of fish continuously fed the finishing diet, which is hence needed as a reference value. However if the reference value has been established for a species and diet once it might be used continually.

The European Food Safety Authority recommends a daily intake of 250 mg EPA+DHA per person (EFSA 2009) and two servings of oily fish per week. A 200 g serving of carp from the 110 FO control and 110 VO/0 FO group contained 1190 mg and 180 mg EPA+DHA, respectively. According to the predictions by the dilution model and experimental values obtained here, we concluded that the finishing feeding treatment needs to be applied for 70 days to achieve the recommended daily value of 250 mg EPA+DHA in two 200 g servings a week (250 mg × 7 days = 1750 mg/2 servings = 875 mg/serving). Reducing FO feeding to this shorter period would significantly reduce fish production costs and lead to more sustainable use of limited FO resources.

Currently common carp is mostly produced in ponds on the basis of natural feed (plankton and benthos) with cereal supplementation. Since there are huge differences in natural productivity among ponds there is also a huge variability of FA composition in carp flesh (Mráz & Pickova 2012). As a consequence there is no standard of quality which makes it difficult to advertise carp as a healthy product. The finishing feeding strategy could therefore be used in production of carp with defined flesh quality to fulfill dietary needs for humans, especially in connection to cardiovascular recovery. Fish farmers could easier control the final carp flesh quality and produce fish with standardized and tailored quality. In line with this they could declare the content of n-3 PUFA and EPA+DHA on the product label which could increase the market value of carp and support consumption of this locally produced fish. From the consumers point of view this would be desirable as they thereby could easier meet the nutritional recommendations. It would also be easier to set up dietary interventions using carp in prevention and treatment of cardiovascular diseases.

In conclusion, the finishing feeding strategy is suggested for the production of common carp with tailored flesh FA composition, for contributing to healthy fat profile of e.g. EPA and DHA content, for cardiovascular disease prevention of the Central Europe population.

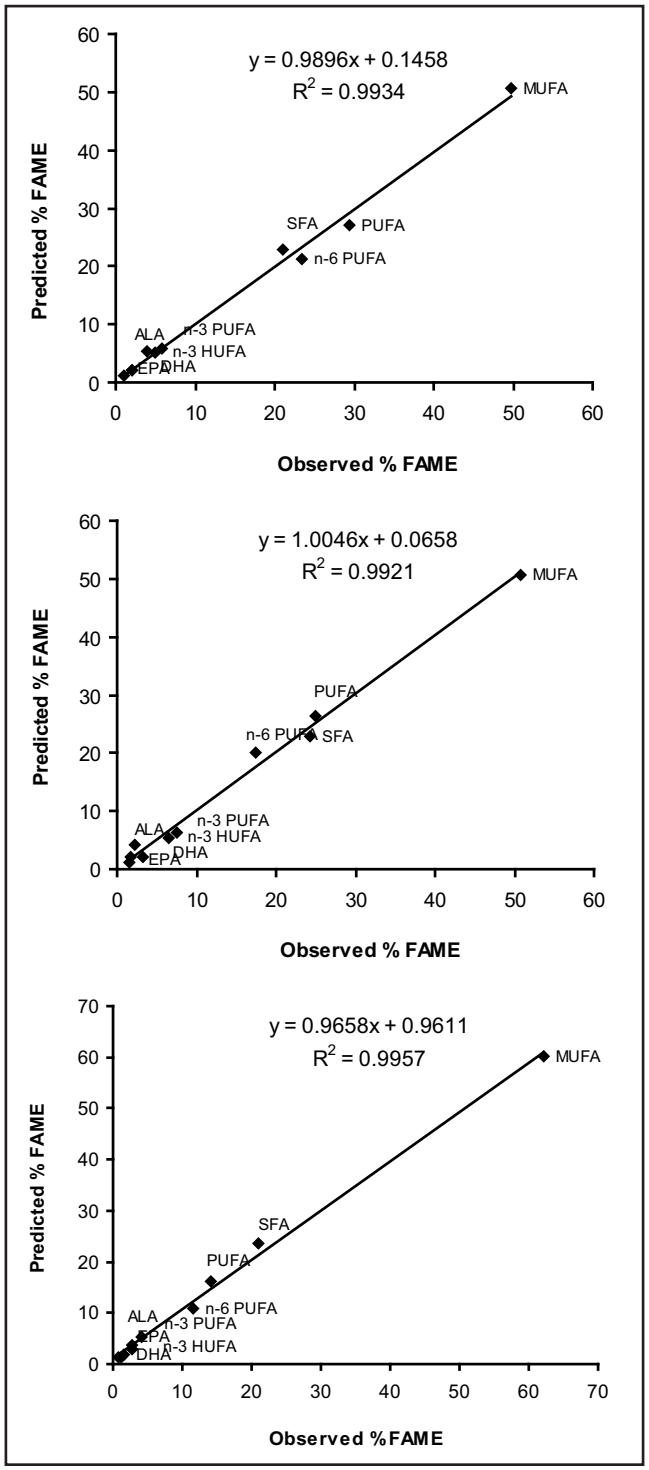

Fig. 4. Prediction plot of fillet fatty acid composition (%) for carp groups A) 80 VO/30 FO; B) 50 VO/60 FO; and C) 80 OO/30 FO. The measured values represent the mean for 6 fish. The thick line shows the regression line. FAME = fatty acid methyl esters, for other abbreviations see Figure 2.

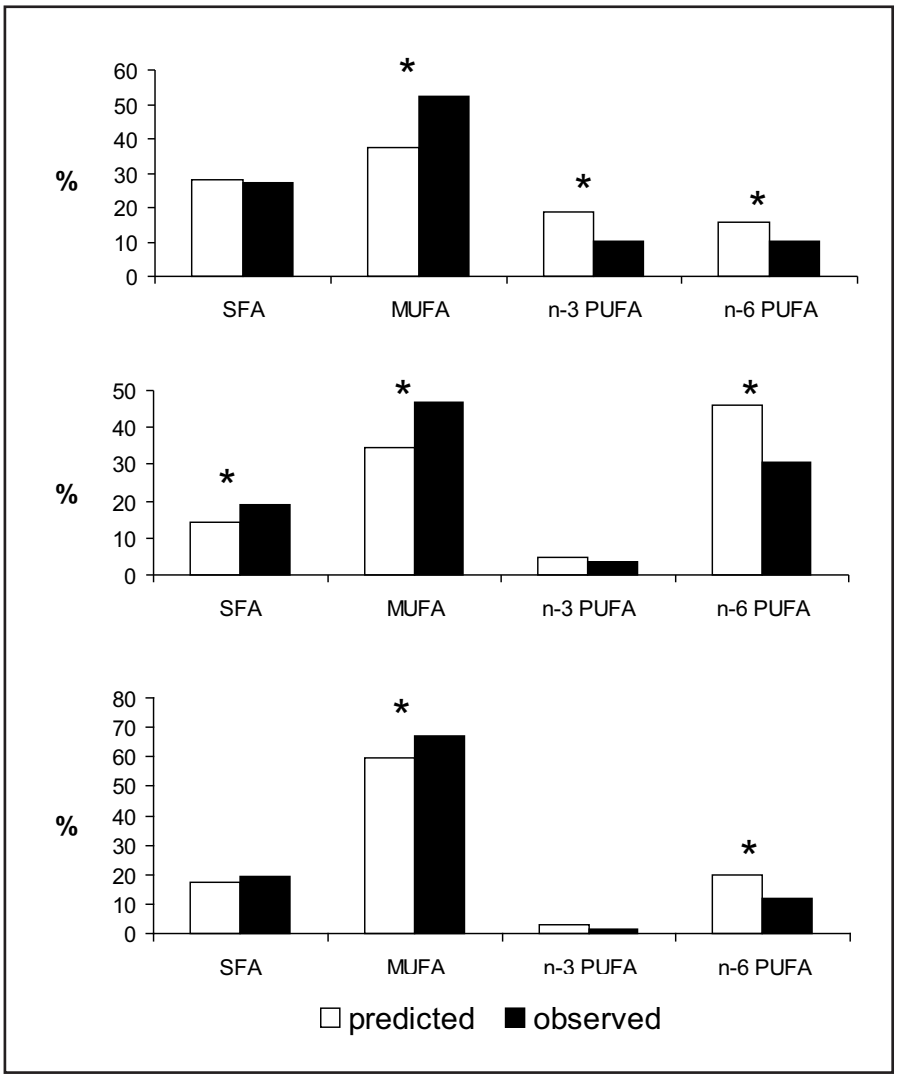

Fig. 5. Observed fatty acid composition (%) in fillet of fish from the carp groups A) 110 FO control; B) 110 VO/0 FO; and C 110 OO/0 FO, compared with the composition predicted by the dilution model when the fatty acid composition of the FO, VO and OO diet, respectively, was used as the reference value. * indicates significant difference ($p<0.05$) between predicted and observed data. For abbreviations see Figure 2.

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Příloha č. 4

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Fillet quality changes as a result of purging of common carp (*Cyprinus carpio* L.) with special regard to weight loss and lipid profile

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ABSTRACT

Purging is a very important part of the rearing process for common carp (*Cyprinus carpio* L.) in Central Europe and is commonly conducted between October and December. Fish are kept in clear water without feeding in order to empty the gut, decrease the entrail proportion and eliminate possible tainted flavour. This leads to weight loss and stored fat mobilisation. This study investigated the effect of a purging period of up to 70 days on lipid content and quality of common carp flesh. Four-year-old, market-size carp (weight 1700–2600 g) from three different production systems (C: cereal supplemented; P: linseed/rapeseed pellet supplemented; N: natural feed) were sampled every 14 days for weight, fillet yield and lipid analysis. Fillet yield was highest after 14 days and decreased thereafter. Throughout the experiment, fillet fat content decreased continuously in groups C and P, but remained stable in group N. Initially, carp from groups C and P mainly metabolised monounsaturated fatty acids (MUFAs), but with prolonged starvation fish from all groups started to metabolise more polyunsaturated fatty acids (PUFAs). After 70 days of purging, all groups showed almost identical saturated FA (SFA), MUFA and PUFA values. Our conclusion is that carp are able to metabolise selected FA for their energy needs when they are in good condition and have surplus fat stores. However, when body fat content is low, they may metabolise all FA types equally to sustain metabolic functions.

1. Introduction

Common carp (*Cyprinus carpio*) is one of the most commonly reared fish globally, with a production volume of around 3 000 000 tons annually (FAO, 2010). Carp for consumption in Central Europe are traditionally harvested during late autumn and kept in concrete coated ponds with fresh water flow for some weeks before being sold, a process known as purging. During this time the fish are not fed, so that the digestive tract is emptied and unpleasant odours are eliminated (Einen et al., 1998). Purging is necessary to achieve good product sensory quality. Common carp in natural conditions decrease feeding and activity with decreasing water temperature in winter to save energy. The optimal temperature range for carp is 20–28 °C, and in general carp stop feeding in the range 12–4 °C and stop movement at water temperatures below 6–4 °C in natural conditions (Bauer and Schlott, 2004).

In preparation for the winter starvation period, carp naturally store fat as reserve energy in muscle and abdominal wall tissues, mainly in the form of energy-rich triacylglycerols (TAGs). During the winter starvation period these TAG reserves are metabolised gradually for maintenance of the organism (Csengeri, 1996).

Lipids have an important function as an energy source in the body (McCue, 2010). Different fatty acids (FAs) also have metabolically important functions in the body, and are involved in the determination of the physical and chemical properties and capacities of biological membranes (Wiseman, 1996). They also serve as precursors in the synthesis of several different chemical messengers and eicosanoid hormones, as well as other regulating factors (Horrobin, 1995; Kinsella, 1988). In general, TAG serves mainly as an energy source, whereas phospholipids (PLs) are mainly constituents of biological membranes (Sargent et al., 1999).

Under semi-intensive rearing conditions, carp have access to natural feed (plankton and benthos) in the pond and are also fed a supplement, often cereals. While cereals are rich in carbohydrates with moderate levels of fat and n−6 polyunsaturated fatty acids (PUFAs), plankton and benthos contain high amounts of n−3 PUFA (Bell et al., 1994; Domaizon et al., 2000). The carbohydrate-rich diet leads to a high muscle fat content (Mraz and Pickova, 2009), more than 10% in intensively reared carp (Keshavanath et al., 2002), by de novo synthesis of FA (Henderson, 1996). In addition, cereals contain n−6 PUFA, which are generally not present in the natural diet of fish in such amounts and therefore affect the lipid composition of fish tissues. Mraz and Pickova

Abbreviations: BF_3, boron trifluoride methanol complex; CF, condition factor; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FFA, free fatty acid; FAME, fatty acid methyl ester; MUFA, monounsaturated fatty acid; PL, phospholipid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TAG, triacylglycerol; TLC, thin layer chromatography.

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(2009) suggested that cereals cause an increase in n−6 PUFA in carp, while the proportions of n−3 PUFA decrease. Mraz et al. (2012) studied the effect of three different production systems (supplementation with cereals or pellets containing rapeseed cake, and a natural diet only) on lipid composition in common carp and found a significantly higher content of n−3 PUFA in fish fed rapeseed pellets compared with fish fed cereals. This and other studies (Pickova and Mørkøre, 2007; Runge et al., 1987; Schwarz, 1996) indicate the possibility of influencing fish lipid composition towards a higher content of n−3 PUFA, which is favourable from a human nutrition perspective (Calder and Yaqoob, 2009; Leaf and Weber, 1987; Simopoulos, 2002, 2008).

However, few previous studies have examined changes in lipid content and FA composition during the purging period of common carp. Csengeri (1996) and Vacha et al. (2007) observed some changes in FA composition during a long-lasting purging period in carp supplemented with different cereals. There was a slight increase in n−3 PUFA in the groups fed cereal, while n−3 PUFA decreased in the control group kept in natural conditions without supplemental feeding before purging. In a study on common carp, Csengeri (1996) concluded that monounsaturated fatty acids (MUFAs), especially oleic acid (18:1 n−9), are mainly utilised for energy production during prolonged starvation, while PUFAs are partly preserved. Similar results have been published for other fish species, for example channel catfish (*Ictalurus punctatus*; Luo et al., 2009), Atlantic salmon (*Salmo salar*; Einen et al., 1998), Murray cod (*Maccullochella peelii peelii*; Palmeri et al., 2008a, 2009a) and hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*; De Silva et al., 1997). Different reduced feed ratio levels in rainbow trout (*Oncorhynchus mykiss*) were studied by Kiessling et al. (1989), who found that higher diet restriction resulted in higher n−3 PUFA percentage in fish flesh. Decreased muscle fat content has been reported in brown trout (*Salmo trutta*) starved for 2 months (Regost et al., 2001).

Previous studies suggest that the lipid content and composition of the edible parts of different fish species are affected during starvation (Palmeri et al., 2008b, 2009b; Thanuthong et al., 2012). In addition, previous nutrition most likely plays an important role in the changes (Tucker, 2000). The aim of the present study was to explore the effect of purging on fillet fat content and FA composition in common carp from three different production systems — supplementation with cereal; supplementation with rapeseed/linseed pellets; and natural feed only.

2. Materials and methods

2.1. Experimental design

Duplicate groups of 4-year-old, market-size common carp were reared in three different production systems for one season (April–September) in the experimental unit of the Faculty of Fisheries and Protection of Waters in Vodňany, Czech Republic. The production systems involved three different types of feed: natural feed only (N); supplementation with cereal (C) and supplementation with rapeseed/linseed pellets (P). Each treatment was carried out in two ponds to balance the effect of the pond environment.

After harvesting, 80 individuals were randomly chosen from each group, labelled by groups with visible implant elastomer (VIE, Northwest Marine Technology, Ltd., USA) and placed in a storage pond with continuous inflow of fresh river water. The pond was 8 m × 4 m × 1.3 m deep, with stony walls and gravel on the bottom. Water temperature measured with a temperature datalogger Minikin I (EMS, Brno, Czech Republic) decreased continually during the experimental period (18.5 °C at the beginning; 2.5 °C at the end; Fig. 1). Dissolved oxygen (O_2) concentration and pH were recorded regularly twice a week (O_2 varied between 6 and 8.5 mg L^{-1}; pH 7.1–7.6). Condition factor (CF) was calculated on each sampling day as the ratio of individual weight (W, grams) to body length (BL, cm = distance between edge of the head and base of tail fin) as:

$$CF = \left(W * BL^{-3}\right) * 100.$$

On days 0, 14, 28, 42, 56 and 70, a subsample of 10 fish from each group were weighed and 6 fish from each treatment (3 each from the two ponds of the same treatment) were killed for sampling. The fish were stunned by a blow to the head and then the gills were cut. A 4 cm wide strip of the fillet (containing white muscle, red muscle and adipose tissue with skin) was taken from each fish at the same position, in the fillet behind the dorsal fin. These fillet samples were packed in aluminium foil and immediately frozen in liquid nitrogen. All samples were stored at −80 °C until further analysis.

2.2. Lipid analysis

Strips of fillets with skin were minced in a table cutter to ensure that all edible parts were represented in the sample analysed. All chemicals and solvents were purchased from Merck (Darmstadt, Germany). Lipid extraction was performed according to Hara and Radin (1978) with minor modifications. Briefly, 1 g of sample was weighed and homogenised in HIP (hexane–isopropanol 3:2, v/v). The homogenate was transferred to a centrifuge tube and 6.5 mL 6.67% Na_2SO_4 was added to separate lipid and non-lipid phases. After centrifugation, the total lipid phase (upper phase) was transferred into pre-weighed tubes and evaporated under nitrogen (for about 1 h). Total lipid content was determined gravimetrically.

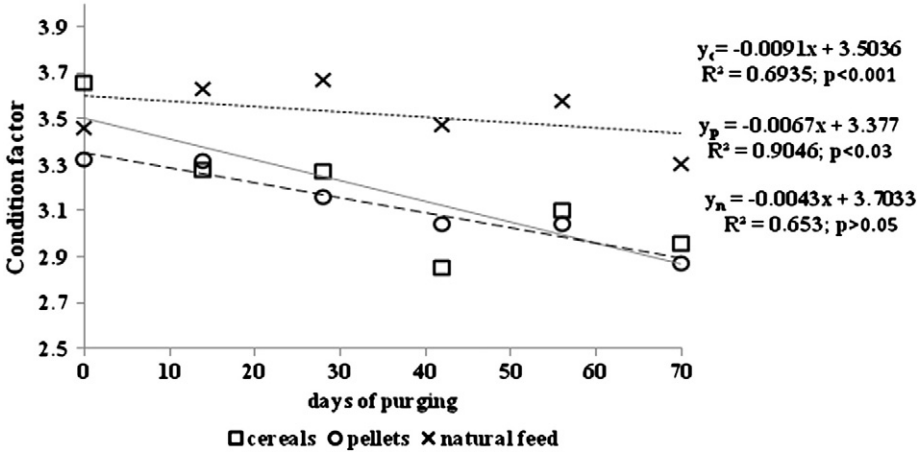

Fig. 1. Condition factor (CF) in carp in the group supplemented with cereals; the group supplemented with rapeseed/linseed pellets; and the group fed natural feed only during the purging period; P value indicates regression dependence within tested groups during purging period (mean values; n = 10).

Table 1
Average weight [g] of common carp in groups C, P and N during the purging period (mean ± standard deviation; n = 10); actual water temperature [°C] at the sampling times.

Group	Days of purging						
	1	14	28	42	56	70	*P value*
C	2690 ± 178[a]	2410 ± 146[a]	2400 ± 135[a]	2100 ± 116[a]	2270 ± 178[a]	2160 ± 122[a]	<0.001
P	2400 ± 176[b]	2380 ± 145[a]	2330 ± 121[a]	2170 ± 112[a]	2190 ± 156[a]	2050 ± 124[a]	<0.001
N	1750 ± 163[c]	1720 ± 160[b]	1760 ± 151[b]	1650 ± 101[b]	1700 ± 276[b]	1590 ± 226[b]	<0.05
t	18.5	13.5	6.9	7.9	1.2	2.5	

Different letters within the same day indicate significant difference ($P < 0.05$) among the groups; *P value* indicates regression dependence within tested groups; Abbreviations: t: temperature; C: cereal fed; P: pellet fed; N: natural fed.

2.3. Lipid class composition and separation

Lipid class composition was analysed according to Olsen and Henderson (1989) with minor modifications as described by Mraz and Pickova (2009). A Camag ATS 4 automatic TLC sampler was used to apply the samples dissolved in hexane (conc. 1 μg/μL) in 4-mm lines on pre-coated silica gel 60 TLC plates (20 × 10 cm; 0.20 mm layer; Merck, Darmstadt, Germany). The lipids were separated using a Camag ADC 2 developing chamber as described above. Derivatisation was performed by dipping the plate in solution phosphoric acid/ethanol followed by heating in an oven at 150 °C for 10 min. The proportions of the different classes of lipid (PLs—phospholipids, sterols, FFAs—free fatty acids, DAGs—diacylglycerols and TAGs—triacylglycerols) were densitometrically measured using a Camag TLC scanner 3. Lipid classes were identified by comparison against an external standard (TLC 18-4A, Nu-Check Prep, Elysian, USA).

2.4. Fatty acid analysis

Total lipids were separated into TAG and PL fractions using thin layer chromatography (TLC) on precoated TLC silica plates (20 × 10 cm Merck, Darmstadt, Germany). The lipids were separated using a Camag ADC 2 developing chamber with hexane-diethyl ether-acetic acid (85:15:1, v/v) as the mobile phase. Total PL and TAG were scraped from the plate and extracted with methanol and chloroform according to Pickova et al. (1997). Fatty acid methyl esters (FAMEs) were prepared with BF_3 following the method of Appelqvist (1968).

FAMEs were analysed using a gas chromatograph (Varian CP 3800; Stockholm, Sweden) equipped with an ionisation detector (FID), split injector and silica capillary column BPX 70 (SGE, Austin, TX) (Fredriksson Eriksson and Pickova, 2007). Helium was used as carrier gas at a flow rate of 0.8 mL min^{-1} and nitrogen was used as make-up gas. Retention time of the different FA was identified by comparison with a standard mixture (GLC-68A, Nu-check Prep, Inc., Elysian, MO). For quantification of the FA, an internal standard (15-methylheptadecanoate; Larodan Fine Chemicals AB, Malmö, Sweden) was used. FAs were expressed as percent of total identified FA.

2.5. Statistical analysis

All statistical analyses were performed using the Statistica CZ 10.0 software package. A proper regression analysis was performed to find the differences within experimental group during purging time. One-way analysis of variance (ANOVA) and Tukey's HSD test were used for the determination of differences among treatments, as well as for regression and correlation analysis. Differences were assumed to be statistically significant at $P < 0.05$.

3. Results

3.1. Water temperature, weight, fillet yield and condition factor

The mortality rate during the purging period in the experiment was in total 6.3% (14/240 fish). Water temperature decreased continuously from 18.5 °C to 2.5 °C at the end of experiment.

The average body weight at the start of experiment was 1750 ± 470 g in group N, 2690 ± 410 g in group C and 2404 ± 391 g in group P. With prolonged starvation time, total body weight decreased significantly in all groups. After 70 days of purging, the lowest weight decline was observed in group N (−9.2%; −161 g), followed by group P (−14.6%; −352 g) and the largest decline was measured in group C (−19.6%; −529 g) (Table 1).

Together with decreasing average weight, condition factor (CF) decreased during the purging period (Fig. 1). These decline is significant for groups C and P. Fillet yield (with skin) increased temporarily between days 1 and 14 in groups C and P, and then gradually decreased throughout the purging period (group C: 48.5%, 51% and 45.2%; group P: 46.1%, 48.7%, and 44.1% on days 1, 14 and 70, respectively). There were almost no changes in fillet yield in group N during the whole purging period (44.1% on day 1, 44.8% on day 70).

3.2. Fat content, fatty acid composition and lipid classes

There was a significant ($P < 0.01$) reduction in fat content in groups C and P during the experiment, while minor changes (non-significant) were observed in group N (Table 2). The largest decrease was measured in group C (−62%), followed by group P (−48%), and the smallest in group N (−9%).

The FA composition (mg/100 g^{-1} fillet) at the start of the experiment and after 14, 28, 42, 56 and 70 days of purging is presented in Table 3. Changes in percentage content of the main FA groups (MUFA, PUFA, n−3 PUFA) throughout the whole experiment are shown in Fig. 2a, b and c, respectively. The proportion of saturated FA (SFA) varied between 24.2 ± 0.92% and 28.6 ± 0.81%. The MUFA content decreased significantly ($P < 0.01$) in group C (Fig. 2a), from initially 54.2 ± 2.19% to 46.9 ± 4.49% at day 70. A decrease in MUFA was also observed in

Table 2
Average fat content [%] of common carp in groups C, P and N during the purging period (mean ± standard deviation; n = 6).

Group	Days of purging						
	1	14	28	42	56	70	*P value*
C	8.68 ± 2.8[a]	8.33 ± 3.4[a]	5.66 ± 1.1[a]	6.65 ± 3.8[a]	7.10 ± 2.4[a]	3.30 ± 1.4	<0.01
P	7.32 ± 4.5[ab]	6.58 ± 3.6[ab]	3.16 ± 1.3[b]	3.19 ± 1.7[b]	3.23 ± 2.3[b]	3.46 ± 2.1	<0.01
N	3.51 ± 0.8[b]	3.59 ± 2.1[b]	3.42 ± 0.9[b]	2.34 ± 1.1[b]	3.54 ± 1.2[b]	3.16 ± 0.9	>0.05

Different letters within the same day indicate significant difference ($P < 0.05$) among the groups, *P value* indicates regression dependence during purging days. Abbreviations: C: cereal fed, P: pellet fed, N: natural fed.

Table 3
Fatty acid composition of total lipid from the edible part of groups C, P and N carp fillet (mg/100 g^{-1} fillet).
At days 1, 14, 28, 42, and 56 and after 70 days of purging (mean ± standard deviation; n = 6).

Group	Sampling day	Fatty acid											
		14:0	15:0	16:0	16:1 (n−7)	18:0	18:1 (n−9)	18:1 (n−7)	18:2 (n−6)	18:3 (n−3)	18:4 (n−3)	20:3 (n−6)	20:4 (n−6)
C	day 1	109 ± 37^{a}	25 ± 9	1411 ± 507^{a}	21 ± 12	454 ± 140^{a}	3503 ± 1057^{a}	250 ± 95	583 ± 206	327 ± 157	51 ± 22^{a}	23 ± 8	37 ± 24
	day 14	87 ± 29^{a}	18 ± 5	1365 ± 491^{a}	531 ± 331^{a}	420 ± 169	3072 ± 1341^{a}	235 ± 97	505 ± 200	235 ± 93^{ab}	57 ± 28^{a}	20 ± 7	67 ± 13
	day 28	64 ± 37^{a}	13 ± 7	1044 ± 363^{a}	347 ± 180^{a}	212 ± 107^{a}	2576 ± 777^{a}	133 ± 67^{a}	330 ± 165	177 ± 90	82 ± 38^{a}	13 ± 7	31 ± 12
	day 42	91 ± 52^{a}	18 ± 9^{a}	1212 ± 635^{a}	572 ± 353^{a}	334 ± 175^{a}	2481 ± 1370^{a}	207 ± 114^{a}	429 ± 243	205 ± 102^{a}	104 ± 52	18 ± 8	11 ± 5^{a}
	day 56	75 ± 47^{a}	17 ± 10^{a}	928 ± 545^{a}	393 ± 228^{a}	291 ± 109^{a}	2122 ± 1280^{a}	173 ± 82^{a}	447 ± 212	220 ± 103	113 ± 66	16 ± 6	11 ± 7
	day 70	38 ± 30	9 ± 6	439 ± 298	157 ± 62	125 ± 92	849 ± 622	74 ± 49	216 ± 121	116 ± 64	29 ± 13	10 ± 5	47 ± 22
	P value	<0.001	<0.001	<0.001	<0.04	<0.001	<0.001	<0.001	>0.05	<0.05	<0.002	>0.05	>0.05
P	day 1	78 ± 38^{ab}	25 ± 13	1187 ± 612^{ab}	33 ± 20	255 ± 119^{ab}	2430 ± 1514^{b}	229 ± 124	870 ± 348	517 ± 211	30 ± 22^{ab}	27 ± 15	49 ± 22
	day 14	62 ± 31^{ab}	17 ± 7	956 ± 403^{ab}	346 ± 104^{ab}	280 ± 116	1956 ± 904^{ab}	186 ± 95	726 ± 266	386 ± 119^{a}	45 ± 20^{ab}	26 ± 11	84 ± 31
	day 28	34 ± 12^{b}	9 ± 5	513 ± 211^{b}	179 ± 79^{b}	131 ± 41^{b}	923 ± 357^{b}	84 ± 33^{b}	325 ± 131	194 ± 99	51 ± 21^{b}	13 ± 5	21 ± 8
	day 42	30 ± 9^{b}	9 ± 3^{b}	477 ± 233^{b}	150 ± 69^{b}	140 ± 91^{b}	973 ± 417^{b}	88 ± 47^{ab}	318 ± 174	167 ± 62^{ab}	62 ± 42	17 ± 9	8 ± 3^{ab}
	day 56	32 ± 15^{b}	9 ± 2^{b}	485 ± 230^{b}	170 ± 58^{b}	136 ± 77^{b}	950 ± 506^{b}	87 ± 32^{b}	353 ± 150	188 ± 111	71 ± 30	14 ± 7	11 ± 6
	day 70	33 ± 16	8 ± 3	508 ± 173	168 ± 109	158 ± 91	949 ± 375	86 ± 39	347 ± 141	211 ± 88	47 ± 18	17 ± 5	74 ± 30
	P value	<0.03	<0.02	<0.04	>0.05	>0.05	<0.04	<0.03	>0.05	>0.05	>0.05	>0.05	>0.05
N	day 1	51 ± 12^{b}	15 ± 5	633 ± 147^{b}	25 ± 13	147 ± 60^{b}	1166 ± 127^{b}	131 ± 22	376 ± 91	165 ± 73	14 ± 2^{b}	16 ± 2	10 ± 4
	day 14	33 ± 14^{b}	9 ± 5	505 ± 281^{b}	168 ± 65^{b}	183 ± 98	1058 ± 457^{b}	106 ± 53	372 ± 159	125 ± 59^{b}	21 ± 9^{b}	18 ± 4	86 ± 16
	day 28	43 ± 15^{b}	13 ± 5	525 ± 117^{b}	235 ± 57^{b}	134 ± 26^{b}	984 ± 232^{b}	110 ± 34^{ab}	367 ± 238	194 ± 82	52 ± 16^{b}	13 ± 7	9 ± 4
	day 42	23 ± 7^{b}	6 ± 3^{b}	356 ± 137^{b}	111 ± 32^{b}	121 ± 61^{b}	696 ± 337^{b}	65 ± 26^{b}	218 ± 108	66 ± 22^{b}	54 ± 26	13 ± 6	5 ± 2^{b}
	day 56	35 ± 11^{b}	8 ± 2^{ab}	456 ± 221^{b}	199 ± 84^{ab}	140 ± 55^{b}	951 ± 396^{b}	91 ± 49^{ab}	239 ± 125	112 ± 68	53 ± 21	10 ± 5	6 ± 2
	day 70	29 ± 14	7 ± 3	387 ± 116	105 ± 58	122 ± 55	844 ± 316	82 ± 25	245 ± 114	110 ± 43	19 ± 4	10 ± 4	43 ± 21
	P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

At day 1 and after 14 and 70 days of purging (mean ± standard deviation; n = 6).

Group	Sampling day	Fatty acid								
		20:5 (n−3)	22:5 (n−3)	22:6 (n−3)	SFA	MUFA	PUFA	n−3 PUFA	n−6 PUFA	n−3/n−6
C	day 1	148 ± 69	46 ± 24	73 ± 32	2034 ± 703^{a}	3976 ± 1203^{a}	1364 ± 518	680 ± 309	685 ± 220	0.99
	day 14	100 ± 31	36 ± 13	63 ± 7	1923 ± 700^{a}	4023 ± 1813^{a}	1135 ± 402	503 ± 174	632 ± 232	0.80
	day 28	90 ± 56	31 ± 11	57 ± 22	1786 ± 532^{a}	3085 ± 1033^{a}	937 ± 420	437 ± 224	400 ± 207	1.09
	day 42	117 ± 54	39 ± 16	68 ± 16	1716 ± 898^{a}	3293 ± 1849^{a}	1027 ± 491	532 ± 223	495 ± 272	1.07
	day 56	102 ± 50	34 ± 13	57 ± 21	1371 ± 517^{a}	2719 ± 1225^{a}	1032 ± 536	524 ± 330	507 ± 329	1.03
	day 70	61 ± 31	24 ± 11	50 ± 23	624 ± 331	1135 ± 616	577 ± 298	286 ± 146	291 ± 155	0.98
	P value	<0.02	>0.05	>0.05	<0.001	<0.001	<0.05	<0.05	>0.05	>0.05
P	day 1	130 ± 46	49 ± 21	109 ± 43	1588 ± 713^{ab}	2782 ± 991^{b}	1853 ± 642	862 ± 314	991 ± 444	0.87
	day 14	101 ± 28	43 ± 15	128 ± 55	1344 ± 526^{ab}	2642 ± 1200^{b}	1611 ± 533	725 ± 284	886 ± 324	0.82
	day 28	54 ± 33	24 ± 11	62 ± 31	715 ± 277^{b}	1207 ± 474^{b}	771 ± 335	484 ± 189	487 ± 155	0.99
	day 42	71 ± 28	33 ± 19	92 ± 47	689 ± 341^{b}	1233 ± 740^{b}	796 ± 400	428 ± 201	368 ± 202	1.16
	day 56	67 ± 29	27 ± 11	72 ± 45	697 ± 269^{b}	1227 ± 733^{b}	828 ± 402	425 ± 223	403 ± 175	1.05
	day 70	73 ± 23	33 ± 9	102 ± 41	722 ± 203	1294 ± 553	937 ± 228	473 ± 209	464 ± 177	1.02
	P value	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05
N	day 1	106 ± 35	44 ± 15	107 ± 40	765 ± 92^{b}	1346 ± 127^{b}	870 ± 145	441 ± 127	429 ± 86	1.03
	day 14	73 ± 37	38 ± 15	104 ± 42	747 ± 317^{b}	1427 ± 322^{b}	882 ± 279	371 ± 96	511 ± 188	0.73
	day 28	75 ± 24	30 ± 6	61 ± 17	756 ± 173^{b}	1360 ± 316^{b}	797 ± 369	381 ± 119	416 ± 158	0.92
	day 42	72 ± 46	34 ± 13	91 ± 39	530 ± 217^{b}	1090 ± 398^{b}	573 ± 214	317 ± 111	256 ± 130	1.24
	day 56	67 ± 24	27 ± 10	55 ± 21	668 ± 295^{b}	1256 ± 560^{b}	589 ± 246	313 ± 98	276 ± 102	1.13
	day 70	56 ± 31	21 ± 13	64 ± 31	558 ± 214	1088 ± 420	594 ± 134	277 ± 129	317 ± 182	0.87
	P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Mean values with different superscripts within different purging days differ significantly ($P < 0.05$) among the groups, *P value* indicates regression dependence within fatty acid during purging; Abbreviations: C: cereal fed; P: pellet fed, N: natural fed; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

group P (Fig. 2b) but was not statistically significant. Almost no changes were observed in group N (Fig. 2c), where the proportion of MUFA varied between 45.2 ± 3.41% and 48.2 ± 2.44% during the whole purging period. At the same time, the content of MUFA (mg/100 g^{-1} fillet; Table 3) was significantly different between the experimental groups at day 1 and after 14, 28, 42 and 56 days. There were no significant differences among the groups after the 70-day purging period. The proportion of PUFA increased linearly in groups C (significantly) and P (non-significantly) and, in contrast, there was a trend of slightly decreasing PUFA in group N (29.1 ± 3.97% at the beginning; 26.9 ± 4.23% at the end) (Fig. 3). There were no significant differences in total amount of PUFA (mg/100 g^{-1} fillet) among the groups during purging period. The similar trend as in total PUFA was observed for the proportion of n−3 PUFA, which increased significantly in groups C and P, while it was unchanged in group N (Fig. 2).

The composition of the lipid classes confirmed the negative correlation between increasing fat content and percentage of PL and, conversely, the positive correlation between increasing fat content and TAG. The percentages of different lipid classes in all groups and the respective changes are presented in Table 4. When the data were expressed as percentage of total lipids, the proportion of PL seemed to increase in groups C and P, since TAG content decreased. However, when expressed as total amount (mg/100 g^{-1} fillet), PL in group C remained stable until day 28, and decreased rapidly after that, while in group N it remained relatively stable until day 42. Total PL decreased from day 14 in group P. Differences among the groups are evident till day 28; from day 42 there are no significant differences in the amount of PL (Fig. 3).

With prolonged purging time, FA composition in the TAG lipid fraction (Table 5a) showed significant changes in terms of MUFA and PUFA (including n−3 PUFA) in group C. Changes are evident also in group P,

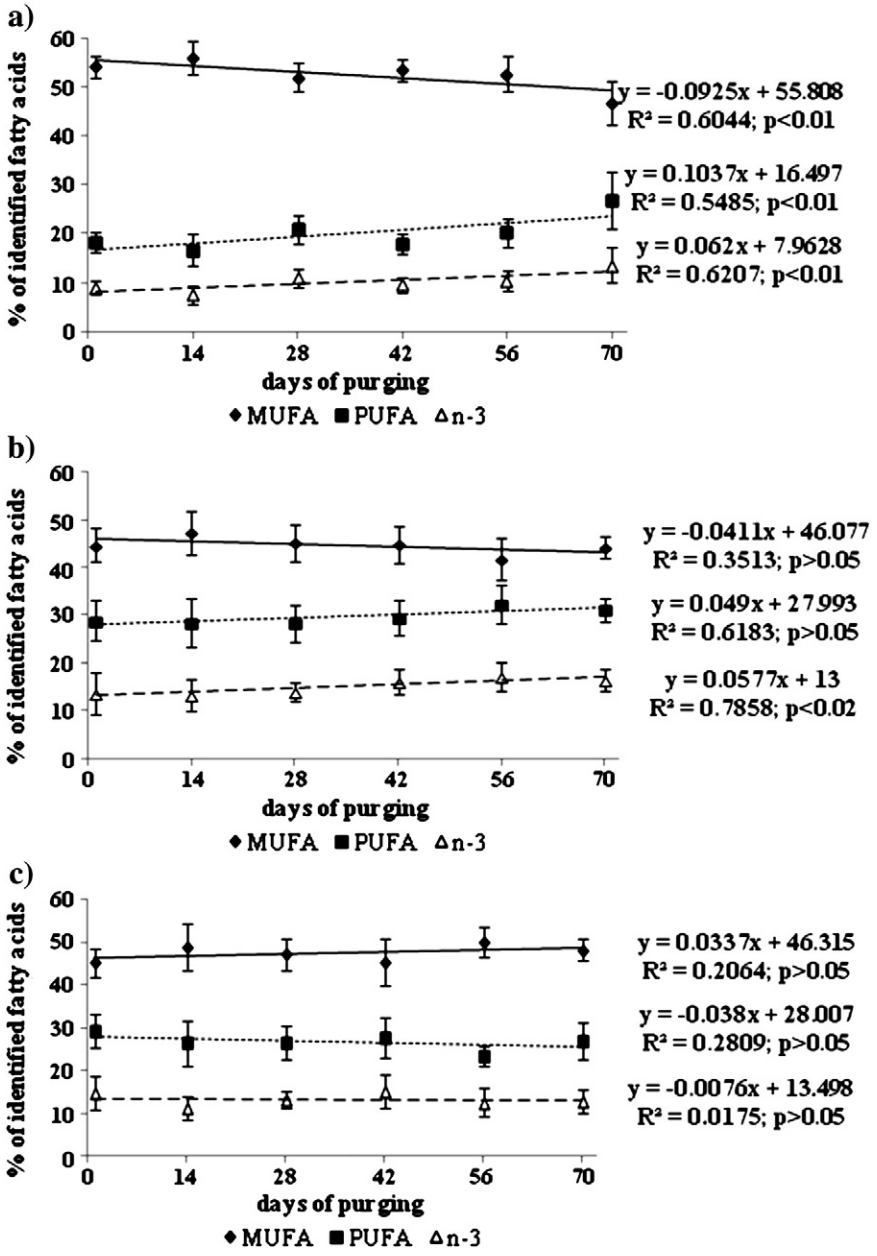

Fig. 2. a, b, c. Percentage of MUFA, PUFA and n−3 PUFA in (a) the group supplemented with cereals; (b) the group supplemented with rapeseed/linseed pellets; and (c) the group fed natural feed only during the purging period; P value indicates regression dependence within tested group during purging period. Abbreviations: MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; n−3 PUFA: sum of 18:3 n−3, 18:4 n−3, 20:3 n−3, 20:5 n−3, 22:5 n−3, 22:6 n−3.

Table 4
Lipid class composition (%) in fillets from groups C, P and N carp during the purging period (mean ± standard deviation; n = 6).

Group	Day 1	Day 14	Day 28	Day 42	Day 56	Day 70	*P value*	Lipid class
C	8.69 ± 1.6^{b}	10.6 ± 2.4	12.7 ± 3.9	9.18 ± 1.7^{b}	5.55 ± 1.6^{b}	9.29 ± 2.5	>0.05	PL
	8.54 ± 0.8	7.91 ± 1.6	10.2 ± 1.6	7.14 ± 0.8^{b}	6.47 ± 1.1^{b}	7.93 ± 1.9	>0.05	STEROLS
	3.16 ± 0.2	nd	nd	nd	nd	2.54 ± 0.7	>0.05	FFA
	2.08 ± 0.7	2.04 ± 1.0	2.30 ± 0.9^{a}	2.17 ± 0.2	0.91 ± 0.2^{b}	1.34 ± 0.4	>0.05	DAG
	80.2 ± 3.3^{a}	79.2 ± 5.4	75.3 ± 6.8	81.5 ± 2.5^{a}	85.9 ± 3.0^{a}	80.4 ± 6.2	>0.05	TAG
P	8.79 ± 2.9^{b}	9.40 ± 3.6	14.2 ± 2.4	17.1 ± 6.4^{ab}	14.1 ± 8.1^{a}	12.3 ± 6.2	>0.05	PL
	10.4 ± 4.2	8.47 ± 2.2	9.76 ± 0.6	11.0 ± 3.0^{ab}	10.2 ± 2.8^{a}	10.5 ± 4.4	>0.05	STEROLS
	nd	nd	nd	2.76 ± 0.8	2.15 ± 0.6	3.20 ± 0.7	>0.05	FFA
	2.31 ± 0.9	1.31 ± 0.3	1.27 ± 0.3^{b}	2.15 ± 0.5	1.95 ± 0.8^{a}	1.43 ± 0.7	>0.05	DAG
	79.4 ± 5.5^{ab}	80.7 ± 6.6	74.7 ± 3.1	71.5 ± 12.2^{ab}	73.5 ± 11.1^{b}	74.7 ± 11.3	>0.05	TAG
N	13.7 ± 3.7^{a}	12.8 ± 4.0	12.9 ± 2.1	19.5 ± 6.3^{a}	8.98 ± 2.3^{ab}	9.65 ± 2.3	>0.05	PL
	11.3 ± 2.2	9.91 ± 2.7	9.56 ± 0.7	12.9 ± 3.3^{a}	8.42 ± 1.9^{ab}	8.25 ± 1.8	>0.05	STEROLS
	nd	1.72 ± 0.2	nd	nd	2.42 ± 0.3	2.91 ± 1.2	>0.05	FFA
	2.07 ± 0.8	1.68 ± 0.6	1.23 ± 0.2^{b}	2.54 ± 0.7	1.38 ± 0.4^{ab}	1.28 ± 0.5	>0.05	DAG
	71.9 ± 6.2^{b}	75.0 ± 6.9	76.5 ± 3.1	64.3 ± 10.3^{b}	79.6 ± 3.8^{ab}	78.9 ± 5.0	>0.05	TAG

Mean values with different superscripts within different purging days differ significantly (P < 0.05) among the groups, *P value* indicates regression dependence within fatty acid during purging; Abbreviations: PL: phospholipid; DAG: diacylglycerol; FFA: free fatty acid; TAG: triacylglycerol; nd: not detected; C: cereal fed; P: pellet fed; N: natural fed.

but they are not significant. No changes were observed in group N. The total amount (Fig. 3) and FA composition in the PL fraction (Table 5b) changed mostly in group C, where alterations in percentage content of SFA, PUFA (including n−3 and n−3 long-chain PUFA) and MUFA/PUFA ratio were confirmed. There was a significant decrease in SFA in group P, together with a trend for increasing proportion of PUFA. Unexpectedly, in group N the percentage of PUFA, n−3 PUFA, n−3 long-chain PUFA, EPA and DHA decreased in the first 14 days and then increased back to the initial proportions.

4. Discussion

4.1. Changes in weight, fillet yield and condition factor

For most of the Czech population carp is the traditional Christmas Eve dinner. In order to provide the market with carp within 2 or 3 weeks before Christmas, the harvest time has to be adjusted to the weather conditions and icing of the ponds. This results in some fish being kept in purging conditions for longer than others. In order to evaluate the possible effects of all alternate purging treatments, this experiment was carried out in the period September–December. In the present study purging started at 18.5 °C, which is relatively high, and then the water temperature dropped to 14.6 °C after 4 days and reached 11.9 °C after 18 days. It has been reported that as stocking density is generally high in purging ponds, fish activity will increase (Bauer and Schlott, 2004). However, as the temperature dropped rapidly after the start of our experiment, the fish were not exposed to stressful conditions for long.

In general, weight loss has been observed in purged carp. During normal overwintering, the weight loss has been reported in different studies to be 3% (Bauer and Schlott, 2004), 5–10% (Geldhauser and Gerstner, 2003), and 14–24% (Blasco et al., 1992). In the present study, we found a total weight loss of 9.2%, 14.6% and 19.6% in groups N, P and C, respectively, during purging (Table 1).

Fillet yield is closely related to weight loss. In the present study fillet yield was still >45% at the last sampling point, which was slightly

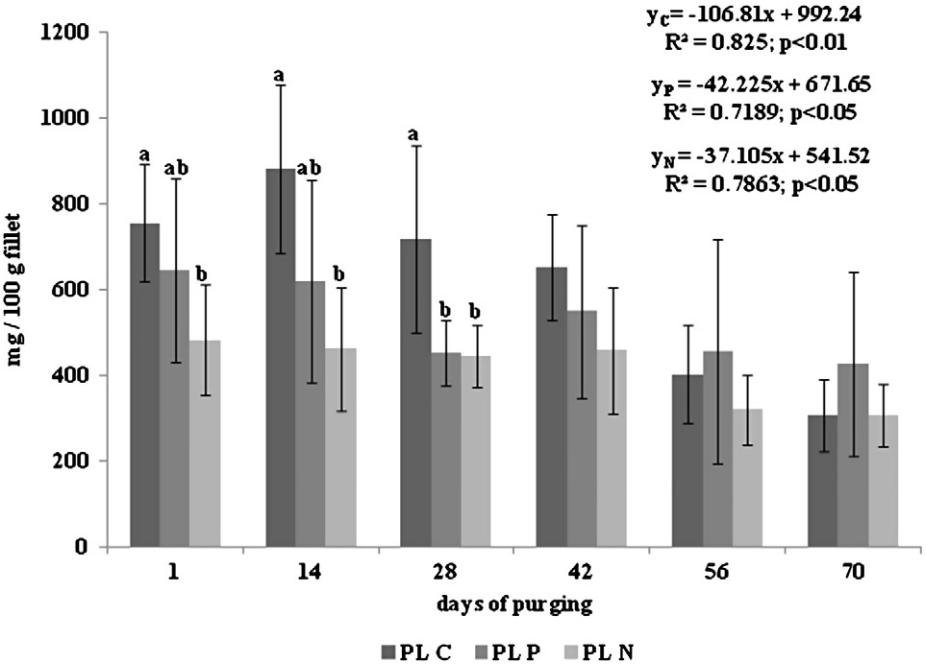

Fig. 3. Calculated total values of phospholipids, with the lines showing changes over time in averages in each group; P value indicates regression dependence within tested group during purging period. Abbreviations: PL: phospholipid; C: cereal group, P: pellet group, N: natural feed group.

Table 5a
Percentage of fatty acid in triacylglycerol fraction of groups C, P and N common carp purged for 70 days (mean ± standard deviation; n = 6); comparison between days 1, 14 and 70.

Group	Sampling day	Fatty acids							
		EPA + DHA	SFA	MUFA	PUFA	n−3 PUFA	n−3 LC PUFA	n−3/n−6	MUFA/PUFA
C	day 1	1.68 ± 0.3	26.2 ± 1.0	58.1 ± 1.9^{a}	15.7 ± 2.5^{b}	7.00 ± 1.5^{b}	2.23 ± 0.4	0.81 ± 0.2	3.84 ± 0.8^{a}
	day 14	1.71 ± 0.3	26.4 ± 0.9	57.7 ± 2.4^{a}	15.8 ± 2.3^{b}	6.68 ± 1.3^{b}	2.30 ± 0.4	0.72 ± 0.1^{ab}	3.75 ± 0.7^{a}
	day 28	1.71 ± 0.2^{ab}	26.1 ± 1.0	57.1 ± 2.6^{a}	16.8 ± 2.2^{b}	7.06 ± 1.0^{b}	2.35 ± 0.3	0.74 ± 0.1	3.49 ± 0.6^{a}
	day 42	1.75 ± 0.4	27.7 ± 0.5^{a}	57.1 ± 1.5	15.3 ± 1.5^{c}	6.74 ± 1.1^{ab}	2.32 ± 0.5	0.80 ± 0.1	3.78 ± 0.5^{a}
	day 56	1.84 ± 0.7	25.6 ± 1.5	56.8 ± 3.2^{a}	17.6 ± 3.7^{b}	7.53 ± 2.3^{b}	2.43 ± 0.9^{b}	0.73 ± 0.1^{b}	3.41 ± 0.9^{a}
	day 70	2.19 ± 0.6	25.9 ± 1.1	53.8 ± 3.0	20.4 ± 4.0	9.27 ± 2.0	3.05 ± 0.8	0.85 ± 0.2	2.77 ± 0.7
	P value	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	>0.05	<0.05
P	day 1	2.37 ± 1.1	24.4 ± 1.7	47.4 ± 4.4^{b}	28.2 ± 4.3^{a}	13.8 ± 3.6^{a}	3.34 ± 1.4	1.00 ± 0.4	1.75 ± 0.5^{b}
	day 14	2.24 ± 1.0	24.8 ± 1.9	48.4 ± 3.9^{b}	26.8 ± 4.1^{a}	12.1 ± 2.2^{a}	3.13 ± 1.3	0.85 ± 0.2^{a}	1.87 ± 0.4^{b}
	day 28	1.43 ± 0.6^{b}	24.7 ± 1.4	51.9 ± 2.6^{b}	23.4 ± 3.4^{a}	9.44 ± 2.4^{a}	2.07 ± 0.8	0.68 ± 0.2	2.30 ± 0.6^{b}
	day 42	1.46 ± 0.7	24.3 ± 1.4^{b}	53.4 ± 3.5	22.3 ± 2.4^{a}	9.05 ± 2.5^{a}	2.10 ± 0.9	0.70 ± 0.2	2.44 ± 0.4^{b}
	day 56	2.73 ± 0.5	25.2 ± 1.2	44.7 ± 3.1^{b}	30.1 ± 4.0^{a}	14.9 ± 1.9^{a}	3.90 ± 0.6^{a}	1.00 ± 0.1^{a}	1.52 ± 0.3^{b}
	day 70	1.53 ± 0.7	24.6 ± 1.6	54.2 ± 4.5	21.2 ± 4.3	8.62 ± 3.5	2.21 ± 1.0	0.69 ± 0.3	2.72 ± 0.8
	P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
N	day 1	1.95 ± 1.0	25.0 ± 2.1	53.1 ± 4.4^{a}	21.9 ± 4.4^{ab}	8.84 ± 3.5^{b}	2.69 ± 1.2	0.69 ± 0.3	2.56 ± 0.7^{b}
	day 14	1.50 ± 0.3	24.9 ± 2.1	54.0 ± 2.8^{ab}	21.2 ± 3.8^{ab}	8.06 ± 1.6^{b}	2.08 ± 0.4	0.62 ± 0.1^{b}	2.65 ± 0.6^{b}
	day 28	2.38 ± 0.7^{a}	25.3 ± 2.4	51.6 ± 5.0^{ab}	23.1 ± 5.0^{a}	9.33 ± 2.4^{ab}	3.22 ± 1.0	0.73 ± 0.3	2.36 ± 0.6^{b}
	day 42	1.50 ± 0.4	24.5 ± 1.9^{b}	56.5 ± 2.1	19.0 ± 0.9^{b}	6.49 ± 1.1^{b}	2.26 ± 0.5	0.53 ± 0.1	2.98 ± 0.2^{b}
	day 56	1.82 ± 0.6	24.7 ± 1.1	56.4 ± 1.4^{a}	18.9 ± 1.2^{b}	7.65 ± 1.8^{b}	2.51 ± 0.9^{b}	0.72 ± 0.3^{b}	3.00 ± 0.3^{a}
	day 70	2.04 ± 0.8	24.6 ± 1.0	54.9 ± 2.9	20.6 ± 2.7	8.10 ± 2.5	2.79 ± 1.1	0.66 ± 0.2	2.73 ± 0.5
	P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Mean values with different superscripts within different purging days differ significantly (P < 0.05) among the groups, *P value* indicates regression dependence within fatty acid during purging; Abbreviations: C: cereal fed; P: pellet fed; N: natural fed, EPA + DHA: eicosapentaenoic and docosahexaenoic acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; n−3 long-chain PUFAs are fatty acids with more than 20 carbon atoms and with at least 3 double bonds (20:3 n−3; 20:5 n−3; 22:5 n−3; 22:6 n−3).

higher than the value reported earlier by Kocour et al. (2007) of 41.1% for 3-year-old carp with average weight around 1500 g after harvest. This difference is most likely due to the higher start weight of the 4-year-old carp (body weight 1700–2600 g) used in the present study, resulting in an increased muscle content in these carp. As expected (Einen et al., 1998), percentage fillet yield increased in the first days of purging (days 1–14), after the gut had emptied and thus the relative proportion of muscle increased by 2.5% in groups C and P. No increase in fillet yield was found in group N. This is most probably due to different rearing practices. While the carp in groups C and P had continuous access to feed, resulting in a constantly filled gut, the fish kept in natural conditions in group N had to seek their feed and therefore most likely had a less full gut at the time of harvest. Similar results have been reported by Oberle et al. (1997), who found that carp kept in natural conditions did not reach a similar fillet yield to diet-supplemented carp after short-term purging.

The decrease in the CF value during the purging period reflected the decrease in weight at unchanged BL of purged carp (Fig. 1). Similarly, Bauer and Schlott (2004) reported a decrease in CF in overwintering carp.

Table 5b
Percentage of fatty acid in phospholipid fraction of groups C, P and N common carp purged for 70 days (mean ± standard deviation; n = 6); comparison between days 1, 14 and 70.

Group	Sampling day	Fatty acids							
		EPA + DHA	SFA	MUFA	PUFA	n−3 PUFA	n−3 LC PUFA	n−3/n−6	MUFA/PUFA
C	day 1	14.7 ± 2.2^{b}	32.9 ± 0.3^{ab}	33.9 ± 5.2^{a}	33.3 ± 5.4^{b}	19.7 ± 3.2^{b}	17.8 ± 2.7^{b}	1.46 ± 0.1	1.07 ± 0.3^{a}
	day 14	17.6 ± 2.2	32.7 ± 1.4	28.2 ± 5.4	39.0 ± 4.5	23.4 ± 2.8	21.6 ± 2.5	1.50 ± 0.1^{a}	0.75 ± 0.2
	day 28	19.5 ± 1.2	30.1 ± 2.3	27.3 ± 1.8^{a}	42.6 ± 4.1	25.5 ± 1.9	23.6 ± 1.5	1.52 ± 0.2	0.65 ± 0.1^{a}
	day 42	18.8 ± 2.8	30.6 ± 4.0	29.7 ± 3.3^{a}	39.6 ± 5.9^{b}	24.6 ± 3.8	22.8 ± 3.4	1.65 ± 0.2	0.78 ± 0.2^{a}
	day 56	17.7 ± 3.5	29.5 ± 3.0	31.3 ± 4.8^{a}	39.3 ± 6.0^{b}	23.7 ± 4.5	21.7 ± 4.1	1.51 ± 0.2	0.84 ± 0.3^{a}
	day 70	20.0 ± 1.5	27.4 ± 3.7	27.4 ± 1.8	45.2 ± 5.2	27.1 ± 2.1	24.4 ± 1.7	1.55 ± 0.3	0.62 ± 0.1
	P value	<0.01	<0.001	>0.05	<0.01	<0.01	<0.01	>0.05	<0.05
P	day 1	16.6 ± 5.0^{ab}	34.8 ± 3.7^{a}	24.8 ± 4.4^{b}	39.7 ± 6.8^{ab}	23.6 ± 5.4^{ab}	20.0 ± 5.7^{ab}	1.47 ± 0.3	0.65 ± 0.2^{b}
	day 14	16.5 ± 6.5	34.6 ± 5.0	26.1 ± 5.1	39.4 ± 9.5	23.1 ± 6.6	19.4 ± 7.1	1.41 ± 0.2^{ab}	0.75 ± 0.4
	day 28	20.3 ± 2.2	27.5 ± 2.6	24.3 ± 2.0^{b}	48.2 ± 2.9	27.6 ± 2.6	24.5 ± 2.5	1.36 ± 0.2	0.51 ± 0.1^{b}
	day 42	19.9 ± 1.7	29.4 ± 3.9	23.4 ± 1.1^{b}	47.3 ± 3.5^{a}	27.3 ± 2.3	24.1 ± 2.2	1.39 ± 0.2	0.50 ± 0.1^{b}
	day 56	18.9 ± 1.3	29.5 ± 4.2	23.4 ± 1.4^{b}	47.1 ± 3.5^{a}	26.4 ± 1.8	23.1 ± 1.7	1.29 ± 0.2	0.50 ± 0.1^{b}
	day 70	19.0 ± 3.2	27.5 ± 4.0	26.1 ± 2.7	46.4 ± 4.6	26.1 ± 2.8	23.0 ± 3.4	1.31 ± 0.2	0.57 ± 0.1
	P value	>0.05	<0.01	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05
N	day 1	21.3 ± 3.1^{a}	29.7 ± 2.4^{ab}	22.4 ± 3.1^{b}	47.8 ± 2.4^{a}	28.7 ± 3.8^{a}	26.1 ± 4.0^{a}	1.57 ± 0.5	0.47 ± 0.1^{b}
	day 14	16.3 ± 2.7	32.4 ± 4.3	25.1 ± 3.0	42.5 ± 5.8	23.2 ± 3.2	20.2 ± 2.9	1.20 ± 0.1^{b}	0.61 ± 0.1
	day 28	20.9 ± 2.0	29.6 ± 4.8	23.5 ± 1.3^{b}	46.9 ± 4.2	28.0 ± 2.8	25.7 ± 2.5	1.50 ± 0.2	0.50 ± 0.1^{b}
	day 42	21.9 ± 2.0	26.5 ± 3.9	24.8 ± 3.1^{b}	48.7 ± 3.1^{a}	28.6 ± 2.4	26.5 ± 2.2	1.43 ± 0.1	0.51 ± 0.1^{b}
	day 56	21.7 ± 4.0	27.3 ± 3.9	25.4 ± 2.8^{b}	47.3 ± 4.2^{a}	29.1 ± 5.0	26.9 ± 4.8	1.65 ± 0.5	0.54 ± 0.1^{b}
	day 70	20.5 ± 1.5	28.0 ± 4.1	25.2 ± 2.3	46.8 ± 2.6	27.32.3	25.0 ± 1.9	1.41 ± 0.2	0.54 ± 0.1
	P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Mean values with different superscripts within different purging days differ significantly (P < 0.05) among the groups, *P value* indicates regression dependence within fatty acid during purging; Abbreviations: C: cereal fed; P: pellet fed; N: natural fed, EPA + DHA: eicosapentaenoic and docosahexaenoic acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; n−3 long-chain PUFAs are fatty acids with more than 20 carbon atoms and with at least 3 double bonds (20:3 n−3; 20:5 n−3; 22:5 n−3; 22:6 n−3).

4.2. Fat content and fatty acid composition

The muscle fat content decreased significantly in groups C and P, where the highest fat content was found at the beginning of the purging period (8.68 ± 2.8% and 7.32 ± 4.5% respectively; Table 2). Decreased fat stores have also been observed in other studies (Einen et al., 1998; Liu et al., 2011).

The carp from group N, in the natural pond environment, did not show any significant decrease in fat content. The variation in fat content between different fish and sampling dates was high, so the fact that different individuals were measured on each sampling occasion may explain the non-significant weight loss in group N fish. Similarly, Palmeri et al. (2009a) did not find a significant fat loss in low-fat Murray cod during starvation ($P > 0.05$).

Extruded linseed and pressed rapeseed cake (mouldings) are a relatively cheap and easily available source of 18:3 n−3 for fish nutrition (Pickova and Mørkøre, 2007) and were part of the mixture fed to group P in this experiment. The presence of 18:3 n−3 in the pellets was reflected in the FA composition of the carp muscle. At the beginning of purging there was a 1.58-fold higher content of 18:3 n−3 in the muscle of carp from group P compared with group C and a 3.13-fold higher content compared with carp from group N. The effect of diet was also evident in the group C, where a high content of MUFA, especially 18:1 n−9, was observed. The opposite was found in the group N fish, reflecting the FA composition of the prey with high levels of PUFA according to our initial hypothesis and findings by Vacha et al. (2007) and Mraz et al. (2012).

During starvation, free fatty acids (FFAs) are released from TAG as a substrate for β-oxidation in the mitochondria (Reshef et al., 2003). The decrease in TAG and the higher stability of PL found in our study are in line with results reported by Henderson and Tocher (1987). In a study by Kiessling et al. (2001) performed on rainbow trout, excess dietary energy was found to be stored in the form of TAG, as was also the case of the cereal-supplemented group, resulting in the highest percentage of TAG in that group at most sampling dates (Table 4).

When the identified proportion of PL was calculated as absolute amounts per portion of fish, these amounts remained stable until days 14, 28 and 42 of sampling in groups P, C and N, respectively. While these are calculated amounts rather than actual values, they correspond well to the actual values and are suggested to give a good picture of the point when the fish start to use PL. We suggest that this is also the point when the fish start to catabolise not only surplus fat, but also muscle mass to meet energy needs. In addition, we suggest that at this point the fish also start to metabolise PUFA.

During β-oxidation of FA from TAG, in general fish utilise FA selectively in order to save the metabolically essential long-chain PUFA and first use the less important FA as fuel (Kiessling and Kiessling, 1993).

In our study there was a continuous increase in the relative content of n−3 PUFA in the muscle of carp from group C, which can be explained by the gradual degradation of MUFA (Jezierska et al., 1982), especially 18:1 n−9, while PUFAs are protected as described by Csengeri (1996). This could be due to the fluidity of biological membranes being increased and carp surviving better at low water temperatures. Similar results have been reported by Palmeri et al. (2008b) in a study on Murray cod.

The FA composition of the TAG and PL fractions of purged carp from group C showed a clear increase in the proportion of PUFA, including n−3 PUFA, EPA and DHA, to the detriment of MUFA (Tables 5a and 5b). These changes were pronounced in PL, combined with a significant decrease in the MUFA/PUFA ratio. This suggests that carp preferentially metabolise MUFA, while PUFAs are protected as suggested by Kiessling and Kiessling (1993) for rainbow trout. At the point when the carp started to metabolise the PUFA, they seemed to metabolise n−6 and n−3 equally. Again, this is in line with Kiessling and Kiessling (1993), who found similar oxidation rates for 18:2 n−6 and 18:3 n−3 in the mitochondria of rainbow trout.

In addition, we suggest that fish have a certain metabolically defined composition of FA that is necessary for functionality and that once that level is reached, fish start to catabolise all FA equally to preserve the relative composition. This theory is supported by the fact that after the longer purging period, the FA composition did not differ more between the groups, despite the significant differences at the beginning of the experiment. With decreasing water temperature, prolonged starvation and metabolism of fat stores, the FA composition gradually equalised in the PL fraction and after 70 days of purging, all groups showed almost identical values of SFA, MUFA, PUFA (including n−3, n−3 long-chain PUFA, EPA and DHA).

In relation to human nutrition, it is well known that fish are an important source of beneficial n−3 FA (Simopoulos, 2002). The European Food Safety Authority (EFSA, 2009) recommends a daily intake of n−3 PUFA for the general population of 2 g and an EPA + DHA intake of 250 mg. One portion (200 g) of carp from groups C, P or N purged for 14 days, which time we concluded as one with the best nutritional values, contained 1.06 g n−3 PUFA and 326 mg EPA + DHA, 1.72 g PUFA and 453 mg EPA + DHA, or 0.75 g PUFA and 354 mg EPA + DHA, respectively. This means that the best choice in terms of human nutrition seems to be carp from group P purged no longer than 14 days.

5. Conclusions

Lipid analyses of purged common carp reared in three different production systems showed that the type of diet prior to purging significantly affected the flesh quality of the fish. Supplementation with rapeseed/linseed pellets in the growing period resulted in a nutritionally beneficial FA flesh composition. The purged carp were able to selectively metabolise FA for energy needs early in the purging period, and when the carp were supplemented with cereals or rapeseed/linseed pellets, which had higher CF, they mainly used MUFA as their energy source. However with prolonged purging and loss of surplus fat, the fish from all groups started to metabolise long-chain PUFA, leading to a decrease in nutritionally valuable n−3 PUFA. Therefore a purging period should be long enough to eliminate possible unpleasant odours and flavours, but as short as possible from a practical handling point of view to preserve the beneficial FA composition of n−3 enriched carp. For this reason, we recommend that carp supplemented with linseed/rapeseed pellets (group P) should not be purged no longer than 14 days. More studies are needed to identify the mechanisms behind the selective FA metabolism in purged carp. This study provided valuable information about FA metabolism in carp that can be used in the further development of feeds and rearing systems.

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Příloha č. 5

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Rapid measurements of fat content in live and slaughtered common carp (*Cyprinus carpio* L.)

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Abstract The objective of this study on common carp (*Cyprinus carpio*) was to obtain and predict the first cross-validated data of the fat content on market size carps using a non-invasive or non-destructive method in situ. The carps (1680 ± 388 g; $n = 136$) used were from a semi-intensive system and were on a different diet (cereal, pelleted and extruded diet). For the evaluation of the fat content, a Fish Fatmeter FM 692 from Distell.com. (FFM) and a manual measurement of back fat height using a digital calliper were used. For the prediction model, the following basic body measurements (variables) were used: total body length, body length, body height, the width of the body, and the circuit of the body. The body weight, weight of intestines, weight of gonads, weight of hepatopancreas, and fillet yield (%) were measured, and the Fulton coefficient was calculated. The study was focussed on evaluating the applicability of these methods and the accuracy of the obtained result, respectively. Results showed that all the rapid methods had a strong correlation. Multiple regression models with forward selection of variables were used throughout. The final prediction model between predicted and observed values for the fat content for FFM and calliper being adjusted index of determination is shown here ($R^2_{adj} = 0.88$; 5 variables and $R^2_{adj} = 0.91$; 7 variables), respectively.

Keywords *Cyprinus carpio* · Fat measurement · Fish Fatmeter · Prediction equation · Rapid method

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Introduction

The pond farming of common carp (*Cyprinus carpio* L.) is of great importance in global aquaculture and the production of farmed common carp was slightly over 4.0 million tonnes of the total global fresh water aquaculture production in 2014 (FAO 2015). The biggest producer of carp in the world is China. In 2002 China produced ~70 % of the total global fresh water production of common carp (FAO 2015). Much of the production takes place in Europe ~145 thousand tonnes, especially in Central and Eastern Europe, as an integral part of the socio-cultural milieu (Edwards 2007), a well-known production system and traditional food (Linhart et al. 2002). The increasing demands for fish of the growing world population could be met by an increased production of fish in aquaculture, and it is essential to produce a suitable quality of fish and fish products which are acceptable to the consumer's expectations and tastes.

Beside texture, the lipid content is one of the main factors affecting fish flesh quality. In general, carp supplemented with cereals or pelleted feed has a higher fat content compared to carp depending only on natural feed (zooplankton and zoobenthos) (Urbánek et al. 2010; Mráz et al. 2012; Zajic et al. 2013; Másílko et al. 2015). Moreover, some studies found out that the lipid content may vary widely (Oberle et al. 1997; Pfeifer and Füllner 2005; Varga et al. 2013; Zajic et al. 2013) which is associated with different pond management strategy and pond production system (Fauconneau et al. 1995; Anderson and De Silva 2003; Mráz et al. 2012; Zajic et al. 2013; Másílko et al. 2015). A higher amount of lipid content has shown to adversely affect sensory properties of carp flesh (Aas and Oberle 2009), and the consequent impact is a lower price of carps in Bavaria (Oberle; personal communication).

Several different methods exist for estimating the lipid content. The most preferred are laboratory methods which are nowadays one of the most precise methods. Laboratory analyses are destructive methods and needs a location, equipment, a lengthy time for preparing samples, their evaluation and it is costly. Recently, several rapid methods have been used for estimating the lipid content of fish which are mostly non-invasive or non-destructive or both and it is not that time consuming. An example of this is computerized chromatography (CT) which is a non-invasive technique and may be used for estimation of fat content in several fish species, including salmonids (Rye 1991) or cyprinids (Romvari et al. 2002; Hancz et al. 2003). Other rapid measurements of fat content in salmonids that are being used are different types of near infrared spectroscopy (NIR) or visible near infrared spectroscopy (VNIRS) in fillet or in live fish (e.g. Solberg et al. 2003; Brown et al. 2014). Another non-invasive and non-destructive rapid method for estimation of fat content is using a Distell Fish Fatmeter. This instrument was compared to chemical analysis in some fish species such as Atlantic salmon (*Salmo salar*), Pacific salmon (*Oncorhynchus spp.*), Hering (*Clupea harengus*), river catfish (*Pangasiodon hypophthalamus*) with very high and positive correlation (Sigurgisladottir et al. 1997; Vogt et al. 2002; Crossin and Hinch 2005; Sang et al. 2009). However, a Distell Fish Fatmeter has an advantage to be used on a live fish. There are no available real data to compare a Fish Fatmeter and chemical analysis for carp.

The objective of this study was to obtain the first cross-validated prediction on the fat content of a live carp using a non-invasive or non-destructive method in situ. For testing, the Distell Fish Fatmeter was used. The next rapid method was to investigate the cross-validated prediction on the fat content according to manual measurement height of back fat using a digital calliper on slaughtered carps.

Materials and methods

Fish sample

Carps were harvested from a semi-intensive system (cereal, pelleted and extruded diet CD, PD, ED, respectively). The market size carps were harvested from the southern and western area of the Czech Republic (Table 1). Carps were slaughtered in the processing plant of the Faculty of Fisheries and Protection of Waters in České Budějovice, Czech Republic. The basic parameters were measured and calculated for each fish. *Longitudo totalis* (LT), standard length (SL), body height (BH), the width of the body (WB), and circuit of the body (CB) were measured using a roller (mm). Body weight (m), weight of intestines (WI), weight of gonads (WG), and weight of hepatopancreas (WH) were measured using a digital weight (KERN, PFB 6000-1, Germany, ±0.1 g); fillet yield $(\%)$ was calculated $:= \left(\frac{100 \times m \text{ of fillets}}{m}\right)$; and Fulton coefficient was calculated according to formula: $(\mathrm{FC}) = \left(\frac{m \times 100}{SL^3}\right)$, where m is weight (g) and SL is standard length in cm. All fish were filleted by hand by the same person to ensure consistency.

Fat content

Laboratory analyses

Whole fillets with skin were minced in a table cutter to ensure that all edible parts were represented in the sample analysed. Lipid extraction was performed with hexane-isopropanol according to Hara and Radin (1978) with slight modifications described by Zajic et al. (2013).

Fish Fatmeter (FFM)

Carps were measured for fat content by a Fish Fatmeter, UK, Model 692 Fish Fatmeter (Distell.com., Scotland). One person, to ensure consistency, did measurements at 4 positions (Fig. 1) at each side of live carps. The FFM was calibrated.

Calliper measurements

During the slaughtering (filleting) of fish, the dorsal parts were exposed, where the layer of fat was visible. After removal of the head and filleting of carp, the thickness of back fat was measured using a digital calliper (16 EWR-NA, Germany) 2 cm caudal direction from

Table 1 Fish samples used for multiple linear regression model

Geographic location	Type of diet	Number of fish	Fish weight (mean ± SD) (g)
South Bohemia	Cereal	11	2268 ± 264
South Bohemia	Extruded	16	1990 ± 252
South Bohemia	Pelleted	33	1733 ± 139
Plzeň region	Cereal	36	1474 ± 194
Plzeň region	Pelleted	40	1557 ± 483

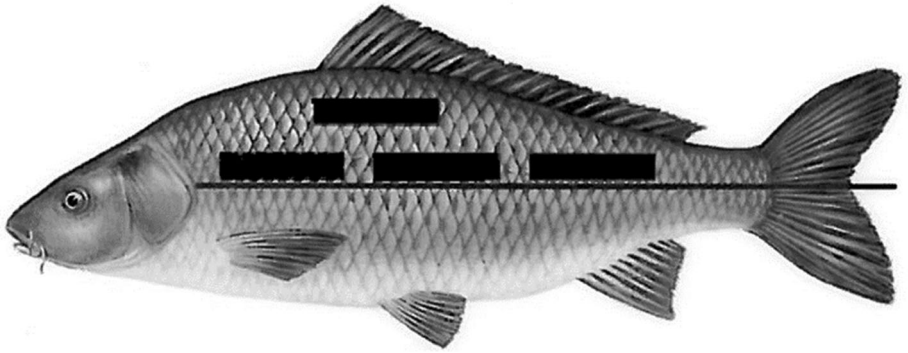

Fig. 1 Four measurements made on each side of the fish

the head of the dorsal parts of the carp skeleton. All samples of carp were measured by the same person to ensure consistency.

All samples analysed by chemical analysis were analysed by "rapid" methods. To evaluate the comparative benefit of these methods, observed results (FFM and calliper) were compared to the predicted data (chemical analysis) by linear regression analysis. In the present study, these criteria were determined: the correlation coefficient between predicted and observed data, regression models for the prediction of chemical analysis by FFM and calliper method. In terms of practice perspective, capital equipment costs, the speed of measurement and manual user training requirement was considered, although these parameters are difficult to specify adequately and objectively.

Statistical analysis

For fat content prediction was used regression analysis. More concretely was used "search for the best subset approach" by exhaustive search algorithm (Miller 1990). An index of determination as criterion for the best model was used. For calibration models were used classical linear regression models, but due to present heteroscedasticity (tested by Beusch-Pagan test) was used consequently weighted least square (WLS) methods instead classical ordinary linear regression (OLS). In results were provided both methods for these calibration models. For determination of correlation between different methods, classical Pearson's correlation coefficient was used.

With the aim to evaluate and choose the most accurate approach for fat content determination in calibrations models, the root-mean-square error of prediction (RMSEP) and adjusted index of determination (R^2_{adj}) as quality criteria was used. The RMSEP was defined as

$$RMSEP = \left(\frac{\sum_{i=1}^{N} (y_i - \widehat{y}_i)^2}{N} \right)^{1/2}$$

where y_i and $\widehat{y}_i$ are reference (analytical) and evaluated method (prediction obtained by regression based on fat meter or physical measurement of back fat measured by calliper) of fat content, respectively, for our sample $i = 1, 2, \ldots, n$. All data were analysed using statistical program R 3.2.0 (R Development Core Team 2015).

Results and discussion

Correlation coefficient

Based on our results, a statistically significant high correlation coefficients between chemical analysis, FMM and calliper, respectively, for determination of fat content (Table 2) were observed. The correlation coefficient between the predicted values of chemical analysis and observed values of calliper was 0.76 ($R^2 = 0.58$), whereas the correlation coefficient 0.88 ($R^2 = 0.78$) between predicted values of chemical analysis and observed values of FMM was higher compared to calliper method. The correlation between observed and predicted fillet fat values were lower compared to the study of Crossin and Hinch (2005), who reported a strong positive correlation 0.98 ($R^2 = 0.96$, four position above the lateral line, $n = 117$) for Pacific salmon (Crossin and Hinch 2005). Sang et al. (2009) described a very similar correlation coefficient 0.86 ($R^2 = 0.75$, one person did the measurement at nine positions on each size, $n = 50$) between predicted and observed values for fat content for river catfish using the same FFM that have been used in our study. In this study Sang et al. (2009) used a different chemical analysis method for predicted data described in Bligh and Dyer (1959). Likewise Vogt et al. (2002) determined a tight correlation 0.84 ($R^2 = 0.70$, one position on each side between the head and the dorsal fin, $n = 60$) between observed values using a FFM and predicted values (chemical analysis) for herring. In this study Vogt et al. (2002) used the Soxhlet method for determination of fat content according to the standard procedure ISO 1444 (1973).

The lower correlation coefficient compared to salmonids fish species might be due to higher variability of fat content in carp flesh, which is associated with a different pond management strategy and pond production system. The lipid content may vary widely from 1 to 16.6 % (Oberle et al. 1997; Pfeifer and Füllner 2005; Varga et al. 2013; Zajic et al. 2013).

Associated regression graphs have been shown in Fig. 2, and the regression result (OLS and WLS) for fat content prediction based on FFM observation is provided in Tables 3 and 4, for calliper is shown in Fig. 3, Tables 5 and 6, respectively. With regard to the reference between the analytical (chemical method) and "rapid" method, the best predictive methods were those with a slope equal to one and intercept term equal to zero. According to these criteria, the FFM method is more precise compared to the use of calliper for estimation of fat content.

Regression model for fat prediction

The regression models developed to predict chemical analysis results from these two rapid methods are shown in Table 7. For better prediction, we used potentially suitable variables

Table 2 Correlation coefficient between different methods

	Chemical analysis	FFM	Calliper
Chemical analysis	–	0.8864[a]	0.7678[a]
FFM	0.8864[a]	–	0.89317[a]
Calliper	0.7678[a]	0.89317[a]	–

[a] Statistically significant $\alpha = 0.01$

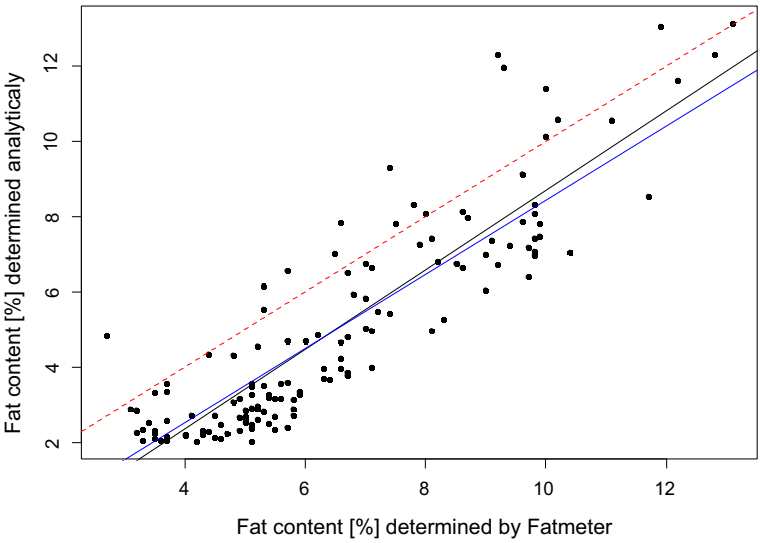

Fig. 2 Calibration plot for fat content determination—FFM versus reference analytical method. The *red dashed diagonal line* is the *target line*. The two regression models are OLS (*black line*) and WLS (*blue line*). (Color figure online)

Table 3 Regression result (OLS) for fat content prediction based on FFM observation

Explanatory variable	Estimate	SE of estimate	*t*	*p* value
Intercept	−1.889	0.3292	−5.739	6.07×10^{-8}
Fat content by FFM	1.0578	0.0477	22.175	$<2 \times 10^{-16}$
Residual standard error	1.298	134 *df*		
F-test	491.7	1 a 134 *df*		2.2×10^{-16}
R^2adj	0.7843			
AIC	460.8125			
Studentized Breusch-Pagan test	4.3639	1 *df*		0.03671
RMSEP	1.288			

AIC Akaike information criterion, *RMSEP* root-mean-square error of prediction, R^2adj adjusted index of determination

Table 4 Regression result (WLS) for fat content prediction based on FFM observation

Explanatory variable	Estimate	SE of estimate	*t*	*p* value
Intercept	−1.41824	0.31004	−4.574	1.08×10^{-5}
Fat content FFM	0.0985	0.05083	19.387	$<2 \times 10^{-16}$
Residual standard error	0.5182	134 *df*		
F-test	375.8	1 a 134 *df*		2.2×10^{-16}
R^2adj	0.7352			
AIC	456.9907			
RMSEP	1.299			

AIC Akaike information criterion, *RMSEP* root-mean-square error of prediction, R^2adj adjusted index of determination

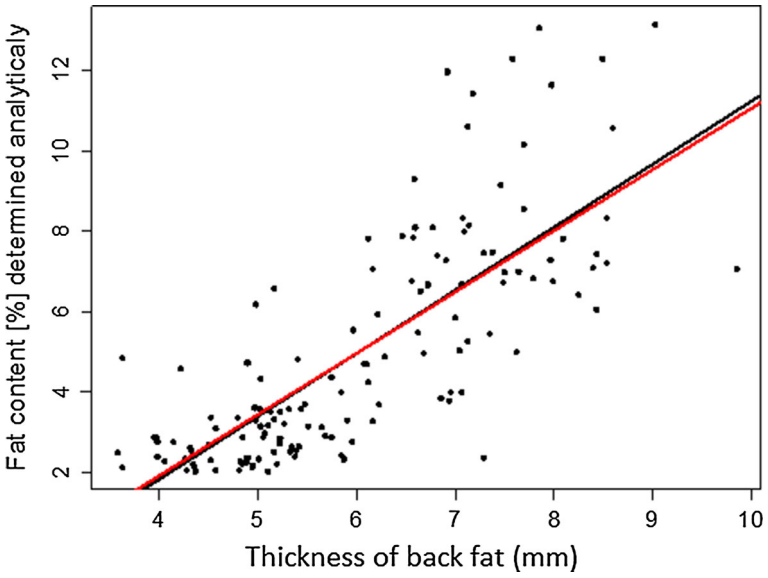

Fig. 3 Calibration plot for fat content determination—calliper versus reference analytical method. The *red diagonal line* is the *target line*. (Color figure online)

Table 5 Regression result (OLS) for fat content prediction based on calliper measurement

Explanatory variable	Estimate	S.E. of estimate	*t*	*p* value
Intercept	−4.4766	0.6989	−6.405	2.35×10^{-9}
Fat content by calliper	1.5713	0.1133	13.872	$< 2 \times 10^{-16}$
Residual standard error	1.797	134 *df*		
F-test	192.4	1 a 134		$<2.2 \times 10^{-16}$
R^2adj	0.5864			
AIC	549.3068			
Studentized Breusch-Pagan test	19.4431	1 *df*		1.036×10^{-5}
RMSEP	1.7833			

AIC Akaike information criterion, *RMSEP* root-mean-square error of prediction, R^2adj adjusted index of determination

Table 6 Regression result (WLS) for fat content prediction based on calliper measurement

Explanatory variable	Estimate	S.E. of estimate	*t*	*p* value
Intercept	−4.1795	0.6425	−6.505	1.42×10^{-9}
Fat content by calliper	1.5220	0.1095	13.896	2×10^{-16}
Residual standard error	0.7004	134 *df*		
F-test	193.1	1 a 134 *df*		
R^2adj	0.5873			
AIC	533.7071			
RMSEP	1.784			

AIC Akaike information criterion, *RMSEP* root-mean-square error of prediction, R^2adj adjusted index of determination

Table 7 Regression models for the prediction of chemical method by FFM and by calliper measurement

Method	Regression model	R^2_{adj}	RMSEP
FFM (OLS)	−1.889 + 1.0578FFM	0.7843	1.288097
FFM (WLS)	−1.418 + 0.0985FFM	0.7352	1.299153
Calliper (OLS)	−4.4766 + 1.5713Calliper	0.5864	1.783385
Calliper (WLS)	−4.1795 + 1.522Calliper	0.5893	1.784648

RMSEP root-mean-square error of prediction, R^2adj adjusted index of determination

Table 8 Potentially best multiple linear regression models for different complexity of regression model for FFM

Model (included variables)	Regression model for fat prediction	R^2_{adj}
FFM	−1.889 + 1.0578FFM	0.7843
FFM, WB	−3.367 + 0.91001FFM + 0.02077WB	0.8163
FFM, WB, WI	−3.820465 + 0.9334FFM + 0.0287WB − 0.00147WI	0.8138
FFM, WB, WI, m	−3.81158 + 0.9418FFM + 0.031WB − 0.00081WI − 0.00024BW00024m	0.8108
FFM, WB, WI, m, WH	−3.379 + 0.926FFM + 0.024WB − 0.0011WI − 0.00017BW00017m + 0.0027WH	0.8082
FFM, WB, WI, m, WH, WG	−3.5726 + 0.976FFM + 0.055WB + 0.0124WI − 0.002BW002m − 0.015WH − 0.0047WG	0.8792

FFM fat meter (%), *WB* width of the body (mm), *WI* weight of intestine (g), *m* body weight (g), *WH* weight of hepatopancreas (g), *WG* weight of gonads (g)

such as diet and morphometric characteristics, which are described in the materials and methods, and fat content determined by FFM and calliper, respectively. Tables 8 and 9 present the adjusted index of determination for the best multiple linear regression models with different complexity (in terms of number of explanatory variables) determined by the exhaustive search for FFM and calliper. Among the variables, volume had a large effect in terms of increasing R^2_{adj}. The preferred model for FFM had another 5 variables (WB, WI, m, WH and WG) and explains 88 % of the variation in fat content. For calliper, the preferred model had 7 variables in equation (WH, WB, BH, CD, ED, PD and WG), which explained 91 % of the variation in fat content. In some studies were reported similar prediction models with different variables but mostly for fillet weight and fillet yield of different fish species (e.g. Rutten et al. 2004; Sang et al. 2009). Sang et al. (2009) found out 5 variables in the equation for the final model, which explained 86 % of the variation in fillet weight and 4 variables for the final model explaining 77 % of the variation for fillet yield of river catfish. As well Rutten et al. (2004) found out 5 variables for the best model in the equation for fillet yield and 4 variables for the best model for fillet yield for tilapia.

Practical application

In addition there are some practical considerations of these rapid methods of fat prediction such as how much the instrument and analysis cost, difficulty of use, requirements for skill of users, time per analysis. A relative order of priority of the methods is shown in Table 10.

Table 9 Potentially best multiple linear regression models for different complexity of regression model for calliper

Model (included variables)	Regression model for fat prediction	R^2_{adj}
Calliper	−4,4766 + 1.5713Calliper	0.5864
Calliper, WH	−2.9635 + 0.9837Calliper + 0.01199WH	0.8073
Calliper, WH, CB	−2.09294 + 1.0082Calliper + 0.0132WH − 0.003113CB	0.8046
Calliper, WH, WB, BH	−2.5634 + 1.01058Calliper + 0.010932WH + 0.0335WB − 0.02322BH	0.8055
Calliper, WH, WB, BH, CD, ED, PD	−2.0877 + 1.01691Calliper + 0.00896WH + 0.02422WB − 0.01857BH − 0.1CD−0.4629ED − 0.18902PD	0.8019
Calliper, WH, WB, BH, CD, ED, PD, WG	1.051 + 1.119Calliper + 0.0168WH + 0.0823WB − 0.0872BH + 0.0258CD − 0.37198ED − 0.14846PD + 0.0064WG	0.9078

WH weight of hepatopancreas (g), *CB* circumference of the fish body (mm), *WB* width of the body (mm), *BH* body height (mm), *WG* weight of gonads (g), *CD* cereal diet (1), *ED* extruded diet (1), *PD* pelleted diet (1)

Table 10 Relative order of priority of the methods in terms of practical use

Scaling factor	Relative order of priority (lowest to greatest)*
Cost per instrument	Calliper < FFM < chemical analysis
Cost per analysis	Calliper < FFM < chemical analysis
Time consuming	FFM < calliper < chemical analysis
Requirements for skills	Calliper < FFM < chemical analysis
Precision	Chemical analysis < FFM < calliper

* These factors are according to authorś personal experience and the classification is subjected

The calliper method is very simple to estimate the fat content and very cheap, but there is a potentially lower precision due to the possible damage of the layer of back fat on the skeleton during the filleting. The chemical analysis is full laboratory and achieves the level of analytical precision. On the other hand, laboratory analysis is more time consuming compared to FMM or calliper. The FFM is easy to use, and the big advantage is pre-calibration for different fish species. Moreover, the FFM is a unique instrument and might be used on a live fish because of the non-invasive and non-destructive application and the time per analysis is the fastest form compared to other methods. This was confirmed by Vogt et al. (2002) who reported similar relative ranking of different rapid methods on the basic of practical consideration. In the study of Vogt et al. (2002), the lowest time per analysis was measured for the Torry Fat meter, while the greatest time per analysis was stated for laboratory analysis. In the same study, the greatest required operation skill level was stated for laboratory analysis, while the lowest operation skill level was stated for the Torry Fat meter (Vogt et al. 2002), which is in accordance with our findings.

Conclusion

It might be concluded, that it is possible to measure or estimate fat content on marketable size carps using the tested rapid methods in situ. The FFM is non-destructive and non-invasive in use and for this reason can be used equally well on live or dead fish. In

addition the investigation of fat content is quite fast. Moreover, using the FFM and calliper is fully convenient for fat determination. The inclusion of other explanatory variables is relatively redundant, because of more time consumption to estimate other parameters (variables). But nevertheless, for more precise fat prediction, inclusion of morphological parameters could be useful and increase the adjusted index of determination according to the multiple linear regression model. Using the calliper might be useful in practice too, due to its minimal manufactory costs, speed of fat content evaluation with a high fidelity.

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Příloha č. 6

Sampels, S., Zajic, T., **Mraz, J.**, 2014. Effects of Frying Fat and Preparation on Carp (*Cyprinus carpio*) Fillet Lipid Composition and Oxidation. Czech Journal of Food Sciences 32, 493-502. (IF 2014 = 0.675)

Effects of Frying Fat and Preparation on Carp (*Cyprinus carpio*) Fillet Lipid Composition and Oxidation

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Abstract

Sampels S., Zajíc T., Mráz J. (2014): **Effects of frying fat and preparation on carp** (*Cyprinus carpio*) **fillet lipid composition and oxidation**. Czech J. Food Sci., **32**: 493–502.

We investigated the changes in omega-3 enriched carp fillets caused by pan frying. The investigated characteristics were fat uptake, fatty acid (FA) composition, and oxidation. Four different fats were used and fillets were fried plain or battered. The fillet fat content increased during frying and FA composition in the fillets reflected the composition of the frying fat. Frying with sunflower oil negatively influenced the nutritional value by decreasing the n-3/n-6 ratio in the fillets. Frying with rapeseed oil preserved the favourable n-3/n-6 ratio without increasing the saturated fatty acids (SFA). Frying with lard and butter preserved the n-3/n-6 ratio but increased the SFA content. No increased oxidation occurred with the use of rapeseed oil. We concluded that using rapeseed oil for fish seemed to preserve the nutritionally valuable composition best.

Keywords: DHA; EPA; TBARS; nutritional quality

List of abbreviations (in the order of appearance): FA = fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; omega 6 = n-6; omega 3 = n-3; FAME = fatty acid methyl esters; TBARS = thiobarbituric acid reactive substances; MDA = malondialdehyde; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; DPA = docosapentaenoic acid

The inclusion of fish and fish products in the human diet at least twice a week is recommended from the nutritional point of view, due to the high content of omega 3 polyunsaturated fatty acids (n-3 PUFA) in marine and fresh water fishes (Steffens 1997; EFSA 2009; EFSA Panel on Dietetic Products 2010). The n-3 PUFA are well known to have positive effects on different health aspects such as, for example, metabolic syndrome, obesity, diabetes, arteriosclerosis, neural and brain development (Storlien *et al.* 1997; Connor 2000; Williams 2000; Calder & Grimble 2002; Richardson 2006). However, today's Western diet contains an increasing content of omega 6 (n-6) PUFA, leading to increased occurrence of cardiovascular and atherosclerotic diseases, type 2 diabetes and obesity (Simopoulos 1999; Ailhaud *et al.* 2006).

Both n-6 and n-3 PUFA are precursors of a variety of divers chemical messengers, regulating factors, and eicosanoids like prostaglandins, leukotriens and related substances, which play important roles in the inflammation and regulation of immunity (Kinsella 1988; Horrobin 1995; Calder 1997, 2001). Since metabolites of n-3 and n-6 PUFA have different, often opposing biological actions and potencies, the intake ratio between n-3 and n-6 PUFA is important (Calder 2001; Schmitz & Ecker 2008). There is a competition between the enzymes involved in the

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elongation and desaturation of n-3 and n-6 FA as well as for the production of their respective metabolites, which will lead to increased n-6 metabolites if n-6 FA are present in a much higher proportion than the n-3 ones. In addition, high amounts of 18:2n-6 decrease the conversion of 18:3n-3 to 20:5n-3 (Simopoulos 2000). The values of 1 to 4 for n-6/n-3 in the diet have been recommended while in today's Western diets this ratio is often 15 to 20 (Simopoulos 2002a).

It is known from earlier studies that the type of preparation has some influence on the final fatty acid (FA) composition of the product and that especially during frying the fat used will have some influence on the FA composition of the final product (Ågren & Hänninen 1993; Ramirez *et al.* 2005; Sioen *et al.* 2006; Weber *et al.* 2008). The effects are partly due to the heat which can cause oxidation, but also due to the fact that during frying the exchange of water and fat will occur in which some water from the surface layer of the fried object is exchanged with the frying fat (Candela *et al.* 1998; Dobarganes *et al.* 2000). In addition, when dealing with an object like fatty fish whose fat, due to its high unsaturation, has a very low melting point and a higher liquidity than the frying fat, a loss may also happen of the original fat due to leakage. Furthermore, the possibility of fat leakage may also depend on the fish fat content (Sioen *et al.* 2006).

Most of the work until now has been done in deep-fat frying whereas only few researchers investigate pan frying (Mai *et al.* 1978; Al-Saghir *et al.* 2004; Sioen *et al.* 2006; Haak *et al.* 2007). On the other hand, pan-frying is one of the most frequently used ways to prepare fish or fish products at home. Therefore, the knowledge of what happens during this process is important when giving nutritional recommendations to consumers.

Many investigators have fried fish in sunflower oil and found increased proportions of 18:2n-6 influencing the n-3/n-6 ratio enormously (Gladyshev *et al.* 2006, 2007). On the other hand, in a study on catfish fried in canola oil 18:3n-3 was increased and thereby also the n-3/n-6 ratio leading to a more valuable nutritional composition (Weber *et al.* 2008).

In the Czech Republic and central Europe, the carp is a commonly consumed fish species and recently an omega 3 rich carp has been patented and is sold on the market as a product with positive effects on health, based on the results from a clinical study (Adámková *et al.* 2011; Mráz *et al.* 2011). The intake of two 200 g portions of this carp rich in n-3 per week improved the blood lipid values of patients recovering from ischemic heart disease (Adámková *et al.* 2011).

There are only very few papers concerning the preparation of carp before the consumption at home. Some authors investigated cooking methods without adding any oil or fat and only one reference was found to the investigation of deep fat frying of whole carp fillets. (Tothmarkus & Sasskiss 1993; De Castro *et al.* 2007; Naseri *et al.* 2010). A very recent publication described for the first time shallow fat frying of silver carp with olive, sunflower, and canola oils (Rahimabadi & Dad 2012).

Especially with the new developed n-3 rich carp is it important to know what types of changes can occur in the fish's FA composition when using different types of frying fat. This is why we chose butter and lard besides sunflower and rapeseed oils as the most commonly used oils in Czech homes. In addition, we fried the fillets plain and battered, which is also a typical way of preparation in Czech homes. Besides FA composition, we also monitored the oxidation due to frying with different fats.

The aim was to observe the combined effects of fat exchange and oxidation for us to be able to make suggestions on the preferable way to prepare carp in order to retain its nutritional value. As the effects may be similar to those with other oily fish species, the results can also have a more general use.

MATERIAL AND METHODS

Fish samples. Twelve 4-year-old market scaly carp (*Cyprinus carpio*) were used, reared for one season (April–September) with a rapeseed/linseed pellet supplementation for an enriched content of n-3 FA.

After harvesting and purging, the fish were killed and filleted. Fillets were frozen in –20°C until the frying experiment. All samples were stored frozen for the same period of time.

Lard, butter, rapeseed oil, and sunflower oil obtained in the local supermarket were used for frying. Before frying, the fish fillets were cut in three similar parts. Front and back parts of the fillets were cut makling about 100 g sized pieces to be fried, while the minor middle part was used as raw control for each fillet. From each corresponding fillet one back and one front part were used to be fried in the same fat source, either with or without a batter. The batter was prepared according to the traditional recipe by first turning the filet piece in wheat flour, then in beaten egg and finally in breadcrumbs. No spices or salt were added. For frying, 50 ml of the fat (butter

and lard were carefully melted before use) were used to fry the two pieces of the same fish and the same treatment (plain or battered) in a Teflon pan with 24 cm in diameter. The amount of fat was chosen so that the bottom of the pan was covered with liquid fat. The fat was heated to approximately 130°C and then the pieces were fried for 4 + 4 min on each side, and then for additional 2 min on each side until reaching a core temperature of 65°C, measured with a meat thermometer. After frying and cooling, the whole pieces were separately minced in a table blender to assure that all edible parts were included equally in the sample. The 2 stripes of raw samples from one fish were combined and minced together. For each fat type, three fish were used resulting in 3 duplicate plain fried pieces, 3 duplicate battered fried pieces, and 3 raw samples. The minced samples were frozen and kept at –80°C until further analysis.

Total fat content and FA composition. The lipid extraction was performed according to Hara and Radin (1978), with a slight modification. The samples were semi-thawed, and sub-samples of approx. 1 g were taken for the extraction from the fish, and of 200 mg from the fats. The samples were homogenised for 3 × 30 s in 10 ml of hexane : isopropanol (3 : 2 v/v) using an Ultra Turrax (T25**;** IKA-Werke Janke & Kunkel GmbH & Co, Staufen, Germany), and 6.5 ml Na_2SO_4 solution (0.47M) was added. The homogenate was left to separate in 4°C for 20 min and the upper phase was then transferred to a new tube and evaporated under N_2. The lipid content of the fillet pieces was determined gravimetrically from this total extracted lipid, which was then dissolved in 1 ml of hexane. The samples were stored at –80°C in normal atmosphere until further analyses.

FA from the total lipids were methylated with boron triflouoride-methanol complex (BF3) (Appelqvist 1968). To each sample, 2 ml of a 0.01M solution of NaOH in dry methanol was added, and the samples were then heated for 10 min at 60°C. Further, 3 ml of BF_3 reagent were added and the samples were reheated at 60°C for 10 minutes. Thereafter, the tubes were cooled in ice water and 2 ml of a 3.42M NaCl solution in water was added to all tubes. The FA methyl esters (FAME) were extracted with 2 ml of hexane, the upper layer was transferred to a new tube and evaporated under nitrogen to dryness. The lipids were dissolved in 0.5 ml of hexane and stored under normal atmosphere at –80°C until gas chromatography analysis.

The FAME were then analysed with a gas chromatograph (Trace Ultra FID; Thermo Scientific, Milan, Italy) equipped with a flame ionisation detector and PVT injector, using a BPX 70 column (SGE Inc., Austin, USA), length 50 m, *i.d*. 0.22 mm, and film thickness 0.25 µm. The GC was programmed with a constant gas flow of 1.2 ml/min and a temperature program which started at 70°C for 0.5 min, followed by a ramp of 30°C/min up to 150°C, a second ramp with a rate of 2°C/min up to 220°C, and a final constant time of 11 min at 220°C. The injector and detector temperatures were programmed at 150 and 250°C, respectively. The injector was programmed in splitless mode, with a splitless time of 0.8 min and a split flow of 25 ml/minute. The peaks were identified by comparing their retention times with those of the standard mixture GLC-68D (Nu-Chek Prep, Elysian, USA) and other authentic standards (Nu-Chek Prep, Elysian, USA; Larodan, Sweden).

Oxidation. The analysis of thiobarbituric acid reactive substances (TBARS) was conducted in the fresh and fried fillets according to a method described by Miller (1998). After reaction in darkness for 15–20 h (overnight) at room temperature (20°C), the reaction complex was detected at a wavelength of 530 nm against the sample blank using a UV-visual spectrophotometer (Specord 210; Analytik Jena, Germany). The results were expressed as malonaldehyde equivalents (MDA) in µg/g.

Statistics and calculation. The averages and standard deviations were calculated in Excel and the statistical evaluation was performed using the Mixed Procedure in SAS (Version 9.1; SAS Institute Inc., Cary, USA). The changes in FA percentages were calculated in Excel.

RESULTS

Total fat content. Fat content is presented in Table 1. The fat content increased significantly due to frying and even more when the fillet was battered before frying. The increase difference between the plain fried and battered fried fillets was significant for lard.

Fatty acid composition. FA composition in raw and fried samples is presented in Table 1. The main FA of the fats are shown in Table 2. After frying the fillets showed an increase in the major FA from the different fats and oils used for frying. When using butter for frying, SFA significantly increased from raw to plain fried and to fried in batter, while MUFA and PUFA decreased. The difference, however, was significant only when the fillets were fried battered. The FA that increased most were C14:0 and C16:0

Table 1. Fat content (% in whole fillet with skin and with batter) and fatty acid composition (% of total identified) in raw and fried fillets of carp (n = 3 in each treatment), fried in different fats, plain or covered with a bread-batter with each fish as their own raw control (averages ± standard deviation)

	Butter			Lard			Rapeseed oil			Sunflower oil		
	raw	fried plain	fried with batter	raw	fried plain	fried with batter	raw	fried plain	fried with batter	raw	fried plain	fried with batter
Total fat	4.69 ± 2.53^{D}	10.5 ± 3.16^{E}	14.1 ± 2.65^{E}	3.07 ± 2.65^{D}	9.82 ± 1.76^{E}	14.0 ± 4.08^{F}	3.74 ± 1.05^{D}	9.47 ± 1.39^{E}	13.1 ± 1.34^{E}	5.07 ± 4.31^{D}	11.9 ± 5.05^{E}	13.9 ± 3.32^{E}
C14:0	1.19 ± 0.09^{D}	5.31 ± 1.08^{Ea}	8.11 ± 1.02^{Fa}	1.04 ± 0.26	1.48 ± 0.08^{b}	1.43 ± 0.05^{b}	1.04 ± 0.17	0.54 ± 0.09^{c}	0.40 ± 0.21^{c}	1.33 ± 0.51^{D}	0.60 ± 0.17^{cDE}	0.42 ± 0.24^{Ec}
C16:0	19.0 ± 1.88^{D}	25.3 ± 1.08^{Ea}	30.3 ± 1.87^{Fa}	18.7 ± 0.22^{D}	23.95 ± 1.52^{Ea}	25.21 ± 0.84^{Eb}	16.8 ± 0.79^{D}	10.4 ± 0.90^{Eb}	10.1 ± 3.30^{Ec}	18.12 ± 1.50^{D}	12.8 ± 1.90^{Eb}	12.3 ± 4.45^{Ec}
C16:1tr	1.14 ± 0.10^{Dab}	0.83 ± 0.07^{D}	0.65 ± 0.11^{Eab}	1.35 ± 0.49^{Dabc}	0.87 ± 0.26^{E}	0.66 ± 0.23^{Ea}	1.01 ± 0.29^{Db}	0.59 ± 0.05^{E}	0.42 ± 0.23^{Eb}	1.59 ± 0.45^{Dc}	0.63 ± 0.27^{E}	0.41 ± 0.29^{Eab}
C16:1	7.72 ± 1.88^{Da}	6.12 ± 1.73^{DEa}	4.66 ± 1.24^{Ea}	4.03 ± 2.60^{b}	3.72 ± 1.03^{b}	3.14 ± 0.85^{ab}	4.53 ± 1.42^{b}	2.87 ± 0.48^{b}	2.18 ± 0.9^{b}	6.75 ± 5.67^{Dab}	3.15 ± 1.80^{Eb}	2.14 ± 2.04^{Fb}
C18:0	5.12 ± 0.27	6.21 ± 1.44^{a}	7.41 ± 1.80^{a}	4.33 ± 1.95^{D}	11.9 ± 2.44^{Eb}	12.3 ± 3.62^{Eb}	5.12 ± 0.51	3.17 ± 0.32^{c}	3.10 ± 0.84^{c}	6.51 ± 2.16^{D}	4.47 ± 0.74^{DEac}	3.84 ± 1.00^{Ec}
C18:1n-9	37.2 ± 4.05^{a}	33.7 ± 4.08^{a}	31.2 ± 2.23^{a}	29.5 ± 3.83^{ab}	33.9 ± 0.93^{a}	35.9 ± 0.60^{b}	36.4 ± 3.20^{Da}	50.5 ± 1.46^{Eb}	49.7 ± 8.3^{Ec}	34.3 ± 8.58^{Db}	34.0 ± 4.45^{Ea}	35.6 ± 10.2^{Eab}
C18:1n-7	3.42 ± 0.15^{D}	2.58 ± 0.31^{DEab}	2.02 ± 0.35^{acE}	2.40 ± 2.08	2.85 ± 0.22^{ab}	2.72 ± 0.21^{a}	3.37 ± 0.33^{D}	3.35 ± 0.12^{aDE}	3.71 ± 1.17^{Db}	2.30 ± 2.01^{D}	2.06 ± 0.47^{Deb}	1.71 ± 0.65^{Ec}
C18:2n-6	9.0 ± 2.16	6.8 ± 1.02^{a}	6.3 ± 1.41^{a}	11.2 ± 1.28	10.5 ± 0.71^{ab}	10.9 ± 0.4^{a}	11.4 ± 1.19	15.6 ± 1.00^{b}	19.2 ± 5.65^{b}	12.2 ± 0.78^{D}	32.8 ± 10.48^{Ec}	40.6 ± 14.18^{Fc}
C18:3n-3	5.92 ± 2.30^{Da}	4.17 ± 1.22^{DEa}	2.96 ± 1.25^{Ea}	8.21 ± 2.07^{Dab}	4.34 ± 1.84^{Ea}	3.08 ± 1.60^{Ea}	6.74 ± 0.81^{ab}	7.79 ± 0.34^{b}	6.99 ± 1.40^{b}	8.46 ± 2.18^{Db}	3.61 ± 1.35^{Ea}	2.41 ± 1.18^{Ea}
C20:1n-9	2.00 ± 0.10^{Dab}	1.39 ± 0.15^{DE}	0.90 ± 0.25^{Eab}	1.23 ± 1.06^{a}	1.17 ± 0.23	0.92 ± 0.16^{ab}	2.53 ± 0.39^{Db}	0.99 ± 0.81^{E}	1.40 ± 0.29^{Ea}	2.46 ± 1.58^{Db}	1.08 ± 0.48^{E}	0.59 ± 0.17^{Eb}
C20:3n-6	0.28 ± 0.08^{Da}	0.21 ± 0.06^{DE}	0.11 ± 0.04^{E}	0.58 ± 0.23^{Db}	0.18 ± 0.07^{E}	0.09 ± 0.08^{E}	0.48 ± 0.07^{Db}	0.11 ± 0.08^{E}	0.05 ± 0.08^{E}	0.40 ± 0.22^{Da}	0.13 ± 0.05^{E}	0.06 ± 0.07^{E}
C20:4n-6	1.89 ± 0.50	1.10 ± 0.18	0.73 ± 0.14	4.80 ± 0.96^{D}	1.13 ± 0.17^{E}	0.88 ± 0.21^{D}	2.29 ± 0.63^{D}	0.68 ± 0.28^{E}	0.42 ± 0.13^{E}	2.96 ± 2.07^{D}	0.73 ± 0.30^{E}	0.45 ± 0.20^{E}
C20:3n-3	0.28 ± 0.12^{a}	0.16 ± 0.07	0.05 ± 0.04	1.13 ± 1.12^{Db}	0.18 ± 0.13^{E}	0.25 ± 0.29^{E}	0.37 ± 0.10^{a}	0.15 ± 0.13	0.06 ± 0.11	0.29 ± 0.25^{a}	0.36 ± 0.19	0.08 ± 0.08
C22:1	0.32 ± 0.17^{ab}	0.27 ± 0.10	0.16 ± 0.07	0.48 ± 0.07^{Db}	0.29 ± 0.18^{DE}	0.15 ± 0.20^{E}	0.15 ± 0.01^{a}	0.15 ± 0.05	0.10 ± 0.06	0.55 ± 0.38^{Db}	0.21 ± 0.08^{E}	0.08 ± 0.09^{E}
C20:5n-3	2.21 ± 0.80^{Da}	1.33 ± 0.29^{E}	0.75 ± 0.28^{E}	4.70 ± 1.23^{Db}	1.51 ± 0.51^{E}	0.90 ± 0.47^{E}	2.63 ± 0.54^{Da}	1.12 ± 0.11^{E}	0.67 ± 0.36^{E}	3.79 ± 1.34^{Dc}	1.30 ± 0.37^{E}	0.65 ± 0.22^{E}
C22:5n-3	0.95 ± 0.29^{Da}	0.54 ± 0.09^{DE}	0.34 ± 0.11^{E}	1.94 ± 0.78^{Db}	0.56 ± 0.08^{E}	0.32 ± 0.09^{E}	1.09 ± 0.27^{Da}	0.38 ± 0.06^{E}	0.21 ± 0.10^{E}	1.63 ± 0.88^{Db}	0.49 ± 0.22^{E}	0.20 ± 0.07^{E}
C22:6n-3	2.03 ± 0.80^{Da}	0.98 ± 0.33^{E}	0.58 ± 0.21^{E}	3.79 ± 1.26^{Db}	0.99 ± 0.24^{E}	0.56 ± 0.23^{E}	2.31 ± 0.70^{Da}	0.83 ± 0.14^{E}	0.38 ± 0.17^{E}	2.41 ± 2.35^{Da}	0.86 ± 0.49^{E}	0.40 ± 0.11^{E}
SFA	25.6 ± 2.03^{Dab}	40.8 ± 2.34^{Ea}	51.3 ± 3.83^{Fa}	24.7 ± 1.74^{Dab}	37.4 ± 3.97^{Ea}	39.1 ± 4.12^{Eb}	23.4 ± 0.60^{Da}	14.6 ± 1.08^{Eb}	14.2 ± 4.62^{Ec}	31.1 ± 10.75^{Db}	18.4 ± 2.66^{Eb}	16.5 ± 3.43^{Ec}
MUFA	52.0 ± 5.82^{Da}	44.8 ± 6.46^{DEa}	39.0 ± 4.09^{Ea}	40.3 ± 4.95^{bc}	42.8 ± 1.17^{a}	43.7 ± 1.57^{a}	48.5 ± 3.78^{Dab}	58.5 ± 0.79^{Eb}	57.6 ± 9.58^{DEb}	35.6 ± 8.46^{c}	40.9 ± 7.38^{a}	38.4 ± 9.37^{a}
PUFA	23.3 ± 7.12^{Da}	15.8 ± 2.79^{DEa}	12.1 ± 3.33^{Ea}	34.9 ± 3.20^{Db}	19.7 ± 2.91^{Eab}	17.2 ± 2.62^{Ea}	28.2 ± 3.20^{ab}	26.9 ± 1.17^{b}	28.3 ± 5.12^{b}	33.4 ± 3.94^{Dab}	40.6 ± 9.88^{DEc}	45.1 ± 12.64^{Ec}
n-3	11.4 ± 4.29^{Da}	7.18 ± 1.70^{Ea}	4.69 ± 1.84^{Fa}	19.8 ± 1.30^{Db}	7.59 ± 2.72^{Ea}	5.10 ± 2.40^{Fa}	13.1 ± 1.53^{Da}	10.3 ± 0.35^{DEb}	8.32 ± 0.71^{Eb}	16.6 ± 2.63^{Db}	6.61 ± 1.65^{Ea}	3.75 ± 1.53^{Fa}
n-6	11.9 ± 2.90	8.50 ± 1.18^{a}	7.29 ± 1.55^{a}	15.1 ± 2.28	12.1 ± 0.79^{ab}	12.1 ± 0.43^{a}	15.0 ± 1.73	16.7 ± 0.83^{b}	19.9 ± 5.79^{b}	16.7 ± 1.79^{D}	33.9 ± 10.14^{Ec}	41.2 ± 13.90^{Ec}
n-3/n-6	0.94 ± 0.15	0.83 ± 0.10	0.62 ± 0.11	1.32 ± 0.15^{D}	0.63 ± 0.23^{E}	0.42 ± 0.19^{E}	0.88 ± 0.03	0.62 ± 0.01	0.46 ± 0.08	0.99 ± 0.13^{D}	0.41 ± 0.54^{E}	0.43 ± 0.85^{E}

$^{D–E}$different capital letters in a row indicate significant difference (P < 0.05) between raw fish, plain fried fish, and fish fried in batter in the same fat; $^{a–c}$different small letters in a row indicate significant differences (P < 0.05) between the raw fish or same frying type across different fats (n = 12); n-6 = omega 6; n-3 = omega 3; SFA = sum of saturated fatty acids; MUFA = sum of monounsaturated fatty acids; PUFA = sum of polyunsaturated fatty acids; n-3/n-6 = ratio between the sum of omega 3 and omega 6 fatty acids

Table 2. Fatty acid composition in the oils and fats used for frying (% of total identified)

	butter	lard	Oil	
			rapeseed	sunflower
C10:0	3.11	nd	nd	nd
C12:0	4.35	0.11	nd	nd
C14:0	13.8	1.56	0.07	0.09
C16:0	38.6	27.8	4.58	6.60
C18:0	10.3	17.4	1.59	3.25
C18:1n-9	22.6	35.7	60.5	26.5
C18:1n-7	0.74	2.42	3.32	0.73
C18:2n-6	2.44	10.2	19.0	60.8
C18:3n-3	0.57	0.84	8.46	0.36

nd = not detected

(Figure 1). The highest increase was in 16:0 which was also the major FA in butter (38.6%).

Frying with lard resulted in significant increase of SFA and significant decrease of PUFA and n-3, while n-6 was stable. The main FA increasing were C16:0 and C18:0 while all unsaturated FA decreased more or less significantly. The decrease in n-3 also caused a significant decrease in the n-3/n-6 ratio.

Frying with rapeseed oil led to significantly decreased SFA and significant increase of MUFA. In the battered fillets after frying even the total n-3 FA were significantly decreased as compared to the raw fillets. However, that decrease was not significant in the plain fried fillets. The main FA increased was 18:1n-9 (Figure 1). Frying with rapeseed oil resulted in an increase of 18:1n-9 up to 50% and up to 8% of 18:3n-3, reflecting the proportions of these FA in the oil. In the oil used, the proportions of these FA were 60 and 8.5%, respectively.

The fillets fried in sunflower oil showed a significant increase in total PUFA and n-6 FA, while SFA and n-3 FA decreased significantly. The FA that increased significantly were 18:2n-6 and 18:1n-9. The significant increase in n-6 also caused a significant decrease in the n-3/n-6 ratio.

The total proportion of n-3 was significantly decreased due to the frying process in all samples, however, in the samples fried with rapeseed oil this decrease was the lowest in percentage (36.5% compared to 48.1, 74.2, and 77.4% in battered samples fried with rapeseed oil, butter, lard, and sunflower oil, respectively). At the same time, the proportion of total n-6 increased slightly in the samples fried with butter and lard and significantly in the samples fried with sunflower oil. 20:5n-3 (eicosapentaenoic acid, EPA), 22:5n-3 (docosapentaenoic acid, DPA) and 22:6n-3 (docosahexaenoic acid, DHA) decreased significantly in all frying treatments independently of the fat used.

20:4n-6 (arachidonic acid, AA) decreased significantly due to frying with lard, rapeseed oil, and sunflower oil, however, not when butter was used.

Oxidation. TBARS were similar among the raw fish samples were similar. Frying increased TBARS in the fish fried with lard and sunflower oils significantly, while butter did not increase TBARS and frying with rapeseed oil only showed a tendency towards increased TBARS values (P = 0.0674). TBARS in the battered fillets were not analysed as the bat-

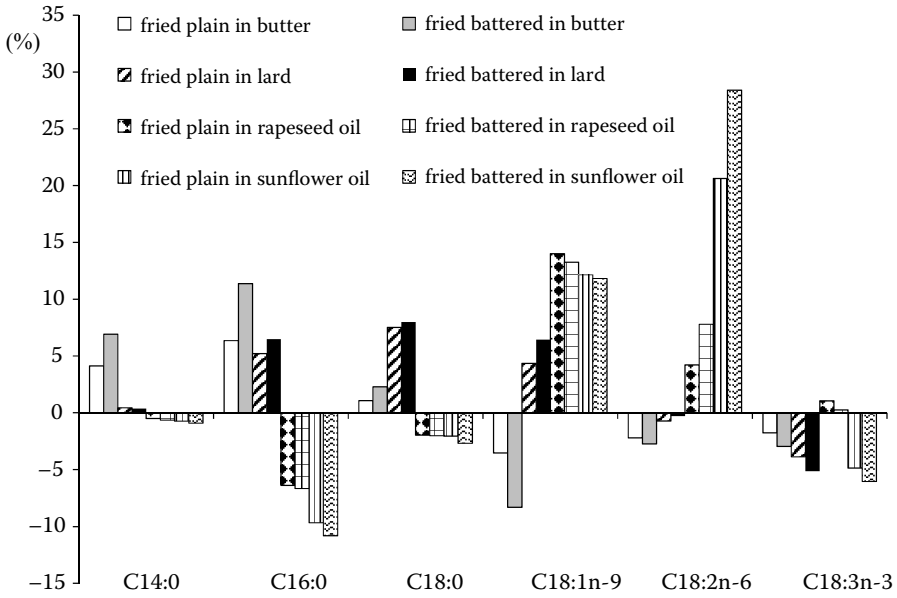

Figure 1. Changes in selected fatty acid percentages in plain or battered carp fillets (n = 3 in each treatment), due to frying in different fats

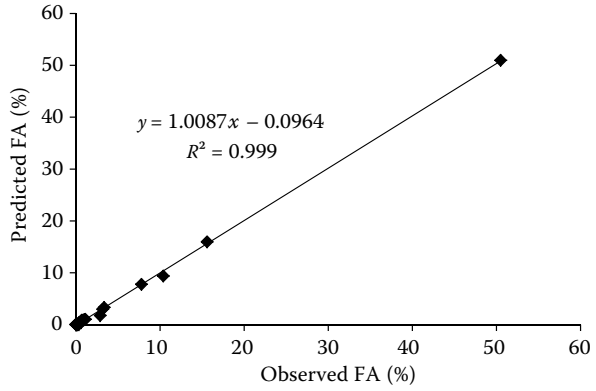

Figure 2. Prediction plot of fillet fatty acid composition (%) according to the dilution model; showing observed versus predicted percentages of fatty acids

ter interfered with the TBA solution leading to a yellowish colour, making the comparison with the standard impossible.

DISCUSSION

In the present study, the main aim was to investigate the effects of pan frying on the nutritional value of carp fillets. The fact that the carp fillets had an enriched content of n-3 PUFA compared to the fillets from normal production created an additional necessity to investigate the effect of frying on this special product.

Total lipid content. During frying, the lipid content in the fillets increased to approximately twice the amount when the fillets were fried plain and up to threefold amount when the fillets were battered. An increase of the lipid content in fish fillets during the preparation has been reported earlier by various researchers with salmon, cod, herring and other fish species (Mai *et al.* 1978; Gladyshev *et al.* 2006, 2007; Sioen *et al.* 2006; Weber *et al.* 2008). An even higher increase of the lipid content was found during frying with or without breading in bluegill (*Lepomis macrochirus*) and white sucker (*Catostomus commersoni*) fillets, where the lipid content increased nearly 10 times when fried with breading and fourfold in the white sucker when fried without breading (Mai *et al.* 1978). In the same study, the increase of the lipid content in lake trout (*Savelinus namaycush*), however, was not as drastic (from 8.8% to 9.5%), which probably can be ascribed to the higher lipid content in the trout fillets.

Nevertheless, the increase of the lipid content in the fillets in our study was independent of the fat source. This is in line with earlier results reporting a significant increase in the lipid content in silver catfish (*Rhamdia quelen*) due to panfrying while no variance was found in the lipid content inrelation to frying with different oils (Weber *et al.* 2008). When comparing the different species, it is obvious that in general the lipid content in lean fish like cod increased to a much higher percentage than in fatty fish. Even earlier it was concluded that lean fish take up more fat and the lipid changes are greater compared to more fatty fish (Mai *et al.* 1978).

Consequently, the lipid uptake in fish fillets during pan-frying seems to be negatively correlated with the raw fish fat content. This hypothesis is strengthened by the fact that the fat content in the catfish fillets increased more compared to our carp fillets (Weber *et al.* 2008). In our trial, the plain fried carp had the fat content from 9.5–11.9% while the reported fat content in the fried catfish was 13–14% (Weber *et al.* 2008).

Fatty acid composition. Several significant changes in the FA composition in the fillets were found after frying. For rapeseed oil, the highest increase was found for 18:1n-9. It was discussed that 18:1n-9 is a FA easily taken up as it has a higher viscosity compared to the more unsaturated FA and a higher adherence to the material to be fried (Kalogeropoulos *et al.* 2007). The same authors showed an increased uptake of 18:1n-9 in pan-fried potatoes. Also in catfish an increased uptake of 18:1n-9 from canola oil was found, which is in line with our findings (Weber *et al.* 2008). However, in the fillets fried with lard, it can be observed in the present study that the SFA seem to have an even stronger effect as the lard we used had 18:1n-9 as the major FA but the main increasing FA was 18:0, of which there was only 17.4% compared to 35.7% of 18:1n-9.

In addition, the increase in 18:0 in the fillets fried with lard was relatively higher than that in 16:0 despite the higher content of 16:0 in lard. This could be due to either the higher liquidity of 18:0 and hence an easier uptake into the fillet or the higher content of 16:0 in the raw fillets, as their ratio of these FA in the fillets was approaching to the ratio in lard (16:0/18:0 from 4.3 to 2.0 in the raw and fried fillets, respectively, compared to 0.8 in lard). Furthermore we found a significant increase of 14:0 and a minor increase of 18:0 in the samples fried with butter, despite the fact that the proportion of 18:1n-9 was higher in butter than the proportion of SFA. This would strengthen the hypothesis that the FA are taken up into the fish fillets depending on the FA composition both of the fat used and of the raw fillet

in a way that the final FA composition in the fried fish reflects the FA proportion in the used oil. This might be influenced in addition by the lipid content of the raw fillets with lean fish facilitating a higher uptake of lipid compared with fatty fish.

This hypothesis is supported by the results showing an increase in 18:3n-3 in catfish when frying in canola oil in opposite to our results with carp (Weber *et al.* 2008). This can be explained by the fact that the raw catfish fillets had a lower total fat content and a lower percentage of 18:3n-3 (2.5 and 1.4%, respectively) compared to our carp (3.1–5.1 and 5.9–8.5%, respectively), hence leading to an increased overall uptake of fat and also to a relatively increased proportion of 18:3n-3 reflecting the greater discrepancy in these FA between oil and fish.

Also in the study on shallow- and deep-fat frying of silver carp from Iran the FA composition in the fried fillets reflected the FA composition of the frying fats to a great extent (Rahimabadi & Dad 2012). However, as these authors did not report the total fat content before and after frying, it is difficult to estimate the influence of the fat content.

The significant decrease of EPA, DPA, and DHA in all frying treatments independent of the fat used depends most probably on the fact that these FA were not present in the frying fats and hence could not be taken up. As our results are expressed in proportions of FA and not in the absolute amounts, the decrease is derived only relatively and consequentially from the increase of the major FA from the frying fats.

Concerning the nutritional value of fish in general and especially of our n-3 enriched carp fillets in the present study, it can be stated that frying with lard and sunflower oil resulted in a significant decrease of the n-3/n-6 ratio, while the ratio was stable when the fillets were fried with butter or rapeseed oil. However, the desaturase systems for the metabolism of the parental n-3 and n-6 FA ALA and LA are the same for both n-3 and n-6 FA (De Henauw *et al.* 2007; Palmquist 2009). Even though delta 6 desaturase has a higher affinity for ALA than for LA, due to the much higher dietary intake the n-6 PUFA have been suggested to limit the conversion of LA to eicosapentaenoic acid = 20:5 n-3 (EPA) and DHA) (Palmquist 2009). Thus especially the use of lard and sunflower oil decreased the nutritional value of the fish by changing the n-3/n-6 ratio, as the higher intake of n-6 will shift the human metabolic pathways of n-3 and n-6 FA more towards the n-6 products, which are connected to increased inflammation and platelet aggregation (Simopoulos 2002b). For this reason, a balanced intake of n-6 and n-3 FA is important. This makes butter and rapeseed oil better candidates for fish frying fats. However, as frying with butter resulted in increased SFA in the fillets, the use of butter will also decrease the nutritional value of fish. SFA are also well-known for their negative effects on human health, such as, for example, increased blood cholesterol and prevalence of coronary heart disease (Williams 2000). This general conclusion has been discussed more recently as it is not valid for stearic acid, for example, and has not been well investigated for the medium chain FA (Grundy 1997). However, it is true for palmitic acid 16:0 which was the major FA increased after frying with butter. Following this rationale, as concerns the final FA composition of the fried fish fillets, rapeseed oil showed to be the best choice, preserving the n-3/n-6 ratio in the fillets without increasing the content of SFA at the same time.

Oxidation. Unexpectedly, rapeseed oil proved to have a similar good stability against oxidation as butter. In general, oxidation of oil is positively correlated to the number of double bonds from 18:2n-6 and 18:3n-3 and negatively correlated with 18:1n-9 (Kamal-Eldin 2006). Therefore the question arises if the use of an oil rich in 18:3n-3 will lead to increased oxidation in the final product. In our present study, we could not find an increased oxidation with rapeseed oil compared to sunflower oil (Table 3). Considering the conclusions drawn by Kamal Eldin, this could be explained by the high proportion of 18:1n-9 in the rapeseed oil used in the present study (Kamal-Eldin 2006). As this was 60%, it could have protected the rapeseed oil during frying as it has been

Table 3. TBARS in raw and plain fried pieces from carp fillets (n = 3 in each treatment) fried in different oils (μg malondioaldehyd/g fish; average ± SD)

	Butter	Lard	Rapeseed oil	Dunflower oil
Raw	0.37 ± 0.05	0.61 ± 0.49^{A}	0.44 ± 0.21	0.42 ± 0.31^{A}
Fried plain	0.58 ± 0.22^{a}	2.03 ± 0.55^{Bb}	0.97 ± 0.23^{ac}	1.42 ± 0.40^{Bc}

Different capital letters in a column indicate significant differences ($P < 0.5$) between raw and fried fish (n =12); different small letters in a row indicate significant difference between fillets fried in different fats

shown that 18:1n-9 could act as an inert (Romero & Morton 1977). Also other researchers showed an increased stability of canola, peanut and sunflower oils with increased proportions of 18:1n-9 (O'Keefe *et al.* 1993; Martí-Polvillo *et al.* 1996; Petukhov *et al.* 1999). Consequently, we hypothesise that the high percentage of 18:1n-9 in the rapeseed oil compared to the used sunflower oil and the other fats leads to the good oxidative stability in comparison to the other fats.

The significant increase of oxidation after frying with lard was unexpected as the lard was very rich in SFA that are relatively stable during oxidation. One reason could be that the lard was more oxidised from the beginning due to the processing procedure, which includes heating. Our results of MDA contents suggest that lard was the most oxidised among the fats used in the present trial. However, as described above, the measurement was heavily influenced by the high background absorbance, which we did not manage to extinguish. Further investigation is needed in this area.

CONCLUSIONS

We conclude that frying n-3 rich carp fillets with rapeseed oil preserved the favourable n-3/n-6 ratio without increasing the saturated fats content. It seems that FA uptake in fish is negatively correlated with the raw fish fat content and positively correlated with the FA composition in the frying fat used. Comparing our results with earlier studies in the literature, we expect similar changes of FA composition in other fish species with similar fat contents. However, the concrete mechanisms for the uptake and leakage of FA into and from fish fillets need to be investigated additionally, in order to be able to give recommendations to the public and official institutions dealing with human nutrition for the appropriate frying fat for different species. The frying with rapeseed oil did not increase oxidation compared to frying with sunflower oil, which confirms the suitability of rapeseed oil for pan frying of fish.

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Intake of Carp Meat From Two Aquaculture Production Systems Aimed at Secondary Prevention of Ischemic Heart Disease – a Follow-up Study

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Summary

Our previous study showed that a diet enriched with 400 g of carp per week improved plasma lipids in subjects after aortocoronary bypass (CABG). The aim of the present study is to determine whether the different carp farming systems have an impact on the effects of carp meat in secondary cardiovascular prevention. We examined 3 groups of patients after CABG over a 4-week period of spa treatment (108 persons, 73 males, 35 females, age over 60 years). We found no differences in baseline values of blood pressure or plasma lipids. The patients were given a standard spa diet (controls; N=36) or a diet enriched of 400 g of carp meat per week, enriched omega-3 (N=37) or cereal carp (N=35). Plasma lipid parameters were examined at start and after 4 weeks in a routine laboratory setting. Group consuming omega-3 carp showed the largest decline in total cholesterol, LDL cholesterol, triglycerides and an increase in HDL cholesterol (all $p<0.01$). We found that carp meat from the two production systems showed significantly different effects on plasma lipids. Further trials should be performed to clarify the exact causes of the differences.

Key words

Cardiovascular health • Carp • Fish oil • n-3 PUFA • Nutrition

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Introduction

Fish intake has been associated with lower risk of total and cardiovascular disease mortality in many, but not all studies (Villegas *et al.* 2015). Most of the health benefits of fish consumption are usually attributed to the high content of omega-3 long-chain polyunsaturated fatty acids (n-3 LC PUFA), especially eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), of which fish is the major dietary source (Balk *et al.* 2006). Animal and human studies have demonstrated that n-3 LC PUFAs improve the function of the normal and damaged endothelium, by an increasing nitric oxide availability and the metabolic pathways of cytochrome P450 epoxygenases. These epoxides cause potent vasodilatation and blood pressure reduction. Additionally, the antioxidant, anti-inflammatory, and anti-thrombotic properties of omega-3 fatty acids improve the stabilization of the electrophysiological properties of cardiomyocytes (Colussi *et al.* 2017). Most health authorities recommend two servings of fish per week as part of a healthy balanced diet. The European

Food Safety Authority (EFSA) Scientific Panel on Contaminants in the Food Chain state: "There is evidence that fish consumption, especially of fatty fish (one to two servings a week) benefits the cardiovascular system and is suitable for secondary prevention in manifested coronary heart disease" (EFSA 2005). The EFSA Panel on Dietetic Products, Nutrition and Allergies has set the following Adequate Intake values: linoleic acid (LA) 4 E% (percentage of total dietary energy per day), alpha linolenic acid (ALA) 0.5 E%; EPA+DHA, adults 250 mg; DHA, children 6-24 months 100 mg; pregnancy and lactation, an additional 100-200 mg of DHA (EFSA 2010).

Despite recommendations, fish consumption in Central Europe remains very low, e.g. in the Czech Republic it amounts to only 5.5 kg of fish and fish products per capita per year (Zeniskova and Gall 2009, ÚZEI 2016). Compared to marine fish, freshwater fish which usually contain a lower content of n-3 LC PUFA, are often overlooked as a source (Ackman 2002). Most observation studies and clinical trials have been realized using marine fish or fish oil, but not many articles related to freshwater fish and cardiovascular health are available. Fish also contains other potentially important nutrients such as taurine, selenium and astaxanthin which are understood to displace less healthy components in the diet (Radcliffe *et al.* 2016). In the Lugalawa study, Pauletto *et al.* (1996) compared a group of Tanzanian villagers on a high fish consumption diet (300-600 g daily) with a group on a vegetarian diet. The fish eaters were found to have significantly lower blood pressure and plasma lipid concentrations.

In our previous study (Adamkova *et al.* 2011) we showed that a diet enriched with 2 meals of carp (200 g each portion) per week significantly improved plasma lipids and markers of inflammation (high-sensitive CRP) in the blood of subjects after cardiac revascularization surgery for ischemic heart disease over a four-week period of follow-up spa treatment. The carp meat used in the study had a relatively low amount of n-3 LC PUFA in relation to the high effects observed on plasma lipid parameters, which led us to the hypothesis that it may not have been just lipid quality that was responsible for the effects observed.

Moreover, carp meat fat content and fatty acid composition are highly variable (Mraz and Pickova 2011) and influenced by farming systems (Mraz *et al.* 2012a), nutrition (Mraz *et al.* 2012b), purging (Zajic *et al.* 2013), processing (Sampels *et al.* 2015), cooking (Sampels *et al.* 2014) and other factors. Recently, a patented system (Mraz *et al.* 2011) for the aquaculture production of "omega-3 carp" (carp with increased content of n-3 FA) was developed and successfully tested. This type of omega-3 carp is already available on the market in the Czech Republic and sold under a specific trademark (Fig. 1). However, so far no study has compared the effects of carp meat from different production systems (cereal supplementation x pelleted feed) on cardiovascular prevention.

Fig. 1. Commercial trademark used in the Czech Republic for omega-3 carp (carp with increased content of n-3 FA).

Therefore, the aim of the present study was to confirm the results from our previous trial (Adamkova *et al.* 2011) and to examine whether carp farming systems would have an impact on carp meat health effects as a means towards post-surgery secondary cardiovascular prevention.

Methods

Carps production

The carps used for the intervention study were raised under two different pond production systems by the company Blatenská ryba, s.r.o. (i.e. Blatná Fish, Ltd., South Bohemian Region, Czech Republic). The first group was cultured with a traditional pond culture using supplemental cereal feeding (cereal carp). The other group was produced using the company´s patented technology (Mraz *et al.* 2011) of pond production of carp with increased content of n-3 fatty acids (omega-3 carp). Both groups were cultured in earthen dam fish ponds (2-3 ha) during the period April-September, 2010,

(stocked with 3-year-old carps with an average weight of 1 kg; stocking density 670 individuals/ha) and supplemented three times a week with either triticale (*Triticosecale*) (cereal carp) or special feed KP Len (Výroba krmiv, s.r.o., Stříbrné hory, Czech Republic) containing 12 % rapeseed cake and 20 % extruded linseed (omega-3 carp). Fish were harvested in September (with an average weight of 2.3 kg), purged in small purging ponds for 14 days, filleted, vacuum packed, frozen and delivered to Spa Poděbrady (Czech Republic) for the intervention study.

Ten fillets per group were randomly selected for chemical analysis of carp meat quality. Lipid content and fatty acid composition of carp fillets were analyzed as described in Mraz and Pickova (2009). Briefly, carp fillets were homogenized and 1 g of the aliquot sample used for lipid extraction using the hexane/isopropanol method (Hara and Radin 1978). Fatty acids were methylated (Appelqvist 1968) and analyzed by gas chromatography (Fredriksson Eriksson and Pickova 2007). Fatty acids were identified by comparison using the standard mixture GLC-68A (Nu-Check Prep, Elysian, MN) and retention times. For calculating the absolute amount of individual fatty acids, an internal standard of 15-methylheptadecanoate (Larodan Fine Chemicals AB, Malmö, Sweden) was used.

Intervention study

One hundred and eight patients after aortocoronary bypass in secondary prevention were included. None of these patients smoked during treatment (spa treatment after aortocoronary bypass) and none of the patients enrolled into this study had prescribed a special diet. The patients with verified diabetes mellitus type 2 were excluded. All were medicated with statins, beta-blockers and acetylsalicylic acid by their ambulatory cardiologists. The post-surgery patients underwent recovery for four weeks in the Spa Poděbrady (Czech Republic). During spa treatment, the patients consumed a standard spa diet for patients after cardiac surgery (controls) or a standard diet enriched with meals prepared from 2 x 200 g of carp meat per week using either "omega-3 carp" or "cereal carp". There were no differences in baseline values among the three groups (sex, age, blood pressure, lipid parameters, heart rate and body mass index) (Table 2). Laboratory tests for lipid parameters were performed at the start and at the end of the period. Total cholesterol and triglycerides were determined in fasting blood samples using an enzymatic method (Hoffmann-La Roche, Switzerland). High-density lipoprotein cholesterol concentrations were analyzed (Cobas Mira Plus, Roche, Switzerland) after precipitation of apolipoprotein B-containing particles using the phosphotungstate method. Low density lipoprotein cholesterol (LDL-C) was measured by a direct method using a commercially available kit (Roche Diagnostics, Basel, Switzerland). Blood pressure measurements were taken on the right forearm in the sitting position after at least ten minutes of rest. Three blood pressure readings were obtained, and the mean value of the final last two measurements was used for further analysis.

Statistical analysis

Data were analyzed using the paired t-test, the two-sample t-test, ANOVA and multivariate linear regression analysis. All data are expressed as means ± standard deviations. A p-level <0.05 was considered statistically significant. Statistical analyses were performed without regard to gender, because of our previous studies we know that gender played no role in influencing these monitored parameters.

Results

Carp meat analyses

The carp used in the intervention study had a very distinct meat quality (Table 1). Analysis revealed significantly higher amounts of PUFA and n-3 LC PUFA in omega-3 carp meat, while the cereal carp meat contained a higher proportion of MUFA (mostly oleic acid; 18:1n-9). Moreover, omega-3 carp had a content of beneficial EPA+DHA five times higher than that of cereal carp (53 vs. 262 mg of EPA+DHA per 100 g fillet).

Intervention study

The basic parameters of the study probands at the start of this trial are shown in Table 2.

Changes in the plasma lipid profiles of subjects after 4 weeks of intervention are shown in Table 3. The best results were found in group consuming omega-3 carp, which recorded the highest HDL cholesterol values and the largest decline in total cholesterol, LDL-C and triglycerides. We found no differences in fasting glucose, systolic/diastolic blood pressure or body mass index after spa treatment among the three groups. The lipid parameters after 4 weeks spa-treatment are shown in Table 4.

Five years after the surgery events, all subjects were reassessed using questionnaire survey. Thirty-one

persons responded from group consuming omega-3 carp, 29 from group consuming cereal carp and only 2 from control group. There were no cases of myocardial infarction. In the intervening 5 years, 4 people from groups consuming carps were hospitalized for deteriorated blood pressure. All patients attended regular check-ups. Twenty eight individuals continued to consume 200 g of freshwater fish twice a week.

Table 1. Fillet lipid content and fatty acid composition (% and mg/100 g) of cereal supplemented and omega-3 carp used for the intervention (mean ± SD; n=6).

	Cereal carp %	**Omega-3 carp %**	**Cereal carp mg/100 g**	**Omega-3 carp mg/100 g**
Lipids	6.8 ± 2.1	6.2 ± 2.0	6788 ± 2057	6197 ± 1974
14:0	1.1 ± 0.1	1.4 ± 0.3**	63 ± 17	73 ± 23
15:0	0.1 ± 0.1	0.3 ± 0.2***	4 ± 3	15 ± 6**
16:0	19.4 ± 1.1	18.5 ± 1.1	1128 ± 326	975 ± 300
18:0	6.5 ± 0.6	4.8 ± 0.8**	373 ± 105	254 ± 83
16:1n-7	9.5 ± 1.8	9.3 ± 1.1	569 ± 210	496 ± 172
18:1n-9	48.5 ± 2.8	32.3 ± 8**	2805 ± 819	1757 ± 650*
18:1n-7	3.4 ± 0.2	3.7 ± 0.3	197 ± 56	195 ± 60
20:1n-9	1.6 ± 0.3	1.7 ± 0.3	91 ± 17	92 ± 33
18:2n-6	5.7 ± 0.6	11.8 ± 1.1***	317 ± 63	622 ± 189**
20:2n-6	0.2 ± 0.1	0.3 ± 0.1	9 ± 4	15 ± 5
20:3n-6	0.2 ± 0.1	0.3 ± 0.0**	11 ± 1	16 ± 4*
20:4n-6	0.7 ± 0.3	1.3 ± 0.5*	38 ± 10	66 ± 17**
18:3n-3	0.9 ± 0.2	5.2 ± 1.7***	50 ± 18	262 ± 92***
18:4n-3	0.6 ± 0.1	1.3 ± 0.5*	36 ± 11	66 ± 32
20:5n-3	0.4 ± 0.2	3.2 ± 1.7**	20 ± 11	152 ± 58***
22:5n-3	0.3 ± 0.1	1.0 ± 0.5**	14 ± 4	48 ± 16***
22:6n-3	0.6 ± 0.2	2.4 ± 1.5*	34 ± 4	110 ± 35***
SFA	27.1 ± 0.8	25.4 ± 1.0*	1568 ± 439	1338 ± 401
MUFA	63.3 ± 1.7	47.3 ± 7.1***	3667 ± 1074	2554 ± 860
PUFA	9.6 ± 1.4	27.3 ± 6.4***	534 ± 99	1375 ± 389***
n-6	6.9 ± 1.0	139 ± 1.2***	381 ± 66	726 ± 210**
n-3	2.7 ± 0.5	13.4 ± 5.8**	153 ± 40	649 ± 231***
n-3 LC PUFA	1.3 ± 0.4	6.9 ± 3.8**	67 ± 17	322 ± 115***
EPA+DHA	1.0 ± 0.3	5.6 ± 3.1**	53 ± 13	262 ± 91***

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC PUFA, long chain polyunsaturated fatty acids (20 and more carbons); EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3); *, **, *** – statistically significant difference between the two groups at $p<0.05$; $p<0.01$; $p<0.001$, respectively, independent two-sample Student's t-test.

Discussion

To our best knowledge, our study is the first work aimed at investigating the impact of common carp farming systems on secondary cardiovascular prevention effects.

The results show that consumption of common carp meat with increased content of n-3 LC PUFA has positive effects on plasma lipids in subjects recovering from heart surgery. Therefore, it indicates that carp consumption would be beneficial in general. Fish consumption is generally very low in the Czech Republic (Zeniskova and Gall 2009, ÚZEI 2016) and far below current recommendations. This suggests that it would be beneficial to boost fish consumption in general as well as to increase the content of beneficial fatty acids in locally produced fish and associated products.

Table 2. Basic proband parameters at beginning of the study.

	Omega-3 carp group	Cereal carp group	Controls	p
N	37	35	36	
Males/females	26/11	25/10	22/14	
TC (mmol/l)	5.2 ± 0.43	5.0 ± 0.69	5.2 ± 0.61	n.s.
LDL-C (mmol/l)	2.7 ± 0.46	2.6 ± 0.88	2.5 ± 0.53	n.s.
HDL-C (mmol/l)	0.9 ± 0.21	0.8 ± 0.20	0.9 ± 0.22	n.s.
TG (mmol/l)	2.0 ± 0.57	2.0 ± 0.65	1.7 ± 0.51	n.s.
Glucose (mmol/l)	6.1 ± 0.95	6.1 ± 0.78	6.1 ± 0.97	n.s
SBP (mm Hg)	129.1 ± 10.36	130.2 ± 9.84	130.8 ± 7.65	n.s.
DBP (mm Hg)	80.3 ± 4.26	81.1 ± 4.98	83.2 ± 6.49	n.s.
HR (min)	70.3 ± 4.67	69.9 ± 4.92	70.4 ± 5.02	n.s.
BMI (kg/m²)	27.5 ± 6.38	27.6 ± 7.01	28.0 ± 7.92	n.s.

Controls – standard spa diet for patients after cardiac surgery; cereal carp group – standard diet enriched by cereal carp; omega-3 carp group – standard diet enriched by omega-3 carp. TC, total cholesterol; LDL-C, low density cholesterol; HDL-C, high density cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; BMI, body mass index.

Table 3. Plasma lipid changes in three studied groups of patients after 4 weeks of dietary intervention.

	Omega-3 carp group	Cereal carp group	Controls
N	37	35	36
Total C (mmol/l)	-1.3 ± 0.75***	-0.2 ± 0.97	0.1 ± 0.49
LDL-C (mmol/l)	-0.6 ± 0.37***	-0.1 ± 0.86	0.1 ± 0.22
HDL-C (mmol/l)	0.3 ± 0.16**	0.1 ± 0.24	-0.1 ± 0.18
TG (mmol/l)	-0.5 ± 0.51**	-0.1 ± 0.80	0.4 ± 0.52

Controls – standard spa diet for patients after cardiac surgery; cereal carp group – standard diet enriched by cereal carp; omega-3 carp group – standard diet enriched by omega-3 carp. TC, total cholesterol; LDL-C, low density cholesterol; HDL-C, high density cholesterol; TG, triglycerides. Significantly different from both cereal carp group and controls: ** $p<0.01$, *** $p<0.001$.

Table 4. Plasma lipids of three studied groups of patients after 4 weeks of dietary intervention.

	Omega-3 carp group	Cereal carp group	Controls
N	37	35	36
TC (mmol/l)	3.9 ± 0.63**	4.8 ± 1.0	5.4 ± 0.61
HDL-C (mmol/l)	1.2 ± 0.19**	1.0 ±0.23	0.9 ± 0.16
LDL-C (mmol/l)	2.0 ± 0.49**	2.5 ± 1.0	2.6 ± 0.60
TG (mmol/l)	1.5 ± 0.41*	2.0 ± 0.81	2.1± 0.60

Controls – standard spa diet for patients after cardiac surgery; cereal carp group – standard diet enriched by cereal carp; omega-3 carp group – standard diet enriched by omega-3 carp. TC, total cholesterol; LDL-C, low density cholesterol; HDL-C, high density cholesterol; TG, triglycerides. Significantly different from both cereal carp group and controls: * $p<0.05$, ** $p<0.01$.

The improvement of plasma lipid parameters (a decrease in total cholesterol, LDL cholesterol and triglycerols, and an increase in HDL cholesterol) resulting from the consumption of carp meat, observed by Adamkova *et al.* (2011) and in the current study is in agreement with published studies on sea fish (Balk *et al.* 2006). Fish proteins have been shown to have several positive effects on the different disorders and parameters

of metabolic syndrome. Among the most important findings observed are anti-inflammatory effects (Calder 2015), increased insulin sensitivity and prevention of type 2 diabetes mellitus (T2DM) and obesity (Lavigne *et al.* 2001). Cod protein has been shown to inhibit the development of obesity-linked insulin resistance and glucose intolerance in mice (Lavigne *et al.* 2000, Lavigne *et al.* 2001). Similar effects have been observed in human trials (von Post-Skagegård *et al.* 2006, Ouellet *et al.* 2007, Ouellet *et al.* 2008).

However, the mechanisms underlying these effects are to be investigated yet. One of the possible explanations may be high levels of specific amino acids, which contain low levels of branched amino acids, such as taurine and arginine. Calcitonin salmon, the bioactive part of fish protein, has been shown to inhibit osteoporosis (Chesnut *et al.* 2008) and is a homologue of amylin, a hormone involved in the regulation of satiation and energy expenditure (Osaka *et al.* 2008).

Pilon *et al.* (2011) compared the effects of protein in different fish species (bonito, herring, mackerel and salmon) when included in a high-fat, high-sucrose diet fed to rats. They found that, compared with a casein diet, protein from all fish species exhibited anti-inflammatory action through tumor necrosis factor-α and interleukin-6. In addition, the group fed salmon protein had lower weight gain and reduced fat in epididymal white adipose tissue. This suggests that there might be specific effects linked to protein from different fish species. The bioavailability of fatty acids might also differ depending on lipid structure and food matrix (Mu 2008, Schram *et al.* 2007). The final effect may also symbiotically contribute to other substances in fish.

Recently, there have been many controversies concerning the effects of fatty acids (including omega-3), because inversely risk-related, yet randomized large multicentre trials have not supported this direct relationship (Harris *et al.* 2016). In one large prospective cohort study of healthy women, intake of tuna, dark fish and marine omega-3 fatty acids was not associated with the risk of major CVD (Rhee *et al.* 2017). The National Heart Foundation of Australia recommended that Australian adults should consume 500 mg of omega-3 fatty acids per day for the primary prevention of cardiovascular diseases and 1000 mg of omega-3 fatty acids for the secondary prevention of CVD (Nestel *et al.* 2015).

The median fish intake at 27 g/day in all probands showed an inverse association between total fish intake and total mortality among all probands while the association between intake of fish and total mortality was not significant among participants with chronic disease (Villegas *et al.* 2015). Our probands were given a daily median intake of about 57 g of fish (400 g per week), but only freshwater carp. We have not found any valid trial or study that has focused on carp.

Some recent trials show neutral findings as concerns the effect of long chain fatty acids on the cardiovascular disease event risk. These results may be possibly attributable to e.g. aggressive cardiovascular drug treatment or overshadowing the benefits of these fatty acids (Rice *et al.* 2016). The current recommendation of American Heart Association for CVD secondary prevention (2011) state that "it may be reasonable to recommend omega-3 fatty acids from fish or fish oil (1 g/day)" (Kleber *et al.* 2016), but no trials have confirm any significant benefit from intake of these products for reduction of clinical endpoints, while low plasma concentrations of omega-3 fatty acids precede the development of congestive heart failure (Kleber *et al.* 2016). As of yet, we have been unable to compare these data with data from our groups. For CVD risk, meta-analyses of randomized controlled trials indicate a significant triglyceride-lowering effect for fish and fish oil. Indeed, recent studies of type 2 diabetics indicate that fish and fish oil may improve endothelial function. Some of the heterogeneity in the results pertaining to fish and fish oil in diabetes may be explained by substantial variation in the experimental designs used, including the selection of study populations, the amount of fish, and fish oil administered, as well as the continually improving standards in healthcare (Ward and Hintze 2016). Compared to the Danish population living in Denmark, diabetes mellitus was essentially unknown among the Inuits (Radcliffe *et al.* 2016) inhabiting Greenland which forms an autonomous part of the Kingdom of Denmark.

Omega-3 PUFA can attenuate the immune system response of T cells and macrophages through hitherto unidentified cell surfaces receptors, perhaps by changing the composition of membrane micro-domains, since a recent cross-sectional study on Danish children shows a positive association of mean arterial blood pressure with whole blood DHA (only in boys) (Bonafini *et al.* 2015). The beneficial effects of 1800 mg/day of EPA on CVD risk reduction may relate in part to the lowering of Lp-PLA_2 without adversely affecting LDL-C. In contrast, DHA decreases postprandial TG, but raises

LDL-C. Other observations indicate that these dietary fatty acids have divergent effects on cardiovascular risk markers (Asztalos *et al.* 2016). Data and analysis were compiled for a prospective cohort study of U.S. women participating in the Women's Health Study (1993-2014). As part of the analysis, the Cox proportional hazards model was used to evaluate the association between fish and energy-adjusted omega-3 polyunsaturated fatty acid intake and the risk of major cardiovascular disease, defined as the composite outcome of myocardial infarction, stroke, and cardiovascular death, in 38.392 women in the final (i.e. 96 %) analytic sample. There was no modification of effect in age, BMI or baseline history of hypertension differences. In another study a group of women without history of cardiovascular disease, intake of tuna and dark fish, α-linolenic acid, and marine omega-3 fatty acids were not associated with the risk of major cardiovascular disease (Rhee *et al.* 2017).

Another study on normotensive subjects with highest dietary consumption of omega-3 fatty acids had a risk of hypertension development lower by 27 % as compared with subjects with the lowest intake (Colussi *et al.* 2017).

Patients after myocardial infarction that consumed 1 g/day of omega-3 PUFA for 3.5 years reduced their mortality rate by 20 % and sudden death rate by 45 % as compared to placebo-administered patients (Harris *et al.* 2016). Our follow up study is rather short to confirm these results.

Over recent years, a number of observational and intervention studies that have tried to determine the possible cardiovascular protective effects of omega-3 fatty acids have reported negative findings. However, despite these reports the use of fish oil is beneficial for the prevention of sudden death after myocardial infarction, the publication reports an association between supplementation with omega-3 polyunsaturated acids and higher mortality. But while significant results have been obtained regarding the cardioprotective effect of omega-3 PUFA in native Japanese and Alaskan populations, in other population (Americans) the same effect has not been demonstrated (Colussi *et al.* 2017). A recent report showed the relationship between hypertriglyceridemia and cardiovascular disease, since elevated triglycerides can promote atherogenesis. However, only high-risk patients treated with omega-3 PUFA showed a significant decrease in carotid-femoral pulse wave velocity (Casanova *et al.* 2016).

It is clear, that further research focused on the intake of polyunsaturated acids and their influence on cardiovascular diseases, comorbidities and mortality must be performed in order to explain their effects.

Conclusion

In conclusion, we show that carp meat has positive effects on plasma lipids in the secondary prevention of ischemic heart disease. Furthermore, the significance of these effects is strongly influenced by the carp production system. Cardioprotective effects were obtained with the omega-3 carp, which contains five times the amount of EPA+DHA than that of traditional cereal carp. We believe that carp can be of major importance in combating metabolic disorders in many populations in central continental regions without sea access, as carp can be produced world-wide in large quantities. However, more studies – both animal studies and intervention studies on human subjects – are needed to confirm this.

Conflict of Interest

M.J., P.J. and K.P. are only authors of the Czech Patent nr. 302744, for production of the omega-3 enriched carp. The owner of the patent is the University of South Bohemia in Ceske Budejovice.

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Příloha č. 8

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The consumption of the carp meat and plasma lipids in secondary prevention in the heart ischemic disease patients

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Abstract

OBJECTIVES: Omega-3 fatty acids (FA) have been shown to be protective against cardiovascular diseases (CVD). The effect of the consumption of carp meat on CVD risk factors has not yet been examined in detail. We ascertained the influence of a diet enriched with carp meat with an elevated content of omega-3FA (200 g twice weekly for 4 weeks) in a group of subjects after cardiac revascularization surgery for ischemic heart disease with a follow-up spa treatment.

DESIGN: After cardiac revascularization surgery, the probands consumed either a standard spa diet (56 individuals, 41 males, 15 females, age 41–80 years) or a diet enriched with two portions of carp meat (87 individuals, 64 males, 23 females, age 50–82 years). The differences in body mass index (kg/m^2), blood pressure, plasma lipids and C-reactive protein (CRP) of the groups were analyzed.

RESULTS: In the group with a higher consumption of carp meat, significantly greater improvements in lipid parameters in comparison to the standard spa diet were detected (total cholesterol $p<0.001$, triglycerides $p<0.001$, LDL-C $p<0.001$, CRP $p<0.001$, HDL-C $p<0.001$). No differences between these groups in blood pressure and body mass index were found.

CONCLUSION: We conclude that the diet enriched with carp meat significantly improved plasma lipid parameters in patients after major cardiac revascularization surgery.

Abbreviations:

BMI	- body mass index
CRP	- C reactive protein
CVD	- cardiovascular disease
DBP	- diastolic blood pressure
DHA	- docosahexaenoic acid
DPA	- docosapentaenoic acid
HDL-C	- high density lipoprotein cholesterol
LC PUFA	- long chain polyunsaturated fatty acids
LDL-C	- low density lipoprotein cholesterol
SBP	- systolic blood pressure
SFA	- saturated fatty acids
TC	- total cholesterol
TG	- triglycerides

ORIGINAL ARTICLE

INTRODUCTION

Cardiovascular diseases (CVD) cause the majority of the deaths of the adult inhabitants of the Czech Republic. Since Danish researchers discovered the low incidence of CVD in Greenland Eskimos (despite the fact that they consume most of their calories in fat) in 1980 (Cole *et al.* 2009), the intake of fish has been considered to be healthy. The relationship between fish consumption and the risk of CVD was confirmed in a large meta-analysis (Whelton *et al.* 2004), which found that each 20 g/day increase in fish intake was related to a 7% lower risk of CVD mortality.

Fish have been considered to be a healthy dietary product because they are high in protein and have a positive lipid composition (a low percentage of saturated fats) (Smith & Guentzel 2010). Fish fat contains essential omega-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic and docosahexaenoic acids, which are not synthesized in the human body. Nevertheless, their inclusion in the human diet is essential. Omega-3 long chain polyunsaturated fatty acids are important for maintaining the integrity of cell membranes, for promoting prostaglandin synthesis (which is essential for inflammation and blood clotting) and for regulating body cholesterol metabolism (Jabeen *et al.* 2010).

Fish and shellfish farming worldwide is currently the most rapidly growing type of food production. It is important that fish farming develop in a sustainable manner; the replacement of fish meals and fish oils in the aqua feeds used needs to be implemented on a large scale. There is a large potential for carp in the development of Central Europe as an aquacultural region (Kobler 2010). Carp is classified as an intermediate fish because its lipid content is 5–10% of its wet weight (Fontagné *et al.* 2001). However, carp has been interesting to nutritional specialists for a long time due to its relatively lower content of PUFA compared to sea fish. Carp has a beneficial fatty acid composition with regard to human health, and an increase in the consumption of this locally available freshwater fish is very important. In this study, we analyze the effect of regular consumption of carp meat enriched in PUFA on plasma lipids known to be CVD risk parameters. The carp was enriched in PUFA by a special feeding formula containing rapeseed oil (the source of the PUFA) (Mraz & Pickova 2009).

MATERIAL AND METHODS

High-risk adult (age >18 years) CVD patients were enrolled in this study after major cardiac revascularization surgery performed between 12/2009 and 10/2010 at the Institute for Clinical and Experimental Medicine in Prague. The patients were randomized into two groups and underwent a four-week follow-up spa treatment at Spa Poděbrady. All procedures were prescribed by physicians according to the individual requirements of the patients with the exception of the diet interventions; the other treatments within the spa protocol were not different between these two groups.

Dietary intervention was based on a substantial increase of carp meat in the intervention group (87 probands, 64 males), which was compared with the control group (56 probands, 41 males). The detailed characteristics of the individuals are included in the Table 1.

All participants were Caucasians from the Central European Czech population. Written informed consent was obtained from all study participants, and the local ethics committee approved the design of the study according to the Helsinki Declaration (1975).

The 200 g carp filets consumed twice weekly in the intervention group were prepared in vegetable oil from carp fed a special formula with a higher volume of polyunsaturated fatty acids (Mraz *et al.* 2010a) (n-3 PUFA content of 439±146 mg per 100 g of filet), under special supervision by nutrition specialists. This preparation method adhered to the recommendations for the secondary prevention of CVD. The total energy intake was equal in both groups.

Body mass index (kg/m^2) was calculated, and the changes in total cholesterol, low density cholesterol (LDL), high density cholesterol (HDL), triglycerides and C-reactive protein (CRP) were analyzed with routine laboratory tests. The laboratory tests were performed at the start of the diet and after 4 weeks of dietary treatment.

The data were analyzed using the paired t-test, the two-sample t-test, ANOVA and multivariate linear regression analysis. All data are expressed as means ± standard deviations. A p-level <0.05 was considered statistically significant.¨

RESULTS

The basal characteristics of the probands are summarized in Table 1. In general, plasma lipids improved significantly in the group of patients consuming higher amounts of carp compared to the control group. Briefly, total cholesterol decreased by 27% in the experimental group compared to a 2% decrease in the control group (p<0.001), LDL – cholesterol decreased by 26% in comparison to 4% (p<0.001), plasma TG decreased by 26% in comparison to 3% (p<0.001) and plasma HDL cholesterol increased by 30% in comparison to 10% (p<0.001). Detailed changes are summarized in Table 2. Importantly, no differences were found in the body mass indices between the analyzed groups.

DISCUSSION

We provide evidence that the consumption of carp meat containing an elevated content of long chain FAs (LC-PUFA) can be highly beneficial for the improvement of plasma lipids in a Central European population. According to our knowledge, this is the first report of

the effect of carp meat on improved health parameters and how it can substantially influence the recommendations of nutrition specialists. It is well known that herbivorous or omnivorous fish (for example, carp) have lower n-3 PUFAs than sea fish. The standard profile of carp meat in the Czech Republic is not known, but the fatty acid profile in freshwater fishes can be changed by diet (Guillon *et al.* 1995, Mraz *et al.* 2010b).

Traditionally, common carp is cultured by semi-intensive farming in ponds and is based on natural food supplemented with cereals. The lipid composition of filets from these carp is highly variable. There are no standard values for such fish because the lipid composition varies depending on many factors, e.g., fish size, breed, the amount of natural food available in the pond and farming intensity. However, in general, carp fed diets supplemented with cereals have higher lipid contents, high levels of monounsaturated fatty acids, lower levels of polyunsaturated fatty acids and EPA+DHA and a higher n-6/n-3 ratio compared with fish fed natural food only (Csengeri 1996) or with the carp used in our trial. In a previous trial, we found that filets from carp supplemented with cereals contained around 130 mg of n-3 PUFA and 45mg of EPA+DHA per 100 g of filet, with an n-6/n-3 ratio of 2.6 (unpublished results).

The improvement in the plasma lipid parameters (i.e., decreases in serum total cholesterol, low density cholesterol and triglycerides and an increase in high density cholesterol) after the consumption of carp meat in our study is in compliance with published studies on sea fish (Balk *et al.* 2006). The fasting plasma glucose concentrations did not differ significantly between the beginning and the end of the study period; similar results were described previously (Schwab *et al.* 2006). In addition, we have shown highly significant decreases in CRP as a result of the higher consumption of carp meat, probably due to the anti-inflammatory effect of omega 3 FA (Al-Khalifa *et al.* 2007). Importantly, BMI values were stable during the study, which indicates that both diets were isocaloric. These positive effects of omega 3 fatty acids from fish meat are well known, but the positive effect associated with the diet of freshwater fish has not previously been shown.

Because classically farmed carp has a relatively low content of omega3-PUFA compared to sea fish, it is advisable to farm the carp using a new feed blend with a high content of omega-3 FA in order to achieve the recommended values. These feed blends are based on replacing the traditional wheat supplementation (rich in n-6 PUFA) with substances rich in omega 3 PUFA. Previous studies reveal that carp fed with the new formula have an approximately seven times higher content of the protective DHA and DPA compounds (Mraz *et al.* 2010a) than classically farmed fish. In addition, the total levels of omega 6/omega 3 of the commonly farmed carp are about 2, compared to wild fish, which have a ratio less than 1. It was shown that a combination of linseed cake and rapeseed cake in the feed improves the n-3 levels in the cultured carp to levels similar to those in fish fed only plankton (Fontagné *et al.* 2001).

It is well known that carp originated in Asia and that carp account for the majority of all farmed freshwater fish worldwide (Kobler 2010). The importance of locally available freshwater fish in the Central European diet is based not only on the positive effects on plasma lipids but also on the possible positive effects of omega 3 FA on inflammation, psoriasis, aggression, and depression (De Lorgeril & Salen 2002).

We believe that the farmed carp fed the special diet could improve human health conditions because it is known that the lipid composition of farmed fish is more constant and has a beneficial lipid composition compared to wild fish (Cahen *et al.* 2004).

We conclude that the consumption of freshwater fish (carp meat enriched in omega 3 FA) improves lipid parameters and markers of the inflammatory process in patients after major cardiac revascularization surgery and has the potential to improve CVD morbidity.

Tab. 1. Basal characteristic of the groups (baseline data).

	Treated group	Control group	*p*-value
N	87	56	
Age	57.9 ± 10.3	57.3 ± 9.5	n.s.
Males/females	64/23	41/15	
Total C (mmol/l)	5.6 ± 0.6	5.4 ± 0.9	n.s.
LDL-C (mmol/l)	3.0 ± 0.8	2.9 ± 0.9	n.s.
HDL-C (mmol/l)	0.9 ± 0.2	0.9 ± 0.2	n.s.
TG (mmol/l)	2.2 ± 0.7	2.1 ± 0.7	n.s.
CRP (g/l)	33.5 ± 15.4	32.8 ± 14.9	n.s.
BMI (kg/m^2)	29.3 ± 4.6	29.1 ± 4.5	n.s.
Glycaemia (mmol/l)	6.7 ± 1.9	6.6 ± 1.9	n.s.
height (cm)	169.3 ± 9.1	169.2 ± 8.9	n.s.
weight (kg)	84.2 ± 14.2	84.3 ± 14.0	n.s.
SBP (mmHg)	136.5 ± 10.1	135.8 ± 11.3	n.s.
DBP (mm Hg)	87.2 ± 9.4	87.1 ± 10.9	n.s.
apo A1	0.9 ± 0.3	0.8 ± 0.3	n.s.

Tab. 2. Changes of the plasma lipids after intervention (difference final - baseline values).

	Treated	Controls	*p*-value
TC (mmol/l)	–1.50 ± 0.89	–0.21 ± 0.79	0.001
TG (mmol/l)	–0.6 ± 0.53	–0.07 ± 0.38	0.001
HDL C (mmol/l)	+0.25 ± 0.16	+0.07 ± 0.06	0.001
LDL C (mmol/l)	–0.82 ± 0.56	+0.03 ± 0.04	0.001
Glucose (mmol/l)	–2.3 ± 1.8	–1.9 ± 0.8	0.05
CRP (g/l)	–26.3 ± 13.0	–21. ± 11.6	0.01

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