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# ANALÝZA RŮSTU A JEJÍ UPLATNĚNÍ PŘI SELEKCI SKOTU MASNÉHO UŽITKOVÉHO TYPU

## GROWTH ANALYSIS AND ITS APPLICATION TO SELECTION OF CATTLE OF COMMERCIAL MEAT TYPE

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**ABSTRACT:** Based on the values of Richards growth function, parameters of so called linear phase of inflection, a time segment around the growth curve inflection point, were derived in young cattle of some breeds for the age of animals when their live weight is linearly increasing. This phase is described by a time segment, change in live weight and increment of the linear phase of inflection (maximum increment)  $k^*$  expressed by the equation:  $k^* = (y_2^* - y_1^*) / (t_2^* - t_1^*)$ ; it is also the value of the coefficient of linear regression on weight at the time around inflection. Suitability of criteria derived on the basis of Richards growth function was tested in a set of calves of some beef breeds when live weight at birth and at the age of 120 days and 210 days was recorded as a part of performance testing of beef cattle. Data were processed on a set of 664 heifers and 344 young bulls of the breeds Aberdeen Angus (G), Piemontese (P), Blonde d'Aquitaine (Q), Meat Simmental (S), Charolais (T), Hereford (U) and Limousine (Y). The age of heifers at the linear phase of inflection ( $t_1^*$ ) is different at a significance level  $P = 0.01$ . Based on the age when this value is reached, the breeds can be divided into early ones (Q, S, T and P reaching  $t^*$  at the respective age of 24.21, 25.35, 28.01 and 29.43 days and live weights of 65.72, 60.74, 67.42 and 62.75 kg) and late ones (U and G with the linear phase of inflection at the respective age of 40.79 and 45.89 days and live weights of 63.90 and 70.27 kg). Highly significant differences were recorded between heifers of the breeds concerned in the duration of linear phase and in the age of this growth phase termination. The shortest duration was determined in breed S (176.9 days) and the longest in breeds U and P (262.9 and 296.6 days, respectively) at total live weight gains of 191.48, 202.52 and 199.46 kg. There were significant differences (significance of differences at a level  $P = 0.01$ ) between the young bull sets in the age when the linear phase of inflection was reached. Sets Y, P, T and Q can be classified as "early" breeds with the respective age of 48.11, 51.67, 52.63 and 52.94 days and live weights 84.08, 89.62, 96.55 and 95.46 kg. Set U with the age of 69.12 days and live weight 90.38 kg is included in the group of "late" breeds. Duration of the linear phase of inflection was also significant ( $P = 0.01$ ). It was shortest in set Y – 207.28 days with total live weight gain 265.62 kg, and significantly longer in sets U, P and Q with total duration of 253.21, 254.33 and 257 days, respectively, when total weight gains of 286.8, 283.01 and 301.87 kg were reached. The values of the coefficient of regression on live weight in time were different in the groups of heifers and young bulls at the linear phase of inflection ( $k^*$ ), which also indicates average daily weight gain ( $P = 0.01$ ). The lowest weight gain was recorded in heifers of groups P and U (0.747 and 0.862 kg) at the live weight reached at the age  $y^* = 262.22$  and 266.43 kg. Groups G and T had high weight gains while group S had the highest weight gain of all: the respective values  $k^* = 1.129, 1.140$  and  $1.219$  kg. The sets of young bulls had the lowest weight gains also in breeds P and U (1.224 and 1.238 kg). The highest weight gain was determined in groups T and S (1.491 and 1.557 kg). The values of live weight growth calculated from flexible Richards growth function indicate high interbreed variability with significant differences ( $P = 0.05$  and  $P = 0.01$ ). The earlier the onset of the linear phase of inflection, which is characterized by the highest average daily weight gain, the sooner the required live weight is reached. Therefore the value  $t_1^*$ , age of heifers and bulls entering the linear phase of inflection, is considered as an important selection criterion along with the duration of this phase and value  $k^*$ , average weight gain reached in this phase.

**Keywords:** cattle; growth; Richards function; linear phase of inflection

**ABSTRAKT:** Na základě hodnot Richardsovy růstové funkce byly pro mladý skot vybraných plemen odvozeny parametry tzv. lineární fáze inflexe, úseku v okolí inflexního bodu růstové křivky, pro věk zvířat, kdy se živá hmotnost lineárně zvyšuje. Tato fáze je vyjádřena délkou časového úseku, změnou hmotnosti a přírůstkem lineární fáze inflexe (maximální přírůstek)  $k^*$  vyplývající ze vztahu  $k^* = (y_2^* - y_1^*) / (t_2^* - t_1^*)$ , který je i hodnotou koeficientu lineární regrese hmotnosti v čase kolem inflexe. Rozdíly ve věku jaloviček jsou při dosažení lineární fáze inflexe ( $t_1^*$ ) rozdílné s průkazností na hranici  $P = 0,01$ . Na základě věku, kdy sledovaná plemena dosahují této hodnoty, je můžeme rozdělit na skupinu plemen raných (plavé aquitánské, masné simentálské, charolais a piemontese) dosahujících  $t_1^*$  ve 24,21, 25,35, 28,01, resp. 29,43 dnech věku při živé hmotnosti 65,72, 60,47, 67,42, resp. 62,75 kg a plemen pozdních (hereford a aberdeen angus), která dosahují lineární fáze inflexe ve věku 40,79, resp. 45,89 dnů při živé hmotnosti 63,90, resp. 70,27 kg. Rovněž délka lineární fáze a věk ukončení této fáze růstu

byly pro jalovičky uvedených plemen rozdílné s vysokou průkazností. Věk dosažení lineární fáze inflexe byl i mezi soubory býčků významně rozdílný, s průkazností rozdílu na hladině  $P = 0,01$ . Významně průkazný ( $P = 0,01$ ) byl i rozdíl doby trvání lineární fáze inflexe. V práci jsou uvedeny i hodnoty regresního koeficientu živé hmotnosti v čase v době lineární fáze inflexe ( $k^*$ ). Časnější dosažení požadované hmotnosti souvisí s ranějším nástupem lineární fáze inflexe, která je charakteristická nejvyšším průměrným denním přírůstkem. Proto hodnotu  $t^*$ , věk jalovic a býčků, kdy vchází do lineární fáze inflexe, spolu s délkou této fáze a hodnotou  $k^*$ , velikostí průměrného přírůstku, který je v ní dosahován, považujeme za významná selekční kritéria.

**Klíčová slova:** skot; růst; Richardsova funkce; lineární fáze inflexe

## ÚVOD

Růstová křivka, vyjádřená změnami živé hmotnosti v čase, by měla být základem pro selekční a hybridizační programy u všech masných typů hospodářských zvířat. U skotu masného užitkového typu je kontrola užitkovosti v ČR založena na ověření růstové schopnosti telat, a to na základě jejich živé hmotnosti při narození, ve věku 120 dnů, dále v rozmezí 180 až 240 dnů věku (tj. při odstavu) a ve věku jednoho roku (Golda, 1997). Růstová schopnost mladého skotu je vyjadřována porážkovou hmotností dosaženou bez nadměrného ukládání tuku.

Parnell *et al.* (1997) odvodili genetické parametry růstu, selekční zisk aj. použitím divergentní selekce na vyšší přírůstek živé hmotnosti telat plemene angus. Na základě živé hmotnosti a přírůstků Tozser *et al.* (1995) zpracovali selekční model růstu pro plemeno limousine. Arthur *et al.* (1997) u téhož materiálu uvádějí korelace mezi přírůstkem jednoletých telat a živou hmotností při narození a vybranými rozměry těla. Bennet a Gregory (1996) stanovili genetické i fenotypové korelace pro živou hmotnost a přírůstek živé hmotnosti u dvanácti genotypů skotu. Slipka *et al.* (1995) uvádějí korelace pro živou hmotnost jalovic tří genotypů pro věk 6, 9 a 18 měsíců. Green *et al.* (1988) sledovali u vybrané skupiny dojníc vnitřní rozměry pánve ve vztahu k věku a k plemeni. Zjistili průkazné korelace, mající význam při selekci na snadné porody. Baker *et al.* (1988) analyzovali vztah mezi růstovou rychlostí a věkem dosažení pohlavní dospělosti u skotu. Vedle růstu živé hmotnosti sledovali i růst výšky v kříži. Prokázali, že rychlost růstu tohoto rozměru kladně koreluje nejen se zvyšováním živé hmotnosti, ale i s věkem při dosažení pohlavní dospělosti. Růstovou rychlost rozměru výšky v kříži považují za významný zdroj genetické proměnlivosti i proto, že její hodnoty jsou méně ovlivňovány prostředím, než je tomu u živé hmotnosti. Rovněž Heinrichs *et al.* (1992) doporučili vedle živé hmotnosti sledovat i soubor vybraných rozměrů; uvádějí výšku v kohoutku, šířku zádě v kyčlích a délku těla. Tyto ukazatele odrážejí růst skeletu a jsou méně ovlivňovány kondicí zvířat a stupněm nasazení tuku.

Význam sledování růstu vybraných rozměrů spolu s růstem živé hmotnosti potvrdili Kratochvílová *et al.* (1996). Při hodnocení vztahu růstu živé hmotnosti k rozměrům telat černostrakatého skotu ve věku 15 mě-

síců neprokázali pro první období po narození telat těsnější a významnější korelace.

Vhodnost rovnic popisujících růst byla analyzována řadou autorů. Richards (1959), Laird (1966), Fitzhugh (1976), Šiler *et al.* (1981), Hyánek *et al.* (1981) a další diskutovali tvar růstových křivek v souvislosti s tzv. antagonistickou selekcí, tj. s preferencí jedinců s vysokou hodnotou růstové intenzity v autoakcelerační fázi a s nízkou hodnotou asymptoty u jedince v dospělosti. Gompertzovu růstovou funkci využil Meyer (1995) pro odhad genetických parametrů a korelací živé hmotnosti a rozměrů (délky holeně a výšky zádi) u dvou genotypů skotu. Souza a Bianchini-Sobrinho (1994) použili při analýze růstu skotu čtyři nelineární růstové funkce – Bertalanffyho, algebraickou Woodsovu funkci, lineární hyperbolický model a kvadratický logaritmický model.

Selekce na tvar růstové křivky a na její parametry umožňuje specifikovat požadovaný chovný cíl řadou dalších hodnot. Při známé změně tvaru růstové křivky lze odhadnout velikost změny jatečné hmotnosti a s tím korelující změny skladby jatečného těla, jak uvádí Keane (1994). Lopez de Torre *et al.* (1996) na základě exponenciální růstové funkce, Gompertzovy, logistické, Bertalanffyho a hyperbolické funkce zpracovali modelové růstové křivky vybraných masných plemen.

Většina použitých matematických funkcí má charakter logistické křivky s pevným bodem inflexe, což vyznačuje rozsah použitelnosti při hodnocení vývinu zvířat. Z tohoto hlediska růstové křivky Bertalanffyho i logistická jsou méně vhodné pro hodnocení růstu živých organismů. Poloha inflexního bodu (tvarový parametr  $= y^*/A$ ) je u první křivky dána hodnotou 0,296 a u druhé 0,500. I když autoři Lopez de Torre *et al.* (1996) funkci Bertalanffyho považují za nejpřesnější pro zhodnocení růstu živé hmotnosti u jalovic ve věku od 8 do 97 měsíců, vhodnější je Gompertzova funkce s tvarovým parametrem 0,368, kterému se blíží např. průběh růstu živé hmotnosti. Na nepřesnosti hodnocení průběhu růstu touto funkcí upozornili Mikšík *et al.* (1988). Rovněž Perotto *et al.* (1992) poukázali na nepřesnost některých růstových funkcí (monomolekulární, logistická a Gompertzova funkce) při hodnocení průběhu růstu živé hmotnosti a za nejvhodnější považují růstovou funkci Richardsovu.

Čtyřparametrová funkce Richardsova zahrnuje oproti ostatním běžně používaným funkcím ještě tvarový

parametr, který určuje polohu inflexního bodu na růstové křivce z hlediska hmotnosti (tj. poměr  $y^*/A$ ), což odráží biologickou variabilitu tohoto znaku. Ostatní tříparametrové modely mohou odhadovat polohu inflexního bodu pouze z hlediska času (tj. věku zvířat) a vypočítat pak příslušnou hmotnost v tomto bodu ( $y^*$ ), ale poměr  $y^*/A$  je fixní, korelace mezi  $y^*$  a  $A$  je rovna 1.

Podrobnou analýzu této růstové funkce provedl Fitzhugh (1976) a Nath a Moore (1992) ji doplňují o využití první derivace (absolutní a relativní rychlost růstu) a druhé či třetí derivace, kterými lze stanovit délku exponenciální a lineární fáze růstu.

Nevýhodou použití této funkce je vyšší pracnost při zadávání hodnot, na což upozornili např. Kim *et al.* (1996) při hodnocení růstu místní populace skotu. Obtížný je odhad hodnoty horní asymptoty, což někteří autoři obcházejí použitím asymptoty dolní, tj. velikosti hodnoty při narození.

Cílem této práce bylo upozornit na možnost využití jako selekčního kritéria při šlechtění skotu masného užitkového typu hodnot, které lze stanovit z růstové křivky odvozené na základě čtyřparametrové Richardsovy funkce.

## MATERIÁL A METODA

Analýza vhodnosti vybraných ukazatelů pro selekci u masného typu skotu byla provedena u hodnot, které lze stanovit na základě růstové funkce podle Richardse, uvedené v základním tvaru:

$$y_t = A(1 - b e^{-kt})^{1/n} \quad \text{pro } n < 0, A \text{ a } k > 0,$$

kde parametry odhadnuté metodou nejmenších čtverců vyjadřují:

$y_t$  – hmotnost / rozměr těla ve věku  $t$  (ve dnech)

$n$  – 280 dnů věku (počet, kdy  $y_0 = 0$  kg)

$A$  – hodnota horní asymptoty (průměrná hmotnost v dospělosti)

$b$  – časový parametr měřítka, integrační konstanta

$k$  – hodnota růstové intenzity (relativní růstová rychlost dosažení  $A$ )

$n$  – tvarový parametr, který určuje polohu inflexního bodu

a odvozené hodnoty:

– živá hmotnost / rozměr ve věku dosažení inflexe

$$y^* = A / (n + 1)^{1/n}$$

– věk dosažení inflexe

$$t^* = -1/k \cdot \ln \ln b/l$$

Pro každého jedince byly na základě uvedených hodnot růstové křivky odvozeny parametry lineární fáze inflexe, úseku v okolí inflexního bodu růstové křivky, který se vyznačuje lineárním růstem hmotnosti v čase s odchylkou do 5 % koeficientu růstové intenzity této lineární fáze.

Tato fáze je vyjádřena:

1. hodnotou délky časového úseku =  $t_2^* - t_1^*$

2. změnou hmotnosti / rozměru =  $y_2^* - y_1^*$

kde:  $t_2^*, y_2^*$  – věk / rozměr při ukončení lineární fáze inflexe

$t_1^*, y_1^*$  – věk / rozměr počátku lineární fáze inflexe

3. přírůstkem lineární fáze inflexe (maximální přírůstek)  $k^*$  vyplývajícím ze vztahu:

$k^* = (y_2^* - y_1^*) / (t_2^* - t_1^*)$ , který je i hodnotou koeficientu lineární regrese hmotnosti / rozměru v čase.

Analýzou rozptylu a následným mnohonásobným porovnáním metodou LSD byla testována průkaznost rozdílů stanovených hodnot. Stejným písmenem jsou označeny skupiny statisticky průkazně odlišné.

## Experimentální materiál

Ověření vhodnosti kritérií odvozených na základě Richardsovy růstové funkce bylo provedeno v souboru telat vybraných masných plemen, kdy v rámci kontroly užitkovosti masného skotu byla sledována živá hmotnost, s vážením jedinců při narození, ve věku 120 dnů a ve věku 210 dnů. Pro sledování byl získán soubor 664 jaloviček a 344 býčků plemen aberdeen angus (G), piemontese (P), plavé aquitánské (Q), masný simental (S), charolais (T), hereford (U) a limousine (Y).

V tomto souboru byla hodnocena růstová křivka živé hmotnosti a hodnoty z ní odvozené. Data o růstu živé hmotnosti byla získána péčí Českého svazu chovatelů masného skotu z výsledků kontroly užitkovosti v letech 1996 až 1997.

## VÝSLEDKY

Hodnoty růstové křivky podle Richardse charakterizující růst živé hmotnosti telat jsou uvedeny v tab. 1 a 2. Průměrná hodnota horní asymptoty u jaloviček sledovaných plemen kolísá s rozpětím hodnot od 411,8 kg u plemene S do 477,7 kg u plemene G. U býčků byla nejnižší průměrná hodnota horní asymptoty u plemene Y – 570,6 kg a nejvyšší u plemene S – 699,7 kg. Rozdíly mezi plemeny v rámci pohlaví jsou průkazné na hladině  $P = 0,05$ .

Významná průkaznost rozdílů byla zjištěna pro hodnoty koeficientu růstové intenzity ( $k$ ) u jaloviček. Plemenná skupina S s hodnotou koeficientu  $k = 0,00827$  byla významně rozdílná oproti všem dalším souborům jaloviček a skupiny P a U oproti pěti dalším souborům. U býčků byly rozdíly hodnoty  $k$  mezi soubory průkazné, avšak s menší četností rozdílů.

Tvarový parametr ( $n$ ) byl průkazně rozdílný jen mezi býčků souboru G a T na hladině  $P = 0,01$ .

Věk jalovic při dosažení bodu inflexe růstu živé hmotnosti, počátek a konec lineární fáze inflexe a délka této fáze jsou uvedeny v tab. 3, jejich živá hmotnost dosažená v inflexním bodě křivky, počáteční a konečná živá hmotnost této fáze spolu s celkovým přírůstkem v tab. 4. Hodnoty věku jsou v souborech pro všechny ukazatele rozdílné s vysokou průkazností. Nejdříve ze sledovaných souborů / plemen dosahuje hodnoty inflexe skupina S – ve věku 108,9 dnů při živé hmotnosti 150,5 kg. Pozdními jsou v této hodnotě plemena U a P

Tab. 1. Hodnoty Richardsovy růstové funkce pro růst živé hmotnosti jaloviček – The values of Richards growth function for live weight growth in a set of heifers

Skupina <sup>1</sup>	n	A				k				n			
		$\bar{x}$ kg	$s_x$	průkaznost <sup>2</sup>		$\bar{x}$	$s_x$	průkaznost		$\bar{x}$	$s_x$	průkaznost	
				F	LSD			F	LSD			F	LSD
G	63	477,6	54,02		aBC	0,00645	0,00103		ABC	-0,0113	0,00323		
P	15	427,9	118,68		a	0,00494	0,00150		ADEFg	-0,0115	0,00316		
Q	19	447,1	67,80			0,00654	0,00168		DHi	-0,0131	0,00245		
S	20	411,8	84,15	+	Bde	0,00827	0,00218	++	BEHJKL	-0,0130	0,00109	-	
T	175	458,5	83,97		df	0,00693	0,00193		FMJ	-0,0120	0,00312		
U	77	434,8	115,80		Cf	0,00558	0,00163		CiKMN	-0,0115	0,00372		
Y	26	468,6	42,74		e	0,00635	0,00117		gLN	-0,0118	0,00153		
Celkem <sup>3</sup>	664	450,4	92,05			0,00656	0,00185			-0,0117	0,00358		

a... m, n – průkazný rozdíl – significant difference ( $P < 0,05$ )

A... M, N – průkazný rozdíl significant difference ( $P < 0,01$ )

<sup>1</sup>group, <sup>2</sup>significance, <sup>3</sup>total

Tab. 2. Hodnoty Richardsovy růstové funkce pro růst živé hmotnosti býčků – The values of Richards growth function for live weight growth in a set of young bulls

Skupina <sup>1</sup>	n	A				k				n			
		$\bar{x}$ kg	$s_x$	průkaznost <sup>2</sup>		$\bar{x}$	$s_x$	průkaznost		$\bar{x}$	$s_x$	průkaznost	
				F	LSD			F	LSD			F	LSD
G	74	642,0	113,33		a	0,00620	0,00121		a	-0,00824	0,00396		A
P	9	607,9	128,78			0,00570	0,00157		b	-0,01023	0,00219		
Q	17	648,3	159,95			0,00558	0,00130		cD	-0,00974	0,00249		
S	16	699,7	78,77	+	bC	0,00609	0,00092	+		-0,00925	0,00191	+	
T	99	655,2	131,75		de	0,00634	0,00135		cE	-0,00964	0,00286		A
U	67	613,9	151,44		bd	0,00574	0,00158		aEF	-0,00910	0,00261		
Y	18	570,6	92,93		aCe	0,00683	0,00121		bDF	-0,00912	0,00369		
Celkem <sup>3</sup>	344	632,5	131,26			0,00617	0,00138			-0,00916	0,00320		

a... e, f – průkazný rozdíl – significant difference ( $P < 0,05$ )

A... E, F – průkazný rozdíl – significant difference ( $P < 0,01$ )

<sup>1</sup>group, <sup>2</sup>significance, <sup>3</sup>total

s věkem 164,4, resp. 167,6 dnů při živé hmotnosti 159,1 kg, resp. 156,5 kg.

I rozdíly ve věku dosažení lineární fáze inflexe ( $t_1^*$ ) jsou rozdílné s průkazností na hranici  $P = 0,01$ . Na základě věku, kdy sledovaná plemena dosahují tuto hodnotu, je můžeme rozdělit na skupinu plemen raných (Q, S, T a P,  $t_1^* = 24,2, 25,3, 28,0$  a  $29,4$  dnů věku při živé hmotnosti  $y_2^* = 65,7, 60,4, 67,4$  a  $62,7$  kg) a plemen pozdních (U a G, která dosahují lineární fázi inflexe ve věku 40,7, resp. 45,8 dnů při živé hmotnosti 63,9, resp. 70,2 kg).

Rovněž délka lineární fáze a věk ukončení této fáze růstu byla pro jalovičky uvedených plemen rozdílná s vysokou průkazností. Nejkratší doba byla zjištěna u plemene S (176,9 dnů) a nejdelší u plemen U a P (262,9, resp. 296,6 dnů) při celkovém přírůstku živé hmotnosti 191,5, 202,5, resp. 199,4 kg.

Živá hmotnost, kterou soubory jaloviček dosáhly ve věku inflexe, je rozdílná (tab. 4), celková variabilita

živé hmotnosti je vcelku nízká s rozdílem 9,8 kg (mezi souborem jaloviček G a S).

Věk býčků při dosažení inflexního bodu křivky růstu živé hmotnosti a délka lineární fáze inflexe jsou uvedeny v tab. 5 a živá hmotnost býčků ve věku  $t_1^*$ ,  $t_1^*$  a  $t_2^*$  spolu s celkovým přírůstkem v lineární fázi inflexe jsou uvedeny v tab. 6.

Průměrný věk býčků v souborech v bodě inflexe živé hmotnosti byl 167,3 dnů věku. Rozdíly mezi skupinami byly průkazné oproti výrazně pozdnímu plemeni U, s věkem 188,7 dnů. Věk dosažení lineární fáze inflexe byl i mezi soubory býčků významně rozdílný, s průkazností rozdílů na hladině  $P = 0,01$ . K raným plemenům lze řadit soubory Y, P, T a Q, s hodnotou  $t_1^* = 48,1, 51,6, 52,6$  a  $52,9$  dnů s živou hmotností  $y_1^* = 84,1, 89,6, 96,5$  a  $95,5$  kg a do skupiny pozdních plemen pak soubor U s věkem 69,1 dnů při živé hmotnosti 90,4 kg. Významně průkazná ( $P = 0,01$ ) byla i doba trvání lineární fáze inflexe. Nejkratší byla zjištěna

Tab. 3. Věk jalovic při dosažení inflexe živé hmotnosti ( $t^*$ ) a délka lineárního úseku inflexe ( $t_2^* - t_1^*$ ) ve dnech – Heifer age at the time of live weight inflection ( $t^*$ ) and duration of the linear segment of inflection ( $t_2^* - t_1^*$ ) in days

Skupina <sup>1</sup>	$t^*$				$t_1^*$				$t_2^*$				$t_2^* - t_1^*$			
	$\bar{x}$	$s_x$	průkaznost <sup>2</sup>		$\bar{x}$	$s_x$	průkaznost		$\bar{x}$	$s_x$	průkaznost		$\bar{x}$	$s_x$	průkaznost	
			F	LSD			F	LSD			F	LSD			F	LSD
G	147,58	25,9825		ABc	45,89	14,7343		aBCD	261,36	43,0054		ABcD	215,48	35,3918		AbC
P	167,64	65,3548		DEFg	29,43	42,3860		a	324,13	101,9280		Aefgh	296,67	78,8051		ADEFg
Q	128,85	31,7870		DH	24,21	25,8211		BE	246,00	59,8712		EI	221,79	64,4503		DhI
S	108,92	21,8184	++	AEIj	25,35	8,8750	++	Cf	202,25	53,2362	++	BfIKe	176,90	55,3181	++	bEhjKl
T	127,53	38,6690		BFKL	28,01	19,7671		DG	239,36	69,6773		cGjM	211,83	63,6800		FjM
U	164,38	60,2080		cHIKL	40,79	36,9769		EfG	303,16	95,4359		DIKMN	262,90	74,9676		CIKMN
Y	139,30	23,7691		gIjL	34,85	10,9679			256,08	46,0694		Hen	221,23	44,9500		GIN
Celkem <sup>3</sup>	138,38	45,0247			33,15	26,1119			256,45	75,78350			223,65	64,8432		

a... m, n – průkazný rozdíl – significant difference ( $P < 0,05$ )

A... M, N – průkazný rozdíl – significant difference ( $P < 0,01$ )

<sup>1</sup>group, <sup>2</sup>significance, <sup>3</sup>total

Tab. 4. Živá hmotnost jalovic v inflexním bodě křivky růstu ( $y^*$ ) a přírůstek živé hmotnosti v lineárním úseku inflexe ( $y_2^* - y_1^*$ ) v kg – Live weight of heifers at the inflection point of growth curve ( $y^*$ ) and live weight gain in the linear segment of inflection ( $y_2^* - y_1^*$ ) in kg

Skupina <sup>1</sup>	$y^*$				$y_1^*$				$y_2^*$				$y_2^* - y_1^*$			
	$\bar{x}$	$s_x$	průkaznost <sup>2</sup>		$\bar{x}$	$s_x$	průkaznost		$\bar{x}$	$s_x$	průkaznost		$\bar{x}$	$s_x$	průkaznost	
			F	LSD			F	LSD			F	LSD			F	LSD
G	174,73	19,8287		aBC	70,27	7,9308		aBC	292,60	33,2066		aBC	222,33	25,2830		aBC
P	156,55	43,5140		a	62,75	17,4181		a	262,22	72,8912		a	199,46	55,4742		a
Q	163,43	24,8316			65,72	9,8980			273,78	41,6278			208,06	31,7351		
S	150,51	30,7801	+	Bde	60,47	12,4087	+	Bde	251,96	51,6428	+	Bde	191,48	39,2390	+	Bde
T	167,70	30,7479		df	67,42	12,2727		df	280,79	51,5276		df	213,36	39,2613		df
U	159,08	42,5040		Cf	63,90	17,1234		Cf	266,43	71,1172		Cf	202,52	53,9970		Cf
Y	171,37	15,6106		e	68,81	6,2155		e	287,03	26,1613		e	218,22	19,9536		e
Celkem <sup>3</sup>	164,74	33,7301			66,21	13,5128			275,86	56,5090			209,64	43,0010		

a... e, f – průkazný rozdíl – significant difference ( $P < 0,05$ )

A... E, F – průkazný rozdíl – significant difference ( $P < 0,01$ )

<sup>1</sup>group, <sup>2</sup>significance, <sup>3</sup>total

Tab. 5. Věk býčků při dosažení inflexe živé hmotnosti ( $t^*$ ) a délka lineárního úseku inflexe ( $t_2^* - t_1^*$ ) ve dnech – Young bull age at the time of live weight inflection ( $t^*$ ) and duration of the linear segment of inflection ( $t_2^* - t_1^*$ ) in days

Skupina <sup>1</sup>	$t^*$				$t_1^*$				$t_2^*$				$t_2^* - t_1^*$			
	$\bar{x}$	$s_x$	průkaznost <sup>2</sup>		$\bar{x}$	$s_x$	průkaznost		$\bar{x}$	$s_x$	průkaznost		$\bar{x}$	$s_x$	průkaznost	
			F	LSD			F	LSD			F	LSD			F	LSD
G	169,08	38,6230		Ab	61,61	21,0699		aBc	289,19	61,2830		A	227,58	45,4626		aB
P	171,52	46,8877			51,67	16,0000		d	306,00	82,5621			254,33	68,1322		C
Q	174,31	48,0751			52,94	24,5063		E	309,94	80,5237		b	257,00	65,5553		aEd
S	168,40	27,5655	+		60,81	13,4473	++		288,75	45,0947	++		227,94	34,0362	++	
T	158,27	40,7622		C	52,63	20,8569		BF	276,40	65,5236		C	223,77	48,9264		dF
U	188,70	55,3998		ACD	69,12	28,5086		aEFdG	322,33	88,2331		ACD	253,21	64,6076		BFG
Y	146,04	36,6020		bD	48,11	13,2571		cG	255,39	64,7894		bD	207,28	54,3678		CEG
Celkem <sup>3</sup>	167,32	44,5908			57,82	22,9286			289,70	72,3024			231,87	55,1726		

a... f, g – průkazný rozdíl – significant difference ( $P < 0,05$ )

A... F, G – průkazný rozdíl – significant difference ( $P < 0,01$ )

<sup>1</sup>group, <sup>2</sup>significance, <sup>3</sup>total

Tab. 6. Živá hmotnost býčků v inflexním bodě křivky růstu ( $y^*$ ) a přírůstek živé hmotnosti v lineárním úseku inflexe ( $y_2^* - y_1^*$ ) v kg – Live weight of young bulls at the inflection point of growth curve ( $y^*$ ) and live weight gain in the linear segment of inflection ( $y_2^* - y_1^*$ ) in kg

Skupina <sup>1</sup>	$y^*$				$y_1^*$				$y_2^*$				$y_2^* - y_1^*$			
	$\bar{x}$	$s_x$	průkaznost <sup>2</sup>		$\bar{x}$	$s_x$	průkaznost		$\bar{x}$	$s_x$	průkaznost		$\bar{x}$	$s_x$	průkaznost	
			F	LSD			F	LSD			F	LSD			F	LSD
G	235,21	41,5569		a	94,65	16,6667		a	393,64	69,6328		a	298,99	52,9731		a
P	222,50	47,0481			89,62	18,5658			372,64	78,8353			283,01	60,2711		
Q	237,35	58,5859			95,46	23,5581			397,33	97,9696			301,87	74,4150		
S	256,20	28,7536	+	bC	103,03	11,6457	+	bC	428,97	48,2930	+	bC	325,94	36,6522	+	bC
T	239,91	48,2751		de	96,55	19,3133		de	401,62	80,9282		de	305,08	61,6200		de
U	224,84	55,4936		bd	90,38	22,2111		bd	376,38	92,9696		bd	286,00	70,7614		bd
Y	209,00	34,1181		aeC	84,08	13,6237		aCe	349,70	57,2115		aCe	265,62	43,5932		aCe
Celkem <sup>3</sup>	231,62	48,0942			93,17	19,2701			387,71	80,5879			294,54	61,3227		

a... d, e – průkazný rozdíl – significant difference ( $P < 0,05$ )

A... D, E – průkazný rozdíl – significant difference ( $P < 0,01$ )

<sup>1</sup>group, <sup>2</sup>significance, <sup>3</sup>total

Tab. 7. Hodnota regresního koeficientu ( $k^*$ ) lineární fáze inflexe pro obě pohlaví (v kg) – The values of regression coefficient ( $k^*$ ) of the linear phase of inflection for both genders (in kg)

Skupina <sup>1</sup>	Jalovice <sup>2</sup>				Býčci <sup>3</sup>			
	$\bar{x}$	$s_x$	průkaznost <sup>4</sup>		$\bar{x}$	$s_x$	průkaznost	
			F	LSD			F	LSD
G	1,129	0,1147		AB	1,432	0,1746		AbcD
P	0,747	0,1754		ACDFE	1,224	0,1647		AEFg
Q	1,070	0,2638		CgH	1,305	0,3076		bHI
S	1,219	0,2514	++	DgIj	1,557	0,1815	++	eEHJk
T	1,140	0,2395		EK	1,491	0,2394		FIL
U	0,862	0,2204		BHIKL	1,238	0,1927		DJLM
Y	1,089	0,1501		FjL	1,410	0,1947		gkM
Celkem	1,058	0,2379			1,398	0,2428		

a... l, m – průkazný rozdíl – significant difference ( $P < 0,05$ )

A... L, M – průkazný rozdíl – significant difference ( $P < 0,01$ )

<sup>1</sup>group, <sup>2</sup>heifers, <sup>3</sup>bulls, <sup>4</sup>significance

u souboru Y – 207,3 dnů s celkovým přírůstkem živé hmotnosti 265,62 kg. Významně delší byla u souborů U, P a Q s celkovou dobou 253,2, 254,3, resp. 257,0 dnů a celkovým přírůstkem 286,0, 283,0, resp. 301,9 kg.

Hodnoty regresního koeficientu živé hmotnosti v čase v době lineární fáze inflexe ( $k^*$ ), který současně vyjadřuje i průměrný denní přírůstek živé hmotnosti lineární fáze, jsou uvedeny v tab. 7. Mezi hodnotami ve skupinách jaloviček i býčků byly zjištěny průkazné rozdíly, vesměs na hladině  $P = 0,01$ . Nejnižší přírůstek byl u jaloviček skupin P a U, 0,747 kg a 0,862 kg, při dosažené živé hmotnosti ve věku  $y^* = 262,2$  kg a 266,4 kg (tab. 5). Vysoký přírůstek živé hmotnosti byl ve skupinách G a T a nejvyšší u skupiny S, s hodnotami  $k^* = 1,129$ , 1,140, resp. 1,219 kg. I v souborech býčků byla zjištěna nejnižší hodnota přírůstku u skupin P a U – 1,224 kg a 1,238 kg. Nejvyšší přírůstek byl u skupiny T ( $k^* = 1,491$  kg) a S ( $k^* = 1,557$  kg).

## DISKUSE A ZÁVĚR

Při vytváření vhodného masného typu skotu, zejména při selekci vhodných genotypů, nepovažujeme za postačující hodnotit jen růst živé hmotnosti, tj. změnu hodnoty v čase, ale bude vhodné analyzovat i ukazatele, které mohou výstižněji charakterizovat průběh růstu. Tento názor potvrzují výsledky prací dalších autorů. Cunningham *et al.* (1987) hodnotili růst živé hmotnosti podle několika chovných schémat. Zjistili průkazné korelace mezi živou hmotností narozených telat a živou hmotností v jednom roce. Telata jednoho plemene po býčích selektovaných na vysokou živou hmotnost v jednom roce byla při narození průkazně těžší než telata kontrolní skupiny. Avšak ve skupině s rotační plemenitbou se zařazením plemene fríského skotu se při nevýznamném zvýšení počáteční živé hmotnosti zvýšila relativní růstová rychlost ( $k = 0,834$ , resp. 0,795)

spolu s průměrným denním přírůstkem (0,849, resp. 0,713 kg). Selekcí telat na živou hmotnost při odstavu se v populaci může zvyšovat hmotnost telat při narození, jak uvádějí Aaron *et al.* (1987) na základě sledování růstu živé hmotnosti u plemene santa gertrudis. Jak zjistili Antal *et al.* (1992), při selekci na vyšší přírůstek býčků pingavského skotu zaznamenali průkazné zvýšení hmotnosti vnitřního tuku i jeho procentuálního podílu ze živé hmotnosti. Herd (1995) tuto skutečnost potvrdil při divergentní selekci na výši přírůstku mladého skotu plemene angus do jednoho roku věku. Linie selektovaná na vysoký přírůstek měla vyšší podíl tuku ve tkáních a nižší podíl proteinu.

Využití horní asymptoty pro účely selekce zvířat považujeme za značně problematické, neboť její praktické ověření je dlouhodobou záležitostí s významnou variabilitou danou fyziologií zvířete i vlivy vnějšího prostředí. V uvedených souborech jsme zjistili významné rozdíly hodnoty horní asymptoty jen pro některé skupiny. Výsledky, ke kterým jsme dospěli, však mohou být zkresleny i proto, že v souboru byla poslední živá hmotnost zjišťována ve věku 210 dnů. Rovněž tvarový parametr  $n$  byl omezeným počtem vážení ovlivněn do té míry, že rozdíly mezi plemeny jsou vesměs neprůkazné a poměr  $y^*/A$  prakticky odpovídá tříparametrové funkci podle Gompertze.

Časový parametr inflexe ( $t^*$ ) považují Hyánek *et al.* (1981) pro zhodnocení průběhu růstu a tvaru růstové křivky za významný, neboť dává cennou informaci o stupni ranosti vývinu organismu, a lze doplnit, že tento ukazatel je výchozím bodem pro zjištění a hodnocení dalších znaků.

Cílem této práce nebylo hodnocení průběhu růstu jednotlivých plemen, i když výsledky jsou zajímavé, ale rozbor možnosti využití dalších hodnot, které lze na základě vážení v rámci kontroly užitkovosti získat. Hodnoty růstu živé hmotnosti zjištěné na základě flexibilní Richardsovy růstové funkce vykazují vysokou meziplennou variabilitu s rozdíly průkaznými na

hladině  $P = 0,05$  i  $0,01$ . Časnější dosažení požadované hmotnosti souvisí s ranějším nástupem lineární fáze inflexe, která je charakteristická nejvyšším průměrným denním přírůstkem. Proto hodnotu  $t_1^*$  – věk jalovic a býčků, kdy vchází do lineární fáze inflexe, považujeme za jedno z významných selekčních kritérií spolu s délkou této fáze a hodnotou  $k^*$ , velikostí průměrného přírůstku, který je v ní dosahován.

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# IMMUNOCYTOCHEMICAL DETECTION OF HSP70 IN CELL CULTURE OF PIG GRANULOSA

## IMUNOCYTOCHEMICKÁ DETEKCE HSP70 V BUNĚČNÉ KULTUŘE PRASEČÍ GRANULÓZY

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**ABSTRACT:** The current study was undertaken to investigate the presence of heat shock proteins (HSPs) in a monolayer culture of pig ovarian granulosa cells submitted to stress conditions, and to exactly define heat shock (43 °C) during 1, 2, 4, and 6 hours. For the detection of HSPs we used immunocytochemical reaction with a monoclonal antiHSP70 antibody. The adherent granulosa cells growing in the monolayer showed the strongest reaction after 4 and 6 hours' heat shock, especially when the culture was transported, after 6 hours of heat shock, into normal culture conditions (temperature of 37 °C). A very strong positive reaction was exceptionally concentrated within the nuclei. There was a substantially weak reaction within the cytoplasm. Reaction in the control culture cells was minimized and, after 1 hour of heat shock, the presence of HSPs was also very weak. The induction of HSPs into a culture of pig ovarian granulosa cells after heat shock presents a very interesting model for the study of cellular adaptive response to external stress.

**Keywords:** granulosa cells; culture; pig; HSP70; immunocytochemistry

**ABSTRAKT:** Cílem práce bylo zjistit dynamiku změn tepelně šokových proteinů (HSP70) v kultuře prasečích ovariálních granulózních buněk ve formě monolayerů po vystavení definovanému tepelnému šoku (teplotě 43 °C) na dobu 1, 2, 4 a 6 hodin. Adherující rostoucí buňky granulózy ukazovaly nejsilnější reakci HSP70 po tepelném šoku trvajícím 4 a 6 hodin a zejména tehdy, když kultura po šestihodinovém tepelném stresu byla ponechána jednu hodinu v normálních kultivačních podmínkách (37 °C). Pozitivní reakce značné intenzity byla soustředěna zejména v buněčných jádrech, cytoplazma jevila slabší intenzitu reakce. Reakce v buňkách kontrolní kultury byla nepatrná, rovněž po jednohodinovém tepelném šoku byla velmi slabá. Indukce šokových proteinů v kultuře buněk prasečí ovariální granulózy po tepelném šoku se ukazuje být dobrým modelem pro studium buněčné adaptační odpovědi na vnější stres.

**Klíčová slova:** buňky granulózy; kultivace; prase; HSP70; imunocytochemie

### INTRODUCTION

The maturation of oocytes, above all cytoplasmic maturation, influences the cells which are in immediate contact with the oocyte. They are the granulosa cells and *cumulus oophorus* cells. The possibility of influencing the process of maturation of oocytes for example, by cocultivation of complexes of oocytes surrounded by *cumulus oophorus* cells (abbreviation C-O-C) with granulosa cells (Thibault *et al.*, 1987) was indicated. Joint cultivation led to the inhibition of oocyte maturation (Sirard and Bilodeau, 1990 – in the cow; Sato and Ishibashi, 1977 – in the pig) and breakdown of the germinal vesicle did not occur.

During the past decade much progress was made in understanding the molecular, biochemical and cellular consequences of thermal stress (Lindquist, 1986). Nevertheless, the molecular mechanism of thermal-tolerance and the role of HSPs in thermal protection are far from being clear. Evidence of a positive correlation between HSPs, in particular HSP70, and thermal-tolerance is substantial. HSP70 synthesis has been shown in a variety of tissue culture cells, normal tissues and tumours following heat shock and also a positive correlation between HSPs and thermal-tolerance (Li *et al.*, 1995).

We concentrated our attention on the culture of granulosa cells to find how these cells react to heat shock. A good indicator could be heat shock proteins

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because it is known that these proteins are synthesized by cells, especially as a response to heat stress.

Heat shock proteins (HSPs) were first described in the *Drosophila* species (Ritossa, 1962), as a group of highly conservative proteins developed during the evolution of the species which are constitutively expressed or induced by stress.

We focused our attention on one group of stress proteins named HSP70, which contains a whole series of proteins where a numerical index indicates their molecular weight in kiloDaltons. The protein HSP70 is a major member of heat shock proteins and plays an important role in the protection and recovery of cells from stress-induced damage. These proteins act as molecular chaperones, all of which bind ATP *in vitro* (Becker and Craig, 1994). This family of HSP70 plays a unique role during the folding and translocation of newly synthesized proteins. They bind to nascent and denatured proteins. They serve as molecular chaperones, and import proteins into the mitochondria and endoplasmic reticulum.

Hightower (1980) states in his study that excessive intracellular accumulation of abnormally founded proteins initiates the formation of HSPs. The onset of the increased presence of HSPs is probably really connected with the need of the traumatized cell to eliminate denatured damaged proteins. During the last decade research leading to an understanding of the molecular, biochemical and cellular consequences of heat stress has advanced considerably (review Lindquist, 1986).

It must be emphasized that stress proteins also play a certain role in the cell cycle during the process of differentiation and possibly dedifferentiation, because these processes are accompanied by the synthesis of some of them (Chaloupka, 1994). Moreover, it is known that the degree of expression is strongly related to cellular metabolic activity. An example could be the excessive accumulation of some HSPs within the quickly dividing and growing cells as compared with their quiet state, or within the active secretory cells (Welch, 1992). And just such cell types are the follicular granulosa cells, which are, on the one hand, rapidly increasing through mitotic division and, on the other hand, they are secretory elements. Through their secretion activity they are involved in the formation of follicular fluid (*liquor folliculi*). It was also for that reason we chose to investigate granulosa cell culture because it appeared to be a good model for research into the dynamism of the formation of HSPs after heat treatment (stress) precisely defined over several time intervals.

Stress causes ultrastructural changes in the cell, the membrane structures, membrane organelles, the cytoskeleton, ribosomes and the nucleus being particularly affected. After heat treatment, there is a rapid decrease in intracellular pH, followed by a decrease in the ATP level, and there is an increase in the level of calcium ions in the cytosol (Findly *et al.*, 1983; Coss and Linemans, 1996). On the other hand, Drummond *et al.* demonstrated already in 1986 that neither a decrease in

intracellular pH nor an increased level of intracellular calcium can evoke any remarkable alteration in the expression of stress proteins. Increased temperature has an unfavourable influence on the function of the mitochondria, particularly their aerobic metabolism. The transition of the cell to anaerobic metabolism can play a role in its survival after heat shock. Cells damaged by heat stress show an increased dependence on glycolytic energetic metabolism (Nickells and Browder, 1988). Morphological alterations can also be perceived at the ultrastructural level. Mitochondria enlarge, *cristae mitochondriales* lessen and the intercrystal matrix spaces increase (Welch, 1992). Other structures damaged by heat stress are the nucleus and the nucleolus. An accumulation of chromatin granules occurs within the nucleus (Yost and Lindquist, 1986). Amalric *et al.* (1969) and Welch (1992) described an aggregation of mature preribosomes and the formation of ribonucleo-protein complexes. The size and quantity of these complexes were directly dependent on the intensity of the heat shock. Welch (1992) described another morphologic abnormality within the nucleus which was the occurrence of extraordinary intranuclear filaments, probably composed of actin. The elements forming cytoskeleton are also a cytoplasmic formation stricken by heat stress. After heat treatment there is a rearrangement of microtubules and microfilaments towards the nucleus (Thomas *et al.*, 1982) and, dependent on this change, also a regrouping or shift of the mitochondria and other cellular organelles towards the nucleus. Welch (1992) terms all these cytoskeletal changes a breakdown of the cytoskeleton.

A hypothesis was formed, on the basis of scientific data, that cells of the tissues and organs synthesize heat shock proteins as a defence against the effects of heat shock. We wanted to verify this hypothesis by observing and demonstrating HSP70 in pig granulosa cells in culture, which are exposed to an exactly defined heat shock.

## MATERIAL AND METHODS

Porcine ovaries were obtained from a local slaughterhouse from gilts at an unknown phase of the oestrous cycle. The ovaries were transported to the laboratory in a saline solution at 37 °C. Granulosa cells were obtained from follicles 3-5 mm in diameter. Only follicles without any signs of atresia, that means follicles having a clear follicular fluid with visible blood vessels in the follicle walls, were chosen for the experiments. After cutting from the ovary, the follicles were placed in a culture medium and by delicate manipulation the granulosa cells were released from their walls into the medium. In the centrifuge (600 g/10 min) the cells were accumulated in the sediment from which they were transported after washing into a culture medium (according to Pavlok *et al.*, 1989) and cultured in Petri dishes. The initial concentration of granulosa cells was

approximately 2–5 millions/1 ml. The obtained cells were cultured for 48–72 hours at 39 °C in a mixture of air with 5% CO<sub>2</sub> and at 100% humidity. After this time, the granulosa cells formed a monolayer at the bottom of the Petri dishes. The monolayer of granulosa cells was exposed to heat shock at 43 °C for 1, 2, 4 and 6 hours. After the appointed time had elapsed the first series of culture was immediately fixed, and the second series, which contained the cell culture after 2 hours heating and 6 hours heating, was transported to a normal culture medium at a temperature of 39 °C and incubated for 1 hour.

All samples were treated in a standard way. We fixed them in a cooled 4% solution of formaldehyde for 60 minutes, washed them with phosphate buffer (PBS) for 120 minutes and, a 0.1% solution of Triton X100 was then applied for 30 minutes at room temperature. The activity of endogenous peroxidases was blocked with a 0.3% solution of H<sub>2</sub>O<sub>2</sub> in methanol for 30 minutes. Nonspecific bindings were blocked using a nonimmune horse serum. Subsequently, the monolayer of granulosa cells was covered with a specific monoclonal antibody anti-HSP70 (StressGen, Biotechnologies Corp. Canada) in a dilution of 1 : 200 in a humidified chamber at 37 °C for 30 minutes. We then used the Vectastain ABC kit (Vector Laboratories, USA). An anti-mouse biotinylated secondary antibody was applied at 37 °C for 30 minutes, also in a humidified chamber. A peroxidase-labelled avidin-biotinylated horseradish peroxidase complex was applied for 45 minutes at 37 °C and the color of the peroxidase was developed using diaminobenzidine tetrahydrochloride (DAB) as the chromogen. The final reaction precipitate was very fine, deep-brown. A negative control was made for all samples, i.e. the whole process without application of the first specific antibody.

During the final step the samples were dehydrated in an ascending series of alcohol, submerged in xylol and mounted in a mounting medium. Results were ex-

amined and photographed with a light microscope JenaMed II (Carl Zeiss, Jena).

## RESULTS

Two different cell types were present in the cultured pig granulosa – adhering cells and nonadhering, free cells. Adhering cells forming the monolayer were usually spindle-shaped with a spreading central part which contained the nucleus. The cytoplasm of these cells formed short, relatively thick protuberances – Figs. 2, 5, 6, 7. (All figures represent the cell culture from pig granulosa after a cytochemical detection of HSP70.) Nonadhering free cells lost their original polyhedral form and turned into round elements. These free, round

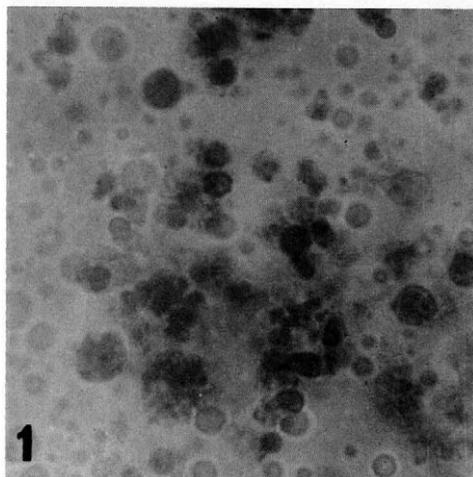


Fig. 1. Control material, cultivation at 39 °C, it is not possible to detect HSP70 (magnification 3 200x)

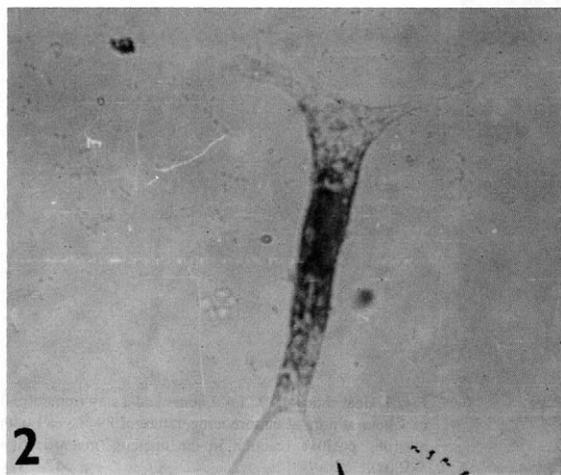


Fig. 2. Heat shock 43 °C for 1 hour, a weak positive reaction in the nuclei (magnification 5 500x)

cells had a tendency to flock (Figs. 1, 3, 8, 9). An accumulation of HSP70 was localized especially in the nucleus and partially in the cytoplasm. After heat shock lasting 1 hour the nuclei contained only a small quantity of positive precipitate which corresponded with the localization of HSP70 (Fig. 2). The situation changed and a quantity of fine positive material was localized in the cell nuclei of both the free cells and the adhering cells after 2 hours heat shock (Fig. 3) and especially after 2 hours of heating with a 1 hour stay at a normal culture temperature (Fig. 4). When the heat shock duration was 4 hours, a reaction proving the presence of HSP70 in the cell nuclei was marked and many vacuoles were also detected in the cytoplasm (Figs. 5, 6). After 6 hours heat shock there was a strong, positive

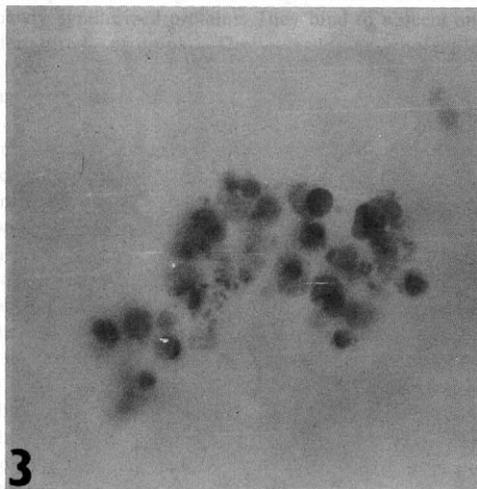


Fig. 3. Heat shock 43 °C for 2 hours, a positive reaction in the nuclei of nonadhering cells (magnification 3 200x)

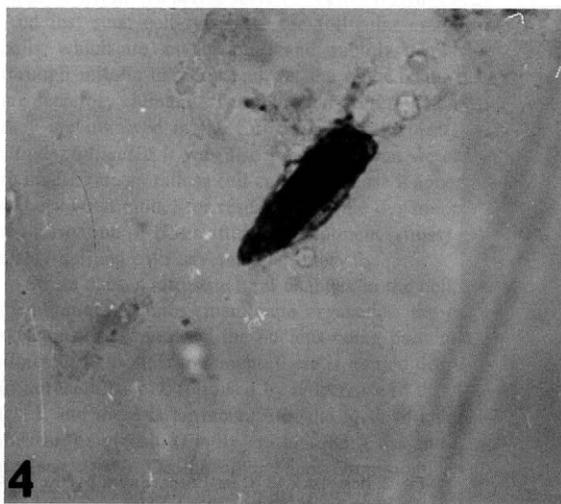


Fig. 4. Heat shock 43 °C for 2 hours and a stay (reparation) of 1 hour at normal culture temperature of 39 °C, cell with a strong positive reaction in the nucleus (magnification 5 500x)

reaction to HSP70, localized in the nucleus (Fig. 7). There was a demonstrable difference of intensity in the immunocytochemical reaction particularly when Fig. 2 was compared with Fig. 7. A stronger, positive reaction in the nuclei and cytoplasm of granulosa cells was detected after 6 hours heat shock with a 1 hour delay in normal culture conditions. This time delay provides a better possibility to observe the dynamism of development of the process of the formation and manifestation of HSPs in the cells. It seems that a return to a normal culture temperature in order to achieve gradual adaptation reinforces HSP70 synthesis in the nuclei as well as in the cytoplasm (Figs. 8, 9).

Round, nonadhering elements increase in parallel with the extended time of heat shock. Likewise, vacuolization proceeds in direct dependence on the duration of heat stress.

Granulosa cells cultured in a normal culture temperature, which were taken as control material, exhibited no positive reaction, either in the nuclei or in the cytoplasm after application of immunocytochemical reaction for detection of HSP70 (Fig. 1).

The situation was the same in the control cultures. After application of immunocytochemical reaction without specific antibody, the results were negative.

The intensity of the HSP70 reaction in the granulosa cell cultures was directly proportional to the duration of heat shock (Table 1).

## DISCUSSION

The presence of HSP70 in cultured granulosa cells was detected immunocytochemically mainly within the nucleus, and in a lesser amount within the cytoplasm after a heat shock of 43 °C. The amount of HSP70 depends on the duration of treatment and it grows in

parallel with the length of heat shock application. We reached this conclusion on the basis of the intensity of the reaction precipitate which grows with the length of time from 1 to 6 hours. A 6-hour application of heat stress was chosen as a time limit sufficient for the synthesis of HSPs. Coss and Linnemans, (1996) state that HSPs synthesize within the tissues after 4 hours of heat application. After stress reaction the occurrence of HSPs is found practically in the whole cell, which means in the nucleus as well as in the cytoplasm. This correlates well with the conclusions of Watanabe *et al.* (1995). These authors declare that stress reaction must be re-

flected in the whole cell because stress affects the whole cell by changing its function and biochemical processes.

The presence of HSP70 in the cell nuclei has quite logical causes connected with the morphological changes within the nucleus. Yost and Lindquist (1986) described an accumulation of chromatin granules which are probably composed of mRNA stock. Welch (1992) found an aggregation of mature preribosomes and a subsequent formation of ribonucleoprotein complexes, their quantity being directly dependent on the intensity of heat stress.

The manifestation of HSP70 in the cytoplasm is much weaker compared with the intensity of its occurrence within the nucleus. At a light microscopy level it is impossible to demonstrate any connection with the well-defined cytoplasmic structures, nevertheless in many places it seems that HSP70 would have a certain bond with the cytoskeleton (Figs. 5, 6, 7). We are thus entering the sphere of speculation because it is known that many great dramatical changes occur within the cytoskeletal net after heat treatment. In normal conditions the filaments are dispersed with relative regularity and form a quite regular network which passes through the cytoplasm. During heat stress, microtubules, microfilaments and intermediate filaments very quickly rearrange themselves in the neighbourhood of the nucleus, and eventually form a close cover around the nucleus (Coss and Linnemans, 1996). The role of the cytoskeleton during heat stress is not known, but we can suppose that the HSPs present try to repair the damaged proteins of the cytoskeleton by binding to them and there is a possibility of indicating them during immunocytochemical detection. In their recent study Liang and MacRae (1997) demonstrated that HSP70 protect the centrosome and probably also intermediate filaments. In an interesting survey Coss and Linnemans (1996) arrived at the conclusion that the cytoskeleton is reconstituted in about 4 hours after heat shock or during its

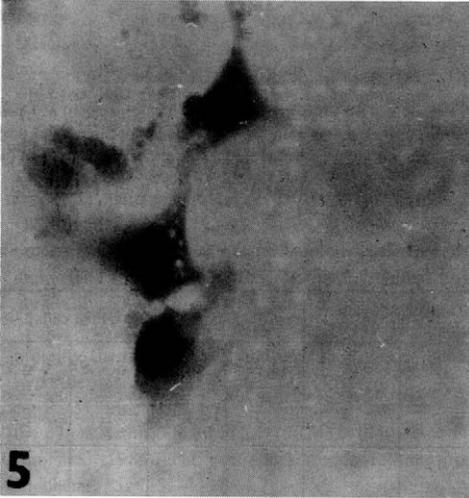


Fig. 5. Heat shock 43 °C for 4 hours, a strong positive reaction in the nuclei and remarkable reaction in the cell with vacuolization of cytoplasm (magnification 5 000x)

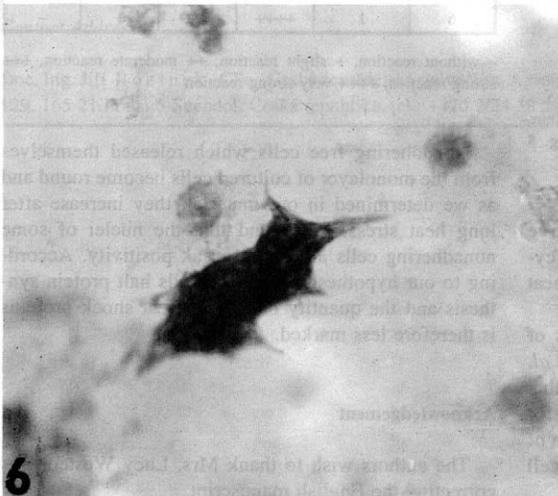


Fig. 6. Heat shock 43 °C for 4 hours. Two associated adhering cells with strong positivity in the nuclei as well as in the cytoplasm (magnification 5 600x)

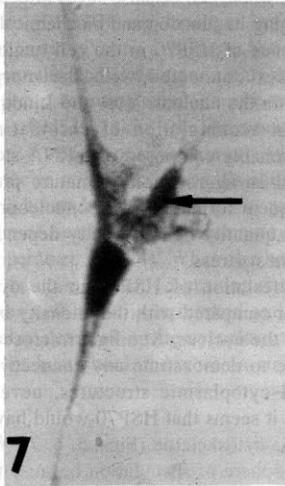


Fig. 7. Heat shock 43 °C for 6 hours, very strong reaction in the nucleus of the adhering cell as well as in the cytoplasm. Arrow – a free cell also with the positive nucleus (magnification 5 000x)

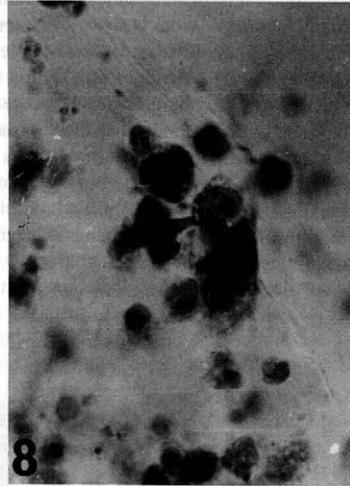


Fig. 8. Heat shock 43 °C for 6 hours and a stay (reparation) of 1 hour at normal culture temperature 39 °C. A graded occurrence of positivity in the nuclei, a vacuolization in cytoplasm is visible, quantity of free elements grows (magnification 4 900x)

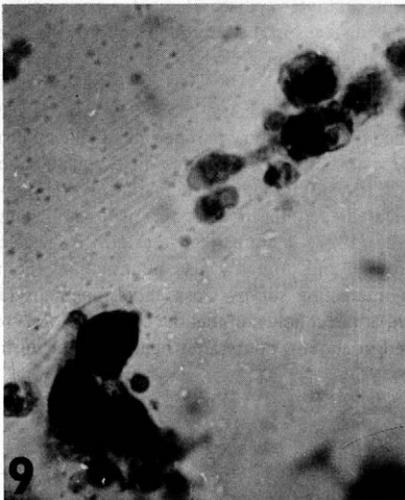


Fig. 9. Heat shock 43 °C and reparation 1 hour at normal culture temperature 39 °C, the same situation as demonstrated in Fig. 8 (magnification 4 900x)

long term course. And we could therefore observe a positive staining of filamentous structures in the cytoplasm of granulosa cells only after long term heat exposure (4 and 6 hours).

We chose a temperature of 43 °C on the basis of data cited in the literature where, for example, Li *et al.* (1995) demonstrated in connection with heat stress that this temperature is critical and the breaking point for the formation of HSPs. During long term application of this temperature, or when it has been exceeded, cell death is inevitable.

Table 1. Evaluation of immunocytochemical reaction for detection of HSP70 in cultured pig granulosa cells after heat stress

Shock temperature 43 °C	Time of cultivation (h)	cultivation temperature 39 °C	Adherent cells		Free cells	
			nucleus	cytoplasm	nucleus	cytoplasm
0			-	-	-	-
1			+/-	-	-	-
2			+	+	-	-
2	1		++	+	+	-
4			++	++	+	-
6			+++	++	+	-
6	1		++++	+++	+	-

- without reaction, + slight reaction, ++ moderate reaction, +++ strong reaction, ++++ very strong reaction

Nonadhering free cells which released themselves from the monolayer of cultured cells become round and as we determined in our material, they increase after long heat stress. We found that the nuclei of some nonadhering cells also show weak positivity. According to our hypothesis these free cells halt protein synthesis and the quantity of present heat shock proteins is therefore less marked.

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# THE ANALYSIS OF SOME SKELETAL MUSCLES IN LAMBS OF DIFFERENT GENOTYPE BY THE DISTRIBUTION FREQUENCY OF MUSCLE FIBRES DIAMETER

## ROZBOR NĚKTERÝCH KOSTERNÍCH SVALŮ U JEHŇAT RŮZNÉHO GENOTYPU PODLE ROZLOŽENÍ ČETNOSTÍ PRŮMĚRŮ SVALOVÝCH VLÁKEN

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**ABSTRACT:** Samples of *m. gluteobiceps*, *m. longissimus lumborum*, *m. psoas major*, *m. semitendinosus*, *m. semimembranosus*, *m. quadriceps femoris* (*m. rectus femoris*) and *m. triceps brachii* (*caput longum*) of 9 male lambs 71 days old, originating from the ewes crossbreds between the Istrian Pramenka breed and Sardinian rams and Sardinian (S), domestic Merino-Württemberg (W) and East Friesian (EF) rams, were investigated by the distribution frequency of muscle fibre diameters with the shift of 0.5 µm. The frequency distribution pointed out the prevalence of fibres of smaller diameter in muscles of W and EF crossbreds having higher body weight and meatiness. The comparison between W and EF crossbreds having similar body weight and meatiness showed that smaller fibre diameters were prevailing in 5 muscles of EF crossbreds and 2 muscles in W crossbreds.

**Keywords:** lambs; crossbred breeds; frequency distribution; diameter of muscle fibres

**ABSTRAKT:** Provedli jsme šetření průměru svalových vláken na základě rozložení četnosti s odstupňováním po 0,5 µm ve vzorcích svalů *m. gluteobiceps*, *m. longissimus lumborum*, *m. psoas major*, *m. semitendinosus*, *m. semimembranosus*, *m. quadriceps femoris* (*m. rectus femoris*) a *m. triceps brachii* (*caput longum*) u beránků pocházejících od matek-kříženek mezi bahnicemi plemene istrijská pramenka a berany sardinského plemene a beranů plemen sardinské (S), domácí württembergské merino (W) a východofrišské (EF). Do studie jsme zařadili 9 beránků, po třech z každé plemenné skupiny, ve věku 71 dní a o hmotnosti 21,74 kg (S), 25,66 kg (W) a 25,43 kg (EF). Vzorky svalů jsme zalili do parafinu, vytvořili jsme řezy o tloušťce 10 µm a obarvili je hematoxylinem a eozinem. V histologických preparátech jsme měřili průměr vláken, podle průměru jsme je rozdělili do skupin odstupňovaných po 0,5 µm a provedli jsme statistickou analýzu. Skupiny vláken jsme dále rozdělili na skupinu jemných vláken (do 30 µm), středních vláken (31–50 µm) a silných vláken (nad 51 µm). Rozdělení četnosti průměrů vláken naznačilo, že u hybridních jehňat po beranech EF převládala ve všech svalech s výjimkou *m. triceps brachii* vlákna malého (26,1–45,6 %) (jemná vlákna) a středního (49–71,6 %) průměru. Ve svalu *m. triceps brachii* převládala vlákna středního (68,2 %) a velkého (20,4 %) průměru (silná vlákna). U hybridních beranů S se dominantní podíl středních a silných vláken vyskytoval v *m. longissimus lumborum* (64,2 % a 20,3 %), *m. semitendinosus* (49,6 % a 38,4 %), *m. quadriceps femoris* (26,4 % a 63,3 %) a v *m. semimembranosus* (42 % a 46 %); dominantní podíl v *m. triceps brachii* (51,5 %) a v *m. gluteobiceps* (54,8 %) jsme zjistili u vláken středního průměru. U kříženců po beranech plemene W jsme dominantní podíl středních a silných vláken zaznamenali v *m. gluteobiceps* (64,6 % a 30,1 %), *m. triceps brachii* (53,3 % a 45,2 %) a *m. semitendinosus* (49,4 % a 44,2 %); v *m. longissimus lumborum* (79,4 %), *m. semimembranosus* (77,5 %) a *m. quadriceps femoris* (70,3 %) převládala střední vlákna a v *m. psoas major* vlákna jemná a střední (31,6 % a 58,1 %). Rozložení četnosti průměrů vláken jednoznačně dokládá převládající podíl jemných vláken ve svalech kříženců W a EF, kteří mají vyšší tělesnou hmotnost a zmasilost. U kříženců s plemenem S o nižší tělesné hmotnosti a zmasilosti převládala silnější vlákna. Vzájemné porovnání kříženců s plemeny W a EF s podobnou tělesnou hmotností a zmasilostí ukázalo, že u kříženců s plemenem EF převládala jemná vlákna v *m. gluteobiceps* (45,6 %), *m. longissimus lumborum* (26,1 %), *m. semitendinosus* (25 %), *m. semimembranosus* (16,9 %) a *m. triceps brachii* (11,4 %) a u kříženců s plemenem W v *m. psoas major* (31,6 %) a *m. quadriceps femoris* (16,4 %), zatímco převládající podíl silných vláken jsme u kříženců plemene W zjistili v *m. gluteobiceps* (30,1 %), *m. semitendinosus* (44,2 %) a *m. triceps brachii* (45,2 %).

**Klíčová slova:** jehňata; hybridní plemena; rozložení četnosti; průměr svalových vláken

## INTRODUCTION

In our former short communication (Gjurčević-Kantura *et al.*, 1998) we investigated histomorphological properties of some muscles of the three-breed crosses originating from crossbreds between Istrian Pramenka ewes and the Sardinian rams, and Sardinian, domestic Merino-Würtemberg and East Friesian rams. At the age of 71 days the lambs originating from the Sardinian rams had significantly lower body and final weight, i. e. they were markedly less meaty than the lambs of the same age originating from domestic Merino-Würtemberg and East Friesian rams (Mikulec *et al.*, 1997). The analysis of the fibers from their skeletal muscles showed marked heterogeneity as concerned fibre diameter and muscle type. Studied muscles (*m. gluteobiceps*, *m. longissimus lumborum*, *m. psoas major*, *m. semitendinosus*, *m. semimembranosus*, *m. quadriceps femoris* – *m. rectus femoris* and *m. triceps brachii* – *caput longum*) contained the fibres of small, medium and large diameters, oxidative, oxidative-glycolytic and glycolytic fibres and slow-twitch and fast-twitch fibres.

The results (Gjurčević-Kantura *et al.*, 1998) have shown that the largest average diameter of the fibres was in the offspring of the Sardinian rams in five muscles and in the offspring of domestic Merino-Würtemberg rams in two muscles. Conversely, the smallest average diameter of the fibres was in the offspring of the East Friesian rams in 5 muscles, in the Sardinian ram progeny in one muscle and in the domestic Merino-Würtemberg ram progeny also in one muscle.

The differences in fibre diameter and type of similar muscles between various crossbreds were not significant (Gjurčević-Kantura *et al.*, 1998) given the fact that all three crossbreds had the same original material, i. e. 75% genomes of the crossbred Sardinian breed and 25% genomes of the crossbred Istrian Pramenka breed. Consequently, grading up with the genome of the Sardinian, domestic Merino-Würtemberg or East Friesian breeds (Mikulec *et al.*, 1997) did not produce any significant differences. Since the lambs of the domestic crossbred Merino-Würtemberg and East Friesian breeds have shown a considerably higher number of muscle fibres in the visual field than the Sardinian offspring, it can be assumed that significant meatiness of the crossbred Merino-Würtemberg and East Friesian breeds vs. crossbred Sardinian breeds was the outcome of the increased number of muscle fibres, rather than of the increased fibre diameter.

The study (Gjurčević-Kantura *et al.*, 1998) has not elucidated entirely the differences in morphological and functional properties of the fibres in the studied muscles of the three-breed crosses. Average diameter of the muscle fibres has not fully shown the differences in the muscle structure and their functional role. Therefore, we studied the muscles, used in the preceding study, from the point of frequency distribution of the fibres (Bego, 1994) to find out the difference between the same muscles in various crossbreds. Frequency dis-

tribution has clearly indicated functional differences between the *pars costalis* and *pars lumbalis* of diaphragm in horses (Bego *et al.*, 1996; Zobundžija *et al.*, 1997), cattle (Zobundžija *et al.*, 1998) and other domestic animals (Gjurčević-Kantura *et al.*, 1996) as well as in humans between *m. masseter* and *m. temporalis* (Valentić-Peruzović *et al.*, 1994).

## MATERIAL AND METHODS

The study was carried out on the same muscles as in the preceding work (Gjurčević-Kantura *et al.*, 1998), i. e. *m. gluteobiceps*, *m. longissimus lumborum*, *m. psoas major*, *m. semitendinosus*, *m. semimembranosus*, *m. quadriceps femoris* (*m. rectus femoris*) and *m. triceps brachii* (*caput longum*) of nine lambs aged 71 days, originating from crossbred ewes of the Istrian Pramenka and Sardinian rams, crossbred with the Sardinian, domestic Merino-Würtemberg and East Friesian rams. The lambs, three each breed group, were kept and fed under the same conditions up to 71 days when they were slaughtered, cut in the slaughterhouse in the usual manner and weighed. Their final weight was 21.74 kg (Sardinian crossbreds), 25.66 kg (crossbreds with domestic Merino-Würtemberg breed) and 25.43 kg (crossbreds with East Friesian breed). Samples of the above mentioned muscles, sized 1 x 1 cm, were taken from the central part of *m. triceps brachii* (*caput longum*) and *m. quadriceps femoris* (*m. rectus femoris*); from *m. longissimus lumborum* and *m. psoas major* at the height of the third lumbar vertebra, and at the height of the lateral joint from *m. gluteobiceps*, *m. semitendinosus* and *m. semimembranosus*. The samples were immersed into paraffin, cut on the microtome into 10 µm slices and stained with hematoxylin and eosine (Romeis, 1968) to show the fibres. So stained microscopic preparations were measured for fibre diameter. There were 3 476 fibres in total. Prior to analysis and based on diameter, the fibres were classified into groups with a shift of 0.5 µm (Bego, 1994) and graphically presented relevant to each muscle and crossbred and analysed statistically by help of Statistica for Windows-release 4.3. The groups of muscle fibres were also classified according to diameter as small fibres up to 30 µm, medium fibres from 31–50 µm and as large fibres over 51 µm.

## RESULTS

The analysis of the muscle fibre diameter showed the differences between the muscles of the same crossbreds and between the same muscles of different crossbreds (Table 1). Fibre diameters of the Sardinian ram progenies ranged from 10–90 µm, of Merino-Würtemberg progenies from 10–85 µm and of the East Friesian progenies from 10–70 µm. These values, however, ranged relevant to the muscle, animal and breed. This

I. Percentage of muscle fibres in lambs breed and diameter

Muscle	Breed	Total number of measured muscle fibres	Fibres according to diameter			Fibres diameter $\mu\text{m}$		
			small fibres (> 30 $\mu\text{m}$ ) %	medium fibres (31–50 $\mu\text{m}$ ) %	large fibres (< 50 $\mu\text{m}$ ) %	average	minimal	maximal
<i>M. gluteobiceps</i>	S	159	23.3	54.8	21.9	37.88	10	75
	W	113	5.3	64.6	30.1	42.65	25	80
	EF	229	45.6	53.3	2.1	30.17	10	55
<i>M. longissimus lumborum</i>	S	168	15.5	64.2	20.3	38.63	12.50	90
	W	146	16.4	79.4	4.2	35.77	15	50
	EF	241	26.1	71.4	2.5	33.88	10	52.50
<i>M. psoas major</i>	S	152	45.4	48.6	6.0	30.30	10	65
	W	136	31.6	58.1	10.3	34.19	10	65
	EF	230	29.6	61.7	8.7	33.92	10	55
<i>M. semitendinosus</i>	S	119	6.8	49.6	38.6	49.25	10	85
	W	172	6.4	49.4	44.2	47.56	15	85
	EF	212	25.0	49.0	26.0	38.14	10	70
<i>M. semimembranosus</i>	S	124	12.0	42.0	46.0	45.60	10	70
	W	145	7.9	77.5	14.6	38.78	17.50	62.50
	EF	151	16.9	68.9	15.2	36.92	20	70
<i>M. quadriceps femoris</i>	S	106	10.3	26.4	63.3	52.36	10	90
	W	122	16.4	70.3	13.3	37.64	17.50	60
	EF	232	14.2	71.6	14.2	38.23	15	60
<i>M. triceps brachii</i>	S	153	4.0	51.3	44.7	47.04	15	90
	W	139	1.5	53.3	45.2	46.68	22.50	75
	EF	229	11.4	68.2	20.4	39.47	10	70

Legend: S = Sardinian ram – Sardinian x Pramenka crossbred; W = Merino-Würtemberg ram – Sardinian x Pramenka crossbred; EF = East Friesian ram – Sardinian x Pramenka crossbred

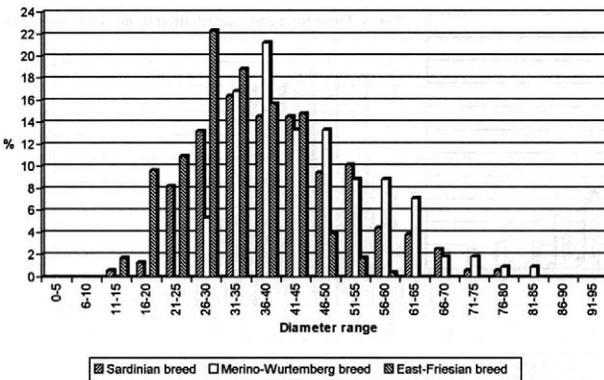


Fig. 1. Fibre frequency distribution of *m. gluteobiceps*

was evidenced by the fibres frequency distribution with a 0.5  $\mu\text{m}$  shift (Figs. 1–7) and the following diameter range: small diameter – up to 30  $\mu\text{m}$ , medium diameter – from 31 to 50  $\mu\text{m}$ , large diameter – over 51  $\mu\text{m}$ .

Fibre diameter of *m. gluteobiceps* of the Sardinian progenies ranged from 10 to 75  $\mu\text{m}$ , of Merino-Würtemberg progenies 25–80  $\mu\text{m}$  and of East Friesian progenies 15–55  $\mu\text{m}$ . Distribution frequency of muscle fibres showed that in these muscles the commonest were small

(45.6%) and medium (53.3%) of East Friesian progenies, medium (54.8%) of Sardinian progenies and medium (64.6%) and large (30.1%) of Merino-Würtemberg diameter fibres (Fig. 1).

In *m. longissimus lumborum* fibres diameter ranged from 12.50 to 90  $\mu\text{m}$  (the Sardinian offsprings), from 15 to 50  $\mu\text{m}$  (Merino-Würtemberg) and 10–52.50  $\mu\text{m}$  (East Friesian). Distribution frequency showed that the commonest in these muscles were medium (64.2%) and

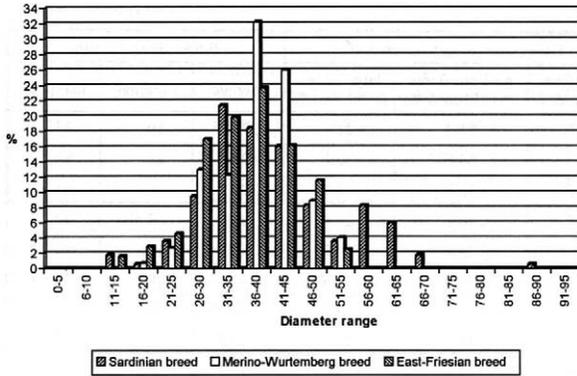


Fig. 2. Fibre frequency distribution of *m. longissimus lumborum*

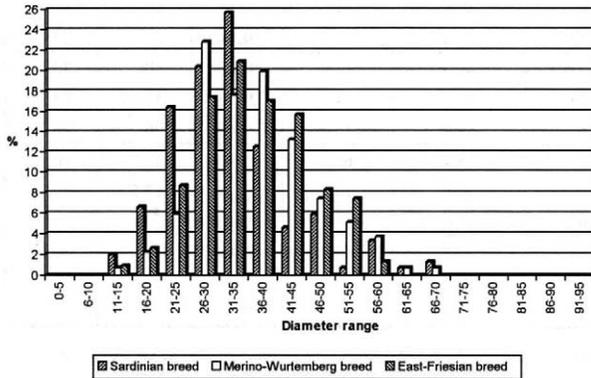


Fig. 3. Fibre frequency distribution of *m. psoas major*

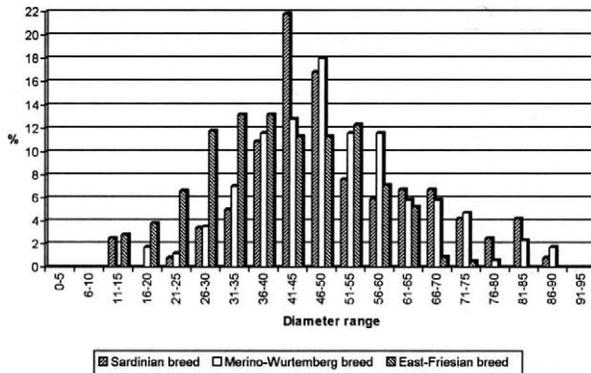


Fig. 4. Fibre frequency distribution of *m. semitendinosus*

large (20.3%) diameter fibres of the crossbred Sardinian breed, small (26.1%) and medium (71.4%) diameter fibres of the crossbred East Friesian breed and medium diameter fibres (79.4%) of the crossbred Merino-Würtemberg breed (Fig. 2).

In *m. psoas major* the diameter of fibres ranged from 10 to 65 µm (Sardinian and Merino-Würtemberg) and from 10 to 55 µm (East Friesian). Distribution frequency of diameters showed that in the crossbred Sar-

dinian breed the commonest were small (45.4%) and medium (48.6%) diameter fibres but in the crossbred Merino-Würtemberg and East Friesian breeds medium (58.1% and 61.7%) diameter fibres were commonest (Fig. 3).

*M. semitendinosus* contained the fibres ranging in diameter from 10 to 85 µm (Sardinian), from 15 to 85 µm (Merino-Würtemberg) and from 10 to 70 µm (East Friesian). Distribution frequency of diameters indicated

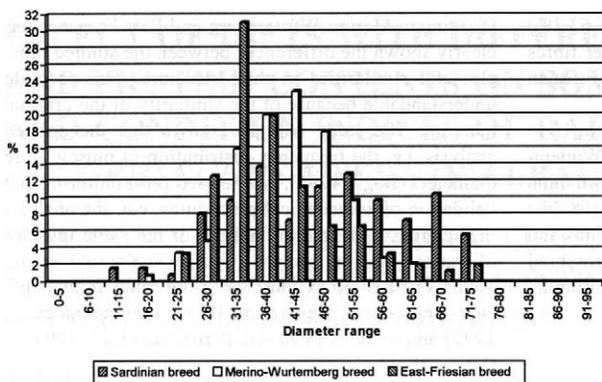


Fig. 5. Fibre frequency distribution of *m. semimembranosus*

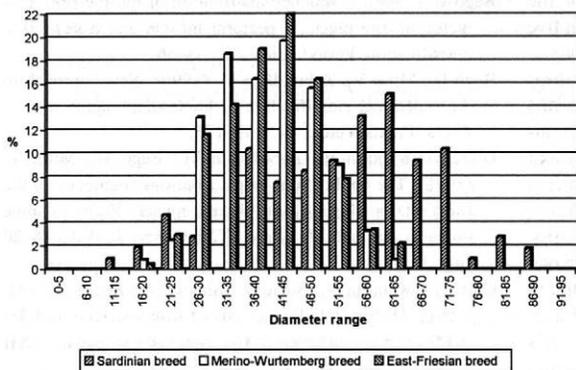


Fig. 6. Fibre frequency distribution of *m. quadriceps femoris*

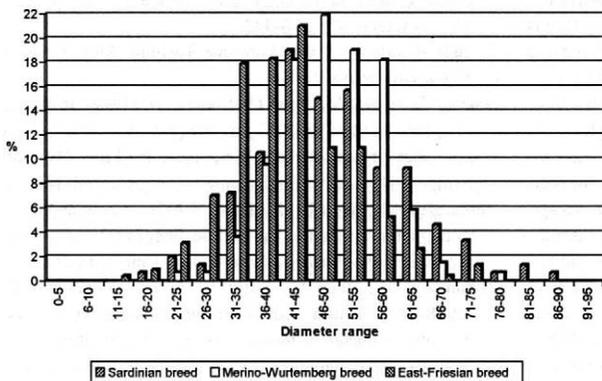


Fig. 7. Fibre frequency distribution of *m. triceps brachii*

prevalence of medium diameter fibres in the crossbred East Friesian (49%) and Sardinian (49.6%) breeds and medium and large diameter fibres in the crossbred of Merino-Wurtemberg (40.4% and 44.2%) breed (Fig. 4).

In *m. semimembranosus* the diameter ranged from 10 to 70 µm (Sardinian), from 17.50 to 62.50 µm (Merino-Wurtemberg) and from 20 to 70 µm (East Friesian). Distribution frequency of diameters showed dominance

of medium diameter fibres in the crossbred East Friesian (68.9%) and Merino-Wurtemberg (77.5%) breeds and medium (42%) and large (46%) diameter fibres in the crossbreds of Sardinian breed (Fig. 5).

Fibre diameter in *m. quadriceps femoris* ranged from 10 to 90 µm (Sardinian), from 17.50 to 60 µm (Merino-Wurtemberg) and from 15 to 60 µm (East Friesian). Distribution frequency of diameter showed prevalence of

medium and large diameter fibres (26.4% and 63.3%) in the Sardinian group and of medium diameter fibres in the Merino-Würtemberg (71.6%) and East Friesian (70.3%) group (Fig. 6).

In *m. triceps brachii* the diameter ranged from 15 to 90 µm (Sardinian), from 22.50 to 75 µm (Merino-Würtemberg) and from 10 to 70 µm (East Friesian). Distribution frequency showed the prevalence of medium (68.2%) diameter fibres in the crossbred East Friesian rams and of medium and large diameter fibres in the crossbred Merino-Würtemberg (53.3% and 45.2%) and Sardinian (51.3% and 44.7%) breeds (Fig. 7).

## DISCUSSION

Although our preceding short communication (Gjurčević-Kantura *et al.*, 1998) had shown that the largest average diameter of muscle fibres occurred in five muscles of the crossbred Sardinian rams (*m. longissimus lumborum*, *m. semitendinosus*, *m. semimembranosus*, *m. quadriceps femoris* and *m. triceps brachii*) and in two muscles of the crossbred Merino-Würtemberg rams (*m. gluteobiceps* and *m. psoas major*) and that the lowest average diameter fibres occurred in five muscles of the crossbred East Friesian rams (*m. gluteobiceps*, *m. longissimus lumborum*, *m. semitendinosus*, *m. semimembranosus* and *m. triceps brachii*) and in one muscle of the crossbred Merino-Würtemberg breed (*m. quadriceps femoris*) and of the crossbred Sardinian breed (*m. psoas major*), distribution frequency analysis of the fibre diameter with a 0.5 µm shift showed somewhat different relations. In the East Friesian group, in all muscles, except in *triceps brachii* (i.e. six muscles of the seven studied), majority fibres were positioned on the left side, i. e. between the fibres of the small and medium diameter. There they were dominant with respect to those of the crossbred Sardinian and Merino-Würtemberg breeds. Conversely, in the crossbred Sardinian rams, a shift in the muscle diameter to the right, i. e. to the fibres of larger diameter, was recorded in five muscles. There, the shift in three muscles was marked (*m. semitendinosus*, *m. quadriceps femoris* and *m. triceps brachii*) and in two muscles less marked (*m. longissimus lumborum* and *m. semimembranosus*). In the remaining two muscles the shift was to the left (*m. psoas major*), i.e. to the small diameter fibres, where small diameter fibres dominated. In the other muscle (*m. gluteobiceps*) there was a slight shift to the left, i. e. to smaller diameter fibres. However, compared to the crossbred Merino-Würtemberg breed, the largest diameter fibres were not so numerous.

Frequency distribution of the muscle fibre diameter in the analysis of the muscles of the three-breed crosses

(Sardinian, Merino-Würtemberg and East Friesian) has clearly shown the differences between the studied muscles and similarities in their structure. This is quite understandable because of the similarity of the original material. The study has also shown that the applied analysis, i.e. the frequency distribution of muscle fibre diameters (Bego, 1994), can be used for monitoring and validation of zootechnical procedures, e.g. the analysis of meatiness, functional analysis of the same muscles of various animal species (Gjurčević-Kantura *et al.*, 1996), or of complex muscles of the same animal species (Bego, 1994; Bego *et al.*, 1996; Zobundžija *et al.*, 1997) and humans (Valentic-Peruzovic *et al.*, 1994).

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# EFFECT OF DIETS WITH DIFFERENT LIPID AND PROTEIN CONTENTS ON SOME BLOOD AND CONDITION INDICES OF RAINBOW TROUT, *ONCORHYNCHUS MYKISS* (WALBAUM)

VLIV DIET S RŮZNÝM OBSAHEM LIPIDŮ A PROTEINŮ NA NĚKTERÉ KREVŇÍ A KONDIČNÍ UKAZATELE PSTRUHA DUHOVÉHO, *ONCORHYNCHUS MYKISS* (WALBAUM)

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**ABSTRACT:** Two pilot experiments were conducted with the fish held in flow-through tanks containing 6 m<sup>3</sup> water. Rainbow trout, *Oncorhynchus mykiss* (Walbaum), at a weight of 324 ± 53.9 g (Experiment A) and 203 ± 17 g (Experiment B), was evaluated as to the action of different quantities of dietary lipids (14 and 18%, Experiment A) and proteins (40 and 45%, Experiment B) on the health, condition, red blood cells and some biochemical indices of the metabolism of protein, carbohydrates, lipids and minerals in the blood plasma. The experimental group of fish fed pellets containing 14% lipids, compared with a control group given granules produced outside the Czech Republic (17.08% fat and 41% crude protein) showed a significant ( $P = 0.05$ ) reduction in the level of triacylglycerols (4.83 vs 7.28 mmol.L<sup>-1</sup>) and a highly significant ( $P = 0.01$ ) reduction of total proteinemia (41.2 vs 50.3 g.L<sup>-1</sup>), cholesterolemia (7.13 vs 9.12 mmol.L<sup>-1</sup>) and aspartate aminotransferase (AST) activities (4.47 vs 7.93  $\mu$ kat.L<sup>-1</sup>). The stock given feed containing 18% fat showed a significant ( $P = 0.05$ ) decrease in the erythrocyte count (1.05 vs 1.16 T.L<sup>-1</sup>), total protein (43.4 vs 50.3 g.L<sup>-1</sup>) and AST activity (5.42 vs 7.93  $\mu$ kat.L<sup>-1</sup>). What was recorded in experiment B over 91 days was only a significant ( $P = 0.05$ ) reduction of plasma protein (37.6 vs 42.8 g.L<sup>-1</sup>) in the experimental group fed pellets with 20% lipids and 40% crude protein, compared with the group of fish given a diet with 18% lipids and 45% crude protein. In both experiments, the statistically significant metabolic response was not recorded between the experimental groups but between the experimental and the control group of fish. This indicates that the chemical composition of the diets (in terms of both presence and ratios of the components) is a factor of primary importance. The results of biochemical tests in both experiments have suggested that the levels of protein and lipid metabolism correlated with the contents of the protein and lipid components in the compound feed. In experiment A, the strongest metabolic response to feed ration composition was recorded in the fish given the feed produced outside the Czech Republic: those fish had the highest levels of total protein, urea, uric acid, creatinine, glucose, cholesterol and triacylglycerols in their blood. The well-balanced results of hematological and biochemical parameters in experiment B correspond with the adequate growth potential and feed conversion rates; at the same time, they have indicated how suitable it is to use these tests for the control of the internal environment of the experimental fish.

**Keywords:** rainbow trout; hematological indices; biochemical indices of blood plasma; hepatosomatic index

**ABSTRAKT:** Ve dvou poloprovězných pokusech v průtočných bazénech o objemu vody 6 m<sup>3</sup> byl u pstruha duhového *Oncorhynchus mykiss* (Walbaum) o hmotnosti 324 ± 53,9 g (pokus A) a 203 ± 17 g (pokus B) vyhodnocen vliv různého množství lipidů (14 a 18 %, pokus A) a proteinů (40 a 45 %, pokus B) v krmivu na zdravotní stav, kondici, červený krevní obraz a některé biochemické ukazatele dusíkového, sacharidového, lipidového a minerálního metabolismu v krevní plazmě. U experimentální skupiny ryb krmené granulí se 14 % lipidů došlo za 83 dní oproti srovnávací skupině ryb konzumujících granulě zahraničního provenience (17,08 % tuku, 41 % dusíkatých látek) k významnému ( $P = 0,05$ ) snížení triacylglycerolů (4,83 vs. 7,28 mmol.L<sup>-1</sup>) a k vysoce významnému ( $P = 0,01$ ) snížení celkové proteinémie (41,2 vs. 50,3 g.L<sup>-1</sup>), cholesterolemie (7,13 vs. 9,12 mmol.L<sup>-1</sup>) a aktivity aspartátaminotransferázy AST (4,47 vs. 7,93  $\mu$ kat.L<sup>-1</sup>). U obsádky dostávající krmivo s 18 %

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tuku byl zaznamenán signifikantní ( $P = 0,05$ ) pokles počtu erytrocytů ( $1,05$  vs.  $1,16 \text{ T.L}^{-1}$ ), celkové bílkoviny ( $43,4$  vs.  $50,3 \text{ g.L}^{-1}$ ) a aktivity AST ( $5,42$  vs.  $7,93 \text{ } \mu\text{kat.L}^{-1}$ ). V pokuse B pak bylo za 91 dní registrováno pouze signifikantní ( $P = 0,05$ ) snížení plazmatických bílkovin ( $37,6$  vs.  $42,8 \text{ g.L}^{-1}$ ) u pokusné skupiny krmené granulí s 20 % lipidů a 40 % dusíkatých látek) oproti srovnávací skupině ryb krmené dietou s 18 % lipidů a 45 % dusíkatých látek. V obou pokusech nebyla zjištěna statisticky průkazná metabolická odezva mezi experimentálními skupinami, nýbrž mezi experimentální a srovnávací skupinou ryb. Tato skutečnost svědčí o rozhodující úloze odlišného komponentového a chemického složení porovnávaných diet. Výsledky biochemických testů v obou pokusech naznačily, že úroveň dusíkového a lipidového metabolismu korelovala s obsahem proteinových a lipidových komponent v krmné směsi. Nejvyšší metabolickou odezvu organismu na složení krmné dávky projevil v pokuse A ryby krmené dietou zahraničního provenience, u nichž se v krevní plazmě nalézaly nejvyšší hodnoty celkové bílkoviny, močoviny, kyseliny močové, kreatininu, glukózy, cholesterolu a triacylglycerolů. Vyrovnané výsledky hematologických a biochemických ukazatelů v pokuse B korespondují se shodnými růstovými vlastnostmi a konverzí krmliva a současně upozornily na účelnost aplikace testů použitých při kontrole vnitřního prostředí pokusných ryb.

**Klíčová slova:** pstruh duhový; červený krevní obraz; biochemické ukazatele krevní plazmy; hepatosomatický index

## INTRODUCTION

In intensive aquaculture, hematological and biochemical examinations are an essential part of the set of auxiliary diagnostic methods of determining health of the fish, allowing to record any differences by which the actual values measured in the fish body may – due to various causes – depart from the physiological equilibrium. However, clinical hematology and biology has also won a strong position within the complex of methods used for the interpretation of feeding experiments conducted to check the biological and production effectiveness of feeds and the components thereof. For rainbow trout, this is documented by papers published by Bělík *et al.* (1978), Dobšínská *et al.* (1980), Řehulka (1984a, b, 1994) and Jirásek *et al.* (1989), whose results have drawn attention to rainbow trout's ability not only to respond sensitively to changes in the nutrient structure of the feed but also to indicate the consequences of ingestion of feed which fails to comply with hygienic requirements. What justifies this method of assessment of the nutritive value of feeds according to the blood indices of the fish is the fact that it allows to learn the changes in the fish body's internal environment and, on that basis, to change the diet in order to optimize the processes of life. In this study we present the results of our hematological and biochemical examinations of the peripheral blood of rainbow trout. This study is a continuation of previous experiments which we presented in our previous paper (Řehulka, Párová, 2000). In these experiments we analyzed rainbow trout's metabolic response to three types of oils under a project aimed at optimizing the production of granular feed, the optimization being based on reduced levels of nitrogen compounds while using different fat components as sources of nutrients and energy.

In this study we wanted to assess the state of health and selected condition, hematological and biochemical indices of the blood plasma in an experimental group of fish fed pellets having equal levels of crude protein but different levels of fat, including three types of oils (experiment A) and in another experimental group of

fish given diet at equal levels of fat but different levels of crude protein (experiment B).

The results presented here are a part of long-term research project aimed at recording and analyzing the biochemical changes occurring within the system governing the fish body's relationship to the external environment and guaranteeing the metabolic integration of the external environment and tissue metabolism.

## MATERIAL AND METHODS

### Experimental diets

In trial A, experimental diet 1 contained 94% of its volume as the basic diet with a 6% addition of oils whereas in experimental diet 2 the basic diet represented 90% of the basic diet and 10% of oils. The oil mixture added to the basic diet was prepared by mixing 77.8% refined cod oil, 10% sunflower oil and 12.2% flax oil. Commercial feed formulated by a foreign firm served as a reference diet (diet 3). The formulation of the basic experimental diet is shown in Table 1. Nutrient and energy contents in experimental diets 1 and 2 and in the reference diet 3 are shown in Table 2.

The composition of experimental diets 4 and 5 in trial B is shown in Table 3. The nutrient levels in the diets, including the reference diet (diet 6), represented by a granular feed formulated by a foreign company, are shown in Table 4. The fat supplement to experimental diets 4 and 5 was represented by refined cod oil. The granules in both the experimental and the reference diets were 4 mm in size in both trials.

### Experimental fish

Juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum), all of the same origin and all in good condition and good health, were used for the experiments. The fish used in experiment A (from September 24 to December 16, 1997) had a starting weight of  $199 \pm 46.3 \text{ g}$  (mean  $\pm$  SD) and final weight of  $324 \pm 53.9 \text{ g}$ . In

Table 1. Formulations of basic experimental diet in experiment A

Ingredients	%
Fish meal	43
Meat-bone meal	19
Soya extrudate	23
Yeasts Vitex	2
Whey powder	4
Feed flour	8.1
Aminovitan Pd <sup>*)</sup>	0.9

<sup>\*)</sup> Composition of Aminovitan Pd supplement (contents per 1 kg):  
 Vitamins: vitamin A 2 500 000 i.u., vitamin D<sub>3</sub> 400 000 i.u., vitamin E 30 000 mg, vitamin K<sub>3</sub> 1 000 mg, vitamin B<sub>1</sub> 2 000 mg, vitamin B<sub>2</sub> 2 000 mg, vitamin B<sub>6</sub> 2 400 mg, vitamin B<sub>12</sub> 5 mg, Niacin 10 000 mg, calcium pantothenate 10 000 mg, choline 100 000 mg, folic acid 500 mg, vitamin C 100 000 mg, biotin 50 mg, inositol 100 000 mg, antioxidant Endox 20 000 mg  
 Amino acids: DL-methionine 100 000 mg, L-lysine HCL 100 000 mg  
 Microelements: cobalt 50 mg, copper 800 mg, iodine 80 mg, manganese 8 400 mg, zinc 8 200 mg, selenium 24 mg

Table 3. Formulations of experimental diets in experiment B

Ingredients in %	Experimental diets		
	4	5	6
Maize meal	7.5	10	-
Wheat extrudate	3	5	-
Yeasts Vitex Q	5	-	-
Soybean meal	7	-	-
Fish meal	27	29	-
Poultry meal	35	40	-
Fish oil	12	12	-
SAL CURB DRY	1	1	-
Biovitan Pd 811	0.5	0.5	-
Supplement of lysine 20%	2	2.5	-

Composition of Aminovitan Pd 811 supplement (contents per 1 kg):  
 Vitamins: vitamin A 2 500 000 i.u., vitamin D<sub>3</sub> 400 000 i.u., vitamin E (alpha-tocopherol) 30 000 mg, vitamin K<sub>3</sub> 1 000 mg, vitamin B<sub>1</sub> 2 000 mg, vitamin B<sub>2</sub> 2 000 mg, vitamin B<sub>6</sub> 2 400 mg, vitamin B<sub>12</sub> 5 mg, Niacin 10 000 mg, calcium pantothenate 10 000 mg, choline 100 000 mg, folic acid 500 mg, vitamin C 100 000 mg, biotin 50 mg, inositol 100 000 mg  
 Amino acids: DL-methionine 100 000 mg, L-lysine HCL 100 000 mg

experiment B, (from June 16 to September 15, 1998) the starting weight of the fish was  $76 \pm 8.2$  g and final weight  $203 \pm 17$  g. Both experiments were performed at the same site, the fish being kept in flow-through concrete tanks 6 m<sup>3</sup> in size. Each tank representing one experimental group (EG) or reference group (CG) was stocked with 100 kg fish. The daily ration ranged between 1 and 1.6% of the weight of the fish (experiment A) and between 1.5 and 2.4% of the weight of the fish (experiment B).

The average physical and chemical characteristics of water during experiments A (B) were as follows: tem-

Table 2. Nutrient content in experimental diets 1, 2 and comparative reference diet 3 in experiment A (chemical analysis)

Composition	Diets		
	1	2	3
Dry matter (%)	92.15	92.91	91.78
Crude protein (% in dry matter)	39.10	38.30	41.00
Crude fat (% in dry matter)	14.40	17.91	17.08
Ash (% in dry matter)	11.20	10.94	6.57
Crude fibre (% in dry matter)	2.83	2.69	4.96
Nitrogen free extract (% in dry matter)	24.62	23.07	22.17
Lysine (% in dry matter)	2.08	2.09	2.43
Methionine and cystine (% in dry matter)	1.45	1.45	1.55
Gross energy (MJ.kg <sup>-1</sup> )	17.7	18.5	18.7
Metabolizable energy (MJ.kg <sup>-1</sup> )	15.8	16.7	16.7

Table 4. Nutrient content in experimental diets 4, 5 and comparative diet 6 in trial B

Composition	Diets		
	4	5	6
Dry matter (%)	90.15	90.41	93.86
Crude protein (% in dry matter)	40.10	44.20	45
Crude fat (% in dry matter)	20.0	18.86	18
Ash (% in dry matter)	10.83	12.38	6.55
Crude fibre (% in dry matter)	2.63	1.57	3.41
NFE (% in dry matter)	16.59	13.40	20.90
Lysine (% in dry matter)	2.90	3.3	2.5
Methionine and cystine (% in dry matter)	1	1.1	0.7
Gross energy (MJ.kg <sup>-1</sup> )	18.8	18.7	19.7
Metabolizable energy (MJ.kg <sup>-1</sup> )	16.8	16.6	17.6

perature 7 °C (11), dissolved O<sub>2</sub> 12.6 mg.L<sup>-1</sup> (12.0), pH 6.8 (6.6), COD<sub>Mn</sub> 9.7 mg O<sub>2</sub>.L<sup>-1</sup> (9.8), phosphates PO<sub>4</sub><sup>3-</sup> - P 0.33 mg.L<sup>-1</sup> (0.26), nitrates NO<sub>3</sub><sup>-</sup> - A 3.90 mg.L<sup>-1</sup> (18.0), nitrites NO<sub>2</sub><sup>-</sup> - N 0.019 mg.L<sup>-1</sup> (0.15) and NH<sub>4</sub><sup>+</sup> 0.23 mg.L<sup>-1</sup> (0.16).

#### Preparation of blood samples

Blood samples were taken immediately after catching the fish. The fish were first anesthetized with Menocaine liver and then the blood sample was taken by puncturing the blood vessels of the caudal peduncle. The sampling was performed 20 hours after the last feeding during the forenoon. To take the samples, ten fish were caught by random selection from each experimental group. Aqueous solution of heparin was used as anticoagulant: the injection needle was rinsed with the solution before each sampling. For the purposes of biochemical examination, the blood was centrifuged after collection and the plasma was separated to vials for subsequent laboratory processing.

## Hematology

The red blood cell count (RBCc  $T.L^{-1}$ ) was determined using Bürkers hemocytometer; the Hayem solution was applied and the erythrocytes were counted in 2 x 20 rectangles. Hematocrit (Hct) was determined in heparinized microhematocrit capillaries in duplicate, using a microhematocrit centrifuge (13 000 rpm for 3 min). Hemoglobin (Hb  $g.L^{-1}$ ) was determined by the standard cyanohemoglobin method. As to the remaining indices of the red blood cell, we determined the mean cell volume (MCV fl), mean cell hemoglobin (MCH pg) and mean cell hemoglobin concentration (MCHC).

The hematological examination was performed on the same date as the blood was sampled.

## Clinical chemistry

The biochemical examination of blood plasma was performed within 24 hours of keeping the plasma at 2 °C. Except the potassium cation ( $K^+$   $mmol.L^{-1}$ ) and sodium cation ( $Na^+$   $mmol.L^{-1}$ ) (flame emission photometry), all indicators were determined on the HITACHI 717 automatic instrument. The following items were also determined: inorganic phosphate (P  $mmol.L^{-1}$ ), calcium ( $Ca^{2+}$   $mmol.L^{-1}$ ), total plasma protein (TPP  $g.L^{-1}$ ), urea (BUN  $mmol.L^{-1}$ ), uric acid (UA  $\mu mol.L^{-1}$ ), creatinine (CREA  $\mu mol.L^{-1}$ ), glucose (GLU  $mmol.L^{-1}$ ), triacyl glycerols (TGL  $mmol.L^{-1}$ ), cholesterol (CHOL  $mmol.L^{-1}$ ) and the activities of alanine aminotransferase (ALT  $\mu kat.L^{-1}$ ) and aspartate aminotransferase (AST  $\mu kat.L^{-1}$ ).

## Assessment of health state and the biometry of organs

To be able to assess reliably the hematological and metabolic tests, all details of each individual's state of health must be known because the pathological, infection or parasitic finding must be taken into account with respect to its potential influence on the blood parameters being examined. Examination of the health of the experimental stocks was performed in monthly intervals, including an examination for the presence of parasites and a patho-anatomic post-mortem examination. The condition coefficient (Fulton) and the Clark formula were calculated at the end of the experiment. In addition, the absolute liver weight (ALW in g) was determined to the nearest of 0.01 g for the calculation of liver somatic index LSI-1 (liver weight to total weight of fish) and of liver somatic index LSI-2 (liver weight to the weight of eviscerated fish).

## Statistical analysis

During the mathematical and statistical processing of the results, the selected sets of the experimental

groups were characterized by arithmetic means, standard deviations and coefficients of variance. Confidence intervals of the arithmetic means of the basic sets were calculated at a significance level of  $P = 0.05$ . The statistical significance of the differences between the arithmetic means was checked by the *t*-test at significance levels of  $P = 0.05$  and  $P = 0.01$ .

## RESULTS AND DISCUSSION

The parasite populations and clinical manifestations of the fish during experiment A and at its end showed no changes, compared to the normal state of health. The initial hematological examination of 10 fishes at a water temperature of 12 °C (dissolved  $O_2$  content of 10.1  $mg.L^{-1}$  and  $O_2$  saturation of 92%) did not indicate any departures from the standard range of physiological values normally recorded in our laboratory for the mentioned age and performance category of fish (RBCc  $1.12 \pm 0.158 T.L^{-1}$ , Hct  $0.434 \pm 0.0384$ , Hb  $73.4 \pm 5.41 g.L^{-1}$ , MCV  $395 \pm 73.5 fl$ , MCH  $66 \pm 9.3 pg$ , MCHC  $0.17 \pm 0.011$ ) (mean  $\pm$  SD).

The metabolic response of the fish body to the composition of feeds with particular respect to the fat component was checked at the end of the experiment by metabolic tests; their results are shown in Table 5.

As to the characteristics of the red blood cell count, attention should mainly be drawn to the significantly ( $P = 0.05$ ) lower value of RBC in EG 1, compared to EG 2 and CG 3. When assessing the protein metabolism, we recorded a significant ( $P = 0.05$ ) and highly significant ( $P = 0.01$ ) decline in total proteinemia in EG 1 and EG 2, compared to the higher and better-balanced values of TPP in CG 3. Cholesterolemia was lower in EG 1 and EG 2 – especially in EG 1 in which the reduction decline was highly significant ( $P = 0.01$ ), compared to CG 3. In the other parameter of lipid metabolism, triacylglycerols, the situation was similar – their level in the blood plasma was significantly ( $P = 0.05$ ) lower in EG1.

What can be considered particularly interesting is AST activity which was higher in the fish given diet 3, compared with both experimental groups, the difference being significant ( $P = 0.05$ ) and highly significant ( $P = 0.01$ ), respectively.

During the testing of high-fat feeds, the tests must include assessment of the state of the liver parenchyma and its biometric characteristics. ALW and LSI-1 and LSI-2 (Table 6) grew with the content of fat in the feed and were significantly ( $P = 0.05$ ) higher in the fish given diet 3 – probably due to greater lipomobilization – but were free of pathological consequences.

Experiment B was aimed at assessing the state of health and changes in the blood of the experimental fish given experimental diets 4 and 5 which had almost the same lipid content but different levels of crude protein. Fish given diet 6 were used to provide reference

Table 5. Hematological and biochemical parameters in 1+ rainbow trout at the end of experiment A (water temperature 2 °C, dissolved O<sub>2</sub> content 14.9 mg.L<sup>-1</sup>, O<sub>2</sub> saturation of water 106%)

Indices		Diets					
		1		2		3	
RBCc	T.L <sup>-1</sup>	1.16 <sup>1</sup> (1-1.32) <sup>3</sup>	0.218 <sup>2</sup> 18.79 <sup>4</sup>	1.05 <sup>a</sup> (0.93-1.17)	0.160 15.24	1.19 (1.1-1.28)	0.118 9.92
Hct		0.399 (0.35-0.448)	0.0644 16.14	0.39 (0.343-0.437)	0.0627 16.08	0.419 (0.394-0.432)	0.0258 6.25
Hb	g.L <sup>-1</sup>	76.6 (69.7-83.5)	9.20 12.01	76.1 (69.2-82.1)	9.14 12.01	79.3 (75.5-83.1)	5.08 6.41
MCV	fl	350 (310-390)	53.7 15.34	371 (349-393)	29.6 7.98	351 (323-379)	37.2 10.6
MCH	pg	67 (61-73)	8 11.94	73 (69-77)	5.4 7.4	67 (62-72)	6.9 10.3
MCHC		0.194 (0.181-0.207)	0.0171 8.81	0.196 (0.186-0.206)	0.0134 6.84	0.192 (0.183-0.201)	0.0121 6.3
TPP	g.L <sup>-1</sup>	41.2 <sup>b</sup> (35.3-47.1)	7.8 18.93	43.4 <sup>a</sup> (37.7-49.1)	17.5 17.44	50.3 (46.9-53.7)	4.47 8.89
BUN	μmol.L <sup>-1</sup>	0.4 (0.3-0.5)	0.1 25	0.3 (0.2-0.4)	0.08 26.67	0.5 (0.4-0.6)	0.13 26
CREA	μmol.L <sup>-1</sup>	18 (16-20)	2.7 15	18 (15-21)	3.6 20	21 (20-22)	1.8 8.57
GLU	mmol.L <sup>-1</sup>	5.1 (4.35-5.85)	0.99 19.41	5.1 (4.1-6.1)	1.28 25.1	6 (3.1-7.5)	2.03 33.83
CHOL	mmol.L <sup>-1</sup>	7.13 <sup>b</sup> (6.12-8.14)	1.335 18.72	7.84 (6.3-9.38)	2.043 26.06	9.12 (8.25-9.99)	1.150 12.61
TGL	mmol.L <sup>-1</sup>	4.83 <sup>a</sup> (3.42-6.24)	1.873 38.78	5.42 (3.23-7.61)	2.91 53.69	7.28 (5.65-8.91)	2.165 29.74
Ca <sup>2+</sup>	mmol.L <sup>-1</sup>	3.19 (2.96-3.42)	0.304 9.53	3.33 (2.86-3.8)	0.62 18.62	3.42 (3.26-3.58)	0.210 6.14
P	mmol.L <sup>-1</sup>	3.97 (3.68-4.26)	0.391 9.85	3.95 (3.67-4.23)	0.371 9.39	4.2 (3.87-4.53)	0.433 10.31
Na <sup>+</sup>	mmol.L <sup>-1</sup>	156 (154-158)	2.2 1.41	156 (154-158)	3.3 2.12	158 (153-163)	6.7 4.24
K <sup>+</sup>	mmol.L <sup>-1</sup>	0.3 (0.25-0.34)	0.06 20	0.5 (0.3-0.7)	0.21 42	0.3 (0.2-0.4)	0.07 23.33
ALT	μkat.L <sup>-1</sup>	0.13 (0.08-0.18)	0.061 46.92	0.15 (0.09-0.21)	0.075 50	0.19 (0.12-0.26)	0.092 48.42
AST	μkat.L <sup>-1</sup>	4.47 <sup>b</sup> (3.16-5.78)	1.735 38.81	5.42 <sup>a</sup> (3.83-7.01)	2.115 39.02	7.93 (6.74-9.12)	1.581 19.94

Note: <sup>1</sup>arithmetic mean, <sup>2</sup>standard deviation <sup>3</sup>confidence interval for parameter  $\mu$  with a 95% probability (Reisenauer, 1965), <sup>4</sup>variance coefficient in %

Values with superscripts <sup>a</sup> and <sup>b</sup> express a significant difference (<sup>a</sup> =  $P = 0.05$ , <sup>b</sup> =  $P = 0.01$ ) between experimental groups 1 or 2 and comparative (reference) group 3

data. No clinical and patho-anatomic changes were recorded during the examination of the fish health, nor were any such changes recorded at the end of the examination. The results of the closing hematological and biochemical examination were well balanced in all parameters except the significantly ( $P = 0.05$ ) lower total proteinemia in EG 4, compared to CG 6. The metabo-

lism of carbohydrates, lipids and minerals was not affected by the diets being tested. Glycemia and the concentration of inorganic phosphate declined and calcium concentration grew during the experiments. Between the start and end of the trials, glucose level significantly ( $P = 0.05$ ) and highly significantly ( $P = 0.01$ ) decreased from  $7.42 \pm 1.957$  mmol.L<sup>-1</sup> to  $4.82 \pm 0.991$  mmol.L<sup>-1</sup>

Table 6. The values of the condition indices for 1+ rainbow trout at the end of experiment A ( $n = 10$ )

Diets	Condition parameters											
	weight (g)		Fulton		Clark		ALW (g)		LSI-1 (%)		LSI-2 (%)	
1	316	25.4	1.65	0.099	1.37	0.089	4.89	1.32	1.54 <sup>a</sup>	0.395	1.86	0.487
	(297-335)	8.04	(1.58-1.72)	6	(1.30-1.44)	6.5	(3.89-5.89)	26.99	(1.24-1.84)	25.65	(1.49-2.23)	26.18
2	341	40.2	1.65	0.135	1.37	0.121	5.48	1.408	1.6 <sup>a</sup>	0.247	1.92 <sup>a</sup>	0.334
	(311-371)	11.79	(1.55-1.75)	8.18	(1.28-1.46)	8.83	(4.42-6.54)	25.69	(1.41-1.79)	15.44	(1.67-2.17)	17.4
3	324	53.9	1.62	0.075	1.34	0.057	6.16	1.54	1.89	0.282	2.28	0.367
	(283-365)	16.64	(1.56-1.68)	4.63	(1.30-1.38)	4.25	(5.00-7.32)	25	(1.68-2.10)	14.92	(2.00-2.56)	16.10

Note: Values with superscripts <sup>a</sup> express a significant difference between the experimental and comparative group ( $P = 0.05$ )

Table 7. Hematological and biochemical parameters in 1+ rainbow trout at the end of trial B (water temperature 9 °C, dissolved O<sub>2</sub> content 9 mg.L<sup>-1</sup>, O<sub>2</sub> saturation 77%)

Indices		Diets					
		4		5		6	
RBCc	T.L <sup>-1</sup>	1.22 (1.08-1.36)	0.185 15.16	1.21 (1.11-1.31)	0.132 10.91	1.20 (1.09-1.31)	0.133 11.08
Hct		0.435 (0.393-0.477)	0.0558 12.83	0.397 (0.365-0.429)	0.0424 10.68	0.408 (0.369-0.447)	0.0479 11.74
Hb	g.L <sup>-1</sup>	71.3 (63.6-79)	10.23 14.35	64.5 (57.9-71.1)	8.79 13.63	63.4 (58.5-68.3)	6.01 9.48
MCV	fl	361 (324-398)	48.9 13.55	330 (306-354)	31.6 9.58	342 (313-371)	36 10.53
MCH	pg	59 (54-64)	6.7 11.36	53 (49-57)	5 9.43	53 (48-58)	6.3 11.89
MCHC		0.164 (0.154-0.174)	0.0136 8.29	0.162 (0.151-0.173)	0.0142 8.77	0.157 (0.145-0.169)	0.0143 9.11
TPP	g.L <sup>-1</sup>	37.6 <sup>a</sup> (33.5-41.7)	5.48 14.57	39.7 (34.3-45.1)	7.12 17.93	42.8 (39.2-46.4)	4.47 10.44
BUN	μmol.L <sup>-1</sup>	0.6 (0.5-0.7)	0.19 31.67	0.6 (0.4-0.8)	0.21 35	0.6 (0.48-0.72)	0.15 25
UA	mmol.L <sup>-1</sup>	5 (2.4-7.6)	3.5 70	5 (2-8)	3.8 76	7 (3-11)	5.2 74.29
CREA	μmol.L <sup>-1</sup>	28 (23-33)	7.1 25.36	28 (25-31)	4.6 16.43	27 (21-33)	7.6 28.15
GLU	mmol.L <sup>-1</sup>	6.27 (3.84-8.7)	2.11 33.65	4.82 (3.68-5.96)	0.991 20.56	4.84 (3.73-5.95)	1.246 25.74
CHOL	mmol.L <sup>-1</sup>	12.65 (10.47-14.83)	1.889 15.01	10.05 (7.9-12.2)	1.873 18.64	8.6 (4.85-12.35)	4.198 48.81
TGL	mmol.L <sup>-1</sup>	4.45 (2.57-6.33)	1.631 36.65	413 (3.51-4.75)	0.538 13.03	5.19 (3.1-7.28)	2.343 45.14
Ca <sup>2+</sup>	mmol.L <sup>-1</sup>	3.38 (3.2-3.56)	0.244 7.22	3.34 (3.11-3.57)	0.304 9.1	3.58 (3.26-3.9)	0.395 11.03
P	mmol.L <sup>-1</sup>	5.73 (4.78-6.68)	1.266 22.09	5.96 (4.94-6.98)	1.354 22.72	5.28 (4.49-6.07)	0.963 18.24
Na <sup>+</sup>	mmol.L <sup>-1</sup>	155 (153-157)	2.7 1.74	155 (153-157)	2.2 1.42	155 (153-157)	2.5 1.61
K <sup>+</sup>	mmol.L <sup>-1</sup>	0.7 (0.3-1.1)	0.5 71.43	0.8 (0.2-1.4)	0.73 91.25	0.5 (0.3-0.7)	0.26 52

Note: Value with superscript <sup>a</sup> express a significant difference ( $P = 0.05$ ) between experimental group 4 and comparative group 6

(EG 5) and  $4.84 \pm 1.246 \text{ mmol.L}^{-1}$  (CG 6). Inorganic phosphate concentration significantly ( $P = 0.05$ ) declined from  $9.89 \pm 4.679 \text{ mmol.L}^{-1}$  to  $5.73 \pm 1.266 \text{ mmol.L}^{-1}$  (EG 4),  $5.96 \pm 1.354$  (EG 5) and  $5.28 \pm 0.963 \text{ mmol.L}^{-1}$  (CG 6). Calcium concentration significantly ( $P = 0.05$ ) grew from  $3.03 \pm 0.191 \text{ mmol.L}^{-1}$  to  $3.38 \pm 0.244 \text{ mmol.L}^{-1}$  (EG 4) and  $3.34 \pm 0.304 \text{ mmol.L}^{-1}$  (EG 5) and highly significantly ( $P = 0.01$ ) to  $3.58 \pm 0.395 \text{ mmol.L}^{-1}$  (CG6). As to the remaining hematological (RBCc, Hct, Hb, MCV, MCH, MCHC) and biochemical parameters (TPP, BUN, UA, CREA, CHOL, TGL,  $\text{Na}^+$ ,  $\text{K}^+$ ), no statistically significant changes occurred between the opening and closing examination.

Evaluating the results of biochemical tests in both experiments, it can be said that the level of protein and lipid metabolism corresponded to the contents of protein and lipid components in the feed. The strongest metabolic response of the fish body to the composition of the feed ration was recorded in experiment A by the fish given diet 3, which had the highest levels of TPP, BUN, CREA, GLU, CHOL and TGL in their blood plasma. For the assessment of lipid metabolism, similar results were recorded, under Czech conditions, by Jirásek *et al.* (1989), who tested five types of feed: in those experiments, the best-growing experimental group of fish, fed the EWOS ST-40 pellets with a much higher content of energy, showed the highest CHOL and TGL levels. Changes in the lipid components of the blood plasma in relation to the different types of feeds were also described by Pravda and Paláčková (1989).

A somewhat different situation was recorded in the case of calcium metabolism. The concentration of calcium ions was lower in the blood plasma of the fish of both groups, though the calcium level in the feed was higher than in diet 3 ( $28.2$  and  $27$  vs  $12.9 \text{ g.kg}^{-1}$ ). The well-balanced results of hematological and biochemical parameters in experiment B are in keeping with the corresponding growth potential and feed conversion rates; they have also indicated how suitable it is to use metabolic tests for the control of the internal environment of the experimental fish in experiments focused on providing an optimum balance of dietary fat at the expense of protein, while respecting the energy require-

ments of rainbow trout and while also respecting the environmental interests, i.e. the need to minimize environmental exposures of the aquatic environment downstream from the trout farms.

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# ASSESSING OF LIMITS FOR THE DEPOSITION OF PROTEINS IN THE BODIES OF PIGS OF FOUR HYBRID COMBINATIONS

## STANOVENÍ MEZE UKLÁDÁNÍ BÍLKOVIN V TĚLE PRASAT ČTYŘ HYBRIDNÍCH KOMBINACÍ

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**ABSTRACT:** The limit for the deposition of proteins was set by increasing levels of protein nutrition (CP, LYS) in metabolic trials on 32 pigs with an average weight of 46 kg, including four hybrid combinations (LW x L x L85; x L x LW<sub>f</sub>; LW x L x DU; LW x L x CMP). We studied the digestibility of N, the utilization of N from N received and digested N and the daily deposits of proteins (CP) in the bodies of pigs. No unambiguous and conclusive differences were found in the digestibility of N between the experimental groups (4 levels of CP and LYS) or between the used hybrid combinations. The level of utilization of N was valid in particular for the genotype of experimental pigs. A limit was set for the deposition of CP at the level of 165 g per head and day for combination 1 (LW x L x L85), at the level of 147–150 g per head and day for combinations 2 and 3 (LW x L x LW<sub>f</sub>; LW x L x DU) and at the level of 120 g per head and day for combination 4 (LW x L x CMP). To ensure intensive nutrition for growing pigs of these hybrid combinations, there is a requirement for LYS of 13.3 g per 1 kg of mixture for combinations 1 and 3, 12.0 g of LYS per 1 kg of mixture for combination 2 and 10.5 g of LYS per 1 kg of mixture for combination 4.

**Keywords:** Large White – father's line of descent; Landrace; Piétrain; Czech Meaty Pig; Duroc; genotype; LYS demand; CP digestibility; N balance

**ABSTRAKT:** V bilančních pokusech s 32 vepřiky o průměrné hmotnosti 46 kg čtyř hybridních kombinací (BU x L x L85; BU x L x BU<sub>o</sub>; BU x L x DU; BU x L x ČVM) byla metodou stupňované úrovně bílkovinné výživy (NL, LYS) stanovena mez ukládání bílkovin. Sledována byla stravitelnost dusíku, využití dusíku z dusíku přijatého a stráveného a denní ukládání bílkovin (NL) v těle prasat. Ve stravitelnosti dusíku nebyly nalezeny jednoznačné a průkazné rozdíly mezi pokusnými skupinami (4 hladiny NL a LYS) ani mezi použitými hybridními kombinacemi. Úroveň využití dusíku byla poplatná především genotypu pokusných prasat. Byla stanovena mez ukládání NL na úrovni 165 g na kus a den u kombinace 1 (BU x L x L85), na úrovni 147–150 g na kus a den u kombinací 2 a 3 (BU x L x BU<sub>o</sub>; BU x L x DU) a na úrovni 120 g na kus a den u kombinace 4 (BU x L x ČVM). Pro intenzivní výživu rostoucích prasat těchto hybridních kombinací činí potřeba LYS u kombinace 1 a 3 – 13,3 g na 1 kg směsi, u kombinace 2 – 12,0 g LYS na 1 kg směsi a u kombinace 4 – 10,5 g LYS na 1 kg směsi.

**Klíčová slova:** bílé ušlechtilé – otcovská linie; landrace; piétrain; české výrazně masné; duroc; genotyp; potřeba LYS; stravitelnost NL; bilance N

### INTRODUCTION

Both, genetic methods and positive influences on environmental factors, result in more efficient pig management. At the same time, the animal genotype needs adequate environmental conditions for the optimum manifestation of genetically fixed abilities. Nutrition is one of the priority factors of the environment; there is an important linkage to the genotype as it determines genetically fixed abilities to deposit nutrients and energy (mainly in the form of proteins and fat), which should correspond to the nutrition level.

The identification of these abilities in relatively homogeneous groups of growing pigs with most commonly used hybrid combinations is desirable not only for effective breeding and improvement work but also for the establishment of objective conditions for the nutrition of these pigs.

The objective of this study was to determine the genetically given limit for the deposition of proteins in the bodies of growing pigs of four hybrid combinations (LW x L x L85; LW x L x LW<sub>f</sub>; LW x L x DU; LW x L x CMP) as an important feature of the genotype characteristics and as a criterion for determining the biologically effective conditions of their nutrition.

It is clear from Prokop's (1994a) results that if we compare two genotypes and two levels of nutrition, it is the genotype which dominates. This is caused by the ability of the more effective genotype to deposit a higher amount of proteins in the body.

The deposition of substances and energy in the body – mainly in the form of CP and fat – mostly depends on the intake of lysine and other amino acids, on energy intake and the mutual relations of both indicators. Hanrahan (1989) demonstrates that it is possible to increase the ratio of lean meat in the body by adding lysine to the mixture for pigs, however, the productivity of the mixtures can only be perfected to a certain point and therefore any further increase in nutrition intensity is ineffective.

Schulz (1993) recommends a daily intake between 220 and 340 g of CP for pigs weighing 20–105 kg.

It appears from what has been mentioned that the contents of CP are subsidiary to the dominant contents of lysine and energy in mixtures for pigs. This secondary implication is relevant although only to a certain point. This is proven by the well-known principle of the ideal protein, i.e. decreasing the contents of CP in the mixture while adding lysine and other essential amino acids (threonine, sulphuric amino acids, tryptophane) in an optimum ratio at the same time. This also makes it possible to decrease the excretion of nitrogen into the environment by 15–20% (Prokop, 1994b). The effect of both these possibilities (higher contents of CP; lower contents of CP + amino acids) was observed on pigs of two different genotypes during fattening by Stahly *et al.* (1993). It is clear from these results that a high-protein diet is more effective for the more efficient genotype, a low-protein diet with the addition of amino acids is more effective for the less efficient genotype.

The above-mentioned information demonstrates the significance of genotype and nutrition and their mutual relations. It is relevant to state what indicators are important for the assessment of fatted pigs. The most important is fattening performance, the nutritional equivalent of which is the productivity of mixtures. This refers to the increase of live weight and to the conversion of mixtures. Another general indicator is the carcass value. Here this refers to the dressing percentage, back fat thickness and the contents of lean meat in the body (instrumentally), or to the ratio of mainly meaty parts as the case may be. The third general indicator is the quality of meat (or fat as well). That can have a variety of physical and chemical criteria. As Ducos *et al.* (1993) suggest, this factor is assessed in France by a defined index of meat quality.

The genetic contingency of high suitability for fattening and carcass value is nutritionally represented by the ability to deposit proteins in the body. This parameter not only provides another defined genotype characteristic but also allows for exact nutrition strategy of these pigs during fattening.

## MATERIAL AND METHODS

Eight pigs of the chosen hybrid combination were included in each of the four metabolic experiments. These pigs were half-siblings (common father, different mothers). All experiments were carried out by the method of Latin square (2 x 4 x 4), i.e. in each group, 2 pigs in four repeated balance periods (8 data entries) with a gradual changing of four experimental groups (mixtures). Every experimental intervention was therefore checked on all eight experimental pigs placed in balance cages which were adapted as needed during the pigs' growth. The average weight of the pigs in the experimental group and the balance period is listed in Tables 2b–5b. Experiment (combination) 3 (LW x L x DU) which was carried out on pigs with an average weight of 60 kg was an exception and even despite a certain error, it was possible to interpolate the results reached (noted in the next chapter and in tables) about the intake of feed mixture to the weight of 46 kg. This is because there is no linear dependence of simple conversion on the feed intake at different weights.

Table 1. The composition of experimental mixtures and their nutritional value

Component	Unit	Mixture – group			
		1	2	3	4
Barley	%	37.20	40.00	40.00	41.50
Wheat	%	51.17	42.58	36.29	28.44
Soyabean meal	%	4.00	7.00	11.00	16.00
Meat and bone meal	%	2.00	4.40	6.00	7.00
Wheat bran	%	–	1.50	3.00	4.00
MKP PV	%	2.70	1.70	1.00	0.50
Premix LYS HCl (20 %)	%	1.68	1.60	1.46	1.31
Premix THR (20 %)	%	0.75	0.72	0.75	0.75
Bioviton P1 plus	%	0.50	0.50	0.50	0.50
		100.00	100.00	100.00	100.00
NL	g/kg	146	172	196	208
ME	MJ/kg	12.9	12.9	13.0	12.9
Fiber	g/kg	45	47	44	49
LYS	g/kg	8.9	10.5	12.0	13.3
THR	g/kg	5.2	5.8	7.1	8.4
MET	g/kg	2.4	2.6	2.9	3.4
CYS	g/kg	2.6	2.9	3.4	3.9
Ca	g/kg	9.6	9.7	9.8	9.6
P	g/kg	6.4	6.9	7.4	7.6
LYS from CP	%	6.1	6.1	6.1	6.4
LYS : ME	–	0.69	0.81	0.92	1.03
THR : LYS	%	58	55	59	63
MET + CYS : LYS	%	56	52	52	55

1 kg of MKP PV contains: 251 g Ca, 72 g P, 1 g Mg and 62 g Na  
 1 kg of premix Bioviton P1 plus contains: 1 400 000 i.u. vitamin A, 250 000 i.u. vitamin D<sub>3</sub>, 3 500 mg vitamin E, 700 mg vitamin B<sub>2</sub>, 300 mg vitamin B<sub>6</sub>, 4 mg vitamin B<sub>12</sub>, 3 000 mg niacin, 2 100 mg calcium pantothenate, 20 000 mg choline, 280 000 mg L-lysine HCl

In all the experiments the same feed mixtures were used. Their contents and nutritive values are listed in Table 1. These mixtures were given to the experimental animals 2x a day, faeces and urine were collected once a day and was always completed at the end of each period. The collected excrements were then weighed, a sample was removed, dried and prepared for other analyses. All analyses for determining the digestibility of nutrients and amino acids were carried out in accordance with Regulation No. 222/1996 of the Ministry of Agriculture of the Czech Republic which determines the requirements for sample collection and the methods of laboratory tests for feed, additives and premixes.

We also monitored the complete balance of N (received N, N output in faeces and N output in urine). The potential utilization of lysine was calculated approximately according to Bikker *et al.* (1994), as a very significant figure for the factorial calculation of its optimum requirement. On the basis of this calculation, we also specify how much lysine was received and how much was deposited. From the basic values we list the daily intake of crude protein and the average intake of feed in balance in all tables for better clarity. All other values mentioned in tables are analytical, energy was converted from the nutrients in the feed mixture.

## RESULTS AND DISCUSSION

In experiment 1 (combination LW x L x L85) which concerns the increasing levels of CP and amino acids, the digestibility of N grows, even if inconclusively.

The utilization of N from the N received does not show any significant differences and that is the reason for the differences shown in the utilization of N from the digested N. However, the important thing is that the absolute retention of CP grows significantly up to group (mixture) 3 and further increase of this value in group 4 is insignificant (Table 2). Therefore, at this level of CP and amino acids, it is possible to say that the limit of utilizing CP has been reached. Considering the real average weight of pigs is 46 kg, this is a very good result.

In experiment 2 (combination LW x L x LW<sub>p</sub>), no significant differences were found in the digestibility of N or in the relative utilization of N. The deposition of CP per head and day grew along with the growing ration of CP and amino acids in mixtures. The limit of utilization of CP was nevertheless reached at the level of the third group; the deposition of CP did not change in group 4 (Table 3).

In experiment 3 (combination LW x L x DU) we used pigs with an average weight of 60 kg, which strongly influenced the results of balance. No significant differences were found in the relative values, i.e. digestibility and relative utilization of N (Table 4). However, the absolute values of CP deposition were high due to the higher intake of experimental mixtures, exceeding 200 g of CP per head and day at the highest levels of CP and amino acids. To facilitate the comparison of results from other experiments, the reached results of CP deposition were interpolated with the weight of pigs of 46 kg. Evidently, in the case of this

Table 2a. Digestibility and balance of N in the combination LW x L x L85 (trial 1)

Indicator	Unit	Groups - mixtures (n = 8)			
		1	2	3	4
		$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Daily intake of CP	g	230.62 ± 19.66 <sup>A</sup>	253.37 ± 23.17	311.14 ± 30.81 <sup>b</sup>	326.63 ± 21.87 <sup>B</sup>
Daily intake of feed	kg	1.58 ± 0.13	1.42 ± 0.13	1.59 ± 0.16	1.57 ± 0.11
Digestibility of N	%	81.74 ± 2.13	82.36 ± 1.30	83.12 ± 0.95	84.48 ± 1.13
Utilization of N from received N	%	52.99 ± 1.21	51.21 ± 2.16	49.23 ± 2.54	52.10 ± 2.01
Utilization of N from N digested	%	63.11 ± 1.16	62.00 ± 2.10	59.19 ± 2.81	61.56 ± 1.87
CP deposited per head and day	g	122.84 ± 12.18 <sup>a</sup>	127.75 ± 9.90 <sup>a</sup>	150.37 ± 13.05	165.21 ± 13.98 <sup>b</sup>
Received lysine	g	14.06 ± 1.19 <sup>aA</sup>	14.86 ± 1.36 <sup>A</sup>	19.05 ± 1.88 <sup>b</sup>	20.89 ± 1.39 <sup>B</sup>
Deposited lysine	g	8.68 ± 0.86 <sup>a</sup>	9.03 ± 0.70 <sup>a</sup>	10.63 ± 0.92	12.09 ± 0.97 <sup>b</sup>
Utilization of lysine	%	61.46 ± 1.40	61.72 ± 2.60	56.85 ± 2.93	57.60 ± 2.22

a, b =  $P < 0.05$ , A, B =  $P < 0.01$

Table 2b. Average live weight of animals in metabolic trials in a group and period (in kg)

Group (mixture)	1	2	3	4
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Average live weight	51.45 ± 3.58	51.90 ± 4.69	52.26 ± 4.66	51.61 ± 3.77
Period	A	B	C	D
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Average live weight	40.25 ± 2.29	47.15 ± 2.43	57.21 ± 2.78	62.61 ± 2.97

Table 3a. Digestibility and balance of N in the combination LW x L x LWO (trial 2)

Indicator	Unit	Groups – mixtures (n = 8)			
		1	2	3	4
		$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Daily intake of CP	g	182.55 ± 11.25 <sup>A</sup>	221.43 ± 17.90	261.22 ± 21.50 <sup>B</sup>	261.83 ± 19.64 <sup>B</sup>
Daily intake of feed	kg	1.25 ± 0.08	1.24 ± 0.10	1.33 ± 0.11	1.26 ± 0.09
Digestibility of N	%	81.92 ± 2.38	81.88 ± 1.34	81.88 ± 1.13	79.72 ± 1.29
Utilization of N from received N	%	57.84 ± 1.07	57.51 ± 1.72	55.80 ± 2.01	55.48 ± 2.24
Utilization of N from N digested	%	70.61 ± 1.17	66.78 ± 3.82	69.00 ± 1.72	69.48 ± 2.10
CP deposited per head and day	g	106.03 ± 7.65 <sup>a</sup>	128.61 ± 12.76	147.34 ± 15.63 <sup>b</sup>	147.83 ± 16.16 <sup>b</sup>
Received lysine	g	11.13 ± 0.69 <sup>A</sup>	12.99 ± 1.05 <sup>a</sup>	15.99 ± 1.32 <sup>B</sup>	16.74 ± 1.26 <sup>B</sup>
Deposited lysine	g	7.50 ± 0.54 <sup>a</sup>	9.09 ± 0.90	10.42 ± 1.11 <sup>b</sup>	10.45 ± 1.14 <sup>b</sup>
Utilization of lysine	%	67.08 ± 1.24	69.31 ± 2.07 <sup>a</sup>	64.44 ± 2.32	61.34 ± 2.48 <sup>b</sup>

a, b =  $P < 0.05$ , A, B =  $P < 0.01$ 

Table 3b. Average live weight of animals in metabolic trials in a group and period (in kg)

Group (mixture)	1	2	3	4
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Average live weight	44.00 ± 2.54	43.85 ± 3.65	44.88 ± 3.35	44.93 ± 3.50
Period	A	B	C	D
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Average live weight	35.70 ± 1.55	40.85 ± 1.77	47.08 ± 2.30	54.03 ± 2.40

Table 4a. Digestibility and balance of N in the combination LW x L x DU (trial 3)

Indicator	Unit	Groups – mixtures (n = 8)			
		1	2	3	4
		$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Daily intake of CP	g	284.51 ± 17.83 <sup>aA</sup>	310.70 ± 18.54 <sup>a</sup>	356.52 ± 21.76 <sup>b</sup>	378.35 ± 23.27 <sup>B</sup> <sup>b</sup>
Daily intake of feed	kg	1.95 ± 0.12	1.74 ± 0.10	1.82 ± 0.11	1.82 ± 0.11
Digestibility of N	%	82.93 ± 0.86	81.57 ± 0.75	81.42 ± 1.01	81.18 ± 1.07
Utilization of N from received N	%	54.06 ± 2.00	55.93 ± 1.66	52.80 ± 3.19	55.16 ± 1.21
Utilization of N from N digested	%	64.94 ± 3.42	68.12 ± 2.25	64.73 ± 3.58	68.05 ± 1.66
CP deposited per head and day	g	152.80 ± 8.53 <sup>A</sup>	172.95 ± 9.56	187.70 ± 15.18	208.86 ± 14.10 <sup>B</sup>
(correlation to the weight of 46 kg)	g	110.0	124.6	135.1	150.0
Received lysine	g	17.34 ± 1.09 <sup>Aa</sup>	18.23 ± 1.09 <sup>A</sup>	21.83 ± 1.33 <sup>b</sup>	24.19 ± 1.49 <sup>B</sup>
Deposited lysine	g	10.80 ± 0.60 <sup>A</sup>	12.23 ± 0.68	13.27 ± 1.07	14.77 ± 2.82 <sup>B</sup>
Utilization of lysine	%	62.70 ± 2.32	67.42 ± 2.01 <sup>a</sup>	60.97 ± 3.69	60.99 ± 1.34 <sup>b</sup>

a, b =  $P < 0.05$ , A, B =  $P < 0.01$ 

Table 4b. Average live weight of animals in metabolic trials in a group and period (in kg)

Group (mixture)	1	2	3	4
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Average live weight	62.09 ± 4.04	61.95 ± 3.96	62.36 ± 3.70	63.88 ± 3.65
Period	A	B	C	D
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Average live weight	50.63 ± 1.48	57.64 ± 1.73	66.25 ± 1.29	75.76 ± 1.38

Table 5a. Digestibility and balance of N in the combination LW x L x CMP (trial 4)

Indicator	Unit	Groups – mixtures (n = 8)			
		1	2	3	4
		$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Daily intake of CP	g	191.22 ± 11.59 <sup>a</sup>	229.65 ± 19.29	245.58 ± 19.51 <sup>b</sup>	249.47 ± 24.63 <sup>b</sup>
Daily intake of feed	kg	1.31 ± 0.08	1.28 ± 0.11	1.25 ± 0.10	1.20 ± 0.12
Digestibility of N	%	79.88 ± 2.65	82.22 ± 1.16	80.26 ± 4.93	83.31 ± 5.16
Utilization of N from received N	%	45.81 ± 1.78 <sup>a</sup>	51.98 ± 1.42 <sup>b</sup>	49.10 ± 2.74	46.28 ± 2.94
Utilization of N from N digested	%	57.37 ± 2.21 <sup>a</sup>	63.20 ± 1.64 <sup>b</sup>	59.78 ± 2.94	56.96 ± 2.68
CP deposited per head and day	g	88.11 ± 7.76 <sup>a</sup>	118.83 ± 9.69 <sup>b</sup>	120.71 ± 11.72 <sup>b</sup>	118.01 ± 16.53
Received lysine	g	11.66 ± 0.71 <sup>a</sup>	13.47 ± 1.13	15.04 ± 1.19 <sup>b</sup>	15.95 ± 1.57 <sup>b</sup>
Deposited lysine	g	6.23 ± 0.55 <sup>a</sup>	8.40 ± 0.69 <sup>b</sup>	8.53 ± 0.83 <sup>b</sup>	8.34 ± 1.17
Utilization of lysine	%	53.13 ± 2.07 <sup>A</sup>	62.65 ± 1.72 <sup>BA</sup>	56.70 ± 3.17	51.17 ± 3.25 <sup>B</sup>

a, b =  $P < 0.05$

Table 5b. Average live weight of animals in metabolic trials in a group and period (in kg)

Group (mixture)	1	2	3	4
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Average live weight	41.66 ± 2.20	42.08 ± 3.39	43.21 ± 4.50	42.63 ± 3.05
Period	A	B	C	D
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Average live weight	32.93 ± 1.77	39.24 ± 1.86	45.39 ± 2.18	52.03 ± 2.45

experiment it is not possible to identify reliably the limit of depositing CP as the course of values basically gets a linear character in the chronology of groups 1–4. It is therefore unclear whether the 150 g value of CP deposition per head and day is final for a pig weighing 46 kg or whether it would further grow in the case of higher intake of CP and amino acids.

In experiment 4 (combination LW x L x CMP) a significant variability was found in the digestibility of N as well as in the relative utilization of N. The significant difference in the relative utilization of N between group 1 and 2 was conclusive (Table 5). The data concerning the deposition of CP demonstrate that the pigs involved were mostly of an average genotype because the limit of utilization was already reached at the second level of CP and amino acids with the CP deposition values in groups 2–4 at 118–120 g per head and day.

It is obvious from all the results that, with the exception of the combination LW x L x CMP (experiment 4), the pigs involved had a genotype of above the average with high growth ability. It is also possible to deduce from the results that the effective level of pigs' nutrition with combination LW x L x L85 and LW x L x DU is reached in mixture 4 (contains 13.3 g of LYS in 1 kg), combination LW x L x LW<sub>f</sub> in mixture 3 (contains 12 g of LYS in 1 kg) and combination LW x L x CMP in mixture 2 (contains 10.5 g of LYS in 1 kg). These facts (experiment 2 and 4) only partially correspond with present recommendations (Šimeček *et al.*,

2000) and they do not even correspond in data concerning super-meaty pigs.

**Key for abbreviations:** LW – Large White; L – Landrace; L85 – line 85 (combination of Piétrain and Czech Meaty Pig); LW<sub>f</sub> – father's line of descent; DU – duroc; CMP – Czech Meat Pig; CP – crude protein; N – nitrogen; LYS – lysine; THR – threonine; MET – methionine; CYS – cystine; TRP – tryptophane

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# THE EFFECT OF FEED MIXTURES WITH DIFFERENT PROTEIN AND FAT CONTENTS ON PRODUCTION AND CHEMICAL COMPOSITION OF THE BODY OF JUVENILE AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

## VLIV KRMNÝCH SMĚSÍ S ROZDÍLNÝM OBSAHEM PROTEINU A TUKU NA PRODUKCI A CHEMICKÉ SLOŽENÍ TĚLA JUVENILNÍHO SUMEČKA AFRICKÉHO (*CLARIAS GARIEPINUS*)

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**ABSTRACT:** The African catfish *Clarias gariepinus* (Burchell, 1822) is considered as the most suitable species for intensive aquaculture in lukewarm waters due to its physiological characteristics and delicious meat. The objective of the paper was to compare the effect of available feeds for the culture of trout, catfish and carp on the growth rate and resultant chemical composition of the body in African catfish. The first experiment was conducted to test two feeds with different contents of proteins and fat (PA2 – declared nutrient content 47% proteins/12.5 % fat, SA2 – 49/14) in fish with two initial weights (12.5 and 20.2 g). The performance parameters were positively influenced by feed with higher protein and fat content (SA2). The values of specific growth rate in experimental fish of both weight categories receiving this feed were higher in the first stage (20 days) by 2.3% (fish with lower initial weight – 3.06%/d) and by 2.9% (fish with higher initial weight – 3.86%/d) than in the other group (SGR 2.99 and 3.75%/d). But the differences in fish growth rate were not statistically significant. A similar trend was observed in the second stage of experiment (22 days). The use of feeds with higher protein and fat content (SA2) resulted in an increase in hepatosomatic index (HSI) in both smaller and larger fish (by 40.1% and 10.6%, respectively) in the first stage of experiment and in higher fat deposition in muscle (by 13.3 and 28.5%, resp.) at the end of experiment. The second experiment was carried out to test feeds for the production of trout (P2 – 42/13), catfish (S2 – 42/10) and carp (K2 – 30/14) in fish with initial weight 76.2 g. In the first stage of experiment (35 days), the best results were determined in fish receiving feed S2 (42/10 – SGR 1.44%/d, FCR 1.04), the worst in fish on diet K2 (30/14 – SGR 0.56%/d, FCR 3.32). Average individual weight of fish receiving feed K2 was demonstrably lower than in the other variants ( $P < 0.01$ ) and was accompanied by the largest decrease in deposition of intramuscular fat (by 38.7% in females, by 29.6% in males) and visceral fat (by 60.6% in males, by 51.3% in females) in comparison with the values determined at the beginning of experiment. In the second stage of experiment (35 days), slower growth rate and lower feed conversion ratio were observed in the first (S2) and in the second variant (P2) while these parameters had higher values in the third variant when feed K2 was replaced by feed S2. The lower feeding intensity (1.5% of fish weight) resulted in further decrease in fat deposition and adipose tissue proportion in fish viscera in all variants.

**Keywords:** *Clariidae*; feeding; nutrition; growth; proteins; fat

**ABSTRAKT:** Cílem práce bylo porovnat vliv dostupných krmiv, určených pro chov pstruha, sumce a kapra, na růst sumečka afrického *Clarias gariepinus* (Burchell, 1822) a na výslednou kvalitu jeho masa. Celkem byly provedeny dva pokusy rozdělené do dvou etap, ve kterých byla denně sledována spotřeba krmiva a týdenní přírůstek ryb. První pokus byl prováděn na rybách o dvou rozdílných počátečních hmotnostech (12,5 a 20,2 g), které byly nasazeny po 60 ks do 70litrových akvárií. Testována byla dvě krmiva s rozdílným obsahem N-látek a tuku (PA2 – deklarovaný obsah živin 47 % N-látek/12,5 % tuku, SA2 – 49/14). V první etapě (20 dní) bylo krmeno intenzitou 4 % hmotnosti obsádky, ve druhé (22 dní) 3 % hmotnosti obsádky. Ve druhém pokusu byly do pokusných nádrží nasazeny ryby o průměrné počáteční hmotnosti 76,2 g (po 30 ks). Testována byla krmiva určená pro chov sumce (S2 – 42/10), pstruha (P2 – 42/13) a kapra (K2 – 30/14). Obě etapy pokusu trvaly 35 dní, přičemž v první etapě bylo krmeno intenzitou 2 % a ve druhé 1,5 % hmotnosti ryb. Vzhledem k nízké produkční účinnosti krmiva K2 bylo toto krmivo ve druhé etapě pokusu nahrazeno krmivem S2. V prvním pokusu se pozitivně projevil vliv krmiva s vyšším obsahem N-látek a tuku (SA2). Pokusné ryby obou hmotnostních kategorií, kterým bylo předkládáno toto krmivo, dosáhly v první etapě o 2,3 % (ryby o nižší počáteční hmotnosti – 3,06 %/d), resp. o 2,9 % (ryby o vyšší počáteční hmotnosti – 3,86 %/d) vyšší specifické rychlosti růstu ve srovnání s druhou variantou (SGR 2,99 a 3,75 %/d). Obě

krmiva vedla k vyšší depozici tuku ve vnitřnostech (o 33,1 %, resp. 51,8 %). Obdobný trend byl pozorován také ve druhé etapě pokusu. Pokusné ryby, kterým bylo předkládáno krmivo s vyšším obsahem N-látek a tuku (SA2), dosáhly ve srovnání s druhou variantou o 5,2 %, resp. 0,4 % vyšší SGR. Rozdíly mezi pokusnými variantami však nebyly ani v jedné etapě statisticky průkazné. Ve všech variantách přitom byla zjištěna vyšší depozice tuku ve svalovině ryb (o 11,0 až 67,4 %) ve srovnání s první etapou. Vyšší uložení tuku ve svalovině bylo na konci pokusu pozorováno u ryb krmených směsí SA2 (o 13,3 % u menších ryb, o 28,5 % u větších ryb) než u ryb, kterým byla předkládána směs PA2. Ve druhém pokusu byly v první etapě dosaženy nejlepší výsledky u ryb krmených směsí S2 (42/10 – SGR 1,44 %/d, FCR 1,04) a nejhorší u ryb krmených směsí K2 (30/14 – SGR 0,56 %/d, FCR 3,32). Průměrná individuální hmotnost ryb krmených směsí K2 byla prokazatelně nižší ve srovnání s ostatními variantami ( $P < 0,01$ ). Ve druhé části pokusu bylo pozorováno v první (S2) a druhé (P2) variantě zpomalení růstu a zhoršení koeficientu konverze krmiva, zatímco ve třetí variantě, ve které bylo krmivo K2 nahrazeno krmivem S2, naopak zlepšení sledovaných ukazatelů. V první etapě druhého pokusu bylo pozorováno ve všech variantách snížení depozice tuku ve svalovině ryb o 2,0 až 38,7 %, přičemž k nejvyššímu snížení došlo u ryb krmených směsí K2 (u jikernaček o 38,7 %, u mlíčáků o 29,6 %). Ve všech variantách bylo rovněž zjištěno snížení depozice tuku ve vnitřnostech. Největší pokles byl zaznamenán opět ve variantě krmené směsí K2 (u mlíčáků o 60,6 %, u jikernaček o 51,3 %). Ve druhé etapě pokusu došlo ke snížení obsahu tuku ve svalovině ryb u jikernaček v první a druhé variantě (o 9,5 a 35,1 %) a u mlíčáků v první a třetí variantě (o 18,9 a 16,9 %). Nižší intenzita krmení (1,5 % hmotnosti ryb) se projevila dalším snížením depozice tuku a podílu tukové tkáně ve vnitřnostech ryb ve všech variantách.

**Klíčová slova:** *Clariidae*; krmení; výživa; růst; N-látky; tuk

## INTRODUCTION

The African catfish *Clarias gariepinus* (Burchell, 1822) is considered as the most suitable species for intensive aquaculture in lukewarm waters due to its physiological characteristics and delicious meat (Wedekind, 1991). Great attention has been focused on its culture in the last 20 years, consisting in breeders interest in the culture of African catfish and its hybrids (Jantrarotai and Boonman, 1996) in intensive aquacultures in many countries of Africa, Europe and Asia.

To ensure the efficient culture of African catfish it is necessary to know its nutritional requirements for contents and sources of dietary proteins, lipids and saccharides. Dietary protein content in the range of 25–49% is recommended for African catfish while its requirements for protein content decrease with growing age and falling temperature (Filipiak *et al.*, 1993b). Diets should be based on proteins from animal sources (Degani *et al.*, 1989). Various authors indicate different requirements for lipid content. Maximum recommended dietary lipid content is mostly up to 10% but Filipiak *et al.* (1993a) determined an optimum need of dietary lipids for African catfish weighing 58 g at a 15% level. Lipid utilization is largely influenced by their source and composition (Hoffman and Prinsloo, 1995). Requirements for saccharide content were tested in a hybrid of African catfish with a related Asian species (*Clarias macrocephalus*). Jantrarotai *et al.* (1994) determined an optimum dietary saccharide to lipid ratio: 3.83–11.24 at lipid content 9.6–4.42%.

Fish meat can be evaluated from several aspects. With respect to storage and processing, intramuscular fat content is a crucial criterion for fish meat evaluation. The African catfish is a fish species with intermediate fat content (1–5%). A lower content is required in fish to be marketed in fresh condition while a higher intramuscular fat content is claimed in fish to be processed

by smoking. Fat content in the African catfish muscle is influenced by feeding intensity, age, sex and other factors (Wedekind, 1991).

The objective of the paper was to evaluate the effect of available feeds for the production of trout, catfish and carp on the growth of African catfish and on the final quality of its meat.

## MATERIAL AND METHOD

Experiments were carried out in experimental breeding facilities of the Institute of Fisheries and Hydrobiology at Mendel University of Agriculture and Forestry at Brno. Experimental fish came from our own culture. Feed consumption was recorded daily and fish increments weekly in the course of experiments. Available feeds produced for the culture of trout, catfish and carp were tested.

The content of dissolved oxygen in water ranged from 60% to 90% saturation, water pH value was 7.5–7.8. The content of ammonia nitrogen in water was lower than 0.5 mg/l, the content of nitrate nitrogen did not exceed 50 mg/l.

### Experiment A

Two feeds of ALMA company were tested in experiment A: they had different contents of proteins and fat but the same size of pellets (1.7–2.0 mm). One feed was to be used for trout production (PA2 – declared nutrient content 47% proteins/12.5% fat, code 6008), the other for catfish production (SA2 – 49/14, code 6408). The experiment on fish with average initial weights 12.5 and 20.2 g had two replications; the fish were stocked into 70 liter aquaria containing 60 individuals each.

Water temperature ranged from 22.9 to 27.9 °C in the course of experiment (mean 25.9 ± 1.4 °C). Experimental fish were fed four times a day every four hours. The experiment had two stages. Feeding intensity was 4% of fish weight in the first stage lasting 20 days. In the second stage of 22 days feeding intensity was 3% of fish weight.

### Experiment B

Feeds of ALMA company were used in experiment B: they are destined for the production of catfish (S2 – 42/10, code 6410), trout (P2 42/13, code 6020) and carp (K2 – 30/14, code 6225), pellet size is 2 mm. Fish of average initial weight 76.2 g were stocked by 30 individuals into two reservoirs of the capacity 280 l, partitioned into two compartments (variants receiving P2 and S2) and into two reservoirs with the capacity 150 l (variants receiving K2). The experiment had two replications.

Water temperature ranged between 22.4 °C and 27.8 °C in the course of experiment (mean 25.3 ± 1.3 °C). Experimental fish were fed three times a day every 5 hours. The experiment had two stages. Feeding intensity was 2% of fish weight in the first stage, and 1.5% in the second stage. As productive efficiency of feed K2 was low, it was replaced by feed S2 in the second stage.

To evaluate fish exterior and condition, condition coefficient:  $C_F = m/dt^3 \cdot 100$  ( $m$  – weight [g],  $dt$  – body length [cm]) and hepatosomatic index:  $HSI = mj/mc \cdot 100$  ( $mj$  – liver weight [g],  $mc$  – total weight [g]) were used.

The growth rate of fish was evaluated by specific growth rate according to the formula:

$$SGR = [(W_f/W_0)^{1/t} - 1] \cdot 100 \text{ [%/d]}$$

where:  $W_f$  – fish weight at the end of experiment  
 $W_0$  – fish weight at the beginning of experiment  
 $t$  – experiment duration (days)

Food conversion ratio was used to compare feed efficiency:  $FCR = \text{weight of consumed feed/fish biomass increment}$ .

Chemical analyses of fish muscle, viscera and liver were carried out in accordance with the Czech standard CSN 46 7092 (1985) in so called mixed samples prepared by homogenization of the tissues. Percentage proportions of dry matter, proteins, fat and ash were examined. Protein content was determined by Kjeldahl's method; proteins were calculated from nitrogen content in the sample, fat content was determined by Soxhlet's method; fat extraction with diethyl ether lasted for 10 hours.

Significance of differences between the means of tested variants was determined by analysis of variance (Snedecor and Cochran, 1969). Subsequent multiple comparison of differences between the means was subject to Scheffe's test.

Table 1. Results of experiment A (12th Sept.–24th Oct. 1995)

Stage	1				2			
Experiment duration (days)	20 (12. 9.–2. 10. 1995)				22 (2. 10.–24. 10. 1995)			
Feed ration (%)	4				3			
Feed type	PA2	SA2	PA2	SA2	PA2	SA2	PA2	SA2
	beginning of 1st stage				beginning of 2nd stage			
Fish number	120	120	120	120	110	110	110	110
Total weight (g)	1 492	1 515	2 507	2 353	2 478	2 538	4 801	4 723
Individual weight (g)	12.43	12.63	20.89	19.61	22.53	23.07	43.65	42.94
	end of 1st stage				end of 2nd stage			
Fish number	120	119	120	120	103	107	107	104
Total weight (g)	2 687	2 743	5 232	5 015	3 974	4 353	8 505	8 140
Individual weight (g)	22.39	23.05	43.60	41.79	38.58	40.68	79.49	78.27
Survival rate (%)	100.00	99.17	100.00	100.00	93.64	97.27	97.27	94.55
Feed consumption (g)	1 197	1 116	2 032	2 023	1 568	1 708	3 280	3 262
Weight gain:								
– total (g)	1 195	1 228	2 725	2 662	1 496	1 815	3 704	3 417
– individual (g)	9.96	10.43	22.71	22.18	16.06	17.61	35.84	35.33
(individual/d)	0.50	0.52	1.14	1.11	0.73	0.80	1.63	1.61
(%/d)	4.00	4.13	5.43	5.66	3.24	3.47	3.73	3.74
SGR	2.99	3.06	3.75	3.86	2.48	2.61	2.76	2.77
FCR	1.00	0.91	0.75	0.76	1.05	0.94	0.89	0.95
FCR/SGR	0.34	0.30	0.20	0.20	0.42	0.36	0.32	0.35
PER	2.04	2.25	2.74	2.69	1.95	2.17	2.30	2.14

## RESULTS

Table 1 shows the results of both stages of experiment A. In the first stage, the fish with lower initial weight receiving SA2 feed had by 3.2% higher percentage increment, by 2.3% higher specific growth rate and by 9% lower feed conversion ratio than the fish on diet PA2. Similar results were also obtained in fish with higher initial weight: percentage increment higher by 4.2% and SGR higher by 2.9%. Only FCR values were lower in the variant receiving feed SA2.

A similar trend was observed in the second stage of experiment. The fish with lower initial weight receiving feed SA2 had by 7.1% higher percentage increment, by 5.2% higher SGR and by 10.5% lower FCR. In fish with higher initial weight, the percentage increment of fish on the same diet was higher by 0.3%, SGR was higher by 0.4% and FCR was lower by 6.7%. The differences in fish growth between the experimental variants were not statistically significant.

The effect of diet on the chemical composition of African catfish body in experiment A is shown by data in Tables 2 and 3. Table 4 gives basic characteristics of the analyzed fish.

In the first stage of experiment A, the use of feed SA2 (49/14) increased fat deposition in the muscle of larger fish (by 9.3%) and protein retention in the muscle of smaller fish of the same age (by 9.9%). Administration of feed PA2 (47/12.5) led to an increase in protein retention in the muscle of smaller fish by 10.5%. Both feeds increased visceral fat deposition (Table 3). The increase in visceral fat content amounted to 51.8% when feed PA2 was used, and to 33.1% for feed SA2. Feed with higher protein and fat contents (SA2) resulted in an increase in HSI in comparison with feed PA2; the index was higher by 40.1% in smaller fish and by 10.6% in larger fish (Table 4).

Protein retention in the muscle of larger fish increased by 6.8% (feed PA2) in the second stage of experiment A in comparison with the preceding stage. All variants showed higher intramuscular fat deposition (by 11.0-67.4%). Higher intramuscular fat deposition was determined in fish receiving feed SA2 (by 13.3% in smaller fish, by 28.5% in larger fish). The high dietary fat content was reflected in an increase in liver fat content by 24.5% (feed PA2) and by 29.7% (feed SA2).

Table 5 shows the results of both stages of experiment B. The best results were achieved by fish receiving

Table 2. Chemical composition of African catfish muscle (experiment A)

Variant	Group	Dry matter (%)	Protein		Fat		Ash	
			dry matter	tissue	dry matter	tissue	dry matter	tissue
Start	12.5 g	22.09	69.63	15.38	22.67	5.01	8.77	1.94
12. 9. 1995	20.0 g	23.42	79.70	18.67	21.65	5.07	7.78	1.82
End of 1st stage 2. 10. 1995								
PA2 4%	smaller fish	23.13	73.48	17.00	18.79	4.35	6.21	1.44
	larger fish	22.97	70.27	16.14	22.13	5.08	5.29	1.22
SA2 4%	smaller fish	22.98	73.55	16.90	15.76	3.62	5.60	1.29
	larger fish	24.51	68.05	16.68	22.59	5.54	5.28	1.29
End of 2nd stage 24. 10. 1995								
PA2 3%	smaller fish	24.77	68.54	16.98	21.60	5.35	8.29	2.05
	larger fish	24.36	70.70	17.23	23.15	5.64	7.02	1.71
SA2 3%	smaller fish	24.45	66.62	16.29	24.79	6.06	6.85	1.67
	larger fish	24.97	65.80	16.43	29.03	7.25	5.70	1.42

Table 3. Fat content in the liver and viscera of African catfish (experiment A)

Variant	Liver			Viscera		
	dry matter	fat		dry matter	fat	
12. 9. 1995	%	dry matter	tissue	%	dry matter	tissue
Start				35.22	57.29	20.18
End of 1st stage 2. 10. 1995						
PA2	30.97	30.99	9.60	51.98	58.94	30.64
SA2	32.71	29.50	9.65	42.61	63.02	26.85
End of 2nd stage 24. 10. 1995						
PA2	39.17	30.52	11.95	40.80	27.44	11.20
SA2	41.11	30.46	12.52	36.47	41.17	15.01

Table 4. Basic characteristics of analyzed fish (experiment A 12th Sept.–24th Oct. 1995)

Feed	N	Weight ± SD	C <sub>F</sub> ± SD	HSI ± SD	Fat ± SD
		(g)	(%)	(%)	(%)
Start	10	11.94 ± 2.52	1.11 ± 0.07		
12. 9. 1995	10	20.09 ± 3.39	1.08 ± 0.08		
4% end of 1st stage 2. 10. 1995					
PA2	10	20.01 ± 8.32	1.17 ± 0.08	1.47 ± 0.57	
	10	42.42 ± 15.34	1.18 ± 0.17	2.08 ± 0.39	
SA2	10	22.17 ± 4.82	1.20 ± 0.16	2.06 ± 0.72	
	10	37.75 ± 12.83	1.16 ± 0.09	2.30 ± 0.70	
3% End of 2nd stage 24. 10. 1995					
PA2	20	38.80 ± 17.79	1.21 ± 0.17	1.58 ± 0.37	–
	20	75.83 ± 34.13	1.23 ± 0.11	1.73 ± 0.31	1.54 ± 0.89
SA2	20	38.96 ± 14.60	1.14 ± 0.10	1.59 ± 0.34	1.48 ± 0.86
	20	66.36 ± 22.61	1.18 ± 0.10	1.57 ± 0.23	1.83 ± 0.82

Table 5. Results of experiment B (30th Oct. 1995–8th Jan. 1996)

Stage	1			2		
Experiment duration (days)	35 (30. 10.–4. 12. 1995)			35 (4. 12. 1995–8. 1. 1996)		
Feed ration (%)	2			+ 1.5		
Feed type	S2	P2	K2	S2	P2	S2
	beginning of 1st stage			beginning of 2nd stage		
Fish number	60	60	60	49	47	46
Total weight (g)	4 698	4 510	4 513	6 214	5 594	3 966
Individual weight (g)	78.3	75.2	75.2	126.8	119.0	86.2
	end of 1st stage			end of 2nd stage		
Fish number	60	59	58	47	45	45
Total weight (g)	7 738	7 149	5 301	7 556	6 830	5 151
Individual weight (g)	129.0	121.2	91.4	160.8	151.8	114.5
Survival rate (%)	100.00	98.33	96.67	95.92	95.74	97.83
Feed consumption (g)	3 168	3 182	2 615	2 703	2 400	1 763
Weight gain:						
– total (g)	3 040	2 639	788	1 342	1 236	1 185
– individual (g)	50.7	46.0	16.2	33.9	32.8	28.2
(individual/d)	1.81	1.64	0.58	0.97	0.94	0.81
(%/d)	2.31	2.19	0.77	0.76	0.79	0.94
SGR	1.44	1.37	0.56	0.68	0.70	0.81
FCR	1.04	1.21	3.32	2.01	1.94	1.49
FCR/SGR	0.73	0.88	5.94	2.96	2.79	1.83
PER	2.29	1.97	1.00	1.18	1.23	1.60

feed S2 for catfish (percentage increment 2.3%, SGR 1.44%/d and FCR 1.04), the worst by fish on diet K2 for carp (percentage increment 0.77%, SGR 0.56%/d and FCR 3.32). Average individual weight of fish in the latter variant was significantly lower than fish weight in the other variants ( $P < 0.05$ ).

In the second stage of experiment B, lower growth rate and lower values of feed conversion ratio were observed in the first (S2) and second (P2) variant while

in the third variant, when feed K2 was replaced by feed S2, there was an improvement of these parameters.

Tables 6 and 7 document the effect of feed on the chemical composition of African catfish body in experiment B. Basic characteristics of analyzed fish are given in Table 8.

Intramuscular fat deposition decreased by 2.0–38.7% in the first stage of experiment B; the highest decrease was observed in fish receiving feed K2 (by 38.7% in

Table 6. Chemical composition of African catfish muscle (experiment B)

Variant	Dry matter (%)	Protein		Fat		Ash		
		dry matter	tissue	dry matter	tissue	dry matter	tissue	
Start 30. 10. 1995								
Females	f	22.14	not determined		22.03	4.88	5.25	1.16
Males	m	22.87			18.01	4.12	5.32	1.22
End of 1st stage 4. 12. 1995								
S2	f	23.89			17.69	4.23	5.59	1.34
P2	f	23.02	not determined		20.76	4.78	4.91	1.13
K2	f	22.16			13.49	2.99		
S2	m	23.47			16.30	3.83	5.57	1.31
P2	m	21.55			14.39	3.10	5.47	1.18
K2	m	22.25			13.04	2.90	5.23	1.16
End of 2nd stage 8. 1. 1996								
S2	f	22.24	76.49	17.01	17.14	3.81	5.51	1.23
P2	f	23.76	78.68	18.69	13.21	3.14	5.92	1.41
S2	f	22.73	77.10	17.52	14.12	3.21	5.46	1.24
S2	m	22.80	78.22	17.83	13.55	3.09	5.95	1.36
P2	m	22.12	79.08	17.49	14.67	3.25	5.66	1.25
S2	m	22.07	78.95	17.42	10.91	2.41	5.87	1.30

Table 7. Fat content in the liver and viscera of African catfish (experiment B)

Variant		Liver			Viscera		
		dry matter %	fat		dry matter %	fat	
			dry matter	tissue		dry matter	tissue
Start 30. 10. 1995							
Females	f	31.50	36.63	11.54	46.67	80.40	37.52
Males	m	33.28	37.69	12.54	44.94	76.63	34.44
End of 1st stage 4. 12. 1995							
S2	f	30.73	29.80	9.16	36.59	54.85	20.07
P2	f	39.76	38.33	15.24	38.80	54.40	21.11
K2	f	33.47	52.72	17.65	38.68	47.23	18.27
S2	m	30.12	31.51	9.49	28.14	50.66	14.26
P2	m	36.67	36.52	13.39	36.83	56.64	20.86
K2	m	33.32	43.46	14.48	30.34	44.74	13.57
End of 2nd stage 8. 1. 1996							
S2	f	31.73	40.95	12.99	26.39	51.43	13.57
P2	f	34.19	35.26	12.06	42.42	41.66	17.67
S2	f	28.77	95.88	27.58	24.93	36.89	9.20
S2	m	31.76	15.17	4.82	35.97	33.50	12.05
P2	m	35.08	33.51	11.76	29.59	39.98	11.83
S2	m	34.65	35.82	12.41	31.39	38.44	12.07

females, by 29.6% in males). Visceral fat deposition was found to decrease in all variants. The highest decrease was also determined in fish receiving feed K2 (by 60.6% in males, by 51.3% in females). HSI increased in all variants while the proportion of adipose tissue in viscera was lower in all variants than at the beginning of experiment. The highest HSI and proportion of adipose tissue in viscera were observed in fish

receiving feed P2, the lowest in fish on diet S2. The higher HSI in males (by 16.3%) was statistically significant unlike in the females (Table 8). Milers had a by 20% higher proportion of adipose tissue in viscera.

In the second stage of experiment B, further decrease in intramuscular fat content was observed in females in the first and second variant (by 9.5 and 35.1%) and in males in the first and third variant (by 18.9 and

Table 8. Basic characteristics of analyzed fish (experiment B 30th Oct. 1995–8th Jan. 1996)

Feed	N	Weight ± SD (g)	C <sub>F</sub> ± SD (%)	HSI ± SD (%)	Fat ± SD (%)	GSI ± SD (%)
Start 30. 10. 1995						
	18	71 ± 11.4	1.13 ± 0.11	1.59 ± 0.29	1.54 ± 0.40	0.20 ± 0.13
End of 1st stage 4. 12. 1995						
S2	12	127 <sup>A</sup> ± 39.0	1.07 ± 0.11	1.74 <sup>A</sup> ± 0.25	1.00 ± 0.31	0.48 ± 0.45
P2	12	130 <sup>A</sup> ± 23.8	1.16 ± 0.10	2.23 <sup>B</sup> ± 0.41	1.36 ± 0.44	0.62 ± 0.63
K2	12	86 <sup>B</sup> ± 15.1	1.06 ± 0.11	1.81 <sup>A</sup> ± 0.37	1.11 ± 0.31	0.44 ± 0.22
Males	18	115 ± 32.0	1.04 ± 0.10	2.07 <sup>a</sup> ± 0.38	1.26 ± 0.42	0.21 <sup>A</sup> ± 0.10
Females	18	113 ± 36.1	1.15 ± 0.10	1.78 <sup>b</sup> ± 0.40	1.05 ± 0.33	0.82 <sup>B</sup> ± 0.50
End of 2nd stage 8. 1. 1996						
S2	8	128 ± 44.3	1.10 ± 0.05	1.54 ± 0.29	0.60 ± 0.30	0.74 ± 0.79
P2	8	119 ± 40.9	1.24 ± 0.14	1.80 ± 0.47	0.90 ± 0.47	1.05 ± 1.44
K2	8	102 ± 25.1	1.12 ± 0.10	1.72 ± 0.35	0.80 ± 0.21	0.83 ± 0.74
Males	12	125 ± 43.7	1.14 ± 0.14	1.88 <sup>a</sup> ± 0.35	0.91 ± 0.33	0.31 <sup>A</sup> ± 0.14
Females	12	108 ± 31.8	1.17 ± 0.09	1.49 <sup>b</sup> ± 0.33	0.62 ± 0.34	1.43 <sup>B</sup> ± 1.24

No statistically significant differences were determined between the means designated by the same letter (A, B for  $P < 0.01$ , a, b for  $P < 0.05$ )

16.9%). The lower feeding intensity was reflected in further decrease in fat deposition and adipose tissue proportion in fish viscera and by HSI decrease in all variants. The higher HSI of males (by 26.2%) was statistically significant. The proportion of adipose tissue in miltier viscera was higher by 46.8% than in females.

## DISCUSSION

Evaluation of suitability of using two feeds with different protein and fat contents (SA2 49/14 and PA2 47/12.5) for production of African catfish with initial weight over 12 g indicated the higher growth rate of fish on diets with higher protein and fat contents. But the differences in fish weight were statistically insignificant. The feeds with higher protein and fat contents administered to fish with initial weight over 40 g did not virtually result in higher growth rate. These results suggest that the requirements of African catfish with weight under 40 g for dietary protein and fat contents are about 49 and 14%, respectively, while in fish with weight over 40 g they are lower than 47 and 12.5%, resp. These values are higher than the results reported by Degani *et al.* (1989) and also by Fagbenro *et al.* (1992) for a related species *Heterobranchus bidorsalis*, who declare an optimum dietary protein content of 40%, but they are comparable with Filipiak's *et al.* (1993a, b) results.

Evaluation of feed suitability for production of African catfish with weight over 100 g demonstrated negligible differences in the studied parameters between feeds for catfish (S2 42/10) and for trout (P2 42/13). The use of feed with low protein content (K2 30/14) for carp production, when all studied parameters showed the worst values, cannot be recommended for

African catfish diets. The lower weight growth of fish receiving feed K2 was statistically highly significant. The reason for lower growth rate was likely a low protein content, and perhaps their source. It is to note that utilization of proteins from plant sources by African catfish is worse (Degani *et al.*, 1988, 1989) unless they have been subject to heat treatment (Balogun and Ologhobo, 1989).

Dry matter content 21.6–25.0%, protein content 15.4–18.7% and fat content 2.4–7.3% were determined in muscles of African catfish by analyses of the chemical composition of its body. Similar dry matter values (22.6–23.8%) were calculated by Wedekind (1991). But he reported higher protein content (19.3–20.25%) and lower fat content (1.6–2.8%). Hoffman *et al.* (1995) determined lower intramuscular protein content in African catfish (16.7–17.6%) similarly like Degani (1988).

The use of feed with higher protein and fat contents was found to influence the intramuscular content of proteins and fat in fish under 100 g in experiment A. Intramuscular protein content was lower while fat content was higher than in fish of the second variant. Increased intramuscular protein and fat contents resulting from the high content of dietary proteins and lipids (Wedekind, 1995) were confirmed in this experiment only in the case of fat. Higher dietary fat and protein contents were also reflected in higher fat deposition in liver and higher proportion of adipose tissue in fish viscera.

The tested feeds were not found to influence the intramuscular dry matter and protein contents of fish over 100 g in experiment B, nevertheless fat content in the muscle of fish receiving feed K2 was lowest both in males and in females. Feed with higher fat proportion (P2) led to an increase in HSI and adipose tissue

proportion in fish viscera in comparison with feed with identical protein content but lower fat proportion (S2).

Among the tested feeds, feed SA2 (49/14) is recommended as most suitable for fish weighing from 12 to 40 g, feed PA2 (47/12.5) for fish from 40 to 80 g and feed S2 (42/10) for fish over 80 g.

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# KVALITA VAJEČNÉ SKOŘÁPKY U VYBRANÝCH BĚLOVAJEČNÝCH A HNĚDOVAJEČNÝCH KOMBINACÍ SLEPIC DOMINANT

## EGG SHELL QUALITY IN SOME WHITE-EGGED AND BROWN-EGGED CROSS COMBINATIONS OF DOMINANT HENS

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**ABSTRACT:** Egg shell quality is one of the most important parameters of technological value of eggs because it influences the economics of egg production to a great extent. The goal of trials was to examine egg shell quality in nine white-egged lines of White Leghorn (025, 026, 028, 031, 032, 033, 036, 038, 039) and in brown-egged final hybrid D 102, which were all selected on a breeding and reproduction farm for Dominant poultry at Dobřenice in the Czech Republic. Laying hens received a common commercial mixture with 14.49% of protein. Egg shell quality was examined between weeks 20 and 44 of layer age and egg analyses were conducted in 28-day intervals. The significantly highest egg weight was determined in brown-egged hybrid D 102 (64.09 g,  $P \leq 0.05$ ) and in white-egged line 033 (63.85 g,  $P \leq 0.05$ ). White-egged line 033 had the highest shell weight (7.21 g,  $P \leq 0.05$ ). There were no significant differences between the genotypes in the values of shell weight to egg weight ratio while the highest shell proportion was determined in lines 031 and 032 – 11.37% and 11.34%, respectively. The significantly highest shell thickness (0.37 mm,  $P \leq 0.05$ ) was observed in line 038 and in brown-egged hybrid D 102. Line 038 had the significantly highest shell strength, which was confirmed by a nondestructive method because the shell deformation was lowest in line 038 – 34.80  $\mu\text{m}$  ( $P \leq 0.05$ ).

**Keywords:** hen; laying type; genotype; egg shell quality

**ABSTRAKT:** Hodnotili jsme kvalitu vaječné skořáčky u devíti bělovaječných linií leghornky bílé (025, 026, 028, 031, 032, 033, 036, 038, 039) a u hnědovaječného finálního hybridu Dominant D 102. Kvalitu skořáčky jsme sledovali mezi 20. až 44. týdnem věku a rozborů jsme prováděli v 28denních intervalech. Nosnicím se podávala běžná obchodní směs o obsahu 14,49 % NL. Průkazně nejvyšší hmotnosti vajec dosáhl hnědovaječný hybrid D 102 a bělovaječná linie 033. Nejvyšší hmotnost skořáčky se zjistila u bělovaječné linie 033. Nejvyšší podíl skořáčky z hmotnosti vejce měly bělovaječné linie 031 a 032. Tloušťka skořáčky byla nejvyšší u bělovaječné linie 038 a u finálního hybridu D 102. Nejvyšší pevnost skořáčky zjištěná destruktivní metodou byla zaznamenána u bělovaječné linie 038. Při použití nedestruktivní metody byla u této linie zjištěna nejnižší deformace skořáčky.

**Klíčová slova:** slepice; nosný typ; genotyp; kvalita vaječné skořáčky

### ÚVOD

Kvalita vaječné skořáčky je jednou z nejdůležitějších vlastností vajec a je dlouhodobě sledovaným ukazatelem při šlechtění nosného typu slepic. Vejce s nízkou kvalitou skořáčky představují pro výrobce konzumních vajec značné ekonomické ztráty. Nestandardní vejce představují 3 až 12 % z celkové snášky. Kvalita skořáčky je charakterizována hmotností a podílem skořáčky, tloušťkou skořáčky a její pevností.

Celková hmotnost skořáčky je přímo úměrná velikosti vajec a tloušťce skořáčky. Mezi tloušťkou skořáčky a její pevností existuje vysoká korelace 0,92–0,97 v závislosti na genotypu (Harms *et al.*, 1990). Cotterill a Geiger (1977) uvádějí, že slepičí vejce o hmotnosti

60,9 g obsahuje 5,8 g skořáčky, což představuje 9,5 % jeho hmotnosti. Halaj (1987) uvádí u vajec hybridu Shaver Starcross 288 hmotnost skořáčky na počátku snášky 4,76 g a na konci 5,88 g. Simeonová *et al.* (1992) uvádějí u vajec průměrný podíl skořáčky 9,49 %, tloušťku skořáčky 0,37 mm a pevnost skořáčky měřenou destruktivní metodou 37,35 N. Skřivan (1990) zjistil u hnědovaječných nosnic Moravia SSL signifikantně nižší podíl skořáčky a nižší tloušťku než u bělovaječného hybridu Shaver Starcross 288. Tůmová *et al.* (1993) udávají u vajec Hixese hnědého významně nižší podíl skořáčky (11,23 % a 11,27 %) oproti bělovaječné kombinaci D-29 (11,93 % a 11,88 %). Naopak tloušťka skořáčky a její pevnost byla průkazně vyšší u Hixese hnědého. Arent *et al.* (1997) uvádějí u někte-

rych kombinací křížení (leghornka bílá x rodajlendka červená, plymutka žíhaná x rodajlendka červená a další) podíl skořápky v rozmezí 10,45–11,17, tloušťku skořápky 0,323–0,356 mm a pevnost 29,64–37,08 N. Podobných výsledků jsme dosáhli i v předcházející práci (Ledvinka *et al.*, 1997).

Stanovení pevnosti vaječné skořápky se provádí buď destruktivní metodou, při které dochází k porušení skořápky tlakem na podélnou osu vejce a kdy jsou používány přístroje různých konstrukcí (Voisey, Mc Donald, 1978; Fagan *et al.*, 1988; Simeonová a Vysloužil, 1993), nebo metodou nedestruktivní (nepřímou) podle autorů Simeonová *et al.* (1992). Při této metodě se vychází ze skutečnosti, že skořápka se tím méně deformuje, čím vyšší je její pevnost a tloušťka. Hodnoty deformace skořápky zjišťované touto metodou se pohybují v krajním rozmezí 14–71  $\mu\text{m}$  (Simeonová *et al.*, 1992), většinou však v rozmezí 28,80–35,16  $\mu\text{m}$  (Simeonová *et al.*, 1995; Simeonová, Kalová, 1993; Halaj a Grofik, 1994).

## MATERIÁL A METODA

Cílem sledování bylo porovnat kvalitu vaječné skořápky u vybraných bělovaječných a hnědovaječných genotypů slepic.

Do pokusu bylo zařazeno devět bělovaječných linií DOMINANT vzniklých z leghornky bílé, označených jako 025, 026, 028, 031, 032, 033, 036, 038, 039, a hnědovaječný finální hybrid DOMINANT D 102. Všem skupinám slepic se zkrmovala běžná obchodní směs o obsahu 14,49% NL.

Složení krmné směsi v %: rybí moučka 1,0, kvasnice 2,0, sójový extrahovaný šrot 15,5, kukuřice 22,0, pšenice 47,8, sušená vojtěška 2,0, aminovitan SK Super 0,5, krmná sůl 0,2, dikalciumfosfát 1,0, mletý vápenec 8,0.

Obsah živin: sušina 89,82 %, dusíkaté látky 14,49 %, 11,03 ME ( $\text{MJ}\cdot\text{kg}^{-1}$ ), tuk 1,88 %, vláknina 4,44 %, vápník 3,18 %, fosfor 0,70 %.

Sledování se uskutečnilo v poloprovazních podmínkách Školního zemědělského podniku Lány, na farmě Suchdol, ve snáškové hale BIOS o rozměrech 30 x 10 m. Nosnice byly ustájeny v třítěžové klecové technologii typu SALMET STS Hostovice. Během pokusu byl používán světelný režim doporučený pro kombinaci Dominant. Intenzita světla v době sledování byla 10 luxů.

Kvalitu vaječné skořápky jsme sledovali mezi 20. až 44. týdnem věku nosnic a rozborů se prováděly v 28denních intervalech. Uskutečnilo se sedm rozborů, při kterých se sledovalo vždy pět vajec každého genotypu, to znamená 35 vajec každého genotypu za celé sledované období.

Byla posuzována hmotnost vajec, hmotnost vaječné skořápky po vysátí, podíl skořápky, tloušťka skořápky a její pevnost nedestruktivní a destruktivní metodou. Ke stanovení pevnosti skořápky nedestruktivní metodou byl použit přístroj Kolumbus M a při destruktivní metodě přístroj Kolumbus 4.

Výsledky byly zpracovány analýzou variance (programem STATGRAPHICS), průkaznost byla stanovena Shéffeho metodou. Statisticky významné rozdíly jsou v tabulkách označeny písmeny. Mezi sledovanými ukazateli byly vypočteny korelace.

## VÝSLEDKY A DISKUSE

Z výsledků (tab. 1 a 2) vyplývá, že významně nejvyšší hmotnosti vajec (64,09 g,  $P \leq 0,05$ ) dosáhl hnědovaječný finální hybrid D 102. Vysokou hmotnost vajec vykazala i bělovaječná linie 033 (63,85 g,  $P \leq 0,05$ ), u které se zároveň zjistila průkazně nejvyšší hmotnost skořápky. Diference mezi hmotností vajec a hmotností skořápky u bělovaječných linií leghornky bílé ukazují

Tab. 1. Ukazatele kvality skořápky – Egg shell quality parameters

Genotyp <sup>1</sup>	Hmotnost vejce <sup>2</sup> (g)	Hmotnost skořápky po vysátí <sup>3</sup> (g)	Podíl skořápky z hmotnosti vejce <sup>4</sup> (%)
025	59,31 <sup>a</sup>	6,61 <sup>a</sup>	11,14
026	60,73 <sup>ab</sup>	7,11 <sup>bcd</sup>	11,94
028	59,89 <sup>ab</sup>	6,98 <sup>bcd</sup>	11,69
031	62,42 <sup>ab</sup>	7,03 <sup>bcd</sup>	11,37
032	61,73 <sup>ab</sup>	6,96 <sup>bcd</sup>	11,34
033	63,85 <sup>ab</sup>	7,21 <sup>d</sup>	11,25
036	62,85 <sup>ab</sup>	6,87 <sup>bcd</sup>	10,83
038	62,32 <sup>ab</sup>	7,14 <sup>cd</sup>	11,51
039	62,55 <sup>ab</sup>	6,79 <sup>ab</sup>	10,86
102	64,09 <sup>b</sup>	7,17 <sup>cd</sup>	11,18

a, b, c, d –  $P \leq 0,05$

<sup>1</sup>genotype, <sup>2</sup>egg weight, <sup>3</sup>shell weight of drawn-off egg, <sup>4</sup>shell to egg weight ratio

Tab. 2. Tloušťka a pevnost skořápky – Egg shell thickness and strength

Genotyp <sup>1</sup>	Tloušťka skořápky <sup>2</sup> (mm)	Pevnost destruktivní metodou <sup>3</sup> (N)	Pevnost nedestruktivní metodou <sup>4</sup> ( $\mu\text{m}$ )
025	0,34 <sup>a</sup>	24,26 <sup>a</sup>	41,34 <sup>b</sup>
026	0,36 <sup>ab</sup>	26,23 <sup>ab</sup>	35,94 <sup>a</sup>
028	0,35 <sup>a</sup>	25,76 <sup>ab</sup>	37,83 <sup>a</sup>
031	0,36 <sup>ab</sup>	26,56 <sup>abc</sup>	36,26 <sup>a</sup>
032	0,34 <sup>a</sup>	25,59 <sup>ab</sup>	38,03 <sup>ab</sup>
033	0,36 <sup>ab</sup>	26,68 <sup>bc</sup>	37,00 <sup>a</sup>
036	0,36 <sup>ab</sup>	27,47 <sup>bc</sup>	36,77 <sup>a</sup>
038	0,37 <sup>b</sup>	28,70 <sup>c</sup>	34,80 <sup>a</sup>
039	0,35 <sup>ab</sup>	27,40 <sup>bc</sup>	35,97 <sup>a</sup>
102	0,37 <sup>b</sup>	27,20 <sup>bc</sup>	35,79 <sup>a</sup>

a, b, c –  $P \leq 0,05$

<sup>1</sup>genotype, <sup>2</sup>shell thickness, <sup>3</sup>destructive-method strength, <sup>4</sup>nondestructive-method strength

Tab. 3. Korelace mezi jednotlivými ukazateli kvality vaječné skořápky – Correlations between the parameters of egg shell quality

	Pevnost skořápky – nedestruktivní metoda <sup>1</sup>	Pevnost skořápky – destruktivní metoda <sup>2</sup>
Podíl skořápky z hmotnosti vejce <sup>3</sup>	0,36	0,22
Tloušťka skořápky <sup>4</sup>	0,61	0,46
Hmotnost skořápky <sup>5</sup>	0,33	0,19
Deformace skořápky <sup>6</sup>	-0,17	-0,062

<sup>1</sup>destructive-method shell strength, <sup>2</sup>nondestructive-method shell strength, <sup>3</sup>shell to egg weight ratio, <sup>4</sup>shell thickness, <sup>5</sup>shell weight, <sup>6</sup>shell deformation

na vysokou možnost selekce a šlechtitelské práce na oba sledované znaky. Nejvyšší hmotnost skořápky byla zjištěna u linie 033 (7,21 g,  $P \leq 0,05$ ), avšak nejvyšší podíl skořápky z hmotnosti vejce vykazaly bělovaječné linie 031 a 032. U výsledků podílu skořápky nebyly zjištěny statisticky významné rozdíly mezi sledovanými genotypy a lze konstatovat, že procentuální podíl skořápky korespondoval s hmotností vajec. Námi zjištěné hodnoty jsou však poněkud vyšší, než uvádějí někteří zahraniční autoři (Cotterill a Geiger, 1977; Wells a Belyavin, 1987; Halaj, 1987; Simeonová *et al.*, 1992). Rozdíly u těchto ukazatelů jsou způsobeny především skutečností, že v literatuře jsou většinou uváděny hodnoty hmotnosti a podílu skořápky po vysušení. Výsledky zjištěné v našem pokusu však jsou téměř totožné s údaji některých našich autorů (Skřivan, 1990; Tůmová *et al.*, 1993; Tůmová a Skřivan, 1994).

Hodnoty tloušťky skořápky vykazují mezi některými liniemi signifikantní rozdíly. Nejvyšší tloušťku skořápky (0,37 mm,  $P \leq 0,05$ ) měly shodně bělovaječná linie 038 a finální hnědovaječný hybrid D 102. Nepotvrdila se tedy často citovaná skutečnost, že bílá vejce mají kvalitnější skořápku než vejce hnědá. Zajímavé srovnání ukazuje použití obou metod zjišťování pevnosti skořápky u stejného souboru vajec. Plně je zde dokládán fakt, že pevnější skořápka se méně deformuje (vychýlí) než skořápka slabší a méně pevná. Nejvyšší pevnost skořápky byla signifikantně zjištěna u bělovaječné linie 038 (28,70 N,  $P \leq 0,05$ ), která při nedestrukční metodě vykazovala nejnížší deformaci (34,80  $\mu\text{m}$ ,  $P \leq 0,05$ ). Naproti tomu nejnižší pevnost skořápky při destruktivní metodě byla zjištěna u bělovaječné linie 025 (24,26 N,  $P \leq 0,05$ ), u které byla zaznamenána největší výchylka (41,34  $\mu\text{m}$ ,  $P \leq 0,05$ ) při metodě nedestrukční.

Pro hodnocení vztahů mezi jednotlivými ukazateli kvality skořápky byly vypočteny korelace (tab. 3). Korelace mezi oběma přístroji v přesnosti měření je 0,55.

Mezi všemi hodnocenými znaky jakosti skořápky byla zjištěna průkazná pozitivní korelace s výjimkou vztahů k deformaci skořápky, u kterých je logicky negativní korelace. Tyto závislosti mezi pevností, tloušťkou či podílem skořápky z hmotnosti vejce dokumentují i výsledky autorů Voisey a Hamilton (1977). Korelace mezi deformací skořápky a pevností vyjadřují přesnost měření.

V našem testování byla hodnota korelací mezi deformací skořápky a pevností 0,062. Tato hodnota byla

stanovena při měření pevnosti destruktivní metodou přístrojem Kolumbus 4, který je přesnější než přístroj Kolumbus M. Rozdíl je dán celkovou konstrukcí těchto přístrojů a způsobem měření. Simeonová *et al.* (1995) stanovili korelační koeficienty pevnosti vaječné skořápky měřené elektronicky k hmotnosti vejce 0,255, k hmotnosti skořápky 0,543, k podílu hmotnosti skořápky z hmotnosti vejce 0,600, k nedestrukční deformaci -0,570 a k tloušťce skořápky 0,635. Korelační vztahy mezi hodnocenými znaky jakosti vajec byly vypočítány u rodajendky bílé a leghornky bílé. K měření byl použit přístroj firmy Geoart Brno. I v tomto případě byla zjištěna mezi hodnocenými znaky jakosti pozitivní korelace s výjimkou vztahů k deformaci skořápky.

Při našem měření byly hodnoty korelací mezi pevností skořápky a dalšími dílčími ukazateli nižší, než uvádějí Simeonová *et al.* (1995). Vyšší korelace mezi kvalitou skořápky a pevností byla zjištěna při měření nedestrukční metodou.

Výsledky ukazují, že obě metody jsou srovnatelné a že nedestrukční metodu lze použít všude tam, kde chceme uchovat vejce celá. Výhody nedestrukční metody vyzdvihují ve své práci i Halaj a Grofík (1994).

Z výsledků je zřejmé, že difference mezi bělovaječnými a hnědovaječnými kombinacemi Dominant se vlivem šlechtitelské práce v poslední době vyrovnávají nejen v ukazatelích kvality vaječné skořápky, ale i v ostatních ukazatelích technologické hodnoty vajec. Tuto tendenci jsme zaznamenali i při hodnocení některých dalších genotypů v našich předcházejících sledováních (Arent *et al.*, 1997, 1999; Ledvinka *et al.*, 1997).

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# SHORT COMMUNICATION

## INOCULATION OF FOREIGN DNA INTO TESTES OF YOUNG COCKS OF DOMESTIC POULTRY (*GALLUS DOMESTICUS*)

### APLIKACE CIZORODÉ DNA DO VARLAT MLADÝCH KOHOUTŮ KURA DOMÁCÍHO (*GALLUS DOMESTICUS*)

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**ABSTRACT:** Only some of the gene transfer techniques can be used as tools for insertion of foreign DNA into the cells of chicken testes. In our experiments a possible transfer of lipofectamine plasmid (linearized plasmid DNA-pGL3 H CMV luc) mixture via direct injection into the testes of the young cocks was verified. In Group 1, testes of 17 cocks were inoculated at the age of 2 weeks, in Group 2, testes of 21 cocks were inoculated at the age of 4 weeks. Twelve days after the inoculation a test for the presence of the inserted plasmid was performed. All analyzed samples except one in Group 2 were found highly positive, by PCR. No positive signals were found with DNA isolated from the testes 6 weeks after inoculation of the testis of both groups nor in the semen of the cocks allowed to grow to adulthood. The transferred plasmid complex has not been integrated into the genomic DNA.

**Keywords:** cocks; testes; inoculation; liposome; DNA; PCR

**ABSTRAKT:** Technika přenosu cizorodé DNA do buněk kohoutích varlat dosud nebyla vyvinuta. Pro přenos cizorodé DNA lze použít pouze omezeně dosud známé techniky přenosu. V experimentech byl ověřován přenos směsi lipofektaminu s plasmidem (linearizovaného plazmidu DNA-pGL3 H CMV luc) cestou přímé injekce do varlat mladých kohoutů. Ve skupině 1 byla provedena aplikace plazmidu do varlat 17 kohoutů ve věku dvou 2 týdnů, ve skupině 2 byla provedena aplikace do varlat 21 kohoutů ve věku čtyř týdnů. Dvanáct dnů po inzerci plazmidového komplexu byla pomocí PCR techniky zjišťována přítomnost/nepřítomnost přenášeného komplexu. Všechny analyzované vzorky kromě jednoho ve skupině 2 byly vysoce pozitivní na přítomnost přenášeného plazmidu. Šest týdnů po aplikaci plazmidu již nebyl zachycen pozitivní signál v DNA izolované z varlat kohoutů. Rovněž všechny vzorky DNA izolované později z ejakulátu dospělých kohoutů byly negativní. Přenášený plazmidový komplex nebyl integrován do genomové DNA.

**Klíčová slova:** kohouti; varlata; inokulace; liposom; DNA; PCR

#### Introduction

The periodic spermatogenic process originating from unipotential stem cells in chickens is probably from Ad spermatogonia cells as described by Lin and Jones (1992). These progenitor cells proceed to the next spermatogonial proliferation stage and then the spermatogonia differentiate into meiotic spermatocyte stages.

Finally, haploid spermatids mature into spermatozoa. Recently, several *in vivo* gene transfer techniques were described as tools for gene therapy (Marshall, 1995) and biological analysis of introduced foreign DNA into whole organs. Instead of virus mediated transfer of foreign DNA which is of limited use, nonviral vectors

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such as the direct injection of foreign DNA, or lipid mediated systems are used, but the transfection efficiency is relatively low. Manthorpe *et al.* (1993) achieved, after a direct injection of pCMV<sub>V</sub>int-lux into the murine muscle, maximal expression within 7–14 days after the injection. Roessler *et al.* (1994) described that after the direct injection of a plasmid-cationic liposome mixture into mouse brain cells, plasmid expression at least 21 days after the transfection. Tsan *et al.* (1995) described that expression of the plasmid liposome-complex lasted for 21 days in the lungs of rats.

A variety of constructs were injected into the muscles, or vessels of mice, rats and dogs, but only a few of these techniques have been used for insertion into testes and or to try to transfect spermatogonial cells as a possible tool for transferring of inserted genetic information via spermatozoa.

In mice, Yamazaki *et al.* (1998) described use of combination of direct insertion of foreign DNA into testis and subsequent *in vivo* electroporation. This system appears to be efficient and convenient system for gene expression during the mouse spermatogenesis. In chickens, only Afanassieff *et al.* (1996) described cock intratesticular inoculation of avian leukosis virus. No evidence of spermatozoa infection was found in contrast to the positive results obtained with retroviral infection of *in vitro* cultured PGCs cells as described by Allilioli *et al.* (1994).

The aim of this study was to verify the possibility to transfect spermatogonial cells of young cocks using a plasmid liposome complex.

## Materials and methods

### Plasmid

Plasmid DNA-pGL3 H CMV luc was previously linearized with BamHI and lipofection was made with lipofectamin (Gibco-BRL). The lipofectamin-plasmid mixture (0.5 µg/µl) was prepared according to the manufacturers guide.

Testes were inoculated by means of fine glass pipette with 4 µl of DNA/lipofectamine solution containing 2 pg of plasmid injected into the testis center. Only left testes were inoculated.

### Animals

White leghorn cocks were used for the experiments. They were divided into two groups.

Group 1 consisted of 17 cocks, inoculated at the age of 2 weeks, Group 2 consisted of 21 cocks inoculated at the age of 4 weeks. Before inoculation, the cocks were anesthetized by intramuscular injection of 0.03 ml of Pentobarbital Sodique 6% (Sanofi com.).

Six cocks from each group were euthanized 12 days and 4 cocks from each group 45 days after inoculation.

Both left and right testes of all these cocks were frozen in liquid nitrogen and then stored at -70 °C.

Ejaculates were collected 3 times from the remaining adult cocks – DNA extraction and analysis from each collected ejaculate was then separately performed.

At the end of the experiment, the remaining 40-week old adult cocks were euthanized.

### DNA extraction and PCR analysis

DNA extraction from both testes was performed as described by Thoraval *et al.* (1995). From these samples, the integration of DNA-pGL3 HMCV luc was analyzed by the PCR method with primers able to amplify a 580 base pair fragment from the luciferase gene:

The primer 49 (forward): 5' AGATCTGAATTCGAA-GACGCCAAA'3 and the primer 56 (reverse): 5' ATGCATGGATCCAGAGGAGTTCATTATCAGTGC ATTG'3 were derived from the luciferase gene sequences.

Two hundred nanograms of DNA template were subjected to 28 cycles of PCR. The three step pattern involved denaturation at 94 °C for 1 minute, primer annealing at 35 °C for 1 minute, and extension at 72 °C for 1 minute.

## Results and discussion

Six from the 17 cocks inoculated at the age of 2 weeks (Group 1) and six from the 21 cocks inoculated at the age of 4 weeks (Group 2) with the plasmid-liposome mixture into the left testes were euthanized 12 days after inoculation. The presence of plasmid was checked by PCR from DNA samples isolated from left and right testis. In Group 1, 3 samples were highly positive in the left testis (Fig. 1, lanes 1, 5 and 6), as well as 3 samples which were also positive in the left testis, although the signal was not so strong (Fig. 1, lanes 2, 8 and 9). No samples of DNA isolated from the right testis were positive (Fig. 1, lanes 3, 4 and 7). In Group 2, 3 samples were highly positive in the left testis (Fig. 1, lanes 12, 13, 15). Three samples were also positive in the left testis although the signal was not so strong (Fig. 1, lanes 10, 11, 15). No samples of DNA isolated from the right testis were positive (Fig. 1, lane 14).

Six weeks after the plasmid inoculation, 4 cocks from each group were euthanized. In all samples from both groups, the plasmid detection by PCR was negative (data not shown).

The remaining cocks (7 in Group 1 and 11 in Group 2) were kept till their sexual maturity. All DNA samples extracted from collected cocks ejaculates were also found to be negative by PCR.

These cocks were then euthanized and DNA was extracted from their whole testes in order to detect the presence of the plasmid. In all samples plasmid detection by PCR was negative (data not shown).



Fig. 1. Analysis of DNA samples from the cocks (Group 1 and group 2) 12 days after inoculation of their testes, for presence of DNA-pGL3 H CMV luc with PCR

DNA from testes of 6 cocks (Group 1) inoculated at the age of 2 weeks (lane 1–9)

DNA from testes of 6 cocks (Group 2) inoculated at the age of 4 weeks (lane 10–16). Positive control: plasmid pGL3 H CMV luc (lane 17). Ladder- $\lambda$ DNA/*HindIII*, pBR/*HaeIII*

DNA from left testes of the cocks: lanes 1, 2, 5, 6, 8, 9, 10, 11, 12, 13, 15 and 16. DNA from right testes of the cocks: lanes 3, 4, 7 and 14

Twelve days after the injection we were able to detect a highly positive response for the presence of inserted pGL3HCMVluc into the left testes. In Group 1, all analyzed cocks were positive by PCR. Half of them had a strong signal, the others had a thin band. No positive samples were detected with DNA extracted from the right testis originating from the same animal indicating that the signal observed was specific. In Group 2, 5 out of 6 analyzed cocks were found to be positive by PCR. Three of them gave a strong signal. No positive signals were found in DNA from testes of chickens euthanastized 45 days after the inoculation nor in semen of adult cocks.

The explanation for the fact that the transferred plasmid-liposome complex can be detected only during 12 days after its inoculation into the testis is that this complex has not been integrated into the genomic DNA. The results correspond with the findings of Tsan *et al.* (1995) using this liposome-plasmid complex for transfection of mammalian lung cells as well as the findings of Roessler *et al.* (1994) which show positive expression of transferred liposome-plasmid complex in the mammalian brain cells at least 21 days after the transfection. Sergienko and Gauze (1995) described preservation of foreign DNA in mammalian liver cells up to 11 days. Manthorpe *et al.* (1993) reported that use of direct injection of circular plasmid DNA into the muscle resulted in its expression in myofiber cells.

Comparing with mammals the chicken testes are situated in the body cavity, just beneath the vertebral column, close to the *vena cava caudalis* and *aorta abdominalis*, hence any manipulation is extremely difficult.

From a practical point of view, it is not possible in chickens to use a combination of DNA injection into semeniferous tubulus and subsequent *in vivo* electroporation, as described in mouse by Yamazaki *et al.* (1998). Use of the testis of very young cocks, hence seemed to give bigger chance for the integration of the plasmid, because of the number of presented cells in a testis and the multiplication stadium of spermatog-

onial cells. At the age of 2 and 4 weeks, the testes of cocks are in the first phase of development when tubules become organized and multiplication of spermatogonia occurs (Johnson, 1986).

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The paper extent shall not exceed 15 typescript pages, including tables, figures and graphs.

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If any abbreviation is used in the paper, it is necessary to mention its full form at least once to avoid misunderstanding. The abbreviations should not be used in the title of the paper nor in the summary.

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**Abstract** is an information selection of the subject and conclusions of the paper, it is not a mere description of the paper. It must present all substantial information contained in the paper. It shall not exceed 170 words. It shall be written in full sentences, not in form of keynotes, and comprise basic numerical data including statistical data. It must contain key words. It should be submitted in English and if possible also in Czech or Slovak.

**Introduction** has to present the main reasons why the study was conducted, and the circumstances of the studied problems should be described in a very brief form.

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