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ANALÝZA NĚKTERÝCH VLIVŮ PŮSOBÍCÍCH NA REPRODUKČNÍ UŽITKOVOST PRASNIC*

ANALYSIS OF SOME FACTORS INFLUENCING REPRODUCTIVE PERFORMANCE IN SOWS

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ABSTRACT: Analysis of factors affecting reproductive performance (parameters of reproduction and natality, SP interval) – year, season, parity, weather characteristics at A.I., sow's father, mated boar – was the principal objective of the study. Anderson and Melampy (1972) found that the number of piglets born increased between the first and fourth litters; as related to age, a decline of litter size was evident after about 4.5 years. Britt *et al.* (1983) noted that although sows of all parities were affected, postweaning anoestrus was more prevalent in primiparous sows during the summer and autumn seasons. It is known that seasonal variations in response to exogenous estrogens may occur in pigs (Cox *et al.*, 1987) and this may be a reason for the differing seasonal responses noted in weaned sows. Daylength is another important factor related to the seasonal factor. Kermabon *et al.* (1995) examined effects of daylength on gonadotropin profiles in lactating sows. Weather 'conditions (environmental temperature) affect ova fertilization. Huhn *et al.* (1995) analysed results of 25 215 first inseminations carried out over a 3-year period. The highest number of piglets born per 100 inseminations occurred for maximum daily temperatures of 10–15 °C; the lowest for temperatures higher than 30 °C. The following reproductive parameters (related to litter size) were controlled and analysed in the breeding and commercial herd of ZOD Žichlínek in the period 1990–1997: born piglets in total, mortality (stillborn piglets), piglets born alive. Data characterizing 4 881 sows – Landrace (L) x White purebred (BU) crosses – were compiled and classified. The following factors were studied: year, season, weather characteristics at A.I., morning temperature at A.I., lunar phase, sow's father, mated boar, parity. The following values characterizing litter size were found: born piglets in total: 10.20 ± 2.76; v = 27.05 – Table 2; piglets born alive: 8.83 ± 2.43; v = 27.49 – Table 2. As for the mentioned litter size parameters, differences between individual effects related to year, season, temperature at A.I., sow's father, and boar were highly significant ($P < 0.01$) and significant ($P < 0.05$) in two cases. Differences between effects related to weather characteristics, lunar phase at A.I. and parity were nonsignificant ($P > 0.05$) – Table 1. The highest total number of born piglets and the highest proportion of piglets born alive were recorded in the period September–April, the lowest in the period May–August. November and December are the optimum months for farrowing. Sow's father and mated boar factors had significant ($P < 0.05$) or highly significant ($P < 0.01$) effects on litter size parameters. The same herd and the same population of crossbred (successfully inseminated) sows (n = 2 246) was used for studying effects of year, season, parity, weather characteristics at A.I., morning temperature at A.I., lunar phase at A.I. on SP interval (farrowing–conception interval). Mean SP interval 43.37 ± 18.95 days (coefficient of variation 45.78%) was found (Table 6). SP was shorter in the period March–August (36.83–41.32 days) than SP in the period September–February (42.21–56.05 days) – Table 7. Highly significant differences ($P < 0.01$) between effects were found in the case of temperature at A.I. Any tendency indicating significant effects of temperature (temperature range) on SP was not found. The longest SP was associated with the first farrowing (67.24 days), a gradual decrease within 38.98 days (8th farrowing) was noted – Table. 8.

Keywords: sows; reproduction; service period; natality; factors of external and internal environment; sow's father factor; mated boar factor

ABSTRAKT: Cílem práce byla analýza některých vlivů působících na reprodukční výkonnost prasnic (ukazatele reprodukce a natality, resp. mortality selat po narození a délky servis periody) – vlivy roku, ročního období, pořadí vrhu, charakteru počasí při inseminaci, otce matky a připařovaného plemeníka. U 4 881 prasnic-kříženek plemen landrase (L) x bílé ušlechtilé (BU) byly v rozmnožovacím a užitkovém stádě Sázava ZOD Žichlínek, okr. Ústí nad Orlicí, v letech 1990–1997 sledovány a následně analyzovány tyto ukazatele reprodukce v jednom vrhu prasnice (vlastnosti související s velikostí vrhu): počet narozených selat celkem, počet mrtvě narozených selat (mortalita), počet živě narozených selat. Data byla analyzována s ohledem na faktory rok, roční období (v měsících), charakter počasí při inseminaci, ranní teplota při inseminaci, fáze měsíce,

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otec prasnice, připařovaný plemeník a pořadí vrhu. Počasí bylo členěno podle převažujícího charakteru počasí ve dni provedené inseminace. Pro ukazatele velikosti vrhu byly zjištěny tyto parametry: počet selat narozených = 10,20 ± 2,76 (v = 27,05), počet selat živě narozených = 8,83 ± 2,43 (v = 27,49). Rozdíly mezi efekty uvnitř faktoru roku, ročního období, teploty při inseminaci, otce prasnice a připařovaného plemeníka byly pro uvedené ukazatele velikosti vrhu zpravidla vysoce významné ($P < 0,01$) a ve dvou případech významné ($P < 0,05$). Rozdíly mezi efekty uvnitř faktoru počasí při inseminaci, fáze měsíce při inseminaci a pořadí vrhu byly nevýznamné ($P > 0,05$). Z analýzy faktoru ročního období vyplývá, že od září do dubna se vyskytovaly u počtu narozených a živě narozených selat nejvyšší hodnoty. Od května do srpna byly tyto hodnoty nejnižší. Nepříznivější měsíce pro oprasení byly listopad a prosinec. Faktor otce prasnice a připařovaného plemeníka měly významný ($P < 0,05$) až vysoce významný vliv ($P < 0,01$) na ukazatele velikosti vrhu. Ve stejném stádě a u stejné populace hybridních prasnic (n = 2 246) byl při úspěšné inseminaci analyzován vliv faktoru roku, ročního období, pořadí vrhu, charakteru počasí při inseminaci, ranní teploty při inseminaci a fáze měsíce při inseminaci na délku servis periody (časové období od oprasení do zabřeznutí). Průměrná délka servis periody byla 43,37 ± 18,95 dnů s variačním koeficientem 45,78 %. V měsících březen až srpen byla délka service periody kratší (v rozpětí 36,83 až 41,32 dnů) než v měsících září až únor (v rozpětí od 42,21 do 56,05 dnů). Byly zjištěny vysoce významné rozdíly ($P < 0,01$) mezi efekty uvnitř faktoru teplota při inseminaci. Z analýzy nevyplývá tendence, která by naznačovala, že teploty v určitém teplotním rozpětí ovlivňují výrazně délku servis periody. Nejdelší servis perioda byla zjištěna v prvním vrhu (67,24 dnů) a postupně klesala až na hodnotu 38,98 dnů v 8. vrhu.

Klíčová slova: prasnice; reprodukce; servis perioda; natalita; faktory vnitřního a vnějšího prostředí; faktor otce; faktor připařovaného plemeníka

ÚVOD

Jedním z hlavních faktorů v efektivnosti chovu prasat je reprodukční výkonnost prasnic. Na reprodukční užitkovost prasnic má vliv řada faktorů. Cílem práce je analýza některých vlivů působících na ukazatele reprodukce a natality, resp. mortality selat po narození – vlivy roku, ročního období, pořadí vrhu, charakteru počasí při inseminaci, otce matky a připařovaného plemeníka.

Za významný faktor reprodukčních schopností prasnic je považováno pořadí vrhu. Anderson a Melampy (1972) uvádějí narůstající počet narozených selat mezi prvním až čtvrtým vrhem; ve vztahu k věku uvádějí pokles po cca 4,5 letech. Britt *et al.* (1983), Martinat-Botté *et al.* (1984) a Clark a Leman (1986) udávají na základě analýzy velkého souboru dat nejnižší četnost vrhu při první březosti; maxima je dosahováno ve třetí, čtvrté a páté březosti; dále se udržuje na konstantní úrovni, případně postupně s věkem mírně klesá.

Britt *et al.* (1983) zjistili vliv sezony na reprodukční schopnost prasnic bez ohledu na paritu; nejvíce případů anestru po odstavu však zaznamenali u primipar. Je známo, že u prasat může dojít k sezonnímu kolísání reakce na exogenní estrogeny (Cox *et al.*, 1987), což může být příčinou variabilních sezonních reakcí prasnic. Aumaitre *et al.* (1976) uvádějí u prasnic oprasených v období červenec až září o 10 dní delší interval mezi odstavem a zabřeznutím než u prasnic oprasených ve zbývající části roku. Rovněž v USA byl zjištěn delší interval u prasnic oprasených v období červen až srpen (Hurtgen, Leman, 1981). Obecně lze konstatovat, že reprodukční schopnost prasnic chovaných v chladnějších oblastech a v tropických pásmech se liší; v chladnějších oblastech nejsou uváděné výkyvy zjišťovány (Pepper, Taylor, 1981).

Dalším faktorem je délka světelného dne, která úzce souvisí s faktorem sezony. Kermabon *et al.* (1995) sledovali vliv délky světelného dne na hladiny gonadotropinu u laktujících prasnic. Bylo prokázáno, že při kratší délce dne se průkazně zvýšil podíl prasnic říjících se dříve po odstavu než v případě delších denních cyklů. Rovněž Prunier *et al.* (1994) uvádějí vliv světelného režimu na interval mezi odstavem a říjí. V podmínkách ČR se vlivem světla na reprodukci prasnic zabýval Říha (1997). Uvádí, že ve stájích je často nedostatečná intenzita světla a samotné změny délky světelného dne nejsou dostatečné k tomu, aby stabilně navodily rytmus sekvence metabolismu, a tím ovlivnily hypotalamo-hypofyzární aktivitu.

Je nesporné, že počasí, se kterým úzce souvisí teplota prostředí, má vliv na oplození vajíček prasnice. Huhn *et al.* (1995) analyzovali výsledky 25 215 prvních inseminací provedených v průběhu tří let. Zjistili nejvyšší počet narozených selat na 100 inseminací v případě maximálních denních teplot 10 až 15 °C, nejnižší v případě teplot přesahujících 30 °C. Vliv teploty na reprodukční schopnosti prasnic se projevuje sníženým příjmem krmiva a následně opožděným nástupem pohlavních funkcí (Prunier *et al.*, 1994; Říha, 1997). Složitým faktorem z hlediska vlivu na velikost vrhu je interval, resp. inseminační interval, který je definován jako časové období od posledního porodu po první zapuštění, resp. první inseminaci. Retrospektivní studie vlivu některých faktorů na velikost vrhů, realizovaná ve Francii (Le Cozler *et al.*, 1997), byla zpracována na základě analýzy dat z 1 881 stád u 46 523 prasnic. Průměrný interval mezi odstavem a koncepcí (27,5 dní) byl kratší než hodnota uváděná např. před 20 lety (Aumaitre *et al.*, 1976); tato skutečnost může být rovněž důsledkem současného systému brakování prasnic (Dagorn *et al.*, 1996). Existují důkazy, že délka intervalu má průkazný vliv na četnost vrhu. Tak například Dewey *et al.* (1994) uvádějí, že menší velikost vrhu ve vztahu k délce intervalu odstav–koncepce je možné vysvětlit méně kvalitní ovulací anebo vyšší embryonální

mortalitou. Kemp a Souede (1996) se však domnívají, že negativní účinky dlouhého intervalu by mohly být rovněž důsledkem neoptimálního načasování inseminace nebo zapuštění ve vztahu k ovulaci a nemusely by být způsobeny slabší fertilitou prasnic. Weitze *et al.* (1994) zjistili u prasnic říjících se dříve po odstavu průkazně delší říje než u prasnic říjících se později.

Morrow *et al.* (1992) uvádějí, že delší laktace má za následek vyšší četnost vrhu. Xue *et al.* (1993) zjistili, že procento prasnic říjících se do šesti dnů do odstavu selat je ovlivněno délkou laktace. Stejní autoři však nezjistili průkazný vliv délky laktace na délku inseminačního intervalu. Nejnižší hodnota intervalu mezi odstavem a koncepcí byla zjištěna u laktace trvající cca 28 dní (Allrich *et al.*, 1979; Aumaitre, Dagorn, 1982; Xue *et al.*, 1993). Stein *et al.* (1990) a Dewey *et al.* (1994) zjistili průměrnou délku laktace (27,2 dní), která odpovídá hodnotám uvedeným v práci autorů Le Cozler *et al.* (1997). Pro odpovídající velikosti následného vrhu je optimální odstav selat ve čtyřech týdnech věku (Dewey *et al.*, 1994). Xue *et al.* (1993) uvádějí, že optimální délka laktace pro dané stádo je rovněž ovlivněna – alespoň částečně – systémem chovu. Výsledky francouzských studií naznačily, že je tento předpoklad správný (Le Cozler *et al.*, 1997; Dagorn *et al.*, 1996). Podle současných norem EU (1991) není dovoleno odstavovat selata dříve než za tři týdny po narození; ranější odstav je povolen pouze ve výjimečných případech. Na základě shromážděných výsledků lze předpokládat při prodloužení délky laktace z 19 na 29 dní zvýšení velikosti vrhu o 0,34 selete u druhého vrhu a o 0,62 selete u třetího a následujících vrhů. U druhého vrhu byl zjištěn lineární nárůst četnosti vrhu v závislosti na délce laktace, u následujících vrhů nešlo o lineární vztah; optimální hodnoty byly zjištěny pro délku laktace 21 až 28 dní.

MATERIÁL A METODY

U 4 881 prasnic-kříženek plemen landrase (L) x bílé ušlechtilé (BU) byly v rozmnožovacím a užitkovém stádě Sázava ZOD Žichlínek, okres Ústí nad Orlicí, v letech 1990–1997 sledovány a následně analyzovány tyto ukazatele reprodukce v jednom vrhu prasnice bez ohledu na pořadí říje po odstavu selat (vlastnosti související s velikostí vrhu): počet narozených selat celkem, počet mrtvě narozených selat (mortalita 1 – v kusech a procentech), počet živě narozených selat. Pro počet narozených a živě narozených selat byly vypočítány průměry efektů uvnitř faktorů metodou nejmenších čtverců. Mortalita 1 byla vypočtena jako rozdíl mezi vypočítanými průměry. Prasnice byly inseminovány kanci plemene ČVM a odstav selat se prováděl v intervalu 27–33 dnů po narození. U části užitkového chovu byla při 2. reinseminaci prováděna nepravá heterospermie – u části prasnic byla čtvrteční reinseminace provedena jiným kancem téhož plemene než 1. inseminace (úterý) a 1. reinseminace (středa)). Data byla analyzována s ohledem na faktory: rok, roční období (je uvedeno v měsících), charakter počasí při inseminaci, ranní teplota při inseminaci, fáze měsíce, otec prasnice, připařovaný plemeník, pořadí vrhu.

Počasí bylo členěno podle převažujícího charakteru počasí ve dni provedené inseminace. Jednalo se o šest tříd: jasno, polojasno, zataženo, přeháňky, déšť, sníh. Fáze měsíce byly tříděny do čtyř tříd: narůstání, úplněk, ubývání, nov. Teplota byla zjišťována na farmě venkovním teploměrem a byla odečítána mezi 7. a 8. hodinou ráno.

K analýze dat byl použit tento model:

$$y_{ijklmnopq} = \mu + a_i + b_j + c_k + d_l + e_m + f_n + \gamma_o + \delta_p + e_{ijklmnopq}$$

kde: $y_{ijklmnopq}$ – pozorovaná hodnota
m – celkový průměr
a_i – fixní efekt roku
b_j – fixní efekt ročního období
c_k – fixní efekt počasí
d_l – fixní efekt ranní venkovní teploty (°C)
e_m – fixní efekt fáze měsíce
f_n – fixní efekt pořadí vrhu
g_o – náhodný efekt otce prasnice
d_p – náhodný efekt připařovaného plemeníka
$e_{ijklmnopq}$ – reziduální chyba

Dále byl ve stejném stádě a u stejné populace hybridních prasnic (v letech 1990–1997 u 2 246 prasnic) při úspěšné inseminaci analyzován vliv faktoru roku, ročního období, pořadí vrhu, charakteru počasí při inseminaci, ranní teploty při inseminaci a fáze měsíce při inseminaci na délku servis periody (časové období od oprasení do zabřeznutí).

K analýze dat byl použit tento model:

$$y_{ijklmnop} = \mu + a_i + b_j + c_k + d_l + e_m + f_n + \gamma_o + e_{ijklmnop}$$

kde: $y_{ijklmnop}$ – pozorovaná hodnota
m – celkový průměr
a_i – fixní efekt roku
b_j – fixní efekt ročního období
c_k – fixní efekt počasí
d_l – fixní efekt ranní venkovní teploty (°C)
e_m – fixní efekt fáze měsíce
f_n – fixní efekt pořadí vrhu
g_o – náhodný efekt otce prasnice
$e_{ijklmnop}$ – reziduální chyba

Všechny vlastnosti byly analyzovány metodou nejmenších čtverců GLM (SAS/STAT, Release verze 6.11, 1998).

VÝSLEDKY A DISKUSE

V tab. 1 jsou uvedeny počty efektů uvnitř faktorů pro ukazatele velikosti vrhu a významnosti rozdílů mezi efekty uvnitř faktorů pro ukazatele velikosti vrhu. Při analýze metodou nejmenších čtverců byly zjištěny průkazné vlivy ($P < 0,05$) pro ukazatele teplota při inseminaci a připařovaný plemeník na počet narozených selat a vysoce průkazné vlivy ($P < 0,01$) faktorů roku, ročního období, otce prasnice, teploty při inseminaci a připařovaného plemeníka na počet narozených, resp. živě narozených selat. Neprůkazné ($P > 0,05$) byly vli-

Tab. 1. Počet efektů a významnost rozdílů mezi efekty uvnitř faktorů pro ukazatele velikosti vrhu – The number of effects and significance of differences between effects within the factors related to litter size parameters

Faktor[1] n = 4 881	Počet efektů uvnitř faktoru[10]	Počet narozených selat[11]	Z toho živě narozených selat[12]
Rok[2]	7	3,50**	5,99**
Roční období[3]	12	11,59**	20,21**
Počasí při inseminaci[4]	6	0,98	0,86
Teplota při inseminaci[5]	42	1,55*	1,73**
Fáze měsíce při inseminaci[6]	4	0,77	0,93
Pořadí vrhu[7]	15	1,02	3,22
Otec prasnice[8]	89	2,23**	2,13**
Připařovaný plemeník[9]	132	1,37*	1,52**

Hladiny významnosti: * $P < 0,05$; ** $P < 0,01$
Significance levels: * $P < 0.05$; ** $P < 0.01$

[1]factor, [2]year, [3]year season, [4]weather at A.I., [5]temperature at A.I., [6]lunar phase at A.I., [7]parity, [8]sow's father, [9]mated boar, [10]number of effects within the factor, [11]number of piglets born, [12]of them: live-born ones

Tab. 2. Průměr, směrodatná odchylka a variační koeficient pro ukazatele velikosti vrhu – Mean, standard deviation and coefficient of variation for litter size parameters

Počet pozorování[1] n = 4881	$\bar{x}$	$s_{\bar{x}}$	v
Ukazatel[2]	ks	ks	%
Počet narozených selat[3]	10,20	2,76	27,05
Z toho živě narozených selat[4]	8,83	2,43	27,49

[1]number of observations, [2]parameter, [3]number of piglets born, [4]of them: live-born ones

vy charakteru počasí, fáze měsíce a pořadí vrhu na sledované ukazatele natality.

V průměru se narodilo na vrh 10,20 ± 2,76 selat, z toho živě 8,83 ± 2,43 selat; hodnota variačního koeficientu byla cca 27 % (tab. 2). Úroveň natality je z pohledu ČR na poměrně dobré úrovni (Čeřovský *et al.*, 1998 a další).

Rok měl vysoce významný vliv na ukazatele velikosti vrhu ($P < 0,01$). V roce 1992 byl zaznamenán nejvyšší průměrný počet narozených selat. V letech 1993 a 1997 byl zjištěn nejnižší průměrný počet narozených selat, zatímco v letech 1994 až 1995 byl počet narozených selat v rozpětí 10,01 až 10,49. Nejvyšší počet živě narozených selat byl v roce 1998 (10,00), při nejnižší mortalitě narozených selat. Mortalita činila pouze 0,42 narozeného (4,03 %) selete. V letech 1992–1993 činila mortalita selat přes 14 %. I když byla mortalita narozených selat v roce 1994 o něco nižší (12,88 %), zůstal počet živě narozených selat v letech 1993–1994 také nejnižší, stejně jako u narozených selat v letech 1993–1994 (tab. 3).

Vliv pořadí vrhu na ukazatele natality nebyl průkazný ($P > 0,05$). Tento vliv se patrně kombinuje s jinými efekty – teplota, zlepšení technologie a management stáda, zlepšení výživy atd. (Prunier *et al.*, 1994; Huhn *et al.*, 1995; Dagorn *et al.*, 1996). Podobně tomu bylo i v průběhu let ve sledovaném chovu.

V tab. 4 jsou uvedeny průměry a standardní chyby, získané metodou nejmenších čtverců, ukazatelů veli-

Tab. 3. Průměry (LSM) a standardní chyby (SE), získané metodou nejmenších čtverců, ukazatelů velikosti vrhu pro efekty roku – Means (LSM) and standard errors (SE) of litter size parameters for the effects of year, as calculated by a least-squares method

Rok[1]	Počet[2]				Mortalita[5] 1	
	narozených selat[3]		živě narozených selat[4]			
	LSM	SE	LSM	SE	ks	%
1992	11,12	0,28	9,53	0,25	1,59	14,30
1993	9,70	0,39	8,33	0,34	1,37	14,12
1994	10,01	0,35	8,72	0,31	1,29	12,88
1995	10,49	0,35	9,26	0,31	1,23	11,73
1996	10,40	0,36	9,19	0,32	1,21	11,63
1997	9,53	0,42	9,53	0,37	1,29	11,92
1998	10,42	0,28	10,00	0,25	0,42	4,03

[1]year, [2]number of, [3]piglets born, [4]live-born piglets, [5]mortality

Tab. 4. Průměry (LSM) a standardní chyby (SE) získané metodou nejmenších čtverců ukazatelů velikosti vrhu pro měsíce uvnitř roku – Means (LSM) and standard errors (SE) of litter size parameters for months within the year, as calculated by a least-squares method

Roční období (měsíc)[1]	Počet[2]				Mortalita[5] 1	
	narozených selat[3]		živě narozených selat[4]			
	LSM	SE	LSM	SE	ks	%
1	10,76	0,67	9,75	0,59	1,01	9,39
2	10,42	0,67	9,56	0,59	0,86	8,25
3	10,96	0,67	9,86	0,59	1,10	10,04
4	10,23	0,67	9,16	0,59	1,07	10,46
5	9,83	0,67	8,72	0,59	1,63	16,59
6	10,03	0,68	8,74	0,60	1,29	12,86
7	9,67	0,68	8,41	0,60	1,26	13,03
8	10,06	0,67	8,81	0,59	1,25	12,43
9	10,36	0,68	9,41	0,60	0,95	9,17
10	10,30	0,67	9,36	0,59	0,94	9,13
11	11,12	0,67	10,20	0,59	0,92	8,27
12	11,33	0,68	10,40	0,59	0,93	8,21

[1]year season (month), [2]number of, [3]piglets born, [4]live-born piglets, [5]mortality

Tab. 5. Významnost rozdílů mezi efekty uvnitř faktorů pro délku servis periody – Significance of differences between effects within the factors for SP interval

Faktor[1] n = 2 246	Počet efektů uvnitř faktoru[9]	Servis perioda (dny)[10]
Rok[2]	6	2,08
Roční období[3]	12	6,14**
Počasí při inseminaci[4]	6	1,66
Teplota při inseminaci[5]	40	2,01**
Fáze měsíce při inseminaci[6]	4	0,85
Pořadí vrhu[7]	18	8,44**
Otec prasnice[8]	55	1,70**

Hladina významnosti: ** $P < 0,01$
Significance level: ** $P < 0.01$

For 1–8 see Table 1, [9]number of effects within the factor, [10]service period (days)

Tab. 7. Průměry (LSM) a standardní chyby (SE) získané metodou nejmenších čtverců pro délku servis periody pro měsíce uvnitř ročního období – Means (LSM) and standard errors (SE) for service period interval related to months within the year season, as calculated by a least-squares method

Roční období[1]	Servis perioda (dny)[2]	
	LSM	SE
1	45,10	4,58
2	42,21	4,65
3	37,30	4,60
4	40,07	4,60
5	36,83	4,64
6	41,32	4,67
7	40,03	4,89
8	39,16	4,69
9	44,57	4,60
10	47,45	4,51
11	49,67	4,46
12	52,05	4,52

[1]year season, [2]service period (days)

kosti vrhu pro roční období (měsíce uvnitř roku). Faktor ročního období vykázal vysoce významný vliv na ukazatele velikosti vrhu ($P < 0,01$). Od září do dubna se vyskytovaly u počtu narozených, živě narozených a odstavených selat nejvyšší hodnoty. Od května do srpna byly tyto hodnoty naopak nejnižší. Znamená to, že prasnice oprasené v letním období měly v průměru všechny hodnoty ukazatelů velikosti vrhu nižší. Nejpříznivější měsíce pro oprasení jsou listopad a prosinec. Uvedené zjištění je v souladu s poznatky našich i zahraničních autorů (Říha, 1997; Čeřovský *et al.*, 1998; Říha *et al.*, 1999; Pepper, Taylor, 1981; Prunier *et al.*, 1994; Kermabon *et al.*, 1995) a rovněž koresponduje s výsledky uvedenými v naší předchozí práci (Říha *et al.*, 1999). V našich podmínkách činilo procento jalovosti prasnic, které byly inseminovány v květnu až

Tab. 6. Průměr, směrodatná odchylka a variační koeficient pro délku servis periody – Mean, standard deviation and coefficient of variation for SP interval

Faktor[1] n = 2 246	Průměr (dny)[3]	Směrodatná odchylka (dny)[4]	Variační koeficient[5] (%)
Servis perioda[2]	43,37	19,85	45,78

[1]factor, [2]service period, [3]mean (days), [4]standard deviation (days), [5]coefficient of variation

Tab. 8. Průměry (LSM) a standardní chyby (SE) získané metodou nejmenších čtverců pro délku servis periody pro pořadí vrhu s uvedením četnosti prasnic uvnitř vrhu – Means (LSM) and standard errors (SE) for service period interval related to parity and sow frequency within the litter, as calculated by a least-squares method

Pořadí vrhu[1]	Servis perioda (dny)[2]		Četnost prasnic[3]	
	LSM	SE	ks	%
1	67,24	4,39	199	8,9
2	48,78	4,58	284	12,6
3	48,26	4,18	264	11,8
4	47,49	4.,9	270	12
5	45,43	4,00	305	13,6
6	41,12	3,97	298	13,3
7	41,55	3,9	251	11,2
8	38,98	4,28	108	4,8
9	40,42	4,39	87	3,9
10	34,7	4,58	69	3,1
11	39,69	5,08	45	2,0
12	37,84	5,55	31	1,4
13	35,6	6,46	19	0,8
14	46,15	7,81	11	0,5
15	29,86	15,48	2	0,1
16	35,82	15,59	2	0,1
18	22,72	21,15	1	0,0

[1]parity, [2]service period (days), [3]sow frequency

říjnu, 13 až 15 %, zatímco z prasnic inseminovaných v listopadu až dubnu bylo pouze 8 až 12 % jalových. Odpovídá to i údajům v literatuře o kombinovaném vlivu světla a teploty na reprodukční schopnost prasnic (Prunier *et al.*, 1994). Jedním z atributů tohoto efektu je kolísání reprodukčních schopností v závislosti na působení exogenních estrogenů (Cox *et al.*, 1987).

Největší počet mrtvě narozených selat byl rovněž zaznamenán v měsících květen až srpen. Mortalitu selat od narození do odstavu nebylo možné v užitkovém chovu vyhodnotit.

Charakter počasí při inseminaci a fáze měsíce při inseminaci (tab. 1) neměly průkazný vliv na ukazatele velikosti vrhu ($P > 0,05$). Teplota při inseminaci měla významný až vysoce významný vliv ($P < 0,05$; $P < 0,01$) na ukazatele velikosti vrhu (tab. 1).

Pořadí vrhu (tab. 1) nemělo významný vliv ($P > 0,05$) na ukazatele velikosti vrhu. Tento výsledek je na

první pohled překvapující, vzhledem k tomu, že se všeobecně uvádí zvyšování velikosti vrhu od prvního vrhu až po zhruba třetí až pátý vrh a že se posléze se zvyšujícím pořadím vrhu četnost vrhu opět snižuje (Anderson, Melampy, 1972; Britt *et al.*, 1983; Martinat-Botté *et al.*, 1984; Clark a Leman, 1986). Je třeba mít na zřeteli okolnost, že se jednalo o sledování v provozních podmínkách a že nebyl počet prasnic ve všech sledovaných vrzích stejný.

Z tab. 1 vyplývá, že faktor otce prasnice a faktor připařovaného plemeníka měly významný až vysoce významný vliv na všechny ukazatele četnosti vrhu ($P < 0,05$; $P < 0,01$). Tento poznatek je v souladu se zjištěními, která uvádějí Johnson *et al.* (1978) a Webb (1995). Existují genetické zdroje pro zvyšování velikosti vrhu.

V tab. 5 je uvedena charakteristika souboru s členěním na počet efektů uvnitř faktorů pro ukazatel délky servis periody. V tab. 6 jsou uvedeny základní charakteristiky.

Významnost rozdílů mezi efekty uvnitř faktorů pro délku service periody je uvedena v tab. 5. Roční období a teplota při inseminaci měly průkazný vliv na oba ukazatele, stejně jako v případě ukazatelů velikosti vrhu ($P < 0,01$). Avšak na rozdíl od ukazatelů velikosti vrhu mělo pořadí vrhu vysoce významný vliv na délku servis periody. Otec prasnice měl významný vliv na oba ukazatele ($P < 0,01$). Ostatní sledované faktory byly statisticky nevýznamné ($P > 0,05$).

Roční období (tab. 7) mělo vysoce významný vliv ($P < 0,01$) na délku servis periody. V měsících březen až srpen byla servis perioda kratší (v rozpětí 36,83 až 41,32 dnů) než v měsících září až únor (od 42,21 do 52,05 dnů). Naše zjištění je v rozporu s poznatky autorů Aumaitre *et al.* (1976) a Hurtgen a Leman (1981). Aumaitre *et al.* (1976) uvádějí u prasnic oprasených v období červenec–září o 10 dní delší interval mezi odstavem a zabřeznutím než u prasnic oprasených ve zbývající části roku. Rovněž v USA byl zjištěn delší interval u prasnic oprasených v období červen–srpen (Hurtgen, Leman, 1981). Je zřejmé, že s ročním obdobím kolísá délka světelného dne. Kermabon *et al.* (1995) zjistili, že v případě krátkých denních cyklů se zvyšuje podíl říjících prasnic po odstavu v porovnání s dlouhými denními cykly. Prunier *et al.* (1994) prokázali vliv světelného režimu na reprodukční schopnost prasnic, vyjádřenou délkou intervalu mezi odstavem a říjí, resp. zabřeznutím.

V tab. 8 jsou uvedeny průměry (LSM) a standardní chyby (SE), získané metodou nejmenších čtverců, pro délku servis periody pro pořadí vrhu s uvedením četnosti prasnic uvnitř vrhu. Nejdelší servis perioda je na prvním vrhu (67,24 dnů) a postupně klesá až na hodnotu 38,98 dnů na 8. vrhu. Délka servis periody je zpravidla v 9. vrhu a vrzích s vyšším pořadí přibližně stejná nebo nepatrně nižší než v 8. vrhu. Tato informace má omezenou vypovídací schopnost pro relativně nižší četnost prasnic v těchto vrzích, což se odráží rovněž ve vyšší standardní chybě. Naše zjištění odpovídá výsledkům pro reprodukční schopnost prasnic na různých vrzích uvedeným v pracích autorů Říha *et al.* (1999) a Čeřovský *et al.* (1998).

Otec prasnic měl vysoce významný vliv na délku servis periody (tab. 5). Existuje genetická možnost zkracovat servis periodu zařazením plemeníků prověřených podle potomstva do plemenitby. Tím ovšem lze zkracovat i generační interval. Selekční pokrok je příznivě ovlivněn jak zvyšováním spolehlivosti odhadu plemenné hodnoty, tak i zkracováním délky generačního intervalu (Jakubec *et al.*, 1998).

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CIRCADIAN BIORHYTHM OF MELATONIN LEVEL IN SWORDTAIL (*XIPHOPHORUS HELLERI*)

CIRKADIÁNNÍ BIORYTMUS HLADINY MELATONINU U MEČOVKY (*XIPHOPHORUS HELLERI*)

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ABSTRACT: Many biological features of fish of the genus *Xiphophorus* are very suitable for use of these organisms in different domains of biological research, e. g. developmental biology, genetics, oncology and others. The aim of this study was to monitor the melatonin level in swordtail (*Xiphophorus helleri*) under different light conditions: LD 12:12, 16:8, 20:4 (L: 13.00–9.00), constant light and constant dark. Melatonin level was detected by radioimmunoanalysis (RIA). 30 adult individuals on average were used in each experiment. Fish of „Berlin" line were used in one experiment only. Its number was also 30 individuals. For comparison of melatonin levels in brain, eyes and intestine a group of 45 individuals was used. Daily course of melatonin production in *Xiphophorus helleri* under the light regime LD 12:12 (L: 6.00–18.00) suggests the existence of circadian rhythm. Other modifications of light regime indicate the influence of light conditions, and constant conditions also indicate the presence of an endogenous rhythm. Significantly higher ($P < 0.05$) values of melatonin level in males compared to females under light regime LD 20:4 (L: 13.00–9.00) were found. In other cases including light conditions LD 12:12 the differences between both sexes were not significant. The difference between melatonin levels in fish of original form and individuals of "Berlin" line under light conditions LD 12:12 was significant ($P < 0.05$, higher in line "Berlin"). Positive correlations between melatonin level in the brain, eyes and gastrointestinal tract were found.

Keywords: swordtail (*Xiphophorus helleri*); melatonin; light regime

ABSTRAKT: Ryby rodu *Xiphophorus* jsou výhodné modelové organismy v řadě biologických věd – vývojové biologii, genetice, onkologii a dalších. Z endokrinologických studií dosud nebyly známy zákonitosti sekrece melatoninu u tohoto druhu ryby. Cílem studie bylo sledování denního průběhu hladin melatoninu u mečovky (*Xiphophorus helleri*) ve světelných podmínkách LD 12:12, 16:8, 20:4 (L: 13.00–9.00 hod.), stálém světle a stálé tmě. K práci byly použity ryby původní formy a vybrané akvarijní linie, tzv. berlínské, téhož druhu. K pokusům byly vybrány skupiny o průměrném počtu 30 jedinců. Berlínská linie byla použita pouze v jednom experimentu, taktéž v počtu 30 kusů. Pro srovnání hodnot obsahu melatoninu v mozku, očích a střevě byla vybrána skupina 45 jedinců. Hladina melatoninu byla sledována v mozku a očích dohromady, ke zjištění lokalizace sekrece u vybraných skupin jedinců v hlavě mozku a očích zvlášť a u skupiny jedinců orientačně i ve střevě. Obsah melatoninu byl ve vzorcích stanoven pomocí radioimunoanalýzy (RIA). V podmínkách světelného režimu LD 12:12 byla naznačena existence cirkadiálního rytmu produkce melatoninu. V případě synchronizace k modifikovaným světelným režimům byl patrný vliv délky fotoperiody. V případě konstantních podmínek byla naznačena možnost existence endogenního biorytmu. V případě světelného režimu LD 20:4 (L: 13.00–9.00 hod.) byla nalezena statisticky významně ($P < 0,05$) vyšší hladina melatoninu u samců než u samic, ve světelných podmínkách LD 12:12 byly zjištěny statisticky významně ($P < 0,05$) vyšší hodnoty hladiny melatoninu u linie „Berlin" než u ryb původní formy. Mezi obsahem melatoninu v mozku, očích a střevě pokusných ryb byly zjištěny pozitivní korelace.

Klíčová slova: mečovka (*Xiphophorus helleri*); melatonin; světelný režim

INTRODUCTION

The viviparous fish of the *Poeciliidae* family have many specific biological features. One of the important genera of this family is *Xiphophorus* HECKEL, in which 17 species are known. These are viviparous fish of the small body size. Body growth of males is terminated by sexual maturity. One of the widely distributed

species of genus *Xiphophorus* (swordtail) is *Xiphophorus helleri*. The area of distribution of this species is Mexico (and other tropical and subtropical areas where it has been introduced) and its considerable adaptability to various environmental conditions resulted in morphologically different natural populations, and many domestic lines.

Many biological features of the genus *Xiphophorus* are very advantageous for the use of these organisms in different domains of biological research, e. g. developmental biology, genetics, oncology and others. Basic biological features of these fishes are exemplified by *Xiphophorus maculatus* Schreibman *et al.* (1990): internal insemination by copulation, internal fertilization, sperm storage inside the female, follicular gestation, multiple broods from a single insemination, free-swimming newborn, usually 20 to 40 animals per brood, 28 days between broods on the year-round basis, age at maturity genetically determined (2 to 12 months) at P locus linked to body pigment genes, sexually dimorphic body structure, gonopodium development, index of androgen production, gametogenesis until death, average life span of 2.5 years.

Length of the light part of daily cycle is called photoperiod. Organisms which react to changes of photoperiod length by some changes of physiological functions are called photoperiodic organisms. The photoperiod is a highly important environmental factor, which can influence physiological functions, e. g. reproduction. Melatonin is one of the signals formed by the pineal organ of vertebrates. This hormone is involved, as an internal timer (in original terminology often referred to as "Zeitgeber"), in the control of various circadian and seasonal rhythms. In all vertebrates investigated so far, the LD cycle (it is a relation between the light and dark part of 24 hours cycle) is the principal environmental factor controlling melatonin secretion. Melatonin biosynthesis is low during daytime and high during nighttime. Melatonin influences a number of physiological functions including reproduction (Illnerová, 1995; Illnerová and Sumová, 1997). The influence of crowding of pigment particles in melanophores and other pigment cells is known in lower vertebrates. This function influences the colour of fish skin (Nayudu and Hunter, 1979; Fujii *et al.*, 1992).

Biosynthesis takes place in photoreceptors cells in the pineals of lower vertebrates. Their structure and function are analogous to retinal photoreceptors. The afferent nerves bring the light information from the pineal gland to sensoric areas of brain stem, but light influences the synthesis of melatonin. Rhythmicity of melatonin synthesis is endogenous (Falcon and Collin, 1989). Until now there have been a few studies of melatonin secretion in fish in comparison with mammals and birds.

A simplified radioimmunoassay (RIA) of plasma melatonin was used in the common carp (*Cyprinus carpio*) (Kezuka *et al.*, 1988). Results of this study suggest that melatonin is an important hormone in photoperiodism and circadian rhythm in fish. The results of an *in vitro* study focused on the function of epiphysis cells in pike (*Esox lucius*) indicate that an oscillator, which is synchronized with photoperiod, controls the activity of N-acetyltransferase activities and melatonin secretion rhythms (Falcon *et al.*, 1989). Melatonin secretion from the superfused goldfish (*Carassius auratus*) pineal gland is directly photosensitive. Goldfish pineal gland contains a circadian oscillator which generates rhythms in melatonin secretion (Iigo *et al.*, 1991).

The dependence of rhythmicity in melatonin secretion on the light-dark cycle was demonstrated on *in vitro* isolated rainbow trout pineals: increase of secretion in dark and decrease in light (Max and Menaker, 1992), and *Zacco temminckii* pineals (Takabatake and Iga, 1991). At the same time the influence of temperature was examined: a higher temperature increases melatonin secretion in dark and positively influences response sensitivity to the light.

Plasma melatonin levels were detected by RIA in rainbow trout (*Oncorhynchus mykiss*) kept under a photoperiod LD 8:16 and receiving light pulses during the late scotophase. In other experiments plasma melatonin levels were measured at hourly intervals in rainbow trout, kept under three different skeleton photoperiods: 8L:2D:2L:12D, 8L:7D:2L:7D and 8L:12D:2L:2D. The results suggest that melatonin secretion in the species used is not under endogenous circadian control like in other vertebrates: light pulses during photophase decreased the melatonin level. This level was increased after the beginning of scotophase, after 90 minutes it reached the values of the last scotophase (Alvarino *et al.*, 1993a, b). Analogous results were presented by Bolliet *et al.* (1996): *In vitro* melatonin secretion in pineal organs was measured in nine wild freshwater and six marine teleost species cultured at constant temperature and under different photic conditions. Results of these experiments suggest that most fish possess endogenous intrapineal oscillators driving the rhythm of melatonin production, with the exception of rainbow trout. The light/dark cycle influences the rhythmic production of melatonin by the rainbow trout pineal organ through a modulation of the serotonin N-acetyltransferase activity. Melatonin release, determined in superfused organs under natural conditions of illumination, was stimulated during the light period of a light/dark cycle by adding an analogue of cAMP or a phosphodiesterase inhibitor (Thibault *et al.*, 1993).

The parallelism between pineal and circulating melatonin patterns suggests that the lateral eyes of the brook trout (*Salvelinus fontinalis*) have no significant endocrine function as far as the melatonin rhythm in the blood is concerned. Circadian variations in melatonin levels in the blood and pineal organ showed similar patterns, higher values during the dark period, whereas melatonin concentrations in the retina increased slightly in the first half of the light period. Light exposure of 1 h duration at mid-dark decreased melatonin levels in the blood and pineal organ in an intensity-

dependent manner, whereas retinal melatonin levels increased with increasing light intensities (Zachmann *et al.*, 1992). Participation of eyes and pineal melatonin secretion in *Zacco temminckii* was examined under light conditions LD 12:12. Melatonin contents in the blood during a dark period were relatively inhibited by blinding.

Pinealectomy could change colour through the removal of a melatonin source, but it did not affect the colour changes during the light and dark period. Only experimentally pinealectomized fish in the constant dark (DD) showed darkening responses in contrast with normal fish. Pinealectomy of blind animals caused darkening responses during both light and dark periods (Takabatake *et al.*, 1992). The retina has been classically regarded as a mediator between light and the central pacemaker, which is located at the suprachiasmatic nucleus of vertebrates. Sánchez-Vázquez *et al.* (1997) examined the melatonin plasma and eye levels of a marine species of sea bass (*Dicentrarchus labrax*). Melatonin in the eyes exhibited an inverse profile, with high levels during daytime and low levels at night. This suggests that melatonin in plasma and the eyes may act independently of the sea bass physiology. Melatonin causes a significant reduction of noradrenaline levels in *Channa punctatus* held on LD 16:8 (temperature 22 °C) and on the LD 12:12 (temperature 17 °C), while the hypothalamic dopamine level did not change in either group (Khan and Joy, 1988).

The nocturnal rise in melatonin secretion was associated with an increase of cAMP production with an entry of Ca super (2^+) ions through L-type voltage dependent channels. It is shown that two inhibitors of calciproteins, W 7 and calmidazolium, inhibit melatonin secretion and, to a lesser extent, cAMP levels in cultured trout pineal photoreceptors (Begay *et al.*, 1994).

Circadian melatonin levels in the retina and gastrointestinal tract in sturgeon *Acipenser fulvescens*, rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) showed similar patterns. There were marked interspecific differences in gastrointestinal tract (GIT) melatonin levels (Bubenik and Pang, 1997).

MATERIAL AND METHODS

The original form (designated as "Wild") of *Xiphophorus helleri* from Mexico and an aquarium line designated as "Berlin" (which exists about 80 years) were used.

Basic characteristics of *Xiphophorus helleri*

Viviparous fish, originally widely distributed in nature in Mexico, were secondarily transported by humans to other tropical and subtropical regions. It became important for aquarium breeding and contributed to the development of aquaristics. In artificial breeding, genetic variability was a major biological prerequisite for development of many types of coloration and other morphological characteristics; for instance, changes in the shape of the tail and back fins, and body size. These and other qualities may be used in biological research, especially in genetics, physiology, developmental biology, oncology and other disciplines.

Characteristics of the lines used

The morphology of the form designated as Wild corresponds to the characteristics of the original form: females achieve total length of 80 mm, color is olive-green with several thin black strips and one red strip on sides. In advanced stages of pregnancy besides their increased abdominal volume, females have a distinct dark spot, so called pregnancy spot, in a caudal portion of the abdomen (pigmentation of the embryo eyes visible through the abdominal wall). Sexual dimorphism of external morphology is developed. In original forms, males achieve only 70 mm. An about 15–20 mm long sword is developed on the lower border of the tail fin of males at sexual maturity.

The line designated as "Berlin" did not have the basic swordtail coloring. It is light or dark red colored with differences between the black spots on body and fins. Black spots are formed by special cells called macromelanophores.

Breeding conditions

Aquaria with 30 liters of water with no substrate on the bottom were used for breeding. Young fish were kept in larger tanks with 80 liters of water. Tanks were planted with loose-floating plants and plants in pot. Only natural light was used. The average temperature was 22 °C, ranging from 21 °C to 24 °C. Tanks were aerated (to obtain 100% oxygen saturation) and those with a larger number of fish were equipped with filters.

Fish were fed live zooplankton twice a week. For the rest of the week, they were fed a commercial aquarium fish mixture; in winter, the diet was supplemented also with frozen plankton.

Gravid females at advanced stages of gravidity were moved into "birth tanks" in order to allow newly hatched fish to safely escape from mother's reach after birth (prevention of cannibalism). Slanting pieces of glass with a slot were used as birth tanks, placed into small tanks or plastic birth cages. After birth, females were returned into their original aquarium and four weeks later, they were moved into birth tanks again.

Individual lines of fish were kept separately (prevention of possible inter-linear competition). Their population densities, expressed as numbers of individuals per aquarium space, were set as different densities might influence growth characteristics.

Synchronization of circadian rhythms

Experimental fish were kept in aquaria with 50 liters of water. The aquaria were situated in the laboratory without natural daylight. Intensity of artificial light at the water surface in aquaria was 200 lx. Water temperature was 22–24 °C. Synchronization lasted for 21 days. Light schedules: LD 12:12 [12 hours of light (L) from 6.00–18.00, 12 hours of darkness (D) from 18.00–6.00], 16:8 (L: 3.00–19.00), 20:4 (L: 13.00–9.00 h.), LL (constant light), DD (48 hours of dark after 21 days of synchronization to natural light regime LD 12:12).

Sample collection

Tissues were sampled either in 3 hours intervals or in 4 hours intervals, in one series in 1 hour intervals, in order to find out the exact time of melatonin level changes. The fish were killed by carbon dioxide (CO_2) in a glass with soda-water, with the same temperature as water temperature in aquarium. After killing the body size of fish was measured and brain with eyes, or brain and eyes separately, were isolated. This preparation was carried out in a laboratory with aquaria, in the same light conditions as the fish were exposed instantaneously – in the dark part of daily cycle in the conditions of damped red light. Samples were immediately put in test-tubes and frozen at a temperature –30 °C.

Melatonin level detection

Melatonin levels were detected by radioimmunoanalysis (RIA) after extraction with dichloromethane (CH_2Cl_2) as described by Humlová (1992). Melatonin was measured by a direct radioimmunoassay (Frazer *et al.*, 1983): [^{3}H] melatonin, specific activity 3.15 Tbq/mmol, was purchased from the Radiochemical Centre (Amersham, UK). The antiserum, batch G/S 704-8483, was kindly provided by dr. J. Arendt via Stockgrand Ltd., Department of Biochemistry, University of Surrey. Samples of 25 and 100 microg melatonin per assay had interassay variations of 13 and 9%, respectively. The limit of assay detection was 6 pg per 500 microliters of sample. The value of 0 pg/ml was arbitrarily assigned to all baseline daytimes valued which were below the level of detection. Tissue extracts were dissolved in assay buffer, incubated with labelled ligand (about 10 000 cpm/tube) and antiserum (dilution 1 : 4 000) overnight. Free and bound radioactivity was separated by absorption on dextran-charcoal and centrifugation. Antibody-bound radioactivity in supernatant was measured in Bray scintillation fluid. Samples were assayed in duplicate and read against the calibration curve based on melatonin (3–100 pg/assay).

30 adult animals were used on average in each experiment. Fish of "Berlin" line were used in one experiment only. Their number was also 30 individuals. To compare melatonin levels in brain, eyes and intestines a group of 45 individuals was used. Mean values of melatonin level and also the standard deviation (S_d) were calculated. Single points in figures are mean values calculated from values of individual melatonin level. Error bars refer to the standard error (S_e) values. $S_e = S_d/\sqrt{n}$. Statistical significance was calculated at the level P: $P < 0.05$. Correlation between melatonin levels in adults and in embryos were calculated and also correlation between melatonin level values in brain, eyes and intestines.

RESULTS

Melatonin levels under light conditions LD 12:12 (L: 6.00–18.00 h) (Fig. 1)

The daily melatonin levels in *Xiphophorus helleri* under the light regime LD 12:12 document the existence of circadian rhythm. This figure shows the massive

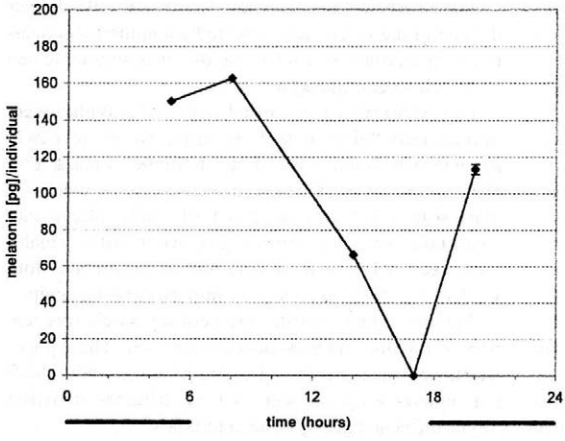

Fig. 1. Melatonin levels in *Xiphophorus helleri* under light conditions LD 12:12

Legend to Figs. 1–7:
— melatonin level
Black bars below abscisa indicate dark period of LD cycle

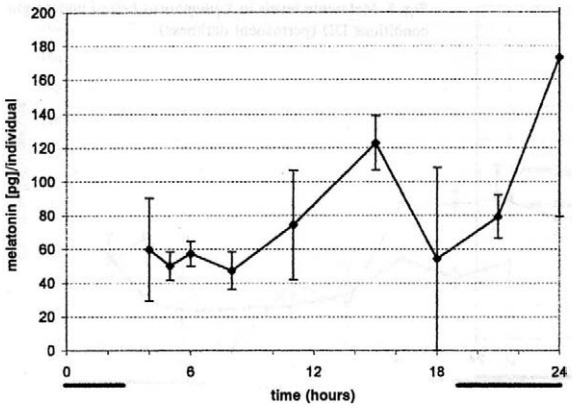

Fig. 2. Melatonin levels in *Xiphophorus helleri* under light conditions LD 16:8

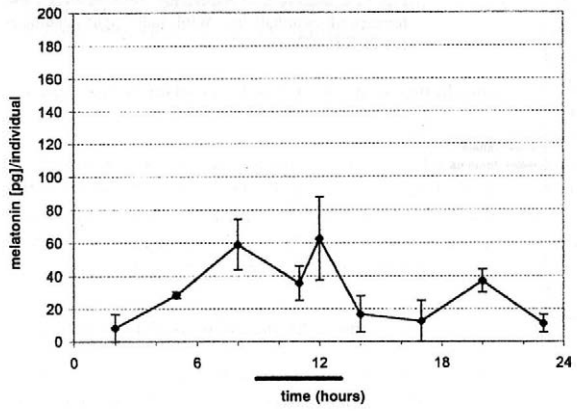

Fig. 3. Melatonin levels in *Xiphophorus helleri* under light conditions LD 20:4 (L: 13.00–9.00)

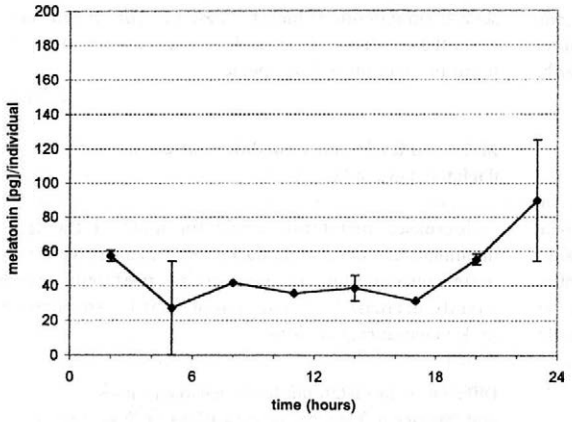

Fig. 4. Melatonin levels in *Xiphophorus helleri* under light conditions LL (permanent light)

production of melatonin with apparent minimum at 17.00 h. A marked evening increase can be detected in 1 hour after light off. In 2 hours after light on, the level of melatonin was still high. The first significant decrease was detected betwen 8.00–14.00.

Melatonin levels at light/dark phase ratio LD 16:8 (L: 3.00–19.00 h) (Fig. 2)

Under these light conditions, the night increase was observed already within 2 hours after light off and

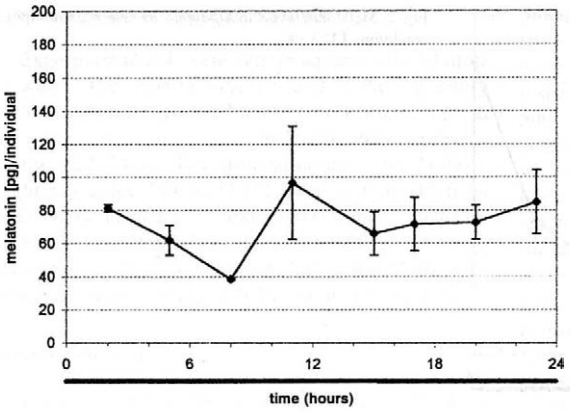

Fig. 5. Melatonin levels in *Xiphophorus helleri* under light conditions DD (permanent darkness)

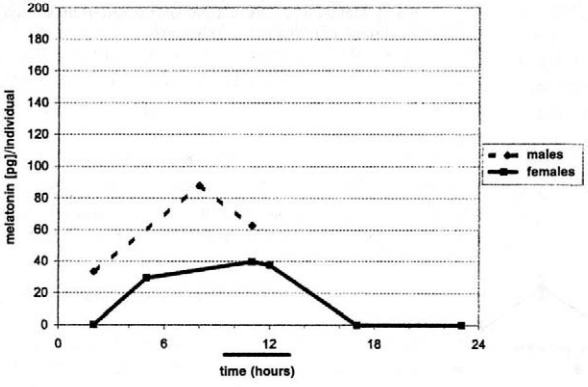

Fig. 6. Comparison of melatonin levels in males and females of swordtail line Wild under light conditions LD 20:4 (L: 13.00–9.00)

a peak occurred at midnight. The first decrease was detected already 2 hours after light on. An additional peak of melatonin in photophase appeared at 15.00 h.

Melatonin levels at light/dark phase ratio LD 20:4 (L: 13.00–9.00 h) (Fig. 3)

When the light regime was changed in such a way that scotophase occurred in daytime, melatonin peak concentration was shifted to dark hours (9.00–13.00 h). Start of melatonin level increase was indicated before light was switched off and a decrease occurred immediately after light was switched on. Another smaller second peak was found at 20.00 h.

Melatonin levels under conditions of permanent light (Fig. 4)

Constant light influenced daily melatonin levels in the following manner: the lowest melatonin concentrations were determined during the subjective day from 5.00 to 17.00 h. At night hours an increase was observed (maximum value at 23.00 h). This result suggests the existence of an endogenous rhythm of melatonin production in this species.

Melatonin levels under conditions of permanent darkness (48 hours) (Fig. 5)

Increased melatonin levels for most of the time document that permanent darkness influences the melatonin concentration. In the morning melatonin levels slightly decreased to minimum at 8.00 h. An increase took place during midday.

Differences in melatonin levels between males and females in the used modifications of light regime (Fig. 6)

Under light conditions LD 12:12 (L: 7.00–19.00 h) the differences between males and females in the daily melatonin levels were not significant. Significantly higher ($P < 0.05$) values of melatonin level in males were found in a small collection under light conditions

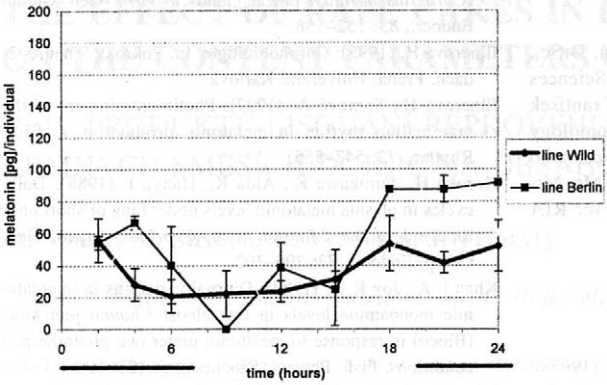

Fig. 7. Comparison of melatonin levels between swordtail line Wild and Berlin under light conditions LD 12:12

LD 20:4 (L: 13.00–9.00). In other cases the differences between both sexes were not significant.

Comparison of melatonin levels between swordtail lines (Fig. 7)

This comparison was possible only under light conditions LD 12:12 (L: 7.00–19.00 h). In this experiment individuals of the wild form and of "Berlin" line were used at the same time. The melatonin levels were significantly higher ($P < 0.05$) in the "Berlin" line.

Localization of melatonin concentration

Results proved analogous melatonin levels in brain, eyes and intestines in the used groups of fish. There were positive correlations between melatonin levels detected in the brain and eyes, the brain and intestines and the intestines and eyes.

DISCUSSION

There is not much information in the literature about melatonin concentrations in *Poeciliidae* family. The results of this study suggest that regular melatonin level patterns exist in circadian cycle in this species, similarly like in other organisms, e. g. laboratory mammals (Illnerová, 1995; Illnerová and Sumová, 1997). Maximum levels were found during scotophase.

Bolliet *et al.* (1996) studied pineal melatonin levels in nine fresh water and six marine fish species. Rhythmical changes in melatonin secretion were found in pineals of all these organisms except rainbow trout (*Oncorhynchus mykiss*) under light/dark alternation conditions and in permanent dark conditions. In most of the species studied, similar or higher maximum melatonin levels were detected under permanent dark conditions than under light/dark alternation. Rhythmic melatonin secretion in rainbow trout (*Oncorhynchus mykiss*) is evidently controlled by light impulses. In fish, as in most other vertebrates, some interspecific differences may exist in the manner of melatonin production control. This aspect is little understood in fish so far.

These facts correspond with the results of Iigo *et al.* (1991), who studied melatonin levels in fish as a light dependent function after studying melatonin production in *in vitro* cultured pineals of *Carassius auratus*. Similarly, Alvarino *et al.* (1993a) and Max and Menacher (1992) described melatonin secretion in the rainbow trout (*Oncorhynchus mykiss*) as a direct response to the dark period, which is not related to endogenous circadian control like in other vertebrates. Analogous results were also found in the taxonomically distant species *Zacco temminckii* (Takabatake and Iga, 1991).

Our results of melatonin level study in swordtail *Xiphophorus helleri* suggest that it belongs to species with endogenous rhythm, but with some plasticity of melatonin levels under the influence of light regime.

Our results also document that the waveform of the rhythm of melatonin production depends on photoperiod. In fish maintained under LD 12:12, the duration of a high night value of melatonin seems to be longer than in fish maintained under either LD 16:8 or LD 20:4. This finding would correspond to what has been observed in rats (Illnerová, 1995; Illnerová and Sumová, 1997), but more detailed studies would be necessary to fully confirm this fact.

Results concerning localization of melatonin production correspond to the data of Takabatake *et al.* (1992), who worked with *Zacco temminckii*. On the contrary the retina of brook trout (*Salvelinus fontinalis*) probably does not have an endocrine function (Zachman *et al.*, 1992). Significant correlations between melatonin levels in brain, eyes and gastrointestinal tract (GIT) were found. Melatonin secretion in the gastrointestinal tract and retina corresponds to results reported by Bubenik and Pang (1997) in sturgeon *Acipenser fulvescens*, rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*). There were marked interspecific differences in GIT melatonin levels.

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THE EFFECT OF RAPE CAKES IN FEED MIXTURES ON THE CONTENT PARAMETERS OF COW MILK*

VLIV PRODUKTŮ LISOVÁNÍ ŘEPKOVÉHO SEMENE V PRODUKČNÍCH KRMNÝCH SMĚSÍCH DOJNIC NA OBSAHOVÉ UKAZATELE MLÉKA

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ABSTRACT: In a trial performed on 15 dairy cows of Czech Pied cattle feed mixtures for lactating cows with a high proportion of oil cakes from different types of rapeseed pressing (cold pressing and hot pressing) were assessed (mixture S1 with 15% of rape cakes from cold pressing, mixture S2 with 25% of rape cakes from hot pressing, against control mixture with 20% of soybean meal). One of the objectives of the trial was to assess the effect of experimental mixtures on the composition of milk, especially from the viewpoint of fat, protein, casein, solids and solids-non-fat content. The content of milk fat was nearly identical for both experimental groups and control (4.33% for the control group, 4.36% for S1, and 4.33% for S2). The fat yield was higher for the experimental groups than for the control group (+44.9 g/day with S1, +69.19 g/day with S2, $P > 0.05$). The experimental groups tended to show lower proportions of proteins (–0.10% with S1, –0.07% with S2, $P > 0.05$), these differences were statistically insignificant. The yield of proteins was 591 g/day for S1, 618 g/day for S2 and 573 g/day for the control group ($P > 0.05$). In a direct relation to the total content of proteins both experimental groups showed a statistically significant reduction in the content of casein (–0.20% with S1 and –0.17% with S2, $P < 0.01$). The casein yield was 501 g/day for S1, 526 g/day for S2 and 506 g/day for the control group ($P > 0.05$). The proportion of casein in the total protein content in milk was assessed, and equal results for both experimental groups and a statistically significant reduction in comparison to the control mixture were found (–0.03 with S1 and S2 equally, $P < 0.01$). Neither the content nor the yield of solids and non-fat solids in milk were significantly affected by the tested components.

Keywords: rape cakes; cold pressing; hot pressing; feed mixtures for lactating cows; milk fat; proteins; casein; solids; solids-non-fat

ABSTRAKT: V krmném pokusu s 15 dojnicemi českého strakatého skotu byly hodnoceny produkční krmné směsi s vysokým zastoupením řepkových výlisků lisovaných za studena (lisování oleje za účelem výroby metylesteru řepkového oleje) a řepkových pokrutin (technologie lisování při teplotě 105 °C). Směs S1 obsahovala 15 % řepkových výlisků, směs S2 25 % řepkových pokrutin a do směsi kontrolní bylo zařazeno 20 % sójového extrahovaného šrotu (podrobné složení směsí znázorňuje tab. 1). Náhrada sójového extrahovaného šrotu produkty lisování řepkového semene činila u směsi S1 75 % a u směsi S2 85 %. Průměrný denní příjem produktů lisování řepkového semene z krmných směsí činil u skupiny S1 0,67 kg sušiny/ks a u skupiny S2 1,09 kg sušiny/ks (tab. 4). Jedním z cílů pokusu bylo sledování vlivu pokusných směsí na složení mléka – obsah tuku, bílkovin, kazeinu, sušiny a tukuprosté sušiny. Obsah mléčného tuku byl u všech skupin téměř shodný (kontrola 4,33 %, S1 4,36 %, S2 4,33 %), produkce tuku byla oproti kontrole S0 u pokusných skupin vyšší (S1 +45 g/den, S2 +69 g/den, $P > 0,05$; průměrné hodnoty rozborů mléka jsou uvedeny v tab. 2). U pokusných skupin S1 a S2 se projevila tendence k nižšímu obsahu bílkovin (S1 –0,10 %, S2 –0,07 %, $P > 0,05$), rozdíly byly statisticky neprůkazné. Produkce bílkovin, vypočtená z obsahu bílkovin v 1 kg mléka a produkce mléka v kg, byla u skupiny S1 591 g/den, u skupiny S2 618 g/den a u skupiny kontrolní 573 g/den ($P > 0,05$). V úzkém vztahu k obsahu celkových bílkovin bylo u obou pokusných skupin statisticky průkazné snížení obsahu kazeinu (S1 –0,20 %, S2 –0,17 %; $P < 0,01$). Produkce kazeinu byla vyrovnaná, u skupiny S1 501 g/den, u skupiny S2 526 g/den a u kontrolní skupiny 506 g/den ($P > 0.05$). Dynamika průběhu obsahu a produkce tuku, bílkovin, kazeinu a tukuprosté sušiny je znázorněna na obr. 1 až 4. Podíl kazeinu z celkových bílkovin mléka byl u obou pokusných skupin shodný, ve srovnání s kontrolou průkazně nižší (S1, S2 –0,03; $P < 0,01$). Během pokusu nedošlo k žádným statisticky průkazným změnám obsahu a produkce sušiny a tukuprosté sušiny mléka.

Klíčová slova: řepkové výlisky za studena; řepkové pokrutiny; produkční krmné směsi pro dojnice; mléčný tuk; bílkoviny; kazein; sušina mléka; tukuprostá sušina mléka

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INTRODUCTION

The objective of this work was to define the effect of cold pressed and hot pressed rape cakes in dairy cow feed on the content and yield of the basic milk components. The assessment was based on data obtained by the project of the National Agency for Agricultural Research (No. 7013 "Research into the Relationship between the Content of Rapeseed Feeds in the Assortment of Feed Mixtures for Dairy Cows and their Performance, Economy of Production and Composition of Milk").

As a result of an increased production of rapeseed in the Czech Republic the oil processing industry produces a large amount of rapeseed feed elements. In addition to the traditional rapeseed meal and hot pressed rape cakes, the production of cold pressed rape cakes has increased recently in relation to the growth of rape processing outside the food industry. This has brought about a chance to replace a significant proportion of soybean protein in feed mixtures for dairy cows by rapeseed feeds. However, besides the question of the economy of such a substitution, there is a question how the change would affect milk composition.

Rapeseed meal has recently been the most common rapeseed component of the feed of dairy cows. The comparison of rapeseed and soybean meal has shown that after the substitution of the latter by the former the energetic value of the feed mixture remained within an acceptable range. The content of crude protein in rapeseed meal and cakes was lower. The biological nutritive value of the meal and the cakes was defined by the sum of essential amino acids. The index of the sum of essential amino acids in rapeseed meal "00" and cakes was 78.07 and 63.31, respectively (for soybean meal 100%) (Lichvár, 1997). Traditional formulations for complementary feed mixtures for dairy cows usually contain up to 15% of rapeseed meal (Zeman *et al.*, 1998). There is still a considerable mistrust in rapeseed feed despite its high nutritive value, which is even more true about rape cakes.

Numerous trials focusing on rapeseed meal have proved that even with regard to the content of anti-quality components a higher proportion of rapeseed meal in the diet can be recommended without any significant negative effects on the health state, reproduction and performance of cows (Zech, 1995; Gutzwiller, 1996). No significant negative effects were observed in milk fat and protein content and milk yield reported by various authors dealing with rapeseed meal "00" in the diet of dairy cows with regard to their performance (Zech, 1995), 30% of rapeseed meal in feed mixtures for lactating cows (Münger, 1996), 70% of rapeseed meal in the protein concentrate of feed mixture, average daily intake of rapeseed meal 1.4 kg/head (Eyer, 1996), average daily intake of rapeseed meal 2.0 kg/head). As for the proteins, Zech (1995) even found a significant increase in their content in milk.

The assessment of rapeseed feed cannot be confined to anti-quality components and their content only, though. There are also the above-mentioned differences in the value and digestibility of nutrients and the amino acid composition. Comparisons of the nutritive value and chemical composition of rape cakes and rapeseed „00" meal have shown better digestibility of nutrients (crude fibre in particular) of the rape cakes, their higher proportion of usable lysine and their higher nutritive value resulting from a higher fat content and better digestibility of the cake nutrients. Regarding the differences in the nutritive value of different rapeseed feeds a parallel can be drawn between the effect of a higher proportion of rape cakes on the performance of dairy cows and that of full-fat rapeseed meal. Trials with higher proportions of full-fat rapeseed meal "00" (rape seed "00") in feed mixtures for dairy cows, in comparison to the results of the above experiments with rapeseed meal, yielded the following different conclusions: In LVVG (1993) a significant reduction of the fat content in milk, an inclination towards a lower protein content and a higher milk yield were observed (with 15% of rapeseed in the mixture for lactating cows, or 1.3 kg/head/day). Jahreis *et al.* (1996) reported a significant decrease of the fat and protein content in milk and a significant increase in milk yield (1.0 kg/head/day of rapeseed).

According to available literature, the way how the feed mixtures with a higher proportion of rape cakes affect milk composition and performance of dairy cows is comparable to the results of trials with rape seed "00" (full-fat rape seed meal). Pajtáš *et al.* (1995) detected a significant increase of milk yield in trials with cold pressed rape cakes, but no significant changes in the fat and protein content (the average consumption of rape cakes was 1.46 kg/head/day), Jahreis *et al.* (1996) reported a significant reduction of fat and protein in milk when the diet contained 2.50 kg/head/day of cold pressed rape cakes, together with a significant increase in dairy cows' performance, LVVG (1995) performed their own trials with dairy cows and daily intake of 1.7 kg/head of cold pressed rape cakes (or 30% of rape cakes in the mixture for dairy cows), finding no significant changes in the fat or protein content in milk nor in milk yield. Part of Jahreis's *et al.* trial (1996) was application of a half of the usual portion of rape cakes (1.25 kg/head/day), which did not confirm the significant change in the dairy cows' performance resulting from the first part of the trial.

The comparable literature results mentioned above show that an increased proportion of rape cakes in feed mixtures can result in a slight reduction of fat and protein content in milk on the one hand, and on the other in increased milk yield. Despite this, such a comparison of the effects of higher proportions of rape cakes in feed mixtures for dairy cows is by no means unambiguous. As for the changes in the composition of milk the authors mostly concentrate on the fat component

(regarded from the angle of changes in the fatty acid composition of milk fat) and less on the protein content. Our trial focused on investigations into the effect of higher proportions of rape cakes in feed mixtures for dairy cows, giving more weight to the total content of milk proteins and casein.

MATERIAL AND METHOD

The trial was performed on fifteen dairy cows of Czech Pied cattle (or rather F_1 generation of Czech Pied x Montbéliard), individually stabled. The test animals were selected according to their live weight and performance. According to the Vebster classification the cows were classified 3 at the beginning of the trial, which means a good state of nutrition. The basic diet was based on corn silage (with 33.4% of dry matter) and alfalfa hay. The composition of the proposed feed mixtures and their nutritive values are summarised in Table 1. The dosage of mixtures for milk yield exceeding the usual performance of the basic diet was 0.45 kg per 1 kg of milk. Residues of feed were weighed and recorded daily. In the course of the trial laboratory analyses of the feed were performed in regular intervals. The amount of milk from the test animals was recorded daily. Samples of milk were extracted on the 50th, 70th, 100th, 130th, 160th, 200th, 250th, 270th and 305th day after calving and the content of solids, fat, total proteins, casein, and milk density were measured. Dry matter was determined by weighing, fat was determined using the acidobutyrometric method (according to Gerber), casein was determined by the Pfeiffer method (Czech Commercial Standard No. 57 0530), protein content was determined by titration (protein titre of milk). The proposed feed mixtures were based on barley, corn, wheat, oats and protein components (flax, soybean meal, cold pressed rape cakes 15.82% fat/DM, and hot pressed rape cakes 13.93% fat/DM). Rape cakes were obtained from rape oil production by cold pressing (feed mixture S1) and hot pressing at 105 °C (feed mixture S2). As for the minerals, the DOM 3 (Agricultural Cooperative at Moutnice) minerals and vitamin mix and feeding salt were used. The feed mixtures for dairy cows were designed as isonitrogenous (CP content 17%). Because of a higher proportion of fat in rape cakes the experimental mixtures showed higher energetic values than the control mixture (+0.28 MJ NEL for S1, +0.31 MJ NEL for S2).

Table 1. Ingredient and chemical formulation of the presented mixtures for lactating cows

Ingredient	Mixture for lactating cows		
	S0	S1	S2
	% of DM		
Barley	15.0	15.0	10.0
Corn	35.0	35.0	30.0
Wheat	12.0	12.0	12.0
Oats	12.0	12.0	12.0
Flax	3.0	3.0	5.0
Soybean meal	20.0	5.0	3.0
Rapeseed cakes	0.0	15.0	0.0
Rapeseed expellers	0.0	0.0	25.0
MKP-DOM 3[1)]	2.5	2.5	2.5
Feeding salt	0.5	0.5	0.5
Total	100.0	100.0	100.0
Chemical analysis	S0	S1	S2
	g/kg		
DM	880.00	890.00	880.00
CP	172.00	170.00	169.00
PDIN	118.95	108.87	127.35
PDIE	118.09	104.34	106.27
Ca	4.96	4.54	4.93
P	5.40	5.45	5.96
NEL, MJ	7.05	7.33	7.36

[1)] calcium 162.5 g/kg, phosphorus 34.7 g/kg, sodium 39.0 g/kg, magnesium 74.9 g/kg, zinc 193 mg/kg, manganese 216 mg/kg, iron 542 mg/kg, cobalt 20 mg/kg, iodine 20 mg/kg, vitamin A 17 500 i.u./kg, vitamin D 3 500 i.u./kg, vitamin E 40 mg/kg

RESULTS AND DISCUSSION

Rape cakes were used in the experimental feed mixtures as replacement of a part of soybean meal. The proportion of soybean meal in the S0 control mixture was 200 g/kg (Table 1). In the S1 feed mixture with cold pressed rape cakes 75% of soybean meal was replaced by the cakes. The average daily intake of rape cakes in S1 was 0.67 kg/head, which resulted in the daily intake of 106 g/head/day of rape oil. In the S2 feed mixture with hot pressed rape cakes, 85% of soybean meal was replaced by the cakes. The average daily intake of rape cakes in S2 was 1.09 kg/head, which resulted in the daily intake of 152 g/head/day of rape oil. The average daily dry matter intake was 20.17 kg/head for S0, 20.84 kg/head for S1and 20.56 kg/head for S2 (Table 2).

For the average values of the content and yield of fat, proteins, casein, non-fat solids and solids see Table 3. The fat content in milk yielded after application of S1 and S2 was virtually identical (4.36% and 4.34%, respectively) and did not differ from the control group either (S0 4.33%) ($P > 0.05$). In both experimental groups there was a trend towards higher daily yield of

Table 2. Average daily nutrient intake from the diets

Group	DM	CP	NEL	PDIN	PDIE	Fat		CF
	kg	kg	MJ	kg	kg	kg	% DM	kg
S0	20.17	2.65	121.99	1.69	1.74	0.61	3.03	4.32
S1	20.84	2.68	127.77	1.72	1.75	0.73	3.52	4.45
S2	20.56	2.76	125.90	1.78	1.73	0.70	3.43	4.46

Table 3. Average milk composition and production of milk components

Characteristics		S0		S1		S2	
		mean	s.e.	mean	s.e.	mean	s.e.
Protein content	(%)	3.29	0.04	3.19	0.03	3.22	0.03
Protein yield	(g/d)	573.38	24.09	590.52	16.81	617.92	23.86
Casein content	(%)	2.91^{A}	0.04	2.71^{B}	0.03	2.74^{B}	0.03
Casein yield	(g/d)	505.69	21.07	500.88	12.81	526.07	21.13
Casein / Protein	(%)	0.88^{A}	0.01	0.85^{B}	0.01	0.85^{B}	0.01
Fat content	(%)	4.33	0.09	4.36	0.09	4.34	0.06
Fat yield	(g/d)	757.75	31.53	802.66	23.36	826.96	33.23
Solids content	(%)	13.80	0.14	13.74	0.16	13.82	0.11
Solids yield	(g/d)	2 409.39	99.93	2 542.66	68.38	2 622.76	102.82
Solids-non-fat content	(%)	9.47	0.09	9.38	0.11	9.49	0.09
Solids-non-fat yield	(g/d)	1 653.10	70.79	1 739.25	49.41	1 822.47	74.17

$^{A, B}$ Treatment means of rows differ significantly ($P < 0.01$)

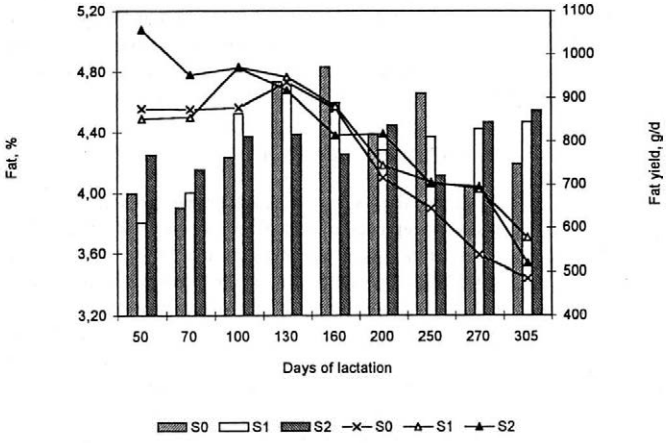

Fig. 1. Dynamics of milk fat content (bar chart) and fat yield (line chart)

milk fat, namely +45 g with S1 and +69 g with S2 ($P > 0.05$). Fig. 1 shows the dynamics of milk fat content and daily yield in the form of a flow chart.

A higher fat content in diets has negative effects on ruminal fermentation and lowers microbial activity, which results in decreased digestibility of crude fibre and organic matter and changed ratio of propionic and acetic acid (Sommer, 1997). Acetic acid is the main precursor of milk fat in the milk gland. Acetic acid is produced in the rumen from structural saccharides in the course of rumen fermentation, or results from beta oxidation of fatty acids in fat tissues of dairy cows (Kudrna *et al.*, 1998). A lack of milk fat precursors can also result in a decreased fat content in milk. Very high proportions of rapeseed feed (rape cakes and rape seed itself) can have a negative effect on fat content in milk. In a trial with dairy cows Pajtáš *et al.* (1993) replaced one third of the grain in a feed mixture by cold pressed rape cakes with oil content of 15.5%. For the average daily rape cakes consumption of 1.46 kg/head/day they did not find any significant changes in the content of fat, proteins and lactose. On the contrary, Jahreis *et al.* (1996), who replaced 2.50 kg of feed mixture by rape cakes (with fat content of 16.0%), found a significant reduction of milk fat and protein content in milk. A similar result was achieved in the second part of the same trial, where 1 kg of full-fat rapeseed meal was used instead of the cakes. The effect of additional lipids on fat content in milk was not constant. Doreau and Chilliard (1992) suggested that it depended on an increase in the concentration of long-chain fatty acids in the diet and a decrease in the synthesis of medium- and short-chain fatty acids in the milk gland. However, there is a general trend of additional lipids lowering milk protein content, and especially casein content.

In our trial the intake of rapeseed feed linked with the intake of rape oil (Table 4) in the course of lactation did not probably reach the level which is able to affect the milk fat content. This partly corresponds to some of the published analyses of effects of rape cakes inclu-

sion in the feed of dairy cows. See for example LVVG (1995) (average daily intake of cakes 1.75 kg/head), Jahreis *et al.* (1996) (the trial variant with 1.25 kg/head of cakes), and Lossmann *et al.* (1996) (a trial with 15% of rape cakes in the mixture). In none of the above trials any significant effect on fat content has been reported.

There was a trend towards a certain decrease in protein content in the milk of the experimental dairy cows (Table 3). With the control group the average protein content was 3.29%. With the S1 group (cold pressed rape cakes) the protein content was 3.19%, i.e. 0.10% less than in the control group, which was statistically insignificant. A similar result was found with the S2 group (hot pressed cakes), where the average content was 3.22%, i.e. 0.07% less than in the control group ($P = 0.0671$). The trend towards a lower protein content, however, did not manifest itself in the lower protein yield of either of the experimental groups. Rather to the contrary, the protein yield was higher with both groups, that is to say by 17.13 g/head/day with S1 and by 44.53 g/head/day with S2, even if still statistically insignificant ($P > 0.05$). Fig. 2 shows the dynamics of protein content and yield in the form of a flow chart. A trend towards a continuous increase in protein content up to days 200–250 of the trial is evident from the flow chart. Then the protein content kept decreasing.

A decrease in a protein and fat content is sometimes explained as the effect of high intake of rape oil on nutrient digestibility in the fore-stomach of dairy cows (Münger, 1998; Jahreis *et al.*, 1996; and others). The extent of this effect depends on the kind of bulky feed used, on the proportion of easily fermenting saccharides, and on the sort of fats and fatty acids used. A good example could be two trials with comparable intakes of rape cake oil (LVVG, 1995 – 444 g/head/day

Table 4. Average daily intake of feed mixtures (kg DM/head)

Group	Mixture intake	Fat intake	Rape feed intake	Rape oil intake
S0	4.10	0.144	0	0
S1	4.47	0.257	0.67	0.106
S2	4.37	0.235	1.09	0.152

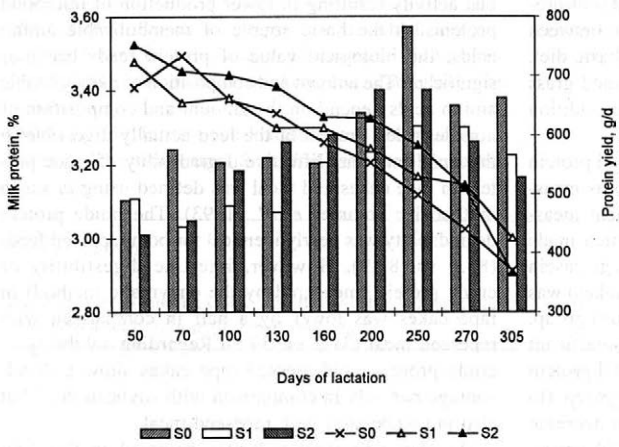

Fig. 2. Dynamics of milk protein content (bar chart) and protein yield (line chart)

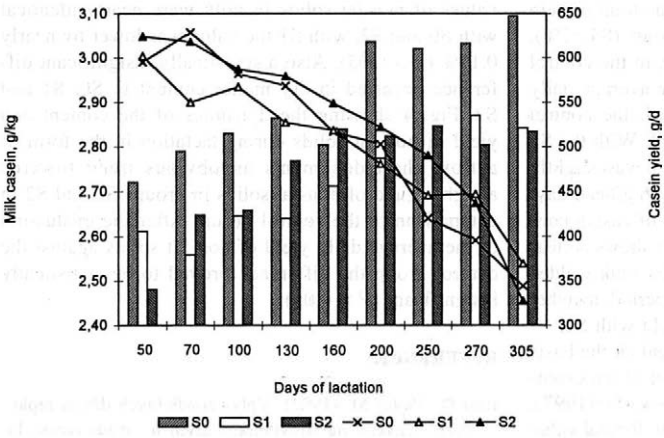

Fig. 3. Dynamics of milk casein content (bar chart) and casein yield (line chart)

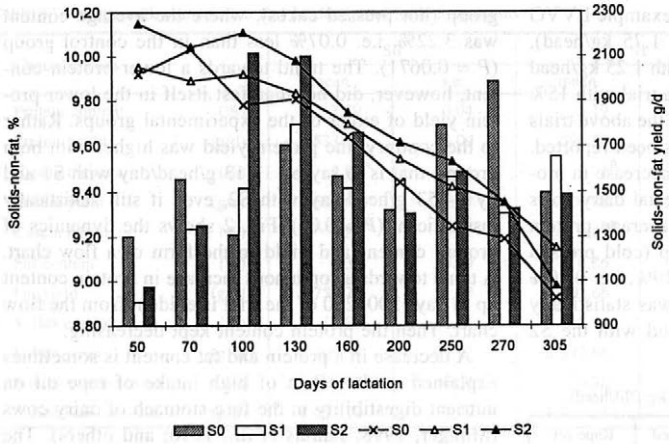

Fig. 4. Dynamics of content (bar chart) and yield of non-fat solids (line chart)

in average, and Jahreis *et al.*, 1996 – 400 g/head/day of rape oil). Although in the case of LVVG the oil intake from the cakes was 11% higher, no significant decrease in fat or protein content was observed, and even a trend towards a certain increase in fat and protein content was found. The main difference between the two trials lay in the composition of the basic diet, which, in the case of the LVVG trial, contained grass silage, and mangel in one of the trial groups, in addition to corn silage and hay.

In the course of our trial it was not only total protein content in milk but also casein content that was measured (see Table 3). The average casein content measured in the S0 control group was 2.91%, which made 88.40% of the total protein content. The average casein content value found with the S1 group (rape cakes) was 2.71%, which was 0.20% less than in the control group. This difference was therefore statistically significant ($P < 0.01$). The proportion of casein in the total protein content decreased by 3.53% with the S1 group (to 84.87%) ($P < 0.01$). A statistically significant decrease in casein content was also found with the S2 group, where the average casein content was 2.74% (–0.17%; $P < 0.01$). The proportion of casein in the total protein content was identical with the S1 group (84.87%), which was significantly 3.53% less than in the control group ($P < 0.01$). With the S1 group the average daily yield of casein was nearly identical with the control group (501 vs. 506 g/head/day; $P > 0.05$). With the S2 group the average daily yield of casein was slightly higher than with the control group (+19.96 g/head/day; $P > 0.05$). Fig. 3 showing the dynamics of casein content and yield in the form of a flow chart shows a clear trend towards a lower casein content in the milk yielded by groups S1 and S2 during the lactation period, together with a trend towards a higher casein yield with S2.

Biological value of rape cakes and meal on the basis of lysine and methionine content and sum of ten essential amino acids (EAA) was defined by Sommer (1997), using an index of the EAA sum and the biological value of soybean meal (index 100%). His investigation resulted in the EAA index of 78.07 for rapeseed meal and 63.31 for rape cakes. With the assumption of a negative impact on ruminal fermentation and a decrease in microbial activity resulting in lower production of microbial proteins as the basic source of metabolisable amino acids, the biological value of protein feeds becomes significant. The amount and composition of metabolisable amino acids depend on the amount and composition of non-degraded protein of the feed actually digestible in the small intestine. Effective degradability of crude protein in rape cakes and meal was defined using *in sacco* method by Sommer *et al.* (1993). The crude protein degradability was nearly identical for both rapeseed feeds (83.6 vs. 83.9). However, intestine digestibility of crude protein (measured by the enzymatic method) in rape cakes was lower by a half in comparison with rapeseed meal (31.2 vs. 59.5). Regarding solubility of crude protein, cold-pressed rape cakes show a disadvantage not only in comparison with soybean meal but also in comparison with rapeseed meal.

Another important indicator assessed in this trial was non-fat solids (see Table 3). While the average values of non-fat solids in milk were nearly identical with S0 and S2, with S1 the value was lower by nearly 0.10% ($P > 0.05$). Also a statistically insignificant difference occurred in dry matter content in S0, S1 and S2. Fig. 4 showing the dynamics of the content and yield of non-fat solids during lactation in the form of a flow chart documents an obvious trend towards a higher yield of non-fat solids in groups S1 and S2 in comparison to the control group. After the evaluation of the average daily yield of non-fat solids against the control group the difference proved to be statistically insignificant ($P > 0.05$).

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THE EFFECT OF *SACCHAROMYCES CEREVISIAE* Sc47 ON CHICKEN BROILER PERFORMANCE AND NITROGEN OUTPUT*

VLIV *SACCHAROMYCES CEREVISIAE* Sc47 NA UŽITKOVOST KUŘECÍCH BROJLERŮ A VYLUČOVÁNÍ DUSÍKU

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ABSTRACT: A feeding comparative trial and a metabolic trial with chicken broilers were conducted to study the effect of continual applications of a probiotic (levels a_0 – control, a_1 – 100 g of probiotic/100 kg feeds, a_2 – 200 g of probiotic/100 kg feeds) containing the Sc47 strain of the yeasts *Saccharomyces cerevisiae* to BR1 feed mixtures of standard formulation and to BR2 feed mixtures with two different levels of crude protein (b_0 – 21.97% CP and b_1 – 19.49% CP), on broiler live weight, feed consumption in kg per 1 kg weight gain, nitrogen retention, fat, nitrogen-free extract (NFE) and fiber digestibility. N output in chicken droppings per 1 kg weight gain was another important parameter under observation. A total of 240 cockerels of ROSS hybrid were included in a feeding comparative trial. A metabolic trial was carried out on 48 cockerels from the 22nd day of age. The live weight of broilers at 21 days of age was not statistically significantly influenced by the levels of the probiotic while it was higher than in the control by 1.8% and 1.38%, respectively, in groups a1 and a2 at 42 days of age. The difference was not statistically significant. Positive effects of both levels of the probiotic were observed in cockerels receiving BR2 mixtures with a lower level of crude protein. The consumption of BR1 mixture was reduced by both levels of the probiotic in comparison with control (by 1.72% in a_1, by 1.19% in a_2). The applications of probiotics did not statistically significantly influence the consumption of BR2 mixture. A lower level of crude protein in BR2 mixtures resulted in significantly ($P < 0.05$) to highly significantly ($P < 0.01$) higher feed consumption per 1 kg weight gain (by 2.25% to 5.75% higher against control). The effect of both levels of the probiotic on feed consumption in kg per 1 kg weight gain was more pronounced when it was applied to feeds with a lower CP level. Slaughter yield was not statistically significantly influenced by applications of the probiotic and different CP levels in BR2 mixtures. The highest N retention on average (+5.25%) was determined in groups of broilers receiving BR2 mixtures with 200 g probiotic/100 g feed (a_2). But the difference was not statistically significant. Statistically significantly higher N retention ($P < 0.05$) was determined for BR2 mixtures with a lower CP level than for those with a higher CP level. The highest coefficient of fiber digestibility on average was also calculated for groups a_2. The difference from the control (+16.23%) was not statistically significant. Both levels of the probiotic decreased N output in droppings per 1 kg weight gain (5.79% in a1, 9.95% in a_2), but the difference was not statistically significant. The effect of the lower CP level in BR2 mixture on N output in droppings per 1 kg weight gain was statistically highly significant ($P < 0.01$) (b_1 – 13.59 against b_0). The examination of A x B interaction showed an increase in N retention for feeds with both CP levels after the application 200 g probiotic/100 kg feed; the coefficient of fiber digestibility increased only in the groups that received the probiotic in feeds with a higher CP level; N output in droppings decreased as a result of the lower CP level in BR2 mixtures as well as of the increasing levels of probiotics. The experimental results indicate positive (statistically insignificant) effects of probiotic applications on broiler performance at a lower crude protein level and suggest a possibility of reducing the environmental nitrogen load.

Keywords: probiotics; *Saccharomyces cerevisiae* Sc47; poultry broilers; growth; feed consumption; nitrogen retention; nitrogen output in droppings

ABSTRAKT: V krmném srovnávacím a v bilančním pokusu s kuřecími brojlery byl studován vliv kontinuální aplikace probiotického preparátu založeného na kmeni kvasinek *Saccharomyces cerevisiae* Sc47 v krmných směsích BR1 standardního složení a v krmných směsích BR2 se dvěma rozdílnými hladinami N-látek (21,97 % NL a 19,49 % NL) na hmotnost kuřat, spotřebu směsí na 1 kg přírůstku, retenci dusíku, stravitelnost tuku, BNLV (bezdusíkatých látek výtažkových) a vlákniny.

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Zároveň byl kladen důraz na sledování množství dusíku vyloučeného exkrementy kuřat na kg přírůstku. Do krmného srovnávacího pokusu bylo zařazeno 240 kohoutků hybrida ROSS. Do bilančního pokusu bylo zařazeno celkem 48 kohoutků od 22. dne věku ve 12 klecích. Krmný srovnávací pokus probíhal v období výkrmu směsmi BR1 (1.–21. den) jako jednofaktoriální s opakováním podle vzorce $F_A(3)$ x 80 pro hmotnost kuřat a $F_A(3)$ x 8 pro spotřebu směsí na 1 kg přírůstku. V období výkrmu kuřat směsmi BR2 (22.–42. den věku) byly pokusy, jak krmný, tak bilanční, vedeny jako dvoufaktoriální s opakováním podle vzorce $F_A(3)$ x $F_B(2)$ x 40 pro hmotnost, $F_A(3)$ x $F_B(2)$ x 4 pro spotřebu směsí a $F_A(3)$ x $F_B(2)$ x 8 pro bilanci. F_A představoval hladiny probiotického preparátu: a_0 – kontrola, a_1 – 100 g preparátu/100 kg směsí, a_2 – 200 g preparátu/100 kg směsí. F_B představoval krmné směsi BR2 s rozdílnou hladinou N-látek: b_0 – směsi BR2 s hladinou 21,97 % NL a 11,86 MJ ME/kg, b_1 – směsi BR2 s hladinou 19,49 % NL a 12,14 MJ ME/kg. Hmotnost kuřat ve 21. dni věku (tab. 2) nebyla statisticky významně ovlivněna použitými hladinami probiotického preparátu. Hmotnost kuřat ve 42. dni věku byla ve skupině a_1 o 1,8 % a ve skupině a_2 o 1,38 % vyšší oproti kontrole. Rozdíl nebyl statisticky významný. Pozitivní vliv obou hladin preparátu byl zaznamenán u kohoutků krmených směsmi BR2 s nižší hladinou N-látek (tab. 3 – skupiny a_1b_1, a_2b_1). Obě hladiny probiotického preparátu na bázi *Saccharomyces cerevisiae* Sc47 snížily spotřebu směsí BR1 na 1 kg přírůstku (a_1 o 1,72 % nižší, a_2 o 1,19 % nižší oproti kontrole). Spotřeba směsí BR2 nebyla použitými preparáty statisticky významně ovlivněna. Nižší hladina N-látek ve směsích BR2 měla za následek průkazně ($P < 0,05$) až vysoce průkazně ($P < 0,01$) vyšší spotřebu směsí na 1 kg přírůstku (o 2,25% až 5,75% vyšší oproti kontrole – tab. 4). Účinek obou hladin preparátu na spotřebu krmných směsí na 1 kg přírůstku se výrazněji projevil při jeho aplikaci do směsí s nižší hladinou NL (tab. 5). Jatečná výtěžnost nebyla aplikací preparátu a rozdílnými hladinami NL ve směsích BR2 statisticky významně ovlivněna. Průměrně nejvyšší retence dusíku (+5,25 %) byla zjištěna u skupin kuřat krmených směsmi BR2 s hladinou 200 g preparátu/100 kg směsi (a_2). Rozdíl však nebyl statisticky významný (tab. 8). U směsí BR2 s nižší hladinou NL byla zaznamenána statisticky průkazně vyšší retence dusíku ($P < 0,05$) než u směsí BR2 s vyšší hladinou NL (b_1 +5,31 % oproti b_0). Průměrně nejvyšší koeficient stravitelnosti vlákniny byl zjištěn také u skupin a_2. Zjištěný rozdíl (+16,23 % oproti kontrole) nebyl statisticky významný. Obě hladiny probiotického preparátu snižovaly podíl dusíku vyloučeného exkrementy na 1 kg přírůstku (a_1 o 5,79 % nižší, a_2 o 9,95 % nižší oproti kontrole). Rozdíl však nebyl statisticky významný. Statisticky vysoce průkazný ($P < 0,01$) byl vliv nižší hladiny NL v BR2 na podíl dusíku vyloučeného exkrementy na 1 kg přírůstku (b_1 – 13,59 % oproti b_0). Při sledování interakce A x B (tab. 9) se retence dusíku zvyšovala ve směsích s vyšší i nižší hladinou NL při použití hladiny 200 g preparátu/100 kg směsi; koeficient stravitelnosti vlákniny se zvýšil pouze ve skupinách, které dostávaly probiotikum ve směsích s vyšší hladinou NL; množství dusíku vylučovaného exkrementy na 1 kg přírůstku se snižovalo jak s nižší hladinou NL ve směsích BR2, tak se zvyšujícími se hladinami probiotických preparátů. Výsledky pokusu naznačují pozitivní (statisticky nevýznamný) vliv sledovaného preparátu na užitkovost brojlerů při nižší úrovni dusíkatých látek a upozorňují na určitou schopnost přispět ke snížení zátěže životního prostředí dusíkatými látkami.

Klíčová slova: probiotika; *Saccharomyces cerevisiae* Sc47; drůbeží brojleři; růst; spotřeba směsí; retence dusíku; vylučování dusíku exkrementy

INTRODUCTION

Probiotic supplements help stabilize the intestinal microflora, consequently they improve the animal health and increase performance. The principles of their action in poultry were described e.g. by Jernigan *et al.* (1985), Vanbelle (1990), Barrow (1992), Jin *et al.* (1997), etc.

Applications of probiotics can be a way how to improve the conversion of dietary crude protein (CP). Increasing the protein efficiency ratio (PER) of feeds for farm animals can help reduce environmental contamination with nitrogen from animal excrements and result in a better profit and loss balance of animal husbandry. The effects of probiotics on nitrogen retention in poultry were studied e.g. by Kumprecht and Zobač (1998), Bhatt *et al.* (1995), Mohan *et al.* (1996), etc.

BIOSAF Sc47 (Société Industrielle LESAFFRE, France) is a probiotic preparation containing strain Sc47 of live yeasts *Saccharomyces cerevisiae*. They are facultatively anaerobic microorganisms. They are not natural inhabitants of the digestive tract because they do not adhere to the intestinal epithelium and their multiplication in the digestive tract is low. They are obviously transient microorganisms. Saccharides are transformed by their action while CO_2 and alcohol are produced. They produce amino acids, B-vitamins, lipids, enzymes (invertases, hydrolases, maltases, acid phosphatases, galactosidases, proteases, peptidases) in a neutral pH environment. Their cellular membrane is rich in oligosaccharides acting as selective sugars and promoting the growth of beneficial bacteria in the intestines that stimulate the host's local immunity system (Lyons and Bourne, 1995). Some strains of *Saccharomyces cerevisiae* are antagonistic in their live form to pathogenic microorganisms (Gedek, 1990, 1991).

Available literary sources indicate that there is a lot of work to be done to examine in detail applications of *Saccharomyces cerevisiae*, strain Sc47, to diets for chicken broilers.

MATERIAL AND METHODS

The objective of this paper was to study the effects of continual applications of a probiotic (at two dietary levels) containing *Saccharomyces cerevisiae* Sc47

Table 1. Formulation and nutrient contents in broiler starter diets BR1 and broiler production diets BR2

Ingredient		Diets		
		BR1 (%)	BR2 (%)	
			b_0	b_1
Fish meal		5	1	1
Meat-bone meal		3	2	2
Yeast		1	1	1
Soybean meal		25	25	20
Corn		40	40	40
Wheat		22	27	27
Wheat starch		–	–	5
Mineral feed additive DV[1]		3	3	3
BIOVITAN BR1 Super without anticoccidials[2]		1	–	–
BIOVITAN BR2-Super without anticoccidials[3]		-	1	1
Total		100	100	100
Dry matter	%	88.74	88.57	89.51
Crude protein (N x 6.25)	%	24.38	21.97	19.49
Fat	%	3.12	2.86	2.78
Fiber	%	3.25	3.37	3.03
Ash	%	7.22	6.25	5.95
Nitrogen-free extract	%	50.77	54.12	58.24
Metabolizable energy	MJ/kg	11.78	11.86	12.14

[1]Mineral feed additive DV (Mikrop Čebín, a.s.) contains per 1 kg: feed limestone, feed salt, monocalcium phosphate, dicalcium phosphate, mineral premix, wheat flour

[2]Biovitan BR1 Super (Biofaktory Praha, s.r.o.) contains per 1 kg of premix: vitamin A_1 250 000 m.j., vitamin D_3 350 000 m.j., vitamin E (alpha-tocopherol) 5 000 mg, vitamin K_3 300 mg, vitamin B_1 200 mg, vitamin B_2 500 mg, vitamin B_6 450 mg, vitamin B_{12} 2.5 mg, niacin 4 000 mg, calcium pantothenate 250 mg, biotin 10 mg, folic acid 100 mg, choline 30 000 mg, antioxidant 10 000 mg, DL-methionine 200 000 mg, L-lysine HCl 200 000 mg

[3]Biovitan BR2 Super (Biofaktory Praha, spol. s r.o.) contains per 1 kg of premix: vitamin A_1 250 000 m.j., vitamin D_3 250 000 m.j., vitamin E (alpha-tocopherol) 3 500 mg, vitamin K_3 250 mg, vitamin B_1 200 mg, vitamin B_2 500 mg, vitamin B_6 300 mg, vitamin B_{12} 2 mg, niacin 2 500 mg, calcium pantothenate 1 000 mg, biotin 10 mg, choline 20 000 mg, antioxidant 10 000 mg, DL-methionine 180 000 mg, L-lysine HCl 185 000 mg

(BIOSAF Sc47 manufactured by Société Industrielle LESAFFRE, France) on broiler live weight, feed consumption per 1 kg weight gain (FCR), N retention, digestibility of fat, fiber, nitrogen-free extract (NFE), and on N output in droppings per 1 kg weight gain. 1 g of BIOSAF Sc47 probiotic contains 8.10^8 yeasts (CFU/g). The probiotic was applied to feed mixture BR1 of standard formulation and to feed mixture BR2 with two levels of crude protein.

To achieve the goal defined for this research, a feeding comparative trial and a metabolic trial were carried out on cockerels of ROSS hybrid supplied by the company Líhně kuřat MACH Litomyšl (Chick Hatcheries).

A total of 240 straight-run cockerels were included in a feeding comparative trial; they were kept in cages of an experimental poultry-house of the Research Institute of Animal Nutrition, s.r.o., at Pohořelice. The number of cockerels per cage was 20 from 1 to 21 days of age, and 10 individuals from 22 to 42 days of age.

A group metabolic trial with 48 chicken broilers was conducted parallelly to the feeding comparative trial. Four cockerels were kept per metabolic cage in which continual collection of droppings was possible.

Broilers included in the feeding comparative trial received a starter - feed mixture BR1 from 1 to 21 days of age. From 22 to 42 days of age they were given production feed mixture BR2 with different levels of crude protein. Table 1 shows formulations of these diets.

The feeding comparative trial, which was carried out in a starter period (feed mixtures BR1, days of age 1–21), had a one-factor design with replications according to formula $F_A(3) \times 80$ for broiler live weight in g, and $F_A(3) \times 8$ for feed consumption in kg per 1 kg weight gain.

In the production period (feed mixtures BR2, days of age 22–42) the feeding and the metabolic trial had a two-factor design with replications according to formula $F_A(3) \times F_B(2) \times 40$ for live weight, $F_A(3) \times F_B(2) \times 4$ for feed consumption, $F_A(3) \times F_B(2) \times 8$ for metabolic studies.

F_A was a factor of probiotic levels:

a_0 – control (without probiotic)

a_1 – 100 g of *Saccharomyces cerevisiae* Sc47 based probiotic/100 kg feed

a_2 – 200 g of *Saccharomyces cerevisiae* Sc47 based probiotic/100 kg feed

F_B was a factor of feed mixtures BR2 with different levels of crude protein:

b_0 – BR2 mixtures with 21.97% CP and 11.86 MJ ME/kg

b_1 – BR2 mixtures with 19.49% CP and 12.14 MJ ME/kg

Broiler live weight in g was recorded in a feeding comparative trial. Broilers were weighed at 1, 21, 35 and 42 days of age. The consumption of BR1 and BR2 mixtures was continually recorded by weighing. Feed consumption per 1 kg weight gain was determined for growth periods of 1–21, 22–35 and 36–42 days of age. Finally, total feed consumption per 1 kg weight gain was calculated for production periods of 1–35 and 1–42 days of age. Check broilers were sacrificed at the end of the feeding comparative trial (day 42 of age) and consumer slaughter yield in % was determined.

Chemical analyses of feeds and droppings for the content of dry matter, crude protein, fat, fiber, nitrogen-free extract and ash were carried out in chemical laboratories of the RIAN s.r.o. at Pohořelice in accordance with methodology published in a Gazette of the Ministry of Agriculture of the Czech Republic in April 1997. Air temperatures and atmospheric humidity were controlled in keeping with regulations for broiler starting and production. Continuous lighting of the experimental house was used. Broiler mortality was recorded at regular intervals and analyzed by a veterinarian. All

data were processed by one- and two-factor analysis of variance with replication according to Snedecor and Cochran (1969).

RESULTS

Table 2 documents the effect of two levels of *Saccharomyces cerevisiae* Sc47 based probiotic which was applied to feed mixture BR1 of standard formulation and to feed mixture BR2 with two levels of crude protein (21.97% and 19.49%), on broiler live weight. Interaction of the effect of two probiotic levels in feed with 21.97% CP and 19.49% CP is shown in Table 3.

The experimental levels of the probiotic did not statistically significantly influence broiler live weight (g) at 21 days of age. The values of broiler live weights were in the range of natural variability.

Broiler weight at 42 days of age in the groups that received *Saccharomyces cerevisiae* Sc47 at doses of 100 g probiotic/100 kg BR2 and 200 g probiotic/100 kg BR2 was higher by 1.80% and 1.38%, respectively, than in the control. The differences were not statistically significant.

The average live weight of cockerels receiving BR2 mixtures with 19.49% CP was lower by 2.28% at 42 days of age than that of cockerels on diet with 21.97% crude protein. The difference was not statistically significant.

Positive effects of *Saccharomyces cerevisiae* Sc47 applied at doses of 100 g probiotic/100 kg BR2 and 200 g probiotic/100 kg BR2 were observed in cockerels receiving feeds with the lower crude protein level (19.49% CP). Average live weights of cockerels administered feeds with 100 g and 200 g probiotic/100 kg feed, respectively, were higher by 3.47% and 2.82% at

Table 2. The effects of two different levels of *Saccharomyces cerevisiae* Sc47 based probiotic preparation and BR2 diets with different crude protein contents on chicken broiler weight

Parameter	Unit	Probiotic preparation			Crude protein content	
		a_0	a_1	a_2	b_0	b_1
n		78	78	77	115	118
Weight on day 1	g	52	52	51	–	–
Weight on day 21	g	602	611	603	–	–
S.D.	g	± 98	± 86	± 69	–	–
Index	%	100.00	101.50	100.17	–	–
Weight on day 35	g	1 419	1 436	1 422	1 446	1 406
S.D.	g	± 223	± 214	± 191	± 216	± 201
Index	%	100.00	101.20	100.21	100.00	97.23
Weight on day 42	g	1 887	1 921	1 913	1 929	1 885
S.D.	g	± 269	± 233	± 227	± 262	± 222
Index	%	100.00	101.80	101.38	100.00	97.72

Legend for Tables 2–9:
a_0 – control
a_1 – 100 g of *Saccharomyces cerevisiae* Sc47 based probiotic preparation/100 kg of feed
a_2 – 200 g of *Saccharomyces cerevisiae* Sc47 based probiotic preparation/100 kg of feed
b_0 – BR2 diets with 21.97% crude protein and 11.86 MJ ME/kg
b_1 – BR2 diets with 19.49% crude protein and 12.14 MJ ME/kg
Capital letters designate the values significantly different at $P < 0.01$
Small letters designate the values significantly different at $P < 0.05$
Digits designate the values significantly different at $P < 0.1$

Table 3. Interactions of the effects of two different levels of *Saccharomyces cerevisiae* Sc47 based probiotic preparation in BR2 diets with 21.97% and 19.49% crude protein on chicken broiler weight

Parameter	Unit	21.97% crude protein			19.49% crude protein		
		a_0b_0	a_1b_0	a_2b_0	a_0b_1	a_1b_1	a_2b_1
n		39	38	38	39	40	39
Weight on day 35	g	1 452	1 443	1 441	1 385	1 429	1 404
Index I	%	100.00	99.38	99.24	95.39	98.42	96.63
Index II	%	100.00	99.38	99.24	100.00	103.18	101.30
Weight on day 42	g	1 928	1 931	1 928	1 846	1 910	1 898
Index I	%	100.00	100.16	100.00	95.75	99.07	98.44
Index II	%	100.00	100.16	100.00	100.00	103.47	102.82

42 days of age than in the control while the differences were not statistically significant.

Table 4 shows the effect of two *Saccharomyces cerevisiae* Sc47 levels and two crude protein levels in BR2 mixtures on feed consumption in kg per 1 kg weight gain. Interaction of the effect of two *Saccharomyces cerevisiae* Sc47 levels in BR2 mixtures with different CP levels on feed consumption in kg per 1 kg weight gain is documented in Table 5.

The consumption of BR1 mixtures in kg per 1 kg weight gain was reduced by the application of *Saccharomyces cerevisiae* Sc47 based probiotic to BR1 mixtures at ($P < 0.1$). Feed consumption per 1 kg weight gain in the group receiving BR1 mixture with 100 g probiotic/100 kg of feed was lower by 1.72% than in the control while it was lower by 1.19% in the group receiving 200 g probiotic/100 kg of feed. There was no significant difference between the experimental groups.

The consumption of BR2 mixtures was not statistically significantly influenced by the application of probiotics, the values were in the range of natural variability.

Feed consumption in kg per 1 kg weight gain was significantly ($P < 0.05$) to highly significantly ($P < 0.01$) higher in groups receiving BR2 mixtures with 19.49% crude protein than in groups of chicken broilers on BR2 diets with 21.97% crude protein. The difference was highly significant ($P < 0.01$) between 22 and 35 days

Table 4. The effects of two different levels of *Saccharomyces cerevisiae* Sc47 based probiotic preparation and BR2 diets with different crud protein contents on feed consumption in kg per 1 kg of weight gain

Parameter	Unit	Probiotic preparation			Crude protein content	
		a_0	a_1	a_2	b_0	b_1
Number of groups		8	8	8	12	12
BR1 feed consumption – days 1–21	kg	1.510[1]	1.484[2]	1.492[2]	–	–
S.D.	kg	± 0.013	± 0.016	± 0.021	–	–
Index	%	100.00	98.28	98.81	–	–
BR2 feed consumption – days 22–35	kg	1.948	1.971	1.952	1.904[A]	2.010[B]
S.D.	kg	± 0.122	± 0.082	± 0.055	± 0.078	± 0.061
Index	%	100.00	101.19	100.21	100.00	105.57
BR2 feed consumption – days 22–42	kg	2.059	2.051	2.043	2.017[a]	2.085[b]
S.D.	kg	± 0.109	± 0.055	± 0.042	± 0.066	± 0.061
Index	%	100.00	99.61	99.22	100.00	103.37
BR1 + BR2 feed consumption – days 1–35	kg	1.768	1.774	1.768	1.742[a]	1.797[b]
S.D.	kg	± 0.064	± 0.041	± 0.030	± 0.038	± 0.033
Index	%	100.00	100.34	100.00	100.00	103.16
BR1 + BR2 feed consumption – days 1–42	kg	1.891	1.882	1.882	1.864[b]	1.906[a]
S.D.	kg	± 0.069	± 0.035	± 0.030	± 0.041	± 0.041
Index	%	100.00	99.52	99.52	100.00	102.25

Table 5. Interactions of the effects of two different levels of *Saccharomyces cerevisiae* Sc47 based probiotic preparation in BR2 diets with 21.97% and 19.49% crude protein on feed consumption in kg per 1 kg of weight gain

Parameter	Unit	21.97% crude protein			19.49% crude protein		
		a_0b_0	a_1b_0	a_2b_0	a_0b_1	a_1b_1	a_2b_1
Number of groups		4	4	4	4	4	4
BR2 feed consumption – days 22–35	kg	1.866	1.934	1.913	2.029	2.009	1.991
Index I	%	100.00	103.64	102.52	108.74	107.66	106.70
Index II	%	100.00	103.64	102.52	100.00	99.01	98.13
BR2 feed consumption – days 22–42	kg	1.989	2.047	2.016	2.129	2.056	2.071
Index I	%	100.00	102.92	101.36	107.04	103.37	104.12
Index II	%	100.00	102.92	101.36	100.00	96.57	97.28
BR1 + BR2 feed consumption – days 1–35	kg	1.727	1.757	1.743	1.809	1.791	1.793
Index I	%	100.00	101.74	100.93	104.75	103.71	103.82
Index II	%	100.00	101.74	100.93	100.00	99.00	99.12
BR1 + BR2 feed consumption – days 1–42	kg	1.849	1.882	1.861	1.934	1.883	1.903
Index I	%	100.00	101.78	100.65	104.60	101.84	102.92
Index II	%	100.00	101.78	100.65	100.00	97.36	98.40

Table 6. The effects of two different levels of *Saccharomyces cerevisiae* Sc47 based probiotic preparation and BR2 diets with different crude protein contents on chicken broiler slaughter yield

Parameter	Unit	Probiotic preparation			Crude protein content	
		a_0	a_1	a_2	b_0	b_1
n		16	16	16	24	24
Slaughter yield	%	74.91	74.78	74.76	74.28	74.95
S.D.	%	± 1.34	± 1.16	± 2.13	± 1.92	± 1.13
Index	%	100.00	99.83	99.80	100.00	100.90

Table 7. Interactions of the effects of two different levels of *Saccharomyces cerevisiae* Sc47 based probiotic preparation in BR2 diets with 21.97% and 19.49% crude protein on chicken broiler slaughter yield

Parameter	Unit	21.97% crude protein			19.49% crude protein		
		a_0b_0	a_1b_0	a_2b_0	a_0b_1	a_1b_1	a_2b_1
n		8	8	8	8	8	8
Slaughter yield	%	74.63	74.74	73.46	75.18	74.81	74.86
Index I	%	100.00	100.15	98.43	100.74	100.24	100.31
Index II	%	100.00	100.15	98.43	100.00	99.51	99.57

of age. Total final feed consumption per 1 kg weight gain (1–42 days of age) was higher by 2.25% in groups receiving BR2 mixtures with 19.49% CP than in groups on diets with 21.97% CP.

The effect of *Saccharomyces cerevisiae* Sc47 applications on feed consumption per 1 kg weight gain was higher in feeds with 19.49% CP and 12.14 MJ ME / kg beginning with the level of 100 g probiotic/100 kg feed (Table 5). No differences were observed between control and experimental groups after this probiotic was applied to feeds with 21.97% CP and 11.86 MJ ME/kg.

Tables 6 and 7 show the effect of two levels of *Saccharomyces cerevisiae* Sc47 and two levels of crude protein including A x B interaction on slaughter yield of chicken broilers in %.

The yeasts *Saccharomyces cerevisiae* Sc47 applied at a level of either 100 g or 200 g of probiotic per 100 kg feed did not statistically significantly influence the values of slaughter yield. Neither was the effect of different contents of crude protein in BR2 mixtures on slaughter yield of chicken broilers statistically significant, and the values were in the range of natural variability.

Table 8 shows the effect of two *Saccharomyces cerevisiae* Sc47 levels and two crude protein levels in BR2 mixtures on N retention, coefficients of fiber, fat and NFE digestibility and N output in droppings per 1 kg weight gain in chicken broilers. Table 9 contains data on A x B interaction in relation to the above-mentioned indicators.

The highest N retention on average was determined in groups of chicken broilers receiving BR2 mixtures with 200 g of *Saccharomyces cerevisiae* Sc47 based probiotic/100 kg feed. The difference +5.25% was not statistically significant. Cockerels on diets with 19.49% CP and 12.14 MJ ME/kg had by 5.31% higher N retention at ($P < 0.05$) than cockerels receiving BR2 mixtures with 21.97% CP and 11.86 MJ ME/kg.

The highest coefficient of fiber digestibility on average, +16.23% against control, was also calculated for groups of chicken broilers on diets with 200 g probiotic/100 kg BR2 mixture. But the difference was not statistically significant. A difference ($P < 0.1$) in fiber digestibility was determined between the groups of chicken broilers receiving BR2 mixtures with 19.49% CP (–11.52%) and those receiving feeds with 21.97% CP.

The coefficients of fat digestibility were not influenced statistically significantly either by the probiotic levels or by different CP levels in BR2 mixtures, and all values were in the range of natural variability.

An increase in the value of NFE digestibility at ($P < 0.1$) was observed in the groups of chicken broilers receiving mixtures with 200 g probiotic/100 kg. A difference between the experimental and control group was 2.40%. Groups of chickens on diets with 19.49% CP and 12.14 MJ ME/kg had NFE digestibility higher by 2.22% than those receiving feeds with 21.97% CP and 11.86 MJ ME/kg.

The lowest average N output in droppings per 1 kg weight gain (–9.95%) against control was determined in groups of chicken broilers receiving feeds with 200 g probiotic/100 kg. This difference was 5.79% in groups of chickens on diets with 100 g probiotic/100 kg. N output per 1 kg weight gain in droppings of chickens on diets with 19.49% CP and 12.14 MJ ME/kg was lower by 13.59% than in those receiving mixtures with 21.97% CP and 11.87 MJ ME/kg while the differences were statistically significant ($P < 0.01$).

An examination of A x B interaction showed that N retention increased in feed mixtures with higher and lower crude protein levels when the level of 200 g probiotic per 100 kg feed was used.

Table 8. The effects of two different levels of *Saccharomyces cerevisiae* Sc47 based probiotic preparation and BR2 diets with different crude protein contents on nitrogen retention, digestibility of fat, fiber, nitrogen-free extract and N output in droppings per 1 kg of weight gain in broilers

Parameter	Unit	Probiotic preparation			Protein content	
		a_0	a_1	a_2	b_0	b_1
Number of determinations		6	6	6	9	9
N retention	%	62.07	62.97	65.33	61.82^{a}	65.1^{b}
S.D.	%	± 2.88	± 3.37	± 2.13	± 3.13	± 1.88
Index	%	100.00	101.45	105.25	100.00	105.31
Fiber digestibility	%	33.14	34.63	38.52	37.59^{1}	33.26^{2}
S.D.	%	± 3.28	± 4.73	± 6.54	± 5.47	± 4.30
Index	%	100.00	104.50	116.23	100.00	88.48
Fat digestibility	%	83.33	82.33	86.04	82.83	84.97
S.D.	%	± 1.51	± 5.37	± 2.35	± 4.36	± 2.62
Index	%	100.00	98.80	103.25	100.00	102.58
Nitrogen-free extract digestibility	%	80.52^{1}	81.11	82.46^{2}	80.48^{a}	82.24^{b}
S.D.	%	± 1.39	± 2.05	± 1.01	± 1.86	± 0.87
Index	%	100.00	100.73	102.40	100.00	102.22
N output per 1 kg of weight gain	g/kg	30.76	28.98	27.70	31.27^{A}	27.02^{B}
S.D.	g/kg	± 3.78	± 2.91	± 2.87	± 2.01	± 2.95
Index	%	100.00	94.21	90.05	100.00	86.41

Table 9. Interactions of the effects of two different levels of *Saccharomyces cerevisiae* Sc47 in BR2 diets with 21.97% and 19.49% crude protein on nitrogen retention, digestibility of fat, fiber, nitrogen-free extract and nitrogen output in droppings per 1 kg of weight gain in broilers

Parameter	Unit	21.97% crude protein			19.49% crude protein		
		a_0b_0	a_1b_0	a_2b_0	a_0b_1	a_1b_1	a_2b_1
Number of determinations		3	3	3	3	3	3
N retention	%	60.48	60.72	64.24	63.66	65.21	66.42
Index I	%	100.00	100.40	106.22	105.26	107.82	109.82
Index II	%	100.00	100.40	106.22	100.00	102.43	104.33
Fiber digestibility	%	32.96	36.40	43.42	33.31	32.86	33.61
Index I	%	100.00	110.44	131.74	101.06	99.70	101.97
Index II	%	100.00	110.44	131.74	100.00	98.65	100.90
Fat digestibility	%	83.20	80.25	85.06	83.47	84.42	87.02
Index I	%	100.00	96.45	102.24	100.00	101.14	104.25
Index II	%	100.00	96.45	102.24	100.00	101.14	104.25
Nitrogen-free extract digestibility	%	79.46	79.66	82.32	81.57	82.55	82.59
Index I	%	100.00	100.25	103.60	102.66	103.89	103.94
Index II	%	100.00	100.25	103.60	100.00	101.20	101.25
N output per 1 kg of weight gain	g/kg	32.39	31.57	29.85	29.13	26.39	25.55
Index I	%	100.00	97.47	92.16	89.94	81.48	78.88
Index II	%	100.00	97.47	92.16	100.00	90.59	87.71

The coefficient of fiber digestibility was substantially higher in the groups of chicken broilers receiving feeds with 21.97% CP and 11.86 MJ ME/kg at both levels of the probiotic. Fiber digestibility was not influenced when the probiotic was applied to mixtures with 19.49% CP and 12.14 MJ ME /kg.

The coefficient of fat digestibility was not influenced by applications of the probiotics to feed mixtures regardless of a higher or lower level of crude proteins.

N output in droppings per 1 kg weight gain decreased at a lower dietary crude protein level and with increasing levels of the probiotic in BR2 mixtures.

DISCUSSION

As indicated by experimental results, live weight and the related feed consumption per 1 kg weight gain were positively influenced by continual applications of both levels of the *Saccharomyces cerevisiae* Sc47 based probiotic. Even though the differences in live weight between control groups and groups with probiotic applications were not statistically significant, the difference in feed consumption was at a significance level ($P < 0.05$). The effect of CP level on live weight was not statistically significant, but the negative effect of a reduced CP level (19.49% CP) in BR2 mixture on feed consumption per 1 kg weight gain was statistically significant to highly significant.

The positive effect of the probiotic was higher in the groups receiving BR2 mixtures with a lower CP level (19.49% CP). The live weight of chicken broilers at 42 days of age was by 3.47% (a_1b_1) and 2.82% (a_2b_1) higher than in the control. Consistently to the live weight, these groups had good results of feed consumption per 1 kg weight gain.

The yeasts *Saccharomyces cerevisiae* are likely to have positive effects on the digestive tract microflora. Their cellular membrane is rich in oligosaccharides, acting as selective sugars and supporting the growth of beneficial bacteria in the gut (Lyons and Bourne, 1995). They are also important producers of B-vitamins, which can have stimulative effects on animal growth. Positive effects of applications of *Saccharomyces cerevisiae* based probiotics on live weight of chicken broilers and feed consumption were also reported by Kumprecht *et al.* (1994), Kumprecht and Zobač (1998), Kočíová *et al.* (1990) and Bhatt *et al.* (1995).

Especially nitrogen retention and fiber digestibility underlie growth and feed consumption. As indicated by the results of our experiments, there was a relationship between higher N retention and lower N output in droppings per 1 kg weight gain.

The positive effect of applications of the *Saccharomyces cerevisiae* Sc47 based probiotic on N retention (+5.25% against control), fiber digestibility coefficient (+16.23% against control) and N output in droppings per 1 kg weight gain (9.95% against control) was higher at the level of 200 g probiotic/100 kg feed. But the differences were not statistically significant. The lower CP level in BR2 mixtures had a statistically significant ($P < 0.05$) positive effect on N retention and a statistically highly significant ($P < 0.01$) positive effect on N output in droppings per 1 kg weight gain.

The positive effect of 200 g probiotic/100 kg feed on N retention was observed both in BR2 mixtures with a lower CP level (+4.33% against control) and in feeds higher in CP (+6.22% against control). A decrease in N output in droppings per 1 kg weight gain in chicken broilers was more pronounced for both levels of the probiotic in BR2 mixtures with a reduced CP level (9.41% and 12.29% against control).

The positive effect of *Saccharomyces cerevisiae* on nitrogen retention can partly be explained by their production of proteases and peptidases. Our results are in agreement with the evidence of Bhatt *et al.* (1995), who recorded an improvement of nitrogen retention in chicken broilers that received strain Y3 of *Saccharomyces cerevisiae*.

Kumprecht *et al.* (1994) reported the effect of *Saccharomyces cerevisiae* var. *elipsoideus* applications that resulted in an increase in cellulase activity in the cecal chyme.

It is possible to establish a relationship between increases in fiber digestibility and N retention and a reduction in feed consumption per 1 kg weight gain.

As indicated by experimentation results, the positive effect of *Saccharomyces cerevisiae* Sc47 was recorded particularly when they were applied to BR2 mixtures with a lower CP content (19.49%).

The yeasts *Saccharomyces cerevisiae* Sc47 improve commercial parameters of chicken broilers, and they can also positively contribute to a reduction in N output in animal excrements, so decreasing the environmental load.

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INFLUENCE OF LYSINE ON WEIGHT GAIN OF CARP FRY (*CYPRINUS CARPIO*) IN CAGE AND FISHPOND FARMING

VLIV LYZINU NA HMOTNOSTNÍ PŘÍRŮSTEK PLŮDKU KAPRA (*CYPRINUS CARPIO*) PŘI KLECOVÉM A RYBNIČNÍM ODCHOVU

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ABSTRACT: The paper shows research results on the effect of the synthetic amino acid lysine added to feed mixtures on breeding results for carp fry bred in cages and fishponds. The experiments in cages and fishponds were carried out in three replications. Three different types of feed mixtures were tested: positive control feed mixture (33.1% protein and 1.8% lysine), experimental feed mixture (28% protein and 1.3% lysine) and negative control feed mixture (27.96% protein and 1.3% lysine). For cage breeding, identical weight gain (positive control 22.8 $g.ind^{-1}$, experimental group 22.7 $g.ind^{-1}$) and feeding coefficient results (positive control 2.10, experimental group 2.11) were found in the positive control and the experimental group. Significantly lower weight gain results (17.9 $g.ind^{-1}$) and feeding coefficient (2.68) were found in the negative control group. Other breeding parameters (SGR, PER) were significantly better in the positive control and the experimental groups in comparison with the negative control group. For fishpond farming, significantly better results for weight gain, feeding coefficient, SGR and PER values were determined for the control and experimental groups in comparison with the negative control ($P < 0.01$). There were no statistically significant differences between positive control and experimental groups ($P > 0.05$).

Keywords: *Cyprinus carpio*; lysine; feeding; cages; fishponds

ABSTRAKT: V práci jsou uvedeny výsledky výzkumu zabývajícího se vlivem přídavku syntetické aminokyseliny do krmných směsí na výsledky odchovu kapřího plůdku v klecích a v chovných rybnících. Pokusy v klecích a v chovných rybnících jsme prováděli ve třech opakováních. Ověřovali jsme tři různé typy krmných směsí: krmnou směs pro pozitivní kontrolu (33,1 % proteinů a 1,8 % lyzinu), pokusnou krmnou směs (28 % proteinů a 1,3 % lyzinu) a krmnou směs pro negativní kontrolu (27,96 % proteinů a 1,3 % lyzinu). Při klecovém odchovu jsme u pozitivní kontroly a pokusné skupiny zaznamenali shodné výsledky, pokud se jedná o hmotnostní přírůstek (pozitivní kontrola 22,8 g/kus, pokusná skupina 22,7 g/kus) a o krmný koeficient (pozitivní kontrola 2,10, pokusná skupina 2,11). U negativní kontroly jsme zjistili významně nižší hmotnostní přírůstky (17,9 g/kus) a hodnoty krmného koeficientu (2,68). Ve srovnání s negativní kontrolou byly hodnoty ostatních chovných parametrů (SGR, PER) významně vyšší u pozitivní kontroly a v pokusné skupině. V chovných rybnících jsme dosáhli významně lepších hodnot hmotnostního přírůstku, krmného koeficientu a parametrů SGR a PER v kontrolní a pokusné skupině než u negativní kontroly ($P < 0,01$). Mezi pozitivní kontrolou a pokusnou skupinou nebyly zjištěny statisticky významné rozdíly ($P > 0,05$).

Klíčová slova: *Cyprinus carpio*; lyzin; výkrm; klece; chovné rybníky

INTRODUCTION

The composition of fish feed is one of the relevant factors that directly influence the efficiency of both fish and fry farming. The use of quality diet results in much better feed utilisation per unit of weight gain. Furthermore, the use of higher quality diet reduces fish losses and veterinary expenses.

Out of the different nutrients contained in diet, proteins are of the greatest importance, in terms of both quantity and quality. However, the price of proteins needs to be taken into account, since they are an expensive ingredient. Depending on the category, the lowest recommended level of protein for carp ranges between 30 to 38% (Viola and Arieli, 1982; Watanabe *et al.*, 1987; Takeuchi *et al.*, 1988).

Proteins are a source of all essential and non-essential amino acids. The former partly break down and are used as a source of energy. Protein related energy is, however, very expensive, but certain cost reductions

can be obtained by increasing the lipid level in diet (De Silva *et al.*, 1991; Bogut *et al.*, 1999).

Many factors influence the protein, i.e. amino acid requirements of fish, the most important of which are: age, protein content in diet, partial deficit of one of the essential amino acids, content of non-essential amino acids in diet, content of energy in diet and biological availability of essential amino acids.

It is a well-known fact that of all kinds of fish, carp has the highest lysine requirement (Viola and Lahav, 1991). The high requirement for lysine could be met by using a plant-based diet rich in protein, soybean being the most suitable one. Despite of the high level of lysine, a diet based on soybean could prove questionable. To allow the carp to utilize proteins contained in soybean, it needs to be correctly heat- treated.

On the other hand, carp does not have gastric digestion, so the breakdown of proteins and the release of lysine depends on the influence of intestinal trypsin. However, even in the correctly heat-treated soybean, a certain amount of lysine cannot be utilized because it is firmly bound in different compounds (Dabrowski, 1983; Abel *et al.*, 1984). Another disadvantage of using soybean for feeding fish fry is a high content (over 25%) of complex carbohydrates in soybean. Such compounds are difficult to digest and thus reduce the energetic value of diets. Unlike carbohydrates in soybean, those contained in corn and wheat are utilized in a high percentage (Degani *et al.*, 1979), especially if they were heat-processed (Párová, 1998). Since the basic diet ingredient in this research was soybean grits, our aim was to investigate the influence of adding the synthetic amino acid lysine on weight gain of carp fry.

MATERIAL AND METHODS

Fish farming. The effect of addition of the synthetic amino acid lysine to diet on weight gain of carp fry was studied on Grudnjak fish farm near Orahovica from 22 July to 26 August 1998. Research was conducted parallelly in two experiments in cages and fish ponds.

In the first experiment (Table 3), a cage platform with nine 120 x 50 x 50 cm cages was set up in the fish pond of approximately 600 m^2. The experiment was conducted in three treatments, each in three replications. The investigations were carried out in three treatments, each in three replications. 100 randomly chosen individuals of carp fry (O^+) of average individual weight 11.9–12.7 g were stocked in each of the nine cages. The total ichthyomass (Table 5) ranged between 1 210 g and 1 240 g at the beginning of the experiment. The utilizable volume of each of the nine cages was 0.3 m^{-3}. Water was let in the fish pond one day before the stocking of fish fry in cages.

In the second experiment carp fry was of average individual weight of 12.3–12.5 g. In each of the three fish ponds of the capacity 650 m^2, there were 5 000 individuals of carp fry, or 61.5–62.5 kg total ichthyomass (Table 5). Before the experiment each of the fish ponds was disinfected with 150 kg of calcium hydrocarbonate $Ca(HCO_3)$ and fertilised with 350 kg of chicken fertiliser. Water was let in the fish pond eight days before the stocking of fish.

Feed mixtures. Three different types of feed were tested in cages and fish ponds. In the first test group (positive control) the fish were fed pelleted feed containing 33.1% of proteins and 1.8% of lysine from a particular feed. The second test group of fish in cages and fishponds were fed pelleted feed containing 28% of proteins with addition of 0.5% of synthetic lysine in order to balance the amount of lysine against the first test group (positive control). In the third test group (negative control) the fish in fish ponds and cages were fed pelleted feed containing 27.9% of protein with 1.3% of lysine contained in the feed. During the investigation period, the fish in cages were fed four times a day: at 8 a.m., 12 p.m., 3 p.m., and 6 p.m., whereas the fish in fish ponds was fed three times a day: at 8 a.m., 12 p.m. and 4 p.m.

Table 1. Feed mixture formulation (in percentage)

Diet	Positive control	Experimental group (lysine supplemented)	Negative control
Ingredient			
Fish meal	10.0	10.0	10.0
Meat meal	6.0	6.0	6.0
Soybean meal	37.0	22.0	22.0
Yeast	3.0	3.0	3.0
Wheat meal	25.0	40.0	40.0
Wheat bran	140	13.5	14.0
Dicalcium phosphate	2.0	2.0	2.0
Premix*	1.0	1.0	1.0
Binder	2.0	2.0	2.0
Lysine – HCl	–	0.5	–

* Premix: vitamin A 1 500 IU, vitamin D_3 1 000 IU, vitamin E 240 mg, vitamin K 12 mg, vitamin B_1 24 mg, vitamin B_2 32 mg, vitamin B_6 24 mg, vitamin B_{12} 0.3 mg, vitamin C 600 mg, nicotinic acid 144 mg, pantothenic acid 96 mg, biotin 0.2 mg, folic acid 4 mg, choline 1 600 mg, Inositol 200 mg

Table 2. The composition of tested feed mixtures (in % dry matter)

Chemical composition	Positive control	Experimental group (lysine supplemented)	Negative control
Dry matter	90.46	90.47	90.48
Crude protein + lysine	33.10	28.00	27.96
Fat	3.20	3.10	3.00
Fiber	4.70	4.34	4.34
Ash	8.10	7.40	7.38
NFE	41.36	47.65	47.80
Lysine	1.80	1.80	1.30

Daily portions were calculated according to feeding tables (Csengery, 1992), based on control fishing, forecast fish losses and physical and chemical water parameters.

Composition and chemical analysis of diets are shown in Table 1 and 2.

Analyses. Water content in pelleted feed was determined by drying feed samples at 105 °C untill constant weight was reached. Ash content was determined by burning the feed in a muffle furnace at 550 °C. Fat content was determined using the Soxhlet method, and crude protein (N. 6.25) according to Kjeldahl. Lysine concentration in pelleted feed mixtures was determined by column chromatography in Degussa company (Hanau, Switzerland), with the help of the Valpovka Feed Factory.

Data processing. Specific growth rate (SGR) was calculated according to the formula:

$$SGR = [(\ln w_t - \ln w_0) / t].100 \ (\% \ day^{-1})$$

where: $\ln w_t$ – final mean individual weight
$\ln w_0$ – initial mean individual weight
t – duration of the experiment

Feed conversion ratio (FCR) = feed consumption / wet weight gain ($g.g^{-1}$)

Specific feeding rate (SFR) was determined from the relation:

$$SFR = FCR.\ SGR \ (\% \ day^{-1})$$

Protein efficiency ratio (PER) was calculated according to the formula:

PER = live weight gain (g) / crude protein fed (g)

Quantitave biomass of zooplankton ($g.m^{-3}$) was measured on the stocking day, and subsequently in seven-day intervals after control fishing.

Water temperature and concentration of dissolved oxygen were registered by oxymeter every day, and other water parameters were measured every week using the APHA method (1980).

Excell for Windows 98 was used for the statistical data analysis.

RESULTS AND DISCUSSION

Zooplankton biomass at stocking ranged between 89.3 and 91.3 $g.m^{-3}$. During the experiment zooplankton biomass ranged from 26.7 to 31.2 $g.m^{-3}$, decreasing to 14.4–15.2 $g.m^{-3}$ at the end of the experiment.

Table 3. Values of individual and total biomass at stocking and final fishing in cages

Group	Cage No.	Stocked			Fished out		
		numbers of fish	x ($g.ind^{-1}$)	Σ (g)	numbers of fish	x ($g.ind^{-1}$)	Σ (g)
Positive control	1	100	12.1	1 210	95	34.8	3 306
	2	100	12.5	1 250	96	35.3	3 389
	3	100	12.3	1 230	94	35.2	3 309
Experimental control	4	100	11.9	1 190	98	34.5	3 381
	5	100	12.4	1 240	94	35.1	3 299
	6	100	12	1 200	96	34.9	3 350
Negative control	7	100	12.3	1 230	99	31.2	3 089
	8	100	12.2	1 220	90	30.5	2 745
	9	100	12.7	1 270	92	29.3	2 696

Table 4. Yield of individual and total biomass, feed consumption, SFR, FCR, SGR and PER in cages

Group	Cage No.	Gain		Feed consumption ($g.ind^{-1}$)	SFR (% $day.ind^{-1}$)	FCR ($g.g^{-1}$)	SGR (% day)	PER
		x ($g.ind^{-1}$)	Σ (g)					
Positive control	1	22.7 ± 3.16	2 156	48.10	6.18 ± 0.27	2.11 ± 0.24	2.930	1.42 ± 0.18
	2	22.8 ± 2.99	2 189	48.10	6.05 ± 0.27	2.10 ± 0.18	2.883	1.43 ± 0.15
	3	22.9 ± 3.54	2 153	48.10	6.13 ± 0.25	2.10 ± 0.21	2.920	1.44 ± 0.13
Experimental control	4	22.6 ± 3.84	2 215	48.10	6.26 ± 0.33	2.12 ± 0.34	2.956	1.67 ± 0.24
	5	22.7 ± 4.05	2 134	48.10	6.09 ± 0.28	2.11 ± 0.32	2.890	1.68 ± 0.21
	6	22.9 ± 4.16	2 198	48.10	6.22 ± 0.27	2.10 ± 0.30	2.965	1.70 ± 0.17
Negative control	7	18.9 ± 3.52	1 871	48.10	6.56 ± 0.95	2.54 ± 0.46	2.585	1.40 ± 0.22
	8	18.3 ± 3.27	1 647	48.10	6.66 ± 0.81	2.62 ± 0.41	2.545	1.36 ± 0.20
	9	16.6 ± 4.11	1 527	48.10	6.71 ± 1.16	2.89 ± 0.52	2.322	1.23 ± 0.24

Physical and chemical water parameters varied in favourable limits for carp fry breeding during the experiment. Average water temperature was 24.7 °C (20.1–29.4 °C) in July and 24.3 °C (19.7–28.9 °C) in August. Concentrations of dissolved oxygen ranged from 4.3 $mg.l^{-1}$ in fish ponds to 6.7 $mg.l^{-1}$ in cages in the morning, and 8.3–10.1 $mg.l^{-1}$ in the afternoon and evening. Concentration of carbon dioxide ranged from 4.1 to 12.7 $mg.l^{-1}$, ammonia ions ($N\text{-}NH_4^+$) from 0.12 to 0.31 $mg.l^{-1}$, and nitrate ions $N\text{-}NO_3^-$ from 0.15 to 1.14 $mg.l.l^{-1}$. Organic water contamination varied from 20.4 $mg.l^{-1}$ in cages to 46.8 $mg.l^{-1}$ in fish ponds, whereas water pH was 7.5–8.71.

At the beginning of our research, the average carp fry weight in all groups (Table 3 and 5) was balanced, and ranged between 11.9 to 12.7 $g.ind^{-1}$ in cages, and 12.3 to 12,5 $g.ind^{-1}$ in fish ponds. The differences between groups were not statistically significant ($P > 0.05$).

The results of research on the influence of the addition of synthetic amino acid lysine on production results of carp fry bred in cages and fishponds are shown in Tables 3, 4, 5 and 6. The lowest average weight of carp fry bred in cages was found in the negative control group (30.3 $g.ind^{-1}$), and the highest (35.1 $g.ind^{-1}$) in the positive control group fed pelleted feed mixture containing 33% of protein, which is 4.8 $g.ind^{-1}$ or 13.67% more than in the negative control group. Pelleted feed mixture used for feeding the fish in the positive control group contained 33% of protein and 1.8% of lysine from the feed used in feed mixtures. Insignificantly lower average individual weight (34.8 $g.ind^{-1}$) in comparison with the positive control group was found in the experimental group, where fish received the feed mixture containing 5% less protein, but with addition of 0.5% of synthetic amino acid lysine. It can be concluded from the results showing the average individual weight that the amount of 0.5% of synthetic amino acid lysine, with the same level of protein as in the negative control group, influenced the weight gain intensity, which resulted in higher carp fry weight. The differences in average carp fry weight between the experimental and the negative control groups were highly significant ($P < 0.01$). Statistically significant differences regarding the average individual weight and weight gain between the positive control group and the experimental group were not determined ($P > 0.05$).

The results shown in Table 5 and 6 indicate the efficiency of lysine addition to feed mixture used for feeding carp fry bred in fish ponds. The average individual weight in the negative control group was lower by 8.9 $g.ind^{-1}$ or 17.7% than in the experimental group. Insignificantly higher weight (50.9 $g.ind^{-1}$) was determined in the positive control group. The statistical analysis of lysine efficiency indicates that the differences in weight and weight gain between the experimental and the control group are highly significant ($P < 0.01$). A comparison of the average individual weight and weight gain in cage and fish pond farming yields significant differences despite the use of the same diet. Those differences could be explained by the presence of natural diet in fish ponds. Regarding nutritive values, natural diet is ideal, especially for younger fish categories.

The differences in the average weight between the test group and the negative control group can be explained from research results by Their *et al.* (1994), who determined that the deficit of only one amino acid in the diet can result in incomplete utilisation of all the others. Therefore, they cannot be fully utilised as protein-building material, but are used as sources of energy. Similar results regarding weight gain and protein savings were obtained by Viola and Lahav (1991) for consumer carp bred in fish ponds. Higher weight gain, protein savings and lower pollution of water by nitrogen were determined by Stibranyiová and Párová (1996), due to the addition of more essential amino acids to feed mixtures used for feeding African catfish.

Daily weight gain of carp fry bred in cages in the positive and the experimental group were identical and amounted to 0.63 $g.ind^{-1}$.

Table 5. Values of individual and total biomass at stocking and final fishing in fish ponds

Group	Fish ponds	Stocked			Fished out		
		numbers of fish	x ($g.ind^{-1}$)	Σ (kg)	numbers of fish	x ($g.ind^{-1}$)	Σ (kg)
Positive control	1	5 000	12.5	62.5	4 370	50.9	222.4
Experimental group	2	5 000	12.4	62	4 280	50.2	214.8
Negative control	3	5 000	12.3	61.5	4 210	41.3	173.8

Table 6. Yield of individual and total biomass, feed consumption, SFR, FCR, SGR and PER in fish ponds

Group	Fish ponds	Gain		Feed consumption (kg)	SFR	FCR	SGR	PER
		x ($g.ind^{-1}$)	Σ (kg)					
Positive control	1	38.4	167.8	38.4	5.30	1.36	3.900	2.22
Experimental group	2	37.8	161.8	37.8	5.48	1.41	3.884	2.53
Negative control	3	29.0	122.1	29.0	6.29	1.87	3.364	1.91

Table 7. Statistical significance of difference between the means

Group	Statistical significance of difference				
	gain ($g.ind^{-1}$)	SFR (% $day.ind^{-1}$)	FCR ($g.g^{-1}$)	SGR (% $day.ind^{-1}$)	PER
In cages					
PC : EG	-0.577692^{ns}	-1.5112^{ns}	-0.69749^{ns}	0.256074^{ns}	-3.9445^{**}
PC : NC	5.17091^{**}	-2.5683^{*}	-5.18768^{**}	5.249512^{**}	1.88648^{ns}
EG : NC	5.24805^{**}	-2.0426^{*}	-4.26001^{**}	5.515433^{**}	4.80833^{**}
Fish ponds					
PC : EG	0.6258^{ns}	0.424^{ns}	-0.01^{ns}	0.312348^{ns}	-4.7374^{**}
PC : NC	8.456317^{**}	-3.659^{**}	-6.85994^{**}	7.34978^{**}	2.90619^{**}
EG : NC	7.149575^{**}	-4.2378^{**}	-6.68844^{**}	6.52988^{**}	6.72484^{**}

PC – positive control, EG – experimental group, NC – negative control

Daily weight gain in the negative control group was lower by 0.14 $g.day^{-1}$ or 22.22%. Higher daily weight gain despite the same diet was observed in fish ponds, which indicated that carp fry consumed both pelleted feed mixture and natural diet. The differences in weight gain found in our research correspond to those reported by Noble *et al.* (1998), who determined that soybean based diet used for feeding carp fry in laboratories did not give satisfactory results in comparison with using the same diet in fishponds. Similar results were obtained by Degani *et al.* (1997) and Yamamoto *et al.* (1988). The comparison of results showing the consumption of diet per 1 gram of weight gain for different groups in cage farming shows that the carp fry fed lysine supplemented diet utilised the diet better. The feeding coefficient in the test group in comparison with the negative control group was higher by 21.26%. Higher feeding coefficients were recorded in fish pond farming, probably due to the availability of both natural and additional diets. Escaffre *et al.* (1997) stress that more than 60% of soybean in diet results in the decrease of growth and worse diet utilisation, despite of the synthetic amino acid supplementation.

Carp fry fed lysine supplemented diet had the specific growth rate (SGR) higher by 15.53% in cages and by 13.40% in fish ponds in comparison with the negative control group. The given results indicate a positive influence of lysine addition to feed mixtures composed mainly of soybean grits and that it is a reasonable measure.

The efficiency of feeding carp fry with lysine supplemented feed mixtures is confirmed by the analysis of PER values. The protein utilisation in the test group in comparison with the positive control group and the negative control group was higher by 14.88% and 20.83%, respectively. The results refer to cage farming of carp fry. The utilisation of protein from feed mixtures in fish pond farming of carp fry was similar to the results obtained for cage farming. The highlighted differences are statistically highly significant ($P < 0.01$).

The results of our research indicate a positive effect of lysine addition to pelleted feed mixtures on weight gain of carp fry and protein savings, in both cage and fish pond farming.

In cage farming of carp fry the price of feed in USD per 1 kg of weight gain was 0.79 in the positive control group, 0.76 in the experimental group, and 0.92 in the negative control group. The price of feed per 1 kg of weight gain for fish in fish ponds was significantly lower and it was 0.47 USD in the positive control group, 0.45 USD in the experimental group, and 0.56 USD in the negative control group.

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THE EFFECT OF BIOLOGICAL PRESERVATIVES, TREATMENT AND DIFFERENT LEVELS OF DRY BIOMASS ON PROTEOLYSIS AND CONTENT OF BIOGENIC AMINES IN ALFALFA SILAGE

VLIV BIOLOGICKÝCH KONZERVAČNÍCH PŘÍPRAVKŮ, STUPNĚ NARUŠENÍ A ROZDÍLNÉ SUŠINY HMOTY NA PROTEOLÝZU A OBSAH BIOGENNÍCH AMINŮ VE VOJTĚŠKOVÉ SILÁŽI

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ABSTRACT: The effect of preservatives (formic acid and Bactozyme) and chops length on proteolysis and content of biogenic amines was studied in alfalfa silages with different dry matter contents (25%, 35%, 45% and 55%). Greatest changes in the parameters under study were observed in alfalfa silage with 25% dry matter content. The effect on the degree of proteolysis and content of biogenic amines decreased with increasing dry matter contents. While proteolysis was inhibited by short chops (6 mm) in alfalfa silages, the content of biogenic amines in control variant and in Bactozyme-treated variant increased. Average content of biogenic amines was at the same level in the variant treated with formic acid, but their content was lower (185.0 mg/kg) at 25% dry matter in alfalfa silage with short chops in comparison with silage made from long chops (346.0 mg/kg). The content of biogenic amines decreased in relation to increasing dry matter contents and type of preservative. Tyramine accounted for the highest proportion in the total content of biogenic amines in alfalfa silage (ca. 65%).

Keywords: alfalfa; silage; degree of wilting; mechanical treatment; proteolysis; biogenic amines

ABSTRAKT: U vojtěškových siláží o různé sušině (25 %, 35 %, 45 % a 55 %) byl studován vliv konzervačních přípravků (kyselina mravenčí a Bactozym) a délky řezanky na stupeň proteolýzy a obsah biogenních aminů. Největší změny sledovaných ukazatelů byly zjištěny při sušině vojtěškové siláže 25 %. Při zvyšující se sušině byl zjištěn snižující se vliv na stupeň proteolýzy a obsah biogenních aminů. Zatímco krátká řezanka (6 mm) u vojtěškových siláží potlačila stupeň proteolýzy, obsah biogenních aminů u kontrolní varianty a u varianty ošetřené Bactozymem se zvýšil. U varianty ošetřené kyselinou mravenčí byl zjištěn průměrný obsah biogenních aminů na stejné úrovni, avšak při sušině 25 % byl jejich obsah nižší (185,0 mg/kg) u vojtěškové siláže s krátkou řezankou oproti siláži s dlouhou řezankou (346,0 mg/kg). Obsah biogenních aminů klesal v závislosti na zvyšujícím se obsahu sušiny a použitém konzervačním prostředku. Největší podíl z celkového obsahu biogenních aminů ve vojtěškové siláži tvořil tyramin (cca 65 %).

Klíčová slova: vojtěška; siláž; stupeň zavadnutí; mechanická úprava; proteolýza; biogenní aminy

INTRODUCTION

The volume of ensiled feeds has recently increased, but hay production and feeding of green forage have declined. About 64 Mt hay dry matter and 83 Mt silage dry matter were produced in Western Europe in 1980 while it was 48 Mt hay and 104.5 Mt silage in 1990 (Wilkinson and Stark, 1992). Silages have become the main component of diets for ruminants in Europe due to the advantages of ensiling over hay production.

Proteolysis, metabolism of amino acids and their role at ensiling were described in a monograph (McDonald *et al.*, 1991). Changes in the concentrations of proteolytic enzymes during alfalfa ensiling were reported by McKersie *et al.* (1981). Proteins are decomposed during silage fermentation to nitrogen in form of soluble organic matters, including free amino acids, amides, glutamine and asparagine, as well as low quantities of amines, chlorophyll, urea products and low-molecular peptides. Amino acid catabolism is stimulated by interactions of plant and microbial enzymes (Oshima and McDonald, 1978), so amino acid degradation during a fermentation process is not homogeneous. Concentrations of glutamine acid, arginine and asparagine acid are usually decreased by protein degradation in silages while concentrations of gamma-aminobutyric acid and ornithine are increased (Lessard *et al.*, 1978; Oshima *et al.*, 1979; Heron *et al.*, 1986). Ornithine is a product of arginine degradation similarly like glutamine acids is decarboxylated to gamma-ami-

nobutyric acid. Amino acids with branched chains (valine, leucine and isoleucine) are indicators of good silage preservation. Free amino acids in ensiled biomass are decomposed by help of plant proteases and by the action of *Clostridium* bacteria and some lactic bacteria, to ammonia through deamination or to biogenic amines through decarboxylation (Oshima and McDonald, 1978; Heron *et al.*, 1986). Amines determined in silages can be either volatile amines (Jackson, 1964) or biogenic amines (Voos, 1966). Biogenic amines are toxic compounds inhibiting microbial fermentation in rumen if they are present at higher quantities, which have negative impacts on animal health (Joosten, 1988).

The objective of the paper was to determine degree of proteolysis and content of amino acids in alfalfa silage treated with formic acid and biological preservative Bactozyme in relation to the degree of wilting and chops length.

MATERIAL AND METHODS

The first cut of alfalfa was carried out on 24th May 1996. Cut swath was left to wilt in the field and harvested at dry matter content I ca. 25%, II 35%, III 45% and IV 55%. Alfalfa wilting in the field was continually monitored. When the required content of alfalfa dry matter was achieved, the respective quantity of alfalfa was chopped with a stationary chopper set up for chops lengths 6 and 12 mm (short chops – 6 mm – SC, long chops – 12 mm – LC). The chopped biomass was treated with the following preservatives applied by spraying.

1. Control without preservative (4 l/t H_2O)
2. Bactozyme MEDIPHARM CZ, Hustopeče near Brno
   - bacterial component (15.10^9 CFU/g *Lactobacillus plantarum*, *Lactobacillus casei*, *Enterococcus faecium* M 74 and *Pediococcus* spp.) – 10 g/t wilted forage
   - enzymatic component (25 000 nkat/ml cellulases and hemicellulases and 4 000 nkat/ml glucoseoxidases) – 100 ml/t wilted forage
3. Formic acid 4 l/t wilted forage

Three variants of alfalfa silages with four contents of dry matter were prepared. Alfalfa biomass was ensiled after treatment into tube-shaped minisilos at three replications. The tubes were hermetically sealed after filling and placed into a thermobox at a temperature of 28 ± 2 °C. The minisilos were opened after 90 days of fermentation and samples for chemical analyses were taken. These parameters were determined in alfalfa silage samples: dry matter, fiber, water-soluble carbohydrates (liquid chromatography), crude protein (Kjehldahl method), proteins as a proportion of crude protein after precipitation with trichloroacetic acid (TCA), biogenic amines – histamine, tyramine, putrescine and cadaverine (liquid chromatography), formaline titration (Hartman, 1980), NH_3-N and ammonia (Conway method). Computerized data-processing was carried out by standard programs.

RESULTS AND DISCUSSION

Table 1 shows data on dry matter and organic nutrient contents in alfalfa in relation to the degree of wilting. The values of crude protein content gradually decreased from 220.0 g/kg to 172.9 g/kg with increasing dry matter contents from 256.0 g/kg and 258.6 g/kg, respectively, to 534.2 g/kg and 566.0 g/kg. They documented crude protein losses of 5.2%, 12.3% and 20.8% in long chops, expressed as percentage losses of crude protein at the tested dry matter contents. The respective percentage losses of alfalfa short chops were 8.6%, 12.6% and 12.2%. No correlations were determined in the other parameters. Contents of nitrogen-free extract and water-soluble carbohydrates were higher: it documents fast wilting while no loss of organic nutrients by aeration was observed.

Evaluation of crude protein fractions

Alfalfa silages of all variants were examined for their contents of crude protein, proteins, free amino acids, proteolysis and proportion of ammonia nitrogen in total nitrogen (NH_3-N). Crude protein fractions (Table 2) were evaluated in relation to the degree of wilting (dry matter content) of alfalfa silages, chops length and effect of the type of preservative. The largest differences were determined at 25% dry matter content, when proteolysis was substantially inhibited by a chemical preservative (formic acid) ($P < 0.05$) and crude protein breakdown was reduced statistically significantly by the biological preservative Bactozyme against the control ($P < 0.05$). The proportion of ammonia nitrogen (NH_3-N) in total nitrogen and contents of free amino-acids in test silages were the most sensitive indicators of crude protein breakdown inhibition. Positive effects of preservatives reducing free amino acid proportions were observed at all dry matter contents. A statistically significant decrease ($P < 0.05$) in free amino acid content was determined in silages at dry matter contents ca. 25%, 35% and 45%, the content of free amino acids at 55% dry matter was statistically insignificantly lower. Jones *et al.* (1996) also stated that determination of free amino acids was the best indicator of proteolysis in grass silages. Fairbairn *et al.* (1992) reported a decrease in free amino acid concentrations in alfalfa silage treated with formic acid.

Evaluation of biogenic amine contents in silages

Table 3 shows the contents of biogenic amines studied in these experiments histamine, tyramine, putrescine and cadaverine. Tyramine proportion was the highest of all biogenic amines under study (ca. 65%). The greatest changes in biogenic amine contents were determined at 25% dry matter. Total content of control variant amounted to 1 265 mg/kg, that of the Bactozyme-treated variant was 909 mg/kg, and the variant treated with formic acid had the lowest content of 271 mg/kg.

Table 1. Chemical composition of alfalfa at different degrees of wilting before ensiling in a laboratory experiment

		I (25% DM)		II (35% DM)		III (45% DM)		IV (55% DM)	
		LC	SC	LC	SC	LC	SC	LC	SC
Dry matter	g/kg	256.0	258.6	326.9	369.7	472.9	428.3	566.0	534.2
Crude protein	g/kg DM	218.3	220.0	206.9	201.1	191.4	192.2	172.9	193.1
Crude protein losses	%			5.2	8.6	12.3	12.6	20.8	12.2
Fiber	g/kg DM	321.5	321.9	306.8	329.0	359.7	313.1	357.4	338.9
BNLV	g/kg DM	333.7	329.9	356.1	341.0	315.9	366.5	349.6	344.4
WSC	g/kg DM	59.4	53.2	57.0	40.4	67.7	83.2	62.1	59.7
WSC	% of biomass	1.5	1.4	1.9	1.5	3.2	3.6	3.5	3.2

LC – long chops, SC – short chops
BNLV – nitrogen-free extract, WSC – water-soluble carbohydrates

Table 2. Contents of crude protein fractions (%) in alfalfa silage in relation to wilting, type of preservative and chops length

		I (25% DM)		II (35% DM)		III (45% DM)		IV (55% DM)	
		LC	SC	LC	SC	LC	SC	LC	SC
Crude protein	K	12.5 a	14.3 a	20.8	20.7	18.0	18.0	18.2	19.1
	B	16.8 b	21.5 b	20.8	20.8	18.6	18.8	18.3	18.6
	M	22.0 c	21.2 b	20.6	20.2	18.2	17.7	18.2	18.4
Proteins	K	31.6 a	35.4 a	50.3	58.4 b	44.1	49.2 b	40.5	45.5 b
	B	41.1 b	51.9 b	50.6	50.6 a	46.4	46.4 ab	35.0	34.4 a
	M	47.9 c	50.9 b	50.5	50.8 a	41.2	40.4 a	35.3	37.5 ab
Free amino acids	K	11.1 b	12.9 c	5.8 c	4.1	5.7 b	5.3	4.7	2.8
	B	8.8 b	6.4 b	4.1 b	3.7	5.0 ab	5.8	3.9	3.1
	M	1.7 a	2.6 a	2.4 a	3.1	4.1 a	4.5	3.9	4.4
Degree of proteolysis	K	79.0 b	87.8 c	28.2 c	18.9 ab	15.4 b	19.0 b	18.7 b	17.9 b
	B	64.9 b	38.5 b	21.7 b	20.6 b	11.0 a	17.3 ab	14.9 a	11.6 a
	M	17.4 a	14.3 a	14.1 a	14.5 a	11.3 a	13.1 a	15.5 a	15.1 ab
NH_3-N	K	159 c	151 c	43 c	30 b	41 b	31 c	34 b	31 b
	B	87 b	44 b	27 b	26 b	18 a	26 b	26 a	20 a
	M	15 a	12 a	13 a	18 a	20 a	25 a	28 a	30 b

LC – long chops, SC – short chops
K – control, B – Bactozyme, M – formic acid
Means with the same subscript in the row are not significantly different ($P < 0.05$)

The contents of biogenic amines and their sum gradually decreased with increasing dry matter contents. Positive effects of the used preservatives reducing biogenic amine contents in alfalfa silages were demonstrated by the results of experiments. Van Os (1996) reported that contents of biogenic amines statistically significantly decreased in grass silage with high dry matter content and treated with formic acid or lactic bacteria *L. plantarum* (10^7 CFU.g^{-1}). Similar relations were observed in alfalfa silage in our experiments but when the effect of biomass treatment (long or short chops) before ensiling was investigated, higher contents of biogenic amines were demonstrated in silages from short chops in alfalfa silages of control variant and Bactozyme-treated variant. This finding can be explained by greater mechanical breakdown of plant tissue connected with a release of plant proteins; it can result in their higher degradation and production of biogenic amines as indicated by the results of these experiments. It will be necessary to repeat these experiments to confirm the results. An increase in proteolysis was observed in control variant only at the lowest dry matter content (25%), proteolysis in the other variants was reduced by the effect of short chops in comparison to silages with long chops (Table 4).

CONCLUSION

A laboratory experiment demonstrated that crude protein breakdown was inhibited by increased dry matter content and application of chemical and biological preservatives. The lowest crude protein breakdown at low dry matter content was determined after treatment with formic acid. The breakdown of crude protein was also reduced by the biological preparation Bactozyme. Contents of biogenic amines decreased in relation to the degree of wilting (to dry matter content in silage) and to the type of preservative. The content of biogenic amines was negatively influenced by short chops in control and Bactozyme-treated variants. The content of biogenic amines was not influenced by chops length in the variant treated with formic acid but at 25% dry matter content in silage the content of biogenic amines was lower in short chops as a result of formic acid treatment.

Table 3. Contents of biogenic amines in alfalfa silage after 90 days of fermentation (mg/kg dry matter)

	Biogenic amines	Control		Bactozyme		Formic acid	
		LC	SC	LC	SC	LC	SC
I (25% DM)	histamine	101.3	149.0	93.3	145.3	22.3	5.0
	tyramine	842.3	1081.7	262.3	844.7	42.7	32.3
	putrescine	143.3 ab	177.3 ab	201.7 ab	154.7 ab	208.3 b	106.3 a
	cadaverine	25.0	10.0	63.3	52.0	72.7	53.0
II (35% DM)	histamine	7.3	3.7	9.0	8.0	6.0	7.3
	tyramine	301.3 ab	134.3 ab	446.7 bc	486.7 c	52.0 a	46.7 a
	putrescine	143.0	61.0	173.3	61.0	79.0	103.7
	cadaverine	60.0	70.0	70.3	63.0	76.7	59.3
III (45% DM)	histamine	5.7	6.7	7.3	7.0	5.7	3.7
	tyramine	109.0	339.7	344.3	50.3	91.7	108.3
	putrescine	72.3	62.3	72.3	66.3	55.7	109.0
	cadaverine	47.7	50.7	43.3	46.3	42.0	68.7
IV (55% DM)	histamine	1.1	1.9	2.7	2.5	2.0	1.6
	tyramine	39.0	57.3	69.3	38.3	69.7	31.1
	putrescine	58.3	47.7	52.3	60.0	53.6	41.3
	cadaverine	45.7	43.7	48.0	47.3	34.0	29.7

LC – long chops, SC – short chops
Means with the same subscript in the row are not significantly different ($P < 0.05$)

Table 4. Contents of total biogenic amines in alfalfa silages in relation to dry matter content and type of preservative

Biogenic amines	Control		Bactozyme		Formic acid	
	LC	SC	LC	SC	LC	SC
I 25% DM	1112.0	1418.0	620.7 a	1196.7 b	346.0 b	185.0 a
II 35% DM	269 a	511.7 b	612.0	699.3	213.7	217.0
III 45% DM	234.7	459.3	170.0 a	474.0 b	195.0	289.7
IV 55% DM	144.3	150.0	148.3	172.3	96.3 a	159.3 b

LC – long chops, SC – short chops
Means with the same subscript in the row are not significantly different ($P < 0.05$)

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SHORT COMMUNICATION

POLYMORPHISM IN THE PORCINE *NGFB* GENE DETECTED BY DGGE AND ITS LINKAGE MAPPING*

POLYMORFISMUS *NGFB* GENU PRASETE, DETEKOVANÝ DGGE, A JEHO VAZBOVÉ MAPOVÁNÍ

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ABSTRACT: The porcine nerve growth factor beta gene (*NGFB*) belongs to a conserved syntenic group on chromosome 4. The objective of this study was to find a simply detectable polymorphism and use it in genetic studies. The new set of PCR primers was used to amplify a 448 bp fragment of the gene. Polymorphism was studied by denaturing gradient gel electrophoresis (DGGE). One hundred and fifty-four pigs belonging to eight breeds were studied and two alleles, *A* and *B*, were detected. Frequencies of *B* allele were: Large White – 0.50, Landrace – 0.96, Piétrain – 0.81 and Black Pied Přeštice – 0.83. Czech Meat Pig (14 pigs), Hampshire (6), Duroc (2) and Meishan (8) were monomorphic for allele *B*. Data from three Hohenheim PiGMaP F_2 pedigrees were used for linkage mapping. The sex average locations of the *NGFB* gene were (in Kosambi cM) 101.8 (Wild Boar x Piétrain), 95.5 (Meishan x Piétrain) and 89.5 (Wild Boar x Meishan), respectively.

Keywords: nerve growth factor beta; pig; denaturing gradient gel electrophoresis; linkage analysis

ABSTRAKT: Gen nervového růstového faktoru beta (*NGFB*) je součástí syntenické skupiny lokalizované u prasete na chromozomu 4. Cílem práce bylo nalézt jednoduše detekovatelný polymorfismus využitelný pro genetické analýzy. S využitím nové sady PCR primerů byl amplifikován fragment o velikosti 448 pb. Polymorfismus byl studován pomocí denaturační gradientové gelové elektroforézy (DGGE). Bylo testováno 154 zvířat osmi plemen, u kterých byl zjištěn výskyt dvou alel, *A* a *B*. Frekvence alely *B* byla u plemene bílé ušlechtilé 0,50, landrase 0,96, piétrain 0,81 a přeštické černostrakaté 0,83. Plemena české výrazně masné (14 ks), hampshire (6), duroc (2) a meishan (8) byla monomorfní pro alelu *B*. Výsledky testování tří F_2 rodin (PiGMaP, Hohenheim) byly využity pro vazbovou analýzu a vypočtená lokalizace genu *NGFB* (bez ohledu na pohlaví) v Kosambi cM byla 101,8 (divoké prase x piétrain), 95,5 (meishan x piétrain) a 89,5 (divoké prase x meishan).

Klíčová slova: nervový růstový faktor beta; prase; denaturační gradientová gelová elektroforéza; vazbová analýza

Current gene maps of the pig encompass more than 1700 loci, of which about 500 loci represent genes (http://www.ri.bbsrc.ac.uk/pigmap/pigbase/pigbase.html). These maps are inevitable for mapping of QTLs, especially those that control performance traits, immune response and genetic diseases. On the porcine chromosome 4 QTLs for fattening traits and carcass traits have been mapped (Andersson *et al.*, 1994; Knott *et al.*, 1998; Walling *et al.*, 1998; Geldermann *et al.*, 1999). Although the QTL intervals in various families differ, basically it appears that the QTLs may be expected in the chromosome region approximately 4q1.4–q2.3. In this region several Type I genes have been mapped cytogenetically – *ATP1A1*, *ATP1B1*, *IVL*, *NGFB* (see Yerle *et al.*, 1997), *CD1* (Garrido *et al.*, 1998), *TSHB* (Kojima *et al.*, 1996), *PKLR* (Marklund *et al.*, 1998) and *V-ATPase* (Hui *et al.*, 1999). Effort has been devoted to map the genes by linkage analysis (Archibald *et al.*,

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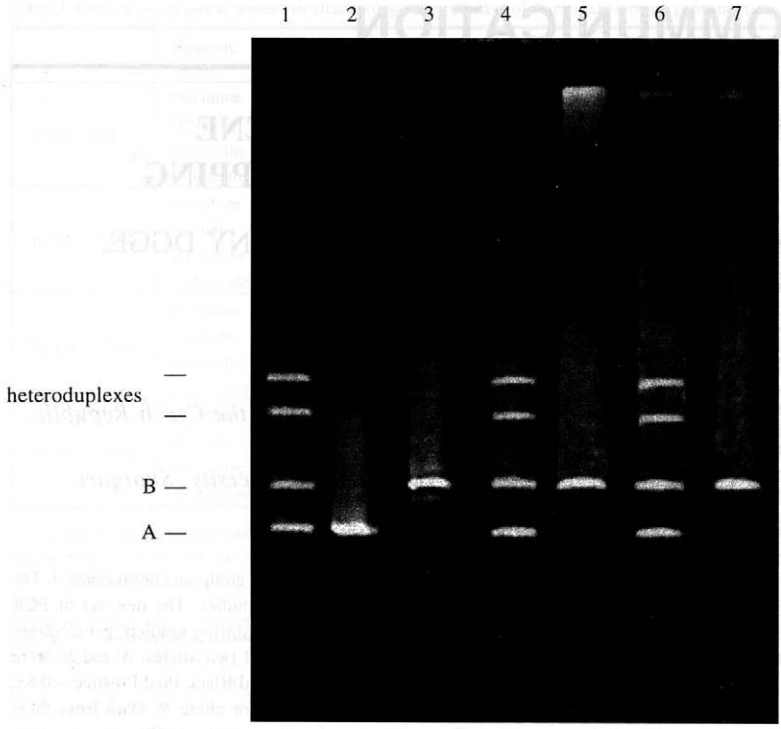

Fig. 1. An enlarged photograph of a part of the polyacrylamide gel showing three genotypes of the porcine *NGFB* gene

Lanes 1, 4 and 6 – heterozygotes *AB* (note the presence of heteroduplexes); lane 2 – homozygote *AA*; lanes 3, 5 and 7 - homozygotes *BB*

1995; Marklund *et al.*, 1996; Rohrer *et al.*, 1996) and identify further Type I genes in this region to facilitate the exact location or identification of responsible QTL loci.

In this study we aimed at the application of a reliable and practical method, denaturing gradient gel electrophoresis (DGGE), for testing of polymorphism in the nerve growth factor beta (*NGFB*) gene. Allele frequencies in different breeds were determined and the gene was mapped by linkage analysis in the Hohenheim F_2 pedigrees (Geldermann *et al.*, 1996).

Nerve growth factor (NGF) is a polypeptide involved in the regulation of growth and differentiation of sympathetic and certain sensory neurons (see Levi-Montalcini, 1987). NGF consists of 3 types of subunits, alpha, beta and gamma, which specifically interact to form a 130 000-molecular weight complex. This complex contains 2 identical 118-amino acid beta-chains, which are solely responsible for nerve growth stimulating activity of NGF. The porcine *NGFB* gene was mapped to chromosome region 4q1.6-q2.3 by radioactive *in situ* hybridization and an *Msp*I RFLP was identified (Lahbib-Mansais *et al.*, 1994).

One hundred and fifty-four pigs belonging to eight breeds (Large White, Landrace, Duroc, Hampshire, Piétrain, Black Pied Přeštice, Czech Meat Pig, Meishan) and a total of 996 F_2 animals (613 informative for *NGFB*) from three F_2 Hohenheim pedigrees – Wild Boar x Piétrain (W x P), Meishan x Piétrain (M x P) and Wild Boar x Meishan (W x M) (Geldermann *et al.*, 1996) were used in this study.

Table 1. Allele frequencies in the porcine *NGFB* gene

Breed	*n*	Allele *A*	Allele *B*
Large White	14	0.500	0.500
Czech Meat Pig	14	0.000	1.000
Duroc	2	0.000	1.000
Hampshire	6	0.000	1.000
Landrace	86	0.035	0.965
Piétrain	18	0.194	0.806
Black Pied Přeštice	6	0.167	0.833
Meishan	8	0.000	1.000

The new set of PCR primers was designed from DNA sequence of the porcine nerve growth factor beta gene (Lahbib-Mansais *et al.*, 1994, GenBank accession no. L31898) to amplify a 448 bp fragment by PCR:

Forward primer: 5'- GCA TAC AGG CAG AAC CGC ACA C -3'

Reverse primer: 5'- TTC ACC TCT CCC AAC ACC ATC AC -3'

PCR was performed in 15 µl reactions using 50 ng genomic DNA, standard PCR buffer, 2 mM $MgCl_2$, 200 µM each dNTP, 5 pmol each primer, and 0.3 U *Taq*

Table 2. Linkage mapping of the *NGFB* gene on porcine chromosome 4 in three Hohenheim pedigrees. Distances from the most proximal marker, *Sw489*, are given in Kosambi cM

Locus	Pedigree W x P			Pedigree M x P			Pedigree W x M		
	average	female	male	average	female	male	average	female	male
S0073	78.7	89.7	79.0	74.6	90.4	61.0	73.7	79.4	71.9
EAL	91.1	105.0	84.2	90.8	113.8	71.0	85.8	96.3	79.0
NGFB	**101.8**	**119.4**	**84.2**	**95.5**	**119.2**	**75.2**	**89.5**	**100.9**	**81.4**
Sw2435	106.4	127.3	87.9	107.1	139.4	78.5	97.5	115.3	84.7
Total length of chromosome 4	133.3	154.7	115.2	129.6	160.5	102.2	126.3	142.7	114.8

polymerase. Denaturing at 95 °C for 2 min was followed by 35 cycles of 94 °C (30 s), 60 °C (30 s) and 72 °C (45 s), with final elongation step at 72 °C (7 min). Identity of the PCR product with the porcine *NGFB* gene was verified by restriction analysis.

Polymorphism in the PCR products was detected by DGGE, using DCode^{TM} Universal Detection System (Bio-Rad Laboratories, Hercules, CA, USA). The denaturing gradient needed to produce separation of alleles was determined using perpendicular DGGE, and this gradient was then applied to parallel denaturing gels. The optimal time for producing allele separation was determined using a time-series analysis. The samples were loaded on a 6% polyacrylamide gel (acrylamide/bis 37.5 : 1) size 16 x 16 x 0.2 cm containing a linear 50–70% denaturant gradient (100% denaturant = 7 M urea, 40% formamide) and separated for 2.5 h at a constant 130 V in 1x TAE buffer at 57 °C. The gels were stained with ethidium bromide and photographed.

Two-point and multipoint linkage analysis on the Hohenheim W x P, M x P and W x M pedigrees was performed with LOD score method using the CRIMAP package (Green *et al.*, 1990).

By using DGGE we found polymorphism in the 448 bp PCR fragment of the porcine *NGFB* gene (Fig. 1). Two mobility variants, *A* (fast) and *B* (slow), and additional slowly migrating bands of heteroduplexes (in heterozygotes) were observed. Codominant inheritance was confirmed in the Hohenheim pedigrees W x P, M x P and W x M.

As our fragment contained a 126 bp sequence of Lahbib-Mansais *et al.* (1994) in which they described an *Msp*I polymorphism, we digested our PCR product with *Hpa*II (an isoschizomer of *Msp*I) and observed polymorphism, too. Although we were not able to reliably distinguish, after restriction, one homozygote from the heterozygote, there is a probability that the DGGE polymorphism may correspond with the *Hpa*II (or *Msp*I) polymorphism. However, the possibility of two polymorphic sites within the same fragment cannot be excluded.

The frequencies of the alleles detected by DGGE in 154 unrelated pigs of eight breeds are given in Table 1. It can be seen that, with the exception of Large White, allele *B* has a higher frequency and some breeds are monomorphic for this allele.

Multipoint linkage analysis on Hohenheim F_2 pedigrees gave the order of genes on chromosome 4:.... *S0073* – *EAL* – ***NGFB*** – *Sw2435*. Locations on the chromosome and distances (in Kosambi cM) of the linked genes for the three families studied are presented in Table 2. Rohrer *et al.* (1996) mapped *NGFB* in position 88 cM in a relatively small USDA MARC family, but they did not include the *EAL* gene.

The linkage map of the porcine chromosome 4 with the *NGFB* gene has been used for mapping QTLs in the Hohenheim F_2 pedigrees.

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