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ODHAD GENETICKÝCH PARAMETRŮ PRO ZNAKY LINEÁRNÍHO POPISU A HODNOCENÍ ZEVNĚJŠKU ČESKÉHO STRAKATÉHO SKOTU*

GENETIC PARAMETER ESTIMATES FOR LINEARLY DESCRIBED TRAITS AND CONFORMATION EVALUATION OF CZECH PIED CATTLE

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ABSTRACT: The aim of the study was to estimate genetic parameters for conformation traits and to analyze relations between the conformation of Czech Pied first-calvers and their production characteristics. Totally 16 148 cows were linearly scored and type classified. Heritability estimates (h^2) for descriptive traits and conformation charactericties are given in Table I. The highest h^2 were estimated for body capacity (h^2 = 0.45), muscularity (h^2 = 0.41), stature (h^2 = 0.38), pin width (h^2 = 0.35) and body depth (h^2 = 0.34). The highest h^2 for descriptive traits of udder were estimated for rear udder conformation (h^2 = 0.35), fore teat placement (h^2 = 0.33), median suspensory (h^2 = 0.27) and teat length (h^2 = 0.27). The lowest h^2 were estimated for traits of feet and legs. Heritability estimates for general conformation characteristics ranged from 0.24 to 0.45. Genetic (r_g) and phenotypic (r_p) correlations between different linearly described traits are shown in Table II. The highest genetic correlations were found between body capacity and body depth (r_g = 0.69), production form and body depth (r_g = 0.68), stature and body capacity (r_g = 0.63), muscularity and body depth (r_g = 0.58), muscularity and body capacity (r_g = 0.53). From udder traits the closest relationship was found between fore and rear udder conformation (r_g = 0.50). Genetic and phenotypic correlations between different general conformation characteristics are given in Table III. High genetic correlations were found between production type and other traits (r_g = 0.65–0.85). Genetic correlations between linearly described traits, different general conformation characteristics and body measures are presented in Table IV. With respect to the evaluation of production type close relationships were found for body depth (r_g = 0.75), production form (r_g = 0.71) and muscularity (r_g = 0.64). Genetic correlations between linearly described traits, general conformation characteristics, body measures and cows performance in the 1st lactation (305 days) are given in Table V. With respect to production characteristics, from linearly described traits the highest genetic correlations were found for body capacity, stature, body depth and feet and legs conformation. Regarding body capacity r_g ranged from 0.54 for protein production in kg to 0.49 for milk production. A significant positive relationship was observed between percentage of protein in milk and body capacity (r_g = 0.37) and muscularity (r_g = 0.32). Stature and body capacity of first-calvers are basically expressed by body measurements, therefore there can be found similar trends like for corresponding linearly described traits.

Keywords: Czech Pied cattle; first-calvers; production; conformation; heritability; genetic correlation

ABSTRAKT: U 16 148 prvotelek českého strakatého skotu byly analyzovány vztahy mezi utvářením exteriéru a užitkovými vlastnostmi. Současně byly odhadnuty genetické parametry pro znaky zevnějšku a výsledné charakteristiky hodnocení exteriéru. Nejvyšší hodnoty koeficientu dědivosti (h^2) byly odhadnuty u kapacity těla (h^2 = 0,45), osvalení (h^2 = 0,41), velikosti (h^2 = 0,38), šířky zádě (h^2 = 0,35) a hloubky těla (h^2 = 0,34). U popisovaných znaků vemene byly hodnoty h^2 pro utváření zadních čtvrtí na úrovni 0,35, pro rozmístění předních struků 0,33 a pro utváření závěsného vazu 0,27. Nejnižší hodnoty h^2 byly odhadnuty pro utváření končetin (0,12–0,18). U souhrnných charakteristik se pohybují hodnoty h^2 v rozmezí 0,24 až 0,45. Nejvyšší hodnoty genetické korelace (r_g) mezi jednotlivými popisovanými znaky byly zjištěny mezi hloubkou hrudníku a kapacitou těla, resp. ušlechtilostí (r_g = 0,69, resp. 0,68), hloubkou hrudníku a osvalením (r_g = 0,58), velikostí a kapacitou těla (r_g = 0,63). U znaků vemene byla odhadnuta nejvyšší hodnota r_g mezi utvářením předních a zadních čtvrtí (0,50). Nejtěsnější vztah mezi charakteristikami hodnocení zevnějšku byl zjištěn mezi užitkovým typem a ostatními ukazateli (r_g = 0,65–0,85). U ukazatelů produkce byly nejvyšší hodnoty r_g odhadnuty pro kapacitu těla (r_g = 0,54 pro kg bílkovin, r_g = 0,49 pro produkci mléka). Významný pozitivní vztah byl zaznamenán mezi procentuálním obsahem bílkovin v mléce a kapacitou těla, resp. osvalením (r_g = 0,37, resp. 0,32).

Klíčová slova: český strakatý skot; prvotelky; užitkovost; exteriér; koeficient dědivosti; genetické korelace

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ÚVOD

Vývoj systémů hodnocení exteriéru skotu je nedílnou součástí organizovaného šlechtění. Nejvýraznější pokrok přineslo zavedení lineárně popisného systému hodnocení zevnějšku, který je používán u černostrakatého skotu (Diers, 1992). Pro plemena s kombinovanou užitkovostí byl vyvinut popisně hodnotící systém (Gottschalk, 1987), který byl experimentálně ověřován i v České republice. I přes jeho některé nedostatky byl lineárně popisný systém zaveden i u českého strakatého skotu, jak uvádějí Bouška *et al.* (1991).

Moxley (1984) uvádí, že hlavními znaky při selekci dojnic musí být mléčná produkce, její kvalita a exteriér zvířat, který prakticky umožňuje opakování vysoké užitkovosti i v následujících laktacích. Teoretická účinnost selekce je dána odhadem genetických parametrů pro uvažované znaky exteriéru. Odhad genetických parametrů pro vybrané znaky zevnějšku u německého strakatého skotu (fleckvieh) provedl Sieber (1985), u českého strakatého skotu Bouška *et al.* (1991). Obdobnou problematikou se zabývali u pěti plemen dojeného skotu v USA Norman *et al.* (1988) a u švýcarského hnědého skotu Vušinovič *et al.* (1994). Chceme-li účinně selektovat na znaky exteriéru zvířat, je potřebně znát vzájemné vztahy mezi jednotlivými znaky zevnějšku i vztahy k produkčním vlastnostem. Touto problematikou se zabývali Sieber (1986), Sekerden *et al.* (1995a, b) a Juszczak (1995).

Cílem práce bylo provést odhad genetických parametrů pro znaky zevnějšku českého strakatého skotu a současně i analýzu vztahů mezi utvářením exteriéru plemenic a jejich užitkovými vlastnostmi, jako důležitý podklad pro efektivní selekci.

MATERIÁL A METODA

Lineární popis a hodnocení exteriéru byly u prvotelek českého strakatého skotu provedeny podle metodiky (1993) vybranými bonitéry zevnějšku. Měřením byly zjišťovány čtyři základní tělesné rozměry: výška v kohoutku, výška v kříži, obvod hrudníku a délka trupu. Délka trupu je definována jako rozměr měřený páskovou mírou od nejvyššího bodu kohoutku k okraji kosti křížové (spojnice kyčelních hrbolů). Lineární popis byl pomocí devítibodové stupnice proveden u těchto znaků: ušlechtilost, osvalení, velikost, kapacita těla, hloubka hrudníku, šířka zádě, sklon zádě, postoj zadních končetin, utváření paznehtů, utváření předních čtvrtí vemene, utváření zadních čtvrtí vemene, výraznost závěsného vazu, rozmístění předních struků a délka struků. Na lineární popis jednotlivých exteriérových znaků navazovalo celkové hodnocení zevnějšku prvotelek českého strakatého skotu, které zahrnovalo posouzení těchto pěti dílčích charakteristik: užitkový typ, kapacita, stavba těla, utváření končetin a utváření vemene. Pro hodnocení těchto dílčích charakteristik byl použit 100bodový systém hodnocení zevnějšku.

Do zpracování byly zařazeny údaje o utváření exteriéru 16 148 prvotelek českého strakatého skotu, dcer 951 plemeníků, u kterých byla známa užitkovost za 1. laktaci. Pro korekci jednotlivých efektů a odhad genetických parametrů pomocí vícefaktorové analýzy variance a kovariance bylo použito statistického programu (Harvey, 1987) a byla zvolena tato modelová rovnice:

$$y_{ijklmn} = \mu + o_i + S_i + P_j + M_l + K_m + \alpha(x - \bar{x}) + e_{ijklmn}$$

kde: y_{ijklmn} – hodnocení znaku, vlastnosti
μ – střední hodnota
o_i – náhodný efekt otce
S_i – pevný efekt roku a období otelení
P_j – pevný efekt plemenné skupiny otce
M_l – pevný efekt plemenné skupiny matky
K_m – pevný efekt klasifikátora (regionu)
$\alpha(x - \bar{x})$ – lineární regrese na věk při 1. otelení
e_{ijklmn} – zbytková chyba

Genetické parametry byly odhadnuty přes komponenty otce:

$$h^2 = [(1/NR1) \cdot \sigma_s^2]/[(1\text{-}NW)/NR1) \cdot \sigma_s^2) + \sigma_e^2$$

kde: σ_s^2 – variance genotypová
σ_e^2 – variance prostředová
NR1 – proměnlivost mezi skupinami
NW – proměnlivost uvnitř skupin

$$r_{gxgy} = cov_{(xy)}/\sqrt{\sigma_x^2 \cdot \sigma_y^2}$$

kde: - σ_x^2, σ_y^2 – genotypová variance znaků *x* a *y*

VÝSLEDKY A DISKUSE

Odhad koeficientů dědivosti h^2 pro znaky lineárního popisu a hodnocené dílčí souhrnné charakteristiky zevnějšku prvotelek českého strakatého skotu je uveden v tab. I. U lineárně popisovaných znaků byly nejvyšší hodnoty h^2 odhadnuty pro kapacitu těla (0,45), osvalení (0,41), velikost (0,38), šířku zádě (0,35) a hloubku hrudníku (0,34). U popisovaných znaků vemene byly nejvyšší hodnoty koeficientu heritability odhadnuty u utváření zadních čtvrtí vemene (0,35), rozmístění předních struků (0,33), utváření závěsného vazu (0,27) a délky struků (0,27). Pro oba ukazatele utváření končetin byly odhadnuty nižší hodnoty h^2 – u postoje zadních končetin 0,18 a u utváření paznehtů 0,12. U souhrnných charakteristik zevnějšku byly odhadnuty koeficienty dědivosti v rozmezí h^2 = 0,24–0,45. Zjištěné výsledky korespondují s údaji jiných autorů. Sieber (1986) uvádí u německého strakatého skotu h^2 pro velikost 0,40, pro osvalení 0,28 a pro stavbu těla 0,24. Vušinovič *et al.* (1994) odhadli u švýcarského hnědého skotu nižší koeficient heritability pro osvalení (0,15); u dalších znaků (velikost 0,40, hloubka těla 0,41, postoj zadních končetin 0,21, znaky utváření vemene 0,33–0,46) se naše zjištění významně neodlišují.

Genetické (r_g) a fenotypové (r_p) korelace mezi jednotlivými lineárně popisovanými znaky jsou uvedeny

I. Odhadnuté koeficienty heriability pro znaky popisu (charakteristiky hodnocení) – Estimates of heritability coefficients for descriptive traits (evaluation characteristics)

	h^2	s_h^2
Ušlechtilost[1]	0,30	0,02
Osvalení[2]	0,41	0,03
Velikost[3]	0,38	0,03
Kapacita těla[4]	0,45	0,03
Hloubka hrudníku[5]	0,34	0,03
Šířka zádě[6]	0,35	0,03
Sklon zádě[7]	0,23	0,02
Postoj zadních končetin[8]	0,18	0,02
Paznehty[9]	0,12	0,02
Vemeno – přední čtvrť[10]	0,26	0,02
Vemeno – zadní čtvrť[11]	0,35	0,03
Vemeno – závěsný vaz[12]	0,27	0,02
Rozmístění předních struků[13]	0,33	0,03
Délka struků[14]	0,27	0,02
Užitkový typ[15]	0,43	0,03
Kapacita[16]	0,45	0,03
Stavba těla[17]	0,30	0,02
Končetiny[18]	0,24	0,02
Vemeno[19]	0,35	0,03

[1]thoroughbreeding, [2]muscularity, [3]stature, [4]body capacity, [5]chest depth, [6]pin width, [7]pin angle, [8]posture of hind legs, [9]hooves, [10]udder – forequarter, [11]udder – hindquarter, [12]udder – median suspensory, [13]fore teat placement, [14]teat length, [15]production type, [16]capacity, [17]body conformation, [18]legs, [19]udder

III. Genetické (r_g) a fenotypové (r_p) korelace mezi charakteristikami hodnocení – Genetic (r_g) and phenotype (r_g) correlations between the evaluation characteristics

	Hodnocení[1]	1	2	3	4	5
1	užitkový typ[2]		0,72	0,65	0,45	0,45
2	kapacita[3]	0,84		0,52	0,30	0,26
3	stavba těla[4]	0,85	0,68		0,52	0,40
4	končetiny[5]	0,79	0,54	0,76		0,37
5	vemeno[6]	0,65	0,49	0,73	0,61	

r_g – pod diagonálou – below diagonal
r_p – nad diagonálou – above diagonal

[1]evaluation, [2]production type, [3]capacity, [4]body conformation, [5]legs, [6]udder

v tab. II. Nejvyšší genetické korelace byly zjištěny mezi kapacitou těla a hloubkou hrudníku (r_g = 0,69), ušlechtilostí a hloubkou hrudníku (r_g = 0,68), velikostí a kapacitou těla (r_g = 0,63), osvalením a hloubkou hrudníku (r_g = 0,58), osvalením a kapacitou těla (r_g = 0,53). U znaků vemene byl nejtěsnější vztah zjištěn mezi utvářením předních a zadních čtvrtí (r_g = 0,50) a mezi utvářením zadních čtvrtí a závěsného vazu (r_g = 0,45).

Genetické a fenotypové korelace mezi jednotlivými charakteristikami hodnocení zevnějšku jsou uvedeny v tab. III. Vysoké hodnoty genetické korelace byly zjištěny mezi užitkovým typem a ostatními charakteristikami (r_g = 0,65–0,85). U dalších výsledků byly nejvyšší hodnoty zaznamenány mezi tělesnou stavbou a utvářením končetin i vemene (r_g = 0,76, resp. 0,73).

Genetické korelace mezi lineárně popisovanými znaky, jednotlivými charakteristikami hodnocení a tělesnými rozměry jsou uvedeny v tab. IV. Nejtěsnější vztah lineárně popisovaných znaků má k hodnocení užitkového typu hloubka hrudníku (r_g = 0,75), ušlech-

II. Genetické (r_g) a fenotypové (r_p) korelace mezi lineárně popisovanými znaky – Genetic (r_g) and phenotype (r_p) correlations between the linear descriptive traits

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	ušlechtilost[1]		0,49	0,27	0,35	0,47	0,29	–0,11	–0,19	0,14	0,28	0,24	0,11	0,05	0,01
2	osvalení[2]	0,56		0,23	0,52	0,56	0,35	–0,07	–0,13	0,13	0,23	0,08	0,03	–0,02	0,07
3	velikost[3]	0,31	0,30		0,49	0,36	0,36	0,13	–0,07	0,11	0,03	0,04	0,03	0,00	0,08
4	kapacita těla[4]	0,34	0,53	0,63		0,67	0,38	0,01	–0,03	0,07	0,16	0,07	0,02	–0,02	0,07
5	hloubka hrudníku[5]	0,68	0,58	0,44	0,69		0,41	–0,02	–0,04	0,08	0,24	0,19	0,09	–0,00	0,07
6	šířka zádě[6]	0,41	0,43	0,41	0,38	0,49		–0,04	–0,02	0,09	0,14	0,12	0,06	0,01	0,08
7	sklon zádě[7]	–0,00	0,05	0,27	0,17	0,19	–0,02		0,05	–0,05	–0,12	–0,10	–0,03	–0,06	0,01
8	postoj zadních končetin[8]	–0,08	–0,12	–0,05	–0,01	0,03	0,01	–0,15		–0,23	–0,04	–0,03	0,01	0,02	0,01
9	paznehty[9]	0,24	0,41	0,16	0,11	0,23	0,20	0,04	–0,35		0,06	0,05	0,04	0,02	0,01
10	vemeno – přední čtvrť[10]	0,43	0,27	0,08	0,20	0,37	0,31	–0,11	0,06	0,21		0,39	0,16	0,10	–0,03
11	vemeno – zadní čtvrť[11]	0,41	–0,03	0,10	0,03	0,35	0,26	–0,05	0,06	0,12	0,50		0,34	0,08	0,01
12	vemeno – závěsný vaz[12]	0,28	0,07	0,08	0,04	0,17	0,16	–0,01	0,11	0,17	0,29	0,45		0,09	0,00
13	rozmístění předních struků[13]	0,19	0,07	–0,06	0,01	0,06	0,11	–0,18	0,14	0,13	0,27	0,13	0,27		–0,10
14	délka struků[14]	0,10	0,20	0,03	0,02	0,04	0,13	–0,02	–0,01	0,05	–0,02	–0,06	–0,18	–0,11	

r_g – pod diagonálou – below diagonal
r_p – nad diagonálou – above diagonal

For 1–14 see Tab. I

IV. Genetické (r_g) korelace mezi znaky popisu, tělesnými rozměry a charakteristikami hodnocení exteriéru – Genetic (r_g) correlations between descriptive traits, body measures and evaluation characteristics of body conformation

Znaky popisu a tělesné rozměry[19]	Hodnocení[20]				
	užitkový typ[21]	kapacita těla[22]	stavba těla[23]	končetiny[24]	vemeno[25]
Ušlechtilost[1]	0,71	0,53	0,73	0,62	0,51
Osvalení[2]	0,64	0,52	0,40	0,40	0,16
Velikost[3]	0,45	0,70	0,28	0,15	0,12
Hloubka hrudníku[5]	0,75	0,78	0,64	0,48	0,45
Šířka zádě[6]	0,50	0,48	0,46	0,24	0,26
Sklon zádě[7]	0,02	0,16	–0,08	–0,08	–0,14
Postoj zadních končetin[8]	–0,14	–0,05	–0,00	–0,31	0,10
Paznehty[9]	0,38	0,24	0,27	0,43	0,19
Vemeno – přední čtvrť[10]	0,44	0,31	0,46	0,36	0,75
Vemeno – zadní čtvrť[11]	0,39	0,29	0,47	0,44	0,61
Vemeno – závěsný vaz[12]	0,19	0,19	0,31	0,26	0,37
Rozmístění předních struků[13]	0,10	0,05	0,10	0,09	0,30
Délka struků[14]	0,01	0,01	–0,04	–0,01	–0,24
Výška v kohoutku[15]	0,45	0,69	0,29	0,14	0,13
Výška v kříži[16]	0,44	0,69	0,27	0,14	0,10
Obvod hrudníku[17]	0,53	0,71	0,32	0,12	0,22
Délka těla[18]	0,32	0,50	0,24	0,10	–0,00

For 1–14 see Tab. I; [15]height at withers, [16]height in hips, [17]chest girth, [18]body length, [19]descriptive traits and body measures, [20]evaluation, [21]production type, [22]body capacity, [23]body conformation, [24]legs, [25]udder

V. Genetické (r_g) korelace mezi znaky popisu, hodnocením, tělesnými rozměry a užitkovostí v 1. normované laktaci – Genetic (r_g) correlations between descriptive traits, evaluation, body measures and performance in the 1st standardized lactation

Ukazatel	Užitkovost v 1. normované laktaci[24]				
	mléko[25] kg	tuk[26] kg	tuk %	bílkoviny[27] kg	bílkoviny %
Ušlechtilost[1]	0,23	0,21	–0,01	0,24	0,08
Osvalení[2]	0,16	0,21	0,18	0,23	0,32
Velikost[3]	0,41	0,42	0,13	0,45	0,26
Kapacita těla[4]	0,49	0,50	0,16	0,54	0,37
Hloubka hrudníku[5]	0,35	0,34	0,04	0,37	0,17
Šířka zádě[6]	0,17	0,18	0,05	0,19	0,13
Sklon zádě[7]	0,14	0,14	0,01	0,16	0,12
Postoj zadních končetin[8]	–0,01	–0,02	–0,04	–0,02	–0,01
Paznehty[9]	0,06	0,09	0,07	0,10	0,15
Vemeno – přední čtvrť[10]	0,30	0,27	–0,02	0,29	0,03
Vemeno – zadní čtvrť[11]	0,32	0,27	–0,11	0,29	–0,07
Vemeno – závěsný vaz[12]	0,25	0,25	0,03	0,24	0,05
Rozmístění předních struků[13]	0,02	0,05	0,10	0,03	0,04
Délka struků[14]	–0,10	–0,07	0,04	–0,10	–0,05
Užitkový typ[15]	0,30	0,27	–0,02	0,33	0,19
Kapacita[16]	0,47	0,45	0,03	0,51	0,26
Stavba těla[17]	0,20	0,16	–0,10	0,22	0,09
Končetiny[18]	0,09	0,02	–0,16	0,09	0,02
Vemeno[19]	0,28	0,22	–0,11	0,27	0,04
Výška v kohoutku[20]	0,44	0,43	0,07	0,46	0,22
Výška v kříži[21]	0,42	0,43	0,13	0,45	0,27
Obvod hrudníku[22]	0,47	0,49	0,16	0,52	0,35
Délka těla[23]	0,35	0,37	0,12	0,36	0,13

For 1–19 see Tab. I; [20]height at withers, [21]height in hips, [22]chest girth, [23]body length, [24]performance in the 1st standardized lactation, [25]milk, [26]fat, [27]proteins

tilost (r_g = 0,71) a osvalení (r_g = 0,64), u kapacity těla pak hloubka hrudníku (r_g = 0,78) a velikost (r_g = 0,70). Stavba těla nejvíce koreluje s ušlechtilostí (r_g = 0,73) a hloubkou hrudníku (r_g = 0,64).

Pro celkové hodnocení utváření vemene se jako nejvýznamnější lineárně popisované znaky logicky projevily utváření předních čtvrtí vemene (r_g = 0,75) a utváření zadních čtvrtí vemene (r_g = 0,61).

Velmi významná je z hlediska efektivní selekce znalost vztahů mezi utvářením exteriéru a užitkovými vlastnostmi plemenic. Genetické korelace mezi znaky lineárního popisu, hodnocením celkových charakteristik, tělesnými rozměry a užitkovostí plemenic v 1. normované laktaci jsou uvedeny v tab. V. Mezi ukazateli produkce a lineárně popisovanými znaky zevnějšku prvotelek byly zjištěny nejvyšší genetické korelace pro kapacitu těla, velikost, hloubku hrudníku a utváření zadních čtvrtí vemene. Mezi produkcí mléčných bílkovin, jako nejdůležitějšího ukazatele užitkovosti prvotelek byly odhadnuty genetické korelace na úrovni r_g = 0,54 pro kapacitu těla, r_g = 0,45 pro velikost, r_g = 0,37 pro hloubku hrudníku a r_g = 0,29 pro utváření zadních čtvrtí vemene. Pro kvalitu produkce, která je především charakterizována procentuálním obsahem bílkovin v mléce, byl nejvýznamnější pozitivní vztah zaznamenán pro kapacitu těla (r_g = 0,37) a osvalení (r_g = 0,32). Obdobné trendy byly zjištěny mezi hodnocením celkových charakteristik zevnějšku a užitkovými vlastnostmi plemenic. Nejvyšší genetické korelace byly odhadnuty mezi produkcí bílkovin a kapacitou (r_g = 0,51) a mezi produkcí mléka a kapacitou (r_g = 0,47). Tělesné rozměry v podstatě charakterizují velikost a kapacitu těla prvotelek, takže jsou zjišťovány obdobné trendy jako u odpovídajících lineárně popisovaných znaků.

Námi zjištěné výsledky potvrzuje Juszczak (1995), který rovněž zjistil pozitivní vztah mezi velikostí, tělesnou kapacitou a produkcí mléka. Stejně tak i Sekerden *et al.* (1995a, b) uvádějí pozitivní korelace mezi znaky vemene a užitkovostí. U zadních čtvrtí vemene ve vztahu k produkci mléka v 1. nornované laktaci uvádějí hodnotu r = 0,31, u předních čtvrtí pak r = 0,50, což představuje mírně vyšší hodnoty oproti našim výsledkům. Z hodnocení vztahů mezi utvářením zevnějšku a užitkovostí prvotelek vyplývá určitá ekonomická váha jednotlivých znaků, která spolu s výsledky odhadu genetických parametrů u důležitých znaků exteriéru dává základní teoretický předpoklad pro možnost zpřesnění selekce plemenic využitím znalostí o utváření jejich zevnějšku.

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PRESENT POSSIBILITIES OF OBJECTIFIED ELECTRONIC MEASUREMENT OF EQUINE LOCOMOTIVE POTENTIAL IN THE CZECH REPUBLIC

SOUČASNÉ MOŽNOSTI OBJEKTIVNÍHO MĚŘENÍ POHYBOVÉHO POTENCIÁLU KONÍ V ČESKÉ REPUBLICE

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ABSTRACT: An electronic device has been developed which helps to substantially objectify the so-far manual method of measuring the quantitative element of locomotion in a horse until now used in the Czech Republic. The device can be used with all horse breeds. It operates on the principle of a special sensor, fixed to the sole part of a hoof. When it touches the ground, it transmits a numerical signal. The signal reaches a portable computer via a repeater station and it is processed by software there and evaluated. Reading is made on a sandy or grassy testing track exactly defined by electronic gates. It is possible to change the length of the track as needed. Special software has also been developed for the evaluation of horses tested, ranking them according to a ten-credit scale expressing the locomotive potential of the observed equine gait as compared with a set standard. The results are documented in the illustrative graphs enclosed. The device makes it possible to observe the tested gait of a horse simultaneously on all the four limbs. Besides the usual parameters of a given gait, it is also possible to observe the regularity or irregularity of the basic gaits, such as "amble", and the like. For a routine use, more large-scale testing is made in dependence on breeds, with the aim to bring standards up-to-date and to improve criteria for effective selection.

Keywords: horses; mechanics of motion; locomotion

ABSTRAKT: Bylo vyvinuto elektronické přístrojové vybavení výrazně objektivizující ruční způsob měření kvantitativní složky lokomoce koně, dosud využívaný v ČR. Zařízení je využitelné pro všechna plemena koní. Pracuje na principu speciálního čidla upevněného na chodidlové části kopyta, které při dotyku s podložkou vysílá číslicový signál přes retranslační stanici do přenosného počítače, kde je signál softwarově zpracováván a vyhodnocován. Měření je prováděno na pískové nebo travnaté zkušební dráze, přesně definované elektronickými závorami. Délka dráhy může být různě měněna. Dále byl vytvořen speciální vyhodnocovací software umožňující vyhodnocení konkrétních testovaných koní podle desetibodové stupnice vyjadřující úroveň pohybového potenciálu sledovaného chodu koně vzhledem k stanovenému standardu. Výsledky jsou dokumentovány na přiložených ilustračních grafech. Zařízení umožňuje sledování testovaného chodu koně současně na všech čtyřech končetinách. Vedle běžně sledovaných parametrů příslušného chodu je umožněno sledování pravidelnosti, ale i nepravidelnosti základních chodů, jako je „mimochod", „nákrok" apod. Pro rutinní využití je prováděno další plošné testování podle plemen s cílem aktualizace standardů a upřesnění kritérií pro efektivní selekci.

Klíčová slova: koně; mechanika pohybu; lokomoce

INTRODUCTION

The mechanics of equine locomotion plays one of the most decisive roles in its practical use. This role is even stronger in special, namely sport and racing, use of a horse, which is predominant in most breeds today.

The mechanics of locomotion can be methodically divided into two elements. The quantitative element (sway, rhythm, action, motion balance, regularity) is usually judged subjectively by an assessor (a jury) in different testing disciplines. The other element is quantitative (length of step, speed, frequency) which may be measured and evaluated by more or less exact methods.

The possibility to estimate objectively the mechanics of equine locomotion, especially its quantitative element, constitutes a precondition for estimating the locomotive potential or working abilities of horses with a genetic impact.

The mechanics of locomotion in the breeding of horses with high performance is therefore becoming one of the basic preconditions of a successful breed and plays an important role in the process of breeding.

The mechanics of equine locomotion and interrelations between the mentioned properties have been studied by a number of authors. In the Czech Republic it was above all Dušek (1973, 1974a, b, 1981) and Dušek *et al.* (1970), who give a summary of his own and foreign works related to the given issue and establishes a method of measuring the quantitative element of locomotion measured by a subject, including proposed standards for selected horse breeds.

Evaluation is based on an analysis of the locomotive potential of a horse made in a special test over a 100-m long track. The method evaluates the interrelation of all the three variables, i.e. speed, step length and its frequency. From values measured, standards were set for the usual breeds, except the English full-blooded and the trotter (Dušek, 1977). Measuring the quantitative elements of the mechanics of locomotion has become part of evaluation in performance tests of young horses before they are introduced into the breed.

Systematic and non-systematic faults occur when performing measurements. Namely Dušek *et al.* (1970) and Dušek (1973) tried to alleviate these faults and proposed some corrections to be made at the start and end of the measured track. However, not even after the alternations the methods are quite satisfactory.

In our approach we have concentrated mainly at eliminating the shortcomings of the presently used method of measuring the quantitative element of the mechanics of equine locomotion as an auxiliary selection criterion. In the framework of a research solution, co-financed by the Czech Ministry of Agriculture, development was started of a device which would minimize the subjective element in measuring horses and gradually enable to consider other, so far unmeasured, components or signs of equine locomotion.

The presented state is the result of research of a number of years in different concepts of apparatuses. The following procedure indicates the essence of our latest proposed variant.

MATERIAL AND METHOD

The aim of the task was to increase precision in time measuring, bring to minimum any systematic or non-systematic faults, minimize the influence of a subjective factor in measuring and to make possible consideration of some other effects, such as regularity of gaits (which belongs to the qualitative element), the length of steps, etc. This concerns mainly signs and properties which cannot be evaluated in the manual measuring method. The solution is also to provide computer processed measured values and their evaluation.

In its next stage, the research will concentrate on standardisation of curves of data measured for the basic equine gaits using an objectified method. The aim is to make use of the results in breeding and selective work in horse breeds included in a research project.

In the first stage of our research, dealt with here, our efforts were concentrated in three spheres and were to result in:

1. Electronic reading of a signal from the direct impact of one and/or all four limbs simultaneously in all basic equine gaits and its telemetric reception in a computer.
2. Electronic reading of the measured time of the length of the course of the observed limb impact, and/or of all the four limbs, including an objective determination of the start and the finish of the test track.
3. Development of software which would enable processing the transmitted signal, later standardisation and classification of measurement results from a specific individual as compared with a standard value. The result thus obtained should be defined simply and it should be possible to use it in the selective and breeding process of observed horse breeds.

The results obtained from the device developed have so far been verified on horses of warm-blooded breeds and the English full-blooded horse. Development and verification took place in the Research Institute of Horse Breeding in Slatiňany and on the Research Grounds of the Czech Association of Steeplechase, the Race Course in Pardubice. First results of the new method of measurement were presented by Jelínek *et al.* (1995a).

RESULTS AND DISCUSSION

The method of present-day measurement of the quantitative element of the mechanics is based in principle on counting by sight the horse's steps over a 100-m long direct track in relation to increasing speed of gait under observation. This calculation results in credits given on the basis of a set evaluating scale with regard to the standardised curve of the gait of a horse under observation.

The mentioned procedure, used traditionally in testing systems also in other countries, is not quite satisfactory even after the above mentioned additional corrections. One of the shortcomings being inaccuracy, in some cases even faults without any possibility to judge objectively the regularity of gaits, the lengths of individual steps, etc.

With regard to the above facts we have started to develop and design a new equipment for the already mentioned three spheres.

Device for reading a signal from limb impact and its transmission into a computer

Electronic sensors of different structures were tested and developed; they worked on different principles and at different places during work on a horse. Their detailed description is given by Jelínek and Volenec

(1990). We do not consider effective to give a detailed description in this study because none of the structures tested showed sufficient reliability during tests and precision in practical measuring. The final solution is described by Jelínek *et al.* (1994, 1995b).

The final sensor structure, designed by us, works in direct contact of the sole area of a horse's hoof with the surface of the testing track. The sensor is fixed by a special system which keeps it in a constant position even at the top speeds of race gallop.

Signal is recorded when a tensometric unit reacts to the load of hoof pressure onto the base. The sensor consists of a power supply unit and a miniature transmitter which sends the signal over an aerial into a repeater station which amplifies it and transmits it into a computer placed at a control post close to the testing track.

Signals from sensors are transferred in the form of a numerical code and sent, all together, through a single high-frequency channel to a stationary receiver in a small portable computer.

The repeater station, which was developed together with the sensor, is placed in the seat pad and the force of the signal transmitted guarantees to cover the necessary distance. The station can simultaneously receive independent signals from all four limbs.

The device of the said configuration makes it possible to follow on a computer screen the course of hoof load in real time. Data measured are immediately stored into the memory. After field measuring is completed, data may be analysed independently and evaluated. The analysis results in objective parameters describing the mechanics of a given horse.

When measuring the contact of limbs with the track surface, there are, understandably, quite marked pressures, especially during gallop impacts when the total weight of the horse is borne by a single front leg, depending which leg the horse uses in gallop. Our present experience from practical tests with some 30 horses in principle only allows measuring on a sandy testing track. This fact limits, to a certain degree, the use of the developed sensor for some breeds that cannot be measured under the saddle, without some distortion, or measured free, e.g. cold-blooded breeds in which this is still required under the amended standard. Measuring in sulky would thus result in a significant systematic fault which would distort and undermine the measured parameters.

The mentioned restrictions make us work even more at technically improving the sensor structure and the manner of reading the signal in order to overcome the problems. Today, the development is in a stage of practical verification. The first results (from about 20 horses) indicate the possibility of recording on a grassy surface. However, not even will such an adjusted manner of reading the signal most likely make it possible to determine the specific objective distribution of load of the different limbs during a horse's motion. It is however a guarantee of the possibility of measuring shoed horses of all breeds and does not have any other specific requirements as to the structure of the surface of the testing track.

The original intent of the task was, inter alia, to make it possible to judge the relative load per specific limbs. This was to help study the distribution of weight and the movement of the centre of gravity at the different stages of somatic development, in natural and artificial balance, during different stages of training and practical use of horses. Results of the study of data obtained from different surfaces of testing tracks showed however showed that the course of measured load of a localised impact onto the base displayed such a high variability that the load cannot be objectively and without any significant faults calibrated. The bases of these faults are the significantly changing conditions of the testing track during the individual impacts of limbs and the physiological reaction of the horse, caused by the influence of the level of artificial balance achieved, as well as the reaction of the higher nerve activity to the external environment.

As regards the structure of our solution itself, we can say that this is a unique principle of measuring. In international literature we can only find indirect methods of measuring, utilising an accelerometric device, or a camera or a video-camera. The first mentioned principle is based on recording and analysing inertial forces at two levels; cranio-caudal and dorso-ventral. This method is namely practised in France and is utilised mainly for analysing the quality of locomotion in people and, lately, in horses. Besides studying and analysing locomotion, it is directed to predicting the suitability of horses for sport (jump, training, trotters) or to pin-pointing limping in horses. The method and results of accelerometry in the last years were namely presented by Barrey *et al.* (1994, 1995), Barrey (1995), as well as Kennedy *et al.* (1995).

Electronic reading of the measured time of the length of the course of the observed limb impact, and/or of all the four limbs, including determination of the start and the finish of the test track

A necessary requirement for an objective measuring of time and length of step of individual limbs is an independent, electronic measuring of start and finish bound to data obtaining. For this purpose, two modified infra-sensors with special lenses are used. They are placed along the sides of the testing track and they register motion only in a narrow sector around the starting and finishing lines. The passage of a horse is indicated to the nearest ca. 10 cm.

The signal travelling by air is intercepted, by means of a specially designed device, directly by a portable computer and processed as part of the software evaluation of the measured data. The result is then displayed and impacts of the different limbs are also represented. An example of such representation can be seen in graphical examples Nos. 1 to 3.

Software for reading and evaluating results

We have developed special software for evaluation – it enables numerical and graphical processing of the data of motion and impact of the different limbs of a horse. The software makes it possible to evaluate specific steps or jumps of a horse.

Besides a colour display of motion, it also shows the following basic parameters for each limb under measurement:

1. The number of measurements
2. Total time in seconds for measured steps between start and finish
3. Speed of horse motion in the measured gait
4. Average step length in the measured gait in seconds and also converted into meters
5. Average limb frequency over the measured track per minute
6. Decisive deviation of tested step lengths for estimating the regularity of manner of movement of the limb under observation
7. Numbers of identical steps in length made by a given limb over the track under observation in seconds of a time interval and also converted into meters.

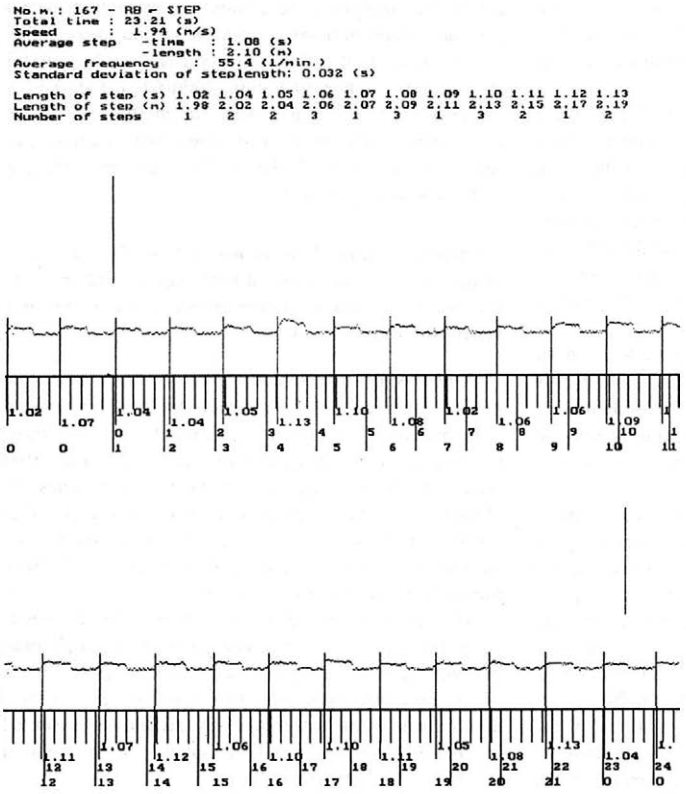
Practical illustration can be seen in Figs. 1–3.

For each basic kind of horse gait software graphical illustration is made for all the four limbs, or rather sensors, together. This makes it possible to consider if the succession of legs is correct and to judge the overall regularity of succession of limb impacts. An example can be seen in Fig. 4.

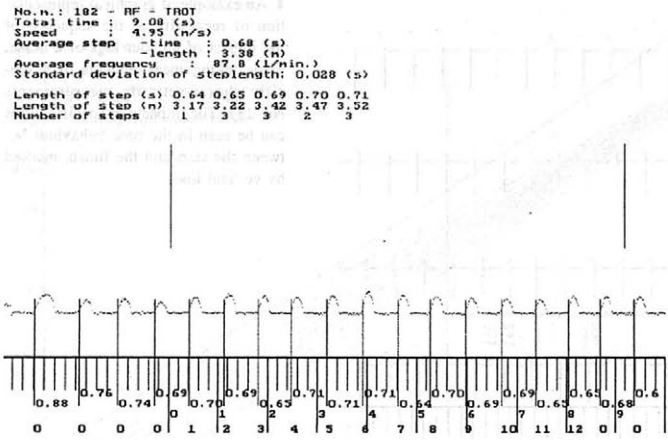
We have developed special software for evaluating and standardising gait measurements in specific horses. The software enables us to speedily obtain results with the help of a computer. Its weak point is that it has so far worked with a single-channel reception only, i.e. for a single horse limb.

An example of a step evaluation can be seen in Fig. 5, Fig. 6 for trotting and 7 for gallop. The heavy central line represents the course of the calculated standard. When evaluating the quantitative element of the mechanics of motion, length of step was considered, i.e. from one single impact of a limb to the next one. The length depends on the speed of the motion of the horse, or rather of the limb observed. Earlier experience from observing the results justifies us to state that the dependence is linear in the case of step and trot while that of gallop is quadratic.

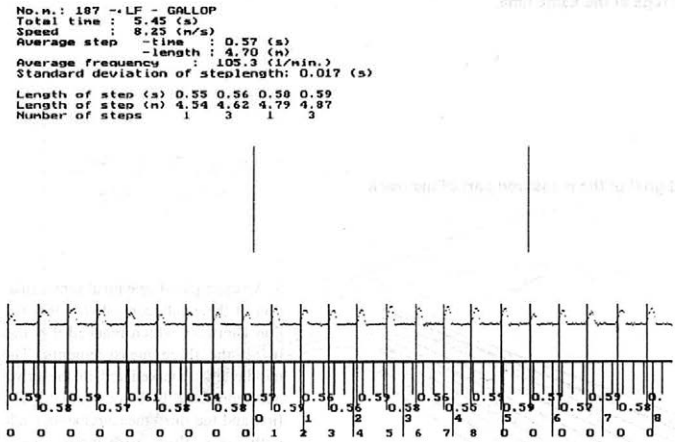
Each horse and kind of step were measured in different speeds. The values were used to construct the function (linear or quadratic) which expresses the de-



1. An example of graphical representation of step for set of measurements No. 167. Parameters can be seen in the graph legend and description. Vertical lines over step course indicate the start and finish



2. An example of graphical representation of trot for set of measurements No. 182. Parameters can be seen in the graph legend and description. Vertical lines over step course indicate the start and finish



3. An example of graphical representation of gallop for set of measurements No. 187. Parameters can be seen in the graph legend and description. Vertical lines over step course indicate the start and finish

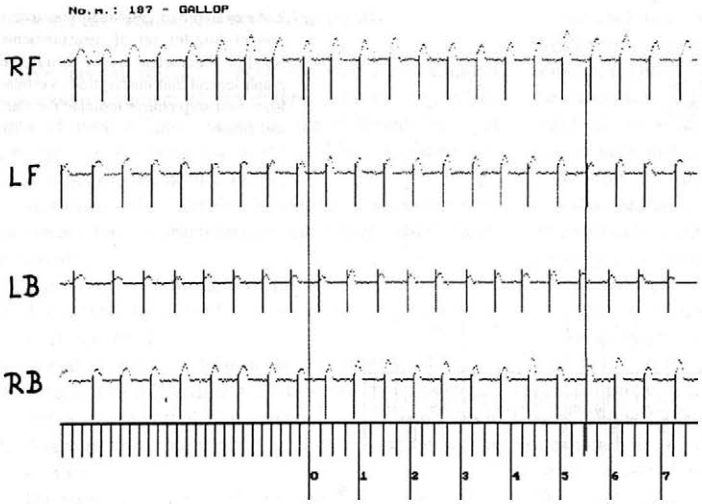
pendence of step length on horse speed and the frequency of the observed limb. The method of the least squares was used. From this function, "theoretical values" are deduced in speeds we have selected.

From the theoretical values, basic statistic values were calculated for each speed, i.e. mean value and standard deviation which we made use of the calculation of decile zones for each appropriate theoretical speed.

From the limits of these calculated zones, functions were obtained again by means of the least squares method. These functions determine appropriate decile zones in the full extent of speeds in each equine gait. The zones served as a limit for awarding credits to specific horses within a ten-credit scale. Decile zones were created on the basis of normal division quantiles. Kolmogorov-Smirnov test was used to verify normality in selected speeds.

It is evident from the results that we have managed to find a satisfactory technical electronic device as well as processing software. This significantly improves objectification of the method and manner of measuring used until now for the quantitative element of the mechanics of equine motion as used in our conditions and to expand evaluation of some other characteristics of horse locomotion, which was the target of the first stage of our research.

The equipment enables to determine the length of each single step over the test track in a relatively accurate and simple way as compared to manual measuring. It allows to characterise the regularity of gaits in the form of standard deviations in measured horses, which are automatically calculated for each tested individual together with other gait characteristics and classifies it into a ten-credit scale depending on its motion potential for the given gait in comparison with the breed



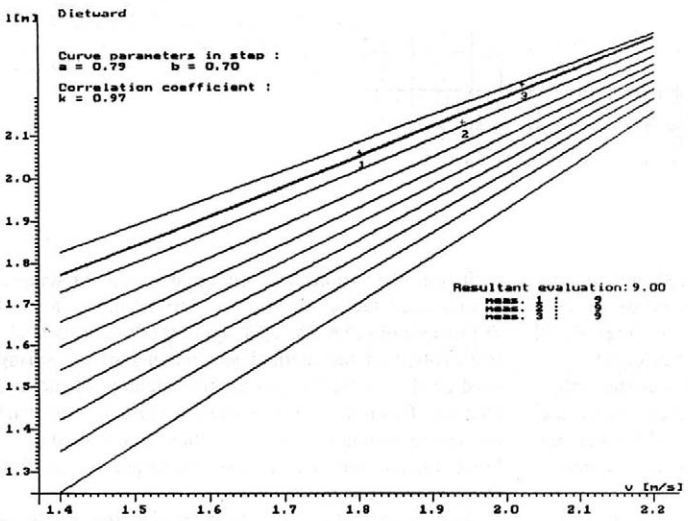
4. An example of graphical representation of regularity of the sequence of the impact of all four legs of a horse, while taking measurements of a specific horse-set of measurements No. 187. The impacts of specific limbs can be seen in the time behaviour between the start and the finish, marked by vertical lines

Graphic exhibit of measure of all four legs at the same time.

Legend:

RF - right front leg
LF - left front leg
LB - left back leg
RB - right back leg

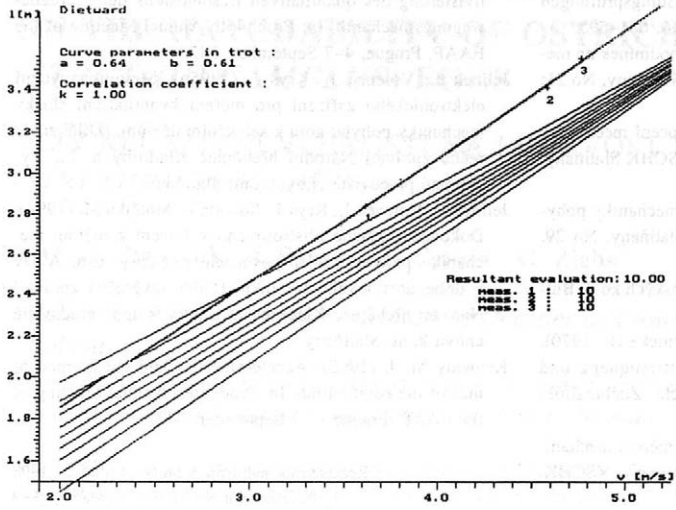
The vertical long lines mean start and goal of the measured part of the track.



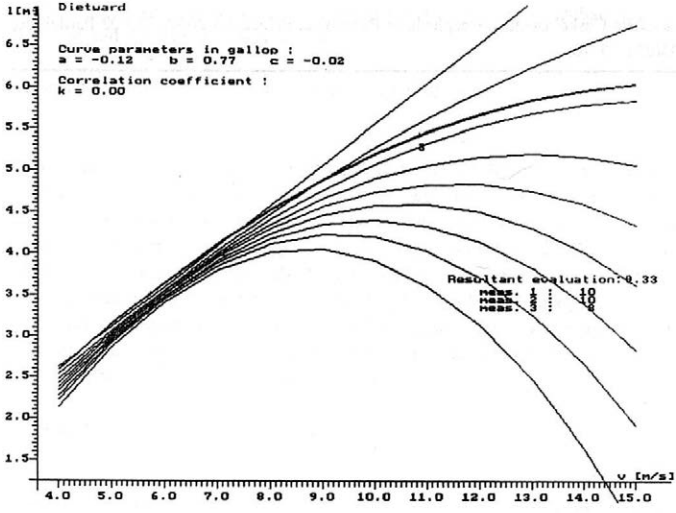
5. An example of graphical representation of the final score of step for stallion Dietward which reached 9 points in all the three measurements. The middle line (marked red in the original) represents the breed standard. The first and the third measurements made with the stallion in different speeds (marked by numbers with interpolated regression) show the position of the stallion under evaluation as compared with the standard. Parameters can be seen in the graph legend and description.

standard (see illustrative Figs. 5 to 7). The already mentioned shortcomings are that the software has so far worked only over a single channel, a problem we shall tackle in the next period.

Using the contemporary manner of recording four channels, it is possible to identify inborn irregularities of gait, such as partial or complete "amble" etc. It is not always necessary to make the measuring over an



6. An example of graphical representation of the final score of trot for stallion Dietward which reached 10 points in all the three measurements. The middle line (marked red in the original) represents the breed standard. The first and the third measurements made with the stallion in different speeds (marked by numbers with interpolated regression) show the excellent position of the stallion under evaluation as compared with the standard. Parameters can be seen in the graph legend and description.



7. An example of graphical representation of the final score of gallop for stallion Dietward which reached 9.33 points in all the three measurements. The middle line (marked red in the original) represents the breed standard. The first and the third measurements made with the stallion in different speeds (marked by numbers with interpolated regression) show the very good position of the stallion under evaluation as compared with the standard. Parameters can be seen in the graph legend and description.

identical 100-m long track. It is also possible to exclude poor-quality riders from the test, incorrect sets of horse measurements, etc.

We have started a large-scale measurement of an adequate number of horses of appropriate breeds for later routine utilisation of the device in selective and breeding practice. After data are obtained, standards may be amended and criteria set for an effective selection, which is the subject of further research. Selection strategy should be based on the updating of the basic genetic parameters re-evaluated according to this method of measuring.

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EFFECT OF RELATIVE HUMIDITY ON THE HATCHABILITY OF OSTRICH (*STRUTHIO CAMELUS*) EGGS

VLIV RELATIVNÍ VLHKOSTI NA LÍHNIVOST VAJEC PŠTROSA (*STRUTHIO CAMELUS*)

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ABSTRACT: Two hundred forty ostrich eggs, divided into 4 batches of 60 each, were incubated in multi-stage PasReform incubators at the same dry bulb temperature (36.4 °C), but at four different initial levels (50, 40, 30, 25%) of relative humidity (RH). The overall hatchability was 54% of eggs set and 76% of fertile eggs. The hatchability of fertile eggs was 65, 72, 75 and 82% for batch I (50% RH), II (40% RH), III (30% RH) and IV (25% RH). Weight losses during incubation ranged from 10.18% in batch I to 13.51% in batch IV. Eggs from the batch incubated at the highest initial RH (I) showed chicks with the poorest vitality, the greatest number of malpositioned chicks and the most chicks with unabsorbed yolk sacs. Present results suggest that a wide range of RH (25–40%) can be applied to incubate ostrich eggs provided that egg weight, egg shell thickness and porosity are taken into account.

Keywords: ostrich eggs; relative humidity; hatchability

ABSTRAKT: Ve dvou vícestupňových inkubátorech PasReform jsme inkubovali 240 pštrosích vajec rozdělených do čtyř várek po 60 kusech při shodné teplotě suchého teploměru (36,4 °C), ale při čtyřech rozdílných počátečních hladinách (50, 40, 30, 25 %) relativní vlhkosti (RV). Celková líhnivost dosahovala 54 % z násadových vajec a 76 % z oplodněných vajec. Líhnivost oplodněných vajec činila 65 % v I. várce (50% RV), 72 % ve II. várce (40% RV), 75 % v III. várce (30% RV) a 82% ve IV. várce (25% RV). Ztráty hmotnosti se během inkubace pohybovaly od 10,18 % v I. várce do 13,51 % ve IV. várce. Z vajec várky, kterou jsme inkubovali při nejvyšší počáteční RV (I), se vylíhla kuřata s nejhorší vitalitou, nejvyšší počet kuřat s nesprávnou polohou a u většiny kuřat nedošlo k absorpci žloutkového vaku. Naše výsledky naznačují, že k inkubaci pštrosích vajec lze použít široké rozpětí relativní vlhkosti (25–40 %), jestliže bude brána v úvahu hmotnost vajec a dále tloušťka a pórovitost vaječné skořápky.

Klíčová slova: pštrosí vejce; relativní vlhkost; líhnivost

INTRODUCTION

One of the important factors limiting the expansion of the ostrich industry is the low hatchability (30–90%) of fertile ostrich eggs (Phibley *et al.*, 1991; Jost, 1994; Deeming, 1995). While 15% of the initial egg mass is the mean value for water loss for most bird eggs (Ar, Rahn, 1980), ostrich eggs have a mean weight loss of 13–14% in the nature (Burger, Bertram, 1981; Jarvis *et al.*, 1985; Swart *et al.*, 1987). Such low values can only be achieved in artificial conditions by having unusually low incubation humidities (Deeming, 1995). Due to the low eggshell conductance of ostrich eggs relative to other bird species, Christensen *et al.* (1996) suggest a humidity lower than 25% for optimal hatchability. However, these low values are sometimes difficult to achieve, particularly in areas of high natural ambient humidity (Deeming *et al.*, 1993).

The present investigation was aimed at examining the effect of four levels of relative humidity on hatchability during artificial incubation of ostrich eggs.

MATERIAL AND METHODS

Two hundred forty eggs, from females all in their third year of laying, were incubated at the Garczyn farm in Poland. Eggs were sanitised in paraformaldehyde vapours (per 1 m^3 of chamber 17 mg $KMNO_4$ was added to 25 ml of 40% formaline and 21 ml of water)

after collection, stored for a maximum of 7 days at 12–15 °C and sanitised again before setting. Four batches (I, II, III, IV) of 60 eggs each were incubated in multi-stage PasReform incubators. Eggs were set in metal-framed egg trays at an angle of 45° and kept at a temperature of 25 °C for 24 h before increasing the incubation temperature to 36.4 °C. Eggs were automatically turned through an angle of 90° once every hour. Temperature and humidity in the setter were electronically controlled and continuously monitored. While dry bulb temperature was kept at 36.4 °C for all four batches, initial relative humidity was maintained at 50, 40, 30 and 25% for batch I, II, III and IV, respectively.

All eggs were candled (PasReform halogen lamp MR16-12 V) and weighed (electronic balance Precisa 5000D) on days 14, 28 and 39 of incubation. On day 39 viable eggs were transferred into the hatcher and placed horizontally (without turning) in plastic baskets lined with artificial grass. The dry bulb temperature in the hatcher was lowered by 0.4 °C. Eggs in the hatcher were examined every 2–3 hours in the hatcher in order to record (whenever possible) the time of internal and external pipping and final hatching. If the chicks pipped externally, but showed no progress for over 24 hours, eggshells were cracked around the equator to facilitate hatching. On day 45 all unhatched eggs were opened. Eggs which proved to be infertile, or in which the embryo died during incubation, were carefully examined in order to confirm the lack of embryonic development, or to determine the age, position and any obvious abnormalities of the embryo.

Results were analysed statistically using one-way ANOVA analysis procedures of the Statistical Analysis System (SAS, 1985). Means with a significant F ratio were separated by Duncan's multiple range test (Duncan, 1955).

RESULTS

Hatchability and embryonic mortality

Hatchability of fertile eggs increased as RH decreased (Table I). The highest number of dead embryos was found in batch I. Mortality peaks were determined by candling during the last trimester (29–38 days) of incubation and after transfer of eggs to the hatcher (> 39 day). After opening the eggs that remained unhatched on day 45 a careful examination of the dead embryos demonstrated a considerable volume of foetal water in the subcutaneous tissues of the head, neck and around the stomach in the form of gelatinous tumours. The yolk sacs were usually overhydrated and absorbed poorly or not at all. The symptoms occurred most often in batch I (70% of total dead embryos), in several cases in batch II (30%), but rarely in batch III and IV (10%). Moreover, 58% of all dead-in-shell embryos were malpositioned (the highest number in batch I). Single cases were also observed of underdeveloped eye-ball, crooked beak and umbilicus hernia (personal observation).

Incubation and chick characteristics

No differences ($P > 0.05$) were found in initial egg weight between the batches (Table II). In agreement with initial egg weight, chick weight at hatching did not differ ($P > 0.05$) between the batches. While no specific pattern was observed in weight loss at day 14, weight loss at day 28 and 39 increased as RH decreased.

Time to both internal and external pipping was shorter ($P < 0.05$) at RH's of 30 and 25% than at 50 and 40%. Chicks were allowed to hatch naturally, with as little assistance as possible. As a rule the chicks broke the shell in the vicinity of the equator (85%) – the greatest number in batch III, the smallest number in batch I (personal observation). In some cases in batches II and IV the shell was not broken with the beak but with the right leg, in this case help was necessary.

The hatching process proved the longest ($P < 0.05$) in batch I. The hatching of the first eggs was completed on day 40, but the majority of chicks hatched between days 42–43. The mean length of incubation did not differ ($P > 0.05$) between either 50 and 40% RH or 30 and 25% RH.

After hatching, each chick was carefully examined and the umbilical cord was disinfected with 7% iodine.

I. Hatchability and embryonic mortalities of ostrich eggs

Item	Batch			
	I (60)	II (60)	III (60)	IV (60)
Infertile eggs	23	17	20	9
Number of dead embryos at day 14	3	1	2	2
Number of dead embryos between days 15 and 28	2	1	1	0
Number of dead embryos between days 29 and 38	4	4	4	5
Number of dead-in-shell embryos from day 39	4	3	3	2
Hatchability of all eggs set (%)	40.0	56.6	50.0	70.0
Hatchability of fertile eggs (%)	64.9	72.3	75.0	82.3

I – 50% RH, II – 40% RH, III – 30% RH, IV – 25% RH

II. Incubation results of ostrich eggs

Item		Batch			
		I (60) mean ± SD	II (60) mean ± SD	III (60) mean ± SD	IV (60) mean ± SD
Initial egg weight	(g)	1639 ± 59	1611 ± 128	1633 ± 115	1601 ± 116
Weight loss at day 14	(%)	$3.68^{a,b}$ ± 1.0	4.51^{a} ± 1.42	4.24 ± 0.84	4.84^{b} ± 0.82
Weight loss at day 28	(%)	7.44^{a} ± 1.4	8.00^{b} ± 2.13	8.22^{c}± 1.58	$9.38^{a,b,c}$ ± 1.48
Weight loss at day 39	(%)	$10.18^{a,b}$ ± 1.6	11.00^{c} ± 3.06	12.34^{a} ± 2.01	$13.51^{b,c}$ ± 1.98
Time to internal pipping	(h)	$989^{a,b}$ ± 15	$992^{c,d}$ ± 17	$975^{a,c}$ ± 18	$973^{b,d}$ ± 16
Time to external pipping	(h)	$1010^{a,b}$ ± 15	$1008^{c,d}$ ± 18	$992^{a,c}$ ± 18	$986^{b,d}$ ± 17
Mean hatching time	(min)	$1285^{a,b,c}$ ± 218	1090^{a} ± 277	1074^{b} ± 306	1083^{c} ± 305
Mean length of incubation	(h)	$1031^{a,b}$ ± 18	$1026^{c,d}$ ± 20	$1009^{a,c}$ ± 19	$1004^{b,d}$ ± 18
Chick weight at hatching	(g)	1075 ± 33	1060 ± 98	1046 ± 83	1037 ± 76

Within rows means bearing the same letter are different at $P < 0.05$
I – 50% RH, II – 40% RH, III – 30% RH, IV – 25% RH

Chicks from batch I generally showed the lowest vitality. In 70% of day old chicks oedema was found, particularly on the legs and neck (the data was not shown in Table III). Chicks in this batch had considerable troubles in leaving the shell and 50% of them had to be assisted (Table III).

Thus, chicks from batch I had to be kept in the hatcher at least 10–14 hours longer than those from the other batches in order to dry and to avoid any post--hatching health problems, such as pneumonia or colds. Apart from oedema and low vitality, the hatching difficulties in batch I were caused by the highest rate of malposition. The most common malposition was "head placed in the small end" (malposition II), where the embryo was inverted within the egg with the head in the end away from the air space (Landauer, 1967). In this position only 15% of chicks pipped internally and none hatched without assistance. Similar trends were also observed in the percentage of deformed chicks hatched in individual batches (Table III). In batch I a high percentage of deformed chicks showed unabsorbed yolk sacs.

DISCUSSION

Egg characteristics and hatchability

The mean initial ostrich egg weight in this study (over 1600 g) is higher than the values of 1453 g (Brown *et al.*, 1996) and 1382 g (Jost, 1994) reported for ostriches from South Africa and Namibia, respectively. In the present study the mean weight of the chicks hatched in all batches was comparatively high caused by a large initial egg weight and small weight losses during incubation. However, the correlation of chick weight to initial weight of eggs set (63%) was in the same order as 60–68% reported in other studies (Foggin, 1992; Deeming, Ayres, 1994).

Means of 54% of all eggs and 76% of fertile eggs in the present study are higher than hatchability results of 24–60% for ostrich eggs in the United Kingdom (Deeming *et al.*, 1993; Deeming, 1996a, b) and 50% for South Africa (Smith *et al.*, 1995).

III. Number of assisted and malpositioned chicks during hatching and number of deformed day old chicks

Item		Batch			
		I	II	III	IV
Assisted chicks during hatching	(%)	50.0	35.3	36.7	35.7
Malpositioned chicks	(%)	25.0	14.7	10.0	14.3
"Head in small end" position	(%)	33.3	40.0	33.3	50.0
Deformed chicks*	(%)	16.7	8.8	10.0	9.5
Chicks with unabsorbed yolk sacs	(%)	75.0	66.7	66.7	50.0

* Chicks with unabsorbed yolk sacs, clearly visible defects as blindness, adenoma, crooked beak, leg deformities as bowed or twisted legs, paralysed chicks, strong myopathy
I – 50% RH, II – 40% RH, III – 30% RH, IV – 25% RH

Weight loss

Weight losses during incubation, particularly in batches I and II, are lower than the values of 12–15% reported during artificial incubation of ostrich eggs (Bertram, Burger, 1981; Jarvis *et al.*, 1985; Hicks, 1992; Stewart, 1992), but in agreement with the values of 11–13.2% (Huchzermeyer, 1994; Bertram, 1996) reported for natural incubation where air humidity ranged between 39% and 52%. Inadequate weight loss results in a low hatchability as the chicks are oedematous (Stewart, 1992). In eggs which lose an insufficient volume of water during incubation the embryos die probably through hypoxia, induced by low shell conductance (Deeming, 1995). Christensen *et al.* (1996) indicated that, as egg shell conductance is lower in ostriches than in the other avian species, in order to obtain a loss of initial egg weight of 15% the incubation humidity ought to be below 25%. According to Deeming *et al.* (1993) and Brown *et al.* (1996) hatching at a weight loss below 10% produces oedematous chicks that are sluggish and frequently have unabsorbed yolk sacs. The failure of eggs to lose water results in the embryo storing excess water in the muscles and under the skin, observed as a characteristic oedema (Davis *et al.*, 1988). Anasarca is a common problem in ostrich eggs which fail to hatch (Ley *et al.*, 1986).

The problems discussed have appeared also in the present investigations. Batch I with a weight loss of 10.18% at day 39 showed the greatest number of chicks with oedema and unabsorbed yolk sacs.

Assistance of chicks during incubation

Thirty-five to 50% of chicks needed assistance during hatching. This could be due to malposition, low vitality of chicks and a weight loss during incubation lower than that recommended. Ostrich chicks should be allowed to pip and hatch unaided. Unfortunately, the practice of helping chicks is quite common. Thus, the efforts to keep alive chicks which needed assistance at hatching result in the production and maintenance of poor quality birds (Deeming, Ayres, 1994).

Malpositions

In the present study a comparatively large number of malpositioned embryos hatched was observed, especially in batch I (25%). However, Phibley *et al.* (1991) observed a variation of 38–75% of malpositioned chicks between the farms. Malpositioning of embryos may be caused by the position of the egg during incubation, by inadequate turning, by genetic factors and by insufficient water loss (Landauer, 1967; Foggin, 1992; Jensen *et al.*, 1992). The percentage of malpositions observed in the present study corresponds to that described by Deeming (1995), but it is worth emphasising that this problem has been treated with caution as the assessment of malposition in ostriches is still not unequivocal (Brown *et al.*, 1996).

The hatching results presented here suggest that ostrich eggs can be incubated at a wide range of 25–40% RH with acceptable results. However, although satisfactory, the weight loss at 40 % RH was only 11%. The results indicate that a level of RH reaching 50% is obviously too high. Weight losses of 11–15% are optimal for satisfactory hatching results. However, the high variation of ostrich egg quality (egg weight, thickness and porosity of the shell) is important with respect to determining hatchability parameters for ostrich eggs and need further investigation.

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ARTIFICIAL PROPAGATION OF EUROPEAN PERCH (*PERCA FLUVIATILIS* L.) BY MEANS OF A GnRH ANALOGUE

UMĚLÝ VÝTĚR OKOUNA ŘÍČNÍHO (*PERCA FLUVIATILIS* L.) POMOCÍ ANALOGU GnRH

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ABSTRACT: Mature females were administered intramuscularly a single dose of a mammalian GnRH analogue ([D-Ala^6]GnRHProNHEt) and/or an extract of dehydrated carp pituitary dissolved in physiological solution (0.9 g.l^{-1} NaCl). Males were not treated at all. In the first experiment, carp pituitary (at doses of 0.75, 1.5, 3 and 6 mg.kg^{-1}) was administered to 4 groups (n = 5) of females placed in submerged cages (without males and without replicates). The first control group was injected with physiological saline and the second control group with GnRH analogue (100 μg.kg^{-1}). Mean temperature was 16.1 ± 1.1 °C. Ovulation did not occur either in females treated with pituitary or in those treated with physiological saline. Females treated with GnRH did ovulate and they were artificially stripped out. The number of stripped eggs was 40.7 ± 18.4 10^3 eggs per 1 female. Relative working fecundity in this group was 128.6 ± 53.7 10^3 eggs.kg^{-1}. The second experiment was performed with artificial propagation of females placed in cages without males, after hormonally induced ovulation by means of a single 100 μg.kg^{-1} dose of GnRH analogue. Twenty five females (80%) out of 35 fish injected were artificially stripped out. Relative working fecundity was 102.1 ± 49.5 10^3 eggs.kg^{-1}. Relative weight of stripped eggs was 18.7 ± 3.7%. The third experiment was performed with two groups of 7 females each, treated with 100 μg.kg^{-1} GnRHa and placed in cages (without replicates) in order to compare the effect of presence/absence of males on ovulation and performance of spawning. Among the females stocked with males at a 1 : 1 ratio, 57% of females spawned successfully. In the absence of males, 100% females ovulated and spawned successfully. The relative working fecundity was 64.7 ± 14.5 and 52.2 ± 26.5 10^3 eggs.kg^{-1}, respectively. No significant differences ($P < 0.05$) were found in fecundity indices between the groups of spawned females. The estimated fertilisation rate ranged within 60–95%. No desticking of eggs was performed, eggs were hatched in aerated cages placed in a flow-through trough. The management of methods of controlled reproduction in perch will contribute to the production of stockfish for open waters, as well as for ponds with market production of this species.

Keywords: European perch; *Perca fluviatilis* L.; hormonal induction of spawning; semiartificial spawning; artificial propagation; GnRH analogue; carp pituitary; fecundity

ABSTRAKT: Pohlavně zralým jikernačkám byl jednorázově injekčně intramuskulárně podáván analog savčího GnRH ([D-Ala^6]GnRHProNHEt) nebo extrakt dehydrované kapří hypofýzy ve fyziologickém roztoku (0,9 g.l^{-1} NaCl). Mlíčáci injikováni nebyli. V prvním pokusu byla jikernačkám umístěným v ponořených klecích (bez mlíčáků) ve 4 skupinách bez opakování podána kapří hypofýza (v dávkách 0,75; 1.5; 3 a 6 mg.kg^{-1}). První kontrolní skupině byl injikován fyziologický roztok a druhé kontrolní skupině GnRHa (100 μg.kg^{-1}), při průměrné teplotě 16,1 ± 1,1 °C. K ovulaci nedošlo u žádné z jikernaček injikovaných hypofýzou a fyziologickým roztokem. Jikernačky injikované GnRHa ovulovaly a byly uměle vytřeny. U skupiny injikované GnRHa činila relativní pracovní plodnost 128,6 ± 53,7 10^3 ks.kg^{-1}. Ve druhém pokusu byl proveden umělý výtěr jikernaček umístěných v klecích (bez mlíčáků) po hormonálně indukované ovulaci pomocí jednorázově podaného GnRHa v dávce 100 μg.kg^{-1}. Ze 35 injikovaných bylo 28 jikernaček (80 %) uměle vytřeno. Absolutní pracovní plodnost jikernaček byla 40,7 ± 18,4 10^3. Relativní pracovní plodnost činila 102,1 ± 49,5 10^3 ks.kg^{-1}. Relativní hmotnost vytřených jiker dosáhla hodnoty 18,7 ± 3,7 %. Ve třetím pokusu byl ve 2 skupinách po 7 jikernačkách injikovaných GnRHa (100 μg.kg^{-1}) a umístěných v klecích (bez opakování) porovnáván vliv přítomnosti či nepřítomnosti mlíčáků na dosažení ovulace, resp. uskutečnění výtěru. U jikernaček umístěných v poměru 1 : 1 s mlíčáky došlo k úspěšnému výtěru 57 % jikernaček. Bez přítomnosti mlíčáků ovulovalo a bylo vytřeno 100 % jikernaček. Průměrná relativní pracovní plodnost vytřených jikernaček dosáhla 64,7 ± 14,5 a 52,2 ± 26,5 10^3 ks.kg^{-1}. Nebyly zjištěny statisticky signifikantní rozdíly v ukazatelích plodnosti mezi skupinami vytřených jikernaček. Odhadem stanovená oplozenost se pohybovala v rozpětí 60 až 95 %. Odlepkování jiker nebylo prováděno, jikry byly líhnuty v klíckách se vzduchováním zavěšených v průtočném žlabu. Zvládnutí metod řízené reprodukce okouna říčního přispěje k produkci násadového materiálu pro nasazování nejen do volných vod, ale i do rybníků s tržní produkcí tohoto druhu.

Klíčová slova: okoun říční; *Perca fluviatilis* L.; hormonální indukce ovulace; poloumělý výtěr; umělý výtěr; analog GnRH; kapří hypofýza; plodnost

INTRODUCTION

Flajšhans and Göndör (1989) described spawning of perch in a fibre-glass trough. Females ovulated without hormonal induction at temperatures ranging within 13.5–18 °C. Fertilized eggs were incubated in fine-mesh cages hanging in aerated tanks. Kucharzcyk *et al.* (1996) performed artificial spawning of perch after hormonal induction of ovulation either by means of human chorionic gonadotropin (hCG), or of carp pituitary with or without additives of hCG. Ovulation, collection of sperm, as well as fry hatching were successful after three times repeated injection of carp pituitary with hCG. Skrypczak *et al.* (1998) described hormonal induction ovulation using Ovopel (GnRHa containing pellets). Szcerbowski *et al.* (1998) studied the use of hCG and/or Ovopel on ovulation with success.

Kouřil *et al.* (1995, 1997) tested successfully a possibility to induce semiartificial spawning of perch using mammalian GnRH analogue ([D-Ala^6]GnRHProNHEt) at 15.4 °C. Compared to other fish species, a relatively high dose (125 $\mu g.kg^{-1}$) of GnRH analogue was necessary to induce spawning of females. Altogether 78.0 ± 19.1% females spawned. No spawning was achieved with control group treated with physiological saline. Using lower doses of GnRH analogue (5 and 25 $\mu g.kg^{-1}$), a significantly lesser amount of females spawned (11.0 ± 19.1 and 27.6 ± 9.2%, respectively). Males were not treated. Effect of environment temperature on the spawning success after hormonally induced semiartificial spawning of perch by means of the above-mentioned GnRH analogue at a dose of 100 $\mu g.kg^{-1}$ was tested by Kouřil and Linhart (1996, 1997a, b), also describing a spawning behaviour of perch. The highest success was gained at 16.3 ± 1,0 °C with 85% females spawned (3.7 d after injection). At 13.3 ± 0.6 °C, 14.7 ± 0.7 °C and 17.9 ± 0.9 °C, only 55–65% females spawned. Information on recent results of research on semiartificial- and artificial reproduction of perch, including results reviewed in this paper, was summarised by Kouřil *et al.* (1998). The way of perch reproduction, reproduction cycle control and information on gamete quality of perch, *Perca fluviatilis* and closely related North American yellow perch, *P. flavescens* were reported by Goubier (1995).

Gamete maturation in yellow perch is also affected by a change of photoperiod (Kayes, Calbert, 1979). A method of reproduction based on an ecological stimulation of natural spawning of this species in ponds and tanks was described by West and Leonard (1978 – cit. Goubier, 1995). Kayes, Calbert (1979) and Dabrowski *et al.* (1994) performed a hormonally-induced semiartificial spawning of this species in tanks and Kayes (1977) gained a successful hormonally-stimulated artificial propagation. The effect of application of steroid hormones on the course of the final phase of maturation (Goetz, Bergman, 1978; Goetz *et al.*, 1989), as well as their effect on *in vitro* oocyte maturation (Goetz, Theofan, 1978) were studied in yellow perch.

According to Švátora (1987), water temperature is an important factor affecting sex maturation and initiation of spawning in perch. Gillet *et al.* (1995) carried out long-term studies of the effect of water temperature and of female size on the date of propagation in this species. Sulistyo *et al.* (1998) described the course of annual sex cycle of perch from a lake locality. They stated changes in gonadosomatic, hepatosomatic and viscerosomatic indices, in oocyte size, gonad histology and levels of sex steroids in relation to water temperature and duration of the daylight. Spawning behaviour of perch was described by Dalimier *et al.* (1982), Hergenrader (1969) and Konovalova (1965).

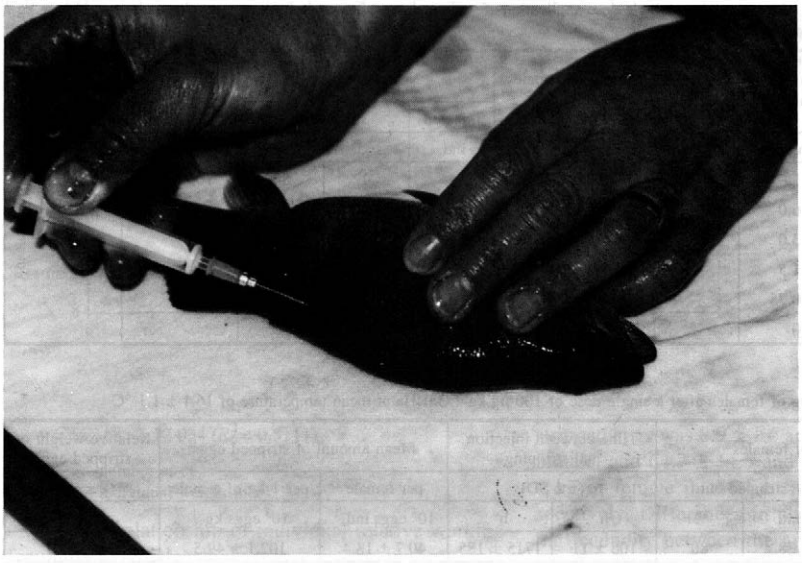
Fecundity of perch from natural localities was studied by several authors in several countries (Backiel, Zawisza, 1988; Craig, 1974; Evtyukhova-Rekstin, 1962; Petrowski, 1960; Stehlík, 1968; Švátora, 1987; Thorpe, 1977; Treasurer, 1981; Zacharova, 1955; Zeh *et al.*, 1989). The values of absolute fecundity of perch females range within 0.95–300 thousand eggs.

The goal of our experiments was to verify a dose relation of GnRH analogue and of carp pituitary, as well as a temperature effect on the induction of ovulation and semiartificial spawning and artificial propagation of perch as methods of controlled reproduction which might be feasible for production of stocking material for the needs of market fish culture as well as for stocking the open waters.

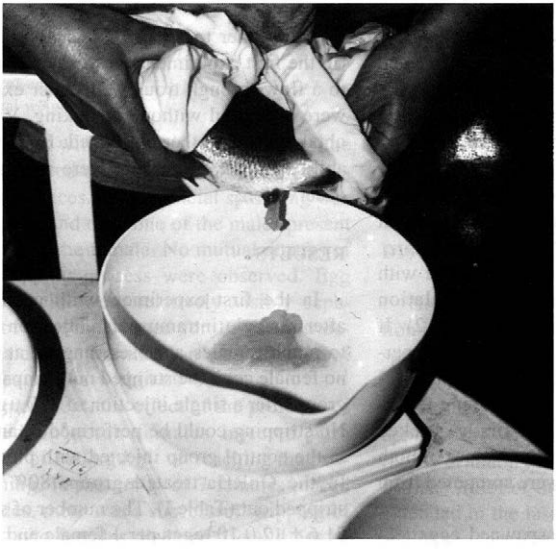
MATERIAL AND METHODS

The experiments were performed on Aquaculture Continentale fish farm in Missilac, Bretagne, France during the second half of March 1997. Adult perch were used, starting with 1+ age, with individual weights ranging within 20–945 g, of good health and originating from an extensive pond culture. Prior to the initiation of experiments, brood perch of both sexes were kept together in a cage on flow-through of recirculated water of 13–15 °C for approximately one week. Tanks with cages were placed in a plastic-foil tunnel with light regime 12hD/12hN, indirect outdoor illumination was complemented with indoor artificial illumination. Fish weighing, intramuscular application of preparations used, as well as artificial stripping were performed upon anaesthetised fish, using 2-phenoxyethanol of 0.5 $ml.l^{-1}$ concentration for about 2 min exposure. A superactive GnRH analogue ([D-Ala^6] GnRHProNHEt) and/or carp pituitary were used for hormonal induction of ovulation and artificial stripping. Pituitary used was acetone-dehydrated common carp pituitary of 3.74 mg mean weight which was homogenised prior to injection. Either of the preparations was administered in sterile physiological saline (0.9 $g.l^{-1}$ NaCl) as a single dose intramuscular injection into the dorsal part (Fig. 1). Males were neither anaesthetised nor injected.

Experimental fish were stocked in groups into firm plastic-mesh cages or plastic baskets placed in a con-



1. Intramuscular injication GnRHa of perch female



2. Artificial stripping of perch female.

crete trough or in fibre-glass tanks. Every cage/basket was stocked with one group of females. The troughs and fibre-glass tanks were provided with a mild flow--through of recirculated water adjusted in sprinkled biological filters with supplementation by spring water. Water temperature required was maintained in each tank by means of thermostat-controlled electric heaters.

Dissolved oxygen content in water was measured several times daily. It ranged within 7.0–8.5 $mg.l^{-1}$ in all experiments. The pH level was measured once per day. It ranged within 7.38–7.45 in all experiments. Water temperature was checked in approximately 4 h intervals.

Six groups of 5 females each were used for the first experiment. Four groups were treated with carp pituitary (doses of 0.75, 1.5, 3 and 6 $mg.kg^{-1}$). Females were identified according to their total length and body length recorded at injection time and after spawning. The first control group was treated with physiological saline and the second control group was administered a GnRH analogue (100 $\mu g.kg^{-1}$). In the second experiment, 35 females placed into cages in groups of 5 fish per cage in the absence of males were injected identically with a dose of 100 $\mu g.kg^{-1}$. When reaching the ovulation, artificial stripping was performed. Both experiments were carried out at mean temperature 16.1 ±

I. Results of artificial stripping of females after a single dose application of various doses of carp pituitary compared to a dose of 100 μg.kg^{-1} GnRHa at mean temperature of 16.1 ± 1.1 °C

Group	Preparation	Dose		Weight of females ($\bar{x}$ ± SD)	No. of females			Time interval between injection and stripping ($\bar{x}$ ± SD)	
					injected	stripped out			
		mg.kg^{-1}	μg.kg^{-1}	g	ind.	ind.	%	h	h°
1	aGnRH	–	100	493 ± 275	5	4	80	105 ± 10	1687 ± 166
2	CP	6.0	–	385 ± 178	5	0	0		
3	CP	3.0	–	486 ± 291	5	0	0		
4	CP	1.5	–	431 ± 145	5	0	0		
5	CP	0.75	–	447 ± 190	5	0	0		
6	CP	0	–	405 ± 122	5	0	0		

II. Results of artificial stripping of females after a single dose of 100 μg.kg^{-1} GnRHa at mean temperature of 16.1 ± 1.1 °C

Weight of females	No. of females			Time between injection and stripping		Mean amount of stripped eggs		Relative weight of stripped eggs
($\bar{x}$ ± SD)	injected	stripped out		($\bar{x}$ ± SD)		per female	per 1 kg of female	($\bar{x}$ ± SD)
g	ind.	ind.	%	h	h°	10^3 eggs.ind^{-1}	10^3 eggs.kg^{-1}	%
378 ± 177	35	28	80	106 ± 11	1715 ± 185	40.7 ± 18.4	102.1 ± 49.5	18.7 ± 3.7

1.1 °C. In the third experiment without replication, both groups containing 7 females each were injected with a dose of 100 μg.kg^{-1}. Males at a 1 : 1 ratio were added to the first group, while no males were added to the second group placed in a separated cage. The first group spawned semiartificially while an artificial stripping was performed with the second group after reaching the ovulation. The experiment was performed at mean temperature of 17.0 ± 1.0 °C.

Prior to artificial stripping, females registered with enlarged papilla were anaesthetised and ovulation checked by mild pressing the ventral part (Fig. 2). If ovulation was registered, artificial stripping was performed. The fecundity of semiartificially spawned females was checked volumetrically. The working fecundity of artificially stripped females was firstly checked gravimetrically and then volumetrically. Values of both the relative and absolute fecundity were computed from these data.

The value of relative weight of spawned eggs (%) was gained as a percentage ratio of the weight of spawned not-inseminated and not-swollen eggs to the female body weight registered by injection treatment. The stripped eggs were inseminated by milt obtained by direct stripping the males to the eggs.

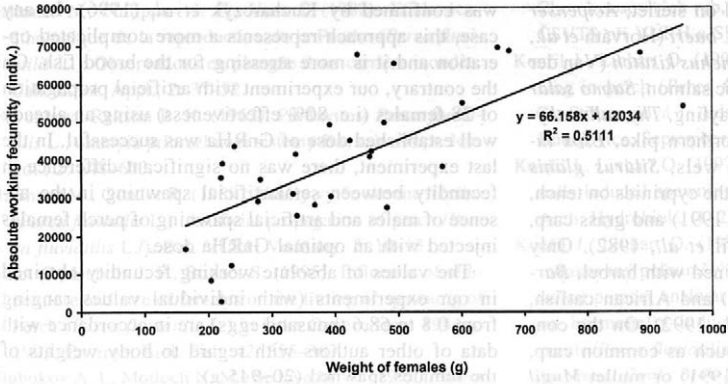
In all experiments, the duration of time interval from injection to spawning or to the artificial stripping in days (d) and in degree days (d°) was evaluated individually for each female. The spawned females were caught when they terminated spawning. Egg fertilisation rate was estimated. In order to test the differences statistically, ANOVA test (Tukey's multiple comparison test) and *t* test were used.

The spawned/stripped and fertilised eggs from both the semiartificial and artificial reproduction were stocked either to aerated aquaria with water exchange (in the first experiment) or to small aerated cages fixed to a flow-through trough (all other experiments). Eggs were incubated without desticking. Viable sac fry was obtained and further cultivated. Egg incubation, hatchability and larvae rearing were not the subject of this observation.

RESULTS

In the first experiment with artificial propagation after a single intramuscular injection of carp pituitary to perch females at doses ranging at 0.75–6 mg.kg^{-1}, no female could be stripped out compared to the control group after a single injection of 100 μg.kg^{-1} of GnRHa. No stripping could be performed in any of the females in the control group injected with physiological saline. In the GnRHa-treated group, 80% of females was stripped out (Table I). The number of stripped eggs was 51.6 ± 12.0 10^3 eggs per 1 female and 128.6 ± 53.7 10^3 eggs per 1 kg of female.

In the second experiment, artificial propagation of females was performed after ovulation hormonally induced by means of GnRHa administration at a single dose of 100 μg.kg^{-1}. Twenty-eight fish (i.e. 80% out of 35 females injected) ovulated and they were successfully artificially propagated (Table II). Ovulated eggs were inseminated by artificially stripped milt and further incubated. The number of stripped eggs was 40.7 ± 18.4 10^3 eggs per 1 female and 102.1 ± 49.5 10^3 eggs per 1 kg of female. Relative weight of stripped eggs was 18.7 ± 3.7%. A linear relationship of individual absolute working fecundity to female weight (Fig. 3) was registered in the sample of 28 ovulating females. This relationship can be expressed as follows:



3. Relationship of individual absolute working fecundity of females injected with GnRHa at a dose of 100 μg.kg^{-1} on individual female weight

$$y = 66.158x + 12\,034\ (R^2 = 0.5111)$$

where: y – number of eggs
x – individual weight in g

A linear relationship of individual relative working fecundity to female weight was registered in the sample of 28 ovulating females. This relationship can be expressed as follows:

$$y = -41.284x + 115\,801\ (R^2 = 0.0425)$$

where: y – number of eggs
x – individual weight in g

In the experiments with semiartificial spawning the spawning itself occurred during day and/or night without noticeable differences. Semiartificial spawning was based on pair mating and only one of the males present was always mating to the female. No mutual attacks of fish during the mating process were observed. Egg batches of single pairs were relatively visibly separated. Duration of spawning lasted for 10–60 min depending on female size. Fertilisation rate of eggs of individual females ranged within 60–95% .

In the third experiment, the effect of the presence of males was tested on perch females injected with a GnRHa at a dose of 100 μg.kg^{-1} on reaching the ovulation and spawning. In the group of 7 females placed together with males, there was a successful stripping of 4 females (57%), two females died and 1 could not be stripped out. In the group of females in the absence of males, 100% of fish ovulated and they were successfully artificially stripped (Table III). The number of stripped eggs was 8.9 ± 1.6 and 9.8 ± 10.2 10^3 eggs per female, and 64.7 ± 14.5 and 52.2 ± 26.5 10^3 eggs per 1 kg of female in the first and the second group, respectively. Differences in absolute and relative working fecundity between the groups of females were insignificant ($P > 0.05$). The estimated fertilisation rate of eggs of single females ranged within 60–95%.

It was registered within the experiments that a sufficient anaesthetic concentration was 0.5 ml.l^{-1} of 2-phenoxyethanol. Despite of using anaesthetics in females, as well as of minimum fish handling, there was a remarkably high mortality of fish, mainly of males. Females [illegible] to a lesser extent, usually after spawning due to surface fungal infection.

DISCUSSION

Hormonal induction of both ovulation and spermiation is being used in artificial reproduction of many fish species. Apart from methods using application of fish gonadotropins or gonadotropin-containing pituitary at various degrees of purification, approaches based on application of either human chorionic gonadotropin (hCG) or mainly of synthetically produced mammalian or fish GnRH or their superactive analogues have been extended in the last 10–15 years. A mammalian LHRH analogue (specified as des-Gly^{10}[D-Ala^6]-luteinization hormone releasing hormone, ethylamide or [D-Ala^6] GnRHProNHEt) belongs to the most frequently used preparations of this group. When applied solely, this

III. Comparison of the results of semiartificial spawning and artificial stripping of females after a single dose of 100 μg.kg^{-1} GnRHa at mean temperature 17.0 ± 1.0 °C

Reproduction	Weight of females	Females			Time between injection and spawning/stripping		Mean amount of spawned/stripped eggs		Relative weight of spawned/stripped eggs
	($\bar{x}$ ± SD)	No. of injected	No. of spawned/stripped		($\bar{x}$ ± SD)		per ovulating female	per 1 kg of ovulating female	
	g	ind.	ind.	%	h	h°	10^3 eggs.ind^{-1}	10^3 eggs.kg^{-1}	%
Semiartificial	137 ± 56	7	4	57	93 ± 6	1585 ± 99	8.9 ± 1.6	64.7 ± 14.5	–
Artificial	161 ± 139	7	7	100	82 ± 10	1395 ± 165	9.8 ± 10.2	52.2 ± 26.5	16.6

hormone was successfully tested on sterlet, *Acipenser ruthenus*, on Siberian sturgeon, *A. baeri* (Horváth *et al.*, 1986), on coho salmon, *Oncorhynchus kisutch* (Van der Kraag *et al.*, 1983) and on Atlantic salmon, *Salmo salar* (Crim *et al.*, 1983), further on grayling, *Thymallus thymallus* (Kouřil, Barth, 1989), northern pike, *Esox lucius* (Pecha *et al.*, 1992) and wels, *Silurus glanis* (Kouřil *et al.*, 1987, 1996), from the cyprinids on tench, *Tinca tinca* (Kouřil *et al.*, 1986, 1991) and grass carp, *Ctenopharyngodon idella* (Kouřil *et al.*, 1982). Only partly positive results were obtained with barbel, *Barbus barbus* (Kouřil *et al.*, 1988) and African catfish, *Clarias gariepinus* (Kouřil *et al.*, 1992). On the contrary, with some other species such as common carp, *Cyprinus carpio* (Kouřil *et al.*, 1991) or mullet *Mugil so-iuy* (Glubokov *et al.*, 1994), it was found necessary to perform injection application of a dopamine inhibitor along with the GnRH in order to reach ovulation.

Kouřil *et al.* (1995, 1997) verified a possibility of hormonally induced semiartificial spawning of perch by means of a single application of GnRHa. Kucharczyk *et al.* (1996), Skrypczak *et al.* (1998) and Szczerbowski *et al.* (1998) reached ovulation in perch and performed artificial propagation of perch by means of a replicated injection application of either hCG or carp pituitary with or without hCG or GnRHa. A successful hormonally induced ovulation of North American perch *P. flavescens*, long-term kept in aquaria under constant temperature and photoperiod, was described by Dabrowski *et al.* (1994). Males were intraperitoneally injected with a single dose of GnRHa ([D-Ala^6] GnRHProNHEt). One group of females was administered sole GnRHa at two doses within 48 hours, the other was injected with GnRHa and contemporarily with pimozide, a dopaminergic inhibitor.

In our case there was registered a relatively high optimal single dose of GnRHa (125 $\mu g.kg^{-1}$) in order to reach ovulation. There was a highly significant difference in the number of stripped females between this dose and those of 5 and 25 $\mu g.kg^{-1}$ although the latter are sufficient to induce ovulation in many fish species such as e.g. tench, grayling, wels, northern pike (Kouřil *et al.*, 1982, 1986, 1989; Kouřil, Barth, 1989; Pecha *et al.*, 1992) when applied at a single dose.

Earlier maturation of perch and its spawning in the second half of March is associated with shorter and milder winter in Bretagne compared to the climate of the Czech Republic. Within the temperature range from the point of view of the number of spawned females, the optimum temperature was found as 16.3 °C while a lesser amount of injected females spawned/were stripped out at lower (13.3 and 14.7 °C) and/or higher (17.9 °C) temperatures.

Although rather surprisingly negative, there was an important fact that ovulation was not induced in females treated with carp pituitary at single doses ranging within 0.75–6 $mg.kg^{-1}$ (on the contrary to a control group treated with GnRHa). A successful application of carp pituitary along with hCG at 2 or 3 partial doses was confirmed by Kucharczyk *et al.* (1996). In any case, this approach represents a more complicated operation and it is more stressing for the brood fish. On the contrary, our experiment with artificial propagation of 28 females (i.e. 80% effectiveness) using an already well established dose of GnRHa was successful. In the last experiment, there was no significant difference in fecundity between semiartificial spawning in the absence of males and artificial spawning of perch females injected with an optimal GnRHa dose.

The values of absolute working fecundity obtained in our experiments (with individual values ranging from 0.8 to 68.6 thousand eggs) are in accordance with data of other authors with regard to body weights of the females spawned (20–945 g).

A higher sensitivity of perch to the anaesthetic 2-phenoxylethanole was observed. A sufficient dose inducing anaesthesis was 0.5 $ml.l^{-1}$ contrary to the recommended one of 1.0 $ml.l^{-1}$. High mortality was found with the brood perch and it was mainly a high mortality of males although the males were less exposed to handling than the females. There may be a different sensitivity of males and females to handling stress.

Management of the techniques of semiartificial and artificial reproduction makes prerequisites for production of stock fish for stocking the open waters as well as ponds for marketable production of this fish species.

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EVALUATION OF CALCIUM, PHOSPHORUS, ENERGY AND PROTEIN INTAKE IN FATTENED BULLS*

HODNOCENÍ PŘÍJMU VÁPNÍKU, FOSFORU, ENERGIE A PROTEINU U VYKRMOVANÝCH BÝKŮ

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ABSTRACT: Our aim was to evaluate the consumption of Ca, P, energy and protein in fattened bulls. The database contained classic balance trials conducted in the Research Institute of Animal Nutrition, Ltd., in Pohořelice. Trials contained 126 individual balance determinations of digestibility and were carried out on Czech Pied (>75%) bulls. The rations consisted of maize silage and mixtures of our own production (grain meals, alfalfa meal, rape cake, molasses, urea and mineral supplements). Gross energy, metabolisable energy, net energy and PDI content of rations and their daily intake were calculated. Deg and dsi values were taken over from literature. Average weights of the bulls in the individual periods varied between 251 and 467 kg, with daily gain ranging from 0.99 to 1.42 kg. The values apparent digestibility of calcium in the individual periods varied too, ranging from 1.1 to 45.9%. Higher apparent digestibility values were determined with phosphorus (21.8–55.1%). The average q was 51.4%, the average k_{mf} was 0.56. With the average weight of 364 kg and the average gain of 1.12 kg/day, the average daily intake of Ca was 59.0 g, P 32.1 g, dry matter 7.58 kg, crude protein 1.08 kg, crude fibre 1.74 kg, NEV 39.3 MJ, PDIN 671 g, PDIE 583 g.

Keywords: fattened bulls; calcium; phosphorus; energy; protein

ABSTRAKT: Cílem práce bylo hodnocení příjmu Ca, P, energie a proteinu u vykrmovaných býků. Databáze obsahovala klasické bilanční pokusy provedené ve Výzkumném ústavu výživy zvířat, s. r. o., Pohořelice. Pokusy obsahovaly 126 individuálních bilančních stanovení stravitelnosti, která byla provedena na bycích českého strakatého plemene (>75 %). Krmné dávky byly založeny na kukuřičné siláži a směsi vlastní výroby (obilní šroty, vojtěšková moučka, řepkové pokrutiny, melasa, močovina a minerální doplňky). Byl vypočítán obsah bruttoenergie, metabolizovatelné energie, nettoenergie a PDI v dávkách a jejich denní příjem. Hodnoty deg a dsi byly převzaty z literatury. Průměrná hmotnost býků v jednotlivých periodách kolísala mezi 251 a 467 kg s denním přírůstkem v rozsahu 0,99 až 1,42 kg. Hodnoty zdánlivé stravitelnosti vápníku v jednotlivých periodách kolísaly od 1,1 do 45,9 %. U fosforu byly nalezeny vyšší hodnoty zdánlivé stravitelnosti (21,8–55,1 %). Průměrné q bylo 51,4 %, průměrné k_{mf} 0,56. Při průměrné hmotnosti 364 kg, průměrném přírůstku 1,12 kg/den byl zjištěný průměrný denní příjem Ca 59,0 g, P 32,1 g, sušiny 7,58 kg, dusíkatých látek 1,08 kg, vlákniny 1,74 kg, NEV 39,3 MJ, PDIN 671 g a PDIE 583 g.

Klíčová slova: vykrmovaní býci; vápník; fosfor; energie; protein

INTRODUCTION

Consumption of Ca and P minerals in fattened bulls is one of the main factors affecting the performance of fattened bulls. The given daily requirement of Ca in fattened bulls ranges between 34 and 74 g (depending on their weight and gain) according to AFRC (1991), Vencl (1991) and Šimek *et al.* (1995). The same sources mention P requirement per day between 22 and 47 g. Apparent digestibility coefficients vary from 16 to 55% with Ca and from 10 to 60% with P (Dedek *et al.*, 1976; Field *et al.*, 1985; Hartmans, 1986).

In the last 20 years new energy and protein systems have been developed and applied in ruminant nutrition. The new systems of energy evaluation in fattened bulls (e.g. Van Es, 1978; Vermorel, 1987) are based on the calculation of net energy (NEV) of feeds from metabolisable energy (ME). The utilization of ME depends on the ME concentration in the ration and on the level of feeding APL (Van der Honing, Alderman, 1988). The utilization of protein in ruminats depends on the degradation of crude protein (CP) in the rumen, on the microbial protein synthesis and on the digestibility of nondegraded and microbial protein in the intestine (INRA, 1988; Vencl, 1991).

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Our aim was to evaluate the consumption of Ca, P, energy and protein. The database contained four classic balance trials using bulls stabled in the Research Institute of Animal Nutrition, Ltd., Pohořelice. Some aspects of the database were discussed in Třináctý *et al.* (1993, 1994).

MATERIALS AND METHODS

The four trials, each of them involving two periods (Tab. I), contained 126 individual classic balances, carried out in Czech Pied (75%) bulls. The balance trials were carried out according to Schiemann (1981). The rations consisted of maize silage and mixtures (grain meals, alfalfa meal, rape cake, molasses, urea and mineral supplements), the rations were calculated according to ČSN 46 7070.

Samples of feeds, refusals and faeces were mineralized and solved in HCl, then calcium was determined by atomic absorption spectrophotometry and phosphorus by the photometric method.

GE of rations was calculated according to Schiemann *et al.* (1971), the same equations had also been used by Van Es (1978):

$$GE = 0.0242 \times CP + 0.0366 \times CFat + 0.0209 \times CF + 0.017 \times NFE$$

ME was calculated according to Schiemann *et al.* (1971):

$$ME = 0.0159 \times DCP + 0.0377 \times DCFat + 0.0138 \times DCF + 0.0147 \times DNFE - 0.00063 \times sugars$$

As the content of sugars in our rations did not reach 8%, this correction was not applied. NEV was calculated according to Van Es (1978):

$$NEV = ME \times k_{mf} \text{ (with APL 1.5)}$$

PDI calculation was made according to Vérité *et al.* (1987):

$$PDIA = 1.11 \times CP \times (1 - deg) \times dsi$$
$$PDIMN = 0.64 \times CP \times (deg - 0.1)$$
$$PDIME = 0.093 \times FOM$$
$$FOM = DOM - CFat - UDP - FP$$
$$PDIN = PDIA + PDIMN$$
$$PDIE = PDIA + PDIME$$

Deg and dsi values were taken from the publication of Vencl (1991). In our case the value of FP 100 g/kg was used.

Abbreviations

APL – animal production level, expressed as a multiple of maintenance
CF – crude fibre
CFat – crude fat
CP – crude protein
DCF – digestible crude fibre
DCFat – digestible crude fat
DCP – digestible crude protein
deg – degradability of CP in rumen
DM – dry matter
DNFE – digestible nitrogen free extract
DOM – digestible organic matter
dsi – true digestibility of nondegradable protein in small intestine
FOM – fermentable organic matter
FP – fermentation products in silage
GE – gross energy
k_{mf} – efficiency of utilization of ME for maintenance and gain
ME – metabolizable energy
NEV – net energy for growth and fattening
NFE – nitrogen free extract
OM – organic matter
PDIA – undegraded protein of feed true digestible in intestine
PDIE – true digestible protein in intestine when energy in rumen is limited
PDIME – proteosynthesis of microbial protein from energy achieved in rumen
PDIMN – proteosynthesis of microbial protein from nitrogen achieved in rumen
PDIN – true digestible protein in intestine when nitrogen in rumen is limited
q – ratio of ME to GE, metabolizability of gross energy (%)
UDP – undegradable protein of feed

Calculation and statistics: The analysis of variance and the regression analyses of the results were made with Statgraphic, ver. 5.1 software. The regression analyses were based on the following formula: $y = a + bx_1 + cx_2$, where a is constant, b and c are variables, x_1 is weight (kg), x_2 is gain (kg).

RESULTS AND DISCUSSION

The average weight, the daily gain and the consumption of nutrients in the eight periods of the four trials are mentioned in Tab. I. The average weight of the bulls ranged from 251 to 467 kg in the individual periods with the daily gain within the range of 0.99 to 1.42 kg. The daily consumption of calcium was significantly higher ($P < 0.05$) in the group with the higher average weight (the second periods) only in the first trial (57.2 vs. 66.8 g/day). Significantly higher ($P < 0.05$) consumption of phosphorus was found in trials 3 and 4. Except for the first trial there were large differences ($P < 0.05$) in consumption of CP between the periods in individual trials, the average consumption being 1.08 kg/day. The average daily intakes of DM, CF and OM were different ($P < 0.05$) comparing the first and the second period of each trial.

The apparent digestibility coefficients of Ca and P generally show lower values in the second periods. Two significant ($P < 0.05$) differences appeared with each of the two minerals (Tab. II). The values of the apparent digestibility of calcium in the individual periods range from 1.1 to 45.9%. Except for the value of 1.1% they are in agreement with the values mentioned by Dedek *et al.* (1976), Hartmans (1986) and Field *et al.* (1985). Higher apparent digestibility values (21.8–55.1%) were found with phosphorus. The above mentioned authors mention values 10–60%, which means no difference in comparison to our values. Relatively

I. Average weight, gain and daily consumption of nutrients in fattened bulls in individual periods, analysis of variance

Trial	Period	Number	Average weight	Daily weight gain	Daily consumption of nutrients					
					Ca	P	DM	CP	CF	OM
			(kg)	(kg)	(g)	(g)	(kg)	(kg)	(kg)	(kg)
1	1	16	424[a]	0.99	57.2[a]	30.6	8.08[a]	1.11	1.83[a]	7.32[a]
	2	16	482[b]	0.99	66.8[b]	31.1	8.98[b]	1.15	2.19[b]	8.24[b]
2	1	15	270[a]	1.03	56.4	33.5	6.09[a]	1.03[a]	1.17[a]	5.51[a]
	2	15	424[b]	1.04	57.9	32.2	7.39[b]	1.16[b]	1.41[b]	6.82[b]
3	1	16	279[a]	1.42[a]	66.0[a]	35.8[a]	7.57[a]	0.96[a]	1.78[a]	6.80[a]
	2	16	467[b]	1.26[b]	57.5[b]	43.9[b]	9.68[b]	1.21[b]	2.26[b]	8.80[b]
4	1	16	251[a]	1.09	51.8	19.6[a]	5.80[a]	0.94[a]	1.41[a]	5.26[a]
	2	16	314[b]	1.13	58.3	30.2[b]	6.91[b]	1.10[b]	1.84[b]	6.21[b]
Mean	–	–	364	1.12	59.0	32.1	7.58	1.08	1.74	6.89
S.e.	–	–	1.9	0.012	0.59	0.29	0.047	0.008	0.012	0.043

Means in the same trial with different superscripts differ ($P < 0.05$)

II. Apparent digestibility coefficients of nutrients and Ca/P ratio, analysis of variance

Trial	Period	Apparent digestibility coefficients and Ca/P ratio						
		Ca	P	DM	CP	CF	OM	Ca/P
		(%)	(%)	(%)	(%)	(%)	(%)	Ca (g)/P (g)
1	1	23.3	43.8	62.3	62.3	55.3[a]	63.0[a]	1.87[a]
	2	15.6	41.1	63.8	65.9	64.3[b]	67.0[b]	2.15[b]
2	1	45.9	44.1[a]	64.9	67.4	61.1	65.8	1.69
	2	38.3	21.8[b]	66.7	67.1	62.2	68.8	1.81
3	1	27.2[a]	55.1[a]	66.9	61.1	65.0	67.6	1.84[a]
	2	1.1[b]	35.6[b]	65.4	59.8	62.6	66.8	1.30[b]
4	1	32.1[a]	44.9	64.0	72.8	63.3	65.7	2.66[a]
	2	16.7[b]	51.3	63.3	70.2	64.5	64.7	1.94[b]
Mean	–	24.7	42.4	64.6	65.8	62.3	66.2	1.91
S.e.	–	0.85	0.99	0.31	0.33	0.40	0.29	0.012

Means in the same trial with different superscripts differ ($P < 0.05$)

small differences in values were found with DM and CP apparent digestibility coefficients, a significant difference ($P < 0.05$) only appearing in a single case with CF and OM. In two of the second periods significantly lower values ($P < 0.05$) were obtained in the Ca/P ratio. Labuda *et al.* (1973) mentioned that the optimum utilization of Ca and P occurred when the Ca/P ratio was 1–2 : 1. When one of these minerals was in abundance, their utilization decreased. The blood serum of cows revealed the ratio of 1.64–1.8:1 (Limpoka *et al.*, 1983). Lapshin *et al.* (1995) recommended the ratio 1.6–1.7 : 1 for heifers. According to INRA (1988) the recommended ratio for fattened bulls and 150 kg of weight was 1.87 : 1, while for 575 kg it decreased to 1.56 : 1. In our database the obtained average value of Ca/P is 1.91, which means a slightly higher value. We suppose that the reason was a higher level of Ca consumption.

The values of q and k_{mf}, mentioned in Tab. III, do not vary too much, with q two significant differences ($P < 0.05$) were obtained, with k_{mf} there are no significant differences ($P > 0.05$). The average q is 51.4%, which is lower than what Van Es (1978) mentions as the average value for bulls in the Netherlands (60 %). AFRC (1991) related the daily requirement of Ca and P to q metabolizability. While with Ca 50–70% was considered suitable, with P it was 60–70%. Our average value of 51.4% is also relatively low in this context. The consumption of NEV units was significantly higher ($P < 0.05$) in three of the second periods (trials 1, 2 and 3) with the total average of 39.3 MJ/day. On the contrary, the consumption of calculated PDIA only significantly increased ($P < 0.05$) in a single second period, while in the case of the PDIMN and PDIN values a significant increase appeared in three trials ($P < 0.05$), in PDIME and PDIE it was the case in all four trials. Except for trial 3, period 2, in all periods the intake of PDIMN was higher than that of PDIME. The average intakes of PDIMN and PDIME (421 vs. 333 g) proved that in our type of rations had the growing rumen microorganisms lack of energy. This fact was the reason why the average PDIE intake was lower than that of PDIN (583 vs. 671 g/day).

III. Values q and k_{mf}, consumption of NEV and PDI values (in a day)

Trial	Period	Energy and protein values							
		q (%)	k_{mf}	NEV (MJ/day)	PDIA (g)	PDIMN (g)	PDIME (g)	PDIN (g)	PDIE (g)
1	1	49.0[a]	0.55	38.6[a]	234	440	328[a]	673	561[a]
	2	52.4[b]	0.57	48.6[b]	238	459	404[b]	698	642[b]
2	1	51.0[a]	0.56	31.0[a]	258	404[a]	256[a]	662[a]	514[a]
	2	54.3[b]	0.58	42.2[b]	289	458[b]	338[b]	748[b]	627[b]
3	1	53.1	0.58	40.7[a]	265[a]	334[a]	331[a]	599[a]	596[a]
	2	52.0	0.57	50.5[b]	331[b]	423[b]	428[b]	755[b]	759[b]
4	1	50.2	0.56	29.1	178	391[a]	266[a]	569[a]	444[a]
	2	49.0	0.55	33.1	208	458[b]	311[b]	665[b]	518[b]
Mean	–	51.4	0.56	39.3	250	421	333	671	583
S.e.	–	0.23	0.001	0.39	3.3	2.8	3.0	5.4	5.2

Means in the same trial with different superscripts differ ($P < 0.05$)

IV. Regression analysis of mineral and organic nutrients consumption its dependence on weight and daily gain of fattened bulls

Nutrient consumption		Constant a	Significance ($P < 0.05$)	Variable b	Significance ($P < 0.05$)	Variable c	Significance ($P < 0.05$)	R
Ca	(g/day)	24.77	+	0.0319	+	20.20	+	0.58
P	(g/day)	–3.80	–	0.0414	+	18.61	+	0.69
DM	(kg/day)	0.223	–	0.0131	+	2.32	+	0.90
CP	(kg/day)	0.585	+	0.00103	+	0.109	+	0.75
DCP	(kg/day)	0.570	+	0.000426	+	–11.87	–	0.41
CF	(kg/day)	0.00663	–	0.00292	+	0.601	+	0.73
OM	(kg/day)	0.180	–	0.0123	+	1.99	+	0.91

Means in the same trial with different superscripts differ ($P < 0.05$)

The regression analysis of Ca, P and organic nutrient consumption (Tab. IV) showed that the a constant was only significant ($P < 0.05$) with Ca, CP and DCP. Coefficients b and c were generally significant ($P < 0.05$) with all nutrients, except for the c value in DCP (–11.87). What follows from these results is that in our database the consumption of all nutrients (except DCP) rose ($P < 0.05$) through the periods with increase in weight and gain. The R correlation coefficient showed the highest value with OM (0.91) and DM (0.90). R was also relatively high with Ca and P (0.58 and 0.69).

Tab. V shows the results of the regression analysis of apparent digestibility coefficients and the Ca/P consumption ratio. We have observed that almost all of the nutrients (except for OM) had negative values of the b variable (for weight). This means that the digestibility of all of the nutrients (except for OM) decreased when the weight of the animals and their consumption of feeds increased. But at the same time the b variable was only found significant ($P < 0.05$) for Ca, P, CP and the Ca/P ratio. As for the c variable (for gain) this was only true with P and CP, while all of the a constants were significant ($P < 0.05$). The value of R was low with these regressions, ranging between 0.12 and 0.46, and so we have to take the above conclusions as only tentative. On the contrary, a relatively high R value was obtained in the regression analysis of energy and protein consumption (Tab. VI). Even the a constant was only significant ($P < 0.05$) in two cases (PDIMN and PDIN), while the b and c variables were significant ($P < 0.05$) for all of the calculated units, which is in agreement with the results shown in Tab. IV.

For the purpose of comparison the weight of 350 kg and the daily gain of 1.2 kg were chosen and the consumption of the individual nutrients was calculated for these values using the equations obtained from the regression analysis (see Tab. IV and VI). The results were compared with the data found in literature (Tab. VII). The calculated value of 60 g/day Ca consumption is substantially higher than that mentioned in AFRC (1991), ČSN 46 7070 (1982) and so on. This high consumption value can account for the low values of Ca digestibility (with the average value of 24.7%), because it is known that when the consumption of Ca increases the digestibility of it decreases (Šimek *et al.* 1995). While the calculated consumption of P (33 g/day) is comparable with values mentioned by ČSN 46 7070 (1982) and INRA (1988) and does not much differ from values of AFRC (1991), Landis (1984) and Sommer (1994), the value of NRC (1994) is substantially lower (18 g/day).

The intake of DM is comparable with all available sources of data, only the value mentioned in NRC

V. Regression analysis of apparent digestibility coefficients and consumption of Ca/P ratio, its dependence on weight and gain of fattened bulls

Coefficients of digestibility and Ca/P ratio		Constant a	Significance ($P < 0.05$)	Variable b	Significance ($P < 0.05$)	Variable c	Significance ($P < 0.05$)	R
Ca	(%)	67.60	+	–0.0799	+	–12.31	–	0.46
P	(%)	49.12	+	–0.0549	+	11.84	+	0.41
DM	(%)	61.45	+	–0.000194	–	2.91	–	0.15
CP	(%)	83.14	+	–0.0233	+	–7.93	+	0.45
CF	(%)	60.52	+	–0.00569	–	3.43	–	0.17
OM	(%)	62.98	+	0.00342	–	1.72	–	0.12
Ca/P ratio		2.98	+	–0.00164	+	–0.425	–	0.41

Means in the same trial with different superscripts differ ($P < 0.05$).

VI. Regression analysis of energy and protein system values, their dependence on weight and gain of fattened bulls

Energy and protein parameters		Constant a	Significance ($P < 0.05$)	Variable b	Significance ($P < 0.05$)	Variable c	Significance ($P < 0.05$)	R
NEV	(MJ/day)	–3.61	–	0.0763	+	13.48	+	0.84
PDIA	(g/day)	–24.94	–	0.340	+	134.68	+	0.67
PDIMN	(g/day)	359.13	+	0.308	+	–44.81	+	0.60
PDIME	(g/day)	3.82	–	0.581	+	105.62	+	0.84
PDIN	(g/day)	334.20	+	0.648	+	89.87	+	0.70
PDIE	(g/day)	–21.12	–	0.921	+	240.30	+	0.85

Means in the same trial with different superscripts differ ($P < 0.05$).

VII. Calculated values of Ca, P, DM, NEV, PDI, CP, and CF, comparison with literature data. All values related to 350 kg weight, 1.2 kg gain per day

Nutrient	Units	Calculated data (our values[8])	Data in literature
Ca	g/day	60	33[1]; 43[3]; 45[4]; 46[5]; 30[6]; 38[7]; 34[10]
P	g/day	33	29[1]; 33[3]; 32[4]; 29[5]; 18[6]; 28[7]; 24[10]
DM	kg/day	7.59	7.9[3]; 6.38[5]; 8.4[6]; 7.66[7,10,11]
NEV	MJ/day	39.3	45.0[4]; 48.8[7]; 49.6[9]; 45.8[10]; 50.5[11]
PDI	g/day	590	585[4]; 586[7]; 620[10]; 611[11]
CP	g/day	1076	902[2]; 1080[3]; 857[5]; 923[7]; 891[10]; 982[11]
CF	kg/day	1.75	1.25[3]; 1.37[7]; 1.23[11]

[1]AFRC (1991), [2]Ausschuss für Bedarfsnormen (1986), [3]ČSN 46 7070 (1982), [4]INRA (1988), [5]Landis (1984), [6]NRC (1994), [7]Sommer (1994), [8]values calculated from regression analysis (Tables IV and VI), [9]Van Es (1978), [10]Vencl (1991), [11]Zeman (1999)

(1994) is slightly higher. On the contrary, larger differences were found in the NEV intake. Our calculated value of 39.3 MJ/day differs from data mentioned in literature, which are 45.0 MJ/day (INRA, 1988) and higher. We can suppose that the real q metabolizability and/or the efficiency of utilization of ME k_{mf} were higher than what we calculated according to formulas of Schiemann *et al.* (1971) and Van Es (1978).

As already discussed (Tab. III), the low values of PDIE (or PDIME, respectively) in our database did not make higher gain possible and so, following INRA (1988), our PDIE values are presented as PDI values in the results. Our value of 590 g of PDI/day calculated in Tab. VII for the weight of 350 kg and the gain of 1.2 kg is in good agreement with the requirements mentioned in literature: 585 g/day (INRA, 1988) and 586 g/day (Sommer, 1994). A slightly higher value is mentioned by Vencl (1991), though. The calculated value of 1076 g/day for CP is relatively high with respect to literature data and is only comparable with ČSN 46 7070 (1080 g/day). Other sources recommend a lower amount of CP, for example Ausschuss für Bedarfsnormen's (1986) 902 g/day and Landis's (1984) 857 g/day.

The calculated amount of CF consumed in our trials was 1.75 kg/day, which is substantially more than the 1.25 kg/day mentioned by ČSN 46 7070 (1982) or the 1.37 kg/day to be found in Sommer (1994). This high value of CF might be one of the reasons why the gain achieved in our trials was not higher. This item is not usually mentioned in available data sources.

We can conclude that the values of calcium apparent digestibility in individual periods ranged between 1.1

and 45.9%. With phosphorus the apparent digestibility values were 21.8–55.1%. With the average weight of 364 kg and the average gain of 1.12 kg/day, the average daily consumption of P was 32.1 g, of dry matter 7.58 kg, of NEV 39.3 MJ, of PDIN 671 g and of PDIE 583 g. Our database was characterized by low metabolizability (average q was 51.4%), high average daily consumption of Ca (59.0 g), high daily consumption of crude fiber and crude protein (1.08 and 1.74 kg).

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PREDIKCE STŘEVNÍ STRAVITELNOSTI PROTEINU NEDEGRADOVANÉHO V BACHORU KOMBINOVANOU ENZYMATICKOU METODOU*

PREDICTION OF INTESTINAL DIGESTIBILITY OF PROTEIN UNDEGRADABLE IN RUMEN BY A COMBINED ENZYMATIC METHOD

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ABSTRACT: The experimentation objective was to test a combined enzymatic method of determining intestinal digestibility of protein undegradable in rumen. The combined enzymatic method has two stages. The first stage, simulating degradation in rumen, is based on modification of our own methods (Tománková, Kopečný, 1995), the second stage, determining intestinal digestibility, is based on modification of the method developed by Antoniewicz *et al.* (1992). Modification of the classical enzymatic method consisted in replacement of the first stage, i.e. 16-hour incubation of feed in cows rumen, by incubation of feed in so called resultant incubation medium. Incubation times 0, 1, 16 and 24 hours were tested. The method was tested on a set of feeds (n = 13) comprising bulk feeds, grains and feeds from animal sources (Table I). In order to improve protease access to proteins amylase was used for feed incubation in incubation medium with bromelain in starchy feed (corn, barley and wheat grain). Two methods of determination were checked: with feed pre-drying and without it. The relations between values determined by the two test methods and values determined by a mobile bag method in the given set of feeds were expressed by linear regression equations (Table II) with calculated values of correlation coefficients (r) and standard deviations (% RSD). Figs. 1 and 2 show the relationship between digestibility of undegradable protein in the mobile bag method and digestibility in combined enzymatic methods. The values of both correlation coefficients show a high level of relationship closeness. The value r (r = 0.912) is a little higher in the method with pre-drying than in the method without it (r = 0.893); on the contrary, %RSD = 5.48 is lower than in the other case (%RSD = 6.02). Therefore the method with feed pre-drying, and higher r and lower %RSD was preferred. Experimental data show that this combined enzymatic method is appropriate from feed pre-incubation in incubation medium with bromelain in time intervals of 1 and 16 hours, and is not apt for intervals of 0 and 24 hours.

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

ABSTRAKT: Cílem práce bylo ověřit experimentální metodu stanovení střevní stravitelnosti proteinu nedegradovaného v bachoru přežvýkavců v čistě laboratorních podmínkách metodami využívajícími proteolytické enzymy, bez použití kanylovaného zvířete. Skupina krmiv (n = 13) zahrnovala krmiva objemná, jadrná a živočišného původu. Klasická enzymatická metoda střevní stravitelnosti byla modifikována tak, že v první fázi byla 16hodinová inkubace krmiva v bachoru zvířete nahrazena inkubací krmiva v proteolytickém enzymu bromelainu v časových intervalech 0, 1, 16 a 24 hodin, na které pak navazovala druhá fáze stanovení, 24hodinová inkubace nezdegradovaného zbytku krmiva enzymem pankreatinem. U škrobnatého krmiva (zrno kukuřice, pšenice a ječmene) byla inkubována s bromelainem zároveň amyláza, a to pro zlepšení přístupu proteázy k proteinům. Na základě porovnání s hodnotami stravitelnosti získanými metodou mobile bag na kanylovaných zvířatech byly vypočteny regresní rovnice. Ze získaných výsledků je patrné, že tato kombinovaná enzymatická metoda je vhodná při preinkubaci krmiva v inkubačním roztoku s bromelainem v časových intervalech 1 a 16 hodin a nehodí se pro intervaly 0 a 24 hodin.

Klíčová slova: přežvýkavci; degradace proteinu; střevní stravitelnost; *in vitro* metody; proteolytické enzymy

ÚVOD

Současná kritéria hodnocení dusíkatých látek v krmivu podle systému PDI jsou založena na využitelnosti zdrojů dusíku v tenkém střevě přežvýkavců. Systém PDI zahrnuje všechny nové poznatky o metabolických proměnách dusíkatých látek a sleduje různé aspekty hodnocení proteinu krmiva, zejména bachorovou mi-

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krobiální degradabilitu a střevní stravitelnost proteinu, který nebyl zdegradován v bachoru a prochází do tenkého střeva. Základní a referenční metodou pro stanovení rozsahu degradace proteinu krmiva v bachoru je metoda stanovení *in vivo*, která je pracná a náročná. Proto se používají metody stanovení proteinové rozpustnosti (Madsen, Hvelplund, 1985; Susmel *et al.*, 1993), *in sacco* metody s korekcí na výtokovou rychlost krmiva (Orskov, McDonald, 1979), které vyžadují zvířata s bachorovou kanylou, a metody *in vitro*. V podmínkách *in vitro* bylo ověřováno použití různých proteolytických enzymů jako bromelain (Poos-Floyd *et al.*, 1985; Tománková, Kopečný, 1995), *Streptomyces griseus* – Pronase E (Aufrére *et al.*, 1991) a jiné. Porovnávání různých metod *in vitro* s metodami *in sacco* dává možnost vzniku predikčních rovnic, výpočtů korelačních koeficientů a parametrů těchto rovnic (Aufrére *et al.*, 1991; Michalet-Doreau *et al.*, 1992). Hodnoty střevní stravitelnosti dusíkatých látek nedegradovaných v bachoru se stanovují metodou mobile bag na zvířatech s bachorovou a duodenální kanylou (Hvelplund *et al.*, 1992; Frydrych, 1992; Homolka *et al.*, 1996). Tyto metody jsou časově a technicky náročné. Proto se neustále vyvíjejí metody *in vitro* s použitím proteolytických enzymů (Antoniewicz *et al.*, 1992; Van Straalen *et al.*, 1993; Calsamiglia, Stern, 1995; Tománková, Homolka, 1997). I na enzymatické stanovení se používá krmivo preinkubované v bachoru kanylovaného zvířete po dobu 12 nebo 16 hodin, vlastní enzymatický postup odstraňuje inkubaci vzorku krmiva v dolní části trávicího traktu zvířete vybaveného duodenální kanylou.

Naším úkolem bylo ověřit kombinovanou enzymatickou metodu, ve které bychom nevyužívali kanylovaná zvířata a plně nahradili podmínky inkubace krmiva v bachoru inkubací v roztoku enzymu s pufrem, který by co nejvíc simuloval bachorovou inkubaci. Současně jsme provedli srovnání s metodou mobile bag.

MATERIÁL A METODA

Testovaná krmiva a experimentální postup

V experimentu jsme testovali soubor krmiv (n = 13), který zahrnoval krmiva objemná, jadrná i krmiva živočišného původu. V tab. I je uveden přehled ověřovaných krmiv, obsah sušiny a dusíku v krmivech a jejich reziduích, degradovatelnost po 16hodinové inkubaci krmiva v enzymatickém roztoku s bromelainem a údaje stravitelnosti v procentech podle jednotlivých metod.

Jako základní metodu pro porovnání ověřované metody a pro odvození regresních rovnic jsme použili metodu mobile bag (Frydrych, 1992; Homolka *et al.*, 1996) využívající kanylovaná zvířata. Byly použity čtyři krávy černostrakatého plemene, z toho dvě, vybavené bachorovou kanylou, sloužily k přípravě nedegradovaných zbytků. Nedegradované zbytky po 16hodinové bachorové inkubaci byly vkládány v malých polyesterových sáčcích preinkubovaných v pepsinu a HCl do tenkého střeva dvou krav s duodenálními kanylami.

Kombinovaná enzymatická metoda

Kombinovaná enzymatická metoda se skládá ze dvou částí. První část, simulující degradaci v bachoru, je založena na úpravě vlastních metodik (Tománková, Kopečný, 1995). Druhá část, stanovující střevní stravitelnost, je založena na modifikaci metody autorů Antoniewicz *et al.* (1992). Modifikace klasické enzymatické metody spočívala v nahrazení první fáze metody, tj. 16hodinové inkubace krmiva v bachoru krávy, inkubací krmiva v tzv. výsledném inkubačním roztoku. Testovány byly časy inkubace 0, 1, 16 a 24 hodin. Inkubační roztok se skládal ze 100 mMol fosfátového pufru o pH 7,2, s 50 mMol cysteinu, 1 mMol EDTA, 1 mg/ml chloramfenikolu a 0,060 mg/ml bromelainu (Sigma, 2–4 U/mg proteinu). U jadrných krmiv (kukuřice, pšenice, ječmen) s vysokým obsahem škrobu, který limituje hranici přístupnosti proteinu krmiva, jsme použili α-amylázu (Bolamylasa, spec. aktivita 1,05 mg maltózy/h/mg, Lachema, Česká republika) v množství 2,5 mg na 1 ml enzymatického roztoku. Krmivo se inkubovalo v inkubačním roztoku při teplotě 39 °C. Takto připravené a preinkubované krmivo jsme použili pro druhou fázi stanovení střevní stravitelnosti nedegradovaného proteinu, založenou na inkubaci v enzymatickém roztoku s pancreatinem.

Ověřovali jsme dvě metody stanovení, s předsušením a bez předsušení krmiva.

I. pokus (metoda s předsušením)

1. Inkubace původního zhomogenizovaného krmiva (velikost částic 1 mm) ve výsledném inkubačním roztoku s bromelainem za účelem získání nedegradovaných zbytků.

2. Vysušení nezdegradovaného zbytku krmiva při teplotě 55 °C po dobu 24 hodin a stanovení obsahu sušiny a dusíku v nezdegradovaných zbytcích.

3. Stanovení stravitelnosti nedegradovaných zbytků skládající se ze dvou částí:

V první části proběhla inkubace nedegradovaných, vysušených a znovu zhomogenizovaných zbytků ve 2% roztoku pepsinu v 0,1N HCL při teplotě 39 °C po dobu 2 hodin ve vodní lázni. Ve druhé části, po centrifugaci vzorků a odsání supernatantu, se krmivo důkladně promylo destilovanou vodou a 0,1M fosfátovým pufrem o pH 7,4. Po promytí se supernatant znovu odsál a k sedimentu se přidal enzymatický roztok s pancreatinem (aktivita 4 x USP, koncentrace 600 mg/l fosfátového pufru). Inkubace trvala 24 hodin při teplotě 39 °C. Po ukončení inkubace byl nestrávený zbytek centrifugován, filtrován, třikrát promyt destilovanou vodou a zmineralizován podle Kjeldahla. Dusík byl stanoven spektrofotometricky, po reakci s Nesslerovým činidlem

I. Přehled ověřovaných krmiv, obsah sušiny a dusíku v původních krmivech a jejich reziduích (hodnoty Deg a Dsi stanovené podle jednotlivých metod) – An overview of tested feeds, contents of dry matter and nitrogen in original feeds and their residues (values Deg and Dsi determined by the separate methods)

Číslo[1]	Krmivo[2]	DPi (h)	Sušina[3] (%)	N (% sušiny)	Deg (%)	Stravitelnost[4] (%)			
						SP	BP	KM	MB
1	lněné pokrutiny[5]	0	92,4	5,64	68,1	89,0		85,5	85,4
		1	91,9	4,72		83,7			
		16	94,6	3,50		63,9	73,8		
2	řepkový šrot[6]	0	93,9	5,99	76,7	90,6		76,3	80,4
		1	92,5	4,54		71,7			
		16	90,8	3,20		66,5	62,1		
3	masokostní moučka[7]	0	95,5	7,92	66,1	81,2		64,5	72,4
		1	94,8	6,55		66,8			
		16	95,7	4,85		57,8	67,6		
4	krevní šrot[8]	0	92,02	4,22	62,7	83,1		86,8	89,7
		1	92,49	3,81		77,3			
		16	94,56	2,85		75,9	73,4		
5	rybí moučka[9]	0	93,14	12,33	55,4	83,2		86,2	94,5
		1	93,38	11,12		79,9			
		16	94,08	11,16		76,2	86,0		
6	siláž z cukrových skrojků[10]	0	93,65	1,75	67,4	79,0		60,7	69,7
		1	95,43	1,49		53,3			
		16	95,54	1,26		40,7	12,3		
7	luční porost[11]	0	93,36	1,27	73,5	76,9		77,7	68,2
		1	93,83	0,77		65,8			
		16	93,15	0,52		34,0	7,6		
8	GPS ječmen[12]	0	92,85	1,60	80,6	89,2		62,9	68,3
		1	93,19	0,53		70,4			
		16	93,91	0,44		54,9	28,6		
9	kukuřice – zrno[13]	0	91,43	1,56	56,7	74,1		92,8	94,6
		1	91,73	1,35		82,3			
		16	91,07	1,02		72,9	91,6		
10	ječmen – zrno[14]	0	90,00	2,11	69,1	88,2		85,5	88,7
		1	92,13	1,60		83,6			
		16	92,02	1,08		72,8	70,9		
11	jetel zelený[15]	0	94,12	2,51	76,9	82,7		79,1	85,8
		1	96,28	2,22		73,7			
		16	92,42	1,46		65,0	81,3		
		24	93,41	1,20		35,1			
12	sójový extrahovaný šrot[16]	0	92,41	7,84	81,7	95,6		96,7	99,0
		1	95,90	6,19		92,5			
		16	92,53	4,62		86,2	92,7		
		24	92,68	3,03		79,7			
13	pšenice – zrno[17]	0	90,55	2,16	80,9	89,6		78,9	88,1
		1	91,01	1,05		77,6			
		16	90,42	0,72		75,5	79,3		
		24	90,70	0,54		72,9			

DPi = doba preinkubace krmiva – time of feed preincubation
N = hodnota dusíku v krmivu po preinkubaci – nitrogen value of feed after preincubation
Deg = degradovatelnost po 16hodinové preinkubaci v enzymatickém roztoku s bromelainem – degradability after 16-hour preincubation in enzymatic medium with bromelain
SP = metoda s předsušením krmiva – method with feed pre-drying
BP = metoda bez předsušení krmiva – method without feed pre-drying
KM = klasická enzymatická metoda – classical enzymatic method
MB = mobile bag metoda – mobile bag method
GPS = silážované drtě – crushed silages

[1]no., [2]feed, [3]dry matter, [4]digestibility, [5]linseed cake, [6]rapeseed meal, [7]meat and bone meal, [8]blood coarse meal, [9]fish meal, [10]silage of sugar beet tops, [11]grassland, [12]GPS – barley, [13]corn – grain, [14]barley – grain, [15]green clover, [16]soybean meal, [17]wheat – grain

(AOAC, 1980). Každý vzorek byl inkubován v trojím opakování.

Výpočet: Obsah dusíku stanovený ve zbytku po konečné inkubaci byl vyjádřen jako procento z dusíku navážky nezdegradovaného zbytku po inkubaci v roztoku s bromelainem. Reciproká hodnota vyjadřuje procento stravitelnosti.

II. pokus (metoda bez předsušení)

1. Stanovení obsahu sušiny a dusíku v původních krmivech.

2. 16hodinová inkubace původního zhomogenizovaného krmiva (velikost částic 1 mm) ve výsledném inkubačním roztoku s bromelainem za účelem získání nedegradovaných zbytků.

3. Okamžité navázání přechodu krmiva z inkubačního roztoku s bromelainem do inkubačního 0.1M fosfátového roztoku s pancreatinem o pH 7,4. Vzorky krmiv však musíme po vyjmutí z prvního inkubačního roztoku pětkrát důkladně promýt destilovanou vodou a odsát supernatant. Následuje ihned inkubace v inkubačním roztoku s pancreatinem, postupem popsaným výše. Konečná fáze je stejná jako při stanovení metodou s předsušením krmiva.

Výpočet: Střevní stravitelnost proteinu byla vypočtena podle vzorce:

$$Dsi = 100 - [(100 - Cs)/(100 - Deg) \times 100]$$

kde: Dsi – střevní stravitelnost nedegradovaného proteinu v bachoru
Cs – celková stravitelnost
Deg – degradovatelnost proteinu v bachoru

Vztah hodnot stanovených dvěma ověřovanými metodami a hodnot stanovených metodou mobile bag u daného souboru krmiv byl vyjádřen rovnicemi lineární regrese (tab. II) a byly vypočteny hodnoty korelačních koeficientů a směrodatných odchylek.

VÝSLEDKY A DISKUSE

Izolované proteázy mohou mít odlišné účinky na strukturu proteinu než proteázy bachorové mikroflóry, a proto je důležitým předpokladem aplikace ověřovaných metod odvození regresních rovnic závislosti hodnot stanovených metodou mobile bag na hodnotách získaných enzymaticky.

Závislost mezi stravitelností nedegradovaného proteinu stanovenou metodou mobilních sáčků a stravitelností stanovenou kombinovanými enzymatickými metodami v daném souboru je zobrazena na obr. 1 a 2. Hodnoty obou korelačních koeficientů (r) poukazují na vysoký stupeň těsnosti vztahu. U metody s předsušením, po 16hodinové preinkubaci, je korelační koeficient o něco vyšší (r = 0,912) než u metody bez předsušení (r = 0,893) a naopak směrodatná odchylka RSD je nižší (% RSD = 5,48) než ve druhém případě (% RDS = 6,02). Proto jsme se zaměřili na metodu s předsušením s vyšším r a nižší RSD.

II. Lineární regrese mezi hodnotami zjištěnými kombinovanými enzymatickými metodami a metodou mobile bag – Linear regression of values determined by combined enzymatic methods and by the mobile bag method

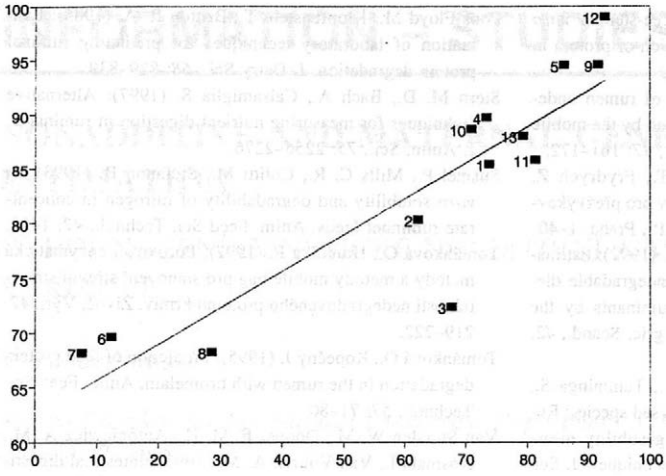
Kombinované metody (doba preinkubace)[1]	n	a	b	r	% RSD
Bez předsušení[2] (16)	13	62,36	0,33	0,893	6,016
S předsušením[3] (0)	13	65,38	0,19	0,091	16,139
S předsušením (1)	13	14,30	0,92	0,860	6,826
S předsušením (16)	13	40,85	0,66	0,912	5,481

n = počet testovaných krmiv – number of tested feeds
a, b = parametry rovnice – equation parameters
r = korelační koeficient – correlation coefficient
RSD = reziduální směrodatná odchylka – residual standard deviation

[1]combined methods (preincubation time), [2]without pre-drying, [3]with pre-drying

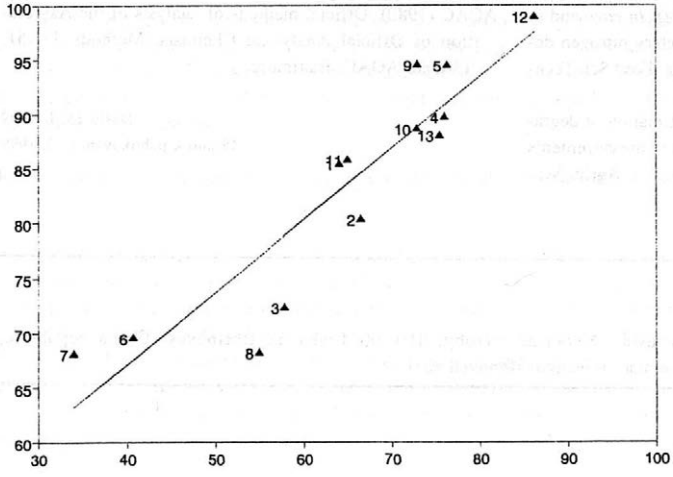
Odzkoušena byla preinkubace krmiva v inkubačním roztoku s bromelainem v různých časových intervalech – 0 (bez inkubace), 1, 16 a 24 hodin. Metoda s předsušením je vhodná pro preinkubaci krmiva v inkubačním roztoku s bromelainem v časovém intervalu 1 a 16 hodin (tab. II) a nehodí se pro interval 0 a 24 hodin. Při 24hodinové preinkubaci výrazně klesaly hodnoty střevní stravitelnosti proteinu, a proto nebylo dokončeno stanovení všech krmiv. Při nulovém intervalu preinkubace vykazovaly hodnoty střevní stravitelnosti velkou variabilitu ve vztahu k hodnotám stanovených metodou mobile bag a korelační pole bylo tak široké, že korelační koeficient byl téměř nulový (tab. II). Calsamiglia a Stern (1995) stanovili *in vitro* střevní stravitelnost nedegradovaného proteinu (Dsi) u krmiv živočišného původu. Testovali i vliv preinkubace krmiva (12 a 16 a 18 hodin) v bachoru na hodnotu Dsi. Čas preinkubace neovlivňoval hodnotu Dsi u krevní a u sójové moučky. U péřové, masokostní a rybí moučky delší čas preinkubace snižoval hodnotu Dsi. Nepatrný pokles Dsi se v našem případě potvrdil u sójové a krevní moučky. Mezi intervalem 1 a 16 hodin poklesla Dsi jen o 1,7 % u krevní moučky a o 7,2 % u sójové moučky. U masokostní moučky značně poklesla Dsi z 81 % na 57 % mezi nulovou a 16hodinovou inkubací, tj. o 30 %. Nepotvrdili jsme pokles Dsi u rybí moučky. Kaitho *et al.* (1998) sledovali úbytek suché hmoty a dusíku v sušených letorostech některých listnáčů v časových intervalech 16 a 24 hodin inkubace v bachoru. Střevní stravitelnost reziduí sledovali metodou mobile bag u 16 a 24hodinových reziduí a *in vitro* metodou jen při 16hodinové inkubaci. Tato metoda poněkud nadhodnocovala střevní stravitelnost nedegradovaného proteinu.

V našem případě vykazuje kombinovaná enzymatická metoda nižší hodnoty střevní stravitelnosti nedegradovaného proteinu, ale poskytuje přesnost v řazení krmiv, zvláště při 16hodinové inkubaci v inkubačním roztoku s bromelainem. Vhodná je především pro měření relativních rozdílů mezi krmivy a pro jejich seřa-



1. Srovnání hodnot střevní stravitelnosti nedegradovaného proteinu v bachoru (Dsi) získaných kombinovanou enzymatickou metodou (bez předsušení) a mobile bag metodou – Comparison of the values of intestinal digestibility of protein undegradable in rumen (Dsi) provided by combined enzymatic method (without pre-drying) and mobile bag method
$y = 62.36 + 0.33x$; $r = 0.893$

Pro obr. 1 a 2: osa x – kombinovaná enzymatická střevní stravitelnost (%); osa y – střevní stravitelnost metodou mobile bag (%)
For Figs. 1 and 2: x-axis – combined enzymatic intestinal digestibility (%); y-axis – intestinal digestibility by mobile bag method (%)



2. Srovnání hodnot střevní stravitelnosti nedegradovaného proteinu v bachoru (Dsi) získaných kombinovanou enzymatickou metodou (s předsušením) a mobile bag metodou – Comparison of the values of intestinal digestibility of protein undegradable in rumen (Dsi) provided by combined enzymatic method (with pre-drying) and mobile bag method
$y = 40.85 + 0.66x$; $r = 0.912$

zení (např. testace hybridů trav), méně vhodná je pro získání absolutních hodnot stravitelnosti nedegradovaného proteinu. Vztah mezi hodnotami získanými kombinovanou metodou a hodnotami získanými metodou mobile bag je lineární. Výraznější rozdíly u objemných krmiv vyvolávají otázku o správnosti lineárního vztahu a zároveň poskytují možnost použití metody pro vytvoření skupin krmiv a jejich případné hodnocení formou vícenásobné regrese.

Přehled vývoje různých alternativních metod predikce bachorového a střevního trávení u přežvýkavců popsali Stern *et al.* (1997). Pokračuje vývoj *in vitro* metod, které i přes svou nenáročnost, rychlost a jednoduchost zaručují poměrně přesné výsledky. Pro aplikaci kombinované enzymatické metody stanovení střevní stravitelnosti nedegradovaného proteinu v bachoru je nutné stanovit tyto hodnoty u dalších krmiv, a tak rozšířením sledovaného souboru zpřesnit parametry regresních rovnic.

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NONADDITIVE AND MATERNAL GENETIC VARIANCE ESTIMATION

VYHODNOCENÍ GENETICKÉ NEADITIVNÍ A MATERNÁLNÍ VARIANCE

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ABSTRACT: The aim of this review is to present some models and algorithms to estimate the direct additive, non-additive and maternal variance components. In the first part, the general questions on intra- and interlocus interactions are shown. Sources of specific maternal (indirect) and cytoplasmic variability are briefly characterised. The next section contains a literature review on the estimates of the genetic variance in different livestock species. The so-called unitrait total merit model (including known genetic effects) with its variance-covariance structure as well as currently applied approaches to estimate genetic parameters are described. In the final section some practical implications of the estimation of non-additive and maternal variances are discussed.

Keywords: variance components; nonadditive genetic effects; maternal effects; animal model

ABSTRAKT: Cílem práce bylo představení modelů a algoritmů vyhodnocení komponent genetické variance přímé aditivní, neaditivní a mateřské. V první části byly představeny teoretické základy různých forem proměnlivosti genetických efektů. Byly charakterizovány zdroje nepřímých mateřských a také cytoplazmatických vlivů a gametického potisku (imprinting). Byl proveden přehled literatury zabývající se vyhodnocením těchto variancí získaných pro užitkové vlastnosti u různých druhů hospodářských zvířat. Dále práce obsahuje popis jednoproměnného lineárního modelu, který zahrnuje všechny dříve uváděné genetické efekty (včetně struktury variančně-kovarianční matice), a také charakteristiku v současné době používaných přístupů k vyhodnocení genetických parametrů. Následně byly představeny praktické implikace spojené s odhadem genetických neaditivních a mateřských komponent variance.

Klíčová slova: komponenty rozptylu; neaditivní genetické efekty; maternální efekty; animal model

INTRODUCTION

In conventional approaches, non-additive and specific genetic effects are usually connected with cross-breeding experiments. Mating of individuals from different breeds (for instance, in the diallelic cross experiment) may lead to a large expression of interaction within and between the loci as well as to specific genetic effects. On the basis of reciprocal crosses between large Shire horses and small Shetland ponies, Walton and Hammond (1938) showed maternal (indirect) influences on the growth of offspring. An evaluation of additive and non-additive gene actions is based on some linear functions of genotypic mean estimates (e.g. Hill, 1982). Models to estimate these genetic effects have been developed by many authors (Cockerham, 1954; Jakubec *et al.*, 1991; Jakubec, 1993). On the other hand, studies have been conducted on the available field livestock data-sets, and evidence concerning relatively large non-additive and maternal variability of performance traits (Tempelman, Burnside, 1991; Wei, Van der Werf, 1993; Fuerst, Soelkner, 1994).

The prediction of genetic merits requires knowledge of the magnitude of respective (co)variance of these effects. Therefore, in the mixed model methodology, the genetic effects are treated as random.

The objective of this review is to present methodological approaches for the estimation of non-additive and maternal genetic variance components under an animal model.

THEORETICAL BACKGROUNDS

A majority of livestock and poultry performance traits are formed in the course of a relative long physiological process. In consequence, they are determined by many genes as well as environmental factors.

In an infinite number of loci (the so-called infinitesimal model), a genetic effect can be divided into direct and maternal (indirect) components. The direct genetic effects are partitioned into additive effects (no interaction of intralocus and interloci), dominance effects (interaction of allele effects at the same locus), and epistatic effects (interlocus interactions, e.g. additive by additive, additive by dominance, dominance by dominance, etc.). A maternal effect may be defined as any influence on a progeny phenotype attributable to the dam, other than the nuclear genes of progeny. These effects are classified into prenatal ones (e.g. uterine component), which may be both genetically and environmentally determined. Thus, these indirect (maternal) genotype effects are divided into the same components as mentioned above (additive, dominance, additive by additive, etc.). The confounding of the two contributions from the dam is combined with the possibility of a genetic correlation between the respective direct and maternal effects (Willham, 1980).

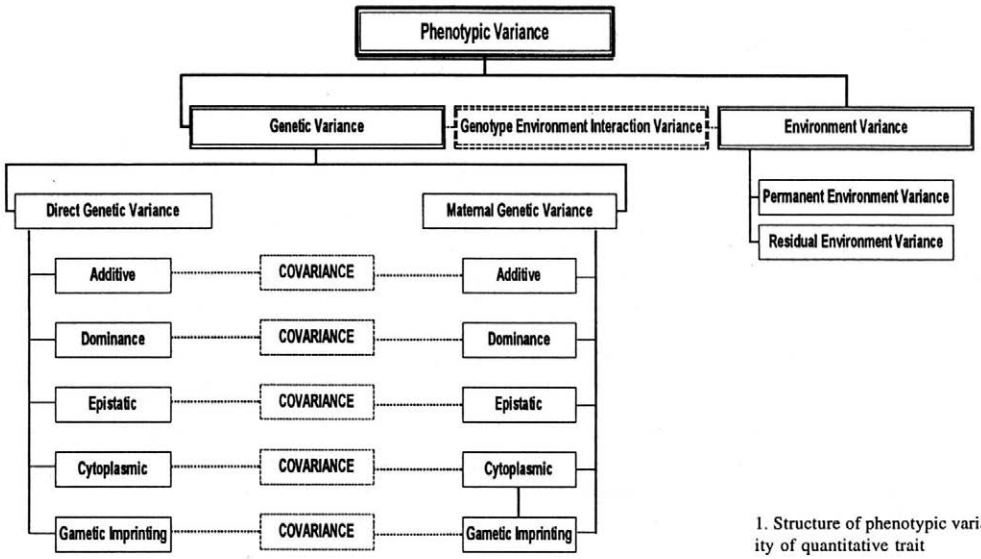
Another source of maternal effects are the mitochondrial DNA (mtDNA) (Wagner, 1972). Ovum cytoplasm contains about one hundred thousand copies of mtDNA (whereas a male cell contains 70–100 copies). Hence, the mitochondrial genotype is transmitted only from the female parent to the ensuing offspring (Gyllensten *et al.*, 1985), although limited biparental inheritance of mtDNA was established in mice (Gyllensten *et al.*, 1991). Molecular variation in mtDNA of several livestock species (bovine, horse, pig, sheep, dog) was demonstrated by many authors (Loftus *et al.*, 1994; Marklund *et al.*, 1995; Takeda *et al.*, 1995; Okumura *et al.*, 1996). A polymorphism of maternal lineages seemed to result, first of all, from a mutational event as well as an interaction of extranuclear by intranuclear DNA. It should be stressed that the first evidence of maternal effects for several performance traits (from field collected data) was registered from differences in heritability estimates obtained by daughter – dam regression and paternal half-sibs correlations (see e.g. Van Vleck, Bradford, 1965).

Genomic (or gametic) imprinting is a biological phenomenon discovered in the mid-80's. The term refers to a differential expression activity of a gene depending on whether it has been passed through the male or the female germ line. The expression of a gene may be blocked by methylation of DNA. The investigation carried out by De Vries *et al.* (1994) indicates a considerable proportion of genomic imprinting variance in total variance of backfat thickness and growth rate in pig. A structure of phenotypic variability is shown in Fig. 1. Several studies have shown these genetic influences on productive and reproductive traits in livestock. Some results are summarised in Table I. It should be stressed that both genetic evaluation and variance estimation are based on the theory of covariances (relationships) between the relatives. Coefficients of relationships of gene actions are given in Table II.

GENETIC MODELS

Sire and sire-dam model

The first approaches to estimate the nonadditive and maternal effects variances are based on simple sire and



1. Structure of phenotypic variability of quantitative trait

I. Previous estimates of non-additive and maternal genetic variances of livestock

Reference	Species	Trait	Variance as ratio of phenotypic							Method
			h^2	h_a^2	h_d^2	h_{aa}^2	h_m^2	h_{am}^2	h_c^2	
Allaire and Henderson (1965)	dairy cattle	milk	0.24	0.11	0.16	0.50				noniterative
		fat	0.18	0.23	0.24	–0.18				
Tempelman and Burnside (1990)	dairy cattle	milk		0.40	0.06					REML-SDM
Fuerst and Soelkner (1994)	dairy cattle	milk	0.28	0.18	0.08	0.33				REML-SDM
Tess and MacNeil (1994)	beef cattle	birth weight		0.35			0.14	0.02	0.004	REML-AM
Snyman *et al.* (1995)	sheep	body weight		0.22			0.09			REML-AM
Koerhuis and Thompson (1997)	broiler chicken	body weight		0.32			0.04	–0.06		REML-AM
Wei and Van der Werf (1993)	laying hen	egg number		0.52	0.11					REML-AM
Szwaczkowski (1995)	laying hen	age at sexual maturity		0.2391			0.0001	0.0005	0.0014	REML-AM

Note on symbols:
REML-SDM – REML method under a sire-dam model, REML-AM – REML method under an animal model
h^2 – heritability estimates obtained from simple model
$h_a^2 = \sigma_a^2 / \sigma_p^2$ (σ_a^2 is the direct additive genetic variance, σ_p^2 is the phenotypic variance)
$h_d^2 = \sigma_d^2 / \sigma_p^2$ (σ_d^2 is the direct dominance genetic variance)
$h_{aa}^2 = \sigma_{aa}^2 / \sigma_p^2$ (σ_{aa}^2 is the direct additive by additive genetic variance)
$h_m^2 = \sigma_m^2 / \sigma_p^2$ (σ_m^2 is the maternal additive genetic variance)
$h_{am}^2 = \sigma_{am} / \sigma_p^2$ (σ_{am}^2 is the covariance between direct additive and maternal additive effects)
$h_c^2 = \sigma_c^2 / \sigma_p^2$ (σ_c^2 is the cytoplasmic variance)

II. Coefficients of additive, nonadditive and cytoplasmic relationships (Van Raden *et al.*, 1992)

Relatives	Relationship coefficients of			
	additive	dominance	additive by additive	cytoplasmic
Identicals	1	1	1	1
Full-sibs	0.5	0.25	0.25	1
Parent – progeny	0.5	0	0.25	1/0[a]
Three-quarter sibs	0.3125	0.0625	0.0977	1
Half-sib (sire groups)	0.25	0	0.625	0

[a] 1 for dam – progeny; 0 for sire – progeny

sire-dam models (see e.g. Wężyk, 1970). Thus, respective variance components are estimated by the so-called three Henderson's methods (Henderson, 1953). The approach was developed by Lin, Lee (1989) and Tempelman, Burnside (1990), who applied the REML method to evaluate non-additive variance components. Thus, upon maximization of the log likelihood, heritability (in a narrow sense) was estimated as four times the sire variance divided by the total variance for both hierarchical and cross-classified models (see Table III), whereas the ratio of dominance genetic variance to total variance was evaluated as four times the dam-within--sire variance minus four times the sire variance (from a hierarchical model). However, in the model assumed epistatic, maternal and cytoplasmic variances as well as common environmental variance between the full sibs are negligible. From a practical point of view the procedure may supply approximate information about nonadditive genetic variability. Moreover, the heritability estimates may be obtained from the sire, the dam or jointly from the sire and dam variance components. They lead to biased heritability estimates in a narrow sense. Hence, the applicability of this approach to the evaluation of nonadditive and maternal genetic variances is also limited.

Total-merit genetic model

From a theoretical point of view, all genetic effects can be included in a model. Thus:

$$\mathbf{y} = \mathbf{X}\beta + \sum_{i=1}^{l} \mathbf{Z}_i\mathbf{u}_i + \sum_{i=1}^{l} \mathbf{W}_i\mathbf{m}_i + \mathbf{S}_d\mathbf{c}_d + \mathbf{S}_d\mathbf{c}_m + \mathbf{T}_d\mathbf{f}_d + \mathbf{T}_m\mathbf{f}_m + \mathbf{e}$$

where: $\mathbf{y}$ is the *nx1* observation vector (one observation per individual); β is a *px1* vector of environment (fixed) effects; $\mathbf{u}_i$ is a *qx1* (*q* is the number of evaluated individuals) vector of *i*-th random direct genetic effects (i.e. $\mathbf{u}_1$ is a *qx1* vector of random direct additive genetic effects, $\mathbf{u}_2$ is a *qx1* vector of random direct dominance genetic effects, $\mathbf{u}_3$ is a *qx1* vector of random direct

III. Causal components of sire and dam variance (Tempelman, Burnside, 1990)

Sire/dam component	Causal components
Sire	$\frac{1}{4}\sigma_a^2+\frac{1}{16}\sigma_{aa}^2$
Dam within sire variance (hierarchical model)	$\frac{1}{4}\sigma_a^2+\frac{1}{4}\sigma_d^2+\frac{3}{16}\sigma_{aa}^2+\frac{1}{8}\sigma_{ad}^2+\frac{1}{16}\sigma_{dd}^2+\sigma_m^2+\sigma_c^2+\sigma_{ec}$
Dam variance (cross-classified model)	$\frac{1}{4}\sigma_a^2+\frac{1}{16}\sigma_{aa}^2+\sigma_m^2+\sigma_c^2+\sigma_{ec}$

Note on symbols: σ_a^2 – additive variance, σ_{aa}^2 – additive by additive variance, σ_d^2 – dominance variance, σ_{ad}^2 – additive by dominance variance, σ_{dd}^2 – dominance by dominance variance, σ_m – maternal variance, σ_c^2 – cytoplasmic variance, σ_{ec} – environmental covariance

additive by additive genetic effects, $\mathbf{u}_4$ is a *qx1* vector of random direct additive by dominance genetic effects, etc.); $\mathbf{m}_i$ is a *qx1* vector of *i*-th random maternal (indirect) effects (as above); $\mathbf{c}_d$ is a *cx1* (*c* is the number of maternal lineages) vector of random direct cytoplasmic effects; $\mathbf{c}_m$ is a *cx1* vector of random maternal cytoplasmic effects; $\mathbf{f}_d$ is a *dx1* (*d* is a number of gamete effects of evaluated individuals, hence $d = 2q$) vector of random direct gametic imprinting effects; $\mathbf{f}_m$ is a *dx1* vector of random maternal gametic imprinting effects; $\mathbf{e}$ is a *nx1* vector of residuals; $\mathbf{X}$ is the *nxp* incidence matrix associating $\mathbf{b}$ with $\mathbf{y}$; $\mathbf{Z}_i$, $\mathbf{W}_i$, $\mathbf{S}_d$, $\mathbf{S}_m$, $\mathbf{T}_d$ and $\mathbf{T}_m$ are the *nxq*, *nxq*, *nxc*, *nxc*, *nxd*, *nxd* incidence matrices for the respective (above mentioned) genetic effects. The first and second moments of the model (genetic part) are assumed to be:

$$E\begin{bmatrix}u_1\\m_1\\u_2\\m_2\\u_3\\m_3\\\cdot\\\cdot\\\cdot\\u_1\\m_1\\c_d\\c_m\\f_d\\f_m\end{bmatrix}=\begin{bmatrix}0\\0\\0\\0\\0\\0\\\cdot\\\cdot\\\cdot\\0\\0\\0\\0\\0\\0\end{bmatrix}\quad Var\begin{bmatrix}u_1\\m_1\\u_2\\m_2\\u_3\\m_3\\\cdot\\\cdot\\\cdot\\u_1\\m_1\\c_d\\c_m\\f_d\\f_m\end{bmatrix}=\begin{bmatrix}G_1\sigma_{u_1}^2 & G_1\sigma_{u_1m_1} & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 & 0 & 0\\ G_1\sigma_{u_1m_1} & G_1\sigma_{m_1}^2 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 & 0 & 0\\ 0 & 0 & G_2\sigma_{u_2}^2 & G_2\sigma_{u_2m_2} & 0 & 0 & \cdots & 0 & 0 & 0 & 0 & 0 & 0\\ 0 & 0 & G_2\sigma_{u_2m_2} & G_2\sigma_{m_2}^2 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & G_3\sigma_{u_3}^2 & G_3\sigma_{u_3m_3} & \cdots & 0 & 0 & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & G_3\sigma_{u_3m_3} & G_3\sigma_{m_3}^2 & \cdots & 0 & 0 & 0 & 0 & 0 & 0\\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdots & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot\\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdots & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot\\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdots & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot\\ 0 & 0 & 0 & 0 & 0 & 0 & \cdots & G_f\sigma_{u_f}^2 & G_f\sigma_{u_fm_f} & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & 0 & \cdots & G_f\sigma_{u_fm_f} & G_f\sigma_{m_f}^2 & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & I_c\sigma_{c_d}^2 & I_c\sigma_{c_{dm}} & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & I_c\sigma_{c_{dm}} & I_c\sigma_{c_m}^2 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 & F\sigma_{f_d}^2 & F\sigma_{f_{dm}}\\ 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 & F\sigma_{f_{dm}} & F\sigma_{f_m}^2\end{bmatrix}$$

Additionally, the residual effects are assumed as independent ($\mathrm{Var(e)} = \mathbf{I}\sigma_e^2$) and uncorrelated with other random effects.

Hence, $\mathrm{E(y)} = \mathbf{X}\beta$ and $\mathrm{Var(y)} = \mathbf{V}$

with

$$\mathbf{V}=\sum_{i=1}^{l}\mathbf{Z}_i\mathbf{G}_i\mathbf{Z}'_i\sigma_{u_i}^2+\sum_{i=1}^{l}\mathbf{W}_i\mathbf{G}_i\mathbf{W}'_i\sigma_{m_i}^2+\sum_{i=1}^{l}\mathbf{Z}_i\mathbf{G}_i\mathbf{W}_i\sigma_{u_im_i}+\sum_{i=1}^{l}\mathbf{W}_i\mathbf{G}_i\mathbf{Z}'_i\sigma_{u_im_i}+\mathbf{S}'_d\mathbf{S}_d\sigma_{c_d}^2+\mathbf{S}'_m\mathbf{S}_m\sigma_{c_m}^2+\mathbf{S}'_d\mathbf{S}_m\sigma_{c_{dm}}^2+\mathbf{S}_m\mathbf{S}'_d\sigma_{c_{dm}}^2+$$

$$+\mathbf{T}'_d\mathbf{F}\mathbf{T}_d\sigma_{f_d}^2+\mathbf{T}'_m\mathbf{F}\mathbf{T}_m\sigma_{f_m}^2+\mathbf{T}'_d\mathbf{F}\mathbf{T}_m\sigma_{f_{dm}}^2+\mathbf{T}_m\mathbf{F}\mathbf{T}_d\sigma_{f_{dm}}^2+\mathbf{I}\sigma_e^2$$

where:

$\sigma_{u_i}^2$ – *i*-th direct genetic variance (additive variance, dominance variance, additive by additive, additive by dominance, dominance by dominance variance, etc.)

$\sigma_{m_i}^2$ – *i*-th maternal genetic variance (as above)

$\sigma_{u_im_i}$ – *i*-th covariance between the respective direct and maternal effects (e.g. additive direct and additive maternal effects, etc.)

$\sigma^2_{c_d}$ – direct cytoplasmic variance
$\sigma^2_{c_m}$ – maternal cytoplasmic variance
$\sigma_{c_{dm}}$ – covariance between direct and maternal cytoplasmic effects
$\sigma^2_{f_d}$ – direct gametic imprinting variance
$\sigma^2_{f_m}$ – maternal gametic imprinting variance
$\sigma_{f_{dm}}$ – covariance between direct and maternal gametic imprinting effects
σ^2_r – residual variance
$\mathbf{G_i}$ – *qxq* respective relationship matrix, where: $\mathbf{G_1}$ is the *qxq* additive (**A**), $\mathbf{G_2}$ is the *qxq* dominance (**D**), $\mathbf{G_3}$ is the *qxq* additive by additive (**A#A**) relationship matrices, etc; # is the Hadamard product); The off-diagonal elements of additive relationship matrix (**A**, here the so-called $\mathbf{G_1}$) are the additive relationship coefficients between the included individuals. Whereas the diagonal elements are one plus inbreeding coefficients of individuals. All nonadditive genetic relationship can be generated from the knowledge **A**.
F – *dxd* gametic relationship matrix. As already mentioned, the order of **F** is twice that of above relationship matrices (Schaeffer *et al.*, 1989).

Though there is evidence that mtDNA evolves more rapidly than nuclear DNA, identical mitochondrial genome was assumed for all descendens of one dam (founder). Thus, null covariances between maternal lineages were assumed (see e.g. Boettcher *et al.*, 1996b). Hence, the respective variance-covariance matrix is expressed as an identity ($\mathbf{I_c}$).

When cytoplasmic and gametic imprinting effects are included in the model, the variance matrices are as follows: $\mathbf{I_c}\sigma^2_c$ and $\mathbf{F}\sigma^2_f$, respectively.

These relationship matrices can only be easily formed and inverted for small data-sets. For larger populations inverses of the additive, dominance, additive by additive and the so-called gametic relationship matrices can be obtained by using recurrence algorithms described by Quaas (1976), Van Raden, Hoeschele (1991), Hoeschele, Van Raden (1991) and Schaeffer *et al.* (1989).

Algorithms to estimate the genetic parameters

There are many algorithms available to estimate the variance components (Hofer, 1998). The first ones were described by Henderson (1953). In the Henderson III method, a random dam effect can be nested (or sometimes crossclassified) in random sire effects. On the other hand, when the dominance and/or maternal effects are significant, the dam variance component is overestimated and the occurrence of the additive by additive effects influences the sire variance. New approaches were developed in the 1970's to analyse unbalanced data. These include, first of all, the restricted maximum likelihood – REML (Patterson, Thompson 1971), minimum variance quadratic unbiased estimation – MIVQUE (Rao, 1971). Impediments to the widespread use of these methods (particularly under an animal model) resulted from their relatively large computational requirements. Hence, easier numerical algorithms were developed (see Wężyk, Szwaczkowski, 1997), for instance Henderson's IV method, Schaeffer's pseudo-expectation procedure and the tilde-hat procedure. Moreover, several alternative numerical algorithms to obtain the REML estimates were also described, e.g. Henderson's algorithm, the expectation-maximization (EM) procedure and the derivative-free algorithm. However, when many variance components are estimated, the applicability of these algorithms to very large data-sets is limited. Thus, the method R (Reverter *et al.*, 1994) is recommended. The method has an advantage that the inverse of coefficient matrix is not required. However, the statistical properties of this method are still not very clear. Recently, an alternative method to estimate variance components is Gibbs sampling. It should be stressed that the use of this approach has several advantages (Van Tassel *et al.*, 1995) such as: no solution to the mixed model equations is needed; possibility of analysis of data-sets larger than those using REML due to simple sparse matrix techniques; direct and exact estimates of variance components. Moreover, Gibbs sampling is well suited for use on microcomputers and workstations because relatively little information needs to be stored in memory.

Some practical implications and related problems

Genetic evaluation of livestock is still based on the additive model. As already mentioned, many authors indicate the advantages of including nonadditive and maternal effects in a genetic model. Wei and Van der Werf (1993) reported that estimation of these variances implicates an unbiased estimation of heritability in a narrow sense, a more precise prediction of additive effects, and the use of nonadditive and maternal effects through a crossbreeding strategy. So, consequences of ignoring the nonadditive effects are different for various groups of individuals. They are largest for animals whose relationship contains relatively large nonadditive relationships (for instance, groups of full sibs) (Misztal *et al.*, 1995). If nonadditive effects are ignored in a single record model, they are confounded with and subsequently predicted as additive genetic and residual effects. Generally, using nonadditive, maternal and cytoplasmic effects leads to an increase of genetic gain in livestock population. The results obtained by other authors (De Stefano, Hoeschele, 1992; Boettcher *et al.*, 1996a) basically concern the influence of single genetic effects on selection efficiency. De Stefano and Hoeschele (1992) reported that the extra genetic gain due to selection using dominance and inbreeding ranged from 1.5% to 8% of the phenotypic standard deviation. Boettcher *et al.* (1996a) showed a response of about 2 kilograms of milk per year when cytoplasmic effects (the cytoplasmic variance was at 10% of the phenotypic one) were included in the model.

From a practical point of view, including all genetic effects into the model is both impossible and unfounded. Misztal *et al.* (1995) specify the conditions for including nonadditive effects into genetic evaluations. This might occur if: the population contains enough respective relationships (nonadditive, signifi-

cant differences between the maternal lineages) to allow the prediction of genetic effects; nonadditive and maternal variances are of sufficient size; dissemination mechanisms exist for particular genetic evaluations; computations are manageable. Van Raden *et al.* (1992) concluded that the use of new reproductive technology (e.g. embryo transfer) leads to increasing nonadditive relationships. On the other hand, the above authors found that REML analysis of all US Holstein data could provide estimates of dominance and additive by additive variance with standard errors of approximately 1% of the phenotypic variance, whereas variances of higher order interlocus interactions would have standard deviations above 10%. Moreover, particular small genetic effects may be confounded with other genetic and environmental effects.

As already mentioned, another problem in the estimation of many variance components in an animal model are the very large computational requirements, following from the number of predicted genetic effects and the number of recorded and base individuals. Misztal *et al.* (1995) reported that estimation of the dominance variance required about 20 times more data than estimation of the additive variance, and estimation of the additive by additive variance about 380 times more data. However Salehi and James (1997) concluded that the detection of cytoplasmic variance was more likely with data spread over 20 generations, while the power of the detection was reduced with fewer data. On the other hand, Tess and MacNeil (1994) suggested inclusion of the cytoplasmic effects as fixed. Thus, residual standard deviations were reduced by less than 0.1% only.

How is a genetic effect (variance component) significant? The evaluation is performed by comparison of the values of maximised likelihood logarithms. The statistical procedure is based on the following ratio test: minus two times the difference between the maximised likelihood logarithms of two models (without and within the genetic effect) was tested against the chi-square distribution with one degree of freedom (Dobson, 1990).

It seems that development of new reproductive techniques and knowledge of livestock genomes will influence the extention of genetic models as well as detection of new numerical approaches to solving current and future problems.

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