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Kaláb J. (1995): Changes in milk production during the sexual cycle. In: Hekel K. (ed.): *Lactation in Cattle*. Academic Press, London. 876–888.

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Relationship between MHS status and plasma cortisol concentration in individually confined pigs

N. SIARD¹, M. KOVAC¹, J. LADEWIG², I. ŠTUHEC¹

¹Zootechnical Department, Biotechnical Faculty, University of Ljubljana, Domžale, Slovenia

²Institute of Animal Husbandry and Animal Behaviour, Trenthorst, Westerau, Germany

Present Address: Royal Veterinary and Agricultural University, Division of Ethology and Health, Frederiksberg C, Denmark

ABSTRACT: The degree of stress was estimated in two individual housing conditions (large pens with straw bedding, small metabolic cages with slatted floor without straw) and in two malignant hyperthermia syndrome (MHS) genotypes (*NN* and *Nn*) by means of plasma cortisol concentration. MHS genotype was determined by a DNA-based test. Three replications were done, each including eight German Landrace barrows, four (2 *NN*, 2 *Nn*) housed in pens and four (2 *NN*, 2 *Nn*) in metabolic cages (24 animals in total). Blood samples of all animals were collected simultaneously every 15 minutes between 8.00 and 11.00 a.m. on days 8, 22 and 36 after the insertion of intravenous catheters. The animals in metabolic cages had higher plasma cortisol values than the animals in pens, which indicates that extreme confinement is a severe stressor. *NN* animals had higher plasma cortisol values than *Nn* animals. This indicates a relationship between MHS and the adrenal function.

Keywords: animal welfare; pigs; housing; malignant hyperthermia; plasma cortisol

Space allowance is an important factor in adaptation to the environment. Confinement, lasting for weeks, can chronically increase plasma cortisol concentration (e.g. Warnier and Paquay, 1983; Štuhec, 1984; Becker *et al.*, 1985a; Janssens *et al.*, 1994). The examples of extreme confinement are metabolic cages, often used in nutrition experiments. Other important factors in adaptation are social contacts and control of the environment. Social isolation increased plasma cortisol concentration (Štuhec, 1984) and social isolation together with reduced visual control of the environment increased cortisol response to exogenous ACTH (Janssens *et al.*, 1994). Lower basal cortisol concentrations were observed in a positive human-animal relationship, indicating that positive contacts can obviate some of the negative effects of the environment (Pedersen *et al.*, 1998). Another important factor in reducing plasma cortisol concentration is previous ex-

perience with the stressful environment. Only an initial increase in plasma cortisol concentration was observed in lambs in metabolic cages that had previous experience with metabolic cages and were habituated to the contact with human beings (Kapp *et al.*, 1997).

Malignant hyperthermia syndrome (MHS) is an acute reaction to environmental disturbances, e.g. transport, high ambient temperature, or mixing with other animals. It is characterised by hyperthermia and muscle rigidity, often followed by sudden death within minutes. It is inherited by a single recessive gene (*n*). The relationship between MHS and adrenal function is not clear: none was found by Aberle *et al.* (1976) and von Borell and Ladewig (1989) while Schaefer *et al.* (1990) and Nyberg *et al.* (1988) reported a relationship but the results were not in agreement. MHS status was determined by the halothane test in these studies.

The errors can occur when MHS genotype is determined by the halothane test (Webb *et al.*, 1987; Dovč, 1992) and they can partly describe the reason for the discrepancies in the mentioned results. The development of a highly reliable DNA-based test eliminated these problems (Fujii *et al.*, 1991).

The aim of the study was to evaluate the difference in plasma cortisol concentrations between two MHS genotypes (*NN* and *Nn*). A lot of studies have been done in order to explain the relationship between MHS and adrenal function, but there was a problem of correct genotyping and furthermore of distinguishing *NN* and *Nn* animals. To avoid that problem we used a highly reliable DNA-test. The pigs were housed individually – in metabolic cages and in large straw bedded individual pens.

MATERIAL AND METHODS

Animals and housing

The experiment was conducted in three replications, each including eight German Landrace barrows housed individually, four in pens (1.98 × 1.93 m) and four in metabolic cages. In total 24 animals were included in the experiment. Pens bedded with straw were separated by solid wooden partitions. In metabolic cages without straw, the animals were able to stand up and lie down, but not to turn around. The MHS genotype was determined by a DNA-based test (Fujii *et al.*, 1991). Two pigs in each housing type were dominant homozygous (*NN*) and two were heterozygous (*Nn*).

Pigs were housed in the experimental environment 14 days before intravenous catheters for blood sampling were inserted. The animals had no previous experience with the two housing conditions. Health condition of the animals was followed by daily measurement of body temperature. Dry meal and water were available *ad libitum*. The illumination and ventilation were natural.

Collection of samples

Blood samples were collected from the external jugular veins through catheters that were inserted at the age of 143 ± 5 days (body weight 63.0 ± 4.0 kg). Blood samples were collected from all animals on

days 8, 22 and 36 after the insertion between 8.00 h and 11.00 h in 15 min intervals. Pigs were always handled by a familiar person. Blood samples were centrifuged immediately at 2 500 × g for 15 min and plasma samples were stored at –20°C.

Cortisol analysis

The total plasma cortisol concentration was quantified by means of RIA as described by Ladewig and Smidt (1989). The intra- and inter-assay variability was up to 10%.

Statistics

Data on cortisol concentration in plasma (y_{ijklmn}) were logarithmically transformed to achieve normality. The model comprised three replications (R_i), two housing conditions (H_j), two MHS genotypes (G_k), individual animals (A_{ijkl}) and sampling day (D_{im}). Possible changes of cortisol concentration within sampling day (trial) were described by linear regression (b_{im}). The model also included two and three level interactions, the ones that showed the significant effects in the previous analysis:

$$y_{ijklmn} = \mu + R_i + H_j + G_k + A_{ijkl} + D_{im} + b_{im}(x_{imn} - 5.5) + RH_{ij} + RG_{ik} + RHG_{ijk} + e_{ijklmn}$$

RESULTS

Considerable variability between and within animals was observed. Variability was lowest in replication three (Figure 1). This is reflected in the effect of animal and in the effect of replication being highly significant (Table 1).

All of the effects included in the model were statistically significant (Table 1); however, only main effects are discussed.

The results demonstrate that pigs in metabolic cages had significantly higher cortisol values than the pigs in individual pens ($P = 0.049$; Tables 1 and 2). In addition, the difference between the two MHS genotypes was highly significant ($P = 0.006$; Table 1). *NN* animals had higher cortisol values than *Nn* animals (Table 2).

An increase in cortisol values in a 4-week period (from day one to day three) was observed within the three replications. The differences were highly

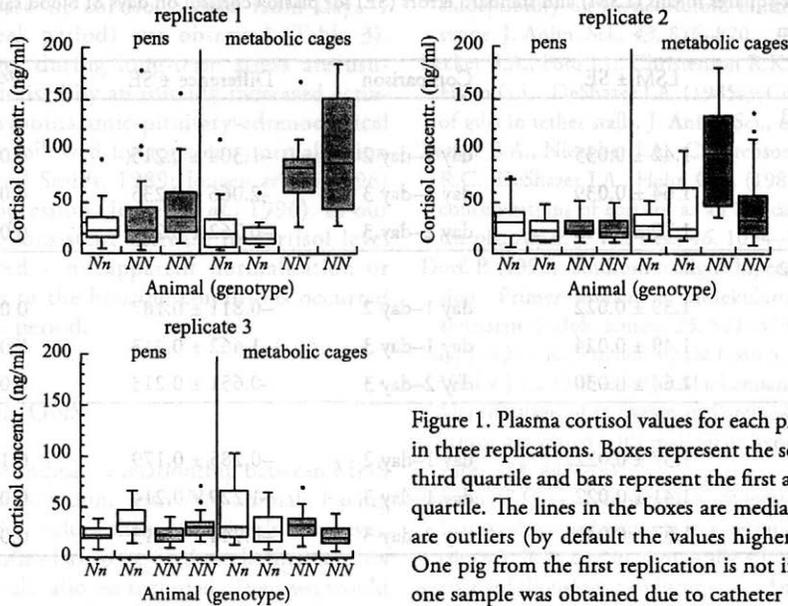


Figure 1. Plasma cortisol values for each pig respectively in three replications. Boxes represent the second and the third quartile and bars represent the first and the fourth quartile. The lines in the boxes are medians, the points are outliers (by default the values higher than 3 SD). One pig from the first replication is not included (only one sample was obtained due to catheter dysfunction)

Table 1. Statistical significance of the effects on plasma cortisol concentration

Effects	Degrees of freedom	P-value	Effects	Degrees of freedom	P-value
Replication	2	0.0001	Trial – linear regression	9	0.0001
Housing condition	1	0.0492	Replication × housing	2	0.0001
MHS genotype	1	0.0064	Replication × genotype	2	0.0001
Animal	12	0.0001	Replication × housing × genotype	3	0.0001
Sampling day	6	0.0001			

Table 2. Least-squares means (LSM) and standard errors (SE) for plasma cortisol in two housing conditions and in two MHS genotypes

Effects	LSM ± SE	Difference
Housing		
Individual pens	1.50 ± 0.022	-0.048 ± 0.025
Metabolic cages	1.55 ± 0.012	
MHS genotype		
NN	1.56 ± 0.012	0.067 ± 0.025
Nn	1.49 ± 0.022	

significant ($P = 0.0001$; Table 1), except between day one and day two in the third replication (Table 3).

DISCUSSION

High variability between and within the animals was observed (Figure 1). Janssens *et al.* (1994) also reported large differences in cortisol values between the animals and von Borell and Ladewig (1989) showed that adrenocortical reactivity is an individual characteristic.

Social isolation and reduced visual control of the environment are considered stressful (Štuhec, 1984; Janssens *et al.*, 1994). Such situation was experienced by animals in individual pens and in metabolic cages. In cages, visual contact and visual control of the environment were less reduced than in the pens. However, pigs in cages had higher plasma cortisol concentrations than pigs in pens (Table 2), indicating that in the extreme confinement, lasting for weeks, the animals were

Table 3. Least-squares means (LSM) and standard errors (SE) for plasma cortisol on days of blood sampling within the replication

Day	LSM ± SE	Comparison	Difference ± SE	P-value
Replication 1				
1	1.42 ± 0.035	day 1–day 2	–1.304 ± 0.215	0.0001
2	1.64 ± 0.039	day 1–day 3	–2.066 ± 0.235	0.0001
3	1.79 ± 0.042	day 2–day 3	–0.762 ± 0.250	0.002
Replication 2				
1	1.39 ± 0.022	day 1–day 2	–0.811 ± 0.187	0.0001
2	1.49 ± 0.024	day 1–day 3	–1.462 ± 0.213	0.0001
3	1.64 ± 0.030	day 2–day 3	–0.651 ± 0.213	0.002
Replication 3				
1	1.37 ± 0.022	day 1–day 2	–0.285 ± 0.179	0.11
2	1.41 ± 0.022	day 1–day 3	–1.229 ± 0.214	0.0001
3	1.57 ± 0.030	day 2–day 3	–0.944 ± 0.213	0.0001

experiencing more stressful conditions. Becker *et al.* (1985b) reported a higher increase in serum cortisol in confined than in electrically stimulated and heat-stressed pigs.

Animals with *NN* genotype for MHS had higher cortisol values than *Nn* animals (Table 2). The results are not directly comparable with the results of other studies, using phenotypic tests for MHS genotyping and therefore distinguishing only stress-susceptible (*nn*) and stress-resistant (*NN* and *Nn*) animals. As far as we know, only Nyberg *et al.* (1988) and Schaefer *et al.* (1990) distinguished all three MHS genotypes; the first by halothane and blood typing for three marker loci and the second by using genetically known lines (*NN* and *nn*) and their crossbreeds (*Nn*). In the study of Nyberg *et al.* (1988) a small difference was found between the three genotypes after exposure to stress (transport). However, two weeks after transport and at slaughter, transported *NN* animals showed an increase in cortisol concentration and *nn* animals showed a decrease; *Nn* pigs showed no difference in this regard. In the study of Schaefer *et al.* (1990), *nn* animals had twice as high basal cortisol levels as *NN* animals and crossbreeds (*Nn*) showed intermediate levels. A lower adrenal response to endogenous (Marple *et al.*, 1972) and exogenous (Sebranek *et al.*, 1973) ACTH was reported in susceptible (*nn*) pigs – however, in the study of Marple *et al.* (1972) only in non-stressful conditions. In various

stressful environments, the differences between susceptible (*nn*) and resistant (*NN* and *Nn*) pigs were not consistent. On the other hand, Mitchell and Heffron (1981) found a similar cortisol secretion in susceptible (*nn*) and resistant (*NN* and *Nn*) pigs in non-stressful situations and reduced ability to secrete cortisol in susceptible (*nn*) pigs in stressful conditions. Marple and Cassens (1973) suggested increased production and utilisation of cortisol instead of reduced ability to secrete cortisol in susceptible (*nn*) animals. Our results indicate a relationship between *NN* genotype and high plasma cortisol concentration in stressful conditions. In agreement with the results of Nyberg *et al.* (1988) and Schaefer *et al.* (1990), they also show that distinguishing stress-resistant (*NN* and *Nn*) and stress-susceptible (*nn*) animals is not sufficient since the differences in plasma cortisol values were found between resistant pigs. However, it is important that in the mentioned studies, susceptible (*nn*) and resistant (*NN* and *Nn*) animals were of different breeds. In the study of Aberle *et al.* (1976), plasma cortisol concentrations varied significantly between breeds. Therefore it is possible that the differences in cortisol values reflected the differences between breeds not related to the MHS status. Thus, Aberle *et al.* (1976) and von Borell and Ladewig (1989) found no relationship between MHS status and adrenal function in the same breed. These findings are not in agreement with our results.

An increase in cortisol values from days 1 to 3 (4-week period) was observed (Table 3). The changes during long-term stress are usually characterised by an initially increased activity of the hypothalamic-pituitary-adrenocortical (HPA) axis followed by apparent normalisation (Ladewig and Smidt, 1989; Jensen *et al.*, 1996) or even suppression (Jensen *et al.*, 1996). In our research, a consistent increase in cortisol level was observed – no apparent normalisation or habituation to the housing conditions occurred in a 4-week period.

CONCLUSIONS

The results indicate a relationship between MHS and adrenal function, with *Nn* animals having higher cortisol values than *Nn* animals. However, the relationship has to be further clarified and *nn* animals should also be included. Then we would know what impact the selection against *n* allele has on the adrenal function in pigs, i.e. on their ability to cope with the stressors.

Higher cortisol values in pigs in metabolic cages than in individual pens indicate that extreme confinement, lasting for weeks, is a severe stressor – more stressful than social isolation and reduced visual control of the environment. Therefore, the confinement in metabolic cages, lasting for weeks, compromises the welfare of the pigs. This is important to take into consideration since metabolic cages are often used in the experiments when urine and faeces have to be collected.

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ABSTRAKT

Závislost mezi genotypem MHS a koncentrací kortizolu v krevní plazmě u individuálně ustájených prasat

U dvou genotypů (*NN* a *Nn*) syndromu maligní hypertermie (MHS) byl pomocí koncentrace kortizolu v krevní plazmě hodnocen stupeň stresu ve dvou typech ustájení (velké kotce s podestýlkou ze slámy, malé metabolické klece s roštovou podlahou bez slámy). Genotyp MHS byl stanoven analýzou DNA. Pokus měl tři opakování, každé opakování zahrnovalo osm kanečků plemene německá landrase, z nichž čtyři (2 *NN*, 2 *Nn*) byli ustájeni v kotcích a čtyři (2 *NN*, 2 *Nn*) v metabolických klecích (celkem 24 zvířat). Vzorky krve byly odebírány souběžně od všech zvířat každých 15 minut mezi 8. a 11. hodinou dopoledne 8., 22. a 36. den intravenózními kanylemi. Zvířata v metabolických klecích měla vyšší hodnoty kortizolu v krevní plazmě než zvířata v kotcích, což dokládá, že extrémní omezení pohybu je silný stresor. Zvířata genotypu *NN* měla vyšší hodnoty kortizolu v krevní plazmě než zvířata genotypu *Nn*. Naznačuje to vztah mezi MHS a funkcí nadledvinek.

Klíčová slova: pohoda zvířat; prasata; ustájení; maligní hypertermie; kortizol v krevní plazmě

Corresponding Author

Nataša Siard, Zootechnical Department, Biotechnical Faculty, University of Ljubljana, Groblje 3, SI-1230 Domžale, Slovenia
E-mail: natasa.siard@bfro.uni-lj.si

The effect of different incubation temperatures on the incubation performance of ostrich (*Struthio camelus*) eggs

A. IPEK, U. SAHAN, B. YILMAZ

Animal Science Department, Agricultural Faculty, Uludag University, Bursa, Turkey

ABSTRACT: This research was carried out with the aim of determining the effect of different incubation temperatures on the incubation performance of ostrich eggs. The total of 282 eggs were incubated in a defined developmental period at 36.0, 36.6 and 37.2°C in three fully automatic programmable incubators that were available on the farm (12 times at one week intervals) during the laying period. The temperature applied during the growth period in artificial incubation of ostrich eggs significantly affected the hatchability of fertile eggs, embryo mortalities and incubation periods ($P < 0.01$). Egg weight loss increased with increased temperature ($P < 0.05$). In this study early and late embryonic mortality rates in eggs incubated at a high temperature were found to be high ($P < 0.01$). Eye abnormality was observed in four of the chicks hatched at 37.2°C.

Keywords: incubation; temperature; ostrich (*Struthio camelus*)

Incubation conditions are the most important factors affecting the hatchability of ostrich eggs. As is known, temperature, humidity, ventilation and rotation during the incubation period markedly affect the hatchability of fertile eggs and chick quality. The most dramatic effect of these factors on the hatchability of fertile eggs is temperature.

The temperature experienced by a developing embryo depends on three factors; incubator temperature, ability of heat to pass between the incubator and the embryo and metabolic heat production of the embryo itself (French, 1997).

The effect of temperature on the hatchability of fertile eggs was investigated by many researchers (Kosin, 1964; Landauer, 1967; Lundy, 1969; Meir and Ar, 1990). The incubation temperature of ostrich eggs under natural conditions was determined to be 36.0°C (Swart *et al.*, 1987). Egg temperature varies greatly among the species in natural incubation (Kosin, 1964; Wilson *et al.*, 1979). Landauer (1967) reported that the increase in temperature during incubation was very critical for chick embryos. Moreover, it was reported that growth was retarded or ceased and the incidence of poor second quality chicks increased as the temperature

was raised (Wilson, 1991). This study was carried out with the aim of determining the effects of different incubation temperatures of ostrich eggs on hatchability.

MATERIAL AND METHODS

The eggs used in the trial were from breeding ostriches raised in South Africa. The nests of ostriches were checked twice a day for the presence of eggs and eggs were collected; as soon as they were laid, each egg was coded by the date when they were laid and transferred to a storage room. The eggs were weighed individually on an electronic balance to the nearest ± 0.01 g. The eggs were stored for 7 days at 15–18°C and 80% relative humidity. The total of 282 eggs whose weights ranged between 1 320 and 1 480 g were used in the trial. Thereafter, they were incubated at temperatures 36.0, 36.6 and 37.2°C in three fully automatic incubators. Eggs were set in the incubators 12 times during the laying period. Relative humidity of 30% was provided for these eggs in the incubators for 38 days and then it was maintained at 40% until hatching. They were

turned at an angle of 45° every hour. Fertility was determined via candling on the 14th day of incubation. The eggs were candled on the 38th day of incubation again, and those exhibiting embryonic mortality were determined and removed from the machines. They were opened to determine the time of embryonic mortality. Viable eggs were weighed using an electronic balance to the nearest 0.01 g in order to determine the weight loss during the incubation period. The incubation period was determined and the hatched chicks were checked for deformities.

The effect of different temperatures on the hatchability of fertile eggs, hatchability of total eggs and the incidence of embryonic mortality and deformed chicks were revealed. Moreover, the relations between different temperatures and egg weight loss, chick hatch weight and length of the incubation period were determined. Trials were subjected to an analysis of variance (Minitab – personal communication, 1989), utilizing ANOVA procedures for balanced data. Analyses for percentage data were conducted after an arc sine transformation of the data. Significant differences between treatment means were determined by Duncan's multiple range test.

RESULTS

The effects of different temperatures on hatchability characteristics are shown in Table 1.

Data obtained by the research indicated that the temperature applied during the growth period in artificial incubation of ostrich eggs significantly affected the hatchability of fertile eggs, embryo mortalities and incubation periods ($P < 0.01$). Hatchability of fertile eggs declined with temperature, for maximum hatchability a lower temperature than 37.2°C is desirable in the incubation period. Embryonic mortality tended to increase at 37.2°C ($P < 0.01$). Egg weight loss increased with increased temperature ($P < 0.05$). Deformed chicks were observed only at 37.2°C. Length of incubation was affected by the temperature ($P < 0.05$).

DISCUSSION

The effects of temperature on embryonic development and duration of incubation period depend on the temperature (low or high), age of the embryo, duration of exposure and their interactions, as well as humidity and type of incubator (Wilson, 1991). The

Table 1. The effect of different temperatures on the hatchability characteristics (mean \pm SEM)

	Temperature (°C)			
	36.0	36.6	37.2	
Total eggs (<i>n</i>)	92	90	93	
Egg weight (g)	1 420.2 \pm 42.7	1 409.2 \pm 38.8	1 427.5 \pm 53.9	N.S.
Fertility	67.4 \pm 1.9	66.6 \pm 1.3	67.7 \pm 2.3	N.S.
Hatchability of fertile eggs	70.9 \pm 1.1a	71.6 \pm 1.9a	57.1 \pm 1.7b	**
Hatchability of total eggs	47.8 \pm 1.2a	47.8 \pm 1.2a	38.7 \pm 1.1b	**
Early term embryonic mortality	8.1 \pm 1.3b	8.3 \pm 1.6b	15.9 \pm 1.9a	**
Middle term embryonic mortality	4.8 \pm 1.0b	5.0 \pm 1.2 b	6.3 \pm 1.3a	**
Late term embryonic mortality	16.1 \pm 1.5b	15.0 \pm 1.3 b	20.6 \pm 1.7a	**
Number of deformed chicks (<i>n</i>)	–	–	4	
Egg weight loss	12.4 \pm 0.2c	13.7 \pm 0.3b	15.5 \pm 0.5a	*
Incubation period (<i>d</i>)	42.6 \pm 1.1b	41.8 \pm 0.9b	40.8 \pm 0.8a	*

a, b, c = means in the same line with no common superscript are significantly different at the $P < 0.05$, $P < 0.01$ level

* $P < 0.05$, ** $P < 0.01$

optimum incubation temperature for ostrich eggs has not been precisely established so far. Deeming *et al.* (1993) reported that ostrich eggs could be incubated at temperatures between 35–37°C. The incubation of eggs at a variety of temperatures between 36.0 and 36.7°C showed that incubation at 36.4°C allowed 50% of all chicks to hatch within 42 days (Ar *et al.*, 1996). However, few studies have reported the influence of high temperature on hatchability, weight loss of the embryo and length of the incubation period of ostrich eggs.

Smith *et al.* (1995) determined the hatchability to be 44% when applying a temperature of 37.3°C to the ostrich eggs during their developmental period. In the present study the hatchability of eggs subjected to a temperature of 37.2°C was shown to decline significantly.

A higher temperature inside the eggs could increase the gradient of temperature between the embryo's body and the incubator, leading in turn to more heat dissipated by evaporation and thus to a higher weight loss before hatching (Gildersleeve, 1984). In this study, the weight loss from the eggs incubated at 37.2°C was rather higher than at 36.0 and 36.6°C.

Decuyper and Michels (1992) noted that older embryos were more affected by low temperatures. In the present study the late embryonic mortality rate in the eggs incubated at 37.2°C temperature was found to be high.

The incidence of congenital deformities was generally excessive; the incubator temperature was implicated as a potential cause (Deeming, 1993; Stewart, 1996). Eye abnormalities were observed in four of the chicks hatched at 37.2°C. These findings are in accordance with the presented results.

Swart and Rahn (1988) reported that the incubation period for ostriches ranged between 40 and 44 days, 42 days being optimal. Jarvis *et al.* (1985) found out that the incubation period was prolonged by 2–3 days when ostrich eggs were incubated at 35°C compared with those incubated at 36.0°C while Deeming *et al.* (1993) determined that the incubation period was shortened by 3 days by raising the temperature from 36.0 to 37.0°C in single-stage incubators. Similarly, Stewart (1996) found out that every 0.6°C increase in temperature shortened the incubation period by 1 day in ostrich eggs. This researcher reported that the incubation period was 2 days shorter in the eggs incubated at 37.2°C. This result is in accordance with the results of our research.

Eggs at different developmental stages are incubated in the machines during weekly settings due to the difficulties of obtaining a sufficient number of eggs especially at the beginning and at the end of the laying period (Ar, 1996). Essentially, one should try to incubate the ostrich eggs in single-stage machines for high hatchability and optimum incubation period. The presence of embryos at different developmental stages being together in the multi-stage machines makes the temperature factor even more important (Deeming *et al.*, 1993). This research indicates that the temperature is of great importance in the incubation of ostrich eggs, even a slight increase in the temperature of the incubator significantly affects the hatchability results. Therefore it is important to keep the temperature at the optimum level and avoid the temperature fluctuations in the artificial incubation of ostrich eggs. For the greatest success of incubation, the adjustment of the temperature should not be more than 36.6°C in the incubator.

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ABSTRAKT

Vliv různých inkubačních teplot na inkubaci vajec pštrosa (*Struthio camelus*)

Cílem výzkumu bylo stanovení vlivu různé inkubační teploty při inkubaci pštrosích vajec. V průběhu snáškového období bylo inkubováno celkem 282 vajec při teplotě 36,0, 36,6 a 37,2 °C ve třech plně automatizovaných programovatelných inkubátorech, které byly na farmě k dispozici. Teplota použitá při umělé inkubaci pštrosích vajec významně ovlivnila líhivost oplodněných vajec, embryonální mortalitu a dobu inkubace ($P < 0,01$). Úbytek hmotnosti vajec se zvyšoval s vyšší teplotou ($P < 0,05$). V této studii jsme zaznamenali vysokou rannou a pozdní embryonální mortalitu vajec inkubovaných při vysoké teplotě ($P < 0,01$). U čtyř kuřat jsme při teplotě 37,2 °C zjistili oční abnormality.

Klíčová slova: inkubace; teplota; pštros (*Struthio camelus*)

Corresponding Author

Prof. Dr. Aydin Ipek, Uludag University, Agricultural Faculty, Department of Animal Science, 16059 Gorukle, Bursa, Turkey
Tel. +90 224 442 89 70, fax +90 224 442 81 52, e-mail: bilgehan@uludag.edu.tr

Radiation hybrid mapping of the porcine *AMPD1*, *PKLR* and *CHRNA2* genes to chromosome 4

A. STRATIL¹, M. VAN POUCKE², L. J. PEELMAN², M. KOPEČNÝ¹

¹Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Liběchov, Czech Republic

²Department of Animal Nutrition, Genetics, Breeding and Ethology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

ABSTRACT: Three porcine genes, *AMPD1* (encoding muscle isoform of adenosine monophosphate deaminase), *PKLR* (encoding liver and red blood cell isozymes of pyruvate kinase) and *CHRNA2* (encoding neuronal nicotinic acetylcholine receptor, beta 2 polypeptide), were assigned to the radiation hybrid map of chromosome 4 using PCR amplification on clones of the IMpRH panel. *PKLR* was mapped between markers *SW270* and *SW512*, *CHRNA2* between *SW512* and *SW2435*, and *AMPD1* was mapped more distally from *SW512* than *CHRNA2*. The positions and order of the genes are in good agreement with the porcine linkage map. Their orthologues are located on human chromosome 1 in reverse order. A fragment of the porcine *CHRNA2* gene was cloned and sequenced for the first time.

Keywords: pig; radiation hybrid mapping; *AMPD1*; *PKLR*; *CHRNA2*; DNA sequence

In recent years numerous QTL studies in pigs have been performed and many of them have mapped QTLs to chromosome 4. There are QTLs for growth, fat deposition, length of small intestine, carcass composition, carcass length, fat deposition traits, muscling traits, muscle mass, etc. (e.g. Andersson *et al.*, 1994; Marklund *et al.*, 1999; Paszek *et al.*, 1999; De Koning *et al.*, 2001; Knott *et al.*, 2002; Geldermann *et al.*, 2003). However, genes responsible for the effects have not been identified yet and the chromosome segments have not been studied adequately for Type I genes. Efforts have been devoted to study and map genes within this region to facilitate refinement of cytogenetic, radiation hybrid and linkage maps with the prospect of detailed mapping or positional cloning of QTLs, or selection of candidate genes for a trait of interest.

The assignment of genes using radiation hybrid (RH) panels is an efficient way to map genes and markers as well as to integrate the linkage and

cytogenetic maps of a species (Yerle *et al.*, 1998; Hawken *et al.*, 1999). RH mapping enhances linkage map reliability because of unambiguous determination of marker order and provides a powerful tool for fine mapping (Yerle *et al.*, 1998).

In this study we have RH mapped three genes (*AMPD1*, *PKLR* and *CHRNA2*) from the human chromosome 1 region that is orthologous to the porcine chromosome 4 region (Goureau *et al.*, 1996), where numerous QTLs have been detected. Porcine *AMPD1* and *PKLR* were mapped earlier by linkage analysis (Stratil *et al.*, 2000; Knoll *et al.*, 2000) while porcine *CHRNA2* has not been studied yet.

AMPD1 encodes the M (muscle) isoform of adenosine monophosphate deaminase (EC 3.5.4.6) and *PKLR* encodes the L (liver) and R (red blood cell) isozymes of pyruvate kinase (EC 2.7.1.40). *CHRNA2* (neuronal nicotinic acetylcholine receptor, beta 2 polypeptide) is one of the nicotinic acetylcholine receptors that are members of a super-

family of ligand-gated ion channels that mediate fast signal transmission at synapses (Groot Kormelink and Luyten, 1997). Rempel *et al.* (1998) assigned the human *CHRNA2* gene to chromosome 1q21.

MATERIAL AND METHODS

Radiation hybrid panel

Radiation hybrid mapping was performed using the INRA-University of Minnesota porcine Radiation Hybrid panel (IMpRH) (Yerle *et al.*, 1998; Hawken *et al.*, 1999). A panel of 90 hybrid clones was screened by PCR. The results were analysed by the IMpRH mapping tool available at the IMpRH server (<http://imprh.toulouse.inra.fr>). A radiation hybrid map was built using the RHMAP3.0 statistical package (Lange *et al.*, 1995). Analyses were performed under the equal retention probability model. Two-point distances were calculated between all markers using the RH2PT program. Linkage groups were defined using a lod score threshold of 4.8. Multipoint analyses were then performed using RHMAXLIK.

Polymerase chain reaction (PCR)

AMPD1. PCR was performed using the conditions described by Stratil *et al.* (2000). Pair 2 primers (Forward: 5'-GAA GAA CAA TCC TCA CCG GGA CTT-3'; Reverse: 5'-GGC TGC GTG GAT ATG GGT GTC-3') were used and a fragment of ~1.3 kb was amplified.

PKLR. PCR was performed according to Knoll *et al.* (2000). The primers (Forward: 5'-GGA GGC TKC CTT CAA GTG CTG T-3'; Reverse: 5'-RAG CAA GGG GAA GAC TCC TCG G-3'; K stands for G or T, and R for A or G) were used to amplify an 827-bp fragment.

CHRNA2. To amplify a fragment of porcine *CHRNA2* PCR primers (Pair 1) were designed using the human genomic sequence (EMBL accession number AF077186; Lueders *et al.*, 1999), exons 4 and 5. The Pair 1 primer sequences were as follows: Forward: 5'-CTC CCA GAT GTG GTC CTG TAC-3'; Reverse: 5'-CGG CGA ATG ATG AAG TCA TAC-3'. PCR was carried out in 25 µl containing 100 ng porcine genomic DNA, reaction buffer, 2.0 mM MgCl₂, 2% dimethyl sulphoxide (DMSO), 200 µM of each dNTP, 10 pmol of each

primer and 1.0 U LA polymerase (Top Bio, Prague, Czech Republic). Amplification conditions were 2 min at 95°C followed by 35 cycles at 94°C (45 sec), 56°C (45 sec) and 68°C (1.5 min), with a final extension at 68°C (7 min). Two fragments (~100 bp and ~100 bp) were observed after agarose gel electrophoresis. The ~100-bp fragment was cloned (pUC18; *Escherichia coli* DH5α) and sequenced using an ALFexpress Sequencing System (Pharmacia Biotech, Uppsala, Sweden). A new pair of primers (Pair 2) was designed from the determined porcine sequence: Forward: 5'-GGA GGA TTG GGC AGG ATC AAA-3'; Reverse: 5'-GTC GAT CTC CGT GCG GTC ATA-3'. The PCR conditions were slightly modified (concentration of MgCl₂ was 1.5 mM, annealing temperature 55°C, time of amplification 1 min, and 30 cycles were performed). A single fragment of ~670 bp was amplified.

RESULTS AND DISCUSSION

PCR amplification and sequencing of *CHRNA2*

The larger PCR fragment (~100 bp) of porcine *CHRNA2* amplified with the Pair 1 primers was cloned and sequenced. The sequence is homologous to parts of exons 4 and 5 and intervening intron of human *CHRNA2* (EMBL AF077186). The porcine *CHRNA2* sequence, without the Pair 1 primer sequences, has been deposited in the EMBL database under accession number AJ557775. The porcine exon sequences are 91% identical with the corresponding human exon sequences, and the deduced porcine amino acid sequence is 99% identical with the human sequence. The Pair 2 primers (porcine-specific) were designed from the porcine nucleotide sequence and used for RH mapping (for details, see Material and Methods).

RH mapping of *PKLR*, *CHRNA2* and *AMPD1*

The positions of *PKLR*, *CHRNA2* and *AMPD1* on the IMpRH map are shown in Figure 1.

PKLR maps to chromosome 4 between *SW270* (28 cR; lod score = 9.7) and *SW512* (67 cR; lod score = 4.27).

CHRNA2 and *AMPD1* map to the same region on chromosome 4, between *SW512* and *SW2435*,

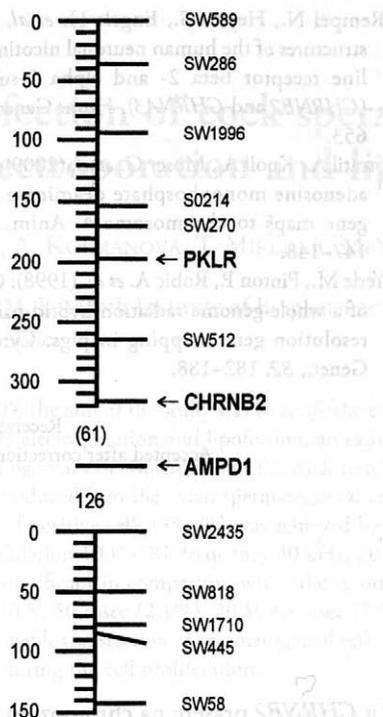


Figure 1. A partial RH map of porcine chromosome 4 showing the positions of *PKLR*, *CHRN2* and *AMPD1*; distances are in centiRays (cR)

according to the multipoint analysis. According to the 2-pt analysis *CHRN2* is most significantly linked with *SW512* (52 cR; lod score = 5.85). However, *AMPD1* is located slightly farther and because of the lack of markers in this region the distance to the closest known marker, *SW512*, is 113 cR (lod score = 1.6). Because a lod score of 4.8 was chosen as evidence for linkage (Hawken *et al.*, 1999), *AMPD1* is shown as an unlinked marker in Figure 1.

The position and orientation of the porcine *PKLR* and *AMPD1* genes on the RH map are in good agreement with the results of linkage mapping (Knoll *et al.*, 2000). In the Hohenheim Meishan × Piétrain pedigree (Geldermann *et al.*, 1999) the distance between the two genes is 13.2 Kosambi cM. Linkage assignment is not available for *CHRN2* as no useful polymorphism was found in the PCR fragment. However, when compared with the order of the genes on human chromosome 1, the order corresponds to *PKLR* – *CHRN2* – *AMPD1* (considering the reverse order as the porcine chromo-

some 4 segment is inverted when compared with the orthologous chromosome 1 segment).

In summary, three new genes have been added to the RH map of porcine chromosome 4 that are located in the QTL region for a number of carcass and growth traits (Geldermann *et al.*, 2003). These genes can be considered candidate genes or included in a set of markers for detailed QTL mapping.

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ABSTRAKT

Radiační hybridní mapování genů *AMPD1*, *PKLR* a *CHRNA2* prasete na chromozom 4

Tři geny prasete, *AMPD1* (kódující M izoformu adenosinmonofosfátdeaminázy), *PKLR* (kódující L /jaterní/ a R /erytrocytární/ izoenzymy pyruvátkinázy) a *CHRNA2* (kódující beta-2 podjednotku cholinergního receptoru), byly lokalizovány na RH mapu chromozomu 4 pomocí PCR amplifikace klonů IMpRH panelu. *PKLR* byl mapován mezi markry *SW270* a *SW512*, *CHRNA2* mezi *SW512* a *SW2435*, a *AMPD1* byl lokalizován dále od *SW512* než *CHRNA2*. Polohy a pořadí genů jsou ve shodě s vazbovou mapou prasete. Jejich orthology jsou lokalizovány na lidském chromozomu 1 v obráceném pořadí. Fragment genu *CHRNA2* prasete byl klonován a sekvencován poprvé.

Klíčová slova: prase; radiační hybridní mapování; *AMPD1*; *PKLR*; *CHRNA2*; DNA sekvence

Corresponding Author

Doc. Ing. Antonín Stratil, DrSc., Ústav živočišné fyziologie a genetiky, Akademie věd České republiky,
277 21 Liběchov, Česká republika
Tel. +420 315 63 95 31, fax +420 315 69 71 86, e-mail: stratil@iapg.cas.cz

Transfection of cock spermatogonial cells via electroporation and lipofection

J. KALINA, A. KOLMANOVÁ, T. MIKUŠ, A. MIČÁKOVÁ, P. TREFIL

BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, a.s., Jílové near Prague, Czech Republic

ABSTRACT: The aim of the study was to verify the efficiency of transfection of spermatogonial cells via electroporation. Using electroporation and lipofection, an expression vector carrying the marker gene GFP was transfected into spermatogonial cells isolated from the cock testes. The obtained results show that foreign DNA (pEGFP-C1 plasmid) introduced into the avian spermatogonial cells can consequently express the pEGFP protein. The highest number of positive cells (35.8%) was achieved by the following electroporation parameters: total volts 400 V, percent modulation 100%, RF frequency 40 kHz, 20 msec burst duration, number of bursts 1. These results were statistically significant in comparison with other groups of transfected cells where different important parameters were used: 10 V, 50 msec (2.1%), 20 V, 40 msec (3.9%), 200 V, 30 msec (4.5%), 400 V, 5 msec (5.3%). In the experiment, stable transfection of spermatogonial cells was achieved, and the expression of GFP was observed within one month during the cell proliferation.

Keywords: poultry; spermatogonial cells; pEGFP-C1; transfection, lipofection; electroporation

Cell transformation is an essential process for molecular, cellular and genetic research mainly in creating transgenic animals.

One of the most important categories of cell transformation experiments is transfection – the introduction of foreign DNA into a recipient eukaryotic cell with subsequent integration into chromosomal DNA of the recipient eukaryotic cell (Etches *et al.*, 1997). Liposome-mediated transfection (lipofection) is a simple and powerful technique for DNA transfer into cultured cells (Chiang, 1991; Dickinson, 2002). Lipofection is suitable for the transfection of primary cells with either circular or linear DNA for stable and transient transfection. Disadvantages consist in possible toxicity (Lamb, 1993).

The underlying mechanism is not completely understood, but it is known that the positively charged liposomes form aggregates with negatively charged DNA. The aggregates carrying a positive net charge are then attracted to the negatively polarized cell membrane and enter the cells via endocytosis (Buchberger, 1996).

Transfection experiments can be performed in two ways: transient transfection is usually followed by the gene expression driven from unintegrated foreign DNA. On the other hand, stable transfection requires foreign DNA to integrate stably into the host cell chromosomes and to replicate together with the host genome. Other methods have been developed to transport molecules (that are normally prohibited from crossing the cell membrane into the cytoplasm or nucleus of pluripotent cells): via microparticle bombardment (Muramatsu, 1997), electroporation (Aritomi, 2000), via retrovirus (Nagano, 2000), and virofection-mediated transfection (Flamant *et al.*, 1994). Instead of the virus-mediated transfer of foreign DNA, the nonviral vectors such as direct injection of foreign DNA or lipid-mediated systems are used but the transfection efficiency is relatively low (Thoraval *et al.*, 2000).

Electroporation – the application of an electric field to cells creates temporary pores in the cell membrane. The pores allow exogenous molecules

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to cross into the cytoplasm or the nucleus (Rae and Levis, 2002).

Electroporation parameters are total volts, percent modulation, DC amplitude, RF frequency, burst duration, number of bursts and burst interval. When the transfection method is chosen, the next task consists in optimization of these parameters (Templeton, 1997).

The purpose of this study was to achieve the successful *in vitro* transfection of chicken spermatogonial cells using the method of electroporation and lipofection.

MATERIAL AND METHODS

Isolation of cells

Spermatogonial cells were isolated from testes of mature avian males (White Leghorn). The testes were removed from the body cavity and washed in PBS (Phosphate Buffered Saline). A part of the testis tissue (3 × 3 mm), without *tunica albuginea*, was sieved through the crowded grid, and separated cells were collected into a Petri dish with the M 199 medium (Sigma).

The density and physiognomy were evaluated. Cell densities from 10^5 to 10^7 were used for transfection experiments. After diluting the cells into a correct cell concentration (10^5 to 10^7 /ml), the transfection was carried out.

The vector pEGFP-C1 (Clontech) was used for the expression of EGFP protein in the cells of interest as a transfection marker. EGFP is a gene coding the red-shifted variant of wild-type GFP – Green Fluorescent Protein-(1-3) from *Aequorea victoria*, which has been optimized for brighter fluorescence and higher expression in cells. Excitation maximum is 488 nm, emission maximum 507 nm. The length of the insert is 4.7 kb and consists of 238 amino acids. The optimal ratio of the lipofection reagent – plasmid used for transfection was 3 : 1. The final concentration of the plasmid in spermatogonial cell suspension was 1.25 µg/1 ml.

Lipofection

In our experiment, the Lipofectin® Reagent (GIBCO BRL®) – liposome formulation of the cationic lipid N-[1-(2,3-dioleoyloxy)propyl]/n,n,n-trimethyl ammonium chloride (DOTMA) and

dioleoyl phosphatidylethanolamine (DOPE) was used.

For transfection, the circular pEGFP-C1 plasmid was applied. The solution of Lipofectin-plasmid (ratio 3 : 1) reacted together for 10 minutes minimally.

Electroporation

100 µl of the medium (M 199, Sigma) containing a correct cell concentration (10^5 to 10^7 /ml) of cells were put into the electroporator cuvette (Bio Rad Pulsertm) Cuvette, 0.1-cm gap) and the solution of Lipofectin-plasmid (ratio 3 : 1) was added. The cuvette was inserted into the shocking chamber of an electroporator machine (BioRad Gene Pulser® II) where the requested programme was installed. The parameters to optimize the use of the RF Gene Pulser module are voltage, burst duration, percent modulation, radio frequency, number of bursts and burst interval. The following electroporation parameters were tested in the experiments: group 1 (10 V, 50 msec), group 2 (20 V, 40 msec), group 3 (200 V, 30 msec), group 4 (400 V, 5 msec), group 5 (400 V, 10 msec), group 6 (400 V, 20 msec). Other parameters were constant: percentage of modulation 100%, RF frequency 40 kHz, number of burst 1.

All the experiments were performed at room temperature. The cuvettes with a gap of 0.1 cm for electroporation were used and samples were in a high-resistance medium.

Cell culture and evaluation

After electroporation, the cells were removed from the cuvette to a Petri dish containing the outgrowth medium M 199 (Sigma) with Earle's salt enriched with L-glutamine and NaHCO₃ supplemented with 10% of Foetal Bovine Serum (GibcoBRL), 2% of Chicken Serum (GibcoBRL) and antibiotics (Gentamycin Solution, Sigma Cell Culture™, working concentration 50 mg/l). Thereafter, spermatogonial cells were placed on a chicken fibroblast monolayer inactivated by gamma radiation. The cells were incubated in the thermostat (Sanyo, CO₂ incubator MCO-17 AIC) in a Petri dish (Nunc®), diameter 35 mm, at 37°C and 5% CO₂. The length of incubation was about one month. Cell cultures were checked under a fluorescent microscope (Olympus IX50, U-RFL-T) with

the excitation wavelength of 488 nm and emission wavelength of 507 nm, magnification 400 \times , and gene expression was analysed.

The χ -test was used for a statistical comparison of the percentage of GFP positive spermatogonial cells with different electroporation conditions (Likeš and Machek, 1983).

RESULTS

The best results of the transfection of spermatogonial cells (31.5% and 35.8% of positive cells) were achieved in 5-day-old cultures of spermatogonial cells (Figure 1).

In this case, the following electroporation parameters were used: 400 V, 100% modulation, RF frequency 40 kHz, 10 and 20 msec burst duration, number of bursts 1. The final concentration of plasmid in 1 ml of the spermatogonial cell suspension was 1.25 μ g.

The ratio of plasmid : lipofectin was 1 : 3. The percentage of positive cells in other groups (1–4) was low and statistically significant ($P < 0.01$) – see Table 1.

We managed to observe GFP positive cells in our cultures within one month. During this period, we noticed a gradual decrease of GFP positive cells after each trypsination of cells.

DISCUSSION

Several methods were tested to acquire the most effective stable transfection.

Table 1. Comparison of different electroporation conditions for transfection of spermatogonial cells (5 days after electroporation)

Group No.	Positive cells (%)
1. 10 V, 50 msec	2.1 ^A
2. 20 V, 40 msec	3.9 ^A
3. 200 V, 30 msec	4.5 ^A
4. 400 V, 5 msec	5.3 ^A
5. 400 V, 10 msec	31.5 ^B
6. 400 V, 20 msec	35.8 ^B

There were no statistically significant differences in the percentage of positive spermatogonial cells between groups 1–4 and 5–6. The highest percentage of positive spermatogonial cells (31.5% and 35.8%) was achieved in groups 5 and 6 and these groups were statistically significant as compared with groups 1–4. All the cell cultures were checked for 5 days after electroporation

Optimum electroporation parameters were important in order to obtain reliable and standardized cell culture and to find optimal transfection conditions. Successful transfection consists in a high level GFP expression in the cell population. The purpose is to maximize the number of cells that are viable after transformation and are able to incorporate an exogenous molecule of interest into the cytoplasm or nucleus. Fluorescent proteins seem to be an ideal marker for studying the cell morphology. During the electroporation process, the voltage acting on the cell membrane should be high enough to cause a localized dielectric breakdown of the membrane

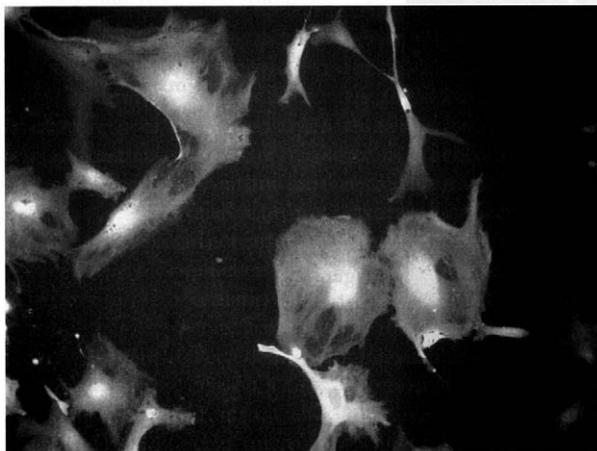


Figure 1. Five-days-old spermatogonial cells ($\times 200$) producing green fluorescent protein transfected by the Liposome-plasmid pEGFP-C1 complex using electroporation with the following parameters (400 V, 100% modulation, 40 kHz modulation, 20 msec, 1 \times). After transfection spermatogonial cells were placed on an inactivated (via gamma radiation) chicken fibroblast monolayer

and to create pores that allow compounds to enter the cells. In our laboratory, we were able to use maximum 400 V for the cell transfection, which was the peak parameter of the electroporator machine. The most significant parameter was the total time for which the voltage was applied – burst duration. We applied the maximum of 20 msec because a higher value of the burst duration leads to cell death.

Hong *et al.* (1998) described the electroporation of gonadal primordial germ cells (gPGCs) that resulted in 80% transfection efficiency, compared to 14% efficiency observed when liposomes were used. These results indicated that electroporation was a suitable method of transfection of avian gPGCs for the goal of producing transgenic poultry.

In our experiment with spermatogonial cells, the maximum of 35.8% of positive cells was achieved using the set of electroporation parameters of 400 V and 20 msec. In this case, the expression was predominantly stable – the fluorescent protein was visible for a period of more than one month in the dividing and growing cell culture (Figure 2). We are convinced that the lower level of positive cells after transfection is associated with the specific life cycle of spermatogonial cells and their division.

Other important factors affecting transformation efficiency are tissue culture conditions characteristic of the exogenous molecule, transfection reagent and tools used for transformation (Ostresh, 1999). Further significant variables for successful transfection are DNA quality, transfection enhancing reagents, plasmid size, pH of the vehicle liquid, osmolarity, salinity, cell type. Cell growth factors,

cell density and Log phase of the growth curve have an important influence on successful transfection of cells. The other attributes should be considered when optimization of the transformation method is undertaken (Ostresh, 1999).

Efficient transfection was obtained with a low cellular density of 10^5 cells per 35-mm well (Lascombe *et al.*, 1996). Ostresh (1999) recommends the cell density of 10^5 to 10^7 for transfection. The use of the density of 10^6 of spermatogonial cells per 35-mm well and 1.0–1.6 micrograms of plasmid per 1 ml of the cell suspension was considered suitable in our experiments (Trefil *et al.*, 2002). Brazolot *et al.* (1991) succeeded in transfecting the chicken blastodermal cells by lipofection with a mixture of 6.2 micrograms of lipofectin and 1.55–3.1 micrograms of DNA per 1 ml DMEM. Lascombe *et al.* (1996) described efficient transfection using the following conditions: the value of six for the ratio of lipofectin to DNA, low cellular density (10^5 cells per 35-mm well), time of subculture (48 h before lipofection) and lipofection duration – 12 hours.

Kalina (2002) obtained successful gene transfection of chicken blastodermal cells using the following electroporation parameters: 225–250 V, 100%, 40 KHz, 2 msec burst duration and 1 sec burst duration, 1–2 bursts. The gene expression was manifested by cells emitting green light under the fluorescent microscope 48 hrs after electroporation.

In our experiments, the best results were obtained in transfection of spermatogonial cells (35.8% of positive cells) when the lipofectin-plasmid complex (concentration of plasmid in cell suspension was

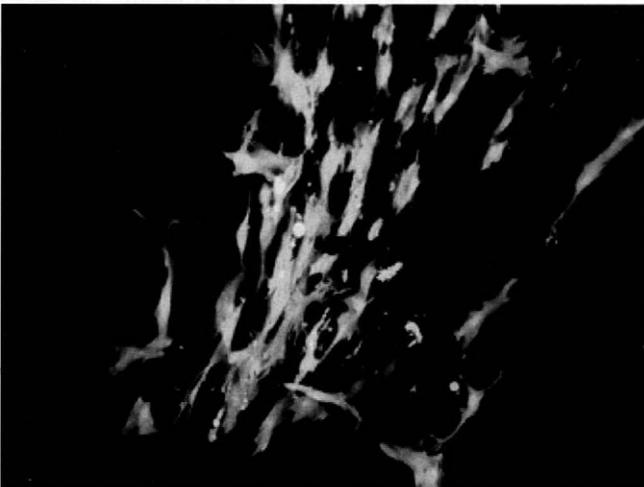


Figure 2. One-month-old spermatogonial cells producing green fluorescent protein ($\times 400$) transfected by the Liposome-plasmid GFP-C1 complex using electroporation with the following parameters (400 V, 100% modulation, 40 kHz, 20 msec, 1 \times)

1.25 µg/ml) was used together with the electroporation following these parameters: total volts 400 V, 100% modulation, RF frequency 40 kHz, 20 msec burst duration and number of bursts 1 (Table 1).

This result seems to be valuable in the process of chicken transgenesis.

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ABSTRAKT

Transfekce kohoutích spermatogoniálních buněk pomocí elektroporace a lipofekce

Cílem práce bylo ověřit účinnost transfekce spermatogoniálních buněk metodou elektroporace. Jako transfekční marker byl použit gen pro GFP. Ten byl pomocí elektroporace a lipofekce transfekován do směsi spermatogoniálních buněk izolovaných z kohoutích varlat. Získané výsledky naznačují, že vnesená cizorodá DNA (pEGFP-C1) do ptačích

spermatogoniálních buněk může exprimovat příslušné proteiny (GFP). Nejvyššího počtu úspěšně transfekovaných buněk bylo dosaženo při použití těchto elektroporačních parametrů: celkové napětí 400 V, modulace 100 %, RF frekvence 40 kHz, délka dávky 20 msec, počet dávek 1. Takto získané výsledky byly statisticky signifikantní v porovnání s ostatními skupinami transfekovaných buněk, kde byly použity odlišné elektroporační parametry: 10 V, 50 msec (2,1 %), 20 V, 40 msec (3,9 %), 200 V, 30 msec (4,5 %), 400 V, 5 msec (5,3 %). V experimentu bylo dosaženo dlouhodobé transfekce spermatogoniálních buněk, exprese GFP byla sledována až po dobu jednoho měsíce.

Klíčová slova: drůbež; spermatogoniální buňky; pEGFP-C1; transfekce; lipofekce; elektroporace

Corresponding Author

Ing. Pavel Trefil, Dr.Sc., BIOPHARM, Výzkumný ústav biofarmacie a veterinárních léčiv, a.s., Pohoří-Chotouň,
252 49 Jílové u Prahy, Česká republika
E-mail: trefil@bri.cz

Analysis of slaughtering value of diploid and triploid population of tench (*Tinca tinca*, Linnaeus 1758)

H. BUCHTOVÁ¹, Z. SVOBODOVÁ^{1,2}, M. FLAJŠHANS², L. VORLOVÁ¹

¹Veterinary and Pharmaceutical University, Brno, Czech Republic

²University of South Bohemia at České Budějovice, Research Institute of Fish Culture and Hydrobiology, Vodňany, Czech Republic

ABSTRACT: The goal of this study was to determine differences in weight (W_{abs}) C-of carcass_{without head, fins and viscera} and waste body parts (H-head_{without gills}, F-fins both paired and unpaired, AF- also abdominal fat in tench of T_{3+} age) and organs (G-gills, V-viscera_{sum}, of them: HP-hepatopancreas, also S-spleen in tench of T_{3+} age) and their ratios in fish weight (W_{rel}) between diploid ($2n$) and triploid ($3n$) tench of the same origin kept in a communal stock in relation to sex (female – F vs. male – M) and age (three-year old tench in spring – T_3 and in autumn – T_{3+}). Analyses of 137 siblings in total were made. Control group consisted of 72 diploid specimens (39 F and 33 M) and experimental group consisted of 65 triploid specimens (38 F and 27 M). The effect of ploidy level was reflected in significantly higher values of W_{abs} (C, H, G, F, V, HP) in triploid females at T_3 and T_{3+} age ($P < 0.001$) and value of W_{abs} (S, AF) in triploid females at T_{3+} age. Triploid females at T_3 age showed significantly higher CW_{rel} ($P < 0.01$) and lower VW_{rel} ($P < 0.001$), those at T_{3+} had significantly higher AFW_{rel} ($P < 0.001$). Triploid males at T_3 had significantly higher W_{abs} (C, H also at T_{3+} , G, F, V, HP) ($P < 0.001$) compared to diploids, due to the effect of ploidy level. The effect of sex was expressed by significantly higher values of CW_{rel} ($P < 0.01$) and FW_{rel} ($P < 0.001$) in diploids at T_3 and T_{3+} age as well as by lower $VW_{abs,rel}$ ($P < 0.001$) of males. Diploid males at T_3 age were also found with significantly lower W_{abs} (H, G) ($P < 0.001$) and those at T_{3+} were found with lower HPW_{abs} . In triploids at T_3 and T_{3+} age, the effect of sex resulted in significantly higher values of W_{abs} (C, H, G, V) ($P < 0.001$) as well as lower FW_{rel} ($P < 0.001$) of females. Triploid females at T_3 age were found with significantly higher HW_{rel} ($P < 0.05$) and those at T_{3+} also with significantly higher W_{abs} (HP, S, AF) ($P < 0.001$) and AFW_{rel} . The weights of carcass (CW_{abs}), body parts (HW_{abs} , FW_{abs}) and organs (GW_{abs} , HPW_{abs}) were found significantly enhanced in relation with age ($P < 0.001$) in all tench groups under study.

Keywords: diploid and triploid tench; genome polyploidy; absolute and relative weight; head; fins; gills; viscera; abdominal fat

Experimental studies focused on waste body parts (head, fins, abdominal fat) and organs of fish (gills, viscera) are rather scarce. Sporadic results of research on these parts of the fish organism are mentioned only marginally as a part of other topical studies.

In tench (*Tinca tinca* L.), Zięcik and Sławiński (1965) dealt among others with observations of

some of these parameters (gonads, liver, soft waste, hard waste) in relation to sex, and a study of Jirásek and Laudát (1984) mentioned the parameters of head, fins, skin, gonads and hepatopancreas. While Zięcik and Sławiński (1965) examined the mean weight of the parts, Jirásek and Laudát (1984) also mentioned their ratio in the fish weight. However,

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the results in these papers were not tested statistically in a sufficient way.

The culture of diploid tench and of chromosomally manipulated triploid tench in the Czech Republic as well as scientific studies aimed at differences between both populations enabled us to assess some of these waste parts of the tench organism also in relation to the ploidy level. This research, seemingly not important for the fish culture practice from the production aspect, cannot be replaced by any other observations if we wish to understand anatomical and physiological differences between intact- and triploid tench. All studies focused in this direction are therefore indispensable and take a specific position in research on aquaculture products.

This study is a part of extensive research on diploid and triploid populations of tench (Flajšhans *et al.*, 1993; Kvasnička and Flajšhans, 1993; Flajšhans, 1997a,b; Svobodová *et al.*, 1998, 2001; Sedláček, 1999) conducted at the University of South Bohemia, Research Institute of Fish Culture and Hydrobiology at Vodňany (USB RIFCH). The USB RIFCH initiated a co-operation with the Faculty of Veterinary Hygiene and Ecology, Veterinary and Pharmaceutical University in Brno. Research on diploid and triploid tench at this workplace (Buchtová and Vorlová, 2002) was focused on their biometric and weight parameters and relevant indices (Buchtová *et al.*, 2003). These authors also experimentally studied parameters associated with nutritional assessment of tench flesh, such as the basic chemical composition of their flesh and quantitative and qualitative assessment of amino-acid composition of muscle proteins. These results have not been published yet.

The goal of this study was to determine differences in the weight (W_{abs}) of waste body parts (head-without gills, fins_{both paired and unpaired}, abdominal fat also in tench of T_{3+} age) and organs (gills, viscera_{sum}), of them: hepatopancreas, also spleen in tench at T_{3+} age) and their ratio in fish weight (W_{rel}) between diploid ($2n$) and triploid ($3n$) tench of the same origin, in relation to sex (female – F vs. male – M) and age (three-year old tench in spring – T_3 and in autumn – T_{3+}).

MATERIAL AND METHODS

Tench (*Tinca tinca*, L.) populations were established at a hatchery of the Department of Fish

Genetics and Breeding at Vodňany, Research Institute of Fish Culture and Hydrobiology of the University of South Bohemia in 1998 by mass propagation as described in Buchtová and Vorlová (2002). Triploidy ($3n$) was induced by means of cold shock following the protocol of Flajšhans and Linhart (2000).

Diploid and triploid larvae were nursed separately in pond monoculture. At the end of the 1st growing season, diploid fry was given a group-mark by means of freeze-branding while triploid fry was left unmarked. Both groups (300 specimens each) were stocked into a 0.2 ha experimental earthen pond for a communal test in monoculture until 2001. Feeding was based upon regularly checked production of natural food. Pollard was administered to tench fry as supplemental feeding, larger fish were given cereals.

Prior to checking (March 27, 2001 and October 10, 2001), fish were harvested and maintained in tanks with original pond water under stress-eliminating conditions (O_2 saturation above 80%, constant water temperature).

Altogether 137 siblings of tench were studied. Control group consisted of 72 diploid specimens (39 females – F, 33 males – M) and experimental group consisted of 65 triploid specimens (38 F, 27 M). The experiment was carried out in two periods. The first one involved 76 specimens of three-year-old tench before the onset of adult period (T_3 , harvested in March 27, 2001) with control group of 37 diploid specimens (19 F, 18 M) and experimental group of 39 triploid specimens (19 F, 20 M). The second period involved 61 specimens of three-year-old tench after the first reproductive cycle (T_{3+} , harvested in October 10, 2001) with control group of 35 diploid specimens (20 F, 15 M) and experimental group of 26 triploid specimens (19 F, 7 M).

The age of fish was determined directly from hatching data in breeding records.

The sex of tench was determined upon expressive sexual dimorphism (120 specimens), according to pathological and anatomical examination of gonads (10 specimens) and according to histological preparations (7 specimens) according to Flajšhans *et al.* (1993) and Kvasnička and Flajšhans (1993). Diploid specimens were identified according to freeze-branded marks on skin.

Ploidy level was checked by means of flow cytometry as relative DNA content in peripheral blood cells sampled by the puncture of caudal vessel (*vena caudalis*). Blood samples were processed for flow

cytometry according to Vindelov and Christensen (1990).

Weight (W_{abs}) was checked for the following waste parts and for their ratio in the fish weight (W_{rel}): H-head_{without gills} (head hereinafter), F-fins_{both paired and unpaired} (fins hereinafter), G-gills, V-viscera_{sum}, HP-hepatopancreas and also AF-abdominal fat and S-spleen in tench at T_3 , age. The weight of abdominal fat of each specimen was determined gravimetrically after preparation from the surface of the viscera.

For a complete review, the study contains statistically processed results (ANOVA, Statgraphics 5.0) of other two parameters – weight of carcass (CW_{abs} , where C is body without head, fins and viscera) and its ratio in fish weight (CW_{rel}).

Tables 1 and 2 are complemented with the values of gonad weight (gonad W_{abs}), gonadosomatic index (GSI) and hepatosomatic index (HSI) with statistical evaluation (ANOVA, Statgraphics 5.0) of the effect of ploidy level and of sex on these parameters in groups of tench at T_3 and T_3 , age according to Buchtová *et al.* (2003). The effect of age (ANOVA, Excel 97) on these parameters according to the same author is given in Figures 1 and 2. The results of checking the effects of ploidy level, sex and age on these parameters will not be discussed in this study.

Basic statistical values (mean, S.D.) of the observed parameters were processed in Excel 97. The evaluation of statistical significance of the results was performed on a static level (separately for T_3

Table 1. Summarized data on the carcass, waste body parts and organs of 3-years-old diploid ($2n$) tench and their artificially induced triploid ($3n$) siblings (March 2001)

Index	F $2n$ ($n = 19$) mean \pm S.D.	M $2n$ ($n = 18$) mean \pm S.D.	F $3n$ ($n = 19$) mean \pm S.D.	M $3n$ ($n = 20$) mean \pm S.D.	Statistical significance
CW_{abs} (g)	92.93 \pm 46.97 ^{ab}	52.33 \pm 15.26 ^a	182.91 \pm 48.83 ^c	131.98 \pm 43.72 ^b	$P < 0.001$
CW_{rel} (%)	65.89 \pm 1.68 ^a	68.24 \pm 1.90 ^b	67.94 \pm 1.90 ^b	68.58 \pm 2.26 ^b	$P < 0.01$
HW_{abs} (g)	19.41 \pm 8.76 ^b	10.59 \pm 2.69 ^a	37.64 \pm 9.99 ^c	24.49 \pm 7.67 ^b	$P < 0.001$
HW_{rel} (%)	14.06 \pm 1.24 ^b	13.99 \pm 1.19 ^{ab}	14.01 \pm 1.03 ^b	12.87 \pm 1.33 ^a	$P < 0.05$
GW_{abs} (g)	3.96 \pm 1.77 ^b	1.93 \pm 0.51 ^a	6.93 \pm 1.73 ^c	5.00 \pm 1.74 ^b	$P < 0.001$
GW_{rel} (%)	2.86 \pm 0.45	2.55 \pm 0.36	2.59 \pm 0.53	2.64 \pm 0.54	–*
FW_{abs} (g)	4.37 \pm 2.33 ^a	3.23 \pm 1.04 ^a	8.92 \pm 2.39 ^b	8.01 \pm 2.35 ^b	$P < 0.001$
FW_{rel} (%)	3.09 \pm 0.32 ^a	4.19 \pm 0.53 ^b	3.32 \pm 0.3 ^a	4.23 \pm 0.48 ^b	$P < 0.001$
VW_{abs} (g)	20.22 \pm 11.46 ^{ad}	8.39 \pm 2.31 ^b	32.22 \pm 8.15 ^c	22.17 \pm 6.62 ^d	$P < 0.001$
VW_{rel} (%)	14.10 \pm 1.49 ^a	11.04 \pm 1.38 ^{bcd}	12.11 \pm 1.67 ^{cd}	11.68 \pm 1.53 ^d	$P < 0.001$
Of that: ^{1,2}					
Gonad W_{abs} (g)	4.18 \pm 2.62 ^b	0.23 \pm 0.14 ^a	1.35 \pm 1.08 ^a	0.38 \pm 0.38 ^a	$P < 0.001$
GSI	2.85 \pm 0.89 ^b	0.28 \pm 0.16 ^a	0.57 \pm 0.54 ^a	0.20 \pm 0.15 ^a	$P < 0.001$
HPW_{abs} (g)	3.24 \pm 2.36 ^{ab}	1.69 \pm 0.61 ^a	6.14 \pm 1.68 ^c	4.65 \pm 0.33 ^{bc}	$P < 0.001$
HSI	2.18 \pm 0.53	2.20 \pm 0.38	2.30 \pm 0.41	2.44 \pm 0.33	–*

¹gonad W_{abs} , GSI and HSI according to Buchtová *et al.* (2003)

² W_{abs} and W_{rel} S-spleen and AF-abdominal fat were not investigated in tench at T_3 age

–*not significantly different at $P < 0.05$

Table 2. Summarized data on the carcass, waste body parts and organs of 3+ years-old diploid (2n) tench and their artificially induced triploid (3n) siblings (October 2001)

Index	F 2n (n = 20) mean ± S.D.	M 2n (n = 15) mean ± S.D.	F 3n (n = 19) mean ± S.D.	M 3n (n = 7) mean ± S.D.	Statistical significance
CW _{abs} (g)	239.30 ± 34.84 ^a	201.05 ± 34.56 ^a	504.82 ± 111.52 ^b	293.52 ± 106.54 ^a	P < 0.001
CW _{rel} (%)	65.54 ± 1.88 ^a	67.94 ± 1.54 ^b	66.94 ± 1.61 ^{ab}	66.13 ± 1.73 ^{ab}	P < 0.01
HW _{abs} (g)	56.67 ± 9.25 ^{ab}	43.23 ± 7.00 ^a	108.84 ± 22.01 ^c	70.35 ± 27.65 ^b	P < 0.001
HW _{rel} (%)	15.54 ± 1.37	14.70 ± 1.38	14.51 ± 1.18	15.75 ± 1.35	–*
GW _{abs} (g)	6.87 ± 1.48 ^a	6.13 ± 1.51 ^a	15.55 ± 4.04 ^b	10.38 ± 5.01 ^a	P < 0.001
GW _{rel} (%)	1.88 ± 0.30	2.06 ± 0.27	2.08 ± 0.34	2.25 ± 0.38	–*
FW _{abs} (g)	9.34 ± 1.66 ^a	12.26 ± 2.58 ^{ab}	20.37 ± 5.61 ^c	15.94 ± 4.31 ^{bc}	P < 0.001
FW _{rel} (%)	2.55 ± 0.20 ^a	4.14 ± 0.41 ^b	2.68 ± 0.28 ^a	3.73 ± 0.61 ^b	P < 0.001
VW _{abs} (g)	53.05 ± 10.02 ^a	33.18 ± 6.86 ^b	108.25 ± 24.49 ^c	53.80 ± 18.96 ^{ab}	P < 0.001
VW _{rel} (%)	14.49 ± 1.54 ^a	11.62 ± 2.06 ^{bc}	14.39 ± 1.48 ^{ad}	12.15 ± 1.08 ^{cd}	P < 0.001
Of that: ^{1,2}					
Gonad W _{abs} (g)	10.13 ± 2.70 ^b	1.43 ± 0.67 ^a	11.17 ± 5.53 ^b	2.72 ± 2.59 ^a	P < 0.001
GSI	2.78 ± 0.69 ^c	0.47 ± 0.17 ^a	1.52 ± 0.78 ^b	0.56 ± 0.36 ^a	P < 0.001
HPW _{abs} (g)	13.80 ± 2.78 ^b	8.59 ± 2.23 ^a	23.98 ± 4.25 ^c	12.81 ± 3.87 ^{ab}	P < 0.001
HSI	3.78 ± 0.60 ^b	2.89 ± 0.48 ^a	3.24 ± 0.52 ^a	2.95 ± 0.30 ^a	P < 0.001
SW _{abs} (g)	1.08 ± 0.50 ^a	0.91 ± 0.35 ^a	2.80 ± 1.35 ^b	1.61 ± 0.49 ^a	P < 0.001
SW _{rel} (%)	0.29 ± 0.10	0.31 ± 0.10	0.37 ± 0.14	0.37 ± 0.08	–*
AFW _{abs} (g)	4.93 ± 2.34 ^a	3.23 ± 1.91 ^a	19.89 ± 8.19 ^b	7.85 ± 5.02 ^a	P < 0.001
AFW _{rel} (%)	1.32 ± 0.50 ^a	1.05 ± 0.47 ^a	2.58 ± 0.85 ^b	1.63 ± 0.66 ^a	P < 0.001

¹gonad W_{abs}, GSI and HSI according to Buchtová *et al.* (2003)

²W_{abs} and W_{rel} S-spleen and AF-abdominal fat were not investigated in tench at T₃ age

–*not significantly different at P < 0.05

and T₃₊) related to ploidy level and sex by means of multifactorial ANOVA in Statgraphics 5.0. The effect of increasing age on the studied parameter was evaluated on a dynamic level (T₃ - T₃₊) separately for each group (F 2n, M 2n, F 3n, M 3n) at * P < 0.05; ** P < 0.01; *** P < 0.001 by means of one-way ANOVA in Excel 97.

RESULTS

The values (mean, S.D.) and statistical significance of the observed parameters in tench at T₃ age in relation to ploidy level (F 2n vs. F 3n, M 2n vs. M 3n) and sex (F 2n vs. M 2n, F 3n vs. M 3n) are given in Table 1.

The weight of carcass (CW_{abs}), body parts (HW_{abs} , FW_{abs} , VW_{abs}) and organs (GW_{abs} , HPW_{abs}) was significantly higher ($P < 0.001$) in both sexes of the triploid population of tench at T_3 age (the effect of ploidy level). The ratios of these parameters (W_{rel}) in the fish weight were not affected by the ploidy level in tench at T_3 age on the given levels of significance, except for highly significant differences in CW_{rel} ($P < 0.01$) and VW_{rel} ($P < 0.001$) in females of T_3 age.

The effect of sex on CW_{abs} in tench at T_3 age was expressed by a significantly higher ($P < 0.001$) value of CW_{abs} in triploid females; while considering CW_{rel} , a significantly higher value was registered for diploid males of T_3 age ($P < 0.01$). Further effects of sex in both ploidy levels of tench at T_3 age resulted in significantly ($P < 0.001$) higher HW_{abs} , GW_{abs} and VW_{abs} of females. In triploids of T_3 age, there was a higher ratio of HW_{rel} in females ($P < 0.05$) while the males of both ploidy levels showed significantly higher FW_{rel} ($P < 0.001$). Diploid females showed significantly higher values of VW_{rel} ($P < 0.01$). Other parameters were not affected by the sex on the given levels of significance.

The values (mean, S.D.) and statistical significance of the given parameters in tench at T_{3+} age

in relation to the ploidy level (F 2n vs. F 3n, M 2n vs. M 3n) and sex (F 2n vs. M 2n, F 3n vs. M 3n) are given in Table 2.

Triploid females of tench at T_{3+} age were found to have significantly ($P < 0.001$) higher carcass weight (CW_{abs}), body parts (HW_{abs} , FW_{abs} , VW_{abs} and AFW_{abs}) and organs (GW_{abs} , HPW_{abs} , SW_{abs}) as the effect of ploidy level. The effect of ploidy level in males at T_{3+} age was proven as significantly higher ($P < 0.001$) HW_{abs} of triploid males. The ratios of W_{rel} of the observed parameters in the fish weight were not found to be affected by the ploidy level in tench at T_{3+} age, except for AFW_{rel} of females where significantly higher values ($P < 0.001$) were registered for triploids.

The effect of sex on the given parameters was found more expressed in triploids at T_{3+} age. There were significantly higher ($P < 0.001$) weights of CW_{abs} , HW_{abs} , organs (GW_{abs} , HPW_{abs} , SW_{abs}) and AFW_{abs} (and its ratio in the fish weight, AFW_{rel}) of triploid females at T_{3+} age. The values of VW_{abs} were found to be significantly higher ($P < 0.001$) in the females of both ploidy levels. In accordance with those at T_3 age, the males of both ploidy levels at T_{3+} age showed a significantly higher ($P < 0.001$) ratio of FW_{rel} . Compared to diploid males at T_{3+}

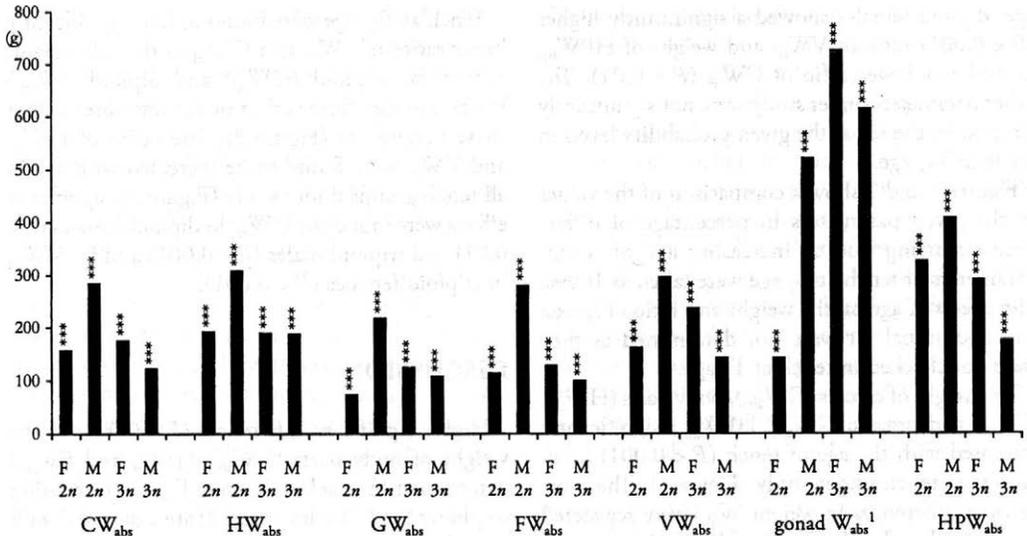


Figure 1. Comparison of the weight W_{abs} of carcass, head, gills, fins, viscera, gonads and hepatopancreas in tench at T_3 (= 100%) and T_{3+} age

¹according to Buchtová *et al.* (2003)

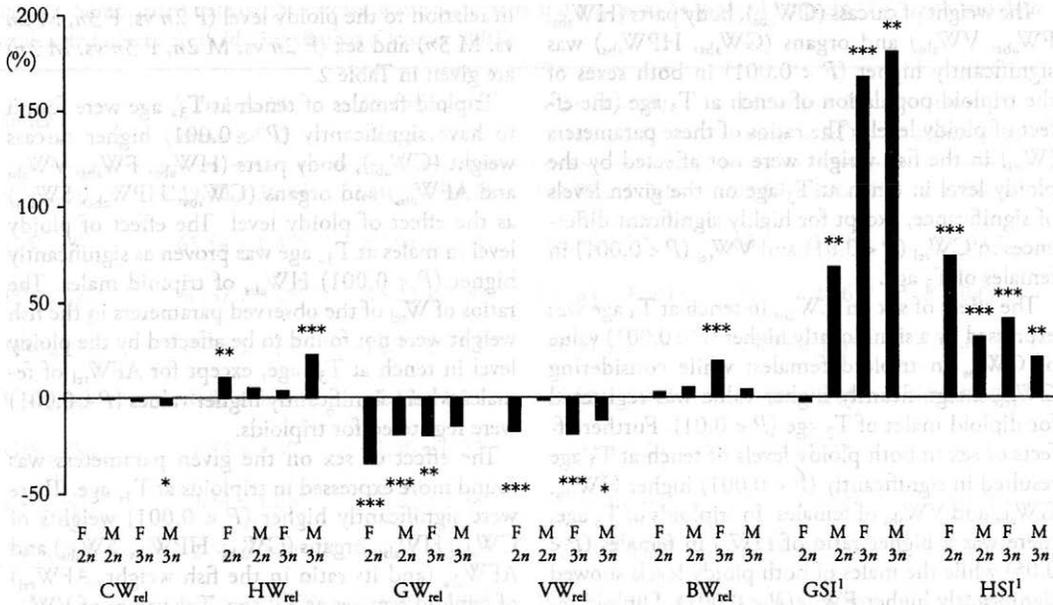


Figure 2. Comparison of the relative weight W_{rel} of carcass, head, gills, fins, viscera, gonads and hepatopancreas in the fish weight in tench at T_3 (= 100%) and T_{3+} age

¹according to Buchtová *et al.* (2003)

age, diploid females showed a significantly higher ($P < 0.001$) ratio of VW_{rel} and weight of HPW_{abs} as well as a lower ratio of CW_{rel} ($P < 0.01$). The other parameters under study were not significantly affected by the sex at the given probability levels in tench at T_{3+} age.

Figures 1 and 2 show a comparison of the values of the given parameters in percentage of difference occurring due to increasing age of tench. Parameters of tench at T_3 age were taken as 100%. The effects of age on the weight and ratio of spleen and abdominal fat were not determined as they were not checked in tench at T_3 age.

The weight of carcass (CW_{abs}), body parts (HW_{abs} , FW_{abs}) and organs (GW_{abs} , HPW_{abs}) significantly increased with the age of tench ($P < 0.001$) in all groups of tench under study (Figure 1). The most marked differences in weight W_{abs} were registered for diploid males during the observed period $T_3 - T_{3+}$.

The ratios of CW_{rel} in the fish weight were nearly the same in all groups of tench, except for a significant decrease ($P < 0.05$) in triploid males (Figure 2).

Tench at T_{3+} age were found to have significantly lower ratios of FW_{rel} and GW_{rel} in the fish weight, except for triploid (GW_{rel}) and diploid (FW_{rel}) males. No significant effect of age was observed for these parameters (Figure 2). The ratios of HW_{rel} and VW_{rel} were found to be increased with age in all tench groups under study (Figure 2): significant effects were found for HW_{rel} in diploid females ($P < 0.01$) and triploid males ($P < 0.001$) and for VW_{rel} in triploid females ($P < 0.001$).

DISCUSSION

Highly significant differences ($P < 0.001$) in the weight of body parts (CW_{abs} , HW_{abs} and FW_{abs}) determined in tench at T_3 and T_{3+} age in relation to ploidy level (Tables 1 and 2) are associated with growth potential characteristics of triploid individuals (accelerated somatic growth and higher final weight). It was recently described for the same groups of tench at T_3 and T_{3+} age by Buchtová *et al.* (2003). This effect was found by Flajšhans *et al.* (1993) for the same species ten years ago. No statis-

tically significant differences were found for CW_{abs} and FW_{abs} between males at T_{3+} , probably due to different growth of diploid and triploid males in these body parts during the observed period $T_3 - T_{3+}$ (Figure 1).

Marked sex dimorphism described e.g. by Baruš and Oliva (1995) which, apart from other signs, was expressed by the larger size and weight of females was a reason for significant differences ($P < 0.001$) in the values of CW_{abs} (between triploids at T_3 and T_{3+}) and HW_{abs} (except for diploids at T_{3+} age) in the groups of tench under study. However, its effect on FW_{abs} of tench at T_3 and T_{3+} age was not proven at the given level of significance (Tables 1 and 2).

Statistical evaluation of objective parameters CW_{rel} , HW_{rel} and FW_{rel} related to the ploidy level did not prove any significant differences in the ratios of these body parts in the fish weight of tench at T_3 and T_{3+} age. Only the triploid females of tench at T_3 age showed significantly higher values ($P < 0.01$) of CW_{rel} , which was in accordance with our observations on dressing percentage (DP) in the same groups of tench, published elsewhere (Buchtová *et al.*, 2003). Differences in CW_{rel} ($P < 0.01$) of diploid tench at T_3 and T_{3+} age related to sex also referred to these results (Tables 1 and 2).

Sex dimorphism was also demonstrated by significant differences ($P < 0.05$) in HW_{rel} of triploid tench at T_3 age. Its most marked expression was found in FW_{rel} , with significantly higher ratio of fins in the fish weight ($P < 0.001$) in both diploid and triploid males at both age categories. In this fish species, sex dimorphism is also reflected by morphological characteristics of ventral fins (Kvasnička and Flajšhans, 1993) according to which the sex of diploid fish can be safely distinguished already since 15 months of age (Baruš and Oliva, 1995).

Highly significant differences in GW_{abs} ($P < 0.001$) in tench at T_3 and T_{3+} age (Tables 1 and 2) related to the ploidy level (except for males at T_{3+} age) and sex (except for diploid tench at T_{3+} age) can also be associated, apart from the fish size, with different requirements of the organism for oxygen supply to tissues. Triploids and females that are assumed to have different metabolism intensity due to higher food uptake have higher oxygen consumption under identical environmental conditions (Hochachka and Mommsen, 1995). If the water saturation with oxygen is sufficient (above 80%), the process of gill respiration is regulated by the intensity of respiratory motions and increased

by the size (weight) of this respiratory organ. No statistically significant differences were registered in the values of GW_{rel} in relation to the ploidy level and sex in tench at T_3 and T_{3+} age.

The weight of internal organs of fish (VW_{abs}) can be affected by various internal factors (age, sex, muscular activity, health) and external factors (water temperature, oxygen content in water, nutrition) (Kouřil *et al.*, 1978). In relation to the ploidy level, triploids of both age categories T_3 and T_{3+} showed significantly higher values of VW_{abs} ($P < 0.001$) in accordance with their growth potential (except for T_{3+} males at the given level of significance). In relation to the sex, higher values were reached by females of both ploidy levels. Diploid females at T_3 and T_{3+} age also had a significantly higher ($P < 0.001$) ratio of VW_{rel} compared to diploid males. These differences in the values of VW_{abs} and VW_{rel} were due to more pronounced differences in the weight of gonads and hepatopancreas in the groups of tench at T_3 and T_{3+} age as well as to those in the weight of abdominal fat in tench at T_{3+} age (Tables 1 and 2).

According to Svobodová (1977) the weight of hepatopancreas highly correlates with the absolute quantity of glycogen deposited in it, and with its ability to retain or release water. The annual proportion of hepatopancreas fluctuates in relation to the seasonal cycle (Kouřil *et al.*, 1978).

In our experiment and in relation to the ploidy level, significantly higher HPW_{abs} ($P < 0.001$) were found for triploids of both age categories (except for males at T_{3+} age), which could probably be associated with higher intensity of food uptake and higher somatic growth of these fish. Neither the content of glycogen in hepatopancreas nor that of water was studied. The effect of sex showed higher values of HPW_{abs} in bigger and heavier diploid and triploid females although a high significance ($P < 0.001$) was found for T_{3+} age only.

Moreover, some other parameters were studied and statistically tested in tench of T_{3+} age. These were $SW_{abs, rel}$ and $AFW_{abs, rel}$. The highest value of SW_{abs} in triploid females at T_{3+} age (Table 2) was the reason for significant differences ($P < 0.001$) in SW_{abs} related to the ploidy level (in females) and sex (in triploids). The values of SW_{rel} were found nearly the same for all tench of T_{3+} age.

The values of AFW_{abs} deposited in the abdominal cavity and of its ratio AFW_{rel} in the fish weight were also found to be the highest in triploid females at T_{3+} age. If compared with the content of

intramuscular fat in the flesh of the same groups of tench of T_{3+} age where no effects of ploidy level or sex were proved (the results of analyses of the basic chemical composition of flesh in identical groups of tench performed by the same authors have not been published yet), we found highly significant differences ($P < 0.001$) in AFW_{abs} and AFW_{rel} between the females at T_{3+} age (the effect of ploidy level) and between the triploids at T_{3+} age (the effect of sex).

Due to the specific composition of fatty acids of fish oils that contain large proportions of PUFA n-3 even in freshwater fish (Steffens *et al.*, 1998; Quiros and Alvarino, 1998; Vácha and Tvřizická, 1998), it is possible to anticipate the presence of these essential fatty acids also in abdominal fat of fish. However, the published data on fat composition concern the intramuscular fat exclusively. No due attention has been paid to qualitative and quantitative analyses of waste abdominal fat of freshwater fish yet although the waste fish fats could become a potential domestic source of PUFA n-3, namely for inland countries.

The effect of increasing age on W_{abs} values of the studied body parts and organs (C, H, F, G, V) was found to be significant for all tench groups ($P < 0.001$) and it was mostly expressed in diploid males (Figure 1). This was in accordance with the assessment of biometric and weight parameters in the same groups of tench (Buchtová *et al.*, 2003).

A significant increase in HW_{rel} by about 22% ($P < 0.001$) was found responsible for a 4% decrease in CW_{rel} ($P < 0.05$) in triploid males as a function of age as it was registered by this team of authors (Figure 2). In diploid females, the increase in HW_{rel} by about 11% ($P < 0.01$) had no effect on the CW_{rel} value.

A decreasing trend of the ratio of gills (GW_{rel}) and fins (FW_{rel}) in the fish weight was found due to changes in the weight parameters (GW_{abs} , FW_{abs} : fish weight) of the studied groups of tench in relation to age. The lower ratio of gills in tench at T_{3+} age could be associated with lower requirements of this category for oxygen consumption compared to T_3 fish. Svobodová *et al.* (1987) dealt with requirements for oxygen consumption in various age categories of common carp (*Cyprinus carpio*, L.). They reported decreasing oxygen requirements by the following indices: $C_1 = 1$; $C_2 = 0.5-0.7$; $C_3 = 0.3-0.4$.

From the anatomical aspects, there was an interesting decrease in the ratio of gills (GW_{rel}) with simultaneous increase in the ratio of head (HW_{rel}) in the fish weight related to age. The increasing ratio of head (HW_{rel}) in higher age categories of tench could probably be associated with increasing values of HW_{abs} due to the higher proportion of flesh and probably also of subcutaneous fat on the head.

This hypothesis was based on the results of the effect of age on the deposition of intramuscular fat in the same groups of diploid and triploid tench at T_3 and T_{3+} age, despite of the fact that the content of fat significantly increased ($P < 0.05$) in the flesh of triploid females only (the results of analyses of the basic chemical composition of flesh in identical groups of tench performed by the same authors have not been published yet).

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ABSTRAKT

Analýza jateční výtěžnosti diploidní a triploidní populace lína obecného (*Tinca tinca*, Linnaeus 1758)

Cílem práce bylo stanovení rozdílů v hmotnosti (W_{abs}) C-očištěného trupu^{bez hlavy, ploutví a vnitřních orgánů} a odpadních částí těl (H-hlava^{bez žaber}, F-plouťve^{párové i nepárové} u línů T_{3+} i AF-vnitřní tuk) a orgánů (G-žebra, V-vnitřní orgány^{suma}, z toho: HP-hepatopankreas, u línů T_{3+} i S-slezina) a jejich podílu na celkové hmotnosti (W_{rel}) mezi diploidními (2n) a triploidními (3n) línými identické genetické specifikace a chovných podmínek v závislosti na pohlaví (F-female vs. M-male) a věku ryb (T_3 vs. T_{3+}). Celkem bylo analyzováno 137 sourozenců lína obecného. Kontrolní skupinu tvořilo 72 línů 2n (39 F a 33 M) a experimentální skupinu 65 línů 3n (38 F a 27 M). Vliv ploidie se projevil u jikernaček T_3 a T_{3+} , vysoce signifikantně ($P < 0,001$) vyššími hodnotami W_{abs} (C, H, G, F, V, HP) a u jikernaček T_{3+} i hodnotami W_{abs} (S, AF) ve prospěch 3n samic. Triploidní jikernačky T_3 vykazovaly vysoce signifikantně vyšší hodnoty CW_{rel} ($P < 0,01$) a nižší hodnoty VW_{rel} ($P < 0,001$), ve věkové kategorii T_{3+} , pak vysoce signifikantně vyšší hodnoty AFW_{rel} ($P < 0,001$). U triploidních mlíčáků T_3 byly zjištěny v důsledku vlivu ploidie vysoce signifikantně ($P < 0,001$) vyšší hodnoty W_{abs} (C, H i u T_{3+} , G, F, V, HP) ve srovnání s M 2n línými. Vliv pohlaví se u 2n línů T_3

a T_{3+} , projevil vysoce signifikantně vyššími hodnotami CW_{rel} ($P < 0,01$) a FW_{rel} ($P < 0,001$) a nižšími hodnotami $VW_{abs, rel}$ ($P < 0,001$) u mlíčáků. U $2n$ mlíčáků T_3 byly zjištěny i vysoce signifikantně ($P < 0,001$) nižší hodnoty W_{abs} (H, G) a ve věkové kategorii T_{3+} i nižší hodnoty HPW_{abs} . U $3n$ línů T_3 a T_{3+} se vliv pohlaví projevil vysoce signifikantně ($P < 0,001$) vyššími hodnotami W_{abs} (C, H, G, V) a nižšími hodnotami FW_{rel} ($P < 0,001$) u triploidních samic. U $3n$ jikernaček T_3 byly zjištěny průkazně ($P < 0,05$) vyšší hodnoty HW_{rel} a u věkové kategorie T_{3+} i vysoce průkazně ($P < 0,001$) vyšší hodnoty W_{abs} (HP, S, AF) a dále hodnoty AFW_{rel} . V závislosti na zvyšujícím se věku línů vzrostla vysoce signifikantně ($P < 0,001$) hmotnost trupu (CW_{abs}), částí těla (HW_{abs} , FW_{abs}) a orgánů (GW_{abs} , HPW_{abs}) u všech sledovaných skupin ryb.

Klíčová slova: diploidní a triploidní lín; genomová polyploidie; hmotnost a podíl; hlava; ploutve; žábra; vnitřní orgány; vnitřnostní tuk

Corresponding Author

Ing. Martin Flajšhans, Jihočeská univerzita v Českých Budějovicích, Výzkumný ústav rybářský a hydrobiologický, 389 25 Vodňany, Česká republika

Tel. +420 383 382 402, fax +420 383 382 396, e-mail: flajshans@vurh.jcu.cz

Post-mortal changes of pH value and lactic acid content in the muscles of pigs and bulls

J. ŠIMEK, L. VORLOVÁ, L. MALOTA, I. STEINHAUSEROVÁ, L. STEINHAUSER

University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

ABSTRACT: The development of post-mortal changes was evaluated in *m. longissimus lumborum et thoracis* (MLLT) and *m. semimembranosus* (SM) of pigs and in *m. longissimus lumborum et thoracis* of bulls; both half-carasses were compared. The lowest value of pH in MLLT of pigs was measured 36 h after slaughter (5.52) in the right side and 48 h in the left side of pork (5.48). The highest concentration of lactic acid was recorded 24 h *post mortem* (109.78 and 109.22 mmol/kg). The decrease in pH values in SM was slower while the lowest values 5.68 and 5.79 were found 48 h *post mortem*. An increase in lactic acid concentration was analogous to MLLT, but the amount of produced acid was lower. Differences between the right and the left side were not statistically significant. Significant differences were found between the muscles ($P < 0.01$). The lowest values of pH in MLLT of bulls were measured 48 h after slaughter (5.43 and 5.42). The maximum concentration of lactic acid was found 72 h after slaughter (113.33 and 116.00 mmol/kg). The differences between both sides of beef were not statistically significant. The correlation coefficients between pH value and lactic acid concentration were -0.87 ($P < 0.01$) for MLLT and -0.78 ($P < 0.01$) for SM in pigs, and -0.96 ($P < 0.01$) for MLLT in bulls.

Keywords: buffering capacity; meat quality; ultimate pH; pigs; bulls

One of the most important post-mortal changes in muscles is a decrease in pH value due to the production of lactic acid. The decrease in pH value from 7.4 to 5.6–5.7 (live muscle) occurs in pig muscles in six to eight hours after slaughter, then pH decreases to 5.3–5.7 (ultimate pH) within 24 h after slaughter (Hedrick *et al.*, 1994). The pH decrease is determined by the physiological condition of muscles at the time of stunning and can be related to lactate production, or to the capacity of the muscle to produce energy in the form of ATP (Henckel *et al.*, 2000). Muscle glycogen is the main metabolic substrate responsible for post-mortem accumulation of lactic acid and thus for normal pH decrease (Immonen *et al.*, 2000). Muscle glycogen concentration at the time of slaughter is one of the most important factors affecting meat quality. Glycogen is a metabolic substrate that fuels post-mortem lactate production, contributing to a pH decrease. The glycogenolysis continues and pH continues to decrease until the reserves of glycogen

are used up or the metabolic processes stop due to enzymatic arrest caused by low pH. The accumulation of lactate in muscles causes the pH to decrease post mortem, and the reserves of glycogen in the muscles at slaughter will determine the value of ultimate pH (Maribo *et al.*, 1999).

The pH value of meat is influenced not only by lactic acid but also by phosphoric acid and high-powered ions. In proof of that, different pH values were found in poultry meat and pork with similar contents of lactic acid. It is caused by a different buffering capacity of meat. Light muscles usually have a notably better buffering capacity than dark muscles. This is consistent because they have a high content of glycolytic enzymes. The end product of glycolytic metabolism is lactic acid, which tends to lower the pH value. Thus white fibres need a more effective buffering mechanism than red ones. Buffering prolongs the time of effective fibre activity. The principal difference in the buffering capacity of different types of muscles is that white fibres

have a higher content of histidine compounds than red ones. Consequent variations in buffering capacity can be explained by variations in the amounts of dipeptides (Puolanne and Kivikari, 2000). The correlation relationship was ascertained between pH and buffering capacity of pork (MLD) (correlation coefficient = -0.43) (Van Lack *et al.*, 2001). Besides buffering capacity of meat, the pH value is also influenced by other acids, especially free amino acids. The total amount of all acids is expressed as titratable acidity of meat. The objective of this study was to determine continuous changes of pH and lactic acid content in the muscles of pigs and bulls after slaughter and the influence of lactic acid on the range of pH values. The course of post-mortal changes was examined in two muscles of pigs and in one muscle of bulls, with concurrent comparison of both half-carcasses with respect to pH and lactic acid concentration.

MATERIAL AND METHODS

Pigs – four final hybrids (Czech Large White \times Landrace) \times (Large White \times Belgian Landrace) of the average weight about 110 kg were used (gilts and barrows). The pigs were housed 12 h before slaughter. Stunning was performed by means of electrical current with electrical tongs in a dazing trap. The lying animals were exsanguinated on a conveyor belt. Thereafter, common slaughter operations and veterinary inspection followed. Meat samples (approximately 2 kg) of both *m. longissimus lumborum et thoracis* (MLLT) and *m. semimembranosus* (SM) were collected at the level of the last rib from each carcass.

Bulls – five crossbred bulls, Czech Pied \times Holstein breed, at the age of 20 to 21 months and of the live weight about 610 kg were selected for the study. The bulls were not housed before slaughter contrary to the pigs. Stunning was carried out mechanically with the gun provided with a confined projectile. After exsanguination of the lying bulls, common slaughter operations and veterinary inspection were made. From each carcass, the sample of muscle (about 2 kg) was taken from *m. lumborum et thoracis* (MLLT) at the level of the ninth rib on both sides.

The samples were stored at 4°C. The pH value of the samples was measured 30 minutes (in pigs only), 1, 6, 12, 24, and 72 h (in bulls only) after slaughter. A sample of muscle (100 g) was collected

at the same time (immediately after pH measurement) in order to establish the amount of lactic acid. This sample was frozen in liquid nitrogen (-196°C) and stored at -85°C .

The pH values were measured with Sentron Argus pH meter with the needle electrode Isfet (Sentron Inc.). Lactic acid concentration was determined spectrophotometrically (340 nm) from the homogenate using a commercial kit manufactured by Boehringer Mannheim (No. 0139084, FRG), which is based on the reaction catalyzed by lactate dehydrogenase and glutamate pyruvate transaminase.

All data were processed by the statistical programme STAT-Plus; the computed average, standard deviation, correlation analysis, and *t*-test were specified (Matoušková *et al.*, 1992).

RESULTS

The average values of pH and lactic acid content in MLLT of pigs at the individual times after slaughter are shown in Table 1. A decrease in pH within 24h *post mortem* was more intensive in the left side than in the right side of pork. The lowest value of pH was measured 36 h after slaughter (5.52) in the right side and 48 h after slaughter in the left side (5.48). The pH values within 12 h after slaughter were more variable. No statistically significant differences in pH values were found between the right and the left half-carcass. The concentration of lactic acid increased similarly in both sides. The highest concentration of lactic acid was recorded 24 h *post mortem* (109.78 and 109.22 mmol/kg). The decrease in lactic acid content was evident 48 h after slaughter. There was a higher variability in lactic acid content than in pH values.

In Table 2, the pH values and lactic acid content in SM of pigs are documented. The pH values were analogous to MLLT 30 min and 1 h after slaughter. The decrease in pH was slower in SM, with the lowest values 5.68 and 5.79 that were higher than in MLLT, detected 48 h *post mortem*. The variability was higher too. The differences between both sides were not statistically significant. The increase in lactic acid concentration was analogous to MLLT, but the amount of produced acid was lower. The highest concentration of lactic acid was determined in the right side 48 hours after slaughter, and in the left side 24 hours after slaughter. Significant differences between both sides were not confirmed.

Table 1. pH values and lactic acid content in *M. longissimus lumborum et thoracis* of pigs

Time post mortem (h)	pH		Lactic acid (mmol/kg)	
	right side	left side	right side	left side
0.5	6.29 ± 0.17	6.34 ± 0.22	66.22 ± 8.33	63.44 ± 10.22
1.0	6.26 ± 0.31	6.27 ± 0.32	75.11 ± 10.22	82.00 ± 4.33
6.0	5.99 ± 0.22	5.89 ± 0.11	92.56 ± 14.33	93.00 ± 12.89
12.0	5.92 ± 0.19	5.68 ± 0.18	107.11 ± 8.33	106.78 ± 9.67
24.0	5.72 ± 0.14	5.66 ± 0.16	109.78 ± 6.78	109.22 ± 10.22
48.0	5.53 ± 0.16	5.48 ± 0.07	104.22 ± 4.33	105.44 ± 4.78

Table 2. pH values and lactic acid content in *M. semimembranosus* of pigs

Time post mortem (h)	pH		Lactic acid (mmol/kg)	
	right side	left side	right side	left side
0.5	6.23 ± 0.13	6.33 ± 0.06	54.67 ± 11.67	56.11 ± 10.00
1.0	6.34 ± 0.31	6.31 ± 0.17	63.22 ± 8.33	61.89 ± 8.00
6.0	6.14 ± 0.24	6.10 ± 0.18	72.78 ± 6.00	71.11 ± 6.89
12.0	5.95 ± 0.16	6.02 ± 0.16	82.44 ± 7.89	83.56 ± 6.11
24.0	6.02 ± 0.22	6.00 ± 0.11	86.44 ± 7.89	92.00 ± 9.78
48.0	5.68 ± 0.03	5.79 ± 0.26	89.78 ± 7.00	91.56 ± 5.33

Table 3. pH values and lactic acid content in *M. longissimus lumborum et thoracis* of bulls

Time post mortem (h)	pH		Lactic acid (mmol/kg)	
	right side	left side	right side	left side
0.5	6.70 ± 0.14	6.54 ± 0.21	43.33 ± 8.43	46.78 ± 14.76
1.0	6.39 ± 0.15	6.37 ± 0.13	56.00 ± 8.40	54.67 ± 16.89
6.0	5.89 ± 0.26	5.93 ± 0.17	82.67 ± 7.46	82.56 ± 5.03
12.0	5.65 ± 0.06	5.65 ± 0.07	105.89 ± 2.52	105.56 ± 4.64
24.0	5.43 ± 0.03	5.42 ± 0.05	109.78 ± 5.91	110.11 ± 3.13
48.0	5.60 ± 0.03	5.58 ± 0.03	113.33 ± 5.52	116.00 ± 5.07

Average values of pH and lactic acid content in MLLT of bulls are given in Table 3. The decrease in pH value in both sides was almost identical and the difference was not statistically significant. The lowest pH value was measured 48 h after slaughter (5.43 and 5.42). Higher variability was found in pH values 12 h after slaughter. The increase in lactic acid concentration was similar in both sides. The highest concentration of lactic acid was determined

72 h after slaughter (113.33 and 116.00 mmol/kg). There were no statistically significant differences between both sides.

DISCUSSION

A decrease in pH values in pigs was typical. This rapid decrease within six hours after slaughter is in

conformity with published data (Stoier *et al.*, 2001). Between the first and the sixth hour after slaughter, the pH value decreased 0.054 and 0.076 units per hour in MLLT as well as 0.040 and 0.042 units in SM. The decrease in pH was lower in the subsequent hours *post mortem* (between 12 and 24 h after slaughter about 0.002 unit per hour in both muscles). The lowest value of pH was measured 48 h after slaughter. The ultimate pH value was reached 24 h after slaughter, but this result is not in concordance with the published data. The published values of pH_{24} were lower than our values. Rosenfold *et al.* (2002) reported the pH_{24} value 5.58 in MLLT and 5.64 in SM. The lower value of pH_{24} was found in SM (5.59) than in MLLT (5.66) according to Henckel *et al.* (2000). We found out the higher pH_{24} value in SM than in MLLT ($P < 0.01$). The differences between the right and the left half-carcass were not statistically significant. Significant differences were detected between the muscles ($P < 0.01$). However, these differences were not significant within one hour after slaughter, which is important for the identification of quality difference when both muscles can be used for pH value measurement or only *m. semimebranosus* which is better accessible in the side of pork.

The very fast increase in lactic acid was documented during the first twelve hours after slaughter. The highest increase was measured between the first and the sixth hour after slaughter, i.e. 3.49 and 2.2 mmol per hour in MLLT, and 1.912 and 1.844 in SM. A subsequent increase in lactic acid concentration was very slow (0.2–0.3 mmol per hour in both muscles). The amount of lactic acid increased within 24 hours after slaughter and then it gradually decreased (except for the right side of SM, where a significant increase was still found). Lactic acid concentration was higher than the published data. Maribo *et al.* (1999) reported 94.5 mmol/kg thirty minutes after slaughter. Differences between both sides were not significant, but statistically significant differences ($P < 0.01$) were demonstrated between the individual muscles again.

A decrease in the pH values is typical of beef muscles. The pH value decreased in the right side at a speed of 0.062 units per hour between the first and the sixth hour after slaughter. In the left side, the pH value decreased more slowly, at a speed of 0.034 units per hour. These documented values were lower than the published data – 0.09 units per hour, within the first to the second hour *post mortem* (Kim *et al.*, 2000). The following decrease

continued equally in both sides. The ultimate pH value was reached 48 h after slaughter, which is in concordance with the published data of pH value about 5.5 (Van Laack *et al.*, 2000). According to Immonen *et al.* (2000), the ultimate pH value was higher 48 h after slaughter (5.87). On the other hand, according to Byrne *et al.* (2000), the value 5.48 was reached twenty-four hours after slaughter. There was an increase in pH value 48 h post mortem. Lactic acid concentration increased very quickly within 24 h after slaughter, with the peak ranging from 4.45 to 4.65 mmol/h between the first and the twelfth hour.

The pH value of meat is not influenced by the presence of lactic acid only. Therefore we computed correlation coefficients for pH and lactic acid content. The correlation coefficients were congruent with the individual muscles, for MLLT -0.87 ($P < 0.01$) and for SM -0.78 ($P < 0.01$). This implies that the pH value in MLLT was influenced to a larger extent by the presence of lactic acid than that in SM. This difference is probably caused by different buffering capacity of both muscles, and/or compounds with buffering capacity. The correlation coefficient of MLLT in bulls was -0.96 ($P < 0.01$).

In pigs as well in bulls, the pH_u value in MLLT was obtained 48 h after slaughter. This value was lower in bulls than in pigs. Rapidity of pH decrease was higher in bulls, particularly between the first and the twelfth hour. The increase in lactic acid concentration was higher in pigs during the first six hours after slaughter. This rapid increase occurred in bulls later. In pigs, a decrease in its concentration was detected 24 h after slaughter, in contrast with bulls, where the increase in lactic acid content continued within 72 h after slaughter. There were no significant differences between the individual muscles of pigs or bulls. The correlation coefficient of pH and lactic acid content was higher in bulls; in pigs it was higher in *m. longissimus lumborum et thoracis* than in *m. semimembranosus*. The results show that the differences are due to different buffering capacity and chemical composition of muscles.

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ABSTRAKT

Postmortální změny pH a obsahu kyseliny mléčné ve svalovině prasat a býků

Cílem práce bylo stanovit průběh pH a obsah kyseliny mléčné ve svalovině prasat a býků po poražení a míru ovlivnění hodnoty pH přítomností kyseliny mléčné. Průběh postmortálních změn byl hodnocen u dvou svalů u prasat (*m. longissimus lumborum et thoracis* a *m. semimembranosus*) a v jednom svalů u býků (*m. longissimus lumborum et thoracis*) a byly porovnány obě půlky jatečně opracovaného těla. Pokles pH v MLLT do 24 hodin *post mortem* byl intenzivnější v levé než v pravé vepřové půlce. Nejnižší hodnota pH byla zaznamenána 36 hodin po poražení (5,52) v pravé půlce a za 48 hodin v levé půlce (5,48). Maximální koncentrace kyseliny mléčné bylo dosaženo za 24 hodin *post mortem* (109,78 a 109,22 mmol/kg). Pokles obsahu kyseliny mléčné je patrný 48 hodin po poražení. Hodnoty pH v SM 30 minut a 1 hodinu po poražení byly podobné jako v MLLT. Pokles pH byl v SM pomalejší a nejnižší hodnoty (5,68 a 5,79) byly nalezeny za 48 hodin *post mortem* a byly vyšší než v MLLT. Zvyšování koncentrace kyseliny mléčné bylo obdobné jako v MLLT, ale množství vytvořené kyseliny bylo nižší. Rozdíly mezi pravou a levou půlkou jatečně opracovaného těla nebyly statisticky významné. Průkazné rozdíly byly nalezeny mezi svaly ($P < 0,01$). Nejnižší hodnoty pH v MLLT býků bylo dosaženo za 48 hodin po poražení (5,43 a 5,42). Maximální koncentrace kyseliny mléčné bylo dosaženo za 72 hodin po poražení (113,33 a 116,00 mmol/kg). Rozdíly mezi půlkami nebyly statisticky průkazné. Korelační koeficienty u prasat byly pro MLLT $-0,87$ ($P < 0,01$) a pro SM $-0,78$ ($P < 0,01$), v MLLT býků $-0,96$ ($P < 0,01$). Rozdíly jsou ovlivněny rozdílnou pufovací schopností a chemickým složením svalů.

Klíčová slova: pufovací schopnost; kvalita masa; pH; prasata; býci

Corresponding Author

Ing. Jaroslav Šimek, Veterinární a farmaceutická univerzita, Ústav hygieny a technologie masa, Palackého 1–3, 612 42 Brno, Česká republika
Tel. +420 541 562 651, fax +420 541 321 230, e-mail: simekj@vfu.cz

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

Slezská 7, 120 56 Prague 2, Czech Republic

Tel.: + 420 227 010 111, Fax: + 420 227 010 116, E-mail: redakce@uzpi.cz

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Comparison of the contents of intramuscular amino acids in different lamb hybrids

J. JANDÁSEK¹, S. KRÁČMAR¹, M. MILERSKI², I. INGR¹

¹Mendel University of Agriculture and Forestry, Brno, Czech Republic

²Research Institute of Animal Production, Prague-Uhřetěves, Czech Republic

ABSTRACT: Contents of amino acids in the *musculus longissimus lumborum et thoracis* were investigated in ram-lambs, crossbreds between Merino ewes and rams of Oxford Down, Texel, Charollais, Suffolk and Merinolandschaf breeds. Lambs ($n = 100$) were slaughtered at the average age of 119.2 days. Average weight of lamb carcasses was 16.3 kg. Samples of muscles were collected 24 h after slaughter and they were analysed by oxidative acid hydrolysis of proteins with HCl ($c = 6$ mol/l). The alkaline hydrolysis of proteins with LiOH ($c = 4.2$ mol/l) was used for the detection of tryptophan content. The chromatographic analysis of a hydrolysate was performed using sodium citrate buffers and ninhydrin detection. The results were evaluated by the analysis of variance using the least squares method. The effects of slaughter date, slaughter age and live weight, litter size, sire breed and age and breed group of dam on amino acid contents were examined. A highly statistically significant ($P < 0.001$) effect of sire breed on the contents of almost all amino acids was found. The higher content of essential and semi-essential amino acids (EAA) compared to non-essential amino acids (NEAA) was detected in all examined hybrid combinations. Glutamic and aspartic acids were found as amino acids with the highest content among the NEAA. In the EAA group lysine, arginine and leucine showed the highest contents. The highest contents of leucine (6.70%), histidine (4.81%), arginine (7.44%) and methionine (2.18%) were detected in the progeny of Texel sires. The progeny of Charollais rams showed the highest content of threonine (4.12%) and cysteine (0.92%), progeny of Merinolandschaf – valine (4.66%) and phenylalanine (4.56%), progeny of Oxford Down – lysine (8.33%) and progeny of Suffolk – isoleucine (4.32%) and tryptophan (1.10%). The highest total content of EAA was detected in the muscles of the progeny after Merinolandschaf sires (47.29%).

Keywords: lamb; essential, semi-essential and non-essential amino acids; sire breed

There is not much information about the contents of amino acids (AA) in dorsal muscles of lamb carcasses. Approximately 90% of AA in the animal body is incorporated in protein molecules and some of them also play an important role in biosynthesis of various nitrogen compounds (Vodrážka, 1996). According to the importance in human nutrition the AA are divided into essential: valine (Val), leucine (Leu), isoleucine (Ile), threonine (Thr), methionine (Met), lysine (Lys), phenylalanine (Phe) and tryptophan (Trp), semi-essential: arginine (Arg) and histidine (His) and non-essential ones: tyrosine (Tyr), aspartic acid

(Asp), serine (Ser), glutamic acid (Glu), proline (Pro), glycine (Gly), alanine (Ala), cysteine (Cys) (Velíšek *et al.*, 1999).

Swartvagherová *et al.* (1996) detected the contents of individual AA in the loin muscles of Merino lambs: Glu – 13.64%, Asp – 9.44%, Lys – 8.00%, Leu – 7.10%, Arg – 5.34%, Met – 1.5%, Thr – 4.29%. Contents of AA in the meat of Mutton Merino × Ille de France were investigated by Jelínek (1988), who found out the values: Glu – 12.71%, Lys – 9.85%, Asp – 7.26%, Ala – 4.62%, and Met ranged between 1.56 and 1.76%. Gajdošík *et al.* (1988) reported the con-

tents Glu – 19.03%, Lys – 9.46%, Asp – 8.62% and Met – 1.46% in muscles of Mutton Merino. Kuchtk *et al.* (1998) examined the AA contents in dorsal muscles of Merino and Charollais lambs. They detected these contents of AA in *m. longissimus thoracis et lumborum* of Merino lambs: Lys – 7.72%, Leu – 7.39%, Arg – 7.28%, Met – 2.87% and Thr – 4.19%. Mikulík *et al.* (1984) found out the contents of tryptophan 0.82%, lysine 9.85% and methionine 2.45% in the muscle *m. longissimus lumborum et thoracis* of young rams – crosses between North-Caucasian Meat-Wool Sheep and Ascanian Merino.

According to Horák *et al.* (1999) the long-term consumption of lamb meat in the Czech Republic ranged between 0.3 and 0.4 kg/capita/year. Taking into account the number of sheep kept in the Czech Republic and the amount of imported lamb meat, the consumption of lamb meat has recently become even lower than 0.1 kg/capita/year.

The objective of this study was to determine the amino acid contents in the muscular tissue (*m. longissimus lumborum et thoracis*) in commercial meat hybrids, the progeny of Merino ewes and sires of Oxford Down, Texel, Charollais, Suffolk and Merinolandschaf breeds.

MATERIAL AND METHODS

An experiment was conducted on carcasses of ram lambs, crosses between Merino ewes and rams of Merinolandschaf [(M, M × ML), (M × ML) × ML] and Oxford Down (OD), Texel (T), Charollais (CH), Suffolk (SF) and Merinolandschaf (ML) breeds. Lambs were fattened indoors together with their dams in the same flock, and they were fed alfalfa hay (*ad libitum*) and concentrate. Animals ($n = 100$) were slaughtered at the average age of 119.2 days and average live weight of 34.6 kg. Average cold carcass weight was 16.3 kg. The samples of muscles (50–100 g) were taken 24 hours *post mortem* from the dorsal muscles (*m. longissimus lumborum et thoracis*) at the level of the 1st–3rd lumbar vertebrae.

The samples of meat were processed by oxidative acid hydrolysis with HCl ($c = 6$ mol/l) (Davišek *et al.*, 1981). For the detection of Trp content the alkaline hydrolysis with LiOH ($c = 4.2$ mol/l) (Kráčmar and Liška, 2002) was used. The chromatographic analysis of hydrolysate samples was performed in the analyser AAA 400 (f. Ingos,

Prague) using Na-citrate buffers and ninhydrin detection.

The procedure PROC GLM from the SAS program package (SAS Institute Inc., 1989) was used for data analysis. The following statistical model was used:

$$Y = SB + LS + DA + BS + \text{age} + lw + e$$

where: Y = observed trait (amino-acid content)
 SB = slaughter batch (fixed effect – 4 levels)
 LS = litter size (fixed effect – 2 levels)
 DA = age and breed group of dam (fixed effect – 3 levels)
 BS = breed of the sire (fixed effect – 5 levels)
 age = age at slaughter (fixed linear regression)
 lw = live weight at slaughter (fixed linear regression)
 e = residual error

RESULTS AND DISCUSSION

The effects of systematic factors of slaughter date, live weight at slaughter, litter size, sire breed, age and breed of dam on AA contents in lamb meat are presented in Table 1. There were not detected any significant influences of slaughter date, slaughter age and live weight of lambs, litter size, age and breed group of dam for the majority of examined amino acids. On the contrary, the sire breed showed statistically significant ($P < 0.001$) effects on the contents of Asp, Met, Thr, Glu, Pro, Gly, Ala, Val, Ile, Leu, Tyr, Phe, Lys and Trp.

Lower contents of nonessential amino acids (NEAA) than essential and semi-essential amino acids (EAA) in the muscle *m. longissimus lumborum et thoracis* were detected for all examined hybrid combinations of lambs (Table 2). Swartvagherová *et al.* (1996) and Mikulík *et al.* (1984) reported the opposite situation; nevertheless these authors examined a different number of amino acids in comparison with this study. Šubrt *et al.* (2002) detected lower contents of essential AA (30.16–34.5%) and semi-essential AA (9.17–11.62%) in the sirloin muscle of bulls of dairy and beef commercial types in comparison with the lambs examined in this study.

In the group of NEAA the highest contents were detected for Glu (11.67–12.74%) and Asp (7.61–8.10%). Lower contents were observed for Ala (5.21–5.48%) and Tyr (4.79–5.38%).

Table 1. *F*-values and levels of statistical significance (in brackets) of the effects of systematic factors on the content of individual amino acids in the *m. longissimus dorsi et thoracis* of lambs

AA	Sire breed	Dam age and breed group	Litter size	Slaughter age	Slaughter live weight	Slaughter date	<i>R</i> ²
Thr	20.97 (<0001)***	0.73 (0.49)	0.59 (0.45)	0.05 (0.83)	2.33 (0.13)	1.08 (0.36)	0.537
Val	8.40 (<0001)***	2.18 (0.12)	0.03 (0.87)	0.90 (0.35)	0.68 (0.41)	0.33 (0.80)	0.346
Ile	3.56 (0.01)**	0.33 (0.72)	0.15 (0.70)	0.49 (0.48)	0.53 (0.47)	1.45 (0.23)	0.212
Leu	15.03 (<0001)***	0.21 (0.81)	0.26 (0.61)	3.80 (0.05)*	0.03 (0.87)	1.23 (0.30)	0.497
Phe	4.63 (0.002)**	0.58 (0.56)	0.15 (0.70)	2.63 (0.11)	0.65 (0.42)	0.50 (0.68)	0.293
His	2.49 (0.05)*	0.13 (0.87)	2.52 (0.12)	0.14 (0.71)	1.87 (0.17)	0.75 (0.52)	0.174
Lys	25.68 (<0001)***	0.19 (0.83)	12.09 (0.001)***	0.40 (0.53)	4.00 (0.05)	0.97 (0.41)	0.621
Arg	2.33 (0.06)	3.95 (0.02)*	3.74 (0.06)	0.03 (0.86)	0.61 (0.44)	1.85 (0.14)	0.263
Trp	4.03 (0.005)**	0.27 (0.77)	0.23 (0.63)	2.19 (0.14)	0.83 (0.37)	2.42 (0.07)	0.245
Cys	3.40 (0.01)**	0.17 (0.42)	1.03 (0.31)	0.17 (0.67)	1.67 (0.20)	2.09 (0.11)	0.259
Met	10.88 (<0001)***	2.60 (0.08)	0.41 (0.53)	0.02 (0.89)	2.73 (0.10)	1.92 (0.13)	0.413
Tyr	19.51 (<0001)***	0.59 (0.56)	0.51 (0.48)	1.36 (0.25)	1.87 (0.18)	2.27 (0.09)	0.527
Asp	16.60 (<0001)***	1.76 (0.18)	1.11 (0.30)	0.08 (0.77)	5.09 (0.03)*	2.03 (0.12)	0.523
Ser	2.11 (0.09)	1.04 (0.36)	4.01 (0.05)	0.03 (0.87)	7.94 (0.01)**	3.29 (0.02)*	0.234
Glu	67.60 (<0001)***	1.78 (0.17)	0 (0.99)	0.16 (0.69)	0.34 (0.56)	0.16 (0.92)	0.782
Pro	33.77 (<0001)***	0.95 (0.39)	0.01 (0.91)	0.85 (0.36)	2.60 (0.11)	1.23 (0.30)	0.648
Gly	34.38 (<0001)***	0.66 (0.52)	0 (0.98)	0.08 (0.78)	0.68 (0.41)	3.97 (0.01)**	0.639
Ala	17.88 (<0001)***	0.76 (0.47)	1.94 (0.17)	0.29 (0.59)	4.93 (0.03)*	3.12 (0.03)*	0.530

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 2. Contents of amino acids (g/16 g N) in *m. longissimus lumborum et thoracis* in lamb hybrids according to sire breed (LSM \pm S.E.)

Sire breed	Oxford Down <i>n</i> = 24	Texel <i>n</i> = 21	Charollais <i>n</i> = 11	Merinolandschaf <i>n</i> = 26	Suffolk <i>n</i> = 18
Thr	3.84 \pm 0.028 A	3.88 \pm 0.030 A	4.12 \pm 0.043 B	4.10 \pm 0.030 B	4.04 \pm 0.036 B
Val	4.50 \pm 0.023 A	4.59 \pm 0.025 Ba	4.64 \pm 0.035 B	4.66 \pm 0.024 Bb	4.65 \pm 0.029 B
Ile	4.32 \pm 0.041 B	4.13 \pm 0.044 A	4.24 \pm 0.063	4.29 \pm 0.043 B	4.32 \pm 0.052 B
Leu	6.31 \pm 0.040 A	6.70 \pm 0.044 C	6.23 \pm 0.062 Aa	6.48 \pm 0.043 B	6.42 \pm 0.051 ABb
Phe	4.51 \pm 0.021 B	4.55 \pm 0.024 B	4.40 \pm 0.033 Aa	4.56 \pm 0.023 B	4.50 \pm 0.028 b
His	4.70 \pm 0.028 A	4.81 \pm 0.031 Bb	4.77 \pm 0.043	4.72 \pm 0.030 a	4.78 \pm 0.036
Lys	8.33 \pm 0.043 C	7.74 \pm 0.047 Aa	7.97 \pm 0.070 B	7.97 \pm 0.046 B	7.87 \pm 0.055 ABb
Arg	7.41 \pm 0.058	7.44 \pm 0.063 b	7.23 \pm 0.090	7.35 \pm 0.062	7.23 \pm 0.074 a
Trp	0.93 \pm 0.043 a	0.91 \pm 0.047 A	1.06 \pm 0.066	1.09 \pm 0.046 Bb	1.10 \pm 0.055 Bb
Met	1.98 \pm 0.021 Aa	2.18 \pm 0.023 Cac	2.07 \pm 0.033 b	2.08 \pm 0.023 B	2.10 \pm 0.027 BCab
Σ EAA	46.82 \pm 0.13B	46.92 \pm 0.14b	46.72 \pm 0.20Aa	47.29 \pm 0.14b	47.00 \pm 0.16
Cys	0.81 \pm 0.023 Aa	0.89 \pm 0.024 b	0.92 \pm 0.035 B	0.89 \pm 0.024 b	0.91 \pm 0.029 B
Tyr	5.38 \pm 0.046 C	4.99 \pm 0.050 ABb	4.79 \pm 0.071 Aa	5.11 \pm 0.049 B	5.00 \pm 0.059 ABb
Asp	7.61 \pm 0.058 Aa	7.61 \pm 0.062 A	8.05 \pm 0.089 B	8.10 \pm 0.061 Ba	7.92 \pm 0.073 b
Ser	3.32 \pm 0.068	3.30 \pm 0.073	3.54 \pm 0.104 B	3.20 \pm 0.071 A	3.37 \pm 0.085
Glu	11.67 \pm 0.057 Aabc	11.83 \pm 0.062 Aabd	12.51 \pm 0.088 BCa	12.74 \pm 0.061 Bb	12.37 \pm 0.072 C
Pro	3.03 \pm 0.021 A	3.14 \pm 0.022 Ca	3.27 \pm 0.032 B	3.32 \pm 0.022 BD	3.22 \pm 0.026 BCb
Gly	3.30 \pm 0.035 A	3.27 \pm 0.038 A	3.69 \pm 0.054 B	3.71 \pm 0.037 BC	3.56 \pm 0.044 BD
Ala	5.21 \pm 0.028 A	5.25 \pm 0.030 A	5.44 \pm 0.043 B	5.48 \pm 0.030 BC	5.36 \pm 0.036 BD
Σ NEAA	40.36 \pm 0.20 A	40.28 \pm 0.22 A	42.22 \pm 0.31 Ba	42.56 \pm 0.21 B	41.72 \pm 0.25 Bb
Σ AA	87.18 \pm 0.23 A	87.20 \pm 0.25 A	88.94 \pm 0.35 Ba	89.86 \pm 0.24 BCb	88.72 \pm 0.29 BD

Σ EAA = sum of essential and semi-essential amino acids

Σ NEAA = sum of nonessential amino acids

Σ AA = sum of all detected amino acids

t-test: A, B, C = $P < 0.01$; a, b, c, d = $P < 0.05$

Contents of Gly, Ser and Pro ranged between 3.03 and 3.71%. Jelínek (1998), Gajdošík *et al.* (1998) and Swartvagherová *et al.* (1996) also found out the highest contents of Glu and Asp out of all NEAA.

Lys (7.74–8.33%), Arg (7.23–7.44%) and Leu (6.23–6.70%) had the highest contents of all EAA. The low contents, usually less than 2%, were detected for Met, Trp and Cys. Jelínek (1988), Swartvagherová *et al.* (1996) and Kuchtík *et al.* (1998) also proved the highest content of EAA lysine, arginine and leucine. Relations between the contents of NEAA and EAA for individual hybrids are shown in Table 2.

Aliphatic sulphur amino acid Met was contained in the highest percentage in the meat samples taken from carcasses of the progeny of Texel sires (2.18%) while in the progeny of Oxford Down sires the lowest content of this amino acid (1.98%) was determined. Contents of Cys were relatively stable and ranged between 0.92 (M × CH) and 0.81 (M × OD). Thus the lowest contents of Met and Cys were found in the muscles of Oxford Down progeny. A relatively low content of Met in the lamb muscular tissue was also found by Jelínek (1998), Gajdošík *et al.* (1988) and Swartvagherová *et al.* (1996). Kuchtík *et al.* (1998) or Mikulík *et al.* (1984) apparently found higher values of Met concentration.

Threonine was detected in the highest percentage content in the progeny of Charollais sires (4.12%) and in the lowest content in the progeny of Oxford Down sires (3.84%). A high content was also observed in muscular tissues of lambs sired by Merinolandschaf (4.10%). Swartvagherová *et al.* (1996) and Kuchtík *et al.* (1998) reported higher Thr contents.

The basic amino acid lysine was determined in the maximum amount in the muscles of M × OD hybrids (8.33%) while the progeny of Merinolandschaf showed the lowest concentration of this amino acid in the muscular tissue (7.74%). The content of Lys in the muscles of other hybrids was similar and ranged between 7.87% and 7.97%. These results are in agreement with the results obtained by Swartvagherová *et al.* (1996) and Kuchtík *et al.* (1998) while Jelínek (1988), Gajdošík *et al.* (1988) or Mikulík *et al.* (1984) found higher values of Lys concentration.

Tryptophan showed the highest content in the progeny of Suffolk sires (1.10%). A high content was also observed in hybrids M × ML (1.09%) and M × CH (1.06%) while lower content was determined for the progeny of Oxford Down (0.93%)

and Texel (0.91%) sires. Mikulík *et al.* (1984) published a lower concentration of Trp in comparison with the findings of our study.

By comparing EAA contents in the individual hybrids we obtained the following results: M × OD hybrid had the highest content of lysine and the lowest contents of methionine, cysteine, threonine, valine and histidine of all examined hybrids. The progeny of Texel sires had the highest content of methionine, arginine, leucine and histidine but the lowest contents of lysine and tryptophan. In muscles of Ch × M hybrids the highest contents of cysteine, threonine and the lowest contents of arginine, leucine, phenylalanine and isoleucine were detected. The progeny of Merinolandschaf sires showed the highest contents of valine and phenylalanine. The last examined hybrid Sf × M had the highest content of tryptophan and isoleucine and the lowest content of arginine. In the muscular tissue of the progeny of Merinolandschaf sires the highest total content of all EAA (47.29%) was detected. The total EAA contents of the hybrids between Merino ewes and rams of meat breeds ranged from 46.72 to 47.29%.

CONCLUSION

We discovered higher contents of essential and semi-essential amino acids than those of non-essential amino acids in all analysed hybrid combinations. The effects of slaughter batch, sire breed, age and breed group of dam, litter size, age and live weight of lambs at slaughter on the amino acid profile in muscular tissue of lambs were analysed. The statistically significant ($P < 0.001$) effect of sire breed was found for a majority of analysed amino acids. The other effects were not usually statistically significant. The progeny of Merinolandschaf sires had the highest percentage of essential amino acids (47.29%).

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ABSTRAKT

Porovnání obsahů vnitrosvalových aminokyselin u jehňat různých hybridních kombinací

Zastoupení aminokyselin ve svalu *musculus longissimus lumborum et thoracis* bylo sledováno u beránků-hybridů merinových matek a beranů plemen oxford down, texel, charollais, suffolk a merinolandschaf. Beránci ($n = 100$) byli poráženi v průměrném věku 119,2 dnů, při průměrné hmotnosti jatečně upraveného těla 16,3 kg. Vzorky masa pro determinaci aminokyselin byly odebrány 24 h po porážce a následně zpracovány oxidativně kyselou hydrolyzou HCl ($c = 6 \text{ mol/l}$), tryptofan alkalickou hydrolyzou LiOH ($c = 4,2 \text{ mol/l}$). Chromatografická analýza byla provedena Na-citrátovými pufrý a ninhydrinovou detekcí. Výsledky byly zpracovány analýzou variance metodou nejmenších čtverců. Byly zohledněny vlivy turnusu porážky, živé hmotnosti, četnosti vrhu, plemene otce a věku a plemenného typu matky na obsah aminokyselin. Velmi vysoce průkazný ($P < 0,001$) vliv na obsah téměř všech aminokyselin byl zjištěn zejména u plemene otce. Bylo zjištěno, že všechny hybridní kombinace vykazovaly vyšší obsah aminokyselin esenciálních a semiesenciálních (EAA) než neesenciálních (NEAA). Z NEAA byl nejvyšší obsah zjištěn u kyseliny glutamové a asparagové. Z EAA to byly lyzin, arginin a leucin. Nejvyšší obsahy u leucinu (6,70 %), histidinu (4,81 %), argininu (7,44 %) a methioninu (2,18 %) byly zjištěny u potomstva beranů plemene texel. Nejvyšší obsahy threoninu (4,12 %) a cysteinu (0,92 %) byly zjištěny u potomstva beranů Charollais. Potomstvo po otcích merinolandschaf se vyznačovalo nejvyšším obsahem valinu (4,66 %) a fenylalaninu (4,56 %), potomstvo oxford down – lyzinu (8,33 %) a potomstvo po beranech suffolk – izoleucinu (4,32 %) a tryptofanu (1,10 %). Potomstvo po beranech merinolandschaf se vyznačovalo nejvyšším celkovým obsahem EAA (47,29 %).

Klíčová slova: jehňata; esenciální, semiesenciální a neesenciální aminokyseliny; otcovská plemena

Corresponding Author

Ing. Josef Jandásek, Ústav technologie potravin, Mendelova zemědělská a lesnická univerzita Brno, Zemědělská 1, 613 00, Brno, Česká republika
Tel. +420 545 133 338, fax +420 545 133 190, e-mail: jandasek@email.cz

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