

CZECH ACADEMY OF AGRICULTURAL SCIENCES

Czech Journal of
ANIMAL SCIENCE

ŽIVOČIŠNÁ VÝROBA



INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

4

VOLUME 47
PRAGUE 2002
ISSN 1212-1819

CZECH JOURNAL OF ANIMAL SCIENCE

An international journal published under the auspices of the Czech Academy of Agricultural Sciences and financed by the Ministry of Agriculture of the Czech Republic

Mezinárodní vědecký časopis vydávaný pod záštitou České akademie zemědělských věd a s finanční podporou Ministerstva zemědělství České republiky

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Aim and scope: The journal publishes scientific papers and reviews dealing with the study of genetics and breeding, physiology, reproduction, nutrition and feeds, technology, ethology and economics of cattle, pig, sheep, goat, poultry, fish and other farm animal management.

The journal is cited in the bibliographical journal *Current Contents – Agriculture, Biology and Environmental Sciences* and abstracted in *Animal Breeding Abstracts*. Abstracts from the journal are comprised in the databases: *Agris*, *CAB Abstracts*, *Current Contents on Diskette – Agriculture, Biology and Environmental Sciences*, *Czech Agricultural Bibliography*, *Food Science and Technology Abstracts*, *Toxline Plus*.

Periodicity: The journal is published monthly (12 issues per year). Volume 47 appearing in 2002.

Acceptance of manuscripts: Two copies of manuscript should be addressed to: Ing. Zdeňka Radošová, Institute of Agricultural and Food Information, Slezká 7, 120 56 Praha 2, Czech Republic, tel.: + 420 2 27 01 03 52, fax: + 420 2 27 01 01 16, e-mail: edit@uzpi.cz.

Subscription information: Subscription orders can be entered only by calendar year (January–December) and should be sent to: Institute of Agricultural and Food Information, Slezká 7, 120 56 Praha 2, Czech Republic. Subscription price for 2002 is 214 USD.

Cíl a odborná náplň: Časopis publikuje původní vědecké práce a studie typu review z oblasti genetiky, šlechtění, fyziologie, reprodukce, výživy a krmení, technologie, etologie a ekonomiky chovu skotu, prasat, ovcí, koz, drůbeže, ryb a dalších druhů hospodářských zvířat.

Časopis je citován v bibliografickém časopise *Current Contents – Agriculture, Biology and Environmental Sciences* a v časopise *Animal Breeding Abstracts*. Abstrakty z časopisu jsou zahrnuty v těchto databázích: *Agris*, *CAB Abstracts*, *Current Contents on Diskette – Agriculture, Biology and Environmental Sciences*, *Czech Agricultural Bibliography*, *Food Science and Technology Abstracts*, *Toxline Plus*.

Periodicita: Časopis vychází měsíčně (12× ročně), ročník 47 vychází v roce 2002.

Přijímání rukopisů: Rukopisy ve dvou kopiích je třeba zaslat na adresu redakce: Ing. Zdeňka Radošová, Ústav zemědělských a potravinářských informací, Slezká 7, 120 56 Praha 2, Česká republika, tel.: + 420 2 27 01 03 52, fax: + 420 2 27 01 01 16, e-mail: edit@uzpi.cz.

Informace o předplatném: Objednávky na předplatné jsou přijímány pouze na celý rok (leden–prosinec) a měly by být zaslané na adresu: Ústav zemědělských a potravinářských informací, vydavatelské oddělení, Slezká 7, 120 56 Praha 2. Cena předplatného pro rok 2002 je 1176 Kč.

Growth curve analysis in cattle from early maturity and mature body size viewpoints

Analýza růstových křivek skotu z hlediska ranosti a tělesné velikosti v dospělosti

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ABSTRACT: The study is focused on the relationships between growth curve and mature body size (weight). One hundred and fifty seven Holstein cows were included in the observation. Body weights were recorded during the rearing period and after the calving. Gompertz growth function was used for estimating growth curve parameters (e.g. mature body weight, maturing rate, degree of maturity etc.). A correlation analysis between the parameters was carried out. The analysed set was divided into three groups according to maturing rate. The differences in body weight, absolute weight gain and relative weight gain were analysed. The correlation analysis revealed a tendency that early maturing cows had lower mature weight. During the rearing period it is possible to distinguish between early and late maturing animals according to relative weight gain in the period from birth to 6 months of age, according to absolute weight gain in the period from 6 to 9 months of age, and according to body weight attained in the period from 9 to 15 months.

Keywords: Holstein cattle; growth; maturing rate; early maturity; mature weight

ABSTRAKT: Studie se zabývá vztahem mezi průběhem růstu a tělesnou velikostí v dospělosti. Byla sledována hmotnost 157 holštýnských krav během jejich odchovu a po otelení. Pro odhad parametrů růstových křivek (hmotnost v dospělosti, rychlost dospívání, stupeň dospělosti aj.) byla zvolena Gompertzova funkce. Byla provedena korelační analýza pro vztah mezi jednotlivými parametry růstových křivek. Soubor krav byl rozdělen do tří skupin podle rychlosti dospívání a byly mezi nimi sledovány rozdíly v dosažené hmotnosti, absolutních a relativních přírůstcích. Korelační analýza ukázala, že dříve dospívající zvířata mají v dospělosti nižší hmotnost. Během odchovu lze různě rychle dospívající zvířata rozlišit podle relativního přírůstku hmotnosti od narození do věku 6 měsíců, podle absolutního přírůstku hmotnosti v období od 6 do 9 měsíců věku a podle dosažené hmotnosti v období od 9 do 15 měsíců věku.

Klíčová slova: holštýnský skot; růst; rychlost dospívání; ranost růstu; hmotnost v dospělosti

Long series of body weights or body size changes observed throughout the life of animals are very difficult to explain. An appropriate way to simplify and describe them using few parameters is to use mathematical models.

Growth models mathematically express the lifetime growth course. To accomplish this purpose, they have to comply with some requirements. The estimated weights have to correspond to actually observed body weights and the parameters have to attain the biologi-

cal values. The individual growth curve parameters, both basic and derived, are suitable phenotypic variables for the assessment of growth course and early maturity.

The general expression of the growth curve is the non-linear four-parameter Richards function, other growth models (e.g. Brody, logistic, Bertalanffy, Gompertz and others) are its special cases. They differ in a fixed position of the inflection point (γ^* , t^*) related to the asymptotic weight (A) that can be located

approximately at $1/3$ of A in Bertalanffy function ($y^*/A = 0.296$) and in Gompertz function ($y^*/A = 0.368$) or at $1/2$ of A in logistic function.

Comparative analyses of growth curves in cattle were reported by Brown *et al.* (1976), Sager (1983), DeNise and Brinks (1985), Perotto *et al.* (1992), and Matthes *et al.* (1996). They agreed that Richards model most appropriately fits to actual data and owing to its general character provided the least sum of squares but its computational difficulty was higher in comparison with the other functions. Describing growth patterns, some authors used the Brody function (Marshall *et al.*, 1984; Jenkins *et al.*, 1991; Koenen and Groen, 1996), others used the Gompertz one because the position of the inflection point at $1/3$ of asymptotic weight was suitable for a shape of the growth curve in cattle (Meyer, 1995; Nešetřilová *et al.*, 1999).

This work is aimed at the analysis of the growth curve parameters and at their possible application in dairy cattle improvement. Although growth is not a selection trait in dairy cattle breeding, there is a possibility to influence the length of non-productive period, i.e. replacement heifer rearing, by growth patterns, which is associated with economic efficiency of the whole herd.

MATERIAL AND METHODS

Body weight records of 157 Holstein cows were collected during their rearing period (quarterly) and after calving. They were born from 1994 to 1995 in a single commercial herd. To estimate the growth curve parameters, Gompertz function was chosen. Its formula is as follows:

$$y_t = A e^{-be^{-kt}}$$

where: A = asymptotic weight (for $t \rightarrow \infty$), generally interpreted as an average mature size independent of short-term fluctuations caused by environmental effects such as food supply

b = scaling parameter which adjusts for the situation where $y_0 \neq 0$ or $t_0 \neq 0$, e.g. when t_0 is taken as birth and postnatal growth is recorded

k = maturing index expressed as a function of the ratio of maximum growth rate to mature size

Fitzhugh (1976) reported the growth curve parameters derived:

a) point of inflection, where growth rate changes from increasing to decreasing. Its co-ordinates are marked with y^* , t^* for weight and age, respectively,

b) degree of maturity – u_t – proportion of mature size attained at age t , $u_t = y_t/A$ (here at birth and at 15 months of age, u_0 and u_{15} , respectively),

c) instantaneous absolute growth rate – v^* – rate at the point of inflection, it is the first derivative of the function, dy/dt ,

d) lifetime absolute maturing rate – v – formula is derived as $v = A^{-1} dy/dt$.

Other observed parameters were age at 75% and at 99% of mature weight ($t_{0.75A}$ and $t_{0.99A}$, respectively).

Based on the correlation analysis, relationships between the growth curve parameters were observed. The cows were divided into groups according to maturing rate (k) and the differences in body weights (BW), in absolute weight gains – $(BW2 - BW1)/(AGE2 - AGE1)$, and in relative weight gains – $(BW2 - BW1)/BW1$, were determined. A linear model was as follows:

$$y_{ij} = \mu + K_i + \epsilon_{ij}$$

where: y = body weights, absolute gains, and relative gains

μ = overall mean

K = fixed effect of the maturing rate, $I = 1$ to 3

ϵ = residual error distributed as $N(0, \sigma^2)$

Classes of the maturing rate effect were compiled using a mean value and standard deviation in such a way that three groups were distinguished:

group of below-average cows ($i = 1$), where

$$k \leq \bar{x} - 0.5 s_x$$

group of average cows ($i = 2$), where

$$\bar{x} - 0.5 s_x < k \leq \bar{x} + 0.5 s_x$$

group of above-average cows ($i = 3$), where

$$k > \bar{x} + 0.5 s_x$$

All calculations were conducted using procedures (NLIN, GLM) of the statistical programme SAS®/STAT (1998).

RESULTS

Basic statistics of the growth curve parameters estimated are given in Table 1. Phenotypic correlation coefficients between the growth curve parameters are given in Table 2.

The highest coefficients between mature body weight (A) and the other parameters were found for maturing index ($r = -0.736$) and for the parameters associated with mature stage of growth, i.e. degree of maturity at 15 month of age, age at 75 and 99% of mature weight ($r = -0.791, 0.783, 0.762$, respectively). The correlation between A and co-ordinates of the inflection point reflects the definite relationship A and y^* determined

Table 1. Basic statistics of the growth curve parameters estimated

Parameter	\bar{x}	s_x
A (kg)	643.35	74.513
b	2.566	0.1972
k (per day)	0.00367	0.000718
t^* (days)	264.29	46.105
y^* (kg)	236.67	27.410
v^* (kg/day)	0.855	0.1277
v (kg/day)	0.581	0.0868
μ_0	0.078	0.0154
μ_{15}	0.612	0.0847
$t_{0.75A}$ (days)	617.67	115.756
$t_{0.99A}$ (days)	1 569.10	309.066

by the functional dependence ($y^* = 0.368A$) and the consequently close relationship between A and t^* ($r = 0.805$). The cows, which matured later, tended to have higher weight at maturity.

Correlation coefficients between age at the inflection point and all the other parameters except those which are related to the beginning (b , μ_0) were high and positive for A , y^* , $t_{0.75A}$, $t_{0.99A}$ ($r = 0.805$, 0.805 , 0.973 ,

0.948 , respectively) and negative for k , v^* , v and μ_{15} ($r = -0.891$, -0.678 , -0.678 , -0.971 , respectively). The cows with the longer accelerated phase had higher mature weights and matured later.

The correlation coefficient between maximal growth intensity (v^*) and age at inflection was high and negative ($r = -0.678$). It means that a steepness of the linear phase was associated with age at inflection. The steeper curve, the lower age and the earlier onset of growth retardation can be expected.

Figures 1, 2, 3 illustrate weight-age, absolute weight gain-age, and relative gain-age data up to the age of 36 months, respectively, in groups distinguished according to maturing rate. The curve shapes in different groups correspond to the relationships between the parameters. The weight curve in the group of earlier maturing animals was steeper and at the end of the observed period did not attain as heavy weight as the group of later maturing animals which grew slowly.

Differences between groups are displayed in Figure 4 that compares the earlier and later maturing groups related to the average group. The largest differences in body weight between groups were found from 9 to 15 months of age (earlier maturing animals were heavier) and at the end of the observed period (earlier maturing animals attained lower body weight).

The absolute weight gain curves reached the peak (maximum growth intensity) at early age and were

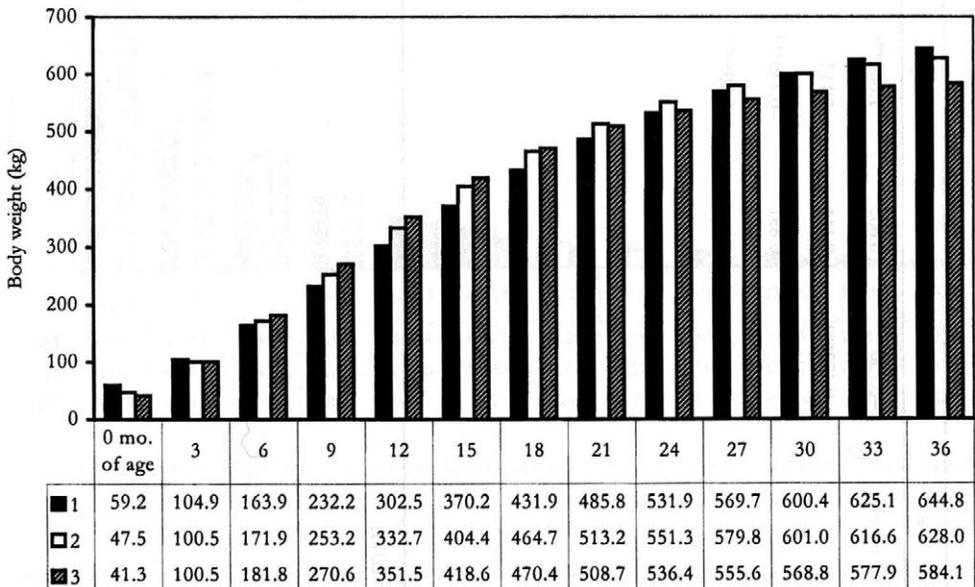


Figure 1. Body weight-age data in groups with below-average (1), average (2) and above-average maturing rate (3)

Table 2. Phenotypic correlation between growth curve parameters

<i>r</i>	<i>b</i>	<i>k</i>	<i>r</i> *	<i>y</i> *	<i>v</i> *	<i>u</i>	<i>u</i> ₀	<i>u</i> ₁₅	<i>t</i> _{0.75A}	<i>t</i> _{0.99A}
<i>A</i>	-0.113	-0.736***	0.805***	1.000***	-0.274***	-0.275***	0.087	-0.791***	0.783***	0.762***
<i>b</i>		0.473***	-0.111	-0.113	0.592***	0.593***	-0.993***	0.285***	-0.330***	-0.413***
<i>k</i>			-0.891***	-0.736***	0.836***	0.836***	-0.459***	0.970***	-0.949***	-0.957***
<i>r</i> *				0.805***	-0.678***	-0.678***	0.095	-0.971***	0.973***	0.948***
<i>y</i> *					-0.274***	-0.275***	0.087	-0.791***	0.783***	0.762***
<i>v</i> *						1.000***	-0.592***	0.775***	-0.778***	-0.807***
<i>v</i>							-0.592***	0.775***	-0.778***	-0.807***
<i>u</i> ₀								-0.270***	0.317***	0.403***
<i>u</i> ₁₅									-0.983***	-0.972***
<i>t</i> _{0.75A}										0.996***

****P* < 0.001

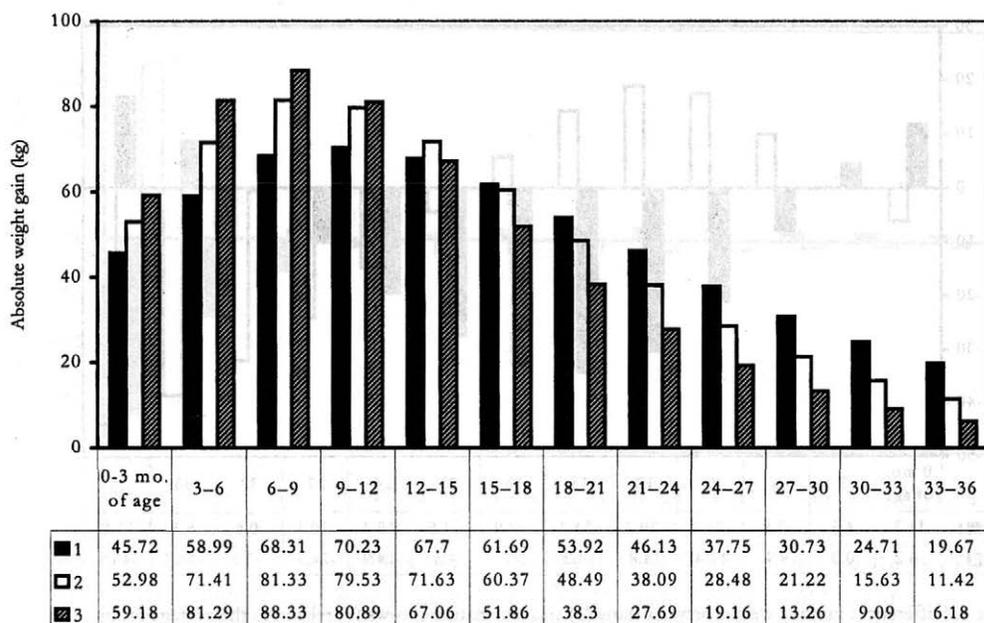


Figure 2. Weight gain-age data in groups with below-average (1), average (2) and above-average maturing rate (3)

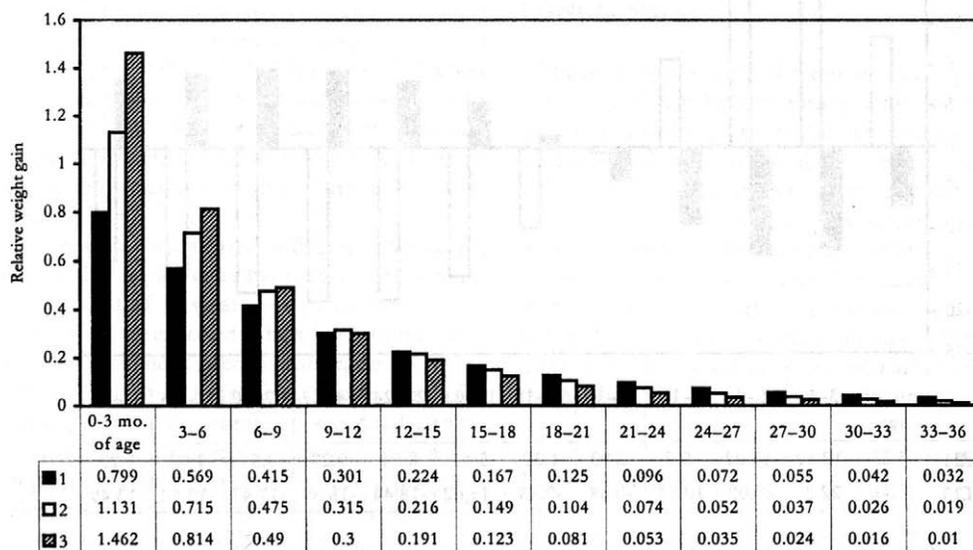


Figure 3. Relative weight gain-age data in groups with below-average (1), average (2) and above-average maturing rate (3)

sharper in earlier maturing animals, and reached the peak at later age and were flatter in later maturing animals (Figure 2). Figure 5 displays differences of

these groups related to the average group. The groups were the most different at the age from 3 to 9 months.

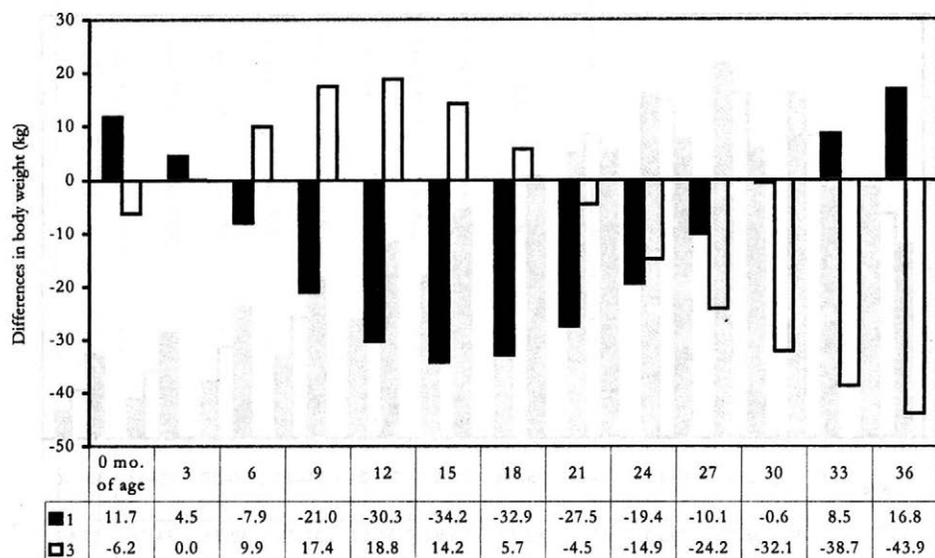


Figure 4. Differences in body weight between early (3) and late maturing cows (1) related to the average group

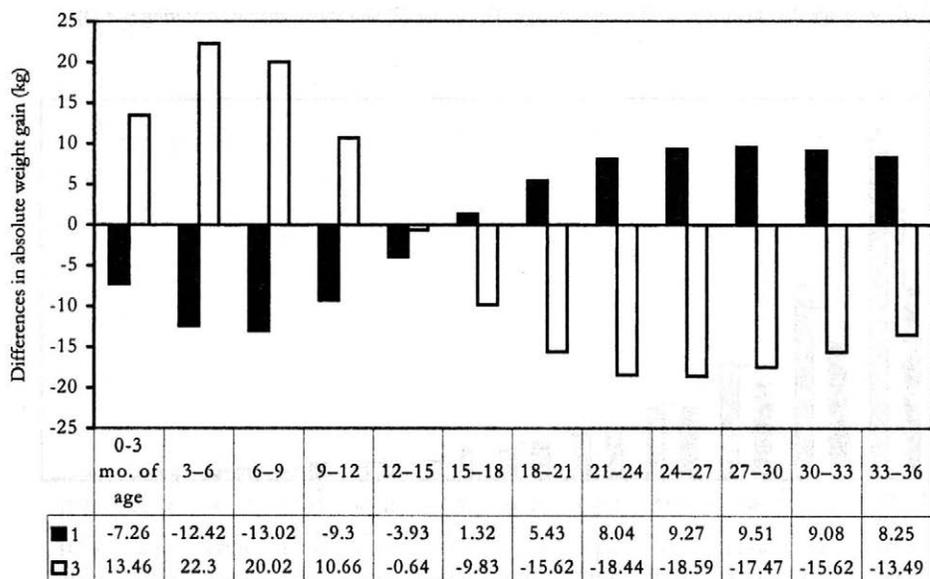


Figure 5. Differences in absolute weight gain between early (3) and late maturing cows (1) related to the average group

The relative weight gain curves were steeper in the earlier maturing group and less descending in the later maturing groups (Figure 3). The largest differences between groups were found at the beginning, i.e. from birth to 6 months of age (Figure 6).

DISCUSSION

The growth curve parameters can be used as phenotypic traits and to analyse relationships between them is possible owing to their biological meaning. Most of

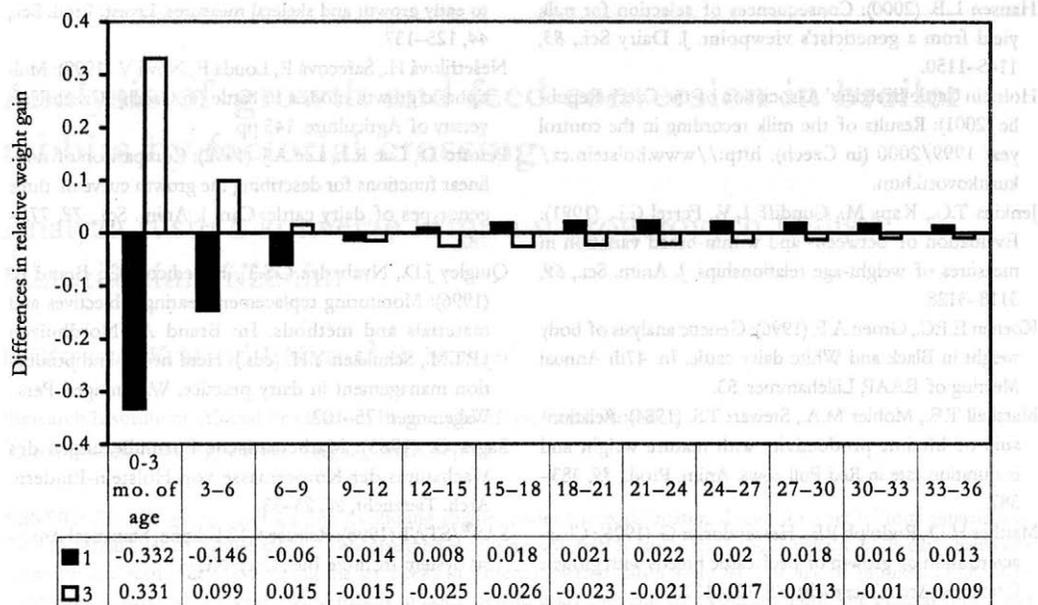


Figure 6. Differences in relative weight gain between early (3) and late maturing cows (1) related to the average group

authors referred to negative and closed correlation between mature size (A) and maturing rate (k), e. g. $r = -0.69$ (Marshall *et al.*, 1984), $r = -0.57$ (DeNise and Brinks, 1985). Thus, the early maturity cannot be linked to a large body size. However, breeding programmes in Holsteins are focused on both. It means that large body frame and early maturity are requested at the same time.

This trend in a breeding policy does not consider the evidence that smaller cows have advantages from the survival point of view, are less prone to metabolic problems and are expected to have lower maintenance feed costs. Continued selection for large body size of Holsteins might not be economically justifiable (Hansen, 2000). Early maturity is requested with respect to the herd effectiveness as a whole, i.e. to reduce non-productive rearing inputs by breeding replacement heifers earlier at the same stage of maturity. Generally, recommended age and body weight at parturition are 24 months and 570 kg, respectively (Quigley *et al.*, 1996). The average age at first calving in Holsteins is 28 months in the Czech Republic (Holstein Cattle Breeders' Association of the Czech Republic, 2001). An opportunity to improve this trait is by utilisation of growth curves and their properties. As reported in this study, it is possible to assess early maturity as soon as the heifers are reared.

CONCLUSIONS

The correlation analysis of the growth curve parameters showed that later maturing cows had higher mature weight. An assessment of the heifer rearing quality according to achieved body weight prefers early maturing heifers and their lower mature weight can be expected. According to body weight gains, it is possible to distinguish between early and later maturing animals at the age of 6–9 months but it is not possible at the age of 9–15 months. At very early age (from birth to 6 months), it is possible to distinguish between differently maturing animals according to their relative weight gains.

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Received: 01–04–20

Accepted after corrections: 02–04–05

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Analysis of growth and feed conversion in broiler rabbits by factorial crossing

Analýza růstu a konverze krmiva u brojlerových králíků faktoriálním křížením

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ABSTRACT: The general and specific combining abilities under a factorial mating design for a set of four paternal and three maternal lines of broiler rabbits were analyzed for the growth performance traits (body weight, body gain, feed consumption and feed efficiency) during the six weeks of fattening period (from 42 to 84 d of age of rabbits). Differences between crossbred rabbits were found mainly in body weight, body gain and feed consumption. The general combining ability (GCA) estimates were significant for body weight, body gain and feed consumption while most estimates of specific combining ability (SCA) were insignificant. The minor differences between crossbreds as well as non-significant estimates of GCA and SCA were found for feed conversion. In spite of the effect of SCA on the growth performance of crossbreds, the striking differences between them focus on GCA rather than on SCA for all the analysed traits.

Keywords: rabbit; growth; feed conversion; factorial mating design

ABSTRAKT: Pro soubor kříženců čtyř otcovských a tří mateřských linií byly odhadnuty obecné a specifické kombinační schopnosti pro živou hmotnost (g), přírůstek těla (g), spotřebu krmiva a konverzi krmiva v jednotlivých týdnech výkrmu i za celou jeho dobu. Pokus se uskutečnil od 42. do 84. dne věku králíků, tj. šesti týdnů výkrmu. Rozdíly mezi kříženci byly nalezeny především v hmotnosti těla, přírůstku hmotnosti a spotřebě krmiva. Obecná kombinační schopnost (GCA) pro hmotnost těla, spotřebu krmiva a přírůstek hmotnosti byla statisticky významná, zatímco většina odhadů specifické kombinační schopnosti byla statisticky nevýznamná (SCA). Malé rozdíly mezi skupinami kříženců, a proto i statisticky nevýznamné odhady GCA a SCA, byly zjištěny pro konverzi krmiva. Rozdíly mezi kříženci stanovené na základě hodnot GCA převýšily v celku rozdíly způsobené SCA pro všechny analyzované znaky.

Klíčová slova: králík; růst; konverze krmiva; faktoriální křížení

Currently, the annual world market production of rabbit meat is estimated around 1 300 000 t, 60 per cent of this production is provided by farm fattening of broiler rabbits (Mach *et al.*, 2001). A broiler rabbit is generally a four-way hybrid. The reasons for using a parental population either in sire or dam position are identical with those valid for other multiparous species of livestock (heterosis, position effect, complementarity). Subsequently, the performance of final hybrids

is determined by the quality of parental populations. The estimation of the combining abilities is an essential presumption for choosing lines for an optimised crossbreeding system. The complete diallel mating and the factorial mating (a special kind of partial diallel mating) are very useful tools for estimating combining abilities. The factorial mating is of special relevance (Wolf *et al.*, 1991) if the parental lines are *a priori* subdivided into paternal and maternal lines. This mat-

ing design was first proposed by Comstock and Robinson (1948, 1952) and is widely known as "North Carolina Design II" (especially among the plant breeders). The principles of estimation of crossbreeding effects in livestock including several aspects of diallel and factorial mating designs were described by Wolf (1987), Jakubec *et al.* (1987) and Wolf *et al.* (1991). In broiler chickens, factorial mating was used by Soukupová *et al.* (1991). The results on factorial mating designs applied to rabbits were published by Mach *et al.* (2000).

With regard to scarce information about the use of a factorial mating design in rabbits, the aim of this study was to analyse the general and specific combining abilities under a factorial mating design for a set of four paternal and three maternal lines of broiler rabbits for growth performance during the fattening period.

MATERIAL AND METHODS

In the year 2000 a factorial mating design with four paternal and three maternal broiler rabbit lines was initiated. The test on 12 groups of crossbreds was carried out at the Department of Genetics and General Animal Science of Czech University of Agriculture in Prague. The six weeks long fattening period started at 42 d and finished at 84 d of age of rabbits. The numbers of tested animals for each combination of lines are listed in Table 1. The live weight (BW) and individual feed consumption were weekly measured till the end of the experiment. The average daily gain (ADG), average daily feed consumption (ADF) and feed conversion (FC) were calculated from data for every experimental week. In addition, total gain (G), total feed consumption (F), feed conversion (FCw) were calculated for the fattening period.

Rabbits were placed into individual cages and were fed *ad libitum* on a commercial pelleted diet containing 16.5% crude protein, 4.5% crude fat, and 15% crude fiber throughout the fattening period.

The analysis was carried out by the ordinary least-squares method in the procedure GLM of statistical software SAS (SAS, 1988). A linear model included the

Table 1. Number of crossbred rabbits

Paternal ↓	Maternal →	Line 14	Line 19	Line 54
Line 24		15	15	15
Line 39		12	15	14
Line 64		12	15	12
Line 59		13	15	10

factor of hybrid. The sex of rabbits was not taken into consideration because of the absence of sexual dimorphism on these traits (Cortier *et al.*, 1969, cited in Brun and Ouhayoun, 1989; Krogmeier and Dzapo, 1991). The estimated least-squares means were used as input data for the program package CBE4, version 4.0 (Wolf, 1995) that was used for estimating the general and specific combining abilities for every group of crossbreds and all analysed traits. The estimation was carried out by weighted least squares using the reciprocals of the variances of the genetic group means as weights. The following model was used:

$$y_{ij} = \mu + g_i^I + g_j^{II} + s_{ij} + e_{ijk}$$

where: y_{ij} = least-squares means for crossbred of line i and line j

μ = overall mean

g_i^I = general combining ability (GCA) of paternal line i

g_j^{II} = general combining ability (GCA) of maternal line j

s_{ij} = specific combining ability (SCA) for combination $i \times j$

e_{ijk} = residual effect

The general combining ability includes the line maternal effect in addition to the direct and line heterosis effects. The specific combining ability is defined similarly like the specific heterosis (Wolf *et al.*, 1991).

RESULTS AND DISCUSSION

Growth performance of crossbred lines during the six-week fattening period

A perusal of Table 2 showed that the variations among crossbred lines were mainly observed in BW. The heaviest one 59 × 54 attained 1 514 g at the beginning and 3 148 g by the end of the fattening period whereas the lowest BW was observed for 24 × 19.

Feed consumption was proportional to the growth of rabbits in parallel to their body weight. The highest ADF was estimated for crossbred 59 × 19 while the lowest for 24 × 19 and 24 × 14. Except for 24 × 14, 64 × 14 and 64 × 54 crossbreds, ADF declined in the last week. In regard to ADG, 59 × 19, 59 × 54 and 64 × 54 crossbred lines showed high values while 24 × 19 showed the lowest value. In the last week, a notable decrease in ADG was detected for 39 × 14 and 39 × 19.

No marked differences were found in feed conversion between all lines. A rapid increase in FC (FC deterioration) was observed in the last week for 24 × 19,

Table 2. Growth performance of crossbred rabbits during the six-week fattening period

Crossbreds	1st week				2nd week				3rd week			
	BW	ADG	ADF	F	BW	ADG	ADF	F	BW	ADG	ADF	F
24 × 14	1 532.7	35.3	123.1	4.4	1 812.7	40.0	143.4	3.7	2 058.0	35.0	136.4	4.0
24 × 19	1 296.0	43.0	116.9	2.9	1 580.0	40.6	119.9	3.0	1 778.0	28.3	114.6	4.2
24 × 54	1 444.0	57.0	135.5	2.5	1 723.3	39.9	162.7	4.1	1 986.0	37.5	157.0	4.2
39 × 14	1 732.9	42.3	143.2	3.4	2 005.0	38.9	159.3	4.3	2 310.0	43.6	181.3	4.5
39 × 19	1 736.7	41.9	137.0	3.3	2 002.7	42.2	148.9	3.8	2 287.3	40.7	158.9	4.1
39 × 54	1 616.4	64.7	158.7	2.5	1 909.3	41.8	180.8	4.5	2 170.0	40.3	170.1	4.7
59 × 14	1 733.1	45.2	166.9	3.9	1 993.8	37.3	139.6	3.9	2 232.4	34.1	155.5	4.8
59 × 19	1 557.3	42.1	177.1	4.5	1 794.0	42.1	129.7	6.4	2 089.3	42.2	182.4	4.6
59 × 54	1 869.0	50.7	167.4	3.9	2 147.0	48.6	164.6	3.7	2 419.0	38.9	166.1	4.4
64 × 14	1 607.5	52.7	148.1	3.0	1 904.2	42.4	178.8	4.4	2 178.3	39.2	167.4	4.4
64 × 19	1 534.7	48.3	136.5	2.9	1 855.3	45.8	139.7	3.1	2 080.7	32.2	141.5	6.5
64 × 54	1 573.3	51.8	141.8	2.9	1 905.8	47.5	159.6	3.6	2 167.5	37.4	151.5	4.1
Crossbreds	4th week				5th week				6st week			
	BW	ADG	ADF	F	BW	ADG	ADF	F	BW	ADG	ADF	F
24 × 14	2 328.7	38.7	159.0	4.2	2 535.3	29.5	162.9	6.0	2 776.0	34.4	178.6	5.4
24 × 19	1 954.7	25.2	122.7	5.3	2 168.0	30.5	145.0	5.1	2 255.3	13.9	128.1	13.3
24 × 54	2 158.0	27.0	147.0	6.2	2 386.0	32.6	166.5	5.5	2 539.3	21.9	141.6	12.2
39 × 14	2 560.7	35.8	182.5	5.3	2 811.4	35.8	187.7	7.0	2 819.3	8.8	159.6	35.0
39 × 19	2 570.7	40.5	180.9	4.6	2 843.3	39.0	189.7	5.1	2 848.7	7.7	163.3	37.0
39 × 54	2 394.3	32.0	164.5	5.5	2 617.1	31.8	203.2	6.7	2 820.7	29.1	181.8	6.9
59 × 14	2 430.0	33.9	176.8	5.7	2 662.3	33.2	195.0	6.2	2 827.7	25.7	188.5	23.8
59 × 19	2 382.7	41.9	203.3	6.0	2 623.3	34.4	207.5	6.6	2 869.3	35.1	125.7	3.7
59 × 54	2 710.0	41.6	192.7	4.9	2 892.0	26.0	207.9	34.2	3 148.0	36.6	203.4	7.6
64 × 14	2 415.8	33.9	166.4	5.1	2 630.0	30.6	171.1	6.8	2 800.0	24.3	182.9	8.8
64 × 19	2 296.0	33.8	150.4	5.8	2 492.7	28.1	153.2	7.1	2 616.0	18.9	132.7	19.6
64 × 54	2 415.0	40.5	152.4	4.1	2 653.3	34.1	176.8	5.6	2 935.8	40.4	198.7	5.4

in bold = outstanding observation

24 × 54, 39 × 14, 39 × 19, 59 × 14 and 64 × 19 with one week earlier for 59 × 54.

14 and 59 × 14 because they grew slowly despite the high feed intake.

Growth performance of crossbred lines for the fattening period

As it could be inferred from Figure 1, the highest body gain was found for 39 × 54, 59 × 54 and 64 × 54 at the end of the experiment. The best feed conversion was recorded for 64 × 54, which could be due to the high body gain and medium feed consumption. A low feed conversion was also detected for 24 × 14, 24 × 19, 24 × 54 and 64 × 19 that grew slowly and consumed less feed. They were also characterised by low body weight. The worst feed conversion was found for 39 ×

Estimation of GCA for parental lines for the fattening period

A positive estimate of GCA for G and negative for F were found for paternal line 64. While maternal line 54 was positive for both traits, paternal line 24 and maternal line 19 were negative. The values of GCA for FCw were low and insignificant for each of paternal lines 24 and 64. Although GCA for BW84 was negative for paternal line 24 and maternal line 19, it was positive for paternal line 59 and maternal line 54 (as shown in Table 3).

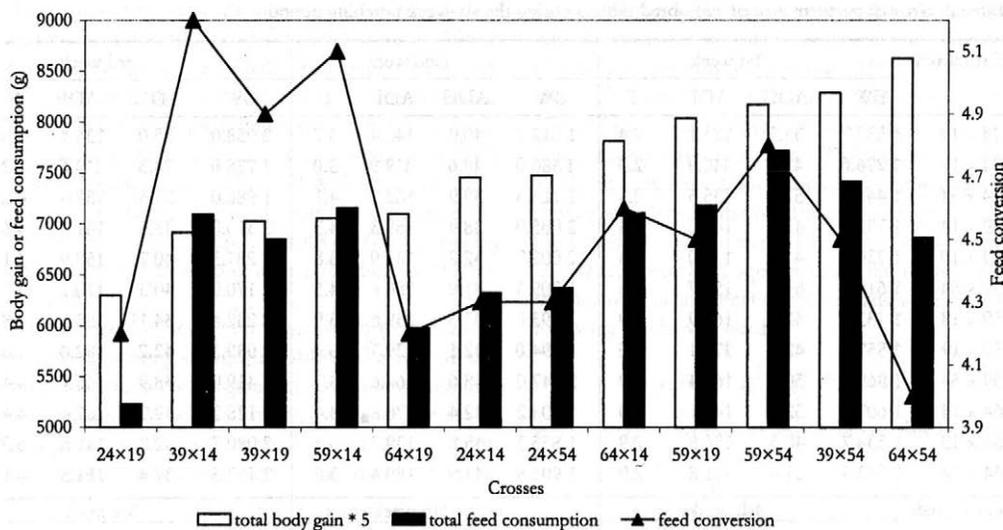


Figure 1. Growth performance of crossbred rabbits for the fattening period

SCA of crossbred lines for the fattening period

The effect of SCA on few lines was apparently produced, e.g. on 24 × 14 and 59 × 19 where it was positive for G, F, BW84. Crossbred 59 × 14 was negative for all of these traits. In spite of the positive estimates of SCA for G and BW84, it was negative for F in crossbred 64 × 54 (as shown in Table 4). In general, the effect of SCA on FCw was very low.

Table 3. The estimates of general combining ability for total daily gain (G), average daily feed consumption (F), feed conversion (FCw), and final body weight (BW84) for the fattening period in parental lines

	G (g)	F (g)	FCw	BW84 (g)
Paternal lines				
24	-88.9**	-798.9**	-0.3	-247.8**
39	-22.2	345.9**	0.3	58.2
59	46.5	577.2**	0.2	177.0**
64	64.7*	-124.2	-0.3	12.6
Maternal lines				
14	-42.5	146.1	0.3	34.4
19	-81.2**	-463.5**	-0.1	-124.0**
54	123.7**	317.4**	-0.2	89.6**

* $P < 0.05$; ** $P < 0.01$

GCA for parental lines during the fattening period

The assessment of GCA pointed out that ADF was positive for paternal line 59 while negative values were recorded for lines 24 and 64 through the last three weeks (Figure 2). On the one hand, the highest values of GCA for BW and ADG were computed for lines

Table 4. The estimates of specific combining ability for total daily gain (G), average daily feed consumption (F), feed conversion (FCw), and final body weight (BW84) for the fattening period in crossbreds

Crossbred	G (g)	F (g)	FCw	BW84 (g)
24 × 14	118.1**	201.6	-0.2	217.9**
24 × 19	-74.0	-280.4	0.03	-144.1**
24 × 54	-44.2	78.8	0.2	-73.8
39 × 14	-56.3	-175.8	0.1	-44.5
39 × 19	4.7	197.9	0.1	143.1**
39 × 54	51.6	-22.1	-0.2	-98.6
59 × 14	-97.2**	-332.8*	0.06	-155.2**
59 × 19	137.4**	289.3	-0.2	45.1
59 × 54	-40.1	43.5	0.1	110.2
64 × 14	35.5	307.0	0.05	-18.1
64 × 19	-68.1	-206.8	0.06	-44.0
64 × 54	32.7	-100.2	-0.1	62.2

* $P < 0.05$; ** $P < 0.01$

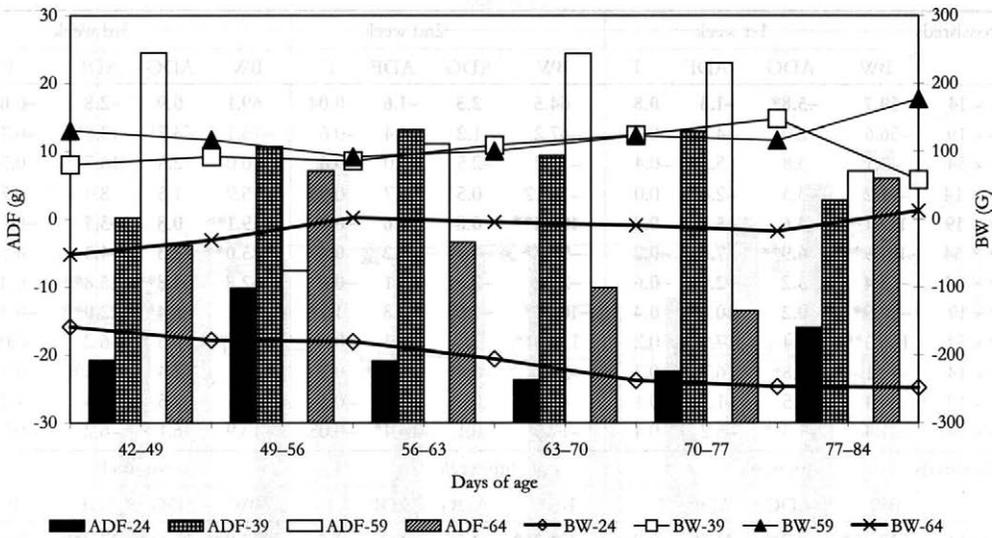


Figure 2. General combining ability (GCA) for feed consumption (ADF-bars) and body weight (BW-lines) for paternal lines of rabbits

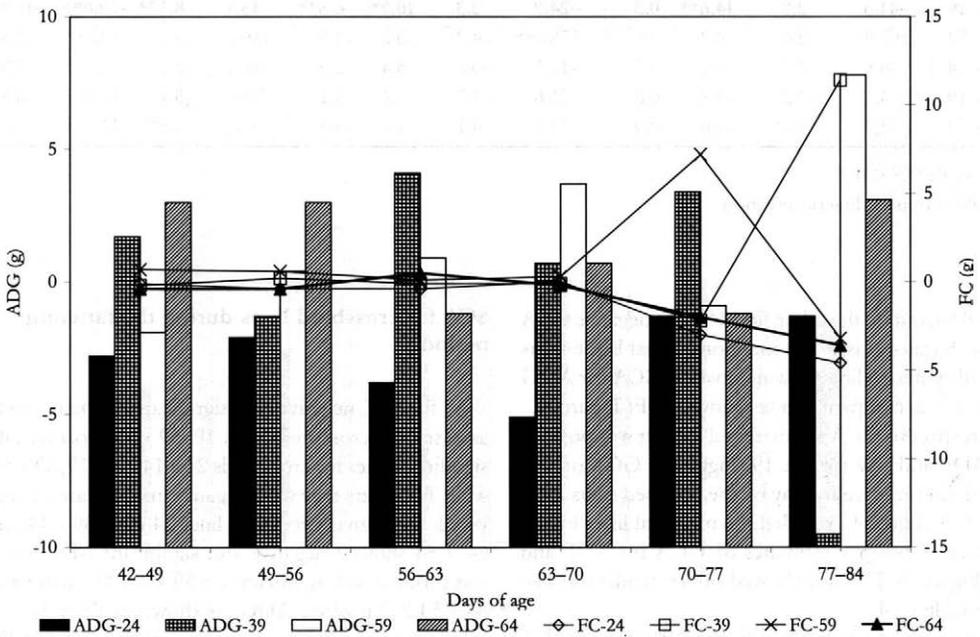


Figure 3. General combining ability (GCA) for daily gain (ADG-bars) and feed conversions (FC-lines) for paternal lines of rabbits

Table 5. The estimates of specific combining ability in crossbreds

Crossbreds	1st week				2nd week				3rd week			
	BW	ADG	ADF	F	BW	ADG	ADF	F	BW	ADG	ADF	F
24 × 14	59.7	-5.8*	-1.3	0.8	64.5	2.5	-1.6	0.04	69.1	0.9	-2.8	-0.05
24 × 19	-56.6	2.0	-4.2	-0.4	-47.2	-1.2	-4.4	-0.6	-75.1	-3.7*	-13.8*	-0.2
24 × 54	-3.1	3.8	5.5	-0.4	-17.2	-2.5	6.0	0.6	6.0	2.8	16.7**	0.3
39 × 14	-11.2	-3.3	-2.4	0.0	-10.12	0.5	-6.7	0.03	5.9	1.5	8.0	0.2
39 × 19	113.1**	-3.6	-5.2	0.2	108.4**	0.8	3.6	-0.4	119.1**	0.8	-3.7	-0.6
39 × 54	-101.9**	6.9**	7.6	-0.2	-98.3*	-1.4	3.2	0.4	-125.0**	-2.3	-4.3	0.5
59 × 14	-35.4	3.2	-2.9	-0.6	-27.2	-2.8	-8.1	-0.8	-62.8	-4.8**	-15.8**	0.4
59 × 19	-90.9*	0.2	10.8*	0.4	-106.1*	-1.0	2.8	1.7*	-70.1	5.4**	22.0**	-0.3
59 × 54	126.3**	-3.4	-7.9	0.2	133.3**	3.7	5.3	-0.9	132.9**	-0.6	-6.2	-0.02
64 × 14	-13.1	5.8*	6.7	-0.3	-27.1	-0.2	16.4**	0.7	-12.1	2.4	10.6	-0.5
64 × 19	34.4	1.5	-1.5	-0.1	45.0	0.2	-2.0	-0.6	26.1	-2.5	-4.4	1.2
64 × 54	-21.4	-7.3**	-5.2	0.4	-17.9	0.1	-14.4*	-0.05	-13.9	5.1	-6.2	-0.7
Crossbreds	4th week				5th week				6st week			
	BW	ADG	ADF	F	BW	ADG	ADF	F	BW	ADG	ADF	F
24 × 14	132.4**	8.2**	11.5*	-0.9	121.9**	-1.5	6.2	2.5	217.9**	12.4**	17.2**	-8.2*
24 × 19	-108.7*	-5.0**	-18.0**	-0.1	-117.2*	-1.2	-6.5	2.1	-144.1**	-3.7	6.6	-0.5
24 × 54	-23.8	-3.2	6.5	1.1	-4.7	2.7	0.3	-4.6	-73.8	-8.7**	-23.8**	8.8*
39 × 14	3.0	-0.5	1.9	0.3	3.9	0.1	-4.5	2.7	-44.5	-5.0*	-20.6**	5.3
39 × 19	145.9**	4.4**	7.2	-0.7	163.6**	2.6	2.9	1.3	143.1**	-1.7	23.0**	7.2
39 × 54	-148.9**	-4.0*	-9.0	0.4	-167.6**	-2.7	1.6	-4.0**	-98.6	6.7**	-2.4	-12.5**
59 × 14	-126.6**	-5.4**	-18.8**	0.3	-113.8*	1.8	-7.1	-7.5*	-155.2**	-5.3**	4.0	8.7*
59 × 19	-41.3	2.8	14.6**	0.3	-24.8	2.3	10.7*	-6.5**	45.1	8.5**	-19.0**	-11.5**
59 × 54	167.9**	2.6	4.2	-0.6	138.6**	-4.2	-3.6	14.0	110.2	-3.2	14.9**	2.8
64 × 14	-8.8	-2.3	5.4	0.3	-12.1	-0.5	5.4	2.3	-18.1	-2.1	-0.5	-5.8
64 × 19	4.1	-2.2	-3.8	0.6	-21.6	-3.7	-7.1	3.1	-44.0	-3.1	-10.8	4.8
64 × 54	4.7	4.6*	-1.6	-0.9	33.7	4.1	1.7	-5.4	62.2	5.3**	11.3	1.0

* $P < 0.05$; ** $P < 0.01$

in bold = outstanding observation

59 and 64, but on the other, line 24 had a negative GCA for both traits. It is worth mentioning that line 64 was the only paternal line showing positive GCA for ADG (Figure 3) accompanied by negative ADF (Figure 2).

In respect to GCA for maternal lines, it was positive for ADF and BW for line 19 (Figure 4). GCA of line 14 did not improve for any of the analysed traits (Figures 4, 5). Line 54 exceeded the maternal lines by the number of positive estimates of GCA for ADF and FC (Figure 5). This line showed similar tendencies like paternal line 64.

Collectively, high and significant estimates of GCA for FC either for paternal or maternal lines were determined in the last two weeks of the fattening period.

SCA for crossbred lines during the fattening period

As for BW, negative and significant estimates were assessed for crossbreds 24 × 19, 59 × 14, positive and significant ones for crossbreds 24 × 14, 39 × 19, 59 × 54, while for others they were negative insignificant or/and very low positive. Crossbred lines 24 × 19, 59 × 14 and 64 × 54 showed negative and significant ADF but it was positive and significant for 59 × 19. The latter and 64 × 54 had positive ADG (as shown in Table 5).

It was quite obvious that the rapid increase in FC (FC deterioration) in the last week led to high and significant SCA. But it started earlier by two weeks for crossbred 59 × 54.

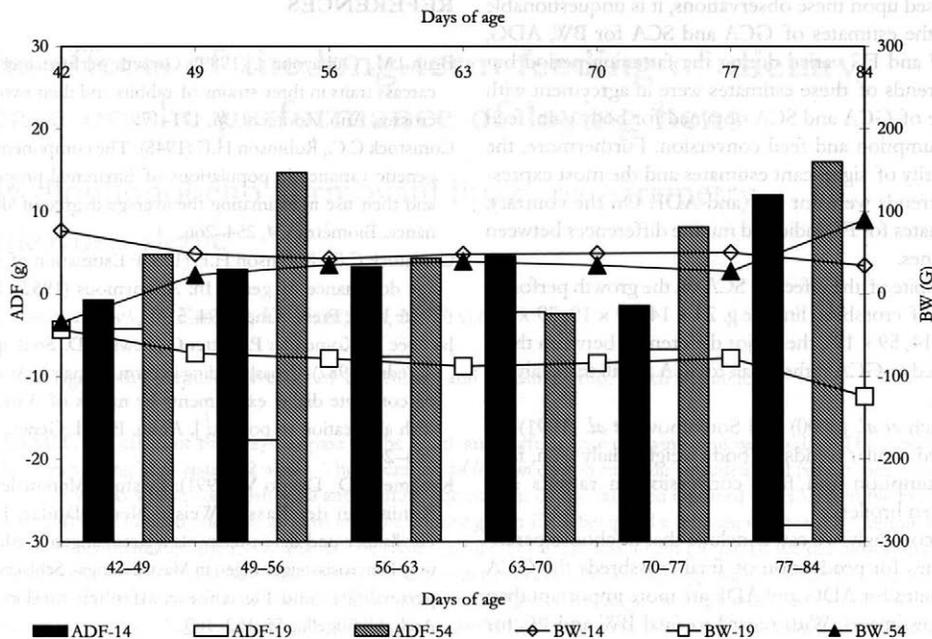


Figure 4. General combining ability (GCA) for feed consumption (ADF-bars) and body weight (BW-lines) for maternal lines of rabbits

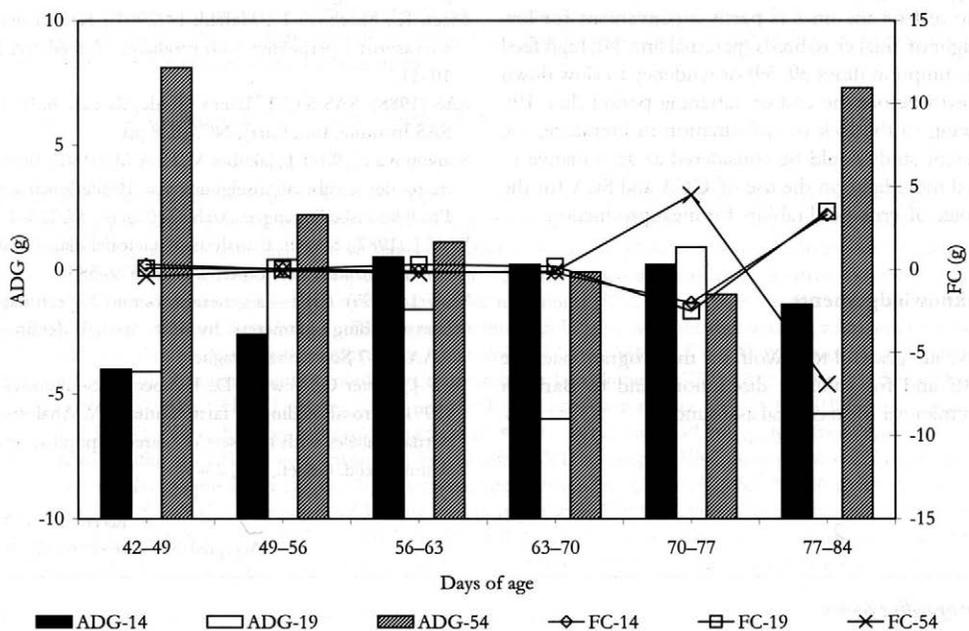


Figure 5. General combining ability (GCA) for daily gain (ADGG-bars) and feed conversions (FC-lines) for maternal lines of rabbits

Based upon these observations, it is unquestionable that the estimates of GCA and SCA for BW, ADG, ADF and FC varied during the fattening period but the trends of these estimates were in agreement with those of GCA and SCA obtained for body gain, feed consumption and feed conversion. Furthermore, the majority of significant estimates and the most expressive trends were for BW and ADF. On the contrary, estimates for FC indicated minute differences between the lines.

In spite of the effect of SCA on the growth performance of crossbred lines (e.g. 24 × 14, 39 × 19, 39 × 54, 59 × 14, 59 × 19), the major differences between them applied to GCA rather than to SCA for all the analysed traits.

Mach *et al.* (2000) and Soukupová *et al.* (1991) reported similar trends of body weight, daily gain, feed consumption and feed conversion in rabbits and chicken broilers.

Accordingly, we can conclude that to choose parental lines for production of final crossbreds the GCA estimates for ADG and ADF are more important than SCA estimates. With regard to final BW and FC for the whole period of fattening, for crossing we propose to use paternal line 64 in sire position and maternal line 54 in dam position because of the high body weight and good feed conversion of their crossbreds. The rest of the lines is partly inconvenient for low weight of final crossbreds (paternal line 24), high feed consumption (lines 39, 59) or tendency to slow down growth before the end of fattening period (line 19). Owing to the lack of information in literature, the current study could be considered as an initiative to shed more light on the use of GCA and SCA for the choice of crossbred rabbits for meat production.

Acknowledgements

We are grateful to J. Wolf for the program package CBE and for valuable discussions and to Barbora Hofmanová for technical assistance.

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Received: 02–02–07

Accepted after corrections: 02–04–12

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The effects of the long-term feeding of dietary lipase on the performance of laying hens

Vliv dlouhodobého zkrmování lipázy na parametry užítkovosti slepic

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ABSTRACT: The effect of the enzyme lipase on the health and performance of laying hens was studied. The experiment with Isabrown laying hens lasted 52 weeks. They were fed *ad libitum* diets containing extruded feed (wheat, pea, rapeseed – 4 : 3 : 3), as well as wheat, corn, fish meal and premix. The content of the extruded rapeseed was 13.5% in the mixtures. The hens (64) were divided into two groups; with the control group EXT being fed a mixture without the addition of the lipase. The experimental group, ENZ was fed the same mixture, but lipase was added (500 g/t). We did not observe any health problems in either group of hens during the experiment period. The average egg weight in the ENZ group was heavier (62.5 ± 0.38 g) than that of the EXT group (61.8 ± 0.39 g). The average daily egg mass production was 54.8 ± 0.69 g/hen/day in the EXT group and 54.6 ± 0.84 g/hen/day in the ENZ group. The ENZ hens had a better coefficient of fat retention (0.92 ± 0.008) than the hens in the EXT group (0.91 ± 0.007). The addition of lipase significantly enhanced the content of the PUFA, the polyunsaturated fatty acids, ($P < 0.01$) in the yolk fat (a figure of 18.2% in comparison with a figure of 16.4% in the EXT group). We observed more haemorrhagic liver conditions in the group fed by lipase and their livers were significantly ($P < 0.05$) heavier (48.3 ± 3.66 g) than in the EXT group (41.1 ± 2.09 g). In addition, the ENZ group of hens had a significantly ($P < 0.05$) lower content of erythrocytes in blood (2.08 ± 0.102 T/l) than in the EXT group (2.27 ± 0.055 T/l). We concluded that dietary lipase had a positive effect on the PUFA content in the yolk fat, but it caused incidence of liver haemorrhages more often.

Keywords: hens; lipase; egg weight; PUFA; liver haemorrhages

ABSTRAKT: Sledovali jsme vliv přidavku krmné lipázy na ukazatele užítkovosti a zdravotní stav slepic. Pokus, který jsme provedli na slepicích hybridní kombinace Isabrown, probíhal 52 týdnů. Slepičky byly krmeny *ad libitum* směsí na bázi extrudovaného krmiva (pšenice, řepkové semeno a hrách – 4 : 3 : 3), pšenice, kukuřice, rybí moučky a premixu. Směsi obsahovaly 13,5 % extrudovaného řepkového semene. Slepičky (64 ks) byly rozděleny do dvou skupin; kontrolní skupina (EXT) byla krmena směsí bez přidavku lipázy. Pokusná skupina ENZ byla krmena stejnou směsí, ale do směsi byla přimíchána lipáza v množství 500 g/t. Během pokusného období jsme nepozorovali výrazné zdravotní problémy u slepic v žádné ze skupin. Průměrná hmotnost vejce ve skupině ENZ byla vyšší ($62,5 \pm 0,38$ g) v porovnání se skupinou EXT ($61,8 \pm 0,39$ g). Průměrná denní produkce vaječné hmoty byla u skupiny EXT $54,8 \pm 0,69$ g/ks/den a $54,6 \pm 0,84$ g/ks/den u skupiny ENZ. U skupiny ENZ jsme zjistili vyšší koeficient retence tuku ($0,92 \pm 0,008$) než u skupiny EXT ($0,91 \pm 0,007$). Přidavek lipázy statisticky průkazně ($P < 0,01$) zvýšil obsah PUFA (polynenasycených mastných kyselin) v tuku žloutku (18,2 % v porovnání se 16,4 % u skupiny EXT). U skupiny ENZ jsme pozorovali častější výskyt hemoragií jater a játra slepic z této skupiny byla statisticky průkazně ($P < 0,05$) těžší ($48,3 \pm 3,66$ g) než u slepic ze skupiny EXT ($41,1 \pm 2,09$ g). U skupiny ENZ byl také statisticky průkazně ($P < 0,05$) nižší obsah erytrocytů v krvi ($2,08 \pm 0,102$ T/l) než u skupiny EXT ($2,27 \pm 0,055$ T/l). Přidavek krmné lipázy ke směsi s vyšším obsahem extrudované řepky měl pozitivní vliv na obsah PUFA v tuku žloutku a pravděpodobně zapříčinil častější výskyt hemoragií jater.

Klíčová slova: slepičky; lipáza; hmotnost vejce; PUFA; hemoragie jater

Supported by the Ministry of Agriculture of the Czech Republic (Grant No. EP/7193) and Ministry of Education, Youth and Sports of the Czech Republic (Grant No. MSM 432100001).

The aim of our experiment was to:

Find the long-term effects of adding lipase to the feed mixture, (with 13.5% of the extruded rapeseed) on the overall performance of the hens.

Analyze the composition of the fatty acids in the yolk fat.

Monitor the overall health of the hens.

The higher the content of the oil in the seeds of the oilseed crops, the higher the amount of natural lipase. The deactivation of the enzymes (lipases) in the feeds can be caused by hydrothermic treatments (extrusion) depending on the physical parameters. The activity of the enzymes (lipases) can also be decreased by the anti-nutritional factors contained in the rapeseed, the non-starch polysaccharides, and the tannins. Rapeseed contains about 0.48% of the tannins in the dry matter (Terril *et al.*, 1992). Tannins and other anti-nutritional factors can also react with the digestive enzymes (with their protein units). This was documented in tests *in vitro*, where there was shown to be an inhibition of cellulase, α -amylase, lipase, and trypsin (Shahidi and Naczki, 1992; Kalač and Míka, 1997). Also Nguz *et al.* (1998) observed strong enzyme inhibition *in vitro* with a low concentration of condensate tannins. Soluble non-starch polysaccharides increase the viscosity of the digesta and decrease the digestive enzyme (amylase, lipase) activities (Almirall *et al.*, 1995). A slight decrease of lipid digestion in chicks caused by tannins and polysaccharides contained in field bean hulls was observed by Longstaff and McNab (1991). Some micro-organisms produce a lipase mixture that is more specific towards the C 2 position (Asahara *et al.*, 1993), where mainly polyunsaturated fatty (*n*-3 series) acids are tied up (Kinsella *et al.*, 1990). This can influence the composition of the digested fat and consequently the composition of the yolk fat.

MATERIAL AND METHODS

Isabrown layers were used in this experiment. The experiment period lasted from 21 to 72 weeks of age of the hens. The composition of the mixtures fed to the hens and the content of the nutrients are shown in Tables 1 and 2. The content of the nutrients was determined according to Ordinance 16/2000 Sb. (2000). The main part of the mixture (45%) consisted of the extruded feed (wheat, pea and rapeseed in ratio 4 : 3 : 3). The mixture contained 13.5% of the extruded rapeseed. The 64 hens, which were used in the experiment, were divided into two groups of 32 hens each. They were kept in four-story cages with two hens

Table 1. Feed mixture formulation

Feed		Group	
		EXT	ENZ
Wheat	(g/kg)	210.0	210.0
Extruded rapeseed feed ⁺	(g/kg)	450.0	450.0
Soybean meal	(g/kg)	60.0	60.0
Corn	(g/kg)	140.0	140.0
Fish meal	(g/kg)	30.0	30.0
Limestone	(g/kg)	77.0	77.0
NaCl	(g/kg)	1.8	1.8
Dicalcium phosphate	(g/kg)	20.4	20.4
Premix	(g/kg)	10.9	10.9
Lipase	(mg/kg)	–	500.0

⁺ Extruded rapeseed feed contained wheat, rapeseed and pea in ratio 4 : 3 : 3

in each cage. The control group EXT was fed the mixture without lipase. The experimental group ENZ was fed the same mixture as the EXT group, but with the addition of lipase (500 g/t). The activity of lipase

Table 2. Dietary nutrient contents per kilogram of dry matter

Nutrient		Group
		EXT + ENZ
Dry matter	(g)	875.0
Protein	(g)	175.3
Methionine	(g)	3.7
Thiosulfuric amino acids	(g)	6.4
Threonine	(g)	6.5
Fiber	(g)	53.8
AME _N	(MJ)	11.6
Ca	(g)	36.5
P	(g)	8.7
Na	(g)	1.5
Mg	(g)	1.5

To the 1 kg of mixture was added by premix followed amount of nutrients: Cu – 4.94 mg, Fe – 142.8 mg, Zn – 60.4 mg, Mn – 59.9 mg, Co – 0.24 mg, I – 3.54 mg, Se – 0.44 mg, vit. A – 11 tis. m.j., vit. D3 – 2 tis. m.j., vit. E – 25.2 mg, vit. K3 – 2.2 mg, vit. B1 – 2.24 mg, vit. B2 – 7.85 mg, vit. B6 – 3.27 mg, vit. B12 – 0.018 mg, biotin – 0.11 mg, B3 – 25.1 mg, folic acid – 1.05 mg, calcium pantothenate – 9.8 mg, cholin – 508.8 mg, L-lysine – 44 mg, methionin – 773.6 mg, threonin – 39.6 mg.

was 300 TLU/kg (total lipase units). The mixtures were fed *ad libitum*. During the 52-week experimental period, we recorded both the number of eggs laid as well as the feed intake in weekly intervals. We weighed the amounts of feed mixtures and the unused feed each week in both groups. All the eggs were weighed and we calculated the daily egg mass production and the feed conversion. From the age of 62 to 72 weeks, we conducted balance assays with the indicator method to find the coefficients of the fat retention. In the assays, we used all hens (2×32); each group was divided into four subgroups and we analyzed eight samples (8×4) of droppings in particular group. The indicator was chromium oxide Cr_2O_3 .

For the evaluation of the health status, we chose (during the 72nd week of age), 16 intensively laying hens from each group. We bled them to determine the total content of lipids and cholesterol in the blood plasma, as well as to determine the number of erythrocytes, leukocytes, and amount of haemoglobin and haematocrit. The total content of lipids and cholesterol were determined by Bio-La tests of Lachema firm. The numbers of erythrocytes and leukocytes were determined in Bürker closet. The values of haematocrit were detected using microhaematocrit tubes. These hens were killed and we evaluated their weight and any damage that we observed visually to their livers. We also noted the content of the fat in the liver according to Ordinance 16/2000 Sb. By using a gas chromatograph, we were able to analyze the content of the particular fatty acids in the yolk fat. All the hens were weighed at the beginning of experiment (21st week of age) and at the end of experiment (72nd week of age) individually.

The results were evaluated by means of variance analysis (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

During the experiment, we did not observe marked health problems in the hens.

We did not find marked differences between the parameters of the performance of the hens (Tables 3a and 3b). The average weight of the eggs was higher in the ENZ group (62.5 ± 0.38 g) than it was in the EXT group (61.8 ± 0.39 g). This was probably a result of better retention of the fat in this group. We calculated the coefficients of the fat retention 0.92 ± 0.008 for the group ENZ, and 0.91 ± 0.007 for the group EXT. However, in a study by Tan *et al.* (2000) better digestibility of fat after addition of lipase was not observed.

The addition of lipase did not have any effect on the final weight of the hens (EXT – $2\,049 \pm 32.8$ g versus ENZ – $2\,023 \pm 28.8$ g). Their average weight changes from week 52 to week 72 were as follows: ENZ – 411 ± 28.9 g/hen, EXT – 435 ± 36.4 g/hen.

The characteristics of the blood at 72 weeks of age are shown in Table 4.

The experiment did not affect the content of total lipids and cholesterol in the blood plasma. The total contents of the lipids in the blood plasma were as follows: 26.68 ± 3.536 g/l in group ENZ and 27.68 ± 2.982 g/l in group EXT. The cholesterol levels were almost the same in both groups, 3.56 and 3.57 mmol/l (EXT and ENZ, respectively). The weight of the livers in the group ENZ was significantly ($P < 0.05$) higher

Table 3a. Parameters of laying performance from 21st to 72nd week of hen age

Parameter	Laying intensity (%)	Egg weight (g)	Egg mass (g/hen/day)
Group	average \pm SE	average \pm SE	average \pm SE
EXT	88.3 ± 0.94	61.8 ± 0.39	54.8 ± 0.69
ENZ	87.1 ± 1.30	62.5 ± 0.38	54.6 ± 0.84

Table 3b. Parameters of laying performance from 21st to 72nd week of hen age

Parameter	Feed intake (g/hen/day)	Conversion (kg/kg)
Group	average \pm SE	average
EXT	112.3 ± 1.05	2.05
ENZ	111.4 ± 0.96	2.04

SE = standard error

Table 4. Characteristics of hens blood in 72 week of age

Group	Cholesterol (mmol/l)	Total lipids (g/l)
	average \pm SE	average \pm SE
EXT	3.56 \pm 0.352	27.68 \pm 2.982
ENZ	3.57 \pm 0.420	26.68 \pm 3.536
	Erythrocytes (T/l)	Leukocytes (G/l)
	average \pm SE	average \pm SE
EXT	2.27 \pm 0.055 ^b	21.74 \pm 1.332
ENZ	2.08 \pm 0.102 ^a	21.76 \pm 1.676
	Haemoglobin (g/l)	Haematocrit (%)
	average \pm SE	average \pm SE
EXT	84.41 \pm 2.789	31.1 \pm 1.13
ENZ	76.23 \pm 4.517	28.2 \pm 1.64

a, b = means of the same order designated by different letters are significantly different from each other ($P < 0.05$)

(48.3 \pm 3.66 g versus 41.1 \pm 2.09 g) in comparison with the group EXT (Figure 1). There wasn't a significant difference in the percentage of fat in the livers, ENZ 4.83 \pm 0.359%, and EXT 5.63 \pm 0.317%. The lower content of the fat in the livers in the group ENZ was

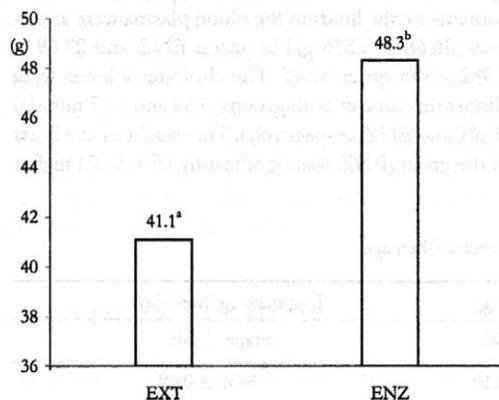


Figure 1. The effect of lipase addition on liver weight in 72 week of hens age

a, b = means of the same order designated by different letters are significantly different from each other ($P < 0.05$)

probably caused by a more frequent incidence of haemorrhages. In some cases, the older haemorrhages were replaced by ligament. The number of erythrocytes was statistically ($P < 0.05$) lower in the group ENZ and consequently there were lower contents of haemoglobin and haematocrit. The number of erythrocytes was, in the group EXT, 2.27 \pm 0.055 T/l, and in the group ENZ it was 2.08 \pm 0.102 T/l. The amount of haemoglobin in the group EXT was 84.41 \pm 2.789 g/l, and in the group ENZ it was 76.23 \pm 4.517 g/l. The haematocrit in the group EXT was 31.1 \pm 1.13% and in the group ENZ 28.2 \pm 1.64%. The experiment had no effect on the number of leucocytes.

We studied whether the addition of lipase had some effect on the composition of the fatty acids contained in the yolk fat (Figure 2). In the content of saturated fatty acids there wasn't any difference between the groups (ENZ – 23.3 \pm 0.27%, EXT – 23.6 \pm 0.20%). We detected significantly ($P < 0.05$) lower amounts of monounsaturated fatty acids in the yolk fat of ENZ (58.5 \pm 0.73%) in comparison with the group EXT (60.0 \pm 0.16%). We found highly significant ($P < 0.01$) differences between the groups in the percentage content of the polyunsaturated fatty acids (PUFA), which was caused by the addition of lipase. The content of the PUFA in the yolk fat was in the group ENZ

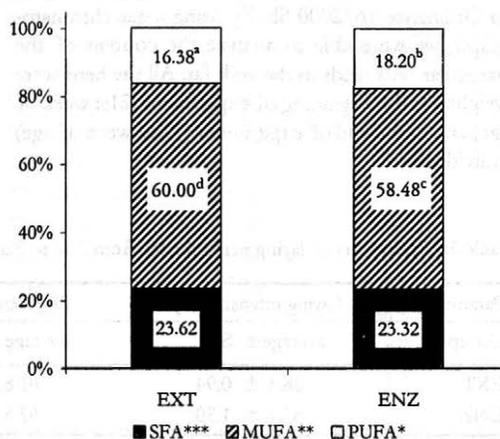


Figure 2. The effect of lipase addition on fatty acids composition in yolk fat

* PUFA = polyunsaturated fatty acids

** MUFA = monounsaturated fatty acids

*** SFA = saturated fatty acids

a, b = means of the same order designated by different letters are significantly different from each other ($P < 0.05$)

c, d = means of the same order designated by different letters are significantly different from each other ($P < 0.05$)

18.2 ± 0.54% and in the group EXT 16.4 ± 0.19%. Our results indicate that there was a better retention of essential fatty acids, linoleic and linolenic, after the lipase application. This fact is supported by the significantly higher ($P < 0.05$) amount of these fatty acids in the yolk fat; linoleic acid ($n-6$) being ENZ – 13.6 ± 0.52% versus the EXT group at – 12.1 ± 0.15% and linolenic acid ($n-3$) ENZ – 1.7 ± 0.06% versus EXT – 1.5 ± 0.04%.

The percentage content of the polyunsaturated fatty acids in the yolk fat was enhanced by the long-term feeding of microbial lipase (500 g/t) in the feed mixture for the hens. However, we observed a higher incidence of liver haemorrhages when the hens were 72 weeks of age. The addition of lipase did not have any effect on the selected parameters of performance.

Acknowledgement

Authors thank Ing. Vladimír Šiške, CSc. Alltech, Inc. for grant of lipase and Mrs. Judy McPherson for language correction.

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Received: 01–08–29

Accepted after corrections: 02–04–15

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Impact of copper and iron additives in feed on productivity of layers and technological characteristics of eggs

Vliv přídavku mědi a železa do krmných směsí na užítkovost nosnic a technologické vlastnosti vajec

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ABSTRACT: The impact of non-antibiotic biostimulative preparations including copper and iron was studied in feed mixture in relation to efficiency of layers and technological qualities of eggs. Two months experiment was carried in the midst of lay time period in three floor cages type Salmet on central floor, in order to eliminate environmental impact. 28 cells with 4 hens (112 hens) were used as a control. Control was fed standard feed mixture, second group (112 hens) was fed standard feed enriched by copper and iron. Egg production was recorded monthly for duration of the experiment. Egg weight was evaluated every month gravimetrically in three consequent days. Consumption of feed mixture was measured every month in representative sample. Technological quality of eggs was determined monthly. Supplement of iron and copper was monitored in feed mixture. Increase in egg production of 2.89% for total period was recorded in the supplemented group. This was associated with reduced daily consumption of feed from 122 to 120 g. Consumption of feed mix per 1 egg was reduced from 0.147 kg to 0.141 kg, or per 1 kg egg matter from 2.274 kg to 2.142 kg, respectively. Thickness of shell, value of shell solidity testing, indices of yolk and albumen, as well as colour of yolk were not significantly influenced.

Keywords: layers; copper; iron; lay of eggs; technological quality of eggs

ABSTRAKT: Byl sledován vliv neantibiotického biostimulačního přídavku, obsahujícího měď a železo, v krmných směsích na užítkovost nosnic a technologické vlastnosti vajec. Pokus se uskutečnil v průběhu dvou měsíců uprostřed snáškového období v třítážových klecích typu Salmet, ve střední etáži pro eliminaci vlivu prostředí. V kontrolní skupině bylo 28 buněk po čtyřech slepicích (112 slepic), které byly krmeny standardní krmnou směsí, v pokusné skupině byl stejný počet slepic, které byly krmeny standardní směsí obohacenou přídavkem železa a mědi. Snáška byla vyhodnocena v kusech za každý pokusný měsíc a ještě za celé sledované období. Hmotnost vajec byla vyhodnocena každý měsíc metodou vážení všech vajec ve třech po sobě jdoucích dnech. Spotřeba krmiva byla sledována po měsících a za celé pokusné období. Technologická kvalita vajec byla stanovena na reprezentativních vzorcích každý měsíc pokusu. Sledovaný přídavek železa a mědi v krmné směsí zvýšil snášku průběžně za celé období o 2,89 % a snížil spotřebu na 1 KD ze 122 g na 120 g. Nižší byla i spotřeba krmné směsí na jedno vejce (0,141 kg oproti 0,147 kg) i na 1 kg vaječné hmoty (2,142 kg oproti 2,274 kg). Hodnoty tloušťky skořápky, hodnoty pevnostních zkoušek skořápky, indexů žloutků i bílků a barva žloutků byly ovlivněny nepodstatně.

Klíčová slova: nosnice; měď; železo; snáška; technologické kvality vajec

Beside other factors iron is important in nutrition of hens, e.g. by its impact on blood formation. Sufficiency of iron prevents leukaemia. It has also important function in red/ox processes taking part in many enzymatic processes as component of haemoglobin (Rous *et al.*, 1971). Content of iron in hen blood is 45–55 mg per 1 kg living mass. Majority is in blood, which contains 10–12 times more iron than other organs (Ferenčík *et al.*, 2000). Iron from ruined erythrocytes is not excreted from the body. Hemosiderin and ferritin are main forms in which it deposits in spleen and in reticule histiocytar tissue, from which it could be released by demand of metabolism, for production new erythrocytes in bone marrow (Sova *et al.*, 1990). Iron is as essential for living function, as it is essential for many subsequent physiologic and biochemical steps (electron transport and connected formation of energetic “stocks” – ATP). It is active in respiratory chains of mitochondrias. Iron is also necessary for storage, activation and transport of oxygen, and its deactivation, for synthesis of nucleotides and many another functions (Harper, 1977; Ferenčík *et al.*, 2000). The degree of iron utilisation is significantly dependent on presence of copper, which activates not only production of haemoglobin but is also active as a catalyst of the metabolism of protein. It increases egg production and has positive impact on development of young animals. Iron helps transfer to bone marrow, causing the stimulation of maturation of erythrocytes. As a part of group oxidative and reductive enzymes, it is active in respiration of tissues. It determines the activity of non-stable hypofysal hormones. (Jindra, 1985; Sova *et al.*, 1990). 1.5–2 mg of copper per 1 kg tissue (without fats) was recorded in body of adult animals. It is concentrated particularly in liver, with considerable amount in kidney, brain, muscles, spleen and blood (Mehner and Harfiel, 1983). Majority of iron consumed in feed mixtures, recommended in norms, fluctuates depending on variety of poultry from 20–30 mg per kg feed mixture, in water poultry 50–80 mg per kg. The exigencie of copper in feed mixture varies in relation to variety of poultry from 3–15 mg per kg. World norm NRC (norm recommended consumption) (Sine, 1994) is higher compared to Czech norm ČSN 46 7070 (Sine, 1983) or CAAS (Czech Academy of Agricultural Sciences) (Sine, 1993). The effect of cupric sulphate pentahydrate on duck weight increases was studied in Department of Pig and Poultry. Positive effect on muscle fortification was observed above all in early phases of development (Rous *et al.*, 1968; Rous and Holoubek, 1968; Podhorský *et al.*, 1970). Similar conclusions were formed in publications studying various

forms of copper (Pesti and Bakalli, 1996; Ewing *et al.*, 1998; Miles *et al.*, 1998). Same effect of copper supplement in feeding of fertile hens was detected especially with synergetic effect of copper and iron repeatedly (Holoubek *et al.*, 1994a, b). The impact of cupric supplement on feed mixture studied Zelenka and Fajmonová (1999). Their results did not confirmed lower demand on copper content with increased ages, described by Georgijevsky *et al.* (1982) – cit. Zelenka and Fajmonová (1999). Zelenka and Fajmonová (1999) on the contrary demonstrated enhances of copper in chicken organisms related to increases of body weight. Skřivan *et al.* (1999, 2000) found that supplement of cupric sulphate pentahydrate enhanced living weight of female broilers by 8.5%, and male broilers by 2.8%. The feed consumption per unit of body weight gain decreased. Simultaneously was observed the reduction of cholesterol in tissues of chicken fed with cupric supplement. (Konjufca *et al.*, 1997; Komprdá *et al.*, 1999). Remarkable is the observation by Podhorský *et al.*, (1970) where the tissue of wild ducks had significantly higher copper content than tissue of domestic ducks (e.g. variety “Peking’s white”). Jackson and Stevenson (1981) studied the impact of various sources of copper (oxide vs. sulphate) on production of eggs and fatty acids in lipidic portion of tissues. The quality of eggs is predominantly the factor of egg weight, thickness and solidity of eggshell, shape index, the rate of yolk to albumen, and eventually the colour of yolk. Egg weight reflects many factors (Halaj and Arpášová, 1995). The heredity of weight ranges from $h^2 = 0.51–0.63$ (Rous, 1972; Izat *et al.*, 1985; Kníže, 1994). The economy of egg production could be affected highly by eggshell quality. Eggshell weight correlates to size of egg and thickness. Significant correlation between thickness of eggshell and solidity is 0.92–0.97 in relation to genotype (Harms *et al.*, 1990). Damage caused by low quality of eggshell, particularly low solidity as described in countries of European Union (EU) and in USA varied between 7–10% (Saprykin and Rjabokon, 1987). Damages observed in Czech Republic were even higher (Jelínek, 1996).

MATERIAL AND METHODS

The effect of biostimulative non-antibiotic preparation on hen organism, excessively strained by intensive egg production, was examined in this study. In addition, the hypothesis that preparation would prevent incidence of anaemia and will satisfy all ecological criteria was also investigated. Main components of the bios-

timulator are iron and copper, extended by wheat flour, partly fortified by enzyme amylase, and complex saccharase with citric acid.

Characteristic of the biopreparate is given on the table:

Component	Content % min.
Dry matter	92
Nitrogenous matter NM	11.5
Reductive matter	17.5
Ash	6.5
Iron	0.84
Copper	0.09

During two months, (February – March 1999) the testing of preparations was conducted on egg-layers brought in September 1998, approximately in the middle of laying period. They were placed in same comparable (2nd) floor of three-floor battery cage type Salmer made in STS Hostivice.

Egg-layers cv. Dominant D 102, 286–345 days old (136–195 days of laying period) were used in experiment. 56 units each occupied by four fertile hens (224 hens) were used. These hens were divided into two groups. First group was fed with commercially produced feed mixture (N) with supplement of 0.5% biostimulative non-antibiotic addition. Second group, control was fed with unsupplemented commercial feed mixture (N). Both groups were fed and watered *ad libitum*. During the whole time of experiment, standard light regime recommended for egg layers was used.

Characterisation of feed mixture N component	Content (g/kg)
Dry matter	855
Nitrogenous matter (NM)	172
Calcium	34
Fibre	34
Phosphorus	5.1
Metabolised energy (ME)	11.2 (Mj/kg)

Parameters studied:

1. Egg production per month and during the total experimental period.
2. Egg weight per month in three consequent days approximately in the middle of month (15–17th day of month).
3. Egg matter per month and for the whole experiment.
4. Consumption of feed per egg, per day and per unit of egg matter for experimental period.

5. Egg quality. Analyses of eggs were conducted monthly always during two consequent days on batches of 20 eggs in experimental and control group. Main technological qualities were tested simultaneously with analyses of egg quality: egg shape index, index of yolk, index of albumen, strength of eggshell, eggshell solidity and yolk colour (by La Roche scale).

RESULTS AND DISCUSSION

No losses were observed during the experiment. Egg production in first month was $88.17 \pm 0.552\%$ in supplemented group, and only $86.51 \pm 0.592\%$ in control (difference is 1.7% and is significant in level $P_{(0.05)}$).

Next month was characterised by reduced egg production in both groups, however difference between experimental and control was increased. Experimental group produced $83.15 \pm 0.624\%$; control group only $79.15 \pm 0.652\%$. The difference (4%) was significant. Overall difference was for the two months of experiment was approximately 2.9%, (Tables 1, 2, 3). This difference was also significant. Egg weight was tested by gravimetric method for all eggs produced in three consequent days once per month in both groups. The results show negligible increase of egg weight between first and second month of experiment in supplemented group (+0.03 g) while decreases of 1.59 g were observed in control. This decrease could be caused either by lower content of some amino acid, probably lysine, in feed mixture, or by low consumption or biopreparation, which cause high N-matter utilisation and restriction of the utility of ME in feed mixture.

In Tables 4, 5 are presented values, which demonstrate positive impact of biopreparate on egg matter production, but the results, which describe the impact on mean egg weight, was not significant.

Data on feed conversion are presented in Table 6, these results are significant.

Compared to values from other laboratories (Sine, 1997) the results are more progressive. Even if the result could be affected by choice two months of highest egg production (by the screening of experimental time), supplement of biopreparate enriched by iron and copper is very effective.

None of technological parameters was affected by supplement of biopreparation used (Tables 7, 8).

Table 9 make evident that the addition of copper and iron to feed mixture has not significant impact on the quality of albumen. Only the little non-significant positive impact on the level $P_{(0.05)}$ was observed.

Table 1. View of egg production in experimental and control egg layers during 1st month of experiment

Day	Experimental group		Control	
	Egg production (pieces)	Egg production (%)	Egg production (pieces)	Egg production (%)
1	100	89.29	102	84.82
2	102	91.07	101	90.18
3	102	91.07	107	95.54
4	102	91.07	102	91.07
5	106	94.64	104	92.86
6	101	90.18	105	93.75
7	107	95.54	105	93.75
8	101	90.18	96	85.71
9	98	87.50	99	88.39
10	90	80.36	76	67.86
11	110	98.21	112	100.00
12	100	89.29	100	89.29
13	95	84.82	96	85.71
14	95	84.82	93	83.04
15	100	89.29	95	84.82
16	91	81.25	96	85.71
17	95	84.82	90	80.36
18	102	91.07	98	87.50
19	102	91.07	95	84.82
20	90	80.36	87	77.68
21	100	89.29	96	85.71
22	98	87.50	91	81.25
23	96	85.71	101	90.18
24	102	91.07	101	90.18
25	92	82.14	89	79.46
26	96	87.51	94	83.93
27	96	87.51	92	82.14
28	96	87.51	92	82.14

Total (pieces) 2 765

Average 88.170

Variance $s = 15.460$ Standard error $s_{\bar{x}} = 0.552$ $\nu = 17.530$ Significant difference for avg. 1.66 in a level $P_{(0.05)}$

Total (pieces) 2 713

Average 86.510

Variance $s = 16.589$ Standard error $s_{\bar{x}} = 0.592$ $\nu = 19.180$

Table 2. View of egg production in experimental and control egg layers during 2nd month of experiment

Day	Experimental group		Control	
	Egg production (pieces)	Egg production (%)	Egg production (pieces)	Egg production (%)
1	89	79.46	94	83.93
2	91	81.25	100	89.29
3	96	85.71	92	82.14
4	89	79.46	90	80.36
5	86	76.79	88	78.57
6	90	80.36	70	62.50
7	92	82.14	80	71.43
8	96	85.71	98	87.50
9	98	87.50	94	83.93
10	87	77.68	88	78.57
11	90	80.36	96	85.71
12	98	87.50	88	78.57
13	97	86.61	88	78.57
14	95	84.82	88	78.57
15	98	87.50	94	83.93
16	96	85.71	96	85.71
17	87	77.68	80	71.43
18	95	84.82	86	76.79
19	95	84.82	96	85.71
20	98	87.50	92	82.14
21	93	83.04	88	78.57
22	92	82.14	90	80.36
23	87	77.68	90	80.36
24	95	84.82	86	76.79
25	91	81.25	90	80.36
26	91	81.25	86	76.79
27	95	84.82	88	78.57
28	96	85.71	80	71.43
29	92	82.14	82	73.21
30	99	88.39	86	76.79
31	103	91.96	84	75.00

Total (pieces) 2 887

Average 83.150

Variance $s = 18.382$ Standard error $s_{\bar{x}} = 0.624$ $\nu = 22.110$ Significant difference for avg. 4.00 in a level $P_{(0.05)}$

Total (pieces) 2 748

Average 79.150

Variance $s = 19.214$ Standard error $s_{\bar{x}} = 0.652$ $\nu = 24.280$

Table 3. Egg production during total laying period (%)

Experimental group				Control			
average	variance s	standard error $s_{\bar{x}}$	ν	average	variance s	standard error $s_{\bar{x}}$	ν
85.83	17.236	0.424	20.15	82.64	18.382	0.452	22.24

Significant difference for avg. 2.89 in a level $P_{(0.05)}$

Tables summarise the values obtained by determination of egg weight per feed consumption

Table 4. The weight of egg during 1st and 2nd month and total experiment (g)

Experimental group					Control				
period	average (g)	variance s	standard error $s_{\bar{x}}$	ν	average (g)	variance s	standard error $s_{\bar{x}}$	ν	minimal difference $D_{\lambda \min}$.
February	65.50	4.533	0.267	6.92	65.71	4.633	0.276	7.05	0.21
March	65.53	4.582	0.190	6.99	64.12	5.096	0.224	7.95	1.41
Total	65.52	4.563	0.155	6.96	64.68	4.993	0.177	7.72	0.84

Significant difference for avg. 2.89 in a level $P_{(0.05)}$

Table 5. Egg matter per unit of feed. Comparison per experimental month and total period (averages)

Experimental group				Control		
period	daily egg production (pieces)	avg. egg weight (g)	egg matter per unit of feed (FD)	daily egg production (pieces)	avg. egg weight (g)	egg matter per unit of feed (FD)
February	0.787	65.60	57.77	0.772	65.71	56.84
March	0.742	65.53	54.32	0.707	64.12	50.78
Total	0.765	65.52	56.02	0.740	64.68	53.43

Table 6. Feed consumption per day, per egg, and per unit of egg matter

Period	Feeding days	Experimental group				Control			
		feed consumption				feed consumption			
		total (kg)	per unit of feed (1 FD)	per 1 egg	per 1 kg of egg matter	total (kg)	per unit of feed (1 FD)	per 1 egg	per 1 kg of egg matter
February	3 136	380	0.121	0.137	2.095	382	0.122	0.140	2.146
March	3 472	415	0.119	0.144	2.184	423	0.122	0.153	2.404
Total	6 608	795	0.120	0.141	2.142	805	0.122	0.147	2.274

Table 7. Shape index of egg (length : width) – averages measured ($n = 20$ pieces)

Date	Experimental group			Control		
	$\bar{x} \pm s_{\bar{x}}$	s	ν	$\bar{x} \pm s_{\bar{x}}$	s	ν
16. 2.	1.30 \pm 0.026	0.118	9.08	1.29 \pm 0.013	0.057	4.42
17. 2.	1.29 \pm 0.012	0.052	4.03	1.27 \pm 0.009	0.042	3.31
16. 3.	1.28 \pm 0.011	0.047	3.67	1.30 \pm 0.008	0.035	2.69
17. 3.	1.29 \pm 0.011	0.051	3.95	1.29 \pm 0.017	0.078	6.05
Total	1.29 \pm 0.008	0.072	5.58	1.29 \pm 0.014	0.126	9.77

Table 8. Egg yolk index – averages ($n = 20$ pieces)

Date	Experimental group			Control		
	$\bar{x} \pm s_{\bar{x}}$	s	ν	$\bar{x} \pm s_{\bar{x}}$	s	ν
16. 2.	0.56 \pm 0.004	0.020	3.57	0.55 \pm 0.008	0.038	6.91
17. 2.	0.49 \pm 0.006	0.028	5.71	0.50 \pm 0.004	0.016	3.20
16. 3.	0.47 \pm 0.004	0.020	4.26	0.44 \pm 0.015	0.065	14.77
17. 3.	0.46 \pm 0.006	0.026	5.65	0.43 \pm 0.010	0.043	10.00
Total	0.49 \pm 0.005	0.046	9.39	0.48 \pm 0.007	0.067	13.96

During the assessment of the egg yolk index significant difference was observed only on 16.3. at ($P_{(0.05)}$) level and 17.3. at ($P_{(0.01)}$) level

Table 9. Albumen index – averages ($n = 20$ pieces)

Date	Experimental group			Control		
	$\bar{x} \pm s_{\bar{x}}$	s	ν	$\bar{x} \pm s_{\bar{x}}$	s	ν
16. 2.	0.12 \pm 0.006	0.025	20.83	0.12 \pm 0.006	0.027	22.50
17. 2.	0.10 \pm 0.004	0.020	20.00	0.11 \pm 0.006	0.026	23.64
16. 3.	0.09 \pm 0.005	0.022	24.44	0.09 \pm 0.004	0.020	22.22
17. 3.	0.11 \pm 0.003	0.015	13.64	0.09 \pm 0.004	0.019	21.11
Total	0.10 \pm 0.002	0.022	22.00	0.10 \pm 0.003	0.025	25.00

Table 10. Comparison of eggshell thickness – averages (mm)

Date	Experimental group			Control		
	$\bar{x} \pm s_{\bar{x}}$	s	ν	$\bar{x} \pm s_{\bar{x}}$	s	ν
16. 2.	0.34 \pm 0.009	0.040	11.76	0.35 \pm 0.006	0.029	8.29
17. 2.	0.35 \pm 0.006	0.027	7.71	0.35 \pm 0.006	0.028	8.00
16. 3.	0.35 \pm 0.008	0.034	9.71	0.36 \pm 0.006	0.026	7.22
17. 3.	0.37 \pm 0.005	0.021	5.68	0.36 \pm 0.007	0.032	8.89
Total	0.35 \pm 0.004	0.032	9.14	0.36 \pm 0.003	0.029	8.06

Table 11. Eggshell solidity (N/cm²) comparison

Date	Experimental group			Control		
	$\bar{x} \pm s_x$	<i>s</i>	<i>v</i>	$\bar{x} \pm s_x$	<i>s</i>	<i>v</i>
16. 2.	31.10 ± 0.811	3.626	11.66	28.05 ± 1.445	6.460	23.03
17. 2.	30.40 ± 0.866	3.872	12.74	29.36 ± 0.944	4.221	14.38
16. 3.	32.65 ± 1.288	5.761	17.64	31.75 ± 0.948	4.241	13.36
17. 3.	30.15 ± 0.892	3.990	13.23	29.75 ± 1.192	5.330	17.92
Total	31.08 ± 0.494	4.420	14.22	29.73 ± 0.584	5.222	17.56

Table 12. Yolk colour (according to La Roche)

Date	Experimental group			Control		
	$\bar{x} \pm s_x$	<i>s</i>	<i>v</i>	$\bar{x} \pm s_x$	<i>s</i>	<i>v</i>
16. 2.	3.45 ± 0.185	0.826	23.94	3.45 ± 0.170	0.756	22.00
17. 2.	3.35 ± 0.150	0.671	20.03	3.25 ± 0.099	0.444	13.66
16. 3.	3.45 ± 0.114	0.510	14.78	3.40 ± 0.112	0.503	14.79
17. 3.	3.20 ± 0.092	0.410	12.81	3.05 ± 0.050	0.224	7.34
Total	3.36 ± 0.069	0.621	18.48	3.29 ± 0.059	0.532	16.17

The supplement of iron and copper did not affected the values of eggshell, but some non significant diminution of eggshell thickness were observed (Table 10.)

The supplement of iron and copper positively affected the values of eggshell solidity. Some exceptional diminution of eggshell thickness was observed as very interesting (Table 11).

Non-significant yolk colour improvement indicates that the addition of copper and iron had not negative effect on this parameter (Table 12).

In conclusion it can be stated, that the supplement of iron and copper is suitable for wide practice for egg production for human consumption. Additional studies seem to be necessary for production of eggs for hatching.

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Received: 01–10–23

Accepted after corrections: 02–04–16

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Estimation of beef carcass conformation carried out at a high-performance abattoir line and based on an impedance method

Odhad zmasilosti jatečně upraveného těla skotu impedanční metodou na vysoce výkonné porážkové lince

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ABSTRACT: Using a device (based on a 4-electrode method) for measuring the values of biological tissue impedance at 2 and 120 kHz, the impedance of 293 beef carcasses was identified during the current operation of a high-performance, modern abattoir line. An adapter with variable distance of needle electrodes and a computer-controlled measuring process enabled a fast impedance scanning process. The values recorded by measurements (impedance – imp2, 120 (kHz); warm carcass weight – CW; distance of measuring electrodes – D) and the values subsequently derived were estimated as independent variables, and subsequently their correlation to the estimated commercial classification values (conformation, fatness) was ascertained. The measurements revealed a relationship ($r = 0.8$) between conformation and impedance at the frequency of 120 kHz; and a high correlation between “electric volume” ($D^2/\text{imp}120$) at the frequency of 120 kHz and the warm carcass weight (CW), expressed by the correlation coefficient $r = 0.827$. Using a stepwise regression analysis, regression formulas for conformation estimation were sought for, and the best model with the coefficient of correlation/determination ($r = 0.867/r^2 = 0.752$) was found for the following independent variables: imp2, $D^2/\text{imp}120$, CW and D. The calculated correlation coefficients (r) are lower than those obtained by former measurements using individually inserted electrodes.

Keywords: bio-electrical impedance; beef carcass; estimation of commercial grading; conformation; fatness

ABSTRACT: Pomocí přístroje měřícího 4-elektrodovou metodou impedanci biologických tkání při frekvenci 2 a 120 kHz byla změněna impedance 293 kusů skotu během běžného provozu vysoce výkonné porážkové linky moderních jatek. Rychlé snímání impedance umožnil držák s proměnnou vzdáleností jehlových elektrod a řízení měřícího procesu počítačem. Změřené hodnoty (impedance – imp2, imp120 (kHz); hmotnost teplého jatečně upraveného těla – CW, vzdálenost měřících elektrod – D) a z nich odvozené veličiny byly vyhodnocovány jako nezávislé proměnné a byly zjišťovány jejich korelace na odhadovaných hodnotách obchodních tříd (zmasilost, protučnost). Byla zjištěna závislost zmasilosti na impedanci při frekvenci 120 kHz ($r = 0,8$) a korelace „elektrického objemu“ ($D^2/\text{imp}120$) při frekvenci 120 kHz na hmotnosti teplého jatečně upraveného těla (CW), dobrou těsnost vyjadřuje korelační koeficient $r = 0,827$. Pomocí „stepwise“ regresní analýzy byly hledány regresní rovnice pro odhad zmasilosti a nejlepší model s koeficientem korelace/determinace ($r = 0,867/r^2 = 0,752$) byl nalezen pro tyto nezávislé proměnné: imp2, $D^2/\text{imp}120$, CW a D. Dosažené korelační koeficienty (r) jsou nižší než u dřívějších měření s individuálně vpichovanými elektrodami.

Klíčová slova: bioelektrická impedance; jatečně upravené tělo skotu; odhad obchodní třídy; zmasilost; protučnost

The estimation of bio-physical characteristics of livestock and carcasses is carried out using the method of bio-electric impedance analysis (BIA), serving for indirect detection of the livestock conformation and its carcasses. Research and pilot applications were reported, e.g. by Cosgrove *et al.* (1988), Swantek (1991, 1992) and Marchello (1994). Lukaski (1987) offered an exhaustive description of the methods for determination of the human body composition. The paper comprised both the traditional and new methods, including BIA, applied in human medicine for purposes of estimating fat-free mass (FFM-kg), total body water, extracellular water and protein, etc. The same problem was a subject of the papers by Lukaski *et al.* (1986) and Thomas *et al.* (1992). As for the fundamental reference literature, it is Lukaski *et al.* (1985), describing the methods of tetrapolar impedance analysis and its application to estimation of fat-free mass of the human body. There are no reference publications dealing with the direct determination of body fat. The authors determine these values only on the basis of calculations from an absolute estimation of FFM and subsequent calculation from the known warm carcass weight. The use of BIA for estimating the conformation class according to EU standards was mentioned by Bohušlávěk (2000) – particularly the use of a classic impedance analyser with the individual insertion of needle electrodes to the fore and hind limbs of the carcass. The purpose of this paper is to describe experiments carried out with respect to the BIA method applied at a high-performance abattoir line in full operation. The objective of the experiments was to verify the applicability of the bio-electric impedance method for an estimate of conformation and fatness classification according to the EU.

MATERIAL AND METHODS

Description of measuring device

For purposes of experiments carried out at an abattoir line with an output of 800 cattle carcasses per shift, the author's centre developed a device for the measurement of impedance, with a sampling period shorter than 1 s and the possibility of a PC-controlled measuring process. The device makes use of measuring current of 1 mA and two frequencies, i.e. 2 kHz and 120 kHz, and it is based on the 4-electrode measuring method, Lukaski *et al.* (1985), Swantek and Lukaski, 1991) which helps eliminate the polarization effect and electrode contact resistance, thus enabling to carry out measurements within the potential linear course. The

entire measuring system consists of three independent components:

- telescopic electrode adapters
- automatic impedance measuring set
- master PC unit

The impedance measurement under full operation of the abattoir line does not allow the positioning of individual needle electrodes in the carcass limbs as used by Swantek *et al.* (1992), Hegarty *et al.* (1998), Bohušlávěk (2000). The measurement is carried out by help of a specially developed telescopic adapter with needle electrodes that enables the fast positioning of all electrodes on a carcass, together with further adjustments and measurement of the distances. The impedance measuring set and the computer unit communicate via an optically divided interface, RS232C, and also the input circuits of the device are divided by an optical coupling. The aforesaid solution enables to absolutely eliminate all effects of disturbing voltage. The master PC unit software ensures automated transmission, processing and storage of data measured or entered using the keyboard.

Selection of measuring points

The previously applied needle insertion points, Bohušlávěk (2000), are no longer convenient for the new method of electrode positioning; that is why new points were chosen, based on previous tests, easily accessible from the medial (dividing) part of the carcass. The distance of the pairs of measuring and current electrodes was pre-set at a spacing of 80 mm. For purposes of an experiment, the following easily definable points were selected:

- lower electrodes: position in the gap between the 1st and 2nd rib and current electrodes in front of the 1st rib
- upper electrodes: located 50 mm above the pubic bone (*os pubis*) in the muscle and current electrodes by the 80 mm spacing higher. For the construction diagram and the position of the adapter on a carcass see Figure 1.

Description of the carcass set

The measuring system was verified at an abattoir in Pfarrkirchen (Germany), where 293 beef carcasses (cow) were measured during a part of a shift. The measurement was carried out on left half-carcass. The physical and electrical characteristics for carcasses of

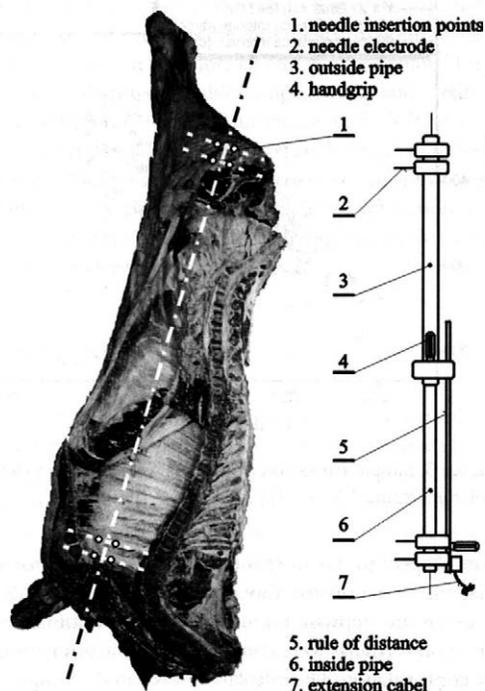


Figure 1. Construction scheme and the position of the adapter on a carcass

the group of evaluated animals ($n = 293$) are summarized in Table 1.

Processing of results, statistical evaluation

After the completion of the measured and estimated values, the acquired data were completed with calculated values. The D^2/imp value is derived from the

measured impedance and the distance of electrodes and evaluated according to Nyboer *et al.* (1943), Lukaski *et al.* (1985, 1986), Swantek *et al.* (1992), Hegarty *et al.* (1998), etc., as electric volume, with generally very good correlation to carcass weight. Another value calculated is the impedance ratio, $imp2/imp120$, which was expected to be related to fatness. The results of experimental measurements were processed using the WINSTAT application. An analysis of correlations was carried out, together with regression analysis for the best relationships. The data were analysed to determine the relationships between conformation and the impedance measurements. Estimation formulae for conformation class were calculated by multiple regression analysis and stepwise regression was used to develop the optimized multiple regression equation. Presented values for r and r^2 are all adjusted coefficients and indicate the proportion of variance attributable to the x variable (δ).

RESULTS AND DISCUSSION

For the results of correlation analyses see Table 2. Similarly like in previous experiments of Bohuslavěk (2000), the results indicate a close relationship between conformation, weight and impedance. As concerns the impedance at higher frequency, i.e. at 120 kHz, the correlation coefficient was $r = 0.8$, which is a proof of the fact that impedance at high frequencies depends on the total carcass mass, similarly like conformation, as presented by Branscheid *et al.* (1999), Bohuslavěk (2002). In addition, the relationship between “electrical volume” ($D^2/imp120$) at the frequency of 120 kHz is significant, and the higher dependence is expressed by the correlation coefficient $r = 0.827$. In total, the ascertained correlations are lower than the correlations acquired from experiments with manually and indi-

Table 1. Physical and electrical characteristics of the group of evaluated animals ($n = 293$)

	Conformation* (15)	Fatness* (15)	Carcass weight (kg)	imp120 (ohm)	imp2 (ohm)	$D^2/imp120$ (cm^2/ohm)
Max	14	12	497.4	111	259	395
Min	5	2	190.6	60	114	184
Mean (\bar{x})	9	7	367.2	75	143	285
S	1.54	1.89	51.09	9.97	24.35	35.74
s/\bar{x} (%)	17.1	27.0	13.9	13.2	17.0	12.5

*grading system EUROP (divided into 15 subclasses) for conformation and fatness, respectively

imp2 = impedance at a frequency 2 kHz, imp120 = impedance at a frequency 120 kHz

D = distance of measuring electrodes

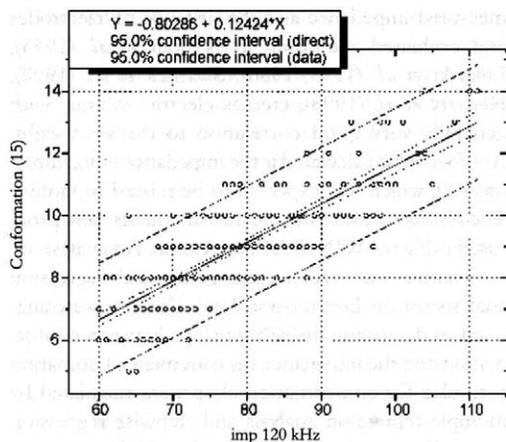


Figure 2. Simple regression on conformation of impedance with frequency 120kHz ($r = 0.8$)

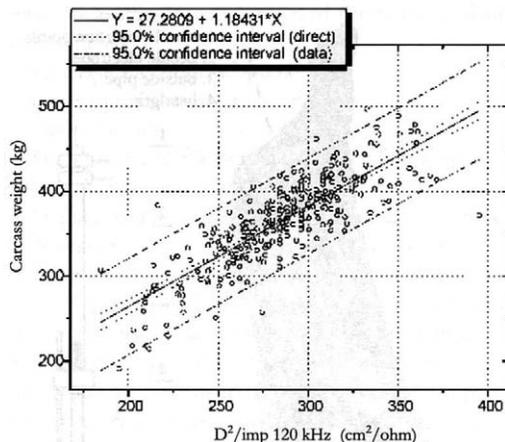


Figure 3. Simple regression on carcass weight CW of electric volume $D^2/imp120$ ($r = 0.827$)

vidually inserted electrodes, as described by Bohušlávěk (2000). This is particularly due to a higher variance of the measured values, caused by the difficulties in inserting needle electrodes, and the subsequently imperfect insertion of electrodes in tissues during a short operation time. Errors were also caused by the frequent puncture of electrodes directly into carcass bones. Low correlation values are reported for the relationship between impedance ratio $imp2/imp120$ and fatness; however, it is impossible to draw final conclusions owing to the considerable variance of the measured values. A simple regression analysis was carried out

with respect to the aforesaid best relationships. For a diagram of regression functions see Figures 2 and 3.

Using the stepwise regression analysis, optimal regression formulas were determined for the estimation of conformation. The calculation was based on impedance measured at two frequencies (i.e. at 2 kHz, 120 kHz) in combination with other physical values (CW – carcass weight, D – distance between measuring electrodes). The formula with the highest coefficient of correlation/determination ($r = 0.867/r^2 = 0.752$) was determined for the following independent variables: $imp2$ (kHz); $D^2/imp120$; $imp2/imp120$ and

Table 2. Correlations between the commercial grades for beef carcasses and electrical or physical characteristics

	$imp2$ (ohm)	$imp120$ (ohm)	$D^2/imp120$ (cm^2/ohm)	$imp120/D$ (ohm/cm)	$imp2/imp120$ (cm/cm)	Carcass weight (kg)
Conformation* (15)	0.666	0.802	-0.609	0.765	0.024	-0.726
Fatness* (15)	0.094	-0.016	0.109	-0.086	0.233	0.314
Carcass weight (kg)	-0.627	-0.645	0.828	-0.8	-0.221	1

Table 3. Regressions models for EUROP-conformation of beef carcasses ($n = 293$)

Model No.	Independent variables	Equations; Dependent variable = Conformation***	r/r^2 * (RSD)**
1	$imp2$; $D^2/imp120$; $imp2/imp120$; CW	Conf. (15) = $5.56 \cdot 10^{-2} \cdot imp120 + 2.06 \cdot 10^{-2} \cdot D^2/imp120$ $- imp2/imp120 \cdot 6.69 - CW \cdot 2.15 \cdot 10^{-2} + 15.38$	0.892/0.795 [0.705]
2	$imp120$; CW; D	Conf. (15) = $5.94 \cdot 10^{-2} \cdot imp120 + 5.11 \cdot 10^{-2} \cdot D -$ $-1.8 \cdot 10^{-2} \cdot CW$	0.867/0.752 [0.774]

* r/r^2 = coefficient of correlation/determination

** (RSD) = standard error of estimation

***conformation classes (divided into 15 subclasses, P⁻ to E⁺)

CW. In order to compare the results with data acquired on the basis of previous measurements of Bohušlák (2000), regression dependence was also determined for the following three variables: imp120, CW and D. For the calculation of both relations see Table 3. The acquired correlation coefficients (r) are lower than those of the earlier measurements carried out with the use of individually inserted electrodes. The explanation of possible reasons is identical to that of the evaluation correlation dependence.

CONCLUSION

The “automatic” measuring system of bio-electric impedance, developed for purposes of objective classification of beef half-carasses, confirmed and verified the potential of needle electrodes used during measurements carried out in the operation. It was possible to derive a regression formula for an estimate of carcass conformation on the basis of the acquired values. Needle electrodes were seen as less reliable with respect to their insertion and considerable variance of the values measured in repeated cycles. During the following stages, attention will be focused on improving the sensor electrodes, their optimum localization on a beef half-carasses and automatic measurement of the distance between electrodes.

Acknowledgement

I am indebted to Prof. C. Augustini and Prof. W. Branscheid, Head of the Institute for Meat Production and Market Research in BAFF Kulmbach for making possible the research experiments in an abattoir in Pfarrkirchen (Germany) and professional beef EUROP-classification by inspectors of the Institute.

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Received: 01–12–18

Accepted after corrections: 02–04–09

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Heavy metal distribution in the ecosystems of the upper course of the Jihlava River

Koncentrace těžkých kovů v ekosystémech horního toku řeky Jihlavy

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ABSTRACT: In October 1999, the concentrations of Hg, Cd, Pb, Cr, Cu, Zn and Ni were monitored in three localities of the upper course of the Jihlava River. The river water, sediments of the riverbed, zoobenthos samples and body tissues of the fish (gills, gonads, skin, dorsal muscles) were analysed. The chub (*Leuciscus cephalus*) was used as the ichthyo-indicator. At the same time the stability of the fish community was evaluated using electrofishing and applying basic ichthyologic methods. In spite of the relatively heavy pollution of the investigated river section (particularly with Pb, Cd, Hg, Cr and Cu), the concentration of mercury and lead in the muscle tissue of the chubs increased only slightly (i.e. < 0.139 mg/kg and < 0.636 mg/kg, respectively). The concentrations of cadmium, lead and zinc were higher in the skin of all the fish (i.e. < 0.132 mg/kg, < 0.597 mg/kg and < 87.30 mg/kg, respectively). The concentration of cadmium (< 0.162 mg/kg) and of lead (< 0.658 mg/kg) was higher in the gonads and the concentration of cadmium (< 0.204 mg/kg), lead (< 0.805 mg/kg) and zinc (< 63.83 mg/kg) was higher in the gills. The index of diversity of the fish community was 1.175–1.743, equitability index 0.455–0.486, abundance 1 748.1–6 396.9 fish/ha and biomass 88.2–284.1 kg/ha.

Keywords: heavy metal; fish; chub; Jihlava River; Czech Republic

ABSTRAKT: V říjnu 1999 byla zjišťována koncentrace Hg, Cd, Pb, Cr, Cu, Zn a Ni na třech lokalitách horního toku řeky Jihlavy. Analýzy byly prováděny v říční vodě, sedimentech dna, vzorcích zoobentosu a v tělních tkáních ryb (žábry, gonády, kůže, hřbetní svalovina). Jako ichthyoindikátor byl použit jelec tloušť (*Leuciscus cephalus*). Současně byla pomocí elektrolovu a základních ichthyologických metod hodnocena stabilita rybího společenstva. Cílem sledování, zahájeného v roce 1999, je postupně zmapovat distribuci těchto specifických polutantů v ekosystému celého toku řeky Jihlavy, která protéká významnými městskými a průmyslovými aglomeracemi a současně je intenzivně využívána ke sportovnímu rybolovu. Studie se nezaměřuje pouze na monitoring obsahu vybraných těžkých kovů v rybí svalovině z hlediska případných rizik pro konzumenty těchto ryb. Sledování jejich distribuce ve vodním prostředí, v potravním řetězci a důležitých rybích orgánech a tkáních umožní kvalifikované posouzení vlivu tohoto znečištění na prosperitu rybích společenstev a populací v dlouhodobějším horizontu. Úsek řeky Jihlavy sledovaný v roce 1999 (mezi říčními kilometry 156,7 a 92,1) svým charakterem odpovídá parmovému pásmu. Lokalita č. 1 se nacházela níže po toku pod masokombinátem Kostelec nad Jihlavou, lokalita č. 2 pod městem Jihlava a lokalita č. 3 pod městem Třebíč. Na základě analýz vzorků zoobentosu byla na lokalitách 1 a 2 kvalita vody vyhodnocena jako α -mezosaprobni, na lokalitě 3 jako β -mezosaprobni. Index saprobity postupně klesal od lokality 1 (3,10) k lokalitě 3 (2,26). Na všech sledovaných lokalitách byly ve vodním prostředí zaznamenány výrazně zvýšené koncentrace sledovaných kovů, zejména Pb, Cd, Hg, Cr a Cu. Obsah těchto kovů v sedimentech dna a zoobentosu výrazně vzrůstal od lokality 1 k lokalitám 2 a 3. V případě Hg, Cd, Pb a Cu byl nejvyšší na lokalitě 2, u Cr a Zn (jen sedimenty) na lokalitě 3. Na lokalitě č. 1 vykázal nejvyšší koncentraci v sedimentech a zoobentosu Ni. Ve svalové tkáni jelců tloušťů však byla zjištěna jen mírně zvýšená koncentrace rtuti ($\leq 0,139$ mg/kg) a olova ($\leq 0,636$ mg/kg). V kůži ryb ze všech lokalit byla zaznamenána zvýšená koncentrace kadmia ($\leq 0,132$ mg/kg), olova

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Research Project No. 43210001).

($\leq 0,597$ mg/kg) a zinku ($\leq 87,30$ mg/kg). V gonádách potom kadmia ($\leq 0,162$ mg/kg) a olova ($\leq 0,658$ mg/kg). A konečně v žábách v případě kadmia ($\leq 0,204$ mg/kg), olova ($\leq 0,805$ mg/kg) a zinku ($\leq 63,83$ mg/kg). Rybí společenstvo bylo charakterizováno výskytem 6–12 rybích druhů, indexem diverzity 1,175–1,743, indexem ekvitivity 0,455–0,486, abundancí 1 748,1–6 396,9 ks/ha a biomasou 88,2–284,1 kg/ha. Relativně nejstabilnější stav rybího společenstva s 12 zastoupenými rybími druhy, indexem diverzity 1,743 a indexem ekvitivity 0,486, ale s nejnižší biomasou 88,2 kg/ha, byl zjištěn na lokalitě 3.

Klíčová slova: těžké kovy; ryby; jelec tloušť; řeka Jihlava; Česká republika

In 1950–1989 the air, soil and water in the Czech Republic were heavily, and virtually uncontrollably, contaminated with pollutants of industrial, agricultural and communal origin, which culminated in 1970–1980. This situation improved considerably after 1989 in connection with the political and economic changes. In the past 10 years, the contamination of surface waters decreased markedly (by 50%), but it is still relatively high, especially due to the long-term after-effects of ecological pollution.

Heavy anthropogenic pollution severely impairs the environment of the fish and, along with the disastrous mortality rate causes chronic environmental stress with grave consequences for the physiological functions of the fish organism. To this effect, the occurrence of heavy metals, along with strong eutrophication, presents a serious problem. Heavy metals are subjected to bioaccumulation in the food chain. At the top of the food chain are fish, particularly predatory fish and long-living benthophagous species. In ecological terms, fish are irreplaceable bio-indicators of the degree of damage of the water environment. However, it is also important to monitor the contamination of fish with heavy metals because frequent consumption of the contaminated fish, especially in families of anglers, presents a very serious health risk. At the present time more than 300 000 anglers are registered in the Czech Republic who catch about 4 000 tons of fish every year in surface streams and consume these fish at home.

In the Czech Republic, heavy metal residues in fish and in the water environment had not been systematically monitored until the past 10 years. For orientation purposes, Vostradovsky *et al.* (1982) monitored mercury concentrations in fish in the Berounka River and Svobodova *et al.* (1988) in fish of the Zelivka water reservoir. Penaz *et al.* (1979, 1980) studied the content of mercury in the basic components of the ecosystems and in the fish muscles of the Jihlava River near Hrubšice and Mohelno. Spurny and Mares (1991) monitored the concentration of Zn, Cr, Pb, Cd and Hg in seven fish species of the Jihlava River (Dalesice and Mohelno reservoirs). Svobodova *et al.* (1993)

monitored foreign substances in fish of the Labe (Elbe) River. Spurny and Mares (1995) monitored the distribution of heavy metals (Hg, Cd, Pb, As, Cr, Cu, Zn, Ni, Fe, Mn, Se and Mo) in the gills, skin, intestine content, intestine wall, kidneys, gonads, liver and dorsal muscles in five fish species of the Austrian and Czech section of the Dyje River. Kockova *et al.* (1996) carried out heavy metal content in benthic organisms (zoobenthos and periphyton) and river bottom sediments along the whole of the Thaya River within the period 1992–1994. Svobodova *et al.* (1999) published data on the bioaccumulation of mercury in various fish species from the Orlik and Kamyk water reservoirs on the Vltava River. Jurajda (1999) monitored the content of Pb, Cd, Hg and 10 organic pollutants (including PCBs and DDT) in fish in the Morava and Dyje rivers.

In the present study our focus was on the distribution of heavy metals in the ecosystem of the upper course of the Jihlava River (barbel zone), which flows through important town and industrial agglomerations; the Jihlava River was generally considered to be one of the most heavily mercury-polluted Czech rivers (Moldan *et al.*, 1990). The concentrations of Hg, Cd, Pb, Cr, Cu, Zn and Ni were monitored in samples of the water, sediments of the river bottom, composite samples of the zoobenthos and in selected body tissues of the chub (*Leuciscus cephalus*). As an indicator, chub is considered a model fish species because it appears in sufficient number virtually in all the river zones, lives a relatively long life and in terms of its choice of food is omnivorous. At the same time, we studied the species diversity and ecological stability of the entire fish community in the river section.

MATERIAL AND METHODS

Throughout October 1999, we conducted systematic ichthyological research by way of electrofishing in three localities of the upper course of the Jihlava River to find the abundance, biomass and species diversity of the fish community. From each locality, we took 7 samples of the chub for laboratory analyses of heavy

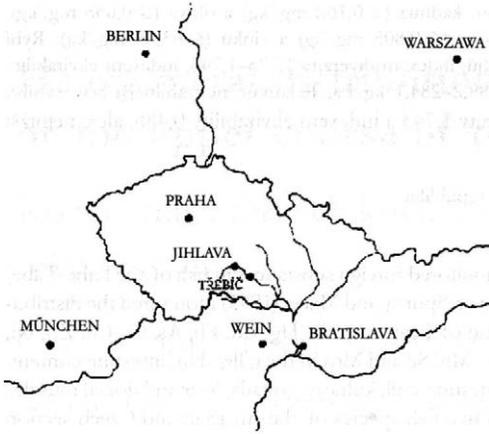


Figure 1. Location of the studied river

metals. At the same time, we used portable equipment to measure the water temperature, pH value, conductivity and the concentration of dissolved oxygen. For follow-up laboratory analyses of heavy metal concentrations, we took samples of the water, sediments of the river bottom and of the zoobenthos. The zoobenthos was further sampled to assess the degree of the saprobity based on saprobity indexes of the represented species.

As to the character of the monitored river section, it is a barbel zone, for the most part with fast flowing water, stony to gravel bottom, in places with large

stones in the riverbed. The width of the riverbed ranges between 9.5 and 16.2 m. Locality No. 1 (river kilometre 156.7) lies in the highest altitude, 28 km from the headwater; and the quality of the water is already contaminated by the large meat-processing plant. Locality No. 2 (river kilometre 123.8) lies downstream below the large industrial town Jihlava (53 000 population; engineering, metallurgical, electrical, building, wood-processing, textile and food industries). Locality No. 3 (river kilometre 92.1) is situated below the discharge of the sewage disposal plant of another fairly large industrial town Třebíč (40 000 population; boot-and-shoe, textile, food, wood processing and engineering industries). A one-day excursion was devoted to each of these three localities.

Samples of the fish tissues for heavy metal analyses were taken in the laboratory immediately after returning from the field excursions. For the individual assessment of heavy metals, samples of the dorsal muscle without skin, gill tissue (cut off from the gill arch), gonads and skin without scales were taken from 7 chubs from each locality. These tissue samples were immediately frozen and kept in the freezer in a temperature of -18°C together with the water, sediment and zoobenthos samples until further assessment. To determine the age of the chubs, 5–8 scales were taken as samples from each fish.

The concentration of heavy metals was analysed after dry mineralising of the fish tissues using the Czech mineralising equipment APION (producer

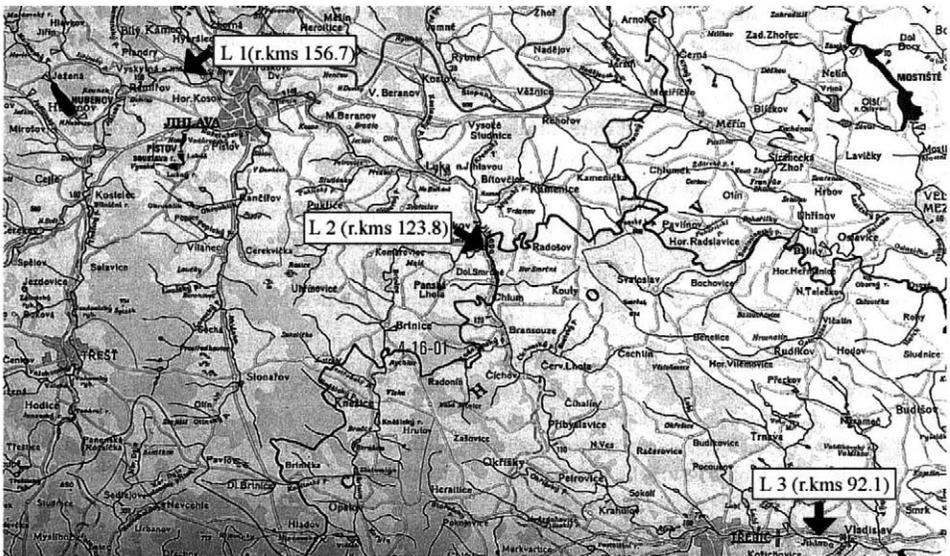


Figure 2. Map of localities

Tessek Prague). Dry mineralising consists in drying the sample in special bowls at 105°C for 2 hours. After cooling the bowls were transferred to the mineralising oven where the samples were incinerated in a mixture of oxidation gases (NO_x , O_3 , O_2) for 12 hours under program-controlled temperatures increasing from 20 to 400°C. The mineralised samples were then put into a solution of 0.5 M p.a. HNO_3 , filtrated and the solution replenished to 25 ml.

Mercury was analysed directly in the samples of water, sediments, zoobenthos and fish tissues on a TMA 254 apparatus where sample mineralising is part of the analytical process.

The concentrations of Cd, Pb, Cr, Cu, Ni and Zn in the samples were analysed by the AAS method with electro-thermal atomisation on the SPECTR AA-30 apparatus (producer Varion) with electro-thermal atomiser GTA-96. The same method was used to determine the elements in the mineralised sediments, zoobenthos and fish tissues, with the exception of Cu and Zn, which were determined using the method of flame AAS on the AA 3000 apparatus (producer Perkin Elmer). A deuterium lamp (on the grounds of interferences caused by the matrix) was used in both apparatuses and for elements determined by the AAS method to correct the background. Standards KS 1014 and KS 1015 from UKZUZ Brno were using to verify the analytical methods of heavy metals.

The resulting concentrations of heavy metals in the body tissues of the chub (Table 1) were statistically processed using variance analysis (ANOVA) and then tested using the method of Scheffe. Small and capital letters are used in the table to indicate the statistically significant differences (for significance $P \leq 0.05 - a, b$; for $P \leq 0.01 - A, B$). The results are presented in mean values and standard deviations (mean values \pm SD).

RESULTS

Table 2 gives the basic characteristics of the water environment of the studied localities of the Jihlava River. In all the localities, the concentrations of dissolved oxygen and pH value of the water ranged within physiological values normal for fish species of the barbel zone. The water conductivity, characterising the content of dissolved substances, increased downstream river from 320 μS in locality 1 to 485 μS in locality 3. The trend of organic contamination was the opposite, the saprobity index gradually decreased from the 1st to the 3rd locality, and in the 3rd locality the alphamesosaprobity passed into betamesosaprobity. In

this river section the fish community was relatively the most stable, with 12 fish species, index of diversity 1.743 and equitability index 0.486, but the lowest biomass, i.e. 88.2 kg/ha (Table 3).

Table 4 shows the results of analyses of heavy metals in the water environment of the studied river section (water, bed sediments, composite samples of the zoobenthos). The concentrations of heavy metals were found to be considerably higher in all the localities, particularly lead, cadmium, mercury, chromium and copper. The content of these metals in the sediments and zoobenthos increased considerably from locality 1 to localities 2 and 3. The highest concentrations of Hg, Cd, Pb and Cu were monitored in locality 2, Cr and Zn (only sediments) in locality 3. In locality 1 the sediments and zoobenthos showed the highest concentration of Ni.

Table 1 presents the average concentrations of heavy metals in the gill, gonads, skin and muscle tissues of the chub. The statutory limit for mercury (0.1 mg/kg for non-predatory fish species) was slightly exceeded in localities 2 and 3 (0.136 and 0.139 mg/kg, respectively) and was statistically significantly higher than in locality 1. In all localities, the concentration of cadmium in the muscles of the chub was in the upper admissible limit. The limit concentration of lead in the muscle tissue of the chub was exceeded in locality 1 (0.594 mg/kg) and 3 (0.636 mg/kg, statistically highly significant difference compared to locality 2). The statutory limits for concentrations of the other metals (Cr, Cu, Zn and Ni) in the muscle tissues were not exceeded; the concentrations were not even near the limit values. The differences in the concentrations among the localities were statistically significant to highly statistically significant.

In the other fish tissues, the statutory limit of cadmium was exceeded in the skin of fish in all localities and in locality 2 also in the gonads (0.162 mg/kg, a significantly higher concentration than in locality 1). The statutory limit for lead was exceeded in the skin and gonads of the chub in all the localities; the highest concentration was detected in gonads of fish in locality 3 (0.658 mg/kg). The statutory limit for zinc was exceeded in the skin of fish of all localities; the concentration was the highest in locality 2 (87.30 mg/kg). Considerably higher concentrations of heavy metals (with the exception of mercury) were detected in the gill tissues of the analysed fish in all the localities; these data corresponded with concentrations found in the water of the localities (with the exception of chromium and nickel). Generally, the heaviest contamination of body tissues of the fish with heavy metals was

Table 1. Concentrations of analysed heavy metals (in mg/kg of wet matter) in the body tissues of the chub (*Leuciscus cephalus*) in 3 sections of the Jihlava River

Section	Tissue	Hg	Cd	Pb	Cr	Cu	Zn	Ni
1 age 3+ – 8+ average 6+	gills	0.019 ± 0.006 ^a	0.133 ± 0.026	0.712 ± 0.123	0.220 ± 0.076 ^B	0.762 ± 0.128 ^A	56.82 ± 15.23	0.628 ± 0.233
	gonads	0.013 ± 0.003	0.087 ± 0.030 ^a	0.566 ± 0.124	0.120 ± 0.048	1.188 ± 0.460	33.83 ± 11.57 ^{AB}	0.164 ± 0.079 ^b
	skin	0.018 ± 0.008 ^A	0.104 ± 0.009	0.572 ± 0.045	0.681 ± 0.284 ^B	0.764 ± 0.194 ^A	56.82 ± 26.68 ^a	0.244 ± 0.118
	muscle	0.078 ± 0.026^a	0.090 ± 0.012	0.594 ± 0.046^{AB}	0.224 ± 0.069^B	0.419 ± 0.161^a	5.574 ± 0.637	0.138 ± 0.048 ^{AB}
2 age 6+ – 8+ average 7+	gills	0.028 ± 0.005 ^{ab}	0.159 ± 0.020	0.701 ± 0.010	0.074 ± 0.028 ^A	1.234 ± 0.119 ^B	63.83 ± 17.86	0.783 ± 0.426
	gonads	0.023 ± 0.009	0.162 ± 0.076 ^b	0.549 ± 0.080	0.061 ± 0.036	1.306 ± 0.677	21.46 ± 2.01 ^A	0.045 ± 0.040 ^a
	skin	0.040 ± 0.014 ^B	0.132 ± 0.032	0.511 ± 0.065	0.259 ± 0.133 ^A	1.270 ± 0.241 ^B	87.30 ± 16.92 ^b	0.446 ± 0.369
	muscle	0.139 ± 0.043^b	0.093 ± 0.008	0.493 ± 0.094^A	0.015 ± 0.011^A	0.735 ± 0.298 ^{ab}	3.990 ± 0.885	0.042 ± 0.043 ^A
3 age 6+ – 8+ average 7+	gills	0.032 ± 0.013 ^b	0.204 ± 0.080	0.805 ± 0.097	0.106 ± 0.052 ^A	1.243 ± 0.230 ^B	62.35 ± 9.21	1.033 ± 0.418
	gonads	0.021 ± 0.008	0.097 ± 0.026 ^{ab}	0.658 ± 0.309	0.058 ± 0.042	1.127 ± 0.501	47.18 ± 16.65 ^B	0.133 ± 0.108 ^{ab}
	skin	0.033 ± 0.010 ^{AB}	0.118 ± 0.019	0.597 ± 0.158	0.113 ± 0.073 ^A	1.264 ± 0.333 ^B	60.55 ± 11.21 ^{ab}	0.370 ± 0.228
	muscle	0.136 ± 0.032^b	0.098 ± 0.025	0.636 ± 0.053^B	0.089 ± 0.056^A	0.907 ± 0.391 ^b	4.883 ± 1.277	0.163 ± 0.140 ^B
Czech hygienic limit ⁺		0.1	0.1	0.5	4.0	10.0	50.0	0.5

⁺Decreed by the Ministry of Health of the Czech Republic (Law Gazette No. 298/1997)

No significant differences were found between the values indicated by the same letters. In case of their total absence in any of the examined parameters, the values are not indicated. Small letters and capitals are used for indicating the significance of differences at the level of $P < 0.05$ and $P < 0.01$, respectively

Table 2. Environmental characteristics of the investigated course of the Jihlava River

Section (r.kms)	Sampling date	Temperature (°C)		pH	Oxygen (mg/l)	Conductivity (µS)	Saprobity
		air	water				
1 (156.7)	19. 10. 1999	10.0	6.7	6.87	9.30	320	3.10 alphameso
2 (123.8)	20. 10. 1999	8.0	5.5	6.7	9.30	410	2.63 alphameso
3 (92.1)	26. 10. 1999	17.0	8.8	7.11	8.36	485	2.26 betameso

Table 3. Characterization of fish communities in 3 sections of the Jihlava River

Section (river kilometres)	1 (156.7)	2 (123.8)	3 (92.1)
Number of fish	848	249	396
Number of species	6	10	12
H'	1.175	1.519	1.743
E	0.455	0.457	0.486
Abundance (fish/ha)	6 396.9	1 810.4	1 748.1
Biomass (kg/ha)	205.5	284.1	88.2

H' = the diversity index (Shannon and Weaver, 1963)

E = the equitability index (Sheldon, 1969)

Table 4. Heavy metal concentrations, analysed in the river environment in 3 sections of the Jihlava River

Section	Specimen	Hg	Cd	Pb	Cr	Cu	Zn	Ni
1	water	3.000	0.830	11.400	DL	40.000	17.000	DL
	sediments	0.043	0.618	9.525	8.195	4.485	46.480	8.276
	zoobenthos	0.020	0.211	0.722	0.712	5.155	44.870	2.444
2	water	2.000	1.040	10.300	DL	29.000	20.000	DL
	sediments	0.287	0.701	19.458	8.713	14.333	80.290	7.190
	zoobenthos	0.035	0.209	4.491	1.507	5.738	39.080	1.714
3	water	1.000	1.250	11.400	DL	24.000	DL	DL
	sediments	0.108	0.638	18.013	15.438	12.974	102.840	6.390
	zoobenthos	0.054	0.126	1.920	1.595	5.198	34.120	0.942

DL = concentration below the detection limit (DIs in water: Cr, Zn \geq 5.0 µg/l; Ni \geq 10.0 µg/l). Heavy metal concentration in water is presented in µg/l, in sediments and zoobenthos in mg/kg of the wet matter

detected in localities 2 and 3, i.e. downstream below the towns Jihlava and Třebíč.

In most cases, the variance coefficient of average concentrations of heavy metals in the body tissues of the chub ranged between 7.70 and 46.91%. Substantially higher was only the variability of chromium (31.08 to 72.88%) and nickel (34.74–101.21%) concentrations.

DISCUSSION

In the water environment of the upper course of the Jihlava River, the concentrations of heavy metals, namely Pb, Cd, Hg, Cr and Cu, were substantially increased. The concentrations of these metals markedly increased in the sediments and zoobenthos organisms of the localities lying further downstream below the

towns of Jihlava and Trebic. Penaz *et al.* (1980) determined the mercury content in bottom materials of the Jihlava River near Hrubcice (r.kms 46.5) and Mohelno (r.kms 58.5) between 0.013 and 0.023 mg/kg of the wet matter and in benthic invertebrates 0.025–0.077 mg/kg. Our results in sediments of the upper course of the Jihlava River were higher (0.043–0.287 mg Hg/kg) but corresponded with contents in zoobenthos (0.020–0.054 mg Hg/kg). Kockova *et al.* (1996) analysed the heavy metal content in Thaya River bottom sediments 0.05–0.20 mg/kg of the wet matter for mercury and lead, 0.04–0.98 mg/kg for cadmium and 0.22 mg/kg (near Breclav) and 1.28 mg/kg (near Dobersberg), respectively for chromium. These authors found the concentration of mercury in zoobenthos of the Thaya River 0.12 mg/kg (near Eibenstein), of cadmium 5.7 mg/kg (near Vitis) and of chromium between 9.2 and 15.3 mg/kg (near Devet Mlynu, Hevlin and Breclav). The concentration of mercury in the muscle tissues of the chub was slightly higher in localities 2 and 3 (0.136 and 0.139 mg/kg, resp.), the concentration of lead in localities 1 and 3 (0.594 and 0.636 mg/kg, resp.), and the concentration of cadmium approached the statutory limit (0.090–0.098 mg/kg) in all the three localities. Penaz *et al.* (1979) determined the total mercury content in the muscular tissue of the chub within 0.14–0.22 mg/kg (Jihlava River near Hrubcice and Mohelno) with the highest values in oldest specimens.

The concentrations of heavy metals in the muscle tissues of the fish exceeded the valid Czech statutory limits only slightly and were considerably lower than data monitored in fish from Czech rivers in the previous years. For instance, Vostradovsky *et al.* (1982) found that the concentration of mercury in the muscles of fish from the Berounka River ranged between 0.3 and 0.5 mg/kg, Svobodova *et al.* (1993) monitored as much as 1.60 mg/kg in the muscle tissues of 4-year-old perch from the Labe (Elbe) River near Celakovice. In chubs (7–9 years old) from the lower course of the Dyje River Spurny and Mares (1995) detected concentrations of mercury in the muscles ranging between 0.15 and 0.24 mg/kg, and 0.43 mg/kg in a 9-year-old barbel (a distinctly benthophagous species). On the other hand, Jurajda (1999) detected a concentration of mercury ranging between 0.08 and 0.195 mg/kg, lead between 0.043 and 0.434 mg/kg and cadmium between 0.008 and 0.065 mg/kg in the muscle tissues of the chub ($n = 7-8$) in 7 localities of the Morava River.

The relatively heavy pollution of the upper course of the Jihlava River with heavy metals will probably increase these concentrations in the fish living in lo-

calities with a higher accumulation of the pollutants, such as dam reservoirs. In the Dalesice reservoir (400 ha) on the Jihlava River about 10 km downstream below locality 3, Spurny and Mares (1991) monitored concentrations of mercury in the muscles of perch (4–5 years old) ranging between 0.63 and 0.78 mg/kg and in the muscles of a 7-year-old roach 0.48 mg/kg.

The higher concentrations of heavy metals (Cd, Pb, Zn, Cu and Ni) in the water environment result in higher concentrations of these metals in the skin and gill tissues of the chub in the monitored section of the Jihlava River, particularly in localities 2 and 3 (below Jihlava and Trebic). Therefore, analyses of these tissues, especially gills, have a high bio-indicative value for the quality of the water environment, because the pollutants enter the tissues directly, not by way of the food chain. In the skin of the chub from the upper course of the River Dyje (Czech branch) Spurny and Mares (1995) detected concentrations of 0.94 mg/kg lead, 4.26 mg/kg nickel, and zinc as high as 213.07 mg/kg.

The increased concentrations of the majority of heavy metals in the gonads of the chub (for instance in locality 3 the concentration of lead was 0.658 mg per kg) may have an adverse effect on natural reproduction of fish in this section of the river. The ecological stability of the fish community based on basic ichthyological methods did not reach optimal values even in locality 3 where the results were the most favourable. In the fish community of the barbel zone of the Becva River Spurny *et al.* (2000) found the index diversity as high as 2.646 and equitability index 0.758 (in locality 3 of the Jihlava River it was 1.743 and 0.486, respectively). The biomass of the fish community, which was 88.2–284.1 kg/ha in the studied section of the Jihlava River, was also more in the lower range found in the extra-trout sections of Czech rivers (160–740 kg/ha).

CONCLUSION

Monitoring the levels of heavy metals and other specific pollutants in the water environment, including fish, is important not only in terms of the ecologic indication, but in countries where angling is more common it is particularly important in terms of the health protection of potential consumers of the contaminated fish. It is therefore important to monitor the content of residues of the hazardous pollutants not only in the fish muscles, but also in the skin and gonads that are consumed together with the meat. In the dan-

gerous regions, it would be wiser to avoid consumption of these edible parts of the fish body. The contamination of the water environment should therefore be monitored over the entire territory of the Czech Republic and the results regularly published. This is essential also to ensure the restoration and protection of the fish communities, negatively affected for a very long time, where these substances cause chronic stress leading to reproductive, immune, growth and other disorders, which could finally lead to the extinction and disappearance of the more sensitive species from their natural ichthyocenoses.

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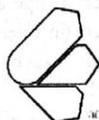
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Received: 01–04–23

Accepted after corrections: 02–04–16

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