CZECH ACADEMY OF AGRICULTURAL SCIENCES

Czech Journal of ANIMAL SCIENCE

ŽIVOČIŠNÁ VÝROBA

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

5

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

For information on Czech J. Anim. Sci. and full papers from Vol. 47 visit http://www.cazv.cz

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, Slezská 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, Slezská 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, Slezská 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 zaslány na adresu: Ústav zemědělských a potravinářských informací, vydavatelské oddělení, Slezská 7, 120 56 Praha 2. Cena předplatného pro rok 2002 je 1176 Kč.

MK ČR E 4886

The effect of selected factors at insemination on reproduction of Holstein cows

Vliv vybraných faktorů v době inseminace na reprodukci dojnic holštýnsko-fríského plemene

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ABSTRACT: The effects of year of insemination, lactation number, size of left and right ovaries, incidence of ovarian follicles, oestrus signs intensity, degree of body condition at insemination, and average daily milk yield in the week of insemination on conception rate in cows were studied in 442 Holstein cows in their 1st–5th lactations in 1997–2000. 1 085 inseminations were evaluated. Pregnancy and incidence of ovarian cysts were diagnosed ultrasonically at 28–42 days after insemination. A linear model of the least squares analysis PROC GLM from the SAS STAT program range was used for the statistical analysis. The daily milk yield averaged 28.02 kg. The effect of body condition at insemination on conception was significant ($P < 0.05$). The conception rate increased with the increasing body condition at insemination, the highest conception rate being obtained for cows awarded 3–4 points on a 5-point scale. In over-fat cows scoring 5 points, the conception rate was 16.6%. The lowest incidence of ovarian cysts (31.80–33.33%) was found in cows scoring 2–3 points for body condition, the incidence of 37.80–41.67% having occurred in cows scoring 4–5 points.

Keywords: cattle; body condition; reproduction; oestrus signs; ovaries; milk yield; insemination

ABSTRAKT: Hodnocení vlivu vybraných faktorů na výsledky zabřezávání dojnic se uskutečnilo v letech 1997 až 2000. Do sledovaného souboru bylo zařazeno 442 dojnic holštýnsko-fríského plemene na 1. až 5. laktaci. Bylo vyhodnoceno 1 085 inseminací u sledovaného souboru dojnic. Vyšetření březosti (%Z) a výskytu ovariálních cyst (%C) bylo provedeno sonograficky 28. až 42. den po inseminaci. K vyhodnocení výsledků byly použity tyto faktory: rok ve kterém byla inseminace provedena, pořadí laktace, velikost levého i pravého vaječníku, výskyt folikulu na vaječnících, intenzita vnějších příznaků říje, stupeň tělesné kondice při inseminaci a průměrná mléčná užitkovost dojnice v týdnu, ve kterém byla inseminována. Statistické vyhodnocení bylo provedeno lineárním modelem metody nejmenších čtverců PROC GLM programového vybavení SAS STAT. Průměrná denní užitkovost sledovaných dojnic dosáhla 28,02 litru mléka za den. Vliv stupně tělesné kondice krav v době inseminace na zabřezávání krav byl průkazný ($P \leq 0,05$). Se zvyšujícím se stupněm tělesné kondice v době inseminace se zvyšovalo procento zabřezlých plemenic. Nejvyšší procento zabřezlých bylo zjištěno u dojnic s tělesnou kondicí stupně 3 a 4 v době inseminace. U skupiny dojnic ztučnělých se stupněm tělesné kondice 5 bylo pozorováno průkazně nižší zabřezávání – 16,6 %. Nejnižší frekvence výskytu ovariálních cyst v rozmezí 31,80 až 33,33 % byla zjištěna u dojnic se stupněm tělesné kondice 2 až 3. U krav s vyšším stupněm tělesné kondice 4 až 5 byl výskyt ovariálních cyst vyšší – 37,80–41,67 %.

Klíčová slova : skot; tělesná kondice; reprodukce; příznaky říje; vaječníky; mléčná užitkovost v den inseminace

Reproduction is an area in which reserves can be identified world-wide. Many authors have dealt with the effect of a number of factors on conception rate. Some results indicated a negative genetic correlation between dairy performance and reproductive indices, implying that a successful selection aimed at high milk

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Projects MSM No. 412100001 and MSM No. 412100003) and by the Ministry of Agriculture of the Czech Republic (Project NAZV No. 1234/2001/01).

yields may result in lowering the reproductive capacity (Pryce *et al.*, 2000).

Royal *et al.* (2000) and Beam and Butler (1997) established that, in the course of the experimental period, conception rate to 1st insemination decreased by 1 and 0.5%, respectively. Webb *et al.* (1997) drew the attention to the relationship between nutritional requirements for lactating cows and a successful conception.

The degree of body condition at insemination is an important factor for a successful conception. The effect of a negative energy balance at the onset of lactation on health, metabolism and reproductive capacity was studied by Louda and Tér (1982), Canfield and Butler (1990), Lucy *et al.* (1991), Veerkamp *et al.* (1994, 1998), Nielsen (1999), Řezáč *et al.* (2000), and Pryce *et al.* (2000).

A mobilisation of physical reserves, with ensuing decrease in body weight, is necessary for a successful course of lactation (Waltner *et al.*, 1993) but a decrease in body condition during the first 10 weeks after calving by more than 1 degree may result in some cows in an adverse effect on conception (Son *et al.*, 1996). An incidence of anoestrus was observed in high-lactating cows in which body condition during the first stages of lactation dropped by 0.75–1.0 degree (Studer, 1998).

Louda and Tér (1982) found that a decrease in body weight by more than 500 g daily in Czech Pied cows resulted in a highly significant increase in the duration of service period ($r = 0.196$, $P < 0.01$). At a correct level of nutrition and a proper feeding technique, the differences in body condition of cows in the same stage of lactation should not be greater than 1 degree (Podmanický *et al.*, 2000). Brocard and Chenais (2000) pointed out the importance of body condition at calving for production and reproduction results in the first period of lactation. Body weight decreasing to 45 days after calving had an adverse effect on conception rate to 1st insemination, and resulted in a marked increase in service period and calving interval (Suriyasathaporn *et al.*, 1998). In cows in later lactations, a decrease in body condition during the dry period and after calving resulted in a significant incidence of inactive ovaries and long service periods (Markusfeld *et al.*, 1997). Podmanický *et al.* (2000) found a positive relationship between body condition during the dry period and the incidence of ovarian cysts during the following lactation; Waltner *et al.* (1993) did not prove this.

Markusfeld *et al.* (1997) found that a superior body condition at calving resulted in a low incidence of anoestrus but not, however, in a higher conception rate to 1st insemination. An extreme body condition – fat or thin – resulted in a frequent incidence of dystocia and a low conception rate (Podmanický *et al.*, 2000). A positive relationship between the onset of ovarian activity and mobilisation of body reserves at $r = 0.88$ ($P < 0.01$) was determined by Benaich *et al.* (1999). To the contrary, Morrison *et al.* (1999) found that the incidence of postpartum luteal activity in cows does not differ significantly among cows with different body condition. Sepúvelda *et al.* (2000) pointed out a negative correlation between body condition at calving and the onset of ovarian activity ($r = -0.64$, $P < 0.001$) or the interval to the 1st postpartum ovulation ($r = -0.59$, $P < 0.001$).

The importance of body condition during the dry period, at calving and during the first stages of lactation, in relation to conception rate, was pointed out by Von Euw *et al.* (2000), Vollema *et al.* (2000), Studer (1998), Pryce *et al.* (2000) and Říha *et al.* (2000).

Our aim was to evaluate the effects of selected factors at insemination on conception rate and incidence of ovarian cysts in Holstein cows.

MATERIAL AND METHODS

An evaluation of selected factors infuencing conception rate in cows was carried out in 1997–2000. The experimental group comprised 442 Holstein cows in their 1st–5th lactations that had been re-inseminated during that period. The allocation of cows into different groups depending on the examined factors is given in Table 1. The average daily milk yield of the experimental cows was 5.63 litres milk higher than the average of their particular herd. The pregnancy after artificial inseminations for the whole herd was 23.33–56.82% in 1997–2000. 1085 inseminations were taken into account. Pregnancy diagnosis (% CR) and incidence of ovarian cysts (% OC) were determined ultrasonically 28–42 days after insemination.

The following factors were considered for the statistical evaluation:

- year of insemination (group 1: 1997; group 2: 1998; group 3: 1999; group 4: 2000)
- lactation number (group 1: 1st lactation to group 5: 5th lactation)
- the condition of reproductive organs at insemination, *i.e.* the size of left and right ovaries (group 1: up to 1 cm; group 2: 1 to 3 cm; group 3: over 3 cm)
- the incidence of ovarian follicles (group 1: no follicles; group 2: left ovary follicle; group 3: right ovary follicle; group 4: follicles on both ovaries)

Table 1. Frequency of cows grouped according to traits

Trait	Group (*n*)				
	1	2	3	4	5
Year (YR)	160	349	301	275	–
Lactation number (LN)	259	359	395	63	9
Size of left ovary (LO)	641	407	37	–	–
Size of right ovary (RO)	494	520	71	–	–
Incidence of follicle (OF)	40	304	636	105	–
Intensity of oestrus signs (OS)	204	470	411	–	–
Degree of body condition (BC)	23	267	607	154	34
Daily milk yield (MY)	184	687	214	–	–

– oestrus signs intensity on a 3-point scale (group 1: weak; group 2: medium; group 3: strong)
– degree of body condition at insemination on a 5-point scale (group 1: poor to group 5: excellent)
– the average daily milk yield of the cow in the week of insemination (group 1: lower than $x - s_d$, group 2: $x - s_d$ to x; group 3: x to $x + s_d$; group 4: greater than $x + s_d$).

Higher number of factors were taken into account during the initial statistical evaluation. Based on the significance test of the results, a combination of only significant factors was chosen. The effect of these factors was the subject of the following evaluation.

The statistical evaluation was carried out using a linear model of the least squares method PROC GLM within the SAS STAT programs. The linear model was:

$$Y_{ijklmnop} = \mu + a_i + b_j + c_k + d_l + f_m + g_n + h_o + e_{ijklmnop}$$

where: $Y_{ijklmnop}$ = result of evaluated insemination
μ = mean of the reproduction result
a_i = effect of the year of insemination (YR)
b_j = effect of the lactation number (LN)
c_k = effect of the condition of reproductive organs at insemination, *i.e.* the size of left and right ovaries (LO, RO)
d_l = effect of the incidence of ovarian follicles (OF)
f_m = effect of the oestrus signs intensity on a 3-point scale (OS)
g_n = effect of the degree of body condition at insemination on a 5-point scale (BC)
h_o = effect of the average daily milk yield of the cow in the week of insemination (MY)
$e_{ijklmnop}$ = effect of the residual error

RESULTS AND DISCUSSION

Only those factors that had a significant effect on dairy performance were included. The statistical significance of the final model used was at $P < 0.05$. The principal statistical characteristics of the evaluated data are given in Table 2. The average stage of lactation at insemination and at the evaluation of body condition was 190.03 days. The average daily milk yield of the studied cows was 28.02 litres. Similar results at a similar performance and stage of lactation were obtained by Von Euw *et al.* (2000), Vollema *et al.* (2000), and Suriyasathaporn *et al.* (1998). Body condition at insemination averaged 2.86 points. A similar degree of body condition was reported by Von Euw *et al.* (2000), and Vollema *et al.* (2000). Body temperature of cows ranged from 37.6 to 40.10°C, averaging 38.65°C at insemination. This figure was slightly higher than that given by Vollema *et al.* (2000). When evaluating the size of ovaries on a 3-point scale, the right ovaries averaged 0.17 points more at oestrus than left ovaries. We ob-

Table 2. Principal statistical characteristics of the experimental groups

Trait	x	s_d	Min	Max
Lactation number	2.26	0.91	1.00	6.00
Size of left ovary	1.44	0.55	1.00	3.00
Size of right ovary	1.61	0.61	1.00	3.00
Quality of uterine tonus	2.18	0.71	1.00	3.00
Intensity of oestrussigns	2.19	0.73	1.00	3.00
Degree of body condition	2.86	0.62	1.00	5.00
Daily milk yield	28.02	8.75	11.40	62.30

served a tendency for an above-average development of the right ovaries. A functional follicle was observed more often on right ovaries (58.67%) than on left ovaries. In only 9.69% of cows were detected functional follicles on both ovaries at insemination, and in 3.60% of cows a functional follicle was absent. Sturman *et al.* (2000) published information that, in practice and at 95% accuracy, some 16% of cows checked for oestrus by an insemination technician were suggested for a later insemination. 44% of these were diagnosed pregnant after an insemination at the following oestrus. Oestrus signs intensity was average and amounted to 2.19 points at insemination. Uterine tonus was average and amounted to 2.18 point at insemination. Signs of oestrus and uterine tonus therefore indicated at good-to-excellent signs of oestrus.

The conception rate and the incidence of cystic ovaries in relation to the effect of the individual factors are given in Table 3. From the point of view of time sequence, the worst results in conception were obtained in 2000, pregnancy rate after all inseminations being 32.7%. The differences from the other years were +5.07% for 1997, +5.02% for 1998, and +4.14% for 1999, the differences being statistically significant at $P < 0.05$. The reasons for these differences could not be explained in simple terms as the effect of year comprised several partial factors. The most significant of these was the effect of nutrition differing in quality among the years. In 2000, however, we found a lower incidence of ovarian cysts after insemination (22.37%). The differences between years in the incidence of ovarian cysts ranged from 11.99% to 16.38%, significant at $P < 0.05$ (Table 3).

The effect of lactation number on reproductive performance was significant at $P < 0.05$. Conception rate decreased somewhat with increasing lactation number. The decrease from the 1st to the 4th lactation reached 6.2%. The highest incidence of ovarian cysts (36.96%) was found in cows in their 3rd lactations. This information is shown in Table 3. The highest percentage of cows returning to service (31.15%) in which no oestrus had been detected following the insemination in question, occurred in the 4th lactation. Our results correspond with those of other authors. For example, a similar tendency was established by McCullough and DeLorenzo (1996), and Eicker (1996).

For ovary size, the highest conception rate (37.59%) was found in cows with ovaries from 1 to 3 cm at insemination, and the lowest (19.44%) in cows with ovaries over 3 cm ($P < 0.05$). The highest incidence of ovarian cysts after insemination (47.22%, $P < 0.05$) was found in cows with the largest ovaries, compared with 13.37% in cows with ovaries measuring up to 1 cm, and 31.94% in cows with ovaries measuring 1–3 cm (Table 3). The percentage of cows returning to service was similar in cows with ovaries up to 1 cm and 1 to 3 cm. In cows with ovaries greater than 3 cm the percentage returning to oestrus was 19.44, the difference from other groups being significant ($P < 0.05$).

In cows in which, at insemination, we had detected a follicle on one of the ovaries, conception rate was 35.24–36.51%, compared with cows in which we had not detected a follicle and in which the conception rate was 30.77% ($P > 0.05$). Table 3 include this results. A similar study was conducted by Sheldon and Dobson (2000) who evaluated the incidence and size of follicles

Table 3. Conception rates (CR, %) and incidence of ovarian cysts (OC, %)

Group		YR	LN	LO	PO	OF	OS	MY
1	CR	37.77	38.99	35.88	36.23	30.77	28.57	29.01
	OC	38.75	27.80	33.85	33.20	33.33	37.44	38.27
2	CR	37.72	37.88	37.59	35.00	36.51	36.59	42.86
	OC	34.36	34.82	31.94	33.85	32.24	31.70	33.86
3	CR	36.84	33.42	30.55	44.29	36.48	39.42	35.60
	OC	34.78	36.96	47.22	34.29	33.33	33.82	30.11
4	CR	32.70	32.79	–	–	35.24	–	29.56
	OC	22.37	29.51	–	–	39.05	–	36.48
5	CR	–	50.00	–	–	–	–	–
	OC	–	33.33	–	–	–	–	–
$P < 0.05$		1 to 3–4	1 to 4–5	1.2–3	–	–	1–2.3	1–2.3; 2–4; 3–4

on ovaries during the first 28 days. Cows in which they detected ovarian follicles averaged shorter service periods.

Further, we determined a significant ($P < 0.05$) effect of overt signs of oestrus on conception rate. The highest percentage conceiving was in those cows which had exhibited the most obvious signs of oestrus. We found no relationship between cystic ovaries and the intensity of signs of oestrus (Table 3). According to Kinsel and Etherington (1998), most cows were inseminated following a visual detection of behavioural changes associated with oestrus. At that, many apparent signs, such as nervousness or sniffing at cows nearby, do not necessarily confirm conclusions on the stage of oestrous cycle. Another possibility of detecting oestrus follows from observing the activity of cows that increases during oestrus by 30–200% (Firk *et al.*, 2000).

The effect of body condition of cows at insemination on conception was significant ($P < 0.05$). The effect of body condition on conception rate is given in Figure 1. Conception rate increased with improving body condition at insemination. The highest conception rate (36.58–36.93%) was found in cows awarded 3–4 points for body condition at insemination. In overfat cows with a 5-point body condition, conception rate was significantly lower (16.6%). The highest percentage of cows returning to service (41.67) was also found in this group. These results are similar to those obtained by Markusfeld *et al.* (1997), Suriyasathaporn *et al.* (1998), Podmanický *et al.* (2000), and Vollema *et al.* (2000). Figure 1 also shows the frequency of the incidence of ovarian cysts in connection with body condition of cows. The lowest incidence of ovarian cysts (31.80–33.33%) was observed in cows classified for body condition as grade 2–3. In cows awarded 4–5 points for body condition, the incidence of ovarian cysts was higher (37.80–41.67%), as shown in Figure 1. A similar trend in the incidence of ovarian cysts was described by Podmanický *et al.* (2000). Compared with this, Waltner *et al.* (1993) did not confirm a similar trend.

The average daily milk yield in the population involved was 28.02 kg, σ =8.75 kg. A lower conception rate and a higher frequency of ovarian cysts were found in cows whose daily milk yield at insemination was σ = 8.75 kg different from the average in both directions. The differences were significant ($P < 0.05$). Conception rate in cows yielding the average milk yield was 35.60–42.86%. Conception rate in cows differing from the average by more than 8.75 kg milk in either direction was 6.59–13.30% lower than in cows yielding the average yields. Detailed information is in Table 3. This indicated that feeding cows for an average milk yield may result in metabolic or health disorders in cows with milk yields differing from the average by more than $\pm \sigma$ = 8.75 kg. The existing genetic correlation between milk yield and reproductive performance in cows, which can be negative, demonstrated that selection aimed at a high milk yield may result in a lower reproductive performance (Pryce *et al.*, 2000). High-yielding cows can exhibit a lower fertility, and a higher incidence of some metabolic and health disorders (Groen *et al.*, 1997). However, as high calving rates are a principal aim of every breeder, low conception

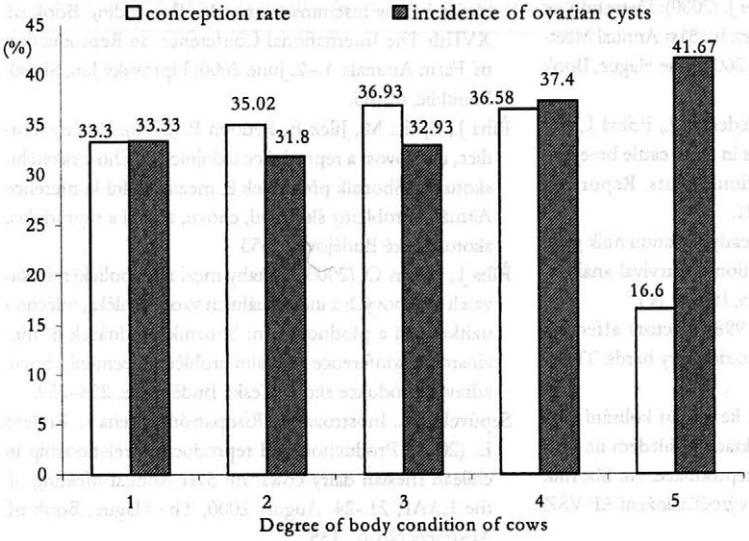

Figure 1. Conception rate and incidence of ovarian cysts in relation to body condition of cows

rates would not discourage farmers from inseminating all their cows (McCullough and DeLorenzo, 1996). Contrary to our conclusions, Harman (1994) published that cows with a milk yield significantly different from the average carry a lower risk of not conceiving than cows with an average milk yield. Říha and Hanuš (2000) published a negative correlation between milk yield and conception rate to 1st insemination ($P < 0.001$). In our investigation, the highest fertility was obtained for cows yielding up to 20 kg milk daily.

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Received: 02–01–16
Accepted after corrections: 02–04–17

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Influence of high environmental temperature on production and haematological and biochemical indexes in broiler chickens

Vliv vyšší teploty prostředí na produkci, hematologické a biochemické ukazatele u brojlerových kuřat

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ABSTRACT: The goal of the experiment was to assess influence of higher environmental temperature on selected haematological indexes (total amount of erythrocytes and leucocytes, value of haematocrit and content of haemoglobin) and biochemical indexes of blood plasma (total amount of proteins, glucose, cholesterol, AST, Ca, P and Mg). The results have confirmed that during fattening, the increased environmental temperature significantly influences some indexes of inner environment and production of broiler chickens. Gradual increase in the air temperature from 16th day of fattening (by 1–5°C) significantly and with high provability ($P \leq 0.01$) decreased weight of the chickens on the 42nd and 62nd day of fattening. This growth depression was accompanied also by significant changes of the inner environment in chickens. The changes within the haematological indexes were characterized by increased value of haemoglobin and by reduced amount of leucocytes in the chickens' blood. These changes within the biochemical indexes were characterized mainly by ($P \leq 0.01$) increase in the molar concentration of plasma glucose and by increased level of plasma cholesterol, of which the differences between the group averages from 42nd day of fattening were tested as highly significant ($P \leq 0.01$). Chickens fed under higher environmental temperature showed also significantly ($P \leq 0.01$) higher concentration of plasma Ca. On the other hand, the aforementioned dependencies were not found within catalytic concentration of AST, nor in P and Mg molar concentrations, though in the course of fattening, some statistically significant differences were shown between the groups.

Keywords: broiler chickens; environmental temperature; live weight; haematological and biochemical indexes of blood plasma

ABSTRAKT: Cílem experimentu bylo posoudit vliv vyšší teploty prostředí na vybrané hematologické (celkový počet erytrocytů a leukocytů, hematokritovou hodnotu a obsah hemoglobinu) a biochemické ukazatele krevní plazmy (celkový protein, glukóza, cholesterol, AST, Ca, P a Mg). Výsledky potvrzují, že zvýšená teplota prostředí v průběhu výkrmu významně ovlivnila některé ukazatele vnitřního prostředí a produkci u brojlerových kuřat. Postupné zvyšování teploty vzduchu od 16. dne výkrmu (o 1–5°C) se vysoce průkazně ($P \leq 0,01$) projevilo snížením živé hmotnosti kuřat ve 42. a 62. dnu výkrmu. Tato růstová deprese byla provázena i významnými změnami ve vnitřním prostředí kuřat. U hematologických ukazatelů byly tyto změny charakterizovány zvýšenou hodnotou hemoglobinu a sníženým počtem leukocytů v krvi kuřat. U biochemických ukazatelů byly tyto změny charakterizovány převážně vysoce průkazným ($P \leq 0,01$) zvýšením molární koncentrace plazmatické glukózy a zvýšenou hladinou plazmatického cholesterolu, u kterého byly rozdíly mezi průměry skupin ve 42. dnu výkrmu testovány jako vysoce průkazné ($P \leq 0,01$). U kuřat vykrmovaných při vyšší teplotě prostředí byly zaznamenány i vysoce průkazně vyšší ($P \leq 0,01$) koncentrace plazmatického Ca. U katalytické koncentrace AST a molárních koncentrací P a Mg i přesto, že v průběhu výkrmu byly prokázány statisticky významné rozdíly mezi průměry skupin, výše uvedené závislosti na teplotě prostředí u těchto ukazatelů nalezeny nebyly.

Klíčová slova: brojlerová kuřata; teplota prostředí; živá hmotnost; hematologické a biochemické ukazatele

The work has been conducted within the research purpose No. 162700004 "Research of ecological and eco-toxicological problems of agrarian ecosystems food chains in production of raw materials and food of animal origin, regarding living environment of population".

The work deals with the influence of gradual increase in environmental temperature on livestock efficiency and changes of inner environment in broiler chickens. It is generally known that external environmental factors have a significant impact on the results of fattening. The air temperature within the living zone of animals is one of the most significant microclimatic factors of environment. The air temperature inside the fattening stations often causes problems in broilers, particularly during summer.

Both low and high air temperatures have negative effects on chickens. Even the early experimental works of Hansen (1988), Arjona *et al.* (1988) and others warned of negative impact of high temperature on chickens. As Manning and Wyatt (1990) proved, the chickens more easily adapt to lower than to higher temperatures. Suchý *et al.* (1993a,b) analysed in details relations between individual microclimatic indexes of stable environment and productivity of broiler chickens. These authors have shown high correlations between the air temperature within the living zone of the chickens and their productivity. The aforementioned authors have found that the more days when the chickens were exposed to temperatures out of optimum, the smaller the average live weight gain was ($r = -0.8797$).

The losses within the productivity of fattened chickens (decrease in growth intensity, increase in morbidity and mortality of chickens) represent outer demonstration of metabolic changes that occur inside the chickens organism. Higher temperatures have impact on homeopoetic processes, as mentioned by Jamadar and Jalnapurkar (1995). In their work, these authors indicate conclusions of studies of effect of high environmental temperature on distribution of Fe in broiler organism. Sahota *et al.* (1994) studied impact of higher temperatures on haematological profile of chickens and laying hens after supplying ascorbic acid in the feed.

Datta *et al.* (1996) found in selected kinds of ducks that the total amounts of erythrocytes and leucocytes and also content of haemoglobin increased in these birds when being exposed to lower or higher temperatures. Deyhim and Teeter (1991) found during their experiments higher haematocrit value in heat stressed chickens.

As Khan and Khan indicate (1993), heat stress causes pathological changes in broilers. These pathological changes are accompanied by a number of biochemical changes. Yang *et al.* (1992) found decrease in content of globulins in broilers' blood and increase in the blood glucose in relation with the increase in environmental temperature. As Ching *et al.* (1992) mentioned, under a heat stress the levels of calcium, sodium and nitrogenous substances in the broilers' blood increase. Suchý *et al.* (1993a,b) noticed significant changes within blood plasma biochemistry in relation with individual microclimate indexes.

The quoted authors realized that except for the impact of microclimate on observed biochemical indexes also some regular changes within blood plasma of broiler chickens occur during fattening (as the chickens grow). These changes are e.g. gradual increase in plasma proteins and gradual decrease in plasma cholesterol level.

The results of this work supplement present knowledge of haematology and biochemistry of blood plasma of broiler chickens during fattening and warn that even relatively small changes of the environmental temperature can have negative impact on metabolism and production of chickens.

MATERIAL AND METHODS

The goal of the experiment was to investigate the impact of increase in temperature on selected (haematological and biochemical) indexes of chicken inner environment during fattening. Seventy experimental one-day-old chickens (35 male birds and 35 female birds) and 70 control one-day-old chickens (35 male birds and 35 female birds) of ROSS 308 meat hybrid of average weight 42 g were selected for the experiment. The chickens were kept in credited experimental stable of the Department of Nutrition, Dietetics, Animal Hygiene and Plant Products of Faculty of Veterinary Hygiene and Ecology of University of Veterinary and Pharmaceutical Sciences in Brno. The stable was equipped with controlled climatic mode and deep bedding. Feeding was performed through tube plastic feeders and the feed consumption was observed daily, drinking water was supplied with automatic watering with continuous control of water consumption. Both groups of chickens were fed by the same feeding mixes: BR 1, BR 2, BR 3 and BR 4 according to the attached scheme and nutrient compositions (Table 1).

The fattening was conducted in accordance with the technological instructions for fattening of ROSS 308 chickens. The experimental group of chickens was subjected to a gradual increase in temperature. From the 16th day of age to the 19th day the temperature increased by 1°C, from the 20th day to the 24th day the temperature increased by 2°C, from the 25th day to the 27th day the temperature increased by 3°C, from

Table 1. Nutrient compositions of complete feed mixes

Index (g/kg)	BR 1 day 1–14	BR 2 day 15–30	BR 3 day 36–52	BR 4 day 53–62
Solids	874.7	885.7	890.3	896.1
Proteins	221.2	203.3	196.3	184.9
Fat	67.9	70.3	80.5	85.3
Fibre	22.6	21.8	18.1	19.5
Ash	58.6	53.8	51.6	53.4
ME (MJ/kg)	12.0	12.2	12.6	12.6
Ca	10.9	9.9	9.9	10.7
P	9.1	6.9	7.3	7.0
Mg	3.1	2.4	2.1	2.1

BR 1, BR 2, BR 3, BR 4 = complete feed mixes

the 28th day to the 31st day the temperature increased by 4°C and from the 32nd day to the end of fattening (62nd day) the temperature increased by 5°C. Simultaneously with the air temperature the relative humidity was observed and maintained at required value: till 42nd day of fattening 60 ± 5% and from 42nd to 52nd day of fattening 70 ± 5% in both experimental and control groups. In the course of fattening the light regime 23 hours of light and 1 hour of dark was used in both chickens groups.

In the course of fattening, the feed consumption, mortality and health conditions of chickens were observed continuously.

On 21st, 42nd, 52nd, and 62nd day of fattening, blood of selected set of 16 chickens of each group (8 male birds and 8 female birds) was tested, taken between 8 and 9 o'clock AM from *vena basilica*. For the purposes of haematological and biochemical examination the blood was stabilized by heparin. Within the haematological examination, the total numbers of erythrocytes (Er) and leucocytes (Le) were determined by flask method of dilution and counting corpuscles using Bürker's chamber, the content of haemoglobin (Hb) was determined using SPECOL-11 photometer and Drabkin's solution at wavelength of 540 nm. The value of haematocrit was determined by the capillary micro-haematocrit method according to Janetzki. Within the biochemical examinations the total amounts of proteins (CB), glucose (Glu), cholesterol (Chol), catalytic concentration of AST, calcium (Ca), phosphorus (P) and magnesium (Mg) in blood plasma were determined.

The aforementioned indexes were determined photometrically using commercially produced Bio-la-test diagnostic sets. Calcium, phosphorus and magnesium were determined using nuclear absorption spectrophotometrical method.

The results were processed using general mathematical-statistical methods and the differences between average values were compared using *t*-test at the level of significance between $P \leq 0.01$ and $P \leq 0.05$.

RESULTS

The results of the study of impact of environmental temperature on the fattened chickens prove that the increased temperature is one of the most significant factors that have impact on chicken inner environment and subsequent impact on their production and health conditions.

Table 2 shows that in the course of fattening, a different development of live weight was observed in the groups (K – control group, P – experimental group). Although to the 21st day of the fattening the experimental group that was until the 19th day kept under identical temperature as the control group, showed ($P \leq 0.05$) higher average weight of chickens – 0.58 kg compared to the control group, the increasing temperature caused depression in the chickens' growth. On 42nd and 62nd day of fattening, a statistically lower average live weight in experimental chickens was proved of high significance ($P \leq 0.01$) – 2.15 kg and 3.71 kg compared to 2.30 kg and 3.96 kg of the control group.

The results of haematological studies indicate that the chickens exposed to higher temperature did not show big differences between average values of the

Table 2. Results of haematological examinations of broiler chickens on individual days of fattening

Index	Group	Day 21		Day 42		Day 52		Day 62	
		$\bar{x}$ (s_{n-1})	td	$\bar{x}$ (s_{n-1})	td	$\bar{x}$ (s_{n-1})	td	$\bar{x}$ (s_{n-1})	td
LV (kg)	control	0.549 (0.071)	*	2.300 (0.243)	**	3.05 (0.221)	–	3.96 (0.281)	**
	experiment	0.577 (0.075)	2.475	2.15 (0.225)	4.067	3.02 (0.225)	0.707	3.71 (0.283)	3.927
Er (T/l)	control	1.95 (0.444)	–	1.81 (0.307)	–	1.75 (0.266)	–	1.66 (0.305)	–
	experiment	1.68 (0.462)	1.682	1.84 (0.227)	0.313	1.94 (0.291)	1.918	1.59 (0.195)	0.774
Hk (l/l)	control	0.280 (0.021)	–	0.28 (0.031)	–	0.30 (0.025)	–	0.30 (0.028)	–
	experiment	0.280 (0.017)	0.000	0.29 (0.025)	1.000	0.30 (0.021)	0.000	0.30 (0.021)	0.000
Hb (g/l)	control	91.20 (10.901)	**	89.49 (11.96)	*	91.41 (12.31)	–	80.22 (16.38)	–
	experiment	76.45 (10.295)	3.935	78.73 (9.30)	2.841	84.12 (9.11)	1.901	79.13 (12.46)	0.212
Le (G/l)	control	14.60 (2.414)	**	18.19 (3.942)	–	17.32 (3.615)	–	19.00 (4.251)	–
	experiment	19.45 (3.885)	4.241	19.88 (3.720)	1.247	20.44 (5.701)	1.849	21.31 (3.484)	1.681

$\bar{x}$ = arithmetic mean, (s_{n-1}) = standard deviation, td = test value according to the Student's t- test

LV = live weight, Er = total erythrocytes, Hk = haematocrit value, Hb = haemoglobin, Le = total leucocytes

* $P \leq 0.05$, ** $P \leq 0.01$

observed indexes. During the fattening, average values of the total amounts of erythrocytes of the control group varied between 1.66 T/l and 1.95 T/l, the average values of the experimental group varied from 1.59 T/l to 1.94 T/l. Within the period of observation, a very narrow range of average values of the haematocrit value was observed. Both groups showed values between 0.280 1/1 and 0.300 1/1. Differences were observed in haemoglobin of the control group, when on 21st and 42nd day of fattening, the determined average values (91.20 g/l and 89.49 g/l) were statistically higher ($P \leq 0.01$ and $P \leq 0.05$), compared to the experimental group of chickens (76.45 g/l and 78.73 g per l). The average values of haemoglobin varied between 80.22 g/l and 91.41 g/l in the control group and between 76.45 g/l and 84.12 g/l in the experimental group.

As to the study of dynamics of total amount of leucocytes, average values ranging from 14.60 G/l to 19.00 G/l were determined in the course of fattening in the control group and values from 19.45 G/l to 21.31 G/l in the experimental group. The experimental group showed higher amount of leucocytes during the whole period of observation, when on 21st day of fattening the difference between the average value of the control group – 14.60 G/l and average value of the experimental group – 19.45 G/l was tested as highly significant ($P \leq 0.01$).

Studying of dynamics of selected biochemical parameters of blood plasma was also a part of the research (Table 3).

The plasma proteins in the course of fattening showed tendencies of gradual increase in average values both in the control (23.13 g/l to 42.13 g/l) and in the experimental (24.18 g/l to 40.48 g/l) group. No statistically significant differences were found between the average values of the control and the experimental group on individual days of fattening.

Statistically significant ($P \leq 0.05$) and highly significant ($P \leq 0.01$) differences were proved between average values of plasma glucose, which was during the

Table 3. Results of biochemical examinations of broiler chickens on individual days of fattening

Index	Group	Day 21		Day 42		Day 52		Day 62	
		$\bar{x}$ (s_{n-1})	td	$\bar{x}$ (s_{n-1})	td	$\bar{x}$ (s_{n-1})	td	$\bar{x}$ (s_{n-1})	td
CB (g/l)	control	25.26 (2.385)	–	23.13 (2.248)	–	38.56 (4.081)	–	42.13 (3.481)	–
	experiment	24.18 (1.540)	1.522	32.31 (1.453)	0.269	37.30 (1.471)	1.162	40.48 (2.850)	1.465
Glu (mmol/l)	control	13.90 (1.027)	**	13.09 (1.015)	*	16.13 (2.043)	**	16.45 (1.080)	**
	experiment	10.59 (0.855)	9.897	12.22 (1.075)	2.352	14.46 (0.956)	2.960	14.76 (1.673)	3.396
Chol (mmol/l)	control	2.11 (0.231)	**	2.29 (2.000)	**	2.80 (0.133)	–	3.19 (0.501)	–
	experiment	1.81 (0.153)	4.326	2.08 (0.173)	3.184	2.76 (0.212)	0.641	2.95 (0.120)	1.867
AST (μkat/l)	control	0.82 (0.035)	**	0.96 (0.094)	–	0.985 (0.086)	**	1.03 (0.057)	–
	experiment	0.75 (0.071)	3.478	0.94 (0.063)	0.693	1.070 (0.076)	2.924	0.982 (0.123)	1.411
Ca (mmol/l)	control	2.52 (2.293)	–	3.11 (0.498)	**	2.77 (0.206)	**	3.80 (0.064)	**
	experiment	2.46 (0.124)	0.756	2.49 (0.135)	4.786	2.34 (0.120)	7.267	2.86 (0.138)	24.425
P (mmol/l)	control	5.27 (1.265)	–	3.17 (0.957)	–	2.78 (0.632)	**	4.72 (1.288)	*
	experiment	4.76 (1.695)	0.964	3.64 (0.605)	1.663	5.40 (0.852)	9.879	3.79 (0.827)	2.429
Mg (mmol/l)	control	0.83 (0.060)	–	0.84 (0.067)	**	0.82 (0.071)	–	0.83 (0.076)	–
	experiment	0.80 (0.050)	1.511	0.77 (0.044)	3.457	0.84 (0.059)	0.854	0.80 (0.040)	1.397

$\bar{x}$ = arithmetic mean, (s_{n-1}) = standard deviation, td = test value according to the Student's *t*-test
CB = total plasma proteins, Glu = plasma glucose, Chol = plasma cholesterol, AST = catalytic concentration of AST, Ca = plasma calcium, P = plasma phosphorus, Mg = plasma magnesium
* $P \leq 0.05$, ** $P \leq 0.01$

observation period always significantly lower in the experimental group. Similarly as the plasma proteins, also the plasma glucose showed characteristic gradual increase, practically during the whole period of fattening, both in the chickens of the control group (13.09 to 16.45 mmol/l) and in the chickens of the experimental group (10.59 mmol/l to 14.76 mmol/l).

Higher average levels of plasma cholesterol in the control groups were observed during the whole period of fattening. The average values of plasma cholesterol gradually increased during the fattening both in the control group (2.11 mmol/l to 3.19 mmol/l) and in the experimental group (1.81 mmol/l to 2.95 mmol/l) as well. On 21st and 42nd day of fattening, the differences between average values of the control group (2.11 mmol/l and 2.29 mmol/l) and the experimental group (1.81 mmol/l and 2.08 mmol/l) were tested as highly significant ($P \leq 0.01$).

Except for the 42nd day of fattening (1.07 μkat/l), the catalytic concentration of AST also showed increase both in the control group (0.82 μkat/l to

1.03 μkat/l) and in the experimental group (0.75 μkat/l to 0.98 μkat/l).

The examination of the blood plasma within the biochemical indexes was also focused on the dynamics of the basic bone-forming elements: Ca, P and Mg.

Significantly, lower levels of plasma Ca were recorded in the experimental group. The average values of plasma Ca varied between 2.52 mmol/l and 3.80 mmol/l in the control group and between 2.34 mmol/l and 2.86 mmol/l in the experimental group. On 42nd, 52nd and 62nd day of fattening, these differences were tested as highly significant ($P \leq 0.01$).

The results within the plasma P show that the levels of plasma P varied during the observation period within a quite wide range of average values, from 2.78 mmol/l to 5.27 mmol/l in the control group and from 3.64 mmol/l to 5.40 mmol/l in the experimental group. Though on 52nd and 62nd day of fattening, a statistically highly significant ($P \leq 0.01$) and statistically significant ($P \leq 0.05$) provability of the differences were found between the average values of both groups, these differences cannot be considered as a result of the experimental intervention.

When studying dynamics of changes of plasma Mg levels, we found that the average levels of plasma Mg during the fattening varied in relatively narrow ranges (0.82–0.84 mmol/l) in the control group, and (0.77–0.84 mmol/l) in the experimental group, except for the 42nd day, when a statistically highly provable lower average value was found in the experimental group (0.77 mmol/l), compared to the control group (0.84 mmol/l).

As to the nutrition, the experimental group showed during the 62 days of fattening lower consumption of feed – 2.21 kg of feed mix per 1 kg of live weight increase, compared to the control group that showed the feed conversion in amount of 2.27 kg. On the contrary, in the experimental group the higher water consumption 20.09 l per a bird compared with control birds (14.88 l per a bird) during the 62 days of fattening was observed.

The experimental group that was fed under higher temperature, showed higher mortality; 7 of 70 chickens died (10.00%), compared to the control group, where only 4 of 70 chickens died (5.71%).

DISCUSSION

The results of studying the impact of higher environmental temperature on organisms of fattened chickens show that the increased air temperature had a significant impact on the inter-medial metabolism of chickens. These metabolic changes were represented by reduced growth intensity and increased mortality of chickens during the fattening. The results of the experiments prove that even a small increase in environmental temperature (in the course of the fattening by 1–5°C) has a significant negative impact on the efficiency of the fattening. These conclusions are in agreement with results reported by Hansen (1988), Arjona *et al.* (1988) and Manning and Wyatt (1990).

The results of haematological examinations proved that total amount of haemoglobin decreased and total amount of leucocytes increased in the blood of the experimental birds that were fattened under higher environmental temperature. Datta *et al.* (1996) noticed similar increase in the amount of leucocytes in blood of ducks that were exposed to higher environmental temperature, though in contrast to our results, the quoted authors observed after the higher temperature exposure of ducks also an increase in the amount of erythrocytes and haemoglobin in the ducks. Based on the experiment that we performed, we can say that increased environmental temperature has a negative impact on the haemoglobin synthesis, which can result in reduced intensity of the overall metabolism. Lower levels of haemoglobin in the chicken blood can relate to the effect of higher environmental temperature on changes in distribution of iron in the organisms of broilers, these changes are described by Jamadar and Jalnapurkar (1995). This can influence also the homeopoetic processes. On the other hand, the increase in leucocytes in the blood of the chickens can be in direct relation to the heat stress the chickens experienced.

The chickens fattened under higher environmental temperature showed also some significant changes within the biochemical profile of their blood plasma. All biochemical changes that we have noticed in the course of fattening can be divided into two groups. First group include biochemical changes that occur regularly during the fattening. These changes are characteristic for gradual increase of the level of total plasma proteins, plasma glucose, plasma cholesterol and catalytic concentration of AST. Suchý *et al.* (1993a,b) reported similar regularities within the changes of biochemical profile of blood plasma in broiler chickens. The second group include biochemical changes related to the heat exposure of chickens. While the concentration of plasma proteins did not change, concentrations of plasma glucose and cholesterol in the blood plasma were significantly influenced. The chickens exposed to the higher temperature

showed significantly or highly significantly lower level of plasma glucose during the whole period of fattening, which indicates reduction of chicken energetic metabolism. Similarly, the chickens exposed to higher environmental temperature had also lower levels of cholesterol in their blood during the fattening. Except for 52nd day of the fattening, the aforementioned metabolic changes were accompanied also by lower catalytic concentration AST in the blood plasma. Based on the aforementioned results, it is clear that a number of biochemical changes occur within the metabolism of chickens fattened under an increased air temperature. These changes have been mentioned already by Suchý *et al.* (1993a,b) in their work.

Ching *et al.* (1992) state within their study of mineral metabolism of broilers that, under a heat stress, the levels of calcium, potassium and nitrogenous substances increase. Our work has not confirmed such relations as chickens were not under acute stress but our results are consequent to adaptation reaction.

The aforementioned changes in the metabolism of chickens, also found in our work, indicate that a long-term impact of higher environmental temperature significantly reduces inter-medial metabolism in fattened chickens. That might be besides decreased feed consumption a significant reason for worsening productivity (weight increase) and increased mortality of the fattened chickens. The achieved results support the conclusions published by Suchý *et al.* (1993a,b) and by Manning and Wyatt (1990).

In conclusion, it can be stated that the air temperature is one of the most significant abiotic factors that can significantly influence metabolism of chickens and thus the whole effect of fattening.

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Received: 02–01–07
Accepted after corrections: 02–04–26

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Primary humoral response in Siberian sturgeon after exposure to anti-furunculosis bacterin

Primární humorální reakce jesetera sibiřského po vakcinaci bakterinem proti furunkulóze

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ABSTRACT: This study demonstrated the primary effects of humoral response in sturgeon juveniles exposed to a vaccine based on both *Aeromonas salmonicida* O-antigen and whole formalin-treated *Aeromonas sobria*. An experiment was carried out on Siberian sturgeon with mean initial body weight 40 ± 4 g at 2 months of age, reared in a recirculation system at water temperature 22°C. Intact fish was immersed in a diluted (1 : 10) vaccine suspension for 5 minutes. The control fish was bathed without supplement. The blood from the fish was taken 11 times at 1-week intervals. The titres of serum anti-*Aeromonas salmonicida* antibodies, agglutinating rough bacteria, as well as the levels of γ-globulin fraction and total proteins were determined in the serum. One week after bath vaccination there was an increase in the agglutinin titres to the level of 2.9 ± 0.5 to 7.0 ± 2.0. The 2.4–3.7-fold increase in titres was observed in exposed fish ($P < 0.001$) until week 6. Elevated ($P < 0.01$) γ-globulin levels (by 50–100% during the first 7 weeks) and total protein contents (until week 10) in experimental fish in comparison with control juveniles were also determined ($P < 0.01$). The data document that the immune system of 2-month sturgeons is fully developed, and the dose of bacterin and other conditions were sufficient to stimulate the humoral response.

Keywords: sturgeon; vaccination; multivalent bacterin; antibodies; γ-globulins; total protein; serum

ABSTRAKT: V této studii byly prokázány primární účinky humorální reakce u juvenilních jeseterů, kteří byli vakcinováni O-antigenem kmene *Aeromonas salmonicida* a celým, formalinem usmrceným kmenem *Aeromonas sobria*. Pokus byl proveden s jeseterem sibiřským o průměrné počáteční tělesné hmotnosti 40 ± 4 g a ve věku 2 měsíců, jehož odchov probíhal v recirkulačním systému při teplotě vody 22 °C. Ryby jsme ponořili na dobu 5 minut do ředěné (1 : 10) suspenze vakcíny. Kontrolní jedinci byly ponořeny do lázně bez vakcíny. Vzorky krve ryb jsme odebrali 11× v týdenních intervalech. V séru jsme stanovili titry sérových protilátek proti *Aeromonas salmonicida*, které aglutinují bakterie, a hladiny frakce γ-globulinu a celkových proteinů. Za jeden týden po vakcinaci došlo ke zvýšení titrů aglutininů na úroveň 2,9 ± 0,5 až 7,0 ± 2,0. Do 6. týdne jsme u vakcinovaných ryb pozorovali 2,4 – 3,7násobné zvýšení titrů ($P < 0,001$). U pokusných ryb jsme rovněž zaznamenali zvýšené ($P < 0,01$) hladiny γ-globulinu (o 50–100 % během prvních 7 týdnů) a celkových proteinů (do 10. týdne) ve srovnání s juvenilní kontrolou ($P < 0,01$). Tyto hodnoty potvrdily, že imunitní systém dvouměsíčních jeseterů je plně vyvinut a dávka bakterinu a ostatní podmínky byly postačující pro stimulaci humorální reakce.

Klíčová slova: jeseter; vakcinace; multivalentní bakterin; protilátky; γ-globuliny; celkové proteiny; sérum

Immunisation of sturgeons against microbial pathogens has not developed as it has for the cultured cyprinids, salmonids, channel catfish and some other species. Immunisation procedures will be effective only if enough information about the basic properties of immune system in a given species is available. Implementation of vaccination in sturgeon breeding is mainly hampered by the fact that available data describing the effect of different bacterins on immune response in cultured sturgeons are yet scarce. *Aeromonas*

salmonicida is one of the most important bacterial agents in aquaculture because the host range of this bacteria includes a wide spectrum of cold-water, warm-water and marine fish species (Tatner, 1990; Kozińska, 1999; Dumontet *et al.*, 2000). This pathogen isolated from healthy fish is responsible for triggering subclinical infection to progress to endemic outbreaks under different external factors (handling stress, high densities related stress, thermal shock) because of furunculosis and associated diseases (Austin and Austin, 1987; Kozińska and Antychowicz, 1996; Kozińska, 1999). For the successful control of infection, immune protection has been described as an effective health intervention in fish (Hasting and Ellis, 1990; Anderson and Jeney, 1991; Youkhimenko *et al.*, 1996; Michel and Hollebecq, 1998; O'Dowd *et al.*, 1999 and others).

It is not the first approach to get an insight into the post-vaccination response in *Acipenseriformes* (Kolman *et al.*, 1999a,b). It was shown that the administration of some species of chemically killed bacteria in vaccine caused a higher response than the application of outer membrane antigen (Kolman *et al.*, 1999a). The use of treated psychrophilic bacteria *A. salmonicida* and *A. hydrophila* is not safe, however, because of their diverse virulence (McCarthy and Rawle, 1975; Kozińska, 1999). That is why this experiment continued previous studies on sturgeons immunised against furunculosis by help of acellular soluble antigen prepared from *Aeromonas salmonicida* together with an appropriate multi-antigenic whole formalin-treated *A. sobria*. Mesophilic *A. sobria* is less pathogenic to fish, at least in Polish conditions (Kozińska, 1999), but it possesses an immunogenic potency because of the multiple antigenic nature of this invader. Research in sturgeon is mainly focused on making antigens safer and more accessible that will amplify the immune response to protect the fish. The present work was initiated to examine a primary humoral response in sturgeon challenged with the same soluble extracellular product of *A. salmonicida* immunogen and the complex particulate immunogen (*Aeromonas sobria*) which as a formalin-treated organism could be used to interact with specific immune response to *A. salmonicida* O-antigen.

MATERIAL AND METHODS

After an adaptation period the studies were carried out on Siberian sturgeon fry aged 2 months and of average body weight 40 ± 4 g reared in tanks, operated in a water recirculation system, with complex water treatment devices installed in. The devices ensured the following conditions: water temperature 22°C; pH 7.5–8.0; oxygen concentration of about 70% saturation; total ammonium 0.15 mg/l; nitrites 0.20 mg/l. The fry in good health was divided into 2 groups, 200 fish in each, and placed in separate tanks (two tanks for each group). The pellets produced commercially by a Danish firm Aller-Molle were given to sturgeons from automatic feeders according the scheme proposed by Kolman (1999).

The vaccine was prepared on the basis of *A. salmonicida* O-antigen and an isolate of *Aeromonas sobria* chemically killed. *Aeromonas salmonicida* O-antigen was supplied by the National Fish Health Research Laboratory in Kearneysville (U.S.A). Dried O-antigen at the dose of 30 mg was dissolved in 500 ml water. *A. sobria* isolates were cultured on nutrient agar. The bacterial isolate contained a dead (formalin-treated) strain of *A. sobria* with 600×10^6 bacterial cells per 1 ml of suspension. Dissolved O-antigen and suspension of bacterial cells were mixed (1 : 1). The bath for an exposed group was prepared dissolving 1 l of the vaccine in 9 l of basin water. The control fish were immersed in water without supplement. Fish were bathed for 5 min. After the bath procedure the exposed fish were rinsed using basin water.

Blood was collected 11 times at weekly intervals, from 10 fish each time in each group. Before blood sampling, the fish were anaesthetised with Propiscin (ethamidate solution). Blood samples of not more than 0.5 cm^3 were obtained by a cardial puncture and were left to clot at 4°C. The sera were decanted after centrifuging complete samples for 10 min at 5 000 rot./min and assayed in duplicate for the following indices. The specificity and kinetics of the humoral immune response of Siberian sturgeon to single exposure to vaccine were investigated by the serum antibody quantification, using virulent (rough) *A. salmonicida* strain as the particulate antigen. Two-fold serial dilutions (in phosphate buffered fish saline) were prepared in duplicate from 0.025 cm^3 aliquots of sera and were incubated with the known number of rough strain *A. salmonicida* cells. A complete serodiagnostic procedure was followed according to the method described by Cossarini-Dunier *et al.* (1987). Analyses of total protein and γ-globulin fraction contents in the serum were based on the biuret micromethod described earlier (Kolman *et al.*, 2000). For statistical analyses, means and standard deviations were calculated for all test values using Student's *t*-test. Differences in means ($n = 10$ fish for each group value) were considered statistically significant at $P < 0.05$ (*) and highly significant at $P < 0.01$ (**).

RESULTS

The somatic growth (above 5-fold) of sturgeon exposed to bacterin was not significantly higher than that of the control. The detection of humoral antibody (Ab) in sturgeon juveniles showed that the elevated level of soluble anti-*A. salmonicida* antibodies was determined in the blood serum of the studied fish at successive weeks after bath (Figure 1). One week after bath vaccination there was a statistically significant ($P < 0.001$) increase in the specific agglutinating serum antibodies titres to the mean level of 2.9 ± 0.5 to 7 ± 2.0. Agglutinating serum Ab titres increased (2.4–3.7-fold) until week 6, when maximal level was observed (about 8.2 ± 1.3). During the last 5 weeks, although the differences ($P < 0.001$) between the groups were maintained by the end of the studies (Figure 1), a decreasing tendency of changes was observed.

Moreover, after 1 week there was a significant ($P < 0.01$) increase in γ-globulin content in the serum to 6.04 ± 1.31 to 10.03 ± 2.51g/l. The increasing (about 50–100%) trend was maintained until week 7. The differences were not significant because of the individual differences in the groups (Figure 2). At last, one week after vaccine exposure, a significant increase in concentrations of serum total protein to 20 ± 1.9 to

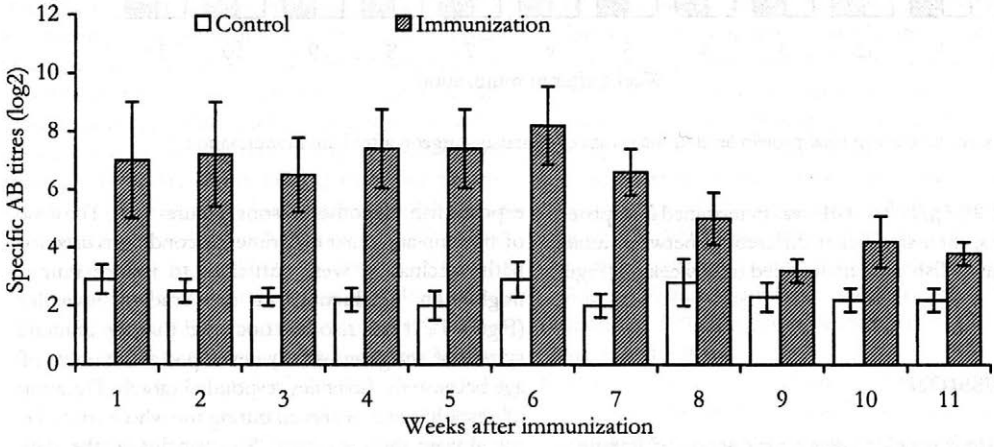

Figure 1. An increase in serum anti-*Aeromonas salmonicida* antibody titres after bath immunisation in Siberian sturgeon

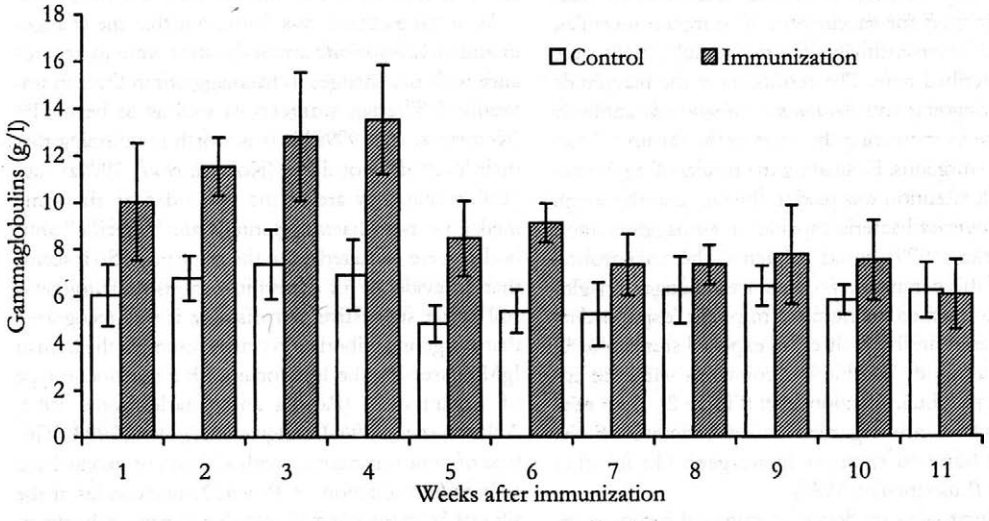

Figure 2. An increase in serum γ-globulins content in Siberian sturgeon juveniles after bath immunisation

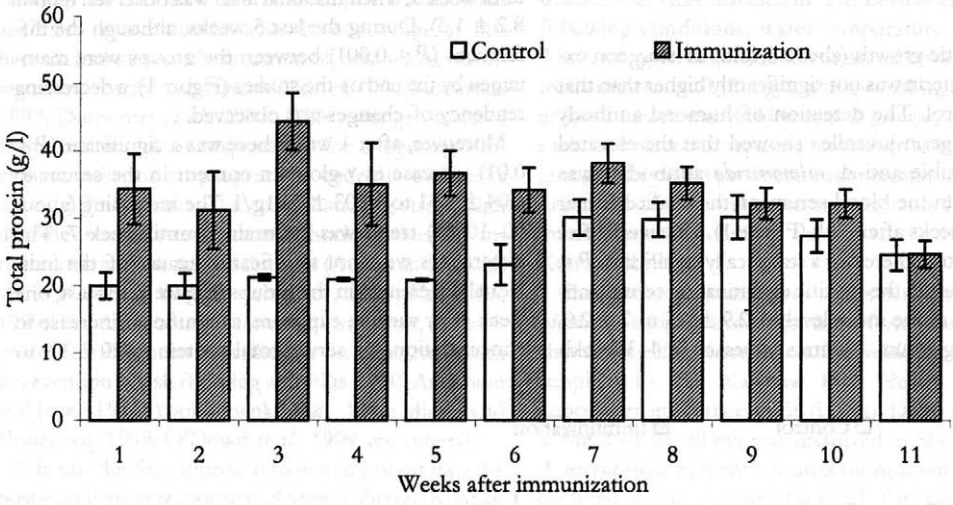

Figure 3. An increase in total protein level in the serum of Siberian sturgeon after bath immunisation

34.27 ± 9.57 g/l ($P < 0.01$) was determined in exposed juveniles, then significant differences between intact and control fish were maintained until week 10 (Figure 3).

DISCUSSION

This study provided data on the action of immunogens against furunculosis in juvenile sturgeons reared in a water recirculation system. Our data documented that the applied direct immersion technique was successfully used for vaccination of sturgeon juveniles. Delayed hypersensitivity effects (Landolt, 1989) were also described here. The results show the magnitude of the ongoing anti-*Aeromonas salmonicida* antibody response by increasing the titres in the serum of vaccinated sturgeons. Evaluating the results of agglutination test, attention was paid to the fact that the rough *A. salmonicida* bacteria capable of autoagglutination (Kozińska, 1999) could influence the background level of this parameter. So, enhanced changes in γ-globulins confirmed the humoral response (expressed by the value of antibody titres) in exposed sturgeons. As noted, antibody production coincides with the enhanced γ-globulin fraction level (Figure 2). Both relevant indices are regarded as an estimate of the specific humoral response in sturgeon like in other animals (Lukyanenko, 1989).

The increasing tendency of humoral response reported here was caused by the antigenic stimulation of exposed fish, and other reasons (Figures 1–3). The dose of bacterin and other experimental conditions used for bath vaccination were sufficient to induce immunoglobulin production in the studied juveniles (Figure 1). It can also be concluded that the immune system of sturgeons is fully developed at 2 months of age because the juveniles responded rapidly. Duration of response was observed during the whole study, i.e. for almost three months. So, principally, the data documented that the joint application of soluble *A. salmonicida* O-antigen and formalin-treated whole *A. sobria* in immersion was immunogenic for sturgeons.

As noted earlier, it was found out that the changes in anti-*A. salmonicida* antibody titres were in accordance with the changes in haemagglutinin titres in immunized Siberian sturgeon as well as in bester F3 (Kolman *et al.*, 1999a,b). It is worth mentioning that their levels did not differ (Kolman *et al.*, 1999a) suggesting that they are as the two sides of the same medal, i.e. both haemagglutinins and "specific" antibodies were recruited from the same pool. So it seems that the evidence for a specific response in sturgeon is still rather suggestive. Surprisingly, it was recognised that sturgeon antibody molecules resemble the human IgM possessing the IgH locus with a translocon type of organisation (Partula and Charlemagne, 1993; Adkison *et al.*, 1996; Lundqvist *et al.*, 1996, 1998). This type of gene organisation with addition of exonuclease activity (introduction of P- and N-nucleotides at the sites of rearrangement) is capable to ensure the diversifying mechanisms that will generate different clones

of antibodies. If so, the production of monoclonal antibodies would provide possibilities for the recognition of lymphoid cell subpopulations in sturgeon.

In the environment of poikilothermic sturgeons, there are high counts and variety of potential microbial pathogens, capable of undergoing mutation at a high rate. That is why it seems that the germ-line coding guided by evolutionary selection for specific recognition mechanisms for each potential invader would not be possible. In reality, instead (of developing those mechanisms), upstream to such direction of adaptation, the fish were "equipped" with IgMs that are characterised by low specificity. On the other hand, those molecules are attributed to stronger avidity and antigen-complexed antibodies of class IgM are capable of activating a complement system to the largest extent in comparison with immunoglobulins of other classes. It is interesting that the complement activity in sturgeon is much higher than in teleost species (Lukyanenko, 1989), maybe because of different spatial structure of sturgeon antibodies and (following from this circumstance) higher maximum functional valence of their antibodies. Current research on intraperitoneal vaccination in sturgeons resulted in the activation of a range of non-specific defence mechanisms (Kolman *et al.*, 1999a,b), suggesting participation and cooperation between lymphoid and phagocytic cells. Such cooperation has been expected especially in the case of animals immunised *via immersion* as at the present work, because of the considerable accumulation of phagocytic cells in the pharyngeal and gill regions of the fish (Manning, 1981; Fange, 1986; Ivanova, 1995).

Without doubt, immuno-physiological study in *Acipenseriformes* remains a very fascinating prospect because sturgeons with their long length of life (both individual and historical) may have evolved especially efficient defence mechanisms than other animals.

Acknowledgement

The author would like to acknowledge his especial gratitude to Prof. A. K. Siwicki for research fellowship.

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Received: 01–09–20
Accepted after corrections: 02–04–16

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Study on a relation between estrogen receptor (*ESR*) gene polymorphism and some pig reproduction performance characters in Polish Landrace breed

Studium vztahu mezi polymorfismem genu receptoru estrogenu (*ESR*) a některými znaky reprodukce u prasat plemene landrase polská

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ABSTRACT: The study was carried out in a pedigree herd of 8 boars and 207 sows of the Polish Landrace breed. The polymorphism of the *ESR* gene was found by means of the PCR-RFLP method. The amplification resulted in a product of 120 base pairs. The product was then digested with the *Pvu*II restriction enzyme. The two alleles of the *ESR* gene were identified – ESR^A allele – 120 bp, ESR^B – 65 and 55 bp. The frequency of ESR^A was 0.9419, whereas that of ESR^B was 0.0581. The frequency of the AA genotype was 0.8837, whereas that of AB was 0.1163. The *ESR* BB genotype was not found in the pig population under study. The study focused on the relation between *ESR*/*Pvu*II genotypes and the number of teats on the left and on the right side, total number of teats in sows, number of piglets in the 1st, 2nd, 3rd, 4th and 5th litters and farrowing age of the first five litters. The initial data for calculations were transformed from the discrete scale to the normal one. The analysis of the relation between the *ESR* genotypes and reproduction performance characters in the pedigree pigs in five subsequent litters showed small and statistically insignificant differences between the sows of *ESR* AA and *ESR* AB genotypes.

Keywords: estrogen receptors; gene polymorphism; pig; reproduction

ABSTRAKT: Sledování se uskutečnilo ve šlechtitelském chovu (8 kanců a 207 prasnic) plemene polská landrase. Polymorfismus genu *ESR* byl zjišťován PCR-RFLP. PCR produkt o 120 bp byl štěpen enzymem *Pvu*II. Zjistili jsme alelu ESR^A = 120 bp, ESR^B = 65 a 55 bp. Frekvence ESR^A byla 0,9419 a ESR^B 0,0581. Frekvence genotypu AA dosáhla hodnoty 0,8837 a frekvence AB 0,1163. Genotyp *ESR* BB jsme ve sledované populaci prasat nezjistili. U prasnic bylo sledování zaměřeno na vztah mezi genotypy *ESR*/*Pvu*II a počtem struků na levé i na pravé straně, počtem selat v 1., 2., 3., 4. a 5. vrhu a věkem prasnic. Pro výpočty jsme provedli transformaci původních údajů z nespojitého na normální rozložení. Analýza vztahu mezi genotypy *ESR* a znaky reprodukce v pěti vrzích ukázala statisticky neprůkazné rozdíly mezi prasnicemi genotypů *ESR* AA a *ESR* AB.

Klíčová slova: prase; receptor estrogenu; polymorfismus; reprodukce

Reproduction plays a key role in the successful production of farm animals, specially in the case of so called multiple-litter animals, e.g. livestock.

The size of a litter is crucial since the cost of producing a piglet is inversely proportional to the size of a litter in sows. Selection for the size of a litter is

rather difficult because this character is of low heritability.

One of the methods used to identify genetic factors that influence quantitative characters is the analysis of the polymorphism of a gene regarded as "gene-candidate", the products of which (e.g. hormones) take part in physiological processes leading to the expression of a certain quantitative character. Genes that determine the synthesis of hormones control metabolism thus influencing the productivity of animals and moreover they can help improve significant characters.

The *ESR* gene located at the end of the first chromosome branch in pigs as "gene-candidate" of reproduction traits (Rothschild *et al.*, 1995).

The ESRs in females are found in endometrium cells and their quantity in sows increases after 60 days of pregnancy (Vallet and Christenson, 1996). The mutation within the *ESR* gene results in changes leading to disturbances of reproduction. Such mutations in human beings may result in occasional abortion and in transgenic mice with the non-active *ESR* gene significant phenotype changes in the reproduction system were reported (Dvořák *et al.*, 1998).

The purpose of this study was to find the frequency of the mutation of *ESR* gene identified with *Pvu*II enzyme as well as a possible relation between *ESR* genotypes and reproductive characters in the herd of sows of the Polish Landrace breed.

MATERIAL AND METHOD

The study was conducted on a pedigree herd of Polish Landrace maintained on a farm in the north-west of Poland and consisting of 207 sows and eight boars.

ESR genotypes were determined by means of the PCR-RFLP method. The fragment of DNA of 120 bp was amplified through starter sequences described by Short *et al.* (1997): forward primer 5'-CCT GTT TTT ACA GTG ACT TTT ACA GAG-3' and reverse primer 5"-CAC TTC GAG GGT CAG TCC AAT TAG-3'. The following amplification parameters were applied: 94°C for 5 minutes followed by 35 cycles: 94°C for 40 seconds, 55°C for 60 seconds, 72°C for 40 seconds. The reaction was completed by the final synthesis; 72°C for 5 minutes. The PCR products were digested with 5 units of the *Pvu*II restriction enzyme (MBI Fermentas) – 3 hours at 37°C. The restriction fragments of DNA were separated by means of electrophoresis in 3.5–4% agarose gel stained with ethidium bromide. After the electrophoresis the gels were analyzed in the UV rays. The restriction fragments were determined and compared with the pUC19/*Msp*I marker (Figure 1).

The data on the performance of sows were taken from the herd book and they included the information on the number of teats on the left and right side, total number of teats, number of piglets in certain litters (males and females) and age of sows on the farrowing day.

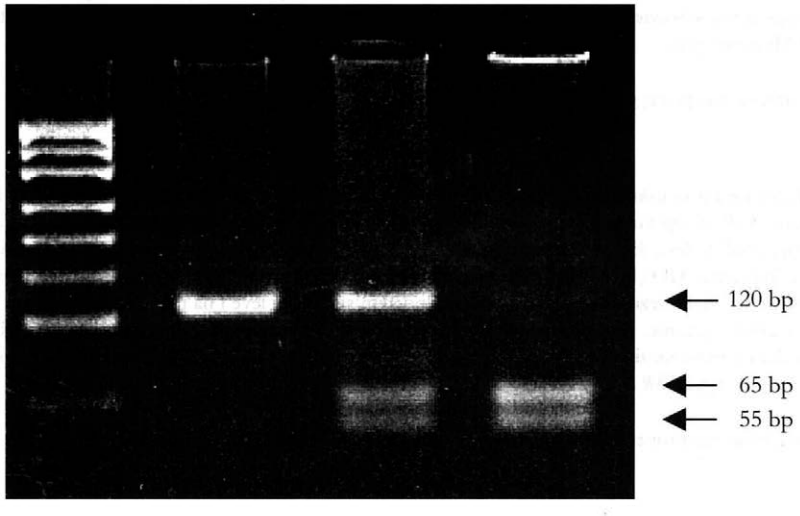

Figure 1. PCR-RFLP fragments of porcine *ESR* gene digested with *Pvu*II. AA, BB – homozygotic genotypes; AB – heterozygotic genotype; M – pUC19/*Msp*I DNA marker

Table 1. Frequency of genotypes and alleles of *ESR* gene in the studied population of pigs

Group		*ESR* genotype				*ESR* allele	
		AA	AB	BB	Total	ESR^A	ESR^B
Boars	*n*	7	1	–	8	0.9375	0.0625
	frequency	0.8750	0.1250	–	1.000		
Sows	*n*	183	24	–	207	0.9420	0.0580
	frequency	0.8841	0.1159	–	1.000		
Total	*n*	190	25	–	215	0.9419	0.0581
	frequency	0.8837	0.1163	–	1.000		

Table 2. Means and standard deviations for teat number in sows carrying different *ESR* genotypes

ESR genoype	Number of sows	Number of left-side teats		Number of right-side teats		Total number of teats	
		mean	SD	mean	SD	mean	SD
AA	183	7.46	0.49	7.28	0.53	15.13	0.78
AB	24	7.30	0.48	7.12	0.56	14.97	0.91
Total	207	7.44	0.49	7.27	0.53	15.11	0.79

No significant differences were found between genotypes

Table 3. Number of piglets in the first five litters of sows with different *ESR* genotypes

ESR genotype	Litter	Number of sows	Number of piglets in a litter						Age at farrowing (days)	
			total		males		females			
			mean	SD	mean	SD	mean	SD	mean	SD
AA	I	183	10.39	1.46	6.46	3.11	5.51	2.80	347.9	47.5
AB		24	10.07	1.29	6.43	3.47	5.07	1.97	350.8	54.6
Total		207	10.36	1.44	6.46	3.15	5.46	2.71	348.2	48.2
AA	II	165	10.42	0.91	4.78	1.34	5.61	1.70	522.1	53.8
AB		20	10.07	1.67	3.94	1.21	6.36	1.82	544.0	88.8
Total		185	10.38	0.97	4.69	1.35	5.69	1.73	524.5	58.7
AA	III	134	10.62	1.14	5.01	1.33	5.67	1.97	731.5	68.7
AB		16	10.44	0.64	5.08	1.03	4.96	1.68	761.6	95.8
Total		150	10.60	1.10	5.01	1.30	5.59	1.95	734.7	72.2
AA	IV	113	10.07	0.98	4.52	1.17	5.54	1.85	895.2	74.8
AB		14	10.01	1.13	4.15	1.02	5.98	1.63	957.0**	117.7
Total		127	10.07	1.00	4.48	1.16	5.59	1.82	902.0	82.3
AA	V	88	10.12	0.99	4.98	1.31	5.20	1.87	1 090.1	82.2
AB		12	10.10	0.96	5.49	1.71	4.23	1.67	1 108.5	106.2
Total		100	10.12	0.98	5.04	1.37	5.08	1.87	1 092.3	85.1
AA	I–V	88	50.80	2.25	28.74	3.84	22.12	4.94		
AB		12	50.05	1.68	28.95	2.87	21.11	2.75		
Total		100	50.71	2.20	28.76	3.72	22.00	4.73		

*$P \leq 0.05$; **$P \leq 0.01$

The data on the number of teats and piglets per litter were transformed from the discrete scale to the quantitative one according to the procedure described by Snell (1964) and corrected in terms of year/season of litter birth by means the multiplier correction (Żuk, 1989) and finally the relation between *ESR* genotypes and reproduction performance characters in sows was analyzed by the analysis of variance. The significance of differences was checked by multiple Duncan's test along with the Statistica '99 calculation package procedure.

RESULTS AND DISCUSSION

The PCR reaction resulted in a product of 120 bp that was digested with the *Pvu*II restriction enzyme. The above process produced one band on agarose gel at the level of 120 bp, i.e. *ESR*/*Pvu*II AA genotype or three bands of 120, 65 and 55 bp *ESR*/*Pvu*II AB genotype, respectively as compared with the pUC19/*Msp*I molecular marker (Figure 1).

Two *ESR* genotypes were identified in the studied pedigree herd of 207 sows and 8 boars of the Polish Landrace breed: AA and AB genotypes. The frequency of AA genotype was 0.8837 and that of AB was 0.1163 (Table 1). The *ESR*/*Pvu*II BB genotype was not found in the herd concerned. The distribution of *ESR*/*Pvu*II genotypes within the studied population did not differ significantly from that expected according to Hardy-Weinberg rule.

The frequency of *ESR*/*Pvu*II AA genotype was 0.862, that of AB genotype – 0.128 and that of BB – 0.010, as reported by Dvořák *et al.* (1999) in the study concerning 114 sows of the Polish Landrace bred in Poland. The same breed kept in the Czech Republic produced the following frequency of *ESR* genotypes: AA – 0.799, AB – 0.185 and BB – 0.016.

The number of teats on the left and right side and total number of teats were higher in sows of the *ESR* AA genotype, but the differences were small and they were not significant (Table 2). Short *et al.* (1997) also showed a higher number of teats in sows of the *ESR*/*Pvu*II AA genotype and the difference was confirmed statistically at $P \leq 0.05$.

Table 3 presents the number of piglets in the 1st, 2nd, 3rd, 4th and 5th litters and the age of sows of the Polish Landrace breed on the day of farrowing of the first five litters versus the *ESR*/*Pvu*II genotype. The higher average number of piglets in each of the five subsequent litters was observed in sows of *ESR*/*Pvu*II – AA genotype, but the reported differences were too small and were not confirmed statistically. Similar results for the herd of F_1 sows (Zlotnicka Pied × Polish Large White) were obtained by Korwin-Kossakowska *et al.* (1999). Short *et al.* (1997), however, proved the statistically significant ($P \leq 0.01$) advantage of *ESR*/*Pvu*II BB sows in the number of piglets delivered in the first and subsequent litters of sows in four commercial synthetic lines PIC.

The average age of sows at the first farrowing in the herd concerned was 348.2 days (± 42 days) and was similar to the age reported by other authors in herds of the Polish Landrace breed, i.e. 347 days (Grudniewska, 1998) and 381.74 days (Czarnecki, 1976).

CONCLUSIONS

The study on the herd of the Polish Landrace pigs discovered two alleles of the *ESR*/*Pvu*II gene, namely ESR^A and ESR^B, that decide on the occurrence of two genotypes, i.e. *ESR* AA and AB (the frequency of 0.8837 and 0.1163 respectively). The *ESR* BB genotype was not found in the herd concerned.

The analysis of the relation between genotypes and the reproductive performance characters showed a small difference in favor of the *ESR* AA that was nor confirmed statistically.

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Received: 01–09–18
Accepted after corrections: 02–04–16

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Analysis of relations between polymorphism in steroid 21-hydroxylase gene (*CYP21*) and quantitative and qualitative characters of boar semen

Analýza závislostí mezi polymorfismem v genu steroidní 21-hydroxylázy (*CYP21*) a kvantitativními a kvalitativními znaky kančího spermatu

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ABSTRACT: The steroid 21-hydroxylase gene (*CYP21*) located on chromosome 7 in pigs in region SLA class III in the DNA segment between regions SLA class I and SLA class II is regarded as a "gene-candidate" of reproductive characters. The purpose of this study was to find a relation between the frequency of mutation in the *CYP21* gene in boars and qualitative and quantitative characters of the semen under study. The study included 88 boars from which the semen was taken to inseminate sows. The *CYP21* polymorphism was determined by means of the PCR-RFLP method. The amplification product was digested with the *Hae*III endonuclease. Two alleles of the *CYP21* were identified: $CYP21^A$ allele – 448 bp and $CYP21^B$ allele – 350 and 98 bp. The frequency of $CYP21^A$ was 0.1989, whereas that of $CYP21^B$ was 0.8011. Two *CYP21* genotypes were found in the boars under study. The frequency of the AB genotype was 0.3978 and that of BB was 0.6022. An analysis of the relation between the *CYP21* genotypes and boar semen characters showed statistically significant predominance of boars with the AB genotype in terms of the percentage of normozoosperms, number of normozoosperms in a dose and higher number of sperm cells in the insemination dose.

Keywords: *Sus scrofa*; steroid 21-hydroxylase gene (*CYP21*); semen characters

ABSTRAKT: Gen steroidní 21-hydroxylázy (*CYP21*) lokalizovaný u prasete na chromozomu 7 v oblasti III. třídy SLA, na segmentu DNA mezi oblastmi SLA I. a SLA II. třídy je považován za kandidátní gen pro reprodukční znaky. Účelem této studie bylo sledování závislostí mezi výskytem mutace v genu *CYP21* a kvalitativními a kvantitativními znaky spermatu u kanců. Do studie bylo zahrnuto 88 kanců používaných k inseminaci prasnic. Polymorfismus *CYP21* jsme stanovili metodou PCR-RFLP. Produkt amplifikace byl štěpen *Hae*III endonukleázou. Zjistili jsme dvě alely *CYP21*: alelu $CYP21^A$ – 448 bp a alelu $CYP21^B$ – 350 a 98 bp, s frekvencemi 0,1989 a 0,8011. U kanců byly identifikovány pouze dva genotypy *CYP21* s frekvencemi AB = 0,3978 a BB = 0,6022. Analýza asociací genotypů *CYP21* se znaky spermatu ukázala statisticky průkazně lepší parametry u kanců s genotypem AB v procentickém zastoupení normozoospermií, počtu normozoospermií a spermatozoidů v inseminační dávce.

Klíčová slova: *Sus scrofa*; gen steroidní 21-hydroxylázy (*CYP21*); znaky spermatu

Reproduction is an important factor that affects the profitability of animal production. Reproduction characters are influenced by a number of genes and environmental factors that modify the phenotype value of the characters. The progress in research on the pig genome made it possible to identify the polymorphic loci of individual genes that control the reproduction characters and may influence the reproduction of animals. The *CYP21* is located on pig chromosome 7 (Geffrotin *et al.*, 1987) in region SLA class III (Geffrotin *et al.*, 1990) in the DNA segment between regions SLA class I and SLA class II (Geffrotin *et al.*, 1991). It consists of about 3 050 bp, contains 10 exons separated by introns and codes for protein of 492 amino acids (Burghelle-Mayeur *et al.*, 1992).

One of the most important enzymatic complexes that take part in the synthesis of adrenal steroids is steroid 21-hydroxylase (Kupczyk *et al.*, 1996). It takes part in the synthesis of mineralcorticoids and glucocorticoids. It converts substrates of 17-hydroxyprogesterone and progesterone into 11-deoxycorticosterone, thus leading to the synthesis of cortisol and aldosterone (Geffrotin *et al.*, 1990). The steroid 21-hydroxylase contains cytochrome P450 reductase and cytochrome 450_{21} (Vinson *et al.*, 1992). The deficit of cytochrome 450_{21} prevents the synthesis of glucocorticoids and mineralcorticoids and leads to the hypersecretion of adrenal androgens (Wilson and Grifin, 1994; New *et al.*, 1994). Changes in *CYP21* gene together with its regulation sequences can dramatically change the physiology of farm animals and consequently affect the productivity (Geffrotin *et al.*, 1990). The molecular polymorphism in *CYP21* gene was confirmed by the RFLP technique using a fragment of swine *CYP21* gene as a probe and discovered to exhibit quite a high frequency (Geffrotin *et al.*, 1991). According to Burghelle-Mayeur *et al.* (1992) the normal functioning of *CYP21* is essential in the whole life and can significantly affect the expression of certain characters. That is why the *CYP21* in pigs that is located within SLA class III can greatly influence characters that influence some reproduction performance characters in pigs.

The purpose of this study was to find the frequency of mutations in the *CYP21* gene and to attempt to determine relations between *CYP21* genotypes and qualitative and quantitative characters of the boar semen.

MATERIAL AND METHOD

The study was carried out in a pedigree herd of 88 boars of different breeds kept at pedigree stations. The semen was taken from boars to inseminate sows kept at the same stations (Table 1).

DNA for the study was obtained from the whole blood sampled into vacuum test tubes containing the K_3EDTA as an anticoagulant. The isolation was carried out by means of the Master Pure kit of Epicentre Technologies. Such an isolation procedure produced DNA of 75–85 μg/ml and about 90% purity.

Genotypes of the *CYP21* were determined by the PCR-RFLP method. The fragment of DNA of 1 271 bp was amplified through starter sequences described by Knoll *et al.*, 1998: forward primer 5'-TGG CAG GCC TAC TGA GTT CA-3' and reverse primer 5'-GCA GAC GCA GCA CCT CAG CAA TG-3'. The following amplification parameters were applied: 95°C for 2 minutes followed by 35 cycles: 95°C for 1 minute, 55°C for 60 seconds, 62°C for 1 minute, 72°C for 4 minutes. The reaction was completed by the final synthesis; 72°C for 5 minutes. The PCR product was digested with 6 units of *Hae*III endonuclease for 3 hours at 37°C. The restriction fragments of DNA were separated by electrophoresis in 1.5% agarose gel colored with ethidium bromide. After the electrophoresis the gels were analyzed in UV rays.

The data collected on the reproduction performance of boars concerning semen characters such as semen volume, sperm concentration, percentage of normozoosperms, number of normozoosperms in ejaculate, number of normozoosperms in an insemination dose, number of insemination doses. The data covered 10 297 ejaculates. 2 049 ejaculates taken from boars of 221–420 days of age were subjected to analysis due to eliminate the influence of age on the characters under study. The period of semen collection was divided into two seasons: season I – 1 March to 30 September (spring-summer season), season II – 1 October to 29 February (autumn-winter season).

The statistical analysis within quantitative characters versus the *CYP21* genotypes was carried out by means

Table 1. Number of boars in breed groups

No.	Breed	Number of boars
1	Polish Landrace (PL)	24
2	Polish Large White (PLW)	13
3	Pietrain (P)	6
4	Duroc × Pietrain (D × P)	14
5	Pietrain × Hampshire (P × H)	17
6	Duroc × Hampshire (D × H)	4
6	Synthetic line (L-890 – 2; L-990 – 3; P076 – 2) (SL)	7
8	PIC	3
Total		88

of the least-squares method (Harvey, 1987) according to the following model:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + d_l + (ab)_{ij} + (ac)_{ik} + (ad)_{il} + e_{ijklm}$$

where: Y_{ijkl} = character value for ith-*CYP21* genotype, jth-year, kth-season, lth-breed

μ = mean for the population

a_i = constant effect of ith-*CYP21* genotype ($i = 1, 2$)

b_j = constant effect of jth year ($j = 1, 2, ...6$)

c_k = constant effect of kth season ($k = 1, 2$)

d_l = constant effect of lth boar breed ($l = 1, 2, ...8$)

$(ab)_{ij}$ = effect of *CYP21* genotype × year interaction

$(ac)_{ik}$ = effect of *CYP21* genotype × season interaction

$(ad)_{il}$ = effect of *CYP21* genotype × breed interaction

e_{ijklm} = error

The values of characters under study were expressed by least-square means and their standard errors.

RESULTS AND DISCUSSION

The PCR reaction resulted in a product of 1 271 bp that was digested with the *Hae*III restriction enzyme at 37°C for 3 hours and then subjected to electrophoresis. The above process produced 12 restriction fragments of 7 to 448 base pairs on the agarose gel. The polymorphic site for *Hae*III endonuclease was located in a fragment of 448 base pairs at position 1 762. The restriction fragments of 448, 350 and 98 bp – *CYP21* genotype AB and 350 and 98 bp – *CYP21* genotype BB were obtained compared to the DNA pUC19/*Msp*I molecular marker.

Two *CYP21* alleles were identified in the boar herd under study: $CYP21^A$ allele and $CYP21^B$ allele that controlled two genotypes, namely *CYP21* AB and *CYP21* BB. $CYP21^A$ exhibited the frequency of 0.1989, whereas the frequency of $CYP21^B$ was 0.8011 (Table 2). The highest frequency of $CYP21^A$ was found in Pietrain boars (0.3333) and the crosses of Duroc and Hampshire (D × H) exhibited none of the allele concerned.

CYP21 AA was not found in the tested breeds. The frequency of *CYP21* AB was 0.3978, and that of *CYP21* BB was 0.6022. The frequency of *CYP21* AB in certain breeds ranged from 0.2857 (synthetic lines) to 0.6667 (Pietrain), and that of *CYP21* BB from 0.3333 (Pietrain) to 1.0000 (D × H) – see Table 2. *CYP21* AB was frequently found in the boars of Polish Large White (PLW) breed (0.5385) and in D × P crossbreds (0.5000). *CYP21* BB was frequently identified in the boars of synthetic lines (SL), 0.7143, and of Polish Landrace (PL) breed, 0.7083 – see Table 2.

Table 2. The frequency of *CYP21* genotypes and alleles in the herd of boars under study

Group		*CYP21* genotype				*CYP21* allele	
		AA	AB	BB	Total	$CYP21^A$	$CYP21^B$
PL	*n*	–	7	17	24	0.1458	0.8542
	frequency	0.0000	0.2917	0.7083	1.0000		
PLW	*n*	–	7	6	13	0.2692	0.7308
	frequency	0.0000	0.5385	0.4615	1.0000		
P	*n*	–	4	2	6	0.3333	0.6667
	frequency	0.0000	0.6667	0.3333	1.0000		
D × P	*n*	–	7	7	14	0.2500	0.7500
	frequency	0.0000	0.5000	0.5000	1.0000		
P × H	*n*	–	7	10	17	0.2058	0.7942
	frequency	0.0000	0.4118	0.5882	1.0000		
D × H	*n*	–	–	4	4	0.0000	1.0000
	frequency	0.0000	0.0000	1.0000	1.0000		
SL	*n*	–	2	5	7	0.1428	0.8572
	frequency	0.0000	0.2857	0.7143	1.0000		
PIC	*n*	–	1	2	3	0.1667	0.8333
	frequency	0.0000	0.3333	0.6667	1.0000		
Total	*n*	–	35	53	88	0.1989	0.8011
	frequency	0.0000	0.3978	0.6022	1.0000		

Table 3. Significance of the influence of factors covered in statistical model on examined characters of boars semen

Character	*CYP21* genotype	Year	Season	Breed	Interaction		
					CYP21 genotype × year	*CYP21* genotype × season	*CYP21* genotype × breed
DF	1	5	1	7	4	1	6
Ejaculate volume	0.119	6.596**	5.568*	22.209**	4.388**	0.685	3.277**
Sperm concentration	0.936	8.422**	3.024	40.388**	4.681**	0.043	9.832**
Normozoospermia percentage	31.968**	44.739**	4.377*	24.671**	6.009**	3,653	14.715**
Number of normozoospermia in ejaculate	6.603**	5.806**	5.037*	36.079**	0.907	0.649	5.533**
Number of sperms in insemination dose	10.922**	16.877**	11.902**	11.625**	3.575**	0.051	6.307**
Number of insemination doses	0.625	8.237**	4.892*	18.795**	1.099	0.163	3.685**
Boar age	0.681	35.104**	14.563**	16.925**	17.255**	27.662**	9.958**

*significance of differences at $P \leq 0.05$; **significance of differences at $P \leq 0.01$

Table 4. Values of the studied semen characters in reference to *CYP21* genotype

Character		*CYP21* genotype			Total
		AB	BB	*P*	
1. Number of ejaculates		753	1 296	–	2 049
2. Semen volume (cm^3)	LSM	228.6	226.6	n.s.	227.6
	SE	5.2	4.6	n.s.	4.0
3. Sperm concentration (mln/cm^3)	LSM	540.2	532.6	n.s.	536.4
	SE	7.7	6.3		5.5
4. Normozoosperms	LSM	75.7	73.9	**	74.8
	SE	0.3	0.2		0.2
5. Number of live sperms in ejaculate (bln)	LSM	91.7	85.9	**	88.8
	SE	2.1	1.8		1.6
6. Number of sperms in insemination dose (bln)	LSM	4.0	3.9	**	4.0
	SE	0.03	0.03		0.02
7. Number of insemination doses	LSM	23.6	23.1	n.s.	23.4
	SE	0.6	0.5		0.4
8. Boar age (days)	LSM	321.0	323.6	n.s.	322.3
	SE	3.0	2.6		2.3

**$P \leq 0.01$; n.s. = non-significant

The observed frequency of *CYP21* genotypes corresponded with that calculated according to Hardy-Weinberg formula.

Relations between the *CYP21*/*Hae*III polymorphism and the semen volume (cm^3), ejaculate sperm concentration (mln/cm^3), percentage of normozoosperms, number of normozoosperms in ejaculate (bln), number of sperm cells in an insemination dose (bln), number of insemination doses and boar age at semen collection were subjected to an analysis, however, the ejaculates taken from boars aged 221–420 days were considered. The analysis showed that the semen qualitative and quantitative characters were influenced by the year ($P \leq 0.01$), breed ($P \leq 0.01$) and semen collection season ($P \leq 0.05$ and $P \leq 0.001$). Some statistically significant interactions were also found, namely: *CYP21* genotype × year, *CYP21* genotype × breed. The interactions such as *CYP21* genotype × season were not statistically significant for the boar semen (Table 3). All factors concerned were considered in a statistical model that helped identify the relations between *CYP21* genotypes and boar semen characters.

Table 4 presents average values of the boar semen characters and their standard errors versus the *CYP* 21/*Hae*III genotype. The total number of analyzed ejaculates was 2 049 and they were taken from 88 boars aged 221–420 days that were kept at the reproduction station. 753 out of the total number of ejaculates were taken from boars of the *CYP21* AB genotype, and 1 296 ejaculates came from boars of the *CYP21* BB homozygotes.

The average semen volume for all boars under study was 227.6 cm^3 and fell within the range of 50–245 cm^3 reported by Dubiel *et al.* (1985a,b) and Wilk (1986) – 140 cm^3 minimum for boars of that age. A larger average volume was found in the boars of *CYP21* AB genotype, the difference, however, was small and statistically non-significant. The average sperm concentration in ejaculates under study was 536.4 × 10^6. A slightly higher concentration than for the whole herd was found in ejaculates from the boars of *CYP21* AB genotype (540.2 × 10^6), the average for the *CYP21* BB animals being 532.6 × 10^6. The differences were small and statistically non-significant (Table 4).

The percentage of normozoosperms in the boar semen under study was 74.8%, which corresponded to the data reported by Wilk (1986) – 60% minimum and Grudniewska (1998) – 70%. The statistically significantly ($P \leq 0.01$) higher percentage was found in the semen of *CYP21* AB boars (75.7%). The value for *CYP21* BB boars was 73.9%.

The average number of normozoosperms in the semen was 88.9 × 10^9. The semen from *CYP21* AB boars exhibited a significantly ($P \leq 0.01$) higher number of normozoosperms in the ejaculate (91.7 × 10^9) than in the semen from *CYP21* BB boars (85.9 × 10^9).

The average number of sperm cells in an insemination dose in boars under study was 4.0 × 10^9. The doses from *CYP21* AB boars contained 4.0 × 10^9

sperm cells and the number was statistically significantly ($P \leq 0.01$) higher than the number found in the *CYP21* BB boars (3.9×10^9).

One ejaculate produced 23.4 insemination doses on average. The *CYP21* AB boar ejaculates produced 23.6 doses whereas the *CYP21* BB produced 23.1 doses. The differences in the number of insemination doses from one ejaculate were not confirmed statistically.

CONCLUSIONS

The preliminary study showed that *CYP21/Hae*III AB boars produced ejaculates of larger volume, concentration of sperm cells and higher percentage and number of normozoosperms in the ejaculate. A higher number of insemination doses containing more sperm cells was obtained. The statistically significant ($P \leq 0.01$) predominance of the *CYP21* AB genotype should be noticed as far as the percentage of normozoosperms, number of normozoosperms per ejaculate and number of sperm cells in an insemination dose are concerned .

The study suggests a possibility of using the existing polymorphism in the *CYP21* gene identified with *Hae*III enzyme to improve some reproduction characters of boars within the qualitative and quantitative characters of semen. The results, however, should be verified by further research on a larger number of animals despite the predominance of *CYP21/Hae*III AB shown in terms of the percentage of normozoosperms, number of normozoosperms per ejaculate and number of sperm cells in an insemination dose.

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Received: 01–09–20
Accepted after corrections: 02–04–16

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Occurrence of *Bacillus cereus* and *Bacillus licheniformis* strains in the course of UHT milk production

Výskyt kmenů *Bacillus cereus* a *Bacillus licheniformis* v procesu výroby UHT mléka

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ABSTRACT: Transfer of *Bacillus cereus* and *Bacillus licheniformis* strains from milk into final products (UHT milk) was studied. Phase milk samples were collected during the whole time of milk processing (one milk batch) in collaboration with the dairy. Representative *B. cereus* and *B. licheniformis* strains were characterised and compared by ribotyping with *Eco*RI enzyme and a probe complementary to *Escherichia coli* 16S and 23S rRNA as well as by phenotyping. According to 26 phenotypical traits the *B. licheniformis* strains formed almost a homogeneous group. In contrast, *B. cereus* strains exhibited variable results in several tests. The hybridisation profiles divided the analysed strains into two groups in full accordance with their species classification. Band patterns of *B. cereus* strains showed similarities ranging from 77 to 97%; this similarity values correlated with biochemical test results as well. *B. licheniformis* strains exhibited band pattern similarities from 82 to 100%. The hybridisation profiles of *B. licheniformis* strains B79 (pasteurized milk) were absolutely identical (100% similarity). Their phenotypical test results were identical as well. These results imply the identity of the strains isolated in the course of UHT milk production.

Keywords: *Bacillus cereus*; *Bacillus licheniformis*; raw milk; pasteurised milk; UHT milk; ribotyping

ABSTRAKT: Byl sledován možný průnik kmenů *Bacillus cereus* a *Bacillus licheniformis* ze syrového mléka do finálního výrobku, konkrétně UHT mléka. Ve spolupráci s mlékárnou byl proveden odběr fázových vzorků mléka během celého procesu zpracování jedné šarže mléka. Reprezentativní kmeny obou druhů byly charakterizovány a srovnány ribotypizací s enzymem *Eco*RI a sondou komplementární k *E. coli* 16S a 23S rRNA. Zatímco kmeny *B. licheniformis* tvořily na základě 26 fenotypových vlastností téměř homogenní skupinu, kmeny *B. cereus* se vzájemně lišily. Získané hybridizační profily rozdělily všechny analyzované kmeny do dvou skupin, které odpovídaly jejich druhovému zařazení. Hybridizační profily zástupců *B. cereus* vykazovaly vzájemně 77 až 97% podobnost, která rovněž korelovala s výsledky biochemického testování. Podobnost hybridizačních profilů kmenů *B. licheniformis* byla v rozmezí 82 až 100 %. Hybridizační profily kmenů *B. licheniformis* B79 (pasterizované mléko) a B82 (UHT mléko) byly naprosto totožné (100% shoda), stejně jako i výsledky biochemických a fyziologických testů. Podporují domněnku o identitě kmenů izolovaných v procesu výroby UHT mléka.

Klíčová slova: *Bacillus ceerus*; *Bacillus licheniformis*; syrové; pasterované a UHT mléko; ribotypizace

The detection of spore-forming microorganisms in cow's raw milk is more difficult because of their occurrence in the phase of spores. Devitalisation of milk samples in laboratory conditions (Anonymus, 1997) or practical pasteurisation in dairies enable germination of these spores resulting in relatively easier isolation

Supported by the Ministry of Agriculture of the Czech Republic (Project No. EP9058).

of these bacteria on special media (Vyletělová *et al.*, 2001). Their occurrence in final products is mostly caused by raw milk contamination and by subsequent transfer of spores in the course of milk processing. UHT temperature should extirpate vegetative cells as well as bacterial spores. Therefore sporadic occurrence of these food pathogens in UHT milk is usually attributed to milk contamination during technological processing.

Comparison of biochemical and physiological traits with the results of various molecular methods was used for *Bacillus* strains isolated from raw milk and from final products to specify the origin or identity of these food pathogens (Herman *et al.*, 1997, 1998; Nilsson *et al.*, 1998; Godič-Torkar and Smole-Možina, 2000; Helgason *et al.*, 2000; Svensson *et al.*, 2000).

Monitoring of the transfer of *B. cereus* and *B. licheniformis* strains from raw milk to final products (UHT milk), testing of ribotyping applicability to species classification and confirmation of *B. cereus* and *B. licheniformis* strains identity were the principal objectives of the present study.

MATERIAL AND METHODS

Sampling and processing of samples

Milk samples (one batch) were collected (250 ml in sterile samplers) in the course of the whole production process (transport – final product) from the following sampling sites: tank RMT_x 100 000 l – raw milk; surge tank before the pasteur – raw milk; cream behind the centrifuge; milk behind the pasteur; 1.5% milk fat; pasteurised milk in the tank before UHT; surge tank before UHT; final products (8 boxes) after UHT (138°C, 4 s); 8 final products (UHT milk) after thermostat testing (37°C, 14 days). Milk samples were frozen, transported to the laboratory and analysed.

Thermostat testing of final products

Samples of UHT milk consumer's package were cultivated in an incubator (37°C). Milk samples (200 ml) were taken after 14 day-incubation; samples were frozen immediately and transported to the laboratory for microbiological analyses.

Isolation of *Bacillus* strains

Modified MYP Agar complemented with Egg Yolk emulsion and Polymyxin B sulphate (HiMedia) and Milk Agar (Merck) were used for the isolation of *Bacillus* strains. Milk samples were devitalised (85°C, 10 min) and consequently cultivated 3 days at 30°C as described by Havlová *et al.* (1993).

Morphological characteristics

Colony morphology on blood agar and production of spores on the nutrient agar complemented with 10 mg $MnSO_4 \cdot H_2O$/1 l were studied for 1–7 days.

Biochemical and physiological characteristics

Tested strains were cultivated on blood agar (24 h, 37°C). Conventional tests described by Gordon *et al.* (1973) were used for production of catalase, haemolysis, urease, production of acetoin, indole, reduction of nitrates, hydrolysis of esculin, starch and tyrosine, growth at 5, 40, 50 and 55°C, growth in 7 and 10% NaCl, and for acidification of carbohydrates. Arginine dihydrolase was tested according to Brooks and Sodeman (1974), ONPG according to Lowe (1962), phosphatase, hydrolysis of gelatine and Tween 80 according to Páčová and Kocur (1978, 1984). Growth on commercial media Simon's Citrate Agar (Oxoid), *Bacillus cereus* Agar Base (HiMedia) and BBL Anaerobic Agar was characterised.

Identification was made according to previously published differentiation tables (Páčová *et al.*, 1996) on the basis to 26 phenotypical traits and morphological characteristics.

Ribotyping

Ribotyping with *Eco*RI restriction enzyme and the probe complementary to *Escherichia coli* 16S and 23S rRNA was made according to Švec *et al.* (2001). Lambda DNA cleaved by *Eco*RI and *Hin*dIII (Promega) was used as a molecular weight marker. Band-pattern analysis and cluster analysis were carried out using GelCompar II software (Applied Maths, Belgium). *B. cereus* CCM 2010^T and *B. licheniformis* CCM 2145^T obtained from the Czech Collection of Microorganisms were used as reference strains.

RESULTS AND DISCUSSION

Proteolytic and lipolytic enzymes affect the quality and sensory properties of foods. These groups of

enzymes are also produced by various *Bacillus* species; therefore the microbiological control of milk product quality includes monitoring of this genus. Bacilli are classified as health-risky microorganisms that are able to produce thermoresistant endospores facilitating the transfer of bacilli to final milk products.

Aerobic growth together with positive catalase reaction enable to distinguish this genus easily from other gram-positive bacteria producing endospores.

As for milk and milk products, *B. licheniformis*, *B. subtilis* and *B. cereus* species are the most frequently isolated bacilli (Griffith and Phillips, 1990; Crielly *et al.*, 1994; Páčová *et al.*, 1996). In total, 158 strains were isolated from 396 milk samples in the period 1999–2000. Hundred and two strains (64.6%) were identified as *B. licheniformis*, 43 (27.2%) as *B. cereus*, the remaining 8.2 % were classified as different species of *Bacillus* and *Paenibacillus* (Vyletělová *et al.*, 2001). Therefore, the two most frequent species – *B. licheniformis* and *B. cereus* – were chosen to monitor the transfer of spore-forming bacteria from raw milk to the final product (UHT milk).

B. licheniformis strains were found in milk samples collected from all sampling sites. In contrast *B. cereus* strains were seldom isolated from raw milk, pasteurised milk and UHT milk (Table 1).

These two species are represented by facultatively anaerobic rods belonging to the 1st morphological group with oval spores not swelling the cell. Vegetative cells of *B. licheniformis* differ from *B. cereus* cells (large rods, in strings); they are smaller and form irregular clusters. Significant differences are also found in colony morphology. *B. licheniformis* strains form almost rough colonies adhering to the agar, with mucose droplets in young cultures. In contrast, typical colonies of *B. cereus* are round, matt and granular with greenish tint on the blood agar. In addition to the above described morphology, the following tests can be used for their species differentiation: production of lecithinase, growth at 50°C and in 10% NaCl, ONPG test, and acidification of mannitol and xylose (Table 2).

In regard to biochemical and physiological traits, all tested strains of *B. licheniformis* formed a relatively homogeneous group; inability to utilise citrate (strains B77 and B78) and negative arginine dihydrolase activity (strain B75) were the only variable results obtained. *B. cereus* strains were characterised by variability in the following characteristics: Simmon's citrate, arginine dihydrolase, growth in 7% NaCl, acidification of cellobiose. Strain B71 was completely atypical: it did not produce acetoin and lecithinase. The negative egg-yolk reaction exhibited by *B. cereus* strain is a remarkable trait because the production of lecithinase forms a basis for selectivity of media recommended for the isolation of this species (Anonymous, 1997).

Ribotyping was made to confirm the species identification and identity of *B. cereus* and *B. licheniformis* strains isolated in the course of UHT milk production.

Ribotyping with restriction enzyme *Eco*RI separated unequivocally representative strains of *B. cereus* and *B. licheniformis* into two groups (Figure 1) in full agreement with species identification based on biochemical and physiological tests. Band patterns of the reference type strains (*B. cereus* CCM 2010^T and *B. licheniformis* CCM 2145^T) showed high coincidences with both species-specific clusters.

Similarities of analysed *B. cereus* strains ranged from 77 to 97% (Figure 1). The highest difference was found for strain B71 isolated from raw milk in tank RMT_x (77% similarity with the type culture of *B. cereus* CCM 2010^T). This result is in full agreement with pheno-

Table 1. Total counts (CFU/ml) of *B. cereus* and *B. licheniformis* in phase samples

Sampling site	*B. cereus*	*B. licheniformis*
Tank RMT_x, 100 000 l – raw milk	10	30
Surge tank before pasteur – raw milk		30
Cream behind centrifuge		20
Milk behind pasteur, 1.5% milk fat		20
Pasteurised milk in tank before UHT		50
Surge tank before UHT	10	80
Final products (boxes) after UHT (138°C, 4 s)	10	10
Final products – UHT milk after thermostat testing (37°C, 14 days)		10

Table 2. Characteristics of the tested strains of *B. cereus* and *B. licheniformis*

Strain No.	LEC	VPT	SCI	ADH	ONP	HEM	TWE	TYR	C50	C55	NAC7	NAC10	MAN	XYL	CEL	Sampling site*	Identification
B 70	+	+	–	+	–	+	sl.	+	–	–	sl.	–	–	–	+	6	*B. cereus*
B 71	–	–	+	+	–	+	+	+	–	–	–	–	–	–	–	1	
B 72	+	+	–	–	–	+	+	+	–	–	–	–	–	–	+	7	
B 73	+	+	+	+	–	+	+	sl.	–	–	sl.	–	–	–	sl.	1	
B 75	–	+	+	–	+	–	–	–	+	+	+	+	+	+	+	1	*B. licheniformis*
B 76	–	+	+	+	+	–	–	–	+	+	+	+	+	+	+	2	
B 77	–	+	–	+	+	–	–	–	+	+	+	sl.	+	+	+	3	
B 78	–	+	–	+	+	–	–	–	+	+	+	+	+	+	+	4	
B 79	–	+	+	+	+	–	–	–	+	+	+	+	+	+	+	5	
B 80	–	+	+	+	+	–	–	–	+	+	+	+	+	+	+	6	
B 81	–	+	+	+	+	–	–	–	+	+	+	+	+	+	+	7	
B 82	–	+	+	+	+	–	–	–	+	+	+	+	+	+	+	8	

* = according to Table 1

+ = positive; – = negative; sl. = slightly positive

LEC = lecithinase; VPT = acetoin production; SCI = Simmons' citrate; ADH = arginine dihydrolase; ONP = β-galaktosidase; HEM = haemolysis; TWE = hydrolysis of Tween 80; TYR = hydrolysis of tyrosine; C50 and C55 = growth at 50 and 55°C; NAC7 and NAC10 = growth in 7 and 10% NaCl; MAN = mannitol; XYL = xylose; CEL = cellobiose

Positive reaction: catalase, phosphatase, reduction of nitrates, hydrolysis of gelatine, starch, casein and esculin, growth at 40°C, growth under anaerobic conditions, acidification of glucose and fructose

Negative reaction: urease, production of indole, growth at 5°C, acidification of lactose and inositol

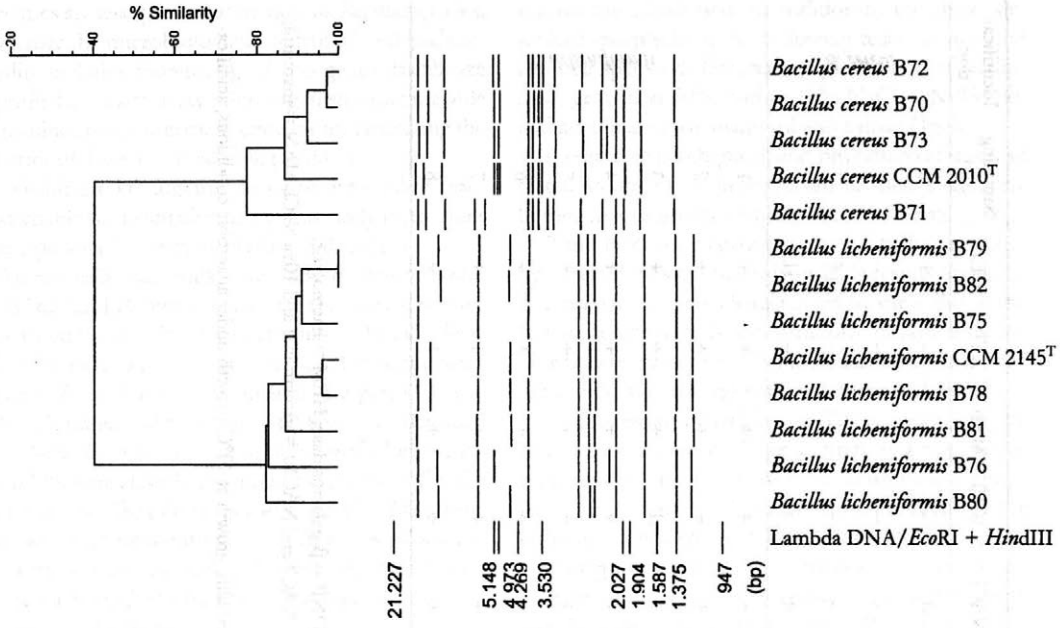

Figure 1. Band-pattern profiles of *B. cereus* and *B. licheniformis* strains. The dendrogram was constructed with Jaccard coeficients using UPGMA clustering method

typic characteristics (atypical reaction in VP test and lecithinase as discussed above). In contrast, another strain isolated from the same sample (B70) showed high similarity with the other strains isolated from pasteurised milk and UHT milk (97 and 92%). The results of ribotyping and biotyping of these two strains (B70 a B71) confirmed the occurrence of two different *B. cereus* strains in the same milk sample.

Similarity of analysed *B. licheniformis* strains ranged from 82 to 100%. Band-pattern profiles of strains B79 (pasteurised milk) and B82 (UHT milk) were identical. However, our results imply that these strains are identical strains passing through the studied technological process. This hypothesis (strain identity) should be confirmed by ribotyping with more restriction enzymes or by other methods based on the genomic DNA analysis.

Although only a small number of strains was analysed in this study, it is evident that these two species give well detectable and easily distinguishable band-pattern profiles (Figure 1). It seems that ribotyping with *Eco*RI is a useful method for the of species identification of *B. cereus* and *B. licheniformis* mainly in case atypical results of biochemical test were obtained.

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Received: 02–01–08
Accepted after corrections: 02–04–16

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Meat content in pigs estimated by various methods and compared with objective lean meat content

Porovnanie odhadovaného podielu svaloviny u ošípaných rôznymi metódami s objektívne zisteným podielom svaloviny

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ABSTRACT: The aim was to determine the expression ability of apparatuses (Piglog-105, SonoMark SM-100 and Aloka 500) and Two points (TP) method, used for estimation of lean meat content (LMC) of pigs. The weight of muscular substance, fat tissue with skin and bones was assessed by total dissection of the carcass side. LMC represents the proportion of muscular substance weight from the right carcass side weight. The results of apparatuses were compared with total dissection value of pig carcasses. Slaughter weight in 128 pigs was on average 104.5 kg. Average back fat thickness was 2.28 cm and eye muscle area was 43.61 cm^2. Lean meat content from dissection was 55.67%. Piglog-105 apparatus estimated lean meat content by 0.36% higher and SonoMark SM-100 by 1.49% higher than total carcass content. The new regression equation for Aloka 500 showed LMC 55.83%. From special regression equations (sex, production type), only the regression for meat type is recommended. Lean meat content estimated by Two points method was 55.48%. Correlation coefficient between estimated LMC (Piglog, SonoMark, Aloka, TP) and objective LMC was 0.776, 0.759, 0.894 and 0.793, respectively.

Keywords: pigs; carcass quality; lean meat content; Aloka

ABSTRAKT: Cieľom práce bolo zistiť vypovedaciu schopnosť prístrojov (Piglog-105, SonoMark SM-100 and Aloka 500) a Dvojbodovej metódy používaných na odhad podielu svaloviny (PS) u ošípaných. Z celkovej rozrábky jatočnej polovice sa zistila hmotnosť svaloviny, tuku s kožou a kostí. Skutočný podiel svaloviny sa vypočítal ako percentuálna časť hmotnosti svaloviny z hmotnosti pravej jatočnej polovice. Odhadované podiely svaloviny boli porovnávané zo skutočným podielom svaloviny. Priemerná hmotnosť zabíjaných zvierat bola 104,5 kg. Priemerná hrúbka chrbtovej slaniny bola 2,28 cm a plocha najdlhšieho chrbtového svalu 43,61 cm^2. Skutočný podiel svaloviny dosiahol hodnotu 55,67 %. Piglog-105 odhadol *in vivo* PS o 0,36 % viac a SonoMark SM-100 o 1,49 % viac ako bol skutočný podiel svaloviny. Prístroj Aloka 500 s novonavrhnutou regresnou rovnicou odhadol PS na úrovni 55,83 %. Zo špeciálnych rovníc (pohlavie, úžitkový typ) sa odporúča len regresia pre ošípané mäsového úžitkového typu. *Post mortem* Dvojbodovou metódou sa odhadol 55,48 % podiel svaloviny. Korelačná závislosť medzi odhadovanými podielmi svaloviny (Piglog, SonoMark, Aloka, Dvojbodová metóda) a skutočným podielom svaloviny bola na úrovni 0,776; 0,759; 0,894 a 0,793.

Kľúčové slová: ošípané; kvalita jatočného tela; podiel svaloviny; Aloka

Miles and Fursey (1974) used the sonographic apparatus for carcass quality evaluation for the first time. The basis of carcass quality evaluating is to find out the total lean meat content (LMC) and content of subcutaneous fat. The content of subcutaneous fat is highly negatively correlated with the lean meat content. This content can be found by some backfat thickness measures. More recently also the muscle thickness was used for more exact estimation of carcass quality. Zhang *et al.* (1993) determined the precise localisation

points of measurement. The highest correlation between lean meat content and point of measurement was found in the place of last ribs 5–8 cm lateral from middle line.

For the estimation of lean meat content nonapparative methods post mortem are also used. The LSQ method for evaluation of LMC was introduced in Austria (Pfeiffer and Falkenberg, 1972). Since 1987 the Two points method has been used in Germany. Bach and Sack (1987) described its principle, the basis of which is the regression equation with fat and muscle thickness on the carcass cut.

In the European Union the pig carcasses are classified in S-EUROP classes by the prescription No. 3127/94. The limit for measuring systems is the Residual Standard Deviation RSD = 2.5 and R^2 = 0.64.

To assess the accuracy or expression ability of aparatus used to determine the lean meat content in live pigs and manual method in pig carcasses and to compare them with the results of total analysis of pig carcass sides. It was the aim to propose a new equation for apparatus, which has not a build in regression to estimate the proportion of lean meat content.

MATERIAL AND METHODS

Experimental animals

One hundred and twenty eight pigs were in the experiment. They were raised in identical conditions and fed the complete feed mixture for pigs. They were killed in the experimental slaughterhouse at RIAP Nitra after achieving the live weight of approx. 105 kg after 12 hours starvation. The lean meat content (LMC) was assessed by the apparatus Piglog 105, SonoMark SM-100 and Aloka 500 one day before the slaughter and by Two points method *post mortem.*

Methods of measurement and estimations *in vivo* and *post mortem*

Following measures were determined in live animals:

- thickness of backfat with skin 1 – Piglog (PS1) and SonoMark (SS1) between 3rd and 4th lumbar vertebra 7 cm (8 cm) aside from the middle line (cm)
- thickness of backfat with skin 2 – Piglog (PS2) and SonoMark (SS2) between 3rd and 4th last but one *dorsal vertebra* 7 cm (6 cm) aside from middle line (cm)
- *musculus longissimus dorsi* thickness Piglog (PM1) and SonoMark (SM1) in the same place as backfat 2 (cm)
- thickness of backfat with skin (Aloka) after the last dorsal vertebra 7 cm aside the middle line (mm) (x_1)
- *musculus longissimus dorsi* thickness (Aloka) after the last dorsal vertebra 7 cm aside the middle line (mm) (x_2)
- eye-muscle area (Aloka) after the last dorsal vertebra 7 cm aside the middle line (mm^2) (x_3)
- backfat thickness (TP1) – the thinnest fat thickness with skin above *musculus gluteus medius* (mm)
- muscle thickness (TP2) between cranial top of *musculus gluteus medius* and dorsal edge of spinal chord canal (mm)

Repetitions of individual measures were done until the indication appeared for three times. The indication was then confirmed and incorporated into the regression of the apparatus. The apparatus Piglog and SonoMark estimated the lean meat content in carcass alive from the determined measures by means of the built in regression equations. As there is no incorporated regression equation in the echocamera Aloka the lean meat content was estimated by the new proposed linear regression equation. For estimation, the LMC by Two points method we used the original german equation.

Linear regression analysis

The method of linear regression analysis in general form was used for calculation:

$$y_i = a + b_1x_1 + ...+ b_kx_k + e_i \; i = 1...n$$

The proposal of optimum model was done by means of the so-called step-wise variable selection-backward procedure. Multicollinearity between independent variables was removed by excluding some variables with high correlation coefficient. The coefficient of determination with correction to the number of parameters (R^2 adjusted) and residual standard deviation (RSD) level of significance of t-test values of individual parameters were used to estimate the suitability of the method in regression model. Some of them are marked as high leverage points (HLP), which differ in the values x_i or in their combinations, in the model from all observations. The difference of standard errors of estimates and P-level of coefficients variable from the original equation is detected after they are

sorted out and after repeated calculation of the regression. The unchanged parameters show that HLP do not affect the accuracy of the estimated model of regression.

The right carcass side was dissected according to the standard STN 46 61 64 after 24 hours cooling, and the individual basic parts were weighed. The portion of valuable lean meat cuts (VMC) was calculated out of the proportion of weight of shoulder, neck, loin and leg to the weight of the carcass side. Objective lean meat content (LMC) was determined based on total dissection of leg, shoulder, loin, neck, belly and fore and hind knees to lean meat, bones and fat with skin. The real lean meat content was calculated out of lean meat weight converted to the right carcass side.

RESULTS AND DISCUSSION

The experimental 128 pigs of repro-meat and meat type achieved the parameters given in Table 1.

Estimate of lean meat content

56.03% LMC *in vivo* was estimated by the apparatus PIGLOG-105 one day before slaughter. It is 0.36% more when compared with the objective LMC. The apparatus SONOMARK SM-100 estimated 57.16% LMC. The estimated value is 1.49% higher than the LMC determined by dissection. ALOKA has no incorporated regression equation to calculate LMC so two regression equations were calculated. As one model of equation has in the value of invariable a higher standard error than the second one, and the level of significance of *t*-test in one parameter was above the level $P = 0.05$ at almost the same values R^2 (adj.) (0.795 or 0.8019) and RSD (1.7313 or 1.7055), the universal equation was determined as follows:

$$y = 51.692591 - (0.533407\, x_1) + (0.003238\, x_3)$$

Three (23, 36 and 102) out of 128 observations were labelled as high leverage points at the calculation of the equation. After they were sorted out and after a repeated calculation the new regression does not differ markedly in values of standard errors in coefficients of variables from the values at *y* and values of *P*-level remained unchanged as well. Therefore, the values of high leverage points do not influence the accuracy of the proposed model markedly so it can be used to estimate LMC by the echocamera Aloka. The measures detected by the Aloka 500 were introduced and 55.83% LMC were estimated in the evaluated set of pigs, this value is larger only by 0.16% than the real one. Turlington (1990) determined for the sonographic apparatuses $R^2 = 0.40$–0.93, Sather *et al.* (1991) for Aloka 210 $R^2 = 0.6$, Strzelecki *et al.* (1998) for Aloka 256 $R^2 = 0.77$ and Gresham *et al.* (1994) $R^2 = 0.87$.

Correlation coefficients

The level of correlation coefficients between the estimated and determined LMC portion or percentage VMC is also one of the possibilities to determine the expression ability of the used apparatus. The correlation coefficient between the portion of valuable meat cuts and LMC portion achieved the value $r = 0.96$. The linear correlation coefficient between the LMC estimated by Piglog and LMC determined by dissection is $r = 0.776$. Correlation between backfat thickness measured by Piglog and objective LMC was $r = -0.753$ to -0.807, that is higher than the one detected by Adamczyk and Duniec (1994) $r = -0.40$ to -0.58. The correlation

Table 1. Basic parameters of the whole evaluated set in pigs

Trait ($n = 128$)	Average	SD	Min.	Max.
Average live weight (kg)	104.5	3.41	80	124
Average weight of carcass side (kg)	41.58	3.16	34.58	49.22
Valuable lean meat cuts (%)	51.9	3.49	44.1	59.89
Lean meat content (%)	55.67	3.76	46.47	63.04
Average back fat thickness (cm)	2.28	0.46	0.9	3.67
Eye-muscle area (cm^2)	43.61	6.11	30	60

coefficient of the LMC estimated by SonoMark apparatus was $r = 0.759$ to the determined LMC. Kiray (1995) ascertained for SonoMark $r = 0.74$. The correlation of the total LMC estimated by the Aloka apparatus with the proposed regression was $r = 0.894$ to the real LMC. This is higher than $r = 0.60$, found by Chien (1994). Correlation coefficient between objective LMC and estimated by the Two points method was 0.793. Lagin *et al.* (1995) determined correlation between objective LMC and estimated meatiness by TP $r = 0.791$.

Estimates of LMC portion in female and male pigs

The group of 64 gilts achieved the total portion of LMC 57.72% at the live weight before slaughter 105.93 kg. The meatiness 57.06% was estimated by the universal equation y for Aloka 500. The regression equation only for gilts was constructed in similar way based on different ability of subcutaneous fat deposition. Two regression equations were calculated from the determined data. The following equation achieved better parameters:

$$y_P = 55.452682 - 0.545093^* x_1 + 0.00248^* x_3$$

with R^2 (adj.) = 0.7592 and RSD = 1.4275. The proportion of LMC 57.72% was estimated by means of y_P equation in gilts. The estimate of LMC portion in gilts by a special equation for gilts only, is identical with real LMC portion, and it is lower by 0.66% compared with the original equation. Simple linear correlations (Table 2) were calculated between LMC portions, which were highly dependent (for $y_P - r = 0.877$ and for $y - r = 0.875$).

The weight of males (boars and castrated boars) was 105.63 kg before slaughter. LMC portion in 64 animals was 54.49%. The meatiness 55.11% was estimated by the universal equation for Aloka 500. Similarly to gilts, there were proposed two regression equations from which:

$$y_s = 52.085848 - 0.0487694^* x_1 + 0.,002765^* x_3$$

with R^2 (adj.) = 0.7174 and RSD = 1.8676 was more precise. LMC portion was estimated 54.5% after the measures were incorporated into the given equation y_s. It is by 0.61% less when compared with the estimation by universal equation for Aloka. Linear correlation (Table 2) between LMC portions was highly dependent for both equations ($y_s - r = 0.854$ or $y - r = 0.854$).

Based on previous results it can be stated that special regression equations for individual sexes are not necessary, the universal regression equation y is sufficient. However, it is necessary to note that the calculation was performed with insufficient number of pigs (less that 120 animals).

Estimates of LMC portion in repro-meat and meat types

The group of 48 pigs of repro-meat type (LMC below 54%), weight before slaughter 106.23 kg, achieved the total LMC 50.59%.

Two regression equations were proposed, from which:

$$y_k = 49.479289 - 0.30155^* x_1 + 0.002143^* x_3$$

with R^2(adj.) = 0.4445 and RSD = 1.577825 was the more precise one. The portion of total LMC 50.35% was estimated on its basis. Simple linear correlations of the estimated LMC portions of both equations to the determined portions are high and almost identical. The universal y equation is sufficient to estimate the LMC portion by Aloka 500 in the repro-meat type of pigs.

The group with 80 pigs of meat type (LMC over 54%) achieved total LMC portion 57.56% at the weight before slaughter of 104.28 kg. The equation with three

Table 2. Linear correlation dependence and level of significance of Student's *t*-test of portions estimated by Aloka 500 in both sexes of pigs on the basis of different regression equations to the determined LMC portion

Lean meat content estimated by Aloka 500	Dissection r	P
Female – regression y_f	0.877	0.997
– regression y	0.875	0.875
Male – regression y_m	0.8538	0.998
– regression y	0.8536	0.378

Table 3. Linear correlation dependence and level of significance of Student's *t*-test of portions estimated by Aloka 500 in different commercial types of pigs based on different regression equations to the portion of LMC determined from dissection

Lean meat content estimated by Aloka 500	Dissection r	P
Repro-meat type		
– regression y_{r-m}	0.699	0.286
– regression y	0.697	0.089
Meat type		
– regression y_t	0.684	0.427
– regression y	0.591	0.087

independent variables is more precise out of the proposed two equations. Its form is as follows:

$$y_m = 52.187021 - 0.464943^* x_1 + 0.109554^* x_2 + 0.001599^* x_3$$

The portion of total LMC 57.51% was estimated on its basis. The equation achieved RSD = 1.432, and the corrected coefficient of determination R^2(adj.) = 0.5899. The phenotypic correlation dependence (Table 3) of the estimated portions of LMC by means of the equation y_m is narrower than with y ($r = 0.684$, or $r = 0.591$). Based on the results it is possible to recommend ($n = 80$) the apparatus Aloka 500 with the regression equation y_m for the meat type to estimate the LMC *in vivo*. Wood and Robinson (1989) recommended incorporating the eye-muscle area to the regression equation for meat type pigs.

CONCLUSION

Apparatus Piglog 105 and SonoMark SM-100 estimate LMC with small errors that have not substantially influenced their utilisation in pig practice. Two points method is applicable for estimation of pig lean meat content in small abattoirs. From the above mentioned sonographic apparatus ALOKA 500 is the most precise, therefore it is recommended especially for scientific use.

From special regression equations (sex, production type), only the regression for meat type is recommended.

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Received: 01–10–11
Accepted after corrections: 02–04–26

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Volume 47, No. 5 May 2002

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Vědecký časopis CZECH JOURNAL OF ANIMAL SCIENCE (ŽIVOČIŠNÁ VÝROBA) ● Vydává Česká akademie zemědělských věd – Ústav zemědělských a potravinářských informací ● Redakce: Slezská 7, 120 56 Praha 2, tel.: +420 2 27 01 03 52, fax: +420 2 27 01 01 16, e-mail: edit@uzpi.cz ● Předáno do tisku 18. 6. 2002 ● © Ústav zemědělských a potravinářských informací, Praha 2002

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