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Influence of high environmental temperatures and evaporative cooling on some physiological, hematological and biochemical parameters in high-yielding dairy cows

Vliv vysokých teplot prostředí a evaporačního ochlazování na vybrané fyziologické, hematologické a biochemické ukazatele u vysokoprodukčních dojnic

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ABSTRACT: The objective of this study was to ascertain if regular cooling by sprinkling could influence the heat stress in high-yielding dairy cows and which of monitored parameters are suitable heat stress indicators. The scheme of experiment included a week acclimatization, progressive rise of air temperatures, hot period (HP), progressive fall of air temperatures, cool period (CP) and the same cycle with water evaporative cooling by sprinkler (HPC – hot period with cooling). High ambient temperatures caused homeostasis disturbance, a significant increase ($P < 0.05$) in rectal temperature (from 37.3 to 39.3°C), respiration (from 28 to 81 breath/min) and pulse rate (from 64 to 81 pulse/min). The hematocrite value and erythrocyte number rose significantly at the beginning of HP, from 32.48 to 41.6 l/l and from 6.84 to 8.18 T/l, respectively ($P < 0.05$). In addition, total protein values increased from 68.95 to 76.75 g/l in HP ($P < 0.05$). Then their gradual decrease occurred, caused by their enhanced utilization for gluconeogenesis, as confirmed by significant urea increase. In HP urea levels rose from 4.08 to 4.84 mmol/l ($P < 0.05$). The hemoconcentration caused a significant increase in glucose (from 2.898 to 3.35 mmol/l, $P < 0.05$), and then a quick decrease to 2.91 mmol/l occurred ($P < 0.05$). Glucose concentration changes had the opposite course than urea concentration changes. Cooling by sprinklers (HPC) improved some physiological and hematological parameters, but not the biochemical parameters. The respiratory and pulse rate rose significantly as in HP ($P < 0.05$), to 30 breath/min and 68 pulse/min, but rectal temperature increased only slightly to 38.1°C. A decrease in glucose concentration occurred again at the beginning of HPC (2.82 mmol/l) and there was no significant difference between levels at the beginning of HP and HPC. The significant urea growth occurred ($P < 0.05$), when comparing urea levels in CP (4.08 to 4.84 mmol/l) and in HPC (6.56 to 6.45 mmol/l). We recommend rectal temperature measurement for a general condition check. A comparison of total protein and urea levels can be used to judge the metabolic situation.

Keywords: dairy cows; heat stress; water evaporative cooling; rectal temperature; respiratory and pulse rate; blood samples

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ABSTRAKT: Cílem této práce bylo zjistit, zda dokáže pravidelné ochlazování sprchováním ovlivnit tepelný stres u vysokoprodukčních dojnic a které ze sledovaných parametrů jsou vhodnými ukazateli tepelného stresu. Schéma experimentu zahrnovalo týden aklimatizace, progresivní růst teploty vzduchu, horké období (HP), progresivní pokles teploty vzduchu, chladné období a stejný cyklus s ochlazováním se sprchováním vodou (HPC). Vysoké teploty prostředí způsobily narušení homeostázy, průkazný nárůst ($P < 0,05$) rektální teploty (z 37,3 na 39,3 °C), počtu dechů (z 28 na 81 dechů/min) i tepové frekvence (z 64 na 81 tepů/min). Na začátku HP se signifikantně zvýšily z 32,48 na 41,6 l/l hematokritové hodnoty, počet erytrocytů z 6,84 na 8,18 T/l ($P < 0,05$). Také hodnoty celkové bílkoviny vzrostly z 68,95 na 76,75 g/l v HP ($P < 0,05$). Pak nastal postupný pokles hodnot způsobený jejich zvýšenou utižitelností pro glukoneogenezi, jak dokazuje nárůst močoviny. Při HP hladina močoviny vzrostla z 4,08 na 4,84 mmol/l ($P < 0,05$). Hemokoncentrace způsobila průkazné zvýšení glukózy (z 2,89 na 3,35 mmol/l, $P < 0,05$), pak nastal rychlý pokles na 2,91 mmol/l ($P < 0,05$). Změny koncentrace glukózy měly opačný průběh než změny u močoviny. Ochlazování sprchováním (HPC) zmírnilo některé změny fyziologických a hematologických, méně biochemických ukazatelů. Dechová a tepová frekvence se průkazně zvýšila jako při HP ($P < 0,05$) na 30 dechů za min a 68 pulsů za min, ale rektální teplota stoupla jen mírně na 38,1 °C. Na začátku HPC se znovu projevil pokles koncentrace glukózy (2,82 mmol/l), rozdíl mezi její hladinou při HP a HPC nebyl průkazný. Při srovnání hladin močoviny při CP (4,08 až 4,84 mmol/l) a HPC (6,56 až 6,45 mmol/l) byl prokázán signifikantní rozdíl ($P < 0,05$). Pro posouzení celkového stavu organismu doporučujeme měření rektální teploty. Srovnání hladin celkové bílkoviny a močoviny je možné použít pro zvážení metabolického stavu.

Klíčová slova: dojnice; tepelný stres; ochlazování vodou; rektální teplota; dechová a tepová frekvence; vzorky krve

The most sensitive cattle category to high ambient temperatures are lactating dairy cows, because they produce much more heat than non-lactating ones (Blackshaw and Blackshaw, 1994), especially dairy cows in an early stage of lactation (Johnson *et al.*, 1987).

If the organism does not succeed in maintaining body temperature in physiological range, hyperthermia occurs. Metabolic intermediate products cumulate in blood and hemoconcentration rises with a plasma volume decrease. The hyperthermic stress influence depressively the milk yield, decreases the food intake, worsens the health condition and affects the behaviour of cows. Heart activity and protein content in milk decrease, respiratory rate, body temperature, drinking frequency and partially water intake increase in dairy cows (Shearer and Beede, 1990).

The acclimatization has a great importance for resistance to high temperatures. Most of physiological variables stabilize after about 10–15 days of exposure to heat stress (Ronchi *et al.*, 1999). Changes in environmental temperatures are often very sudden and an adaptation reaction proceeds only seldom without a negative impact on performance. That is why various methods of heat stress alleviation are used in practice.

Cooling can alleviate the heat stress and can improve thermal balance and both productive and reproductive performances in dairy cows (Lee *et al.*, 1999). Omar *et al.* (1996) found out, that

red blood cell number and hematocrite value were reduced by cooling, while white blood cell number was increased. However, Abdel-Same and Ibrahim (1992) described a significant rise in hemoglobin concentration and hematocrite value.

Amadori *et al.* (1997) suggested that the measurement of clinical and hematological parameters could be used to evaluate welfare in cattle. The respiratory rate can be used as an indicator of heat stress (Gaughan *et al.*, 2000). However, a change in respiratory character can happen during the stress. For example, Bianca (1971) described fast and shallow respiration in the first phase and slightly slower but deeper respiration in the second phase of the heat stress.

Our aim was to find what parameters are suitable for heat stress appreciation and whether and how much the regular cooling manages to alleviate and moderate the heat stress in high-yielding dairy cows. Selected physiological, hematological and biochemical parameters were used for the assessment, to make the range of monitored functions in animals as wide as possible, and, at the same time, in respect to published and recommended data in references.

MATERIAL AND METHODS

Four healthy Holstein dairy cows, 2–3 months post calving; with daily average milk-yield, 27.81 kg (2nd to 3rd lactation) were used in the

experiment. These dairy cows were housed in experimental conditions of climatic chamber (controlled temperature, relative air humidity and air flow), in free-stalls, fed by total mix ration (TMR – hay, corn silage, grain feed, straw and mineral supplements *ad libitum*) according to milk yield.

The scheme of experiment included a week of acclimatization, progressive rise of temperatures, hot period (HP), progressive fall of temperatures, cool period (CP) and the same cycle with water evaporative cooling by sprinkler (HPC – hot period with cooling) (Figure 1). The cooling was provided every 20 minutes for 50 seconds (4 nozzles) at the temperatures above 21°C in all chamber. The Figure 1 shows, when the measurements were made and blood samples collected from *v. jugularis*.

The assessed parameters were: rectal temperature (by digital thermometer), respiratory and pulse rate (by auscultation). Hematological parameters analysis – erythrocyte and leucocyte number, hematocrite value, and hemoglobin concentration was carried out using Coulter Counter model ZF (Coulter Electronics Ltd, England). Differential cell method was counted in light microscope. Blood serum was used for biochemical detection – glucose, urea and total protein concentrations, ALT (alanine aminotransferase) and AST (aspartat aminotrans-

ferase) enzymatic activities were determined. The obtained values were statistically processed by *F*-test and *T*-test.

RESULTS

Marked changes in physiological parameters occurred during ambient temperatures of 32°C in hot period (HP). The respiratory rate increased 2.6×, from 28 to 81 breath/min, pulse rate was significantly increased as well – from 64 to 81 pulse/min ($P < 0.05$). The body temperature also enhanced, especially at the end of the first hyperthermic period without cooling (HP) (from 37.3 to 39.3°C at average). In the hot period with cooling (HPC), the respiratory and pulse rate were approximately on the same high level as in heat without cooling (HP), 30 breath/min and 68 pulse/min, but rectal temperature increased only slightly to 38.1°C (Figure 2).

The hematocrite value and erythrocyte number rose significantly at the beginning of HP, from 32.48 to 41.6 l/l and from 6.84 to 8.18 T/l, respectively ($P < 0.05$). The rise in the both parameters was not significant in HPC. There were significant differences between hematocrite values (41.6 and

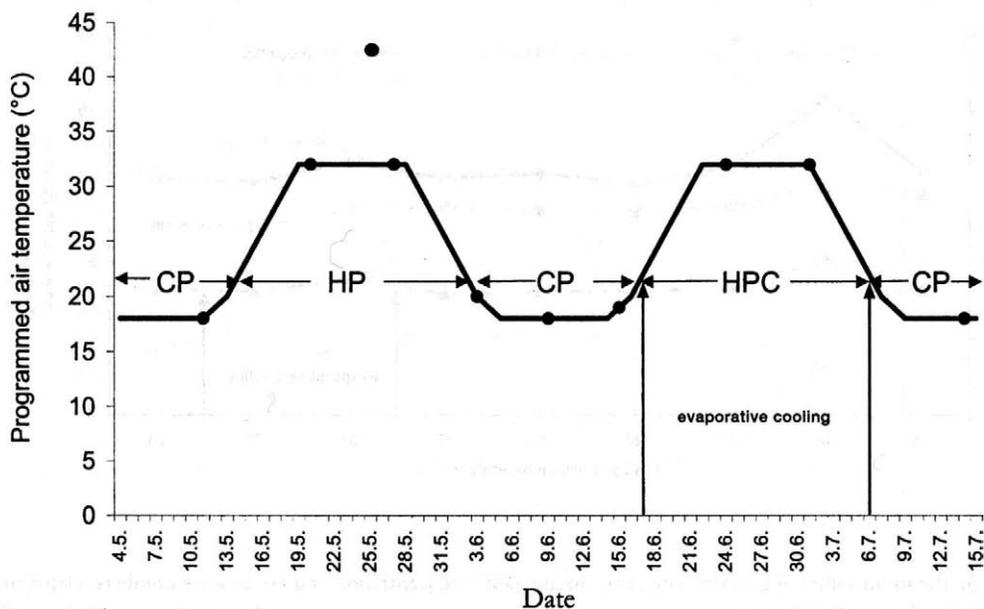


Figure 1. The experiment scheme

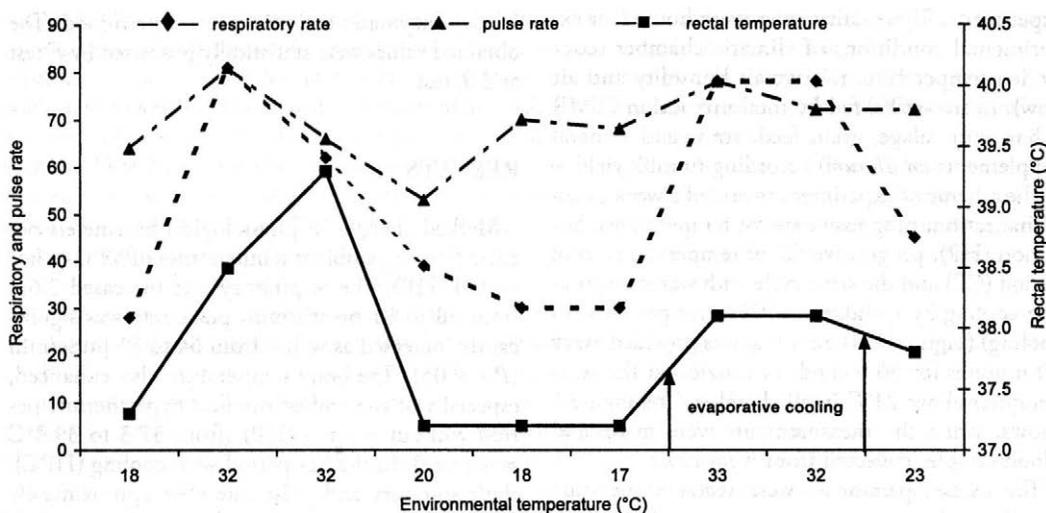


Figure 2. The mean values of rectal temperature, respiratory and pulse rates related to environmental temperatures and cooling

33.23 l/l) and erythrocyte number (8.18 and 7.09 T/l) compared in HP and HPC ($P < 0.05$). The hemoglobin concentration did not show significant differences (Figure 3).

At first, the leucocyte number decreased significantly in HP until a period of lower ambient temperatures (from 8.32 to 5.02 G/l, $P < 0.05$). Then, in the cool period of experiment (CP), a significant

increase to 8.22 G/l was noticed ($P < 0.05$), in the following HPC their number was maintained on the similar level with non-significant differences (Figure 4).

A decrease in white blood cell number after HP was probably due to significant fall of lymphocyte number – their relative distribution significantly decreased from 64 to 46% ($P < 0.05$) in the middle

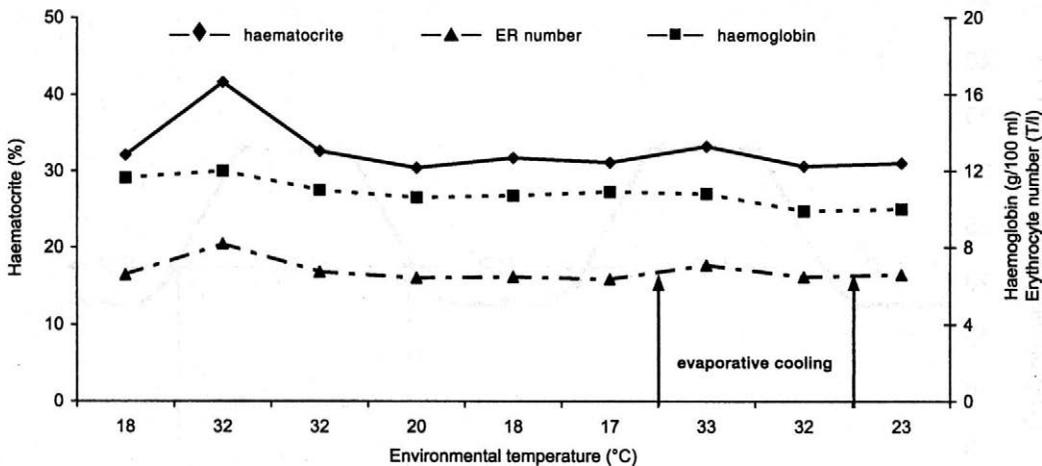


Figure 3. The mean values of haematocrite value, haemoglobin concentration and erythrocyte numbers related to environmental temperatures and cooling

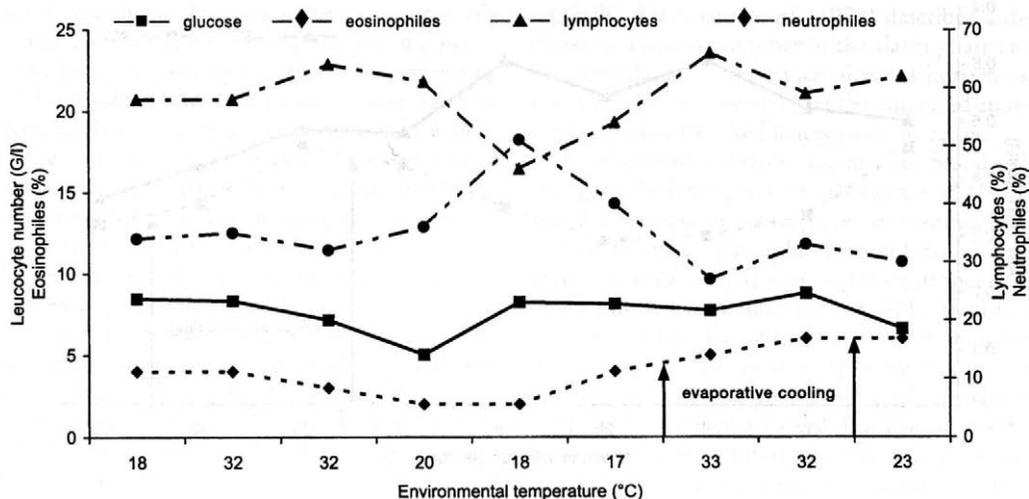


Figure 4. The mean values of leucocyte, lymphocyte, neutrophile and eosinophile numbers related to environmental temperatures and cooling

of cool period after HP. At the same time, a relative increase in neutrophile number occurred (from 32 to 51%, $P < 0.05$), their number decreased again to 27% in HPC. The growth of eosinophile number to 6% in last two measurements was caused by their marked increase in one dairy cow. The basophile number has always been below one percentage;

the monocyte number was between 2–4%, only in dairy cow No.1 it increased to 5, or 6% when quality of milk decreased (the milk quantity and quality were tested as well – nonpublished data).

Total protein concentration in blood serum significantly increased from 68.95 to 76.5 g/l in HP ($P < 0.05$), in the cool period it decrease to

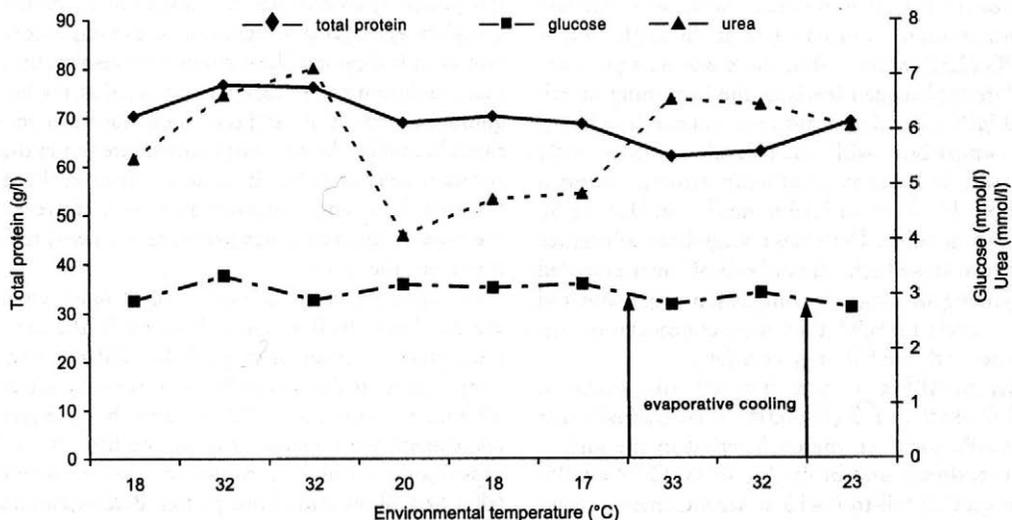


Figure 5. The mean values of total protein, urea and glucose levels related to environmental temperatures and cooling

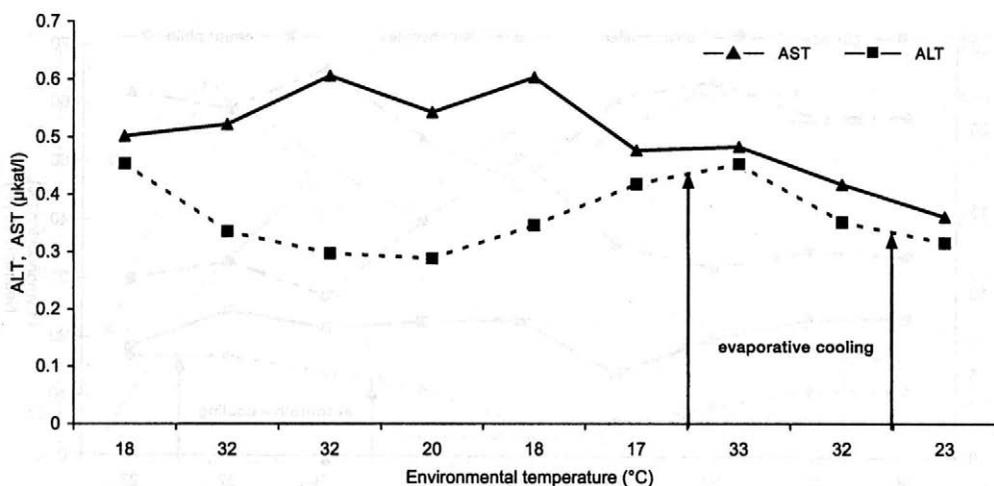


Figure 6. The mean values of AST and ALT levels related to environmental temperatures and cooling

69.03 g/l ($P < 0.05$), and a further significant reduction to 62.97 g/l was manifested in HPC ($P < 0.05$) far below the reference range. Total protein values increased again to 69.05 g/l when the ambient temperature was reduced at the end of experiment (Figure 5).

At the beginning of HP a significant increase from 2.98 to 3.35 mmol/l in glucose concentration occurred, then (still in HP) a quick decrease to 2.91 mmol/l occurred (both $P < 0.05$). Glycaemia stabilised on the value above 3 mmol/l in the cool period of the experiment. A decrease in glucose concentration occurred again at the beginning of HPC (2.82 mmol/l), but there was no significant difference between levels at the beginning of HP and HPC. The significant urea increase ($P < 0.05$), in comparison with their levels (4.08 to 4.84 mmol/l) in lower ambient temperatures, expressed in both HP (6.64 to 7.13 mmol/l) and HPC (6.56 to 6.45 mmol/l). There was no significant difference between these high values, both of them exceeded physiological range. A comparison of glucose and urea levels revealed that urea concentration increases with the fall of glucose level.

Serum ALT activity at first fell from 0.454 to 0.297 μ kat/l in HP ($P < 0.05$), then gradually rose to 0.482 μ kat/l in the cool period in the middle of experiment and beginning of HPC ($P < 0.05$) and quickly fell to 0.416 at second measurement in HPC ($P < 0.05$) and to 0.36 μ kat/l at the end of experiment. Serum AST activity increased from

0.502 to 0.605 μ kat/l at high temperatures, after HP it had a decreasing trend at the end of experiment (from 0.602 to 0.36 μ kat/l, $P < 0.05$) (Figure 6).

DISCUSSION

It is known that the heat stress increases rectal temperature, as well as respiratory and pulse rate in cattle (Marai *et al.*, 1997; Itoh *et al.*, 1998). Eigenberg *et al.* (2000) reported that respiratory rate positively correlates with ambient temperature. Gaughan *et al.* (2000) suggested to use respiratory rate as an indicator of heat stress. Our results show that maximum respiratory rate occurred at the beginning of exposition, and then there was a progressive fall, though the ambient temperature was at the constant level of 32°C. It could be deduced from this that the intensity of stress decreased. However, the measurement of other parameters showed that it was not the case.

For example, rectal temperature of dairy cows increased even by 0.8°C in 7 days of HP. The thermoregulatory mechanism probably failed, rectal temperature in dairy cow No.1 even increased to 40.5°C. Ronchi *et al.* (1999) found that the rectal temperature increased during the first days of exposition to a high level and then progressively fell down till the end of hot period. Blackshaw and Blackshaw (1994) also recorded a step rise of the rectal temperature and respiratory rate on the first

day of experiment. These parameters then gradually declined and heart activity slightly slowed down.

The pulse rate was significantly increased during HP, but its significant decrease after 7 days was even more marked at the beginning of the cold period. In one of dairy cows, the pulse rate fell down to 42 beats in a minute, which was half the number of the value measured at the beginning of HP. Neuheimer-Thoneick *et al.* (1988) observed a long-term effect of temperature of 30°C on dairy cows and found out a pulse rate decrease by about 11.3%.

A recurrent enhancement of pulse rate to original levels occurred again at the end of cool period. Then only a mild increase followed in HPC, which evidenced a positive effect of cooling. Lee *et al.* (1999) also demonstrated that cooling could reduce the heat stress and improve heat balance and dairy cow performance. Cooling decreases rectal temperature, respiratory and pulse rate (Omar *et al.*, 1996; Trout *et al.*, 1998).

Hematocrite value, hemoglobin concentration and erythrocyte number rise, especially at the beginning of HP, evidenced a rise of blood concentration. This hemoconcentration was initially induced by an increase in erythrocyte number, later by plasma dehydration (Bianca, 1971). Toharmat and Kume (1997) did not find any significant difference between hematocrite value and hemoglobin concentration in hot and cool weather. In further work, however, Toharmat *et al.* (1998) described an increase in hematocrite values and hemoglobin concentration in summer. Lee *et al.* (1976) described a significant decrease in hematocrite value in dairy cows exposed to high temperatures. This hematocrite and red blood cells decrease were perhaps caused by a rise in erythrocyte destruction, hemodilution effect could also participate here, because more water was transported in circulatory system for evaporative cooling.

Cooling by sprinkling prevented marked changes in red blood picture, only non-significant increase in hematocrite value and erythrocyte number occurred at the beginning of HPC. Some articles, however, reported that cooling of heat stressed dairy cows increased blood hemoglobin concentration (Abounaga *et al.*, 1989; Abdel-Samee and Ibrahim, 1992).

The leucocyte number significantly decreased during HP until the period of lower ambient temperatures, then a significant rise in leucocyte number followed in this cool period of experiment and was maintained on the same level in the follow-

ing HPC. Hartmann *et al.* (1974) described a decrease in leucocyte number in the alarm phase and leucocytosis in the resistance phase of heat stress. Lee *et al.* (1976) observed that the enhanced ambient temperature evoked leucocytosis in cattle.

The proportional distribution of white blood cells changes at high temperatures. Brouček *et al.* (1984) found a decrease in neutrophiles and eosinophiles and an increase in lymphocytes and monocytes. Hartman *et al.* (1974) also found neutropenia in calves exposed to temperature of 36°C, then leucocytosis caused by neutrophiles and monocytes in resistance phase. In our experiment, a rise in relative neutrophile number and a fall in relative lymphocyte number occurred, but in cool period, in HPC, the neutrophile number fell again. Lee *et al.* (1976) observed a non-significant growth of eosinophile number in dairy cows exposed to high temperature. The high temperatures in HP led to a decrease in eosinophile number at first, their number increased in the cool period. There were great individual differences among eosinophile numbers; the results were not significant. Because the quality of milk was also tested (composition and cell count – nonpublished data), some cows were treated with antibiotics. These facts could also play a role in the differences, although only healthy dairy cows were used in the experiment. It was the heat stress, which caused a severe affection of their health condition.

At the beginning of HP, the hemoconcentration occurred, which is also evident from an increase in total protein level in blood serum. Patel *et al.* (1990) confirmed that the serum protein increase could be an indicator of hemoconcentration. Probably with stress progress the protein utilisation increased. It can be connected with metabolic changes during the heat stress – limited feed-intake and protein catabolism. For example, high ambient temperatures decreased total proteins and non-protein nitrogen in dairy cows soon after parturition in the experiment described by Brouček *et al.* (1985).

However, the total protein levels were even more decreased in HPC, their increase only occurred in repeated temperature fall at the end of the experiment. Abounaga *et al.* (1989) and Abdel-Samee and Ibrahim (1992) demonstrated on the contrary that sprinkling increased total protein levels. Hematocrite levels and red blood cell number, however, did not confirm hemodilution.

Glucose level changes in serum confirmed the metabolic change as well. The significant rise in

glucose levels at the beginning of HP was related to the hemoconcentration, but every heat stress led to a slight but significant fall of glycaemia. There are many articles confirming the glucose decrease during the heat stress (Abeni *et al.*, 1993; Marai *et al.*, 1995; Ronchi *et al.*, 1995; Itoh *et al.*, 1998).

Aboulnaga *et al.* (1989) described an enhancement in glucose levels by cooling in heat stressed dairy cows. In our experiment, however, this effect was not proved, on the contrary, glycaemia fall happened, which was significant to glucose levels in cool period but not to levels in HP. Comparing glucose and urea concentrations in serum, it is evident that glucose concentration decreases, the urea levels increase. This may be due to enhanced protein utilization with gluconeogenesis. An acute heat stress, which is present at least 3 days, results in protein catabolism, decrease in total proteins and increase in non-protein nitrogen and creatinine in blood serum (Vercoe, 1974). Ronchi *et al.* (1997) recorded that the heat stress reduced glucose in blood ($P < 0.001$), increased urea ($P < 0.001$) and creatinine as a result of muscular catabolism for energy supply.

In comparison with cool period a significant growth of urea concentrations was manifested in HPC as well. Also Aboulnaga *et al.* (1989) found an urea level increase in cooled dairy cows. In our experiment, a significant difference between urea levels in HP and HPC was not proved.

During artificial heat stress or during summer an increase of transaminase enzyme activities occurred in serum and water sprinkling improved these parameters (Marai *et al.*, 1995, 1997). High ambient temperatures enhanced significantly AST and ALT activities (Boots *et al.*, 1970) even in exotic crosses (Singh and Bhattacharyya, 1984). On the contrary, according to Ronchi *et al.* (1999) hepatic enzyme activities decreased during heat exposition.

ALT and AST activities exceeded the physiological range in our experimental dairy cows. ALT activity gradually rose in cool period in the middle of the experiment and fell again in HPC. AST activity enhanced in HP and remained above physiological range even after temperature decrease, only HPC brought about its fall. The increase in enzymatic transaminase activities was caused by rise in gluconeogenesis load. Marai *et al.* (1995, 1997) also recorded the increase in transaminase enzyme activities in high ambient summer temperatures as their deleterious effects on thyroid, liver and kidney functions.

CONCLUSION

The high ambient temperatures affected homeostasis in dairy cows. The heat stress increased respiratory and pulse rates and rectal temperature. The maximum respiratory and pulse rates were at the beginning of HP, then fell progressively, though the ambient temperature was at constant level of 32°C. On the contrary, the rectal temperature of dairy cows increased by 0.8°C. Therefore we do not consider respiratory and pulse rates to be suitable indicators of heat stress intensity.

Hemoconcentration also occurred, which was proved by hematocrite enhancement and increase in red blood cell number and total proteins in blood serum. The metabolism changes were followed by urea level increase. We deduced from the obtained results that urea level monitoring could be the suitable parameter for heat stress intensity check.

In the second period of this experiment, with cooling of dairy cows by sprinklers, only a mild growth of physiological and haematological parameters occurred, leucocyte number did not change at the same time. Further fall of protein levels occurred while urea concentration increased. ALT and AST activity changes corresponded with the increase in protein utilization – higher glucose demand required an enhanced gluconeogenesis for glycaemia preservation. It means that evaporative cooling reduces heat stress, but it has only a small influence on metabolic symptoms at high temperatures.

The parameter changes were the most intensive in dairy cows with the highest milk yield and evaporative cooling had the best effect on them.

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An influence of different growth ability of Czech Large White gilts and Landrace gilts on their reproductive performance

Vliv různé růstové schopnosti prasniček plemen bílé ušlechtilé a landrase na jejich reprodukční užitkovost

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ABSTRACT: 8 835 sows of Czech Large White (CLW) and 3 090 Landrace (L) sows were monitored during the years 1994–1997. An average daily gain was determined during performance test (methodology of field test) and it was converted for weight of 90 kg. Sows of both studied breeds were placed to groups by the average daily gain with following intervals: 470 g and less, 471–500 g, 501–530 g, 531–560 g, 561–590 g, 591–620 g, 621 g and more. During the years 1994–1997 an average daily gain in performance test was rising – from 528.78 to 562.25 g (+6%) in Czech Large White gilts and from 521.51 g to 570.07 g (+8.5%) in Landrace gilts. With growing average daily gain, a number of piglets in litter was rising. In group of Czech Large White sows with the lowest average daily gain (470 g and less) we found lower number of total born piglets (10.72), number of alive born piglets (10.00) and number of weaned piglets (9.10) compared to a group with the highest average daily gain (621 g and more), where the sows reached 11.20 of total born piglets, 10.40 of alive born and 9.16 of weaned piglets. A similar trend we found in groups of Landrace sows. Statistically conclusive differences were found in Czech Large White sows among an average daily gain and a number of total born piglets ($P < 0.01$) and a number of alive born piglets ($P < 0.05$). In Landrace sows, we determined very high statistically conclusive differences ($P < 0.001$) among an average daily gain and a number of total and alive born piglets. We evaluated a coefficient of heritability for number of total, alive born and weaned piglets of Czech Large White and Landrace and correlative coefficients among an average daily gain and reproductive traits of both analysed breeds, which were in interval $r = 0.0804–0.1572$.

Keywords: sow; reproduction; average daily gain; correlation; heritability

ABSTRAKT: Bylo sledováno 8 835 ks prasnic plemene bílé ušlechtilé (BU) a 3 090 prasnic plemene landrase (L) v roce 1994–1997. Průměrný denní přírůstek byl zjišťován při provádění zkoušek vlastní užitkovosti, podle metodiky polní testace a byl přepočten na jednotnou hmotnost 90 kg. Podle průměrného denního přírůstku byly prasnice obou sledovaných plemen rozděleny do následujících intervalů: 470 g a méně, 471–500 g, 501–530 g, 531–560 g, 561–590 g, 591–620 g a 621 g a více. Průměrný denní přírůstek ve vlastní užitkovosti zaznamenal ve sledovaném období (1994–1997) vzestup u prasniček plemene BU z 528,78 g na 562,25 g (+6,0 %) a u prasniček plemene L z 521,51 g na 570,07 g (+8,5 %). Se zvyšujícími se hodnotami průměrného denního přírůstku byl zjištěn růst počtu selat ve vrhu. U skupiny prasnic plemene BU s nejnižším průměrným denním přírůstkem (470 g a méně) byl zjištěn nižší počet všech narozených selat (10,72), počet živě narozených selat (10,00) a odchovaných (9,10) oproti skupině s nejvyšším průměrným denním přírůstkem (621 g a více), kde bylo dosaženo 11,20 všech narozených, 10,40 živě

narozených selat a 9,16 odchovaných selat. Obdobný trend byl zaznamenán i u skupin prasníc plemene landrase. Statisticky průkazné rozdíly byly zjištěny u prasníc plemene BU mezi průměrným denním přírůstkem a počtem všech narozených selat ($P < 0,01$) a počtem živě narozených selat ($P < 0,05$). U prasníc plemene L byla zjištěna velmi vysoká průkaznost ($P < 0,001$) mezi průměrným denním přírůstkem a počtem všech a živě narozených selat. U prasniček plemene BU došlo ke snížení věku při prvním zapuštění z 282 dní (470 g a méně) na 242,9 dne (621 g a více) a u prasniček plemene landrase byl obdobný pokles z 271,3 na 234,6 dne. Dále byl vypočítán koeficient heritability pro počet všech narozených, živě narozených a dochovaných selat plemene BU, L a korelační koeficienty mezi průměrným denním přírůstkem a ukazateli plodnosti, které se u obou analyzovaných plemen pohybovaly v rozmezí $r = 0,0804-0,1572$.

Klíčová slova: prasnice; reprodukce; průměrný denní přírůstek; korelace; heritabilita

Growth and evolution of organism is one of the most important manifestations of life. It's complicated biological operation, which is in close relation with meat production and it is quite visible in young animals. Growth is a typical mark of polygene character; genotype and environmental factors share on its manifestation.

Stagnation of an average daily gain of gilts after the finishing performance test it had a statistically conclusive influence on a higher age during the first mating and it had a negative influence on insertion gilts to breeding (Čeřovský, 1996). Kernerová *et al.* (1998) found an acceptable level of growth intensity to 560 g (Czech Large White gilts). Klusáček *et al.* (1987) evaluated 2 783 Czech Large White sows. Gilts with an average daily gain from 525 g to 600 g had in the first three litters higher reproductive efficiency than gilts with an average daily gain from 475 g to 525 g. Hartog and Vestegen (1990) found, that limited growth after weaning increases the age of gilts during the start of their sexual activity. Wolfová *et al.* (1998) analysed an efficiency structure of dam breeds. During the years 1990–1996, a gain (in field test) was increasing by 23 g in Landrace breed. It represents accrual of 4 g per year. A similar situation was in Czech Large White gilts.

Many authors mention, fertility is growing to 3rd–4th litter and then it is decreasing (Whittemoore *et al.*, 1995, etc.).

Dufek (1986) determined coefficients of heritability for an average daily gain of Czech Large White and Landrace 0.728 ± 0.07 and 0.643 ± 0.10 . Gabriš *et al.* (1986) determined coefficients of heritability of Landrace boars $h^2 = 0.5752 \pm 0.2264$ for this trait in 1982. Petříček *et al.* (1991) determined for average daily gain $h^2 = 0.34 \pm 0.054$. Skorupski *et al.* (1996) determined it for average

daily gain of CLW and L lower ($h^2 = 0.20$ and 0.18). Coefficient heritability of fertility is usually mentioned in interval $h^2 = 0.0-0.2$ (Siewerdt *et al.*, 1995; Kayaba *et al.*, 1989, etc.).

Wick (1996) found correlation among fattening ability and natal size of litter for Czech Large White gilts $r = -0.028$ and for Landrace gilts $r = -0.47$. Popesu-Vifor *et al.* (1986) determined genetic correlation between body weight in 101 days of age and an average daily gain ($r_G = 0.82$), between age during the first farrowing and a size of litter ($r_G = -0.64$), between natal size of litter and size of litter in age of 21 days ($r_G = 0.78$) and between size of litter in age of 21 days and weight of litter in age of 21 days ($r_G = 0.66$). Dufek (1986) found genetic correlative coefficients between number of total and alive born piglets $r_G = 0.878$; between number of total born and weaned piglets $r_G = 0.602$ and between number of alive born and weaned piglets $r_G = 0.706$.

MATERIAL AND METHODS

Data for evaluation were taken from control of efficiency and heritability of pigs (ČSN 46 6164) and Pig Breeders Association in Bohemia and Moravia. Parts of performance test and control of reproductive traits from nucleus herds were used. Sows, which farrowed after mating, were monitored in our study. The number of evaluated sows during the years 1994–1997 was 8 835 Czech Large White sows (CLW) and 3 090 Landrace sows (L), respectively. An average daily gain from birth until 90 kg weight was determined during performance test (methodology of field test – ČSN 46 6164). The sows of both breeds were placed to groups by an average daily gain with following intervals:

470 g and less, 471–500 g, 501–530 g, 531–560 g, 561–590 g, 591–620 g, 621 g and more. Next, we statistically evaluated reproductive traits of studied sows; these phenotypic values were drawn from control of reproductive traits efficiency: number of total born piglets, number of alive born piglets, and number of weaned piglets. Number of days from birth until the first successful mating was another studied trait.

An analyse of systematic influences acting on phenotypic variance of studied traits was guided on a base of a model with these effects:

$$y_{ijkl} = \mu + CH_i + P_j + V_k + e_{ijkl}$$

where: y = evaluated parameter
 μ = average of population
 CH = effect of breeding
 P = effect of average daily gain
 V = effect of litter sequence
 e = residuum

For following testing of differences a Tukey test was used.

Determination of correlative coefficients (r) was done with one factor analyse of covariance with an influence of father as a source of variability.

Presumption of coefficients of heritability (b^2) were done by using analyse of triple hierarchical sorting variance and were determined after groups of half siblings along father.

$$y_{ijkl} = \mu + a_i + b_j + c_k + e_{ijkl}$$

Source of variability: a – breeding, b – sire, c – dam, e – residuum

RESULTS AND DISCUSSION

Monitoring of phenotypic levels of productive traits it is one of the basic tasks during the study of genetic trend of population improvement. In Tables 1 and 2, there is an evolution of level of an average daily gain of gilts in field test (in g). Both breeds show an increasing tendency during the years 1994–1997. In Czech Large White gilts was a growth over 33.47 g (6%), in Landrace gilts there was a growth over 48.56 g (8.5%). Variation interval was low. The highest increase of an average daily gain was in L gilts between the years 1996–1997 (25.94 g). The highest increase of an average daily gain was in CLW gilts between the years 1996–1997 (14.37 g). Wolfová *et al.* (1998)

Table 1. Basic statistical characterization of average daily gain (in g) of CLW gilts during the years 1994–1997

Year	n	Average	s_x^2	s_x	v_x	s_x	Min.	Max.
1994	771	528.78	2 186.17	46.76	0.0884	1.69	359.00	673.00
1995	2 558	537.91	2 247.21	47.40	0.0881	0.94	348.00	768.00
1996	2 782	547.88	2 230.00	47.22	0.0862	0.90	401.00	857.00
1997	2 724	562.25	2 026.33	45.01	0.0801	0.86	420.00	721.00
Total	8 835	547.76	2 292.68	47.88	0.0874	0.51	348.00	857.00

Table 2. Basic statistical characterization of average daily gain (in g) of L gilts during the years 1994–1997

Year	n	Average	s_x^2	s_x	v_x	s_x	Min.	Max.
1994	301	521.51	2 660.61	51.58	0.0989	2.98	505.00	721.00
1995	960	538.11	2 613.98	51.13	0.0950	1.65	400.00	725.00
1996	959	544.13	2 526.31	50.26	0.0924	1.62	418.00	751.00
1997	870	570.07	2 955.73	54.37	0.0954	1.84	410.00	770.00
Total	3 090	547.36	2 927.62	54.11	0.0989	0.97	400.00	770.00

Table 3. Phenotypic level of reproductive traits according to an average daily gain of CLW sows

Trait	<i>n</i>	Average	Total piglets	Alive born	Weaned
470g and less	419	449.49	10.72	10.00	9.01
471–500 g	918	487.99	10.78	10.01	8.89
501–530 g	1 895	516.16	10.72	10.00	8.82
531–560 g	2 202	545.23	10.81	10.05	9.01
561–590 g	1 797	574.96	10.93	10.14	9.02
591–620 g	1 041	603.72	11.01	10.25	9.17
621 g and more	563	644.28	11.20	10.40	9.16

Table 4. Phenotypic level of reproductive traits according to an average daily gain of L sows

Trait	<i>n</i>	Average	Total piglets	Alive born	Weaned
470 g and less	209	454.11	10.16	9.64	8.83
471–500 g	402	487.46	10.13	9.44	8.76
501–530 g	636	515.99	10.11	9.64	8.76
531–560 g	673	544.67	10.28	10.00	8.91
561–590 g	499	574.94	10.66	10.00	9.11
591–620 g	384	604.13	10.81	10.15	9.11
621 g and more	287	650.98	11.14	10.43	9.27

Table 5. Results of analyses of variance for number of total, alive born and weaned piglets of CLW sows

Trait	Sum of squares	Level of width	Average square	Stat. <i>F</i>	Significance
Total piglets	6 695.864	6	1 115.977	2.916	0.008**
Alive born	4 543.802	6	757.300	2.286	0.033*
Weaned	2 164.253	6	360.709	0.828	0.548

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 6. Results of analyses of variance for number of total, alive born and weaned piglets of L sows

Trait	Sum of squares	Level of width	Average square	Stat. <i>F</i>	Significance
Total piglets	9 283.738	6	1 547.290	4.297	0.001***
Alive born	7 789.522	6	1 298.254	4.091	0.001***
Weaned	2 081.951	6	346.992	0.895	0.497

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

analysed structure of efficiency of dam breeds and they mention similar results. They noted the highest growth of gain during the years 1992–1996.

If we compare data in Tables 3 and 4, we can see increasing number of piglets in the first litter (total, alive born and weaned) in dependence on increasing average daily gain in field test of both studied breeds. Statistical importance of dependence of average daily gain and number of piglets in the first litter (CLW and L) are given in Tables 5 and 6. For a number of total born piglets it was highly statistically conclusive ($P < 0.01$) and for number of alive born piglets it was statistically conclusive ($P < 0.05$). An influence of an average daily gain on number of weaned piglets wasn't statistically conclusive. An influence of an average daily gain on number of total and alive born piglets of Landrace sows was statistically conclusive ($P < 0.001$). We can say, that higher growing abilities have a good influence on reproductive traits. An influence of an average daily gain on number of weaned piglets of the same breed wasn't statistically conclusive. Kernerová *et al.* (1998) mention an optimal level of growth intensity of gilts, it is no more than 560 g. We can agree with this conclusion, but only in part, because we found increasing reproductive efficiency also with higher gain. We can agree with Klusáček *et al.* (1987), that Czech Large White gilts with lower growth intensity had lower numbers of piglets.

In Figure 1, there is a different level of average daily gain and age during the first mating. We can see an influence of decline of age during the first mating on growing daily gain. An age during the first mating was reduced from 282.79 days to 242.94 days in CLW gilts and from 271.31 to 234.62 days in L gilts. It's evident, that L gilts had a lower age during the first mating with all levels of gain. Hartog and Vestegen (1990) mention the same conclusion, they mention, restricted growth after weaning delays start of sexual activity. Čeřovský (1996) mentions that stagnation of gilt's gain after the performance test lengthens a period from birth until the first mating.

Basic statistical characterization of chosen reproductive traits of both breeds according to order of litter (the first – the seventh and more), are shown in Tables 7 and 8. An average daily gain was reduced from 548.42 g on the first litter to 533.72 g on the sixth litters in CLW sows and from 547.32 g on the first litter to 516.14 g on the seventh and the next litters in L sows. Coefficients of variance were favourable and well balanced in a scope of order of litters. The highest number of total born piglets we found in fifth litters in CLW sows (12.09) and in sixth litters in L sows (11.50). Number of alive born piglets reached the highest values in the fifth litters in both breeds (11.05 and 10.56). The highest number of weaned piglets was in the third litters in both breeds (9.65). Sows with higher daily gain

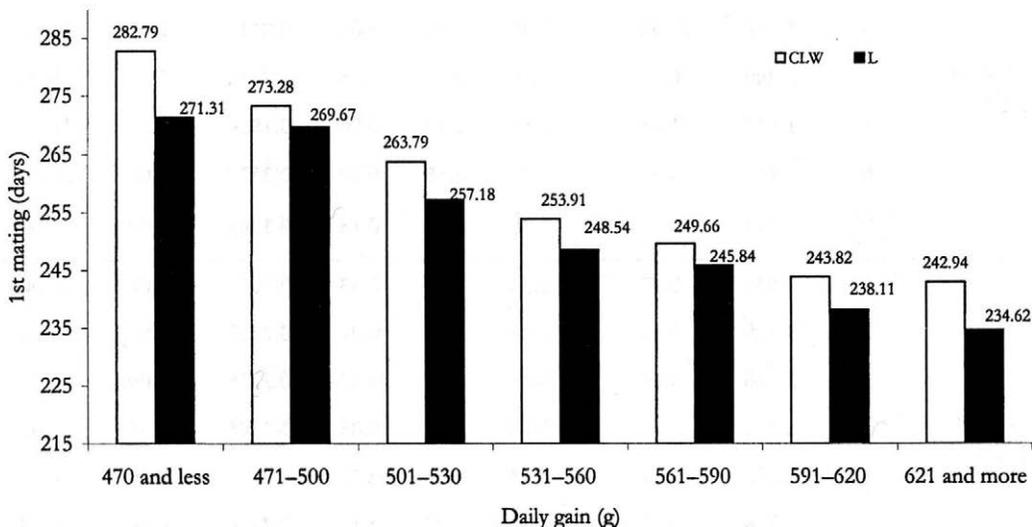


Figure 1. An influence of an average daily gain on age during the first mating of CLW and L gilts

Table 7. Basic statistical characterization of chosen reproductive traits of CLW sows according to order of litter and dependence on average daily gain

Trait	Order of litter	<i>n</i>	Average	s_x^2	s_x	$s_{\bar{x}}$	v_x	Min.	Max.
Daily gain (g)	1	8 835	548.42	2 229.88	47.22	0.52	0.0861	348.00	857.00
	2	5 365	544.26	2 293.07	47.89	0.65	0.0880	348.00	857.00
	3	3 526	540.37	2 252.81	47.46	0.80	0.0878	348.00	857.00
	4	2 246	538.11	2 236.98	47.30	1.00	0.0879	348.00	857.00
	5	1 377	535.10	2 233.34	47.26	1.27	0.0883	348.00	698.00
	6	718	533.72	2 076.79	45.57	1.70	0.0854	348.00	671.00
	7 and more	322	534.48	2 095.90	45.78	2.55	0.0857	368.00	647.00
Total born piglets	1	8 835	10.50	5.62	2.37	0.00	0.2257	1.00	19.00
	2	5 365	11.23	6.13	2.48	0.03	0.2204	1.00	21.00
	3	3 526	11.81	6.58	2.57	0.04	0.2172	2.00	24.00
	4	2 246	11.89	6.84	2.61	0.06	0.2199	4.00	21.00
	5	1 377	12.09	6.16	2.48	0.07	0.2053	3.00	20.00
	6	718	11.93	7.39	2.72	0.10	0.2278	3.00	21.00
	7 and more	322	11.54	6.94	2.63	0.15	0.2284	2.00	18.00
Alive born piglets	1	8 835	9.81	4.92	2.22	0.02	0.2262	1.00	18.00
	2	5 365	10.53	5.17	2.27	0.03	0.2160	2.00	20.00
	3	3 526	10.95	5.39	2.32	0.04	0.2119	2.00	20.00
	4	2 246	10.98	5.39	2.32	0.05	0.2114	3.00	19.00
	5	1 377	11.05	4.86	2.20	0.06	0.1994	3.00	18.00
	6	718	10.86	5.56	2.36	0.09	0.2170	3.00	20.00
	7 and more	322	10.45	5.30	2.30	0.13	0.2203	2.00	16.00
Weaned piglets	1	8 835	8.77	6.33	2.52	0.03	0.2871	0.00	18.00
	2	5 365	9.42	6.85	2.62	0.04	0.2777	0.00	18.00
	3	3 526	9.65	6.87	2.62	0.04	0.2715	0.00	17.00
	4	2 246	9.63	6.78	2.60	0.05	0.2705	0.00	17.00
	5	1 377	9.51	7.13	2.67	0.07	0.2808	0.00	16.00
	6	718	9.05	9.55	3.09	0.12	0.3416	0.00	16.00
	7 and more	322	8.01	14.26	3.78	0.21	0.4713	0.00	15.00

Table 8. Basic statistical characterization of chosen reproductive traits of L sows according to order of litter and dependence on average daily gain

Trait	Order of litter	<i>n</i>	Average	s_x^2	s_x	\bar{s}_x	v_x	Min.	Max.
Daily gain (g)	1	3 090	547.32	2 979.89	54.59	1.02	0.0997	400.00	770.00
	2	1 801	540.13	2 651.59	51.49	1.21	0.0953	400.00	726.00
	3	1 160	535.60	2 573.03	50.73	1.49	0.0947	400.00	721.00
	4	708	531.42	2 381.89	48.80	1.83	0.0918	427.00	721.00
	5	421	528.18	2 192.87	46.83	2.28	0.0887	427.00	655.00
	6	218	525.38	2 436.00	49.36	3.34	0.0939	434.00	677.00
	7 and more	95	516.14	2 311.78	48.08	4.93	0.0932	435.00	622.00
Total born piglets	1	3 090	10.21	5.23	2.29	0.04	0.2239	2.00	18.00
	2	1 801	10.52	6.20	2.49	0.06	0.2366	2.00	19.00
	3	1 160	11.12	5.96	2.44	0.07	0.2195	3.00	19.00
	4	708	11.22	6.17	2.48	0.09	0.2214	2.00	20.00
	5	421	11.30	6.85	2.62	0.13	0.2317	3.00	20.00
	6	218	11.50	6.44	2.54	0.17	0.2207	5.00	19.00
	7 and more	95	11.20	7.10	2.66	0.27	0.2379	4.00	18.00
Alive born piglets	1	3 090	9.59	4.76	2.18	0.04	0.2277	2.00	17.00
	2	1 801	9.94	5.49	2.34	0.06	0.2359	1.00	18.00
	3	1 160	10.46	5.48	2.34	0.07	0.2237	1.00	17.00
	4	708	10.50	5.01	2.24	0.08	0.2131	2.00	19.00
	5	421	10.56	5.43	2.33	0.11	0.2207	3.00	17.00
	6	218	10.54	5.29	2.30	0.16	0.2182	5.00	18.00
	7 and more	95	10.48	6.36	2.52	0.26	0.2405	3.00	16.00
Weaned piglets	1	3 090	8.78	5.34	2.31	0.04	0.2633	0.00	15.00
	2	1 801	9.17	6.12	2.47	0.06	0.2699	0.00	15.00
	3	1 160	9.65	5.66	2.38	0.07	0.2465	0.00	16.00
	4	708	9.54	6.52	2.55	0.10	0.2678	0.00	15.00
	5	421	9.58	5.39	2.32	0.11	0.2424	0.00	16.00
	6	218	9.19	8.27	2.88	0.19	0.3128	0.00	14.00
	7 and more	95	8.00	15.15	3.89	0.40	0.4865	0.00	15.00

Table 9. Coefficients of heritability of average daily gain and reproductive traits of CLW and L breeds

Trait	CLW		L	
	h^2	s_b^2	h^2	s_b^2
Average daily gain	0.3689	0.11	0.4926	0.05
Number of total born piglets	0.1556	0.09	0.1274	0.04
Number of alive born piglets	0.1064	0.08	0.1029	0.04
Number of weaned piglets	0.0804	0.09	0.1123	0.03

Table 10. Coefficients of correlation between number of piglets and an average daily gain of CLW breed

Character	Character		
	1	2	3
1			
2	0.0354**		
3	0.0481**	0.8977**	
4	0.0316**	0.6105**	0.6880**

1 = average daily gain in g, 2 = number of total born piglets, 3 = number of alive born piglets, 4 = number of weaned piglets

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

Table 11. Coefficients of correlation between number of piglets and an average daily gain of L breed

Character	Character		
	1	2	3
1			
2	0.1572**		
3	0.1549**	0.9186**	
4	0.0804**	0.6696**	0.7259**

1 = average daily gain in g, 2 = number of total born piglets, 3 = number of alive born piglets, 4 = number of weaned piglets

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

reached less litters, so it's possible to suppose, that fast growing gilts have shorter reproductive life.

An influence of breeding and order of litter were the other factors, which had a statistically conclusive influence ($P < 0.001$) on the number of total, alive born and weaned piglets of CLW and L sows.

Coefficients of heritability for an average daily gain in field test and for number of total and alive

born and weaned piglets of both breeds are in Table 9. Determined values 0.37; 0.16; 0.11; 0.08 correspond with information from literature (Gabriš *et al.*, 1986, etc.). Skorupski *et al.* (1996) mention quite lower values of LW and L ($h^2 = 0.20$ and 0.18).

Coefficients of correlation, which were found among an average daily gain and traits of repro-

duction (number of total, alive born and weaned piglets) in both analysed breeds reached values close to zero (Tables 10 and 11). Coefficients of correlation, which were found among reproductive traits in CLW and L sows, reached high positive values and were highly statistically conclusive.

CONCLUSION

On the base of our results of growth ability (average daily gain during performance test) we can claim that during the years 1994–1997 an average daily gains of CLW and L gilts increased by 33.47 g (6.0%) and 48.56 g (8.5%), respectively.

After following analyses of reproductive data of sows of both breeds (in groups according to phenotypic level of average daily gains in performance test) we calculated that sows of both breeds with higher gains reached higher number of total, alive and weaned piglets. These facts were confirmed by correlation coefficients on a level $r = 0.0804$ – 0.1572 .

On the base of these results we can mention that an actual level of average daily gains of gilts in performance test, gains had no influence on decrease of phenotypic level of reproduction.

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Studies on the relationship between the body weight, trunk length and pelt size in arctic foxes

Studium závislosti mezi živou hmotností, délkou těla a velikostí kožešiny u polárních lišek

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ABSTRACT: The aim of the present research was to determine the relationship between the live body weight, trunk length and pelt size in arctic foxes. The studies were conducted on 257 arctic foxes. Measurements of the body weight and trunk length were taken directly before slaughter. After slaughter, pelts were subjected to standard processing. Dried pelts were measured and the values obtained were compared with those concerning the body weight and trunk length of a given animal. Statistical differences between males and females were determined. The multiple regression method was employed to calculate the coefficients of correlation between the body weight, trunk length, and pelt length. Multiple regression formulas were derived to estimate the pelt size (trait Y) before slaughter based on the body weight (trait X_1) and trunk length (trait X_2). Those equations make it easier for breeders to determine the expected size of pelts in a given season before animals are slaughtered, and to estimate their market value.

Keywords: arctic fox; body weight; size; pelt; correlation

ABSTRAKT: Cílem výzkumu bylo stanovení závislosti mezi živou hmotností, délkou těla a velikostí kožešiny u polárních lišek. Sledování se uskutečnilo na 257 polárních liškách. Zjišťování živé hmotnosti a délky těla probíhalo těsně před utracením zvířat. Pak došlo ke standardnímu zpracování kožešin. Měření jsme provedli na vysušených kožešinách a získané hodnoty jsme porovnali s hodnotami živé hmotnosti a délky těla daného zvířete. Byly stanoveny statistické rozdíly mezi samci a samicemi. Pro výpočet koeficientů korelace mezi živou hmotností, délkou těla a délkou kožešiny jsme použili metodu mnohonásobné regrese. Na základě živé hmotnosti (znak X_1) a délky těla (znak X_2) jsme odvodili rovnice mnohonásobné regrese pro odhad velikosti kožešiny (znak Y) před utracením. Tyto rovnice umožňují chovatelům snazší stanovení očekávané velikosti kožešin v dané sezoně před utracením zvířat a odhad jejich tržní hodnoty.

Klíčová slova: polární liška; živá hmotnost; velikost; kožešina; korelace

The correlation between various performance traits in fur-bearing animals has been the subject of numerous papers (Moller, 1992; Nielsen *et al.*, 1994; Nurominen and Sepponen, 1996; Piórkowska, 1996, 1998; Leoschke and Michals, 2000; Lorek *et al.*, 2001). The price of pelts first of all depends on their length. It is common knowledge that there is a positive correlation between the length of trunk and pelt (Leoschke and Michals, 2000). However,

the effect of body weight on this parameter has not been yet fully investigated. The results obtained for particular species are often contradictory – differences were noted even between various populations within the same species. According to Therkildsen (1989), the pelt size depends not only on the trunk length and body weight of animals, but also on the way the cuts are made during fur removal, the stretching force and the way the pelt is formed on

the blocks. Because fox breeding is dominated by arctic foxes, the aim of the present studies was to calculate the coefficients of correlation between the body weight, trunk length, and pelt length in arctic foxes. Multiple regression equations were derived to determine the expected size of pelts in a given season before slaughter, and to estimate their market value based on zoometric measurements taken for a representative group of farm animals.

MATERIAL AND METHODS

The studies aimed at determining the relationship between the live body weight, trunk length and pelt size in arctic foxes, were conducted at the beginning of December, under production conditions. Two hundred and fifty seven arctic foxes: 126 males and 131 females constituted the experimental material. Measurements were taken during two working days and concerned foxes slaughtered on these days, selected at random. Measurements of body weight and trunk length were taken directly before slaughter. The foxes were measured with a measuring tape, accurate to 1 cm, from the tip of the nose to the base of the tail. Their body weight was determined accurate to 10 g. The pelts obtained were marked with numbered metal ear studs and subjected to standard processing. After drying the pelts were measured with a measuring tape, accurate to 1 cm, from the tip of the nose to the base of the tail. The values obtained were compared with those concerning the body weight

and trunk length of a given animal. The results were elaborated statistically, taking into account the sex of animals. Statistical differences between males and females were determined by an analysis of variance for one-factor non-orthogonal designs. This allowed finding if coefficients of correlation can be calculated, and regression equations formulated, for the whole population, or males and females separately. Then the multiple regression method was applied to determine the coefficients of correlation between body weight, trunk length, and pelt length, separately for males and females. Multiple regression formulas were also derived, to estimate the pelt size (trait Y) before slaughter based on body weight (trait X_1) and trunk length (trait X_2). The results were analyzed statistically using computer program Statistica Software PL.

RESULTS AND DISCUSSION

Table 1 presents the characteristics of live measurements concerning the body weight, trunk length, and pelt length in arctic foxes, separately for males and females. A statistical analysis of the data collected shows highly statistically significant differences between males and females in all of the traits examined. Cholewa (1983) found out that differences in the structure and size of particular body parts of arctic foxes indicate sexual dimorphism, so the indices concerning their constitution should be considered separately for males and females. In our own investigations, there was a statistical dif-

Table 1. Characteristics of live measurements (body weight and trunk length) and pelt length in polar foxes

Specification	Statistical measures	Males	Females
	n	126	131
Body weight (kg)	\bar{x}	12.80**	9.26**
	range	(10.0–16.5)	(7.7–13.5)
	v	12.28	19.19
Trunk length (cm)	\bar{x}	70.46**	66.27**
	range	(66–75)	(60–79)
	v	3.13	4.56
Pelt length (cm)	\bar{x}	119.03**	108.32**
	range	(108–130)	(90–124)
	v	3.85	5.56

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

Table 2. Coefficients of correlation between body weight, trunk length and length of pelts obtained from male and female foxes

Trait	Trunk length – X_2	Pelt length – Y
Male foxes		
Body weight – X_1	0.509**	0.587**
Trunk length – X_2		0.534**
Female foxes		
Body weight – X_1	0.583**	0.754**
Trunk length – X_2		0.616**

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

ference between the average body weight of males – 12.80 kg and females – 9.26 kg (Table 1). The research carried out by Lorek and Gugolek (1992) did not confirm that there were statistical differences between the body weight of males and females. Kubacki (2000) gives body weights of arctic foxes before slaughter without sex differentiation. They range from 5.54 kg to 11.42 kg. The differences in body weight result from various origins of animals – the highest body weight were noted in the case of Finnish foxes. In our own studies, the experimental foxes also belonged to the Finnish type, which explains their high body weights. These foxes are characterized by larger size and are used for improvement of domestic fox population. The average trunk length of experimental animals was 70.46 cm in males and 66.27 cm in females. The average length of their pelts was 119.03 cm (commercial category “000”) and 108.32 cm (commercial category “00”), respectively. Table 2 presents the coefficients of correlation between the traits included in Table 1. In the experimental population of arctic foxes, the highest correlation coefficient for males was observed between body weight and pelt length – 0.587. The coefficient of correlation between trunk length and pelt length was 0.539, whereas that between body weight and trunk length – 0.509. The research conducted by other authors show considerable differences in the coefficients of correlation between the traits examined in such species of fur-bearing animals as raccoon dogs, common foxes, and minks (Therkildsen, 1989; Piórkowska, 1998; Lorek *et al.*, 2001). In the group of females the highest correlation was noted between body weight and pelt length – 0.754,

lower between trunk length and pelt length – 0.616, and the lowest between body weight and trunk length. Compared with the group of males, these coefficients are higher, especially with regards to the correlation between body weight and pelt length. The studies on minks confirm a correlation between the parameters analyzed. Therkildsen (1989) proved that there was a correlation between the body weight of both males (0.89) and females (0.92) and the length of their pelts after forming. Different results were obtained by Nurominen and Sepponen (1996). According to them, experimental minks given more feed achieved higher slaughter weights – males by 329 g, females by 227 g – than control ones. However, their pelts were not longer than those from control animals, characterized by lower body weights. This suggests that this solution is not always positive, especially when fattening is continued after the growth period. It seems interesting to compare the length of trunk and “raw” pelt. Therkildsen (1989), who investigated the relation between the length of “raw” and formed pelts in minks, found out that those pelts of both females and males became longer, on average by 20% and 13.5%, respectively. In the present experiment, pelts of males became longer by 48 cm, whereas those of females by 42 cm. Expressed in the form of percentages (assuming that trunk length constitutes 100%), an increase in pelt length was equal to 69% in males and 63% in females. In the studies on arctic foxes conducted by Piórkowska (1996) the coefficient of correlation between body weight and trunk length was 0.520, i.e. lower than that achieved in the group of females in the present experiment, and similar to that noted in the group

Table 3. Regression equations for estimating the pelt length (cm) in foxes on the basis of live measurements: body weight and trunk length

Equation No.	Multiple regression equation	S_y	R^2
1.	arctic foxes – males $Y = 1.24 X_1 + 0.66 X_2 + 56.61$	3.51	0.42
2.	arctic foxes – females $Y = 2.03 X_1 + 0.53 X_2 + 54.29$	3.76	0.62

Y – pelt length (cm)

X_1 – body weight (kg)

X_2 – trunk length (cm)

S_y – standard error of estimation (cm)

R^2 – coefficient of determination

of males. The correlation between body weight and pelt length in the above studies was 0.620. In raccoon dogs the coefficient of correlation between body weight and pelt length was 0.227, whereas that between trunk length and pelt length – 0.449. They were relatively low, compared with those calculated for foxes. This is probably connected with a different constitution of raccoon dogs, which are stocky and capable of depositing fatty tissue in autumn (Piórkowska, 1998). The correlation coefficients included in Table 2 provided the basis for deriving multiple regression equations (Table 3), in which the value estimated was the length of dried pelts (variable Y), determined by means of *in vivo* measurements: body weight (X_1) and trunk length (X_2). The paper presents two equations calculated for males and females separately. These equations are easy to apply, as they require taking two simple measurements only, which does not cause fur quality deterioration. Their standard error of estimation was 3.51 cm in females and 3.76 for males. The coefficients of determination (R^2) between Y , X_1 and X_2 were at a level of 0.42 and 0.62.

CONCLUSIONS

High values of the correlation coefficients allowed to derive multiple regression equations, which may be applied to determine the pelt length in the population of arctic foxes examined in present studies, on the basis of live measurements of their body weight and trunk length. These equations make it

easier for breeders to determine the expected size of pelts in a given season before slaughter, and to estimate their market value.

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Effect of feeding pellets to arctic foxes on their performance and selected morphological-biochemical blood indices

Vliv podávání pelet polárním liškám na jejich užitkovost a na vybrané morfologicko-biochemické ukazatele krve

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ABSTRACT: The studies on the effect of dry pelleted feed on the performance of arctic foxes and some of their morphological-biochemical blood indices were divided into two stages. In stage one 200 foxes aged 8 weeks were allocated to two equal groups. The control group received traditional feed. The experimental factor was pelleted feed given to the experimental group. This stage also included: the determination of the body weight of foxes at the beginning and at the end of the studies, the evaluation of conformation and pelt quality, the determination of morphological and biochemical blood indices. The indices of reproduction performance of the experimental animals were determined in stage two. The research results show that feeding pellets to arctic foxes caused a decrease in their final body weight and pelt length, but improved the *in vivo* and post-slaughter parameters of fur quality. The experimental factor affected neither the morphological-biochemical blood indices analyzed in the experiment, nor the reproduction performance of foxes.

Keywords: arctic fox; pellets; feeding; growth; pelt; blood indices

ABSTRAKT: Sledování vlivu suchého krmiva ve formě pelet na užitkovost polárních lišek a vybrané morfologicko-biochemické ukazatele krve jsme rozdělili do dvou etap. V první etapě bylo 200 lišek ve věku osm týdnů rozděleno do dvou stejných skupin. Kontrolní skupina dostávala tradiční krmivo. Pokusným faktorem bylo krmivo ve formě pelet podávané pokusné skupině. Tato etapa zahrnovala: stanovení živé hmotnosti lišek na začátku a na konci sledování, hodnocení exteriéru a kvality kožešiny, stanovení morfologických a biochemických ukazatelů krve. Ve druhé etapě jsme hodnotili ukazatele reprodukce pokusných zvířat. Výsledky našeho výzkumu ukazují, že podávání pelet polárním liškám vedlo ke snížení jejich konečné živé hmotnosti a délky kožešiny, ale zlepšilo kvalitativní parametry kožešiny *in vivo* a po utracení zvířat. Pokusný faktor neovlivnil morfologicko-biochemické ukazatele krve analyzované v tomto pokusu, ani reprodukční schopnost lišek.

Klíčová slova: polární liška; pelety; výživa; růst; kožešina; ukazatele krve

Allain *et al.* (1980), Leoschke (1981) and Sławoń (1981) were among the first to present the results of feeding dry complete diets to arctic foxes and minks. These studies were continued by Hillemann

(1985) and Taranov (1985). Wójcik *et al.* (1987) investigated the effect of dry feed on the metabolic blood profile in foxes. Laplante (1987) proved that the cost of pellets was by 15–20% lower than

that of traditional feed. Weiss (1987) reported that feeding pellets to foxes and minks allows to achieve shorter, but high-quality pelts. Rouvinen *et al.* (1992) focused on various techniques of feeding pellets, taking into consideration their waste. Interesting results concerning feeding pellets to minks and foxes were obtained by Mee (1994) and Zou *et al.* (1997). Szymeczko *et al.* (1999) determined the hematological blood indices in arctic foxes given dry feed. In the research conducted at the University of Warmia and Mazury in Olsztyn, Lorek *et al.* (1999) and Gugolek *et al.* (1997) investigated the effect of dry pelleted feed on the performance, health state and nutrient digestibility

in arctic foxes. The present paper is a continuation of these studies.

MATERIAL AND METHODS

The studies were divided into two stages. In stage one 200 foxes aged 8 weeks were allocated to two equal groups, 50 males and 50 females in each. They were kept in pairs in conventional production cages. Group I (control) received traditional feed. Pelleted feed was produced according to the procedure presented in Table 1. The physical parameters of pellets were: length 5–10 mm, diameter

Table 1. Composition and indices of nutritive value of rations

Specification	Group			
	I		II	
	growing period (July–September)	period of hair coat development (October–November)	growing period (July–September)	period of hair coat development (October–November)
Kind of feed (%)				
hard poultry offal	25	30	–	–
cod offal	35	20	–	–
herring offal	10	10	–	–
meat and bone meal	5	–	–	–
extruded barley	25	20	20	20
steamed potatoes	–	20	–	–
meat meal	–	–	20	20
fish meal	–	–	10	10
blood meal	–	–	7	5
rape oil	–	–	8	5
soybean oil meal	–	–	15	15
extruded corn	–	–	20	25
Total	100	100	100	100
Dry matter	34	34	93	93
Digestible components (%)				
protein	12.1	10.0	26.9	22.8
fat	4.2	3.9	10.6	8.9
carbohydrates	8.3	11.0	20.3	23.7
Metabolizable energy (MJ/kg)	5.35	5.32	13.25	11.82
Protein –energy ratio (g/MJ)	22	19	20	19
Energy (%)				
protein	42	36	40	37
fat	31	29	33	29
carbohydrates	27	35	27	34

4 mm. Group II (experimental) was fed from self-pouring feeders. The research period was divided into two parts: growth (June – September) and fur development (October – November). Body weight was determined at 8 and 26 weeks of age, exact to 0.1 kg. Fully developed furs were evaluated after slaughter, according to the Standards of Arctic Fox Conformation Evaluation, determining pelt length and fur category, including the flesh side. Blood (ca. 5 ml) for morphological and biochemical examinations was collected from 10 males and 10 females selected at random in each of the groups, at 8 and 22 weeks of age. The analyses included the determination of: the red blood cell count (RBC),

the mean red blood cell volume (MCV), the mean hemoglobin mass in a red blood cell (MCH), the mean hemoglobin concentration in a red blood cell (MCHC), the hematocrit value (HCT), the level of hemoglobin (HBG), the white blood cell count (WBC), the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Laboratory analyses were made by commonly applied methods. In stage two the reproduction results of 10 males and 20 females selected at random from each group were determined. The females were mated three times. When the reproduction period was over, the following data were collected: for males – the number of mated females and

Table 2. Performance indices of arctic foxes

Specification	Statistical measures	Group			
		I	II		
Body weight (kg)	<i>n</i>	100	100		
	age 8 weeks	\bar{x}	1.48	1.40	
		<i>v</i>	10.99	13.54	
	age 26 weeks	\bar{x}	8.82*	8.35*	
		<i>v</i>	8.71	9.38	
		<i>n</i>	100	100	
Body length in age 26 weeks (cm)	\bar{x}	66.03*	65.29*		
	<i>v</i>	2.82	4.45		
	External conformation (points)	<i>n</i>	100	100	
		animal length	\bar{x}	5.87	5.75
			<i>v</i>	6.90	10.26
		colour type	\bar{x}	2.96	2.92
		<i>v</i>	6.80	7.28	
fur purity		\bar{x}	1.90	1.72	
	<i>v</i>	28.30	24.66		
fur quality	\bar{x}	6.05*	6.72*		
	<i>v</i>	13.65	16.30		
total	\bar{x}	16.78	17.11		
	<i>v</i>	9.52	8.14		
Pelt evaluation	<i>n</i>	70	70		
	size (cm)	\bar{x}	94.17**	92.62**	
		<i>v</i>	6.31	10.83	
	hair coat category	\bar{x}	1.71	1.49	
	<i>v</i>	42.57	33.46		

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

the number of mating sessions; for females – the number of females: mated and after kitting, the number of kits: born and weaned. The results were analyzed statistically using computer program Statistica Software PL.

RESULTS AND DISCUSSION

Table 1 presents the composition and indices of the nutritive value of diets. They were prepared according to the requirements of growing foxes, taking into account the period of growth and winter fur development. Component selection in traditional diets was typical of the domestic feed market. Pellets were made using the components described in other papers on feeding dry diets to carnivorous fur-bearing animals (Sławoń, 1981, 1991; Lorek *et al.*, 1999; Szymeczko *et al.*, 1999). The nutrient and metabolizable energy content of both kinds of diet was differentiated, which resulted from their different dry matter content. It follows that only indices of their nutritive value could be compared.

These indices were similar and consistent with the recommendations included in relevant norms (Barabasz *et al.*, 1994). The initial body weight of foxes from both groups was similar. The final body weight (at the age of 26 weeks) shows that foxes from the control group were on average by 47 g heavier. These results confirm those obtained by authors in previous investigations (Lorek *et al.*, 1999). According to Weiss (1988), lower body weight of foxes given pellets is caused by disturbances in the water balance or considerable feed losses. Leoschke (1981) and Laplante (1987) obtained lower body weight gains in experimental groups of minks fed pellets. On the other hand, Allain *et al.* (1980) and Zou *et al.* (1997) noted higher gains and bigger pelt sizes in animals fed dry diets. Foxes from the control group received higher scores for size and conformation, which was confirmed by trunk length measurement – they were by 0.7 cm longer. The feeding method had no effect on fur color and colour purity, as they are determined genetically. Fur quality was better in group II – the difference was statistically significant. The score for conformation

Table 3. Morphological indices and enzymatic activity of blood

Specification	Statistical measures	8 weeks/group		22 weeks/group	
		I	II	I	II
RBC ($10^{12}/l$)	<i>n</i>	20	20	20	20
	\bar{x}	6.28	6.38	8.09	8.57
	<i>v</i>	7.03	2.99	9.16	8.52
MCV (fl)	\bar{x}	48.78	48.03	47.70	47.45
	<i>v</i>	2.77	3.21	2.09	3.07
MCH (pg)	\bar{x}	17.20	17.51	17.36	17.29
	<i>v</i>	4.12	4.35	2.38	4.38
MCHC (g/dl)	\bar{x}	35.28	36.49	36.40	36.43
	<i>v</i>	3.10	2.13	1.83	2.02
HCT (l/l)	\bar{x}	0.31	0.31	0.40	0.40
	<i>v</i>	7.92	4.25	13.07	5.75
HBG (g/dl)	\bar{x}	10.76	11.16	14.07	14.76
	<i>v</i>	5.91	5.31	9.15	4.84
WBC ($10^9/l$)	\bar{x}	17.85	14.05	19.16	19.99
	<i>v</i>	26.54	20.95	22.70	29.57
AST (U/l)	\bar{x}	1.14	0.97	1.08	1.10
	<i>v</i>	42.30	25.70	40.00	27.30
ALT (U/l)	\bar{x}	11.97	12.14	6.13	5.33
	<i>x</i>	27.41	32.40	23.72	31.22

was also higher in this group. The pelts obtained in the control group were by 1.5 cm longer, which corresponded with higher body weights and better conformation. It was found that pelleted feed had a positive effect on fur quality, as the fur category in the control group was 1.71, approaching category II, whereas in the experimental group it was 1.49, close to category I (Table 2). Professional literature on the topic provides information about better fur quality in minks and foxes given pellets (Leoschke, 1981; Laplante, 1987; Weiss, 1988; Lorek *et al.*, 1999). Table 3 presents the blood morphological indices of arctic foxes. The results show that there were no statistically significant differences between the groups, neither at the beginning (8 weeks of age) nor at the end of the studies (22 weeks of age). Only some erythrocyte parameters increased in the peripheral blood of older foxes, which was connected with erythropoiesis. No statistically significant differences were observed in blood enzymatic activity between the groups. Also Szymeczko *et al.* (1998, 1999) reported no effect of dry feed on the morphological and biochemical indices of peripheral

blood. Table 4 shows the reproduction indices of foxes fed pellets during growth and development. An analysis of the sexual activity of males indicates no differences between the groups. Males from the control group were on average mated with 2.61 females, whereas those from the experimental one – with 2.74. The mean number of mating sessions was 7.83 in group I and 8.23 – in group II. The percentage of females: mated and after kitting, is also typical (Brzozowski, 1993). No statistical differences were found in the average number of kits born in both groups – 6.70 in group II and 7.31 in group I. These values are similar to those reported by other authors (Einarsson, 1986; Lohi, 1993; Brzozowski, 1993). There were no statistical differences regarding the number of kits, either. The average number of kits reared in the control group was 4.89, and in the experimental one – 5.06. Similar results were obtained by Lohi (1993) and Wang and Sun (2000). The rearing ratio may be affected by various non genetic factors, including the feeding system. In the population examined, the rearing ratio was 75% in the experimental group,

Table 4. Reproduction results of arctic foxes

Specification	Statistical measures	Group	
		I	II
Males	<i>n</i>	10	10
number of mated females	\bar{x}	2.61	2.74
	<i>v</i>	37.60	29.76
number of mating sessions	\bar{x}	7.83	8.23
	<i>v</i>	41.34	54.98
Females	<i>n</i>	20	20
mated	%	100	100
	<i>n</i>	18	17
	%	90	85
after kitting	<i>n</i>	16	17
	%	80	85
	<i>n</i>	117	114
number of born pups	<i>x</i>	7.31	6.70
	<i>v</i>	47.42	53.42
	<i>n</i>	78	86
number of reared pups	<i>x</i>	4.89	5.06
	<i>v</i>	67.87	70.09
Rearing ratio	%	67	75

and 67% in the control one. In the studies conducted by Wang and Sun (2000), this ratio varied from 52% to 81%.

CONCLUSIONS

Feeding pellets to arctic foxes in the period of growth and development caused a decrease in their final body weight and pelt length, but improved the *in vivo* and post-slaughter parameters of fur quality.

The experimental factor did not affect the morphological and biochemical blood indices analyzed in the experiment.

Feeding pellets to arctic foxes during growth and development had no negative effect on their reproduction performance.

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Content of metals in tissues of marketable common carp and in bottom sediments of selected ponds of South and West Bohemia

Obsah kovů v tkáních kaprů a v sedimentech dna rybníků jižních a západních Čech

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ABSTRACT: The goal of this work was to assess the status of selected ponds of South and West Bohemia, considering the content of metals in tissues of common carp and in bottom sediments. Ponds Regent, Tovaryš, Dřemliny, Horusický and Bezdrev were checked in autumns 2000–2001. Seven specimens of marketable common carp were sampled from every pond for analyses of muscle, liver, kidney and gonads. Content of Hg, As, Cd, Pb, Cr, Ni, Cu, and Zn was checked in fish tissues and in bottom sediments. Contents of metals in tissues of marketable common carp ranged below the hygienic limits valid for the respective metals in the Czech Republic. There was one exception, a slightly increased content of nickel (above 0.5 mg/kg) in muscle of 2 common carp specimens. No significant difference was found among the ponds in content of Cd, Pb, Cr, Ni and Cu in fish muscle. Significantly higher ($P < 0.01$) values of Hg and Zn were found in muscle of common carp from Tovaryš pond compared to values found in other ponds. Significantly higher arsenic content ($P < 0.01$) was found in muscle of common carp from Dřemliny and Bezdrev ponds, compared to those found in common carp from Regent pond. Significantly higher ($P < 0.01$) Hg content was detected in muscle, of Cd and Zn in kidney, of Cu in liver compared to contents of these metals in other tissues. Significantly higher content ($P < 0.01$) of copper and zinc was found in eggs compared to testes. Content of metals in dry matter of bottom sediment from the ponds under study ranged within the interval characteristic for relatively not loaded ponds under the conditions of Czech Republic. Increased content of Pb, Cu and Zn was registered only in bottom sediment of Tovaryš pond. This paper contributes to the assessment of quality of common carp as the main marketable fish in the Czech Republic. It contributes to the strategy of food safety.

Keywords: *Cyprinus carpio* L.; muscle; liver; kidney; gonads; hygienic limit; risk elements; AAS

ABSTRAKT: Cílem práce bylo posoudit stav vybraných rybníků jižních a západních Čech z hlediska obsahu kovů v tkáních kaprů a v sedimentech dna. V podzimním období roku 2000 až 2001 byly vyšetřovány rybníky Regent, Tovaryš, Dřemliny, Horusický a Bezdrev. Z každého rybníka bylo odebráno sedm tržních kaprů, analyzovány byly vzorky svaloviny, jater, ledvin a gonád. V tkáních ryb a v sedimentu dna byl sledován obsah Hg, As, Cd, Pb, Cr,

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Ni, Cu a Zn. Obsah kovů ve svalovině tržních kaprů se pohyboval pod hygienickými limity platnými v ČR pro jednotlivé kovy. Výjimkou byl mírně zvýšený obsah niklu (nad 0,5 mg/kg) ve svalovině dvou kaprů. Mezi jednotlivými rybníky nebyl signifikantní rozdíl v obsahu Cd, Pb, Cr, Ni a Cu ve svalovině kaprů. Signifikantně vyšší ($P < 0,01$) hodnoty Hg a Zn byly zjištěny ve svalovině kaprů z rybníka Tovaryš ve srovnání s hodnotami z dalších sledovaných rybníků. Ve svalovině kaprů z rybníka Dřemliny a Bezdrev byl zjištěn signifikantně vyšší obsah arzenu ($P < 0,01$) ve srovnání s hodnotami naměřenými u kaprů z rybníka Regent. Signifikantně vyšší ($P < 0,01$) obsah Hg byl naměřen ve svalovině, Cd a Zn v ledvinách, Cu v játrech ve srovnání s obsahem těchto kovů v ostatních tkáních. Signifikantně vyšší obsah ($P < 0,01$) mědi a zinku byl zjištěn v jikrách ve srovnání s mlíčem. Obsah kovů v sušíně sedimentů dna sledovaných rybníků se pohyboval v rozmezí hodnot charakteristických pro poměrně nezatížené rybníky v podmínkách ČR. Pouze v sedimentu dna rybníka Tovaryš byl zjištěn zvýšený obsah Pb, Cu a Zn. Předložená práce je příspěvkem ke zhodnocení kvality kaprů – hlavních zástupců tržních ryb na území ČR. Přispívá k naplňování strategie bezpečnosti potravin.

Klíčová slova: *Cyprinus carpio* L.; svalovina; játra; ledviny; gonády; hygienický limit; rizikové faktory; AAS

Food hygiene currently becomes a priority within the EU. It is evidenced by accepting a huge amount of regulations focused on veterinary hygiene of food, establishment of the European authority for sanitary food safety (EFSA) and others. This fact is either a reaction to the problems with food hygiene in EU countries (listerioses, BSE, dioxine intoxications, etc.), or getting to know the necessity of solving the problems of food hygiene, mainly at the level of their safety. The Czech Republic, under the effect of getting closer to EU, develops a great effort in following the current trend in the development of food hygiene in EU countries. This effort is focused on the field of strategy of food safety (agreement of the government of the Czech Republic No.1320/2001).

Large amounts of foreign substances enter the environment due to anthropogenic activities. They usually end in surface waters and in organisms living in them. From the point of view of food safety, this brings the necessity to pay an increased attention to marketable fish, either for local market, or for export. With the common carp being the major marketable fish in the Czech Republic in 87–90% (Vácha, 1998; Vostradovský, 2002), attention must be focused to this species cultivated in ponds.

With regards to the metals, major attention has been previously paid to mercury. Svobodová *et al.* (1975) dealt with the problems of mercury content in pond environmental components, checking the total mercury content in muscle of common carp and in bottom sediments of ponds filled from Blanice river in the region of Vodňany. Total mercury content in tissues of common carp and in other components of pond biocoenoses in the region

of Třeboň and Pardubice was stated by Svobodová *et al.* (1982). Total mercury content in muscle of marketable common carp from ponds at Pohořelice (Mašek *et al.*, 1977), South Bohemia (Hruška, 1979), South Moravia (Svobodová and Boháčková, 1979) and North Moravia (Šubrtová and Pavelka, 1988) was always found below 0.1 mg/kg muscle, i.e. below the hygienic limit value.

Much less, attention was paid to other toxic metals as lead and cadmium, compared to mercury. Svobodová *et al.* (1985) determined in muscle of common carp from Velká Podvinice pond (close to a road with dense traffic) lead values ranging from BLD (below the limit of determination) to 0.17 mg/kg muscle, in that from Velký Ústavní pond (far from dense traffic) values ranging in 0.036–0.10 mg/kg. These values were below the hygienic limit for lead. Šubrtová and Pavelka (1988) came to the same conclusion when they analyzed 116 specimens of common carp from North Moravian ponds and found values of lead ranging in 0.02–0.33 mg/kg muscle. Drbal and Bican (1981) stated for muscle of marketable common carp from Vodňany, Třeboň and Klatovy region mean value of lead content 0.28 mg/kg dry matter. Mašek *et al.* (1977) found in muscle of common carp from Pohořelice ponds mean amount of cadmium 0.05 mg/kg muscle. In contrast, Šubrtová and Pavelka (1988) reported high values of cadmium in muscle of common carp from ponds in Ostrava, mean value was 0.213 mg/kg fresh tissue.

The first large-scale monitoring of the content of foreign substances, including metals, was performed on pond common carp in 1991–1993.

Attention was focused on marketable common carp from 9 stabilization ponds and from 4 ponds without any evident source of pollution. There were mostly ponds in South and West Bohemia (Svobodová *et al.*, 1999). A repeated examination of tissues of marketable common carp and of bottom sediments from Dřemliny (Randák *et al.*, 2001a) and Buzický ponds (Randák *et al.*, 2001b) was performed in 1999. Examination in 1991–1993 and in 1999 showed that apart from negligible exceptions, hygienic limits for content of metals in muscle of marketable common carp were not exceeded. The ponds under study were stated to be relatively unloaded with metals.

This paper deals with the results of checking the content of metals in tissues of marketable common carp and in bottom sediments of 5 ponds of South and West Bohemia in 2000–2001. It is linked to the previous monitoring and extended with the determination of the content of arsenic. It is a contribution to the assessment of fish as a safe food, and thus to fulfilling the strategy of food safety.

MATERIAL AND METHODS

Monitoring the content of metals in tissues of marketable common carp (*Cyprinus carpio* L.) and in bottom sediments was performed in autumns of 2000 and 2001 during harvests of the following ponds (Figure 1).

Regent (52 ha) – owned by the Czech Fish Culture, Ltd., Mariánské Lázně. It is situated in cadastre of Holubín village, filled from three independent inflows, last pond in a pond system. A semiintensification pond managed in two-year cycle. A tarmacadam plant is situated nearby the pond. Although it is not in a direct contact with the pond, it is in its gravity field. Common carp is the main species produced (70 t of marketable fish harvested), followed by tench, predatory and herbivorous species.

Tovaryš (16 ha) – owned by Land Fund, hired by Kormorán plus Ltd. It is situated in cadastre of Sepekov village. Filled from Milevský and Farkův brooks. A semiintensification pond managed in an-

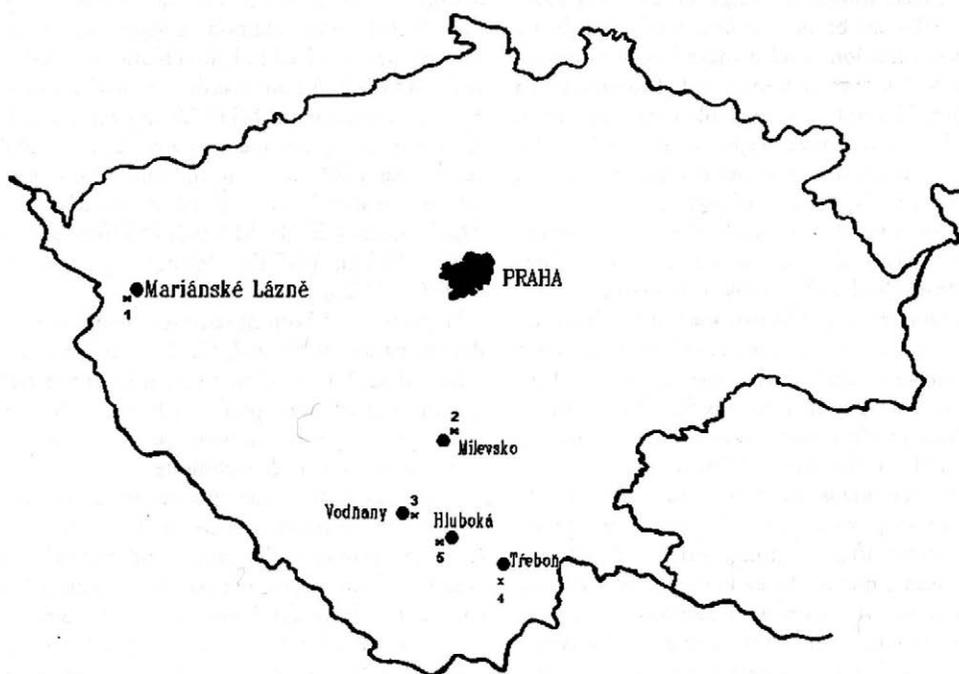


Figure 1. Map of ponds under study in the region of South and West Bohemia (1 – Regent, 2 – Tovaryš, 3 – Dřemliny, 4 – Horusický, 5 – Bezdrev)

nual cycle. Potential sources of industrial pollution in the neighborhood (air-conditioning works at Milevsko and a tarmacadam plant) are not in a direct contact with the pond but in its drainage area. Common carp is the main species produced (10 t of marketable fish harvested), followed by tench, pike and grass carp.

Dřemliny (57 ha) – owned by the town Vodňany. It is situated in cadastral of Vodňany town and Čičenice village. Filled from amelioration system and from overflows of Strpský and Čažárka ponds. Until 1997, it has been used for accumulation of communal and food processing plant waste waters, partly flowing through Čažárka stabilization pond. Later on, waste waters were purified at a purification unit and purification was finished in Čažárka pond. The pond is managed in annual cycle with the majority of common carp stock (over 90%). Ninety tons of common carp are harvested every autumn, while ten tons are harvested during summer catches. Further species produced here involve tench, herbivorous and predatory species.

Horusický (438 ha) – owned by the Fish Culture Třeboň, Ltd. It is situated in cadastral of Horusice village. Filled from three independent inflows, Zlatá stoka, Bukovský brook and Bošilecký pond. It is a semiintensification pond managed in two-year cycle, stocked with ca. 330 thousand of common carp stockfish. Harvest of marketable common carp is about 500 t, supplemental species produced involve tench, predatory and herbivorous species. There is no evidence of any source of pollution.

Bezdrav (434 ha) – owned by the Pond construction Hluboká Ltd. It is in cadastral of Zliv village. Filled from Bezdravský brook. It is a semiintensification pond managed in two-year cycle. Common carp is the main species produced (harvest of 450 t of marketable carp), further species produced involve predator and herbivorous fish. The pond accumulates purified waste waters from purification units at Zliv, Češňovice and Pištěn.

Seven specimens of three- to four-year-old common carp were sampled from every pond. Immediately after catching, fish were weighed and tissues (muscle, liver, kidney, gonads) were sampled. Muscle samples of common carp from all ponds under study were sampled and analyzed individually. The same protocol was used for samples of liver, kidney and gonads from Regent pond. For common carp from the other ponds studied, 2 pooled samples of liver, kidney and gonads were

taken and analyzed (1 pooled sample of males and 1 pooled sample of females). Samples were placed in microtene bags, labeled and put into a freezing box at -18°C . Bottom sediments were sampled by means of benthos sampler from various part of the pond. A pooled sample was made and put in a freezing box at -18°C as well.

Tissue samples of marketable common carp and samples of bottom sediments were analyzed for the content of mercury, arsenic, lead, cadmium, chrome, nickel, copper and zinc. Determination of the total mercury content in fish tissues and in bottom sediments was performed by means of AAS method using a single-purpose mercury analyzer AMA-254 (ALTEC Ltd.). Determination of Pb, Cd, Cr, Cu and Ni in fish tissues was performed by means of AAS electrothermic technique, Zn was determined by means of AAS flame technique (all on Z-5000 apparatus from Perkin Elmer) after mineralization. Mineralization of fish tissues for determination of metals was carried out in laboratory autoclaves with microwave heating, using nitric acid and hydrogen peroxide (Uniclever apparatus from Plasmatronica). Samples for the determination of arsenic were processed as above and burnt in muffle oven (450°C) with addition of magnesium nitrate. The ash was dissolved in hydrochloric acid, As^{V} was reduced to As^{III} . Arsenic was determined by means of hydride technique on MHS-20 apparatus attached to atomic absorption spectrometer. Accuracy of the results was validated using the following standard reference materials: CRM No 278 (mussel tissue of *Mytilus edulis* – BCR); MA-B-3/TM (fish homogenate – IAEA); TORT-2 (lobster hepatopancreas – NRC-CNRC).

Preparation of bottom sediment samples for the determination of Pb, Cd, Cr, Ni, Cu, Zn and As followed ISO 114 66 standard using aqua regia. Determination was performed by AAS flame technique except for As, which was determined by means of AAS hydride technique.

Statistical comparison was made for values of content of the respective metals in muscle of fish from the ponds under study and for values of content of the respective metals in analyzed tissues of fish from the Regent pond. Results were tested using ANOVA in Statgraphics software, and MS-EXCEL 7.0. In order to interpret the results, hygienic limits concerning fish quoted in the Decree of the Ministry of Health of the Czech Republic No. 298/1997 Sb. were used, as

well as reference values of metal content in bottom sediments gained from ponds relatively not-loaded with foreign substances (Svobodová *et al.*, 1996).

RESULTS

Values of metal contents in tissues of marketable common carp from ponds under study are given in Tables 1 (Regent pond), 2 (Tovaryš pond), 3

(Dřemliny pond), 4 (Horusický pond) and 5 (Bezdrav pond). Comparison of metal contents in tissues of marketable common carp from ponds under study is shown on Figure 2. Table 6 reports metal contents in dry matter of bottom sediments from ponds under study.

Mercury

Table 1. Content of metals in tissues of marketable common carp from Regent pond (sampled on 12. 10. 2000)

Tissue	n		Fish weight (g)	Hg	As	Cd	Pb	Cr	Ni	Cu	Zn
				mg/kg ww							
Muscle	7	mean	3 235.7	0.031	0.073	0.0014	< 0.05	0.069	0.059	0.26	3.2
		SD	427.7	0.013	0.019	0.0011	–	0.049	0.058	0.06	0.5
Liver	7	mean	3 235.7	0.007	0.042	0.019	< 0.05	0.071	0.092	5.06	64.4
		SD	427.7	0.001	0.009	0.006	–	0.025	0.068	1.15	32.7
Kidney	7	mean	3 235.7	0.010	0.062	0.062	< 0.05	0.090	0.177	1.24	159.2
		SD	427.7	0.003	0.015	0.023	–	0.049	0.106	0.11	40.4
Eggs	4	mean	3 238.8	0.002	0.060	0.001	< 0.05	0.094	0.114	1.05	55.1
		SD	587.5	0.001	0.025	0.001	–	0.024	0.021	0.18	12.5
Testes	3	mean	3 231.7	0.003	0.030	< 0.001	< 0.05	0.172	0.136	0.17	7.1
		SD	176.2	0.001	0.009	–	–	0.082	0.061	0.04	1.0

ww = wet weight

Table 2. Content of metals in tissues of marketable common carp from Tovaryš pond (sampled on 8. 10. 2001)

Tissue	n		Fish weight (g)	Hg	As	Cd	Pb	Cr	Ni	Cu	Zn
				mg/kg ww							
Muscle	7	mean	1 688.6	0.048	0.116	< 0.001	< 0.05	0.058	0.180	0.12	6.5
		SD	181.1	0.006	0.018	–	–	0.024	0.084	0.02	1.3
Liver	(sample 1)	female	1 672.5	0.009	0.065	0.003	< 0.050	0.113	0.142	1.381	78.2
	(sample 2)	male	1 710.0	0.010	0.084	0.003	< 0.050	0.109	0.456	3.595	82.7
Kidney	(sample 1)	female	1 672.5	0.011	0.120	0.032	< 0.050	0.076	0.227	0.819	247.0
	(sample 2)	male	1 710.0	0.011	0.091	0.026	< 0.050	0.062	0.234	0.935	214.1
Eggs	(sample 1)	female	1 672.5	0.005	0.033	< 0.001	< 0.05	0.050	0.125	0.96	82.9
Testes	(sample 2)	male	1 710.0	0.003	0.037	< 0.001	< 0.05	0.052	0.112	0.26	12.4

Pooled sample 1 = fish No. 2, 5, 7

Pooled sample 2 = fish No. 1, 3, 4, 6

ww = wet weight

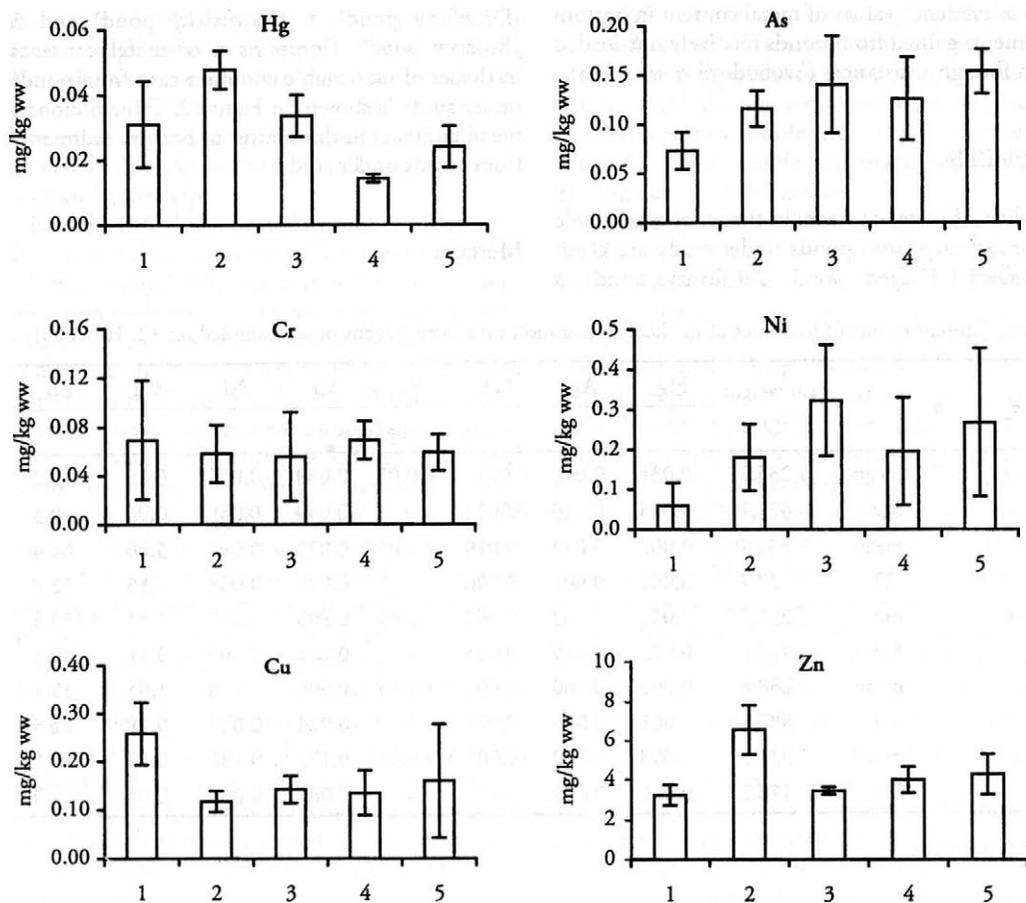


Figure 2. Comparison of the contents of metals in muscle of marketable common carp from the ponds under study (1 – Regent, 2 – Tovaryš, 3 – Dřemliny, 4 – Horusický, 5 – Bezdrev)

Total mercury content in muscle of marketable common carp in all ponds under study was below 0.1 mg/kg (valid hygienic limit). The highest values of total mercury content were found in muscle of common carp from Tovaryš pond, the lowest ones in muscle of marketable common carp from Horusický and Bezdrev ponds. Highly significant differences ($P < 0.01$) in mercury content in muscle were found among common carp from ponds Horusický – Tovaryš, Bezdrev – Tovaryš and Horusický – Dřemliny. Significantly higher value ($P < 0.05$) in total mercury content was found in common carp from Tovaryš pond compared to that in Dřemliny pond. Considering the distribution of mercury in individual tissues, the highest values were detected in muscle, followed by kidney

and liver while the lowest values were in gonads. Difference in mercury content in muscle compared to other tissues of fish from the Regent pond was significant ($P < 0.01$). Mercury content in eggs and in testes was nearly identical.

The sequence of ponds according to total mercury content in dry matter of bottom sediments is similar to that according to mercury content in muscle. It means the highest values in Tovaryš pond and the lowest ones in Horusický and Bezdrev ponds.

Arsenic

The content of arsenic in muscle of marketable common carp from all ponds under study was

Table 3. Content of metals in tissues of marketable common carp from Dřemliny pond (sampled on 12. 10. 2001)

Tissue	n	Fish weight (g)	Hg	As	Cd	Pb	Cr	Ni	Cu	Zn	
			mg/kg ww								
Muscle	7	mean	1 980	0.034	0.141	< 0.001	< 0.05	0.056	0.323	0.14	3.5
		SD	276.5	0.006	0.049	–	–	0.037	0.139	0.03	0.2
Liver	(sample 1)	female	1 775.0	0.007	0.043	0.014	< 0.050	0.070	0.265	4.424	43.0
		male	2 133.8	0.006	0.052	0.009	< 0.050	0.054	0.320	2.508	59.3
Kidney	(sample 1)	female	1 775.0	0.009	0.068	0.058	< 0.050	0.070	0.389	1.139	203.3
		male	2 133.8	0.012	0.117	0.044	< 0.050	0.038	0.358	1.100	194.6
Eggs	(sample 1)	female	1 775.0	0.003	0.031	< 0.001	< 0.05	0.057	0.111	1.16	71.2
Testes	(sample 2)	male	2 133.8	0.003	0.034	< 0.001	< 0.05	0.059	0.092	0.30	7.5

Pooled sample 1 = fish No. 2, 5, 7

Pooled sample 2 = fish No. 1, 3, 4, 6

ww = wet weight

Table 4. Content of metals in tissues of marketable common carp from Horusický pond (sampled on 17. 10. 2001)

Tissue	n	Fish weight (g)	Hg	As	Cd	Pb	Cr	Ni	Cu	Zn	
			mg/kg ww								
Muscle	7	mean	2 190.0	0.014	0.126	< 0.001	< 0.05	0.069	0.196	0.14	4.0
		SD	166.8	0.001	0.042	–	–	0.016	0.135	0.05	0.7
Liver	(sample 1)	female	2 355.0	0.005	0.072	0.023	< 0.050	0.039	0.095	4.913	106.3
		male	2 124.0	0.005	0.064	0.006	< 0.050	0.071	0.121	2.321	97.5
Kidney	(sample 1)	female	2 355.0	0.008	0.086	0.085	< 0.050	0.042	0.451	1.112	310.7
		male	2 124.0	0.009	0.067	0.053	< 0.050	0.025	0.210	0.950	303.3
Eggs	(sample 1)	female	2 355.0	0.002	0.029	< 0.001	< 0.05	0.088	0.190	0.69	102.6
Testes	(sample 2)	male	2 124.0	0.002	0.044	< 0.001	< 0.05	0.045	0.148	0.25	12.0

Pooled sample 1 = fish No. 2, 5, 7

Pooled sample 2 = fish No. 1, 3, 4, 6

ww = wet weight

below 1 mg/kg (valid hygienic limit). The highest values of arsenic content were registered in muscle of common carp from Bezdrev and Dřemliny ponds, the lowest ones in muscle of common carp from the Regent pond. Difference between values of arsenic in muscle of common carp from Regent and Bezdrev ponds was significant ($P < 0.01$), as well as between Regent and Dřemliny ponds. The sequence of tissues of marketable common carp ac-

ording to the arsenic content is as follows: muscle > kidney > liver > gonads. The arsenic content in muscle of fish from the Regent pond was significantly higher ($P < 0.05$) compared to that in their liver. Insignificant difference was found between arsenic content in eggs and in testes.

Content of arsenic in bottom sediments of ponds under study was relatively equalized and ranged in 6.6–19.2 mg/kg dry matter. The lowest

Table 5. Content of metals in tissues of marketable common carp from Bezdrev pond (sampled on 23. 10. 2001)

Tissue	n	Fish weight (g)	Hg	As	Cd	Pb	Cr	Ni	Cu	Zn
			mg/kg ww							
Muscle	7 mean	2 469.3	0.024	0.154	< 0.001	< 0.050	0.059	0.267	0.16	4.3
	SD	204.6	0.006	0.023	–	–	0.015	0.186	0.12	1.0
Liver	(sample 1) female	2 337.5	0.006	0.094	0.021	< 0.050	0.037	0.181	7.773	96.2
	(sample 2) male	2 522.0	0.010	0.123	0.015	–	0.054	0.154	4.953	116.1
Kidney	(sample 1) female	2 337.5	0.008	0.108	0.102	< 0.050	0.163	0.412	1.313	294.8
	(sample 2) male	2 522.0	0.012	0.131	0.064	< 0.050	0.037	0.254	1.156	328.7
Eggs	(sample 1) female	2 337.5	0.002	0.077	< 0.001	< 0.05	0.098	0.268	0.98	91.7
Testes	(sample 2) male	2 522.0	0.004	0.060	< 0.001	< 0.05	0.056	0.120	0.33	24.0

Pooled sample 1 = fish No. 2, 5, 7

Pooled sample 2 = fish No. 1, 3, 4, 6

ww = wet weight

Table 6. Content of metals in dry matter of bottom sediments from the ponds under study

Locality	Date of sampling	Hg	As	Cd	Pb	Cr	Ni	Cu	Zn
		mg/kg							
Regent	12. 10. 2000	0.131	14.8	0.78	21.3	22.2	32.1	32.8	153.2
Tovaryš	08. 10. 2001	0.358	19.2	1.24	51.2	149.5	63.1	49.9	440.9
Dřemliny	06. 11. 2001	0.063	12.0	< 0.3	29.6	29.4	13.1	13.1	50.6
Horusický	17. 10. 2001	0.047	10.3	0.78	26.0	36.4	19.0	21.2	84.0
Bezdrev	23. 10. 2001	0.033	6.6	< 0.3	6.5	10.3	7.7	4.2	20.7

value was detected in bottom sediment of Bezdrev pond while the highest one in bottom sediment of Tovaryš pond.

Cadmium

The content of cadmium in muscle of marketable common carp from all ponds under study was markedly below 0.1 mg/kg (valid hygienic limit). Moreover, this content was even below the limit of determination (0.001 mg/kg) in muscle of all fish from Tovaryš, Dřemliny, Horusický and Bezdrev

ponds. Low determinable values were found in muscle of common carp from Regent pond. The sequence of tissues of marketable common carp from Regent pond is, according to cadmium content, as follows: kidney > liver > muscle > gonads. Cadmium content in kidney of common carp from Regent pond was significantly higher ($P < 0.01$) compared to that in muscle, liver and in gonads, cadmium content in liver was significantly higher ($P < 0.01$) than that in muscle and gonads. Cadmium content in eggs and in testes was nearly identical and below the limit of determination (0.001 mg/kg) practically in all cases.

Values of cadmium content in bottom sediments of Dřemliny and Bezdrev ponds were below the limit of determination (0.03 mg/kg dry matter), sediments of other ponds did not exceed 1.24 mg/kg dry matter, which was registered in Tovaryš pond.

Lead

The content of lead in muscle of marketable common carp from all ponds under study was markedly below 0.5 mg/kg (valid hygienic limit). All measured values of lead content in muscle and in other tissues of marketable common carp from the ponds under study were below the limit of determination (0.05 mg/kg).

The sequence of ponds monitored according to the content of lead in dry matter of bottom sediments was as followed: Tovaryš > Dřemliny > Horusický > Regent > Bezdrev. The highest registered value was 51.2 mg/kg dry matter (Tovaryš pond), the lowest one was 6.5 mg/kg dry matter (Bezdrev pond).

Chrome

The content of chrome in muscle of marketable common carp from all ponds under study was below 0.3 mg/kg (earlier valid hygienic limit). Values of chrome content in muscle of common carp from individual ponds were nearly at the same level, without any significant differences. Similarly, no significant differences were found for the values of chrome content in individual tissues (muscle, liver, kidney, eggs and testes) of common carp from the Regent pond.

The highest content of chrome was found in bottom sediment of Tovaryš pond (149.5 mg/kg dry matter), the lowest one in bottom sediment of Bezdrev pond (10.3 mg/kg dry matter). Sequence of the ponds studied according to the content of chrome in dry matter of pond bottom sediments was as followed: Tovaryš > Horusický > Dřemliny > Regent > Bezdrev.

Nickel

The content of nickel in muscle of marketable common carp from all ponds under study did not exceed the value of 0.5 mg/kg (valid hygienic limit). Slightly higher values were detected for 1 specimen

of common carp from Dřemliny pond and for another 1 specimen of common carp from Bezdrev pond only. The sequence of ponds studied according to the content of nickel in muscle of marketable common carp was as followed: Dřemliny > Bezdrev > Horusický > Tovaryš > Regent. No significant difference was found in values of nickel content in muscle of marketable common carp from the individual ponds studied. Similarly, no significant difference was found for content of nickel in various tissues of common carp from Regent pond.

The highest content of nickel was found in bottom sediment of Tovaryš pond (63.1 mg/kg dry matter), the lowest one in bottom sediment of Bezdrev pond (7.7 mg/kg dry matter). Sequence of the ponds studied according to the content of nickel in dry matter of pond bottom sediments was as followed: Tovaryš > Regent > Horusický > Dřemliny > Bezdrev.

Copper

The content of copper in muscle of marketable common carp from all ponds under study was markedly below 10 mg/kg (valid hygienic limit). The highest value was detected for muscle of common carp from Regent pond, values found in muscle of fish from other ponds were equal. Values of copper content in muscle of marketable fish from all ponds studied were not significantly different. Considering the values of copper content in various tissues of fish, they were in the following sequence: liver > kidney > eggs > testes > muscle. Values of copper content in liver were significantly higher ($P < 0.01$) compared to those found in muscle, kidney and gonads. Values of copper content in kidney were significantly higher ($P < 0.05$) than those found in muscle. Significantly higher ($P < 0.01$) copper content was found in eggs compared to testes.

Sequence of ponds according to copper content in dry matter of their bottom sediments were as followed: Tovaryš > Regent > Horusický > Dřemliny > Bezdrev. The highest value was detected in bottom sediment of Tovaryš pond (49.9 mg/kg dry matter), the lowest one in Bezdrev pond (4.2 mg/kg dry matter).

Zinc

The content of zinc in muscle of marketable common carp from all ponds under study was markedly

below 50 mg/kg (valid hygienic limit). The highest value of zinc content was found in muscle of fish from Tovaryš pond. Values of zinc content found in muscle of fish from the other ponds (Regent, Dřemliny, Horusický, Bezdrev) were practically the same and significantly lower ($P < 0.01$) compared to that found for muscle of fish in Tovaryš pond. Sequence of tissues of marketable common carp according to zinc content was as followed: kidney > liver > eggs > testes > muscle. Significant difference ($P < 0.01$) was found in zinc content of the following tissues: kidney – muscle, kidney – liver, kidney – gonads and liver – muscle. Significantly higher ($P < 0.01$) content of zinc was found in eggs compared to testes.

The highest value of zinc content was found in bottom sediment of Tovaryš pond (440.9 mg/kg dry matter), the lowest one from Bezdrev pond (20.7 mg/kg dry matter). Sequence of ponds according to content of zinc in dry matter of their bottom sediments was as followed: Tovaryš > Regent > Horusický > Dřemliny > Bezdrev.

DISCUSSION

Bottom sediments and tissues of fish are the main indicators of loading the pond environment with metals (Svobodová *et al.*, 1996). These two components of aquatic ecosystem were used for assessment of 5 selected ponds in South and West Bohemia. Common carp was used as indicator fish, as it has been cultivated in all studied ponds either in 1-year-cycle (Tovaryš and Dřemliny ponds), or in 2-year-cycle (Regent, Horusický and Bezdrev ponds). Common carp utilized natural feed in these ponds and they were supplemented with cereals.

The detected content of metals in muscle of marketable common carp from the ponds under study was below the hygienic limits valid for the particular metals, except for a slightly increased content of nickel (above 0.5 mg/kg) in muscle of two common carp specimens. No significant difference was found in content of Cd, Pb, Cr, Ni and/or Cu in muscle of common carp among the ponds studied. Significantly higher values ($P < 0.01$) of Hg and Zn were found in muscle of common carp from Tovaryš pond compared to values from other ponds under study. Significantly higher content of arsenic ($P < 0.01$) was found in muscle of common carp from Dřemliny and Bezdrev ponds, compared to

values found in common carp from Regent pond. Comparing these results of metal content in muscle of marketable fish to those of the wide-scale monitoring of 13 ponds of South and West Bohemia in 1991–1993 (Svobodová *et al.*, 1999), we find them nearly the same. Based on results of the monitoring in 1991–1993, it was proposed to use so-called “reference intervals” of values of metal content in muscle of common carp from pond environment relatively not loaded with these elements: Hg – 0.020 to 0.060 mg/kg, Cd – 0.0025 to 0.0150 mg/kg, Pb – 0.010 to 0.030 mg/kg, Cr – 0.03 to 0.07 mg/kg, Ni – 0.1 to 0.2 mg/kg, Cu – 0.2 to 0.5 mg/kg, Zn – 4 to 8 mg/kg. Values found in 2000 and 2001 in 5 selected ponds ranged within the stated intervals, except for slightly increased values of chrome content (Regent pond) and nickel content (Dřemliny and Bezdrev ponds).

Further interpretation of the results gained is limited due to the lack of literature data. It is not possible to make a comparison of content of metals in muscle of common carp kept in ponds with those from open waters. Open waters are stocked with common carp reared in ponds until the age of 2–4 years and for this reason, common carp is not included among indicator species in open waters. Data from abroad on metal content in tissues of common carp are also minimal. Marketable common carp is mostly produced in ponds in Central and Eastern Europe (Vácha, 1998).

Distribution of the content of metals in the studied tissues of marketable common carp is not even. Significantly higher ($P < 0.01$) content of Hg was found in muscle, of Cd and Zn in kidney, of Cu in liver, compared to their contents in other tissues. The sequence of tissues according to the content of individual metals refers to that reported for common carp (Rehboldt *et al.*, 1976; Svobodová *et al.*, 1982, 1999; Shine *et al.*, 1998; Goldstein and DeWeese, 1999; Jeng *et al.*, 1999; De Smet *et al.*, 2001), for other cyprinids (Seymore *et al.*, 1996; Vigh *et al.*, 1996), as well as for other fish species (Sastri and Shukla, 1993; Deb and Santra, 1997; Pip and Stepaniuk, 1997; Shine *et al.*, 1998; Szarek-Gwiazda, 1999).

Except for Cu and Zn, no significant difference was found in metal content in eggs and testes. Significantly higher content ($P < 0.01$) of copper and zinc was found in eggs compared to testes. Marquez *et al.* (1998) stated, in accordance with our results, higher content of copper and zinc in

tissues of female gonads of *Notothenia coriiceps* compared to testes. Seymore *et al.* (1996) also stated higher content of zinc in ovaries than in testes of *Barbus marequensis*.

Large monitoring of metal content in dry matter of pond bottom sediments was performed in the Czech Republic in 1991–1993 (Svobodová *et al.*, 1996). Values found can be considered characteristic for ponds relatively not loaded, under conditions of the Czech Republic: Hg – 0.05 to 0.5 mg/kg, Cd – 0.01 to 0.3 mg/kg, Pb – 5–30 mg/kg, Cr – 40 to 150 mg/kg, Ni – 15 to 70 mg/kg, Cu – 5 to 40 mg/kg, Zn – 25 to 150 mg/kg, dry matter of sediment. Content of metals in dry matter of bottom sediment from the five ponds of South and West Bohemia under study ranged within the interval characteristic for relatively not loaded ponds. Increased content of Pb, Cu and Zn was registered only in bottom sediment of Tovaryš pond. Comparing the content of metals in dry matter of bottom sediments of ponds under study, it was evident, that the highest contents of all metals were found in Tovaryš pond (with sources of industrial pollution). However, contents of metals in muscle of marketable common carp from this pond were not higher than those from other ponds, except for Hg and Zn. It might be due also to the fact that the Tovaryš pond is managed in an annual cycle. This means that fish are kept here for about 7 months as a maximum, contrary to ponds managed in two-year-cycle (Regent, Horusický and Bezdrév) where the fish are kept for about 19 months.

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Simultaneous analysis of sex determination and κ -casein genotypes from bovine preimplantation embryos

Simultánna analýza pohlavia a genotypov κ -kazeínu z preimplantačného embrya hovädzieho dobytku

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ABSTRACT: Sex determination and κ -casein genotypes of preimplantation bovine embryo were simultaneously analyzed from one blastomere removed from the 8–16 cell stage embryo. Microaspirated blastomere was lysed and subsequently was subjected to the multiplex PCR. The efficiency of sexing and κ -casein genotyping in the multiplex PCR was 82%, based on the amplification of Y-specific locus using κ -casein internal standard. Digestion of the PCR product with *Hind III* allowed the differentiation of κ -casein *A* and *B* alleles and did not digest the sex related fragment. Both Y-specific and κ -casein genotypes were detected by electrophoretic separation on the same agarose gel. This study shows that bovine preimplantation embryos can be successfully tested for sex and κ -casein genotypes simultaneously. This method can decrease the cost of the sample analysis and speed up the determination of economically interesting genetic markers.

Keywords: bovine; embryo; sex determination; κ -casein

ABSTRAKT: Determinácia pohlavia a genotypov κ -kazeínového génu boli simultánne analyzované z jednej blastoméry získanej z 8- až 16-bunkového vývojového štádia embrya hovädzieho dobytku. Mikroaspirovaná blastoméra bola lyzovaná a následne bola uskutočnená multiplex PCR. Úspešnosť určenia pohlavia a genotypov κ -kazeínového génu metódou multiplex PCR, na základe amplifikácie Y-špecifického lokusu a κ -kazeínu ako kontroly PCR ("internal standard") bola 82 %. Štiepenie PCR produktu pomocou *Hind III* umožňuje rozlíšenie alely *A* a *B* κ -kazeínového génu, pričom sa neštiepi fragment determinujúci pohlavie. Všetky kombinácie zistených genotypov boli detegované elektroforetickou separáciou na agarózovom géli. Táto práca ukázala, že preimplantačné hovädzie embryo môže byť úspešne použité na simultánnu analýzu pohlavia a genotypov κ -kazeínu. Táto metóda môže znížiť cenu analyzovanej vzorky a zvýšiť rýchlosť determinácie ekonomicky významných genetických markerov.

Kľúčové slová: hovädzí dobytok; embryo; stanovenie pohlavie; κ -kazeín

Predetermined sex of bovine embryos in combination with genetic polymorphism of marker gene would be of economical importance in modern cattle breeding. Characteristic of embryos, based on genotyping of a few blastomeres is a faster way to obtain the offspring with desired genotype, to

short the generation interval and to decrease cost of the breeding program.

In several studies on sex determination and MAS genotyping, a few cells from bisection on a bovine embryo were used. After transfer to recipients, bisected blastocysts were capable of normal develop-

ment (Bredbacka *et al.*, 1994; Carbonneau *et al.*, 1997). It was also shown that removing only one cell from the 16 to 32 cell stage bovine embryo did not alter subsequent development of the embryo *in vitro* and the material obtained was sufficient for successful sex determination by the PCR method (Machaty *et al.*, 1993).

Using a single cattle cell for genotyping might be less invasive to the embryo than a large biopsy. However, the low amount of the DNA available for subsequent characterization by PCR amplification permits the determination of only a limited number of loci. We aimed at examining how this method works in the case of multiple genotype analysis (sex and κ -casein loci) using DNA isolated from one microaspirated bovine blastomere.

In the present study, we applied multiplex PCR to bovine blastomeres and demonstrated that multiple analyses can be performed accurately. This method can decrease the cost of the sample analysis and increase the speed of determination of sex and κ -casein gene.

MATERIAL AND METHODS

Embryo production

These embryos were produced *in vitro* using IVM and IVF procedures of oocytes of Holstein breed as described previously (Menck *et al.*, 1997). All the embryos were provided from INRA (BDB, Jouy en Josas, France).

Biopsy procedure

Four days after *in vitro* fertilization, a random selected 61 embryos at the 8–16 cell stage were maintained in B2 medium supplemented with 10% FCS at 39°C under 5% CO₂ in air. Before biopsy, the embryos were washed 3 times in M199-Hepes medium (Life Technologies, Paisly, United Kingdom) supplemented with 0.3% ovine serum albumin. Biopsies were performed in medium M199-Hepes containing 7 µg/ml of cytochalasin B (Sigma, St. Louis, USA). Microaspiration of one blastomere was made with a micropipette (the diameter of tip approximately 35 µm) by gentle suction under an Olympus microscope using two micromanipulators (Alcatel, Annency, France). Biopsed embryos were transferred individually in B2 medium (10%

FCS, 5% CO₂, 39°C) and cultured to the hatched blastocyst stage.

Cell lysis

Isolated single blastomeres were washed individually in PBS and then lysed at 56°C for 1 h in 20 µl of 1× PCR buffer containing 0.20 µg/µl proteinase K (Sigma, Norwalk, USA) overlaid with 15 µl of mineral oil. Proteinase K was then deactivated at 96°C for 10 minutes.

Multiplex polymerase chain reaction

The sex of bovine embryos was determined by multiplex PCR using simultaneous analyses of the Y-specific and the κ -casein loci as an internal amplification control of DNA quality. A final volume of the reaction mixture was 30 µl. Briefly, 20 µl of the lysed product was used as a template. PCR mixture further contained 1× PCR buffer, 1.5 mM MgCl₂, 200 µM dNTPs, 200 µM primers specific for κ -casein (5'-GCT GAG CAG GTA TCC TAG TTA T-3' and 5'-CTT CTT TGA TGT CTC CTT AGA G-3'; Vašíček *et al.*, 1995) and Y-specific locus (5'-CCT CAG CTG CTT GAA AGT TC-3' and 5'-GAT CTG TAA CTG CAA ACC TGG-3'; Chrenek *et al.*, 2001) and 1 U of Taq DNA polymerase. After the first denaturation step at 95°C for 4 min, samples were subjected to 35 cycles of denaturation at 94°C for 40 s, annealing at 53°C for 40 s and extension at 72°C for 40 s, followed by a 5 min extension in the last cycle. In case of negative control reactions, no DNA template was used. In case of positive control, DNA isolated from spermatozoa (Chrenek, 1997) of bull used for *in vitro* fertilization was used.

PCR products of the κ -casein gene (15 µl) were digested by 5 U of restriction enzyme *Hind III* for at least 3 h at 37°C. The fragments were separated using 2.5% agarose gels containing 0.5 µg/ml ethidium bromide and visualized by UV transilluminator (256 nm).

RESULTS AND DISCUSSION

The sex determination of bovine embryo from microaspirated one blastomere (Figure 1) amplification of the κ -casein gene was used as an internal

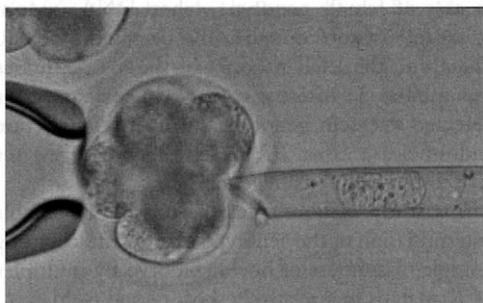


Figure 1. Microaspiration of a single blastomere from bovine preimplantation embryos at 8–16 cell stage

control. A 308 bp long PCR product specific for the amplified region of Y-chromosome was expected to be found only in the case of male genotypes

(Figure 2). Embryo samples without the male-specific band should indicate female sex. The Y-chromosome specific fragment was amplified in 23 of

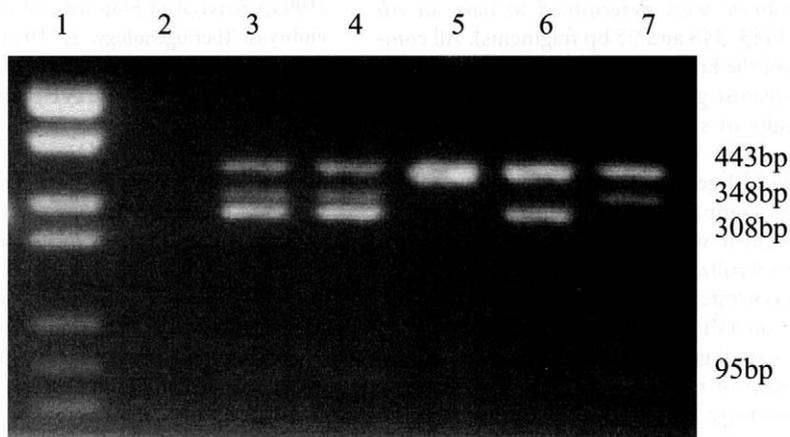


Figure 2. Agarose gel electrophoresis of multiplex PCR. Y and κ -casein fragments were amplified from multiplex-PCR samples of bovine microaspirated embryo. Lane 1 – DNA molecular weight marker (pUC/Sau 3AI); lane 2 – negative control; lanes 3, 4 – embryos with fragment pattern (308 bp) indicating male-sex and κ -casein AB genotype (443, 348 and 95bp); lanes 5 – embryos without the male-specific band indicating female sex, the AA genotype (443bp) of κ -casein is also presents; lane 6 – embryos with male sex genotype and AA genotype of κ -casein gene; lane 7 – embryos with female sex genotype and AB genotype of κ -casein gene

Table 1. The frequency of analyzed loci from bovine microaspirated embryos

Locus	No. analyzed embryos	Genetic variant	Frequency of genetic variants (%)
Sex	50/61 (82%)	male	23/50 (46%)
		female	27/50 (54%)
κ -casein	50/61 (82%)	AA	30/50 (60%)
		AB	20/50 (40%)

50 embryos tested (Table 1). In all of these samples, the analysis was validated by the amplification of the internal control. In the remaining 27 embryos, the Y-chromosome band was absent. The κ -casein specific fragment was visible in 27 of these samples. Hence, these 27 embryos were designated to carry the female genotype. To confirm that the absence of sex related fragment is not due to the absence of DNA in the sample, the PCR product of the κ -casein gene was used as an internal control.

Digestion of the PCR product with *Hind III* allowed the differentiation of κ -casein *A* and *B* alleles and did not digest the sex related fragment. Analysis of κ -casein gene was successful in 50 from 61 (82%) analyzed embryos. Thirty (60%) embryos were determined to carry an *AA* genotype (Table 1), resulting in one fragment 443 bp long and 20 (40%) embryos were determined to have an *AB* genotype (443, 348 and 95 bp fragments). All combinations of the known genotypes were detected on the same agarose gel.

The results of standard PCR analysis obtained using the DNA isolated from the spermatozoa showed the *AA* genotype for κ -casein gene. These results were compared with multiplex PCR results, where one allele was inherited from the bull used for *in vitro* fertilization (not shown).

We demonstrated that DNA content of one blastomere of an IVF bovine embryo is sufficient to genotype several genes by multiplex PCR analysis. The first step of this method is an isolation of a single blastomere by microaspiration from bovine embryos at the 8–16 cell stage and subsequent lysis of blastomere. In the second step, all lysed products and mixed with two sets of primers specific for κ -casein and Y-specific locus were subjected to the multiplex PCR.

Generally, there is no theoretical limit for the number of loci investigated in one step. On the other hand, the number of loci that could be simultaneously investigate by multiplex PCR depend on the temperature of annealing of the used set of primers, restriction endonucleases used, their compatibility with the buffers and a size of fragments. Thus, allele-specific amplification of nine loci was simultaneously achieved by PCR (Chamberlain *et al.*, 1988). From two to three loci were usually analyzed by multiplex PCR (Iakubova *et al.*, 1994; Lindersson *et al.*, 1995; Chrenek *et al.*, 1998).

The absence of amplification of the Y-chromosome specific band might indicate female sex. However, there is a risk of obtaining false results, mainly

in case of female genotype, when DNA amount in sample is not enough to be detected by PCR. Therefore, the PCR product of the κ -casein gene was used as an internal control. In this study, we detected κ -casein genotypes using the restriction endonuclease *Hind III*, which did not digest sex related fragment (male genotype).

This study demonstrates the possibility reliable determination of the genotype of different loci using a single blastomere of bovine embryo by multiplex PCR analysis in a relatively short period (4 h).

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