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Effect of oral administration of iron microemulsion on the erythrocyte profile of suckling piglets in comparison with parenteral application of iron dextran

Účinek perorální aplikace mikroemulze železa na parametry červeného krevního obrazu sajících selat ve srovnání s parenterální aplikací dextransu železa

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ABSTRACT: The aim of this study was to investigate the effect of oral application of Fe^{3+} microemulsion on the erythrocyte profile of suckling piglets compared with parenteral application of Fe^{3+} dextran. Piglets in group 1 ($n = 24$) were given 230 mg Fe^{3+} microemulsion immediately after birth. Piglets in group 2 ($n = 25$) were given 200 mg Fe^{3+} dextran *i.m.* on the 3rd day of life. The piglets had free access to prestarter and starter. Erythrocyte count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), body weight (BW) and daily weight gain (DWG) were determined. On day 7 RBC, Hb, PCV and were significantly higher in group 1 compared with group 2 ($P < 0.01$). On day 14, 21, 28 and 35, no significant differences were found between the two groups and all examined indices were comparable with physiological values. The effect of oral application of Fe^{3+} microemulsion on the erythrocyte profile under conditions of this trial was comparable with parenteral application of Fe^{3+} dextran.

Keywords: anaemia; haemoglobin concentration; erythrocyte count; packed cell volume

ABSTRAKT: Cílem práce bylo zjistit účinek perorální aplikace Fe^{3+} ve formě mikroemulze na parametry červeného krevního obrazu sajících selat ve srovnání s parenterální aplikací dextransu železa. U skupiny 1 ($n = 24$) bylo selatům aplikováno 230 mg Fe^{3+} ve formě mikroemulze ihned po narození. Selatům ve skupině 2 ($n = 25$) bylo aplikováno 200 mg Fe^{3+} vázaného na dextran *i.m.* třetí den života. Selata měla volný přístup k prestartéru a startéru. Stanovovány byly tyto parametry: počet erytrocytů (RBC), koncentrace hemoglobinu (Hb), hematokrit (PCV), střední objem erytrocytu (MCV), střední koncentrace hemoglobinu (MCH), střední barevná koncentrace (MCHC), tělesná hmotnost a denní přírůstek hmotnosti. Sedmý den byl počet erytrocytů, koncentrace hemoglobinu a hodnota hematokritu ve skupině 1 signifikantně vyšší ve srovnání se skupinou 2 ($P < 0.01$); 14., 21., 28. a 35. den nebyly nalezeny signifikantní rozdíly mezi oběma skupinami a všechny vyšetřené parametry byly srovnatelné s fyziologickými hodnotami. Účinek perorální aplikace mikroemulze železa (Fe^{3+}) na parametry červeného krevního obrazu za podmínek této studie byl srovnatelný s *i. m.* aplikací dextransu Fe^{3+} .

Klíčová slova: anémie; koncentrace hemoglobinu; počet erytrocytů; hematokrit

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Grant No. 161700002).

A piglet is born with limited iron reserve (50 mg Fe) (Venn *et al.*, 1947). In order to synthesise haemoglobin required for the piglet to prevent development of anaemia, 7–10 mg of iron per day is needed (Venn *et al.*, 1947). The sow's milk provides 1 mg Fe per day only (Kleinbeck and McGlone, 1999). Without iron supplementation, suckling piglets develop anaemia after 10–14 days postpartum (Framstad and Sjaatad, 1991; Zimmermann, 1995). Iron can be given orally or by injection. Newborn animals will absorb macromolecules intact from the small intestine by pinocytosis (Baustad, 1974). Macromolecules are transported directly to the lymphatic circulatory system and stored in spleen, liver, etc. (Klobasa *et al.*, 1991). Iron bound to macromolecules must be administered as soon as possible after birth, maximally within 6 hours (Iben, 1998; Heinritzi and Plonait, 1997) or 8 hours (Lemacher and Bostedt, 1994) after birth. If it is given later, absorption decreases significantly because of intestinal shuts (Iben, 1998).

The aim of this study was to investigate the effect of oral application of Fe^{3+} microemulsion on the erythrocyte profile of suckling piglets compared with parenteral application of Fe^{3+} dextran.

MATERIAL AND METHODS

Experimental design

Litters were kept with sows in farrowing crates on the concrete floor until weaning. All piglets were individually tattooed with a number in the ear. The piglets were not subjected to castration or tail docking. Altogether 49 piglets (6 litters) were divided randomly into 2 groups (split litters). Group 1 had 24 piglets, group 2 had 25 piglets. The design involving split litters minimised the effect of the sow. Piglets in group 1 were given 230 mg Fe^{3+} orally each in the form of microemulsion immediately after birth. The application was done using a syringe with plastic tube. To ensure proper iron application, the plastic tube was put deep into the pharynx and the swallowing movements of piglets were controlled. Because the lymphatic resorption of particles depends on their size, the size of oil drops in the used preparation was $< 1.5 \mu\text{m}$. Water soluble Fe^{3+} dextran complex is bound to oil drops if a vegetable emulsifier is used. The iron micro-

emulsion contained: water, vegetable oil, vegetable emulsifier and Fe^{3+} dextran complex. One ml of microemulsion contained 115 mg Fe^{3+} . The piglets in group 2 were injected with 200 mg Fe^{3+} dextran (*i.m.*) on the 3rd day of life. From day 3 to day 10, piglets were offered prestarter (100 mg Fe/kg). Starter (248 mg Fe/kg) was offered to all litters *ad libitum* (from day 10–35). Piglets in all litters were weaned on day 28.

Sampling

Blood from the piglets was collected on days 7, 14, 21, 28 and 35. Blood (1.5 ml) was collected from the *cranial vena cava*, using ethylenediaminetetraacetic acid (EDTA) as an anticoagulant.

Haematological examination

Blood samples were analysed for the following indices within the day of sampling: erythrocyte count (RBC), haemoglobin concentration (Hb), packed cell volume (PVC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC). Erythrocytes were counted in Bürker's chamber using Haym's solution. Haemoglobin concentration was measured spectrophotometrically using the cyanomethaemoglobin method. Packed cell volume was measured using standard capillary tubes and centrifugation in a microhematocrit Janetzki TH12 centrifuge. MCV, MCH and MCHC were calculated.

Body weight (BW) and daily weight gain (DWG)

The piglets were weighed at birth (day 1) and on days 7, 14, 21, 28, 35. Daily weight gain of piglets in each group after one week was calculated.

Statistical evaluation

The results were statistically evaluated by analysis of variance (ANOVA). All results are presented as mean values and standard deviations of each index.

RESULTS

RBC (Figure 1)

Significantly lower RBC was found in group 2 on day 7, compared with group 1 ($P < 0.01$). From day 7 to day 35 RBC had an increasing tendency and no significant differences between groups 1 and 2 were found during the trial.

Hb (Figure 2)

Hb in group 1 on day 7 was significantly higher ($P < 0.01$) compared with group 2. From day 7 to day 14, there was a significant increase of Hb in group 2 ($P < 0.01$). Hb in group 1 reached the

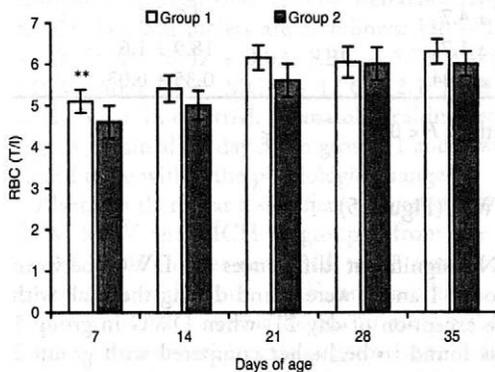


Figure 1. Red blood cell count during the trial (RBC)
** $P < 0.01$

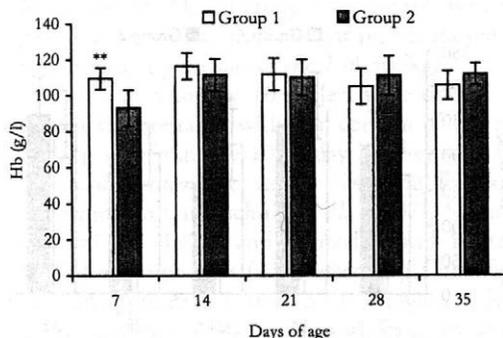


Figure 2. Haemoglobin concentration during the trial (Hb); ** $P < 0.01$

maximum value on day 14 (116 g/l). From day 14, Hb in group 1 was decreasing and reached the minimum value on day 28 (105 g/l). The difference between day 14 and 28 was statistically significant ($P < 0.05$). Differences in Hb between groups 1 and 2 on days 14, 21, 28 and 35 were found to be statistically insignificant.

PVC (Figure 3)

PCV in group 1 on day 7 was significantly higher ($P < 0.01$) compared with group 2. From day 7 to day 14, there was a significant increase of PCV in group 2 ($P < 0.05$). PCV in group 1 reached the maximum value on day 14 (0.36). From day 14, PCV in group 1 was decreasing and reached the minimum value on day 28 (0.32). The difference between days 14 and 28 was found to be statistically significant ($P < 0.01$). Differences in PCV between groups 1 and 2 on days 14, 21, 28 and 35 were found to be statistically insignificant.

MCV, MCH (Table 1)

On day 7, MCV in group 2 was significantly lower compared with group 1 ($P < 0.05$). MCV and MCH in group 1 decreased from day 14 to day 28 significantly (both $P < 0.01$). Differences in MCV and MCH between groups 1 and 2 on days 14, 21, 28 and 35 were found to be statistically insignificant during the trial.

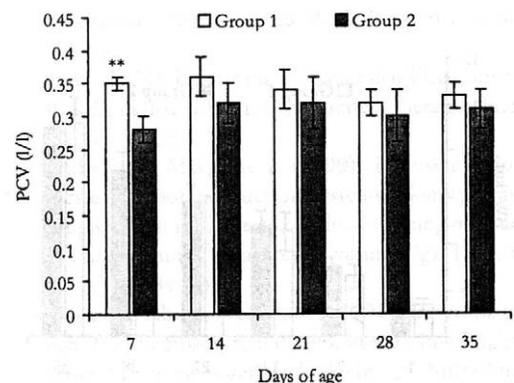


Figure 3. Packed cell volume during the trial (PCV)
** $P < 0.01$

Table 1. The values of MCV, MCH and MCHC during the trial

Day of age	Indices	Group 1 (n = 24)	Group 2 (n = 25)
		mean ± SD	mean ± SD
7	MCV (fl)	68 ± 3.2 ^a	62 ± 5.6 ^b
	MCH (pg)	21.3 ± 1.28	20.86 ± 2.56
	MCHC (g/l)	0.31 ± 0.19	0.33 ± 0.03
14	CV (fl)	66.3 ± 5.6	64.8 ± 7.8
	MCH (pg)	21.6 ± 2.3	22.5 ± 2.1
	MCHC (g/l)	0.32 ± 0.02	0.32 ± 0.03
21	MCV (fl)	56 ± 4.7	58 ± 7
	MCH (pg)	18.2 ± 1.6	19.9 ± 2
	MCHC (g/l)	0.32 ± 0.03	0.35 ± 0.04
28	MCV (fl)	52.5 ± 5.3	49.8 ± 8
	MCH (pg)	18.2 ± 1.9	18.4 ± 2.5
	MCHC (g/l)	0.34 ± 0.03	0.36 ± 0.04
35	MCV (fl)	52.6 ± 4.7	53.7 ± 5
	MCH (pg)	18.3 ± 1.7	18.9 ± 1.6
	MCHC (g/l)	0.33 ± 0.04	0.35 ± 0.03

Groups with different alphabetic superscripts differ significantly at $P < 0.05$

MCHC (Table 1)

No significant differences in MCHC between groups 1 and 2 were found during the trial.

BW (Figure 4)

Differences in BW between groups 1 and 2 were found to be statistically insignificant during the trial.

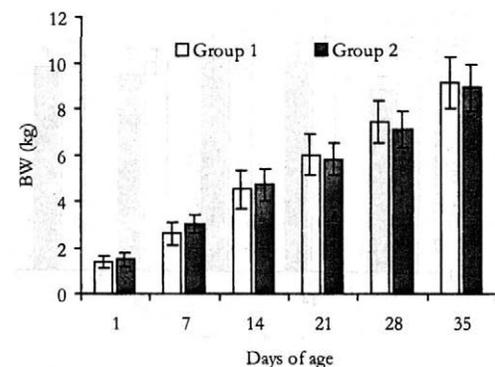


Figure 4. Body weight during the trial (BW)

DWG (Figure 5)

No significant differences in DWG between groups 1 and 2 were found during the trial, with the exception of day 21, when DWG in group 1 was found to be higher compared with group 2 ($P < 0.05$).

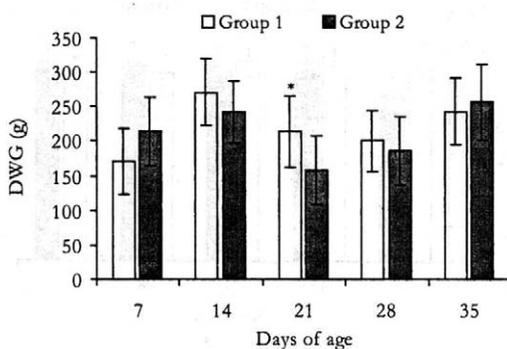


Figure 5. Daily weight gain during the trial (DWG)
* $P < 0.05$

DISCUSSION

On day 7 Hb, PCV and RBC in group 2 (Fe^{3+} dextran, *i. m.* day 3) were significantly lower compared with group 1 (Fe^{3+} microemulsion). The same difference was recorded by Iben (1998) in Hb and PCV on day 10. For the rest of the trial, *i.e.* on days 14, 21, 28 and 35, all haematological indexes between groups 1 and 2 were statistically comparable. No statistically significant differences were found between the two groups in DWG and BW, with the exception of day 21, when DWG in group 1 were found to be higher compared with group 2 ($P < 0.05$).

Lower Hb, PCV and RBC in group 2 on day 7 did not cause lower BW compared with group 1.

Reference ranges for 35 days old piglets were investigated by Egeli *et al.* (1998). Reference ranges for 35 days old piglets are as follows: Hb – 101 ± 10 ; PCV – 0.32 ± 0.03 ; RBC – 5.79 ± 0.68 ; MCV – 56.5 ± 6.6 ; MCH – 17.6 ± 2.1 ; MCHC – 311 ± 10 . In our trial, haematological indices of piglets obtained on day 35 in groups 1 and 2 were found to be within the physiological range.

Although there was a significant decrease of Hb, PCV, MCV and MCH in group 1 from day 14 to 28, those indices remained to be statistically comparable with group 2. The anaemic limit, *i.e.* the point when anaemia begins to exert a detrimental effect on weight gain or gives rise to clinical symptoms of anaemia, is set by most authors at a haemoglobin concentration of 80 g/l (Furugouri, 1975; van Kempen, 1987). Neither in group 1 nor in group 2 did any piglet become anaemic (*i.e.* no piglet had Hb lower than 80 g/l). After weaning at 35 days of age, no further decrease of Hb, PCV, MCV and MCHC in group 1 was found. We suggest that it is due to the fact that piglets started to feed intensively on starter (Fe 248 mg/kg).

From day 3 to day 10 piglets were offered *ad libitum* the prestarter with iron content of 100 mg Fe^{2+} /kg and from day 10 to day 35 they were offered *ad libitum* the starter (248 mg Fe^{2+} /kg). According to Glawischig *et al.* (1987), Schmitz and Müller (1973) and Lemacher and Bostedt (1995) an important factor after single oral Fe^{2+} dextran application is free access of piglets to feed containing iron. As far as the authors know, there has been published only one paper dealing with application of Fe^{3+} microemulsion (Iben, 1998). Our results differ from those found by Iben (1998). Iben (1998) concludes that a single oral dose of

Fe^{3+} microemulsion was not sufficient to prevent the iron deficiency anaemia and the second dose of iron via injection on the 10th day of life was necessary. Haematological indices in his study were examined till the 10th day of life of piglets only, and no creep feed was offered to the piglets.

CONCLUSION

We conclude that a single oral application of Fe^{3+} microemulsion to suckling piglets was efficient enough to prevent anaemia under the conditions of this trial and it resulted in the growth intensity of piglets that was comparable with piglets that were given Fe^{3+} dextran parenterally.

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Impact of genetic groups and crossbred information on the prediction of breeding values in pig sire breeds

Vliv genetických skupin a informací o křížencích na predikci plemenných hodnot u otcovských plemen prasat

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ABSTRACT: The investigation was based on a data set from six pig sire breeds with 11 039 station-tested purebred, 65 313 field-tested purebred and 17 004 field-tested crossbred animals. Four four-trait animal models were studied: (i) without crossbred information, without genetic groups (cb-gg), (ii) with crossbred information, without genetic groups (CB-gg), (iii) without crossbred information, with genetic groups (cb-GG), (iv) with crossbred information and with genetic groups (CB-GG). The traits were lifetime daily gain and lean meat percentage in the field test and average daily gain from 30 to 100 kg live weight and weight of valuable cuts on station. The total breeding value (TBV) was calculated from daily gain on field and valuable cuts on station. For comparing the prediction accuracy of the different models, the mean standard error of the predicted breeding values was expressed in standard deviations of the distribution of predicted breeding values. Under this point of view, the model cb-GG was the best one. The introduction of genetic groups in the basic model cb-gg had a greater impact on ranking boars than the introduction of crossbred information. Imported boars ranked better in models with genetic groups than in models without genetic groups.

Keywords: pig; sire breeds; breeding value estimation; multitrait animal model; genetic groups; crossbred information

ABSTRAKT: Základem všech analýz byl datový soubor šesti otcovských plemen zahrnující údaje od 11 039 čistokrevných zvířat ze staničního testu, 65 313 čistokrevných zvířat z polního testu a 17 004 hybridních kanců z polního testu. Byly zkoumány čtyři čtyřznakové animal modely: a) bez kříženců a bez genetických skupin (cb-gg), b) s kříženci a bez genetických skupin (CB-gg), c) bez kříženců a s genetickými skupinami (cb-GG), d) s kříženci a s genetickými skupinami (CB-GG). Hodnotily se znaky: celoživotní přírůstek a podíl libového masa v polním testu, přírůstek od 30 do 100 kg živé hmotnosti a hmotnost hlavních masitých částí ve staničním testu. Celková plemenná hodnota (TBV) zahrnovala přírůstek v polním testu a hmotnost hlavních masitých částí. Jako kritérium pro srovnání přesnosti predikce jednotlivých modelů sloužila průměrná standardní chyba predikované plemenné hodnoty vyjádřená ve standardních odchylkách rozdělení plemenných hodnot. Podle tohoto kritéria byl nejlepší model cb-GG. Zahrnutí genetických skupin do základního modelu cb-gg mělo větší vliv na pořadí kanců než

zahrnutí informací od kříženců. Importovaní kanci získali v modelech s genetickými skupinami lepší pořadí než v modelech bez nich.

Klíčová slova: prasata; otcovská plemena; odhad plemenné hodnoty; víceznakový animal model; genetické skupiny; informace od kříženců

Compared with most Western countries and pig breeding organisations, a greater number of sire breeds is used in the Czech Republic similarly as in the Slovak Republic. The number of animals in some of the sire breeds is consequently relatively low. In addition to purebred information, data from field-tested crossbred boars are available. It is expected that the inclusion of these data in the genetic evaluation of purebred animals might increase the accuracy of the predicted breeding values of the latter. The first aim of the present paper is therefore to propose a model which uses additional information from field-tested crossbred boars and to analyse the influence of this additional information on the accuracy and the standard error of the predicted breeding values.

A further problem to be solved is the breeding value estimation of imported boars. As there is no international genetic evaluation in pigs, the only way of improving the breeding value estimation of imported boars is the introduction of genetic groups (phantom parents – Westell *et al.*, 1988; Quaas, 1988). The second aim of the present investigation is therefore to propose genetic groups for the sire breeds bred in the Czech Republic and to analyse the influence of these groups on the ranking of imported boars as well as on the accuracy and standard error of the predicted breeding values.

MATERIAL AND METHODS

The investigation was based on a data set with 11 039 station-tested purebred, 65 313 field-tested purebred and 17 004 field-tested crossbred animals. The purebred animals belonged to the breeds Duroc (D), Belgian Landrace (BL), Hampshire (H), Pietrain (P), Czech Meat Pig (CMP) and to a sire line of Large White (LW). The crossbred animals were any combination between these six breeds. The station-test data were collected from November 1989 to January 2001 and the field-test data from September 1990 to January 2001 (purebreds) or to April 2001 (crossbreds). All available information on pedigree data was used (approximately to birth year 1985).

The following traits were analysed:

- average daily gain in the field test from birth to end of test (ADGF, in g/day)
- lean meat percentage in the field test measured by PIGLOG (LMF, in %)
- average daily gain from 30 to 100 kg live weight on station (ADGS, in g/day)
- weight of trimmed valuable cuts (neck, shoulder, ham and loin) in the half-carcass measured on station (VCS, in kg).

Four basic types of calculations were carried out:

- cb-gg = without crossbred information, without genetic groups in the model
- cb-GG = without crossbred information, with genetic groups
- CB-gg = with crossbred information, without genetic groups
- CB-GG = with crossbred information and with genetic groups.

Breeding values were predicted using PEST (Groeneveld *et al.*, 1990). Approximate solutions were calculated by iterative methods, demanding a standardised maximal change between two subsequent solutions of 0.001 and restricting the number of iterations to 2000. Furthermore, the exact solutions were calculated including the prediction error variances. Genetic parameters estimated by Wolf *et al.* (2000) were used in all calculations.

The average of the predicted breeding values of all animals born in 1995 was calculated and this value was subtracted from the originally calculated breeding values. The total breeding value (TBV_n) or aggregated genotype of the n th animal was calculated as follows:

$$TBV_n = 8BV_{ADGF,n} - 600BV_{VCS,n}$$

where: $BV_{ADGF,n}$ and $BV_{VCS,n}$ are the predicted breeding values of the n th animal for the traits ADGF and VCS, respectively

The standard error of prediction for the total breeding value $SEP(TBV_n)$ was achieved from the equation

$$SEP(TBV_n) = \sqrt{8^2 PEV_{ADGF,n} + 600^2 PEV_{VCS,n} + 2 \times 8 \times 600 COV_{ADGF-VCS,n}}$$

where: $PEV_{ADGF,n}$ and $PEV_{VCS,n}$ = the prediction error variances of the breeding values of the n th animal for the traits ADGF and VCS, respectively

$COV_{ADGF-VCS,n}$ = the genetic covariance between ADGF and VCS

A value of -1.845 was inserted for the genetic covariance (Wolf *et al.*, 2000).

The factors included in the individual statistical models are summarised in Table 1. The factor

“breed” used in calculations without crossbred information was replaced by five covariables representing the gene proportions of five breeds in calculations with crossbred information. That

Table 1. Statistical models for the PEST runs

Factor	Type of effect	ADGF	LMF	ADGS	VCS
All models					
Year-season (station test)	F	–	–	x	x
Year-season (field test)	F	x	x	–	–
Sex	F	x	x	x	x
Test station	F	–	–	x	x
Feeding (<i>ad libitum</i> or restricted)	F	x	x	–	–
Live weight at slaughter	C	–	–	–	x
Live weight at end of test	C	–	x	–	–
Herd	R	x	x	x	x
Litter	R	x	x	x	x
Animal	A	x	x	x	x
Models without crossbred information (cb-gg, cb-GG)					
Breed	F	x	x	x	x
Models with crossbred information (CB-gg, CB-GG)					
Gene proportion from Duroc	C	x	x	x	x
Gene proportion from Belgian Landrace	C	x	x	x	x
Gene proportion from Hampshire	C	x	x	x	x
Gene proportion from Pietrain	C	x	x	x	x
Gene proportion from sire line of Large White	C	x	x	x	x
Models with genetic groups (cb-GG, CB-GG)					
Genetic groups	F	x	x	x	x

Traits:

ADGF – average daily gain in the field test, LMF – lean meat percentage in the field test, ADGS – average daily gain on station, VCS – weight of valuable cuts on station

Types of effects:

F – fixed, C – covariable, R – random, A – random with relationship matrix;

Types of calculations:

cb-gg – model without crossbred information and genetic groups, CB-gg – model with crossbred information and without genetic groups, cb-GG – model without crossbred information and with genetic groups, CB-GG – model with crossbred information and genetic groups

means, in the first case the breed effects were estimated as several levels of one factor and in the second case the breed effects were estimated as regression coefficients. As the gene proportions of all six breeds sum up to one in each animal, the effect of the sixth breed (CMP) was set zero and the estimated breed effects can be interpreted as deviations from the effect of CMP. The genetic

groups were formed on the basis of the birth year, the origin and the breed or breed group of the animal (Table 2) and were modelled as phantom parents (Westell *et al.*, 1988; Quaas, 1988). Equal coding was used for phantom sires and phantom dams.

The number of equations was in all runs approximately half a million. The inclusion of ge-

Table 2. Genetic groups

Group number	Description of the genetic group	Frequency in sire position	Frequency in dam position
6090	Animals from native population with unknown parents and unknown year of birth or year of birth ≤ 1980	2 310	1 661
6095	All imports with unknown year of birth or year of birth ≤ 1980	34	35
8490	Animals from native population with unknown parents and year of birth from 1981 to 1984	173	172
8495	All imports with year of birth from 1981 to 1984 (nearly all from Belgium)	96	96
8999	Mostly animals from native population, a very low number of imports from the USA and Great Britain, year of birth from 1985 to 1989	115	152
9290	Animals from native population with unknown parents and year of birth from 1990 to 1992	122	120
9590	Animals from native population with unknown parents and year of birth from 1993 to 1995	97	107
9243	Imports from Austria and Germany, year of birth from 1989 to 1992	103	103
9843	Imports from Austria and Germany, year of birth ≥ 1993 (open group)	40	40
9344	Imports from Great Britain, year of birth from 1990 to 1993	55	55
9444	Imports from Great Britain, year of birth 1994	86	86
9844	Imports from Great Britain, year of birth from 1995 (open group)	65	69
9345	Imports from Denmark, year of birth from 1990 to 1993	55	55
9545	Imports from Denmark, year of birth from 1994 to 1995	89	89
9845	Imports from Denmark, Sweden and Norway, year of birth from 1996 (open group)	38	38
9546	Imports from Sweden, year of birth from 1989 to 1995	70	70
9495	Imports from the USA, France, Italy and Switzerland, year of birth from 1990 to 1994	20	20
9895	All imports with year of birth from 1995 which are not from Austria, Germany, Great Britain, Denmark, Sweden or Norway (open group)	10	10

Table 3. Number of equations, number of iterations to reach the convergence criterion of a standardised maximal change of 0.001 in the approximate solution and CPU-time for the exact solution

symbol	Model		Number of equations	Number of iterations for approximate solution	CPU-time for exact solution
	crossbred information	genetic groups			
cb-gg	no	no	445 360	588	47 min
CB-gg	yes	no	541 584	562	1 h 14 min
cb-GG	no	yes	445 432	1 190	48 min
CB-GG	yes	yes	541 656	>2 000	1 h 11 min

See Table 1 for explanation of symbols for models

netic groups in the models increased the number of equations by 72 only (18 genetic groups \times 4 traits), but led to a considerably worse convergence of the system – compare model cb-gg versus cb-GG and CB-gg versus CB-GG (Table 3). The inclusion of crossbred information increased the number of equations by approximately 20% without influencing the number of iterations in the absence of genetic groups (compare cb-gg versus CB-gg) but with a clear increase in the number of iterations in the presence of genetic groups (cb-GG versus CB-GG). The CPU-time for the exact solution was nearly equal without and with genetic groups (cb-gg versus cb-GG and CB-gg versus CB-GG), but increased by about 50% when adding crossbred information (cb-gg versus CB-gg and cb-GG versus CB-GG).

The results from PEST (the predicted breeding values) were further analysed using the procedures MEANS, CORR and RANK of SAS[®] (SAS Institute Inc., 1990).

RESULTS

A very good agreement between the approximate and exact results was found for the predicted breeding values of all traits including the total breeding value and for all four types of calculations. The correlations between the predicted breeding values from the approximate and exact solutions was greater than 0.99 with one exception (trait ADGS, calculation without crossbreds and with genetic groups). The use of an approximate solution with the convergence criterion as given in Material and Methods in routine genetic evaluations is therefore fully justified and sufficient.

As in the present paper different models are to be compared on the basis of the accuracy of the breeding value estimation, only results from the exact solutions will be treated with in the following text.

The introduction of genetic groups in the model caused an increase in the variation of predicted breeding values expressed in higher standard deviations and larger ranges (models cb-gg and CB-gg versus cb-GG and CB-GG, compare Table 4). On the other hand, the inclusion of crossbred information yielded only minor or no changes in the variability of breeding values. The standard error of the predicted breeding values (SEP) had slightly lower means in the calculation cb-gg than in CB-gg. However, when using genetic groups, the introduction of crossbred information increased on average the SEP (except in ADGS). For comparing the models with each other, the SEP was expressed in standard deviations of the distribution of predicted breeding values. Under this point of view, the model cb-GG was the best one for LMF and VCS, the model CB-gg was best for ADGF and the model CB-GG was best for ADGS, very closely followed by cb-GG. Considering the total breeding value, a decision for the model cb-GG can be recommended.

The results for the set of living boars ($n = 651$) were in good agreement with the results of the whole set of purebred animals ($n = 86\ 845$). The prediction error variances for this data set were on average slightly lower than those for the whole data set. The conclusion that model cb-GG should be recommended was confirmed.

The highest correlations (0.99 and greater in LMF, ADGS, VCS and the total breeding value) of the predicted breeding values from the whole data set occurred between the models cb-GG and

Table 4. Standard deviation and range of the predicted breeding values and mean of the standard error of predicted breeding values (SEP) in absolute values and expressed as proportion of the standard deviation of the distribution of the predicted breeding values

Model	All purebred animals ($n = 86\ 845$)			Living boars ($n = 651$)		
	standard deviation of the distribution of predicted breeding values	range of the distribution of predicted breeding values	mean of the SEP	mean of the SEP expressed in standard deviations	mean of the SEP	mean of the SEP expressed in standard deviations
Average daily gain in the field test						
cb-gg	15.11	153.3	17.25	1.142	16.69	1.105
CB-gg	15.78	194.4	17.03	1.079	16.34	1.035
cb-GG	16.18	164.1	19.25	1.190	19.01	1.175
CB-GG	16.69	200.8	24.07	1.442	23.73	1.422
Lean meat percentage in the field test						
cb-gg	0.878	8.22	0.966	1.100	0.828	0.943
CB-gg	0.902	8.51	0.954	1.058	0.808	0.896
cb-GG	1.224	9.19	1.241	1.014	1.142	0.933
CB-GG	1.264	10.37	1.394	1.103	1.301	1.029
Average daily gain on station						
cb-gg	22.40	270.5	39.64	1.770	40.45	1.806
CB-gg	22.55	270.2	39.61	1.756	40.41	1.792
cb-GG	33.71	337.6	48.57	1.441	50.32	1.493
CB-GG	33.65	337.8	48.26	1.434	49.97	1.485
Weight of valuable cuts on station						
cb-gg	0.4286	3.523	0.4932	1.151	0.4623	1.079
CB-gg	0.4323	3.648	0.4904	1.134	0.4574	1.058
cb-GG	0.7850	4.480	0.6011	0.766	0.5872	0.748
CB-GG	0.7868	5.739	0.7143	0.908	0.7003	0.890
Total breeding value						
cb-gg	307.1	2449	298.1	0.971	277.3	0.903
CB-gg	306.2	2771	295.6	0.965	272.9	0.891
cb-GG	521.1	3279	368.9	0.708	359.9	0.691
CB-GG	508.7	3966	450.6	0.886	441.4	0.868

See Table 1 for explanation of symbols for model

CB-GG (Table 5). The correlations between models cb-gg and CB-gg were nearly as high as the former ones. However, the correlations between two models, where one model included genetic groups and the second did not, were on average clearly lower (around 0.8). Differences between traits were observed. All correlations were greater than 0.9 in ADGF, the trait with the largest amount of avail-

able information. The second highest correlations were observed for LMF, the second trait from the field test. The lowest correlations were found for the station-test traits ADGS and VCS. The correlations observed for the data set of living boars showed the same tendencies as the correlations for the whole data set, but had nearly throughout slightly lower values.

Table 5. Correlations between breeding values from different models for all four traits and the total breeding value (TBV). First line: complete data set of purebred animals ($n = 86\ 845$), second line: data set of living boars ($n = 651$)

Combination of models	Correlation for trait				
	ADGF	LMF	ADGS	VCS	TBV
cb-gg/CB-gg	0.940	0.978	0.993	0.989	0.981
	0.914	0.946	0.987	0.971	0.960
cb-gg/cb-GG	0.969	0.872	0.785	0.804	0.818
	0.964	0.880	0.735	0.709	0.785
cb-gg/CB-GG	0.913	0.849	0.784	0.798	0.816
	0.867	0.806	0.722	0.699	0.760
CB-gg/cb-GG	0.911	0.881	0.780	0.819	0.813
	0.888	0.889	0.743	0.720	0.777
CB-gg/CB-GG	0.974	0.884	0.788	0.823	0.831
	0.969	0.904	0.752	0.754	0.819
cb-GG/CB-GG	0.936	0.990	0.997	0.997	0.993
	0.901	0.960	0.991	0.981	0.970

See Table 1 for explanation of symbols for models and for abbreviations of traits

Table 6. Mean top percent of the data set of living boars ($n = 651$) calculated from the distribution of breeding values for the whole set of evaluated purebred animals ($n = 86845$)

Trait	Top percent for calculation			
	cb-gg	CB-gg	cb-GG	CB-GG
ADGF	27.14	30.86	19.11	23.85
LMF	12.14	11.87	10.62	11.00
ADGS	13.74	13.57	11.42	11.78
VCS	31.75	32.72	15.19	15.86
Total breeding value	13.08	13.32	9.48	9.79

See Table 1 for explanation of symbols for models and for abbreviations of traits

The choice of the type of calculation had considerable influence on the average breeding values of the data set of living boars compared to the average breeding values of the complete data set of purebred animals (Table 6). The average breeding values of the data set of living boars were expressed as top percent. A top percent of q means that $q\%$ of the animals had higher breeding values and $(100 - q)\%$ of the animals showed lower breeding values than the given animal or group of animals. As expected, the data set of living boars had clearly higher values as the mean of the whole population for all models.

The inclusion of genetic groups in the model raised the average position of living boars by 1 to 2% in the traits LMF and ADGS, by 7 to 8% in ADGF, by approximately 15% in VCS and by about 4% in the total breeding value. The use of crossbred information in addition to purebred information changed the position of living boars on average by less than 1% (except in ADGF). The model cb-GG yields the best ranking of living boars for all traits and the total breeding value.

The ranking of 20 boars which had the best total breeding values in model cb-GG is shown

Table 7. Ranks of living boars for the total breeding value within the data set of living boars ($n = 651$) and top percent in the whole data set ($n = 86845$)

Boar	Rank in the data set of living boars				Top percent in the whole data set (%)			
	cb-gg	CB-gg	cb-GG	CB-GG	cb-gg	CB-gg	cb-GG	CB-GG
1	85	127	1	12	0.95	1.69	0.01	0.10
2	36	11	2	1	0.31	0.05	0.01	0.01
3	6	5	3	5	0.02	0.02	0.01	0.02
4	9	9	4	7	0.03	0.04	0.02	0.03
5	39	72	5	19	0.37	0.76	0.03	0.22
6	11	10	6	6	0.07	0.04	0.03	0.03
7	204	196	7	18	3.26	3.02	0.08	0.19
8	168	149	8	15	2.75	2.06	0.08	0.17
9	300	225	9	17	6.42	3.56	0.10	0.18
10	10	7	10	10	0.03	0.03	0.11	0.09
11	148	20	11	2	2.18	0.12	0.11	0.02
12	57	67	12	14	0.72	0.72	0.13	0.16
13	15	14	13	13	0.09	0.08	0.13	0.15
14	159	181	14	26	2.48	2.75	0.15	0.36
15	384	135	15	4	10.52	1.86	0.15	0.02
16	38	80	16	25	0.35	0.85	0.15	0.36
17	35	18	17	9	0.30	0.10	0.16	0.07
18	138	17	18	3	1.92	0.10	0.16	0.02
19	73	65	19	16	0.83	0.69	0.17	0.17
20	480	340	20	11	16.41	8.50	0.18	0.10

See Table 1 for explanation of symbols for models

Table 8. Frequency of boars with a given year of birth among the first 20 boars when ranking was carried out on the total breeding value for the given model

Model	Frequency of boars with year of birth					
	1995	1996	1997	1998	1999	2000
cb-gg	1	-	1	-	5	13
CB-gg	2	-	2	-	4	12
cb-GG	3	-	1	3	3	10
CB-GG	3	-	1	2	4	10

See Table 1 for explanation of symbols for modes

for all models in Table 7. There were considerable changes in rank especially between models without genetic groups on the one hand and models with genetic groups on the other hand. Adding crossbred information to the model with genetic groups (cb-GG) had relatively low impact on ranking boars.

Expressed in top percent of the whole population, the maximal change between models cb-GG and CB-GG was 0.21%. Comparing models cb-gg and CB-gg, the difference was greater than 1.5% in five boars. That means that the introduction of crossbred information had a considerably greater impact

Table 9. Top percent of the total breeding values of imported boars calculated from the distribution of breeding values for the whole set of evaluated purebred animals ($n = 86845$)

Boar	Top percent for model			
	cb-gg	CB-gg	cb-GG	CB-GG
1	0.95	1.69	0.01	0.10
2	0.31	0.05	0.01	0.01
3	0.37	0.76	0.03	0.22
4	0.07	0.07	0.03	0.03
5	16.41	8.50	0.18	0.10
6	16.80	21.28	0.90	1.70
7	12.64	16.41	1.48	1.79
8	1.87	0.71	1.63	0.77
9	28.40	10.51	2.66	1.11
10	7.62	7.73	5.23	6.18
11	24.95	54.12	23.49	36.84

See Table 1 for explanation of symbols for modes

on ranking boars in models without genetic groups than in models with genetic groups. The number of imported boars among the first 20 ones was 1 for model cb-gg, 2 for model CB-gg and 5 for models cb-GG and CB-GG.

Table 8 reflects the age structure of the best 20 boars for different models. In the models without genetic groups, 80 to 90% of the best boars were born in 1999 or 2000, in models with genetic groups the percentage of best boars born in 1999 or 2000 was 65 to 70% only.

The top percent of the total breeding values of imported boars calculated from the distribution of breeding values for the whole data set of purebred animals are summarised in Table 9. All imported boars had clearly better values for the models with genetic groups. The observed changes in top percent were considerable especially in boars 5 and 6 (from about 16% to less than 1%).

DISCUSSION

Cantet and Fernando (1995) proposed an algorithm for breeding value estimation with additive animal models for crosses from two populations. They defined the both crossbred populations and the F_1 cross as three genetic groups and allowed for different genetic variances for each group. In

several papers dealing with variance component estimation or genetic evaluation of pigs, this philosophy was applied and purebred and crossbred performances were treated as two different traits (Brandt, 1994; Brandt and Täubert, 1998; Bijma and van Arendonk, 1998; Fischer, 1998; Merks and Hanenberg, 1998; Lutaaya *et al.*, 2001, 2002). All these papers were based on data from two purebred populations and their crossbred.

As Cantet and Fernando (1995) pointed out, their method can be extended to more than two purebred populations. But the number of genetic variances to be estimated for one trait would be as high as 21 for six purebred populations and in a four-trait animal model, the number of additive genetic (co)variance components would reach a value of 210. Hence, the application of such a procedure which treats observations of the same trait on different populations as different traits seems not feasible for a multi-breed system with a large number of breeds and breed combinations. Therefore, a purely additive model was used treating purebred and crossbred performance as one trait. This seems to be justified for practical purposes, because only production traits with moderate to high heritabilities were evaluated. Very high genetic correlations between crossbred and purebred performances were reported especially for traits referring to lean meat content (Schmutz, 1996; Brandt and Täubert,

1998; Fischer, 1998). Culbertson *et al.* (1998) and Lutaaya *et al.* (2001) reported low or zero dominance variances for backfat thickness, a trait very closely related to VCS and LMF. But in lifetime daily gain, dominance variances different from zero were estimated (Lutaaya *et al.*, 2001).

Though the number of crossbred animals was as large as 18% of all tested animals, their inclusion in the model decreased the standard error of the total breeding value only by 1%, i.e. to 99% of its original value. This low effect of additional information on the precision of the total breeding value can be explained in part by the fact that the crossbred boars are only field-tested, whereas a station-test trait (VCS) is the most important trait for the total breeding value. The influence of crossbred information on the standard errors of the predicted breeding values for field-test traits is significantly higher – they are decreased to about 95% of their original values (model cb-gg). Furthermore, using a simple additive model may not be fully adequate for the given situation and non-additive effects may be one reason for the small effect of the crossbred information on the precision of the predicted breeding values. Nevertheless, non-additive models will not be feasible for the given data structure.

As shown in Results, genetic group effects have important consequences on ranking animals. If too few genetic groups are defined, differences in genetic level among origins may not be adequately modelled (Rodriguez *et al.*, 1996). On the other hand, a complex grouping strategy forming many genetic groups with few animals may cause problems with estimability of group effects (Quaas, 1988). Tholen (1999) compared two grouping strategies for a multi-breed system in pigs. The first strategy was only based on the age of the animals, the second procedure considered the age and the origin of the animals. He concluded that the second grouping strategy (which was applied also in the present paper) should be preferred.

Furthermore, a sex-specific formation of genetic groups was rejected. When a sex-specific definition is used, there can be a complete confounding between the sire and the dam parent groups for animals with both parents unknown causing problems in getting a solution. At the same time the number of animals in the individual genetic groups will be reduced and problems in the convergence of the system of equations for breeding value prediction may occur.

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Controlled spawning of pikeperch, *Stizostedion lucioperca* (L.), in lake cages

Řízený výtěr candáta obecného, *Stizostedion lucioperca* (L.)

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ABSTRACT: The aim of the study was to determine the effectiveness of the cage method in pikeperch propagation with and without hormonal stimulation. The study was carried out in 1999–2000 in the Pojezierze Mazurskie, north-eastern Poland. A total of 276 fishes were studied that were caught at natural spawning grounds using a trap gear. The effects of propagation without hormonal stimulation, expressed as the number of ovulating females, were highly significantly dependent ($P < 0.05$) on the stage of oocyte maturity; they ranged from 0% (stage I) to 100% (maturity stage IV), with an average value of about 29%. The application of 400 IU of human chorionic gonadotropin (HCG) per kg of female body weight synchronized the maturation process, facilitated reproduction in less mature fish (especially those in stage I), and resulted in enhanced spawning effectiveness in which the percentage of resting females was 88%. Hormonal stimulation significantly ($P < 0.05$) shortened the length of the time the fish were held in spawning cages, which allowed their multiple use during the spawning season. The amount of obtained eggs ranged from 60.0×10^3 to 682.5×10^3 and it was positively correlated with female body weight ($R^2 = 0.6212$). A similar, high correlation ($R^2 = 0.633$) was confirmed between body weight and the number of eggs in the eyed stage. The survival rate until the eyed stage was 84% on average.

Keywords: *Stizostedion lucioperca* (L.); spawning; human chorionic gonadotropin (HCG); cage culture

ABSTRAKT: Cílem studie bylo určit účinnost metody řízeného výtěru candáta v ponořených klecích s pomocí a bez pomoci hormonální stimulace. Pokusy byly realizovány v letech 1999 až 2000 na Mazurských jezerech v severovýchodním Polsku. Do studie bylo zahrnuto celkem 276 jedinců odlovených na přirozených trdlišťích do vězenců. Účinnost hormonální stimulace vyjádřená počtem vytřených jikernaček, byla vysoce významná ($P < 0,05$) závislá na stavu jejich pohlavní zralosti; podíl ovulovaných jikernaček kolísala od 0 % (stadium zralosti I) do 100 % (stadium zralosti IV) s průměrnou hodnotou kolem 29 %. Podání 400 IU humánního choriogonadotropinu (HCG) na 1 kg hmotnosti jikernaček synchronizovalo proces dozrávání, usnadnilo reprodukci u nedostatečně připravených jikernaček (zejména ve stadiu zralosti I) a umožnilo dosažení celkové úspěšnosti výtěru 88 % jikernaček. Hormonální stimulace významně ($P < 0,05$) zkrátila časový interval do výtěru, což umožnilo opakované využití ponořených klecí. Jednotlivé snůšky jiker obsahovaly $60,0 \times 10^3$ až $682,5 \times 10^3$ jiker a jejich počet pozitivně koreloval s hmotností jikernaček ($R^2 = 0,6212$). Vysoká korelace ($R^2 = 0,633$) byla potvrzena mezi hmotností jikernaček a počtem jiker ve stadiu očních bodů. Přežití vytřených jiker do stadia očních bodů dosahovalo v průměru 84 %.

Klíčová slova: candát obecný; výtěr; humánní choriogonadotropin; klece

Pikeperch, *Stizostedion lucioperca* (Linnaeus, 1758) (Percidae), is a highly valuable commercial fish species due to its rapid growth rate and appetizing taste (Szczerbowski, 1995). In addition, biomanipulation procedures can be applied to this species, including modification of the trophic structures of aquatic ecosystems in order to improve water quality, especially in dam reservoirs (Shapiro *et al.*, 1975; Mills *et al.*, 1987). For these reasons and due to significant fluctuations in the abundance of year-classes (Nagiec, 1977), attempts have been made for decades to stabilize the production of this species through controlled propagation and the cultivation of stocking material (Kielczewski, 1939; Korycki, 1976; Schlumberger and Proteau, 1996; Steffens *et al.*, 1996).

The oldest method of pikeperch propagation is natural spawning (Berka and Hamáčková, 1980; Horvath *et al.*, 1984; Steffens *et al.*, 1996). Spawners are kept in ponds until the catch of summer fry, which usually lasts from five to six weeks in the Polish climate (Korycki, 1976). This type of spawning is not very effective, nor does it allow the prediction of the final production results (Wojda *et al.*, 1994). Better results are obtained through controlled spawning that is conducted in earthen, concrete, or near-lake ponds (Horvath *et al.*, 1984; Schlumberger and Proteau, 1996). In short, this method is aimed at obtaining fertilized eggs from previously prepared nests made of either natural materials such as pine branches, small roots from willow or alder trees, sedge roots or synthetic materials (Korycki, 1976; Berka and Hamáčková, 1980; Salminen and Ruuhijärvi, 1991). After reproduction, spawners are caught and moved to reservoirs or a hatchery; spawning nests are left in spawning ponds (Zakes, 1997). This method of propagation is used in many European countries, including Hungary and Germany (Horvath *et al.*, 1984; Steffens *et al.*, 1996). In Finland, the pikeperch propagation is carried out in lakes in floating cylindrical cages with nests made of artificial substrate placed on the bottom (Salminen *et al.*, 1992; Ruuhijärvi and Hyvärinen, 1996).

In recent years, significant progress has been made in developing techniques for the artificial propagation of many fish species. The knowledge of physiological processes that occur in fish has facilitated the introduction of substances that stimulate maturation and reproduction (Peter *et al.*, 1991). Substances that stimulate the hypothalamus, hypophysis and gonads are among them.

The most commonly used substances stimulating fish maturity include carp pituitary extract (CPE), human chorionic gonadotropin (HCG) and luteinizing hormone-releasing hormone (LH-RH) or super-active analogs (LH-RHa).

The controlled propagation of pikeperch is difficult because of the sensitivity of this species to stress and any kind of manipulation; therefore, attempts to stimulate the reproduction of this species hormonally are rare. Most commonly, CPE, HCG or a combination of these two substances are applied (Antalfi, 1979; Steffens *et al.*, 1996; Zakes and Demska-Zakes, 1999). According to Steffens *et al.* (1996), in addition to hormonal stimulation, pikeperch requires simultaneous thermal stimulation. Therefore, the effective reproduction of this species is possible only in hatcheries that are equipped with appropriate technical facilities, especially systems for thermoregulation.

The aim of this study was to determine the effectiveness of the propagation of pikeperch obtained from lakes during the pre-spawning period and subsequently placed in lake cages, and to confirm the appropriateness of simultaneous hormonal treatment.

MATERIAL AND METHODS

Materials

The studies on pikeperch propagation in lake cages were carried out on the Mikolajki Fish Farm in north-eastern Poland in 1999–2000. The spawners were caught on natural spawning grounds using a trap gear (fyke-nets, river traps). The caught fish were placed in water-filled containers and transported to the fisheries station; this operation lasted from 30 minutes to one hour. A total of 276 fishes were used in the present study. The average body weight of females in 1999 was 1.04 kg, and in 2000 it was 1.63 kg. The average body weight of males was 1.20 kg, and was more balanced in both years (Table 1).

Equipment

The studies were carried out in standard lake cages measuring 2 × 2 × 2 m and made of 10 mm mesh bar netting that were mounted to piers. In 1999, 14 cages were used – ten for spawning and

Table 1. Characteristics of pikeperch spawners

Years	Females			Males		
	<i>n</i>	body weight (kg) range	average	<i>n</i>	body weight (kg) range	average
1999	54	0.71–2.64	1.04	65	0.80–3.40	1.22
2000	64	0.90–3.60	1.63	93	0.72–3.40	1.20

four for manipulation while in 2000 a total of 22 cages were used – 18 for spawning and four for manipulation.

Depending on the number of mature females, one or two spawning nests made of rice straw according to the recommendations of Salminen *et al.* (1992) were placed in each of the spawning cages. The sizes of nests varied; for females that weighed over 1.5 kg they measured 0.6 × 0.6 m, and for smaller females they were 0.5 × 0.5 m in size.

Procedure

After the spawners were brought to the fisheries station, they were sorted according to sex and weighed. The females were put under general anesthesia using Propiscin at a dose of 1.0–2.0 ml/l (Szkudlarek and Zakes, 1996). The oocytes were harvested using a catheter and preserved in a solution of ethanol, formalin and glacial acetic acid (6 : 3 : 1, v/v). The percentages of cells in the subsequent stages were determined for the oocytes that completed vitellogenesis: I – with a centrally located nucleus, II – with a migrating nucleus, III – with a nucleus that shifted towards the periphery of the cell, IV – after the breakdown of the nucleus morphological structure (GVBD) (Brzuska and Bieniarz, 1977).

Females were divided into four groups according to the degree of maturity. The first group consisted of fish with oocytes only in stage I or with this stage dominating at more than 60%. The second group consisted of fish with oocytes in stage II. The third group consisted of spawners in maturity stage III, and the fourth group contained mature individuals with oocytes in maturity stage IV.

Prior to placing the spawners in cages, they were marked with plastic tags mounted to their spinal dorsal fins; this allowed the specimens to be easily identified. After marking, the fish were bathed in

a saline solution of 200 g NaCl per 10 l of water for two minutes. The females from groups I and II were placed separately in manipulation cages. The fish in the experimental groups were hormonally stimulated using HCG. The hormone was injected peritoneally at a dosage of 400 IU per kg of female body weight. The degree of oocyte maturity was controlled every two days and when the females reached maturity stage III, they were moved to spawning cages. Females from groups III and IV were placed directly into spawning cages; however, some spawners with oocytes in maturity stage III were stimulated hormonally. Depending on the number of fish, one or two sets of spawners composed of one female and two males were placed in one cage. The nests and the water temperature were checked twice daily at 07.00 and at 19.00. The resting females were caught, weighed and their commercial fecundity (the number of eggs obtained during controlled cage spawning) was determined gravimetrically. Nests with eggs were moved to incubation cages where they were kept until the eyed stage when their survival rate was determined.

Statistical analysis

The statistical analysis of the data was conducted using the STATISTICA program. The data on the number of ovulating females in the control groups and those that were hormonally stimulated were analyzed using logistic regression. The time of fish captivity in cages until egg release was analyzed using analysis of variance (ANOVA). If significant differences ($P < 0.05$) were detected, further analysis was carried out using the LSD test. The dependences between body weight and commercial fecundity and body weight and the number of eggs in the eyed stage were tested by regression analysis.

RESULTS

Spawning time

Due to the low water temperature in 1999, the fecundity studies began on 8 May while in 2000 they began on 26 April. In 1999, the water temperature ranged from 8.1 to 15.6°C, and in 2000 from 13.8 to 18.1°C (Figure 1). The thermal conditions in 1999 resulted in a longer spawning season; it significantly impacted spawner condition and health. Symptoms of fungal infections were observed in the majority of fish and especially in females in maturity stages I and II. Nevertheless, the spawner survival rate in both years was similar, 98 and 99%, respectively.

Despite of different thermal conditions, the fish residence time in the cages until spawning was similar in both years. It was significantly dependent on the degree of female maturity ($P < 0.001$) in both the control and experimental groups. The impact of hormonal stimulation on the maturation

and ovulation processes, and thus on the fish residence time in the cages, was also significant ($P < 0.05$; Table 2). Both in 1999 and 2000, no female in stage I (control group) began reproduction. After six to nine days in the cages, oocytes with a centrally located nucleus (50–70%) or with one that moved slightly towards the cell periphery (30–50%) were observed in their gonads. Spawners that were hormonally treated reproduced between the fifth and eighth day (Table 2).

Of the females in stage II from the control group, spawning occurred between day five and seven in 1999 and day four and seven in 2000. When hormonal injections were applied, significant acceleration ($P < 0.001$) and greater synchronization of spawning was observed. In such cases, spawning began within two to five days. In group III, the impact of hormonal stimulation on maturation was observed to a lesser extent although it was also statistically significant ($P < 0.05$); females injected with HCG started reproduction within one to three days, and spawners from the control groups within

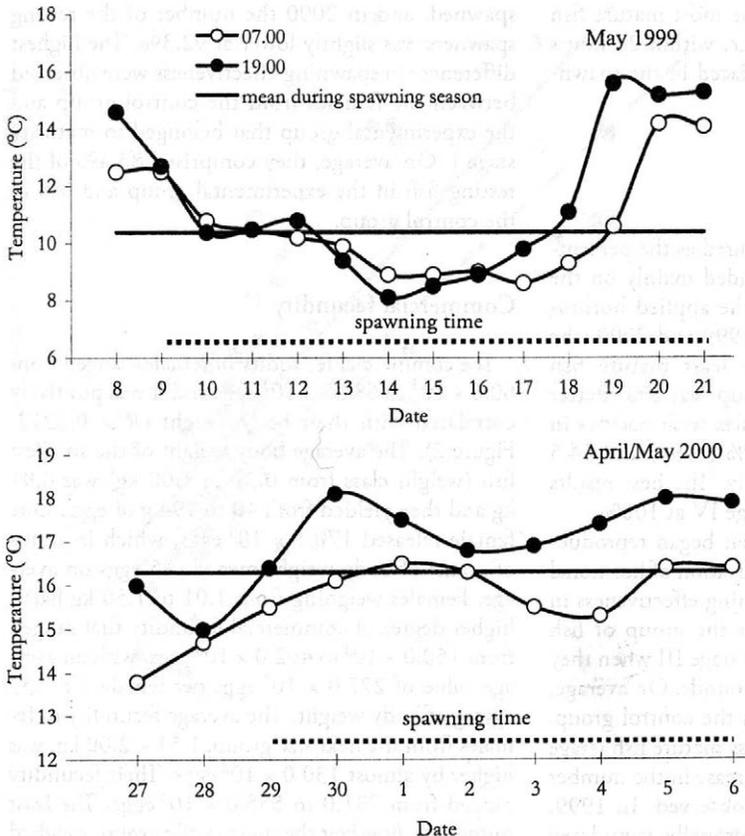


Figure 1. Water temperature and pikeperch spawning in 1999–2000

Table 2. Results of pikeperch spawning in cages from 1999–2000

Preparation used	Number of females			% of stripped females			Caging time (days)		
	1999	2000	total	1999	2000	average*	1999	2000	average*
I stage of maturity									
Control	14	22	36	0.0	0.0	0.0 ^A	–	–	–
HCG	1	6	7	100.0	66.7	83.4 ^B	5	5–8	6.2
II stage of maturity									
Control	13	11	24	23.1	54.5	38.8 ^A	5–7	4–7	5.4 ^A
HCG	8	13	21	100.0	92.3	96.2 ^B	4–5	2–5	3.6 ^B
III stage of maturity									
Control	13	4	17	69.2	50.0	59.6 ^A	2–4	2–3	2.8 ^A
HCG	5	5	10	80.0	80.0	80.0 ^A	1–3	1–3	1.7 ^B
IV stage of maturity									
Control	–	3	3	–	100.0	100.0	–	1	1.0
HCG	–	–	–	–	–	–	–	–	–

* data with the same superscripts in the same column are not significantly different ($P > 0.05$)

one to four days (Table 2). The most mature fish started spawning the earliest, i.e. within 24 hours from the moment they were placed in the spawning cages.

Spawning percentage

Spawning effectiveness, measured as the percentage of spawned females, depended mainly on the degree of their maturity and the applied hormonal stimulation (Table 2). In 1999 and 2000, the spawning effectiveness of the least mature fish (stage I) from the control group was 0%. Better results were obtained for females with oocytes in stages II or III, 23.1 and 69.2% (1999) and 54.5 and 50.0% (2000), respectively. The best results were obtained for fish from stage IV at 100%.

The percentage of females that began reproducing increased thanks to the application of hormonal injections (Table 2). The spawning effectiveness in both study years was 80% for the group of fish whose oocytes were in maturity stage III when they were caught at the spawning grounds. On average, this was 20.4% higher than in the control group. Additionally, in the group of less mature fish (stage II), a statistically significant increase in the number of females that spawned was observed. In 1999, all the females that were hormonally stimulated

spawned, and in 2000 the number of the resting spawners was slightly lower at 92.3%. The highest differences in spawning effectiveness were observed between the females from the control group and the experimental group that belonged to maturity stage I. On average, they comprised 83.4% of the resting fish in the experimental group and 0% in the control group.

Commercial fecundity

The commercial fecundity of females ranged from 60.0×10^3 to 682.5×10^3 eggs and it was positively correlated with their body weight ($R^2 = 0.6212$; Figure 2). The average body weight of the smallest fish (weight class from 0.71 to 1.00 kg) was 0.91 kg and they yielded from 40 to 194 g of eggs. One female released 170.1×10^3 eggs, which in terms of grams of body weight meant 185 eggs on average. Females weighing from 1.01 to 1.50 kg had a higher degree of commercial fecundity that ranged from 150.0×10^3 to 492.0×10^3 eggs, with an average value of 277.0×10^3 eggs per female, i.e. 235 eggs/g of body weight. The average fecundity of females from the next size group, 1.51–2.00 kg, was higher by almost 130.0×10^3 eggs. Their fecundity ranged from 231.0 to 555.0×10^3 eggs. The least numerous, however the most fertile group, weighed

over 2.01 kg (from 2.13 to 3.01 kg). On average, the fecundity of this group was 513.6×10^3 eggs at an average body weight of 2.54 kg.

In both study years, the survival rate until the eyed stage was similar and ranged from 82 to 86% of the number of released eggs. The number of eggs in the eyed stage obtained from particular females ranged from 50.1×10^3 to 566.5×10^3 eggs and it was positively correlated with their body weight ($R^2 = 0.633$), and thus with their commercial fecundity (Figure 2). Females weighing from 0.71

to 1.00 kg yielded on average 143.2×10^3 eggs, i.e. 155 eggs per g of female weight. The highest average number of eggs in the eyed stage was obtained from fish from the three consecutive size classes, i.e. 240.4×10^3 , 343.5×10^3 and 433.3×10^3 eggs. These values, recalculated into grams of female body weight in size classes 1.01–1.50 kg and 1.51–2.00 kg, were similar at 199 and 201 eggs, respectively. In the group of the largest fish (>2.01 kg) about 171 eggs in the eyed stage/g of female spawner weight were obtained.

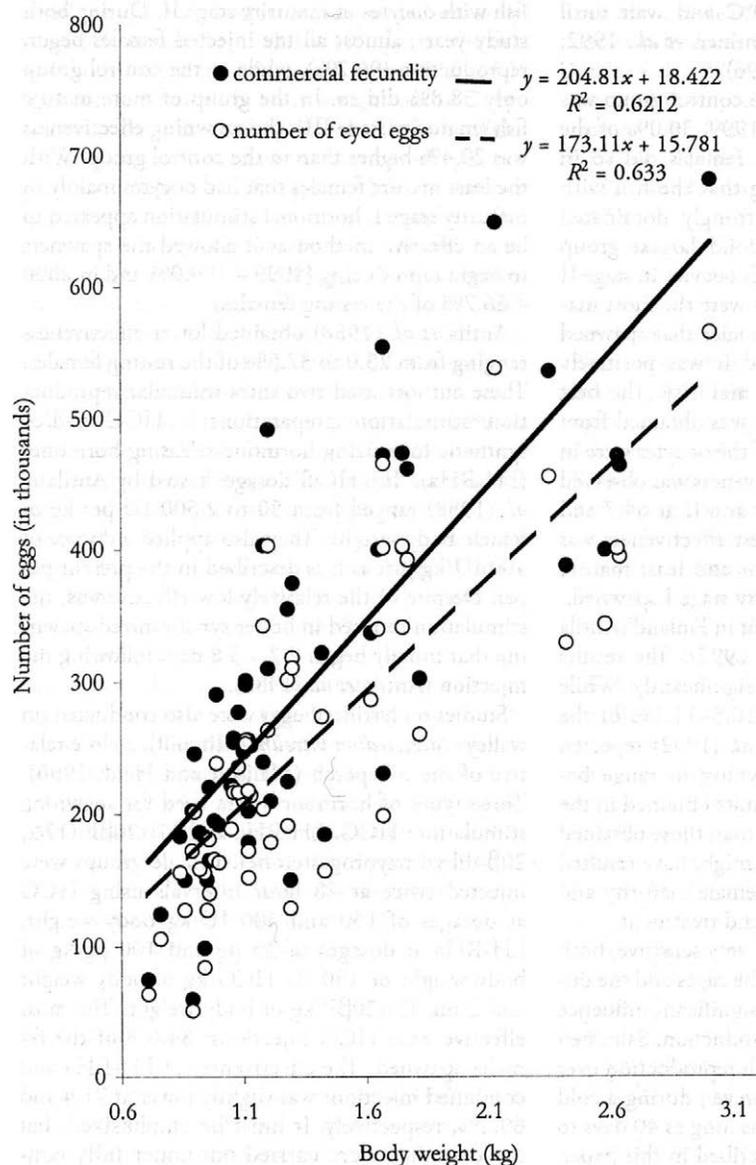


Figure 2. Dependence between pikeperch female body weight and commercial fecundity and the number of eggs in the eyed stage

DISCUSSION

Despite of different thermal conditions in the two study years, the effectiveness of reproduction based on the total number of spawned females was similar; in 1999 it was 46.3% and in 2000 it was 48.4%. Only during the first days of the study in 1999 was the water temperature higher than 10°C; thus, the reproduction period was longer and the spawners were more susceptible to infection. However, spawning was not halted. This conflicts with the theory that pikeperch stop spawning at water temperatures below 10°C and wait until the temperature increases (Salminen *et al.*, 1992; Schlumberger and Proteau, 1996).

Spawning effectiveness in the control group was similar in 1999 and 2000. In 1999, 30.0% of the females spawned, and 27.5% females did so in 2000. It is worth emphasizing that the fish with oocytes in stage I (45.0%) strongly dominated among the spawners. The second largest group (30.0%) involved spawners with oocytes in stage II and the least numerous (3.8%) were the most mature fish. The percentage of females that spawned varied in different groups and it was positively correlated with the degree of maturity. The best effectiveness (100% spawning) was obtained from spawners in which over 50% of the oocytes were in GVBD stage. The lowest effectiveness was observed in less mature fish in stages III and II at 64.7 and 37.5%, respectively. The poorest effectiveness was recorded in the most abundant and least mature group; no females from maturity stage I spawned.

Similar studies were carried out in Finland (Antila *et al.*, 1988; Salminen *et al.*, 1992). The results presented in both papers vary significantly. While Antila *et al.* (1988) reported 10.5–11.1% of the females spawned, Salminen *et al.* (1992) reported the effectiveness of cage spawning to range between 70.0 and 90.0%. The results obtained in the present study are slightly lower than those obtained by Salminen *et al.* (1992). This might have resulted from the different degrees of female maturity and the means of spawner catches and treatment.

Since pikeperch spawners are very sensitive, both the time of their placement in the cages and the duration of their captivity have a significant influence on the effectiveness of their reproduction. Salminen *et al.* (1992) observed pikeperch reproduction over a period of 5.9–13.1 days; however, during a cold spring season, females needed as long as 40 days to spawn. During the studies described in this paper,

the time after which the non-hormonally treated fish began spawning was strongly dependent on their maturity stage and ranged from one to eight days. Most frequently, the females began reproduction after two to four days.

After hormonal stimulation, the female fish spawned faster; it was confirmed statistically. The spawning effectiveness also increased significantly. In 1999, 92.8% females released eggs after they were injected with HCG, and 83.3% of the females did so in 2000. Excellent results, with 96.2% of the females having spawned, were obtained for fish with oocytes in maturity stage II. During both study years, almost all the injected females begun reproduction (96.2%), while in the control group only 38.8% did so. In the group of more mature fish (maturity stage III), the spawning effectiveness was 20.4% higher than in the control group. With the least mature females that had oocytes mainly in maturity stage I, hormonal stimulation appeared to be an effective method as it allowed the spawners to begin reproducing (1999 – 100.0% and in 2000 – 66.7% of the resting females).

Antila *et al.* (1988) obtained lower effectiveness ranging from 25.0 to 37.5% of the resting females. These authors used two intra-muscular reproduction stimulation preparations – HCG and/or synthetic luteinizing hormone-releasing hormones (LH-RHa). The HCG dosages tested by Antila *et al.* (1988) ranged from 50 to 2,500 IU per kg of female body weight. They also applied a dosage of 400 IU/kg just as it is described in the present paper. Despite of the relatively low effectiveness, the stimulation resulted in better synchronized spawning that usually began 2.7 – 3.8 days following the injection (Antila *et al.*, 1988).

Studies on fertilized eggs were also conducted on walleye *Stizostedion vitreum* (Mitchill), a close relative of the pikeperch (Malison and Held, 1996). Three types of hormones were used for spawning stimulation: HCG, LH-RHa and 17 α 20 β P (17 α , 20 β -dihydroxyprogesterone). Female groups were injected twice at 48 hour intervals using HCG at dosages of 150 and 500 IU/kg body weight, LH-RHa at dosages of 35 μ g and 100 μ g/kg of body weight or 150 IU HCG/kg of body weight and 2 mg 17 α 20 β P/kg of body weight. The most effective were HCG injections; 84.6% of the females spawned. The effectiveness of LH-RHa and combined injections was slightly lower at 71.4 and 69.2%, respectively. It must be emphasized that these studies were carried out under fully con-

trolled conditions with thermal stimulation of reproduction.

In addition to the positive results of hormonal stimulation, negative effects also occurred. Increased mortality was observed in spawners that were hormonally stimulated (Antila *et al.*, 1988). After five days, the mortality ranged from 31.2 to 36.3% while in the control group it was 10.8 to 11.5%. In the present studies, spawner mortality was between 1–2% and was the same in both the control and the experimental groups.

The commercial fecundity of the studied females in all groups was similar and depended on body weight. It ranged from 60.0×10^3 eggs in the fish with body weight from 0.71 to 1 kg to 682.5×10^3 eggs for females with body weight above 2 kg. Spawners from the 1–2 kg size class had the highest fecundity expressed as the number of eggs per g of body weight at 235 eggs/g while the heavier spawners (> 2 kg) showed lower fecundity. The lowest fecundity was observed in the fish with body weight below 1 kg (185 eggs/g). These values are very similar to those obtained by Deelder and Willemsen (1964) and Korycki (1976). According to Schlumberger and Proteau (1996), the survival rate of eggs obtained from hormonally and thermally stimulated propagation ranges from 80 to 90% until hatching. Thus, the 82–86% survival rate until the eyed stage obtained in the present study can be regarded as very satisfactory.

Keeping the above in mind and considering effectiveness measured as the percentage of resting females, low mortality and high spawning synchronization that facilitates the multiple use of cages during the spawning season the hormonally stimulated pikeperch reproduction method in lake cages as very effective. Good propagation results can also be obtained without the use of hormonal stimulants, but mature spawners, with oocytes in maturity stages III and IV, should be selected. In order to determine with high probability the ability to spawn and the spawning effectiveness as well as to estimate cage and spawning nest requirements, it is necessary to determine the maturity stage of spawners after they are caught.

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Effect of crossing indigenous Awassi sheep breed with mutton and prolific sire breeds on the growth performance of lambs in a subtropical region

Vliv křížení místního plemene ovce Awassi s berany masných a plodných plemen na růstovou schopnost jehňat v subtropické oblasti

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ABSTRACT: The objective of this study was to evaluate the effect of crossing on body weight and growth ability of lambs from birth to weaning, including the effect of litter size, sex, dam age, dam weight at mating, dam weight after lambing and year of rearing. The study was conducted on the Awassi sheep flock and their crossbreds with Charollais and Romanov breeds maintained at the Agriculture Centre for Research and Production at Jordan University of Science and Technology, Irbid. In 1999 and 2000 the live weight was determined in 192 lambs (70 Awassi, 63 Awassi × Charollais F₁ crossbred and 59 Awassi × Romanov F₁ crossbred) at birth and subsequently every fortnight until weaning by weighing on digital scales to the nearest 0.1 kg. Average live weight of lambs at birth was 4.20 ± 1.15 kg and at the age of 15, 30, 45 and 60 days 8.72 ± 2.12 kg, 12.12 ± 2.77 kg, 15.50 ± 3.70 kg and 19.27 ± 4.59 kg, respectively. ADG of lambs from birth until weaning was 248 ± 0.07 g. Genotype of lambs and litter size affected ADG, live weight of lambs at birth, 15, 30, 45 and 60 days significantly ($P \leq 0.05$ –0.001). Investigations of the effect of sex on live weight of lambs at birth and at 60 days showed that the differences between males and females were statistically significant ($P \leq 0.05$ –0.01). Differences in ADG and live weight at 15, 30 and 45 days according to dam age were significant ($P \leq 0.05$ –0.01). The effect of dam weight at mating on live weight of lambs at birth, ADG until weaning and live weight at weaning was not confirmed. The effect of dam weight at lambing on ADG, live weight of lambs at birth, at 45 days of age and until weaning was not confirmed. Regressions of BW, ADG and weight at 15, 30, 45 and 60 days age of lambs on dam weight after lambing were significant ($P \leq 0.001$). Likewise, live weight of lambs at birth, ADG until weaning and live weight at 15, 30 a 45 days of age were affected by the seasons of lambing ($P \leq 0.01$ –0.001).

Keywords: Awassi; Charollais; Romanov; growth ability; systematic effects; crossing

ABSTRAKT: Cílem práce bylo sledovat a vyhodnotit vliv křížení na růstové schopnosti jehňat v Jordánsku. Bylo provedeno zhodnocení růstové schopnosti jehňat od narození do odstavu ve věku 60 dnů. Kromě genotypu byl sledován také vliv četnosti vrhu, pohlaví jehňat, věku matky, hmotnosti matky při připouštění, hmotnosti matky při obahnění a roku chovu na živou hmotnost a průměrné denní přírůstky jehňat od narození do 60 dnů věku. Pokus se uskutečnil ve výzkumné stanici na zemědělské fakultě Jordánské univerzity v Irbidu. Do pokusu bylo zařazeno celkem 192 jehňat narozených v letech 1999 a 2000; 70 plemene Awassi, 63 F₁ (Awassi × Charollais), 59 F₁ (Awassi

× Romanov). Jehňata od narození až do doby odstavu byla ustájena s matkami v ovčíně a měla k dispozici 0,1 kg jaderné krmné směsi, mateřské mléko, krmnou sůl ad libitum a vodu. U všech jehňat byla zjišťována živá hmotnost vážením na digitální váze s přesností 0,1 kg při narození a dále pak každých 14 dnů až do odstavu. Na základě zjištěných údajů byl proveden přepočít živé hmotnosti na věk 15, 30, 45 a 60 dnů metodou lineární interpolace. Zjištěné údaje byly zpracovány matematicko-statistickým programem – SAS, podle modelových rovnic s pevnými efekty metodou nejmenších čtverců. Průměrná živá hmotnost jehňat při narození byla $4,20 \pm 1,15$ kg; a v 15, 30, 45 a v 60 dnech věku (při odstavu) $8,72 \pm 2,12$ kg, $12,12 \pm 2,77$ kg, $15,50 \pm 3,70$ kg a $19,27 \pm 4,59$ kg, respekt. Průměrné denní přírůstky jehňat od narození až do odstavu činily $248 \pm 0,07$ g. Při hodnocení *F*-testem byl zjištěn vliv genotypu jehněte na průměrné denní přírůstky a živou hmotnost jehňat při narození ve 14, 28, 42 a 60 dnech věku (při odstavu) ($P \leq 0,05$ – $0,001$). Nejlepší živou hmotnost a průměrné denní přírůstky od narození do odstavu vykázaly jehňata – kříženci F_1 Awassi × Romanov. Četnost vrhu velmi průkazně ovlivňuje přírůstky a živou hmotnost při narození a v 15, 30, 45 a 60 dnech věku jehňat ($P \leq 0,001$). Sledování vlivu pohlaví na živou hmotnost při narození a při odstavu byly statisticky významné ($P \leq 0,001$ a $0,05$). Rozdíly v živé hmotnosti a v přírůstcích mezi pohlavími v 15, 30 a 45 dnech věku však nebyly významné. Při hodnocení *F*-testem byl zjištěn vysoce významný vliv věku matky na průměrný denní přírůstek a živou hmotnost jehňat v 15, 30, 45 dnech věku ($P \leq 0,001$). Vliv živé hmotnosti matky při připuštění na živou hmotnost a průměrný denní přírůstek jehňat od narození do odstavu nebyl zjištěn. Živá hmotnost matky po obahnění vysoce průkazně ovlivnila živou hmotnost a průměrný denní přírůstek jehňat v 15, 30 a 45 dnech věku ($P \leq 0,001$). Rozdíly v živé hmotnosti jehňat při narození a průměrné denní přírůstky do 45 dnů věku byly v závislosti na vlivu roku chovu vysoce průkazné ($P \leq 0,001$). Regrese živé hmotnosti v 15, 30, 45 a 60 dnech věku na živou hmotnost jehňat při narození byla průkazná, avšak u průměrných denních přírůstků do 15, 30, 45 a 60 dnů věku nebyl její vliv prokázán. Regrese živé hmotnosti jehňat a průměrných denních přírůstků v 15, 30, 45 a 60 dnech věku na živou hmotnost matek po obahnění byla statisticky průkazná ($P \leq 0,01$).

Klíčová slova: ovce; Awassi; Charollais; Romanov; růstová schopnost; systematické vlivy; křížení

Interest in sheep meat production has increased over the last few years, particularly lamb meat with lower fat content, which coincides with consumer interest (Simm, 1987; Woodward and Wheelock, 1990; Momani Shaker *et al.*, 1996). Increasing mutton production is influenced by litter size and growth intensity of lambs from birth to weaning to rapidly achieve slaughter weight. Two alternative methods of production development exist: using the knowledge of genetics (selection of local breed and crossbreeding with exotic breeds) and improvement of environmental conditions (management and feed quality).

Many authors reported that it is relatively easy and quick to increase fecundity and growth ability of lambs to an optimum level by means of crossing domestic breeds with prolific and mutton breeds (Romanov, Finnish sheep, Charollais, Texel, etc.), as well as forming synthetic breeds or lines (Margetin *et al.*, 1988; Momani Shaker *et al.*, 1994; El Fadili *et al.*, 1999; Horák *et al.*, 2000).

The real growth and development of lambs in the period after birth is a prerequisite for satisfactory efficiency in further phases of rearing and breeding

(Korn and Horstman, 1987; Momani Shaker *et al.*, 1995; Said *et al.*, 2000). These authors indicated that the increase in lamb weight during the period of rearing is affected mainly by lamb sex, litter size and dam age.

The objective of this study was to evaluate the effect of crossing Awassi ewes with Charollais and Romanov sire breeds on body weight and growth ability of lambs from birth to weaning, including the effect of litter size, sex, dam age, dam weight at mating, dam weight after lambing and year of rearing. Regression on birth weight and regression on dam weight at mating and after lambing were also evaluated.

MATERIAL AND METHODS

The study was conducted at the Agriculture Centre for Research and Production at Jordan University of Science and Technology, Irbid. The campus is located in the northern part of Jordan at 36° north and 590 m above sea level. The average rainfall is about 220–230 mm/year.

In 1999 and 2000 the live weight was determined in 192 lambs (70 Awassi, 63 Awassi × Charollais F_1 crossbreds and 59 Awassi × Romanov F_1 crossbreds) at birth and subsequently every fortnight until weaning by weighing on digital scales to the nearest 0.1 kg. Ewes with their lambs were housed in open-sided pens with free access to shade, water and salt block.

Lambs were weaned approximately at 60 days of age. During the pre-weaning period, lambs were supplemented with a concentrate mixture. Lambs received about 0.1 kg/day of concentrate mixture until weaning.

Weights of ewes at mating were determined before insemination and lambing weights of ewes were determined approximately 2 hours after lambing.

During the lambing season ewes received 1.6–2.5 kg/day of concentrate mixture per head.

Ingredient composition and chemical analysis of nutrient content in feed ration are presented in Tables 1 and 2.

Applying the acquired data the method of linear interpolation was used to convert the live weights to age 15, 30, 45 and 60 days (weaning age). Average daily gain (ADG) of lambs from birth to weaning was also evaluated.

To terminate the test, the acquired data were processed by a mathematical and statistical program (SAS) according to the model equations with fixed effects by the least squares method.

Model 1

$$y_{ijkmnop} = \mu + G_i + S_j + L_k + E_m + W_n + Z_o + B_p + e_{ijkmnop}$$

Model 2

$$y_{ijkmnop} = \mu + G_i + S_j + L_k + E_m + B_p + b1(BW_{ijkmnop} - BW) + b2(MW_{ijkmnop} - MW) + b3(ML_{ijkmnop} - ML) + e_{ijkmnop}$$

where: $y_{ijkmnop}$ = live weight of lamb

μ = overall mean

G_i = effect of the i th genotype of lambs ($i = A, A \times Ch, A \times R$)

S_j = effect of the j th sex ($j =$ male and female)

L_k = effect of the k type of birth ($k =$ single and twins)

E_m = effect of the m th age of dam ($m = 2$ years, 3 years, 5 years, 6 years, 7 years and 8 years)

W_n = effect of the n th weight of dam at mating ($n = 30$ –40 kg, 41–50 kg, 51–60 kg and 61–70 kg)

Z_o = effect of the o th weight of dam at lambing ($o = 30$ –40 kg, 41–50 kg, 51–60 kg and 61–70 kg)

B_p = effect of the p th year ($p = 1999$ and 2000)

$b1(BW_{ijkmnop} - BW)$ = regression on lamb birth weight

$b2(MW_{ijkmnop} - MW)$ = regression on dam weight at mating

$b3(ML_{ijkmnop} - ML)$ = regression on dam weight after lambing

$e_{ijkmnop}$ = residual errors distribution $N(0, \sigma^2)$

Table 1. Formulation and proportions of individual ingredients in feed ration (%)

Ingredient	Proportion (%)
Barley	49.46
Alfalfa	14.84
Soybean meal	9.89
Wheat straw	9.89
Wheat bran	14.84
DCP	0.49
Minerals and vitamins	0.10
Salt	0.49

Evaluation of the live weight and growth ability of lambs involved the effects of genotype of lambs, litter size, sex, dam age, dam weight at mating, dam weight after lambing and year of rearing. Regressions of live weights of lambs until weaning on lamb birth weight (BW), on dam weight at mating and dam weight after lambing were also evaluated.

RESULTS AND DISCUSSION

Tables 3 and 4 show ADG and live weight at birth (BW), 15, 30, 45 and 60 days age as depending upon lamb genotype, litter size, sex, dam age,

Table 2. Chemical analysis of nutrient content in feed ration

Dry matter (%)	Fat (%)	Crude protein (%)	Ash (%)	Fiber (%)	ME (MJ/kg)
91.33	2.37	17.12	3.47	5.01	12.60

dam weight at mating, dam weight after lambing and year of rearing.

Average live weight of lambs at birth was 4.20 ± 1.15 kg and at the age of 15, 30, 45 and 60 days 8.72 ± 2.12 kg, 12.12 ± 2.77 kg, 15.50 ± 3.70 kg and 19.27 ± 4.59 kg, respectively. ADG of lambs from birth until weaning was 248 ± 0.07 g.

Effect of lamb genotype

Genotype of lambs affected ADG, BW and live weight of lambs at 15, 30, 45 and 60 days significantly ($P \leq 0.05$ – 0.001). Crossbred lambs F_1 (A \times Ch and A \times R) showed higher growth intensity in all investigated parameters as compared with the pure Awassi lambs.

Live birth and weaning weights of lambs were 4.37 ± 0.16 kg and 17.84 ± 0.68 kg in crossbreds F_1 A \times CH, 3.85 ± 0.16 kg and 18.94 ± 0.69 kg in crossbreds F_1 A \times R, and 3.58 ± 0.24 kg and 13.13 ± 1.08 kg in Awassi lambs ($P \leq 0.001$).

The highest ADG until 15 days of age was found in F_1 A \times Ch (225 ± 0.01 g) while the ADG until weaning age was highest in crossbreds F_1 A \times R (243 ± 0.01 g) compared with crossbreds F_1 A \times Ch (224 ± 0.01 g) and in Awassi lambs (146 ± 0.02 g).

Better indicators of the crossbreds compared with the pure lambs were observed due to the significant genetic and geographical difference of exotic sire breeds (Charollais and Romanov) and probably due to the suggested influence of heterosis. Similar results were reported by Meyer *et al.* (1978), Mavrogenis and Louca (1979) and Gohler and Walther (1981), who found that crossbreds had faster growth and higher live weight at 140 days of age as compared with purebred lambs.

Effect of litter size

Another important criterion that affects the live weight and growth intensity of lambs is the litter size. Live birth weight of lambs and weights at 15, 30, 45 and 60 days of age were significantly influenced ($P \leq 0.001$) by litter size. When comparing

ADG in relation to the litter size, differences were also significant between singles and twins.

Live birth weight of lambs was 36.34% higher in singles compared with twins. The weight of single lambs at 60 days was 18.34 ± 0.67 kg compared with 14.95 ± 0.85 kg in twins. The differences were statistically significant ($P \leq 0.001$). These results are in agreement with those reported by Abdul-Rahman *et al.* (1986), who found that single born Awassi lambs were 0.44 kg heavier at birth than twin born lambs. This difference increased to 3.98 kg at weaning in favour of singles. Mohammed *et al.* (1987) reported that single lambs of the Karadi breed had significantly higher weights at birth than twins, but the differences diminished during further growth. Momani Shaker *et al.* (1995) stated that the weight of single lambs was 10.50% higher at birth and 13.31% higher at the age of 70 days when compared with twins.

Effect of sex

Another important criterion that affects the live weight and growth intensity of lambs is sex. Many authors confirmed that sex has an important effect on the growth: Horák *et al.* (1987), Korn and Horstman (1987), Momani Shaker *et al.* (1995), Said *et al.* (2000).

Investigations of the effect of sex on BW and live weight of lambs at 60 days of age showed that the differences between males and females were statistically significant ($P \leq 0.05$ – 0.01). Live BW of males was 18.01% higher than in females and at weaning the difference between the average weight of males and females was 10.77% higher in favour of males. The differences in live weight and weight gains between the males and females at 15, 30 and 45 days of age were not significant.

Said *et al.* (2000) reported that rams of the Awassi breed had significantly higher BW and weaning weight, and significantly higher ADG compared with females. Also Momani Shaker *et al.* (1995) found that birth weight of Charollais ram lambs was 2.92% higher than in females and at the age of 130 days, the difference was as much as 13.48%.

Table 3. Means, standard deviations and *F* values for live weight at birth, at 15, 30, 45 days and at weaning as depending upon the particular effects

Indicator	<i>n</i>	Birth weight (kg)	Weight at 15 days (kg)	Weight at 30 days (kg)	Weight at 45 days (kg)	Weaning (60) weight (kg)
Mean	192	4.20 ± 1.15	8.72 ± 2.12	12.12 ± 2.77	15.50 ± 3.70	19.27 ± 4.59
<i>F</i> value		9.62***	23.67***	14.43***	11.96***	8.67***
Genotype						
<i>F</i> value		8.22**	3.33*	6.38**	12.28***	9.40 ± 16.42***
A ^a	70	3.58 ± 0.24 ^b	6.91 ± 0.36 ^{bc}	10.97 ± 0.80 ^{bc}	0.78 ^{bc}	13.13 ± 1.08 ^{bc}
A × Ch ^b	63	4.37 ± 0.16 ^{ac}	7.78 ± 0.22 ^a	14.30 ± 0.50 ^a	13.54 ± 0.53 ^a	17.84 ± 0.68 ^a
A × R ^c	59	3.85 ± 0.16 ^b	7.55 ± 0.36 ^a	14.62 ± 0.51 ^a	13.18 ± 0.50 ^a	18.94 ± 0.69 ^a
Type of birth						
<i>F</i> value		53.22***	17.44***	12.43***	9.21***	16.56***
Single ^a	132	4.54 ± 0.16 ^b	7.99 ± 0.22 ^b	11.24 ± 0.35 ^b	14.23 ± 0.50 ^b	18.34 ± 0.67 ^b
Twin ^b	60	3.33 ± 0.18 ^a	6.84 ± 0.28 ^a	9.71 ± 0.44 ^a	12.36 ± 0.63 ^a	14.95 ± 0.85 ^a
Sex						
<i>F</i> value		19.72***	0.50	0.08	0.33	6.50**
Male ^a	97	4.26 ± 0.17 ^b	7.34 ± 0.23	10.42 ± 0.37	13.44 ± 0.52	17.49 ± 0.71 ^b
Female ^b	95	3.61 ± 0.16 ^a	7.49 ± 0.24	10.53 ± 0.38	13.15 ± 0.55	15.79 ± 0.73 ^a
Age of dam						
<i>F</i> value		0.55	7.50***	7.26***	3.79**	1.58
2 years ^a	22	4.07 ± 0.23	8.25 ± 0.32 ^{bef}	11.62 ± 0.50 ^{bef}	14.18 ± 0.72 ^{ef}	17.03 ± 0.97
3 years ^b	25	4.13 ± 0.24	7.18 ± 0.34 ^{ad}	9.69 ± 0.53 ^{ad}	13.12 ± 0.76 ^{ef}	16.22 ± 1.01
5 years ^c	36	3.80 ± 0.22	8.03 ± 0.31 ^{ef}	12.01 ± 0.49 ^{ef}	14.98 ± 0.69 ^{ef}	18.23 ± 0.94 ^f
6 years ^d	43	4.10 ± 0.18	7.97 ± 0.25 ^{bef}	10.71 ± 0.39 ^{bef}	13.19 ± 0.56 ^{ef}	16.58 ± 0.75
7 years ^e	20	3.82 ± 0.28	6.75 ± 0.40 ^{acd}	9.72 ± 0.63 ^{acd}	12.46 ± 0.90 ^{acde}	16.73 ± 1.22
8 years ^f	22	3.68 ± 0.31	6.32 ± 0.44 ^{acd}	9.28 ± 0.69 ^{acd}	11.83 ± 0.98 ^{acde}	15.06 ± 1.32 ^c
Ewe weight at mating						
<i>F</i> value		0.13	3.80*	1.39	0.62	0.56
30–40 kg ^a	21	3.96 ± 0.23	8.07 ± 0.32 ^d	10.90 ± 0.50	13.56 ± 0.72	17.00 ± 0.97
41–50 kg ^b	69	3.94 ± 0.15	7.96 ± 0.23 ^d	10.88 ± 0.36	13.47 ± 0.51	16.84 ± 0.68
51–60 kg ^c	49	4.02 ± 0.17	7.64 ± 0.24 ^d	10.99 ± 0.37	13.87 ± 0.53	17.37 ± 0.72 ^d
61–70 kg ^d	5	3.81 ± 0.42	5.99 ± 0.59 ^{abc}	9.14 ± 0.94	12.27 ± 1.34	15.35 ± 1.80 ^c
Ewe weight after lambing						
<i>F</i> value		1.38	5.90**	4.54**	2.86*	1.69
30–40 kg ^a	12	3.95 ± 0.30	6.43 ± 0.43 ^{cd}	8.83 ± 0.67 ^{bcd}	11.38 ± 0.96 ^{cd}	15.38 ± 1.29
41–50 kg ^b	31	3.86 ± 0.22	7.10 ± 0.31 ^{cd}	10.24 ± 0.49 ^{acd}	13.10 ± 0.70	15.86 ± 0.94
51–60 kg ^c	62	3.76 ± 0.19	7.74 ± 0.28 ^{abd}	11.27 ± 0.44 ^{ab}	14.10 ± 0.62 ^a	17.67 ± 0.83 ^d
61–70 kg ^d	39	4.17 ± 0.18	8.38 ± 0.26 ^{abc}	11.56 ± 0.40 ^{ab}	14.59 ± 0.58 ^a	17.66 ± 0.77 ^c
Year						
<i>F</i> value		22.95***	5.50**	0.01	2.97	2.52
1999 ^a	97	3.37 ± 0.21 ^b	6.98 ± 0.33 ^b	10.44 ± 0.52 ^b	12.58 ± 0.74	16.26 ± 0.99
2000 ^b	95	4.50 ± 0.16 ^a	7.85 ± 0.22 ^a	10.51 ± 0.35 ^a	14.01 ± 0.49	17.02 ± 0.66
Regression on weight at birth						
<i>F</i> value	192	–	64.29***	39.86***	24.10***	9.87**
Regression on dam weight						
<i>F</i> value	92	0.35	1.06	0.74	0.33	0.43
Regression on dam weight after lambing						
<i>F</i> value	192	2.17*	8.18***	10.13***	10.25***	8.68***

P* ≤ 0.05; *P* ≤ 0.01; ****P* ≤ 0.001

Table 4. Means, standard deviations and *F* values for average daily weight gain from birth to 15, 30, 45 days and to weaning as depending upon the particular effects

Indicator	<i>n</i>	Growth until 15 days of age (g)	Growth until 30 days of age (g)	Growth until 45 days of age (g)	Growth until 60 days (weaning) of age (g)
Mean	192	287 ± 0.09	257 ± 0.08	246 ± 0.07	248 ± 0.07
<i>F</i> value		7.04***	6.11***	6.28***	5.96***
Genotype					
<i>F</i> value		3.37*	6.38***	11.88***	16.19***
A ^a	70	166 ± 0.02 _{bc}	163 ± 0.02 _{bc}	145 ± 0.02 _{bc}	146 ± 0.02 _{bc}
A × Ch ^b	63	225 ± 0.01 ^a	220 ± 0.01 ^a	219 ± 0.01 ^a	224 ± 0.01 ^a
A × R ^c	59	209 ± 0.01 ^a	223 ± 0.01 ^a	226 ± 0.01 ^a	243 ± 0.01 ^a
Litter size					
<i>F</i> value		17.11***	11.80***	9.01***	16.63***
Single ^a	132	238 ± 0.01 _b	228 ± 0.01 _b	218 ± 0.01 _b	233 ± 0.01 _b
Twin ^b	60	162 ± 0.02 ^a	177 ± 0.01 ^a	176 ± 0.01 ^a	176 ± 0.01 ^a
Sex					
<i>F</i> value		0.50	0.10	0.26	6.56**
Male ^a	97	195 ± 0.01	200 ± 0.01	200 ± 0.01	219 ± 0.01 _b
Female ^b	95	205 ± 0.01	204 ± 0.01	194 ± 0.01	190 ± 0.01 ^a
Age of dam					
<i>F</i> value		7.38***	7.02***	3.80*	1.49
2 years ^a	22	255 ± 0.02 _{bef}	238 ± 0.02 _{bef}	217 ± 0.02 _f	211 ± 0.02
3 years ^b	25	186 ± 0.02 _{ad}	177 ± 0.02 _{ac}	194 ± 0.02	196 ± 0.02
5 years ^c	36	240 ± 0.02 _{ef}	253 ± 0.02 _{bdef}	233 ± 0.02 _{def}	231 ± 0.02 _f
6 years ^d	43	236 ± 0.02 _{bef}	204 ± 0.01 _c	195 ± 0.01 _c	203 ± 0.02
7 years ^e	20	155 ± 0.03 _{acd}	176 ± 0.02 _{ac}	178 ± 0.02 _c	207 ± 0.02
8 years ^f	22	126 ± 0.03 _{acd}	163 ± 0.02 _{ac}	164 ± 0.02 _{ac}	179 ± 0.02 _c
Ewe weight at mating					
<i>F</i> value		3.80**	1.44	0.64	0.46
30–40 kg ^a	21	243 ± 0.02 _d	217 ± 0.02	202 ± 0.02	210 ± 0.02
41–50 kg ^b	69	236 ± 0.01 _d	216 ± 0.01	201 ± 0.01	208 ± 0.01
51–60 kg ^c	49	216 ± 0.02 _d	218 ± 0.01 _d	210 ± 0.01	215 ± 0.01
61–70 kg ^d	5	105 ± 0.04 _{abc}	156 ± 0.03 _c	173 ± 0.03	184 ± 0.03
Ewe weight after lambing					
<i>F</i> value		5.88***	4.44***148 ± 0.02	2.79*	1.75
30–40 kg ^a	12	135 ± 0.03 _{cd}	_{bcd}	155 ± 0.02 _{cd}	183 ± 0.02
41–50 kg ^b	31	179 ± 0.02 _{cd}	194 ± 0.02 _{acd}	192 ± 0.01	191 ± 0.02
51–60 kg ^c	62	221 ± 0.02 _{abd}	228 ± 0.01 _{ab}	215 ± 0.01 ^a	222 ± 0.01
61–70 kg ^d	39	264 ± 0.02 _{abc}	238 ± 0.01 _{ab}	225 ± 0.01 ^a	221 ± 0.01
Year					
<i>F</i> value		5.50**	0.02	3.05	0.44
1999 ^a	97	171 ± 0.02 _b	200 ± 0.02	181 ± 0.02	198 ± 0.02
2000 ^b	95	229 ± 0.01 ^a	204 ± 0.01	213 ± 0.01	210 ± 0.01
Regression on weight at birth					
<i>F</i> value	192	0.01	1.52	1.77	0.23
Regression on dam weight at mating					
<i>F</i> value	192	0.67	0.55	0.25	0.62
Regression on dam weight after lambing					
<i>F</i> value	192	5.39**	8.26***	8.67***	7.31**

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Effect of dam age

No effect of dam age on BW, ADG until weaning and live weight at weaning were observed. Differences in ADG and live weight at 15, 30 and 45 days according to dam age were significant ($P \leq .05-0.01$).

The lowest live weights of lambs at 45 days of age were from ewes seven and eight years old and the highest were from two and five years old ewes. The differences were statistically significant ($P \leq 0.001$). These results are consistent with the data reported by Momani Shaker *et al.* (1995) in their experiment in the Charollais breed. However, Said *et al.* (2000) found that the age of dams significantly affected live weight and ADG from birth and until weaning in Awassi lambs.

Al-Rawi *et al.* (1982) and Abdul-Rahman *et al.* (1986) reported that the age of dam did not affect the growth of lambs until weaning. Korn and Horstman (1987) and Horák *et al.* (1987) reported that the weight gain of lambs after birth is markedly affected by milk production of dams.

Effect of dam weight at mating

The effect of dam weight at mating on live weight of lambs at birth, average daily gain until weaning and live weight at weaning was not confirmed. According to Krížek *et al.* (1983), live weight of dams significantly affected live weight and ADG of lambs at birth and at the age of 30 and 60 days.

Regressions of BW, ADG and weight at 15, 30, 45 and 60 days age of lambs on dam weight at mating were not significant.

Effect of dam weight after lambing

No effects of dam weight at lambing on ADG, live weight of lambs at birth, at 45 days of age and until weaning were observed, however, ADG and live weight of lambs at 15 and 30 days of age according to dams' weight at lambing were significant ($P \leq 0.05$).

Regressions of BW, ADG and weight at 15, 30, 45 and 60 days age of lambs on dam weight after lambing were significant ($P \leq 0.001$). These results were in accordance with those reported earlier on different breeds by Hermiz (1988), Aziz *et al.* (1989, 1995).

Regression of lamb live weight on dam weight reflects the size of dam and its nutritional condition on prenatal lamb growth. Also, lambs with high milk intake gained faster and were more resistant to diseases and parasites than lambs with low milk intake. Thus, the size of the dam was considered as a major factor that affects the growth of lambs pre and postnatally.

Effect of year

Likewise, live BW, ADG until weaning and live weight at 15, 30 and 45 days of age were affected by the year of lambing ($P \leq 0.01-0.001$).

Live BW of lambs born in 2000 was 33.53% higher than in lambs born in 1999 and the differences at the age of 45 days of lambs according to season were 11.36% higher in favour of the year 2000. The differences were statistically significant ($P \leq 0.001$). The differences in body weight and gain between the seasons of lambing were attributed to the yearly variation in the rain precipitation and its effect on the density, growth and availability of pastures, forage and other feeds. Similarly different climates have been reported to influence milk production of ewes. This indirectly affects the growth of lambs. The results achieved in this study are in congruency with Aziz *et al.* (1989), Said and Al-Rawi (1990), Al-Nidawi (1991) and Said *et al.* (2000), but inconsistent with Momani Shaker *et al.* (1995).

Suitable choice of mutton and prolific breeds and correct method of crossing with the use of excellent performance of the indigenous sheep breed will significantly contribute to an increase in mutton production.

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Comparison of estimated breeding values and daughter yield deviations used in segregation and linkage analyses

Použití predikovaných plemenných hodnot a odchylek užitekosti dcer v segreganční a vazební analýze

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ABSTRACT: Estimated breeding values (EBV's) and daughter yield deviations (DYD's) were used in a segregation and linkage analysis of milk, fat and protein yield. The results were compared focusing on differences in estimated recombination rates and test criteria after using both trait measures. Data analysis was based on a grand daughter design including 591 sons of 5 sires. The sons were typed for several microsatellite markers on chromosome 6. In general lower additive QTL effects were obtained from analyses using DYD's. The estimated recombination rates were similar if the QTL effects could be estimated with high accuracy whether EBV's or DYD's were used. In general, LOD scores using EBV's were higher. A LOD score 3.12 (EBV) and 2.15 (DYD) was obtained for fat yield. The recombination rate was zero between QTL and one marker on chromosome 6 in both cases. For protein yield the LOD scores were 1.78 (EBV) and 1.76 (DYD) while estimating a recombination rate of 0.06 each. If QTL effects are estimated with sufficient accuracy, the results of analyses based on EBV's can provide a good indication of linkage between genetic markers and QTL for sons with a high number of tested daughters are included.

Keywords: milk traits; genetic markers; QTL; breeding values; DYD; segregation analysis; genetic linkage

Abbreviation Key: EBV = estimated breeding value, DYD = daughter yield deviation, MY = milk yield, FY = fat yield, PY = protein yield, QTL = quantitative trait locus, BLUP = best linear unbiased prediction, LH = likelihood, LR = likelihood ratio, LOD = likelihood odds ratio.

ABSTRAKT: Predikované plemenné hodnoty (EBV) a odchylky užitekosti dcer (DYD) byly použity v segreganční a vazební analýze pro znaky množství mléka, tuku a bílkovin. Výsledky byly srovnány se zaměřením na rozdíly v odhadech míry rekombinace a v testovacím kritériu při použití obou měř. Analýza dat se zakládala na grand daughter design s 591 syny od 5 otců. Synové byli typizováni na několik mikrosatelitních markerů na chromozomu 6. Obvykle nižší aditivní QTL efekty byly zjištěny v rozbořech při použití DYD. Jestliže QTL efekty mohly být odhadnuty s vysokou přesností, pak byly odhady míry rekombinace podobné při použití EBV i DYD. LOD skóre bylo obvykle vyšší při použití EBV. LOD skóre pro množství tuku bylo 3,12 a 2,15. Míra rekombinace mezi QTL a jedním markerem na chromozomu 6 byla v obou případech nula. LOD skóre pro množství bílkovin bylo 1,78 a 1,76, odhad míry rekombinace byl v obou případech 0,06. Pokud jsou QTL efekty odhadnuty s dostatečnou přesností a jsou zahrnuti synové s vysokým počtem testovaných dcer, mohou rozboř založené na EBV dobře indikovat vazbu mezi genetickými markery a QTL.

Klíčová slova: mléčná užitekost; genetické markery; QTL; plemenná hodnota; DYD; segreganční analýza; genetická vazba

Before analysing data from dairy cattle breeding aiming at estimation of QTL effects, the question arises what kind of performance measure should be the basis for estimation. Generally a grand daughter design with genotyped sires and sons proven for daughter milk performance is used for QTL mapping in dairy cattle (Weller *et al.*, 1990). Genetic effects were also used for QTL estimation (e.g. Velmala *et al.*, 1999; de Koning *et al.*, 2001). Since estimated breeding values (EBV's) based on an animal model (BLUP) are computed by the national evaluation system routinely. The use of these values especially for a large number of daughters in sires and sons is straightforward. EBV's are available very easily without additional procedures.

What needs to be considered if one uses EBV's as observations in analyses to estimate QTL effects?

EBV derived from a mixed model are regressed and therefore estimates of QTL effects will be biased (Weller, 1997). The EBV of a young sire without any daughter performances is completely determined by the EBV of its parents. The higher the number of daughters, the more independent the EBV of a sire becomes of its parents. In the case of a relatively high heritability (> 0.20) and if the number of daughters exceeds 80, the parental EBV affects the EBV of the son only to a small extent (e.g. Swalve and Bünger, 1997). In such cases, the use of EBV's or DYD's might be equivalent. But in general the use of DYD's is highly recommended (e.g. Swalve and Bünger, 1997 and van Raden and Wiggans, 1991). It should be ensured that each sire is described by only one unregressed value derived from its own offspring. Yield deviation is defined to be the evaluated average of lactation yields of a cow minus the effect of the management group, sire effect and permanent environmental effect.

In the case of dairy bulls DYD is thus defined as the evaluated average of the yield deviations of its daughters corrected for mate performance. DYD's are useful unregressed measures of daughter performances (van Raden and Wiggans, 1991). Here we show and discuss the results of a segregation and linkage analysis based on DYD's in comparison with those based on EBV's.

MATERIAL AND METHODS

Data

A detailed description of the data and microsatellite markers used in our study (Table 1) can be found in Kühn *et al.* (1999). Five families of German Black and White Holsteins following a grand daughter design were used. The DYD of milk yield ranged from 450 kg in family 1 to 1 010 kg in family 5. The data set contained 591 sons with 103 daughters on average belonging to 5 sire families, genotyped for 16 microsatellite markers on the bovine chromosome 6. We concentrate on five markers being informative with respect to the targeted comparison. The goal is to show the differences in recombination rates and test statistic values when comparing the results of two-point analyses based on different markers and the behaviour of DYD and EBV with respect to the results. Another criterion for choosing those five markers was the heterozygosity of the sires and a considerable number of genotyped sons. There is a different number of sons typed for each marker as only sons from heterozygous sires were genotyped at the respective marker. The EBV's for yield traits and the corresponding DYD's were provided by the computer center VIT Verden, Germany, from the

Table 1. Average and standard deviation of traits EBV milk yield, EBV fat yield, EBV protein yield and DYD milk yield, DYD fat yield and DYD protein yield (trait values of 591 sons of 5 sires, each having 73 of 212, 65 of 133, 27 of 73, 17 of 39 and 117 of 134 sons genotyped)

Trait measure	Average	Standard deviation
EBV milk yield	629	517
EBV fat yield	14.5	17.1
EBV protein yield	16.3	13.3
DYD milk yield	367	191
DYD fat yield	13.5	10.1
DYD protein yield	12.2	7.1

routine evaluation in August 1996 because of full data availability for the genotyped animals.

Segregation and linkage analysis

Segregation and linkage analysis was carried out using the maximum likelihood approach according to Liu (1994) and Simianer (1993). A mixed model of inheritance considering a QTL and polygenic component was used. The genetic model was as follows:

$$y_{ij} = \mu + \mu_{Qij} + u_{ij} + e_{ij}$$

where: y_{ij} = son i trait value (EBV or DYD) of sire j
($j = 1$ to 5)

μ = general mean

μ_{Qij} = effect of the assumed individual QTL genotype

u_{ij} = random polygenic effect of son i in sire j

e_{ij} = residual effect

The parameter estimation (frequency of QTL-allele q , additive allelic effect at QTL a , dominance effect d , recombination rate θ between the marker and putative QTL, polygenic heritability h^2) is based on maximum likelihood method.

Three genetic models (M) were used for hypothesis testing:

M1 – model assuming one putative QTL and polygenic inheritance, genetic linkage to one marker is taken into account

M2 – model assuming one putative QTL and polygenic inheritance, free recombination (no linkage) between marker and putative QTL

M3 – polygenic model of inheritance, no QTL assumed

The likelihood (LH) from M1 is compared with the LH of M3 to test for the presence of a QTL and the LH of M1 is compared with the LH of M2 to test for linkage. The linkage was suggested to be confirmed if the LOD score exceeded 3. The QTL effect was assumed to be significant if the likelihood ratio (LR) exceeded 16. According to the χ^2 statistics this corresponds to type I error $\alpha < 0.001$.

RESULTS AND DISCUSSION

Below, we compare the results from analyses using EBV's and DYD's, respectively. We concentrate on

estimated recombination rates θ , LOD scores and likelihood ratios (LR).

The results of analyses are shown in Table 2. In general, estimates for additive QTL effects are lower if DYD's are used as expected due to the lower variances of DYD's.

One or three markers showed LOD scores above zero if EBV's (marker 4) or DYD's (marker 1, 2 and 4) were used for milk yield. Recombination rates varied considerably whether the analysis was based on EBV's or DYD's in this trait. These results might be due to the QTL effect being poorly estimated. Which is indicated by a low LR. The estimated recombination rate regarding fat yield was zero for two markers showing LOD scores 0.78 (EBV) and 0.39 (DYD) for marker 5; as well as 3.12 (EBV) and 2.15 (DYD) for marker 2. The likelihood ratio test statistic (LR) for the latter is the highest obtained in this comparison. Thus, whereas the evidence for linkage is significant using EBV's, we are below the threshold for DYD's. For protein yield, the estimated recombination rate was zero for marker 5 showing LOD scores 0.56 (EBV) and 0.34 (DYD). Higher and quite the same LOD scores were obtained using marker 2, for $\theta = 0.06$ in both cases.

Overall, we got similar results with respect to linkage to involve into discussion whether EBV's or DYD's for fat or protein yield were used in our analyses. The correlation coefficients between EBV's and DYD's regarding yield traits ranged from 0.91 to 0.94, so this result is not surprising.

The use of EBV's resulted in higher LOD scores. From the theoretical point of view, DYD's represent the more appropriate measure. In our study we got one significant result using EBV's with respect to fat yield. It is not clear whether this result is due to analyses based on EBV's being more biased than analyses based on DYD's. This question has not been answered so far. A recent study (Freyer *et al.*, 2002) took such comparison into consideration more extensively. Analysing chromosome 6 marked by 16 microsatellite markers to detect QTL for milk traits they used both DYD and EBV for yield traits. Adapting a computer programme based on the least squares method (Du and Hoeschele, 1999) it could be shown that the suggested positions for putative QTL did not differ whether DYD or EBV was used. Monitoring milk yield and fat yield (Figures 1 and 2) reflects the same shape of the profile by "sharpening" the highest peaks. The only difference is the level of the test statistics and a more peaked

Table 2. Results of segregation and linkage analyses of milk traits and five microsatellite markers in comparison of EBV and DYD

Marker/ Parameter	Milk Trait/Measure						number of sons genotyped per marker
	EBV milk yield	EBV fat yield	EBV protein yield	DYD milk yield	DYD fat yield	DYD protein yield	
Marker 1							
<i>a</i>	342	10.9	11.6	183	8.97	5.19	256
<i>d</i>	295	9.1	-0.1	194	3.9	-0.6	
<i>p</i>	0.22	0.24	0.57	0.16	0.11	0.14	
θ	(0.5)	0.5	0.5	0.48	0.5	0.5	
LR	0.6	-0.1	-0.8	0.02	0.4	1.7	
LOD score	0	0	-0	0.1	0	0	
Marker 2							
<i>a</i>	196	11.6	12.1	162	5.72	1.99	153
<i>d</i>	-758	8.7	-4	-78	6.0	8.01	
<i>p</i>	0.56	0.15	0.5	0.53	0.42	0.07	
θ	-0	-0	0.06	0.16	-0	0.06	
LR	0.34	14.4	8.2	1.4	9.82	0.91	
LOD score	>0	3.12	1.78	0.82	2.15	1.76	
Marker 3							
<i>a</i>	513	17.3	4.6	312	6.7	9.1	153
<i>d</i>	-416	6.1	10.4	-41	-6.5	-16.8	
<i>p</i>	0.98	0.43	0	0.97	0	0.99	
θ	0	0.5	0	0	0.5	0	
LR	-0	0	-0	-0	-0	-0	
LOD score	0	0	0	0	0	0	
Marker 4							
<i>a</i>	306	12.5	9.4	208	8.1	3.2	167
<i>d</i>	41	4.2	2.3	179	-8.6	7.8	
<i>p</i>	0.48	0.21	0.3	0.14	0.04	0.51	
θ	0	0.5	0.28	0.23	0.11	0.17	
LR	1.2	-0	-0	4.4	-0	-0	
LOD score	0.74	-0	-0	0.39	-0	-0	
Marker 5							
<i>a</i>	301	12.6	12.6	120	7.4	28.0	149
<i>d</i>	285	5.7	-3.5	-219	4.2	32.7	
<i>p</i>	0.18	0.21	0.60	0	0.17	0.06	
θ	0.29	-0	-0	0.23	-0	-0	
LR	-0	3.6	2.6	0	1.8	6.8	
LOD score	-0	0.78	0.56	-0	0.39	0.34	

course when using EBV. For protein yield a similar curve as shown for milk yield was obtained differing in the range of the test statistic values. Reflecting the shape of the curve of the DYD analysis the test statistic is higher especially at the noticeable peaks. In the case of such results of analyses it is sure that

chromosome 6 is harbouring more than one QTL for milk traits.

The point is to ask about the consequence of using DYD or EBV in QTL mapping studies. It could be of some interest if the use of EBV's led to definitely false assumptions on linkage whereas

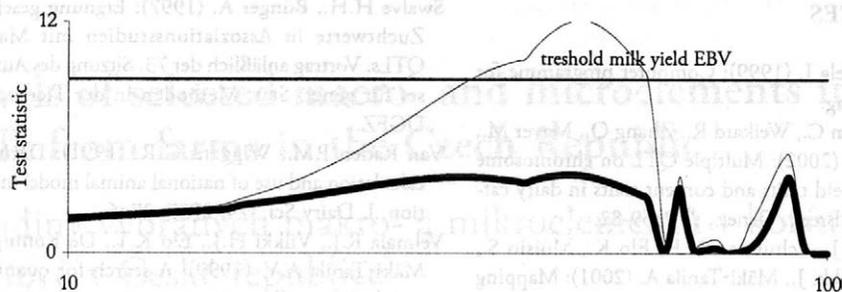


Figure 1. Test statistic profile over a marked map and trait milk yield DYD (dark line) and EBV (thin line), threshold at the chromosome wise 0.05 level (Freyer *et al.*, 2002) in logarithmic map scale (single family)

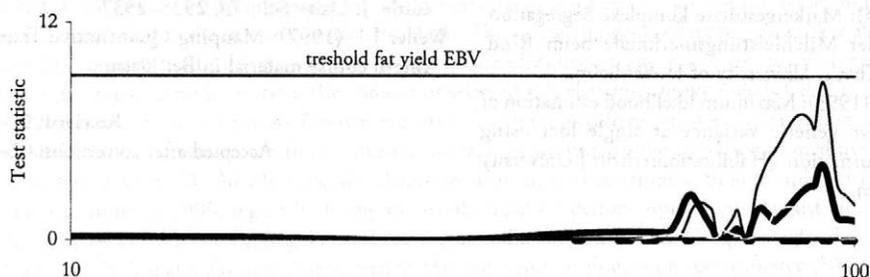


Figure 2. Test statistic profile over a marked map and trait fat yield DYD (dark line) and EBV (thin line), threshold at the chromosome wise 0.05 level (Freyer *et al.*, 2002) in logarithmic map scale (single family)

simultaneously using DYD's does not. This paper may provoke a discussion and possibly a more detailed examination of this subject. Simulation studies could help to finally assess the differences between analyses using DYD's or EBV's. The results from such simulations can be a step forward to clarify possible consequences of bias in MAS if EBV's were used for mapping QTL.

CONCLUSIONS

The DYD of milk traits are more appropriate than EBV for determining genetic linkage between marker loci and quantitative trait loci in dairy cattle as has been underlined by a number of authors. However, from a practical point of view the application of genetic values such as EBV is also accepted. The genetic values are easily available from the routine evaluation and their use is straightforward. We can conclude from our

results that the analysis using EBV is a reflection of the analysis using DYD, partly accompanied by extremely higher test statistic values at the peaks of the profiles which could probably suggest QTL. The estimated QTL positions were mostly the same or they differ within a relatively small interval, respectively. If data on sufficiently large and informative families are used, the success of QTL search mainly depends on the whole marker information. This could be shown repeatedly when comparing the results of DYD and EBV used in different studies based on real data. But simulation studies might be appropriate to clarify the consequences finally.

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Levels of selected macro- and microelements in goat milk from farms in the Czech Republic

Hladiny vybraných makro- a mikroelementů v kozím mléce z chovů v České republice

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ABSTRACT: Goat milk has an important role in the nutrition of children who are allergic to cow milk, and also for the general population in the case of digestion problems. The anticarcinogenic effect of goat milk is also mentioned. Similarly like in other foods, the quality of goat milk is carefully checked and has to comply with general hygienic requirements. In this communication, the content of selected risk elements (heavy metals Cd, Cu, Fe, Pb, Zn) in goat milk collected from two private farms is reported. These farms differed in the concentration of animals and also in the degree of contamination from industrial sources. In addition to the above mentioned five heavy metals, the macroelements Ca, Cl and Mg were also determined in these milk samples. Bulk samples of goat milk were collected four times in 1998, regularly during the whole lactation period (April, June, August and October). For determination of Ca, Cd, Cu, Fe, Mg, Pb, and Zn in goat milk, aliquots of milk (20 g) were dried and decomposed by dry ashing. Ca, Mg and Zn were determined by the flame atomic absorption spectrometry (FAAS), Cu, Cd and Pb by electrothermal atomic absorption spectrometry (ETAAS), respectively. Fe was determined by the modified molecular spectrophotometric method after reaction with potassium thiocyanate (ČSN 83 0530). Chloride ions were determined argentometrically (ČSN 57 0530). In order to control the quality of analytical data, simultaneous analyses of the certified reference material CRM 063R were performed. For statistical evaluation, ANOVA method of one factor analysis of variance by the multiple comparison method and probability level of 95% was used. These results lead to the following conclusions: all average values of the determined chemical elements in milk samples from both farms are below the hygienic limits set in Regulation No. 298/1997 of the Ministry of Health of the Czech Republic. In those cases when the levels of the measured analytes are close to the regulatory limits, a higher number of parallel determinations is required. Geological composition of the given locality along with feeds seem to be more decisive for the resultant contents of contaminating chemical elements in milk than the amount of industrial and traffic emissions in the locality. This can explain the relatively frequent occurrence of variable contents of the measured analytes in milk. We could not find any direct relationship between the content of the elements concerned and the time of sampling. A clearly monotonous increase was observed only in Mg content in milk from both farms during the lactation period. An analogous dependence was obtained also for Fe, but with one exception (high initial Fe content in milk from farm A). On the other hand, the amount of chloride ions in milk from farm B decreased monotonously and significantly during the lactation period. Samples from farm A showed a similar tendency. Despite of certain discrepancies it can be assumed that the levels of Ca, Cu, Zn, Cd and Pb at the beginning of the lactation period are mostly higher than those at its end. The ranges of the contents of macroelements Ca, Mg, and microelements Cd, Pb, Zn, and probably also Cu in goat milk are comparable with those commonly found in cow milk. Slightly increased levels in goat milk were observed in iron and chlorides.

Keywords: goat milk; calcium; magnesium; chlorides; cadmium; lead; zinc; copper; iron; AAS; spectrophotometric determination; precipitation titration; hygienic limit

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ABSTRAKT: Kozí mléko má velký význam ve výživě dětí, které jsou alergické na kravské mléko, a je proto bezpodmínečně nutné, aby jeho kvalita odpovídala platným hygienickým požadavkům. Sledovány byly hladiny Ca, Mg a Cl, Fe, Zn, Cu, Cd a Pb v mléce koz ze dvou kozích farem, nacházejících se v oblastech s rozdílným průmyslovým zatížením. Vzorky mléka byly odebrány celkem čtyřikrát v rovnoměrných intervalech v průběhu laktačního období (duben až říjen). Ca, Mg a Zn byly stanoveny metodou FAAS; Cu, Cd a Pb byly stanoveny metodou ETAAS. Pro stanovení Fe byla využita modifikovaná molekulová spektroskopická metoda v oblasti VIS používaná pro stanovení železa ve vodě (ČSN 83 0530). Obsah chloridových iontů byl stanoven argentometricky podle ČSN 57 0530. Všechny průměrné hladiny sledovaných kontaminujících chemických prvků, a to i z oblasti s vysokým průmyslovým zatížením jsou pod limitními hodnotami uvedenými ve vyhlášce Ministerstvem Zdravotnictví č. 298/1997 Sb. Nelze vyvodit jednoznačné souvislosti mezi obsahem sledovaných analytů a dobou odběru. Lze předpokládat, že až na výjimky (v případě Mg a Fe) je možné očekávat vyšší hladiny sledovaných analytů v kozím mléce na počátku laktačního období, nižší naopak k jeho konci. Rozpětí hodnot obsahů makroelementů Ca, Mg a mikroelementů Zn, Cd, Pb a Cu v kozím mléce je srovnatelné s hodnotami hladin těchto analytů běžně uváděných v mléce kravském. Mírně zvýšený obsah proti obsahům v mléce kravském naznačují stanovené hladiny železa a chloridů.

Klíčová slova: kozí mléko; vápník; hořčík; chloridy; kadmium; olovo; zinek; měď; železo; AAS; fotometrie; argentometrie; hygienický limit

Goat milk has an important role in the nutrition of children who are allergic to cow milk, and also for the general population in the case of digestion problems. The anticarcinogenic effect of goat milk is also discussed. In the Czech Republic, goat breeding has its long tradition. Goats are mostly kept on small private farms where the milk is also processed (mainly goat cheese is made). In the Czech Republic, there were 33 900 goats as to 1 March 1999. The breed structure consists of approximately 90% of the dairy breed Czech White Polled (minimum milk yield 750 kg per year, fat content 3.0%) and another common dairy breed is Czech Brown Polled (average milk yield 650 kg per year, fat content 3.0%) (Štolc *et al.*, 2000). Similarly like in other foods, the quality of goat milk is carefully checked and has to comply with general hygienic requirements.

In its protein and energy content, goat milk is comparable with cow milk. The former is, however, more easily digested due to its different protein composition and finer fat dispersion. Goat milk contains more essential fatty acids, and also more fatty acids with shorter chains. It contains less provitamin A (this accounts for its chalk white color), vitamin B12 and folic acid (Štolc *et al.*, 1998). The amount of mineral substances depends mainly on their content in feeds and considerably varies during the year (Moreno *et al.*, 1993; Fantová, 1997; Rodriguez *et al.*, 1999a). Even the breed and the period of lactation have an influence on the amount of mineral substances in goat milk (Park and Chukwu, 1989; Rodriguez *et al.*, 1999a).

The evaluation of goat milk in comparison with cow milk is not uniform from the viewpoint of macro- and microelements contents. While Gajewska *et al.* (1997) consider the nutrient composition of cow and goat milk being comparable, Coni *et al.* (1996) found considerably higher contents of Cu, Fe, Cr and Ni in cow milk and, on the other hand, considerably lower contents of Ba and Sr in cow milk in comparison with goat milk. The contents of the elements in milk, however, seem to depend on the environment where animals are bred. On the basis of experimental data Rodriguez *et al.* (1999a) derived a mathematical relation between contents of selected elements and the kind of milk. Rodriguez *et al.* (1999b) determined considerably higher contents of Cd and Pb in goat milk in comparison with cow milk.

The levels of selected macroelements as well as copper and zinc in goat milk from farms in the Czech Republic were published by two groups of authors. Dostálová and Snížek (1992) gave the following values: Ca 2.0 g/kg, Mg 0.2 g/kg, K 2.6 g/kg, Na 0.7 g/kg, P 1.2 g/kg, Fe 1.1 mg/kg, Cu 0.6 mg/kg, Zn 4.8 mg/kg. Křížek *et al.* (1992) reported Ca 1.3 g/kg, Mg 0.15 g/kg, K 1.8 g/kg, Na 0.4 g/kg, P 1.6 g/kg, Fe 0.5 mg/kg, Cu 0.4 mg/kg. In comparison with cow milk, goat milk contains more calcium and phosphorus (Kratochvíl *et al.*, 1988).

In the Czech republic as well as worldwide, requirements for the food quality have been increasing continually. Consequently, goat milk, the usage of which is particularly important in the nutrition of children who are allergic to cow milk, and also

for the general population at treatment of peptic disorders, has to comply with those strict requirements. Besides, since anti-cancerous effects of goat milk are discussed, its higher consumption is likely to be expected.

In this communication, the content of selected risk elements (Cd, Cu, Fe, Pb, Zn) in goat milk samples collected from two private farms was examined. These farms differed in the concentration of animals and also in different levels of contamination from industrial sources. In addition to the above-mentioned five heavy metals, macro-elements Ca, Mg and chlorides were also determined. Results are discussed with respect to the hygienic quality of goat milk in comparison with cow milk, and to a possible influence of the farm location and time of sampling.

MATERIAL AND METHODS

Two goat farms, A and B, differing in the concentration of the animals and located in areas with different levels of industrial contamination were chosen for collection of goat milk samples. On both farms, the breed of Czech White Polled an cross-breds with Boer were kept. The lactation period was from April to October. Farm A was located near a natural reserve with no industrial pollution sources in the proximity. Sugar beet was a typical crop in this area. The total number of animals to 1. 9. 1998 was 70 head, including 40 milk goats. The average milk yield in 1997 was 520 liters per year/head. Farm B was surrounded with several industrial enterprises and was close to an international highway with heavy traffic. The typical crop in this area was potato. The total number of animals to 1. 9. 1998 was 170 head, including 143 milk goats. The average milk yield in 1997 was 480 liters per year/head. In summer, the goats on both farms received a majority of necessary nutrients from the pasture. In addition, the animals received pollard (farm A – 0.4 kg per head/day; farm B – 0.2 kg per head/day) and either a mineral feeding supplement (farm A) or feeding salt (farm B). In winter, the diet on both farms consisted mainly of hay and straw (*ad libitum*). On farm A, animals also received potatoes and pollard (1 kg per head/day) and feeding salt. Pollard was produced by the farm owner (mixture of 60% oats, 30% barley, 10% hay). On farm B, barley pollard (0.2 kg per head/day) and silage from sugar beet pulp (0.5 kg per head/day) were fed in

winter. Feeds were manually administered in cribs and mangers on both farms.

For determination of Ca, Cd, Cu, Fe, Mg, Pb, and Zn in goat milk, aliquots of milk (20 g) were dried and mineralized by dry ashing (Mader *et al.*, 1997).

Ca, Mg, and Zn were determined by the flame atomic absorption spectrometry (FAAS) in Varian SpectrAA 300 apparatus in flame acetylene-air at the wavelength 422.7 nm (Ca), 285.2 nm (Mg) and 213.9 nm (Zn), respectively. During measurements of Zn, the background was corrected with a deuterium lamp. In the Ca and Mg determination, 1% solution of lanthanum nitrate was added as a releasing agent.

Cu, Cd, and Pb were determined in Varian SpectrAA 400 atomic absorption spectrometer furnished with GTA-96 electrothermal atomizer. The wavelengths for individual metals were 423.8 nm (Cu), 228.8 nm (Cd) and 283.3 nm (Pb), respectively. The background correction was included in measurements of Cd and Pb and contents of these two elements were evaluated from the peak height using the standard addition to calibration. On the other hand, Cu content was evaluated from the calibration curve based on peak areas. Detailed temperature programs for determination of Cd, Cu and Pb in milk are described in the Technical Report (Mader *et al.*, 2000).

Fe was determined by the modified molecular spectrophotometric method after reaction with potassium thiocyanate (this method is used for the determination of iron in water according to ČSN 83 0530). For measurements, 5 ml aliquots of the digests prepared by dry ashing were used.

Bulk samples of goat milk were collected four times in 1998, regularly during the whole lactation period (April, June, August and October). Each collected bulk sample was mineralized by dry ashing at five parallel replications. Each digest was measured twice.

Chloride ions were determined titrimetrically, again by five parallel determinations, directly in milk samples according to ČSN 57 0530.

RESULTS AND DISCUSSION

For the quality control of analytical data, a simultaneous analysis of the certified reference material CRM 063R (skimmed milk powder) was performed (Table 1). Average values of the deter-

Table 1. The analytical results of certified reference material CRM 063R

		Cd	Pb	Fe	Cu	Zn	Ca	Mg	Cl ⁻
		(mg/kg)							
Analytical values	minimum	< DL*	< DL**	2.11	0.605	48.77	13.41	1.25	9.54
	maximum	2.91	< DL**	2.37	0.675	52.31	13.59	1.36	10.31
Certified values ¹	mean	–	18.5	2.32	0.602	49.0	13.49	1.26	9.94
	uncertainty	–	2.7	0.23	0.019	0.6	0.10	0.02	0.30

¹Certified values are given by the producer of certified reference material CRM 063R (Cd was not certified)

*detection limit for determination of cadmium in CRM – 2.0 µg/kg

**detection limit for determination of lead in CRM – 30.0 µg/kg

mined contents of macro- and microelements for particular dates of sampling are summarized in Figures 1–8. ANOVA method of one-factor analysis of variance by the multiple comparison method and probability level of 95% was used for statistical evaluation.

The results lead to the following conclusions:

- The differences in the levels of particular elements in five parallel determinations are not statistically significant.

- 40% of cadmium content results (7× farm A, 9× farm B) and 7.5% of lead content results (all for farm B during October) are below the detection limit (detection limit of Cd = 0.2 µg/kg; detection limit of Pb = 3.0 µg/kg).

- All determined contents of zinc, copper, cadmium, and iron are sufficiently below the maximum limit values of these contaminating chemical elements in milk as set down by Regulation No. 298/1997 of the Ministry of Health of the Czech Republic (the highest determined value is 1.3×; 3.7×; 4.6× and 17.3× lower, respectively; Table 2). The same result was obtained for all lead content values in milk from farm B and, in the same time, for average lead contents calculated from five parallel determinations in goat milk from farm A. But the determined lead contents

(maximum 0.0265 mg/kg) in goat milk from farm A exceed the maximum limit value in 25% of cases (MTA = 0.02 mg/kg; Table 2). Since no statistically significant differences between the parallel determinations were proved, it is reasonable to assume that a higher number of parallel determinations is always required to evaluate the hygienic capability of food, especially if the levels of determined analytes are very close to the limit values. The fact that all average levels of contaminating chemical elements measured, even from the area of high level of contamination from industrial sources and in the neighborhood of a highway with heavy traffic, are below the hygienic limits set down by the Ministry of Health of the Czech Republic (Regulation No. 298/1997) seems to be positive.

- The average values of five parallel determinations of element contents in samples collected within the same time period are higher in 78% of cases in milk of goats from farm A. 44% of them are significantly higher values. On the other hand, only 9% of values are significantly higher average contents of both macro- and micro-elements in milk of goats from farm A. Only those levels of zinc and lead in milk of goats from farm A show explicit significantly higher average contents. On the contrary, chlorides are the only analyte whose

Table 2. The limit values (mg/kg) of contaminating chemical elements in milk (according to Regulation No. 298/1997 of the Ministry of Health of the Czech Republic)

Element	Cd	Cu	Fe	Pb	Zn
MTA	0.01	0.4	50.0* (TA)	0.02	10.0

MTA = Maximal Tolerable Amount

TA = Tolerable Amount

*limited value for food (general)

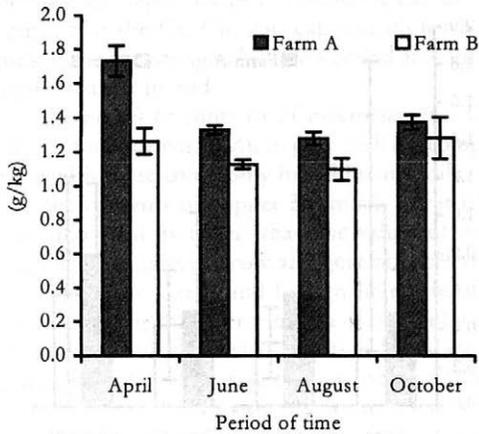


Figure 1. Calcium content in goat milk during lactation

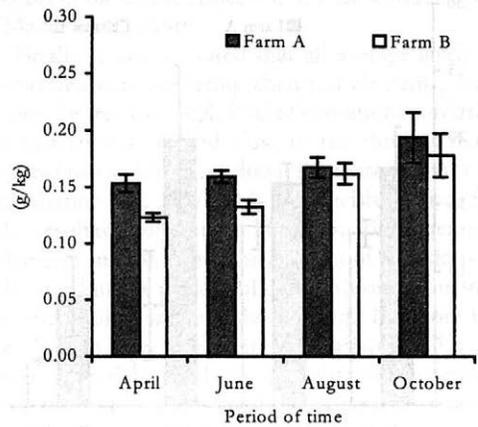


Figure 2. Magnesium content in goat milk during lactation

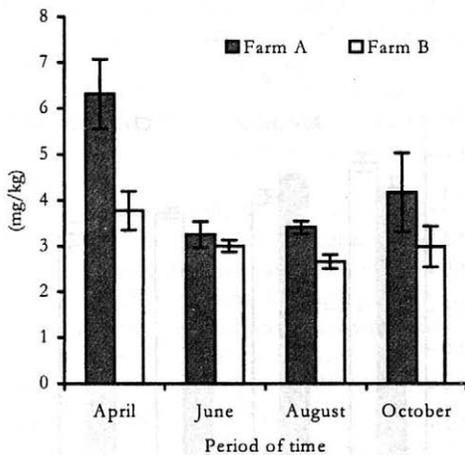


Figure 3. Zinc content in goat milk during lactation

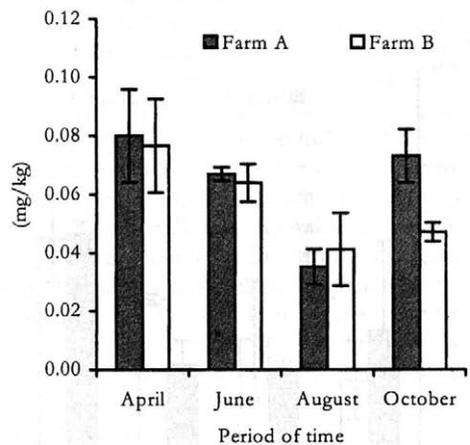


Figure 4. Copper content in goat milk during lactation

average values are mostly (i.e. 75% of cases, 50% significantly) higher in milk of goats from farm B, where the content of iron was also significantly higher in August. The higher average level of some analytes in milk of goats from farm B was found for iron (June), cadmium (April) and copper (August). The findings that the levels of analytes, including contaminating chemical elements except chlorides, are higher in milk of goats from farm A (located near the natural reserve) than in milk of goats from farm B (high level of contamination from industrial sources) tend to suggest that the geological composition of the given locality and especially feed

seem to be more decisive for resultant contents of contaminating chemical elements in milk than the amount of industrial and traffic immissions in this locality. This can explain that the contents of the analytes in milk are very variable.

- On the basis of determination of analytes during various seasons and their statistical evaluation it is not possible to find any direct relationship between the content of the studied elements and the date of sampling.

- As regards the evaluation of the whole lactation it could be said that the contents of measured analytes varied significantly during this period. It

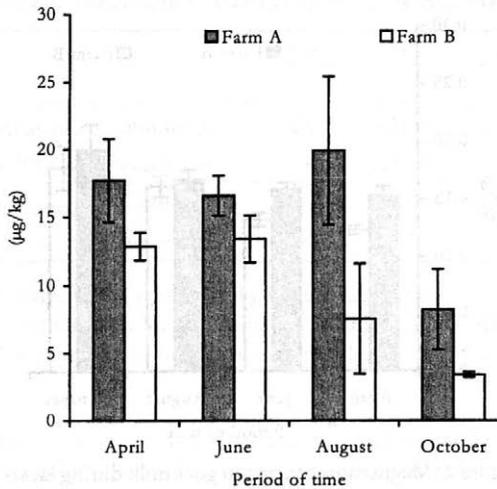


Figure 5. Lead content in goat milk during lactation

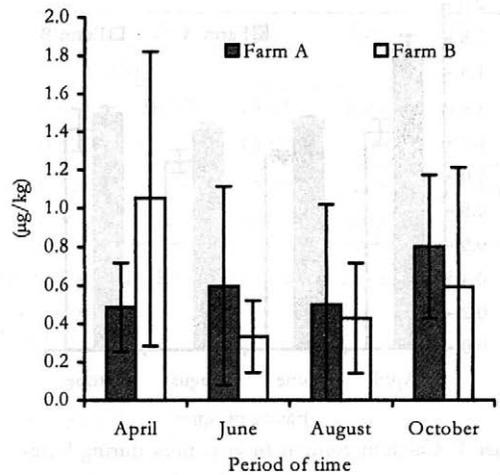


Figure 6. Cadmium content in goat milk during lactation

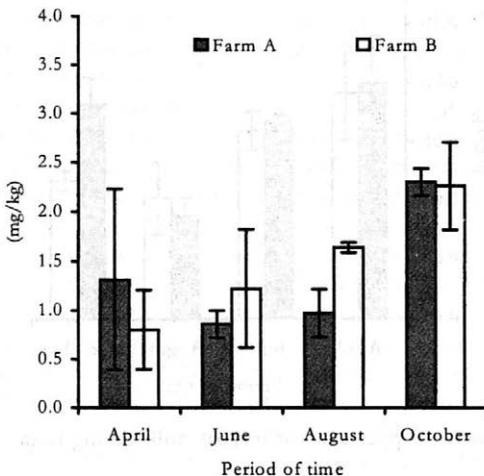


Figure 7. Iron content in goat milk during lactation

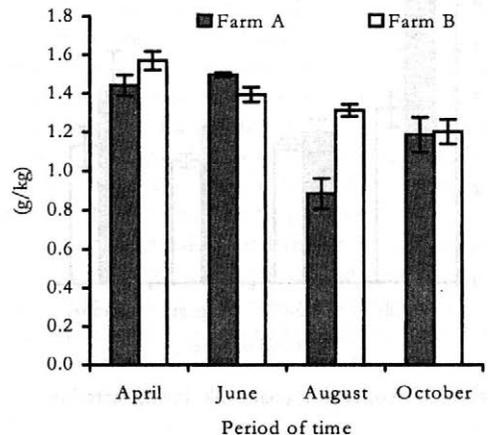


Figure 8. Chloride content in goat milk during lactation

does not always apply to the mutual evaluation of only two samples from different dates of sampling and nor are the trends of various analytes on both farms identical. A clearly monotonous increase was observed only for magnesium content in milk from both farms during the lactation period. An analogous dependence was also obtained for iron, but with one exception of high initial iron content in milk from farm A given by one higher determined value among the five parallel determinations.

• On the other hand, the amount of chloride ions in milk from farm B decreases monotonously and significantly during the lactation period. Samples from farm A show a similar tendency. More than 50% probability of the statistically significant dependence of analyte content upon the date of sampling was obtained also for lead. On the contrary, differences in cadmium levels in milk during the lactation period are absolutely non-significant. It is however influenced by the fact that 40% of determined values is below the detection limit. Despite

of some discrepancies in the results it can be assumed that the Ca, Cu, Zn, Cd, and Pb levels in milk at the beginning of lactation period are mostly higher than at its end.

- The ranges of contents of macroelements Ca, Mg, and microelement Zn in goat milk are comparable with those commonly found in cow milk.

- The contents of copper are much lower (the low limit even by order) than the values of contents of this analyte in cow and goat milk referred to both in the Czech and foreign literature. The reason could be the determination of copper by the method of electrothermal atomization (see Material and methods). Considering the low contents of this analyte in milk, this method is more suitable for its lower detection limit than still commonly used FAAS method. Since the quality of analytical data was proved by a simultaneous analysis of the certified reference material CRM 063R (Table 1), the data on this analyte in milk referred to in the literature are probably overvalued. Determined copper contents in goat milk are comparable with copper contents found in cow milk under the grant project (Mader *et al.*, 2000) mentioned above, where the ETAAS method for Cu determination was also used.

- Determined contents of iron (maximum 2.89 mg/kg) indicate a slightly higher content than the values published for cow milk (Prokš, 1964, gives the higher content 1.1 mg/kg). The values comparable for cow and goat milk were published (e.g. Moreno, 1993 0.67 mg/kg).

- No data were found in available literature for contents of chlorides. Kratochvíl *et al.* (1988) reported the average content of chlorides in cow milk 1 g/l (range 0.8–1.4 g/l). In comparison with cow milk, we found slightly higher contents for chlorides (similarly like for iron) (average content 1.31 g/l, range 0.78–1.62 g/l).

- The minimum and maximum limits of the ranges of contaminating chemical elements lead and cadmium are lower (for Cd even in order) than the limits of these ranges in milk of goats from foreign farms. Rodriguez *et al.*, 1999b reported 18.4 µg/kg as the maximum content of Cd and 40 µg/kg for Pb while in this study the maximum determined content is 1 µg/kg for Cd and 26.5 µg/kg for Pb. The contents of lead and cadmium in goat milk have not been published in the Czech Republic yet. Determined contents of lead and cadmium in goat milk are comparable with contents in cow milk published in the summary

report of the Grant Project NAZV EP 0960006367 (Mader *et al.*, 2000).

Finally it can be stated that all average levels of measured contaminating chemical elements, even from the area with high level of contamination from industrial sources and close to the thoroughfare, are below the limits set down for these analytes by Regulation No. 298/1997. The decisive source for the resultant contents of contaminating chemical elements in milk is not the amount of emissions in the area but the geological composition of the foot in the locality and mainly the feed. Therefore the levels of analytes in milk are very variable. It has not been possible to find any unambiguous interrelation between the content of the elements concerned and the date of sampling, only the clearly monotonously increased magnesium content in milk from both farms during the lactation period was found. On the other hand, the amount of chloride ions in milk from farm B decreases monotonously and significantly during the lactation period; contents of chlorides in milk of goats from farm A show the same tendency. Despite of some discrepancies it can be assumed that the levels of Ca, Cu, Zn, Cd and Pb are mostly higher at the beginning of the lactation period than at its end. The range of contents of the elements concerned, with the exception of copper, in goat milk is comparable with levels of these analytes commonly found in cow milk; the copper content is comparable with the content of this element found in cow milk during the work on the Grant Project NAZV EP 0960006367 (Mader *et al.*, 2000). A slightly increased content in goat milk against cow milk is shown by the determined levels of iron and chlorides.

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