CZECH ACADEMY OF AGRICULTURAL SCIENCES

Czech Journal of ANIMAL SCIENCE

ŽIVOČIŠNÁ VÝROBA

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

6

VOLUME 46
PRAGUE 2001
ISSN 1212-1819

CZECH JOURNAL OF ANIMAL SCIENCE

An international journal published under the authorization by the Ministry of Agriculture and under the direction of the Czech Academy of Agricultural Sciences

Mezinárodní vědecký časopis vydávaný z pověření Ministerstva zemědělství České republiky a pod gescí České akademie zemědělských věd

World Wide Web (URL): http://www.uzpi.cz

Aim and scope: The journal publishes scientific papers and reviews dealing with the study of genetics and breeding, physiology, reproduction, nutrition and feeds, technology, ethology and economics of cattle, pig, sheep, goat, poultry, fish and other farm animal management.
The journal is cited in the bibliographical journal Current Contents – Agriculture, Biology and Environmental Sciences and abstracted in Animal Breeding Abstracts. Abstracts from the journal are comprised in the databases: Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Environ-mental Sciences, Czech Agricultural Bibliography, Food Science and Technology Abstracts, Toxline Plus.

Periodicity: The journal is published monthly (12 issues per year). Volume 46 appearing in 2001.

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

Subscription information: Subscription orders can be entered only by calendar year (January–December) and should be sent to: Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic. Subscription price for 2001 is 195 USD (Europe) and 214 USD (overseas).

Cíl a odborná náplň: Časopis publikuje původní vědecké práce a studie typu review z oblasti genetiky, šlechtění, fyziologie, reprodukce, výživy a krmení, technologie, etologie a ekonomiky chovu skotu, prasat, ovcí, koz, drůbeže, ryb a dalších druhů hospodářských zvířat.
Časopis je citován v bibliografickém časopise Current Contents – Agriculture, Biology and Environmental Sciences a v časopise Animal Breeding Abstracts. Abstrakty z časopisu jsou zahrnuty v těchto databázích: Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Environmental Sciences, Czech Agricultural Bibliography, Food Science and Technology Abstracts,Toxline Plus.

Periodicita: Časopis vychází měsíčně (12× ročně), ročník 46 vychází v roce 2001.

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

Informace o předplatném: Objednávky na předplatné jsou přijímány pouze na celý rok (leden–prosinec) a měly by být zaslány na adresu: Ústav zemědělských a potravinářských informací, vydavatelské oddělení, Slezská 7, 120 56 Praha 2. Cena předplatného pro rok 2001 je 1176 Kč.

F 4886

Relationship between body measurements and reproductive performance of Ossimi and Rahmani ewes

Závislost mezi tělesnými rozměry a reprodukční schopností u ovcí plemene Ossimi a Rahmani

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ABSTRACT: Two different local breeds of Egyptian sheep were subjected to the investigation. The body measurements regarding length, height, weight, ano-genital distance, loin width and back hock distance were determined for 100 Rahmani and Ossimi ewes. In addition, reproductive status in particular with ewe age, age at first service, number of services per conception, lambing interval, twinning rate and lamb weight were recorded. Ewes were subjected to heat detection twice a day for two successive cycles during four different seasons of the year. The results showed that there was a positive correlation between body measurements and reproductive efficiency. Body weight (BW) correlated positively with the other physical body characteristics, $r = 0.78^{**}$, 0.74^{**}, 0.68^{**}, 0.41 and 0.66^{**} for body length (BL), body height (BH), back hock distance (BHD), ano-genital distance (AGD) and loin width (LOW), respectively. On the other hand, reproductive traits correlated positively with each other, especially lambing interval and number of services per conception ($r = 0.97^{**}$). The highly correlated estimates were found between physical body characteristics (BW, BL, BH and BHD) and reproductive status especially for lamb weight (LW) and mothering ability (MA), in which body measurements may impact most of reproductive traits in a direct manner.

Keywords: sheep; reproduction; body measurements; estrus

ABSTRAKT: Do našeho šetření jsme zahrnuli dvě rozdílná domácí plemena egyptských ovcí. U 100 kusů ovcí plemene Rahmani a Ossimi jsme zjišťovali tělesné rozměry: délku, výšku a hmotnost, anogenitální vzdálenost, šířku beder a délku zadního hlezna. Dále jsme zjišťovali reprodukční stav podle věku bahnice, věku při prvním připouštění, počtu připouštění na zabřeznutí, délky mezidobí mezi obahněními a hmotnosti jehňat. Říji jsme u bahnic zjišťovali dvakrát denně ve dvou po sobě následujících cyklech ve čtyřech ročních obdobích. Výsledky ukázaly, že mezi tělesnými rozměry a reprodukční schopností existuje kladná korelace. Tělesná hmotnost (BW) vykazovala kladnou korelaci s ostatními fyzickými tělesnými rozměry, $r = 0{,}78^{**}$, $0{,}74^{**}$, $0{,}68^{**}$, 0,41 a $0{,}66^{**}$ pro tělesnou délku (BL), tělesnou výšku (BH), délku zadního hlezna (BHD), anogenitální vzdálenost (AGD) a šířku beder (LOW). Naproti tomu reprodukční vlastnosti byly ve vzájemné kladné korelaci, zejména pokud se jedná o délku mezidobí mezi obahněními a počet připouštění na zabřeznutí ($r = 0{,}97^{**}$). Odhady s vysokou korelací jsme zjistili mezi fyzickými tělesnými vlastnostmi (BW, BL, BH a BHD) a reprodukčním stavem, zejména u hmotnosti jehňat (LW) a schopnosti matky odchovávat mláďata (MA). Tělesné rozměry mohou mít přímý vliv na většinu reprodukčních vlastností.

Klíčová slova: ovce; reprodukce; tělesné rozměry; estrus

INTRODUCTION

In sheep, the interval between parturition and resumption of ovarian activity has been shown to be influenced by numerous factors such as management of the flock, body measurements and nutrition (Pope *et al.*, 1989). Ossimi and Rahmani are the dominant local breeds in Egypt. The reproductive performance of the ewe can be measured by estimating estrus status, ovulation rate, fecundity, embryo survival, litter size, sexual maturity and estradiol 17-ß level in blood plasma in the earlier postnatal period (Yue, 1996). Most of the above mentioned parameters are mainly correlated with body measurements (Rondon *et al.*, 1996). Body measurements could be affected by the ontogeny of lengthens and deepens of the lamb (Hammond *et al.*, 1984; Sinha and Singh, 1997). Dietary nutri-

ents affect the size, vigor and viability of the newborns and adult ovulation rates in sheep (Robinson, 1996). Furthermore, Rhind *et al.* (1998) demonstrated that there were positive relationships between nutritional levels in both early and adult life and the birth weight of lambs born to these ewes. Syrian Awassi ewes show the seasonality of estrus cycle (Al-Haboby *et al.*, 1999). They found that most of ewes came in estrus during the autumn season. Therefore, this experiment was aimed to study the relationship between body measurements and reproductive performance during adult life and the cyclic ewes status during different seasons of the year.

MATERIAL AND METHODS

Animals and rearing

Animals were divided into two groups: 50 Rahmani and 50 Ossimi. The ewes were housed on an Experimental Farm of Agricultural College, Suez Canal University, Ismailia, Egypt. Ewes were located in an outdoor yard under shade during the daytime. Ewes were fed on green berseem (*Trifolium alexandrinum*) during winter and spring while they were fed on rice straw (treated with 5% ammonia) during summer and autumn. For all seasons, ewes were supplied with 300 g/day concentrate mixture as shown in Table 1. Water supply was available to ewes at any time of the day. The animals were weighed biweekly in the experimental period.

Table 1. Chemical composition of feeding materials used during the experimental period

DM%	Berseem	Ammoniated rice straw *	Concentrate mixture**
Crude protein	17.9	9.8	13.88
Ether extract	3.6	1.22	3.3
Crude fiber	25.1	36.7	9.27
Nitrogen free extract	39.8	36.3	67.14
Ash	13.6	15.98	6.41

* rice straw was treated with 5% ammonia as dry matter

** it contained 40% wheat bran, 46% corn, 10% soybean meal, 2% salt and 2% limestone

Estrus detection

Ewes of each group were subjected to close observation for four successive seasons of the year (spring, summer, autumn and winter) for estrus performance; the ewes were subjected to heat detection twice a day for two successive cycles during four different seasons of the year. Rams for each group (two rams) were used to detect estrus twice daily at 7 a.m. and 7 p.m. The signs of the estrus are the following: redness of vulva, vaginal discharge and receptivity to the male, which was considered the limiting sign for estrus detection.

Body measurements

These parameters were investigated in ewes of each group: body weight (BW/kg), body height (BH/cm), body length (BL/cm), ano-genital distance (AGD/cm), loin width (LOW/cm) and back hock distance (BHD/cm). The above body parameters were determined as follows: body weight by using a kilogram balance, body height (the distance between top of shoulder and hoof), body length (the distance between forehead and tail-head), ano-genital distance(the distance between top of anus opening and end border of vagina opening), loin width (the distance between either side of the fore-rump area) and back hock distance (the distance between top of rump and hock joint). The parameters were taken by one researcher for each season to reduce the personal error.

Reproductive performance characteristics

The following reproductive performance were studied:

Ewe age (EA/month), Number of services per conception (NOSC), Age at first service (AFS/month), Lambing interval (LI/months), Litter size (LS), Lambing weight (LW/kg) and Mothering ability (MA) which was based on the survival rate of lambs until weaning.

Statistical analysis

Data were subjected to statistical analysis using ANOVA test according to Steel and Torrie (1984). The mathematical model which was used as the following formula:

$$Y_{iejkmn} = \mu + BW_i + BH_e + BL_j + AGD_k + LOW_m + + BHD_n + e_{iejkmn}$$

where: μ = overall mean
BW = body weight
BH = body height
BL = body length
AGD = ano-genital distance
LOW = loin width
BHD = back hock distance
E = residual error

All the data were processed and analyzed by using SPSS program according to the model mentioned above to find out the effect of body measurements (independent factors) on the reproductive traits (dependent factors). Correlations between body measurements and reproductive

performance characteristics were carried out to examine these relationships during different seasons of the year according to Snedecor (1973).

RESULTS AND DISCUSSION

Physical body characteristics

Results of this study are presented in Table 2. These data showed that there were no significant differences between the two Egyptian local breeds except for loin width, which was 28.18 ± 1.31 vs. 24.24 ± 0.60 cm for Ossimi and Rahmani ewes, respectively. The other body characteristics were close to each other and did not vary significantly for the rest of the body characteristics as shown in Table 2. The superiority percent for the same body characteristics indicated that Ossimi ewes were superior in body weight (BW), body length (BL), body height (BH): these characteristics were 41.06 ± 1.93, 96.65 ± 1.64, 72.06 ± 1.26 and 38.12 ± 1.44 kg, 94.24 ± 1.28 cm, 68.88 ± 1.1 cm for BW, BL, BH for Ossimi and Rahmani ewes, respectively. The superiority percent was favorable for Rahmani ewes as for the ano-genital distance (AGD) and back hock distance (BHD): these characteristics were 4.94 ± 0.24, 49.00 ± 1.20 and 4.82 ± 0.20 cm, 48.71 ± 1.27 cm for AGD, BHD for Rahmani and Ossimi, respectively.

Effect of body measurements on reproductive performance

The results of this study are presented in Table 3. The most of fecundity status performances of both Ossimi and Rahmani ewes were affected by body weight. The superiority percent showed that Ossimi ewes were superior in lambing weight (LW) while Rahmani ewes were superior in the other reproductive performance: age at first service (AFS), lambing interval (LI), number of services per conception (NOSC) and litter size (LS) as shown in Table 3. These findings can be in agreement with other investigators. They showed that Rahmani ewes had a larger litter size as compared to the other Egyptian sheep breeds (Ashmawy, 1981). The importance of body measurements can be manifested through their effect on both postpartum interval and pregnancy rate. Furthermore, the proper body measurements are necessary to minimize the postpartum anestrus interval and to optimize the conception rate (Houghton *et al.*, 1990). Body weight and viability of ewe are the most important two characteristics which affect the lamb production (Hassan, 1993). Therefore the body weight at any defined age is responsible for either productive or reproductive traits while the nutritional level and quality of feeds play a great role in controlling the endocrine system that regulates reproductive functions (El-Barody *et al.*, 1993). The onset of puberty and estrus

Table 2. Means ± SE of physical body characteristics of Ossimi and Rahmani ewes

Breed		Physical body characteristics					
		BW	BL	BH	AGD	LOW	BHD
	mean	41.06	96.65	72.06	4.82	28.18**	48.71
	minimum	31.00	87.00	66.00	3.50	21.00	42.00
	maximum	58.00	114.00	83.00	7.00	39.00	63.00
Ossimi ^{O}	± SE	1.93	1.64	1.26	0.20	1.31	1.27
	mean	38.12	94.24	68.88	4.94	24.24	49.00
	minimum	28.00	84.00	62.00	2.00	21.00	43.00
	maximum	53.00	105.00	76.00	6.50	29.00	58.00
Rahmani ^{R}	± SE	1.44	1.28	1.10	0.24	0.60	1.20
	mean	39.59	95.44	70.47	4.88	26.21	48.85
	minimum	28.00	84.00	62.00	2.00	21.00	42.00
	maximum	58.00	114.00	83.00	7.00	39.00	63.00
Grand total	± SE	1.21	1.04	0.87	0.15	0.79	0.86
Superiority percent (S %)		7.16^{O}	2.49^{O}	4.41^{O}	2.43^{R}	13.98^{O}	0.59^{R}

BW = body weight (kg) AGD = ano-genital distance (cm) BL = body length (cm)
LOW = loin width (cm) BH = body height (cm) BHD = back-hock distance (cm)
** mean in the column with asterisk superscript differs significantly ($P < 0.01$)

Table 3. Means ± SE of fecundity status of Ossimi and Rahmani ewes

Breed		Reproductive traits					
		EA	AFS	LI	LW	NOSC	LS
	mean	31	10.4	11.8	3.02	1.65	1.29
	minimum	24	9.0	11.0	1.90	1.0	1.0
	maximum	36	11.0	14.0	4.50	3.0	2.0
Ossimi ^{O}	± SE	1.08	0.15	0.24	0.21	0.17	0.11
	mean	30	10.6	11.5	3.00	1.47	1.35
	minimum	24	10.0	11.0	2.00	1.0	1.0
	maximum	36	12.0	12.0	4.50	2.0	2.0
Rahmani ^{R}	± SE	0.96	0.17	0.12	0.17	0.12	0.12
	mean	30	10.5	11.6	3.01	1.56	1.32
	minimum	24	9.0	11.0	1.90	1.0	1.0
	maximum	36	12.0	14.0	4.50	3.0	2.0
Grand total	± SE	0.72	0.11	0.13	0.13	0.10	0.08
Superiority percent (S %)		3.85^{O}	1.89 ^{R}	2.54 ^{R}	0.66 ^{O}	10.91 ^{R}	4.44^{R}

EA = ewe age (months) LW = lamb weight (kg) AFS = age at first service (months)
NOSC = number of services per conception LI = lambing interval (months) LS = litter size (No. of lambs)

in ewe lambs were affected by energy or protein restriction (Foster and Olster, 1985). Adequate nutrition and good body measurements lead to an increase in the ovulation rate of ewes (Thomas *et al.*, 1987). Nutritional restriction and decreased body weight affect the length of anestrus in ewes (Hall *et al.*, 1986).

Cyclic ewes during different seasons of the year

The estrus cycles in both Ossimi and Rahmani ewes in this experiment showed the same trend. It was tonic in winter, autumn, less tonic in summer and very weak in the spring season (Table 4 and Figure 1). Cyclic Rahmani ewes were slightly higher and did not differ significantly from cyclic Ossimi ewes during different seasons of the year. Worthy and Haresign (1983) declared the very low percentage of cyclic ewes during spring. They found that the onset of seasonal anestrus in all animals (sheep) was significantly advanced in ewes adapting to long days, with the end of the breeding season coming at the normal

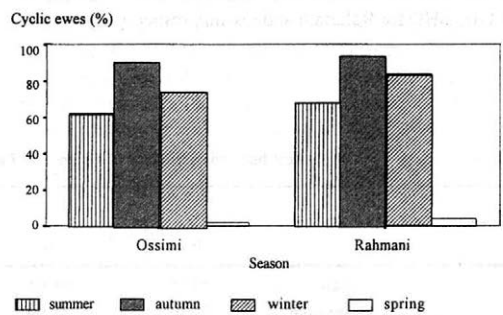

Figure 1. Percent of cyclic ewes during different seasons of the year

Table 4. Number of cyclic Ossimi and Rahmani ewes during the different seasons of the year

Season	Breed	No. of ewes	Cyclic ewes	% of cyclic ewes	Overall mean (%)
Summer	Ossimi	50	31	62.00	65.00
	Rahmani	50	34	68.00	
Autumn	Ossimi	50	45	90.00	92.00
	Rahmani	50	47	94.00	
Winter	Ossimi	50	37	74.00	79.00
	Rahmani	50	42	84.00	
Spring	Ossimi	50	1	2.00	3.00
	Rahmani	50	2	4.00	

Table 5. Estimates of correlations between physical body characteristics in Ossimi and Rahmani ewes

Correlation	Physical traits							
	BW	BL	BH	BHD	AGD	LOW	MA	LW
LI	−0.45**	−0.39*	-0.15	−0.47**	−0.22	−0.32	−0.66**	−0.45**
NOSC	−0.47**	−0.42**	-0.18	−0.49**	−0.26	−0.32	−0.57**	−0.60**
EA	0.87**	0.61**	0.59**	0.44**	0.40*	0.54**	0.57**	0.22
MA	0.44**	0.45**	0.27	0.16	0.11	0.26	–	0.14
LS	−0.10	−0.10	0.02	−0.32	−0.01	−0.24	0.06	−0.74**
AFS	−0.23	0.07	-0.04	−0.01	−0.31	−0.04	−0.14	−0.23

LI = lambing interval
NOSC = number of services per conception
EA = ewe age
MA = mothering ability
LS = litter size
AFS = age at first service
BW = body weight
BL = body length
BH = body height
BHD = back-hock distance
AGD = ano-genital distance
LOW = loin width
LW = lamb weight
* $P < 0.05$, ** $P < 0.01$

time of the year in ewes maintained in short days. Whereas in Egypt, where the light-darkness ratio is not so great, El-Sheikh *et al.* (1980), working with Egyptian Barki, Rahmani and Ossimi sheep, showed that the estrus period tended to be longest in autumn months and shortest in the spring. Matter (1987) reported that Egyptian sheep showed their highest number of cyclic ewes in autumn and the lowest in spring seasons. However, there are large breed differences in the extent of the postpartum anestrus prior to a fertile estrus, reflecting a strong genetic component in addition to seasonal influences (Pope *et al.*, 1989). Rahmani and Ossimi ewes exhibit a long breeding season from June to March and a short anestrus period from March to June (spring) under Egyptian condition. The Manchega sheep is a domesticated Spanish breed that exhibits a long breeding season from June-July to February-March (Comez-Brunet and Lopez-Sebastian, 1991). At the other extreme, the European Mouflon (*Ovis gemelini musimon*) is a primitive sheep originating from the Mediterranean area that exhibits a shorter breeding season (Hafez, 1952), ranging from October to February-May (Santiago-Moreno *et al.*, 1995).

Correlations between body characteristics and reproductive status traits

As regards correlations between physical body characteristics and reproductive traits with each other, these estimates of correlations showed that there were positive estimates of correlations (Table 5); the body weight (BW) was correlated positively with the other physical characteristics (Table 5). Also reproductive characteristics correlated positively with each other especially for lambing interval (LI) and number of services per conception (NOSC) ($r = 0.97$**) as shown in Table 6. In addition, the same situation was observed in ewe age and mothering ability

Table 6. Estimates of correlations between reproductive traits

Correlation	MA	LS	AFS	NOSC
LI	−0.66**	0.26	0.15	0.97**
NOSC	−0.57**	0.40*	0.19	–
EA	0.57**	0.03	−0.26	−0.44**
MA	–	0.06	−0.14	0.57**

LI = lambing interval
LS = litter size
EA = ewe age
MA = mothering ability
AFS = age at first service
NOSC = number of services per conception
* $P < 0.05$, ** $P < 0.01$

($r = 0.57$**), litter size (LS) and NOSC ($r = 0.40$*). The more closely correlated estimates were found between physical body characteristics (BW, BL, BH and BHD) and reproductive status especially for lamb weight (LW) and mothering ability (MA) as shown in Table 5.

It could be concluded that there were no significant differences in most body characteristics between Ossimi and Rahmani ewes except for the superiority of Ossimi ewes in body weight. Rahmani ewes were superior and did not differ significantly in most fecundity traits and especially in the twinning rate and number of cyclic ewes. The Egyptian local breeds Rahmani and Ossimi exhibit a long breeding season with a short anestrus period from March to June. There was no relationship between ewes' body measurements and the number of cyclic ewes during the different seasons of the year.

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Received: 00–10–16
Accepted after corrections: 01–05–17

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Microsatellites on chromosome 6 and their association with milk production traits in Czech Pied cattle

Mikrosatelity na chromosomu 6 a jejich asociace s mléčnou užitkovostí u českého strakatého skotu

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ABSTRACT: Five microsatellite loci (*ILSTS093, BM143, ILSTS097, MB062, BM2320*) located on bovine chromosome 6 were tested as potential markers for milk production traits in five families of the Czech Pied breed. The effect of segregating marker alleles was estimated in 33 sons of 5 grandsires using a granddaughter design. Significant associations between markers within grandsire and breeding values for milk protein and fat yields were detected for four loci (*ILSTS093, BM143, MB062, BM2320*). None of the selected markers showed a significant influence on the breeding value for milk yield. Two new alleles were detected at microsatellite locus *MB062* (162 and 164 bp) and one new allele was identified at microsatellite locus *BM143* (85 bp).

Keywords: cattle; Czech Pied breed; chromosome 6; milk performance; association

ABSTRAKT: Pro testování polymorfismu bylo vybráno pět mikrosatelitních lokusů (*ILSTS093, BM143, ILSTS097, MB062, BM2320*) na bovinním chromozomu 6. Testování bylo provedeno u pěti rodin českého strakatého skotu. Vliv segregujících alel markerů byl odhadnut u 33 synů pěti dědů metodou granddaughter design. Statisticky průkazná korelace mezi markerem děda a plemennou hodnotou pro obsah bílkoviny a tuku byla zjištěna u čtyř lokusů (*ILSTS093, BM143, MB062, BM2320*). Žádný z testovaných markerů neměl vliv na plemennou hodnotu pro množství mléka. Zjištěný polymorfismus byl porovnán s údaji v bovinní mikrosatelitní mapě a byly zjištěny dvě nové alely v lokusu *MB062 (162 a 164 bp)* a rovněž u lokusu *BM143* byl počet alel o jednu vyšší (85 bp).

Klíčová slova: český strakatý skot; chromozom 6; asociace s mléčnou užitkovostí.

INTRODUCTION

Numerous studies identifying genetic polymorphism at the DNA level and the generation of bovine microsatellite maps (Bishop *et al.*, 1994; Barendse *et al.*, 1997) open the way for the use of marker assisted selection (MAS) in breeding schemes. The efficiency of MAS depends on the successful dissection of individual genes which influence production traits by closely linked microsatellite markers (Weller *et al.*, 1990). In the specific case of milk production traits, there is a considerable advantage to sample very large half-sib families. By concentrating on the segregation of the genes originating from the common founder sire in a within-family analysis, the genetic heterogeneity for the milk production traits in the given family can be efficiently reduced.

For milk production traits, an early interest was directed towards analyses of polymorphisms of casein (*CSN*) genes and, consequently, to bovine chromosome 6 (BTA 6), where the casein genes are located. Various polymorphic genetic markers were used to scan the whole chromosome 6 for the existence of QTLs for milk production traits. Georges *et al.* (1995), Spelman *et al.* (1996), Zhang *et al.* (1998) and more recently Kühn *et al.* (1999) and Velmala *et al.* (1999) performed half-sib population analyses using either maximum-likelihood or least squares approaches. Although the above mentioned investigations were performed on different breeds (Dutch, German or American Holstein, Finnish Ayrshire and Norwegian cattle), the obtained results support the existence of QTLs for milk protein and milk fat percentages nearby the markers *BP7* and *BM143*.

Supported by the Ministry of Agriculture of the Czech Republic (Projects M02-99-02 and 0176).

The existence of positive results in QTL mapping on BTA 6 was the main reason for starting investigations on polymorphisms of markers on this chromosome in the Czech Pied breed. The first aim of the present study was to set up a multiplex of informative microsatellites and to check up their main characteristics on a sample of the population. The second goal was to investigate informative markers for their association with the breeding values for milk, protein and fat yields and to recommend selected markers for further investigations and their possible implementation in marker-assisted selection programmes.

MATERIAL AND METHODS

Czech Pied cattle is a dual purpose breed of Simmental type. Five nonrelated elite sires from the lines Bavor, Lom, Primus, Junek and Redaktor and their 33 tested sons (4 to 8 per grandsire) were genotyped for the following microsatellite markers: *ILSTS093, BM143, ILSTS097, MB062* and *BM2320*. In every family 7–18 granddaughters per sire were also genotyped for all five markers.

Primers for loci *BM143, ILSTS093, ILSTS097* and *BM2320* were chosen from the database http://sol.marc.usda.gov. The microsatellite marker *MB062* which is located in intron 3 of the *CSN3* gene was amplified using primers described by Leveziel *et al.* (1994). The reverse primers were 5′end labelled with HEX (*BM2320*), FAM (*BM143, MB062*) and TET (*ILSTS093, ILSTS097*). The PCR reaction was performed in 20 µl, containing 100 ng of genomic DNA, 2.1 µl 10×reagent buffer (100mM TRIS-HCl pH 8.3, 500mM KCl,15mM $MgCl_2$, 0.01%(w/v)gelatin), 10 mM each dNTP and 0.35 µl (5U/1µl) Gold Polymerase (PE Biosystem, New Jersey, USA).

After the first denaturation step at 10 min, 30 cycles of amplification were performed, with denaturation at 94°C, 30 s, annealing at 52°C 30 s and elongation at 72°C 30 s. The last step was extension for 10 min at 72°C. The analysis of PCR products and allelic size computation were performed using ABI PRISM 10 (PE Biosystem) and Gene Scan Analysis software.

For estimating the breeding values of the daughters of genotyped sons, information from the whole population of Czech Pied cattle was used. The data set contained records of milk, fat and protein yields from the first lactation collected from 1991 to 1999. The pedigree was traced back to 1984 for dams; the whole available pedigree information for sires was used.

The single-trait animal models used for all calculations were equal for all traits containing herd-year-season as fixed effect, animal as random effect with relationship matrix and linear and quadratic regression on age at calving and days open. The program PEST (Groeneveld *et al.*, 1990) under LINUX was used.

The effects of the genetic markers were estimated from a granddaughter design. The model of Weller *et al.* (1990) was used:

$$y_{ijkl} = G_i + M_{ij} + S_{ijk} + e_{ijkl}$$

where: y_{ijkl} = breeding value for milk, fat or protein yield of granddaughter *l*, daughter from sire *k* (who is son of grandsire *i*) that received marker allele *j* from grandsire *i*

G_i = effect of grandsire *i*, M_{ij} is the effect of marker allele *j* of grandsire *i*

S_{ijk} = effect of sire *k* with marker genotype *j* from grandsire *i*

e_{ijkl} = residual effect

The equation is the model for a three-level analysis of variance with sires nested in marker alleles and marker alleles nested in grandsires. The calculations were carried out using the procedure GLM of SAS (SAS Institute Inc. 1989a). In total, twelve calculations were carried out (three traits × four markers). The *F*-test in the analysis of variance was carried out on the basis of Type III Sums of Squares (SAS Institute Inc., 1989b).

Table 1 contains some basic information on the structure of the granddaughter design. The five grandsires were heterozygous in two or three markers. The number of sons per grandsire ranged from 4 to 8, the average number of daughters with records per son was between 57 and 70.

RESULTS

Table 2 contains the main characteristics of all five microsatellites typed in 38 sires and 409 cows in comparison with published data from the bovine microsatellite map of Barendse *et al.*(1997). In the population under study two

Table 1. Structure of the granddaughter design

Grandsire	Heterozygous for loci	Number of sons	Number of daughters with records per son		
			minimum	average	maximum
1	*BM143, BM2320*	8	54	66.4	86
2	*ILSTS093, BM143, BM2320*	8	38	56.8	82
3	*BM143, MB062, BM2320*	6	34	66.2	85
4	*ILSTS093, MB062, BM2320*	4	51	67.8	93
5	*BM143, BM2320*	7	41	70.4	97

Table 2. Characteristics of five polymorphic loci in Czech Pied cattle

Locus	Data from the bovine microsatellite map MARC97[1]				Results from the present study				
	relative position (cM)	No. of alleles	size range (bp)	heterozygosity (%)	No. of alleles	size range (bp)	heterozygosity (%)	R/S	PIC
ILSTS093	0.0	18	179–202	83	8	179–197	67.6	0.2776	0.6991
BM143	49.4	12	90–118	62	12	85–113	83.9	0.0440	0.7936
ILSTS097	67.2	3	234–240	48	2	233–239	46.2	0.0217	0.3478
MB062	82.6	6	167–179	79	8	162–178	58.2	0.2027	0.6395
BM2320	120.7	10	128–152	83	12	127–154	76.4	0.4204	0.7654

PIC – polymorphic information content

[1]http://www.marc.usda.gov/cgi-bin/species_chromosome?cattle+06

new alleles (162 and 164 bp) and one new allele (85 bp) were detected at microsatellite loci *MB062* and *BM143*, respectively. At *ILSTS093* and *ILSTS097* lower polymorphisms than expected were found. The heterozygosity estimates of the two last mentioned markers depended also on the family and on the sires' genotype, respectively. The value of polymorphic information content (PIC) was relatively low for *ILSTS097*. But for the remaining four markers, the PIC values were high enough for a possible use of these markers for linkage studies and QTL mapping.

The evaluation of the effects of investigated markers on production traits was influenced by the heterozygosity or homozygosity of the grandsires and sires. Two grandsires were homozygous for three loci (*MB062*, *ILSTS093*, *ILSTS097*) and three grandsires were homozygous for two loci. Due to low PIC, *ILSTS097* was excluded from all subsequent analyses of variance. Similarly, the noninformative sires were not taken into account in these analyses either.

The results of the analyses of variance are summarised in Table 3. No factor showed a significant association

Table 3. Analysis of variance for marker locus *ILSTS093*, *BM143*, *MB062* and *BM2320*

Locus	Factor	*df*	*F*-value for		
			milk yield	fat yield	protein yield
ILSTS093	Grandsire	1	0.59	14.90**	24.38**
	Marker within grandsire	2	0.32	2.25	3.30*
	Sire within marker and grandsire	8	1.16	2.32*	2.64**
	Residual	713			
BM143	Grandsire	3	0.41	0.92	5.72**
	Marker within grandsire	4	0.28	3.09*	10.49**
	Sire within marker and grandsire	21	1.33	2.19**	6.48**
	Residual	1 846			
MB062	Grandsire	1	0.04	8.76**	1.29
	Marker within grandsire	2	0.37	3.05*	7.95**
	Sire within marker and grandsire	6	0.98	1.50	2.44*
	Residual	658			
BM2320	Grandsire	4	0.60	3.89**	7.42**
	Marker within grandsire	5	0.60	1.10	3.39**
	Sire within marker and grandsire	23	1.20	2.65**	6.92**
	Residual	2 113			

* $P < 0.05$

** $P < 0.01$

with the breeding value for milk yield. The grandsire (family) influenced significantly the breeding values for fat and protein yields in three loci in each case. Similarly, there was a significant effect of the sire within grandsire and marker on the breeding values of these two traits for all but one or all loci, respectively.

The marker within grandsire was significant in all four loci for the breeding value of protein yield and significant in two loci (*BM143*, *MB062*) for the breeding value of fat yield. Though the *F*-values cannot be directly compared between markers, it seems that the region between *BM143* and *MB062* might be most interesting for looking for a QTL for protein yield.

DISCUSSION

The results indicate that the investigated marker loci are highly polymorphic in Czech Pied cattle. A valuable result is the detection of two new alleles with a length of 162 and 164 bp, respectively, inside of intron 3 of the *CSN3* gene. This finding extends the results of Leveziel *et al.* (1994), who investigated the same region in nine different breeds and in one crossbred and found only six alleles with size ranging from 167 to 179 bp.

In the present study, four markers exhibited a significant association with breeding values for protein yield and two markers with breeding values for fat yield. In the literature reporting on QTLs on chromosome 6, the marker *BM143* is most frequently considered to be a candidate marker for protein percentage (Spelman *et al.*, 1996; Gomez-Raya *et al.*, 1996; Georges and Anderson, 1996; Velmala *et al.*, 1999 and Kühn *et al.*, 1999). The hypothesis resulting from the present findings that a QTL for protein yield might be located between the microsatellites *BM143* and *MB062* is also supported by the fact that these loci have a significant effect on the fat yield, too. This influence on the fat yield can be a side effect of the influence on the protein yield, as fat and protein yields are generally strongly correlated with each other.

It has been hypothesized by Georges and Anderson (1996) that different breeds may share identity-by-descent QTL alleles, which can be maintained through similar selection goals. This may also be the case for similar QTL alleles on chromosome 6 which occur in different cattle breeds.

The existence of a QTL inside or near the *CSN3* locus is still an open question. Our results indicate that there may also be a QTL on chromosome 6 affecting milk protein and fat yields segregating in Czech Pied cattle. The occurrence of specific alleles in intron 3 of the *CSN3* gene can also be connected with the existence of breed-specific haplotypes, nonrandomly distributed in different breeds and populations (Lien and Rogne, 1993). The evidence of associations between casein haplotypes and milk production traits was clearly demonstrated by Lien *et al.* (1995, 1999).

Acknowledgement

Thanks are due to Václav Čermák (PLEMDAT, Benešov) for making available the data for breeding value estimation.

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Received: 01–02–23
Accepted after corrections: 01–05–29

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Grading versus animal model evaluation in arctic fox (*Alopex lagopus*)

Bodové hodnocení versus hodnocení pomocí animal modelu u lišky polární (*Alopex lagopus*)

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ABSTRACT: Coat and conformation traits (body size, colour type, colour purity, coat density, hair length, general appearance, total score) of 4 306 arctic foxes bred on the Śniaty Fox Farm (Poland) in 1985–1998 were evaluated using the grading standard (based on the point scale) and the BLUP with a single-trait animal model. Pearson correlation coefficients between the trait scores and their estimated additive genetic effects were calculated within each year of the analysis. Moreover, Spearman rank correlation coefficients for 20 animals ranked highest according to the BLUP evaluation and their ranking based on the scores was estimated. Pearson correlation coefficients ranged from –0.128 to 0.970 (most often 0.7–0.8) indicating (with some exceptions in 1991, 1992 and 1998) that the trait scores are usually consistent with the corresponding estimates of additive genetic effects. In contrast, for each trait analysed Spearman rank correlation coefficient was significantly negative (ranging from –0.687 to –0.857, $P \leq 0.01$) showing that the best animals according to the two methods of evaluation did not overlap. Negative and low annual genetic trends estimated for the analysed traits (ranging from –0.00041 to 0.02822 point/year) suggest that the conformation scores as selection criteria are not effective.

Keywords: arctic fox; animal model; grading; genetic trend; selection

ABSTRAKT: V letech 1985 až 1998 byly hodnoceny pomocí klasifikačního standardu (založeného na bodové stupnici) a s použitím jednoznakového animal modelu metody BLUP srst a znaky exteriéru (velikost těla, typ vybarvení, čistota vybarvení, hustota srsti, délka chlupů, celkový vzhled, celkový počet bodů) u 4 603 polárních lišek chovaných na liščí farmě Śniaty (v Polsku). Pro každý rok samostatně byly stanoveny Pearsonovy koeficienty korelace mezi bodovými hodnoceními jednotlivých znaků a odhady aditivity genetických efektů. Dále byly stanoveny Spearmanovy koeficienty korelace pořadí u 20 zvířat, která se umístila nejvýše podle hodnocení BLUP, a podle jejich umístění na základě bodového hodnocení. Pearsonovy korelační koeficienty se pohybovaly v rozmezí –0,128 až 0,970 (nejčastěji 0,7–0,8) a naznačovaly (s výjimkou let 1991, 1992 a 1998), že bodové hodnocení znaků se obvykle shoduje s příslušnými odhady aditivních genetických efektů. Naproti tomu Spearmanův koeficient korelace pořadí nabýval významné záporné hodnoty (od –0,687 do –0,857, $P \leq 0,01$) a naznačoval, že se nejlepší zvířata podle obou způsobů hodnocení nepřekrývala. Záporné a nízké roční genetické trendy, které byly odhadnuty pro jednotlivé analyzované znaky (v rozmezí od –0,00041 do 0,02822 bodu/rok), ukazují, že není vhodné používat bodové hodnocení exteriéru jako selekční kritérium.

Klíčová slova: polární liška; animal model; klasifikace; genetický trend; selekce

INTRODUCTION

The breeding value of animals bred on farms is usually estimated using the mixed model methodology. A mixed analysis of variance model, referred to as an animal model, is most often employed (Engel *et al.*, 1999). The animal model implies a unique type of analytical procedure that incorporates all sources of genetic information (all available records of animal's own performance and its relatives) into the prediction of breeding value, and consequently high accuracy of predictors is achieved (Wright *et al.*, 2000). Identification of individuals with the highest genetic merit is a key point in achieving genetic progress.

Fur animals are not the most important species bred on farms, but in some countries (Norway, Finland, Denmark, Russia, Poland) fur production is of economic importance. The keen competition on the international fur market forces breeders to speed up the genetic progress in fur animal populations. Genetic evaluation based on the mixed model methodology is a good tool to achieve this goal. However, the introduction of this methodology for breeding value estimation has been delayed in farmed fur animals. Although major species of livestock have been selected on the basis of best linear unbiased predictions (BLUPs) of their breeding values (Robinson, 1991) for almost two decades, in fur animal breeding the BLUP with an animal model for routine evaluation was introduced only at the beginning of the nineties (Smëds, 1992). Since that time in Scandinavian countries, where this method is widely used (Sørensen *et al.*, 2000; Hansen *et al.*, 2000), considerable genetic improvement has been achieved.

In Poland one of the most important fur animals is the arctic fox (*Alopex lagopus*). In Polish fox population animals are selected on the basis of conformation evaluation or simplified selection index. The single or multi-trait animal model is not routinely used in practical fox breeding. As far as we know, only Wierzbicki *et al.* (2000) and Jakubczak (2000) have used the mixed model methodology for breeding value estimation in Polish fox population. The mixed model applied by Jakubczak (2000) included three fixed effects (effect of year, effect of sex × dam age interaction and effect of year of birth × whelping season interaction) and one random effect (additive genetic effect). However, the authors estimated breeding values and genetic trends for scientific purposes. In practice, coat and conformation traits are evaluated according to the grading standard (Frindt *et al.*, 1984). Each trait is evaluated on a point scale and the sum of scores gives the total score (Table 1). The individuals with at least one trait assessed negatively (score = 0) are discarded. Arbitrarily assigned scores introduce a large amount of measurement errors, thus the total score is not reliable as a predictor of aggregate breeding value. The problem of subjective evaluation of conformation traits in fur animal populations was raised by Jeżewska and Maciejowski (1983), who suggested that unsatisfactory genetic gain could be brought about by the grading method.

Table 1. Grading standards

Trait	Scale of scores
Old grading standard (until 1996)	
Body size	0–3
Colour type	0–6
Colour purity	0–6
Coat density	0–6
Hair length	0–6
General appearance	0–3
Total score	0–30
New grading standard (from 1997)	
Body size and conformation	0–6
Colour type	0–3
Colour purity	0–5
Coat quality	0–6
Total score	0–20

Table 2. Principles of the construction of simplified selection index

Source of information	Evaluated trait	Grading scale	Index equivalent (points)
Parents	average total score of parents (points)	28.5–30	3
		25.5–28	2
		22.5–25	1
		19.5–22	0.5
Litter the animal was born in	litter size (pups)	≥ 9	3
		7–8	2
		5–6	1
		≤ 4	0
Uniformity of litter the animal originated from	average conformation score of full-sibs (points)	17–18	6
		16–16.9	5
		15–15.9	4
		14–14.9	3
		13–13.9	2
		12–12.9	1
		≤12	0
Body conformation (points)	body length		2–6
	coat colour		2–6
	coat structure		2–6
Maximum index value			30

On larger fox farms where the breeding work is assisted by computers the animals are selected on the basis of a simplified selection index. The principles by which the index is constructed (Kuźniewicz and Filistowicz, 1999) are given in Table 2. When constructing the simplified selection index, some information on relatives is included, hence this type of evaluation should be considered more accurate than the total score. However, the total score is still the basic selection criterion in Polish fox farming.

It can be anticipated that regardless of the method used (total score or simplified selection index) for the breeding value evaluation neither unbiased predictions of an individual's genetic merit nor fast genetic progress will be achieved.

The present study is aimed at comparing the two methods of arctic fox evaluation: the subjective grading based on a point scale currently used in Polish fox population and the BLUP – animal model intended to be implemented for routine evaluation in future.

MATERIAL AND METHODS

Records of 4 306 arctic foxes of blue and white colour type bred on the Śniaty Fox Farm (Poland) in 1985–1998 were analysed. The data were collected in the database of the LISY computer program (Chudoba *et al.*, 1988) developed to assist breeding work on a fox farm. Only individuals with known parents and full information on their performance were taken into account.

Evaluation of foxes was done by a skilled judge according to the grading standard used in Poland until 1996 (Table 1; Frindt *et al.*, 1984). The graded traits were: body size, colour type, colour purity, coat density, hair length, general appearance and the total score. Number of evaluated animals, mean scores and standard deviations of studied traits in the successive years of the analysis are shown in Table 3.

The estimation of additive genetic effects for the traits under study was performed using the BLUP with the following single-trait animal model:

$$y_i = X_i b + Z_i u + e_i$$

where: y_i is vector of observed responses (studied traits), b, u and e are vectors of fixed effects (effect of year, effect of colour type), random genetic effects (breeding values) and random errors, respectively. The structure of available data did not enable to include more fixed effects in the model. X_i and Z_i are design matrices. It is assumed that:

$$u \sim N(0, \sigma_a^2 A)$$

$$e \sim N(0, \sigma_e^2 I)$$

where: u and e are independently distributed, σ_a^2 and σ_e^2 represent additive genetic and environmental variances, and A is additive genetic relationship matrix between all the animals. The following heritability estimates were used in the genetic evaluation: body size – 0.288, colour type – 0.342, colour purity – 0.491, coat density – 0.305, hair length – 0.533, general appearance – 0.226, and total score – 0.374. Detailed description of their estimation was reported by Wierzbicki (2000).

Pearson correlation coefficients between the results of the two methods of fox evaluation (scores versus additive genetic effects) were estimated. Furthermore, Spearman rank correlation coefficients for 20 animals ranked highest according to the BLUP evaluation and their ranking based on the scores were calculated. In order to assess the efficiency of breeding work in the studied fox population over the period of 14 years (1985–1998) genetic trends were estimated. The average annual genetic trends for the studied traits were estimated as a regression of the mean additive genetic effects of animals born in the same year on time. All statistical analyses were performed using the SAS program (1990).

RESULTS AND DISCUSSION

The results of both types of evaluation and Pearson correlation coefficients between them are presented in Table 3. Within each year of the analysis the studied traits are characterised by the mean scores and their standard deviations (results of subjective grading) as well as mean additive genetic effects (results of animal model evaluation).

The correlations between mean scores and mean additive genetic effects were relatively high ranging from 0.7 to 0.8 (on average) although in some years the negative or very low correlations were found (e.g. colour type and general appearance in 1991, $r = -0.062$ and $r = -0.128$, respectively; general appearance in 1992, $r = -0.013$; general appearance in 1998, $r = -0.002$; colour type in 1992, $r = 0.007$; general appearance in 1995 and 1997, $r = 0.027$ and $r = 0.020$, respectively). When the correlations were estimated using all data (not within years) the highest correlation was found for hair length (0.793) and the lowest for body size (0.702).

Comparatively high correlations between the methods of evaluation do not mean that they are similarly accurate. The correlations only indicate that the direction of both evaluations is similar (better animals are evaluated higher) but the accuracy may be markedly different. For example, the 1st and the 10th fox from the ranking list made according to the total score were given 30 points. Based on these scores it is not possible to distinguish which of these two individuals is superior (has higher genetic merit). In contrast, the additive genetic effects of the same individuals estimated for the total score were: 2.8446 and 2.6797, respectively. In consequence of high accuracy of the animal model evaluation, the individuals took 10th

Table 3. Number of animals, mean scores, standard deviations (in brackets), mean additive genetic effects of studied traits and Pearson correlation coefficients between mean score and mean additive genetic effect of each trait in subsequent years of analysis

Year	Number of animals	Trait	Mean score	Mean additive genetic effect	Pearson correlation coefficient
1985	386	body size	2.71 (0.47)	−0.0703	0.815
		colour type	4.73 (1.33)	−0.1683	0.861
		colour purity	4.44 (1.29)	−0.2415	0.799
		coat density	5.29 (0.98)	−0.1408	0.861
		hair length	5.42 (0.83)	−0.0737	0.875
		general appearance	2.89 (0.31)	−0.0016	0.762
		total score	25.49 (2.84)	−0.6962	0.839
1986	463	body size	2.67 (0.50)	−0.0224	0.843
		colour type	5.26 (0.96)	−0.0877	0.862
		colour purity	4.49 (0.89)	−0.1117	0.795
		coat density	5.38 (0.74)	−0.0627	0.877
		hair length	5.69 (0.50)	−0.0218	0.766
		general appearance	2.93 (0.25)	−0.0140	0.775
		total score	26.45 (2.08)	−0.3164	0.879
1987	503	body size	2.43 (0.57)	0.0740	0.872
		colour type	5.45 (0.93)	0.1544	0.804
		colour purity	5.26 (1.02)	−0.0289	0.757
		coat density	5.51 (0.61)	0.0221	0.881
		hair length	5.48 (0.59)	−0.0924	0.853
		general appearance	2.81 (0.39)	0.0108	0.904
		total score	26.91 (2.10)	0.1592	0.692
1988	359	body size	2.56 (0.69)	0.0572	0.890
		colour type	5.82 (0.67)	0.0958	0.797
		colour purity	4.69 (1.16)	0.0664	0.898
		coat density	5.73 (0.51)	0.0456	0.879
		hair length	5.35 (0.72)	0.0496	0.903
		general appearance	2.83 (0.38)	0.0263	0.909
		total score	26.99 (2.01)	0.3503	0.887
1989	273	body size	2.26 (0.71)	−0.0223	0.907
		colour type	5.88 (0.46)	0.1677	0.741
		colour purity	5.69 (0.79)	0.4085	0.604
		coat density	5.14 (0.73)	0.0979	0.839
		hair length	5.47 (0.67)	0.1697	0.880
		general appearance	2.74 (0.46)	−0.0223	0.918
		total score	26.97 (3.03)	0.8204	0.522
1990	175	body size	2.64 (0.55)	0.0398	0.674
		colour type	5.95 (0.29)	0.1054	0.515
		colour purity	5.45 (0.91)	0.2049	0.501
		coat density	5.47 (0.55)	0.0636	0.618
		hair length	5.22 (0.67)	0.0644	0.656
		general appearance	2.89 (0.30)	0.0133	0.241
		total score	26.71 (0.99)	0.5061	0.403
1991	195	body size	2.94 (0.22)	0.0048	0.459
		colour type	5.99 (0.10)	0.0621	−0.062
		colour purity	4.26 (0.78)	0.0936	0.764
		coat density	5.01 (0.39)	−0.0036	0.697
		hair length	5.41 (0.60)	0.0646	0.738
		general appearance	2.99 (0.10)	−0.0182	−0.128
		total score	26.62 (0.92)	0.2225	0.690
1992	346	body size	2.95 (0.24)	−0.0460	0.755
		colour type	5.99 (0.11)	0.0356	0.007
		colour purity	4.08 (0.39)	0.1059	0.470
		coat density	5.07 (0.29)	−0.0374	0.577
		hair length	5.58 (0.49)	0.0712	0.734
		general appearance	2.99 (0.05)	−0.0002	−0.013
		total score	26.67 (0.66)	0.1448	0.543

Table 3 to be continued

Year	Number of animals	Trait	Mean score	Mean additive genetic effect	Pearson correlation coefficient
1993	178	body size	2.94 (0.24)	−0.0009	0.906
		colour type	5.98 (0.15)	0.0173	0.422
		colour purity	4.35 (0.76)	0.0346	−0.559
		coat density	5.08 (0.33)	0.0033	0.638
		hair length	5.67 (0.54)	0.0098	0.721
		general appearance	2.94 (0.23)	−0.0020	0.724
		total score	26.99 (0.80)	0.0759	0.612
1994	283	body size	2.98 (0.13)	−0.0102	0.719
		colour type	5.98 (0.21)	0.0137	0.505
		colour purity	4.23 (0.64)	0.0249	0.788
		Coat density	5.26 (0.47)	0.0041	0.842
		hair length	5.56 (0.50)	0.0327	0.807
		general appearance	2.98 (0.12)	0.0005	0.970
		total score	26.99 (0.82)	0.0808	0.780
1995	352	body size	2.98 (0.14)	0.0009	0.511
		colour type	5.95 (0.30)	0.0218	0.426
		colour purity	4.32 (0.75)	0.1264	0.813
		coat density	5.31 (0.48)	0.0902	0.816
		hair length	5.69 (0.48)	0.0443	0.789
		general appearance	2.99 (0.05)	0.0080	0.027
		total score	27.26 (1.00)	0.3046	0.806
1996	300	body size	2.98 (0.14)	−0.0051	0.862
		colour type	5.89 (0.48)	0.0171	0.709
		colour purity	5.47 (0.87)	0.1946	0.770
		coat density	5.23 (0.44)	0.0926	0.797
		hair length	5.37 (0.58)	0.0322	0.853
		general appearance	2.99 (0.05)	0.0003	0.857
		total score	27.94 (1.09)	0.3581	0.801
1997	303	body size	2.98 (0.13)	−0.0033	0.858
		colour type	5.76 (0.64)	0.0354	0.889
		colour purity	5.01 (0.99)	0.0455	0.906
		coat density	5.02 (0.43)	0.0824	0.894
		hair length	5.57 (0.49)	0.0542	0.886
		general appearance	2.99 (0.06)	0.0044	0.020
		total score	27.36 (0.98)	0.2366	0.861
1998	226	body size	2.99 (0.07)	−0.0071	0.125
		colour type	5.70 (0.78)	−0.0324	0.915
		colour purity	5.42 (0.91)	0.0666	0.935
		coat density	4.98 (0.30)	0.0156	0.859
		hair length	5.56 (0.50)	0.0336	0.916
		general appearance	2.99 (0.07)	0.0039	−0.002
		total score	27.67 (1.12)	0.1002	0.907
Total	4306	body size	2.76 (0.50)	–	0.702
		colour type	5.76 (0.81)	–	0.770
		colour purity	4.74 (1.05)	–	0.719
		coat density	5.29 (0.62)	–	0.766
		hair length	5.51 (0.61)	–	0.793
		general appearance	2.92 (0.27)	–	0.784
		total score	26.88 (1.83)	–	0.764

and 70th positions, respectively, on the ranking list made on the basis of the additive genetic effects.

Low accuracy of the grading results from two main reasons. First, the scales of scores are narrow (especially for body size and general appearance, see Table 1). The narrow scale does not permit to reflect variability of traits leading to biased assessments (Wierzbicki *et al.*, 2000). The general appearance (evaluated on a scale 1–3; animals with score = 0 are culled) is an excellent example supporting this presumption. Pearson correlation coefficients between scores and additive genetic effects of this trait most often had low (in 1990, 1995, 1997) or negative (in 1991, 1992, 1998) values. Similarly the colour type (although evaluated on a scale 0–6, the judge can give 0, 2, 4 or 6) was characterised by low or negative correlations between the obtained scores and estimated additive genetic effects (very low and low in 1990, 1992, 1993, 1994, 1995, and negative in 1991).

The second drawback of the grading is subjectivity of this method. This problem was raised by many authors (Jeżewska and Maciejowski, 1983; Jeżewska *et al.*, 1994; Lohi, 1995; Lohi *et al.*, 1996; Rasmussen, 1996; Reiten, 1977).

Jeżewska and Maciejowski (1983), who carried out the study in populations of mink, arctic and silver fox, found that the repeatability of the grading was low, ranging from 0.250 for the coat density to 0.664 for the total score. According to Reiten (1977) the correlation between successive evaluations which can be achieved by a well trained judge is not higher than 0.50– 0.68.

An interesting study was carried out by Jeżewska *et al.* (1994) in a population of chinchilla. The authors estimated repeatability of conformation grades given by judges to the same 5 animals evaluated three times. Furthermore, the total variation of the grades was partitioned into components. The repeatability of grades ranged from 0.302 (belly coat) to 0.735 (colour purity). Detailed analysis of sources of variation and their contribution to the total variance of grades revealed that only 20.68–53.68% of the total variability of grades was determined by animals, whereas up to 60.34% of the total variance was defined as an error variance. The judges' contribution to the total variance of grades was estimated at 4.69–43.56%. These results clearly indicate that the grading as a subjective method of coat and conformation evaluation is not reliable. Hence, the grades should not be the only criteria when selecting animals.

The estimates of additive genetic effect and the grades of each evaluated trait were used to prepare two ranking lists: 1st ranking according to the results of the animal model evaluation, and the 2nd ranking according to the judge grading. For each studied trait Spearman rank correlation coefficient for 20 animals ranked highest using the BLUP animal model and their positions on the 2nd list was calculated (Table 4). It was found that all correlations were negative and significant ($P \leq 0.01$). The highest discrepancy was observed between evaluations of the colour purity (–0.857) and the lowest between the total evaluations (–0.687).

Table 4. Spearman rank correlation coefficients (R_S)

Trait	R_S
Body size	–0.715**
Colour type	–0.825**
Colour purity	–0.857**
Coat density	–0.733**
Hair length	–0.853**
General appearance	–0.744**
Total score	–0.687**

**$P \leq 0.01$

Spearman rank correlation coefficients sharply contrast with Pearson correlations presented earlier. We can assume that according to the grading, majority of superior animals are ranked higher but we do not know exactly whether the best ones are at the top, in the middle or at the bottom of the ranking list (low accuracy and narrow scale of the grading makes it impossible to distinguish which of two similar individuals is superior and consequently should be higher on the ranking list). The decision (more or less subjective) which individuals should be selected must be taken arbitrarily by a breeder. In contrast, the estimates of additive genetic effects enable to prepare the ranking of animals with high accuracy, and animals are ranked according to their breeding values. Comparison of the two rankings made according to the above rules led to significantly negative Spearman rank correlations because the best animals from the two lists did not overlap (as an example, the positions of the two individuals according to both rankings were presented earlier).

The results of the comparison cannot be related to the other ones since the available literature does not quote the results of similar studies. Only Socha (1996) studied the results of blue fox evaluation comparing rankings according to the grading and selection indexes constructed by the method described by Künzi (1976). Spearman rank correlation coefficients between the total score and the selection index ranged from 0.568 to 0.713 depending on the number of traits included in the index (3, 4 or 5). Regardless of the relatively high correlations the author concluded that the top animals from both lists differed markedly and this discrepancy could lead to different selection decisions.

In the analysed years (1985–1998) the selection criteria in the studied arctic fox population were the total score or the simplified selection index. Only animals with the highest total score or selection index were selected for further breeding. In order to evaluate usefulness of both methods for achieving genetic progress, the genetic trends for the traits under study were estimated (Table 5).

Table 5. Average annual genetic trends (ΔG) for studied traits

Trait	ΔG (point/year)	ΔG (%)
Body size	–0.00041	–0.0153
Colour type	0.00067	0.0124
Colour purity	0.01264	0.2834
Coat density	0.00855	0.1559
Hair length	0.00543	0.1003
General appearance	0.00030	0.0103
Total score	0.02822	0.1085

The annual genetic trends were expressed in absolute terms (point/year) or as percentages of the average trait scores of the foxes born in 1985 (first studied generation). All estimated genetic trends were low ranging from 0.00067 (0.0124%) for the colour type to 0.02822 (0.1085%) for the total score. The genetic trend estimated for the body size was found to be negative (–0.00041; –0.0153%).

The genetic trends have been estimated in majority of domesticated animals. The rates of genetic progress, expressed as percentages of the means, were found within the range from 1% to 2% per year (Falconer and Mackay, 1996).

Jakubczak (2000) estimated the genetic trends in a pastel fox population using the BLUP with a multiple animal model and found the low genetic trends for analysed traits. The annual genetic trend for the total score reached 0.1319 point/year. Socha (1996), who carried out the study in the blue fox population, also found low genetic trends for the conformation traits. The genetic trends ranged from –0.0051 (–0.18%) for the body size to 0.0734 (0.28%) for the total score. However, the author estimated the genetic trends using the method described by Smith (1962), thus the results cannot be compared directly to those obtained using the mixed model methodology.

The low annual genetic trends estimated in the studied arctic fox population as well as unsatisfactory genetic progress in the other fox populations (Jakubczak, 2000; Socha, 1996) suggest that the efficiency of breeding strategies applied in the Polish fox populations is not high. One of the main reasons for which the selection response is very small is a low accuracy of the fox evaluation. In Scandinavian countries where animals are evaluated using the mixed model methodology the selection response is satisfactory. According to Sławoń (1995), 12.5% and 42.5% of fox skins offered by Norwegian and Finnish breeders, respectively, were large whereas only 2.75% of Polish skins were of the large size. In the studied fox population (Wierzbicki, 1999) the most numerous were medium-sized skins (65.3%) whereas the largest ones were very rare (0.2%). A similar situation was found as far as the coat structure was concerned.

The results of the present study and the reports of other authors clearly show disadvantages of the selection criteria currently used in Polish fox population. The introduction of the mixed model methodology for the estimation of a fox genetic merit as well as some other changes which were postulated by Filistowicz *et al.* (1999) are needed for substantial genetic improvement of foxes kept on Polish farms.

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Received: 01–02–15

Accepted after corrections: 01–05–16

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Frequency of *Bacillus* bacteria in raw cow's milk and its relation to other hygienic parameters

Výskyt bakterií rodu *Bacillus* v syrovém kravském mléce a jejich vztah k ostatním hygienickým ukazatelům

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ABSTRACT: Monitoring of *Bacillus* strains presenting hygienic and safety hazards in bulk milk samples was the primary objective of this study. Two sets were formed: 54 samples representing 27 sampling sites in Northern and Central Moravia were included in the Ist set, the IInd set (324 samples from Moravia and Bohemia) was completed in the Central Laboratory at Pardubice. Representative samples (158) of *Bacillus* strains were isolated and identified in the Czech Collection of Microorganisms at Brno (CCM). The following frequencies were found on the species level: *Bacillus licheniformis* (102; 64.6%), *Bacillus cereus* (43; 27.2%), *Bacillus pumilus* (5; 3.2%), *B. subtilis* (2; 1.3%), *B. badius* (1; 0.6%), *B. fusiformis* (1; 0.6%), *B. sphaericus* (1; 0.6%), *Paenibacillus glucanolyticus* (1; 0.6%). Two psychrotolerant strains of *Bacillus cereus* (growth at 4–7°C, negative growth at 40–43°C) were also isolated; they were classified provisionally as *Bacillus weihenstephanensis* according to Lechner *et al.* (1998). As for *B. cereus* strains, 4 intermediary strains characterized by multiplication rates not corresponding to *B. cereus* or to *B. weihenstephanensis* were isolated. These strains grew at 7–10°C, they did not grow at 4°C and 40–43°C. Isolation of *B.stearothermophilus* and *B. sporothermodurans* species was not successful. The nutritive and sensory milk quality can be affected by proteolytic and lipolytic enzymes, produced also by *Bacillus* strains. Production of proteolytic enzymes was evident in 100% and of lipolytic ones in 61–82% strains of *B. licheniformis*. All *B. cereus* strains (100%) produce the proteolytic enzymes whereas lipolytic activity was evident in 12 and 30 strains (28 and 70%). The proteolytic and also lipolytic enzymes were produced (excluding *B. badius, B. sphaericus* and *P. glucanolyticus*) in the remaining strains. Frequencies of the following microorganisms were monitored simultaneously with total *Bacillus* bacteria frequency (TCBa): total count of mesophilic (TCM), thermoresistant (TRM), and sporogenic anaerobic microorganisms (SPAN). Linear regression was used for analyzing relationships between TCBa frequency and other hygienic parameters TCM and TRM. Non-significant correlation coefficients $r = 0.04$ (Ist set) and $r = -0.003$ (IInd set) were found for TCM × TCBa. Significant correlations $r = 0.77$ (I) and $r = 0.15$ (II) – $P \leq 0.001$ and $P \leq 0.01$ – characterized the TCBa ×TRM relation. Significant differences ($P < 0.05$) were found between geometric means of the mesophilic group of TCBa and SPAN (IInd set) by the unpaired *t*-test. Non-significant difference ($P > 0.05$) was found between *B. cereus* and SPAN and between *B. licheniformis* and SPAN. Less frequent captures of psychrotrophic bacilli document hypothesized primary effects of milking hygiene on bacilli frequency in raw milk stored at 4–6°C and less important effects of milk storage and transport. These facts conform to bibliographic references presenting relatively close correlations between spore-forming bacteria frequencies in feedstuffs, faeces, litter, milk, and udder surface. Mostly significant relations of bacilli to TRM and non-significant relations of bacilli to TCM issue from their relatively high or low proportion in the mentioned microbial groups. The ratio index (TCBa/TRM) was $p_i = 0.3$ in both sets; geometric means TRM were $g = 10.34$ (Ist set) and $g = 165.33$ (IInd set). The TRM limit ≤ 2000 CFU/ml corresponds (according to $p_i = 0.3$) to the value 600 TCBa CFU/ml. The higher rates of bacilli associated with the positive SPAN test demonstrate important negative impacts of bacilli on cheese production quality. Evident abundance of bacilli in milk even in the case of negative SPAN capture documents a potential inadequate function of this test under practical conditions. Processed data (CL Pardubice 1997–1999) document that more than 85% analyzed milk samples comply with TCM and TRM

Supported of the Ministry of Agriculture of the Czech Republic (Project No. EP 9058).

frequency standard according to standard ČSN 57 0529 (TCM ≤ 100 ths CFU/ml; TRM ≤ 2000 CFU/ml). The seasonal effects on TRM frequency were not demonstrated whereas data of SPAN show their highest frequency in January and November.

Keywords: raw milk; total number of *Bacillus* bacteria; identification; total count of microorganisms; total count of thermoresistant microorganisms

ABSTRAKT: Byl sledován výskyt hygienicky a zdravotně rizikové skupiny mikroorganismů rodu *Bacillus* v bazénových vzorcích mléka v oblasti severní a střední Moravy (27 odběrných míst, I. soubor 54 vzorků) a oblasti Čech a Moravy (II. soubor 342 vzorků z Centrální laboratoře Pardubice). Celkem bylo izolováno 158 kmenů bacilů, které byly identifikovány v České sbírce mikroorganismů, Brno. Nejčetnější zastoupení měl *Bacillus licheniformis* (102; 64,6 %), dále *Bacillus cereus* (43; 27,2 %), *B. pumilus* (5; 3,2 %), *B. subtilis* (2; 1,3 %), *B. badius* (1; 0,6 %), *B. fusiformis* (1; 0,6 %), *B. sphaericus* (1; 0.6 %), *Paenibacillus glucanolyticus* (1; 0,6 %) – tab. 1. Byly rovněž izolovány dva psychrotolerantní kmeny *B. cereus* (rostly při teplotě 4–7 °C, nerostly při 40 až 43 °C), které byly podle Lechnerové *et al.* (1998) předběžně určeny jako *Bacillus weihenstephanensis*. Mezi kmeny *Bacillus cereus* byly izolovány i čtyři intermediární kmeny, které schopností růstu při různých teplotách neodpovídají druhu *B. cereus* ani druhu *B. weihenstephanensis*. Kmeny rostly při 7–10 °C, ale nerostly při 4 a 40 až 43 °C. Izolace druhů *B. stearothermophilus* a *B. sporothermodurans* nebyla úspěšná. Nutriční a senzorická kvalita mléka může být ovlivněna proteolytickými a lipolytickými enzymy produkovanými i druhy rodu *Bacillus*. Kmeny *B. licheniformis* vykazovaly schopnost tvorby lipolytických enzymů v 61–82 % a proteolytických ve 100 % případů. Všechny kmeny *Bacillus cereus* vytvářely jasnou proteolytickou zónu a pouze ve 12 a 30 případech (28 a 70 %) produkovaly lipolytické enzymy. Ostatní kmeny (vyjma *B. badius, B. sphaericus* a *P. glucanolyticus*) projevovaly schopnost tvorby obou lytických enzymů (tab. 3). Současně s celkovým počtem bakterií rodu *Bacillus* (dále jen TCBa) byl sledován výskyt celkového počtu mezofilních, termorezistentních a sporotvorných anaerobních mikroorganismů (TCM, TRM, SPAN). Mezi výskytem TCBa a hygienickými ukazateli TCM a TRM byla provedena statistická analýza lineární regresí. Korelační koeficient mezi TCM a TCBa byl vzhledem ke způsobu kultivace obou skupin mikroorganismů nevýznamný a činil $r = 0,04$ (I. soubor) a $r = -0,003$ (II. soubor). Zajímavější je vztah mezi TCBa a TRM, který byl významný pro oba soubory a korelační koeficienty byly $r = 0,77$ (I) a $r = 0,15$ (II) na hladinách významnosti $P \leq 0,001$ a $P \leq 0,01$ (tab. 2). Mezi geometrickými průměry mezofilní skupiny TCBa a SPAN hodnot II. souboru byla zjištěna nepárovým *t*-testem významná průkaznost rozdílů ($P < 0,05$). Mezi *B. cereus* a SPAN a *B. licheniformis* a SPAN byla průkaznost rozdílů nevýznamná ($P > 0,05$) – tab. 2. Nízké záchyty psychrotrofních bacilů dokladují, že výskyt bacilů v syrovém mléce v prvovýrobě (úložné teploty 4–6 °C) méně souvisí s uložením a transportem, ale spíše s hygienou dojení, což také potvrzují v literatuře uváděné poměrně těsné korelace mezi výskytem sporotvorných bakterií v krmivech, výkalech, podestýlce, na povrchu vemene a v mléce. Převážně významné vztahy bacilů k TRM a nevýznamné k TCM jsou dány jejich relativně velkým, resp. malým podílem ve zmíněných technologických mikrobiálních skupinách. Poměrný index TCBa/TRM byl pro oba soubory $p_i = 0,3$, geometrické průměry byly $g = 10,34$ (I. soubor) a $g = 165,33$ (II. soubor) – tab. 5. Z uvedené hodnoty p_i lze vypočítat, že limit pro TRM ≤ 2000 CFU/ml koresponduje s hodnotou 600 TCBa CFU/ml. Vyšší záchyty bacilů při pozitivním nálezu SPAN dokládají podstatnou roli bacilů při ohrožení sýrařské produkce. Zřetelné zastoupení bacilů v mléce i při negativním záchytu SPAN dokládá případnou nedostatečnou způsobilost tohoto testu i v praktických podmínkách. Z výsledků CL Pardubice (1997–1999) vyplývá, že více než 85% všech bazénových vzorků mléka vyhovuje požadavkům normy ČSN 57 0529 (TCM ≤ 100 tis. CFU/ml; TRM ≤ 2000 CFU/ml). Výskyt TRM pro jednotlivé roky je vyrovnaný a není odvislý od ročního období. Nejvyšší hodnoty SPAN byly naopak dosahovány v měsících listopad a leden (tab. 4; obr. 1 až 3).

Klíčová slova: syrové mléko; celkový počet bakterií rodu *Bacillus*; identifikace; celkový počet mikroorganismů; celkový počet termorezistentních mikroorganismů

INTRODUCTION

In general, spore-forming microorganisms represent important problematic contaminants of raw milk and milk products above all. Their ability to survive heating to the pasteurization temperature or ultrahigh-temperature (UHT) treatment facilitates their passage into the food-chain resulting in potential incidence of alimentary disorders. *Bacillus licheniformis* (Ist morphologic group), which is also associated with food spoilage (Salkinoja-Salonen *et al.*, 1999), *Bacillus circulans* and *Paenibacillus polymyxa*, formerly *Bacillus polymyxa*, (IInd morphologic group) are the most frequent mesophilic bacteria, *B. stearothermophilus* (IInd morphologic group) is the most frequent thermophilic bacterium. *Bacillus cereus* – the primary initiator of alimentary intoxications caused by dairy product con-

sumption – is the most controlled bacillus in the Czech Republic as well as abroad. Valík *et al.* (2000) mention e.g. 347 patients in a boarding-house (Slovakia, 1984). Larsen and Jörgensen (1997) found 10^3 to 3×10^5 CFU/ml of psychrotolerant strain *B. cereus* in pasteurized milk. Notermans (1997) noted that 7% of the portions of pasteurized milk being consumed in the Netherlands could contain more than 10^5–10^6 *B. cereus* per ml. In Sweden, *B. cereus* is considered as a parameter limiting stable quality of pasteurized milk. The legal count (1000 spores /ml) must not increase during the guarantee period (storage at 8°C) – Christianson *et al.* (1999). Psychrotolerant species *B. cereus* can survive at low temperatures 4–7°C. Griffiths and Phillips (1990) found these microorganisms in 60% raw milk and in 70% pasteurized milk. Ternström *et al.* (1993) found strains *B. cereus* viable at 7°C in 82% pasteurized milk products. In 1998, psychrotolerant strains *B. cereus* (growing at 4–7°C, not growing at 40–43°C) were classified as a new species *Bacillus weihenstephanensis* (Lechner *et al.*, 1998). Some members of the genus *Bacillus* participating in technological milk processing produce highly heat-resistant endospores (HHRS strains). Therefore they can survive and germinate in final dairy products after pasteurization or UHT treatment. Mesophilic strains classified in HHRS group represent a homogeneous group of non-pathogenic bacilli characterized by particular demands necessary for their multiplication (dissimilar from the species identified until now). In 1996, this group was classified as an independent species *Bacillus sporothermodurans* (Petterson *et al.*, 1996).

According to standard ČSN 57 0529 (quality of raw cow's milk) frequencies of psychrotrophic microorganisms (CPP), thermoresistant (TRM) and spore-forming anaerobic bacteria (SPAN) are classified as a complementary trait of hygienic quality. Majority of microbiologic contamination of raw cow's milk is eliminated successfully by pasteurization or UHT. Problems appearing during the successive technological process and during production of specific dairy products are caused by thermostable enzymes of psychrotrophic microflora (Vyletělová *et al.*, 2000) or by enzymes of spore-forming thermoresistant microorganisms. Spore-forming microorganisms (*Bacillus, Clostridium*) in raw milk are hardly detectable without heat activation – they survive in passive form (as spores). Heat treatment of milk contributes to their multiplication by distorting spore walls resulting in bacteria germination. Their active form can produce lytic enzymes or enterotoxins reducing healthy and technological safety of foodstuffs or milk as other raw material.

Screening of *Bacillus* bacteria in raw cow's milk (samples collected in Northern and Central Moravia, samples from various sampling sites of Moravia and Bohemia analyzed in Central Laboratory Pardubice), bacteria identification and analysis of relationships between *Bacillus* bacteria incidence and other hygienic milk parameters were the principal objectives of the presented study.

MATERIAL AND METHODS

Milk sampling, transport, and storage of samples

Pooled milk (Ist set) was taken into sterile samplers containing a preservative agent (30 ml milk, 3 ml Heeschen's agent) – Heeschen *et al.* (1969). Milk samples were transported in thermoboxes with cooling cushions. Samples were analyzed immediately after their receipt or stored in the refrigerator (6.5°C) and analyzed within 2 hours at maximum. The identical system was used for collection and transport of samples into the central laboratory. Samplers with milk intended for analysis of total microorganism count (TCM) on Bactoscan were stored in the freezer (–18°C) after TCM analysis. Frozen samples were transported in thermoboxes to the research laboratory, stored in the freezer (–18°C) and analyzed sequentially.

Sample cultivations

a) Ist sample set was tested by routine methods: total count of mesophilic microorganisms – routine culture medium GTK (Milcom, Tábor) modified according to IDF standards (Havlová *et al.*, 1993) was used. TCM and TRM were cultivated 3 days at 30°C, spore-forming anaerobic bacteria (SPAN) were cultivated 3–5 days at 30°C according to the protocol specified by Havlová *et al.* (1993). Petri dishes with 20–200 CFU/ml and test tubes with positive gas production were classified.

b) IInd sample set from the central laboratory:
- TCM values were measured by Bactoscan;
- CPP analysis: agar broth with GTK or GTKL (Milcom, Tábor), inoculation made by Minipetrifoss apparatus, 10 day-cultivation at 6.5°C;
- analysis of thermoresistant microorganisms: samples were inactivated at 72–75°C (20 min), inoculated (Minipetrifoss), inoculum was embedded (agar broth GTK or GTKL) and cultivated at 30°C (72 h);
- analysis of spore-forming anaerobic bacteria: milk samples were inoculated (0.1 ml) into the broth in the test tube, embedded (1 cm sterile paraffin mixture: 1/2 vaseline : 1/2 paraffin), test tubes were plunged into the water bath (85°C, 10 min). Samples were cooled to 37°C and incubated for 5–7 days (37°C). Broth was prepared from skim-milk poured (by 10 ml) into test tubes and sterilized recurrently (after 24 h) at 120°C (30 min). Broth was heated to 100°C and cooled to 37°C fore inoculation. Test tubes with positive gas production were classified.

c) *Bacillus* strains were isolated on the modified MYP Agar complemented with egg yolk emulsion and Polymyxin B sulphate (HiMedia), and on Milk Agar (Merck) at cultivation temperature 30°C for 3 days for mesophilic strains (Havlová *et al.*, 1993), at 4.0–6.5°C for 10–16 days for psychrotrophic strains (Lechner *et al.*, 1998),

at 55°C for 48 h for thermotolerant and at 60°C for 48 h for thermophilic strains (Phillips and Griffiths, 1986). The Brain Heart Infusion Agar (Oxoid) with vitamin B_{12} and cultivation temperature 37°C for 5 days were used for isolation of *B. sporothermodurans* from milk samples which had been heated to 100°C for 30 min to enrich for HHRS strains (Petterson *et al.*, 1996; Klijn *et al.*, 1997). A total 158 strains of *Bacillus* species was isolated.

Morphological characteristics

Colonial and spore morphology was examined on blood agar and nutrient agar with 10 mg $MnSO_4.H_2O$/l1 for 1–7 days, respectively.

Biochemical and physiological characteristics

Phenotypic tests (production of acetoin, reduction of nitrate; gelatine, Tween 80, esculin, tyrosine and starch-hydrolysis; haemolysis catalase, urease; growth in 7% NaCl, growth at 4, 7, 40, 50, and 55°C, acidification of glucose, mannitol xylose and lactose) were carried out by routine conventional methods (Gordon *et al.*, 1973), arginine dihydrolase by the method of Brooks and Sodeman (1974), ONPG by the method of Lowe (1962), phosphatase production specified by Páčová and Kocur (1978). The following commercial tests were used: Simmons citrate (OXOID CM155), *B. cereus* agar (HiMedia M833) for egg-yolk reaction, medium for anaerobic growth (BBL No. 10926).

Production of proteolytic and lipolytic enzymes

Ability to produce proteolytic and lipolytic enzymes was tested in all strains on Milk Agar (Merck) and on Tributyrin Agar (Merck), respectively. Strains characterized by a distinct zone around colonies were classified as positive ones (Havlová *et al.*, 1993), and compared with the results of gelatine and Tween 80 hydrolysis on medium according to Páčová and Kocur (1984)

Identification of strains

Identification of isolated strains was performed on the basis of 26 classical phenotypic tests. Differential tables and keys according to Gordon *et al.* (1973), Priest *et al.* (1988), Alexander and Priest (1989), Petterson *et al.* (1996), Lechner *et al.* (1998) were used for identification.

Statistical evaluation

With regard to the typical distribution pattern of data sets the results of all microorganism sets were transformed into logarithms – mean values were presented as logarithmic or geometric means. Linear regression was used for statistical evaluation of relationships between total count of *Bacillus* microorganisms (TCBa) and TCM and TRM. Unpaired *t*-test was used for evaluating TCBa × SPAN differences.

Patterns of TCM, TRM, and SPAN

Results of analyses carried out in the Central Laboratory at Pardubice in 1997–1999 were processed and evaluated in Dairy Research Institute Prague – they represent arithmetic means in specific years or months.

RESULTS AND DISCUSSION

Bacteria that produce heat-resistant endospores are classified in 9 genera of group endospore-forming grampositive rods and cocci (Holt *et al.*, 1994). The genus *Bacillus* is the largest and best-known member of this group, aero-

Table 1. Species identification of genus *Bacillus* and their percentage

Strains	Ist set		IInd set		Ist + Ind sets	
	(number)	(%)	(number)	(%)	(number)	(%)
Bacillus licheniformis	16	69.6	86	63.7	102	64.6
Bacillus pumilus	2	8.7	3	2.3	5	3.2
Bacillus subtilis	1	4.3	1	0.7	2	1.3
Bacillus cereus	3	13.1	40	29.6	43	27.2
Bacillus weihenstephanensis			2	1.6	2	1.3
Bacillus badius			1	0.7	1	0.6
Bacillus sphaericus			1	0.7	1	0.6
Bacillus fusiformis	1	4.3			1	0.6
Paenibacillus glucanolyticus			1	0.7	1	0.6

bic or facultatively anaerobic, usually catalase positive, while the other genera, including *Clostridium* spp., are mostly anaerobic and catalase negative. Mesophilic and psychrotrophic *Bacillus* spp. are important contaminants in raw and pasteurized milk. Crielly *et al.* (1994) present *B. licheniformis* and *B. cereus* as most commonly isolated species of *Bacillus* found in milk at all stages of processing. The frequency of identified species of *Bacillus* is listed in Table 1.

Frequency of *Bacillus* bacteria in the Ist set

The identified set of 23 strains (Table 1) formed a homogeneous group of mesophilic species; the highest frequency was mentioned in "*B. subtilis*" group including isolated strains of *B. licheniformis*, *B. subtilis*, and *B. pumilus*. Isolation of mesophilic species found in raw milk corresponds to bibliographic references (Sutherland and Murdock, 1994; Crielly *et al.*, 1994; Páčová *et al.*, 1996). As for the species more resembling psychrotrophic strains initiating alimentary intoxications, *B. cereus* was identified (13%). Non-significant correlations $r = 0.04$ and $r = 0.15$ (Table 2) were found between mesophilic TCBa ×TCM and *B. licheniformis* × TCM, resp. Significant correlations ($P \leq 0.001$) $r = 0.77$ and $r = 0.86$ were recorded in TCBa × TRM and *B. licheniformis* × TRM, resp. (Table 2). The other relationships were not analyzed with regard to low numbers of registered bacilli frequencies.

Frequency of *Bacillus* bacteria in the IInd set

In this set a higher (more than five-fold) number of strains was identified than in the Ist set (Table 1); frequency pattern of specific bacilli was, however, similar. The frequency of mesophilic species of "*B. subtilis*" group amounted to 70.4%; almost similar frequency (as related to the above-mentioned group) was found in *B. licheniformis* (63.7%). Frequency of *B. cereus* increased from 13 to 29.6%. Two psychrotolerant strains (growth at 4°C, no growth at 40–43°C) identified as *Bacillus weihenstephanensis* according to Lechner *et al.* (1998) were isolated in *B. cereus* set. Four intermediate strains of *B. cereus* – *B. weihenstephanensis* (Lechner *et al.*, 1998) were isolated. These strains grew at 7–10°C, but they did not grow at 4°C and 40–43°C. Application of specific molecular-biology methods is necessary for final classification of 2 strains as *B. weihenstephanensis* and for identification of intermediary *B. cereus* strains.

Other representatives of Ist–IIIrd morphologic groups were identified sporadically: *B. badius* (Ist morph. g.), *Paenibacillus glucanolyticus* (excluded from *B. circulans* strains, IInd morph. g.), *B. sphaericus* and *B. fusiformis* (IIIrd morph. g.). The isolation of *B. stearothermophilus* was not successful. The isolation of *B. sporothermodurans* species (group of strains characterized by high resistance of spores to high temperatures – HHRS strains) was not successful either, probably due to the insufficient heating temperature of sample.

Table 2. Results of statistical analysis of Ist and IInd sets

	TCM	TRM	SPAN	
I. set mesophilic				
TCBa	$y = 0.097x + 0.223$	$y = 0.776x + 0.135$		
	$r = 0.04$	$r = 0.77$***	xxx	xxx
	$n = 54$	$n = 54$		
B. licheniformis	$y = 0.121x + 1,083$	$y = 0.84x + 0.206$		
	$r = 0.15$	$r = 0.86$***	xxx	xxx
	$n = 16$	$n = 16$		
II. set mesophilic				
TCBa	$y = -0.005x + 0.74$	$y = 0.1x + 0.491$	$g = 5.599$	negative
	$r = -0.003$	$r = 0.15$**	$g = 7.467$	positive
	$n = 342$	$n = 342$	$t\text{-test}_{153} = 2.28 > t_{tab}1.96$	$P < 0.05$
B. cereus	$y = 0.118x + 0.665$	$y = -0.034x + 1.258$	$g = 9.441$	negative
	$r = 0.11$	$r = -0.12$	$g = 13.646$	positive
	$n = 40$	$n = 40$	$t\text{-test}_{23} = 0.5 < t_{tab}3.77$	$P > 0.05$
B. licheniformis	$y = -0.051x + 1.611$	$y = -0.047x + 1.534$	$g = 2.818$	negative
	$r = -0.06$	$r = -0.06$	$g = 3.897$	positive
	$n = 82$	$n = 86$	$t\text{-test}_{22} = 0.38 < t_{tab}3.79$	$P > 0.05$

n = samples number; g = geometric mean in ths. CFU/ml; r = correlation coefficient; ** = $P \leq 0.01$; *** = $P \leq 0.001$; xxx = wasn't determined

Data describing the mesophilic group were chosen for statistical processing. Psychrotrophic and thermotolerant *Bacillus* bacteria were not evaluated (low frequencies). Similarly like in the previous set, very low correlations $r = -0.003$, $r = 0.11$, $r = -0.06$ were found in TCBa × TCM, *B. cereus* × TCM, and *B. licheniformis* × TCM, respectively (Table 2). Significant ($P \leq 0.01$) correlation $r = 0.15$ was determined in TCBa × TRM (Table 2), non-significant correlations $r = -0.12$ and $r = -0.06$ were found in *B. cereus* × TRM and *B. licheniformis* × TRM, resp. (Table 2). Significance of differences between two geometric means of TCBa sets (or *B. cereus* and *B. licheniformis*) in the case of positive SPAN and negative SPAN was tested, significant difference ($P < 0.05$) was found in TCBa ($t\text{-test}_{128} = 2.28 > t_{tab}$ 1.96). Non-significant differences ($P > 0.05$) were determined in *B. cereus* ($t\text{-test}_{23} = 0.5 < t_{tab}$ 3.77) and *B. licheniformis* ($t\text{-test}_{22} = 0.38 < t_{tab}$ 3.79) (Table 2).

Production of lytic enzymes

Production of proteolytic enzymes was evident in 100% and lipolytic ones in 61–82% strains of *B. licheniformis*. All *B. cereus* strains (100%) produce the proteolytic enzymes whereas lipolytic activity was observed in 12 and 30 strains (28 and 70%). The other strains (excluding *B. badius, B. sphaericus* and *P. glucanolyticus*) were able to produce both lytic enzymes. The results mostly conformed with the results of gelatine and Tween 80 hydrolysis on medium according to Páčová and Kocur (1984) – Table 3.

Patterns of TCM, TRM, and SPAN in 1997–1999

Processed data (CL Pardubice) document that more than 85% of analyzed milk samples comply with EU criteria for TCM, i.e. less than 100 ths. CFU/ml. Almost 70% of milk samples were classified (according to Czech criteria) as prime grade milk (TCM ≤ 50 ths. CFU/ml, ČSN 57 0529). Average TCM value varied from 65 to 79 ths. CFU/ml, the lowest quality was recorded in summer (Figure 1, Table 4). Approximally 86.0–92.5% of pooled milk samples complied with TRM frequency standard (ČSN 57 0529). Seasonal effects on TRM frequency were not demonstrated (Figure 2, Table 4). Figure 3 presents only positive SPAN frequencies amounting to 25% maximally in all monthly controls.

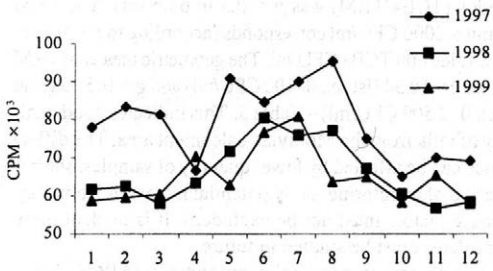

Figure 1. Occurrence of TCM in the individual years (monthly values are TCM averages in the individual months)

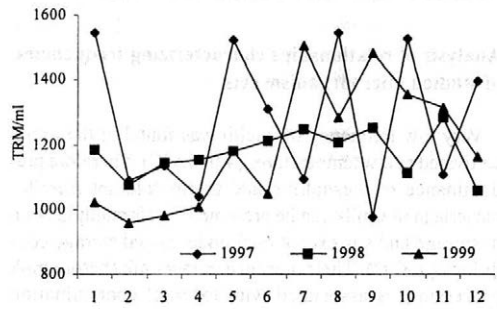

Figure 2. Occurrence of TRM in the individual years (monthly values are TRM averages in the individual months)

Table 3. Production of proteolytic and lipolytic enzymes in Bacillus spp. srains

Strain	Total	Gelatine hydrolysis		Skim milk agar		Tween 80 hydrolysis		Tributyrin agar	
	count	count	%	count	%	count	%	count	%
B. licheniformis	102	102	100	102	100	62	61	84	82
B. pumilus	5	5	100	5	100	1	20	5	100
B. subtilis	2	2	100	2	100	2	100	2	100
B. cereus	43	43	100	43	100	30	70	12	28
B.weihenstephanensis	2	2	100	2	100	2	100	2	100
B. badius	1	1	100	1	100	0	0	0	0
B. sphaericus	1	0	0	0	0	1	100	1	100
B. fusiformis	1	1	100	1	100	1	100	1	100
P. glucanolyticus	1	1	100	1	100	0	0	0	0

Table 4. Summary of TRM and TCM evolution in years 1997, 1998 and 1999

Year	1997	1998	1999
TCM average (in ths. CFU/ml)	79	66	65
Comply with EU standards of TCM (%)	85	90	88
Comply with ČSN standard (prime-quality of TCM; (%))	70	70	70
TRM average (in ths. CFU/ml)	1.3	1.2	1.2
Comply with ČSN standard of TRM (%)	86	93	95

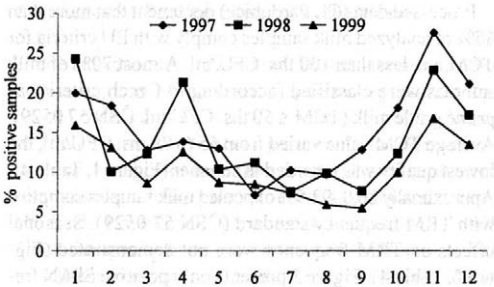

Figure 3. Occurrence of SPAN in the individual years (monthly values are the averages of positive cases of SPAN occurrence)

Table 5. Characteristics of TRM occurrence in the Ist and IInd set

Statistical parameter	Ist set	IInd set
Variation range (CFU/ml)	0–100	0–8 500
Geometric mean g (CFU/ml)	10.34	165.33
Correlation coefficient r (log TRM × log TCBa)	0.77***	0.15**
Ratio index p_i (TCBa/TRM)	0.3	0.3

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

Analysis of relationships characterizing frequencies of studied microorganism sets

Very low frequency of bacilli was found in the group cultivated at low temperatures (4.0–6.5°C). Therefore predominance of mesophilic and thermotolerant *Bacillus* bacteria in raw milk can be presumed. Their multiplication in storage tanks is exceptional under actual storage conditions (4–6°C). Their presence in raw milk (before milk processing) is associated with external contamination (udder surface, technical equipment). Milk storage and transportation do not increase the bacilli frequencies considerably. Technological equipment of dairy plants is another source of potential contamination. The mentioned fact confirms exceptionally close positive correlations (high correlations: 0.69–0.80) between the frequencies of anaerobic spore-forming microorganisms in feedstuffs, faeces, litter, and on udder surface and in milk. Passage of spores through the digestive tract and resulting milk contamination were mentioned as the principal causes (Petersen, 1985 – cit. Kratochvíl, 1991).

The relation between TCBa frequency and TRM was significant (Table 2), as for *B. licheniformis*, the same fact was stated in some cases only. On the contrary, non-significant relations to TCM correspond to the following fact: bacilli and their specific strains represent a relatively low proportion of TCM, but a high proportion of TRM. Therefore they are undesirable for technological milk processing. Dominant position of *B. licheniformis* in bacillary contamination of raw milk (Table 1) confirms the mentioned facts. Thus the analysis of TRM frequency is advisable in the case of composite dairy technologies. The ratio index (TCBa/TRM) was $p_i = 0.3$ in both sets. The TRM limit ≤ 2000 CFU/ml corresponds (according to $p_i = 0.3$) to the value 600 TCBa CFU/ml. The geometric means of TRM were $g = 10.34$ (Ist set; 0–100 CFU/ml) and $g = 165.33$ (IInd set; 0–8 500 CFU/ml) – Table 5. This indicates good quality of milk from the Moravian catchment area. The difference can be affected by lower quantity of samples, shorter period of monitoring, or by potential factors of laboratory (these factors must not be excluded). It is evident these problems must be studied in future.

It is obvious that technological testing of SPAN cannot be adequate for some composite dairy technologies. Geometric means documenting the non-negligible frequency of bacilli (Table 2) even in the case of negative SPAN test support the mentioned hypothesis. Therefore more precise laboratory tests should be applied for studying qualitative problems of cheese production.

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Received: 01–03–08
Accepted after corrections: 01–05–23

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Effect of breed on chemical composition of goat milk

Vliv plemene na chemické složení kozího mléka

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ABSTRACT: Research was focused on the effect of breed (Alpine and Saanen) on changes in the chemical composition of goat milk in the course of five successive lactations. The total number of performed analyses varied depending on the number of monthly checks of milk yield, the number of goats involved and the number of the content-determining analyses: total solids, solids-non-fat, fat, protein, lactose, ash, calcium, phosphorus and acidity level. Daily feed ration consisted of fodder (meadow hay and clover-grass mixture) with the addition of corn silage. The second part of daily ration was a feed mixture for goats containing 14% protein. Average chemical composition of milk (%) of Alpine and Saanen breeds of goat, respectively, was as follows at the start of the lactation period (day 50): total solids (12.29 and 11.76); solids-non-fat (8.57 and 8.20); fat (3.68 and 3.57); protein (2.96 and 2.81); lactose (4.61 and 4.52); ash (0.802 and 0.787); calcium (0.129 and 0.125); phosphorus (0.095 and 0.088) and acidity (7.01°SH and 6.25°SH). At the end of lactation period (day 200), the average content of milk was: total solids (12.01 and 11.91); solids-non-fat (8.26 and 8.33); fat (3.71 and 3.56); protein (3.04 and 3.11); lactose (4.23 and 4.23); ash (0.834 and 0.828); calcium (0.118 and 0.111); phosphorus (0.092 and 0.091) and acidity (7.42°SH and 7.62°SH). It can be said that breed had a significant effect on the content of total solids, solids-non-fat, protein, lactose, ash, calcium and phosphorus, all of which were found to be higher in milk of Alpine breed throughout the five lactations.

Keywords: chemical composition; goat milk; Alpine; Saanen; acidity, stage of lactation

ABSTRAKT: Výzkum jsme zaměřili na vliv plemene (alpského a sánského) na změny chemického složení kozího mléka v průběhu pěti po sobě následujících laktací. Celkový počet prováděných rozborů se lišil podle počtu měsíčních kontrol dojivosti, počtu zahrnutých koz a počtu rozborů stanovujících obsah: celkové sušiny, tukuprosté sušiny, tuku, dusíkatých látek, laktózy, popelovin, vápníku, fosforu a výši kyselosti. Denní krmná dávka se skládala z objemné píce (lučního sena a jetelotravní směsky) a přídavku kukuřičné siláže. Druhou část denní dávky tvořila krmná směs pro kozy s obsahem 14 % N látek. Průměrné chemické složení mléka (%) koz alpského a sánského plemene na začátku laktace (50. den): celková sušina (12,29 a 11,76); tukuprostá sušina (8,57 a 8,20); tuk (3,68 a 3,57); N látky (2,96 a 2,81); laktóza (4,61 a 4,52); popeloviny (0,802 a 0,787); vápník (0,129 a 0,125); fosfor (0,095 a 0,088) a kyselost (7,01°SH a 6,25°SH). Hodnoty průměrného obsahu v mléce na konci laktace (200. den) činily: celková sušina (12,01 a 11,91); tukuprostá sušina (8,26 a 8,33); tuk (3,71 a 3,56); N látky (3,04 a 3,11); laktóza (4,23 a 4,23); popeloviny (0,834 a 0,828); vápník (0,1118a 0,111); fosfor (0,092 a 0,091) a kyselost (7,42 °SH a 7,62 °SH). Lze konstatovat, že plemeno mělo významný vliv na obsah celkové sušiny, tukuprosté sušiny, N látek, popelovin, vápníku a fosforu. Všechny tyto hodnoty byly v průběhu pěti laktací vyšší v mléce alpského plemene.

Klíčová slova: chemické složení; kozí mléko; alpské plemeno; sánské plemeno; kyselost; laktační fáze

INTRODUCTION

Results achieved in goat breeding throughout the world have influenced the revival of this activity in Croatia, where it was not only neglected but also banned by government regulations for years. The newly introduced law on improvement of livestock breeding defines the conditions for goat breeding. The ever increasing demand for goat milk, and the considerable production capacities that goats possess, have contributed both to the development of goat farming and the fact that goat milk production and processing became an important activity in many parts of

Supported by the Ministry of Science and Technology of the Republic of Croatia.

Croatia. Goat milk is mostly processed into soft and semi-hard cheeses. This demanded that traditional, extensive farming be replaced by modern farming methods for dairy goat breeds. Accordingly, Alpine and Saanen dairy breeds have been imported from France and Germany. Currently there are 90 000 goats in Croatia, 20% of that number being covered by selective monitoring, while the percentage is increasing year by year. The new approach to goat farming also demands comprehensive research on the chemical composition, physical properties and quality of goat milk. Although Alpine and Saanen breeds are both quality, high-yielding dairy breeds, certain differences do exists between them. Due to the effect of numerous factors the chemical composition of milk varies considerably during lactation periods. The report on the composition of goat milk during lactations was prepared by Mba *et al.* (1975), Boroš (1986), Csapo *et al.* (1986), Voutsinas *et al.* (1990) and Khaled *et al.* (1999). The aim of this paper is to establish the effect of breed and stage of lactation (beginning, middle and end) on changes in the chemical composition of goat milk.

MATERIAL AND METHODS

The research project focusing on the effect of breed on the chemical composition and individual physical properties of milk covered a part of the population of Alpine and Saanen milk goats during the five lactations. All animals were kept on a farm. The number of Alpine and Saanen goats was as follows: 5 in I, 6 in II, 6 in III, 8 in IV and 8 in V lactation respectively, depending on their health condition. Average age of goats at the beginning of the first lactation was 15 months for Alpine and 13.5 months for Saanen goats. Due to the fact that the age of animals at the start of individual lactations varied, correction was carried out by non-linear function. Goats were housed in separate boxes, each 20 × 5 m in size. Each animal had 2 m^2 of floor area and about 2.5 m^2 of outlet.

All animals were kept under constant similar conditions and were provided with the same care and feed. Daily feed ration comprised roughage (meadow hay and a clover-grass mixture) with the addition of maize grain silage as well as a portion of fodder mixture with 14% protein content. Since the goats produced kids in winter months (February and March), their daily ration at the start of lactation period consisted of hay (*ad libitum*) and 700 g of fodder concentrate per animal. At the start of the vegetation cycle (April and May) the animals received a daily ration of 5–6 kg of cowpea or common vetch. During July, August and September they were also given a clover-grass mixture. A total of 8–10 controls of milking were carried out, depending on the duration of lactation period. The animals were milked manually on the day of the controls, and a proportional sample of milk was taken from each animal from both morning (6 a.m.) and evening (6 p.m.) milking. Milk samples were not conserved but they were kept in a refrigerator at a temperature of +4°C up to the time of analyses. The number of milk samples per month in the course of individual lactations depended on the number of monthly controls of milking capacity and on the number of goats. During the milking period throughout the five lactations, 584 samples were collected. The analyses of milk included total solids, solids-non-fat, fat, protein, lactose, ash, calcium, phosphorus and acidity.

Work methods

The following methods were used for determining the chemical composition of milk: total solids (%) – gravimetric method, drying at 102°C, FIL-IDF, 21B:1987; solids-non-fat (%) – calculation of the difference in total solids and fat content; fat (%) – butyrometric (volumetric) method (Gerber) with calibrated butyrometers, FIL-IDF, 105:1981; crude protein (%) – dye-binding (Amido Black), FIL-IDF, 98A:1985. Pro-Milk apparatus was calibrated against the known sample standards. Protein percentage is obtained from a calibration curve based on the crude protein content established by the Kjeldahl method in 10 samples of milk. Lactose-monohydrate (%) – titrimetric method with chloramine-T, FIL-IDF, 28A:1974; ash (%) gravimetric method – furnace < 550°C, AOAC, 945.46: 1995; calcium (%) – titrimetric, after oxalate precipitation, FIL-IDF, 36A:1992; phosphorus (%) – spectrometric, molybdenum blue, FIL-IDF, 42B:1990. Method used for determination of acidity level (°SH) – titrimetric, Soxhlet-Henkel method.

Statistical data processing

Data processing of results obtained for individual components of milk was based on 2nd degree polynomial:

$$Y_n = \mathrm{S}\ a_n x^n$$

where: a = parameter
x = time

The estimated values of the 50th, 100th, 150th and 200th days of lactation were used to determine the effect of breed and number of lactation on the chemical composition of goat milk, in accordance with the linear model (Harvey, 1975):

$$Y_{ijk} = \mu + A_i + B_j + e_{ijk}$$

where: Y_{ijk} = value of the observed characteristic
μ = mean value corrected to factors A_i and B_j
A_i = fixed effect of the number of lactation (i = 1 ...5)
B_j = fixed effect of breed (j = 1 and 2)
e_{ijk} = unexplained effect

Significance test was conducted by F-test. Statistical values for each lactation were calculated on the basis of

data obtained through the Harvey model. Thus we obtained the least squares means (LSM), standard deviation (SD), standard error (SE) and the coefficient of variation (CV). The analysis of variance enabled us to use *F*-test for checking the effect that individual factors have on specific characteristics. Correlation coefficients between all milk components were also calculated in the course of five lactations.

RESULTS AND DISCUSSION

Total solids

The effect of breed on the average content of total solids for lactation periods is presented in Table 1.

Alpine goat milk had a higher content of total solids than that of the Saanen breed at the beginning (50th day), in the middle (100th and 150th days) and at the end (200th day) of all lactations, although a significantly ($P < 0.01$) higher content was recorded at the start of the lactation period only (50th day). An almost identical total solids content was found in milk of both the Alpine breed, reported by Miletic and Antunac (1987) and Voutsinas *et al.* (1990), and the Saanen breed, reported by Anifantakis and Kandarakis (1980) and Merin *et al.* (1988). No significant differences were found between the Alpine, Saanen and local breeds by Csapo *et al.* (1986) and Kompan *et al.* (1998).

Solids-non-fat

The effect of breed on the average content of solids-non-fat in goat milk for lactation periods is presented in Table 1.

A significant difference ($P < 0.01$) between the breeds was established only at the start of lactation period (50th day), when Alpine goat milk contained 8.57% of solids-non-fat and that of the Saanen breed 8.20%. At the end of lactations (150th and 200th day), Saanen goat milk contained an insignificantly ($P > 0.05$) higher level of solids-non-fat. A lower content of solids-non-fat in Alpine milk was reported by Voutsinas *et al.* (1990) while Anifantakis and Kandarakis (1980) found a similar content in the Saanen breed.

Fat

The effect of breed on the average fat content in goat milk for lactation periods is presented in Table 1.

No significant differences in fat content in the milk of Alpine and Saanen goats were found at the start (50th day), in the middle (100th and 150th day) or at the end (200th day) of lactation periods. At the start of lactation (50th day) milk of both breeds had approximately the same content of fat (Alpine 3.68% and Saanen 3.57%) as it did at the end (200th day) of the lactation period while the Alpine breed displayed an insignificantly higher fat con-

Table 1. Effect of breed on the average content of total solids, solids-non-fat and fat (%) in goat milk during the lactation periods

Day of lactation	50th			100th			150th			200th		
	n	LSM	SE	*n*	LSM	SE	*n*	LSM	SE	*n*	LSM	SE
Total solids												
Alpine	33	12.29[a]	0.11	33	11.44	0.10	33	11.39	0.10	33	12.01	0.12
Saanen	33	11.76[b]	0.11	33	11.24	0.10	33	11.24	0.10	33	11.91	0.12
$\bar{x}$		12.02	0.08		11.34	0.07		11.31	0.07		11.96	0.08
CV		5.28			4.98			4.90			5.51	
Solids-non-fat												
Alpine	33	8.57[a]	0.07	33	8.15	0.06	33	8.03	0.06	33	8.26	0.07
Saanen	33	8.20[b]	0.07	33	8.01	0.06	33	8.05	0.06	33	8.33	0.07
$\bar{x}$		8.38	0.05		8.08	0.04		8.04	0.04		8.30	0.05
CV		4.63			3.90			4.20			4.82	
Fat												
Alpine	33	3.68	0.08	33	3.32	0.07	33	3.33	0.07	33	3.71	0.08
Saanen	33	3.57	0.08	33	3.24	0.07	33	3.17	0.07	33	3.56	0.08
$\bar{x}$		3.63	0.05		3.28	0.05		3.25	0.05		3.63	0.05
CV		11.75			12.69			12.10			11.99	

[a, b] means in a column with different superscript differ ($P < 0.01$)

tent. Both breeds manifested the lowest fat content around the 150th day of lactation, which resulted from the method of feeding, season and lactation stage (Alichanidis and Polychroniadou, 1995). Average fat content in both Alpine and Saanen breeds was almost identical at the start (3.63%) and at the end (3.63%) as well as in the middle of lactation (3.28% and 3.25%, respectively). Throughout all five lactations Alpine goat milk contained more fat ($P > 0.05$) in comparison with the Saanen breed (see Table 1). Findings of a higher content of fat in relation to the Saanen breed were also reported by Boichard *et al.* (1989), Bonsembiante *et al.* (1990), Anonym (1988), Ricordeau (1993), Sung *et al.* (1999). Kompan *et al.* (1998) reported findings of identical fat content in milk of both breeds. On the contrary, Pasquini *et al.* (1996) reported the higher fat content in milk of Saanen goat than in Alpine goat milk (3.35% and 2.74%).

Protein

The effect of breed on the average protein content in goat milk for lactation periods is presented in Table 2.

Significant differences in protein content in milk between the two breeds were established at the start of lactation period (50th day). Alpine goat milk contained more protein (2.96% and 2.78%) at the start (50th and 100th day) while Saanen breed (2.81% and 3.11%) had more protein at the end of lactation periods (150th and 200th day) (Table 2). A higher protein content in milk of Alpine goats in relation to the Saanen breed is also reported by Rakes *et al.* (1981), Anonym (1988), Boichard *et al.* (1989), Bonsembiante *et al.* (1990), Ricordeau (1993), Kompan *et al.* (1998).

Lactose

The effect of breed on the average lactose content in goat milk for lactation periods is presented in Table 2.

A significant difference ($P < 0.05$) between the breeds with regard to lactose content was found only at the start of lactations (50th day), when Alpine goat milk contained more lactose (4.61%) than that of the Saanen breed (4.52%). In the middle of the lactation periods (100th and 150th day) as well as at the end (200th day), milk of both breeds showed approximately the same lactose content ($P > 0.05$) (Table 2). Voutsinas *et al.* (1990) quote 4.3% as the average lactose content in Alpine goat milk in the lactation.

Ash

The effect of breed on the average ash content in goat milk for lactation periods is presented in Table 2.

No significant differences ($P > 0.05$) were established between the two breeds although Alpine goat milk showed a higher ash content (Table 2). Throughout all five lactation periods the ash content in milk of both breeds was very close to even, which is documented by the values of

Table 2. Effect of breed on the average protein, lactose and ash content (%) in goat milk during the lactation periods

Day of lactation	50th			100th			150th			200th		
	n	LSM	SE	*n*	LSM	SE	*n*	LSM	SE	*n*	LSM	SE
Protein												
Alpine	33	2.96[a]	0.04	33	2.78	0.03	33	2.77	0.04	33	3.04	0.05
Saanen	33	2.81[b]	0.04	33	2.72	0.03	33	2.81	0.04	33	3.11	0.05
$\bar{x}$		2.89	0.03		2.75	0.02		2.79	0.03		3.08	0.044
CV		7.51			7.13			7.92			9.21	
Lactose												
Alpine	33	4.61[a]	0.03	33	4.39	0.03	33	4.29	0.03	33	4.23	0.03
Saanen	33	4.52[b]	0.03	33	4.40	0.03	33	4.32	0.03	33	4.23	0.03
$\bar{x}$		4.56	0.02		4.40	0.02		4.31	0.02		4.23	0.02
CV		3.78			4.11			3.37			3.40	
Ash												
Alpine	33	0.802	0.006	33	0.789a	0.005	33	0.794	0.006	33	0.834	0.007
Saanen	33	0.787	0.006	33	0.774b	0.005	33	0.787	0.006	33	0.828	0.007
$\bar{x}$		0.794	0.005		0.782	0.004		0.790	0.004		0.831	0.005
CV		4.63			3.99			3.99			5.07	

[a, b] means in a column with different superscript differ ($P < 0.05$)

Table 3. Effect of breed on the average calcium and phosphorus content (%) and on acidity (°SH) in goat milk during the lactation periods

Day of lactation	50th			100th			150th			200th		
	n	LSM	SE	*n*	LSM	SE	*n*	LSM	SE	*n*	LSM	SE
Calcium												
Alpine	33	0.129	0.002	33	0.116	0.001	33	0.112	0.001	33	0.118a	0.002
Saanen	33	0.125	0.002	33	0.114	0.001	33	0.108	0.001	33	0.111b	0.002
$\bar{x}$		0.127	0.001		0.115	0.001		0.110	0.001		0.115	0.001
CV		7.48			6.21			6.29			7.85	
Phosphorus												
Alpine	33	0.095[a]	0.001	33	0.086[c]	0.001	33	0.085[e]	0.001	33	0.092	0.001
Saanen	33	0.088[b]	0.001	33	0.079[d]	0.001	33	0.081[f]	0.001	33	0.091	0.001
$\bar{x}$		0.092	0.001		0.082	0.001		0.083	0.001		0.092	0.001
CV		7.61			8.55			8.38			8.63	
Acidity												
Alpine	33	7.01[a]	0.108	33	6.49[c]	0.112	33	6.76	0.124	33	7.42	0.141
Saanen	33	6.25[b]	0.108	33	6.08d	0.112	33	6.71	0.124	33	7.62	0.141
$\bar{x}$		6.63	0.077		6.28	0.080		6.73	0.089		7.52	0.1
CV		9.35			10.24			10.67			10.86	

[a, b, c, d] means in a column with different superscript differ ($P < 0.01$)

[e, f] means in a column with different superscript differ ($P < 0.05$)

coefficients of variation (from 3.99% in the middle to 5.07% at the end of lactation periods). Voutsinas *et al.* (1990) found the ash content in milk of the Alpine goat to be 0.76% while Merin *et al.* (1988) when investigating the Saanen breed, established the ash content in their milk as being 0.8%.

Calcium

The effect of breed on the average calcium content in goat milk for lactation periods is presented in Table 3.

A significant difference ($P < 0.05$) between the breeds was established only at the end of lactation periods while the average calcium content throughout the five lactations proved higher in milk of the Alpine breed (from 0.112% on the 150th day to 0.129% on the 50th day).

Phosphorus

The effect of breed on the average phosphorus content in goat milk for lactation periods is presented in Table 3.

Phosphorus content in milk of both breeds was found to be fairly similar throughout the lactation period, but it should be noted that milk from the Alpine breed contained a significantly higher level of phosphorus at the start ($P < 0.01$) and in the middle of the lactation periods in comparison with the Saanen breed. Our results were very similar to those reported by Boroš and Herian (1986).

Acidity

The effect of breed on the average acidity of goat milk for lactation periods is presented in Table 3.

Alpine goat milk showed a significantly ($P < 0.01$) higher acidity at the start and in the middle of lactations (7.0°SH and 6.5°SH) while milk from the Saanen breed had a higher acidity ($P > 0.05$) at the end of the lactation periods (7.6°SH) (Table 3). At the start and in the middle of lactations, milk acidity of Alpine goats (7.0°SH and 6.5°SH) was significantly ($P < 0.01$) higher in comparison with milk of the Saanen breed (6.3°SH and 6.1°SH). At the end of lactations (200th day) no significant difference in milk acidity was established between the breeds although the acidity of milk of Saanen goats was found to be higher.

Coefficients of correlation

Correlation coefficients between individual milk components, as found at the beginning (50th day), in the middle (100th and 150th day) and at the end (200th day) of lactation periods, are presented in Table 4.

In the course of all five lactation periods highly significant ($P < 0.001$) correlations were found between total solids and fats, and proteins and lactose. Highly significant ($P < 0.001$) correlations were also established between the solids-non-fat and protein and lactose, which was coherent with the results reported by Zeng and Escobar (1996). Ash content in milk was in correlation with calcium

Table 10. Coefficients of correlations

	Day of lactation	Solids-non-fat	Fat	Protein	Lactose
Total solids	50	0.78**	0.75**	0.66**	0.41**
	100	0.76**	0.81**	0.67**	0.42**
	150	0.77**	0.72**	0.63**	0.56**
	200	0.74**	0.8**	0.61**	0.46**
Solids-non-fat	50		0.21	0.81**	0.49**
	100		0.25	0.80**	0.61**
	150		0.16	0.75**	0.65**
	200		0.21	0.73**	0.47**
Fat	50			0.23	0.19
	100			0.3*	0.11
	150			0.23	0.23
	200			0.25	0.29*
Protein	50				0.14
	100				0.34*
	150				0.36*
	200				0.09

* $P < 0.01$

** $P < 0.001$

(0.53**, 0.22, 0,34* and 0.46**) and phosphorus (0.39**, 0.43**, 0.56** and 0.33*) contents from the beginning to the end of all five lactation periods.

CONCLUSION

The effect of breed on the content of total solids, solids-non-fat, protein, lactose, ash, calcium and phosphorus was significant, being higher in the Alpine goats milk. The effect of lactation stage on the chemical composition and acidity of goat milk was found to be also significant. The lowest contents of total solids, solids-non-fat, fat, protein, ash, calcium and phosphorus were established in the middle of lactations while the highest levels were found both at the beginning and at the end of individual lactations. The lowest acidity of milk was determined in the middle of lactations, and the highest at their end.

Acknowledgements

The authors wish to thank PhD Miroslav Kaps for his help with statistical analysis and the personnel of the Department of Animal Nutrition for analysis of milk.

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Received: 00–10–03
Accepted after corrections: 01–05–14

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In vivo assessment of meatiness and fattiness of Charollais ram-lambs

Hodnocení zmasilosti a ztučnění na živých zvířatech u beránků charollais

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ABSTRACT: The goal of this study was to verify whether the values of fat and muscle tissue measured ultrasonically (echocamera) on the back of live Charollais lambs could be applicable to estimations of some traits of carcass quality. The results obtained in a set of 83 Charollais ram-lambs show very low ultrasonically measured backfat thickness (1.38 mm, s.d. = 0.54 mm) and percentage of kidney fat in carcass (0.60%, s.d. = 0.33%) which were determined even at the average age of lambs 138.4 days (s.d. = 12.0) and the average live weight 36.7 kg (s.d. = 4.2). On the other hand, the lambs had a large eye-muscle area measured by ultrasound (UMLTLA, 13.01 cm^2, s.d. = 2.25 cm^2) and on carcasses after slaughter (CMLTLA, 13.01 cm^2, s.d. = 2.24 cm^2) as well as very good percentage of meat in leg (77.1%, s.d. = 1.9%). The examined characteristics of carcass quality were adjusted according to the joint effect of flock and year, effect of litter size, age and live weight at slaughter. The effect of live weight had the highest impact on the studied carcass quality traits. UMLTLA and CMLTLA provided the similar residual correlation coefficient to other characteristics of carcass quality measured after slaughter. UMLTLA even more closely correlated with the percentage of meat in leg (r_{rp} = 0.451; $P < 0.001$) than CMLTLA (r_{rp} = 0.407; $P < 0.001$). Residual phenotypic correlation coefficient between UMLTLA and CMLTLA was r_{rp} = 0.687 ($P < 0.001$). The percentage of leg in carcass did not show any significant correlations with other carcass quality traits. Ultrasonically measured backfat thickness was slightly correlated with subjective classification of carcass fattiness (r_{rp} = 0.312; $P < 0.01$) and moderately correlated with percentage of kidney fat in carcass (r_{rp} = 0.501; $P < 0.001$).

Keywords: sheep; Charollais; carcass quality; ultrasonic measurements

ABSTRAKT: Cílem práce bylo ověřit, zda ultrazvuková měření svalové a tukové tkáně na hřbetu živých jehňat charollais mohou být použitelné pro odhady některých znaků jatečné hodnoty. Výsledky získané na souboru 83 beránků charollais v průměrném věku 138,4 dne (s. d. 12,0 dne) a při průměrné živé hmotnosti 36,7 kg (s.d. 4,2 kg) ukazují na velmi nízké hodnoty ultrazvukově měřené tloušťky hřbetního tuku (1,38 mm, s.d. 0,54 mm) a nízký podíl ledvinového tuku v jatečně upraveném těle (0,60 %, s.d. 0,33 %). Na druhé straně plocha hřbetních svalů za posledním žebrem měřená ultrazvukem (UMLTLA, 13,01 cm^2, s.d. 2,25 cm^2) i přímo na jatečně upraveném těle (CMLTLA, 13,01 cm^2, s.d. 2,24 cm^2) , jakož i obsah masa v kýtě (77,1 %, s.d. = 1,9 %) byly vysoké. U hodnocených charakteristik jateční hodnoty byla provedena korekce na vliv stáda a roku, četnosti vrhu, věku a živé hmotnosti při porážce. Efekt živé hmotnosti měl největší vliv na variabilitu sledovaných znaků jateční hodnoty. UMLTLA i CMLTLA vykazovaly podobné hodnoty korelačních koeficientů ve vztahu k ostatním charakteristikám jateční hodnoty měřeným po porážce. UMLTLA byla dokonce v těsnějším vztahu k procentickému obsahu masa v kýtě (r_{rp} = 0,451; $P < 0.001$) než CMLTLA (r_{rp} = 0,407; $P < 0,001$). Mezi UMLTLA a CMLTLA byl zjištěn koeficient reziduální fenotypové korelace ve výši r_{rp} = 0,687 ($P < 0,001$). Podíl kýty v jatečně upraveném těle nevykazoval žádné statisticky významné korelační vztahy k ostatním znakům jateční hodnoty. Tloušťka tuku na hřbetu měřená ultrazvukem byla slabě korelována se subjektivní klasifikací ztučnění jatečně upraveného těla (r_{rp} = 0,312; $P < 0,01$) a středně korelována s procentickým obsahem ledvinového tuku.

Klíčová slova: ovce; charollais; jatečná hodnota; ultrazvukové měření

Supported by the Ministry of Agriculture of Czech Republic (Project No. EP 9329).

INTRODUCTION

The change in production orientation of sheep husbandry has occurred in the Czech Republic in the last decade. This situation evokes the necessity to direct the attention of sheep keepers and breeders at breeding work aimed at improvement of traits determining the level of meat performance in sheep. The carcass quality by the side of reproduction traits and growth intensity also pertains to this group of traits. The consumers demand the excellently conformed carcasses with optimal fattiness (Ellis *et al.*, 1997). The traits connected with carcass quality usually have medium heritability and suitable variability, which is favourable for the genetic improvement of these traits (Croston and Pollot, 1985). On the other hand, the characteristics of carcass quality cannot be measured directly on live animals and there is a reason for use of ultrasound measurements for objective *in vivo* assessments of carcass quality in sheep.

A number of authors were engaged in ultrasonography and related techniques of in vivo assessment of meatiness and fattiness of lambs (Krauth, 1987; Simm and Dingwall,1989; Kadim *et al.*, 1989; Cameron and Bracken, 1992; Fenessy *et al.*, 1993; Milerski *et al.*, 1994; Ringdorfer, 1995; Gut *et al.*, 1996; Cadavez *et al.*, 2000 and others).

Charollais is the most numerous sheep meat breed in the Czech Republic. The process of adaptation of this breed to conditions of the Czech Republic was examined by Kuchtík *et al.* (1996) and Shaker-Momani *et al.* (1995). Horák and Žižlavská (1999) examined the efficiency of Charollais sheep in a system of mixed grazing with cattle.

The main goal of this study was to verify whether the fat and muscle tissue measurements done ultrasonically with echocamera on the back of live Charollais lambs could be applicable for estimations of some traits of carcass quality.

MATERIAL AND METHODS

The ultrasonic measurements of live animals were used for the prediction of carcass quality. Investigations were carried out in 2 flocks in the years 1996–2000. In total 83 purebred Charollais ram-lambs (progeny of 17 sires) were included in the data set. At the end of fattening, the average age of animals was 138.4 (s.d .= 12.0) days and their average live weight was 36.69 (s.d. = 4.18) kg.

The ultrasonic measurements were carried out at the end of the fattening period. The real-time scanner Aloka SSD 210 DX II with the 5 MHz linear probe UST 5813-5 was used for this purpose. The ultrasonic measurements of back cross-section were done on the level of 1st lumbar vertebras. The ultrasonic pictures were recorded with photo-camera from the screen of the machine and then analysed. Measurements of cross-section areas of *musculus longissimus thoracis et lumborum* (UMLTLA) were carried out as well as measurements of the backfat thickness. Subsequently, all measured lambs were slaughtered. After slaughter the carcass weights were recorded and the dressing percentages were computed. After 24 hours of chilling the carcasses were subjectively classified according to SEUROP system of carcass evaluation. Classes for conformation were marked by numbers ($S = 6 .... P = 1$), fattiness classes was marked from 1 (least) to 5 (highest). Also the measurements of carcass rump circumferences were carried out at the level of the *tuberae ischiadicii* of pelvis. Then the kidney fat was separated and weighed and the carcasses were dissected into 7 parts: scrag, middle neck, best end neck, loin, leg, rack and flank (Figure 1). The weights and percentages of these cuts were determined. In addition, the eye-muscle areas were measured by planimetry on the section between the last thoracic and 1st lumbar vertebras (CMLTLA). One leg from each carcass was dissected into meat (muscles + fat) and bones and the percentage of meat in leg was computed.

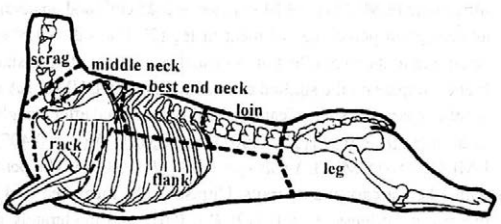

Figure 1. Cuts of lamb

Variance of examined traits was analysed by the least squares method. Software SAS STAT was used for the statistical processing of the data (SAS, 1998). Procedure PROC GLM variant SS4 was used for the estimation and correction of systematic effects. The following statistical model was used for the correction of systematic effects on all examined traits of carcass quality:

$$y_{ijk} = \mu + FY_i + LS_j + a + b + e_{ijk} \qquad \text{(Model 1)}$$

where: y_{ijk} = measured trait
μ = common constant
FY_i = joint effect of flock and year (fixed effect – 6 classes)
LS_j = litter size (fixed effect – 2 classes)
a = linear regression according to live weight
b = linear regression according to age
e_{ijk} = residual error

Phenotypic correlation coefficients (r_p) were computed from the row data. The residual phenotypic correlation coefficients (r_{rp}) between the residual values of examined traits after correcting the data according to Model 1 were computed. Then the multiple residual correlation coefficients (r_{mrp}) between the characteristics of carcass quality that were measured after carcass dissection (percentage

Table 1. Basic statistical characteristics of the data set (n = 83)

Variable	Unit	Mean	S. D.
Age	day	138.4	12.0
Live weight	kg	36.69	4.18
MLD area measured by ultrasound (UMLTLA)	cm^2	13.01	2.25
Back fat measured by ultrasound	mm	1.38	0.54
Dressing percentage	%	46.39	3.52
SEUROP conformation	point	4.07	0.76
SEUROP fattiness	point	2.34	0.62
MLD area measured on carcass (CMLTLA)	cm^2	13.01	2.24
Percentage of legs in the carcass	%	35.15	2.19
Percentage of meat in leg	%	77.14	1.87
Percentage of kidney fat	%	0.60	0.33
Carcass rump circumference	cm	61.59	3.68

of meat in leg, CMLTLA) and group of traits determined on live animals (UMLTLA) or on carcasses before dissection (SEUROP classification of conformation, dressing percentage, rump circumference) were computed.

RESULTS AND DISCUSSION

The mean values and standard deviations of examined traits are presented in Table 1. Dressing percentage and percentage of legs in carcass were approximately on the same level as Kuchtík *et al.* (1996) or Shaker-Momani *et al.* (1995) report for Charollais lambs reared in the conditions of the Czech Republic. Isterdael *et al.* (1985, cit. by Kuchtík *et al.*, 1996) found out higher dressing percentage of Charollais breed in France. Percentage of meat in leg was a slightly higher (77.14%; s.d. = 1.87%) than that reported by Kuchtík *et al.* (1996), who found out average percentage of muscles in leg 69.5% + percentage of fat in leg 5.7% in the set of Charollais lambs at the age 129 days and live weight 32.9 kg on average. Also the mean percentages of legs in carcasses of Charollais lambs presented by Kuchtík *et al.* (1998) were lower (33.2% in average age 148 days) than the values determined in this work (35.15%; s.d. = 2.19%).

There were not found any differences between averages of MLD areas which were measured by ultrasound on live animals (UMLTLA) and MLD areas measured on the carcasses 24 hours after their slaughter (CMLTLA). This finding does not correspond with our results achieved previously on crossbred lambs (Milerski *et al.*, 1994) when the UMLTLA were significantly lower than CMLTLA. On the other hand, Cameron and Smith (1985) and Krauth (1987) did not find out any significant differences between average values from ultrasonic measurements on live animals and the same measurements *post mortem*. It is difficult to compare our values of the eye-muscle area with the results published by other authors because this trait is markedly influenced mostly by the live weights of the examined animals which were very different in various reports. The values of UMLTLA were given by Kempster *et al.* (1982), Krauth (1987), Cameron and Smith (1985), Jensen (1992) and ranged from 7.7 to 17.1 cm^2 at live weight of lambs 32.2–44 kg.

The values of backfat thickness measured by ultrasound were extremely low in the examined set of animals. This fact had influence on the precision of measurement. Only are the results given by Kempster *et al.* (1982) or Gut *et al.* (1996) nearer to a certain extent to these results. The above cited authors found out on average 2.7mm thickness of subcutaneous fat. Cameron and Smith (1985), Krauth (1987) or Ringdorfer (1995) found out the subcutaneous fat thickness on the back of young rams even higher than 7 mm. The percentage of kidney fat in carcass weight was also quite low at the age of almost 140 days and was distinguishably lower than the same characteristic found in other sheep meat breeds (Suffolk 1.09%; Romney 1.06%) at comparable age (136.7 days; 146.8 days) and weight (36.15 kg; 36.01 kg) in the Czech Republic (Milerski, 1998). The individual systematic effects on the performance of investigated traits were also studied (Table 2). The joint effect of year and flock (FY) was statistically highly significant for fattiness characteristics – the subjective carcass fattiness classification and the percentage of kidney fat. Traits connected with meatiness were less influenced by this effect. Nevertheless, it is necessary to correct data of performance recording according to this effect. The effect of litter size was significant only for dressing percentage and for percentage of meat in leg. Surprisingly twins had higher percentage of meat in leg ($l.s.m.$ = 77.4%) than single born lambs ($l.s.m.$ = 76.4%; $P < 0.05$). Age and live weight of lambs are closely correlated with each other. However, the differences in live weight influenced the variability of all examined carcass quality traits to a greater extent than the age of lamb.

The achieved coefficient of determination of model equation was the lowest for percentage of legs in carcass (R^2 = 0.171) and backfat thickness measured by ultrasound (R^2 = 0.244). On the other hand, the used statistical model allowed to explain a high part of variability in the case of carcass rump circumference (R^2 = 0.792), SEUROP fattiness classification (R^2 = 0.664) or percentage of kidney fat (R^2 = 0.619).

Table 3 shows the phenotypic correlation coefficients from row data and the partial phenotypic correlations after correction of the data according to model equation Model 1. The partial correlation coefficients between UMLTLA and indicators of carcass quality that were determined after slaughter were mostly highly significant. The correlation between UMLTLA and CMLTLA was medium (r_p = 0.687). Gut *et al.* (1996) found higher correla-

Table 2. Least squares analysis

Source of variation	D.F.	MLD area measured by ultrasound (UMLTLA)		MLD area measured on carcass (CMLTLA)		Dressing percentage		Percentage of legs in carcass		Percentage of meat in leg	
		mean square	F value	mean square	F value	mean square	F value	mean square	F value	mean square	F value
Flock*Year	4	2.34	0.86^{ns}	3.68	1.64^{ns}	17.30	1.82^{ns}	4.48	1.00^{ns}	3.41	1.80^{ns}
Litter size	1	0.04	0.01^{ns}	0.04	0.02^{ns}	43.94	4.61*	0.34	0.08^{ns}	12.85	6.78**
Live weight	1	165.81	60.92***	171.03	76.03***	79.00	8.29**	56.16	15.57***	90.44	47.69***
Age	1	0.08	0.03^{ns}	0.45	0.20^{ns}	36.70	3.85*	5.80	1.30^{ns}	5.01	2.64^{ns}
R^2		0.519		0.600		0.316		0.171		0.518	
Residual s.d.		1.65 cm^2		1.50 cm^2		3.09%		2.1 %		1.39%	

Table 2. continuation

Source of variation	D.F.	SEUROP meatiness class		SEUROP fattiness class		Back fat measured ultrasonically *in vivo*		Percentage of kidney fat		Rump circumference	
		mean square	F value	mean square	F value	mean square	F value	mean square	F value	mean square	F value
Flock*Year	4	0.54	1.35^{ns}	2.12	14.58***	0.36	1.49^{ns}	0.72	15.41***	32.03	9.86**
Litter size	1	0.30	0.75^{ns}	0.00	0.00^{ns}	0.00	0.01^{ns}	0.05	1.10^{ns}	3.39	1.21^{ns}
Live weight	1	15.37	38.76**	1.84	12.67***	3.20	13.1***	0.50	10.78**	337.6	103.4***
Age	1	0.53	1.35^{ns}	0.00	0.00^{ns}	0.00	0.00^{ns}	0.02	0.37^{ns}	3.14	0.97^{ns}
R^2		0.384		0.664		0.244		0.619		0.792	
Residual s.d.		0.63 point		0.38 point		0.50 mm		0.22%		1.80 cm	

ns = non significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 3. Phenotypic correlations between carcass quality traits computed on the row data (below diagonal) and residual correlations after correction of systematic effects according to MODEL 1 (above diagonal

		1	2	3	4	5	6	7	8	9	10
1	MLD area by ultrasound (UMLTLA)	1	**0.687**	**0.519**	**0.025**	**0.451**	**0.412**	**0.234**	**0.250**	**0.153**	**0.520**
2	MLD area on carcass (CMLTLA)	0.840		**0.519**	**-0.031**	**0.407**	**0.382**	**0.294**	**0.313**	**0.105**	**0.544**
3	Dressing percentage	0.537	0.510		**0.062**	**0.452**	**0.546**	**0.373**	**0.115**	**0.289**	**0.741**
4	Percentage of leg in carcass	0.234	0.210	0.143		**–0.060**	**0.070**	**0.183**	**0.181**	**0.204**	**–0.029**
5	Percentage of meat in leg	0.576	0.639	0.330	0.158		**0.442**	**0.441**	**–0.023**	**0.152**	**0.450**
6	SEUROP conformation classification	0.606	0.573	0.557	0.248	0.583		**0.328**	**0.004**	**0.062**	**0.492**
7	SEUROP fattiness classification	0.471	0.444	0.507	0.131	0.384	0.338		**0.312**	**0.571**	**0.249**
8	Back fat by ultrasound *in vivo*	0.458	0.498	0.151	0.297	0.247	0.195	0.296		**0.501**	**–0.131**
9	Kidney fat percentage	0.441	0.430	0.255	0.224	0.387	0.147	0.651	0.539		**–0.082**
10	Carcass rump circumference	0.771	0.771	0.610	0.376	0.575	0.728	0.320	0.349	0.238	

Residual correlations higher than $r = 0.215$ are significantly different from 0 at the level of significance $P < 0.05$

tion ($r = 824$**) between these traits in a set of 40 lambs slaughtered at the average age 140 days. As can be seen from Table 3, the correlations with other carcass characteristics were comparable for UMLTLA and CMLTLA. As far as the proportion of meat in leg is concerned, the UMLTLA showed even a higher partial correlation coefficient ($r_{rp} = 0.451$) than CMLTLA ($r_{rp} = 0.407$). These facts allow us to consider that ultrasonic measurements of MLD area on live animals are approximately as good predictors of carcass meatiness of Charollais lambs as measurements of MLD area on carcass after slaughter.

The correlation coefficients between the percentage of leg in carcass and other characteristics of carcass quality were very low. Taking into account this fact, the usefulness of the percentage of leg for evaluation of carcass conformation is rather questionable.

On the other hand, the carcass rump circumference showed highly significant correlation relations to the other examined indicators of carcass quality. Especially the correlation between the carcass rump circumference and dressing percentage was high ($r_{rp} = 0.741$).

Ultrasonically measured backfat thickness slightly positively correlated with MLD area measured by ultrasound and on carcasses ($r_{rp} = 0.250$ and $r_{rp} = 0.313$, respectively). Partial phenotypic coefficients of moderate correlation were calculated between the backfat thickness measured on live animals and characteristics of carcass fattiness (SEUROP fattiness classification and percentage of kidney fat). The backfat thickness was not measured directly on carcasses in this study because it was technically very difficult to guarantee accurate measurements of such low backfat thickness (average for ultrasonically measured fat was 1.38 mm). In the previous study correlation coefficient $r = 0.659$ was determined between backfat thickness measured ultrasonically and the same trait measured on carcass (Milerski, 1998). Delfa *et al.* (1996) mention the respective correlations ranging from $r = 0.18$ to $r = 0.33$ in dependence on the site of measurement. On the contrary, Bass *et al.* (1982) found out correlation $r = 0.92$ between the backfat ultrasonic measurements and carcass fat thickness. These different results show the difficulty of backfat thickness measurements in young lambs. However the ultrasonic measurements of backfat thickness could be effective due to the aforementioned positive correlation coefficients with the carcass fattiness characteristics. Lambs with fat thickness higher than 3 mm can be easily detected by this method.

The computed multiple correlation coefficients between the set of characteristics determined before carcass dissection and traits detected after dissection are presented in Table 4. By the use of the set of four traits it is possible to predict the CMLTLA only slightly more accurately than

Table 4. Multiple residual correlation coefficients between traits detectable before carcass dissection and characteristics obtained by carcass dissection

Traits	% of meat in leg	MLTL area in carcass (CMLTLA)
MLTL area measured by ultrasound (UMLTLA)	0.451	0.687
MLTL area measured by ultrasound (UMLTLA) + SEUROP conformation classification	0.499	0.704
MLTL area measured by ultrasound (UMLTLA) + SEUROP conformation classification + rump circumference	0.537	0.711
MLTL area measured by ultrasound (UMLTLA) + SEUROP conformation classification + rump circumference + dressing percentage	0.575	0.721

on the basis the UMLTLA only because the respective correlation coefficients were $r_{mrp} = 0.721$ and $r_{rp} = 0.687$. More beneficial is the use of multiple correlation if the percentage of meat in leg is predicted ($r_{mrp} = 0.575$ and $r_{rp} = 0.451$).

CONCLUSIONS

Very low backfat thickness was observed on Charollais lambs even at the age of almost 140 days. This fact documents the ability of Charollais lambs to be fattened to high weight. MLD area measured by ultrasound seems to be applicable to prediction of some indicators of carcass quality (dressing percentage, proportion of meat in leg, SEUROP classification of carcass conformation, rump circumference), while ultrasonic measurements on the one hand, and measurements of MLD area directly on carcasses on the other hand, showed comparable relations to the aforementioned carcass quality traits detected after slaughter. The ultrasonic measurements offer higher accuracy of carcass quality evaluation in live animals. The use of this method proved to be very suitable especially in selection programmes for sire meat breeds of sheep including Charollais.

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Received: 00–12–05
Accepted after corrections: 01–05–02

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The stability of resistance in a field housefly population, *Musca domestica*, over 60 generations, following the interruption of insecticide selection pressure

Stabilita rezistencie synantrópnej populácie muchy domácej (*Musca domestica*) po prerušení selekčného tlaku insekticídov počas 60 generácií

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ABSTRACT: Our experiments were aimed at the stability of resistance in a field housefly strain to insecticides commonly used to control flies in animal rearing. The stability is expressed by changes in the values of resistance factors and concentrations lethal to 50% of individuals. The stability of resistance to azamethiphos, dimethoate, pirimiphos-methyl, bendiocarb, cypermethrin, deltamethrin and permethrin was investigated over 4 years (60 generations) in a multi resistant "KP" population of flies by a tarsal contact test. This population of flies was kept under standard conditions in an insectary without any insecticide selection pressure. Of all the insecticides investigated, the resistance to bendiocarb was the most persistent. Therefore the first more marked decrease was observed starting with the 35th generation. The resistance to pyrethroids lasted up to the 30th generation. Of the organophosphates tested, the resistance to dimethoate was relatively the most stable. A considerable decrease was observed starting with the 12th generation and the characteristic feature was alternate decrease and increase in LC_{50} values. The resistance to azamethiphos decreased considerably between generations 4 and 13 and starting with the 35th generation the lethal concentration was lower than that for the control strain SRS/WHO. The LC_{50} for pirimiphos-methyl reached the values characteristic of sensitive flies as early as in the 5th generation while the values for generations 6–60 were constantly below those in the susceptible control strain.

Keywords: *Musca domestica*; Diptera; Muscidae; resistance; insecticides; stability

ABSTRAKT: V našich experimentoch sme sledovali stabilitu rezistencie populácie múch, ktorá bola odchytená z chovu ošípaných na vybrané, bežne používané insekticídy v živočíšnej výrobe. Stabilita je vyjadrovaná zmenami hodnôt faktorov rezistencie a LC_{50}. Stabilita rezistencie na insekticídne látky azamethiphos, dimethoate, pirimiphos-methyl, bendiocarb, cypermethrin, deltamethrin a permethrin bola sledovaná v laboratórnych podmienkach počas 4 rokov (60 generácií) u mnohonásobne rezistentnej populácie múch „KP" testom tarzálneho kontaktu, pričom populácia múch bola chovaná v podmienkach bez pôsobenia selekčného tlaku insekticídov. Zo sledovaných látok bola najstabilnejšia rezistencia na bendiocarb, keď prvý výraznejší pokles letálnej koncentrácie bol zaznamenaný až od 35 laboratórnej generácie múch. Rezistencia na pyretroidy bola stabilná do 30 generácie. Z organofosfátov relatívne najstabilnejšou bola rezistencia na dimethoate. Výraznejšia zmena bola zaznamenaná od 12 generácie múch a bola charakterizovaná striedavým poklesom a vzostupom hodnôt LC_{50}. Rezistencia na azamethiphos výrazne poklesla medzi 4 a 13 generáciou a od 35 generácie bola hladina LC_{50} nižšia než u senzitívneho kmeňa SRS/WHO. Hodnoty LC_{50} pre pirimiphos-methyl dosiahli hodnoty citlivých múch už v 5. generácii a od 6. až do 60. generácie boli vždy pod úrovňou kontrolného kmeňa SRS/WHO.

Kľúčové slová: mucha domáca; Diptera; Muscidae; rezistencia; insekticídy; stabilita

INTRODUCTION

Musca domestica Linnaeus, 1761 is one of the insect species most frequently used in experimental studies of physiological, genetic, and biochemical mechanisms in the development of resistance to insecticides. The development of resistance is an example of rapid microevolution caused by artificially produced changes in the frequency of randomly mutated normal selection genes. It develops over several generations exposed to constant

selection pressure by insecticides causing death of a certain portion of the exposed population. Recently published scientific papers focus mostly on the assessment of the rate of development of simple and cross-resistance in flies under laboratory conditions following a strong insecticide pressure (McDonald *et al.*, 1983; Saito *et al.*, 1991; Learmount, 1994; Pap and Toth, 1995). Less attention has been paid to the stability of resistance to commonly used preparations. Once selected for, resistance genes have very lengthy persistence in wild insect populations (Georghiou, 1964). Although the gene frequency of a specific resistance allele may decrease upon removal of insecticide pressure, the persistence of a changed background of residual inheritance in the genome causes the strain to regain its resistance as soon as the insecticide is re-applied. The stability of resistance is an inseparable part of any studies related to resistance and is important from both the theoretical and practical points of view. Our experiments investigated the stability over 60 generations, of resistance in a wild multi-resistant population of *Musca domestica* to 7 insecticide preparations most frequently used to control flies in animal production in the Slovak Republic. The changes in resistance after interruption of selection pressure with the insecticides can be of both scientific and practical importance. The investigations of resistance stability in field populations may give an answer to the question if an insecticide to which resistance has developed can be used effectively again. This knowledge can help to formulate the strategies for fly control programmes.

MATERIAL AND METHODS

Flies tested

A field population of flies denoted "KP" was collected on a pig farm in the Košice district. The flies were kept in an insectary where they reproduced in special cages 20 × 20 × 30 cm in size, made of stainless wire, with a solid bottom on one side. The cage frame was covered by technical tulle of meshes 0.15 × 0.15 cm. The standard of keeping and the conditions of the flies were considerably affected by the larval medium. Our medium consisted of 100g dried yeast, 100g dried milk, 20 g agar and 1 000 ml water (Rupes *et al.*, 1975). The flies were kept at 24–27°C in a relative humidity of 42–57%. Adult flies intended for tests were fed glucose and water. Individual tests were conducted on females of F_1–F_{60} laboratory generations, 4–7 days old. The parent material from a minimum of 1 000 flies (1 : 1 male-female) was used to establish each successive generation. A susceptible strain SRS/WHO – Standard Reference Strain/World Health Organization, kept under identical conditions, was used as a control and obtained from Dr Rupes's laboratory (National Institute of Public Health, Czech Republic).

Determination of LC_{50} values and the resistance factor

A method of tarsal contact (Rupes and Rettich, 1975) was used. Discs of filtration paper (Filtrak 388), 9 cm in diameter (63.5 cm²), were impregnated drop-by-drop either with 0.6 ml (approx. 60 drops) water emulsion or with a suspension of insecticide preparation. They were dried for 24 hours on a table on pinheads to prevent the loss of insecticide. Individual concentrations were selected so that they caused mortality ranging from 0 to 100%. A minimum of 6 different concentrations of preparations was tested each time. Fifteen females of flies were exposed to the impregnated paper for 24 hours while ensuring their access to water for the whole period of testing. The mortality of flies was determined after 24 hours. Controls were carried out simultaneously by exposing flies to a filter paper impregnated with drinking water. In control groups with mortality scale between 5–20% the correction of experimental values was carried out according to the equation by Abbott (1925). The final values of LC_{50} for wild and sensitive flies were calculated using the probit method (Roth *et al.*, 1962) and an adjusted computer programme Plot 50. The LC_{50} values were expressed as the amount of an active ingredient in μg/cm² of the filter paper. All experiments were repeated 3 times. The results are presented as mean values with standard deviations. The resistance factor (RF) shows how many times the resistance of a field strain to the insecticides tested exceeds that of the sensitive insects of the same species. In our experiments we determined it as a ratio of mean values of LC_{50} of the wild "KP" population tested and the corresponding values for the sensitive strain SRS/WHO.

The insecticides used

Filtration paper discs were impregnated with commercial insecticides, the doses of which are expressed in Table 1 as mg of the active ingredient per 63.5 cm². All insecticides are applied as residual sprays except azamethiphos, which is randomly used as a toxic fly bait (SNIP)

Table 1. List of preparations used in the experiments

Preparation	Active ingredient (a. i.)	Concentration used (mg/63.5 cm²)
Actellic 25 EC	pirimiphos-methyl (250 g/l)	6.0–0.0117
Alfacron 50 WP	azamethiphos (500 g/kg)	7.5–0.0073
BI 58 EC	dimethoate (380g/l)	6.7–0.0065
Coopex 25 WP	permethrin (250 g/kg)	0.38–0.0004
Ficam 80 W	bendiocarb (800 g/kg)	1.44–0.0014
Kordon 10 WP	cypermethrin (100 g/kg)	0.6–0.0006
K-Othrine 25 FLOW	deltamethrin (25 g/l)	0.03–0.0001

in the Slovak Republic. New products were used for experiments every year.

RESULTS

The stability of resistance in the flies to azamethiphos is illustrated in Figure 1. While the mean LC_{50} value for 60 generations in the sensitive strain SRS/WHO was 0.788 μg/cm², ranging from 0.746 to 0.811 μg/cm², the field KP strain exhibited a decrease from 10.047 to 0.523 μg/m² over the same time. During he first three generations, the values of LC_{50} were approximately on the same level. A considerable decrease was observed between generations F_4 and F_{13}. Between 13th and 30th generation the values of LC_{50} decreased further to the level observed in the sensitive strain and starting from the 35th generation they were lower than those observed for the strain SRS/WHO.

The overall picture of resistance to dimethoate in the flies investigated is shown in Figure 2. The initial LC_{50} was 2.34 μg/cm² and decreased almost 20 times (Table 2) to 0.119 μg/cm². However, the decrease differed from that observed for azamethiphos. Up to the 11th generation the values of LC_{50} ranged from 2.475 to 1.983 μg/cm². Starting from the 12th generation a gradual varying decrease to the level of the sensitive strain was recorded.

The tested KP population showed low resistance to pirimiphos methyl. The decrease in LC_{50} values is shown in Figure 3. A decrease from the starting value of 1.300 μg per cm² to 0.131 μg/cm² in the 60th generation was observed. The presented values were 5.3 times lower (Table 2) than those in the sensitive strain. A marked decrease almost to the half of the starting value was observed between 2nd and 4th generations. Starting from the 6th generation the LC_{50} values were below those determined in the SRS/WHO strain.

The resistance to bendiocarb in the investigated population (Figure 4) persisted for a long time. Up to the 35th generation the level of LC_{50} showed very little variation. Its values for the tested carbamate decreased considerably from 41.372 μg/cm² to 1.831 μg/cm² between generations F_{35} and F_{60}. The first marked decrease in LC_{50} was observed with the 40th generation and was followed by a rapid decrease from 30.223 to 1.831 μg/cm². However, the mean value of 0.109 μg/cm², determined for the sensitive strain, was not reached.

The decrease in resistance to cypermethrin was the slowest and consisted of several successive stages. The first, stable stage, occurred between generations F_1 and F_{10} and the values of LC_{50} ranged between 1.544 and 1.368 μg/cm² (Figure 5). The second stage, marked by a moderate decrease, was observed between 11th and 13th generation while the third one, characterized by a substantial decrease, lasted from generation 14th to 25th. The decrease in LC_{50} recorded in the above-mentioned stages was not rectilinear but showed some variations, i.e. decline

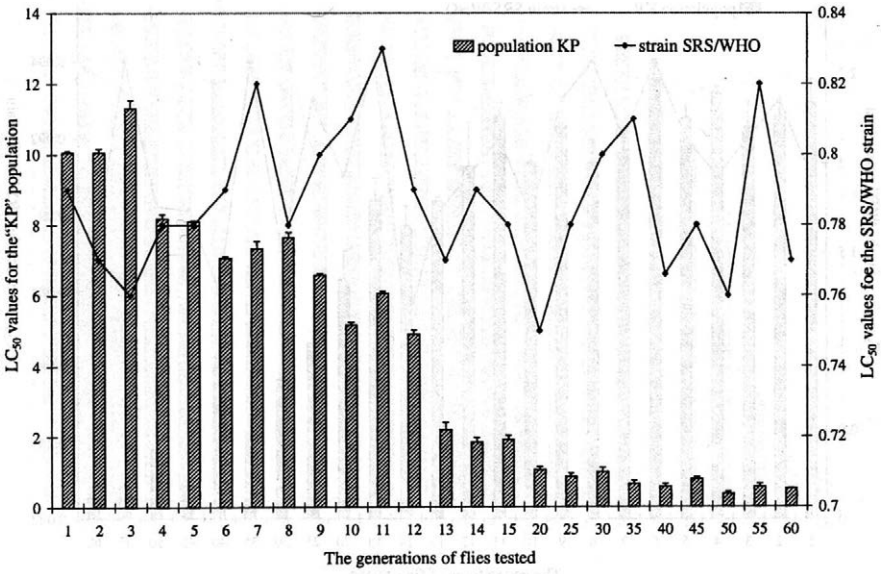

Figure 1. Values of LC_{50} (μg/cm²) for Alfacron 50 WP (a. i. azamethiphos) in the housefly over 60 generations following the interruption of selection pressure

Table 2. Mean values of resistance factors ($R.F_{50}$) of the field "KP" population of flies over 60 generations

Generation of flies	Factor of resistance ($R.F_{50}$)						
	A	Di	P-M	B	C	D	P
1	12.7	25.6	1.9	378.4	180.2	96.0	13.7
2	12.8	24.9	1.5	380.2	180.0	105.9	13.9
3	14.3	24.1	1.5	367.4	173.0	107.5	13.8
4	10.4	26.2	1.5	375.6	176.6	96.0	12.2
5	10.3	24.7	1.1	376.1	159.9	93.0	11.1
6	8.9	27.1	0.71	379.3	160.7	91.5	11.1
7	9.3	22.7	0.46	374.7	155.4	109.4	10.5
8	9.7	21.9	0.83	375.6	170.1	103.9	10.6
9	8.4	24.1	0.48	376.6	160.5	116.9	9.5
10	6.6	22	0.31	375.6	159.6	90.0	11.2
11	7.7	24.8	0.44	369.2	141.6	95.0	9.5
12	6.2	21.7	0.38	371.1	129.6	84.8	10.3
13	2.8	19.6	0.33	372.9	148.2	76.1	10.6
14	2.3	18.1	0.39	367.5	133.8	87.6	10.5
15	2.4	19.7	0.46	360.9	107.4	90.6	9.5
20	1.3	14.8	0.36	361	113.1	92.5	9.1
25	1.0	10.9	0.34	366.5	115.2	91.0	9.1
30	1.2	8.6	0.37	360.5	90.4	86.1	8.8
35	0.83	5.3	0.46	349	47.5	71.1	5.7
40	0.71	10	0.28	277.4	20	69.7	3.5
45	0.9	2.1	0.32	224.1	18.4	27.5	2.2
50	0.47	4.3	0.25	77.8	19.9	27.4	2.7
55	0.71	1.9	0.21	32.1	17.6	14.1	1.3
60	0.66	1.3	0.19	16.5	15.4	9.4	1.1

A – azamethiphos; Di – dimethoate; P-M – pirimiphos-methyl; B – bendiocarb; C – cypermethrin; D – deltamethrin; P – permethrin

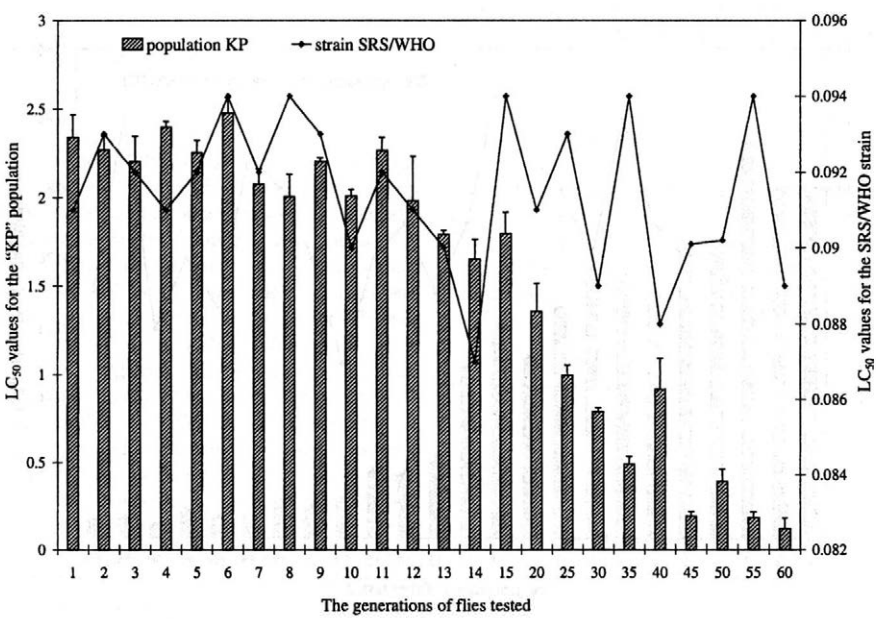

Figure 2. Values of LC_{50} (μg/cm²) for Bi-58 EC (a. i. dimethoate) in the housefly over 60 generations following the interruption of selection pressure

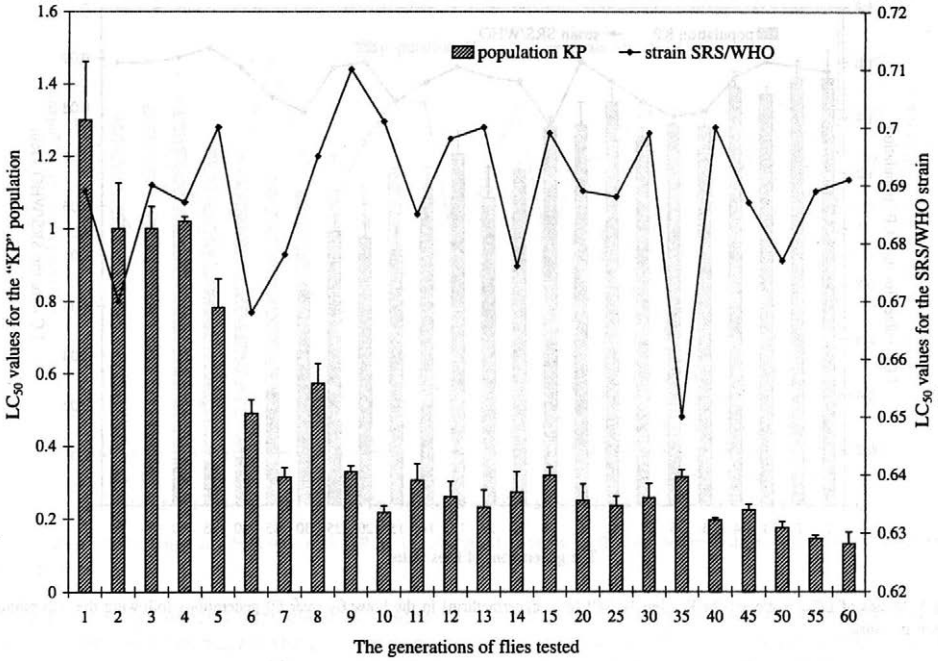

Figure 3. Values of LC_{50} (μg/cm²) for Actellic 50 EC (a. i. pirimiphos-methyl) in the housefly over 60 generations following the interruption of selection pressure

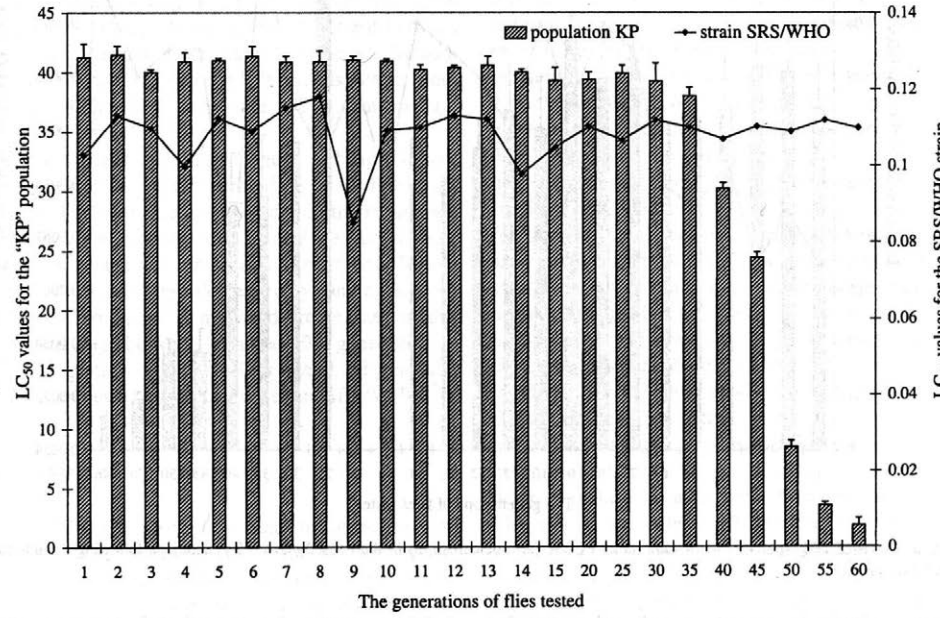

Figure 4. Values of LC_{50} (μg/cm²) for Ficam W (a. i. bendiocarb) in the housefly over 60 generations following the interruption of selection pressure

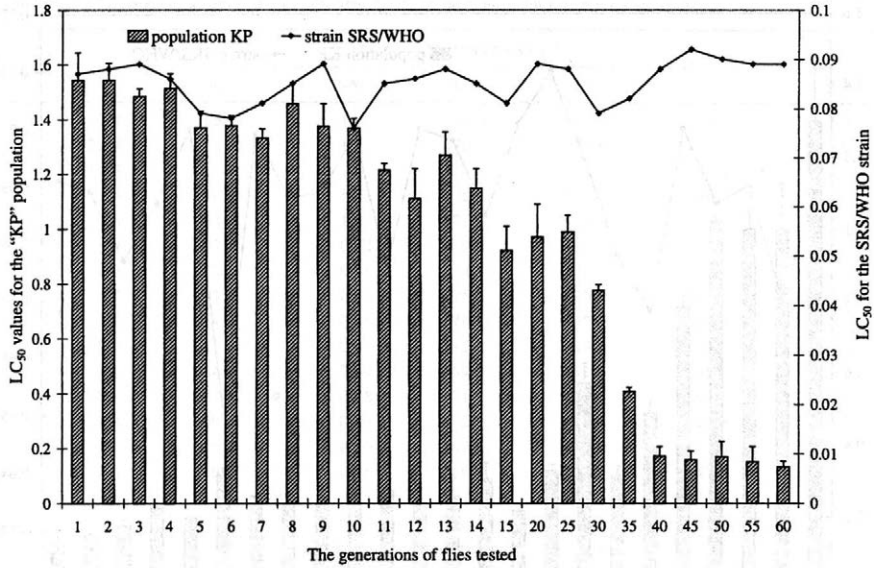

Figure 5. Values of LC_{50} (μg/cm²) for Kordon 10 WP (a. i. cypermethrin) in the housefly over 60 generations following the interruption of selection pressure

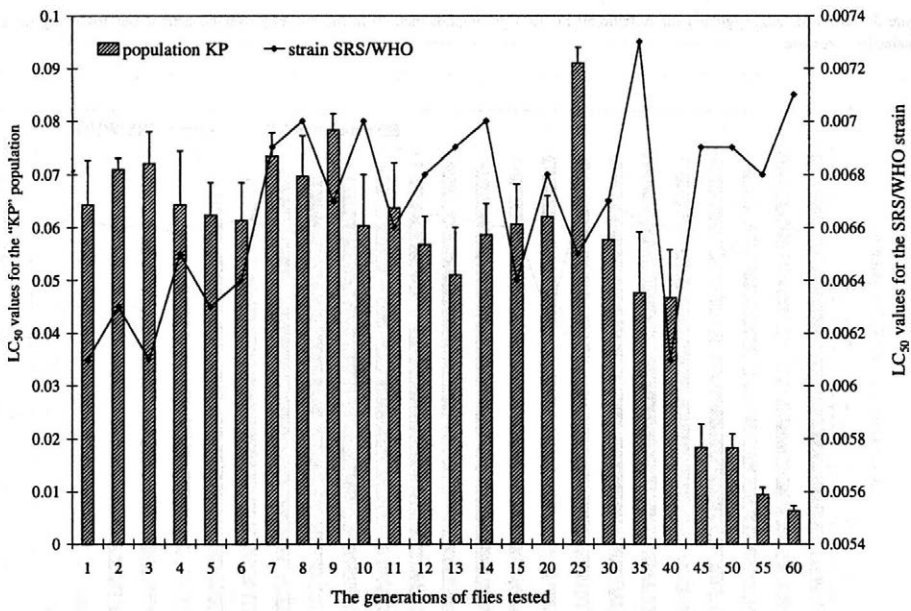

Figure 6. Values of LC_{50} (μg/cm²) for K-Othrine 25 FLOW (a. i. deltamethrin) in the housefly over 60 generations following the interruption of selection pressure

and return of lethal concentrations to the values recorded in the preceding generations, or even higher. In the fourth stage, between 30th and 40th generation, a sharp decline in LC_{50} occurred and after that the LC_{50} levels remained on the same level between 0.170 and 0.132 μg/cm².

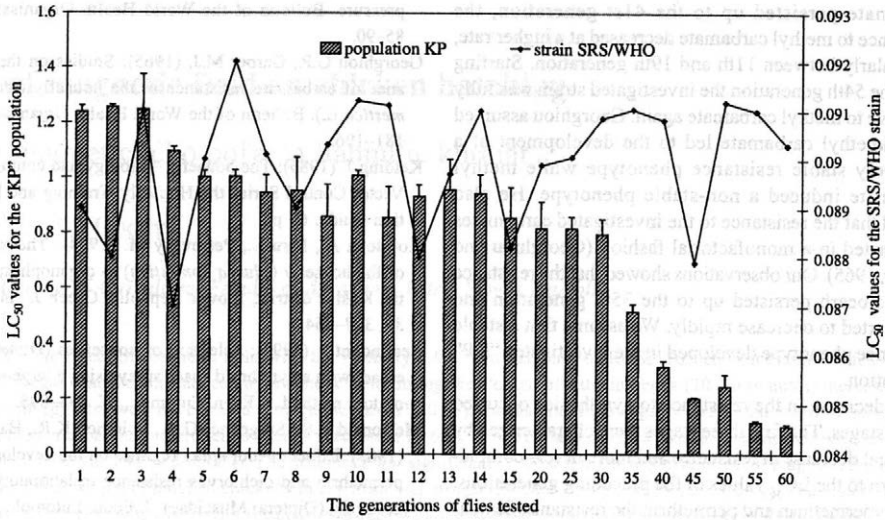

Figure 7. Values of LC_{50} (μg/cm²) for Coopex 25 WP (a. i. permethrin) in the housefly over 60 generations following the interruption of selection pressure

The changes in resistance to deltamethrin (Figure 6) could be divided into five stages. The first one was observed between generations F_1 and F_9, peaking with the 9th generation (LC_{50} = 0.078 μg/cm²). The second stage lasted from 10th to 13th generation and peaked during F_{11} (LC_{50} = 0.064 μg/cm²). The third stage was observed between 14th and 40th generations with peak during the 20th generation (LC_{50} = 0.062 μg/cm²). After that, between 40th and 45th generation, a marked decrease in lethal concentration values was recorded. In the last stage, from generation 45th to 60th, the values of LC_{50} remained unchanged.

The resistance to permethrin (Figure 7) changed in four stages, similar to those in cypermethrin. In the first stage, F_1–F_9, the highest value was recorded in the second generation (LC_{50} = 1.256 μg/cm²). During the second stage, F_{10} to F_{30}, the lethal concentrations were more or less on the same level with a slight decrease only, ranging between 1.009 and 0.796 μg/cm². A marked decrease was recorded in the third stage between 30th and 40th generation. In the last stage (F_{45}–F_{60}), the values decreased gradually always to the level of the sensitive strain SRS/WHO.

DISCUSSION

The stability of housefly resistance to commonly used commercial insecticides differs depending on many factors, most of all on the persistent frequency of genes controlling the principal resistance mechanisms (Keiding, 1986). The stability of resistance was investigated following the interruption of insecticide selection pressure. With the exception of pirimiphos-methyl, for which the lower values of LC_{50} than those for the standard strain were recorded already in the 6th generation, the process regarding susceptibility was slow and long-lasting. The situation which occurred with pirimiphos-methyl was likely to result from the fact that the values of resistance factor (Table 2) were low at the beginning of the experiment (1.9). We ascribe this to low prevalence of resistance to pirimiphos-methyl, which was manifested phenotypically by low values of lethal concentrations and resistance factors. In the area where the field population was collected the factors of resistance to this insecticide ranged from 8 to 10 (Kočišová *et al.*, 1994). Between 1st and 65th generations the sensitivity of flies to pirimiphos-methyl increased 5 times in comparison with the standard sensitive strain. The values of LC_{50} in the 60th generation for the two additional organophosphates investigated, azamethiphos and dimethoate, were also lower than those in the sensitive strain. In the case of azamethiphos the decrease was gradual and the LC_{50} value approached that of the SRS/WHO strain during the 20th generation. On the other hand, the development of resistance is quite rapid. Saito *et al.* (1991) exposed a certain strain to selection pressure by azamethiphos in laboratory and observed 100-fold higher LC_{50} already in generations F_3–F_4 but he did not introduce the initial level of resistance. The decrease in the resistance to dimethoate was not uniform. This can be accounted for by higher prevalence of resistance to dimethoate and also by heterogeneity of the tested population.

The stability of resistance to bendiocarb is comparable to that reported by Georghiou (1964). He investigated the resistance to dimethyl carbamate and methyl carbamate over 62 generations. While the resistance to dimethyl

carbamate persisted up to the 41st generation, the resistance to methyl carbamate decreased at a higher rate, particularly between 11th and 19th generation. Starting from the 54th generation the investigated strain was fully sensitive to methyl carbamate again. Georghiou assumed that dimethyl carbamate led to the development of a relatively stable resistance phenotype while methyl carbamate induced a non-stable phenotype. He also proved that the resistance to the investigated carbamates is inherited in a monofactorial fashion (Georghiou and Garber, 1965). Our observations showed that the resistance to bendiocarb persisted up to the 35th generation and then started to decrease rapidly. We assume that a stable resistance phenotype developed in the investigated "KP" population.

The decrease in the resistance to pyrethroids occurred in 3–4 stages. The first three stages were characterized by a gradual decrease in resistance and more or less complete return to the LC_{50} values of the preceding generations. With cypermethrin and permethrin the resistance decreased markedly after 30 generations, which is comparable with the study of Szabo (1989), and in deltamethrin the decrease was observed after 40 generations. A similar decrease in resistance from 263.6 to 12 over 3 years of keeping under laboratory conditions was observed for a wild Oakland strain (Learmount, 1994), which exhibited initially high resistance to cyanopyrethroid alpha cypermethrin. After the decrease described above, the strain was exposed to selection pressure by cypermethrin again. An increase in LD_{50} from 11.9 to 503 µg per fly was observed as early as after 5 selections. Scott and Georghiou (1985, 1986) stated that the high level of resistance to pyrethroids observed in a LEARN-pyr strain resulted from three different mechanisms of the development of resistance, namely the increased oxidative metabolism, insensitivity of the target site of action (kdr), and decreased penetration of the respective insecticide. We assume that the stability of pyrethroid resistance observed in our study was caused by the three mechanisms mentioned.

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Received: 01–03–12
Accepted after corrections: 01–05–15

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Volume 46, No. 6 June 2001

CONTENTS

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