Effects of different formulae for oral and parenteral administration of iron on somatic growth and selected haematological indexes in piglets

Vliv rozdílné aplikace Fe na somatický růst a vybrané hematologické hodnoty selat

V. KOTRBÁČEK

University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Medicine, Brno, Czech Republic

ABSTRACT: Effects of oral administration of two types of iron-containing pastes and parenteral administration of dextrane-bound iron on somatic growth and selected haematological indexes were compared in 140 piglets, crosses of the Large White and Landrace breeds. The growth rate of piglets treated orally with pastes containing 40 or 90 mg of lactate-bound iron or 100 mg of fumarate-bound iron per dose (1 ml) equalled that of their littermates treated intramuscularly with 200 mg of Ferridextran. The dose of 40 mg of lactate-bound iron was insufficient for maintaining all haematological indexes at the level observed in piglets treated intramuscularly with Ferridextran. A single dose of 90 mg of lactate-bound iron was necessary to induce an effect equal to that of Ferridextran. Mean corpuscular volume and mean corpuscular haemoglobin content were the most sensitive indicators of the level of iron intake. The effect of oral administration of 100 mg fumarate-bound iron on haematological indexes was equal to that of parenteral administration of 200 mg Fe and even stronger on some indexes in the 2nd week of life. It is concluded that oral treatment with iron ions can be as effective as parenteral treatment provided that intestinal functions are normal.

Keywords: iron intake; piglets; growth; haematological indexes

ABSTRAKT: Ve třech experimentech, na celkovém počtu 140 selat plemene BU × LA, jsme sledovali vliv dvou rozdílných Fe past a injekční aplikace Fe na somatický růst a hematologické hodnoty pokusných jedinců. Jednodenní selata dotovaná železem *per os* ve formě pasty obsahující 40, případně 90 mg laktátového Fe a pasty obsahující 100 mg fumaranového Fe v 1 ml, tzn. v jedné aplikační dávce, rostla se stejnou intenzitou, jako jedinci z téhož vrhu ošetření 200 mg *i. m.* aplikovaného Ferridextranu. Vývoj hematologických parametrů ukázal, že 40 mg laktátového Fe nestačilo k trvalému udržení všech hematologických hodnot na úrovni selat dotovaných železem injekčně. K vyrovnání sledovaných údajů v obou jmenovaných skupinách došlo teprve po zvýšení koncentrace Fe na 90 mg v jednorázové aplikační dávce. Na úroveň dotace Fe nejcitlivěji reagoval střední objem erytrocytů a střední obsah hemoglobinu v erytrocytech. Aplikace 100 mg fumaranového Fe na sele se co do účinku na hematologické ukazatele nejen vyrovnala injekci 200 mg Fe, ale ve druhém týdnu života ji v některých hodnotách i převyšovala. Z pokusů uzavíráme, že utilizace Fe iontů podávaných *per os* může být stejně účinná jako forma parenterální. Předpokladem je plně funkční sliznice střeva.

Klíčová slova: příjem železa; růst selat; hematologické hodnoty

INTRODUCTION

Although parenteral administration of iron to piglets is a well-tried method for assuring sufficient iron intake, alternative treatments are sought currently for reasons that are not limited to commercial interests only. The dose of iron administered parenterally may, in some cases, exceed the capacity of the transport, protein and free iron ions induce toxic effects including impairment of blood coagulation, haemorrhages, or circulatory failure. Sudden "oversaturation" of lymphatic tissues by iron can also affect responsiveness of the immune system (Kovářů, 2000 – pers. com.). Although beneficial effects of parenteral treatment prevail, the hazard exists and must be considered.

One of the alternatives is enrichment of prestarter diets with readily absorbable forms of iron. The efficacy of such supplementation has been found insufficient because the consumption of prestarters in the first two weeks after birth is low. Insufficient supply of iron resulted in spending of iron reserves, development of anemia, growth retardation, immunodeficiency, and increased mortality rate (Dubanský *et al.*, 1997a, b).

Another alternative source of iron for piglets is an iron-containing paste. Such product can contain also a probiotic assuring the colonisation of the digestive tract by beneficial lactacidogenic bacteria and can be used as a source of selected minerals and vitamins. Since opinions on the efficacy of such pastes are controversial, we decided to test them under experimental conditions.

The objective of our three consecutive experiments was to obtain data on physiological effects of two iron-containing compounds included into pastes for oral administration and to compare the results of such treatments with those of the conventional parenteral treatment with Ferridextran.

MATERIAL AND METHODS

A group of day-old piglets (Large White × Landrace) received orally iron bound in the unconventional lactate form. In addition to Fe^{2+}, amino acids and lactic acid n-hydrate, the paste also contained 8% of the unicellular green alga *Chlorella kessleri*. The latter component was included into the paste by the manufacturer (Medipharm CZ, Ltd., Hustopeče u Brna, Czech Republic) largely to improve its dietetic value. The administered dose contained 40 mg of iron. Although organic iron was present also in the alga, the contribution to the administered dose was negligible.

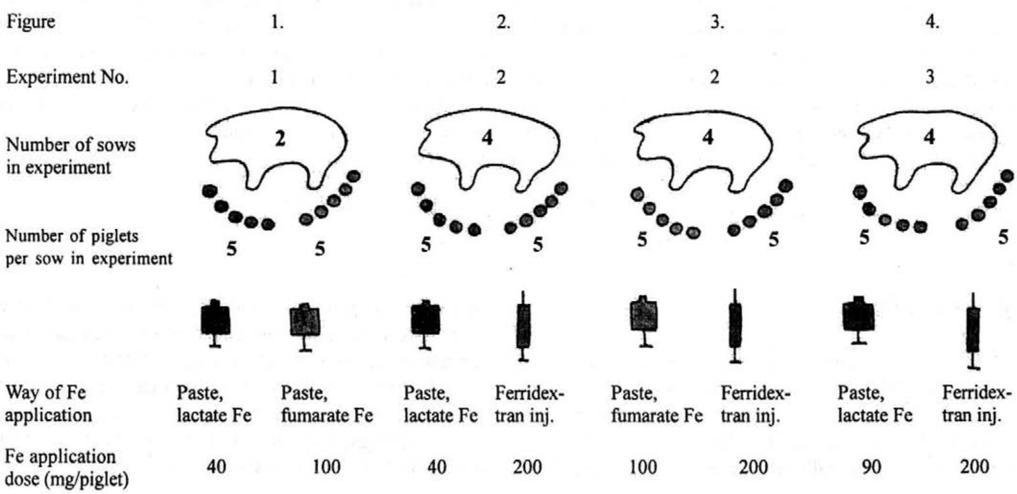
Another group of piglets was treated with a paste containing Fe^{2+} bound in fumarate. The administered dose contained 100 mg of iron. The content of iron in the lactate form was limited by initial difficulties in concentrating iron in the excipient. After mastering this problem, the per dose amount of iron could be increased considerably (see experiment 3).

The design of Experiment 1 is shown in Figure 1. Equal litters of two sows (n = 20) were divided into halves and half-litters of the two sows were pooled into two treatment groups. The two groups were treated with a single dose of the pastes containing lactate-bound iron and fumarate-bound iron, respectively.

The design of Experiment 2 is shown in Figure 2. Again, litters of four sows were divided into halves (n = 5) and groups were formed by pooling half-litters like in Experiment 1. One group was treated orally with a single dose of the paste containing lactate-bound iron and the other parenterally with a single dose of Ferridextran. The same design was applied in litters of another 4 sows (Figure 3). One group was treated orally with the paste containing fumarate-bound iron and the other parenterally with Ferridextran.

The doses of the products corresponded to 40 mg of lactate-bound iron, 100 mg of fumarate-bound iron, and 200 mg of dextrane-bound iron, respectively.

The design of Experiment 3 is shown in Figure 4. Again, equal day-old litters (n = 10) of four sows were divided into halves and treatment groups were formed like in Experiments 1 and 2. One group was treated orally with the paste containing increased concentration of



lactate-bound iron and the other parenterally with Ferridextran. The doses corresponded to 90 mg (oral form) and 200 mg (parenteral form) of iron. In all the three experiments, the pastes were administered to day-old piglets that had been let to suck colostrum several times.

Blood samples were collected from the piglets for the determination of haemoglobin (Hb), packed cell volume (PCV), erythrocyte count (Ery) and blood serum iron concentration. The data were used to calculate mean corpuscular volume (MCV), mean corpuscular haemoglobin content (MCH), and mean corpuscular haemoglobin concentration (MCHC). Further, leukocyte count and phagocytary activity were determined as indicators of nonspecific resistance. The age at sampling is given in the corresponding tables. The obtained data were subjected to Student's t-test.

RESULTS AND DISCUSSION

Experiment 1

No significant between-group differences in growth rate (Table 1) or haematological values (Table 2) were found in Experiment 1. However, the general downward tendency with advancing age was more pronounced in the group treated with lactate-bound iron.

Experiment 2

The results were similar to those obtained in Experiment 1. Growth rate in the piglets treated with the lactate form was comparable with that in the piglets treated with Ferridextran (Table 3). Two piglets of the group treated with Ferridextran died during the experiment. Therefore, the total weight of the group at weaning was lower by 11%.

No difference in growth rate was found between the group treated with the formula containing fumarate-bound iron and that treated with Ferridextran. One piglet died in each group. Therefore, group weights at weaning were equal.

No significant differences in haematological values were found in 13-day-old piglets between the group treated with lactate-bound iron and that treated with Ferridextran, although Hb, PCV, and blood serum iron concentration were somewhat lower in the former. However, this group showed significantly lower MCV and MCH at the age of three weeks. No between-group differences were found for Ery and blood serum iron concentration.

Haematological values in 13-day-old piglets were generally higher in the group treated with fumarate-bound iron than in that treated with Ferridextran. The differences in Hb and PCV were significant (Table 4). Although the blood serum iron concentration was somewhat higher in the group receiving fumarate-bound iron, the difference was nonsignificant.

No between-group differences were found in 21-day-old piglets. The only exception was a higher iron concentration in blood serum in the group receiving fumarate-bound iron.

Table 1. Development of mean live weights of piglets (g) in Experiment 1

Treatment	n	Age (days) 1	10	21
Paste, lactate Fe	10	1 810 ± 296	3 750 ± 704	6 510 ± 974
Paste, fumarate Fe	10	1 790 ± 265	3 840 ± 672	6 530 ± 1 091
Significance of differences		NS	NS	NS

NS = $P > 0.05$ *$P < 0.05$, **$P < 0.01$

Table 2. Development of haematological values in Experiment 1

Age (days)	Treatment	Hb (g/l)	Ht (l/l)	Ery (T/l)	MCH (pg)	MCV (fl)	Fe (mmol/l)
	Paste, lactate Fe	151 ± 15.8	0.34 ± 0.031	5.60 ± 0.600	27 ± 2.3	61± 6.5	
1	Paste, fumarate Fe	151 ± 27.6	0.34 ± 0.060	5.86 ± 1.100	26 ± 4.0	58 ± 10.6	
	Significance of differences	NS	NS	NS	NS	NS	
	Paste, lactate Fe	125 ± 28.0	0.32 ± 0.055	4.15 ± 0.590	30 ± 4.1	77 ± 6.2	
10	Paste, fumarate Fe	132 ± 27.8	0.32 ± 0.054	4.36 ± 0.360	30 ± 6.1	72 ± 9.1	
	Significance of differences	NS	NS	NS	NS	NS	
	Paste, lactate Fe	95 ± 20.4	0.30 ± 0.036	4.97 ± 0.580	19 ± 2.1	60 ± 3.5	6.2 ± 0.84
21	Paste, fumarate Fe	97 ± 15.5	0.31 ± 0.039	4.99 ± 0.520	20 ± 1.5	62 ± 3.3	6.4 ± 1.80
	Significance of differences	NS	NS	NS	NS	NS	NS

NS = $P > 0.05$

Experiment 3

The two groups did not differ in growth rate until weaning (Table 5). The somewhat lower haematological values in 14-day-old piglets treated with lactate-bound iron did not differ significantly from those found in the group treated with Ferridextran (Table 6). The only significant between-group difference was a higher concentration of iron in blood serum at the age of 14 days in the group treated with Ferridextran.

It should be noted, however, that blood serum iron concentration decreased significantly towards the end of the first month of life in the group treated with Ferridextran, while an opposite tendency was observed in the group treated orally. No between-group differences were found in haematological values in 28-day-old piglets.

Worth mentioning is the phagocytary activity of leukocytes indicating the level of nonspecific resistance which was higher by 10% in 14-day-old and by 11.5% in 28-day-old piglets of the orally treated group; the difference was nonsignificant, however (Table 6).

The high growth rate in the first weeks of life increases the demand for iron which is necessary for haemopoiesis and myoglobin synthesis. Such situation can lead to a decrease in some haematological values even in piglets treated with iron-containing drugs. An example thereof is a decrease in erythrocyte count and haemoglobin concentration observed in piglets between days 10 and 20 after birth in spite of standard treatment (200 mg of iron) with Ferridextran (Dubanský *et al.*, 1997a, b). Haematological values in the piglets suffering from iron deficiency and showing low growth rate remained at a low, but more or less constant level.

Similar age-dependent changes were also observed in our rapidly growing piglets in Experiment 1. No significant differences were found between the groups treated with lactate-bound or fumarate-bound iron. Lower values of all indexes under study towards the end of week 3 indicate that a single dose of 40 mg of lactate-bound iron would not apparently meet the demand for iron in rapidly growing piglets for a longer period.

This assumption was confirmed by results of Experiment 2. Growth effects of the treatment with the same

Table 3. Development of mean live weight (g) of piglets in Experiment 2

Treatment	*n*	Age (days)				
		1	13	21	33	Index
Ferridextran inj.	20	1 747 ± 317	4 166 ± 1 092	5 526 ± 1 400	9 194 ± 1 826	100.0
Paste, lactate Fe	20	1 808 ± 289	4 053 ± 836	5 637 ± 1 404	9 200 ± 1 787	100.5
Significance of differences		NS	NS	NS	NS	
Ferridextran inj.	20	1 555 ± 287	3 955 ± 613	5 660 ± 1 028	9 205 ± 1 282	100.0
Paste, fumarate Fe	20	1 716 ± 267	4 116 ± 713	5 863 ± 1 082	9 158 ± 1 759	100.3
Significance of differences		NS	NS	NS	NS	

NS = $P > 0.05$

Table 4. Development of haematological values in Experiment 2

Age (days)	Treatment	Hb (g/l)	Ht (l/l)	Ery (T/l)	MCH (pg)	MCV (fl)	Fe (mmol/l)
13	Ferridextran inj.	81 ± 10.7	0.29 ± 0.033	3.44 ± 0.343	24 ± 2.8	83 ± 7.4	6.7 ± 1.44
	Paste, lactate Fe	79 ± 9.5	0.28 ± 0.029	3.40 ± 0.397	24 ± 3.1	84 ± 9.8	6.0 ± 1.80
	Significance of differences	NS	NS	NS	NS	NS	NS
	Ferridextran inj.	79 ± 10.8	0.28 ± 0.037	3.57 ± 0.330	22 ± 2.7	79 ± 7.0	6.4 ± 1.68
	Paste, fumarate Fe	87 ± 7.8	0.31± 0.022	3.72 ± 0.333	23 ± 1.6	83 ± 6.6	7.5 ± 2.21
	Significance of differences	*	**	NS	NS	NS	NS
21	Ferridextran inj.	87 ± 13.4	0.27 ± 0.033	3.89 ± 0.489	22 ± 2.5	70 ± 5.4	8.1 ± 2.91
	Paste, lactate Fe	80 ± 12.6	0.26 ± 0.030	3.92 ± 0.417	20 ± 2.1	66 ± 5.4	8.0 ± 3.00
	Significance of differences	NS	NS	NS	**	*	NS
	Ferridextran inj.	94 ± 16.1	0.28 ± 0.035	4.26 ± 0.510	22 ± 2.4	65 ± 3.4	8.6 ± 2.91
	Paste, lactate Fe	94 ± 14.5	0.28 ± 0.031	4.25 ± 0.397	22 ± 2.2	66 ± 5.3	12.2 ± 4.46
	Significance of differences	NS	NS	NS	NS	NS	*

NS = $P > 0.05$, *$P < 0.05$, **$P < 0.01$

dose of lactate-bound iron apparently equalled those of Ferridextran. However, haematological values of the two groups were comparable only in 13-day-old piglets. MCV and MCH were significantly lower in 21-day-old piglets treated with lactate-bound iron. Also lower was Hb which is considered a significant indicator of iron availability (Devasthali *et al.*, 1991). However, the differences were nonsignificant owing to a high variance. It is therefore apparent that calculation of MCV and MCH can allow early recognition of shifts in haematological values. As stated by Dubanský *et al.* (1997a), the development of microcytary anaemia is one of the typical manifestations of iron deficiency.

The results of Experiment 2 further indicate that the growth promotion effect of oral administration of fumarate-bound iron was equal to that of parenteral administration of Ferridextran and had even stronger favourable effects on haematological values in 13-day-old piglets. The differences in most of the values disappeared towards the end of 3rd week. Nevertheless, this finding deserves attention considering the fact that the parenterally administered amount of iron was twice as high as the amount administered orally. This result was confirmed in Experiment 3 in which the oral treatment with 90 mg of lactate-bound iron was found equivalent to parenteral treatment with 200 mg of iron as far as weight gains and haematological values were concerned. No significant between-group differences were found in phagocytary activity of leukocytes. However, the causes of increases by 10% in 14-day-old and by 11.5% in 28-day-old piglets treated with the paste containing lactate-bound iron and *Chlorella* should be examined.

Our results contradict the generally accepted opinion that oral treatment with 100 mg of iron is not usually as effective as parenteral treatment with 200 mg of iron and the conclusion derived therefrom is that oral treatment should be repeated. In this connection, some differences in the absorption of iron ions in the organism should be emphasised. The key regulator of absorption and distribution of parenterally administered iron is the blood plasma protein transferrin which transports absorbed iron to bone marrow for direct consumption, i.e.

Table 5. Development of mean live weight (g) of piglets in Experiment 3

Treatment	n	Age (days) 1	14	28	Index
Ferridextran inj.	20	1 645 ± 389	4 635 ± 805	8 111 ± 1 252	100.0
Paste, lactate Fe	20	1 695 ± 276	4 716 ± 880	8 100 ± 1 493	99.9
Significance of differences		NS	NS	NS	

NS = $P > 0.05$

Table 6. Development of haematological values in Experiment 3

Age (days)	Treatment	Hb (g/l)	Ht (l/l)	Ery (T/l)	MCH (pg)	MCV (fl)
	Ferridextran inj.	99 ± 14.4	0.32 ± 0.037	4.15 ± 0.441	24 ± 1.9	77 ± 5.1
14	Paste, lactate Fe	93 ± 17.5	0.30 ± 0.053	4.06 ± 0.606	23 ± 2.1	74 ± 5.7
	Significance of differences	NS	NS	NS	NS	NS
Age (days)	Treatment	MCHC (g/l)	Fe (mmol/l)	Le (G/l)	F.A (%)	F.I.
	Ferridextran inj.	309 ± 15.9	12.9 ± 5.85	10.0 ± 2.32	38.0 ± 11.38	1.27 ± 0.746
14	Paste, lactate Fe	307 ± 17.5	8.2 ± 3.07	10.7 ± 2.42	37.6 ± 17.04	1.40 ± 1.161
	Significance of differences	NS	**	NS	NS	NS
Age (days)	Treatment	Hb (g/l)	Ht (l/l)	Ery (T/l)	MCH (pg)	MCV (fl)
	Ferridextran inj.	91 ± 16.0	0.31 ± 0.040	4.54 ± 0.500	20 ± 2.1	68 ± 3.8
28	Paste, lactate Fe	89 ± 18.5	0.30 ± 0.050	4.45 ± 0.675	20 ± 2.0	67 ± 4.3
	Significance of differences	NS	NS	NS	NS	NS
Age (days)	Treatment	MCHC (g/l)	Fe (mmol/l)	Le (G/l)	F.A (%)	F.I.
	Ferridextran inj.	293 ± 21.1	82 ± 3.31	13.1 ± 4.64	85.4 ± 15.6	8.50 ± 4.266
28	Paste, lactate Fe	298 ± 23.1	9.0 ± 5.40	12.0 ± 3.44	87.4 ± 8.77	9.80 ± 5.689
	Significance of differences	NS	NS	NS	NS	NS

**$P < 0.01$

incorporation into erythrocytes. Excess iron is then stored in the form of ferritin in the bone marrow, the liver and the spleen as a readily utilisable resource. The binding to the protein carrier is necessary because free iron ions would induce toxic effects on tissues and would be removed by glomerular filtration.

Orally administered iron is absorbed by mucosa of the cranial part of the small intestine. Enterocytes bring iron to the blood where it binds with transferrin or albumin. Its further fate is the same as that of parenterally administered iron (Huebers *et al.*, 1986). However, a part of the absorbed iron is retained in enterocytes in the form of reserve mucosal ferritin. Iron present in spontaneously desquamating enterocytes can be reabsorbed from the intestinal contents (Silbernagel and Despopoulos, 1993). These facts emphasise the extraordinary importance of normal intestinal functions in the utilisation of orally administered iron. This does not pertain only to the topical state at the time of treatment, but also to the subsequent four to seven days which are the period of physiological turnover of erythrocytes. During this period, iron bound in intestinal ferritin can be utilised, or lost if diarrhoea develops. These facts must be considered when deciding whether and when oral administration of an iron-containing paste is appropriate. Our decision to treat day-old piglets after several drinkings of colostrum was not a random choice. Le Dividich *et al.* (1997) demonstrated that colostrum intake induces an almost immediate increase in the weight of the small intestine and changes in its structure and functions. The highest relative gain occurred during the first six hours after birth and the major part of this gain comes to the intestinal mucosa. Longitudinal and in-the-width growth of intestinal villi increases the absorption area up to a twofold size. It is apparent that the intestinal mucosa of a day-old piglet can absorb iron. Later treatment is also possible, but we assume that intestinal disorders, which may develop in the postnatal period, affect the utilisation of orally administered iron. Such disorders may also have been the cause of discrepancies between data published by authors who investigated the efficacy of oral iron formulae.

REFERENCES

Devasthali S.D., Gordeuk V.R., Brittenham G.M., Bravo J.R., Hughes M.A., Keating L.J. (1991): Bioavailability of carbonyl iron: A randomized, double-blind study. Eur. J. Haematol., *46*, 272–278.

Dubanský V., Žižlavský M., Drábek J. (1997a): Myeloperoxidázová deficience selat vyvolaná nedostatečným přívodem železa po narození. Veterinářství, *47*, 205–209.

Dubanský V., Žižlavský M., Chloupek J. (1997b): Poruchy buněčné a humorální imunity selat v důsledku nesprávné aplikace Fe. Veterinářství, *47*, 413–417.

Huebers H.A., Brittenham G.M., Csiba E., Finch C.A. (1986): Absorption of carbonyl iron. J. Lab. Clin. Med., *12*, 473–478.

Le Dividich J., Tivey D., Blum J.W., Strullu F., Louat C. (1997): Effect of amount of ingested colostrum on the small intestine growth and lactase activity in the newborn pig. In: Digestive physiology of pigs. EAAP Publication No 88, St. Malo.

Silbernagl S., Despopoulos A. (1993): Atlas fyziologie člověka. Grada Avicenum, Praha. 352 s.

Received: 00–02–10

Accepted after corrections: 00–11–10

Corresponding Author:

Doc. Ing. Václav Kotrbáček, CSc., Veterinární a farmaceutická univerzita, Palackého 1–3, 612 42 Brno, Česká republika
Tel. +420 5 41 56 22 26

Effect of karyotype polymorphism on reproduction of arctic fox (*Alopex lagopus* L.)

Vliv karyotypového polymorfismu na reprodukci polární lišky (*Alopex lagopus* L.)

A. Filistowicz[1], P. Przysiecki[2], M. Zatoń-Dobrowolska[1], A. Zajączkowska[1], M. Świtoński[3]

[1]*Department of Genetics and Animal Husbandry, Agricultural University in Wrocław, Poland*

[2]*Fox Farm, Łubnica, Poland*

[3]*Department of Genetics and Animal Breeding, Agricultural University, Poznań, Poland*

ABSTRACT: Relations were defined between the values of reproductive traits of females (easiness and effectiveness of matings, reproduction maturity age, littering date, length of littering interval, number of pups born and reared in litter and pup losses during rearing) in successive reproduction seasons, considering polymorphic forms ($2n$ = 50, 49 or 48), caused by Robertsonian translocation, of karyotype of arctic fox females. The type of vagina mucus resistiveness curve in females during oestrus in successive reproduction seasons was additionally examined. Repeatability indexes of reproduction traits of females were low (0.191 – number of matings, 0.215 – number of pups born, 0.246 – number of pups reared, 0.248 – littering date, 0.277 – length of littering interval) and medium (0.434 – pup losses, 0.487 – type of vagina mucus resistiveness curve). The frequency of polymorphic karyotype forms in analysed animals differed from its average values on other farms and reached: 10.6% ($2n$ = 48), 38.2% ($2n$ = 49) and 51.2% ($2n$ = 50). Females with fusion karyotype ($2n$ = 48 and $2n$ = 49) were characterized by better reproductive performance, especially by better fecundity and lower pup losses.

Keywords: arctic fox; karyotype polymorphism; Robertsonian translocation; reproduction; *Alopex lagopus*

ABSTRAKT: Sledovali jsme závislosti mezi hodnotami reprodukčních znaků lišek (snadnost a efektivnost páření, věk při dosažení reprodukční zralosti, termín vrhu, délka intervalu mezi vrhy, počet liščat narozených a odchovaných na jeden vrh a ztráty liščat při odchovu) během po sobě jdoucích reprodukčních období, přičemž jsme brali v úvahu polymorfní formy ($2n$ = 50, 49 nebo 48) karyotypu samic polárních lišek způsobené Robertsonovou translokací. Dále jsme zjišťovali typ křivky měrného odporu vaginálního hlenu u lišek v říji během po sobě jdoucích reprodukčních období. Indexy opakovatelnosti reprodukčních znaků lišek byly nízké (0,191 – počet páření, 0,215 – počet narozených liščat, 0,246 – počet odchovaných liščat, 0,248 – termín vrhu, 0,277 – délka intervalu mezi vrhy) a střední (0,434 – ztráty liščat, 0,487 – typ křivky měrného odporu vaginálního hlenu). Četnost polymorfních karyotypových forem u sledovaného chovu se lišila od jejích průměrných hodnot u jiných chovů a dosáhla těchto hodnot: 10,6 % ($2n$ = 48), 38,2 % ($2n$ = 49) a 51,2 % ($2n$ = 50). Lišky s fúzí karyotypu ($2n$ = 48 a $2n$ = 49) dosahovaly lepší reprodukční účinnost, zejména vyšší plodnost a nižší ztráty liščat.

Klíčová slova: polární liška; karyotypový polymorfismus; Robertsonova translokace; reprodukce; *Alopex lagopus*

INTRODUCTION

Arctic fox is an important fur-bearing species. World annual production of arctic fox skins exceeds 4 mln pieces. In the arctic fox population, there exists karyotype polymorphism, caused by Robertsonian translocation, which includes two pairs of acrocentric chromosomes. The distribution of polymorphic karyotype forms of arctic fox in European countries is quite similar. The lowest percentage is represented by animals with 48 chromosomes (on average 21.6%, ranging from 9.8% to 26.2%) and the highest by animals with 49 chromosomes (on average 46.5%, ranging from 38.8% to 48.1%). The frequency of polymorphic forms in the Polish population of arctic fox was: 22.9% ($2n = 48$), 46.1% ($2n = 49$) and 31.0% ($2n = 50$) (Świtoński *et al*., 1991a).

Polish studies (Świtoński, 1981; Świtoński and Nowakowska, 1984; Świtoński *et al*., 1991b) showed that the females homozygous for the translocation ($2n$ = 48 chromosomes) delivered statistically larger litters whereas generally lower fertility characterized the heterozygote ($2n = 49$) ones. Similar results were obtained in Finland and Norway (Christensen and Pedersen, 1982; Mäkinen and Lohi, 1987) and Denmark (Christiansen *et al*., 1986), where larger litters were obtained from parents with karyotypes of 48 and 50 chromosomes than from heterozygous parents.

Norwegian studies (Möller *et al*., 1985) did not confirm the relation between the number of chromosomes and fertility of arctic foxes. Differences in the number of pups in litters from females of different karyotypes turned out not to be significant while the smallest litters were obtained from females of $2n = 48$ karyotypes and the largest from 50 chromosome karyotype females. Different results were presented by Parkanyi *et al*. (1988) in Slovakia; the largest litters were delivered by females of 49 chromosome karyotype, the smallest by females with $2n = 48$ chromosomes and the lowest number of reared pups was observed in females with 50 chromosomes.

This evident lack of univocal results made the authors undertake the studies on a relation between karyotype polymorphism and reproduction performance of arctic fox. It is presumed that the $2n = 48$ chromosome form, as evolutionary younger, should show advantage over the other polymorphic forms. On the other hand, its lower frequency in bred populations of arctic fox suggets that animals with $2n = 48$ chromosomes might be characterized by lower survivability or decreased fertility.

The scope of the study was to analyse fertility traits of females with karyotypes: $2n = 48$, $2n = 49$ and $2n = 50$ chromosomes in successive reproduction seasons, considering easiness and effectiveness of matings, littering dates, age of reproduction maturity, number of pups born and reared, pup losses during rearing and littering interval.

MATERIAL AND METHODS

Females of arctic fox, with known karyotype and origin, born, reared and used in reproduction (in 1985–1993) on fox farm Śniaty (RKS Łubnica), were included into analyses.

Breeding registers gave information about the following traits of each female: birth date, conformation note, number of matings, littering date (number of days since the beginning of the year), number of pups born and reared in litter in successive reproduction seasons. Additionally, age of reproduction maturity (days), littering interval (days) and pup losses recorded. Moreover a part of the females was included in analyses of the curve of electrical resistiveness of vagina mucus during the reproduction season, using an omometer. Measurements of electrical resistiveness of vagina mucus, conducted on four pre-oestrus days, during oestrus and on four post-oestrus days, allowed to determine the type of electrical resistiveness of vagina mucus in successive reproduction seasons. Four types of the curve were distinguished (Przysiecki *et al*., 1991): A – very high resistiveness on oestrus day, compared to pre- and post-oestrus time; B – clearly higher resistiveness on oestrus day, but decidedly lower than in type A; C – similar level of electrical resistiveness of vagina mucus on oestrus day and the days preceding the oestrus day and on the first post-oestrus days and D – nontypical curve, characterized by two days of high resistiveness (two peak curve). The type of vagina mucus resistiveness curve was expressed by a discrete scale: type A – 1 point, type B – 2 points, type C – 3 points and type D – 4 points.

Estimation of reproduction performance included: easiness and effectiveness of mating, type of vagina mucus resistiveness curve, littering date and number of pups born and reared, pup losses during rearing and littering interval. The estimation was performed separately in each of the first three reproduction seasons and coherently – for seasons 4th to 6th and coherently for all six seasons.

The effect of karyotype polymorphism on the estimation of female reproduction performance was determined using the following constant model:

$$Y_{ijk} = \mu + a_i + b_j + e_{ijk}$$

where: μ – mean trait value
a_i – effect of i birth season
b_j – effect of j polymorphic form
e_{ijk} – effect of random error

Repeatability for analysed traits of reproduction performance was assessed according to:

$$r = \sigma_c^2 / \left(\sigma_c^2 + \sigma_e^2\right)$$

where: σ_c^2 – variance of factors common for all individuals' performances
σ_e^2 – variance of effects specific for respective perfor mances of the individual

Variance components were estimated using REML (Restricted Maximum Likelihood Method). Calculations were performed using the VARCOMP procedure of SAS system (SAS, 1998), applying the mixed model:

$$Y_{ijklm} = \mu + a_i + b_j + s_k + c_{jkl} + e_{ijklm}$$

where: μ – population mean
a_i – constant effect of i year
b_j – constant effect of j polymorphic group
s_k – constant effect of k sire group

c_{jkl} – random effect of l individual within j polymorphic group and k sire group
e_{ijklm} – effect of random error

The estimated values of repeatability were transformed from discrete to continuous scale using probit transfiguration (Żuk, 1989).

RESULTS AND DISCUSSION

The frequency of polymorphic karyotype forms in the analysed herd (285 females) significantly differed from average distribution of these forms in other analysed populations (Świtoński *et al.*, 1991a). In the present study the frequency of polymorphic forms was as follows: 10.6% ($2n = 48$), 38.2% ($2n = 49$) and 51.2% ($2n = 50$).

Females with 48 chromosomes conceived for the first time significantly easier than females of other karyotypes (Table 1). In the first reproduction season, on average 1.63 matings were sufficient to conceive females with 48 chromosome karyotype while other females needed on average 2.05 and 2.09 matings, respectively. In the 2nd and 3rd reproduction seasons, more (but not significantly) matings were needed to conceive females with 49 chromosomes. In the first reproduction season, 90.0%, 78.0% and 76.0% of females with karyotypes $2n = 48$, $2n = 49$ and $2n = 50$, respectively, were conceived. In the second reproduction season, the highest percentage of conceived females (97.0%) was in the group of females with 50 chromosome karyotype and the lowest (92.5%) in the group of females with 49 chromosomes. In the third reproduction season, all females with 48 and 49 chromosomes and 98.0% of females with $2n = 50$ conceived. At older age (seasons 4–6), females with $2n = 48$ got pregnant harder than other polymorphic groups (Table 1), but all females from the three polymorphic groups were conceived. Totally, in 6 reproduction seasons, the lowest number (1.96) of matings needed to conceive was determined in females with karyotype $2n = 48$, which differed them significantly from females with $2n = 49$ karyotype and insignificantly from females with karyotype $2n = 50$. Females with 48 chromosomes showed the highest fertility during 6 reproduction seasons (92.5%) and females with 50 chromosomes the lowest (88.0%).

Table 2 shows the curves of vagina mucus resistiveness in successive reproduction seasons. It should be underlined that only a small number of females being used in the 80s was included in this study (Przysiecki *et al.*, 1991). In the first and third seasons, females with karyotype $2n = 48$ had vagina mucus resistiveness curves of type B and C, significantly more frequently than females of the other two polymorphic forms (Table 2). Due to these reasons, the mean value of vagina mucus resistiveness in females with 48 chromosomes over 6 seasons

Table 1. Mean values ($\bar{x}$) and standard deviations (SD) of the number of matings in successive reproduction seasons, considering the chromosome number

Season		Karyotype			Total
		$2n = 48$	$2n = 49$	$2n = 50$	
I	$\bar{x}$	1.63^{AB}	2.05^{A}	2.09^{B}	2.01
	SD	0.61	0.68	0.71	0.70
	N	30	109	146	285
II	$\bar{x}$	2.11	2.16	2.04	2.10
	SD	0.47	0.50	0.57	0.53
	N	18	56	65	139
III	$\bar{x}$	2.12	2.27	2.10	2.17
	SD	0.34	0.55	0.54	0.52
	N	16	40	50	106
IV–V	$\bar{x}$	2.20	2.15	2.13	2.15
	SD	0.41	0.46	0.52	0.47
	N	20	53	54	127
I–VI	$\bar{x}$	1.96^{A}	2.13^{A}	2.09	2.08
	SD	0.55	0.58	0.62	0.60
	N	84	258	315	657

N = group size
A = mean values in rows denoted by the same letters differ significantly at $P \leq 0.05$

Table 2. Mean values ($\bar{x}$) and standard deviations (SD) of the type of vagina mucus resistiveness curve in successive reproduction seasons, considering the chromosome number

Season		Karyotype			Total
		$2n = 48$	$2n = 49$	$2n = 50$	
I	$\bar{x}$	1.73^{AB}	1.25^{A}	1.24^{B}	1.32
	SD	0.80	0.49	0.48	0.56
	N	15	40	46	101
II	$\bar{x}$	1.57	1.35	1.64	1.53
	SD	0.79	0.49	0.84	0.73
	N	7	20	31	58
III	$\bar{x}$	1.67^{AB}	1.29^{A}	1.23^{B}	1.29
	SD	0.71	0.59	0.43	0.48
	N	6	17	22	45
IV–V	$\bar{x}$	1.50	1.51	1.42	1.46
	SD	0.55	0.60	0.58	0.57
	N	6	22	26	54
I–VI	$\bar{x}$	1.56^{AB}	1.33^{A}	1.37^{B}	1.38
	SD	0.76	0.53	0.62	0.60
	N	34	99	125	258

N = group size
A = mean values in rows denoted by the same letters differ significantly at $P \leq 0.05$

differed significantly from the mean values of the other two polymorphic forms (Table 2). Type A of vagina mucus resistiveness curve allows to find an accurate date of mating (Przysiecki *et al.*, 1991). Nevertheless, significant differentiation between the polymorphic forms (Table 2) suggests that females with $2n = 48$ chromosomes, which had less typical curves, also got pregnant easily and in the first season and totally in all six reproduction seasons, got pregnant significantly more easily than females with the other two karyotypes (Table 1).

All three groups of females were characterized by good fecundity (Table 3). The smallest litters were delivered by primiparous females. When including sterile and aborting females (3 females with 48 chromosomes, 24 with 49 chromosomes and 35 with 50 chromosomes), mean litter sizes delivered by primiparous females differed significantly and were: 7.83, 6.70 and 6.44 pups born per litter. Mean sizes of litters delivered by older females were significantly larger than in primiparous females (Table 3), which was caused by intensive culling of females with insufficient fertility and fecundity after the first two reproduction seasons, especially after the first one. After the first reproduction season, 12 (49.0%), 53 (48.6%) and 80 (54.8%) of females with karyotypes of 48, 49 and 50, respectively were fit for further breeding.

The higher values of litter size delivered by females with 48 chromosomes than by females with 49, and especially, with 50 chromosomes were not confirmed statistically, mostly due to high variability of the trait (high SD values) in all three polymorphic groups.

Mean numbers of pups (Table 4) reared by primiparous females with 48, 49 and 50 chromosomes were: 5.63, 6.17 and 5.61. When calculated per one breeding female, the values were 5.07, 4.82 and 4.27. Rate of culling after the first and second season clearly improved the mean size of litters reared in successive reproduction seasons. In the third season and in subsequent seasons (4–6) and totally over six seasons, females with 48 chromosomes reared significantly more pups than their contemporaries with 50 chromosomes (Table 4). Clear differentiation of litter size in the third and subsequent reproduction seasons was caused mostly by the improvement of pup rearing indexes in litters of all females in successive reproduction seasons.

Primiparous females started oestrus distinctly later than older females, which is documented by littering dates (Table 5). Generally, females with 48 chromosomes started oestrus earlier than their contemporaries with other chromosome numbers in each reproduction season, but the differences were not confirmed statistically.

Table 6 shows the values of repeatability of reproductive traits, before and after probit transformation. A comparatively low, but clearly differing from zero, value of repeatability characterized: number of matings, number of pups born and reared, littering date and length of littering interval whereas higher repeatability was estimated for pup rearing losses and type of vagina mucus resistiveness curve. Repeatability values show that early culling of females with insufficient fertility and fecundity is effective and also, that selection based on the original litter size is reasonable and might favour effective selection towards fecundity improvement.

Comparison of the results presented in this study with quite divergent results of other authors suggests a distinct relation between polymorphic karyotype forms and reproductive performance, but their expression

Table 3. Mean values ($\bar{x}$) and standard deviations (SD) of the number of pups delivered by fertile females (a) and calculated per one breeding female (b) in successive reproduction seasons, considering the chromosome number

Karyotype		Season I		Season II		Season III		Seasons IV–VI		Seasons I-VI	
		a	b	a	b	a	b	a	b	a	b
$2n = 48$	$\bar{x}$	8.70	7.83	10.00	9.44	10.56	10.56	10.25	10.25	9.38	9.27
	SD	(4.59)	(5.09)	(3.33)	(4.00)	(3.63)	(3.63)	(3.27)	(3.27)	(3.85)	(4.30)
	N	27	30	17	18	16	16	20	20	80	84
$2n = 49$	$\bar{x}$	8.60	6.70	9.69	9.00	10.37	10.37	9.53	9.53	9.37	8.35
	SD	(3.59)	(4.78)	(2.99)	(3.82)	(3.23)	(3.23)	(2.60)	(2.60)	(3.23)	(4.22)
	N	85	109	52	56	40	40	53	53	230	258
$2n = 50$	$\bar{x}$	8.48	6.44	9.30	9.01	10.00	9.80	9.00	9.00	9.04	7.95
	SD	3.46	(4.72)	(3.13)	(3.47)	(3.17)	(3.44)	(3.24)	(3.24)	(3.230	(4.29)
	N	111	146	64	66	49	50	54	54	278	316
Total	$\bar{x}$	8.55	6.69	9.54	9.06	10.23	10.13	9.42	9.42	9.26	8.28
	SD	(3.65)	(4.78)	(3.09)	(4.00)	(3.24)	(3.37)	(3.00)	(3.00)	(3.37)	(4.28)
	N	223	285	133	140	105	106	127	127	588	658

N = group size

Table 4. Mean values ($\bar{x}$) and standard deviations (SD) of the number of pups delivered by females littering (a) and calculated per one breeding female (b) in successive reproduction seasons, considering the chromosome number

Karyotype		Season I a	Season I b	Season II a	Season II b	Season III a	Season III b	Seasons IV–VI a	Seasons IV–VI b	Seasons I-VI a	Seasons I-VI b
2n = 48	$\bar{x}$	5.63	5.07	8.59	8.11	9.69^A	9.69^A	8.60^A	8.60^A	7.81^A	7.44^A
	SD	(4.74)	(4.56)	(2.60)	(3.23)	(3.61)	(3.61)	(2.28)	(2.28)	(3.78)	(4.05)
	N	27	30	17	18	16	16	20	20	80	84
2n = 49	$\bar{x}$	6.17	4.82	8.15	7.57	8.75^B	8.75^B	7.96^B	7.96^B	7.48^B	6.67^B
	SD	(3.52)	(4.03)	(2.75)	(3.39)	(2.94)	(2.94)	(2.51)	(2.51)	(3.19)	(3.81)
	N	85	109	52	56	40	40	53	53	230	258
2n = 50	$\bar{x}$	5.61	4.27	7.76	7.53	7.84^A	7.68^A	6.85^A	6.85^A	6.76^A	5.97^A
	SD	(3.39)	(3.81)	(2.90)	(3.16)	(3.04)	(3.21)	(2.60)	(2.60)	(3.22)	(3.73)
	N	111	146	64	66	49	50	54	54	278	316
Total	$\bar{x}$	5.83	4.56	8.02	7.62	8.47	8.39	7.59	7.59	7.18	6.41
	SD	(3.58)	(3.97)	(2.80)	(3.25)	(3.14)	(3.23)	(2.59)	(2.59)	(3.31)	(3.83)
	N	223	285	133	140	105	106	127	127	558	658

N = group size
A = mean values in rows denoted by the same letters differ significantly at $P \leq 0.05$

probably depends on the level of herd performance and on direction and intensity of selection.

From the genetical point of view, there are two probable reasons of polymorphic effect of karyotype on reproductive performance of arctic fox. First, females heterozygous for the translocation might show disturbances in chromosome segregation in anaphase of meiosis I, as it was shown for cows and bulls with 59 chromosomes (Gustavsson, 1969; Refsdal, 1976; Dyrendahl and Gustavsson, 1979). This effect had no place in our studies and was not revealed in previous works. On the contrary, Parkanyi *et al.* (1988) and

Table 5. Mean values ($\bar{x}$) and standard deviations (SD) of littering dates of females in succession reproduction seasons, considering the chromosome number

Season		Karyotype 2n = 48	2n = 49	2n = 0	Total
I	$\bar{x}$	145.93	149.15	147.01	147.70
	SD	17.86	17.30	16.45	16.93
	N	29	89	113	231
II	$\bar{x}$	134.53	135.58	133.69	134.53
	SD	12.49	13.35	11.78	12.44
	N	17	52	64	133
III	$\bar{x}$	134.69	135.35	136.65	135.86
	SD	14.11	11.11	11.04	11.49
	N	16	40	49	105
IV–VI	$\bar{x}$	136.75	138.77	140.76	139.30
	SD	9.46	11.40	16.19	13.39
	N	20	53	54	127
I–VI	$\bar{x}$	139.13	141.42	140.94	140.88
	SD	15.01	15.49	15.52	15.43
	N	82	234	280	596

N = group size

Table 6. Values of repeatability coefficients of traits characterising the female reproduction performance

Trait	Repeatability coefficient	
	estimated	after probit transformation
Number of matings	0.147	0.191
Type of vagina mucus resistiveness curve	0.326	0.487
Littering date	0.248	0.248
Number of pups born	0.215	0.215
Number of pups reared	0.246	0.246
Pup losses	0.325	0.277
Length of littering interval	0.277	0.434

Möller *et al.* (1985) reported that females with 49 chromosomes had better reproductive performance than females with $2n = 48$ or 50.

The second aspect, related with karyotype polymorphism, is associated with potential gene linkage caused by centric fusion. The linkage of genes coming from two acrocentric chromosomes, which in karyotype $2n = 50$ segregate independently, might result in the fact that karyotype with fusion ($2n = 48$ or $2n = 49$) might be advantageous in the case of selection towards traits controlled by these linked genes. Numerous studies, which indicated distinctly better fertility and fecundity of females carrying 48 chromosomes (Świtoński, 1981; Świtoński and Nowakowska, 1985; Świtoński *et al.*, 1991b; Christensen and Pedersen, 1982; Mäkinen and Lohi, 1987; Christiansen *et al.*, 1986) or 49 chromosomes (Möller *et al.*, 1985; Parkanyi *et al.*, 1988), and the results of our study (Tables 1–5) support this explanation. If we appreciate that selection towards fertility and fecundity, at least partly effective, has been conducted in the analysed herd for several years, then better fertility and fecundity of both groups with centric fusion (Tables 1–5) is another argument for this speculation. However, the assumed explanation was not supported by distribution of the polymorphic forms, since the highest frequency was of female with $2n = 50$.

The newest results in mapping human, dog, silver fox and arctic fox genomes are very helpful in searching of genes potentially influencing reproductive performance. Yang *et al.* (2000) performed comparative studies on chromosome localisation of microsatellite markers in dog and silver fox genomes. But specially important is the method of comparative chromosome painting, which allows to reveal existing homology between chromosomes of humans, dog, arctic fox and silver fox (Yang *et al.*, 2000; Graphodatsky *et al.*, 2000). It has been shown that human chromosomes 23 and 24 correspond to chromosome 15 and part of chromosome 1 of the dog (Graphodatsky *et al.*, 2000). Canine chromosome 1 contains fragments homologous to parts of human chromosomes 6, 9, 18 and 19, while dog chromosome 15 includes fragments homologous to parts of human chromosomes 1, 4 and 12 (Yang *et al.*, 1999). These results allow to analyse also human genes, the presence of which is expected in chromosomes 23 and 24 of arctic fox, in the context of their potential effect on traits associated with reproduction.

CONCLUSIONS

The frequency of polymorphic karyotype forms (10.6% – $2n = 48$; 38.2% – $2n = 49$ and 51.2% – $2n = 50$) in the analysed animals distinctly differed from distribution of these forms in animals on other farms. Females homozygous for the centric fusion ($2n = 48$) and heterozygous ones ($2n = 49$), were characterised by better reproductive performance.

The authors tend to explain better fertility and fecundity of females with centric fusion by a favourable group of linked genes, coming from two acrocentric chromosomes, which segregate independently during oogenesis in females with 50 chromosomes. Karyotypes carrying fusion ($2n = 48$ and $2n = 49$) turned out to be more favourable in the analysed herd, in which for years selection has been based on birth date and original litter size and where the main criterion of female culling was lowered fertility, insufficient fecundity and big losses of pups.

REFERENCES

Christensen K., Pedersen H. (1982): Variation in chromosome number in the blue fox (*Alopex lagopus*) and its effect on fertility. Hereditas, *97*, 211–215.

Christiansen I., Lohi O., Cleeman-Mitchell T., Norgaard Clausen T., Therkildsen V. (1986): Reproduction of blue foxes (*Alopex lagopus*) with different number chromosomes. Acta Agric. Scand., *37*, 335–339.

Dyrendahl I., Gustavsson I. (1979): Sexual functions, semen characteristics and fertility of bulls carrying 1/29 chromosome translocation. Hereditas, *90*, 281–289.

Graphodatsky A.S., Yang F., O'Brien P.C.M., Serdukova N., Milne B.S., Trifonov V., Ferguson-Smith M.A. (2000): A comparative chromosome map of the arctic fox, red fox and dog defined by chromosome painting and high resolution G-banding. Chromosome Res., *8*, 253–263.

Gustavsson I. (1969): Cytogenetics, distribution and phenotypic effects of a translocation in Swedish cattle. Hereditas, *63*, 68–169.

Mäkinen A., Lohi O. (1987): The litter size in chromosomally polymorphic blue fox. Hereditas, *107*, 115–119.

Möller O.M., Nes N.N., Syed M., Fougner J.A., Norheim K., Smith A.J. (1985): Chromosomal polymorphism in the blue

fox (*Alopex lagopus*) and its effects on fertility. Hereditas, *102*, 159–164.
Parkányi V., Rafay J., Jakubička I., Bárta M. (1988): Chromozómovy polymorfizmus polárnych líšok (*Alopex lagopus*) vo vzťahu k životaschopnosti mláďat. Ved. Práce VÚŽV Nitra, *XXIII*, 123–131.
Przysiecki P., Łoś P., Filistowicz A. (1991): Powtarzalność kształtu krzywej oporności elektrycznej śluzu pochwowego samic lisa polarnego. Zesz. Nauk. Przegl. Hod. *5*, 77–83.
Refsdal A.O. (1976): Low fertility in daughters of bulls with 1/29 translocation. Acta Vet. Scand., *17*, 190–195.
SAS/STAT. (1988). User's Guide. Release 6.03. Edition SAS Institute Inc., Cary, NC, USA.
Świtoński M. (1981): Robertson translocation in the blue fox (*Alopex lagopus*) and its effect on the fertility, Genet. Polon., *22*, 463–474.
Świtoński M., Nowakowska T. (1984): Wpływ polimorfizmu kariotypowego na fenotyp i rozród lisa polarnego, Prace Kom. Nauk Rol. i Kom. Nauk Leśnych, *LVII*, 293–298.
Świtoński M., Lechniak D., Przysiecki P., Filistowicz A., Pietrzak A., Landzwojczak D. (1991a): Ocena przydatności badań nad polimorfizmem kariotypowym w selekcji lisów polarnych. I. Rozprzestrzenienie form polimorficznych oraz ich związek z oceną licencyjną, indeksem osobniczym i tempem wzrostu masy ciała. Zesz. Nauk. Przegl. Hod., *5*, 11–17.
Świtoński M., Lechniak D., Przysiecki P., Filistowicz A., Pietrzak A., Landzwojczak D. (1991b): Ocena przydatności badań nad polimorfizmem kariotypowym w selekcji lisów polarnych. II. Związek z cechami reprodukcyjnymi. Zesz. Nauk. Przegl. Hod. *5*, 18–24.
Yang F., O'Brien P.C.M., Milne B.S., Graphodatsky A.S., Solanky N., Trifonov V., Rens W., Sargan D., Ferguson-Smith M.A. (1999): A complete comparative chromosome map for the dog, red fox and human and its integration with canine genetic maps. Genomics, *62*, 189–202.
Yang F., Milne B.S., Schelling C., Dolf G., Schlapfer J., Świtoński M., Ładoń D., Pieńkowska A., Bosma A.A., Sargan D.R., Ferguson-Smith M.A. (2000): Chromosome identification and assignment of DNA clones in the dog using a red fox and dog comparative map. Chromosome Res., *8*, 93–100.
Żuk B. (1989): Biometria stosowana. PWN, Warszawa.

Received: 00–08–23
Accepted after corrections: 00–11–10

Corresponding Author:

Prof. dr hab. Andrzej Filistowicz, Akademia Rolnicza, Katedra Genetyki i Ogólnej Hodowli Zwierząt, Kożuchowska 7, 51-631 Wrocław, Polska
Tel. +71 3 20 57 57, fax +71 3 20 57 58, e-mail: andrzej@gen.ar.wroc.pl

Modulation of heterotrimeric GTP-binding proteins in immune system and brain

Modulace heterotrimerních GTP-vazebných proteinů v imunitním systému a mozku

H. Kovářů[1,2], A. Fišerová[3], F. Kovářů[4], M. Pospíšil [3], V. Lisá[5]

[1]*Charles University, 1st Medical Faculty, Prague, Czech Republic*
[2]*Czech University of Agriculture, Prague, Czech Republic*
[3]*Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic*
[4]*University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic*
[5]*Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic*

ABSTRACT: We used both *in vitro* and *in vivo* models to study immuno- and neuroimmunomodulator effects on cell signalling at the cellular or organ level of brain (glioma cells) or lymphoid systems. We studied immunostimulators – interleukin 2 (IL2) or ergot alkaloid agroclavine, dopaminergic (D1) agonist, in comparison with psychotropic drugs – antidepressant fluoxetine or citalopram of selective serotonin reuptake inhibitor group (SSRI). We assayed signal transduction via heterotrimeric GTP- binding (G) proteins, and their α subunits Gαq/11, Gαs and Gαi1,2 as well as Gβ subunit levels. The profiles of Gα protein subunits were analyzed using rat natural killer (NK) lymphocytes of RNK16 cell line (CD45+/–), rat C6 glioma cell line and C6 glioma cells transfected (t) with lymphocyte phenotypic markers, prepared by us. We demonstrated similar inhibitory effects of IL2, agroclavine or fluoxetine on RNK16 CD45(–) and C6 glioma cell Gαq/11 subunit levels. Furthermore, adenosine receptor agonist [5′-(N-ethylcarboxamido) adenosine, NECA] effects on Gα subunit profiles of C6 glioma- and RNK16 CD45(–) cells were comparable with fluoxetine action. Different Gα profiles of (t)C6 glioma cells or RNK16 CD45(+) lymphocytes were detected, supporting the idea about microdomain rearrangement role in G protein mediated cell signalling. Finally, antidepressant citalopram induced Gα profile changes of both C6 glioma cells and brains of *in vivo* treated rats were comparable. Furthermore, citalopram induced changes of Ga profiles in the rat brain and the spleen were similar in contrast to distinct thymus response. We can conclude our *in vitro* and in *vivo* study, based on various experimental approaches, that the immune system and the brain can share common properties of molecular regulations during neuro-immunomodulator signal transduction.

Keywords: cell signal transduction; neuroimmunomodulation; G proteins

ABSTRAKT: Na modelu potkana *in vitro* a *in vivo* byly studovány změny hladin heterotrimerních GTP-vazebných (G) proteinů. Byl studován vliv imunostimulačního interleukinu 2 (IL 2) nebo agroklavinu, námelového alkaloidu, a také působení psychofarmak – antidepresiv fluoxetinu nebo citalopramu ze skupiny selektivních inhibitorů serotoninového reuptake (SSRI). Byly sledovány další cíle: 1. profily Gα subjednotek buněk jak mozku (C6 gliomových), tak imunitního systému (přirození zabíječi – NK, linie RNK16) po ovlivnění antidepresivem *in vitro*, vedle působení agonisty adenosinového receptoru (5′-N-etylkarboxamido-adenosin, NECA); 2. možnost alterace exprese Gα subjednotek po působení modulátoru na modifikované povrchové struktury buněčných linií C6 gliomových buněk transfekcí (t) povrchovými znaky lymfocytů [(t) C6 gliomové buňky], tak i RNK16 linie s deficitním CD 45 znakem vedle RNK16 CD45(+). Prokázali jsme podobný inhibiční vliv IL2, agroklavinu a také fluoxetinu zvláště na Gαq/11 subjednotku RNK16 CD 45(–) a C6 gliomových buněk. Působení fluoxetinu nebo NECA na pokles Gαq/11 subjednotky bylo srovnatelné u RNK16 CD45(–) i C6 gliomových buněk. Profily Gα subjednotek u (t) C6 gliomových a RNK16CD45(+) byly odlišné po působení modulátorů vzhledem k výchozím liniím. Byly také prokázány podobnosti v expresi zvláště Galfa (s) subjednotek jak u C6 gliomových buněk, tak i *in vivo* v mozku a slezině vyvolaných citalopramem. Lze uzavřít, že uvedené neuro-imunomodu-

Supported by the Grant Agency of the Czech Republic (Grants No. 310/98/0347, 312/98/K034 and 304/01/0850), MSM 111100001, MSM J03/98:412100003, Int. Sci. Progr. CEZ: J16/98:161700001 FVL VFU Brno.

látory mohou působit podobné změny při přenosu buněčného signálu G proteiny do funkčně odlišných buněk nebo orgánových systémů. Na přenosu signálu v kaskádě z receptoru přes G proteiny na efektorové systémy se podílí i další kooperující membránové struktury.

Klíčová slova: přenos buněčného signálu; neuroimunomodulace; G proteiny

INTRODUCTION

It is evident that cell cultures represent alternative bioassay models to animal experiments. *In vitro* experiments employing established cell lines are used in various immunological, neurobiological or psychopharmacological studies to simulate physiological states. There are many types of effector cells in the immune system, but our attention is focused on natural killer (NK) cells as the most sensitive effector of the immune response. Furthermore NK cells can be involved in pathophysiological mechanisms induced by stress or depression, when NK cytotoxic activity or NK cell levels are decreased (Reynaert *et al.*, 1995). On the other hand, interleukin 2 (IL2) stimulates also NK cell mediated immune events (Al-Aoukaty *et al.*, 1996). Ergot alkaloid agroclavine, dopamine (D1) receptor agonist, participates in stimulatory effects on NK lymphocytes (Fišerová *et al.*, 1997). On the other hand, C6 glioma (astrocytoma) cell line of rat origin is used in neurobiological and psychopharmacological studies as a model of postsynaptic changes induced by psychotropic drugs such as antidepressants (Chen and Rasenick, 1995). Antidepressant effects on C6 glioma cells are studied in acute (up to 24 h) or in chronic assays, representing drug treatment for 5 days, which is adequate to antidepressant administration to rats for 3 weeks (Chen and Rasenick, 1995).

The mentioned immunomodulators or antidepressants represent external signals for cells. Signal transduction by GTP- binding proteins (G proteins) is one of the most widely recognized mechanisms of information signal transfer across the cell membrane induced by first messengers, hormones, cytokines, psychoactive drugs, etc. G proteins are membrane- associated heterotrimeric proteins composed of α and $\beta\gamma$ subunits. They couple membrane receptors (seven transmembrane spanning types) to ligands, bioactive molecules, and G proteins affect effector systems directly, then the signal is processed via signalling pathways within the cell (Spiegel, 1996). Thus various Gα subunits regulate cell signal transduction pathways via effector enzymes, adenylyl cyclase (Gαs, Gαi1,2) and phospholipase C (Gαq/11) (Milligan, 1989, 1993; Spiegel, 1996). Functionally important is Gα subunit with intrinsic GTPase activity but now β subunit and its active role in signalling are suggested in relation to phospholipase C (PLC) (Bristol and Rhee, 1994).

In the family of G protein-coupled receptors, adenosine receptors (P1 purinoceptors) are involved (Lorenzen, 1997; Jacobson, 1998). In both immune system and CNS, adenosine is involved in regulation of growth and differentiation events (Rathbone *et al.*, 1992). Anxiolytic activity and psychiatric or neurological relationship to modulated adenosine receptors have been described (Lorenzen, 1997).

Our aim was to study in vitro effects of both immunostimulators and neuromodulators – psychoactive drugs. We estimated effects of neuroimmunomodulators (IL2 or agroclavine) or antidepressant fluoxetine or citalopram (selective serotonin reuptake inhibitors, SSRI) on Gα and Gβ subunit profiles of rat C6 glioma cells or rat NK lymphocytes of RNK 16 line, CD45(+). Common leucocyte phenotypic CD 45 marker is phosphatase, regulating signal information processed by protein dephosphorylations. Furthermore we used cell lines with transfected (t) lymphocyte markers, (t) C6 glioma cells, or RNK 16, CD45 deficient to study the microdomain arrangement effect on G protein-linked signalling. Possible role of adenosine receptor agonist (5′-N-ethylcarboxamido-adenosine, NECA) on these events was also analysed. Comparative study of antidepressant effect *in vitro* and *in vivo* using a rat model was performed to study changes in the brain, spleen and thymus.

MATERIAL AND METHODS

Animals

We used male Wistar rats with initial weight 180–200 g fed standard ST1 diet and water *ad libitum*. An antidepressant was administered orally each day (5mg/kg) for 3 weeks.

C6 glioma cells

Cells were cultured in MEM medium, pH 7.3 with 5% fetal calf serum under standard conditions. Confluent cultures were exposed to an antidepressant (final 1 µM) for 24 h (acute model) or 5 days (chronic model). Then the cells were washed with PBS, harvested by scraping with rubber policeman in PBS, containing proteolytic inhibitors and cholate membrane extracts were prepared as described previously (Mareš *et al.*, 1991; Kovářů *et al.*, 1997, 1998a).

Transfected (t) C6 glioma cells. The (t) C6 glioma cells were prepared by fusion of original C6 glioma cells with lymphocyte cell surface phenotypic markers from a microsomal fraction of pig thymus origin (postnatal day 60). Fusion was performed by PEG method (Schramm, 1978). Cells were stabilized up to 85th passage and assayed for inserted lymphocyte determinants every two weeks (Kovářů *et al.*, 1994, 1998c). Treatment of cells was the same as described for original C6 glioma cells.

NK cells

RNK 16, CD45(+) and RNK 16 CD45(–) mutant NK cell line were derived from spontaneous rat leukemia (F344 strain) and were a gift of Dr. Reynolds (NIH, Bethesda). Cells were cultured under standard conditions in supplemented RPMI 1640 medium and 10% fetal calf serum. The presence of CD45 membrane marker on the cell line was repeatedly checked by fluorescence activated cell sorter. The modulators were added to cell suspensions (2.10^6/ml) in the following final concentrations: r IL2 – 100 U/ml (Sigma), 1μM agroclavine (Galena, Czech Republic) and 1μM fluoxetine (Elli Lily), 0.1 μM NECA (Sigma) for 17 hours. Cholate membrane extracts of cell cultures were prepared as described above. For other details see (Fišerová *et al.*, 1997; Kovářů *et al.*, 1997).

Gα subunit estimation

Gα changes were analyzed by ELISA technique with our rabbit antibodies to C-terminal dekapeptides of α chains of Gs, Gi1,2 and Gq/11 (Milligan, 1988, 1993) as a commonly used immunoassay based on proportional comparison of various G alpha subunits. ELISA method of competitive inhibiton was performed (Ransnas and Insel, 1989; Lesch and Manji, 1992) and modified by us using Maxisorp microtitration plates (NUNC) for noncovalent peptide binding. ELISA estimations were compared with Western immunoblotting (Kovářů *et al.*, 1997, 1998a).

Statistical analysis

The data are expressed as mean values ± S.D. The differences between experimental samples were evaluated by Student's *t*-test for unpaired values.

RESULTS

Figure 1 summarizes Gα profiles of both RNK16 CD45 (+) and RNK16 CD45(–) cells when they were influenced by IL2 or agroclavine. Both IL2 or agroclavine affected a marked decrease of Gαq/11 (IL2 $p = 0.05$, agroclavine $p < 0.05$) and elevation of Gαs subunits of RNK16 CD45 (–) ($p < 0.05$). On the other hand, agroclavine induced an increase of Gαq/11 ($p = 0.05$) and Gαs levels of RNK16 CD45 (+) ($p < 0.01$). Results show that the increase of Gαs in all assays is not CD45 marker dependent. Besides this, minor changes contribute to total shape of Gα–Gβ subunit profiles of both cell models induced by IL2 and agroclavine.

Figure 2 summarizes effects of fluoxetine and NECA on C6 glioma or (t) C6 glioma cells (acute model) and lymphocytes of RNK16 CD45(–) line. We estimated fluoxetine or NECA induced decrease of Gαq/11 level of

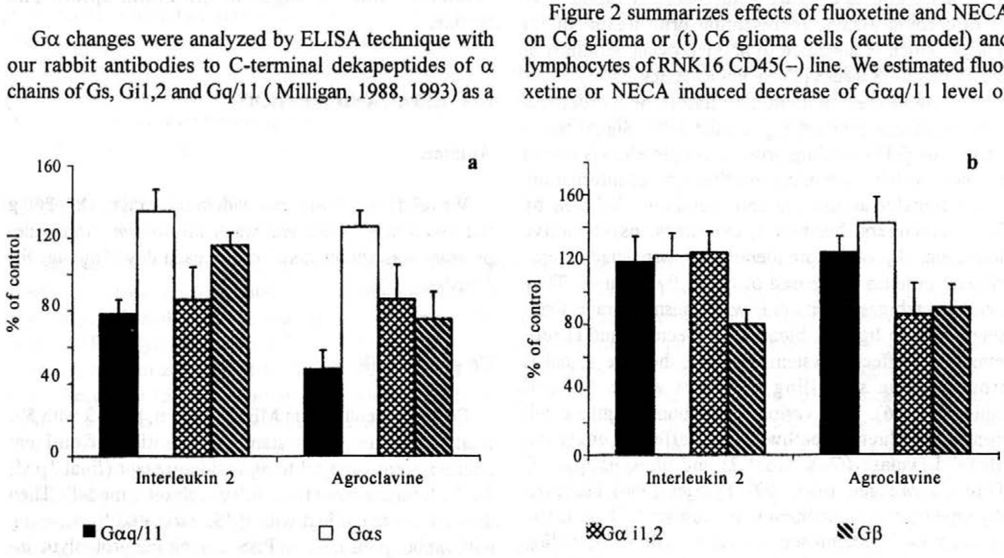


Figure 1. Effect of immunomodulators on RNK16 cells C45(–) – a) and RNK 16 CD45(+) – b) on Gα and Gβ subunit profiles; each result is the mean of 7–9 ELISA measurements in quadruplicates

both C6 glioma ($p < 0.05$) and RNK16 CD45(–) cells (fluoxetine $p < 0.05$, NECA $p = 0.05$) in contrast to slightly changed Gαq/11 or stimulated Gas subunit levels ($p < 0.05$) of (t)C6 glioma cells. Fluoxetine induced increase of C6 glioma cell Gβ level ($p < 0.01$) and no change of Gβ subunit of (t)C6 glioma cells was found.

We analyzed certain lymphocyte phenotypic markers on (t) C6 glioma cells by fluorescence activated cell sorter analysis with the following results (%): CD4 9.8, MHC II 9.7, pan T 16.1 whereas background of original C6 glioma cells was at the range 0–0.5%. Modified (t)C6 glioma cells were CD2 or CD8 lymphocyte marker negative.

Comparative analyses of Gα profiles, summarized in Figure 3, show citalopram effects (SSRI antidepressant) on *in vitro* and *in vivo* brain, spleen and thymus. *In vitro* and *in vivo* analyses of citalopram induced changes in C6 glioma cells or rat brain exhibited a similar increase of Gαs subunit level ($p < 0.05$). The citalopram induced effect was similar in brain and spleen Gαs subunit profiles ($p < 0.05$). A different response in both lymphoid organs was evident, i.e. spleen Gα subunit profile, as a secondary lymphoid organ was quite different from Ga changes in the thymus as a primary lymphoid organ.

DISCUSSION

Immunostimulator effects on RNK 16 cells. We demonstrated immunostimulator (IL2 or agro-clavine) effects on a marked decrease of Gαq/11 level in transmembrane signalling of RNK16 CD45 deficient (–) cells in contrast to elevated Gαq/11 subunit of RNK16, CD45(+). Thus the results can indicate that Gαq/11 subunit changes could be related to some influence by CD45 phenotypic marker, whereas the increased Gα(s) level in both cell systems was not probably CD45 dependent. The CD45 marker is phosphatase, regulating protein dephosphorylations and phosphatase inhibitors involve also okadaic acid (Ramamoorthy *et al.*, 1998). In our preliminary experiments, we studied okadaic acid effects on an immunostimulator (agroclavine or IL2) influencing Gα subunit expression of RNK 16 CD45(+/–) cells in possible relation to the CD45 role. In spite of okadaic acid evoked differences in Gα subunit levels in both cell line subtypes the problem is not resolved and membrane and cytosolic proportion of okadaic acid induced inhibitions could be assayed (Kovářů and Fišerová, in preparation).

Antidepressant effects on RNK 16 and C6 glioma cells. The fluoxetine effect on C6 glioma cells or RNK 16 CD45(–) was associated with markedly decreased Gαq/11 level. On the other hand, transfected (t) C6 glioma cells and RNK16 CD45 (+) displayed different Gα changes. Differences in Gα subunit profiles between fluoxetine evoked C6 glioma- and (t) C6 glioma cells as well as RNK16 CD45(+) vs CD 45(–) cells as postreceptor events can be related to altered Gα subunits containing membrane microdomains. Differences between fluoxetine evoked C6 and (t) C6 glioma cell Gα subunit profiles as postreceptor events can be related to altered Gα subunit containing membrane microdo-mains, as suggested recently (Kovářů *et al.*, 2000).

The effect of fluoxetine on lower membrane Gαq/11 content of C6 glioma cells was confirmed by both translocation of this subunit into cytosol and decreased 1,4,5 IP3 formation by phospholipase C of C6 glioma cells (Kovářů, 1997, 1998b). Effector phospholipase C is regu-

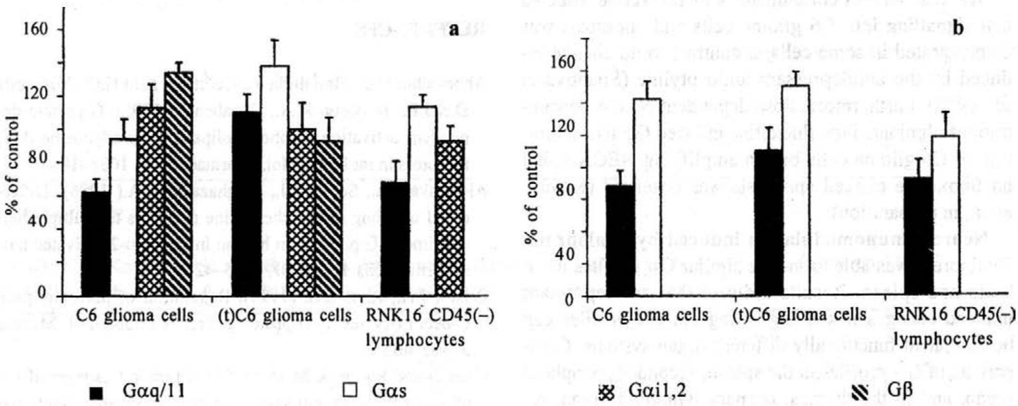


Figure 2. Effect of fluoxetine – a) and NECA – b) on Gα and Gβ subunit profiles; each result is the mean of 8–10 ELISA measurements in quadruplicates

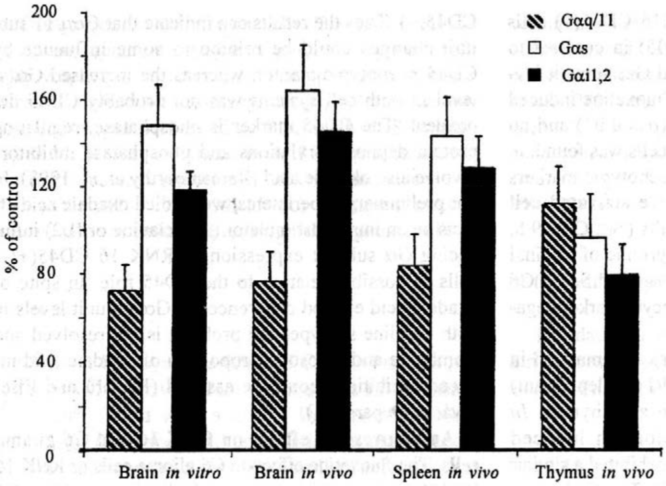


Figure 3. Effect of citalopram *in vitro*, each result is the mean of 7–9 measurements, *in vivo*, n = 6 animals. For other details see Fig. 1

lated by Gαq/11 but Gβ subunit can also be involved in modulation of this enzyme (Bristol and Rhee, 1994).

Fluoxetine or adenosine agonist NECA effects. An other mechanism of neuroimmunomodulation in relation to Gα profiles can be related to adenosine receptor, as analyzed by NECA. Our results (Figure 2) indicated similar effects of fluoxetine or NECA on decreased Gαq/11 subunit of C6 glioma or RNK 16 CD45(–) cells. These agents can act on a similar effector system, because both NECA or fluoxetine effect on C6 glioma cells evoked similar levels of decreased Gαq/11 subunit and lower 2nd messenger 1,4,5 IP3 formation produced by PLC (Kovářů *et al.*, 1997). Recently NECA was estimated as agonist of A3 adenosine receptor and this receptor subtype is able to regulate signalling via PLC (Abracchio *et al.*, 1995; Palmer *et al.*, 1995).

We also studied consequences of fluoxetine affected cell signalling into C6 glioma cells and apoptosis was demonstrated in some cells in contrast to no change induced by the antidepressant amitriptyline (Španová *et al.*, 1997). Furthermore, dose dependent NECA concentrations eliminated the fluoxetine induced Gαq/11 inhibition of C6 glioma cells but an amplifying NECA effect on fluoxetine evoked apoptosis was observed (Kovářů *et al.*, in preparation).

Neuroimmunomodulation induced by citalopram. Citalopram was able to induce similar Gα profiles in rat brain and spleen. Results indicate that antidepressant induced changes in cell signalling via Gα profiles can be similar in functionally different organ systems. Comparison of Gα profiles in the spleen, secondary lymphoid organ, and in the thymus, primary lymphoid organ, reflects probably T-B cell dichotomy. In agreement with this study, we also demonstrated different Gα subunit profiles in the spleeen and thymus during ontogenetical pig development (Kovářů *et al.*, 1998a). We conclude our in vitro and *in vivo* study, based on various experimental approaches, that immune system and brain can share common properties of molecular regulations during transducing neuro-immunomodulator signal(s). Furthermore, our results indicate that membrane microdomain rearrangement involving structures with coregulatory properties can participate in neuro-immunomodulator mediated G protein signalling in cells of brain or immune system. Thus a convergence principle in relation to expression of changes in Gαq/11 mainly of different cell or organ systems to modulatory action by various agents-immunostimulators or antidepressant fluoxetine can be taken in account in our study. In spite of our findings more detailed knowledge remains to be elucidated in this area.

REFERENCES

Abbracchio M.P., Brambilla R., Ceruti S., Kim H.O., Vonlubitz D.K.J.E., Jacobson K.A., Cattabeni F.(1995): G protein-dependent activation of phospholipase C by adenosine A(3) receptors in rat brain. Mol. Pharmacol., *48*, 1038–1045.

Al-Aoukaty A., Schall T.J., Maghazachi A.A.(1996): Differential coupling of CC chemokine receptors to multiple heterotrimeric G proteins in human interleukin-2 activated natural killer cells. Blood, *87*, 4255–4260.

Bristol J.A., Rhee S.G. (1994): Regulation of phospholipase C-beta isozymes by G-proteins. Trends Endocrinol. Metab., *5*, 402–406.

Chen J. and Rasenick M.M. (1995): Chronic treatment of C6 glioma cells with antidepressant drugs increases functional coupling between a G protein (Gs) and adenylyl cyclase. J. Neurochem., *64*, 724–732.

Fišerová A., Kovářů H., Hajduová Z., Mareš V., Starec M., Křen V., Flieger M., Pospíšil M. (1997): Neuroimmunomodulation of natural killer (NK) cells by ergot alkaloid derivates. Physiol. Res., *46*, 119–125.

Jacobson K.A.(1998): Adenosine A3 receptors: novel ligands and paradoxical effects. Trends Pharmacol. Sci., *19*, 184–190.

Kovářů H., Kovářů F., Kozáková H. (1994): Modified C6 glioma line target cells for NK pig lymphocytes. Phys. Res., *43*, 19P.

Kovářů H., Fišerová A., Lisá V., Španová A., Kovářů F., Velek J. (1997): Fluoxetine induced changes in C-6 glioma cells and NK lymphocytes. In: Sikora J., Fišar Z., Petrovický P., Jirák R. (eds.): Biologické podklady psychických poruch. Galén, Praha. 108–112.

Kovářů H., Kovářů F., Žižkovský V., Velek J. (1998a): Developmental changes of trimeric GTP-binding proteins in porcine brain and immune system. Acta Vet., Brno, *67*, 15–20.

Kovářů H., Fišerová A., Španová A., Lisá V., Fišar Z., Velek J. (1998b): Antidepressants as neuroimmunomodulators at postreceptor level. J. Neuroimmunol., *90*, 41.

Kovářů F., Kovářů H., Lisá V., Zendulková D. (1998c): Modified C6 glioma cell targets in effector recognition by pig NK lymphocytes. J. Neuroimmunol., *90*, 101.

Kovářů H., Fišerová A., Kovářů F. (2000): Transmembrane cell signalling by neuro- immunomodulators. Eur. J. Neurosci., *12*, Suppl. 11, 361.

Lesch K.P., Manji H.K. (1992): Signal transducing G proteins and antidepressant drugs: evidence for modulation of alpha subunit gene expression in rat brain. Biol. Psychiat., *32*, 549–579.

Lorenzen A. (1997): Determination of adenosine receptor-G protein coupling. Significance for psychiatric and neurological disorders. In: G protein methods and protocols. Role of G proteins in psychiatric and neurological disorders. Humana Press, Totowa, N. Jersey. 327–352.

Mareš V., Giordano P.A., Pellicciari C., Scherini E., Lisá V., Bottone M.G., Bottiroli G. (1991): Changes in cell cycle and chromatin distribution in C6 glioma cells treated by dibutyryl cyclic AMP. Cell Proliferat., *24*, 569–577.

Milligan G. (1988): Techniques used in the identification and analysis of function of pertussis toxin-sensitive guanine nucleotide binding proteins. Biochem. J., *255*, 1–13.

Milligan G. (1993): Regional distribution and quantitative measurement of the phosphoinisitidase C-linked guanine nucleotide binding proteins G11α and Gqα in rat brain. J. Neurochem., *61*, 845–851.

Palmer T.M., Benovic J.L., Stiles G.L. (1995): Agonist–dependent phosphorylation and desensitization of the rat A (3) adenosine receptor – evidence for Gprotein-coupled receptor kinase mediated mechanism. J. Biol. Chem., *270*, 29607–29613.

Ramamoorthy S., Giovanetti E., Quian Y., Blakely R.D. (1998): Phosphorylation and regulation of antidepressant-sensitive serotonin transporters. J. Biol. Chem., *273*, 2458–2466.

Ransnas L.A, Insel P.A. (1989): Quantitation of a guanine nucleotide binding regulatory protein by an enzyme-linked immunosorbent competition assay. Anal. Biochem., 190.

Rathbone M.P., Middlemiss P.J., Gysbers J.W., DeForge S., Costello P., Del Maestro R.F. (1992): Purine nucleosides and nucleotides stimulate proliferation of a wide range of cell types. In Vitro Cell. Dev. Biol., *28A*, 529–536.

Reynaert C., Janne P., Bosly A., Staquet P., Zdanowicz N., Vause M., Chatelain B. (1995): From health locus of control to immune control: internal locus of control has a buffering effect on natural killer cell activity decrease in major depression. Acta Psychiat. Scand., *92*, 294–300.

Schramm M. (1978): Fusion. Proc. Nat. Acad. Sci., USA, *76*, 1174–1178.

Španová A., Kovářů H., Lisá V., Lukášová E., Rittich B. (1997): Estimation of apoptosis in C6 glioma cells treated with antidepressants. Physiol. Res., *46*, 161–164.

Spiegel A.M. (1996): Defects in G protein-coupled signal transduction in human disease. Ann. Rev. Physiol., *58*, 143–170.

Received: 00–03–16
Accepted after corrections: 00–11–10

Corresponding Author:

RNDr. Hana Kovářů, DrSc., 1. Lékařská fakulta Univerzity Karlovy, Ke Karlovu 11, 120 00 Praha 2, Česká republika
Tel. +420 2 24 96 53 82, fax +420 2 24 91 05 77, e-mail: hkovaru@cesnet.cz

Fattening performance and slaughter value of the offspring of Black and White cows and Welsh Black bulls

Výkrmnost a jatečná hodnota potomstva po černobílých dojnicích a velšských černých býcích

Z. Nogalski, Z. Kijak

Warmia and Masuria University, Department of Cattle Breeding, Olsztyn, Poland

ABSTRACT: The fattening performance and slaughter value of Welsh Black and Black and White crossbred young bulls and heifers were compared to Black and White young bulls and heifers. Fattening was conducted from 181 to 540 days of age, then the animals were slaughtered. The crossbreds had lower daily weight gains and lower final body weight compared to the Black and White cattle, but they also consumed more nutrients per 1 kilogram of weight gain. The crossbreeding of the Black and White breed with bulls of the Welsh Black breed was found to have a positive effect on the slaughter value of crossbreds: they achieved higher dressing percentage and larger eye-muscle area; the shoulders dissected from these animals had a superior tissue composition.

Keywords: beef cattle; crossbreeding; Welsh Black breed; fattening performance; slaughter value

ABSTRAKT: Porovnávali jsme výkrmnost a jatečnou hodnotu býčků a jalovic – kříženců plemene velšské černé a černobílého plemene – s býčky a jalovicemi černobílého plemene. Výkrm se uskutečnil mezi 181. a 540. dnem věku, potom byla zvířata poražena. Kříženci měli ve srovnání s černobílým skotem nižší denní hmotnostní přírůstky a nižší konečnou tělesnou hmotnost, a přitom také spotřebovali více živin na 1 kg přírůstku. Zjistili jsme, že křížením černobílého plemene

INTRODUCTION

Since the Polish Black and White cattle are improved by crossing them with the Holstein-Friesian breed, the primary method to increase beef production, and especially the quality of meat, is by fattening more F_1 crossbred animals.

Good quality beef is tender, juicy, red in colour and good in taste. Dry or tough meat of insufficient acidity is more perishable, and as such it is less suitable for processing or trade (Neumann and Martin 1991; Schwark, 1993). Meat that does not hold water efficiently is also imperfect as it loses much weight during heat treatment and goes off more quickly (Ziemiński *et al.*, 1982). Pure beef breed animals or crossed with dairy breeds produce better quality meat compared to dairy breeds (Klupczyński *et al.*, 1985; Meller and Wroński, 1991; Neumann and Martin, 1991). The decision which beef breed should be selected for mating with dairy cattle depends on economic and natural conditions as well as on the production system and its objective.

The Welsh Black breed is a semi-intensive beef breed. It is kept mainly in the south-west of Great Britain. In the 1970's the British began exporting the Welsh Black cattle to the USA, Canada, Germany, South America and other parts of the world (Averbeck *et al.*, 1990). The Welsh Black, which was produced in the rough climate of Wales, is a medium-sized, frequently horned, hardy breed, with a high efficiency of fodder conversion. The Welsh Black cows are high yielding, so aided by their strong maternal instinct they are capable of raising their offspring quickly and effectively (Rottger, 1993). In Germany, the Welsh Black cattle are reared in remote parts of the country, unsuitable for more intensive agricultural production, or in parks and nature reserves. This practice respects the natural environment, but it also creates a possibility to produce beef at minimum costs (Schwark, 1993; Weiher, 1980). Easy calving is one of the obvious advantages of the breed, so either the Welsh Black bulls are mated with dairy breed heifers or the cows are used for producing beef crossbreds (Frelich and Millar, 1993).

This paper presents the results of one of many experiments conducted at the Department of Cattle Breeding of the University of Agriculture and Technology in Olsztyn, Poland, to evaluate the use of beef breeds for commercial crossing under the natural conditions in the north-east of Poland. The objective of this study was to assess the fattening performance and slaughter value of male and female offspring produced by crossing the Welsh Black with Black and White breeds.

MATERIAL AND METHODS

The study was conducted on 22 F_1 crossbreds of the Black and White cows and the Welsh Black bulls (wb × cb; 11 young bulls and 11 heifers) and 28 Black and White purebreds (cb; 14 young bulls and 14 heifers). The calves were purchased at the age of 3–4 months and the control fattening was carried out from 181 to 540 days of age. The animals were fed grass silage consumed *ad libitum* and supplemented with 2 kg of concentrate and 2 kg of meadow hay. The animals were kept in tie stalls with bedding. The troughs were adjusted to individual feeding. The animals had a continuous access to water and salt lick. The fodder and the leavings were weighed. The nutritive value of fodder was analysed in a laboratory once a month. The body weight and daily gain were checked once a month, by weighing the young bulls and heifers in the morning before feeding. On day 540 of age the following measurements were made: height at withers, width at chest, depth at chest, chest girth, diagonal body length, spiral thigh circumference measure and cannon bone circumference. Massiveness index was computed from the formula: chest girth × 100/ height at withers.

When the fattening was terminated, the animals were fasted for one day and then slaughtered. The carcasses were left to cool for 24 hours and then dissected into cuts according to the method of Chrząszcz and Janicki (1962). Between thoracic vertebras X and XI the eye-muscle area was taken. The right-side shoulder was dissected in great detail. The quality of meat was evaluated by examining the *musculus longissimus dorsi* in the three-rib region, sampled 24 hours after slaughter. The following parameters were determined: the content of dry matter, fat, crude protein and crude ash, physical, chemical and sensory properties according to the commonly applied methods (Grau and Hamm, 1963; Wajda *et al.*, 1992/93). Sensory analysis was carried out by a panel commission; the test included aroma, juiciness, palatability and tenderness at a 5-point scale (Baryłko-Pikelina, 1975).

The data were processed statistically by one factor analysis of variance in a nonorthogonal design, using Winstat computer software.

RESULTS AND DISCUSSION

The initial body weight of the animals, at 180 days of age, did not differ between the groups (Table 1, Figure 1). During fattening we could observe some effects of the genotype and sex on the rate of growth. The pure Black and White bulls and heifers had higher weight gains, so their body weight at 540 days of age was higher in comparison with the wb × cb crossbreds. Mean differences between the bull groups were 0.074 kg in daily gain and 33.4 kg in final body weight. The differences were statistically significant. Mean energy consumption per 1 kg of gain during 365 days of the fattening period was

Table 1. Body weight, daily gain and average intake of nutrients per 1 kg weight gain

Specification		Bulls		Significance of difference	Heifers		Significance of difference
		wb × cb	cb		wb × cb	cb	
Sample size		11	14		11	14	
Body weight at 180 days of age (kg)	$\bar{x}$	154.3	160.7	–	145.8	149.0	–
	v	16.72	3.91		10.36	10.40	
Body weight at 540 days of age (kg)	$\bar{x}$	420.2	453.6	x	394.6	408.7	–
	v	10.55	5.97		8.63	9.13	
Daily gain from 181 to 540 days of age (kg)	$\bar{x}$	0.728	0.802	x	0.687	0.711	–
	v	8.52	8.83		11.50	10.27	
Intake per 1 kg weight gain							
net energy (MJ)	$\bar{x}$	5.59	52.4	–	61.9	59.9	–
	v	10.36	12.18		16.70	14.49	
crude digestible protein (g)	$\bar{x}$	802	780	–	869	829	–
	v	10.32	11.71		15.98	16.10	
dry matter (kg)	$\bar{x}$	9.53	9.19	–	10.6	10.22	–
	v	10.39	12.51		16.23	14.77	

x = significance of difference at $p \leq 0.05$

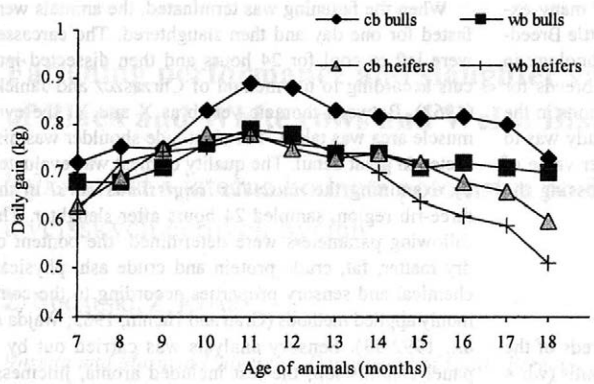


Figure 1. Daily gains of body weight in months of age

higher in the case of the crossbreds, but the differences were not confirmed statistically. As regards the sex, the heifers had lower gains (especially in the second stage of fattening) and higher energy consumption. The relationship between the genotype or sex and the consumption of digestible protein or dry matter per 1kg weight gain resembles the analogous relationship for energy consumption.

Two measurements: height at withers and diagonal body length, were different for the two genotypes irrespective of the sex. The Black and White pure cattle were significantly longer and taller. In the male cattle, the Black and White bulls had significantly larger chest girth and chest depth compared to the crossbred bulls. But as for the heifers, the Black and White animals obtained a significantly higher massiveness index (Table 2), which confirms the massiveness of the body frame of the Welsh Black breed described by other authors (Frelich and Millar, 1993; Rottger, 1993). Sex had an effect on the growth of the animals – the heifers were lower, their bodies were shorter and narrower, and their bones were thinner when compared to the bulls. The differences have confirmed the well-known fact that heifers have a lighter frame and are smaller than bulls.

The results concerning some characteristics of slaughter value reveal large differences between the genetic groups and between the sexes (Table 3). Differences in body weight before slaughter corresponded to differences in weight at the end of fattening. A highly significantly higher slaughter value (3.56%) for bulls

Table 2. Body measurements and massiveness index at 540 days of age

Specification		Bulls		Significance of difference	Heifers		Significance of difference
		wb × cb	cb		wb × cb	cb	
Sample size		11	14		11	14	
Height at withers (cm)	$\bar{x}$	118	122	xx	117	120	x
	v	2.82	2.36		3.53	1.85	
Width of chest (cm)	$\bar{x}$	36	37	–	36	36	–
	v	8.60	6.75		10.90	8.11	
Depth of fore chest (cm)	$\bar{x}$	60	64	xx	59	61	–
	v	10.04	3.17		8.62	3.58	
Chest girth (cm)	$\bar{x}$	169	175	x	168	169	–
	v	6.28	2.29		4.89	3.82	
Diagonal body length (cm)	$\bar{x}$	133	136	x	131	134	x
	v	3.87	2.59		5.03	1.95	
Spiral thigh circumference (cm)	$\bar{x}$	174	174	–	170	169	–
	v	4.38	3.23		4.96	3.39	
Cannon bone cirumference (cm)	$\bar{x}$	20.2	19.7	–	17.8	17.8	–
	v	2.23	5.26		6.31	2.50	
Massiveness index (%)	$\bar{x}$	143.2	143.4	–	143.6	140.8	x
	v	3.87	2.45		5.03	3.52	

x = significance of difference at $p \leq 0.05$, xx = significance of difference at $p \leq 0.01$

and 3.60% for heifers) was recorded in the case of the cross-breds. The sex did not cause any variations in slaughter value, but was related to the fat content. The heifers had a higher deposition of internal fat than the bulls, which can be attributed to the fact that female cattle mature earlier and deposit larger amounts of fat. According to Jasiorowski *et al.* (1993), the eye-muscle area is positively correlated with the content of intermuscular fat, which plays an important role for the evaluation of meat for cooking. The eye-muscle area was significantly larger for the crossbred cattle than for the purebred animals, and for the heifers the difference (9.4 cm^2) was statistically confirmed. The tissue composition of the dissected shoulders was more favourable in the cross-breds. Significant differences were observed between the heifers, where the shoulders of the crossbreds (wb × cb) contained 2.26% more meat and 1.27% more fat, but 0.99% less bone than the shoulders of the pure Black and White breed.

Table 3. Parameters of slaughter value

Specification		Bulls wb × cb	Bulls cb	Significance of difference	Heifers wb × cb	Heifers cb	Significance of difference
Sample size		11	14		11	14	
Weight before slaughter (kg)	$\bar{x}$	388.58	423.9	xx	364	368.9	–
	v	8.58	6.38		8.11	8.40	
Dressing percentage (%)	$\bar{x}$	56.54	52.98	xx	56.90	53.30	xx
	v	2.78	2.64		2.50	2.83	
Internal fat (kg)	$\bar{x}$	11.63	11.26	–	16.33	16.2	–
	v	24.50	12.72		21.40	18.75	
Eye-muscle area (cm^2)	$\bar{x}$	79.7	76.3	–	68.9	59.5	x
	v	13.10	13.15		8.86	12.20	
Proportion of 5 valuable cuts	$\bar{x}$	64.14	63.44	–	63.12	61.98	–
in half-carcass (%)	v	1.93	3.81		4.62	4.83	
Proportion in shoulder (%)	$\bar{x}$	77.25	76.26	–	76.61	74.35	xx
lean	v	2.34	13.11		4.11	2.16	
fat (MJ)	$\bar{x}$	5.54	5.92	–	7.43	8.70	–
	v	21.00	14.17		15.78	14.15	
bones	$\bar{x}$	17.21	17.82	–	15.96	16.95	x
	v	6.25	7.64		4.82	4.71	

x = significance of difference at $p \le 0.05$, xx = significance of difference at $p \le 0.01$

Table 4. Physico-chemical properties and content of dry matter, fat, protein and ash in *muscles longissimus dorsi*

Specification		Bulls wb × cb	Bulls cb	Significance of difference	Heifers wb × cb	Heifers cb	Significance of difference
Sample size		11	14		11	14	
Dry matter (%)	$\bar{x}$	24.53	24.31	–	25.47	26.36	x
	v	3.42	1.97		4.58	3.75	
Fat (%)	$\bar{x}$	1.69	1.48	–	2.26	3.07	xx
	v	18.34	19.59		22.12	14.66	
Crude protein (%)	$\bar{x}$	21.49	20.97	–	21.94	21.81	–
	v	3.77	2.34		2.37	3.30	
Ash (%)	$\bar{x}$	1.13	1.11	–	1.12	1.10	–
	v	4.42	2.70		5.36	1.82	
Water retention (%)	$\bar{x}$	7.55	5.80	–	9.08	8.56	–
	v	37.88	24.23		18.39	16.94	
Colour (%)	$\bar{x}$	12.10	8.30	xx	13.60	11.90	–
	v	18.84	16.06		19.04	20.92	
pH	$\bar{x}$	6.37	6.36	–	5.82	5.52	x
	v	5.49	6.60		5.33	4.53	

x = significance of difference at $p \le 0.05$, xx = significance of difference at $p \le 0.01$

Table 5. Sensory characteristics of meat, points

Specification		Bulls wb × cb	Bulls cb	Significance of difference	Heifers wb × cb	Heifers cb	Significance of difference
Sample size		11	14		11	14	
Aroma – intensity	$\bar{x}$	4.70	4.75	–	4.35	4.60	–
	v	7.45	8.84		7.82	8.48	
Aroma – desirability	$\bar{x}$	4.75	4.85	–	4.35	4.65	x
	v	7.37	7.01		7.82	7.10	
Tenderness	$\bar{x}$	4.45	4.80	–	4.10	4.10	–
	v	14.38	5.21		14.88	9.51	
Juiciness	$\bar{x}$	4.40	4.80	x	4.25	4.15	–
	v	11.59	5.21		11.29	7.95	
Palatability – intensity	$\bar{x}$	4.50	4.85	–	4.20	4.10	–
	v	12.67	4.95		13.81	5.12	
Palatability – desirability	$\bar{x}$	4.55	4.80	–	4.25	4.20	–
	v	12.97	5.20		13.65	8.10	

x = significance of difference at $p \leq 0.05$

The meat produced by the crossbred heifers had a significantly lower dry matter content relative to the meat of the purebred heifers (Table 4). Higher dry matter content is related with higher fat content, which is suggested by the results quoted by other authors (Mielnik, 1990; Wajda *et al.*, 1992). The meat of the Black and White heifers contained 0.81% more fat than the meat of the crossbreds; the difference was highly significant. As regards the bulls, the crossbreds showed a slightly higher fat content. According to Doroszewski's results (1972), the content of fat determined in the experiment was sufficient to maintain the desired culinary value of the meat. No significant differences in the content of protein or ash were recorded. The content of protein was similar to the values cited by Meller and Wroński (1991) and Wajda *et al.* (1992/93). The concentration of the components in the tissue did not vary as much as in some other studies (Klupczyński *et al.*, 1985; Wajda *et al.*, 1992/93).

Acidity and colour of meat are also important indicators of its quality. The meat of the crossbreds had a higher pH value and brighter red colour than the meat of the Black and White purebred cattle. It can be hypothesized that one of the causes of the differences in pH and colour was different sensitivity to stress before slaughter. Regarding the sex, the meat of bulls, regardless of the genotype, was darker in colour and its pH exceeded 6.2, which is the commonly assumed limit value between normal meat and meat with DFD defect (dark, firm, dry meat). Water retention was less favourable in the case of the meat of heifers because the lower pH of this meat impairs electrostatic forces which bind particles of water, so consequently it impairs the capacity of meat to retain water (Klupczyński *et al.*, 1985).

Organoleptic indicators of meat quality were over 4 points, which means the meat was of a very high quality (Table 5). Statistically confirmed differences were found out only for the juiciness of meat from bulls and the aroma of meat from heifers. The meat from the Black and White bulls was significantly more juicy than the meat from the crossbreds, and the meat from the purebred heifers had a more desirable aroma than the meat from the crossbred heifers. As regards the sexes, the meat from bulls scored better in all the sensory tests.

CONCLUSIONS

1. The results failed to prove a favourable effect of crossing the Black and White cattle with the Black Welsh bulls on the fattening performance of the crossbred cattle. The crossbred animals produced lower weight gains and lower slaughter weight. They also consumed more nutrients per 1 kg of weight gain.

2. Slaughter value of crossbred cattle was slightly higher. Relative to purebred cattle, the crossbred animals had a higher dressing percentage, larger eye-muscle area and better tissue composition of the shoulder. No improvement in meat quality was observed in crossbred versus purebred animals.

REFERENCES

Averbeck F., Baltzer J., Daenecke E., Decking J., Gerhardy H., Goebbel T., Hampel G., Lehfeldt W., Plass B., Von Schach J., Woerdemann H., Zachert W. (1990): Welsh Black. Bundesschau Fleischrinder. Grüne Woche, Berlin. 89–93.

Baryłko-Pikielina N. (1975): An Outline of Sensoric Food Analyses. WTN, Warszawa.

Doroszewski B. (1972): Zmienność cech poubojowych wyceny u ras czystych i mieszańców międzyrasowych bydła. IZ Kraków, 2, Rozprawy.

Frelich J., Millar P. (1993): Plemeno skotu Welsh Black. Náš Chov, *53*, 136.

Grau R., Hamm R. (1963): Uber das Wasserbindung des sangetiermuskels. I. Mitteilung: Der Einfluss des pH- Wertes auf die Wasserbindung von der Kleinertem Rindermuskel. Biology, *325*, 1–3.

Jasiorowski H., Kijak Z., Poczynajło S., Wajda S. (1993): Projekt programu rozwoju hodowli bydła mięsnego w Polsce. SGGW, Maszynopis, Warszawa.

Klupczynski J., Meller Z., Sobina I. (1985): Ocena jakosci miesa buhajów rasy czarno-bialej i jej mieszanców z bydlem ras miesnych, opasanych do wieku 18-tu miesiecy z wykorzystaniem jednego sezonu pastwiskowego, Roczn. Inst. Przem. Mies. i Tluszcz., 22/23, 69–78.

Meller Z., Wronski M. (1991): Jakosc miesa mieszanców uzyskanych w wyniku krzyzowania krów rasy czarno-bialej z buhajami typu miesnego, Zesz. Nauk. PTZ, *3*, 261–267.

Mielnik J. (1990): Badania nad użytkowaniem mlecznym i mięsnym mieszańców czterorasowych (cb, cd, c. belg., j) i dwurasowych (cb, ch) w porównaniu z bydłem rasy czarno-bialej. Acta Acad. Agricult. Tech. Olst. Zoot., Rozpr. habil., *33*.

Neumann W., Martin J. (1991): Kreuzungen bringen bessere Schlachtkörperwerte. Schlachtkörperzusammen setzung und Fleischqualität bei verschiedenen Genotypen. Tierzucht, *12*, 533–534.

Röttger J. (1993): Steckbriefe der wichtigsten Fleischrinderrassen. Top agrar extra. Fleischrinderproduktion, 14–18.

Schwark H.J. (1993): Wie haben sich die „neuen" Rassen eingelebt? Neue Landwirtschaft, *1*, 52–54.

Wajda S., Wichłacz H., Bąk T. (1992/93): Wartość rzeźna buhajków żywionych w ostatnich trzech miesiącach przed odstawieniem na rzeź kiszonką, paszą treściwą i dodatkiem tłuszczu lub skwarek. Roczn. Inst. Przem. Mięs. i Tłuszcz., 29/30, 35–49.

Weiher O. (1980): Masthybriden aus der Gebrauchskreuzung bringen Markenfleisch. Tierzucht, *8*, 340–342.

Ziemiński R., Górska I., Szmańko T., Krasnowska G. (1982): Ocena wybranych parametrów jakościowych mięsa mieszańców piemontese x czb i blonde d'aquitaine x czb, Rocz. Nauk. Zoot. Monogr. i Rozpr., *20*, 93–104.

Received: 00–02–22

Accepted after corrections: 00–11–10

Corresponding Author:

Dr. Zenon Nogalski, Chair of Cattle Breeding, Warmia and Masuria University, 10-718 Olsztyn, Poland

Tel. +48 89 523 37 59, Fax +48 89 523 44 13, e-mail: zeno@moskit.uwm.edu.pl

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION
Slezská 7, 120 56 Prague 2, Czech Republic
Tel.: + 420 2 24 25 79 39, Fax: + 420 2 24 25 39 38, E-mail: redakce@uzpi.cz

In this institute scientific journals dealing with the problems of agriculture and related sciences are published on behalf of the Czech Academy of Agricultural Sciences. The periodicals are published in English with abstracts in Czech.

Journal	Number of issues per year	Yearly subscription in USD Europe	overseas
Rostlinná výroba (Plant Production)	12	195,-	214,-
Czech Journal of Animal Science (Živočišná výroba)	12	195,-	214,-
Agricultural Economics (Zemědělská ekonomika)	12	195,-	214,-
Journal of Forest Science	12	195,-	214,-
Veterinární medicína (Veterinary Medicine – Czech)	12	159,-	167,-
Czech Journal of Food Sciences (Potravinářské vědy)	6	92,-	97,-
Plant Protection Science (Ochrana rostlin)	4	62,-	64,-
Czech Journal of Genetics and Plant Breeding (Genetika a šlechtění)	4	62,-	64,-
Horticultural Science (Zahradnictví)	4	62,-	64,-
Research in Agricultural Engineering	4	62,-	64,-

Subscription to these journals be sent to the above-mentioned address.

Changes in ewe milk composition depending on lactation stage and feeding season

Změny ve složení ovčího mléka v závislosti na fázi laktace a krmném období

Z. Antunović[1], Z. Steiner[1], Đ. Senčić[1], M. Mandić[2], T. Klapec[2]

[1]*Faculty of Agriculture,* [2]*Faculty of Food Technology, University of J. J. Strossmayer in Osijek, Croatia*

ABSTRACT: Ewe milk contains relatively a high amount of total solids, fats and proteins but milk composition changes depending on breed, stage of lactation, climatic conditions, feeding, health and herd. The aim of these investigations was to find out the impact of lactation stage and feeding season on ewe milk composition during the lamb sucking period. Biological investigations were carried out with 15 ewes of Würtemberg breed till the 60th lactation day (all ewes were in the fourth and fifth lactation). The investigations comprised two feeding seasons (winter and summer). The winter feeding period started on 1 October whereas the summer one started on 1 May, i.e. in the third month of ewe pregnancy. It finished by their dryness. The ewes were housed in stable boxes and fed grains mixture (300 g daily) containing 60% of oats, 30% of maize and 10% of soybean meal as well as meadow hay *(ad libitum)*. The ewes grazed in the summer feeding period. The stage of lactation and feeding season (winter and summer) considerably affected ewe milk composition in the lamb sucking period (2nd to 60th day). The highest concentrations of basic constituents of ewe milk (fats, proteins, total solids and solids non-fat) were in colostrum (2nd day), except lactose, followed by their decrease on 10th and 30th lactation day and increase on 60th lactation day. The content of macro (Ca, P, K, Na and Mg) and microelements (Fe, Zn and Cu) was also highest in colostrum (2nd day) followed by their milk concentration decreasing and increasing on the 60th lactation day. Ca and Mg concentrations considerably differed depending on the feeding season. In general, higher K and Fe concentrations were determined in the summer whereas P and Na in the winter feeding season. Significant variations were also determined for Zn and Cu concentrations.

Keywords: ewe milk; milk composition; macroelements; microelements; colostrum; stage of lactation; feeding season

ABSTRAKT: Ovčí mléko obsahuje relativně vysoké množství celkové sušiny, tuků a bílkovin, ale složení mléka se mění v závislosti na plemeni, fázi laktace, klimatických podmínkách, výživě, zdravotním stavu a chovu. Cílem našich šetření bylo zjistit vliv fáze laktace a krmného období na složení ovčího mléka během sání jehňat. Biologická pozorování jsme prováděli na 15 bahnicích plemene würtemberg až do 60. dne laktace (všechny bahnice byly ve 4. a 5. laktaci). Pozorování zahrnovala dvě krmná období (zimní a letní). Zimní krmné období začalo 1. října, letní krmné období začalo 1. května, tj. ve 3. měsíci gravidity bahnic. Skončilo jejich zaprahnutím. Bahnice byly ustájeny ve stájových boxech a dostávaly koncentrovanou krmnou směs (300 g denně), která obsahovala 60 % ovsa, 30 % kukuřice a 10 % extrahovaného sojového šrotu. Dále dostávaly luční seno *(ad libitum)*. V letním krmném období byly bahnice na pastvě. Fáze laktace a krmné období (zimní i letní) ovlivnily do značné míry složení ovčího mléka během sání jehňat (od 2. do 60. dne). Nejvyšší koncentrace základních složek ovčího mléka, s výjimkou laktózy (tuků, bílkovin, celkové sušiny a beztukové sušiny), jsme zjistili v kolostru (2. den), potom 10. a 30. den laktace došlo k jejich poklesu a 60. den laktace následoval jejich vzestup. Obsah makroprvků (Ca, P, K, Na a Mg) i mikroprvků (Fe, Zn a Cu) byl nejvyšší rovněž v kolostru (2. den), poté následoval pokles jejich koncentrace v mléku a 60. den jejich vzestup. Koncentrace Ca a Mg byly značně odlišné v závislosti na krmném období. Obecně řečeno, vyšší koncentrace K a Fe jsme zjistili v létě, zatímco v zimním krmném období byly zjištěny vyšší koncentrace P a Na. Významné kolísání jsme zaznamenali u koncentrace Zn a Cu.

Klíčová slova: ovčí mléko; složení mléka; makroprvky; mikroprvky; kolostrum; fáze laktace; krmné období

INTRODUCTION

One of the main ewe products is certainly milk. Although it cannot be compared with cow and recently with goat milk regarding the world production level, its importance is reflected in higher feeding value. Due to ewe milk richness in fats, proteins and minerals it is an especially valuable food of human nutrition. The highest content of fats, proteins, minerals and total solids in ewe milk followed by goat and finally cow milk was determined by Hadjipanayiotou (1995). Ramos and Juares (1981) as well as Storry *et al.* (1983) stated that ewe milk contained more fat, proteins, Ca, Mg and P than cow milk. Milk composition and quality are influenced by a large number of factors such as: breed, climatic conditions, feeding, health and growth of ewe (Bencini and Paulina, 1997). Pugliese *et al.* (2000) stated that milk composition depended on genetic and environmental conditions. The effect of lactation stage on ewe milk composition was determined by Peart (1975), Jelínek *et al.* (1993), Maria and Gabina (1993), Gonzalo *et al.* (1994), Fuertes *et al.* (1998) whereas the season effect was observed by Carta *et al.* (1995).

Since lamb vitality and productivity at the initial increase considerably depend on ewe milk composition, this investigation was aimed at determination of the effect of lactation stage and feeding season on ewe milk composition in the lamb sucking period.

MATERIAL AND METHODS

Selection, keeping and feeding of the ewes

Biological investigations were carried out with 15 ewes of Würtemberg breed till the 60th lactation day. All ewes were in the fourth lactation in winter feeding season whereas in summer feeding season they were in the fifth lactation. Winter feeding period lasted from 1 October, when selected ewes were in the third pregnancy month, to 29 January when they were dry. Ewes were inseminated on 20th day after dryness. Summer feeding period started on 1 May, when ewes were in the third pregnancy month and finished on their dryness on 18 September. The ewes were at the average age of four years, healthy and in good condition. In the winter feeding season the ewes were housed in stable boxes and fed grain mixture (60% of oats, 30% of maize and 10% of soybean meal) 300 g daily and meadow hay (*ad libitum*) whereas in the summer feeding season they were exposed to rotation pasture. On their return to the stable ewes consumed hay *ad libitum*. The ewes were given salt lick and fresh water (*ad libitum*) in both (winter and summer) feeding seasons.

Feed consumption and analysis

Feed samples (mixture of grains, meadow hay and pasture green mass) were taken on 2nd, 10th, 30th and 60th lactation day prior to milk sampling for chemical analysis. Contents of some feed nutrients (Table 1) were analyzed by common chemical methods (AOAC – Association of Offical Agricultural Chemists, Method of Analysis, 10th ed., Washington D. C., 1960).

Crude proteins were determined by Kjehldahl method whereas crude fiber by Henneberg and Stochman method. Ash content was determined by sample burning in a Muffol oven at a temperature of 550°C for two hours and crude fats by Soxhleth method. Dry matter content was determined by sample drying at a temperature of 105°C for 1 hour until constant weight was reached. Then feed samples were destroyed by a wet procedure (Vukadinović and Bertić, 1988) and prepared for determination of mineral composition. Feed mineral composition (Table 2) was seen on an atomic absorption spectrophotometer (Perkin Elmer 1100 B, Norwalk, Connecticut, USA) whereas phosphorus was determined by a spectrophotometric "Blue method" with ammonium molybdate (Vukadinović and Bertić, 1988).

Milk sampling and its analysis

Ewe milk samples were taken two hours after the first lamb sucking (about 10 o'clock a.m.), after ewe feeding,

Table 1. Feed chemical composition (%)

Component	Feeding season					
	winter		summer			
	mixture of grains	meadow hay	green herbage			
			2nd day	10th day	30th day	60th day
Crude proteins	13.25	11.51	25.82	23.45	19.32	16.61
Crude fiber	6.19	29.87	11.80	13.34	20.45	21.20
Crude ash	2.32	8.42	9.06	8.51	9.33	8.59
Crude fat	4.51	4.27	2.11	2.41	2.76	3.30
Dry matter	90.23	91.99	21.14	16.56	18.61	21.00

on 2nd; 10th; 30th and 60th lactation day when ewes were gradually drying off. Prior to milk sampling udders were washed in luke-warm water and disinfected with the product "Plivasept" (Pliva, Croatia) before and after milking. The milking was carried out in special small bottles intended for field milk sampling amounting to 100 ml of milk per ewe during one sampling. Chemical analyses of ewe milk taken in the stage of lactation highly in the laboratory of the milk industry "Mia" Osijek, two hours after sampling whereas the content of some matters (fats, proteins, lactose, total solids and solids non-fat) was determined on an apparatus MILCO SCAN FT 120 (Foss Electric, Hilleroed, Denmark) that was calibrated for ewe milk. Milk samples were digested using a microwave system Star 2 (CEM Corporation, Matthews, North Carolina, USA). On average 5 ml of milk was pipetted into the 200ml cylindrical flask which was then connected to the instrument. The digestion programme given in Table 3 is an adaptation of the programme for milk digestion found in the manual supplied with the instrument. Initial reagents were concentrated HNO_3 (10 ml) and concentrated H_2SO_4 (3 ml). Resulting digestate was diluted to 50 ml with water.

Mineral (Ca, K, Na, Mg, Fe, Cu and Zn) concentration was seen on an atomic absorption spectrophotometer Perkin Elmer 1100 B whereas P concentrations were determined by a spectrophotometric "blue method" with ammonium molybdatae (Vukadinović and Bertić, 1988).

Statistical analysis

Finally, basic statistical processing (one-way analysis of variance) of experimental data on the investigated properties was carried out, using a computer program for the analysis of variance (Vukadinović, 1985).

RESULTS

Basic ewe milk composition

Results of the ewe milk chemical analysis depending on the stage of lactation and feeding season are presented in Table 4.

Basic ewe milk composition during lactation. Statistically highly significantly ($P < 0.01$) higher contents of fats, proteins, total solids and solids non-fat were determined in ewe colostrum during the second lactation day in both feeding seasons compared to measurings performed on 10th, 30th and 60th lactation day. Only colostrum lactose concentrations were the lowest ones (2nd day) in both feeding seasons. Thus, statistically highly significantly higher ($P < 0.01$) lactose concentrations were determined on 10th, 30th and 60th lactation day.

A decrease in most the milk constituents (except lactose) was recorded on 10th lactation day in both feeding seasons.

Table 2. Feed mineral composition

Elements	Feeding season: winter		summer			
	mixture of grains	meadow hay	green herbage			
			2nd day	10th day	30th day	60th day
Macroelements (%)						
Ca	0.624	0.654	1.201	1.093	0.945	1.038
P	0.543	0.349	0.470	0.461	0.526	0.458
K	0.571	2.670	2.395	2.687	2.704	2.191
Na	0.217	0.123	0.192	0.188	0.179	0.201
Mg	0.135	0.130	0.203	0.205	0.250	0.295
N	1.960	1.491	4.130	3.750	3.091	2.658
Microelements (mg/kg)						
Fe	240.38	262.02	367.5	295.2	289.4	468.4
Zn	38.16	28.83	36.85	40.36	43.41	46.64
Cu	12.58	8.94	13.24	12.70	12.38	11.96

Table 3. Microwave digestion programme

	Step 1	2	3
Reagent	–	HNO_3	H_2O_2
Volume (ml)	–	10 ml à 1 ml	20 ml à 1 ml
Temperature (°C)	120	240	200
Time (min)	0	4	8

Ewe milk on 30th day had the lowest values of most constituents (fats, proteins, total solids and solids non-fat) in both feeding seasons.

Ewe milk analysis carried out on 60th lactation day showed an increase in the investigated constituents in both feeding seasons. The highest lactose content was recorded at the end of lactation (60th day) in both feeding seasons.

Basic ewe milk composition during the feeding seasons. As for ewe colostrum analyses (2nd day), no significant differences were determined in constituents between the feeding seasons. Statistically highly significantly ($P < 0.01$) higher content of lactose and solids non-fat compared to winter feeding season was determined in ewe milk on 10th lactation day in summer feeding season.

Statistically significantly ($P < 0.05$) higher content of milk fat and total solids was found on 30th lactation day in winter compared to summer feeding season.

By milk analysis carried out on 60th lactation day highly significantly ($P < 0.01$) higher solids non-fat content was found out, it was also determined that significantly ($P < 0.05$) higher lactose content occurred in summer compared to winter feeding season.

Ewe milk mineral composition

Ewe milk mineral composition in both feeding seasons is presented in Tables 5 and 6.

Minerals in ewe milk during lactation. Statistically highly significantly ($P < 0.01$) higher concentrations of macroelements (Ca, P, K, Na and Mg) compared to later lactations (10th, 30th, and 60th day) were determined in ewe colostrum (2nd day) during both feeding seasons.

Ca concentration decrease in the summer feeding season was recorded by ewe milk analyses performed from 10th to 60th day. A similar trend of Ca concentration decrease was recorded in winter season except measurings on 60th lactation day. Milk P concentrations on 10th day in the winter feeding season were statistically highly significantly ($P < 0.01$) higher compared to 30th and 60th lactation day. Statistically highly significantly ($P < 0.01$) higher Mg concentration was determined in ewe milk on 60th day compared to 10th and 30th day in the winter feeding season. Also, statistically significantly higher ($P < 0.05$) concentration of the same element was found out on 60th day compared to 10th lactation day in the summer feeding season.

Statistically highly significantly($P < 0.01$) higher concentrations of microelements (Fe, Zn and Cu) were determined in ewe colostrum (2nd day) compared to later lactations (10th, 30th and 60th day) in both feeding seasons. No statistically significant differences were determined depending on lactation day by ewe milk analyses of microelements content (Fe, Zn and Cu) performed on 10th, 30th and 60th lactation day in the winter feeding season.

Minerals in ewe milk during the feeding seasons. Similar concentrations of most minerals in both feeding seasons, except statistically significantly ($P < 0.05$) higher Mg and Zn concentrations in the summer feeding season were determined by colostrum analyses between the feeding seasons.

Ca and Mg concentrations considerably differed depending on the feeding season. Generally speaking, higher K and Fe concentrations were determined in summer whereas higher P and Na concentrations were determined in the winter feeding season.

Important variations between the feeding seasons were also determined for Zn and Cu concentrations. Statistically highly significantly ($P < 0.01$) higher Zn and Cu concentration was determined in ewe milk on 10th lactation day in the summer feeding season. Statistically significantly ($P < 0.05$) higher Zn concentration in winter as well as statistically highly significantly ($P < 0.01$) higher Cu concentration in the summer feeding season were determined by ewe milk analysis on 60th lactation day.

DISCUSSION

Basic ewe milk composition

The highest content of all basic constituents, except lactose, was determined in ewe colostrum. It is reasonable since colostrum possesses the highest feeding value. Lactose content appeared to be the lowest in colostrum and increased with lactation stage being the highest on 60th lactation day. The same data were determined by Peart *et al.* (1975) in their colostrum analysis of crossbred ewes (Finnish Landrace x Blackface ewe). Hadjipanayiotou (1995) obtained slightly lower content of fat, proteins and total solids in ewe colostrum on 2nd day whereas lactose content was similar. Colostrum proteins increase is reflected in higher globulin fractions content, especially in gamma globulins (Shubber *et al.* 1979). A decrease in most milk constituents (except lactose) was recorded on 10th lactation day in both feeding seasons. The lowest values of most ewe milk constituents (fats, proteins, total solids and solids non-fat) appeared to be on 30th lactation day in both feeding seasons. This decreasing trend as well as slightly higher variation coefficient can be justified by the highest milk production (Torres-Hernandez, 1980; Fuertes *et al.*, 1998). An increasing trend of the investigated constituents was shown by ewe milk analysis on 60th lactation day in both feeding seasons. An increasing trend of ewe milk fat and proteins was also obtained by Gonzalo *et al.* (1994) on 45th and 75th lactation day. Higher fat content in ewe milk during winter and pro-

Table 4. Content of basic ewe milk components (%)

Components		Feeding season: winter				Feeding season: summer				Significance of differences winter : summer			
		2nd day	10th day	30th day	60th day	2nd day	10th day	30th day	60th day	2nd day	10th day	30th day	60th day
Fats	$\bar{x}$	$10.49^{**2,3,4}$	7.02^{**1}	$6.43^{**1,4}$	$8.05^{**1,3}$	$10.60^{**2,3,4}$	$6.34^{**1,*4}$	$5.52^{**1,4}$	$7.22^{*2,**1,3}$	ns	ns	a	ns
	s	1.49	0.54	0.50	0.52	1.57	0.35	0.71	0.69				
	v	14.20	7.69	7.78	6.46	14.81	5.52	12.86	9.56				
Proteins	$\bar{x}$	$10.51^{**2,3,4}$	4.83^{**1}	4.73^{**1}	5.01^{**1}	$11.06^{**2,3,4}$	5.24^{**1}	4.78^{**1}	5.27^{**1}	ns	ns	ns	ns
	s	0.59	0.85	0.53	0.46	1.30	0.43	0.52	0.94				
	v	5.61	17.60	11.20	9.18	11.75	8.21	10.88	17.84				
Lactose	$\bar{x}$	$3.05^{**2,3,4}$	4.52^{**1}	4.47^{**1}	4.63^{**1}	$3.20^{**2,3,4}$	$5.03^{**1,3}$	$4.61^{**1,2,4}$	$4.91^{**1,3}$	ns	b	ns	a
	s	0.41	0.37	0.47	0.45	0.34	0.18	0.27	0.29				
	v	13.44	8.19	10.51	9.72	10.63	3.58	5.86	5.91				
Solids non-fat	$\bar{x}$	$14.79^{**2,3,4}$	10.31^{**1}	9.98^{**1}	10.34^{**1}	$15.40^{**2,3,4}$	11.13^{**1}	10.14^{**1}	11.12^{**1}	ns	b	ns	b
	s	1.02	0.66	0.47	0.30	0.99	0.33	0.28	0.65				
	v	6.90	6.40	4.71	2.90	6.43	2.96	2.76	5.85				
Total solids	$\bar{x}$	$25.28^{**2,3,4}$	17.33^{**1}	16.41^{**1}	18.39^{**1}	$26.00^{**2,3,4}$	17.47^{**1}	$15.66^{**1,4}$	$18.34^{**1,3}$	ns	ns	a	ns
	s	3.89	0.46	0.61	0.67	2.21	0.29	0.65	0.56				
	v	15.39	2.65	3.72	3.64	8.50	1.66	4.15	3.05				

*(P < 0.05); **(P < 0.01) – depending on the stage of lactation
ns = nonsignificant; a = (*P*< 0.05); b = (*P* < 0.01) – depending on the feeding season
1 – 2nd lactation day; 2 – 10th lactation day; 3 – 30th lactation day, 4–60th lactation day

Table 5. Content of ewe milk macroelements depending on the lactation stage and feeding season (mmol/l)

Macro-elements		Feeding season: winter 2nd day	winter 10th day	winter 30th day	winter 60th day	summer 2nd day	summer 10th day	summer 30th day	summer 60th day	Significance of differences winter : summer 2nd day	10th day	30th day	60th day
Ca	$\bar{x}$	$70.25^{**2,3,4}$	$47.60^{**1,3}$	$33.89^{**1,2,4}$	$46.28^{**1,3}$	$66.50^{**2,3,4}$	$52.29^{**1,3,4}$	$48.35^{**1,2,4}$	$39.68^{**1,2,3}$	ns	b	b	a
	s	2.28	1.74	0.90	0.80	2.06	1.61	0.98	3.13				
	v	3.24	3.66	2.67	1.73	3.10	3.08	2.03	7.90				
P	$\bar{x}$	$54.88^{**2,3,4}$	$47.22^{**1,4,3}$	$37.56^{**1,*2,4}$	$43.77^{**1,*2,3}$	$49.99^{**2,3,*4}$	43.10^{**1}	39.70^{**1}	42.85^{*1}	ns	ns	ns	ns
	s	4.68	3.00	8.09	3.62	2.82	3.80	3.17	5.98				
	v	8.54	6.36	21.53	8.28	5.65	8.82	7.98	13.96				
K	$\bar{x}$	$42.29^{**2,3,4}$	34.16^{**1}	32.85^{**1}	35.43^{**1}	$42.94^{**2,3,4}$	$38.51^{**1,*3}$	$31.36^{**1,4,*2}$	$38.07^{**1,3}$	ns	ns	ns	a
	s	1.27	2.99	5.13	3.83	2.46	5.54	6.42	1.97				
	v	3.00	8.76	15.63	10.82	5.73	14.37	20.46	5.19				
Na	$\bar{x}$	$54.40^{**2,3,4}$	29.15^{**1}	27.29^{**1}	23.31^{**1}	$48.31^{**2,3,4}$	24.11^{**1}	20.31^{**1}	23.30^{**1}	ns	ns	a	ns
	s	11.00	6.06	5.20	5.70	4.10	6.91	3.52	4.70				
	v	20.22	20.77	19.07	19.07	8.48	28.67	17.31	20.15				
Mg	$\bar{x}$	$7.02^{**2,3,4}$	$4.07^{**1,*4}$	$4.57^{**1,*4}$	$5.71^{**1,2,3}$	$7.81^{*2,3,**4}$	$6.13^{*1,4}$	6.55^{*1}	$6.47^{**1,*2}$	a	b	b	a
	s	0.26	0.42	0.74	0.51	0.37	1.17	1.08	0.85				
	v	3.71	10.25	16.12	8.94	4.73	19.08	16.52	13.14				

Table 6. Content of ewe milk microelements depending on lactation stage and feeding season (μmol/l)

Macro-elements		Feeding season: winter 2nd day	winter 10th day	winter 30th day	winter 60th day	summer 2nd day	summer 10th day	summer 30th day	summer 60th day	Significance of differences winter : summer 2nd day	10th day	30th day	60th day
Fe	$\bar{x}$	$8.49^{**2,3,4}$	3.80^{**1}	4.19^{**1}	4.38^{**1}	$7.92^{**2,3,4}$	4.26^{**1}	3.77^{**1}	4.67^{**1}	ns	ns	ns	ns
	s	1.57	1.40	1.03	1.37	2.58	0.52	0.76	0.65				
	v	18.45	36.99	24.51	3.32	32.54	12.25	20.20	13.81				
Zn	$\bar{x}$	$18.69^{**2,3,4}$	10.01^{**1}	8.82^{**1}	9.91^{**1}	$34.80^{**2,3,4}$	$13.59^{**1,3,4}$	$7.95^{**1,2}$	$8.49^{**1,2}$	a	b	ns	a
	s	2.37	1.39	1.21	1.97	12.69	2.58	2.66	1.71				
	v	12.71	13.92	13.75	19.85	36.47	19.01	33.50	20.17				
Cu	$\bar{x}$	$2.62^{**2,3,4}$	0.94^{**1}	0.95^{**1}	0.91^{**1}	$2.45^{**2,3,4}$	$1.57^{**1,3,4}$	$1.12^{**1,2}$	$1.22^{**1,2}$	ns	b	ns	b
	s	0.69	0.24	0.37	0.22	0.35	0.17	0.23	0.29				
	v	26.55	25.60	38.75	23.90	14.35	11.03	20.11	24.23				

*($P < 0.05$); **($P < 0.01$) – depending on the stage of lactation

ns = nonsignificant; a = ($P < 0.05$); b = ($P < 0.01$) – depending on the feeding season

1 – 2nd lactation day; 2 – 10th lactation day; 3 – 30th lactation day, 4 – 60th lactation day

teins in summer feeding can be linked to different chemical of feed composition (Table 1) at ewe lactation.

Effect of the season on milk fat content of Sarda ewe breed was irregular and protein content varied less in the investigation by Carta *et al.* (1995).

Ewe milk mineral composition

It is well known that ewe colostrum (from 1st to 3rd day after lambing) is richest in all minerals (Hadjipanayiotou, 1995) which is indicated by these investigations. Statistically highly significantly ($P < 0.01$) higher concentrations of all macroelements (Ca, P, K, Na and Mg) and microelements (Fe, Zn and Cu) were determined on which 10th, 30th and 60th lactation day. Somewhat higher variation coefficient was determined on 30th lactation day, which is probably due to highest milk production in that period. Similarly to this investigation, Snowder and Glimp (1991) reported P concentration decreasing from the beginning of lactation as well as its slight increase at the lactation end (60th day). Jelínek *et al.* (1993) determined a P concentration decrease at the beginning of the second lactation month and the trend of a slight increase in Mg concentration. Similar Ca, Na, Fe and K concentrations were determined by Gajdušek and Jelinek (1992).

Higher concentrations of Ca, K, Zn and Cu were determined in milk during the summer feeding season whereas slightly higher P and Na concentrations were found out in winter feeding. On the contrary, Fe concentrations did not considerably vary in terms of the feeding season. The reasons for these changes can be seen in mineral composition of feed for ewes in lactation (Table 2).

The most dominant milk microelement was Zn but its concentration as well that of Cu decreased on 10th and 30th day and increased at the end of lactation (60th day). Fe concentration considerably fluctuated. The changes of microelement concentrations during lactation were in accordance with the results reported by Jelinek *et al.* (1993). The same authors stated that microelement concentrations in ewe milk depended on their feed supply and this is probably cause in this investigation in terms of forage minerals variation (Table 2). Apart from the feed effect on ewe milk microelement concentration Coni *et al.* (1996) pointed out a significant impact of the season and environmental conditions.

CONCLUSION

Lactation stage as well as feeding season (both winter and summer) considerably influence ewe milk composition in the lamb suckling period (2nd to 60th day). The highest concentrations of the basic ewe milk constituents (fats, proteins, solids non fat and total solids) were found in colostrum (2nd day) in both feeding seasons, except lactose, followed by their decrease on 10th and 30th lactation day and increase on 60th lactation day. Concentrations of macroelements (Ca, P, K, Na and Mg) and microelements (Fe, Zn and Cu) were found in colostrum (2nd day) followed by their gradual decrease in milk on 10th and 30th day and increase on 60th lactation day. Higher concentrations of Ca, K, Mg, Zn and Cu were revealed in the summer feeding season whereas higher concentrations of P and K were found out in the winter feeding season. The summer feeding season is characterized by slightly better milk composition, that means also by higher lamb vitality and productivity. Thus summer feeding is recommended if quality pasture rich in highly valuable nutrients (proteins and minerals) is available. However, a concentrated part of the ration is required in warm summer months (when pasture is of lower quality).

REFERENCES

Bencini R., Paulina G. (1997): The quality of sheep milk – a review. Wool Technol. Sheep Breeding, *45*, 182–220.

Carta A., Sanna S.R., Casu C. (1995): Estimating lactation curves and seasonal effects for milk, fat and protein in Sarda dairy sheep with test day model. Livest. Prod. Sci., *44*, 37–44.

Coni E., Bocca A., Coppolelli P., Caroli S., Cavallucci C., Marinucci M.T. (1996): Minor and trace element content in sheep and goat milk and dairy products. Food Chem., *57*, 253–260.

Fuertes J.A., Gonzalo C., Carriedo J.A., Sanprimitivo F. (1998): Parameters of test day milk and milk components for dairy ewes. J. Dairy Sci., *81*, 1300–1307.

Gajdůšek S., Jelinek P. (1992): Mutual relationships among components of sheep milk and its rennetability. Živoč. Výr., *37*, 1023–1028.

Gonzalo C., Carriedo J.A., Baro J.A., Primitivo F.S. (1993): Factors influencing variation of test day milk yield, somatic cell count, fat and protein in dairy sheep. J.. Dairy Sci., *77*, 1537–1542.

Hadjipanayiotou M. (1995): Composition of ewe, goat and cow milk and colostrum of ewes and goats. Small Ruminant Res., *18*, 255–262.

Jelínek P., Gajdůšek S., Illek J. (1993): Variations of mineral content in sheep milk during lactation. Živoč. Výr., *38*, 85–96.

Maria G. and Gabina D. (1993): Non-genetic effects on milk production of Latxa ewes. Small Ruminant Res., *12*, 61–67.

Peart J.N. (1975): The yield and composition of the milk of Finnish Landrace x Blackface ewes. II. Ewes and lambs on pasture. J. Agric. Sci. Camb., *85*, 315–324.

Pugliese C., Acciaioli A., Rapaccini S., Parisi G., Franci O. (2000): Evolution of the chemical composition, somatic cell count and renneting properties of the milk of Massese ewes. Small Ruminant Res., *35*, 71–80.

Ramos M., Juarez M. (1981): The composition of ewe's and goat's milk. Bulletin, International Dairy Federation Document, *140*, 5–19.

Shubber A.H., Doxej D.L., Black W.J.M., Fitz-Simons J. (1979): Immunoglobulin levels in ewe colostrum and in lamb serum. Res. Vet. Sci., *27*, 283.

Snowder G.D., Glimp H.A. (1991): Influence of breed, number of sucking lambs, and stage of lactation on ewe milk production and lamb growth under range conditions. J. Anim. Sci., *69*, 923–930.

Storry J.E., Grandison A.S., Millard D., Owen A.J., Ford G.D. (1983): Chemical composition and coagulating properties of renneted milks from different breeds and species of ruminant. J. Dairy Res., *50*, 215–229.

Torres-Hernandez G. (1980), Milk production and progeny growth in crossbred sheep. Dissertation – Abstract – International. B, *40*, 2919.

Vukadinović V. (1985): Primjena mikroračunara u regresijskoj analizi. Znan. Prak. Polj. Tehnol., *15*, 1–2.

Vukadinović V., Bertić B. (1988): Praktikum iz agrokemije i ishrane bilja. Poljoprivredni fakultet Sveučilišta J. J. Strossmayera u Osijeku. 56 pp.

Received: 00–06–08

Accepted after corrections: 00–11–10

Corresponding Author:

Doc. Dr. Sc. Zvonko Antunović, University of J. J. Strossmayer in Osijek, Faculty of Agriculture, P. O. Box 117, Trg sv. Trojstva 3, 31000 Osijek, Croatia
Tel. +385 31 224 200, fax +385 31 207 017, e-mail: zantun@suncokret.pfos.hr

Some carcass traits and chemical composition of different muscle groups in Alpine and Saanen breed kids

Vybrané jatečné znaky a chemické složení různých skupin svalů u kůzlat alpského a sánského plemene

B. Mioč, Vesna Pavić, A. Ivanković

Department of Animal Production, Faculty of Agriculture, University of Zagreb, Croatia

ABSTRACT: The aim of the research was to determine the influence of breed on some carcass traits, as well as on the chemical composition of different muscle groups in Alpine and Saanen breed kids. To that end, 29 male kids (15 Alpine and 14 Saanen) of similar age were slaughtered, and shoulder, back and thigh muscles were taken for analyses. Alpine kids had not only a significantly higher dressing percentage than Saanen kids (48.07% : 45.33%), but also their liver and kidney weight was higher ($P < 0.05$). The basic chemical composition of kid meat varied depending on both the muscle group and the breed. Water percentage ranged from 76.26 to 78.04%, protein percentage was from 19.94 to 20.65%, and ash content from 1.16 to 1.47%. The average content of fat in back muscles of Saanen and Alpine kids was 1.01 and 1.16%, respectively; in thigh muscles it was 1.41 and 1.45%, while the highest fat content was found in shoulder muscles (1.55 and 1.64%). Both breeds had a significantly higher fat content in thigh and shoulder muscles than in back muscles ($P < 0.05$). The two breeds showed no significant differences in the chemical composition of different muscle groups ($P > 0.05$).

Keywords: kids; Alpine; Saanen; carcass traits; muscle; chemical composition

ABSTRAKT: Cílem výzkumu bylo stanovit vliv plemene na vybrané jatečné znaky a na chemické složení různých skupin svalů u kůzlat alpského a sánského plemene. K tomu účelu bylo poraženo 29 kozlíků přibližně stejného věku (15 alpského plemene a 14 sánského plemene) a k analýzám byly odebrány vzorky ze svaloviny plece, hřbetu a kýty.. Kozlíci alpského plemene měli nejenom významně vyšší jatečnou výtěžnost než kozlíci sánského plemene (48,07 % : 45,33 %), ale také vyšší hmotnost jater a ledvin ($P < 0,05$). Základní chemické složení kůzlečího masa se lišilo v závislosti na skupině svalů a na plemeni. Obsah vody se pohyboval od 76,26 do 78,04 %, obsah bílkovin od 19,94 do 20,65 % a obsah popelovin od 1,16 do 1,47 %. Průměrný obsah tuku v zádové svalovině sánských a alpských kůzlat činil 1,01 a 1,16 %; ve stehenní svalovině jeho hodnoty dosahovaly 1,41 a 1,45 %, zatímco nejvyšší obsah tuku jsme zjistili ve svalovině plece (1,55 a 1, 64 %). Obě plemena měla významně vyšší obsah tuku ve stehenní svalovině a ve svalovině plece než v zádové svalovině ($P < 0,05$). U žádného z obou plemen jsme nezjistili významné rozdíly v chemickém složení různých skupin svalů ($P < 0,05$).

Klíčová slova: kůzlata; alpské plemeno; sánské plemeno; jatečné znaky; svalovina; chemické složení

INTRODUCTION

Following the lifting of a long-standing, legally imposed ban on goat breeding, this form of animal husbandry is now becoming increasingly profitable in the Republic of Croatia. The primary reason for goat farming is milk production, which is based on imported breeds (Alpine, Saanen and the German improved Fawen "DBE" breed) characterized not only by a sound genetic potential for milk production, but also by high fertility. A significant portion of income from the production of goat milk is realized through the sale of meat from male and non-breeding female kids. Since in the majority of European countries goats are farmed for milk production, kids are slaughtered at a very early age, with body weight of 6–12 kg. There is a high demand for young kid meat in butchers' establishments throughout Europe and America. The modern consumer prefers meat with as high muscle content as possible, and as it contains less fats and cholesterol, kid meat is a fine alternative to lamb meat (Potchoiba *et al.*, 1990). Kid carcasses are light, delicate, narrow and shallow, with very slight fat layer, as well as being less compact (Pavić and Mioć, 1994). Several researchers (Gaili and Ali, 1985; Casey, 1987) have established that the carcasses of young goats contain small quantities of fat which, together with poor conformation, are the chief characteristics of the carcass. Additionally, most breeds of European origin have a tendency to deposit visceral fat at the expense of carcass fat (Chilliard *et al.*, 1981). However, a distinctly low content of subcutaneous and intramuscular fat found in carcasses of most goat breeds ensures satisfactory storage (Morand-Fehr *et al.*, 1985) and makes some methods of processing difficult. Some authors (Morand-Fehr *et al.*, 1986; Sanz-Sampelayo *et al.*, 1990) believe that both the quality and chemical composition of carcass can be influenced by feed (through increased energy content in meal), by increasing the age of slaughter and by neutering. In other words, carcass yield, as well as its structure and chemical composition depend on a number of factors, the most important of which are: feed, degree of fattening, slaughter age and slaughter weight, breed, neutering, method of keeping the animals, etc. Gall (1982) pointed out the high level of heterogeneity of results with regard to the chemical composition of carcasses in different goat breeds. The chemical composition of individual parts of carcass, organs, tissue and muscles is not the same, and accords with their function. Hogg *et al.* (1992) state that the chemical composition of a kid's thigh differs significantly from the chemical composition of carcass meat in general. The aim of this research was to determine the influence of the breed on some carcass traits of Alpine and Saanen breed kids, as well as the chemical composition of different muscle groups.

MATERIAL AND METHODS

A total of 29 male kids (15 Alpine and 14 Saanen) were slaughtered at the age of 70 days. Up to the moment of slaughter all the kids were kept in identical conditions with regard to feed and accommodation. Immediately after birth the kids were separated from their mothers and were given colostrum pasteurised at 56°C for one hour. The kids subsequently received a milk replacer, ranging from 0.2 litre at the beginning to 0.8 litre per feeding at the end of the fattening period. For the first two weeks the kids were fed three times daily (morning, noon and evening), and then twice daily (morning and evening). Additionally, after 10 days the kids were given meadow hay and pelleted feed mixture. Chemical composition of feed is presented in Table 1.

Table 1. Chemical composition of feed in kid diet (% in D.M.)

Components	Milk replacer	Hay	Pelleted feed
Protein	23.36	12.23	20.80
Fat	14.75	2.79	2.70
Ash	7.80	8.22	7.66
Fibre	–	30.48	4.66
N-free extract	54.09	46.28	64.18
Ca	1.23	–	–
NaCl	2.02	–	–

Each kid was weighed immediately prior to slaughter. Slaughter was performed by the classic method for kids which includes blood letting by parallel cutting of major neck blood vessels (*v. jugularis* externa and *a. carotis communis*), skinning and severing of lower parts of legs, extraction of offal (digestive tract, liver, lungs, heart and spleen). Next, the empty tract, digestive tract, skin with lower legs, lungs and heart, liver and kidneys were weighed individually. Subsequent to weighing the following muscles were removed from the left side of each carcass: shoulder – *m. deltoideus*, *m. subscapularis* and *m. triceps brachii*; back: *m. longissimus dorsi*, *m. spinalis et semispinalis*, *m. trapezius* and *m. romboideus*; thigh: *m. biceps femori*, *m. semitendineus* and *m. gastrocnemius*. Using a clean knife, connective membranes were removed, as were all parts of bone and cartilage. The samples were then minced, homogenized, marked, packed in plastic bags and deep-frozen until needed for analyses. Chemical analyses of samples, which included: supply and laboratory water, dry matter, ash, proteins, fats and N-free extract, were performed in accordance with the Weende method (AOAC, 1984). The results were statistically processed by means of the statistical package SAS STAT 1990. Differences in the indicators manifested on the slaughtering line and in the chemical composition of meat determined between breeds and groups of muscles, corrected to age, were evaluated on the basis of Harvey (1975) linear model:

$$Y_{ij} = b\left(X_{ij} - \bar{x}\right) + P_j + e_{ij}$$

were: Y_{ij} – value of observed characteristic
b – regression coefficient
X_{ij} – age
$\bar{x}$ – least square mean
P_j – breed influence
e_{ij} – unexplained influence

RESULTS AND DISCUSSION

It has been established that breed is the only one of numerous factors having an influence on the yield and quality of kid carcass. In addition to breed, the meat yield in kids and other carcass traits, as well as carcass appearance and quality, are significantly influenced by

Table 2. Carcass traits of Saanen and Alpine breed kids

Traits	LSM	S E	Min.	Max.	CV (%)
			Saanen		
Body weight (kg)	17.02	0.54	15.00	20.8	11.78
Empty body weight (kg)	7.73	0.32	6.18	10.42	15.70
Stomach and intestines (kg)	4.57	0.22	3.05	5.81	18.31
Liver (kg)	0.34	0.02	0.27	0.50	19.96
Kidneys (kg)	0.064	0.002	0.05	0.075	11.14
Lungs and heart (kg)	0.41	0.02	0.24	0.54	21.77
Skin (kg)	1.76	0.04	1.3	1.85	11.22
Dressing percentage (%)	45.33	0.90	41.08	52.18	7.45
			Alpine		
Body weight (kg)	17.72	0.48	15.00	20.5	10.44
Empty body weight (kg)	8.54	0.30	6.30	10.28	13.68
Stomach and intestines (kg)	4.52	0.18	3.50	5.98	15.71
Liver (kg)	0.40*	0.02	0.29	0.51	17.74
Kidneys (kg)	0.071*	0.002	0.055	0.08	12.26
Lungs and heart (kg)	0.41	0.03	0.061	0.54	28.57
Skin (kg)	1.82	0.06	1.45	2.21	15.71
Dressing percentage (%)	48.07*	0.74	42.00	54.03	5.93

*$P < 0.05$

diet, age of slaughter, method and age of weaning, processing method, degree of fattening, conditions of keeping, neutering, etc. (Colomer-Rocher *et al.*, 1987; Blanco *et al.*, 1994; Muir *et al.*, 1995). Alpine breed kids were found to have a significantly higher ($P < 0.05$) dressing percentage than Saanen kids (48.07% : 45.33%), as well as higher liver and kidney weight (Table 2). Our research has shown a considerably lower dressing percentage for both breeds that the values given by Manfredini *et al.* (1988) and Terzano *et al.* (1988). In Saanen breed kids, stomach and intestines accounted for 26.57%, lungs and heart 2.41%, liver 2.0%, kidneys

Table 3. Chemical composition of different muscle groups

Component	leg LSM	leg SE	back LSM	back SE	shoulder LSM	shoulder SE
			Saanen			
Water	78.04	0.28	77.70	0.31	77.77	0.25
Dry matter	21.96	0.28	22.30	0.31	22.23	0.25
Protein	19.94	0.39	20.65	0.42	20.19	0.29
Fat	1.41[a]	0.08	1.01[b]	0.006	1.55[a]	0.19
Ash	1.23	0.05	1.16	0.003	1.19	0.05
N-free extract	55.10	1.08	55.10	0.59	53.37	1.18
			Alpine			
Water	76.62	0.19	76.26	0.23	77.18	0.36
Dry matter	23.38	0.19	23.74	0.23	22.82	0.36
Protein	20.01	0.33	20.55	0.39	20.35	0.33
Fat	1.45[a]	0.60	1.16[b]	0.04	1.64[a]	0.10
Ash	1.44	0.17	1.38	0.09	1.47	0.10
N-free extract	53.73	0.63	53.17	0.59	53.72	0.57

[ab] = means within main effect with different superscripts differ ($P < 0.05$)

0.3%, and skin with lower legs for 10.34% of total body weight. In Alpine breed kids, stomach and intestines accounted for 25.51%, lungs and heart 2.31%, liver 2.26%, kidneys 0.4% and skin with lower legs for 10.27% of total body weight. The above percentages are comparable, to some extent, with the results provided by Riley *et al.* (1989) and Pralom *et al.* (1995), for different breeds and ages of goat.

Chemical composition of muscles in Alpine and Saanen kids is fairly uniform, and there is hardly any difference between the two breeds with regard to protein and fat content in all three groups of investigated muscles (Table 3). The most noticeable difference is in the water content, i.e. of dry matter, but the difference was not significant ($P > 0.05$). The given data accords with the findings of Johnson *et al.* (1995), reporting that the breed and sex have no significant influence on the percentage of water, fat and proteins in *m. longissimus dorsi* in young goats. The fundamental characteristic of the chemical composition in the muscles is the low fat quantity. The lowest fat content in both breeds was found in the back muscles, and in comparison with these the thigh and shoulder muscles were significantly fattier ($P < 0.05$). Similar results with regard to the chemical composition of kid meat from different breeds were obtained by Hogg *et al.* (1989), and Popov-Reljić *et al.* (1995). In their analyses of factors influencing the yield and quality of kid meat the majority of authors agree that the age and slaughter weight, type and quantity of feed, as well as sex, have a greater influence than the breed (Thonney *et al.*, 1987; Warmington and Kirton, 1990; Matsouka *et al.*, 1992). Delfa *et al.* (1995) drew attention to the existence of significant correlations between the live weight of goats and carcass total fat content, as well as between total fat content in the carcass and total muscle quantity (0.94; 0.85; 0.95).

The average fat content determined in carcasses of younger goats totals only about 4% (Owen *et al.*, 1983), and up to 18% in carcasses of adult goats (Anjaneyulu *et al.*, 1985). Similar chemical compositions of goat meat were given by Naude and Hoffmeyer (1981) and Colomer-Rocher *et al.* (1992). The most outstanding property of kid meat is its low content of intramuscular and subcutaneous fat (Owen, 1975; Sanz Sampelayo *et al.*, 1987). Individual parts of the carcass, i.e. muscles, have a different chemical composition. In research dealing with the same matter (Hogg *et al.*, 1992), results showed that the chemical composition of thigh muscles differed significantly from that of the carcass as a whole. On the contrary, the level of protein in kid meat varies much less, remaining almost unchanged in relation to an increase in age and slaughter weight. Matsouka *et al.* (1992) found that the protein level in muscles of adult castrates ranged from 20.3 to 22.2%. Johnson *et al.* (1995) reported that 100 g of adult ram meat contained 19.7% protein. Protein quantities in muscles of kid meat, determined in this research, are fairly similar to the relevant values for adult goats.

REFERENCES

Anjaneyulu A.S.R., Lashmann V., Rao V.K. (1985): Status of meat and milk production from Indian Goats. J. Food Sci. Technol., India, *22*, 151–160.

AOAC. (1984): Official methods of analysis. Association of Official Analytical Chemists, 13th Ed. Washington, DC.

Blanco F.P., Cabezas M.J.G., Garcia M. H., Serrano E.R. (1994): Carcass composition of Florida Sevellana kids. Archiv. Zoot., *43*, 65–174.

Casey N. (1987): Meat production and meat quality from Boer goats. In: Proc. 4th Int. Conf. on Goats, March 8–13, Brasilia (Brazil), vol 1, 211–239.

Chilliard Y., Sauvant D., Bas P., Pascal G., Morand-Fehr P. (1981): Importance relative et activitiés metaboliques des différents tissus adipeux de la Chèvre laitière. In: Nutrition and systems of goat feeding. Itovic-Inra, Paris, France. 81–87.

Colomer-Rocher F., Morand-Fehr P., Kirton A.H. (1987): Standard methods and procedures for goat carcass evaluation, jointing and tissue separation. Livest. Prod. Sci., 67, 149–159.

Colomer-Rocher F., Kirton A.H., Mercer G.J.K., Duganzich D.M. (1992): Carcass composition of New Zealand Saanen goats slaughtered at different weights. Small Rumin. Res., *7*, 161–173.

Delfa R., González C., Teixeira A., Gosalves L. F., Tor M. (1995): Relationships between body fat depots, carcass composition, live weight and body condition scores in Blanca Celtiberica goats. Options Méditerranéennes. Série A, Séminaires Méditerranéens, No. 27, 109–119.

Gaili E.S., Ali A.E. (1985): Meat from Sudan Desert sheep and goats. Part 1: Carcass yield, offals and distribution of carcass tissues. Meat Sci., *13*, 217–227.

Gall C. (1982): Carcass composition. In: Proc. 3rd Int. Conf. on Goat Production and Diseases. University of Arizona, Tucson. 472–487.

Harvey W.R. (1975): Least-squares analysis of data with unequal subclass numbers. Usda, Ars H-4, Beltswille, MD.

Hogg B.W., Catcheside L.M., Mercer G.J.K., Duganzich D.M. (1989): Meat yields and chemical composition of muscle in New Zealand goats. Proc. New Zeal. Soc. Anim. Prod., *49*, 153–156.

Hogg B.W., Mercer G.J.K., Mortimer B.J., Kirton A.H., Duganzich D.M. (1992): Carcass and meat quality attributes of commercial goats in New Zealand. Small Ruminant Res., *8*, 243–256.

Johnson D.D., McGowan C.H., Anous M.R. (1995): Breed type and sex effects on carcass traits, composition and tenderness of young goats. Small Ruminant Res., *17*, 57–63.

Manfredini M., Massari M., Cavani C., Falaschini A.F. (1988): Carcass characteristics of male Alpine kids slaughtered at different weights. Small Ruminant Res., *1*, 49–58.

Matsouka A., Fukuzaki N., Takahashi T., Yamanaka Z. (1992): Carcass traits and chemical composition of meat of castrated male goats. Anim. Sci. and Technol., *63*, 514–519.

Morand-Fehr P., Bas P., Rouzeau A., Hervieu J. (1985): Development and characteristics of adipose deposits in male kids during growth from birth to weaning. Anim. Prod., *41*, 349–357.

Morand-Fehr P., Bas P., Schmidely P., Hervieu J. (1986): Facteurs influeneçant la qualité des carcasses et en particulier son état d'engraissement. 11e Journées de la recherche ovine et caprine. INRA-ITOVIC, Paris, France. 236–252.

Muir J.P., Jordao C., Massaete E.S. (1995): Comparative growth characteristics of goats tethered on native pasture and free-ranged on cultivated pasture. Small Ruminant Res., *17*, 111–116.

Naude R.T., Hofmeyer H.S. (1981): Meat production. In: Goat Production, Academic Press, London, UK. 285–307.

Owen J.E. (1975): The meat-producing characteristics of the indigenous Malawi goat. Trop. Sci., *17*, 123–138.

Owen J.E., Aries Cereceres M.T., Garcia Macias J.A., Nunes Gonzales F.A. (1983): Studies on the Criollo goat of Northern Mexico. Part 1. The effect of body weight on body components and carcass development. Meat Sci., *9*, 191–204.

Pavić V., Mioč B. (1994): Kakvoća jarećeg mesa – čimbenici o kojima ovisi. (Quality of kid meat – dependent factors). Stočarstvo, *48*, 71–75.

Popov-Reljić J., Krajinović M., Kelemen-Mašić D., Cvetković T., Džinić N., Popov S., Kunc V. (1995): Chemical composition of kid meat of the domestic White goat. Acta Vet., *45*, 303–310.

Potchoiba M.J., Lu C.D., Pinkerton F., Sahlu T. (1990): Effect of all-milk diet on weight gain, organ development, carcass characteristics and tissue composition, including fatty acids and cholesterol contents, of growing male goats. Small Ruminant Res., *3*, 583–592.

Pralomkarn W., Saithanoo S., Kochapakdee S., Norton B.W. (1995): Effect of genotype and plane of nutrition on carcass characteristics of Thai Native and Anglo Nubian x Thai Native male goats. Small Ruminant Res., *1*, 21–25.

Riley R.R., Savell J.W., Johnson D.D., Smith G.C., Shelton M. (1989): Carcass grades, rack composition and tenderness of sheep and goats as influenced by market class and breed. Small Ruminant Res., *2*, 273–280.

Sanz Sampelayo M.R., Munoz F. J., Lara L., Gil Extremera F., Boza J. (1987): Factors affecting pre-and post-weaning growth and body composition in kid goats of the Granadina breed. Anim. Prod., *45*, 233–238.

Sanz Sampelayo M.R., Ruiz I., Gill F., Boza J. (1990): Body composition of goat kids during sucking, voluntary feed intake. Brit. J. Nutr., *64*, 611–617.

SAS. (1990): SAS/STAT User's Guide (Release 6.03). SAS Inst. INC., Cary, NC.

Terzano M., Bartocci S., Borghiese E. (1988): La produzione del capretto negli allevamenti intensivi. 4. dati in vita e al macello in soggetti Saanen e Camosciata delle Alpi 35 e 50 giorni di eta. Zoot. Nutr. Anim., *14*, 495–501.

Thonney M.L., Taylor Sr.C.S., McClelling T.H. (1987): Breed and sex differences in equally mature sheep and goats. I. Growth and food intake. Anim. Prod., *45*, 239–260.

Warmington B.G., Kirton A.H. (1990): Genetic and non-genetic influences on growth and carcass traits of goats. Small Ruminant Res., *3*, 147–165.

Received: 00–04–13
Accepted after corrections: 00–11–10

Corresponding Author:

Doc. Dr. sc. Boro Mioč, University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia
Tel. +385 1 239 38 67, fax +385 1 2393 901, e-mail: bmioc@agr.hr

Kongresové centrum Brno, a.s.
Ústav technologie potravin AF MZLU v Brně
Ústav hygieny a technologie masa FVHE VFU v Brně
Odborný časopis Maso

7. KONFERENCE O TECHNOLOGII MASA

Datum: čtvrtek 8. března 2001

Místo: Kongresové centrum Brno, sál A

Konference je součástí doprovodného programu Mezinárodních gastronomických veletrhů (6. až 9. 3. 2001, Výstaviště Brno).

Program konference (09,00 až 13,00 h)

- Aktuálně o evropském problému BSE (Prof. MVDr. Zdeněk Pospíšil, DrSc.)
- Technologické a spotřebitelské využiti hovězího masa (Doc. Ing. Petr Pipek, CSc.)
- Jakostní klasifikace a zpeněžování jatečných prasat v roce 2001 (Ing. Jan Pulkrábek, CSc.)
- Jakostní klasifikace a zpeněžování jatečného skotu v roce 2001 (MVDr. Jaroslav Vrchlabský, CSc.)

Diskuse

- Současné pozice a perspektivy masného průmyslu České republiky (doc. MVDr. Ladislav Steinhauser, CSc.)
- Inovace sortimentu a stabilizace jakosti masných výrobků pro samoobslužný prodej (MVDr. Jan Budig, CSc.)
- Vliv materiálu obalového střeva na obsah vody v trvanlivých salámech v průběhu zrání (MVDr. Josef Kameník, CSc.)
- Krůtí maso – biochemické a hygienické aspekty (MVDr. Lenka Vorlová, PhD.)

Závěr

Odborný garant: Prof. Ing. Ivo Ingr, DrSc., tel. 05/45 13 31 97, fax 05/45 21 20 44,
e-mail: zlato@mendelu.cz

Long-term trends in sport fishery yield from selected reservoirs in the Labe watershed (1958–1998)

Dlouhodobé trendy výlovu ryb sportovním rybolovem ve vybraných údolních nádržích v povodí Labe (1958–1998)

K. Pivnička, M. Rybář

Charles University, Faculty of Sciences, Institute for Environmental Protection, Prague, Czech Republic

ABSTRACT: The biomass of stock carp and its yield and yield of bream, pike, pikeperch, and perch (kg/ha/year) were analyzed in three large (Slapy, Orlík and Lipno) and two small (Hostivař, Džbán) reservoirs in 1958–1998. The biomass of stock carp exceeding 20 kg substantially increase the risk of lower yields in big reservoirs with low fishing pressure. The yield of the common bream amounted 20 to 40 kg in small reservoirs and 10 to 20 kg in large reservoirs. The highest yields of the pike were reported from the Hostivař and Lipno reservoirs (34.6 kg and 12.4 kg, respectively). For pikeperch, the highest yield in small reservoirs was 48 to 55 kg, in large reservoirs at most 5 to 14 kg. The yield of unstocked fish, shows a common trend in different reservoirs since it is influenced by the same short-term as well as middle-term changes of the climate. Synchronous cycles of similar shape were observed in perch yield in the Orlík, Slapy and Hostivař reservoirs, and for the perch and pikeperch in the Orlík Reservoir.

Keywords: reservoirs; yield; cyclic synchronous changes; carp; bream; pike; pikeperch; perch

ABSTRAKT: Velké nádrže na Vltavě byly postaveny koncem 50. let. Do současné doby jsou k dispozici výsledky hospodaření s rybami (násady, výlov). Podobně jsou k dispozici i výlovy a násady ryb ze dvou malých nádrží v Praze (Hostivař, Džbán). Soustředili jsme se především na to jak vysazování ovlivňuje výlov kapra obecného, a dále na dynamiku výlovu dalších druhů ryb (cejn velký, štika obecná, candát obecný a okoun říční). Hodnocen byl pouze výlov sportovních rybářů. U druhů, které nejsou ovlivňovány vysazováním (okoun, do jisté míry štika, candát a cejn) jsme očekávali jistou míru cykličnosti a synchronnosti výlovů, neboť nové ročníky by měly být ovlivněny klimatem v průběhu prvního roku života v různých nádržích srovnatelně. Analyzovány byly násady a úlovky ryb (kg/ha/rok) ze tří velkých nádrží (Slapy, Orlík a Lipno) a ze dvou malých nádrží (Hostivař, Džbán) v povodí Vltavy za období 1958 až 1998. Násady kapra vyšší než 20 kg u velkých nádrží s nízkým rybářským tlakem zvyšují riziko nižší návratnosti. Naopak z malých nádrží (Hostivař a Džbán) při násadách 300 kg bylo ještě uloveno 256 a 328 kg kaprů z hektaru ročně. Úlovky cejna velkého dosáhly v malých nádržích 20 až 40kg, ve velkých nádržích byly o polovinu menší. Nejvíce štik bylo uloveno v nádržích Hostivař a Lipno v období po jejich napuštění (34,6 kg a 12,4 kg). V malých nádržích bylo maximálně vyloveno 48 až 55 kg candátů, ve velkých nádržích maximálně 5 až 14 kg. Celkový výlov ryb bez kapra vykazuje očekávané společné cyklické trendy. U okouna říčního se úlovky cyklicky měnily v nádržích Orlík, Slapy a Hostivař s maximy v začátku 70. a v první polovině 80. let. Společný trend výlovu okouna a candáta byl zjištěn v nádrži Orlík.

Klíčová slova: údolní nádrže; výlov; synchronní cyklické změny; kapr obecný; cejn velký; štika obecná; candát obecný; okoun říční

INTRODUCTION

From a set of 31 lakes and 62 rivers distributed throughout the world it was ascertained that the values of the fish production in reservoirs are intermediate between the production of rivers (in average 273 kg/ha/year) and lakes (82 kg/ha/year) Randall *et al.* (1995). The biomass values estimated from a set of 248 reservoirs of North America in the 1960s to 1980s fluctuated between 20 and 500 kg/ha depending on the MEI (morpho-

Expenses associated with the work were covered from Grant of the Ministry of Education, Youngth and Sports No. J13/98113100004.

edaphic index – 1 to 100), yields from 294 reservoirs fluctuated in average about 23.7 kg/ha/year (Jenkins, 1982). As mentioned by Cyr and Peters (1996), see also Taub (1984), the fish biomass in world reservoirs ranged between 6 and 1 109 kg/ha (data from the IBP Final Report).

The values of fish biomass in reservoirs of the Czech Republic range between 100 and 500 (600) kg (Kubečka *et al.*, 1998; Pivnička, 2000), yield statistics are available in papers by (Lusk and Krčál, 1983; Lusk, 1984; Pivnička,1985, 2000). Large reservoirs on the Vltava River were built at the end of the 1950s and the fish statistics are available for the last 40 years. The same is true of two small reservoirs (Hostivař and Džbán), only the number of years is shorter. Thus, in the present work, we compared the biomass of stock carp and its yield and yield of other fish species from large (Slapy, Orlík, Lipno) and small (Hostivař and Džbán) reservoirs for 1958 to 1998. We particularly considered the relationship between stock carp and carp yield and between the yield of carp and other fish species (bream, *Abramis brama*; pike, *Esox lucius*; pikeperch, *Stizostedion lucioperca* and perch, *Perca fluviatilis*). In species not influenced by stocking (perch and to some extent predators and bream) we expected certain synchronous yield cycles since the YOY should be influenced by the climate in a comparable manner, particularly during climatically extreme years.

MATERIAL AND METHODS

Statistical data on stock fish and fish yield were obtained from the Secretariat of the Czech Sport Fishing Association in Prague and Regional Sport Fishing Association of the City of Prague. For particular reservoirs, data are available for 1958 to 1998 (Lipno, Slapy), 1962 to 1998 (Orlík), 1983 to 1998 (Džbán) and 1965 to 1998 (Hostivař). Only the fish caught by sport fishermen were taken into consideration. The biomass of stock and caught carp as well as the crop of other species (bream, pike, pikeperch, perch) are always in kg/ha/year. Two of the selected reservoirs are small and shallow (Hostivař 43 ha, maximum depth 12 m, Džbán 18 ha, maximum depth 7.5 m), other two reservoirs are large, V-shaped (Slapy 1 390 ha, maximum depth 58 m, Orlík 2 732 ha, maximum depth 74 m), and one reservoir is large and shallow (Lipno 4 870 ha, maximum depth 20 m) (Vlček *et al.*, 1984).

RESULTS AND DISCUSSION

Yield of carp

Carp yield is fully dependent on the amount of stock carp, so in all reservoirs the yield linearly increases with increasing biomass of stock carp. In large reservoirs, the amount of stock carp exceeding 20 kg already increases the risk of lower recapture. In the Orlík Reservoir per 20 kg of stock carp, it is possible to recapture in average 18.7 kg, in the Slapy Reservoir 14.4 kg, and in the Lipno Reservoir only 13.8 kg. Two years of large of stock of carp were not taken into account when estimating the yield from the Lipno Reservoir (marked by large open circles, Figure 1). If these values were included the yield would decrease to 6.8 kg of carp per 20 kg of stock fish. The yield in the Hostivař Reservoir per 20 kg of stock carp reached 77.6 kg in average (stock fish from preceding years), and per 300 kg of stock fish it was 256 kg. The best results were achieved in the smallest reservoir Džbán, where on average, 328 kg of carp were recaptured per 300 kg of stock fish. In addition, the slope of a straight line of the relationship between the biomass of stock fish and fish crop is very close to one (0.97). This means that the stock carp were completely caught even at high values of the stocked biomass. High fishing pressure in the small reservoirs is the main reason for better results of carp recapture.

The yield of the carp during 40 years has been increasing in large reservoirs stepwise from an average value of 2 (4) kg up to 20 kg. The lowest yields were achieved in the mid-1970s, when the amount of stock fish was considerably reduced. Better results achieved later were influenced by higher biomass of stock carp, by their higher average individual weight, and probably also thanks to higher fishing effort.

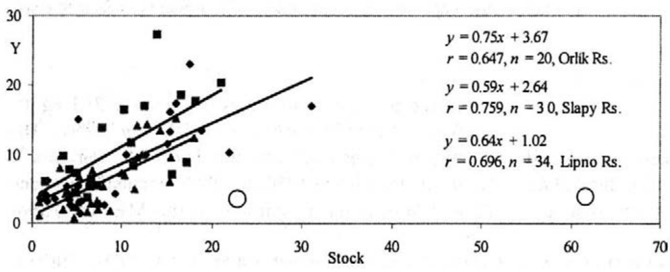


Figure 1. Stock carp and yield – Orlík, Slapy, and Lipno Reservoir (kg/ha/year)

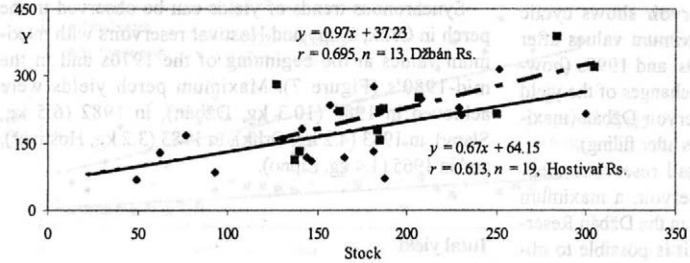


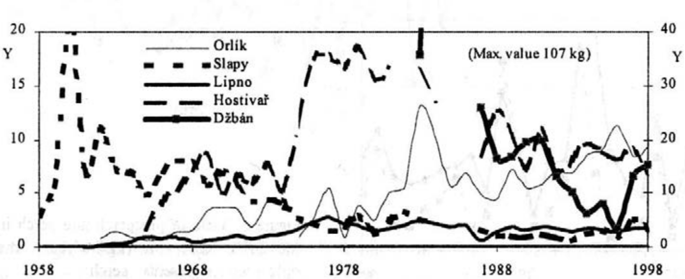
Figure 2. Stock carp and yield – Hostivař and Džbán Reservoir (kg/ha/year)

In the whole period examined the yields of the carp in Hostivař and Džbán reservoirs increased on average from 50 kg up to 200 (300) kg (Figure 2). Carp yields in small reservoirs are influenced by massive stocking and by an essentially higher fishing effort. In small reservoirs, the fishing effort is about 2 500 hours, in large reservoirs only 100 to 200 hours/ha/year (Pivnička, 1985). No data on the growth of stock carp in the reservoirs are available, except for Hostivař, where the annual increments were about 200 g in the 1980s (Pivnička, 1984).

Yield of other fish

The yield of the bream is shown in Figure 3. The highest yields were observed in the Džbán Reservoir in 1984 (107 kg), while in the Hostivař Reservoir they ranged between 30 and 37 kg at the end of 1970s. In 1980s, and at the end of 1990s yields decreased in both reservoirs. In large reservoirs, it is possible to observe a considerable stepwise increase in yields in the Orlík Reservoir (maximum of 13.2 kg in 1983) and, in contrast to this, a decrease in the Slapy Reservoir (maximum in 1960, 20.7 kg) to 1–2 kg in 1990s. The lowest yields of bream were recorded in the Lipno Reservoir, maximum values being achieved in 1977 (2.8 kg). A decrease in bream yields in small reservoirs, starting from the 1980s, can be associated with a stepwise increase in carp yields. However, the decrease in the bream yields in the Slapy Reservoir was accompanied by higher carp yields, and in the Orlík Reservoir the carp and bream yields increased simultaneously. The average individual weight of bream caught in Orlík is significantly lower (0.39 kg) in comparison with the Slapy Reservoir (0.57 kg).

The largest amounts of the pike were caught after filling the Hostivař Reservoir (1965 – 34.6 kg/ha) and Lipno Reservoirs (1961 – 12.4 kg/ha). The yields from the two large and deep reservoirs were much lower (Slapy maximum of 1 kg, Orlík maximum of 5.4 kg in 1963) (Figure 4). A considerable decrease in yields about 5 to 10 years after filling were observed in the Lipno and Orlík.



igure 3. Yield of bream in all reservoirs :udied (kg/ha/year); the right y axis rep-:sents Hostivař and Džbán reservoir

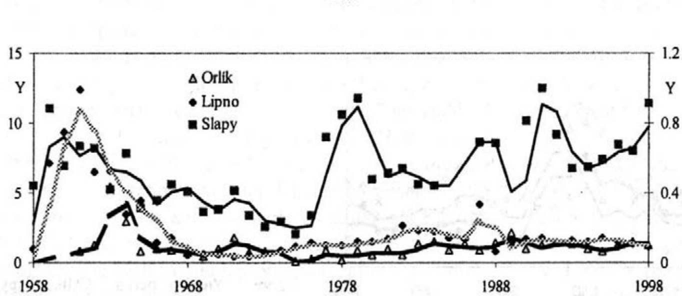


Figure 4. Yield of pike – Orlík, Lipno, and Slapy Reservoir (kg/ha/year); the right y axis represents Slapy Reservoir

In contrast to this, the Slapy Reservoir shows cyclic changes of the pike yields with maximum values after filling, at the end of the 1970s, 1980s, and 1990s (however, always only up to 1 kg). Cyclic changes of the yield can also be observed in the small reservoir Džbán (maximum value in 1988 – 28.1 kg, five years after filling).

The pikeperch yields in both small reservoirs stepwise increased. In the Hostivař Reservoir, a maximum value was achieved in 1992 (55.3 kg), in the Džbán Reservoir in 1998 (48.1 kg). In Hostivař, it is possible to observe yield cycles with increasing amplitude. Out of the large reservoirs, the best results were achieved in the Orlík Reservoir in 1984 (13.7 kg) and in 1989 (13.2 kg) (Figure 5). A similar trend in the yield of the pikeperch and perch in Orlík (Figure 6) is of interest. The lowest yield was reported from Slapy (4.8 kg after filling). Fairly good results were achieved in Lipno, maximum in 1985 (5.6 kg) was shared with the maximum value in the Orlík Reservoir.

Synchronous trends of yields can be observed in the perch in Orlík, Slapy and Hostivař reservoirs with maximum values at the beginning of the 1970s and in the mid-1980's (Figure 7). Maximum perch yields were achieved in 1987 (10.3 kg, Džbán), in 1982 (6.5 kg, Slapy), in 1983 (4.2 kg, Orlík), in 1983 (3.2 kg, Hostivař), and in 1965 (1.4 kg, Lipno).

Total yield

In the Džbán Reservoir, the total fish yield increased annually by 11 kg, however, the yield of the other fish (all fish without carp) was maintained at a level of 75 kg. The total fish yield in the Hostivař Reservoir (Figure 8) stepwise increased on average by 6.9 kg of fish annually, out of this the yield of other fish by 1.8 kg of fish annually. Correlation coefficients of both relationships

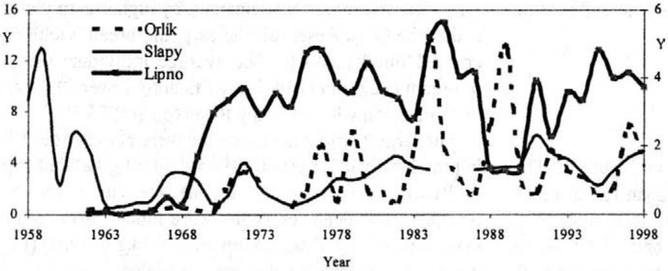


Figure 5. Yield of pikeperch – Orlík, Lipno, and Slapy Reservoir (kg/ha/year); the right *y* axis represents Slapy and Lipno Reservoir

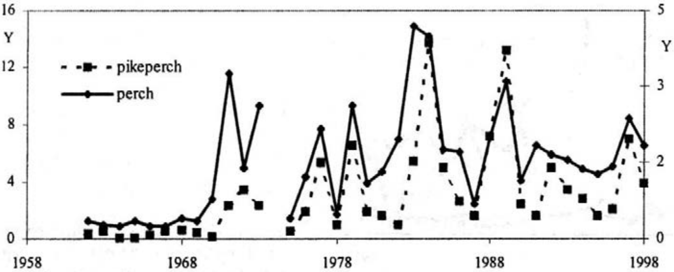


Figure 6. Yield of pikeperch and perch in the Orlík Reservoir (kg/ha/year); the right *y* axis represents perch

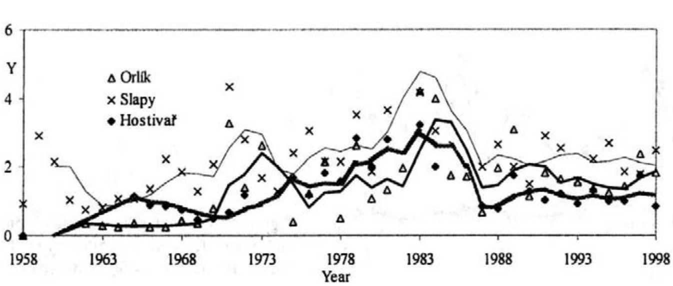


Figure 7. Yield of perch – Orlík, Slapy, and Hostivař Reservoir (kg/ha/year)

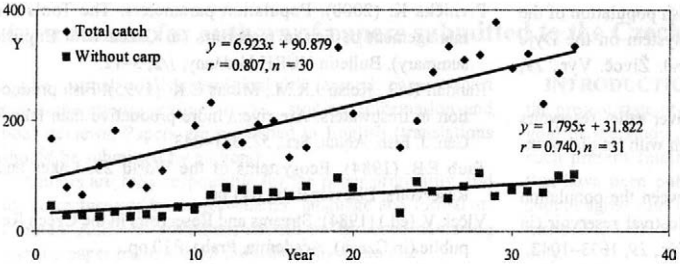


Figure 8. Total yield and yield of other fish (total yield without carp) – Hosti-vař Reservoir (kg/ha/year)

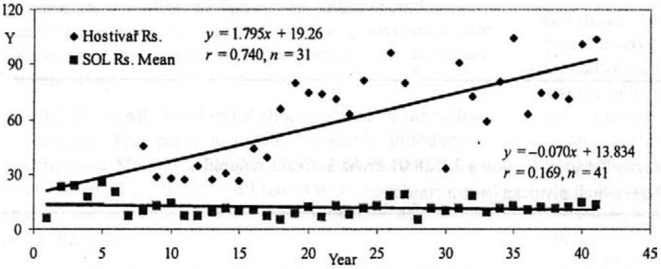


Figure 9. Total yield without carp – Hostivař Reservoir and average total yield without carp for Slapy, Orlík, and Lipno Reservoir, SOL (kg/ha/year)

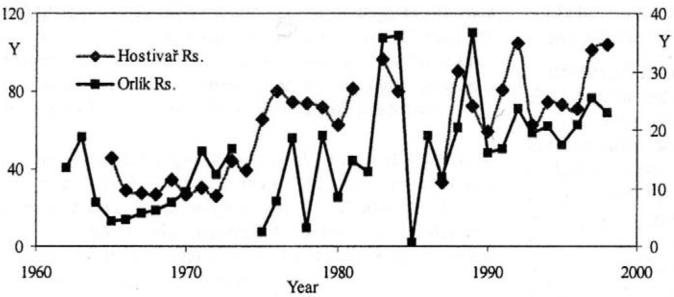


Figure 10. Total yield without carp – Hostivař and Orlík reservoir (kg/ha/year; the right *y* axis represents Orlík Reservoir

are highly significant ($P > 0.001$). The variation of the total yield particularly results from the variation of the stock carp biomass. Yield cycles in the category of the other fish (total yield without carp) can also be observed in the Hostivař Reservoir when compared with the average yield of the other fish for the large reservoirs Slapy, Orlík and Lipno (Figure 9). The yield of other fish in the small and large reservoir (Hostivař and Orlík) can be seen in Figure 10. Also in this case the mutual and synchronous yield cycles can be observed. So the yields of unstocked fish show a mutual trend in different reservoirs since they are influenced by the same short-term as well as middle-term changes of the climate (Beamish and Bouillon, 1993).

REFERENCES

Beamish R.J., Bouillon D.R. (1993): Pacific salmon production trends in relation to climate. Can. J. Fish. Aquat. Sci., *50*, 1002–1016.

Cyr H., Peters R.H. (1996): Biomass-size spectra and the prediction of fish biomass in lakes. Can. J. Fish. Aquat. Sci., *53*, 994–1006.

Jenkins R.M. (1982): The morphoedaphic index and reservoir fish production. Trans. Am. Fish. Soc., *111*, 133–140.

Kubečka J., Šeda J., Duncan A., Matěna J., Ketellars H., Visser P. (1998): Composition and biomass of the fish stocks in various European reservoirs and ecological consequences. Int. Rev. Hydrobiol., *83*, 559–568.

Lusk S. (1984): The management of the fish population of the upper lake of the Nové Mlýny dam systém on the Dyje river (in Czech with English summary). Živoč. Výr., *29*, 1043–1051

Lusk S., Krčál J. (1983): Exploitation of river valley reservoirs in the Dyje river drainage area (in Czech with English summary). Živoč. Výr., *28*, 809–816.

Pivnička K. (1984): The relationship between the population growth of weed fish and carp in the Hostivař reservoir (in Czech with English summary). Živoč. Výr., *29*, 1033–1042.

Pivnička K. (1985): The effectiveness of fishing in selected reservoirs in the Czech Republic (in Czech with English summary). Živoč. Výr., *30*, 927–935.

Pivnička K. (2000): Population parameters. The Tools for management of fish in reservoirs (in Czech with English summary). Bulletin VÚRH Vodňany, *1/2*, 5–12.

Randall R.G., Kelso J.R.M., Minns C.K. (1995): Fish production in freshwaters: Are rivers more productive than lakes? Can. J. Fish. Aquat. Sci., *52*, 631–643.

Taub F.B. (1984): Ecosystems of the World 23. Lakes and Reservoirs. Elsevier Publ. 643 pp.

Vlček V. (ed.) (1984): Streams and Reservoirs in the Czech Republic (in Czech). Academia, Praha. 310 pp.

Received: 00–09–01
Accepted after corrections: 01–01–17

Corresponding Author:

Karel Pivnička, Přírodovědecká fakulta Univerzity Karlovy, Benátská 2, 128 01 Praha 2, Česká republika
Tel. +420 2 21 95 31 09, fax +420 2 24 91 48 03, e-mail pivnicka@natur.cuni.cz

Instructions for authors of papers submitted to the Czech Journal of Animal Science

The journal publishes original full research papers, short communications, review articles, studies, information and book reviews. Papers are published in English (translations should be submitted by authors).

Authors are fully responsible for the paper originality, and for correctness of its subject-matter, language and formal attributes. Authors' statement should be enclosed declaring that the paper has not been published anywhere else.

If the paper is to be published, it should be submitted with an English and Czech (Slovak) abstract. It is the responsibility of the authors to provide for high quality translations. Manuscripts including all figures and tables should be submitted in two hard copies and on the disk. A text editor used for the paper preparation should be mentioned in an accompanying letter.

FULL RESEARCH PAPERS should consist of the following sections: Title page, Abstracts, Keywords, Introduction, Material and Methods, Results, Discussion, Acknowledgements, References, Tables and Figures with legends. The manuscript should not exceed 15 pages in length including all tables, figures, etc.

SHORT COMMUNICATION should consist of the following sections: Title page, Abstracts, Keywords, Main text, Acknowledgements, References, Tables and Figures with legends. The manuscript should not exceed 4 pages in length.

REVIEW ARTICLE summarizes the findings in the field concerned. The manuscript should not exceed 20 pages in length, and the reviewed literature sources must have been published in the last 20 years. Short abstracts in English and in Czech (Slovak) should be submitted with this article.

The title of the paper should be short and informative, of not more than 85 strokes. It must not contain any abbreviations and superfluous words (such as evaluation, study, description, brief results, preliminary information). Do not ever use any subtitles of papers. The title should be given in English and in Czech or in Slovak.

Authors names should have the form of the first name initials and surnames (ladies-authors can use their first names in full).

Grant funding of the paper: besides of the grant number, the full name of grant agency or institution should be given.

Authors **departments, institutions** should be given in an official version in English. If authors are from several institutions, their names should be designated by a digit corresponding to the same digit in a list of institutions.

Abstract should not have more than 150 words. It should contain important information on methods used to solve the problem, clear description of results and their statistical significance, and brief and unambiguous conclusions drawn from the results. References and discussion of results should not be included in the abstract.

Keywords should not repeat nouns used in the title and should describe the studied problem as best as possible.

INTRODUCTION section should provide information on the present state of research in the field concerned and on the goal of the study. References to literary sources document such present findings that are used by the authors, not all that have been published until now. References in the text should agree with those in the list of references. It is recommended to include references to papers from peer periodicals only.

MATERIAL AND METHODS section should provide a clear description of:

- Machines, instruments and equipment, chemicals, diagnostic kits, etc. (model, source or manufacturer, country of origin) and ways of their use.
- Animals/plants (exact definition of the species, number and sources of animals/plants, their inclusion in experiment and control groups, technology of housing/growing, feeding and tending, evaluation of the condition, samplings for examination, sample storage until they are processed).
- Methods (their detailed description or references to papers containing the method description); it is not admissible to cite any paper referring to the method used but not containing its description; it is necessary to describe any modifications of the cited methods in an unambiguous way; if the methods were not employed by the authors themselves, it should be indicated who provided the results (the author's name and institution).
- Methods of result evaluation, statistical data processing and software used.

RESULTS should be processed in a clear way, and if possible, represented graphically or arranged in tables. Parallel documentation of identical results in tables and in figures is not admissible. Statistical processing should be reasoned and perfect. The results should include data making their verification possible (e.g. mean, number of determinations and standard deviation should be given if significance of difference in two means is evaluated). Explicit and clear illustration of statistically significant differences in tables or figures is desirable. It is not possible to include any results that were provided by the procedure neither described nor cited in the Method section.

DISCUSSION can be combined with result presentation into one section or it can be a separate section, but it must not contain any result description in the latter case. Discussion section should contain a comparison of results presented in the paper with the present knowledge, showing clearly what findings are quite new, how the results differ from those presented by other authors or how they coincide with published conclusions. The importance of results should be emphasized, and new problems and the need of their solution should be highlighted in discussion. It should be stated in the last paragraph of the Discussion section whether the goal set in the introductory section was achieved, and what are the authors conclusions.

Acknowledgements make it possible to thank for help with result interpretation, for paper reviewing and for another help with its preparation, for financial or any other aid, for technical assistance, for provision of materials or important information, for examinations carried out by those who did not participate actively and creatively in the study or project, for translation of the text or its language correction, etc. It is also a chance of saying who else besides the authors worked on the paper, who contributed by their ideas, advice, who provided methods or experimental material from other experiments or equipment, etc. But who themselves did not contribute actively and creatively to the solution of the problem.

REFERENCES should contain all papers cited in the text, name is followed by date (if several papers that appeared within a year are cited, letters a, b, c ... should be used both in the text and in the References section). If the paper was written by more authors, all authors should be cited in the list of references, but only the first author plus *et al.* and date should be given in the text. Surnames are printed in small letters, followed by the first name initials, year in brackets and colon, the full title of the paper, official abbreviation of the journal, volume and extent.

Papers published in monographs or proceedings should be cited like this:

Kaláb J. (1995): Changes in milk production during the sexual cycle. In: Hekel K. (ed.): Lactation in Cattle. London, Academic Press: 876-888.

References should consist of peer periodicals. It is not possible to cite abstracts from conferences, research reports, dissertation and habilitation theses, textbooks and monographs without describing or citing experimental papers, popular or daily press and hardly available (in foreign countries unavailable) sources. Only exceptionally can unpublished findings or results be cited in the Discussion section, mentioning the author and using the note (unpublished) or (personal communication).

The papers not referred to in the text cannot be included in the list of references. Examples of references in the text: ... described by Brown (1995) ... described by a number of authors (Brown, 1995; Green and Grey, 1996; Reed *et al.*, 1998) ...

Examples of references in the list (abbreviations of periodicals are given in agreement with Science Citation Index of Current Contents):

Brown J. (1995): Estradiol determination in post-partum sows. J. Endocrinol., *198*,155–169.

Green K.L., Grey M. (1996): Hormones in milk. J. Anim. Res., *29*, 1559–1571.

Reed G., Green K.L., Brown J. (1989): Peptides as biological active compounds. J. Dairy Sci., *144*, 25–38.

Tables and figures should be supplied separately, and references in the text to all tables and figures are necessary. Illustrations should be of printable quality. Photographs and graphs should be mentioned in the text as figures, labelled with sequential numbers. Every figure should have a short and pregnant legend.

Contact Address of one of the authors to whom correspondence will be mailed should be given in Czech or Slovak (in English in foreign authors) at the end of the paper. Besides the address itself, it should contain telephone and fax numbers and e-mail address.

Other requirements

The hard copy of the manuscript should be printed in common fonts, size 12. Other technical requirements (presentation of figures and possibilities of photograph use, recommended text editors, formats of digital versions of figures, etc.) are available in the Editorial Office. The paper manuscript on disk including figures should always be accompanied by mailing two hard copies of the paper. When preparing electronic manuscripts, leave the right-hand margin unjustified and turn the hyphenation option off. Please, use wide margins, double spaces and quarto format. If graphs were produced in Excel (all in the same way), a table used as a basis for graph construction should be supplied as well.

Abbreviations and symbols used in the text should be explained when used for the first time. Units should comply with the SI measure system.

Expert opinion should be read by the authors very carefully; they should revise the manuscript as soon as possible and send it back to the Editorial Office. If the manuscript with reviewers comments was sent to them, it should be returned along with revised manuscript. It is necessary to respond to all comments made by reviewers and to state unambiguously how they were accepted (what alterations were made in the text) or if there are any authors objections to these comments and why they cannot be respected. The authors can disagree with the reviewers' opinion but they should reason their own opinion in written form. Editors will consider authors opinions carefully but they are not obliged to accept their objections.

Proofs should be made within two days of receipt, using common proof-reading marks (their list is available in the Editorial Office). The word *Imprimatur*, date and signature should be placed in the right upper corner of page 1 as a sign of proofreading. It is not possible to make any other alterations in the text except corrections or any alterations that would modify authors' statements or conclusions in the manuscript accepted for publication.

Offprints: Authors will receive 10 free offprints of the paper.