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

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Effects of short-term supplementation of dairy cow diets with surplus selenium and rapeseed meal on milk and blood selenium levels

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ABSTRACT: We studied the effects of short-time dietary supplementation with surplus selenium (Se) on Se concentrations in milk and blood of dairy cows fed a diet containing rapeseed meal. The experiment was designed as a 4 × 4 Latin Square with four three-week experimental periods. Two groups of dairy cows received a mixture containing rapeseed meal (RM) while in another two groups RM was replaced by soybean meal (SM). In each pair of the experimental groups, one mixture was supplemented with selenised yeast (RM_{Se}, SM_{Se}; 0.5 mg of Se per 1 kg of dry matter). The following parameters were evaluated: concentration of Se in milk; concentration of Se in whole blood; activity of glutathione peroxidase (GSH-Px) in whole blood; concentration of iodine in milk; triiodothyronine (T_3) and thyroxine (T_4) plasma concentration. The supplement of Se significantly increased its content in milk of dairy cows fed diets containing either RM (+29.5 µg/l; RM_{Se} vs. RM_0; $P < 0.01$) or SM (+28.0 µg/l; SM_{Se} vs. SM_0; $P < 0.01$). The concentration of Se in blood was not significantly influenced by the supplement of selenised yeast; there was only a tendency to increased Se concentration in cows receiving the Se supplement. GSH-Px activity, T_3 and T_4 concentrations and concentrations of iodine in milk were not influenced by dietary Se supplements. The presence of rapeseed meal in feed mixtures resulted in a reduced content of Se in milk: in groups receiving selenised yeast there was a trend to lower Se concentration by 4.19 µg/l ($P > 0.05$) while in groups without Se supplement the Se contentration was significantly lower by 5.62 µg/l ($P < 0.01$). As far as milk Se was concerned, the interaction of factors under study was statistically insignificant. The intake of RM in the diet resulted in a decreased concentration of iodine in milk both in animals receiving a diet with selenised yeast and without it (–134.0 µg/l, RM_{Se} vs. SM_{Se}, $P < 0.01$ and –145.1 µg/l, RM_0 vs. SM_0, $P < 0.01$, resp.). Feeding of RM did not influence the concentration of Se in blood, GSH-Px activity in blood and plasma concentrations of T_3 and T_4.

Keywords: glucosinolates; iodine; T_3; T_4; glutathione peroxidase

Selenium is an essential functional part of many biologically active proteins called selenoproteins. Selenium is also present in some important enzymes, e.g. glutathione peroxidase (GSH-Px) and iodothyroninedeiodinase (ID). Distribution of selenium in blood proteins is dependent on the amount and chemical form of this trace element supplied in the diet of cattle. In blood proteins of growing calves, selenium is mostly distributed in selenoproteins P and in albumin and glutathione peroxidase. An increased intake of selenium increases its concentration in the blood serum of growing calves (Awadeh *et al.*, 1998), whole blood of calves (Pavlata *et al.*, 2001) and whole blood of

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dairy cows (Awadeh *et al.*, 1998; Knowles *et al.*, 1999; Ortman and Pehrson, 1999).

Data on the effects of cattle diet supplementation with selenium on glutathione peroxidase concentration in blood are varying to a certain extent. In calves, an increased intake of selenium resulted in its increased concentration in blood serum but the concentration of glutathione peroxidase did not change (Awadeh *et al.*, 1998). On the other hand, in heifers (Ortman *et al.*, 1999) and dairy cows (Ortman and Pehrson, 1999) supplementation of their diets with selenium resulted both in its increased concentrations in whole blood and in the increased activity of glutathione peroxidase in erythrocytes. However, it is generally expected that the correlation between the activity of glutathione peroxidase and the concentration of selenium in blood of cattle is very high (Pavlata *et al.*, 2000).

Organic selenium (i.e. selenised yeasts) increases Se concentration in the blood of cattle much more than selenium supplied in the form of inorganic salts (Awadeh *et al.*, 1998; Ortman *et al.*, 1999; Knowles *et al.*, 1999). Selenium is excreted in milk and its concentration is dependent both on the amount and the form of this element supplied in the diet. Supplementation of feeding rations for dairy cows with selenium increases its concentration in milk (Foltys *et al.*, 2001; Ortman and Pehrson, 1999); however, the application of organic selenium is more efficient than its supply in form of inorganic salts (Ortman and Pehrson, 1999; Knowles *et al.*, 1999).

Selenium metabolism is closely related to metabolism of iodine. Iodine is an essential central component of hormones excreted by the thyroid gland but selenium is the second most important microelement in this metabolic process. Selenium is indispensable for an adequate synthesis of thyroid hormones, for their activation and for their metabolism (Kohrle, 1999). The thyroid gland is functionally linked up with a number of selenoproteins, with several forms of glutathione peroxidase, thioredoxinreductase, selenoprotein P and, especially, iodothyroninedeiodinase (Zagrodzki *et al.*, 1998; Kohrle, 1999). An experiment with effects of selenium supplementation on the concentration of thyroid hormones in blood plasma demonstrated that the increased supply of Se resulted in an increased concentration of triiodothyronine and in a higher triiodothyronine to thyroxine ratio; however, this increased intake of selenium did not influence the concentration of thyroxine.

Rapeseed meal contains some anti-quality compounds; the most important of them is the group of compounds generally called glucosinolates. The intake of glucosinolates from rapeseed meal results in hypothyreosis that is characterised by the increased activity of thyroid gland and decreased circulation levels of thyreohormones. It is also known that in dairy cows the supply of rapeseed meal can reduce the concentration of iodine in milk (Emanuelson *et al.*, 1993; Emanuelson, 1994).

The objective of this study was: a) to estimate the effects of surplus organic selenium (i.e. of selenised yeasts) on selenium concentrations in milk and whole blood and on the activity of glutathione peroxidase in whole blood of dairy cows under conditions of a short-term experiment (i.e. within three-week experimental periods); and b) to estimate the effect of rapeseed meal (and/or interactions of this feed with selenium) on selenium concentrations in milk and blood, on concentration of iodine in milk and on concentration of thyroid hormones in blood plasma.

MATERIAL AND METHODS

Eight dairy cows of the Czech Red Pied breed (mean BW 620 ± 44 kg) yielding 22 ± 3 l of milk per day were used in an experiment conducted from January to March 2001 in an experimental stall in the Research Institute of Animal Nutrition, Ltd., Pohořelice. Cows were kept individually and fed twice a day (i.e. at 5:00 a.m. and 3:00 p.m.) a basal diet consisting of maize silage (33% DM) and alfalfa hay plus concentrate feed mixture. The basal diet was calculated both for maintenance and production of 10 litres of milk and concentrates were offered at the amount of 0.45 kg per each litre of milk produced above the basic level of milk performance. The amounts of offered and refused feed were weighed daily. The experiment was designed to involve 4 periods of 21 days, the first 14 days of a period were used to adapt animals to the diet. Samples of milk and blood were collected during the last week of individual periods. The treatments involved four groups of animals given four experimental concentrate feed mixtures (i.e. in a 4 × 4 Latin Square experimental design). Two mixtures contained 270 g/kg of rapeseed meal, either with (RM_{Se}) or without (RM_0) mineral premix containing organic selenium (Se, supplied as selenised yeast, Sel Plex™, Alltech Inc.). The other two

mixtures did not contain RM (it was replaced by soybean meal at amounts respecting the isonitrogeneity of mixtures) and they were also either with (SM_{Se}) or without (SM_0) Se supplement. The content of Se in RM_{Se} and SM_{Se} mixtures was calculated as a surplus amount and corresponded to 250% of the recommended Se daily requirement for dairy cows (Šimek *et al.*, 1994). Ingredients and nutritional composition of experimental feed mixtures are presented in Table 1. The daily Se intake is presented in Table 2.

Samples of forage, feed mixtures and refused feed were collected in the 3rd week of each experimental period. Conventional methods were used to determine basic organic nutrients and minerals: crude protein by Kjeldahl method (Kjel-Foss Automatic 16210), crude fibre by Henneberg-Stohmann method (Fibretec, Tecator), calcium by atomic

Table 1. Ingredient and nutrient composition of feed mixtures

Ingredient		Concentrate feed mixture			
		RM_{Se}[1]	RM_0[2]	SM_{Se}[3]	SM_0[4]
Barley	(g/kg)	100	100	180	180
Maize	(g/kg)	350	350	275	275
Flax	(g/kg)	30	30	30	30
Oat	(g/kg)	80	80	125	125
Wheat	(g/kg)	139	140	99	100
Soybean meal (46% CP)	(g/kg)	–	–	180	180
Rapeseed meal	(g/kg)	270	270	–	–
Dried sugar beet pulp	(g/kg)	–	–	80	80
Feeding salt	(g/kg)	5	5	5	5
Mineral and vitamin supplement*	(g/kg)	25	25	25	25
Selenised yeast	(g/kg)	1	–	1	–
Nutrient		RM_{Se}	RM_0	SM_{Se}	SM_0
DM**	(g/kg)	886	887	884	886
CP**	(g/kg)	159	163	176	174
NEL	(MJ)	7.30	7.31	7.38	7.40
PDIN	(g/kg)	117	117	118	118
PDIE	(g/kg)	108	108	116	116
Crude fibre**	(g/kg)	4.83	4.92	4.67	4.59
Ca**	(g/kg)	6.01	6.01	5.33	5.33
P**	(g/kg)	6.28	6.28	4.99	4.98
Se**	(mg/kg)	1.11	0.08	1.11	0.08

[1]RM_{Se} = feed mixture with rapeseed meal and selenium supplement
[2]RM_0 = feed mixture with rapeseed meal
[3]SM_{Se} = feed mixture with soybean meal and selenium supplement
[4]SM_0 = feed mixture with soybean meal
*Mineral and vitamin supplement: calcium 150 g/kg; phosphorus 50 g/kg; sodium 90 g/kg; magnesium 80 g/kg; iron 2 000 mg/kg; zinc 7 000 mg/kg; manganese 7 000 mg/kg; copper 1 500 mg/kg; iodine 110 mg/kg; selenium 0 mg/kg; cobalt 20 mg/kg; vitamin A 1 000 000 i.u.; vitamin D3 100 000 i.u.; vitamin E 1 000 mg/kg; niacin 4000 mg/kg
** Marked values were obtained from analyses

Table 2. Dietary intake of selenium, iodine and glucosinolates

			Treatment (mean ± SD)			
		n	RM_{Se}	RM_0	SM_{Se}	SM_0
Selenium intake						
Feed mixtures	(mg/d)	2	$8.0^A \pm 1.11$	$0.6^B \pm 0.08$	$7.9^A \pm 1.21$	$0.6^B \pm 0.08$
Total dietary intake	(mg/d)	2	$10.2^A \pm 1.10$	$2.7^B \pm 0.08$	$10.0^A \pm 1.20$	$2.8^B \pm 0.09$
Total dietary intake	(mg/kg DM)	2	$0.5^A \pm 0.04$	$0.1^B \pm 0.00$	$0.5^A \pm 0.04$	$0.1^B \pm 0.00$
Iodine intake						
Feed mixtures	(mg/d)	2	14.8 ± 2.05	14.7 ± 2.10	14.6 ± 2.24	14.5 ± 1.98
Total dietary intake	(mg/d)	2	15.6 ± 2.04	15.5 ± 2.09	15.3 ± 2.24	15.2 ± 1.99
Total dietary intake	(mg/kg DM)	2	0.8 ± 0.07	0.8 ± 0.07	0.8 ± 0.08	0.8 ± 0.07
Glucosinolate intake						
Gluconapin	(mmol/d)	2	6.3 ± 0.87	6.2 ± 0.89	–	–
Glucobrassicanapin	(mmol/d)	2	1.6 ± 0.22	1.6 ± 0.23	–	–
Progoitrin	(mmol/d)	2	14.5 ± 2.01	14.4 ± 2.05	–	–
Total glucosinolates	(mmol/d)	2	22.4 ± 3.09	22.2 ± 3.16	–	–

Means with different superscripts in lines differ significantly ($^{A, B, C}P < 0.01$)

absorption (Perkin-Elmer 4000) and phosphorus by a spectrophotometric method. Feed mixtures were also analysed for the content of glucosinolates (GLS; gluconapin, glucobrassicanapin, progoitrin) using the gas chromatography method (Zukalová and Vašák, 1978). The values of dietary GLS content were converted to total daily GLS intake and are presented in Table 2.

Milk yield was recorded daily. Milk samples were collected during morning milking on Days 16, 17 and 18 in each of the experimental periods. Milk samples were analysed for milk fat by Gerber acidobutyrometric method, total protein (Pro-Milk Mk-II, Foss Electric), whey protein (Pro-Milk Mk-II, Foss Electric, after casein agglomeration), casein (Pro-Milk Mk II: Instruction manual. A/S N., Foss Electric) and lactose by a polarimetric method (according to ČSN 57 0530) and solids-non fat content was calculated from solids content and milk fat content.

Blood was sampled after morning milking on day 19 of each experimental period.

Milk, feed and a part of the blood samples were analysed for Se concentration by atomic absorption after mineralisation by the hydride technique (Unicam 939 spectrometer). The concentration of iodine in milk was analysed after alkaline digestion (Bednář *et al.*, 1964) using Sandell-Kolthoff spectrophotometric method.

The other part of blood samples was used for estimation of glutathione peroxidase activity according to the method described by Paglia and Valentine (1967), using a set supplied by Randox and an automatic analyser (Cobas Mira S, Roche). Concentrations of triiodothyronine (T_3) and total thyroxine (T_4) in blood plasma were measured by means of a radioimmunological method (commercial kits, Immunotech, Prague). The T_3/T_4 ratio was estimated on the basis of plasma concentrations of triiodothyronine and total thyroxine.

The effects of the inclusion of rapeseed and supplemental Se into the diets were tested using the multifactorial analysis of variance (ANOVA). Calculations were carried out using the software MS Excel and Statgraphics (Version 7.0).

RESULTS AND DISCUSSION

Intake of selenium and glucosinolates

In the groups of dairy cows fed diets containing selenised yeasts, the average intake of selenium (Se) was 0.5 mg/kg DM (Table 2). This intake

was 2.5 times higher than the recommended daily intake of Se in dairy cows (Šimek *et al.*, 1994). The intake of most common glucosinolates (GLS) was calculated on the basis of feed mixture analyses. In the group receiving rapeseed meal, the average daily intake of GLS (gluconapin, glucobrassicanapin, progoitrin) was 22.3 mmol (Table 2).

Milk

None of the factors under study showed an effect on milk production or basic milk constituent concentration (Table 3, $P > 0.05$).

The dietary supplement of selenised yeasts highly significantly increased the concentration of Se in milk of both groups of dairy cows (Table 4). In the RM_{Se} and SM_{Se} groups the concentrations of Se were higher by 29.5 µg/l and 28.0 µg/l, respectively, than in RM_0 and SM_0 ($P < 0.01$). Selenium concentrations in milk from non-supplemented dairy cows (i.e. RM_0 and SM_0) corresponded with literary data from cows without Se supplementation (Ortman and Pehrson, 1999). Knowles *et al.* (1999) reported a seasonal variation of milk selenium concentration between 27 and 106 nmol Se/l.

Increased Se levels in milk can be reached within 21 days (Knowles *et al.*, 1999) or 7 days (Ortman and Pehrson, 1999) after the beginning of Se supplementation. In our experiment the Se concentrations in milk were analysed on Day 14 after the beginning of Se supplementation. Ortman and Pehrson (1999) reported that the speed at which the plateau concentration of Se in milk is reached suggests that Se originates from selenoamino acids (selenised yeast) that would displace some of the sulphur-containing amino acids normally used for the synthesis of milk proteins. However, in their experiments after 12 weeks of supplementing the cows with selenised yeast the milk Se plateau was not reached. In a long-term experiment on grazing cows supplemented with Se, Knowles *et al.* (1999) reported that the plateau Se concentration in milk was reached in 60 days after the beginning of Se supplementation.

Due to the increased supply of Se, its daily output in milk and the ratio between its intake and output were higher (Table 5). In groups receiving

Table 3. Mean milk yield and mean basic milk constituents

		n	Treatment (mean ± SD)			
			RM_{Se}	RM_0	SM_{Se}	SM_0
Milk yield	(l/day)	2	20.5 ± 1.69	21.0 ± 2.12	21.4 ± 2.49	20.8 ± 2.10
Milk fat	(%)	2	4.1 ± 0.80	4.1 ± 0.60	4.0 ± 0.65	4.1 ± 0.81
Total protein	(%)	2	3.6 ± 0.37	3.6 ± 0.26	3.7 ± 0.30	3.6 ± 0.39
Whey protein	(%)	2	0.8 ± 0.05	0.7 ± 0.06	0.8 ± 0.18	0.7 ± 0.11
Casein	(%)	2	2.9 ± 0.36	2.9 ± 0.26	2.9 ± 0.24	2.9 ± 0.38
Lactose	(%)	2	5.1 ± 0.21	5.1 ± 0.18	5.1 ± 0.20	5.1 ± 0.19
Solids-non fat	(%)	2	9.3 ± 0.25	9.2 ± 0.26	9.3 ± 0.29	9.2 ± 0.30

Table 4. Concentration of selenium in milk and whole blood

		n	Treatment (mean ± SD)			
			RM_{Se}	RM_0	SM_{Se}	SM_0
Selenium concentration						
Milk	(µg/l)	2	41.95^{A} ± 8.57	12.48^{B} ± 2.69	46.14^{A} ± 8.22	18.10^{C} ± 3.41
Blood	(µg/l)	2	192.0 ± 34.58	184.4 ± 46.56	202.4 ± 31.54	186.3 ± 40.06

Means with different superscripts in lines differ significantly ($^{A, B, C}P < 0.01$)

RM the daily output of Se was higher by 0.6 mg, when RM_{Se} and RM_0 were compared. Similarly, an increased supply of Se in groups fed the diets without RM increased the daily output of Se by 0.6 mg (SM_{Se} vs. SM_0). In the RM_{Se} group, the ratio of Se dietary input to Se output in milk was 1.3 times higher than in RM_0 group. In the SM_{Se} group this ratio was even 2.9 times higher than in SM_0.

The dietary supplement of selenised yeast did not influence the concentration of iodine in milk of experimental dairy cows with and without RM ($P > 0.05$, Table 6); the output of iodine in milk also remained without changes (RM_{Se} vs. RM_0, and/or SM_{Se} vs. SM_0; $P > 0.05$; Table 5).

The supplement of rapeseed meal to feed mixtures decreased Se concentration in milk, viz. in groups with selenised yeast there was a trend to lower Se concentration by 4.19 µg/l (RM_{Se} vs. SM_{Se}; $P < 0.05$; Table 4). In groups without Se supplements, a decrease in Se concentration in milk was observed, viz. by 5.62 µg/l (RM_0 vs. SM_0; $P < 0.01$). Similarly, a decrease in minerals in milk after RM feeding was reported. Trávníček *et al.* (2001) described a decreased iodine content in milk in sheep fed a diet with glucosinolates (rapeseed meal origin) and nitrates. The authors explained the decreased concentrations of iodine in milk as the inhibition of active transport of iodine into the mammary gland due to competitive effects of nitrates and glucosinolates. Zech *et al.* (1995) explained this effect on the basis of an increased renal clearance of iodine due to a higher concentration of thiocyanates in the blood of dairy cows.

As far as the concentration of Se in milk was concerned, the interactions of individual factors were statistically insignificant. The supplement of rapeseed meal to feed mixtures decreased the daily output of Se in milk (–0.1 mg/d in the case of RM_{Se} vs. SM_{Se} and –0.1 mg/d in the case of RM_0 vs. SM_0) while the ratio of its intake to its output in milk increased (+1.7 mg/d for RM_{Se} vs. SM_{Se} and +3.3 for of RM_0 vs. SM_0). The supplement of rapeseed meal to feed mixtures significantly decreased the concentration of iodine in milk; in groups with and without selenised yeast this decrease was 134.0 µg/l (RM_{Se} vs. SM_{Se}; $P < 0.01$) and 145.1 µg/l (RM_0 vs. SM_0; $P < 0.01$), respectively. This supplementation also decreased the daily output of iodine in milk (–2.9 RM_{Se} vs. SM_{Se} and –3.1 RM_0 vs. SM_0).

Concentrations of fat, total protein, whey protein, casein, lactose and solids-non fat were not influenced by any factor under study (Table 3).

Table 5. Selenium and iodine output in milk (calculated values)

		n	Treatment (mean ± SD)			
			RM_{Se}	RM_0	SM_{Se}	SM_0
Selenium output						
Milk	(mg/day)	2	$0.9^{A,a} \pm 0.21$	$0.3^{B,c} \pm 0.08$	$1.0^{A,b} \pm 0.23$	$0.4^{B,d} \pm 0.09$
Intake/output ratio		2	$12.2^{A} \pm 2.62$	$10.9^{a} \pm 2.59$	10.5 ± 2.36	$7.6^{B,b} \pm 1.58$
Iodine output						
Milk	(mg/day)	2	$3.2^{A} \pm 1.42$	$3.2^{A} \pm 1.85$	$6.1^{B} \pm 1.57$	$6.3^{B} \pm 2.28$

Means with different superscripts in lines differ significantly ($^{a,b,c}P < 0.05$; $^{A,B,C}P < 0.01$)

Table 6. Concentration of iodine in milk

		n	Treatment (mean ± SD)			
			RM_{Se}	RM_0	SM_{Se}	SM_0
Iodine concentration						
Milk	(µg/l)	2	$153.4^{A} \pm 68.58$	$153.4^{A} \pm 85.15$	$287.4^{B} \pm 76.54$	$298.5^{B} \pm 91.08$

Means with different superscripts in lines differ significantly ($^{A,B,C}P < 0.01$)

Blood

Selenium concentration in blood was not significantly influenced by its supplements to feed mixtures (Table 4). In groups with selenised yeast there was only a slight tendency to increased Se concentrations. In the RM_{Se} group, its concentration in blood increased by 7.6 µg/l (as compared with RM_0; $P > 0.05$). In the SM_{Se} group, Se concentration in blood increased by 16.1 µg/l (as compared with SM_0; $P > 0.05$). Mean values of Se concentration in blood samples ranged from 184 µg/l (RM_0) to 202 µg/l (SM_{Se}). Gerloff (1992) described Se concentrations of seven herds of dairy cows supplemented with 6 mg of Se per animal/day, which ranged from 58 to 91 ng/ml. The estimated values of Se concentration in blood were by tens of µg/l higher than those reported by Ortman and Pehrson (1999); this corresponded with lower doses of selenised yeast used in their experiments. As far as the evaluation of Se reserves in cattle according to the scale published by Scholz and Fleischer (1996) was concerned, it was concluded that the level of Se in tissues of dairy cows was very high during the whole experiment. The lowest Se concentrations in blood were estimated in the first experimental period; thereafter, only increased Se concentrations in blood were estimated in all groups of dairy cows. This result indicated that Se concentration in blood was stable and that it was not increased by a further surplus intake of Se as it was in the case of its concentration in milk. In experimental periods without the supply of selenised yeast that followed after the periods of Se supplementation no significant decrease of Se in blood was observed. Ortman and Pehrson (1999) supplemented dairy cows with selenised yeast for 12 weeks, the Se concentration in whole blood increased in all the supplemented groups, but it did not reach a plateau in any of the supplemented groups. This means that the three-week experimental periods in our experiment were not sufficient for a restoration of Se concentration in the blood of dairy cows.

The activity of glutathione peroxidase (GSH-Px) in blood was not significantly influenced by Se supplements nor by the presence rapeseed meal in feed mixtures for dairy cows (Table 7, $P > 0.05$). The concentration of selenium in blood can be derived from the activity of GSH-Px in blood because there is a high correlation between these two parameters (Pavlata *et al.*, 2000). In our experiment, the activity of GSH-Px in blood ranged from 656 µcat/l (SM_0) to 721 µcat/l (RM_0). As compared with data published by Pavlata *et al.* (2000), these values were higher but they corresponded to an increased supply of Se. Similarly like in the case of Se concentration in blood, the activity of GSH-Px was lower only in the first experimental period. Thereafter, it was permanently increased, regardless of the experimental treatments.

The concentrations of T_3 in blood plasma were not influenced by the dietary supply of selenium either ($P > 0.05$; Table 7). The supplement of Se was manifested through an increased concentration of T_4 in blood plasma (RM_{Se} +2.1 mmol/l vs. RM_0 and SM_{Se} +3.0 mmol/l vs. SM_0; $P > 0.05$, respectively). Se supplement did not change the T_3 to T_4 ratio ($P > 0.05$).

Rapeseed meal did not influence the concentration of Se in blood (Table 4) and the activity of GSH-Px was not changed by the presence of rapeseed meal in feed mixtures either (Table 7).

The concentration of T_3 in plasma of dairy cows was not influenced by the presence of rapeseed meal in feed mixtures and only a slight decrease in the concentration of T_4 in plasma was observed (–4.1 nmol/l, RM_{Se} vs. SM_{Se}; or –3.2 nmol/l, RM_0 vs. SM_0; $P > 0.05$).

Table 7. Glutathione peroxidase activity in whole blood and thyroidal hormone concentration in plasma

		n	Treatment (mean ± SD)			
			RM_{Se}	RM_0	SM_{Se}	SM_0
$GSH\text{-}P_X$*	(µkat/l)	2	658.8 ± 217.37	721.2 ± 274.37	705.2 ± 197.16	656.0 ± 205.64
Thyroidal hormones						
Triiodothyronine (T 3)	(nmol/l)	2	2.0 ± 0.72	2.0 ± 0.61	2.0 ± 0.72	1.9 ± 0.80
Total thyroxine (T 4)	(nmol/l)	2	51.5 ± 9.30	49.4 ± 7.55	55.6 ± 7.88	52.6 ± 10.64
T 3 to T 4 ratio		2	0.04 ± 0.015	0.04 ± 0.014	0.04 ± 0.013	0.04 ± 0.019

*Glutathione peroxidase activity

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ABSTRAKT

Vliv krátkodobého přídavku nadbytku selenu a řepkového extrahovaného šrotu dojnicím na obsah selenu v mléce a krvi

Byl sledován vliv krátkodobého doplňku selenu v nadbytku na koncentraci selenu (Se) v mléce a krvi dojnic krmených dietou s obsahem řepkového extrahovaného šrotu (ŘEŠ). Pokus byl založen jako 4 × 4 latinský čtverec s třítýdenními pokusnými periodami. Dvěma skupinám dojnic byly krmeny směsi s obsahem ŘEŠ, z nichž jedna

byla dotována doplňkem Se v podobě kvasinek obohacených selenem (RM_{Se}; Sel Plex™; 0,5 mg Se na kg sušiny). Ve směsích dalších dvou skupin dojnic byl ŘEŠ nahrazen sojovým extrahovaným šrotem (SEŠ), směs jedné ze skupin byla taktéž dotována kvasinkami obohacenými selenem (SM_{Se}). Byla sledována koncentrace Se v mléce; koncentrace Se v plné krvi, aktivita glutationperoxidázy (GSH-Px) v plné krvi, koncentrace jodu v mléce, koncentrace trijodthyroninu (T_3) a thyroxinu (T_4) v krevní plazmě dojnic. Přídavek selenem obohacených kvasinek průkazně zvýšil obsah Se v mléce jak u dojnic s ŘEŠ ve směsi (+29,5 μg/l, RM_{Se} vs. RM_0; $P < 0,01$), tak i u dojnic bez ŘEŠ (+28,0 μg/l, SM_{Se} vs. SM_0; $P < 0,01$). Koncentrace Se v krvi nebyla dodatkem selenem obohacených kvasinek průkazně ovlivněna, pouze byla zaznamenána tendence jeho zvýšené koncentrace u dojnic dotovaných Se (+7,6 μg/l, RM_{Se} vs. RM_0; resp. +16,1 μg/l, SM_{Se} vs. SM_0; $P > 0,05$). Aktivita GSH-Px v krvi, koncentrace T_3 a T_4 v plazmě a koncentrace jodu v mléce nebyly dodatkem Se do krmné dávky dojnic ovlivněny. Přítomnost ŘEŠ v krmných směsích dojnic se projevila sníženým obsahem Se v mléce dojnic, ve skupinách s kvasinkami obohacenými selenem ve směsích byla tendence k nižší koncentraci Se o 4,19 μg/l ($P > 0,05$) a ve skupinách bez kvasinek ve směsích byla koncentrace Se průkazně nižší o 5,62 μg/l ($P < 0,01$). Interakce faktorů v případě koncentrace Se v mléce byla statisticky neprůkazná. Příjem ŘEŠ ve směsích měl za následek sníženou koncentraci jodu v mléce, a to jak v případě současné dotace Se (–134,0 μg/l, RM_{Se} vs. SM_{Se}, $P < 0,01$), tak i u skupin bez dodatku Se (–145,1 μg/l, RM_0 vs. SM_0, $P < 0,01$). Koncentrace Se v krvi, aktivita GSH-Px v krvi a koncentrace T_3 a T_4 v plazmě zůstaly vlivem ŘEŠ nezměněny.

Klíčová slova: glukosinoláty; jod; T_3; T_4; glutationperoxidáza

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The effect of oil with elevated content of n-3 polyunsaturated fatty acids (PUFA) on some metabolic and immunological parameters in germ-free and conventional piglets

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ABSTRACT: Oil with n-3 polyunsaturated fatty acids (PUFA) was applied to suckling conventional and germ-free piglets. In an experimental group (EG) of piglets the level of growth factors was significantly higher ($P < 0.05$), determined on the basis of somatomedin in the blood serum. Biochemical indices showed a significant increase in the level of γ-linolenic (GLA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in the blood serum, and at the same time the level of arachidonic acid (AA) decreased. n-3 PUFA affect the fatty acid metabolism and the prostaglandin synthesis. The application of oil with PUFA resulted in a significant increase in actual counts of B lymphocytes in the peripheral blood ($P < 0.05$) of germ-free piglets. Higher individual titres of specific antibodies were also recorded in EG after vaccination. Total counts of leukocytes were higher in EG. A significant increase in the actual counts of CD4 and CD8 lymphocytes in peripheral blood was detected in comparison with control animals. This can be a proposition in the therapy of inflammatory processes and in reducing the risk of infectious diseases in piglets.

Keywords: piglets; n-3 PUFA; fatty acid metabolism; IgM-presenting lymphocytes; CD4, CD8 T lymphocytes

Recently, great attention has been paid to essential polyunsaturated fatty acids (PUFA) and their effect on the nutrition of piglets. An increased intake of n-3 PUFA decreases the level of triacylglycerides, VLDL (very low density lipoproteins), cholesterol and low density lipoproteins (LDL) in the blood plasma of experimental animals. In the plasma lipids, the representation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is increased at the expense of arachidonic acid (AA) (Herold and Kinsella, 1986). In some studies an increase in the high density lipoprotein (HDL) cholesterol is described while the effect of DHA was more pronounced than that of EPA (Nestel *et al.*, 1984). The mechanism of n-3 PUFA action on the plasma lipids has not been explained yet. n-3 PUFAs are expected either to decrease the rate of apoprotein B synthesis (Nestel *et al.*, 1984) or to form VLDL triacylglycerols (Harris *et al.*, 1984).

n-3 PUFAs intervene into the prostaglandin synthesis. This leads to a decrease in aggregation of thrombocytes. Prostaglandins and leucotrienes have an effect on the immune functions and inflammatory reactions. n-3 PUFAs pass the lipoxygenase pathway, whereby mediators with reduced inflammatory activity are formed; on the other hand, n-6 PUFAs are metabolised by cyclo-oxygenase and pro-inflammatory metabolites arise (Vaughn *et al.*, 1994). Essential PUFAs, acting as structural components of cellular membranes and as precursors for eicosanoid production, are important modulators of humoral and cell-mediated immune reactions. The composition of PUFAs in the cells of the immune system indicates their potential effect

on the biological receptors, signal transduction and lymphocyte proliferation.

The aim of this study was to find out the effect of oil with increased content of n-3 PUFAs on biochemical, hematological and immunological changes in the organism of piglets. The production of growth hormones and formation of eicosanoids were also studied using the analysis of blood serum of piglets and comparison of the results of these examinations was carried out in experiments with conventional and germ-free animals.

MATERIAL AND METHODS

Experiments were carried out on conventional and germ-free piglets of the same breed. Eleven conventional piglets (Landrace × Slovak Large White crossbreds) at the age of 4 days, weighing 1.2–2.0 kg, were divided into two groups (control n = 5, experiment n = 6). Eight germ-free piglets, obtained by hysterectomy, bred in sterile isolators, were divided into an experimental (n = 4) and control (n = 4) group.

During a 35-day period after birth, the conventional piglets (n = 6) of the experimental group were given oil containing n-3 PUFAs orally at a dose of 100 mg/kg/day. The composition of oil is in Table 1. The oil dose was increased according to the weight measured in week intervals. The control group was given a physiological solution. All the animals (experimental and control) were immunised intramuscularly with 1 ml of vaccine against Aujeszky's disease (Inavak G1 inj. a.u.v., Mevak, Inc., Nitra, Slovak Republic) in order to determine the effect of n-3 PUFA on the formation of specific antibodies. Revaccination was made with the same dose of vaccine two weeks after the 1st application.

Germ-free experimental piglets (n = 4) were orally administered oil with increased content of n-3 PUFA at the amount of 0.5, 1.0, and 1.5 ml/day in weeks 1, 2, and 3 during 21 days after birth. The control animals were administered a physiological solution at the same dose instead of oil.

Table 1. Chemical composition of oil

5 g of oil contains	
Total n-6 PUFA (g)	0.1
Total n-3 PUFA (g)	1.0
Total unsaturated FA (g)	2.6
Total saturated FA (g)	0.9
Cholesterol (g)	0.005

Table 2. Representation of fatty acids in Sunar milk

Acid	% of volume	Acid	% of volume
Butyric	9.5	Stearic	7.6
Caproic	4.1	Arachic	1.8
Caprylic	0.8	Myristelic	0.9
Capric	3.2	Palmitic	4.3
Lauric	2.9	Oleic	22.4
Myristic	11.5	Linolic	3.1
Palmitic	26.7	Arachidonic	1.0

The food ration consisted of dry, full-fat milk (Sunar, PMV Hradec Králové, Czech Republic) diluted with water at a ratio 1 : 9 (Table 2). Piglets were fed 6 times daily.

The blood sampling was performed from the venous sinus of the piglet eye in weekly intervals.

Lipids were extracted from the serum and modified to bromphenylesters of fatty acids by the method of derivation in acetonitrile (Kašteľ *et al.*, 1999).

Bromphenylesters of fatty acids were determined using the HPLC method (Spectra Physics SP 8 700, Santa Clara, CA, USA) with UV VIS detector at 254 nm.

The growth hormone somatomedin C (IgF_1) was determined in the blood serum by the RIA method.

Subpopulations of T lymphocytes (CD4, CD8) and B lymphocytes were assayed quantitatively on a flow cytometer using mouse anti-pig monoclonal antibodies by the indirect immunofluorescence method, and measured by FAC-Scan (Becton Dickinson, Germany). Primary monoclonal antibodies used in experiments were: CD4 (74-12-4, IgG2b, 1 : 25), CD8 (76-2-11, IgG2a, 1 : 25) all from Czech Academy of Sciences, Prague, Czech Republic; IgM (Kl39.3El, IgG2a, 1 : 25) from University of Bristol, Great Britain. A dot plot of 10 000 cells was used for the analysis, obtained by forward and side scattering of physical character of the lymphocyte population. The results are therefore expressed as the percentage of the lymphocyte population that was positive for specific MoAbs. The absolute number of lymphocytes was calculated by the differential counts of leukocytes.

Student's *t*-test was used for statistical evaluation.

RESULTS

By comparison of the results of experiments with germ-free and conventional animals it was possible to observe more pronounced differences in the individual immune parameters, especially in germ-free animals. Germ-free animals served as an optimal experimental model of digestion physiology and lipid metabolism.

The growth factors, found in the blood serum of conventional and germ-free piglets using the RIA method by somatomedin measurement, indicate their significant increase in the experimental group compared to the control groups of animals ($P < 0.05$; Figure 1).

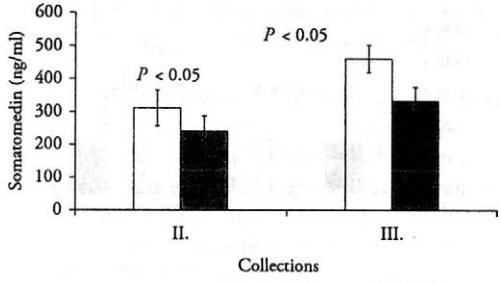

Figure 1. Growth factor in the blood of conventional piglets

After administration of n-3 PUFA to the germ-free experiments, the increased levels of fatty acids in the serum of piglets were found. The level of EPA in the experimental group increased 1.3-times compared to the control group. The level of DHA was significantly increased 1.7-times ($P < 0.05$) in comparison with the control animals, and the concentration of AA decreased 1.5-times compared to the control (Figure 2).

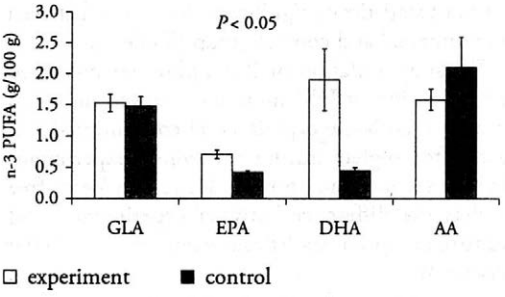

Figure 2. n-3 PUFA in the blood of germ-free piglets

In the peripheral blood of conventional piglets in the experimental group, the mean counts of leukocytes (week 3) and lymphocytes (weeks 1–3; Table 3) were slightly increased, whereas no essential differences were found between conventional and germ-free piglets.

Examination of CD4 and CD8 positive cells revealed a significant increase in the absolute number in the experimental group at the third sampling in germ-free piglets. CD4 and CD8 positive subpopulations of lymphocytes showed increased values in

Table 3. Mean counts of leukocytes and lymphocytes in the peripheral blood of piglets after n-3 PUFA supplementation (means ± SD)

Collections	Lc (1.10^9/l)		Ly (1.10^9/l)	
	E	C	E	C
Germ-free piglets				
1	16.35 ± 2.81	16.79 ± 3.94	3.83 ± 0.64	2.73 ± 0.98
2	12.91 ± 6.03	13.42 ± 1.28	3.84 ± 1.00	2.99 ± 0.70
3	19.13 ± 2.00	16.33 ± 1.39	5.49 ± 1.19	3.70 ± 0.75
Conventional piglets				
1	8.35 ± 2.22	7.12 ± 1.80	5.60 ± 1.38	4.81 ± 1.22
2	12.73 ± 4.04	9.10 ± 2.53	8.76 ± 3.80	5.67 ± 2.13
3	12.13 ± 1.94	11.46 ± 1.17	6.71 ± 1.74	6.09 ± 1.10

Legend: Lc = leukocytes, Ly = lymphocytes, E = experimental group, C = control group

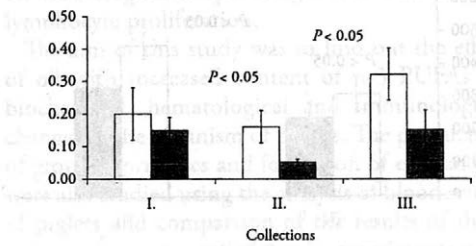

Figure 3. The absolute counts of IgM presenting lymphocytes in the peripheral blood of germ-free piglets

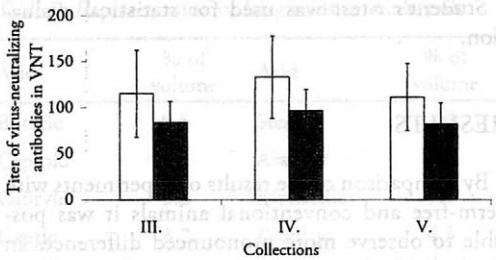

Figure 4. Titres of neutralising serum antibodies to Aujezsky's virus (VNT) in conventional piglets

the experimental group of conventional piglets without statistically significant differences between experimental and control group (Table 4).

The subpopulation of B lymphocytes measured by expression of IgM molecules showed increased values in both the experimental conventional and germ-free piglets during the whole experiment. In the second and third collection in germ-free piglets the difference between experimental and control group was statistically significant ($P < 0.05$; Figure 3).

In conventional animals, the level of specific immunity in a relationship to antibodies against Aujezsky's disease was tested after vaccination with Inavak G 1 (Mevak, Nitra, Slovak Republic). In all three collections after re-vaccination, the higher mean titres of specific antibodies were recorded in the experimental group of piglets compared to the control group of animals. The differences in the mean titres in both groups were not statistically significant regarding the high variability of titres of specific antibodies in individual piglets of both groups. The individual titres of some animals in the experimental group reached the values 1 : 512, while in the control group it was 1 : 128 maximally (Figure 4).

DISCUSSION

The experiment was aimed at observation of biochemical, hematological and immunological changes in the organism of piglets that were caused by supplementation of oil with increased content of n-3 PUFA. The experiments were carried out on conventional and germ-free piglets. Germ-free piglets were used as an optimal experimental model of the digestive tract physiology and lipid metabolism.

Table 4. Effect of n-3 PUFA supplementation on the absolute number of CD4 and CD8 subpopulations of lymphocytes in the peripheral blood of germ-free and conventional piglets (means ± SD)

Collections	CD4 (1.10^9/l)		CD8 (1.10^9/l)	
	E	C	E	C
Germ-free piglets				
1	1.71 ± 0.33	1.18 ± 0.50	0.74 ± 0.20	0.55 ± 0.29
2	1.42 ± 0.35	0.98 ± 0.31	0.89 ± 0.32	0.57 ± 0.29
3	1.28 ± 0.02	1.09 ± 0.08*	1.79 ± 0.49	0.84 ± 0.24*
Conventional piglets				
1	1.70 ± 0.39	1.40 ± 0.32	1.36 ± 0.32	1.02 ± 0.29
2	2.96 ± 1.14	2.23 ± 0.94	3.50 ± 2.13	1.81 ± 0.72
3	2.92 ± 0.99	3.03 ± 0.47	2.37 ± 0.74	2.05 ± 0.48

Legend: CD = cluster of differentiation, E = experimental group, C = control group

*$P < 0.05$

The representation of gamma-linolenic acid and its metabolites in colostrum is related with a low activity of desaturating enzymes in suckling piglets that are not able to synthesize higher PUFAs from the precursor PUFAs at the beginning of their life (Čertík, 1993). Conventional piglets had a higher intake of PUFA from colostrum and mother milk than germ-free piglets from cow milk; that could contribute to the differences in the results between these two groups. The amount of growth factor somatomedin was higher in both experimental groups than that in the control groups, and this resulted in the higher weight of experimental animals compared to the controls. These results are in agreement with the previous study of Kašteľ *et al.* (1999).

In the peripheral blood of the germ-free piglets of the experimental group, there were slightly increased mean counts of leukocytes and lymphocytes. CD4 cells on the third sampling in germ-free piglets showed a significant increase. During the experiment, the subpopulation of CD8 positive cells was slightly increased over the experimental conventional piglets while in germ-free piglets the differences between the animals of control and experimental groups were larger, and in the third sampling they were significant. Sanderson *et al.* (1995), Yaqoob and Calder (1995) reported a decrease in the lymphocyte proliferation in the animals fed the diet with increased amount of n-3 PUFA. Enrichment of immune cells with n-3 PUFA was demonstrated in rodents, chickens and humans (Fritsche *et al.*, 1991; Broard and Pascaud, 1993).

The administration of oil with increased amount of n-3 PUFA to conventional and germ-free piglets induced an increase in the concentration of docosahexaenoic acid and a decrease in arachidonic acid in the experimental group compared to the control; this leads to a decreased production of prostaglandins in coincidence with the literature data (Fritsche *et al.*, 1993). An increased supply of n-3 PUFA leads in the plasma lipids to increasing the representation of APA and DHA at the expense of AA (Herold and Kinsella, 1986). The mechanism of n-3 PUFA action on the plasma lipids has not been solved yet. Eicosapentaenoic acid and other n-3 PUFA extrude arachidonic acid from the membranous phospholipids, and so they influence the lipid metabolism (German *et al.*, 1985).

The administration of n-3 PUFA could favourably influence the treatment of inflammatory processes and increase the resistance of piglets to infections.

In conclusion it can be confirmed that the administration of n-3 PUFA in suckling piglets led to:

- increase in the level of n-3 PUFA in the blood serum,
- changes in the production of eicosanoids,
- increase in the level of the growth factor somatomedin ($P < 0.05$),
- increase in the concentrations of GLA, EPA and DHA in the blood and decrease in AA,
- increase in the numbers of CD4 and CD8 subpopulations of T lymphocytes and IgM presenting lymphocytes in peripheral blood,
- increase in the specific postvaccinal antibodies to Aujeszky's virus.

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ABSTRAKT

Účinok oleja so zvýšeným obsahom n-3 PNMK na niektoré metabolické a imunologické parametre u germ-free a konvenčných ciciakov

Cicajúcim konvenčným a germ-free prasiatkam bol aplikovaný olej s n-3 polynenasýtenými mastnými kyselinami (PNMK). Na základe stanovenia somatomedínu v krvnom sére bola v pokusnej skupine ciciakov signifikantne vyššia hladina rastového faktora. Z biologických ukazovateľov bolo zaznamenané signifikantné zvýšenie koncentrácie kyselín γ-linolenovej (GLA), eikozapentaenovej (EPA), dokozahexaenovej (DHA) a zároveň zníženie koncentrácie kyseliny arachidonovej (AA) v krvnom sére. Celkový počet leukocytov bol v priebehu experimentu vyšší v pokusnej skupine. Po vakcinácii boli u odstavčiat zaznamenané vyššie individuálne titre špecifických protilátok. Bolo dosiahnuté signifikantné zvýšenie absolútnych hodnôt B lymfocytov v periférnej krvi ($P < 0,05$) u konvenčných i germ-free prasiatok oproti kontrolnej skupine. Absolútny počet CD4 a CD8 lymfocytov v periférnej krvi vykazoval signifikantný nárast v porovnaní s kontrolnou skupinou zvierat. n-3 PNMK ovplyvňujú metabolizmus mastných kyselín a syntézu prostaglandínov, čo môže byť prínosom pri terapii zápalových procesov a pri znížení rizika infekčných ochorení u cicajúcich mláďat.

Kľúčové slová: ciciaky; n-3 PNMK; metabolizmus mastných kyselín; IgM lymfocyty; CD4, CD8 lymfocyty

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Effect of pelleting on digestibility and metabolisable energy values of poultry diet

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ABSTRACT: Digestibility of organic nutrients and metabolisable energy values of mash and steam pelleted complete feed mixture were investigated in an experiment with 24 individually followed Ross hybrid male chickens. Altogether four balance periods were compared. Pelleting increased apparent digestibility of all organic nutrients but the difference was significant ($P < 0.001$) only in the case of organic matter and crude fat. In the pelleted diet, percentages of classical metabolisable energy and of nitrogen-corrected apparent metabolisable energy in gross energy were higher by 2.07 and 2.00, respectively, than in the mash diet ($P < 0.001$).

Keywords: chickens; digestibility; metabolisable energy; pelleting of feed

Technological treatment of poultry feed by pelleting is relatively expensive and requires much energy; however, not all effects of this treatment on the nutritional value of processed feed have been fully investigated.

In an experiment performed by Pettersson *et al.* (1991), pelleting increased the water solubility of starch and crude protein but no effect on the solubility of dietary fibre was observed. In an *in vitro* experiment, Mercier and Guilbot (ex Calet, 1965) studied the digestion of maize starch by bacterial amylase using both pelleted and unpelleted maize. In the case of pelleted maize, the enzyme liberated six times more sugar after two hours of amylolysis than in the case of the mash form maize. Bolton (1960) did not find any differences in crude protein, fat and saccharide digestibility between mash and pelleted complete feed mixtures fed to broiler chickens.

In experiments described by Negm (1966), pelleting of feed mixture significantly increased digestibility of crude fat and of crude fibre while digestibility of organic matter, crude protein and nitrogen-free extract remained unchanged. In his experiment, the content of metabolisable energy (ME) increased by 2.17% ($P < 0.05$). Gürocak *et al.* (1973) observed that in the feed mixture with an increased proportion (57%) of maize and, thus, a higher content of energy (12.8 MJ ME/kg) pelleting did not show any marked effect on apparent digestibility; however, in the feed mixture with a lower content of energy (30% maize; 11.3 MJ ME per kg), pelleting resulted in an insignificant increase in digestibility of all organic nutrients. Laying hens fed crumbled pellets showed a slightly higher ($P < 0.05$) total tract digestibility of crude fat and starch than birds receiving the mash (Wahlström *et al.*, 1999).

Hussar and Robblee (1962) and Calet and Albessard (ex Calet, 1965) found the same contents of metabolisable energy (ME) in mash and pelleted feed. McIntosh *et al.* (1962) observed after pelleting an increase in ME by 4.3% in a diet containing 60% of maize. In three experiments reported by Reddy *et al.* (1961), ME contents of the pelleted ration were significantly higher than those of the same diet offered in the mash form. In experiments performed by Bayley *et al.* (1968a,b) the effect of pelleting on ME of complete feed mixtures was not quite explicit. Auckland and Fulton (1972) mentioned that for crumbles the ME value was about one percent higher than that for mash. As

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compared with grinding, pelleting of peas showed a positive effect, especially on the values of nitrogen-corrected apparent metabolisable energy (AME_n) and of starch digestibility (Carre *et al.*, 1991).

The objective of this paper was to study the influence of steam pelleting on the nutritional value of commercial feed mixture.

MATERIAL AND METHODS

The effect of steam pelleting on apparent digestibility of organic nutrients and metabolisable energy values of the feed mixture was studied in four two-day balance periods using 24 Ross hybrid male chickens. At the age of 65 d the chickens were kept in individual balance cages. Chickens were fed a practical-type broiler finisher diet of the following composition (in g/kg): maize meal (600), wheat meal (110), soybean meal (170), sunflower meal (20), meat-and-bone meal (50), torula yeast (30), supplementary premix (10), mineral premix (8), and sodium chloride (2). The mixture contained 949.6 g of organic matter, 216.2 g crude protein, 41.5 g crude fat, 26.6 g crude fibre, 665.3 g nitrogen-free extract and 20.750 MJ gross energy per 1 kg dry matter. In the first part of the experiment, i.e. till the age of 78 days, chickens in even-numbered cages were fed an unprocessed mash diet while those in odd-numbered cages received pellets made of the same batch of feed mixture. In the second part of the experiment, i.e. till the age of 85 days, both diets were exchanged. Pellets with the diameter of 5 mm and crushing strength 5.1 N were manufactured in a commercial technological line Bühler.

The values of apparent digestibility of organic nutrients and metabolisable energy were determined by the total collection method. Excreta were collected daily for each 48 h balance period (Day 75 and 76; 77 and 78; 82 and 83; 84 and 85). This means that each bird received the mash diet in two balance periods and the pelleted diet also in two periods.

The content of dry matter, crude protein, crude fat, crude fibre, ash and nitrogen-free extract in feed and freeze-dried excreta was estimated according to AOAC (2001). The content of uric acid was determined indirectly according to Ekman *et al.* (1949). The combustion heat was determined by oxygen bomb calorimetry.

When calculating coefficients of apparent digestibility, the content of urine was expressed only as the content of uric acid; contents of other urine components were not taken into account. The values of apparent metabolisable energy were calculated either according to the classical formula (AME) or on the basis of a correction for nitrogen equilibrium (AME_n). In the first case, total energy of excreta is subtracted from gross energy of feed while in the second one a correction factor of 36.55 kJ/g retained nitrogen was used (Titus, ex Titus *et al.*, 1959).

Average values of the coefficients of digestibility and metabolisable energy were calculated using data recorded in 24 birds always in two balance periods for the mash diet and two for the pelleted feed. Average coefficients for mash and pelleted diets were compared in individual birds. The paired *t*-test was used to determine the significance of average difference (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Apparent digestibility of organic nutrients of pelleted mixture was higher than that of unpelleted one (Table 1); in organic matter it increased by 1.76% and the corresponding values for crude protein, crude fat, crude fibre and nitrogen-free extract were 1.01%, 12.36%, 0.85% and 0.70%, respectively. Average differences in digestibility of organic matter and crude fat were highly significant ($P < 0.001$) while in other organic nutrients *t*-values did not reach the level of significance. While Bolton (1960) was unable to find any differences in digestibility of organic nutrients in mash and pellets, our results were similar to those mentioned by Negm (1966) and Wahlström *et al.* (1999). In the feed mixture used in the present experiment, maize starch showed the highest proportion in the content of energy. In accordance with the findings mentioned by Carre *et al.* (1991) and *in vitro* by Mercier and Guilbot (ex Calet, 1965), improved digestibility of organic matter present in pelleted mixture can be associated not only with significantly higher digestibility of crude fat but, especially, with higher digestibility of starch.

Pelleting increased the percentage of AME in gross energy of the feed from 70.01 ± 0.415 to 72.08 ± 0.402 and that of AME_n from 66.79 ± 0.369 to 68.79 ± 0.373. The increase in coefficients of metabolisable energy was highly significant ($P < 0.001$). We could not corroborate data presented by Hussar and Robblee (1962) and by

Table 1. Apparent digestibility of nutrients and metabolisable energy values

Determined value	Feed mixture		Average difference ± standard error of the average difference	t-value
	unpelleted	pelleted		
	mean ± standard error of the mean[1)]			
Digestibility of				
organic matter	0.744 ± 0.0036	0.762 ± 0.0039	0.018 ± 0.0033	5.34***
crude protein	0.809 ± 0.0044	0.819± 0.0041	0.010 ± 0.0053	1.89
crude fat	0.752 ± 0.0075	0.876 ± 0.0049	0.124 ± 0.0075	16.48***
crude fibre	0.094 ± 0.0153	0.102 ± 0.0195	0.008 ± 0.0251	0.34
nitrogen-free extract	0.749 ± 0.0478	0.756 ± 0.0049	0.007 ± 0.0052	1.34
Metabolisability coefficients				
AME	0.700 ± 0.0042	0.721 ± 0.0040	0.021 ± 0.0032	6.49***
AME_n	0.668 ± 0.0037	0.688 ± 0.0037	0.020 ± 0.0032	6.31***

AME = apparent metabolisable energy
AME_n = nitrogen-corrected apparent metabolisable energy
[1)] $n = 24$; ***$P < 0.001$

Calet and Albessard (ex Calet, 1965), who observed that pelleting did not influence ME of the feed mixture. Our results agree with data published by Reddy *et al.* (1961), McIntosh *et al.* (1962), Negm (1966), Auckland and Fulton (1972) and Carre *et al.* (1991). This means that steam pelleting of our commercial feed mixture resulted in an increase in its energy content.

An insufficient energy content in the feed mixtures is often a limiting factor for the full use of production capacity of animals. The non-pelleted and pelleted feed mixtures (with 88% DM) used in our experiment contained 12.198 MJ AME_n/kg and 12.563 MJ AME_n/kg, respectively; this means that the content of AME_n in the pelleted diet was higher by approximately 3.0%. The substitution of a part of cereals with feeding fat is a common method used to increase the content of energy in feed mixtures. Provided that wheat and feeding fat contain 12.9 MJ and 35.0 MJ AME_n/kg, respectively, it is possible to say that the effect of pelleting on AME_n content equals to a change in the energy value of feed mixture resulting from the substitution of 1.65% of wheat with feeding fat. This increase in energy content, which of course is not the only positive result of pelleting, compensates a great part of costs associated with pelleting.

The nutritional value observed in mash diet cannot be mechanically transferred to pelleted diet. When formulating diets it is also necessary to take into account if they will be fed unpelleted or in the form of pellets. If the pelleted mixture has a higher energetic value, the content of crude protein must also be increased to preserve the correct energy/protein ratio.

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ABSTRAKT

Vliv granulování na stravitelnost a metabolizovatelnou energii krmné směsi pro kuřata

V pokuse s 24 individuálně sledovanými kohoutky hybridní kombinace Ross jsme ve čtyřech bilančních obdobích porovnávali stravitelnost živin a metabolizovatelnou energii netvarované kompletní krmné směsi a směsi upravené do granulí. Koeficienty bilanční stravitelnosti všech organických živin se při úpravě krmiva granulováním zvýšily, rozdíl však byl průkazný ($P < 0,001$) pouze u organické hmoty a tuku. Procentický podíl klasické metabolizovatelné energie z brutto energie byl u granulovaného krmiva o 2,07 a metabolizovatelné energie opravené na dusíkovou rovnováhu o 2,00 vyšší ($P < 0,001$) než u směsi netvarované.

Klíčová slova: kuřata; stravitelnost; metabolizovatelná energie; granulování krmiv

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Prediction of commercial classification values of beef carcasses by means of the bioelectrical impedance analysis (BIA)

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ABSTRACT: The objective of the experiment was to determine the precision of the prediction of commercial classification values (conformation and fatness) of beef carcasses by means of the bioelectrical impedance analysis (BIA). The method was applied on 441 carcasses using an automatic measuring system with flat elastic electrodes with the surface area of 30 cm^2 located on the outside of the left half-carcass. Resistance and reactance at two frequencies, i.e. 1 kHz and 100 kHz, were registered. A number of impedance values show significant correlations to the conformation of the carcass. Among all evaluated categories, the strongest correlation ($r = 0.86$) was found between conformation and the value D^2/Xcp100k (relationship between the square of the distance between electrodes and the calculated parallel reactance at 100 kHz). Significant relations were also identified for fatness, particularly in the females. The highest correlations were found in values derived from the calculated parallel reactance Xcp1k, measured at the frequency of 1 kHz. The calculated formulae for the prediction of conformation show a considerably high positive index of determination of $R^2 = 0.85$ for all categories. The formula for the prediction of fatness provides a satisfactory determination index of $R^2 = 0.70$ in the female categories.

Keywords: bioelectrical impedance analysis; beef; carcass; classification

Several significant successes have lately been reported in the classification of beef carcasses, or their commercial classification according to quality. This concerns the objectification of these activities thanks to the use of instrumental classification that is sometimes fully automatic; see e.g. Autofom for pig carcasses (Branscheid *et al.*, 1997), BCC 2 (Madsen, 1996; Allen, 1999) or VSB2000 for beef carcasses (Branscheid *et al.*, 1999a,b). Nevertheless, it is useful to look for other methods that will help enhance the parameters of the existing systems or that can be used independently in smaller abattoirs where it is impossible to use the existing costly systems.

The method of bioelectric impedance analysis has been applied in research projects so far. Among the recent publications, there is a study presented by Balcaen *et al.* (2002) examining the ability of the BIA method to estimate carcass lean content of Belgian Blue bulls and to examine the impact of time after slaughter and the location of electrodes. The method was considered relatively precise and practically applicable. Allen *et al.* (2002) examined the accuracy of the BIA method in the estimation of lamb carcass composition. The values of BIA were found to be useless for an accurate estimate. The author does not specify the type of electrodes and the method of processing the measured resistance and reactance values prior to their incorporation in variables for regression models. Hegarty *et al.* (1998) used the multi-frequency method for the same purposes and found on the contrary that the

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BIA measurements taken in warm lamb carcasses explained 87% of the variation of saleable yield with an SEE of 0.74 kg. The measurement of beef carcass composition with the use of an analyser with flat electrodes is described by Bohuslávek and Pipek (2002). For instance, the best regression model for the prediction of loin weight was reported by the coefficient of determination of $R^2 = 0.88$. Based on the positive results obtained by the estimate of beef carcass conformation in Bohuslávek *et al.* (2000) and the estimate of beef carcass composition in Bohuslávek and Pipek (2002), further experimental measurements were carried out with the innovated BIA device within the operating environment of an abattoir. The objective of the experiment was to determine the quality of the prediction of commercial grading with the use of a measuring device with flat electrodes. Another objective was to check the reliability of new electrodes in the measurement carried out at an abattoir line with the capacity of 100 carcasses per hour and the use of compensation for temperature by means of contactless temperature measurement on the surface of beef carcasses.

MATERIAL AND METHODS

The experimental measurements were carried out in the company Südfostleisch, Altenburg, Germany, on 9–11 July, under normal operation. The impedance values were always measured on the left side of moving beef carcasses. Simultaneously, beef carcass classification was in progress on the line, with the use of the installed VBS2000 device, manufactured by E&V, based on the method of video image analysis.

Description of carcass collection

In total, 458 beef carcasses were measured. Of them, 17 carcasses were rejected due to extensive damage during processing and possible distortion due to the contact between the half-carcasses or incorrect operation of the equipment. In total, the evaluation comprised 441 beef carcasses, of them 198 young bulls, 7 bulls, 214 cows and 22 heifers. The physical and electrical characteristics for carcasses of the group of evaluated animals are summarized in Table 1.

Description of measuring method and device

The measurement of bioelectric impedance was carried out with the use of an impedance analyser described in Bohuslávek *et al.* (2002). The entire measuring system comprises three independent parts: 1. Telescopic electrode adapter with an infrared thermometer, 2. Automatic impedance measuring device, 3. Process control PC. For the circuit layout of the measuring system see Figure 1.

Table 1. Characteristics of the group of evaluated 441 animals

Category	198 young bulls + 7 bulls		214 cows		22 heifers	
	mean	SD	mean	SD	mean	SD
CW ... warm carcass weight (kg)	366.20	63.70	281.18	58.13	247.25	53.17
D ... distance of electrodes (cm)	159.24	6.57	160.21	6.38	149.67	8.7
Rp1k (ohm)	133.20	16.03	192.5	24.47	189.02	27.02
Rp100k (ohm)	82.08	11.59	121.39	16.94	117.56	18.69
D^2/Xcp1k (cm^2/ohm)	–7.50	1.66	–5.74	1.55	–5.12	1.29
Conformation subclasses* (15)	9.63	2.6	13.13	1.72	12	1.87
Fatness subclasses* (15)	6.28	1.59	6.39	2.59	6.76	2.28

*Grading system EUROP (divided into 15 subclasses) for conformation and fatness, respectively

Rp1k = parallel resistance at a frequency of 1 kHz

Rp100k = parallel resistance at a frequency of 100 kHz

Xcp1k = parallel reactance at a frequency of 1 kHz

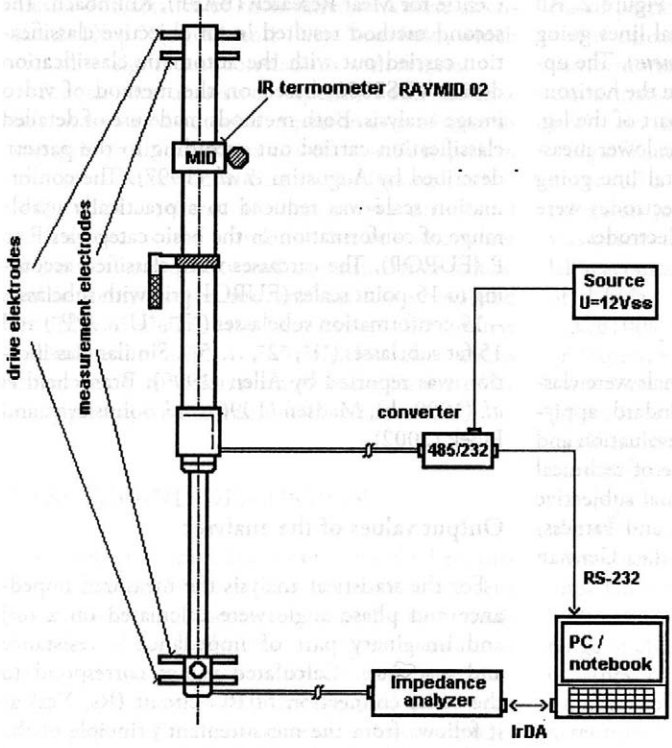

Figure 1. Schematic diagram of measuring system

The functional properties are as follows:
- measurement of impedance and phase angle at the frequency of 1 kHz and 100 kHz
- maximum value of measurement current of 5 mA
- measurement with the use of 4-electrode measuring method
- flat elastic electrodes with a Cu-foil, of the surface area about 30 cm^2 (150 × 20 mm)
- galvanic separation of impedance analyser circuits from the mains
- fast attendance of electrodes on a common adapter
- visual reading of a distance between the measuring electrodes
- automatic measurement of beef carcass (surface) temperature
- connection and communication with PC, measurement process control and archiving of measured data
- the period of measurement, i.e. from the application to the removal of adapter, must be shorter than 3 seconds. This restriction is based on the maximum permissible track of the measured carcass (1 m)

The mechanical design of the electrode adapter, including the method of measuring a distance between the electrodes, was identical to the experiments described by Bohuslávek (2002). The measuring instrument was charged from NiMh batteries and it communicated with the central computer through an IrDA interface. Hence the galvanic selection from the grid was simply provided and higher resistance to the interference was assured. The programme of the central computer used only two frequencies (1 and 10 kHz) for measurements. The programme automatically archived the transmitted data from the analyser (impedance, angle) and the data were manually inserted into the PC (number of measured carcass, distance of electrodes, carcass length) into ASCII tables and Excel format.

Selection of measuring points

The positions of electrodes were selected in order to ensure simple anatomic definition, easy access and good contact of electrodes. Electrodes were situated at the outsides of the left half-carcasses.

For the position see the scheme in Figure 2. All electrodes were placed on the vertical lines going through the heel tendon (*tendo calcaneus*). The upper measuring electrode was placed on the horizontal line going through the broadest part of the leg, i.e. on the *musculus vastus lateralis*, the lower measuring electrode was on the horizontal line going through *tuber olecrani*. The drive electrodes were placed 85 mm from the measuring electrodes.

Reference values

The carcasses of the examined animals were classified according to the SEUROP standard, applying two methods: "subjective" visual evaluation and "objective" classification with the use of technical facilities. The first method is the usual subjective commercial grading (Conformation and Fatness) in the EU by an expert classifier, i.e. German Centre for Meat Research (BAFF), Kulmbach. The second method resulted in an objective classification carried out with the automatic classification device VBS2000, based on the method of video image analysis. Both methods made use of detailed classification carried out according to the pattern described by Augustini *et al.* (1997). The conformation scale was reduced to a practically usable range of conformation in the basic categories E to P (EUROP). The carcasses were classified according to 15-point scales (EUROP grid with subclasses – 15 conformation subclasses ($^{+}E^{-}$, $^{+}U^{-}$, ...$^{+}P^{-}$) and 15 fat subclasses ($^{+}1^{-}$, $^{+}2^{-}$, ...$^{+}5^{-}$). Similar classification was reported by Allen (1999), Branscheid *et al.* (1999a,b), Madsen (1996) and Bohuslávek and Pipek (2002).

1, 4 – drive electrode
2, 3 – measurement electrode

Figure 2. Placement of electrodes on the carcasse surface

Output values of the analyser

For the statistical analysis the measured impedance and phase angle were calculated on a real and imaginary part of impedance – resistance and reactance. Calculated values correspond to the serial connection of RC circuit (Rs, Xcs) as it follows from the measurement principle of the analyser. The simplest biological equivalent model is a single resistor and capacitor in parallel (Vrana, 1989). Therefore the values of serial circuit need to be calculated from the relation that is presented for example in Vrana (1989), Bohuslávek *et al.* (2002) for the resistance and reactance of parallel circuit (Rp, Xcp).

Processing of results, statistical evaluation

The computer-controlled measuring process carried out all measurements of impedance values three times, calculated the mean values and standard deviations and archived all data in the form of tables. This is why it was possible to remove all values exceeding the standard deviation from the impedance analysis data. Some of them were already provided with a note on an incorrect application of electrodes or a mutual contact of the beef half-carcasses. In addition, the impedance data were completed with the calculated values, e.g. the D^2/R_p, L^2/R_p as electrical volume (Nyboer *et al.*, 1943; Lukaski, 1985, 1986; Swantek *et al.*, 1992, Hegarty *et al.*, 1998), with generally very good correlation to carcass weight. Moreover, the relative

values of reactance or their differences were calculated (1Xcp/Xcp100k and 1Xcp-Xcp100k), which can show significant correlations with fatness.

The statistical analysis of impedance and reference data was carried out with respect to a set of animals comprising all categories as well as with respect to two sets divided according to sex (young bulls + bulls, cows + heifers). The analysis was carried out with respect to the correlation between the values of beef carcass quality and the impedance values. Subsequently, regression analysis was completed in order to identify the best regression formulae for the estimation of conformation and fatness categories. Estimation formulae were calculated by multiple stepwise regression analysis.

RESULTS AND DISCUSSION

A number of impedance values for the beef carcass conformation show mutual correlations. For all categories and a separate group of male animals (young bulls + bulls), the strongest correlations (r = 0.86) were identified between conformation and the value D^2/Xcp100k (relationship between the square of the distance between the electrodes and the calculated parallel reactance at 100kHz). For female categories (cows + heifers), the correlations between the conformation and impedance are not so strong, e.g. the highest correlation coefficient r = 0.71 was identified for Xcp1k, i.e. for parallel reactance at the frequency of 1 kHz. This is most likely the result of limitation of the conformation classes of the categories cows + heifers, concentrated particularly in classes R, O and P. As usual, warm carcass weight was highly correlated with conformation. For the correlations between the conformation determined according to BAFF and E&V and impedance values see Table 2.

Significant relations were also found for the fatness of all categories and particularly for the female categories (cows + heifers). The highest correlation was determined for the values derived from parallel reactance Xcp1k, measured at the frequency of 1 kHz. The difference in reactance Xcp1k – Xcp100k also shows a considerable dependence on fatness; e.g. in the category of cows + heifers the correlation coefficient was r = 0.71 for the fatness determined by a BAFF expert. The dependence is significantly better than that in fatness determined according to E&V, therefore this value can serve for the improvement of the E&V estimate. Similarly like in the conformation in female categories, only a low correlation was identified between fatness and impedance values in the male categories (young bulls + bulls). This can also be due to the narrow range of classification, i.e. particularly in the range of fatness concentrated in classes 1, 2 and 3. For a summary of the correlations between the fatness determined according to BAFF and E&V, and impedance values see Table 3. Table 4 shows the correlations of impedance values with beef carcass weight. This is a clear sign of the application of electrical volume, calculated from parallel reactance measured at a frequency of 1 kHz.

Using the outcome of correlation analysis, regression analysis was carried out in order to identify the best regression models – formulae for the estima-

Table 2. Pearson's correlations for conformation determined according to BAFF and E&V

	Category YB+B+C+H		Category YB+B		Category C+H	
	conf. Baff	conf. E&V	conf. Baff	conf. E&V	conf. Baff	conf. E&V
CW	–0.85	–0.85	–0.79	–0.81	**–0.74**	–0.76
Xcp1k	–0.77	0.75	–0.79	–0.79	–0.71	–0.71
D^2/Rp100k	–0.82	–0.81	–0.73	–0.74	–0.60	–0.63
D^2/Xcp100k	**0.87**	**0.86**	**0.83**	**0.83**	0.67	0.69
Xcp100k	–0.77	–0.75	–0.81	–0.79	–0.61	–0.62

Xcp100k = parallel reactance at a frequency of 100 kHz YB = young bulls B = bulls
Rp100k = parallel resistance at a frequency of 100 kHz C = cows H = heifers
all correlation coefficients at a significance level $P < 0.001$

Table 3. Pearson's correlations for fatness determined according to BAFF and E&V

	Category YB+B+C+H		Category YB+B		Category C+H	
	fatness baff	fatness E&V	fatness baff	fatness E&V	fatness baff	fatness E&V
CW	0.49	0.50	0.45	0.29	**0.76**	0.58
Xcp1k	0.52	**0.54**	0.40	0.25	0.70	0.58
Xcp1k/D	0.52	0.53	**0.41**	**0.30**	0.69	0.56
(Xcp1k – Xcp100k)	**0.54**	**0.54**	0.40	0.26	0.71	**0.59**

all correlation coefficients at a significance level $P < 0.001$

Table 4. Pearson's correlations between impedance values and beef carcass weight

	Category YB+B+C+H	Category YB+B	Category C+H
D^2/Xcp1k	**–0.912**	**–0.91**	**–0.87**
D^2/Rp100k	0.894	**0.91**	0.80
D^2/Xcp100k	–0.867	–0.89	–0.71
Xcp1k/D	0.852	0.87	0.83

all correlation coefficients at a significance level $P < 0.001$

Table 5. Regression models for beef carcasses

Model No.	Dependent variables	Independent variables	Equations	r^2/r^{**}	SEE*
1.	Conformation* (15)	CW D D^2/Rp100k D^2/Xcp100k	Conformation = $6.726 - 2.427e^{-02} * CW + 8.487*10^{-02}*D + 2.034*10^{-02} * D^2/Rp100k + 9.652*10^{-02} * D^2/Xcp100k$	0.85/0.92	1.08
2.	Fatness* (15)	CW Rp100k, D (Xcp1k –Xcp100k)	Fatness = $9.057 + 3.87*10^{-2}* CW + 4.93*10^{-2}*Rp100k - 0.102*D + 7.29*10^{-4}*$ (Xcp1k –Xcp100k)	0.70/0.84	1.42

*SEE = standard error of estimation

r^2/r^{**} = coefficient of correlation/determination

all regression coefficients in the equations are significant at $P < 0.001$

tion of conformation and fatness. The formulae were examined for the quality commercial grades determined by the BAFF expert, which can be considered to be more precise. Table 5 shows the best two calculated models, one for the estimate of conformation and the other for fatness. The formula for the estimation of conformation in all categories shows a very high determination index of $R^2 = 0.85$. Model 2 presents a formula for the estimation of fatness in female categories

and shows a satisfactory determination index of $R^2 = 0.70$.

CONCLUSION

The results of the experiment confirmed that the BIA method is suitable for practical use in the instrumental evaluation of the commercial value of beef carcasses. With respect to the satisfactory results of the fatness estimate, it is also possible to expect that BIA can be applied as a complementary device with the VIA system (video image analysis). The estimate of fatness is rather poor for devices on the basis of VIA. So far, no correlation of the measured impedance values has been carried out on the basis of different temperatures of beef carcasses as the used contactless measurement of the beef carcass surface temperature was subject to the negative impact of disturbing factors (differing emissivity, water spraying, cutting at the measuring point, etc.). After the successful solution of this problem, further improvement in the estimate of the commercial value of beef carcass is expected. There is a further potential for development in the estimates of fat ratio, i.e. the fatness class, where it is possible to complement the impedance values by the specific conductivity of muscle tissue and thus compensate the effects of temperature, age or method of feeding.

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ABSTRAKT

Odhad hodnot obchodních tříd jakosti jatečně upravených těl skotu pomocí bioelektrické impedanční analýzy (BIA)

Cílem pokusu bylo vyšetření přesnosti odhadu obchodních tříd jakosti (zmasilosti a protučnělosti) pomocí bioelektrické impedanční analýzy (BIA). Metoda byla aplikována na 441 kusů jatečně upravených těl (JUT) skotu s využitím automatizovaného měřicího systému s pružnými plošnými elektrodami o ploše 30 cm^2, které byly umisťovány na vnější stranu levé poloviny JUT. Biolektrický analyzátor měřil sériovou rezistenci a reaktanci na dvou frekvencích 1 kHz a 100 kHz. analyzátoru. Významné korelační závislosti vykazuje řada impedančních veličin k zmasilosti JUT. Pro všechny zkoumané kategorie zvířat byly nalezeny nejsilnější korelace (r = 0,86) mezi zmasilostí a hodnotou D^2/Xcp100k (poměr kvadrátu distance elektrod a vypočítané paralelní reaktance při 100 kHz). Významné vztahy byly také nalezeny pro protučnělost všech zvířat a především pro samičí kategorie. Nejvyšší korelace vykazují veličiny odvozené z vypočítané paralelní reaktance Xcp1k měřené při frekvenci 1 kHz. Vypočítané rovnice pro odhad zmasilosti vykazují u všech kategorií velmi příznivý index determinace R^2 = 0.85. Rovnice pro odhad protučnělosti u samičích kategorií vykazuje uspokojivý index determinace R^2 = 0.70.

Klíčová slova: bioelektrická impedanční analýza; skot; jatečně upravené tělo; klasifikace

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Growth of bream, *Abramis brama*, in the Croatian section of the Danube

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ABSTRACT: The growth of bream (*Abramis brama*) was analyzed from a commercial catch in the Danube near the mouth of the river Drava, Croatia. The standard length (*L*)-weight (*W*) relationship demonstrated a *b*-value of 3.01. According to bream's stable condition factor (*CF*) of 2.44 ± 0.24 throughout its life, the population was well supplied with food. The back calculated growth in standard length at age *t* (L_t) could be expressed by the formula: $L_t = 57.7\ (1 - e^{-0.087\ (t + 0.885)})$. This growth almost coincides with the average growth of the species and the average absolute length increments during the first ten years confirm it.

Keywords: growth; common bream; *Abramis brama*; Danube; Croatia

In the last two decades the reduction of flooded areas around the middle course of the Danube has been registered. That caused the reduction of spawning and feeding habitats for many fish species. The proportion of benthivores is lowered from 59% to 26% and that of phytophils from 82% to 48% (Maletin and Djukic, 1998). Both, commercial and recreational fisheries are important in this part of the river (Bacalbasa-Dobrovici *et al.*, 1998; Treer *et al.*, 1999). Without commercial fisheries the non-cultured species, such as bream (*Abramis brama*), are never likely to reach the market, as anglers are not allowed to sell their catch. The analysis of commercial harvests in 2001 and 2002 shows that bream is the most abundant fish species and takes about 30% in the mass of the catch in the Croatian part of the Danube.

The growth of bream was studied by many authors (e.g. Kangur, 1996; Specziar *et al.*, 1997; Matenova *et al.*, 1998; Tierney *et al.*, 1999). Živkov *et al.* (1999) defined four principal types of bream length growth: those with asymptotic standard lengths (L_∞) up to 59 cm, typical for cool and northern water basins, but also for stocks with high initial growth rate; those with moderate growth rate with L_∞ between 60 and 80 cm; two types with too high asymptotic values, with no biological significance. The aim of this paper was to investigate bream's growth and population condition in the Croatian part of the Danube where no ichthyological research was done for more than a decade.

MATERIAL AND METHODS

The bream for this investigation were taken out of a commercial catch from the Danube near the mouth of the river Drava, Croatia, on February 12th, 2001. Altogether 59 specimens were caught, out of which 25 were males and 34 females. They were immediately measured for standard length (*L* in cm) and weight (*W* in g). Scales for age determination were taken from above the lateral line below the anterior part of the dorsal fin. Reading of scale rings was done in Scion Image program under a microscope connected to the computer screen by a video camera.

The growth rate of bream was estimated by growth zones on scales and found from back-calculated lengths (Bagenal and Tesch, 1978). The von Bertalanffy growth function (VBGF) was used to fit the values of growth in length, while phi-prime (Φ') was used to study the overall growth performance (Sparre and Venema, 1992):

$$L_t = L_\infty (1 - e^{-K(t-t_0)})$$

$$\Phi' = \ln K + 2 \ln L_\infty$$

where: L_t = standard length at age t
L_∞ = ultimate standard length that an average fish would achieve if it continued to live and grow
K = growth coefficient that determines how fast the fish approaches L_∞
t_0 = hypothetical age for $L_t = 0$
Φ' = overall growth performance

Absolute annual length increments (i_n) and average absolute length increments (i_{1-10}) in cm during the first ten years of life were computed, as suggested by Živkov *et al.* (1999).

To establish the length-weight relationship the commonly used $W = aL^b$ was applied (Ricker, 1975), where W = weight in grams, L = standard length in cm, and a and b are constants. The condition factor (CF) was calculated as follows:

$$CF = W \times L^{-3} \times 100$$

Both sexes were combined in our calculations. In order to make these results possible to compare with those results whose authors used fork length (Hickley and Dexter, 1979), the following equation was established:

$$L = 0.072 + 0.892\ FL\ (P < 0.01)$$

where: L = standard length
FL = fork length in cm

RESULTS AND DISCUSSION

The females caught ranged from 24.7 to 46.1 cm in standard length and males from 23.9 to 43.7 cm. The weight of females ranged between 340 and 2 800 g and that of males between 310 and 2 200 g. The length-weight relationship (Figure 1) demonstrated a b-value of 3.01 ($W = 2.4 \cdot 10^{-5}\ L^{3.01}$; $r = 0.923$; $P < 0.01$). Consequently, the regression between CF and standard length does not exist at all ($r = 0.084$; $P > 0.05$), which stands to prove the statement of Kangur (1996) that the condition of bream is considerably stable during the whole of its lifespan. In the same paper the author concluded that the average CF of 2.09 in the Estonian eutrophic lake Peipsi indicated that the population was well supplied with food. The bream's even higher CF of 2.44 ± 0.24 for the Croatian section of the Danube leads to the same conclusion. Some difference in CF between males (2.33 ± 0.20) and females (2.52 ± 0.23) was registered, but it was not statistically significant ($P > 0.05$), probably due to the winter catch.

The back calculated growth in length of the bream of the Danube, presented in Table 1, which covers 13 age classes, shows large overlaps typical of common bream (Mooij *et al.*, 1996), varying between individuals and between years. It could be expressed by the following formula:

$$L_t = 57.7\ (1 - e^{-0.087\ (t + 0.885)})$$

According to Živkov *et al.* (1999) bream populations with this asymptotic length (L_∞ between

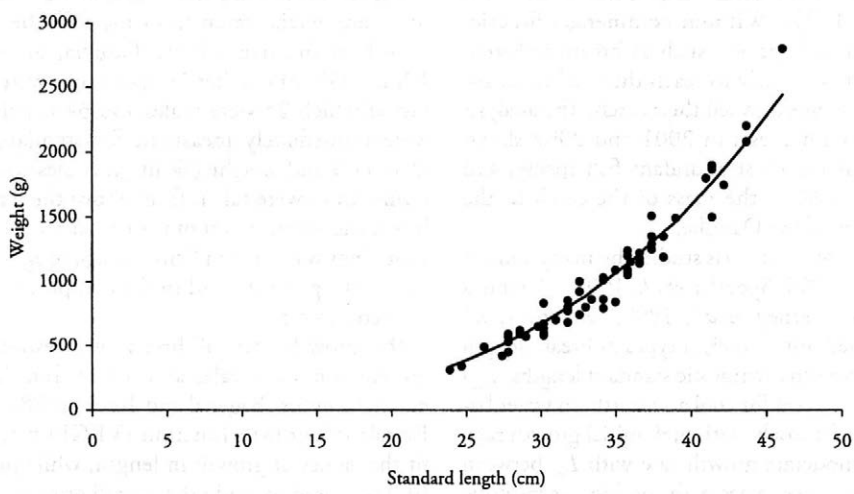

Figure 1. Standard length-weight relationship of common bream from the Croatian section of the Danube

Table 1. Back calculated standard lengths (cm) of common bream in the Croatian section of the Danube

Age group	n	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}	L_{11}	L_{12}	L_{13}	L_{14}	L_{15}	L_{16}
IV	2	6.9	12.3	17.2	21.3												
V	1	9.1	14.5	19.2	22.5	24.7											
VI	9	8.5	13.8	17.9	21.5	24.2	26.5										
VII	6	8.5	14.3	18.6	22.7	25.1	27.3	29.2									
VIII	6	7.6	12.8	16.6	20.1	23.1	25.8	28.2	30.0								
IX	8	8.7	13.7	17.7	21.2	24.0	26.5	28.6	30.6	32.1							
X	3	7.4	12.2	17.2	20.4	23.8	26.4	28.5	30.3	32.0	33.4						
XI	8	8.1	12.6	16.7	20.2	23.2	25.7	27.7	29.7	31.5	33.2	34.8					
XII	8	7.9	12.6	16.6	19.7	22.7	25.1	27.4	29.7	31.7	33.6	35.1	36.5				
XIII	3	9.1	14.2	18.3	22.1	25.9	28.7	30.9	32.7	34.6	36.4	37.9	39.3	40.6			
XIV	2	7.2	12.7	16.2	19.7	22.3	24.6	26.9	28.9	30.9	32.6	34.6	36.7	38.0	39.8		
XV	2	7.6	13.2	18.1	21.4	24.1	26.2	28.3	31.0	32.9	35.3	37.2	38.8	40.2	41.8	43.0	
XVI	1	8.7	14.1	18.7	22.7	25.4	27.4	29.4	31.4	33.4	35.5	37.5	39.5	41.4	42.8	44.1	45.5
Total	59																
Mean		8.1	13.3	17.6	21.2	24.0	26.4	28.5	30.5	32.4	34.3	36.2	38.2	40.1	41.5	43.6	45.5

Table 2. Absolute annual standard length increments (i_n) and average absolute standard length increments ($\bar{i}_{1-10}$) in cm during the first ten years for common bream from the Croatian part of the Danube (this paper), two reference curves (Hartvich and Kubečka, 1989 and Hickley and Dexter, 1979), two fast growing populations (Matenova *et al.*, 1998 and Kangur, 1996) and one slow growing population (Specziar *et al.*, 1997)

Location	Source	i_1	i_2	i_3	i_4	i_5	i_6	i_7	i_8	i_9	i_{10}	$\bar{I}_{1-10}$
Croatian part of the Danube	this paper	8.1	5.2	4.3	3.6	2.8	2.4	2.1	2.0	1.9	1.9	3.43
Average increments reference	Hartvich and Kubečka, 1989	6.11	5.49	4.66	3.99	3.41	2.92	2.52	2.16	1.87	1.62	3.48
Standard growth for British Isles	Hickley and Dexter, 1979	4.53	4.31	4.00	3.77	3.57	3.38	3.20	3.03	2.86	2.36	3.49
De Gijster reservoir, The Netherlands	Matenova *et al.*, 1998	8.2	6.1	6.6	5.4	4.1	3.1	3.9	2.4	1.8	1.7	4.33
Lake Peipsi, Estonia	Kangur, 1996	8.0	5.0	5.0	4.0	5.0	4.0	1.0	3.0	3.0	2.0	4.00
Lake Balaton, Hungary	Specziar *et al.*, 1997	4.2	3.0	3.2	3.3	3.2	3.1	3.2	2.6	1.7	0.7	2.82

Table 3. Comparison of growth parameters for common bream, *Abramis brama*, obtained from different data sources

Location and source	t (years)	Asymptotic standard length in cm (L_∞)	Curvature parameter (k)	Overall growth performance (Φ')
Croatian Danube (this paper)	16	57.7	0.087	5.67
Lake Balaton (Specziar *et al.*, 1997)	10	50.1	0.083	5.34
103 populations (Živkov *et al.*, 1999)	10	62.3	0.0986	5.95
Average lengths reference (Hartvich and Kubečka, 1989)	11	44.5	0.153	5.71

25.5 and 59.0 cm) belong to biological realistic type *b*. They found that 40% of bream stocks belong to this type, which are characterized by rather high growth rates during the first years of life. Similar high initial lengths were found in eutrophic lake Peipsi (Kangur, 1996) and De Gijster reservoir (Matenova *et al.*, 1998). The Croatian section of the Danube is situated near the mouth of the river Drava, close to the vast, rich in zooplankton swamps of National Park Kopački rit. As common bream belongs to the guild of potamal species, which shift from shoreline (eating zooplankton) to mid-channel habitats (eating zoobenthos) during the first year of life (Wolter and Bischoff, 2001), the young-of-the-year bream had fairly good nutritional conditions.

After the fourth year, the growth in length slowed down, but remained constant, like that of the bream from lake Peipsi (Kangur, 1996). Specziar *et al.* (1997) found out that the bream in the Balaton lake kept unchanged growth rate over 22–24 cm of standard length. Compared to reference growth curves for common bream, by the average absolute length increments ($\bar{i}_{1-10}$) in cm during the first ten years (Table 2), the growth of the Danube population ($\bar{i}_{1-10}$ = 3.43) is almost the same as the average growth of the species ($\bar{i}_{1-10}$ = 3.48), presented by Hartvich and Kubečka (1989). Both curves are also very close or rather equal ($\bar{i}_{1-10}$ = 3.49) the bream from the British Isles (Hickley and Dexter, 1979).

The phi-prime of bream in the Croatian Danube is Φ' = 5.67. This value is very close to those obtained from other localities (Table 3). These data confirm the reliability of bream growth curve as the overall growth performance (Φ') has minimum variance within the same species and does not depend on different growth rate (Moreau *et al.*, 1986). However, due to this fact, this index is not suitable for other growth comparisons (Hopkins, 1991; Živkov *et al.*, 1999).

The results of this investigation show that the growth in length of common bream in the Croatian part of the Danube is fairly good and that it nears the average of the species. The condition of the fish is above the average throughout the life. Tierney *et al.* (1999) stated that fish species diversity was greater and total fish biomass was significantly higher in Irish waters with faster growing bream populations. Although there is a lack of thorough information about the ichthyofauna of the Croatian Danube region, such information could indicate its good state, but also needs to be proved by further research. The investigation of Šorić (1999), performed downstream in the Yugoslav part of the Danube, showed similar results on the very different fish species. He concluded that barbel (*Barbus barbus*) in the middle course of the Danube grows at an exceptionally fast rate.

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ABSTRAKT

Růst cejna velkého, *Abramis brama*, v chorvatské části Dunaje

Provedli jsme rozbor růstu cejna velkého (*Abramis brama*) z komerčního úlovku z Dunaje poblíž ústí řeky Drávy v Chorvatsku. Hodnota exponentu *b* vztahu mezi délkou a hmotností byla 3,01. Na základě stabilního koeficientu vyživenosti (*CF*) u cejna velkého dosahujícího hodnoty 2,44 ± 0,24 po celou dobu jeho života lze usoudit, že populace je dostatečně zásobena potravou. Zpětně vypočtený růst délky těla (L_t) ve věku t lze vyjádřit následujícím vztahem: $L_t = 57.7(1 - e^{-0.087\ (t + 0.885)})$. Tento růst je téměř shodný s průměrným růstem tohoto druhu a potvrzují ho i průměrné absolutní délkové přírůstky během prvních deseti let.

Klíčová slova: růst; cejn velký, *Abramis brama*; Dunaj; Chorvatsko

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Relationship between age, milk production and order of goats during automatic milking

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ABSTRACT: 139 Slovak White goats were included in an experiment that was conducted in 3 periods from July to September. The average age and lactation stage of goats at the beginning of the first period were 1 380 days (46 months) and 167 days. We used a parallel milking parlor for eight goats. Two observers recorded the order of goats at entering the milking parlor. The control measurement of milk production was performed on the following day after the observation period. After finishing the experiment, out of the total number of 139 animals 24 goats were selected that entered the parlor as the first (group F) in all observations and 24 goats that entered the parlor as the last (group L). We found significant differences in average daily milk production between the individual periods (1st period 1 209 ml; 2nd period 1 516 ml; 3rd period 1 061 ml). Significant correlations between the order of entry into the milking parlor and milk production were found in all three periods (r = 0.237**; 0.235**; 0.400**). The correlation between the order and age was significant only in the third period (r = 0.244*). Group F consisted of 52% horned goats, group L of 21% only. Group F had 63% of goats at the age of 4–5 years and only 8% animals at the age of 1–2 years. 42% goats of group L were at the age from 4 to 5 years, and 33% at the age from 1 to 2 years. Goats of group F produced more milk than the animals of group L. Significant differences between the groups were observed especially at the third lactation. The results indicate that the behavior of goats when entering the milking parlor is influenced by the age and level of milk production. The age and milk production of goats can have a significant impact on their order at machine milking.

Keywords: goats; milking order; milk production; age

Social order, rank, dominance and hierarchy are terms that are frequently used for the phenomenon that within a pair of animals the behavior of one animal can be inhibited by the other, and for the resultant complex of relationships found in groups of animals. Most people also agree that dominance will have consequences that can be important for the management of domestic animals, just as dominance clearly affects the survival and reproduction of wild animals, but many authors reported difficulties with the phenomenon (Beilharz and Zeeb, 1982). Goats form small but stable groups in the open air and they live at a certain place (Dunbar *et al.*, 1990). Fournier and Festa-Bianchet (1995) studying female mountain goats found that goats were organized in a non-linear but non-random dominance hierarchy, with many reversals in rank. The best morphological predictor of dominance rank was horn length at one year and body weight in the following year. Age was a weaker predictor of dominance status than what was reported for other female ungulates. On the contrary, Cote (2000) found that social rank is strongly related to age and did not decrease for the oldest females. Two major functions have been proposed for social dominance. First, it can allow dominant individuals priority of access to a limited resource. Second, it can reduce the level of aggression in a group. In the production environment for farm animals, the groups are formed by the breeder according to his own needs and criteria. In spite of the gradually changed reactions in these animals under the influence of domestication they have their inborn needs which originate in the wild way of life in

large herds, and as mentioned by Lickliter (1984), they did not lose their fighting mood and aggressiveness. The well-arranged social life creates better preconditions of meeting the necessaries of animals living in a group. It is not necessary to have always an ideal hierarchy in larger herds of goats, however, Fournier and Festa-Bianchet (1995) mentioned that the order of individuals is not incidental. Certain time is necessary to create the social order in the group during which the more dominant goats gain leading position by their behavior (Mowlen, 1988). The incorporation of the individual into the social scale of the group defines its behavior at meeting the other members of the group. The most aggressive animals are those that occupy the highest positions within the social hierarchy (Barroso *et al.*, 2000). It is obvious that the social order can manifest itself markedly in the reduced space in the course of waiting for milking.

There is a number of factors which affect the position of the individual in a herd. Age, large size and horns seem to be the physical factors that most favor dominance. The goats struggle hard for their position, the horned ones are much more aggressive. Such situations occur at milking, too. If the herd is composed of horned individuals, the length of horns and size of body are crucial (Addison and Baker, 1982; Fournier and Festa-Bianchet, 1995). Graser-Herrmann and Sambraus (2001) proved social ranking in three herds of dairy sheep. The social rank was correlated with both the age and the live body weight. Each animal constantly takes up more or less the same position in the order. Settled social relations within the herd affect also the production parameters. Milk performance in relation to the position of animal in the social system of herd was studied by Dúbravská (1996) in goats and by Soffié *et al.* (1976) in dairy cows. The most aggressive animals are those that occupy the highest positions within the social hierarchy (Barroso *et al.*, 2000). It is obvious that the social order can manifest itself markedly in the reduced space in the course of waiting for milking.

One of the parameters of social order in dairy cows is their place in the milking parlor. Possible applications of detailed knowledge of the milking order in farming practice could be realized (Wasilewski, 1999). The order in which goats voluntarily choose to enter the milking parlor could have implications for the speed of throughput of animals. Secondly, an uncharacteristically late entry into the parlor can often be related to health problems and can therefore be used as an indicator, prompting further examination.

Mácha (1983) found the heritability of social order expressed by the place in milking parlor $h^2 = 0.2$ to 0.3. Repeatability at the upper level of heritability was 0.52 to 0.74. The relationship between order during milking and milk yield was determined to be from 0.46 to 0.60. The author also reported that in dairy cows which enter the milking parlor among the first animals a decrease of production is lower compared with the last animals after they are transferred into another stable.

The aim of this study was to test the hypothesis that the order of goats at machine milking is influenced by the age and level of milk production in the later lactations.

MATERIAL AND METHODS

The study was performed on a commercial goat farm in a submontane region. We used 139 goats of the Slovak White Polled race (Saanen type) at the age of 1–5 years (n = 17, 14, 40, 28, 40). The herd was divided into two sub-herds (seventy and sixty-nine animals) at the beginning of the grazing season (second week of May).

The observations were performed in 3 periods during the grazing season with dry and hot weather (July to September). The first period started in 7 weeks after the beginning of the grazing season (after dividing the herd into two sub-herds). In each period there were three observations (totally 9 observations). The interval between the individual periods was 28 days. In the first (July) and second (August) period the animals in two stable sub-herds were grazed and milked separately. During the third (September) period both groups were joined together into one group, grazed and milked together. The average age and lactation stage of goats at the beginning of the first period were 1380 ± 508 days (46 months) and 167 ± 16 days. We used parallel milking parlor AMA 227 for eight goats (Agrostroj Pelhřimov, Czech Republic).

The ethological observations were always performed on three consecutive days. Observations on the order of goat entry into the milking room were made during morning milking from the start of milking (as soon as the first cow entered the parlor) to the end of the milking operation. Two observers recorded the order of goats at entering the milking parlor. All the goats were identified by their ear-tag

numbers. The observers assigned the order number to each goat according to the number of standing in the milking parlor. The first animal got number one, the last animal number eight.

The animals got a concentrate mixture as a supplement at milking in free choice. The feed was added continually so as to ensure that the manger was always full. The control measurement of milk production was performed according to the Instructions for Milk Performance Recording in Goats in the Slovak Republic (Margetin, 1995) on the following day after the observation period.

The studied factors were age (noticed at the first observation), milk production and order of entering the milking parlor. We calculated the average order of milking (from three observations) for the entire period. From those averages we calculated correlations between order and age and between order and milk performance.

During the data processing after the experiments finished, out of the total number of 139 animals 24 goats were selected that went as the first (group F) in all observations and 24 goats that always entered the parlor as the last (L group). Both specially formed groups were compared for the observed factors.

The data were analyzed using an analysis of variance. Significant differences between means were tested by Tukey's test. We also used descriptive methods for mathematical evaluation and regression analysis (Simple regression, model Linear) for the calculation of correlation coefficient. The results were processed with a statistical package STATISTIX (Analytical Software, P.O.Box 12185, Tallahassee, FL 32317-2185, USA).

RESULTS AND DISCUSSION

Evaluation of entire herd

In the herd of 139 goats there were 68 horned ones (48%). Twelve percent of goats were at the age of 1 year, 23% were two years old, 34% three years old, 29% four years old and only 2% were five years old. We found significant differences in the average daily milk production between the individual periods (1st period 1 209 ml; 2nd period 1 516 ml; 3rd period 1 061 ml). We ascribe the increase in average daily production during the second period to the factors of the surroundings (Table 1). The observations were performed in the second period in August when it rained more intensively after a dry period, the conditions on pasture improved and the goats had a better supply of nutrients. We found significant correlations between the order of entering into the milking parlor and milk production (Table 2) in all three periods (r = 0.237**; 0.235**; 0.400**). For the calculated relationships, the correlation between order and age was significant only in the third period (r = 0. 244*).

Table 1. The average of daily milk yield (ml) of the whole herd during periods of observation (n = 139)

Period	$\bar{x}$	$s_{\bar{x}}$	Minimum	Maximum	F
1	1 209	461	240	3 910	33.54***
2	1 516	530	300	3 010	1 : 3*
3	1 061	375	180	2 190	2 : 1, 3***

*P < 0.05; **P < 0.01; ***P < 0.001

Table 2. Correlations between factors and order of goats (n = 139)

Factor	1st period	2nd period	3rd period
Age	0.126	0.074	0.244*
Milk production	0.237**	0.235**	0.400**

*P < 0.05; **P < 0.01

Selected groups

Out of the total number of 139 animals we selected 48 goats after finishing the experiments. They were divided into two groups. The first group (F) consisted of 24 animals that went to the milking parlor as the first in all observations, the group L (last) comprised 24 goats that always entered into the parlor as the last. Eight percent of goats in group F were at the age of 2 years, 29% were three years old, 42% four years old and 21% were five years old. The age composition of group L was as follows: 33% goats were one year old, 4% two years old, 21% three years old, 13% four years old and 29% animals were at the age of five years. The group of the first 24 animals in order (F) comprised

Table 3. The average of daily milk yield (ml) in groups F and L

Order of lactation	Period of observations	Group	n	$\bar{x}$	$s_{\bar{x}}$	F-test	Probability
1	1	F	0	–	–		
		L	8	542	286		
	2	F	0	–	–		
		L	8	734	204		
	3	F	0	–	–		
		L	8	522	191		
2	1	F	2	895	78	0.07	0.8366
		L	1	920	–		
	2	F	2	1 290	28	14.08	0.1658
		L	1	1 160	–		
	3	F	2	1 000	28	3.00	0.3333
		L	1	940	–		
3	1	F	7	1 500	149	23.57***	0.0007
		L	5	1 106	120		
	2	F	7	1 940	430	8.42*	0.0158
		L	5	1 334	202		
	3	F	7	1 261	229	19.32**	0.0013
		L	5	782	90		
4	1	F	10	1 400	297	1.50	0.2457
		L	3	1 140	417		
	2	F	10	1 767	272	6.21*	0.0299
		L	3	1 352	137		
	3	F	10	1 100	305	1.06	0.3248
		L	3	900	240		
5	1	F	5	1 410	465	0.09	0.7697
		L	7	1 342	320		
	2	F	5	1 610	327	0.38	0.5491
		L	7	1 710	235		
	3	F	5	1 478	226	6.16*	0.0325
		L	7	1 263	50		

The first 24 animals in order (F), the last 24 animals in order (L)
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

52% horned goats, the group of the last 24 animals in order (L) 21% only.

Milk production of groups F and L during individual recordings after finishing the observation periods is shown in Table 3. The animals are distributed according to lactations. There were no animals from the 1st lactation in group F. If we disregard the first period at the 2nd lactation for a low number of goats, the milk yield of group F is lower than that of group L in the 2nd period of goats at the 5th lactation only (1 610 ml versus 1 710 ml). Goats of group F produced more milk in all others periods. Significant differences between the groups were determined especially in animals at the third lactation.

It ensues from the evaluation of milking production from the 2nd to 5th lactation (Table 4) that goats at the 2nd lactation performed the least (group F 1 062 ml, group L 1 007 ml). The most productive goats at the all lactations were from group F. A significant difference was found only at the third lactation (1 567 ml against 1 074 ml).

We compared milk yield of goats also during individual periods (July, August, September). The milk production was lower in goats of group L compared with the goats of group F within the individual periods (Table 5). The difference represents 185 ml in the first period, 254 ml in the second one, 193 ml in the third one. The lowest production was found in the third period (September). Group F milked out 1 217 ml on average and group L 1 024 ml. This is in harmony with prolonged lactation. The highest milk performance in both groups was during the second period (group F 1 745 ml and group L 1 491 ml). Marked differences, on the level up to 600 ml, are mainly in the values of maximum production.

Table 4. Milk yield according to the lactation

Lactation	Group	n	$\bar{x}$	$s_{\bar{x}}$	Minimum	Maximum	F-test
2	F	6	1 062	187	840	1 310	4.92***
	L	3	1 007	133	920	1 160	
3	F	21	1 567	401	980	2 570	F : L**
	L	15	1 074	270	670	1 510	
4	F	30	1 422	395	627	2 350	
	L	9	1 131	318	630	1 620	
5	F	15	1 499	338	1 100	2 230	
	L	21	1 438	296	840	1 990	

n = number of milk recordings
*$P < 0.05$; **$P < 0.01$

Table 5. Milk yield according to the period of observation

Period	Group	n	$\bar{x}$	$s_{\bar{x}}$	Minimum	Maximum	F-test
1	F	24	1 389	321	840	2 230	13.96***
	L	16	1 204	294	840	1 620	
2	F	24	1 745	360	1 270	2 570	2F : 3L*
	L	16	1 491	278	1 000	1 990	
3	F	24	1 217	291	627	1 770	
	L	16	1 024	246	630	1 340	

n = number of milk recordings (goats from the first lactation are not included)
*$P < 0.05$; ***$P < 0.001$

We know from practical experience that the highest amount of milk is gained during the first periods of herd milking. We suppose therefore that goats with higher milk production push each other more in the waiting room and enter the parlor sooner than goats with lower milk production. This is ascribed to a dominant position of high-producing goats in the group. However, it is not clear and we did not find it in available scientific literature whether the milking order is manifested also during the second half of lactation when less milk is produced.

The comparison of animals in groups F and L showed that out of 24 goats that came first to be milked (F) 63% were at the age of 4 to 5 years but only 8% at the age of 1 to 2 years. Out of the last 24 goats (L) 42% were at the age of 4 to 5 years, and as much as 33% were one-year old goats. This fact is also confirmed by the positive correlation in each studied period (Table 2). Statistically significant correlation between the order of goats at milking and their age was found in the third period ($r = 0.244^*$) only, when both sub-herds were joined together and the competition was bigger. The composition of groups changed at each milking, therefore the goats had to fight for their position more intensively. Older and more experienced goats were at the advantage. In spite of the fact that the groups were formed accidentally, the more dominant individuals pushed themselves forward at the barriers and pushed the weaker animals away. The stability of female social rank over time is an important element in welfare during milking (Tancin and Brukmaier, 2001).

Husbandry practices in the last two decades have changed drastically. However, little attempt has been made to study how the various changes influence goat behavior and thus milk production. The major change has been to keep goats in bigger herds, and it is important to know how this influences behavior, specifically in the milking rooms. The obtained results confirm the fact that the older goats come first to be milked while the younger goats come later. This finding is also supported by the results of Cote (2000), but Fournier and Festa-Bianchet (1995) insist that the age is not such a significant factor.

The dominance in the group is created to achieve a certain position. As the goats get supplementary concentrates at milking, the dominant animals had a greater possibility to get feed in larger amount than the goats that came to the stand later. Lack of pasture and presence of concentrates in the manger of the stand can be a great motivation for the goats to get a profitable position at milking and to push the weaker individuals away from the order. Miller and Harley (1992) noticed that certain individuals are more able to receive the feed earlier and in particular to choose the feed of better quality compared with the rest of individuals in the given group. Syme *et al.* (1974) pointed out the importance of sufficient amount of feed in relation to the stability of hierarchy and rise of aggressivity at lack of food. Our results can support this opinion. The concentrate mixture is a very strong motivation.

We found a more significant relation by testing the dependence of hierarchy in goats at milking and amount of produced milk. Our results show the earlier lining up of animals with higher milk production compared with animals with lower production that are pushed back at milking. The dependence of the studied factors is statistically significant in all three periods of observations (Table 2). Dúbravská (1996) found out on the contrary that the goats with higher performance were less aggressive and pushing, and they were probably on lower stages in the hierarchy of the herd. A significant relationship between rank and milk yield was found in sheep (Graser-Herrmann and Sambraus, 2001). The authors wrote that East Friesian dairy sheep have quite a marked marching and milking order. Soffié *et al.* (1976) did not find any correlation between the milk production and order at milking with dairy cows either. According to Barroso *et al.* (2000), the dominance of the dairy goat significantly influences its production, with middle-ranking animals the most productive, and those of lower rank, as well as high-status animals, less productive.

We observed markedly lower milk production in goats of group L compared with the goats of group F within the individual periods (Table 5). The obtained results confirmed that age and milk production influence the sequence at entering the milking parlor. However, it is necessary to point out another important fact. The social position of the individual within a group (herd) also markedly influences the intake of feed and its amount. Its lack causes the rise of aggressiveness in all animals. The age and experience presumably play a role. However, the animals with higher position have a better precondition to get the feed. The breeders give the concentrate mixture into mangers as a stimulator during the milking. The lack of pasture during our studies (the hot and dry summer) and the presence of concentrates in mangers were strong

stimuli for goats to get a profitable position during milking and for pushing the weaker and mostly younger individuals aside.

Better access of dominant animals to the concentrates influences in turn the level of their milk production. We wanted to avoid this, therefore, all goats in our experiment had the same possibility to receive the feed in the parlor. Similarly like Sambraus and Keil (1997), who found that dairy goats assumed roughly the same position at each milking, uniformity of ranks was particularly evident at the beginning and at the end of milking.

Except milk performance, age and size of body, horns are also considered important for a good position of goat in the herd (Rathore, 1982). There were 52% horned ones (13) in group F and only 21% (5) in group L in our experiment. But this proved that not all horned animals take advantage of this given fact for a social position. The results of Keil and Sambraus (1996) are interesting in this context. Amongst the horned group of dairy goats, 3 083 cases showing dominant behavior were registered, amongst the hornless group 2 304 cases. We suppose that milking order is closely connected with social dominance. We would like to revert to it in further research, because this relationship has not been scientifically documented. Because the goats used in our experiment were in the second half of lactation, this can be another suggestion for the research on the effect of early lactation on milking order.

The results indicate that the order of goat entry into the milking room is influenced by the age and level of milk production in the later lactation. The age and milk production of goats can have a significant impact on their order at machine milking.

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ABSTRAKT

Vzťah medzi vekom, mliečnou produkciou a poradím kôz počas automatického dojenia

Použili sme 139 kôz plemena slovenské biele. Pozorovania boli prevedené v troch periódach od júla do septembra. Priemerný vek a štádium laktácie boli na začiatku prvej periódy 1 380 a 167 dní. Použili sme paralelnú dojáreň pre 8 kôz. Dvaja pozorovatelia zaznamenávali poradie pri vstupe zvierat do dojárne. Kontrola úžitkovosti sa robila po skončení pozorovania. Po ukončení experimentu sme počas spracovania výsledkov vybrali z celkového počtu 139 zvierat 24 kôz, ktoré vstupovali do dojárne vždy ako prvé (skupina F) a 24 kôz, ktoré vstupovali vždy posledné (skupina L). Zistili sme preukazné rozdiely medzi dojivosťou v jednotlivých periódach (1. perióda 1 209 ml; 2. perióda 1 516 ml; 3. perióda 1 061 ml). Medzi poradím vstupu do dojárne a mliečnou úžitkovosťou boli preukazné korelácie vo všetkých troch periódach (r = 0,237**; 0,235**; 0,400**). Korelačný koeficient medzi poradím a vekom bol preukazný len v tretej perióde (r = 0,244*). V skupine F bolo 52 % kôz rohatých, v skupine L len 21 %. Skupinu F tvorilo 63 % kôz starých 4 až 5 rokov a len 8 % zvierat bolo vo veku 1 až 2 roky. V skupine L bolo 42 % zvierat vo veku 4 až 5 rokov a 33 % vo veku 1 až 2 roky. Kozy skupiny F nadojili viac mlieka než zvieratá skupiny L. Preukazné rozdiely boli najmä medzi zvieratami na tretej laktácii. Výsledky naznačili, že správanie kôz pri vstupe do dojárne je ovplyvnené vekom a úrovňou dojivosti. Vek a mliečna úžitkovosť kôz môže mať preukazný dopad na ich poradie pri strojovom dojení.

Kľúčové slová: kozy; poradie pri dojení; mliečna produkcia; vek

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